

Interference in foraging behaviour of European and American house dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* (Acari: Pyroglyphidae) by catmint, *Nepeta cataria* (Lamiaceae)

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Abstract The European and American house dust mites, *Dermatophagoides pteronyssinus* and *D. farinae*, have a huge impact upon human health worldwide due to being the most important indoor trigger of atopic diseases such as asthma, rhinitis and atopic dermatitis. Preceding studies have shown that the behavioural response of house dust mites towards volatile chemicals from food sources can be assessed using a Y-tube olfactometer assay. In the current study, we used this assay to investigate, for the first time, the ability of the essential oil of the catmint plant, *Nepeta cataria* (Lamiaceae), known to repel other ectoparasites affecting human and animal health, to interfere with the attraction of *D. pteronyssinus* and *D. farinae* towards a standard food source (fish flakes). Two distinct chemotypes (A and B), enriched in the iridoid compounds (4a*S*,7*S*,7a*R*)-nepetalactone and (4a*S*,7*S*,7a*S*)-nepetalactone, and the sesquiterpene (*E*)-(1*R*,9*S*)-caryophyllene, were used. Initial assays with a hexane extract of fish flakes (FF extract) confirmed attraction of mites to this positive control ($P < 0.001$ and $P < 0.05$ for *D. pteronyssinus* and *D. farinae* respectively), but when presented in combination with either *N. cataria* chemotype, tested across a range of doses (10, 1, 0.1 and 0.01 µg), decreasing attraction of mites to their food source was observed as the dose augmented. Our study shows that *N. cataria*, enriched in iridoid nepetalactones and (*E*)-(1*R*,9*S*)-caryophyllene, exhibits potent repellent activity for house dust mites, and has the potential for deployment in control programmes based on interference with normal house dust mite behaviour.

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Introduction

House dust mites belong to the family Pyroglyphidae, of which ten species have been reported to occur in house dust (Platts-Mills et al. 1992). Many other species, including storage mites [e.g. *Acarus siro* (L.) and *Tyrophagus putrescentiae* (Schränk)], can occur in houses and become the predominant population. The European house dust mite, *Dermatophagoides pteronyssinus* (Trouessart), and the American house dust mite, *D. farinae* (Hughes), are two of the most important pyroglyphid mite species because of their cosmopolitan occurrence and abundance in homes (Pollart et al. 1987; Arlian and Morgan 2003), and because they are a major source of multiple potent allergens (Platts-Mills et al. 1992; Stewart 1995; Arlian and Morgan 2003). House dust is a mixture containing many different allergens, but the major allergen is derived from mites, especially *D. pteronyssinus* and *D. farinae*. Exposure to different allergens can elicit asthma attacks in sensitised individuals (Tovey 1992).

Asthma is a chronic inflammatory disease of the respiratory system associated with allergen inhalation. Up to 85% of people with asthma in the United Kingdom are sensitive to house dust mites (Asthma UK, 2000), and the current annual economic cost of this condition in the UK is estimated to be £1.05 billion (WHO 2006). The major causal factors of house dust mite allergens are present in the faecal pellet (Voorhorst et al. 1964; Tovey et al. 1981; Robinson et al. 1997). There are 16 known allergens of the house dust mite (Thomas and Smith 1999). The potent enzymes of the mite have been recorded as causing an allergic-like reaction in the absence of a true allergic response (Machado et al. 1996). The major allergen Derp1 has been found in foetal amniotic fluid at 16–17 week gestation and in the cord blood of some babies at birth (Holloway et al. 2000). The possibility of this phenomenon as a link to allergy, possibly through the immature gut, has been investigated (Holloway et al. 2000). Nesting sites for the mite include carpets, padded sofas and chairs, soft toys and especially bedding. Modern homes with high temperature, high humidity and lack of ventilation have been shown to be ideal breeding grounds (Platts-Mills and Woodfolk 1997). In one study, a house dust collection, including bedding, was found to contain micro-organisms and fungi in abundance as well as colonies of mites in various stages of life (Hay 1991; Hay et al. 1992). The treatment of asthma is both pharmacological, including immunotherapy (Abramson et al. 1995), and non-pharmacological. Non-pharmacological treatment often involves ecological procedures such as elimination of allergens in the patient's surroundings (Colloff 1991).

Control of house dust mite populations has been principally through deployment of synthetic toxicants such as γ -benzene hexachloride (γ -BHC), pirimiphos-methyl, benzyl benzoate and pyrethroids, and the synthetic repellents diethyl-m-toluamide (DEET) and dibutyl phthalate (Pollart et al. 1987; Platts-Mills et al. 1992; Schober et al. 1992). Although partially effective, their repeated use has resulted in the development of acaricide resistance (van Bronswijk and Sinha 1971), produced undesirable effects on non-target organisms, and fostered environmental and human health concerns (Pollart et al. 1987; Hayes and Laws 1991). These problems highlight the need for the development of selective dust mite control alternatives, particularly with volatile action for an easier and more

effective application to the mite populations. Currently available control materials are formulated as foams, powders, solutions or sprays and have only contact action, which, although convenient to use, are not very effective. Alternative compounds or new methods of controlling house dust mites are needed. One source of alternatives lies in botanical compounds that are commonly used as arthropod repellents. Recently, the catmint plant, *Nepeta cataria* L. (Lamiaceae), was shown to be an effective repellent against afro-tropical mosquitoes, ixodid ticks and red poultry mites (Birkett et al. 2011). Two different chemotypes were assessed, one enriched in the cyclopentenoid compound (4a*S*,7*S*,7a*R*)-nepetalactone, and the other enriched in (4a*S*,7*S*,7a*R*)-nepetalactone (4a*S*,7*S*,7a*S*)-nepetalactone and the sesquiterpene (*E*)-(1*R*,9*S*)-caryophyllene (Fig. 1). With a view to developing a novel strategy for house dust mite control, based on deployment of repellent and attractant semiochemicals (e.g. neryl formate, the recently-identified airborne aggregation pheromone (Skelton et al. 2010) in a push–pull strategy (Cook et al. 2007), we assessed the ability of the two previously defined *N. cataria* chemotypes to interfere with foraging behaviour of *Dermatophagoides* spp. using a Y-tube olfactometer assay previously established in our laboratory (Skelton et al. 2007).

Materials and methods

House dust mites

Dermatophagoides pteronyssinus and *D. farinae* were obtained from laboratory cultures maintained at Rothamsted Research, fed on a mixture of Aquarium gold fish flakes (Mars Ireland, Dublin⁴®, Mars Fishcare Europe) and ground yeast cells (Allison, London, United Kingdom), and kept at 23–25°C and 65–75% RH respectively (Skelton et al. 2007, 2010) until required for olfactometer assays. Mites were removed from the colony cells and were kept in other cells providing no food, maintained at 24–25°C, 65–75% RH and left for 2–3 days prior to use.

Preparation of fish flake extract and *Nepeta cataria* essential oils

A fish flake extract was prepared by addition of flakes (0.1 g) to distilled hexane (10 ml) and sonication at room temperature using an ultra-sonic bath (5 min). After leaving overnight at 4°C for 24 h, the mixture was filtered through a Pasteur pipette plugged with cotton wool, with the filtered solution being kept at 4°C until required for behavior assays. Essential oils of *N. cataria* chemotypes (A and B) comprised (A) (4a*S*,7*S*,7a*R*)-nepetalactone: (*E*)-(1*R*,9*S*)-

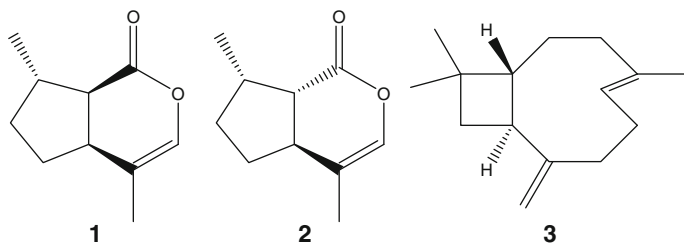


Fig. 1 Structures of the compounds found in *Nepeta cataria* (Lamiaceae) chemotypes A and B. 1 (4a*S*,7*S*,7a*R*)-nepetalactone; 2 (4a*S*,7*S*,7a*S*)-nepetalactone; 3 (*E*)-(1*R*,9*S*)-caryophyllene

caryophyllene (91.95: 8.05 by GC) and (B) (4a*S*,7*S*,7a*R*)-nepetalactone: (4a*S*,7*S*,7a*S*)-nepetalactone: (*E*)-(1*R*,9*S*)-caryophyllene (16.98: 69.83: 13.19), and were prepared by steam distillation as reported previously (Birkett et al. 2011). Solutions of essential oils for chemotypes A and B in distilled hexane were prepared across a range of concentrations (10, 1, 0.1 and 0.01 µg/10 µl) and stored at 4°C until required for behavior assays.

Y-tube olfactometer assay

A Y-tube olfactometer (diameter bore of 2.5 mm, humidified air flow of 15 ml/min) was used to investigate house dust mite behavior in response to a standard positive stimulus, i.e. a fish flake extract, as described previously (Skelton et al. 2007), and the ability of essential oils of *N. cataria* chemotypes A and B to interfere with the biological activity of the fish flake extract. Assays were carried out in a darkened room with a temperature ranging between 23 and 26°C and 65–75% RH. Mites were removed from the colony cell and put in a separate cell 0.5 h prior to assays, to allow acclimatization to assay conditions. Aliquot portions (10 µl) of the fish flake extract and distilled hexane (10 µl) were taken using microcapillary tubes and added to separate paper discs (1.5 cm diameter). Discs were allowed to dry for 1 min and separately placed into the arms of the Y-tube. Single adult mites (either male or female) were picked up with the help of a teasing needle under a microscope and placed one cm before the intersection of the Y-tube. Each assay lasted for ten minutes and mite entries into either the fish flake (treated) arm, hexane (negative control) arm or returns back without making any choice, were recorded. Assays were discarded if the mite failed to move to the intersection and had not moved to either arm. If the mite reached the endpoint (4 cm) of the Y-tube arms before the end of the assay, the assay was curtailed, in order to prevent mite entry into the PTFE tubing. After every attempted assay, each Y-tube apparatus was washed two times with detergent and acetone, and dried in an oven at 160°C to remove any residual volatiles. Treatment and control arms were rotated after each assay.

Assays for checking the effect of fish flakes towards *D. pteronyssinus* and *D. farinae* were repeated 120 and 40 times, respectively. To assess the effect of *N. cataria* A and B chemotypes, the same assay was used as for observing the response of mites towards fish flakes. The stimulus concentrations tested were selected on the basis of those used in our previous studies on *N. cataria* repellency (Birkett et al. 2011) and dust mite behaviour (Skelton et al. 2007, 2010), and were shown in studies elsewhere (Zhu et al. 2009) to be non-toxic to human beings. Aliquots of the fish flake extract (10 µl) and solutions of *N. cataria* (10, 1, 0.1 and 0.01 µg/10 µl) were added to separate filter paper discs (1.5 cm diameter) and prepared in the same way as described above. Assays were replicated 40 times for fish flakes + chemotype A and fish flakes + chemotype B (at each dose tested). The only exception was assays for fish flakes + 10 µg chemotype A, which were performed 80 times. Due to time constraints, chemotype A was only tested with *D. pteronyssinus*, and chemotype B was only tested with *D. farinae*. Because of the preparation and time required for each test, assays were split across several days. For instance, the 40 assays of fish flakes for *D. farinae* spanned four consecutive days (10 assays were performed on each day).

The binomial model for the experiment

The total number of mites that made a choice to either the treated or the negative control arm in the series of Y-tube olfactometer assays on day *i* was denoted by n_i . Each assay

performed on a day, therefore, represented an independent trial of a binomial experiment with n_t total trials per day. Five different doses were tested in the treated arm, including a zero-dose (positive control) corresponding to the fish flake extract. All mites within a day were tested with the same dose and each dose was replicated in 4 days, with the exception of the fish flake extract for *D. pteronyssinus*, which was replicated across six different days.

The observed data comprised the number of mites that chose the treated arm $y_1, \dots, y_N$ out of $n_1, \dots, n_N$ respondent mites on days $1, \dots, N$, respectively. The analysed responses consisted of the proportions of mites choosing the treated arm, $y_1/n_1, \dots, y_N/n_N$ on days $1, \dots, N$. The two mite species were considered separately, and so the total number of observations per analysis was $N = 22$ and $N = 20$ for *D. pteronyssinus* and *D. farinae*, respectively (see Table 1).

Statistical analysis

A binomial generalized linear model (GLM) with logit link was fitted to the data (Dobson 1990) using the GenStat® software package (Payne et al. 2009). To improve the fit of the binomial GLM, the dose variable was transformed into $[\log_{10}(\text{dose} - \varepsilon)]^2$, with $0 \leq \text{dose} \leq 10$ and $\varepsilon = 0.001$. The offset ε was required to deal with the zero dose (positive control), and was chosen by inspection to give an adequate fit to the data. To account for overall differences in background environment, a factor indicating the day of the assay was also included in the model.

The fitted model was then used to predict the dose of *N. cataria* required for producing a 0.5 response of mites (ED_{50}), i.e. the dose at which preference existed to neither the treated nor the negative control arms. It was then ascertained that dose values different to the predicted ED_{50} had an effect on mite preferences; the direction of this effect (i.e. preference to either the treated or the control arm) could then be investigated from a plot of the fitted model. Estimating the ED_{50} established the range of doses that were attractive to *D. pteronyssinus* and *D. farinae* mites and those that were repellent.

To make individual statements of the five tested doses, the response for each of the doses in question was predicted under the fitted model. The estimated mean response for each of the five dose groups was tested against a threshold value of 0.5. If the predicted response (i.e. proportion of mites choosing the treated arm) was statistically significantly greater than 0.5, this indicated that mites presented with that dose preferred the treated over the negative control arm. If the test for a predicted response was statistically significantly less than 0.5, it was concluded that such a dose interfered with the attractant effect of the FF extract such that mites preferred the negative control over the treated arm.

Results

Analyses of the *D. pteronyssinus* and *D. farinae* data sets showed strong evidence that the proportion of mites choosing the treated arm was affected by the dose variable ($P < 0.01$ in all cases). Figures 2a and b present the fitted curves for *D. pteronyssinus* and *D. farinae*, respectively, together with their corresponding 95% confidence interval (CI).

To assess the fit of the model visually, the original data points are shown as open circles. The predicted ED_{50} values (in an offset $\log_{10}$ scale, and with their corresponding 95% CI) were -2.000 ($-2.366, -1.550$) and -2.311 ($-2.784, -1.713$) for *D. pteronyssinus* and *D. farinae*, respectively. The ED_{50} for *D. pteronyssinus* suggested that $\log_{10}$ -doses of *N. cataria* ranging between -2.366 and -1.550 (or 0.003 and $0.027 \mu\text{g}$ in the original

Table 1 Number of mites making a choice to the treated arm, y_i , in a series of Y-tube olfactometer assays at different doses of catmint, *Nepeta cataria* chemotypes A (tested with *Dermatophagoides pteronyssinus*) and B (tested with *D. farinae*)

<i>D. pteronyssinus</i>					<i>D. farinae</i>				
Day, i	Dose [μg]	Number of mites to treated arm, y_i	Number of respondent mites, n_i	Number of non- respondent mites	Day, i	Dose [μg]	Number of mites to treated arm, y_i	Number of respondent mites, n_i	Number of non- respondent mites
1	0	10	14	6	1	0	5	7	3
2	0	9	11	9	2	0	4	6	4
3	0	12	16	4	3	0	5	7	3
4	0	12	16	4	4	0	5	8	2
5	0	12	18	2	5	0.01	3	7	3
6	0	10	14	6	6	0.01	2	5	5
7	0.01	4	8	2	7	0.01	4	9	1
8	0.01	2	5	5	8	0.01	1	3	7
9	0.01	3	5	5	9	0.1	1	4	6
10	0.01	2	4	6	10	0.1	1	4	6
11	0.1	1	4	6	11	0.1	3	8	2
12	0.1	2	6	4	12	0.1	3	9	1
13	0.1	3	8	2	13	1	2	6	4
14	0.1	1	4	6	14	1	2	6	4
15	1	3	7	3	15	1	2	8	2
16	1	2	6	4	16	1	1	7	3
17	1	3	8	2	17	10	1	6	4
18	1	3	8	2	18	10	2	5	5
19	10	2	11	9	19	10	2	7	3
20	10	4	12	8	20	10	2	8	2
21	10	4	11	9					
22	10	3	11	9					

The total number of mites that made a choice to either the treated or the negative control arms (referred to as respondent mites), n_i , and the number of non-respondents are also shown. The responses analysed corresponded to proportions of the form y_i/n_i

scale) would have no effect on the preference of the mites to either the treated or the negative control arms, with 95% confidence. There was, therefore, strong evidence that doses outside this interval would have an effect on mite choices. Of particular interest was the zero dose, which lies outside the 95% confidence interval of the ED_{50} and which, according to Fig. 2a, may be attractive to *D. pteronyssinus* mites. A similar statement could be made for the zero dose for *D. farinae* mites.

To establish the attraction of the fish flake food source and the ability of *N. cataria* to interfere with this putative attraction, a test of the predicted response at each of the doses, 10, 1, 0.1, 0.01 and 0 μg , versus a response of 0.5, was performed (Figs. 3, 4). The proportion of mites choosing the arm treated with the fish flake extract (FF) was significantly greater than 0.5, for both *D. pteronyssinus* and *D. farinae* mites ($P < 0.001$ and $P < 0.05$, respectively). This provided strong evidence of the attraction effect of the FF extract over both species of mites. When *N. cataria* chemotype A and B oils were added to the FF extract, the response to the treatment FF + *N. cataria* (0.01 μg) was not

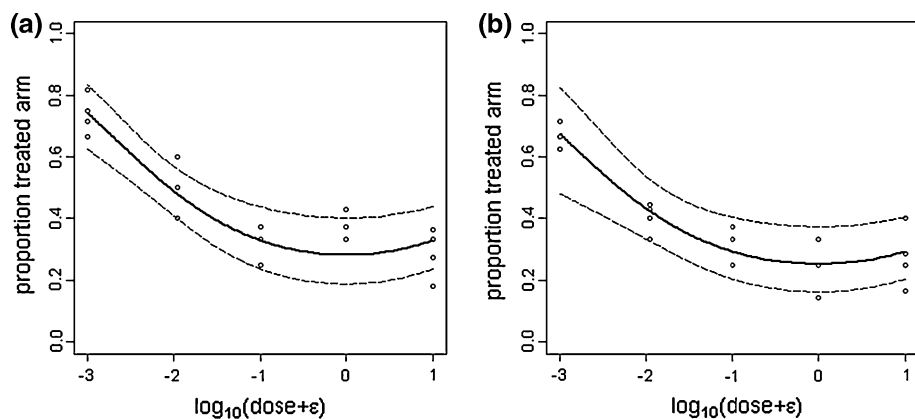


Fig. 2 The fitted binomial generalized linear model showing the predicted proportion of mites choosing the treated arm for a range of doses of catmint, *Nepeta cataria*. Doses are shown in a logarithmic scale offset with a small value $\varepsilon = 0.001$. The dotted curves indicate the 95% confidence interval of the predicted response for (a) European house dust mites, *Dermatophagoides pteronyssinus*, and (b) American house dust mites, *D. farinae*

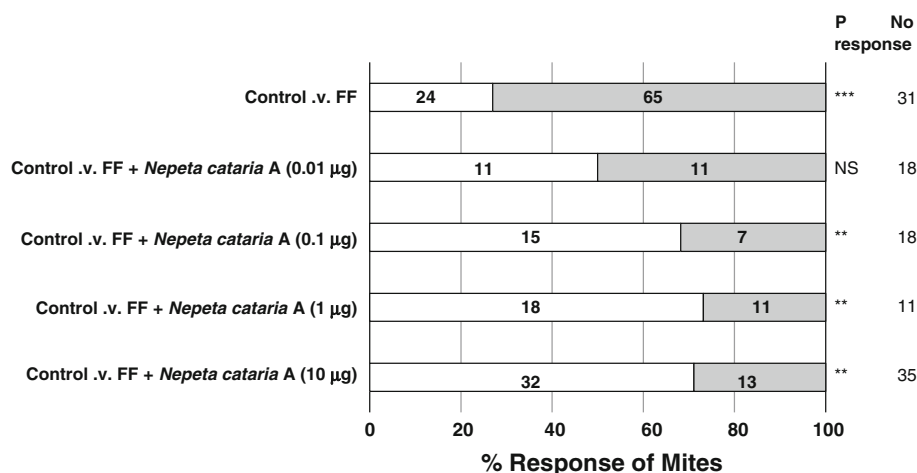


Fig. 3 Accumulated counts of European house dust mites, *Dermatophagoides pteronyssinus*, as recorded during the series of Y-tube olfactometer assays, in response to *Nepeta cataria* chemotype A. Numbers in bars correspond to the total number of mites choosing the control (white area) versus the treated arm (grey area) across all experiments involving the same level of *N. cataria* dose. The accumulated counts of non-responsive mites per dose are also shown. P values in black indicate treatments significantly different to the control, with significance levels ** $P < 0.01$, *** $P < 0.001$ and NS non-significant

significantly different to 0.5 for either species of mites. This was in agreement with the ED_{50} values from above, and confirmed that mites presented with such a dose of *N. cataria* had no preference to either the treated or the negative control arms. The response to all other doses, FF + *N. cataria* (0.1 µg), FF + *N. cataria* (1 µg) and FF + *N. cataria* (10 µg), was significantly less than 0.5 for both mite species ($P < 0.01$ in all three cases for *D. pteronyssinus* and $P < 0.001$ in all cases for *D. farinae*). These results indicated that, when *N. cataria* oils were added to the FF extract at doses of at least 0.1 µg, there was

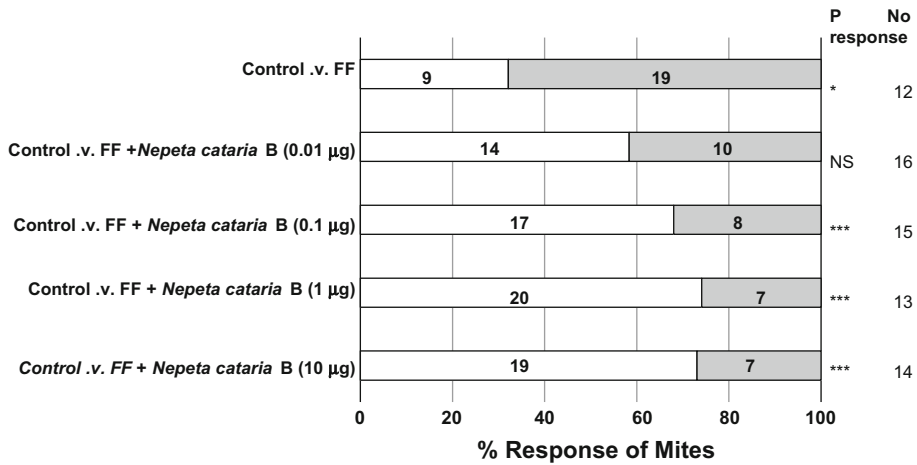


Fig. 4 Accumulated counts of American house dust mites, *Dermatophagoides farinae*, as recorded during the series of Y-tube olfactometer assays, in response to *Nepeta cataria* chemotype B. Numbers in bars correspond to the total number of mites choosing the control (white area) versus the treated arm (grey area) across all experiments involving the same level of *N. cataria* dose. The accumulated counts of non-respondent mites per dose are also shown. *P* values indicate treatments significantly different to the control, with significance levels **P* < 0.05, ****P* < 0.001 and NS non-significant

statistically significant interference with the attraction of both species of mite towards their food source.

Discussion

The main objective of this study was to evaluate whether catmint, *N. cataria*, interferes with house dust mite foraging behaviour, in a similar manner to that reported for afro-tropical mosquitoes, ixodid ticks and red poultry mites (Birkett et al. 2011). Using an established Y-tube olfactometer assay to measure the response of dust mites towards a fish flake food source (Skelton et al. 2007), the data suggest that *N. cataria* has the ability to interfere with food location, even at very low (i.e. nanogram) levels. Whilst both chemotypes tested contain (*E*)-(1*R*,9*S*)-caryophyllene, chemotype A comprises one nepetalactone isomer ((4*aS*,7*S*,7*aR*)-nepetalactone), whilst chemotype B comprises two nepetalactone isomers ((4*aS*,7*S*,7*aR*)-nepetalactone and (4*aS*,7*S*,7*aS*)-nepetalactone). Previous experiments with chemotypes A and B demonstrated repellency of ectoparasites from topically-dosed forearms (Birkett et al. 2011), and so it is likely that the chemotypes will also interfere with location of a natural food source, i.e. skin flakes, by dust mites. Further experiments are required to confirm that this is the case.

Studies that investigate the repellent activity of botanically-derived materials towards ectoparasitic mites are scarce (Birkett et al. 2008), and to our knowledge, this is the first report detailing the ability of *N. cataria* to interfere with house dust mite behavior. Studies elsewhere on investigating the potential for using botanically-derived materials to control mites have focussed predominantly on assessing acaricidal activity (e.g. Choi et al. 2004). Our results demonstrate the potential for using a Y-tube olfactometer assay to assess the ability of botanically-derived materials, and natural products, to interfere with house dust mite behavior at a distance.

In general, plant extracts, essential oils and their constituents are not as potent toxicants as most synthetic dust mite control products, since they are effective against arthropod pests for a relatively short period because of their relatively high volatility. Consequently, testing of the *N. cataria* chemotypes for acaricidal activity fell outside the scope of this study. However, they could be of practical use as highly effective plant-based repellents for personal protection against mites and mite-borne diseases. The lower volatility of *N. cataria* components, compared to traditional repellents such as citronellol, suggests that these highly repellent materials could potentially overcome problems associated with high volatility. Furthermore, *N. cataria* essential oils are readily available and inexpensive to produce (Birkett and Pickett 2003). Further studies are underway to investigate their potential for use as part of a novel strategy for dust mite control, based on deployment of repellent and attractant semiochemicals in a push–pull strategy (Cook et al. 2007), which could incorporate attractive pull components such as the recently identified aggregation pheromone (Skelton et al. 2010).

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