Chemical Senses, 2020, Vol 45, 179–186
doi:10.1093/chemse/bjaa001
Original Article
Advance Access publication 10 January 2020

OXFORD

Original Article

Biosecurity Dogs Detect Live Insects after Training with Odor-Proxy Training Aids: Scent Extract and Dead Specimens

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Editorial Decision 4 January 2020.

Abstract

Detector dogs could be trained to find invasive insect pests at borders before they establish in new areas. However, without access to the live insects themselves, odor training aids are needed to condition dogs to their scent. This proof-of-concept study assessed 2 potential training aids for insect detection: a scent extract and dead specimens of the target species. Using *Musgraveia sulciventris* (Hemiptera: Tessaratomidae) as an experimental model, gas chromatography–mass spectrometry (GC-MS) analyses were carried out to compare the chemical headspaces that make up the odors of live specimens and these 2 training aids. This was then followed by canine scent-detection testing to investigate biosecurity detector dogs' ($n = 4$) responses to training in an ecologically valid context. Both the scent extract and the dead specimens shared the majority of their volatile organic compounds (VOCs) with live insects. Of the dogs trained with scent extract ($n = 2$), both were able to detect the live insects accurately, and of those trained with dead specimens ($n = 2$), one detected the live insects accurately. These findings lend support for these training aids as odor-proxies for live insects—particularly scent extract, which is a relatively novel product with the potential for broad application to facilitate and improve insect-detection training.

Key words: brown marmorated stink bug, canine scent detection, *Halyomorpha halys*, invasive insect, solvent extract, volatile organic compounds

Introduction

Targeted screening with detector dogs could help to prevent incursions by invasive insects that are harmful to native ecosystems and local agriculture. Invasive pests, such as the brown marmorated stink bug *Halyomorpha halys*, can "hitchhike" into new territories hidden inside imported goods and cargo, with potential for rapid expansion in favorable conditions (Jenkins 1996; Hoebeke and Carter 2003; Maistrello et al. 2018). As a preventative measure, detector dogs could be used to screen cargo at points of entry, or in the environment, to find these hidden pests and help to prevent their spread. Currently, dogs are not used in Australian biosecurity to screen for invasive insects, and they are not yet commonly used elsewhere for this purpose. Dogs are effective biosensors and have demonstrated the ability to detect many insect species, including termites, bed bugs, fire ants, and stink bugs (Lewis et al. 1997; Pfiester et al. 2008; Lin et al. 2011; Lee et al. 2014). However, training dogs to detect

an exotic insect species that is not yet endemic in a region poses a problem: the insects are usually not available for training purposes due to biosecurity regulations. Training dogs to detect live insects without using actual live specimens requires a novel approach, as most insect-detection dogs are trained with live targets for all, or at least part, of the training process (Lehnert and Weeks 2016). It also assumes that the dogs will generalize their responses from the training aids to living specimens (for review, see Moser et al. 2019). Therefore, the effectiveness of using only odor-proxy training aids to train for live insect detection warrants investigation.

Two potential insect odor-proxies that could be used as canine training aids are insect scent extract and dead insect specimens. Insect scent extract is a relatively novel approach, and is produced by extracting compounds from live insects into a solvent, specifically pentane; producing what has been described as a "natural pseudo-scent" (Brooks 2001; Pfiester et al. 2008; Lehnert and Weeks 2016). Although promising, research into the use of insect scent extract is still minimal, and it is yet unknown whether it will be adequate for training purposes. Alternatively, dead insects have been used as training aids for insect-detection training, generally with the assumption that they have the same, or very similar, odor to that of live specimens (e.g., Lin et al. 2011). However, empirical evidence is lacking that demonstrates that training with dead specimens alone can translate to the detection of live insects. Conversely, there is some evidence that dogs actively discriminate between live and dead insects for some detection tasks (e.g., Pfiester et al. 2008). As such, both these potential training aids require testing to determine their suitability and efficacy.

The present question of interest for biosecurity operations is whether dogs that have been trained using training aids would detect a novel target—live insects—when they initially encounter them. As a first step in answering this question and investigating the feasibility of training Australia's biosecurity detector dogs to detect the highly invasive insect pest *H. halys*, a related, locally abundant pentatomid species, *Musgraveia sulciventris*, was used for proof-of-concept testing. Two odor-proxy training aids (scent extract and dead insects) were separately evaluated by 1) comparing their chemical odor profiles with that of the live insects, and 2) scent-recognition testing with biosecurity detector dogs in an ecologically valid setting. We hypothesized that live *M. sulciventris* would share most of their headspace volatile organic compounds (VOCs) in common with the 2 training aids previously mentioned: scent extract and dead specimens of the same species. Based on the degree of similarity in VOCs, we anticipated that dogs receiving scent recognition training with either scent extract or dead *M. sulciventris* specimens would show a positive response to living *M. sulciventris* upon their first exposures to them. A positive response would indicate that the training aids had functioned as intended, by having conditioned the dogs to respond to live insect odor. The aim in this instance was not to compare these training aids, but rather to test their efficacy separately to provide more than one training aid option. If successful, these training aids could feasibly be utilized for scent detection training of biosecurity detector dogs for the related *H. halys* and other insect pests.

Materials and methods

Solid-phase microextraction (SPME) samples were collected from the headspaces of live *M. sulciventris* specimens and 2 odor-proxy training aids (scent extract and dead specimens) and were analyzed using gas chromatography–mass spectrometry (GC-MS) to allow comparison of the headspace VOCs of live insects and training aids (see SPME GC-MS methods). Following this, the dogs (n = 4) received scent-recognition training using one of these training aids before testing their responses to live *M. sulciventris* (see Canine scent detection training and testing).

Preparation of odor samples for chemical analysis and canine scent-recognition testing

Live insects

Live *M. sulciventris* were collected from citrus trees (kumquat, grapefruit, lemon, and mandarin) in private residences around New South Wales, Australia, and stored in mesh insect cages with either citrus tree foliage or a living kumquat tree until required for testing. This species was chosen to serve as a proof-of-concept proxy for *H. halys*, a high-risk invasive insect, because of its taxonomical and published chemical similarities (MacLeod et al. 1975; Nixon et al. 2018).

Dead insects

Dead *M. sulciventris* specimens were obtained by collecting freshly deceased captive insects within 24 h of death (captive insects were inspected daily) and storing in glass specimen jars at −18 °C. Specimens were thawed for 10 min before SPME sampling and a minimum of 10 min before presentation to dogs. During training, thawed specimens were used at room temperature for approximately 5 h before being discarded.

Insect scent extract

The procedure for preparing insect scent extract was adapted from that used by Pfiester et al. (2008). Live *M. sulciventris* were immersed in *n*-pentane at a ratio of 1 bug to 10 mL *n*-pentane and washed for 15 min. This ratio allowed just enough liquid to submerge the bugs fully. The insects were removed, and the liquid extract transferred to a glass vial and stored at 2 °C. For headspace sampling, 1 mL of liquid was pipetted onto a filter paper (110 mm diameter; Advantec), and the sample was allowed to stand for 5 min at room temperature for the *n*-pentane to evaporate. For dog training, 1–3 mL of liquid was pipetted onto a piece of filter paper, and a minimum of 2 min (depending on ambient temperature) was allowed for the *n*-pentane to evaporate fully before presentation to the dogs.

SPME GC-MS methods

Analyses of the headspace VOCs of live insects and odor-proxy training aids were determined using headspace SPME GC-MS. Samples were collected on conditioned 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane coated fibers (Supelco, Inc.). Analyses were then performed on a Gas Chromatograph System (model 6890; Hewlett-Packard), coupled with a Mass Selective Detector (model 5973, Hewlett-Packard). The GC was equipped with a Zebron ZB-FFAP 30 m × 0.25 mm, 0.25 µm column (Varian, Inc.). The carrier gas was helium, and the programmed method was as follows: hold oven for 1 min at 50 °C, ramp 10 °C/min, finish at 240 °C for 20 min run time; splitless mode, 7.56 psi, purge flow at 20 mL/min for 2 min.

Triplicate samples of each training aid (dead and extract samples) and controls were carried out, and n = 10 replicates of live insect samples were used to capture the broader range of variances. Each replicate was a reproduction using a new odor sample. Odor samples consisted of 2 live *M. sulciventris*, 1 mL scent extract on filter paper, or 1 dead *M. sulciventris* specimen. These amounts were

chosen based on preliminary testing to detect as many VOCs as possible in comparable volumes. All odor samples were placed into 250 mL glass beakers, to accommodate their size, and sealed with aluminum foil. Control samples were empty beakers or a clean piece of filter paper. Samples were allowed to equilibrate for 30 min at room temperature (approximately 24 °C). The SPME fiber was then exposed to the sample headspace for 15 minutes, held in place by a clamp. The fiber was then immediately injected into the GC injection port. The resulting peak retention times and mass spectra from live bug samples, training aid samples, and the control samples were then compared with determine their similarities.

Data were acquired and analyzed using MSD ChemStation (G1701DA; Agilent), and then processed in OpenChrom (Community Edition Ver.1.3.0) for further analysis. Four algorithms were used in OpenChrom using the software's publically accessible algorithms. These were, in order: a statistics-sensitive nonlinear iterative peak-clipping (SNIP) baseline detection with a detector of 7 and filter of 1; a Savitsky-Golay filter with a smoothing degree of 2 and width of 7; a first-derivative peak detection with a medium threshold level; and a Trapezoid peak integration. Finally, a principal component analysis (PCA), using a singular value decomposition algorithm, was performed in OpenChrom from scans of these files to assess the similarity of VOC composition between live, dead, and extracted samples. Two principal components were calculated and a PCA score plot produced.

Identifying compounds

Compounds found in only one experimental sample, or also found in blank control samples, were removed from analyses. To identify the compounds, the NIST 08 (National Institute of Standards and Technology) mass spectral library was used to identify compounds with match probabilities above 90% (considered an excellent match), and those for which the same identification was matched across several samples with a similar mass spectrum to the library example. For compounds without a confident identification from the NIST library, each compound was assessed by reviewing the molecular ion (MI), base ion (BI), and fragmentation patterns. Where each sample had the same MI, BI, and fragmentation patterns, these were deemed to be the same compound and named Unknown A-U. Following this, standard chemicals (obtained from Sigma–Aldrich Co.) were used for verification and quantification for certain major compounds, including tridecane (91490), dodecane (44010), undecane (94000), (*E*)-oct-2-enal (52464), (*E*)-dec-2-enal (91309), and pentadecane (76509). These were stored at 4 °C under nitrogen. Standards were prepared at 5, 10, 20, 50, and 200 ng/µL, using procedures described in previous research (Nixon et al. 2018).

Canine scent detection training and testing

Ethics statement

Permission to undertake this research with animals was granted by the University of New England Animal Ethics Committee (Authority No. AEC18-049).

Target and control odors

Live specimens, dead specimens, and scent extract of *M. sulciventris* were used as target odors for training and were prepared as described above (see Preparation of odor samples for chemical analysis and canine scent-recognition testing). Also, n = 30 different novel control odors were used (Supplementary Table 3). This included edible products, hygiene products, scented products, and odors associated with storage and experimental set-up (e.g., plastic jars, nitrile gloves, filter paper). Novel control odors that had not been used in training were included in testing lineups. This was to ensure that dogs responded to each odor's characteristics rather than its novelty.

Animals and handlers

Four operational Australian Department of Agriculture Biosecurity Detector Dogs and their handlers participated in the research. All of the dogs (Labrador Retrievers, aged: 3, 6, 8, and 9 years) had been previously trained to detect vegetables, fruits, seeds, fresh plant material, and meat, and were regularly deployed in operational settings in which they screen airport passengers and mail. The dogs used were a sample of convenience selected from dogs that demonstrated reliable scent-detection capability.

Training

Each dog was paired with another of similar general performance and working drive, before randomly allocating 1 dog from each pair to 1 of the 2 training aids. Two dogs were trained using dead specimens, and the other 2 were trained using a scent extract (both of *M. sulciventris*). Scent-recognition training took place in the Department of Agriculture Biosecurity Detector Dog training facility in Brisbane, Australia, under the instruction of the department's Detector Dog Technical Manager, and the same handler handled each dog throughout the experiment. The area used for dog training and testing in the facility was approximately 20 m by 30 m.

For both the initial training and testing, the target odors, control odors, and blanks (empty tins) were presented to dogs in stainless steel tins approximately 17 cm in diameter, closed with lids that had small holes preventing visual or physical access whilst allowing odor exchange. Tins were held in stainless steel "scent planks" (Figure 1). Target and control odors were contained within the tins without other, extraneous containers. After each search, tins were either changed entirely or cleaned to prevent systematic odor differences emerging between targets and nontargets.

The dogs were trained to perform a go/no-go task using positive reinforcement conditioning. They were instructed to sit when they detected the odor of their training aid and to show no response to control odors or blanks, in line with the official Department of Agriculture training protocol for detector dogs. Every sit during a search was recorded as an indication, and moving on to the next odor point without a sit response was recorded as no indication. A sit response was defined as full contact of the hindquarters on the ground, regardless of duration, and responses were determined and recorded by the evaluator. No ambiguous instances, in which the dog did not lower its hindquarters fully to the ground and then moved on, occurred during testing trials. Correct responses were reinforced by giving the dog a food reward along with physical and verbal praise. Each trial throughout the dogs' training included anywhere between zero or several targets, so as to prevent the expectation for a certain number of targets per trial. Each dog underwent an average of 26 training trials with their allocated training aid, lasting between 10 and 60 s each, until they could reliably reach >80% sensitivity and specificity.

Following 3 consecutive days of training, the dogs underwent a blinded training aid scent-recognition test to determine whether they were proficient in detecting the odor of their assigned training aid. This involved each dog performing 3 separate trials with a total of n = 3 targets and n = 29 controls across those trials. The target tins contained either: 6 thawed, dead *M. sulciventris*; or approximately 2 mL of *M. sulciventris* scent extract on filter paper, depending on their assigned training aid.

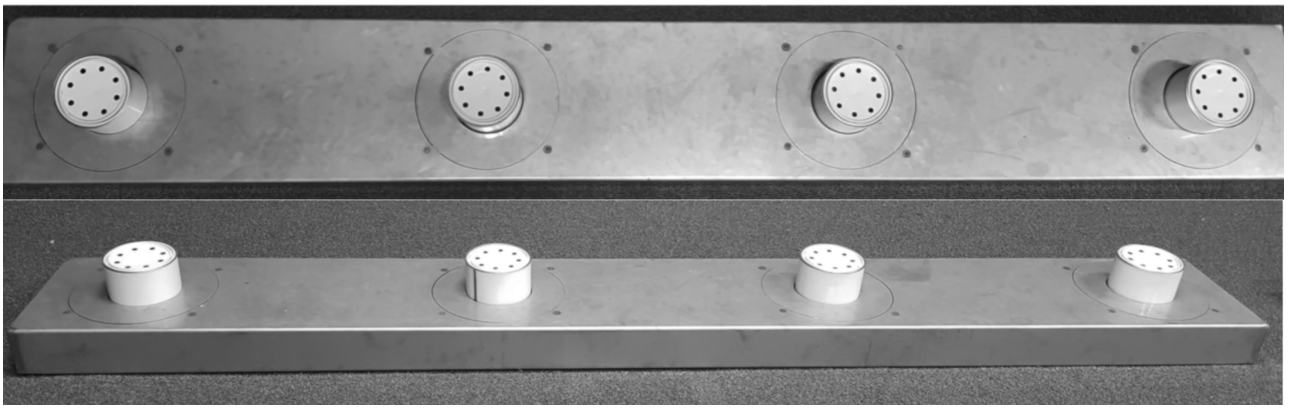

Figure 1. Stainless steel scent plank with odor sample tins. Scent planks were arranged end-to-end in a lineup.

Testing

Scent detection testing was conducted in the same location as the training, using the same scent-plank equipment to ensure that the dogs' performance was reliable and ecologically valid. Generalization testing was carried out with reinforcement of correct responses to avoid compromising the ability of these operational detector dogs to detect insects in their working role.

Each search trial consisted of a handler entering the room with a leashed dog, taking them to a lineup of 8 to 12 stainless steel tins, and giving the dog the command to search. Dogs began at the first odor tin in the lineup, searching each tin separately and either indicating with a sit or not indicating before moving on to the next one. After every tin in the lineup was searched—which took an average of approximately 30 s—the handler and dog exited the room, and the dog was put in a crate, regardless of success or failure. The testing area was out of view of the other dogs and handlers when they were not engaged in a search trial. After each run, samples were moved and all tins were wiped down and had lids changed so as not to leave systematic odor cues for the following dogs.

Each testing lineup contained one target odor among control odors and empty tins. Dogs were previously trained with a changing number of targets in every lineup, between zero and several, to maintain active detection throughout the entire lineup. Testing was performed in a single-blind procedure, in which the handler was blinded to the type of target, the target's location, and the number of targets. A nonblind evaluator provided verbal feedback to the handler from a vantage point several meters away in the same testing room, so as to minimize interference but also provide immediate and accurate feedback. When a dog performed a sit indication, an observing evaluator communicated to the handler whether it was correct or incorrect and the dog was either rewarded with food and praise or instructed to "leave it," accordingly; this immediate feedback on their performance maintained engagement and a consistent working standard from the dogs. Following a false response, dogs continued to sample the rest of the plank. Each odor sample was considered a separate go/no-go decision from the other odor samples.

After undergoing recognition tests of their assigned training aids, dogs then underwent a live insect recognition test to determine whether they were capable of detecting live insects after training with only their assigned training aid. Each dog performed 2 trials with a total of n = 2 targets (containing 6 live *M. sulciventris* each) and n = 14 controls. Exposures to live insect targets for each dog were limited to 2 to reduce the effect of repeated exposure on learning and performance. We observed during training that experienced biosecurity detector dogs could learn to respond to a new odor within as little as 2 to 3 searches. Therefore, fewer searches with a live insect target were used to give a more accurate representation of the dogs' initial responses to the odor purely as a result of the training aid.

Data analysis

Dogs' responses in the blinded tests were entered into a contingency table of true positives, true negatives, false positives, and false negatives. Sensitivity (proportion of targets correctly identified) and specificity (proportion of controls correctly identified) metrics were then calculated. Additionally, 2-sided Fisher's exact tests were calculated using R 3.4.4 (R Core Team 2018) to determine whether positive responses to odor tins were significantly associated with the presence of the target odor.

Results

Volatile compound analyses

Eleven peaks were observed consistently in all chromatograms for the 10 live insect samples. These peaks were labeled "principal" VOCs (Figure 2). Additionally, 34 more peaks were observed in at least 2 or more live insect samples—the presence or absence of these VOCs differed between samples. A complete list of the observed compounds is presented in Supplementary Table 1.

The *M. sulciventris* scent extract shared all 11 of the principal VOCs in common with the live specimens, and 71% of the total VOCs observed in live specimen samples (Figure 3). Additionally, however, 5 new compounds were found in the headspace of the scent extract. Three of the new VOCs were identified as: 2-(2-butoxyethoxy) ethanol-; 2-(2-butoxyethoxy)ethyl acetate; 1-(2,4-dimethylphenyl) ethanone; and 2 were unidentified (Unknown I and T).

Similarly, dead *M. sulciventris* samples shared all 11 of the principal VOCs in common with live specimens, and 77% of the total live specimen VOCs (Figure 3). Dead specimens also appeared to emit 3 additional compounds in minor volumes. Two were identified as (*E*)-hex-2-enoic acid and 1,2-dimethylcyclohexane, and one was unidentified (Unknown B).

Overall, a similar peak pattern was observed across the total ion chromatograms of the 3 different sample groups (Figure 2). Furthermore, a PCA showed no clustering based on the 3 groups of samples and overlap between live specimens and the training aids suggests little variance between the groups (Figure 4). PC1 and PC2 accounted for a total of 72% of the variance between the groups.

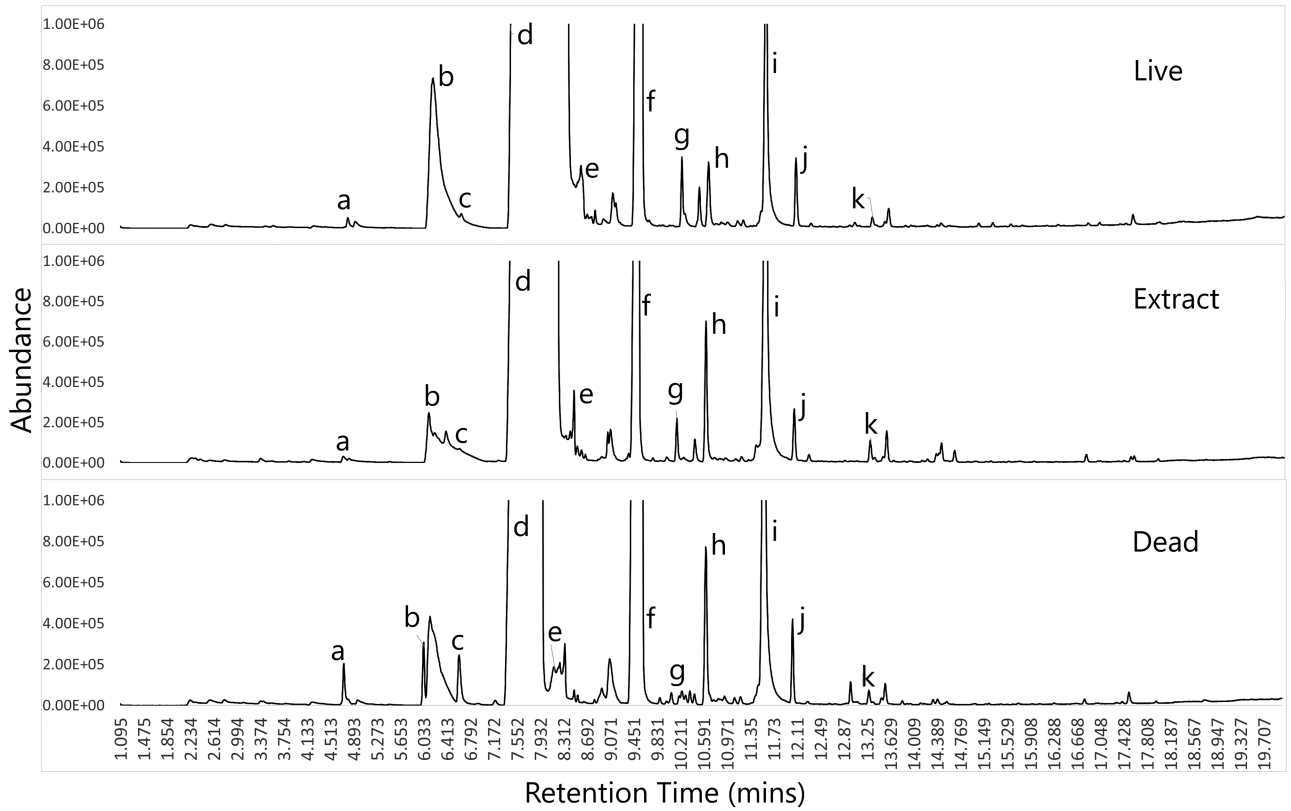

Figure 2. Total ion chromatograms from headspace SPME GC-MS analyses. Each is an example replicate of the headspaces of a live specimen, scent extract, and dead specimen sample of *Musgraveia sulciventris*. Principal peaks (those present in 100% of live samples) are labeled. Principal peaks labeled are (a) undecane; (b) dodecane; (c) (E)-hex-2-enal; (d) tridecane; (e) tridec-1-ene; (f) (E)-oct-2-enal; (g) pentadecane; (h) 4-methylcyclohexan-1-one; (i) (E)-4-oxohex-2-enal; (j) (E)-dec-2-enal; (k) cyclohex-2-ene-1,4-dione.

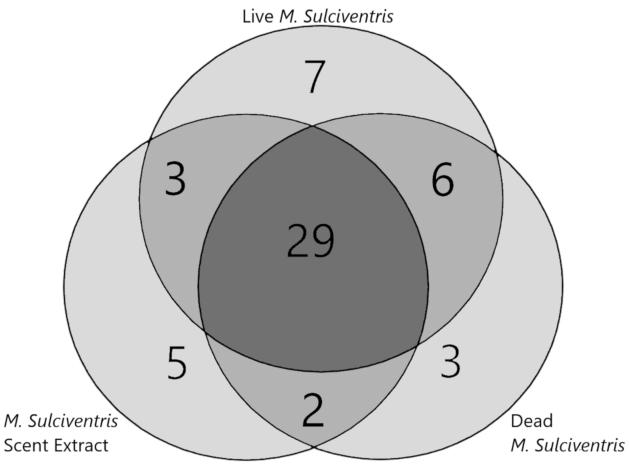

Figure 3. Venn diagram portraying the number of VOCs in common and differing between the groups of samples of *Musgraveia sulciventris* (n = 10) and associated training aids (scent extract [n = 3] and dead specimens [n = 3]) across all replicates. A high number of VOCs in common suggests a similar odor.

A selection of principal compounds was verified and quantified using standard compounds, presented in Supplementary Table 2.

Canine testing results

In blinded recognition tests, dogs demonstrated proficient detection ability (>80% individual sensitivity and specificity scores) for their respective training aids, with each dog achieving 100% sensitivity and at least 83% specificity. Each dog responded to the training aid targets significantly more often than to controls (Table 1).

Both dogs trained with the scent extract detected the live insects with 100% sensitivity and specificity, with each dog demonstrating significantly more positive responses to targets compared with controls (P = 0.008, Fisher's exact test) (Tables 1 and 2). The dogs trained with dead insects had a combined average sensitivity of 75% and specificity of 100%. One dog trained with dead specimens (Dog T) responded to the live insects on both the first and second encounters, responding significantly more often to targets than to controls (P = 0.008, Fisher's exact test) (Tables 1 and 2). However, the other dog trained with dead specimens (Dog W) did not respond upon their first exposure to the live insects, though did respond to the second, and so did not demonstrate statistically significant detection of the live insect target (P = 0.125, Fisher's exact test). Due to the small number of dogs and necessarily limited number of trials per dog, comparisons of the relative effectiveness of the 2 training aids were outside the scope of this study.

Discussion

The findings of our research have provided the first empirical support for scent extract and dead specimens to be used as training aids for the detection of live pentatomid insects. Firstly, both the scent extract of *M. sulciventris* and dead *M. sulciventris* specimens emitted the majority of the same VOCs, including all the principal VOCs, as live *M. sulciventris*, suggesting predominantly similar odors. In

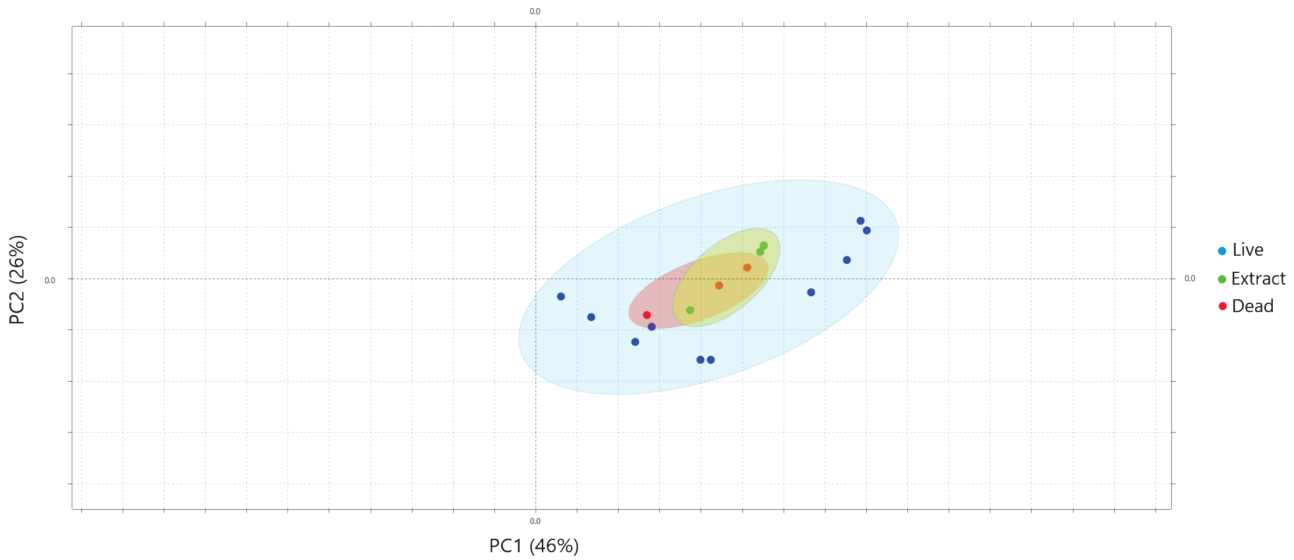

Figure 4. Principal component analysis (PCA) plot of samples from headspace SPME GC-MS scans. Data points are individual scores for each replicate, grouped with ellipses by live *Musgraveia sulciventris* samples (live: $n = 10$), dead *M. sulciventris* samples (dead: $n = 3$), and *M. sulciventris* scent extract samples (extract: $n = 3$). The plot shows a lack of separate clustering of samples by different groups. PC1 accounted for 46% and PC2 accounted for 26% of the variance.

Table 1. Results of canine detection trials, including sensitivity, specificity and *P* values of Fisher's exact tests

Dog	Training aid test			Live insect test		
	Sensitivity (%)	Specificity (%)	*P* value	Sensitivity (%)	Specificity (%)	*P* value
Dog T[a]	100	100	0.0002	100	100	0.0083
Dog W[a]	100	86	0.0071	50	100	0.125
Dog J[b]	100	83	0.0112	100	100	0.0083
Dog A[b]	100	97	0.0008	100	100	0.0083

Targets were the dogs' assigned training aid (either dead specimens or scent extract of *Musgraveia sulciventris*, $n = 3$) for the training aid testing, and $n = 2$ live *M. sulciventris* targets for the live insect testing. Controls ($n = 29$ and $n = 14$, respectively) included edible products, hygiene products, scented products, and odors associated with storage and experimental set-up.

[a]Dog trained with dead specimens.

[b]Dog trained with scent extract.

Table 2. Results of canine detection trials with live insect targets presented in signal detection contingency tables

	Dogs trained with dead insects				Dogs trained with scent extract		
Dog T		Target	Control	Dog J		Target	Control
	Response	2	0		Response	2	0
	No response	0	14		No response	0	14
Dog W		Target	Control	Dog A		Target	Control
	Response	1	0		Response	2	0
	No response	1	14		No response	0	14

Targets were 6 live *Musgraveia sulciventris* specimens.

follow-up testing with biosecurity detector dogs, both dogs trained with scent extract and one trained with dead insects were able to detect live insects accurately upon their first-ever exposures to them.

The headspace chromatograms of both training aids were overall very similar to that of live specimens and shared all of the VOCs that were consistently found in every live insect sample. Scent extract and dead specimens contained the majority of the total VOCs detected in live insect samples—71% and 77%, respectively. Some of these were identified as compounds also observed from *M. sulciventris* in previous research (Park and Sutherland 1962; MacLeod et al. 1975). Notably, both training aids emitted the same principal *E*-2-alkenals as live samples, which are aldehydes considered to be the primary defensive odor compounds of stink bugs (Millar 2004; Blair et al. 2016). Some long-chain hydrocarbons, such as tridecane, were also observed in common; and although these are generally thought to be nearly odorless (Ouellette and Rawn 2015), research has found that mammals do show an olfactory response to them (Ho et al. 2006). However, both training aids each introduced some minor new VOCs that were not found in any live insect sample, which could contribute to a perceptible odor difference. Nevertheless, the considerable

overall similarities in headspace VOCs and minor differences suggest that they may be perceptually similar. This suggests that detector dogs may generalize their training to live insects from either training aid, since animals are most likely to generalize between stimuli with similar perceptual characteristics (Stokes and Baer 1977; Shepard 1987; Moser et al. 2019).

As expected, both biosecurity detector dogs trained with scent extract could successfully detect live insects with high accuracy—100% sensitivity and specificity. This high level of accuracy is consistent with the results of others in similar controlled tests using a simple set-up. To date, only 2 published studies have tested insect scent extract. In one of these studies, Brooks (2001) found that, after being trained with termite scent extract, dogs could detect live termites with 97% sensitivity. Subsequently, Pfiester et al. (2008) found that bed bug-detection dogs responded to a pentane extract of bedbugs in 100% of tests. Therefore, the results in our experiment aligned with expectations for satisfactory detection ability. Of the dogs trained with dead specimens in the present experiment, one achieved 100% sensitivity, while the other achieved only 50% sensitivity, due to not responding upon their first exposure. Overall, most of the tested dogs were capable of learning to respond to live *M. sulciventris* after being trained only with insect scent extract or dead specimens of that species. This provides some promising preliminary evidence for the application of these training aids for the training of biosecurity detector dogs.

However, these results are limited by the use of a small sample of specialized dogs and a limited number of search trials, meaning robust or generalized conclusions should be drawn with caution. On the one hand, using professionally trained, working canines in a simple, controlled set-up allowed a reasonable degree of confidence in their reliability and performance. Moreover, the limited number of live insect trials were necessary to ensure that repeated exposures, and associated learning, did not confound the effect of the training aid itself. On the other hand, the biosecurity detector dogs used in this experiment may have generalized more readily than other, nonspecialized dogs might, due to previous training which involved responding to a large number of related targets. Therefore, further testing of these training aids with a larger sample of novice dogs that are just beginning training is warranted to determine their efficacy more broadly.

Furthermore, this experiment was carried out in an operational setting for ecological validity for this sample of dogs and to inform its immediate translational application. However, researchers have had recent success in using automated apparatus to separate the effect of the handler and context and to ensure accurate and timely feedback in double-blinded scenarios (Edwards 2019). Future research could systematically assess whether these training aids are effective in a controlled laboratory setting with other dog populations before testing their application more broadly.

This experiment sought to investigate the potential efficacy of more than one training aid option for 2 main reasons. Firstly, animals tend to generalize more readily when they are given a higher number of target exemplars of the same category (Ghirlanda and Enquist 2003), and research has suggested that dogs can learn the common components of a series of trained odors, and then respond to new odors containing those same components (for review, see Moser et al. 2019). Therefore, it may be most useful to use a combination of 2 training aids. Secondly, although dead specimens may offer ease of use in a practical sense, there may be circumstances in which using dead specimens may not be appropriate; for example, if discrimination against dead specimens is specifically desired. In these cases, scent extract alone may be a viable alternative. This sample of detector dogs mostly showed a promising ability to generalize to live targets from either training aid, although determining the most effective method of encouraging this generalization requires further testing.

In this experiment, we used *M. sulciventris* as a proof-of-concept model of the efficacy of scent extract and dead specimen training aids in translating to live insect detection of the same species. However, another species of the family Pentatomidae, *H. halys*, is a target insect of interest and high importance for global biosecurity. Based on these results, we hypothesize that we may find success using the same process with *H. halys* since they are a related species that appear to share several important headspace VOCs with *M. sulciventris* (Nixon et al. 2018), and previous research has found that dogs are able to be trained to detect *H. halys* (Lee et al. 2014). Furthermore, following further testing, this method may be applicable to other insect pests that are inaccessible or difficult to maintain.

Supplementary material

Supplementary data are available at *Chemical Senses* online.

Conflicts of interest

None declared.

Funding

This research was funded by the Australian Department of Agriculture's Stronger Biosecurity Quarantine Initiative, and contributes to Ariella Moser's Master of Rural Science thesis undertaken at the University of New England (Armidale, Australia). A.Y.M. is a recipient of an Australian Government Research Training Program stipend scholarship.

Acknowledgments

The authors wish to thank the Australian Department of Agriculture Detector Dog Team, especially Geoffrey O'Neil, Stephen Lorraway, Colleen Eiser, and the participating dog handlers, for their valuable assistance in planning and undertaking the canine experiments, as well as providing their detector dogs. We also wish to thank Calvin Leech for his advice and technical assistance with analyzing the chemical data.

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