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Electrotarsogram responses to synthetic odorants by *Varroa destructor*, a primary parasite of western honey bees (*Apis mellifera*)

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Abstract
Olfaction is a key sensory modality for many arthropods and could be used as a tool in pest management through manipulation of pest behavior. Management of *Varroa destructor*, important parasitic mites of honey bees, could be improved through better understanding of the chemical ecology of this host-parasite relationship. We refined techniques of mounting mites to obtain electrophysiological recordings (electrotarsograms) of their responses to synthetic odor stimuli. Results of 271 electrotarsogram recordings from *V. destructor* revealed responses to 10 odorants relative to solvent controls. Electrotarsogram responses to methyl palmitate, ethyl palmitate, and 2-heptanol were highest at the lowest stimulus loading (10 ng) we tested, suggesting that *V. destructor* may have acute sensitivity to low concentrations of some odors. Results suggest that odorant origin (e.g., methyl oleate from honey bee larvae, geraniol from adult honey bee alarm pheromone, and α-terpineol, a plant secondary metabolite) can influence the degree of electrophysiological response. *Varroa destructor* tended to be more responsive to known attractants and repellents relative to previously unexplored odorants and some repellent terpenes. Electrotarsograms offer the potential for screening odors to determine their importance in *V. destructor* host detection.

Keywords Acari · *Apis mellifera* · Electrophysiology · Electrotarsography · Semiochemicals

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Introduction

Western honey bees (*Apis mellifera* L.; Hymenoptera: Apidae; hereafter honey bees) are the most widely used pollinators of agricultural crops (Morse and Calderone 2000; Klein et al. 2007). *Varroa destructor* Anderson and Trueman (Acari: Varroidae) is an invasive parasitic mite of honey bees originally from southeast Asia. It is currently considered the most economically important and challenging threat to apiculture (Currie et al. 2010; Rosenkranz et al. 2010; Nazzi and LeConte 2016; Ferland et al. 2017). For example, in 2007, up to 85% of overwintering colony mortalities in some parts of Canada were ascribed to *V. destructor*, although globally there is likely greater variability in reported colony mortalities (Currie et al. 2010). Honey bee colony mortalities can be highly variable within and among countries, and the relative proportion of these mortalities attributable to *V. destructor* is unclear (Gray et al. 2019).

Current *V. destructor* management often involves using synthetic miticides, organic acids, essential oils, or non-chemical methods (Calderone 1999; Imdorf et al. 2003; Currie et al. 2010; Ferland et al. 2017). However, miticide-resistant *V. destructor* have repeatedly evolved (Rosenkranz et al. 2010; van der Zee et al. 2012), and other chemical or non-chemical methods can have variable efficacy or cause honey bee worker and queen mortality (Melathopoulos et al. 2000; Underwood and Currie 2003). A possible management alternative involves manipulation of *V. destructor* behavior using in-colony volatiles that are important in the mite's life cycle (Foster and Harris 1997; Yoder and Sammataro 2003; Pernal et al. 2005; Plettner et al. 2017).

Varroa destructor move within and among honey bee colonies primarily by orientation to volatile compounds associated with honey bee hosts (Martin et al. 2001; Frey and Rosenkranz 2014; Nazzi and LeConte 2016). Primary chemosensory organs of *V. destructor* are on the first tarsi, situated in a cluster of sensilla similar in structure to Haller's organ in ticks (Rickli et al. 1992, 1994; Dillier et al. 2003, 2006). There have been few attempts to quantify electrophysiological responses of these sensory organs to putative attractants or repellents (but see Endris and Baker 1993; Dillier et al. 2003).

Varroa destructor alternates between phoretic stages on worker honey bees and reproductive life stages within honey bee brood cells (Boot et al. 1994; Kather et al. 2015). *Varroa destructor* reproductive cycles closely coincide with those of honey bee brood (Boot et al. 1994; Plettner et al. 2017), and honey bee brood odors play a crucial role in *V. destructor* host choice and physiology during *V. destructor* mite reproduction (Trouiller et al. 1992; Milani et al. 2004; Pernal et al. 2005; Nazzi et al. 2006; Frey et al. 2013; Singh et al. 2016). Thus, it may be possible to manage *V. destructor* through manipulation of in-hive honey bee semiochemicals (Donzé et al. 1998; Yoder and Sammataro 2003; Plettner et al. 2017).

Approximately 60 chemicals have been identified that modify *V. destructor* behavior (supplementary Tables S1–S6). However, experiments typically have not tested colony-relevant concentrations of these chemicals (Boot 1994; Rickli et al. 1994; Donzé et al. 1998; Pernal et al. 2005). Quantification of sensory acuity to ecologically relevant odors through concentration-responses may provide a better interpretation of how *V. destructor* navigates colony environments (Del Piccolo et al. 2010).

We tested *V. destructor* responses to several known attractants and repellents using an electrotarsogram (ETG) protocol that we adapted from Endris and Baker (1993). We used the modified ETG protocol with odorants presented in a decadic series of concentrations previously explored in *V. destructor* behavioral research. Odorant concentrations were

selected based upon those found from honey bee and larval cuticle extractions or volatile collections (Calderone and Lin 2001; Martin et al. 2002; Gilley et al. 2006; Schmitt et al. 2007; Thom et al. 2007; Del Piccolo et al. 2010; Carroll and Duehl 2012).

Methods

Mite collection

From June through August 2017 and 2018, six Langstroth bee hives provided by two local beekeepers located in Wolfville (45.0918° N, 64.3598° W) and Berwick (45.0452° N, 64.7347° W), Nova Scotia (NS), Canada were used to rear drone brood via queen-trapping (Human et al. 2013). Collection and maintenance of *V. destructor* followed Dietemann et al. (2013). Drone frames containing brood were transferred from donor colonies to an untreated *V. destructor*-infested queenright colony in Coldbrook, NS (45.0585° N, 64.5925° W). After drone frames were capped, they were collected and transferred to controlled environmental chambers (32 °C, 65% relative humidity; Conviron Model E-16, Controlled Environments, Winnipeg, Manitoba, Canada) at Acadia University. Live adult worker honey bees, at a ratio of 2:1 for every drone, were used to maintain colony structure and *V. destructor* longevity within environmental chambers. Queen mandibular pheromone (Intko Supply, Vancouver, British Columbia, Canada) was applied and allowed to evaporate for 5 min on a glass coverslip every 48 h at a concentration of 0.1 queen equivalents (42.2 ng in 10 µL of 2-propenol) to promote honey bee health and longevity (Grozinger et al. 2007). Adult worker and drone bees were transferred in groups of 10–20 into wooden hoarding cages (17 × 12 × 13 cm) using a vacuum (DCV517B; DeWalt, Baltimore, MD, USA) modified into a bee aspirator (Rogers and Williams, pers. comm.). Transferred bees were then individually examined for phoretic *V. destructor*. *Varroa destructor* were transferred from adult honey bees, using both a moistened paintbrush and aspirator, into 50-mL falcon tubes (Thermo Fisher Scientific, New York, NY, USA) containing 2 × 4 mm moistened filter paper. Five mites were held in each tube. All ETG experiments were performed on *V. destructor* the day they were collected.

Odorant stimuli preparation

ETGs were conducted using a range of odorants that evoke behavioral responses in *V. destructor* mites, and other odorants with unknown attractiveness or repellence (valence) to *V. destructor* mites (Table 1). A subset of these odorants was tested in decadic series (10^0, 10^1, 10^2, and 10^3 ng μL^{-1}) to capture a range of concentrations that *V. destructor* would likely encounter in colony environments (Kraus 1990; Le Conte et al. 1990; Martin et al. 2002; Del Piccolo et al. 2010).

Stimulus cartridges were prepared from serial dilutions of stock compounds using HPLC grade hexane as solvent (Sigma-Aldrich, St. Louis, MO, USA) (Pinnelli et al. 2016; Singh et al. 2016). All compounds were analyzed through gas chromatography mass spectrometry (GCMS) to ensure samples were free of contaminants before developing stimulus cartridges. All serial dilutions were made in 2-mL glass vials sealed with Teflon tape and thereafter stored at −20 °C. Stimulus cartridges were prepared by pipetting diluted compounds onto ethanol-washed filter paper strips cut into 1 × 3 cm pieces (Fisherbrand P8, 90 mm diameter) (Fisher Scientific Company, Ottawa, Ontario, Canada). A stimulus

Table 1 Previous literature examining *Varroa destructor* behavioral responses to putative attractants and repellents tested in this study

Stimulus	Colony origin	Bioassay response	Citation
Methyl oleate	Brood cuticle	Attractant	Trouiller et al. (1992)
Methyl palmitate	Brood cuticle	Attractant	Trouiller et al. (1992)
Ethyl palmitate	Brood cuticle	Attractant	Le Conte et al. (1989)
2-Heptanol	Bee alarm	Repellent	Kraus (1990)
2-Nonanol	Bee alarm	Repellent	Kraus (1990)
Geraniol	Bee alarm	Repellent	Hoppe and Ritter (1988)
2-Heptanone	Bee alarm	NA	Blum (1996)
Benzoic acid	Royal jelly	NS	Nazzi et al. (2004a, b)
Octanoic acid	Royal jelly	Repellent	Nazzi et al. (2009)
Nonanal	Bee cuticle	NA	Torto et al. (2005)
Heptadecane	Bee cuticle	NS	Pernal et al. (2005)
Butyric acid	NA	Attractant	Teal et al. (2014)
α-terpineol	NA	Repellent	Peng et al. (2015)
Citronellol	NA	NA	Endris and Baker (1993)
Trans-nerolidol	NA	NA	Carr and Roe (2016)
Linalool	NA	NA	Tutun et al. (2018)

NA information not available in cited literature, *NS* non-significant findings

cartridge was prepared for each compound at 10^1-, 10^2-, 10^3-, and 10^4-ng loadings, using the decadic series described above. Stimulus cartridges were prepared by loading 10 µL of each dilution of an odorant onto filter papers, individually inserted into disposable borosilicate glass pipettes (Fisherbrand 14.6 cm) and capped with 1-mL plastic pipette tips. Serial concentrations of each odorant were grouped together and wrapped in aluminum foil. All grouped stimuli were placed in freezer bags and stored at −20 °C until use. All odor stimulus cartridges were brought to 25 °C before use. Each odorant series was used on a maximum of four individual *V. destructor* preparations or otherwise stored for a maximum of 4 days at −20 °C before being replaced with new treated filter paper.

Additional odorants were tested at a single stimulus cartridge loading (10^4 ng) to *V. destructor* preparation due to time constraints. *Varroa destructor* ETG responses towards these odorants (α-terpineol, linalool, butyric acid), tea tree and, yarrow essential oils (*Melaleuca alternifolia* Cheel and *Achillea millefolium* L., respectively) were later compared to those tested in decadic series at the same concentration.

Electrotarsography

Mites were chilled for 2–3 s and then mounted on a microscope slide coated in clear dental wax (Electron Microscopy Sciences, Hatfield, PA, USA). A single *V. destructor* was placed on its dorsum without pressing into the dental wax and held in place with two parallel, horizontally-positioned minuten pins (Ento Sphinx, Černá za Bory, Czech Republic) to restrict movement (Fig. 1).

ETG recordings were performed through electrotarsography using a design adapted from single sensillum recordings (Dillier et al. 2003). Changes in electrical potential were

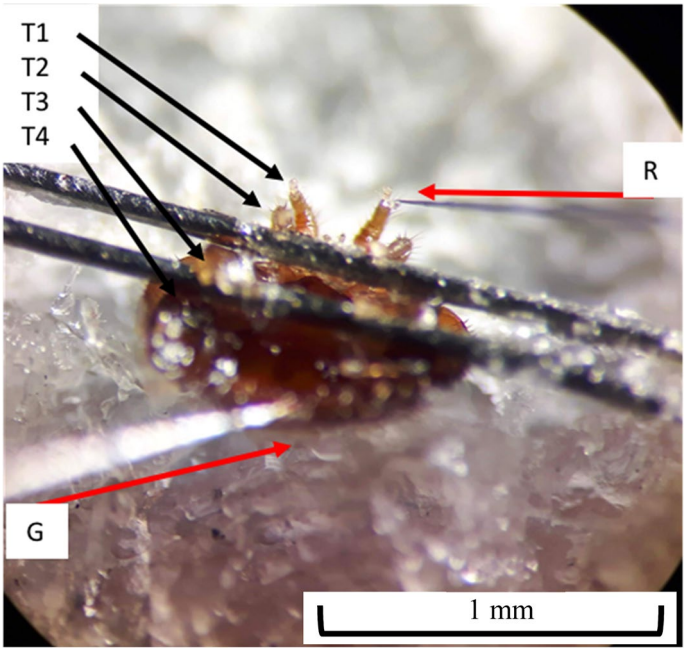

Fig. 1 Electrotarsogram preparation of a live female *Varroa destructor* with T1–T4 indicating tarsi, *R* recording electrode, and *G* ground electrode

measured from either the left or right foretarsus using tungsten recording electrodes. The ETG signal was collected and amplified (Low Cut-off: 0.05 Hz, Offset: 0, Ext amp: 10) by Intelligent Data Acquisition Controller-2 (IDAC-2) (Ockenfels Syntech, Buchenbach, Germany). Before recording, *V. destructor* preparations were positioned in front of humidified airflow (0.5 L min^{-1}) (Endris and Baker 1993). Small amounts of electrode gel (Signagel; Parker Laboratories, Fairfield, NJ, USA) were placed on prepared *V. destructor* anal plates and all tarsi except the foretarsi. Electrode gel placed on tarsi (T2 to T4) further restricted mite movements, improved recording sensitivity and may have reduced preparation desiccation (Syntech 2015). The ground electrode was inserted into the *V. destructor* anus at the base of the anal plate of the ventrum. The recording electrode was inserted just past the apotele of the foretarsus.

Individual stimuli were puffed in random series, with increasing concentrations presented of a stimulus (10^1-, 10^2-, 10^3-, and 10^4-ng loadings), before moving to the next stimulus in sequence. Solvent control stimuli preceded and followed each series of concentrations presented of each odorant. A single odorant puff lasted 0.3 s controlled by a Syntech stimulus controller CS-55 V2.7 (Ockenfels Syntech). Each stimulus cartridge was puffed in intervals of 30 s to allow for preparation recovery (Eliash et al. 2014). *Varroa destructor* preparations were exposed to 11 odorants in increasing concentration series, resulting in 55 individual stimulus recordings per mite including solvent controls. All ETG recordings from an individual *V. destructor* preparation were defined as a single trial. Variability among *V. destructor* preparations in electrical signal quality resulted in incomplete replication of some stimulus recordings. A decline in quality of electrical signal related to a gradual decline in *V. destructor* ETG responses and possible desiccation of preparations through time. To account for among-preparation variability in quality of electrical

connection, all data were normalized to the response to the hexane solvent control, linearly interpolated (see below for justification). Data were later refined to exclude responses less than the solvent control for all *V. destructor* preparations, resulting in an incomplete randomized design (see below for justification).

Statistical analysis

Peak amplitude for each odorant was recorded in mV and collected using GcEad ©2014 software v.1.2.5 (Ockenfels Syntech). Data were analyzed using R statistical software (R Foundation for Statistical Computing 2014). Responses to odor stimuli were identified as the first of two depolarizations (Endris and Baker 1993, Fig. 2). For longer ETG recordings where a depreciation in signal quality is observed through time, a linear interpolated value of responses towards solvent controls was used (Martin et al. 2002). Among-preparation variability in electrical responses relating to electrode placement and arthropod desiccation can occur (Syntech 2015). To account for these sources of variation, an equation adapted from Eliash et al. (2014) was used to calculate normalized responses to stimuli from linear interpolated ETG responses towards hexane solvent controls:

$$Normalized\ response\ (\%) = \frac{Response\ to\ stimulus\ (mV) - Response\ to\ solvent\ (mV)}{Response\ to\ stimulus\ (mV)} \times 100 + 100.$$

Aside from a gradual depreciation of signal quality over time, mite responses towards the solvent control stimulus should remain consistent. A lack of consistency suggests that other variables (e.g., mite preparation death or desiccation, room climatic changes, or poor equipment grounding) may have influenced consistency of solvent control responses (Syntech 2015). Mites that did not show a linear trend in responses to solvent controls due to large inconsistencies among responses (variability > 20 mV among solvent control stimuli) were removed from the analysis ($n = 12$).

We examined normalized responses, which included some mite ETG responses to odorant stimuli that were lower than mite responses to solvent controls. We then filtered normalized data to include only mite ETG responses towards odorant stimuli which were higher than responses towards solvent controls. Both complete and filtered data were subjected to the same statistical testing to account for possible bias in removal of data where

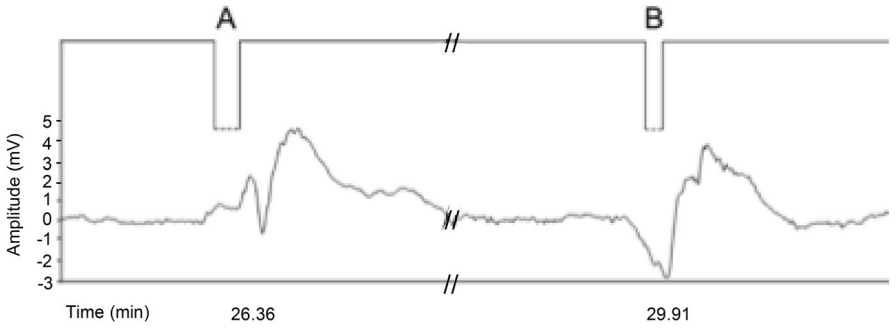

Fig. 2 *Varroa destructor* electrotarsogram responses represented as amplitude (mV) to solvent control (A) and 2-heptanone at 10^3 ng μL^{-1} stimulus concentration (B) with 30 s time intervals between stimulus administration

responses were weaker than to the solvent control. Normalization accounted for differences in among-mite preparation signal sensitivity compared to examining raw amplitude data. Following filtering of ETG responses, we had an unbalanced randomized design. Raw amplitude responses (mV), normalized data, and normalized data with values above solvent controls only were statistically analyzed using the same approaches to identify effect of various data treatments.

Year of data collection was modeled using both raw *V. destructor* ETG responses and normalized data. Data analysis included all mite preparations to capture possible influence of year on quality of mite preparations.

Generalized linear mixed-effects models were used to identify differences among normalized responses in relation to odorants, concentrations, year of study, and their interactions using the ordered quantile normalization transformation (R packages: lme4, emmeans, ggplot2, ggpubr, bestNormalize). Each individual mite was treated as a random effect. Non-significant ($p > 0.05$) interaction terms (odor, concentration, year, and odor × concentration) were sequentially removed from models until only main effects remained. Following identification of significance in responses, either post-hoc pairwise comparisons were made where we report Bonferroni cutoffs, or post-hoc least squares means were compared using Tukey adjustments.

Results

We refined a method for collecting ETG responses from *V. destructor* in which mite preparations sometimes lasted over 60 min. ETG recordings were made from 34 mites; from these, we recorded 1711 stimulus responses. Plotting of initial data indicated variability in the electrical amplitude of responses among mites, with a majority of responses to odorants being less than they were to solvent controls. Data were further filtered to include only *V. destructor* responses to individual odorants that were greater than responses to the control stimulus (Eliash et al. 2014), leaving 289 responses including those to the solvent control from 22 remaining mites. ETG responses that were less than they were to the control stimulus indicate additional noise or other artifacts during stimulus administration. Subsequent analysis involved working with both zeroed responses compared to the control stimulus and un-manipulated data to determine if removal of negative-relative responses changed the outcome of data interpretation.

For 11 compounds, we found no evidence of concentration-dependent responses (n ranging from 2 to 22 for any single concentration; mean normalized *V. destructor* ETG responses for single concentrations ranging from 8.8 to 51.3 mV; for Kruskal-Wallis tests, all $p > 0.06$; Fig. 3). A linear mixed effects model of normalized ETG responses with mite as a random effect indicated a significant effect of odorant ($F_{10,245} = 2.9$, $p = 0.01$), but no effect of concentration ($F_{3,245} = 1.6$, $p = 0.19$) or their interaction ($F_{30,245} = 0.8$, $p = 0.72$). Concentration-response trends were also investigated without data filtering; results indicated a non-significant interaction of concentration × odor ($F_{3,245} = 0.8$, $p = 0.52$) when modeling mite as a random effect but did show a similar effect of odor ($F_{10,245} = 2.2$, $p = 0.01$).

Concentration did not explain significant amounts of variation in the data, and with only four levels could not be included as a random factor (Gelman and Hill 2007). Given that there were no significant differences among concentrations, we pooled data from all concentrations for each compound and simplified our models. The significant

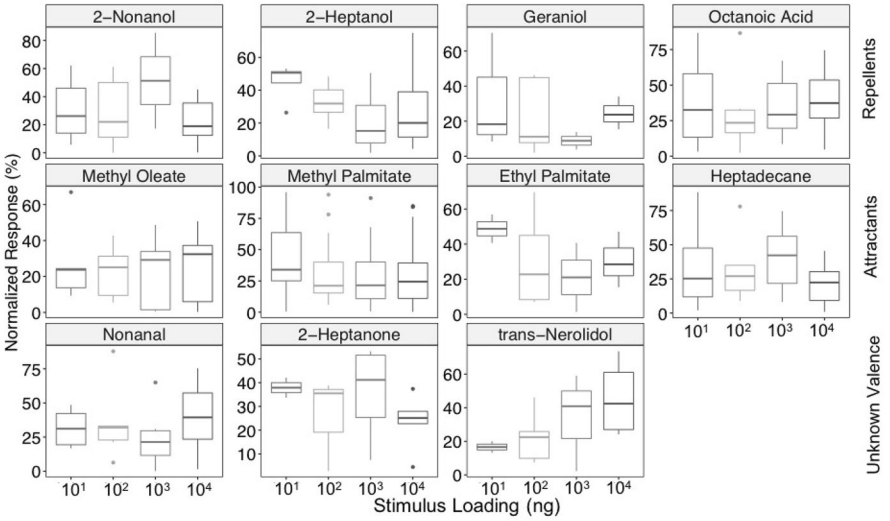

Fig. 3 *Varroa destructor* concentration-dependent responses (10^1, 10^2, 10^3, 10^4 ng in 10 µL stimulus loadings) based on electro-tarsography of putative repellents (top), attractants (middle), and odorants of undetermined valence (bottom); responses were normalized to solvent control; error bars represent standard deviation, dots above and below boxes represent outliers, and boxes contain remaining data points with means indicated by bolded lines

effect of odor ($F_{10,278} = 2.5$, $p = 0.01$) remained when we examined models without concentration and without the interaction of odor × concentration. Pairwise comparisons were not warranted as this would have meant $16 \times 15 = 240$ comparisons with too high a family-wise error rate.

To reduce the number of pairwise comparisons, odors were therefore categorized into functional groups with known valence (Fig. 4). Alcohols with isoprene units (i.e., citronellol, geraniol, linalool, trans-nerolidol, α-terpineol) were more likely to elicit weaker amplitude responses relative to putative attractants ($t = 4.1$, df = 1, $p < 0.001$) and repellents ($t = 3.5$, df = 1, $p = 0.004$). Odors with undescribed *V. destructor* valence that did not contain isoprene units elicited responses weaker than attractants ($t = 2.9$, df = 1, $p = 0.03$), but not repellents ($t = 2.5$, df = 1, $p = 0.09$) and were not significantly different from alcohols with isoprene units ($t = 0.8$, df = 1, $p > 0.99$).

We next tested for differences in responses to odors between years. Absolute and normalized responses indicated a significant effect of year among mite preparations ($F_{1,408} = 18.1$, $p < 0.0001$) and in the interaction of odor × year ($F_{3,403} = 8.7$, $p < 0.0001$). Data collected in 2017 had significantly higher mean (Kruskal–Wallis $\chi^2 = 14$, df = 1, $p < 0.001$) and median (Mood's Median $Z = -7.2$, df = 1, $p < 0.001$) normalized responses compared to those from 2018 across selected odorants (Fig. 5; Supplementary Table S7). Normalized *V. destructor* ETG responses did not have homogeneous variances among odors when stratified by year (Fligner–Killeen $\chi^2 = 692$, df = 18, $p < 0.001$). The average coefficient of variation across odors in 2017 was 0.8 and in 2018 was 1.9, indicating a larger dispersion of normalized ETG responses in 2018.

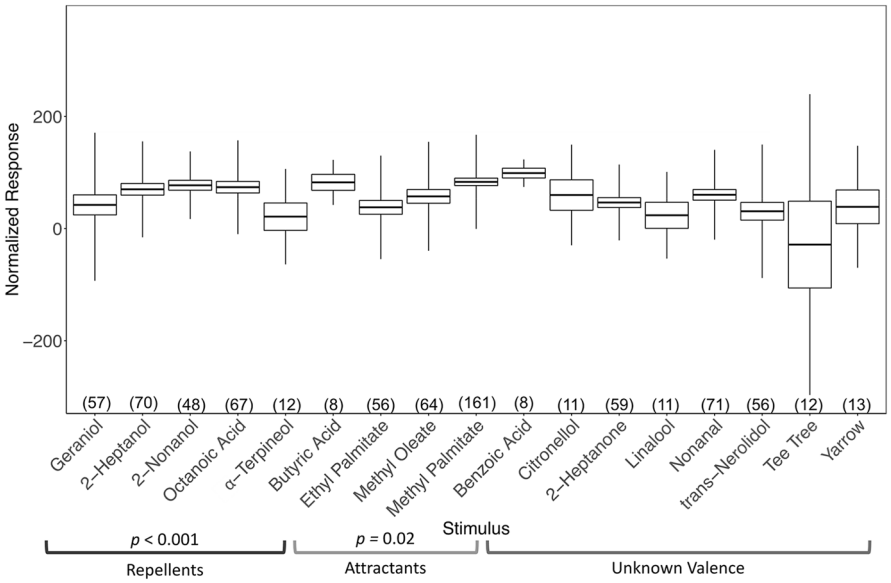

Fig. 4 Comparisons of odors grouped by putative valence of mean *Varroa destructor* normalized electrotarsogram responses. Odors with undescribed valence elicited significantly lower electrophysiological responses from *V. destructor* compared to odors that are putative attractants or repellents using a Bonferroni correction for multiple comparisons (uncorrected $p < 0.001$ for attractants and $p = 0.004$ for repellents towards odors with undescribed valence). Numbers within parentheses denote number of recordings for each stimulus. Stimuli 'Yarrow' and 'Tea-Tree' were essential oils (*Achillea millefolium* L. and *Melaleuca alternifolia* Cheel, respectively) tested at 0.1% v/v; odors are categorized as repellents, attractants, and unknown valence according to Table 1; bolded crossbars represent average normalized response, boxes encompass ± standard error of the mean, and whiskers are ± 1 standard deviation from the mean

Discussion

Using a novel ETG technique, we screened several putative attractants and repellents, enabling us to determine whether *V. destructor* expresses ETG concentration-dependent responses. Results indicated no concentration-response trends, although significant differences were found among odorants relative to solvent control responses. Previous research focusing on *V. destructor* chemosensory-disruptive compounds used electrophysiology on excised foretarsi (Eliash et al. 2014; Singh et al. 2015; Pinnelli et al. 2016). Excised preparations may yield clearer responses given an absence of muscle responses in fresh tissue, but the quality of response from these preparations can decline relatively quickly over time, with preparations providing stable responses for at most 20 min (Eliash et al. 2014). Moreover, *V. destructor* ETG preparations require a 30-s recovery time between stimulus recordings (Eliash et al. 2014), limiting the number of tests that can be done.

Varroa destructor ETGs presented in this and other studies suffer from several sources of variation, which are infrequently addressed (e.g., electrode placement, mite preparation desiccation, mite life cycle stage, and perhaps climatic influences; Eliash et al. 2014; Singh et al. 2016). We propose that reproducible ETG recordings are possible using live *V. destructor* mites, despite mechanical responses due to mite muscle movement. Our method may serve as an alternative approach, depending on electrophysiological setup, laboratory resources, and sufficient numbers of *V. destructor*.

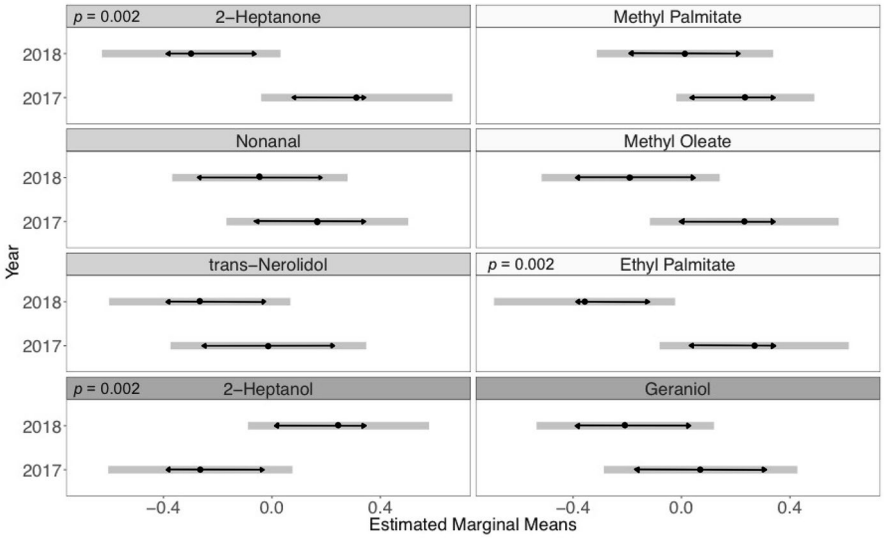

Fig. 5 Estimated marginal means with Tukey adjustment examining the influence of year on *Varroa destructor* electrotarsogram normalized responses with odor as an interaction; odorants selected are those that occur between years with relatively similar sample sizes to avoid sampling bias (putative attractants = white, putative repellents = dark grey, and odors with unknown valence = light grey). Pairwise comparisons (dark arrows) differentiate whether an odorant from a particular year has significantly different estimated marginal means, with grey bars representing confidence limits; see Supplementary Table S7 for sample sizes

Varroa destructor measure approximately 1.5 mm across, with their foretarsi being approximately 0.1 mm long. Removal and manipulation of excised tarsi from *V. destructor* could damage or cover primary chemosensory sensilla (located near the foretarsi apoteles; Rosenkranz et al. 2010). The ETG techniques proposed in this study could double recording time for individual *V. destructor* relative to previous research by using live mite preparations and avoiding damaging or covering sensilla through reduced fine-scale manipulations of foretarsi (Eliash et al. 2014). Comparative studies are required to fully understand the benefits and limitations of each technique.

A lack of concentration-dependent responses in this study was also reported from studies using synthetic odorants with both ETG and single sensillum recordings (Hanes 2015; Singh et al. 2015). We suggest that using live *V. destructor* could produce ETG responses to odorant stimuli for comparison among odors. Improved preparation longevity may provide opportunities to test *V. destructor* responses to various odors via gas chromatography mass spectrometry.

Varroa destructor ETG responses differed significantly among odorants and may be related to the number of odorant receptors that respond to compounds of interest (Dillier et al. 2006). This could indicate the relative importance of individual odorants in host detection (Nazzi et al. 2009; Ziegelmann et al. 2013; Carr and Roe 2016). *Varroa destructor* ETG responses towards odorants that are less than putative attractants could suggest that fewer sensilla are involved in detection of particular odorants, and may relate to sensillar specificity to these odorants (Dillier et al. 2006). 2-heptanone and nonanal have not been tested in previous *V. destructor* research and may be equally important in host

detection because *V. destructor* had similar responses to these as to putative attractants and repellents. Normalized responses towards alcohols with isoprene units were generally less than responses to both attractants and repellents. *Varroa destructor* normalized responses towards putative attractants were significantly greater than responses towards 2-heptanone, trans-nerolidol, α-terpineol, and geraniol (a putative honey bee-emitted repellent with a similar molecular structure to trans-nerolidol and α-terpineol). Interestingly, 2-heptanone is an attractant of small hive beetles (*Aethina tumida*) and trans-nerolidol is an attractant of two-spotted spider mites (*Tetranychus urticae*) and a repellent of brown ear ticks (*Rhipicephalus appendiculatus*) (Torto et al. 2005; Carr and Roe 2016). Moreover, 2-heptanone is found in mandibular glands of guard bees, and 2-heptanone may be associated with chemical communication among honey bees in defending their colony from nest invaders (Breed et al. 2004). 2-heptanone may be relevant to *V. destructor* in avoiding detection by and/or being damaged from honey bees within the colony. The original host of *V. destructor*, *Apis cerana*, has greater aggression to exposed mites than the more recently acquired host *Apis mellifera* (Nazzi and LeConte 2016). Further research should compare honey bee-emitted volatiles between *A. mellifera* and *A. cerana* and associated *V. destructor* behavioral responses. Previous research has reported similar repellent activity of some odorants in *V. destructor* and other acarines (Peng et al. 2015), and this could be an avenue for future research to investigate behavioral importance of acarine attractants to *V. destructor* (Bissinger and Roe 2010; Carr and Roe 2016).

Weaker mean normalized responses were identified among most alcohols with isoprene groups (citronellol, geraniol, linalool, α-terpineol, and trans-nerolidol) when compared to putative attractants and repellents. This leads to questions about the activity of similar compounds on *V. destructor* receptors (Miller et al. 2007; Peng et al. 2015), and whether they have the ability to disrupt or inhibit sensory reception through neurophysiological mechanisms. Several essential oil components were previously examined for behavioral responses from *V. destructor*, including α-terpineol, which has potential for pest management (Kraus et al. 1994; Imdorf et al. 1999; Peng et al. 2015). These findings suggest ETG screening of plant-derived components has potential for identifying novel chemicals which can cause chemosensory disruption of mite behavior by inhibiting peripheral detection of attractive stimuli (Miller et al. 2007). Future *V. destructor* research could explore whether concentration-dependent responses are present using mixtures of putative attractants and semiochemical disruptants (Eliash et al. 2014; Singh et al. 2015).

Varroa *destructor* may have sensitivity to other host odorants, some of which are low volatility (e.g., methyl palmitate, ethyl palmitate), but further research is needed. Boot (1994) and Donzé et al. (1998) speculated that both low volatility and trace components of honey bee larvae are most likely responsible for evoking behavioral responses in *V. destructor*. Trouiller et al. (1992) and Pankiw and Page (2001) reported components of honey bee larvae cuticle extractions range from $< 10^{-1}$ to $> 10^{3}$ ng per individual larva. Higher sensitivity to trace components would be advantageous in *V. destructor* life cycles, particularly those of low volatility under typical colony climatic conditions (e.g., brood methyl- and ethyl-esters; Donzé et al. 1998). Concentrations examined here are comparable to those previously explored in honey bee and *V. destructor* research (Nazzi et al. 2001; Martin et al. 2002; Del Piccolo et al. 2010; Ziegelmann et al. 2013). This indicates a possible overlap in the ranges of detection for *V. destructor* and honey bees (Martin et al. 2002). Single components are often more important than complex blends in honey bee detection, and similar sensitivities may be exhibited by *V. destructor* within the colony environment (Le Conte et al. 1989; Donzé et al. 1998; Keeling et al. 2004; Reinhard et al. 2010). This finding further indicates the importance

of comparative studies that identify differences in responses between hosts and parasites with respect to individual components and mixtures for the development of future in-colony treatment methods particularly in relation to identified *V. destructor* repellents, acaricides, and chemosensory disruptants (Singh et al. 2015; Plettner et al. 2017). Research exploring effects of *V. destructor* semiochemical disruptants suggests that honey bees may recover more quickly and be less susceptible to these synthetic disruptants, although their efficacy and effect on colony dynamics remains to be explored (Eliash et al. 2014; Singh et al. 2015).

The large variability in odor molecular weights across odorants tested likely result in differential relative concentrations presented to *V. destructor* preparations. Differences in odor volatility across the odorants tested here are also likely to be reflected within the colony environment, suggesting that *V. destructor* may have high sensitivities to low concentrations of particular attractants and/or repellents that are relevant to its lifecycle.

Peak amplitude responses varied in shape and strength among *V. destructor* preparations. In several instances, peak shapes were bimodal rather than unimodal, consistent with prior research, and suggests double depolarizations may be both olfactory and mechanical responses occurring in quick succession (Endris and Baker 1993). *Varroa destructor* is covered in many hair-like structures, and these may have mechanosensory capabilities as in other arthropods (Dillier et al. 2006; Ganske and Uhl 2018). Single sensillum recording on live *V. destructor* indicated that electrode placement can greatly influence the quality of responses to odorants (Dillier et al. 2003; Hanes 2015). This was also observed in our data, with between-mite variation in the relative strength of odorant responses to mechanical responses. Electrophysiology using single sensilla recording may provide better signal differentiation between mechanical and odorant responses.

Varroa destructor ETG responses differed between years. It is possible that a relocation of ETG equipment to a more climate-controlled room altered variability in electrophysiological signal (Syntech 2015). Variation in ETG data was explained best by individual varroa mites and could reflect a number of environmental or physiological factors. Normalization of mite responses was important in reducing this variability and was reflected in lower average coefficients of variation between years of study. A possible explanation for pronounced inter-individual variability in this study could relate to receptivity towards brood odors following emergence of adult females which were collected at random from the phoretic stage. Variability in the responsiveness to brood odors of phoretic females was reported elsewhere (Pernal et al. 2005) and could relate to a possible lag time between emergence from a reproductive phase.

Although concentration-dependent responses were not observed in this study, this should be a continued focus in developing solutions for infestation management (Plettner et al. 2017). Identifying functions of individual odorant receptors in *V. destructor* may lead to a better understanding of those in other acarine pests with the potential for cross-application of known semiochemical disruptants (Eliash et al. 2014; Soroker et al. 2019). Improved longevity of preparations using the methods described here could be applied to future *V. destructor* chemical ecology research involving broad-scale screening of honey bee colony volatiles through gas chromatography linked to electrotarsography. Future research should also explore *V. destructor* behavioral responses towards potentially relevant compounds identified here and examine possible crossover in activity with other Acari (Bissinger and Roe 2010; Carr et al. 2013). Identification of shared odorant activities (e.g., repellents, attractants, or disruptants) among Acari could help in understanding mechanisms of semiochemical detection and lead to better methods for screening and testing odorants with behavioral relevance.

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References

Bissinger BW, Roe RM (2010) Tick repellents: past, present, and future. Pestic Biochem Physiol 96:63–79

Blum MS (1996) Semiochemical parsimony in the Arthropoda. Annu Rev Entomol 41:353–374

Boot WJ (1994) Methyl palmitate does not elicit invasion of honeybee brood cells by Varroa mites. Exp Appl Acarol 18:587–592

Boot WJ, Beetsma J, Calis JNM (1994) Behaviour of Varroa mites invading honey bee brood cells. Exp Appl Acarol 18:371–379

Breed MD, Guzmán-Novoa E, Hunt GJ (2004) Defensive behaviour of honey bees: organization, genetics, and comparisons with other bees. Annu Rev Entomol 49:271–298

Calderone NW (1999) Evaluation of formic acid and thymol-based blend of natural products for the fall control of *Varroa jacobsoni* (Acari: Varroidae) in colonies of *Apis mellifera* (Hymenoptera: Apidae). J Econ Entomol 92:253–260

Calderone NW, Lin S (2001) Behavioural responses of *Varroa destructor* (Acari: Varroidae) to extracts of larvae, cocoons and brood food of worker and drone honey bees, *Apis mellifera* (Hymenoptera: Apidae). Physiol Entomol 26:341–350

Carr AL, Roe M (2016) Acarine attractants: chemoreception, bioassay, chemistry and control. Pestic Biochem Physiol 131:60–79

Carr AL, Roe RM, Arellano C, Sonenshine DE, Schal C, Apperson CS (2013) Responses of *Amblyomma americanum* and *Dermacentor variabilis* to odorants that attract haematophagous insects. Med Vet Entomol 27:86–95

Carroll MJ, Duehl AJ (2012) Collection of volatiles from honeybee larvae and adults enclosed on brood frames. Apidologie 43:715–730

Currie RW, Pernal SF, Guzmán-Novoa E (2010) Honey bee colony losses in Canada. J Apic Res 49:104–106

Del Piccolo F, Nazzi F, Vedova G, Della Milani N, Del Piccolo F, Nazzi F, Vedova G, Della Milani N, Del Piccolo F, Nazzi F, Vedova G, Della Milani N (2010) Selection of *Apis mellifera* workers by the parasitic mite *Varroa destructor* using host cuticular hydrocarbons. Parasitology 137:967–973

Dietemann V, Nazzi F, Martin SJ, Anderson DL, Locke B, Delaplane KS, Wauquiez Q, Tannahill C, Frey E, Ziegelmann B, Rosenkranz P, Ellis JD (2013) Standard methods for Varroa research. J Apic Res 52:1–54

Dillier F, Guerin P, Fluri P, Imdorf A, Meyre PB, Diehl P, Bee S (2003) Odour-mediated transfer to brood cells of its honeybee host, *Apis mellifera*, and olfactory cell responses of *Varroa destructor* to volatiles. Swiss Bee Res Cent 1–14

Dillier F-X, Fluri P, Imdorf A (2006) Review of the orientation behaviour in the bee parasitic mite *Varroa destructor*: sensory equipment and cell invasion behaviour. Rev Suisse Zool 113:857–877

Donzé G, Schnyder-Candrian S, Bogdanov S, Diehl P-A, Guerin PM, Kilchenman V, Monachon F (1998) Aliphatic alcohols and aldehydes of the honey bee cocoon induce arrestment behavior in *Varroa jacobsoni* (Acari: Mesostigmata), an ectoparasite of *Apis mellifera*. Arch Insect Biochem 37:129–145

Eliash N, Singh NK, Kamer Y, Pinnelli GR, Plettner E, Soroker V (2014) Can we disrupt the sensing of honey bees by the bee parasite *Varroa destructor*. PLoS ONE 9:1–13

Endris J, Baker R (1993) Action potentials recorded from the foreleg of *Varroa jacobsoni* after olfactory stimulation. Apidologie 24:488–489

Ferland J, Nasr M, Wilson G, Jordan C, Kempers M, Kozak P, Lafreniere R, Maund C, Pernal S, Sproule J, Van Westendorp P (2017) Canadian Association of Professional Apiculturists (CAPA): statement on honey bee wintering losses in Canada (Quebec)

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest management. Annu Rev Entomol 42:123–146

Frey E, Rosenkranz P (2014) Autumn invasion rates of *Varroa destructor* (Mesostigmata: Varroidae) into honey bee (Hymenoptera: Apidae) colonies and the resulting increase in mite populations. J Econ Entomol 107:508–515

Frey E, Odemer R, Blum T, Rosenkranz P (2013) Activation and interruption of the reproduction of *Varroa destructor* is triggered by host signals (*Apis mellifera*). J Invertebr Pathol 113:56–62

Ganske A-S, Uhl G (2018) The sensory equipment of a spider: a morphological survey of different types of sensillum in both sexes of *Argiope bruennichi* (Araneae, Araneidae). Arthropod Struct Dev 47:144–161

Gelman A, Hill J (2007) Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, New York

Gilley DC, DeGrandi-Hoffman G, Hooper JE (2006) Volatile compounds emitted by live European honey bee (*Apis mellifera* L.) queens. J Insect Physiol 52:520–527

Gray A, Brodschneider R, Adjlane N, Ballis A, Brusbardis V, Charrière JD, Chlebo R, Coffey F, Cornelissen M, Amaro da Costa B, Csáki C, Dahle T, Danihlík B, Dražić J, Evans MM, Fedoriak G, Forsythe M, de Graaf I, Gregorc D, Johannesen A, Kauko J, Kristiansen L, Martikkala P, Martín-Hernández M, Medina-Flores R, Mutinelli CA, Patalano F, Petrov S, Raudmets P, Ryzhikov A, Simon-Delso VA, Stevanovic N, Topolska J, Uzunov G, Vejsnaes A, Williams F, Zammit-Mangion A, Soroker V (2019) Loss rates of honey bee colonies during winter 2017/18 in 36 countries participating in the COLOSS survey, including effects of forage sources. J Apic Res 58:479–485

Grozinger CM, Fischer P, Hampton JE (2007) Uncoupling primer and releaser responses to pheromone in honey bees. Naturwissenschaften 94:375–379

Hanes MR (2015) Response of olfactory neurons in *Varroa destructor* mites to attractant and repellent semiochemicals. Acadia University, Wolfville

Hoppe H, Ritter W (1988) The Influence of the Nasonov pheromone on the recognition of house bees and foragers by *Varroa jacobsoni*. Apidologie 19:165–172

Human H, Brodschneider R, Dietemann V, Dively G, Ellis JD, Forsgren E, Fries I, Hatjina F, Hu F-L, Jaffé R, Jensen AB, Köhler A, Magyar JP, Özkýrým A, Pirk CWW, Rose R, Strauss U, Tanner G, Tarpy DR, van der Steen JJM, Vaudo A, Vejsnæs F, Wilde J, Williams GR, Zheng H-Q (2013) Miscellaneous standard methods for *Apis mellifera* research. J Apic Res 52:1–53

Imdorf A, Bogdanov S, Ochoa RI, Calderone NW (1999) Use of essential oils for the control of *Varroa jacobsoni* Oud. in honey bee colonies. Apidologie 30:209–228

Imdorf A, Charrière J, Kilchenmann V, Bogdanov S, Fluri P (2003) Alternative strategy in central Europe for the control of *Varroa destructor* in honey bee colonies. Apiacta 38:258–285

Kather R, Drijfhout FP, Shemilt S, Martin SJ (2015) Evidence for passive chemical camouflage in the parasitic mite *Varroa destructor*. J Chem Ecol 41:178–186

Keeling CI, Plettner E, Slessor KN (2004) Hymenopteran semiochemicals. Top Curr Chem 239:133–177

Klein A-M, Vaissiere BE, Cane JH, Steffan-Dewenter I, Cunningham SA, Kremen C, Tscharntke T (2007) Importance of pollinators in changing landscapes for world crops. Proc R Soc Lond 274:303–313

Kraus B (1990) Effects of honey-bee alarm pheromone compounds on the behaviour of *Varroa jacobsoni*. Apidologie 21:127–134

Kraus B, Koeniger N, Fuchs S (1994) Screening of substances for their effect on *Varroa jacobsoni*: attractiveness, repellency, toxicity and masking effects of ethereal oils. J Apic Res 33:34–43

LeConte Y, Arnold G, Trouiller J, Masson C, Chappe B, Ourisson G (1989) Attraction of the parasitic mite Varroa to the drone larvae of honey bees by simple aliphatic esters. Science 245:638–639

LeConte Y, Arnold G, Trouiller J, Masson C, Chappe B (1990) Identification of a brood pheromone in honeybees. Naturwissenschaften 77:334–336

Martin C, Salvy M, Provost E, Bagneres AG, Roux M, Crauser D, Clement JL, Le Conte Y, Bagnères AG, Roux M, Crauser D, Clement JL, Le Conte Y (2001) Variations in chemical mimicry by the ectoparasitc mite *Varroa jacobsoni* according to the developmental stage of the host honey-bee *Apis mellifera*. Insect Biochem Mol Biol 31:365–379

Martin C, Provost E, Bagnères AG, Roux M, Clément JL, Le Conte Y (2002) Potential mechanism for detection by *Apis mellifera* of the parasitic mite *Varroa destructor* inside sealed brood cells. Physiol Entomol 27:175–188

Melathopoulos AP, Winston ML, Whittington R, Higo H, Doux L (2000) Field evaluation of neem and canola oil for the selective control of the honey bee (Hymenoptera: Apidae) mite parasites *Varroa jacobsoni* (Acari: Varroidae) and *Acarapis woodi* (Acari: Tarsonemidae). J Econ Entomol 93:559–567

Milani N, Della Vedova G, Nazzi F (2004) (Z)-8-heptadecene reduces the reproduction of *Varroa destructor* in brood cells. Apidologie 35:265–273

Miller TA, Lampe DJ, Lauzon CR (2007) Insecticides design using advanced technologies. Springer, Berlin

Morse RA, Calderone NW (2000) The value of honey bees as pollinators of U.S. crops in 2000. Bee Cult 128:31

Nazzi F, LeConte Y (2016) Ecology of *Varroa destructor*, the major ectoparasite of the western honey bee, *Apis mellifera*. Annu Rev Entomol 61:417–432

Nazzi F, Milani N, Vedova GD, Nimis M (2001) Semiochemicals from larval food affect the locomotory behaviour of *Varroa destructor*. Apidologie 32:149–155
Nazzi F, Milani N, Della Vedova G (2004a) A semiochemical from larval food influences the entrance of *Varroa destructor* into brood cells. Apidologie 35:403–410
Nazzi F, Della Vedova G, D'Agaro M (2004b) A semiochemical from brood cells infested by *Varroa destructor* triggers hygienic behaviour in *Apis mellifera*. Apidologie 35:65–70
Nazzi F, Milani N, Vedova G, Della (2006) Attraction of *Varroa destructor* to bee brood cells by cues from larval food. Apimondia 1–4
Nazzi F, Bortolomeazzi R, Della Vedova G, Del Piccolo F, Annoscia D, Milani N (2009) Octanoic acid confers to royal jelly Varroa-repellent properties. Naturwissenschaften 96:309–314
Pankiw T, Page RE (2001) Brood pheromone modulates honeybee (*Apis mellifera* L.) sucrose response thresholds. Behav Ecol Sociobiol 49:206–213
Peng G, Kashio M, Morimoto T, Li T, Zhu J, Tominaga M, Kadowaki T (2015) Plant-derived tick repellents activate the honey bee ectoparasitic mite TRPA1. Cell Rep 12:190–202
Pernal SF, Baird DS, Birmingham AL, Higo HA, Slessor KN, Winston ML (2005) Semiochemicals influencing the host-finding behaviour of *Varroa destructor*. Exp Appl Acarol 37:1–26
Pinnelli GR, Singh NK, Soroker V, Plettner E (2016) Synthesis of enantiopure alicyclic ethers and their activity on the chemosensory organ of the ectoparasite of honey bees, *Varroa destructor*. J Agric Food Chem 64:8653–8658
Plettner E, Eliash N, Singh NK, Pinnelli GR, Soroker V (2017) The chemical ecology of host-parasite interaction as a target of *Varroa destructor* control agents. Apidologie 48:78–92
R Core Team (2018). R: a language and environment for statistical computing (01.0.136). Foundation for Statistical Computing. Available from: https://www.r-project.org/foundation/
Reinhard J, Sinclair M, Srinivasan MV, Claudianos C (2010) Honeybees learn odour mixtures via a selection of key odorants. PLoS ONE 5:1–14
Rickli M, Guerin PM, Diehl PA (1992) Palmitic acid released from honeybee worker larvae attracts the parasitic mite *Varroa jacobsoni* on a servosphere. Naturwissenschaften 79:320–322
Rickli M, Diehl PA, Guerin PM (1994) Cuticle alkanes of honeybee larvae mediate arrestment of bee parasite *Varroa jacobsoni*. J Chem Ecol 20:2437–2453
Rosenkranz P, Aumeier P, Ziegelmann B (2010) Biology and control of *Varroa destructor*. J Invertebr Pathol 103:S96–S119
Schmitt T, Herzner G, Weckerle B, Schreier P, Strohm E (2007) Volatiles of foraging honeybees *Apis mellifera* (Hymenoptera: Apidae) and their potential role as semiochemicals. Apidologie 38:164–170
Singh NK, Eliash N, Kamer Y, Zaidman I, Plettner E, Soroker V (2015) The effect of DEET on chemosensing of the honey bee and its parasite *Varroa destructor*. Apidologie 46:380–391
Singh NK, Eliash N, Stein I, Kamer Y, Ilia Z, Rafaeli A, Soroker V (2016) Identification and gene-silencing of a putative odorant receptor transcription factor in *Varroa destructor*: possible role in olfaction. Insect Mol Biol 25:181–190
Soroker V, Singh NK, Eliash N, Plettner E (2019) Olfaction as a target for control of honeybee parasite mite *Varroa destructor*. In: Picimbon J-F (ed) Olfactory concepts of insect control-alternative to insecticides. Springer, Chicago, pp 117–134
Syntech (2015) Electroantennography: a pratical introduction. Syntech, Kirchzarten. Available from: http://www.ockenfels-syntech.com/wp-content/uploads/EAGpract_man_fin
Teal PEA, Duehl AJ, Carroll MJ (2014) Methods for attracting honey bee parasitic mites. USPC, Washington, DC
Thom C, Gilley DC, Hooper J, Esch HE (2007) The scent of the waggle dance. PLoS Biol 5:1862–1867
Torto B, Suazo A, Alborn H, Tumlinson JH, Teal PEA (2005) Response of the small hive beetle (*Aethina tumida*) to a blend of chemicals identified from honeybee (*Apis mellifera*) volatiles. Apidologie 36:523–532
Torto B, Arbogast RT, Alborn H, Suazo A, Van Engelsdorp D, Boucias D, Tumlinson JH, Teal PEA (2007) Composition of volatiles from fermenting pollen dough and attractiveness to the small hive beetle *Aethina tumida*, a parasite of the honeybee *Apis mellifera*. Apidologie 38:380–389
Trouiller J, Arnold G, Chappe B, Le Conte Y, Masson C (1992) Semiochemical basis of infestation of honey bee brood by *Varroa jacobsoni*. J Chem Ecol 18:2041–2053
Tutun H, Koç N, Kart A (2018) Plant essential oils used against some bee diseases. Turk J Agric Food Sci Technol 6:34
Underwood RM, Currie RW (2003) The effects of temperature and dose of formic acid on treatment efficacy against *Varroa destructor* (Acari: Varroidae), a parasite of *Apis mellifera* (Hymenoptera: Apidae). Exp Appl Acarol 29:303–313

van der Zee R, Pisa L, Andonov S, Brodschneider R, Charrière J-D, Chlebo R, Coffey MF, Crailsheim K, Dahle B, Gajda A, Gray A, Drazic MM, Higes M, Kauko L, Kence A, Kence M, Kezic N, Kiprijanovska H, Kralj J, Kristiansen P, Hernandez RM, Mutinelli F, Nguyen BK, Otten C, Özkırım A, Pernal SF, Peterson M, Ramsay G, Santrac V, Soroker V, Topolska G, Uzunov A, Vejsnæs F, Wei S, Wilkins S (2012) Managed honey bee colony losses in Canada, China, Europe, Israel and Turkey, for the winters of 2008–2009 and 2009–2010. J Apic Res 51:100–114

Yoder JA, Sammataro D (2003) Potential to control Varroa mites (Acari: Varroidae) using chemical ecology. Int J Acarol 29:139–143

Ziegelmann B, Tolasch T, Steidle JLM, Rosenkranz P (2013) The mating behavior of *Varroa destructor* is triggered by a female sex pheromone. part 2: identification and dose-dependent effects of components of the Varroa sex pheromone. Apidologie 44:481–490