



Choice test to determine potential attractants and repellents for the sheep scab mite, *Psoroptes ovis* (Acari: Psoroptidae)

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Abstract

A choice test bioassay was devised to screen compounds as potential semiochemicals (e.g., kairomones or allomones that mediate aggregation, attraction or repellence) for the obligate parasitic mite, *Psoroptes ovis*. The choice test used filter paper discs in a 1:4 test:control ratio and was found to be a reliable, effective and efficient method. Four mammalian lipid components were assessed as potential attractants—linoleic acid, arachidonic acid, methyl myristate and squalene—and the insect/tick repellent DEET for potential repellence. Linoleic acid was significantly attractive to *P. ovis* adult females and has the potential to act as an attractant. Identification of *P. ovis* semiochemicals, especially attractants, would be beneficial in the development of novel control methods and tools for this species. This is essential considering the increase in resistance to the limited prophylactic chemical treatments in the UK, and the high prevalence of scab infections.

Keywords *Psoroptes ovis* · Sheep scab · Attractants · Repellents · Choice tests

Introduction

The sheep scab mite *Psoroptes ovis* (Hering) is an obligate ectoparasite of sheep producing a highly contagious, pruritic and exudative dermatitis initiated by allergens contained in the mite secretory or excretory products (Bates 1981, 2012; Lewis 1997). *Psoroptes ovis* exploits this allergic reaction: the heat and humidity produced by the inflammation forming the micro-climate needed for mite survival and the leakage of serous exudate forming the basis of the mite's nutrition (Bates 1981). This inflammation can become so aggravating that the host sheep spends more time scratching than feeding or resting which can result in emaciation and even death. It is therefore a severe welfare (Tarry 1974; Kirkwood 1980) and economic issue—mainly due to the costs relating to prophylactic treatments, and loss in production efficiency (Nieuwhof and Bishop 2005). However, there are very few chemicals to control these mites and their widespread and intensive use has resulted in reduced effectiveness (Coles and Stafford 1999; Doherty et al. 2018), environmental concerns (Virtue and Clayton 1997) and has affected the

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health of personnel involved in treating sheep (Pilkington et al. 2001; Jamal et al. 2001). Therefore, the development of novel control measures is key to the advancement of best practice management of this debilitating parasite.

Arthropod and insect pests often rely on olfactory signals or semiochemicals for mate and/or host location (Cardé and Millar 2004). The discovery of effective attractants or repellents for these pests could lead to the development of novel control tools. For example, a pheromone-assisted tick control method has been developed with the use of the tick arrestant pheromones guanine, xanthine and hematin, and the acaricide permethrin (Sonenshine 2006). In addition, several astigmatid mites have been found to be attracted to common fatty acids and their esters, possibly as food attractants (Sato et al. 1993), and these compounds have been used to attract house dust mites (Ninomiya and Kawasaki 1988; Skelton et al. 2010). The detection of a mite-produced chemical, tridecane, has also been used for practical purposes in the detection of mite infestations such as *Acarus siro*, *Aeroglyphus robustus* and *Lepidoglyphus destructor* in bin-stored wheat (Tuma et al. 1990). DEET (N,N-diethyl-meta-toluamide) is the well-known insect repellent, that temporarily blocks the neuron receptors that detect CO₂. DEET has proven to be a very effective repellent for biting pests, especially mosquitoes, but also for ticks and the mange mite *Sarcoptes scabiei* (Bissinger and Roe 2010; Fang et al. 2015; Meng et al. 2016) and is an active ingredient in a variety of marketed insect repellent products (<https://www.epa.gov/insect-repellents/deet>).

Understanding how arthropods respond to chemicals requires the development of appropriate tools to measure their behaviour. However, there is no information in the literature on attractants/volatiles or assays/tools for assessing responses of *P. ovis*. Therefore, our initial investigations assessed several different laboratory bioassays designed for other species of Acari/insect (Arlian and Vyszenski-Moher 1995; Carr and Roe 2016; Conte et al. 1989; Stelinski and Siddharth 2013; Vet et al. 1983) with lipid compounds found in skin that are known to attract *S. scabiei*, another obligate parasite (Arlian and Vyszenski-Moher 1995). Assay designs included mite-adapted versions of straight-tube olfactometers, four-armed olfactometer, and various choice test designs with treatment points in a 1:3 test:control ratio, a 2:2 test:control ratio and a 1:4 test:control ratio, and also treatments placed in concentric rings as described by Arlian and Vyszenski-Moher (1995) with *S. scabiei*.

However, for this study the choice test in a 1:4 ratio was chosen as this method was found to be the most reliable, effective and efficient method for determining potential attractants and repellents for *P. ovis*. The tests were repeatable, easy to assess, suitable for use with mites, and allowed for many replicates to be set up simultaneously.

The identification of attractants and or repellents would help in scab management as they could be incorporated into traps that either act as just a lure, a lure and kill, or used with a repellent as a push-pull tool with the repellent aiding the movement of mites on fomites towards an attractant lure trap. Sheep are known to have favoured places for rubbing and scratching which are ideal locations for transfer of mites between sheep especially as mites can remain infective for 15–16 days off host (Meintjes et al. 2002; O'Brien et al. 1994; Smith et al. 1999) thus any posts or rubbing areas where sheep have been scratching, remain residual sources of re-infestation for a considerable length of time. Therefore, an attractant lure placed at these locations could reduce the potential spread of mites between sheep which in turn would help minimise the use of prophylactic chemical treatments to the sheep. The aim of this study was to therefore assess the attractancy of four known mammalian lipid compounds, and the repellence of DEET to adult female mites of *P. ovis* using the developed laboratory choice assay in a 1:4 test:control ratio.

Materials and methods

Mites

Psoroptes ovis were collected from the pelts of experimentally infested ewe sheep. The mites were examined under a binocular microscope at magnification of 10× to 50× and grouped according to sex and stage following the morphological descriptions of Sanders et al. (2000). Adult female mites were used in the experiments.

Preparation of test compounds and their application

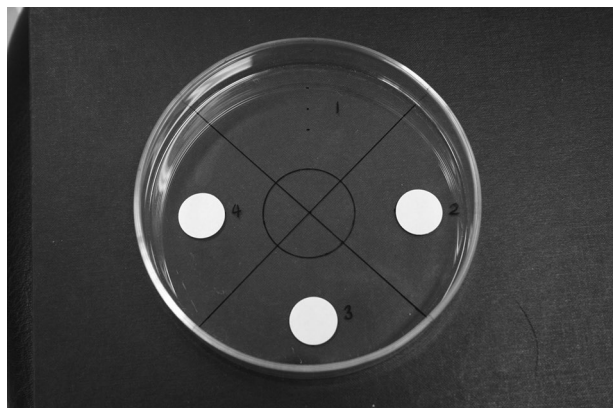
Attractants/repellent

Five volatile attractants were obtained and used in these assays at the following concentrations: 1M linoleic acid (95%, Alfa Aesar L07949), 0.1M arachidonic acid (Calbiochem 181198), 1M methyl myristate (Sigma M-3378), 0.1M squalene (98%, Sigma-Aldrich S3626) and 1M DEET (Sigma-Aldrich 36542). These concentrations were selected from individual dose-response tests undertaken in preliminary experiments.

Multi-choice bioassay

Test arenas (see Fig. 1) comprised of glass Petri dishes (14 cm diameter), muffled in an oven at 500 °C for a minimum of 4 h prior to use. Four treatment areas were marked on the underside of the base of the Petri dish. The centre of each of these areas was marked and filter papers (Whatman No. 1, 20 mm diameter) placed if required. Amounts of 25 µl of the treatment compound dissolved in diethyl ether, or control solution (diethyl ether), were pipetted onto the filter papers accordingly. The treatment points in each dish were allocated a number from 1–4 so that all combinations were represented and to prevent directional bias: 1 = no paper; 2 = blank paper; 3 = solvent; 4 = treatment. After treatment the discs were placed in the corresponding Petri dish and left for 1 h to allow the solvent to evaporate. Thirty adult female *P. ovis* mites were then released in the centre of the arena, the Petri dishes were then closed using a corresponding glass lid and placed in a dark incubator

Fig. 1 Test arena for multi-choice bioassays



(Sanyo MLR-351H) set at 25 °C and 80% RH. There were six replicates per treatment combination.

The mites in each area of each Petri dish were counted after 6 and 24 h. Experiments were repeated with dishes rotated in different directions to preclude any bias from the experimental set-up. Linoleic acid was used as a positive control as this chemical was found to be consistently attractive to *P. ovis* in all the preliminary bioassay designs.

Statistical analysis

A multinomial model was built to investigate whether there was a significant difference in proportion of mites between each of the areas in the test arena, specifically whether there were differences: between paper and no paper, between solvent on paper and paper, and between substance in solvent on paper and solvent on paper. The VGAM package in R (Yee 2010) was applied to fit the model. The significance of the effect of each treatment compared with the previous treatment (no paper, paper, solvent, substance) was estimated by two-tailed t-test derived from the summary of the multinomial model.

Model fit was assessed by examining the standardised residuals associated with the individual observations underlying each estimated difference. A Kolmogorov–Smirnov test applied to each set of residuals showed that each set of residuals was consistent ($p > 0.05$) with a normal distribution with mean of zero and a standard deviation of one. Hence, we concluded that the individual observations of the numbers of mites in each area were consistent with the underlying multinomial model without any other additional significant variation.

Results

There was no mite mortality after the 24-h test period therefore the mites were actively choosing filter papers either at random for behavioural/physical reasons or were actively attracted to or repelled from the papers. Figure 2 shows a summary of the results.

Linoleic acid

Linoleic acid had a significant attractive effect compared to the solvent control after both 6 and 24 h ($p < 0.0001$). Solvent on paper was no more or less attractive than paper ($p > 0.05$), and paper was no more or less attractive than empty arena ($p > 0.05$).

Squalene

Squalene had a weak but significant attractive effect after 24 h compared to the solvent control (adjusted $p = 0.006$). No significant effects were observed after 6 h ($p > 0.05$).

Arachadonic acid

More mites were observed on the paper used in the arachidonic acid experiment than in the empty part of the arena ($p = 0.014$) after 24 h. No other significant effects were observed in this experiment.

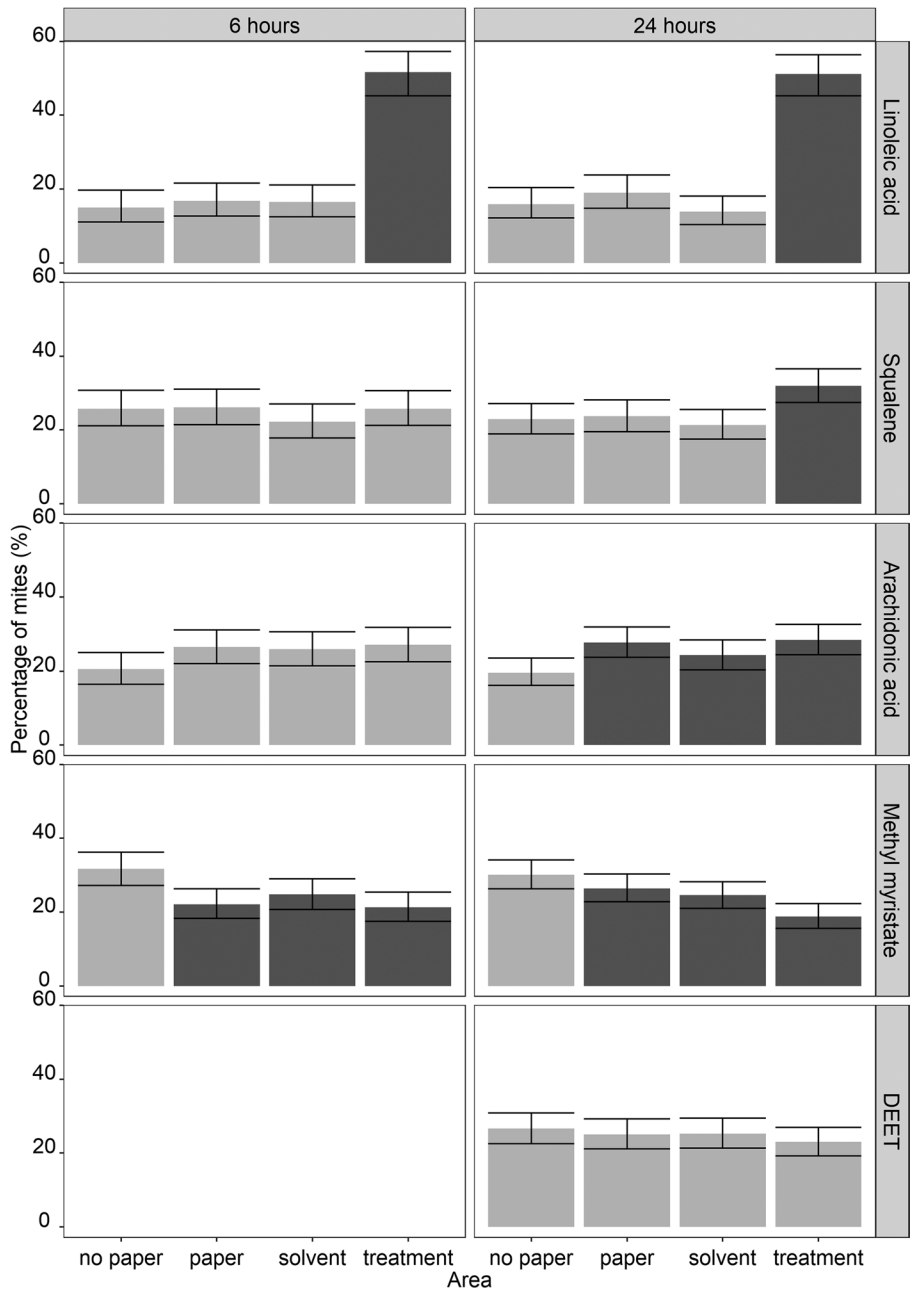


Fig. 2 Mean (+95% confidence intervals) percentage of mites in each area of the experimental area (colour change between adjacent areas indicate a significant difference)

Methyl myristate

Methyl myristate had a significant repulsive effect compared to the solvent after 24 h ($p=0.047$). After 6 h, more mites were observed on the paper used in this experiment than in the empty part of the arena ($p=0.010$). No other significant effects were observed.

DEET

No significant effects were observed in the 24-h DEET experiment.

Discussion

The development of an effective and efficient assay allowed for potential attractants and repellents to be assessed against *P. ovis*. The choice test assay was superior to the olfactometers as the olfactometers became quickly saturated with the more volatile attractants making the mites indecisive in their response often leading to desiccation and unreliable results.

Linoleic acid had the strongest ‘attractive’ response out of the compounds tested. However, the weakened response to the other compounds could be due to the concentrations tested but now that a reliable working bioassay has been devised, multiple concentrations and different volatile attractants and repellents can be assessed. DEET has been reported to be effective against the mange mite *S. scabiei* (Fang et al. 2015); however, Fang et al. (2015) used a commercially formulated product where composition and concentration can vary. In our study we diluted a technical sample to a concentration comparable to the other compounds under test therefore the negative response reported here may be due to concentration and/or the formulation may be key to a response.

The semiochemical compounds tested in this study were host-related (kairomones). It is yet to be determined what types of semiochemicals and which compounds are present and utilised in *P. ovis* ecology, development and behaviour. Ixodid ticks use many kairomones for host identification, e.g., volatiles such as carbon dioxide and ammonia and various oils and acids (Carr and Roe 2016). Therefore, there could be an array of *P. ovis* kairomones produced by sheep as well as conspecific scab mite pheromones waiting to be discovered. The development of this functional choice assay will help in screening potential compounds to identify which, if any, can be used to take forward in pheromone-assisted control methods. This knowledge would help in developing new and innovative control technologies. There are already novel eco-friendly sticky traps for the control of the dog tick *Rhipicephalus sanguineus* which attracts the ticks using known assembly pheromones that are nanoencapsulated onto the sticky trap for slow and safe release (Anish et al. 2017). These traps have been developed with the intention of controlling tick infestations in dog kennels (Anish et al. 2017). Tick decoys have also been developed which have the tick sex pheromone 2,6-dichlorophenol and the acaricide propoxur impregnated into their polyvinyl chloride plastic for the control of ticks on rabbits (Carr and Roe 2016). These decoys have also proven successful over a 3-month trial on cattle (Norval et al. 1992, 1994).

If pheromones could be identified for *P. ovis*, similar traps could be developed for use on fencing/rubbing posts in fields and sheep pens with potentially a small amount of pesticide incorporated so that the mites are both lured and killed. In this study only host related

attractants were tested and so trap development would be limited to off-host fomite locations but if conspecific scab mite pheromones could be identified, decoys might also be developed for use on host.

The development of novel control methods and tools for *P. ovis*, such as traps and lures, is important given the limited chemical treatments available and the increasing incidences of pesticide resistance (Clarke et al. 1996; Doherty et al. 2018; Romero et al. 2008; Synge et al. 1995) and increased cases of sheep scab (Chivers et al. 2018; Rose and Wall 2012). This study has developed an effective assay which would allow for potential semiochemicals to be screened for their use as attractants, repellents and aggregation pheromones for the development of lures against *P. ovis*.

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