IN: A.M. Rimando & S.O. Duke [eds.] Natural Products for Pest Management. ACS Symposium Series #927. American Chemical Society, Washington, DC. 2006.

## Chapter 13

# Natural Insect Repellents: Activity against Mosquitoes and Cockroaches

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Recent research has focused on the repellent properties of extracts from the catnip plant (*Nepeta cataria*) and the Osage orange (*Maclura pomifera*) fruit. This chapter includes results on German cockroach (*Blattella germanica*), and house fly (*Musca domestica*) contact irritancy to catnip essential oil, and its major components, *Z,E*-nepetalactone and *E,Z*-nepetalactone, compared with the commercial standard, N,N-diethyl-*m*-toluamide (DEET). Both species showed high percentage repellency values when exposed to filter paper treated with catnip essential oil or the individual nepetalactone isomers. Of the two nepetalactone isomers evaluated, German cockroaches were most responsive to the *E,Z* isomer. House flies showed similar trends in contact irritancy, responding to surfaces treated with the predominant catnip isomer, *Z,E*-nepetalactone, more intensely than to the catnip essential oil. Catnip and Osage orange essential oils, and a sesquiterpene found in Osage orange, elemol, were evaluated for repellency to the northern house mosquito (*Culex pipiens*) and are presented here. Two mosquito bioassays were used to measure percentage and contact repellecy. Mosquitoes responded initially with high percentage repellency to surfaces treated with catnip essential oil. From the residual repellency study, this trend in repellency by the catnip oil significantly decreased over the 180-minute test period. Elemol, and DEET initially had lower percentage repellency values than catnip essential oil, but did not show the negative relationship between percentage repellency and time, retaining excellent repellency throughout the 3-hour bioassay. Solutions with elemol and DEET exhibited greater significance in contact repellency compared to catnip essential oil. These results show that catnip essential oil is a potent mosquito repellent, but does not provide the same residual effects as the commercial standard, DEET. Elemol, a sesquiterpene extracted from the fruit of the Osage orange, shows excellent promise as a mosquito repellent with comparable activity to DEET in contact and residual repellency.



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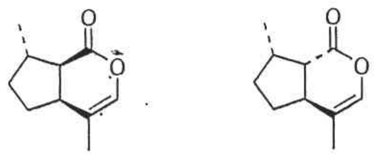
Over the last 20 years there has been intensive effort toward the development of natural products in pest control. Much of this initiative is due to increased regulations on the use of chemicals in insect pest management. Consumers have shown increased interest in and support for products that are safer to human health and more environmentally friendly than many of the traditional chemicals with high acute toxicity and long-lasting residues. Naturally derived biorepellents have been investigated as a group of chemicals that have biological activity and can cause repellent or insecticidal effects without negative impacts on human safety and the environment.

Some of the more common chemicals that have historically been used as mosquito repellents include dimethyl phthalate (DMP), 2-ethyl-1,3-hexanediol (Rutgers 612), dimethyl carbate, benzyl benzoate, butyl 3,4-dihydro-2,2-dimethyl-4-oxo-2H-pyran-6-carboxylate (Indalone), and N,N-diethyl-*m*-toluamide (DEET), which is currently the most widely used and effective mosquito repellent available. Several reports on DEET toxicity, citing encephalopathy in children, anaphylaxis, urticaria syndrome, and hypotension (*1, 2, 3, 4*), have intensified the initiative for developing alternative insect repellents. In recent years, several botanical insect repellents have become available on the market and most commonly include components from at least one of the following extracts: citronella, cinnamon, cedar, eucalyptus, mints, lemongrass, geranium, and soybean (*5*). Neem oil, an extract of the Neem tree, *Azadirachta indica*, is another natural product that has shown repellency of *Anopheles* mosquitoes (*6*).

Many plant oils and extracts have been identified as insect deterrents, repellents or toxins. In addition to economic disadvantages holding back the commercialization of some natural products, one underlying limitation with these botanical materials is that many of them do not offer residual control equivalent to synthetic standards like DEET (*5*). Research in the Pesticide Toxicology Laboratory at Iowa State University has focused on the identification of compounds present in the extracts of two plants, the Osage orange (*Maclura pomifera,* Moraceae) and catnip (*Nepeta cataria,* Lamiaceae). Recent emphasis has been placed on understanding the mechanism of repellency and developing natural products that can offer increased potency and/or residual repellency.

### Catnip

Catnip (*Nepeta cataria*) is an herbaceous mint native to Eurasia and North Africa. Its present distribution includes most of North America, with great wild abundance around the Great Lakes, and commercial production in Alberta, British Columbia, Alaska, Washington, Oregon, and California. The first uses of catnip for insect control are referenced in folklore. Over the past 50 years, experiments have validated its insect repellent activity (*7, 8*). Nepetalactone, the active ingredient present in catnip plant extracts, is known to occur as two isomers: *Z,E* and *E,Z*-nepetalactone (*9*). These two diastereomeric isomers are structurally very similar and differ only in the orientation of substituents across one bond. Past efforts from our lab analyzed the comparative repellency of these nepetalactone isomers. One particular study conducted previously on the German cockroach (*Blattella germanica*) is included in this report.



Z,E- nepetalactone E,Z- nepetalactone

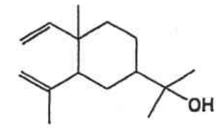
*Figure 1. Z,E and E,Z nepetalactone isomers in catnip.*

### Osage Orange

The Osage orange is another source for natural products with insect repellent properties. *Maclura pomifera,* the osage orange or hedge apple tree, was used by early pioneers in the Midwest for dyes, the wood was used in bow making, trees were planted to create hedge rows (which served as living fences and windbreaks), and fruits were reportedly useful in repelling insects and



spiders. Settlers placed whole fruits in their cupboards to ward off spiders, roaches, and other pests (*10*). Early studies on extracts of the Osage orange fruit focused on effects of two isoflavones, osajin and pomiferin (*11*), and five components of the essential oil obtained by steam distillation (*12*). Elemol, one of the major components of the essential oil, is a sesquiterpene alcohol. This compound has shown significant repellency to several species of insects in our laboratory studies, some of which are presented in this report.



**Elemol**

*Figure 2. Elemol, a sesquiterpene alcohol present in the essential oil of the Osage orange* (Maclura pomifera).

## Repellency Bioassay Methods

### German Cockroach and House Fly Bioassay

A choice-test arena was used to assess irritancy of test solutions to two common household insect pests, the German cockroach (*Blattella germanica*) and the house fly (*Musca domestica*). Catnip essential oil obtained by steam distillation, and the two major components of its essential oil, *Z,E*-nepetalactone and *E,Z*-nepetalactone (isolated from the essential oil by preparative TLC), were evaluated for behavioral effects of **contact irritancy** to the German cockroach (*9*); catnip essential oil and its major constituent *Z,E*-nepetalactone were tested against the house fly. N,N-diethyl-*m*-toluamide (DEET) (Aldrich, St. Louis, MO) served as a positive control for the choice-test arena assay and as a point of comparison for measuring insect behavioral effects that result from current commercial insect repellents. Test solutions ranging from 10% to 0.1% (vol/vol) active ingredient (a.i.) were made up in acetone and then delivered on to a filter paper for solvent evaporation. Resulting rates of a.i. were 1.63 mg/cm$^2$, 815μg/cm$^2$, 163μg/cm$^2$, 81.5μg/cm$^2$, and 16.3μg/cm$^2$. Choice-test arenas for German cockroaches and house flies were constructed from plastic Petri dishes. One-half of a 12.5-cm dia. filter paper was treated with 1 ml test solution, and the other was treated with 1 ml of only solvent (control). Both halves of the filter paper were placed in the choice-test arena. Position of the treated filter paper was randomized using a random-number table. Individual German cockroaches or house flies were placed in each choice-test arena through a centered hole in the lid of the Petri dish and evaluated for a 300-second period. The amount of time the insect spent on the treated and untreated filter papers were recorded and used to calculate a "percentage repellency" value:

Percentage Repellency = ((Time on Untreated - Time on Treated)/ 300) x 100

Ten replicates of each treatment solution were tested for both German cockroaches and house flies. Details of this assay design and some results have previously been described (*8, 13*).

### Mosquito Repellency

*Insects*

A colony of *Culex pipiens,* 10 generations removed from wild mosquitoes collected in Ames, Iowa, was used for testing. The colony was blood-fed on the bobwhite quail, *Colinus virginianus*. Eggs from mosquitoes were dried and stored in an incubator until needed. Eggs were placed in deoxygenated water and two to three drops of a ground TetraMin™ fish food solution were added to the water to feed the larvae. Pupae were removed from the larval pans as they appeared and were placed into mesh-covered paper cups. Following emergence, adult females were tested over a six-day period. The mosquitoes were continually allowed to feed on a cotton ball soaked with 0.3 M sucrose solution. At 1-2 hours before testing began, the cotton balls were removed, and the mosquitoes were preconditioned in the bioassay environmental chamber, held at 26°C, for 1-2 hours.

*Percentage and Contact Repellency Bioassay*

A static-air choice-test apparatus was used to determine the behavioral effects on the insects in this study. The apparatus consisted of a 9 x 60-cm section of glass tubing with a 2-cm hole drilled at the midpoint along the length for central introduction of the insects. All of the testing was conducted in an environmental chamber at 26°C. Treatments included catnip essential oil, obtained by steam distillation previously described by Peterson et al. 2002 (*9*), Osage orange essential oil, obtained by steam distillation of whole fruits previously described by Peterson 2002 (*13*), elemol (Augustus Oils, New Hampshire, England), and DEET (Aldrich, St. Louis, MO) test solutions at 1%, 0.5% and 0.1% concentrations (wt/vol). The test solutions' solvent, hexane, served as a control treatment in this assay. One milliliter of the solution was applied to one half of a 9-cm diameter round filter paper with an area of 63.6



$cm^2$ and then allowed to dry before testing. This resulted in the following rates of exposure: 157, 78.6 and 15.7 $\mu g/cm^2$. Treated filter papers were placed inside the lids of 9-cm glass petri dishes, and placed over the ends of the glass tube. The position of the treated side, to the right or to the left, was selected by using a random-number table. Approximately fifteen unmated adult female mosquitoes were anaesthetized with $CO_2$ and then introduced to the 9 x 60-cm glass cylinder through the centered 2-cm hole. Timing began 2 minutes after mosquito introduction, and mosquito distribution inside the static-air choice-test apparatus was observed over a 180-minute period for each treatment. Mosquito distribution (number of individuals on treated and untreated side) was recorded at 15, 30, 60, 90, 120, and 180-minute timpoints. The data generated by this study was used to examine two measures of mosquito repellency, "percentage repellency" and "contact repellency." Percentage repellency was calculated for with the following formula:

Percentage Repellency = ((Number of Individuals on Untreated Half - Number of Individuals on Treated Half)/15) x 100

Contact repellency was defined in this assay as 100% avoidance of the treated filter paper (no contact). 15, 30, 60, 90, 120, and 180-minute time-points were used to assess contact repellency for individual observations.

The experimental design was a completely randomized design using three replications of each treatment. Analysis of variance was performed on SAS (PROC GLM; SAS Version 8) to identify significant differences of percentage repellency due to treatment, and concentration. Multiple comparisions were completed using Tukey's procedure. Treatment pair-wise comparisons of contact repellency, which included data from the six time-points observed for each treatment, were completed using Fishers Exact (PROC FREQ; SAS Version 8).

*Mosquito Residual Repellency Bioassay*

Aged applications of catnip essential oil, elemol, DEET, and hexane (control) were compared in the static-air choice-test apparatus under the same conditions as described above. The 0.5% and 0.1% (wt/vol) solutions of each test solution were made to yield the same rate of a.i. used in the above mosquito repellency bioassay. Individually treated filter papers were then placed in a fume hood and aged for 0, 30, 60, 120, or 180-minutes, allowing volatization to occur over a set period of time. After the specified ageing period, filter papers were placed on the inside of the 9-cm glass petri dish lids, and then placed over the ends of the glass tube. The position of the treated side was randomized.

Approximately 18 unmated adult female mosquitoes were anaesthetized with $CO_2$ and then introduced to the 9 x 60-cm glass cylinder through the centered 2-cm hole. Timing began 2 minutes after mosquito introduction, and mosquito distribution (number of individuals on treated and untreated sides) inside the static-air choice-test apparatus was recorded after 15 minutes for determination of Percentage Repellency (calculations shown under Percentage and Contact Repellency Bioassay). Experimental design was completely randomized with three replications of each aged test solution. Analysis of variance was used to identify significant differences related to a.i., concentration, and ageing period. Regression analysis was used to examine percentage repellency relationship to filter paper ageing.

## Results

### German Cockroach and House Fly Repellency

The German cockroach and house fly both showed contact irritancy responses to at least one concentration of each test solution evaluated (Table I). German cockroaches gave the highest percentage repellency value response when exposed to the 0.5% solution of *E,Z*-nepetalactone. This percentage repellency response was more than four times the response seen from testing the same concentration of *Z,E*-nepetalactone. In the cockroach experiment, both *Z,E* and *E,Z*-nepetalactone isomers caused an overall higher percentage repellency response at lower concentrations of the respective a.i., compared to treatments with DEET. The house fly responded to the test solutions with a similar trend, although the *E,Z* isomer was not tested. The higher percentage repellency values resulted from exposure to catnip essential oil and to *Z,E*-nepetalactone, ranging from 70-96%, compared to DEET (39%) (Table II).

### Mosquito Repellency

Percentage repellency of catnip and osage orange essential oil, elemol and DEET at 15 minutes is represented in Figures 3, 4, and 5. All compounds tested showed various levels of significance in percentage repellency and contact repellency. The overall concentration effect was not significant ($P$ = 0.4569). Osage orange essential oil represented the lowest values in percentage repellency (<60%) and did not show any significant contact repellency ($P$ = 0.1). Catnip essential oil showed high percentage repellency at the 15-minute time-point at all concentrations tested, including the highest value, 100% from the 0.1% concentration (Figure 5). This was also the most significant level of contact repellency ($P$ <0.0001) resulting from the three concentrations of catnip essential



**Table I. Percentage repellency of catnip essential oil, *Z,E*-nepetalactone, *E,Z*-nepetalactone, DEET and control to the German cockroach, *Blattella germanica*, in the choice-test arena bioassay*.**

| *Treatment* | *Application Rate* | *Percentage Repellency ± SEM* |
|---|---|---|
| Controls | Acetone | 5.2 ± 7.5a |
| | Hexane | 2.9 ± 3.7a |
| DEET | 1.60 mg/cm$^2$ | 58.3 ± 10.5b |
| | 800 μg/cm$^2$ | 25.8 ± 9.5a |
| | 160 μg/cm$^2$ | 20.4 ± 9.2a |
| | 80 μg/cm$^2$ | 15.5 ± 5.4a |
| Catnip Essential Oil | 800 μg/cm$^2$ | 55.6 ± 9.8b |
| | 160 μg/cm$^2$ | 27.7 ± 13.1ab |
| | 80 μg/cm$^2$ | 33.7 ± 15.7ab |
| *Z,E*-Nepetalactone | 800 μg/cm$^2$ | 68.2 ± 5.7b |
| | 160 μg/cm$^2$ | 56.8 ± 7.8b |
| | 80 μg/cm$^2$ | 15.4 ± 6.9a |
| | 16 μg/cm$^2$ | 16.1 ± 7.4a |
| *E,Z*-Nepetalactone | 80 μg/cm$^2$ | 79.4 ± 3.5c |
| | 16 μg/cm$^2$ | 46.4 ± 11.0b |

*Treatments with the same letter are not signigicantly different by least-squares means analysis at $\alpha = 0.05$ (8).

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**Table II. Percentage repellency of DEET, catnip essential oil, *Z,E*-nepetalactone, and control to the house fly, *Musca domestica*, in the choice-test arena bioassay (*13*).**

| *Treatment* | *Application Rate* | *Percentage Repellency* |
|---|---|---|
| Control | - | -5.3 |
| DEET | 800 μg/cm$^2$ | 20.7 |
| | 160 μg/cm$^2$ | 19.3 |
| | 80 μg/cm$^2$ | 38.7 |
| Catnip Essential Oil | 80 μg/cm$^2$ | 63.3 |
| | 160 μg/cm$^2$ | 70.0 |
| | 80 μg/cm$^2$ | 52.7 |
| *Z,E*-Nepetalactone | 800 μg/cm$^2$ | 96.0 |
| | 160 μg/cm$^2$ | 69.3 |
| | 80 μg/cm$^2$ | 87.3 |

oil (Table III). The other concentrations of catnip essential oil varied in contact repellency (0.5% concentration, $P = 0.5$, and 1% concentration, $P = 0.02$). Elemol solutions yielded the second highest set of percentage repellency values of the test solutions, ranging from 81% to 63%. These treatments also resulted in highly significant contact repellency (Table III). The commercially available standard for mosquito repellency, DEET, also showed high percentage repellency values, ranging from 63% to 44%, in addition to high significance for contact repellency.

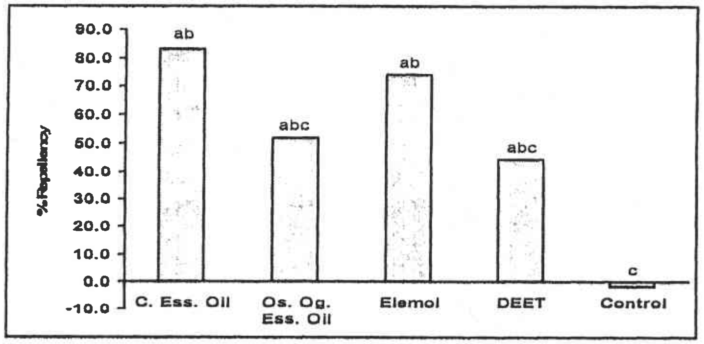


*Figure 3. 15-minute percentage repellency of the northern house mosquito,* Culex pipiens, *in a static-air repellency chamber to 157 µg/cm² application (1% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at α = 0.05.*

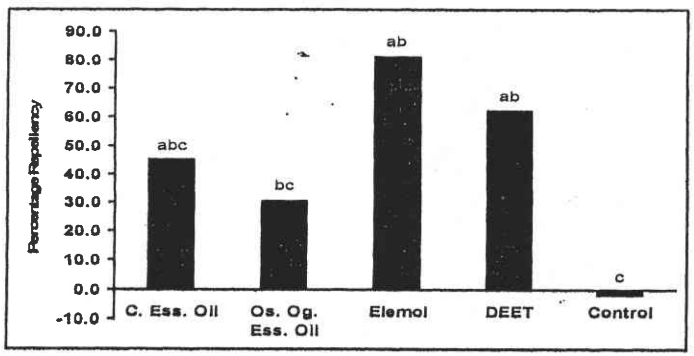


*Figure 4. 15-minute percentage repellency of the northern house mosquito,* Culex pipiens, *in a static-air repellency chamber to 78.6 µg/cm² application (0.5% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at α = 0.05.*



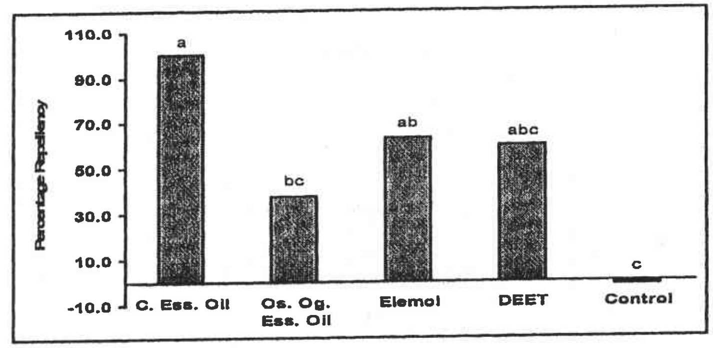


*Figure 5. 15-minute percentage repellency of the northern house mosquito,* Culex pipiens, *in a static-air repellency chamber to 15.7 µg/cm² application (0.1% concentration) of catnip essential oil, elemol, DEET, as well as osage orange essential oil, and a solvent control. Treatments with the same letter are not significantly different by Tukey analysis at α = 0.05.*

**Table III. Contact repellency of the northern house mosquito, *Culex pipiens*, measured at 15, 30, 60, 90, 120, 180-minutes in a static-air repellency chamber to catnip essential oil, Osage orange essential oil, elemol, DEET, and control.***

| *Treatment* | *Application Rate* | *Treatment vs. Control P value* |
|---|---|---|
| Catnip Essential Oil | 157 µg/cm² | 0.02 |
| | 78.6 µg/cm² | 0.5 |
| | 15.7 µg/cm² | <0.001 |
| Osage Orange Essential Oil | 157 µg/cm² | 0.1 |
| | 78.6 µg/cm² | 0.5 |
| | 15.7 µg/cm² | 0.5 |
| Elemol | 157 µg/cm² | <0.001 |
| | 78.6 µg/cm² | <0.001 |
| | 15.7 µg/cm² | <0.001 |
| DEET | 157 µg/cm² | <0.001 |
| | 78.6 µg/cm² | <0.001 |
| | 15.7 µg/cm² | <0.001 |
| Control | - | - |

* *P*-values in the table are from Fisher Exact test.

**Residual Repellency**

Percentage repellency values were high for catnip essential oil, elemol, and DEET solutions immediately following application to the test surface (Table IV). The analysis of variance showed that there was a difference among the three different solutions and the control ($P < 0.0001$), and a significant interaction with treatment solution and time ($P = 0.0019$). The only treatment solutions to show a significant decrease in percentage repellency over time were 0.5% catnip essential oil ($P = 0.02$) and 0.1% catnip essential oil ($P = 0.003$) in which 51% of the variability in the data was explained by this negative linear relationship. Elemol, DEET, and control treatments did not show significant trends in the regression analysis, indicating maintenance of repellency with elemol and DEET over the 3-hour period.

**Table IV. Residual percentage repellency of the northern house mosquito, *Culex pipiens*, to 0, 30, 60, 90, 120, 180-minute aged treatments of 0.5% and 0.1% solutions of catnip essential oil, elemol, DEET, and control in a static-air repellency chamber.**

| *Treatment* | *Application Rate* | *Percentage Repellency Over Time* | | | | |
|---|---|---|---|---|---|---|
| | | *0 min* | *30 min* | *60 min* | *120 min* | *180 min* |
| Catnip Essential Oil | 78.6 μg/cm² | 71.5 | 88.6 | 59.8 | 24 | 31.9 |
| | 15.7 μg/cm² | 88.8 | 37 | 40.7 | 22.2 | 7.4 |
| Elemol | 78.6 μg/cm² | 84.7 | 76.5 | 96.5 | 80.8 | 76.5 |
| | 15.7 μg/cm² | 35.0 | 30.8 | 49 | 20.7 | 44.8 |
| DEET | 78.6 μg/cm² | 74.0 | 37 | 59 | 77.7 | 74 |
| | 15.7 μg/cm² | 54.9 | 23.1 | 45.7 | 39 | 70.6 |
| Control | - | -6.1 | -9.3 | 1.3 | 25.5 | -9.1 |

## Conclusions

Bioassays in a choice-test arena were used to assess cockroach and house fly irritancy responses. The use of deterents is a valuable tool for pest control, particularly when used with an integrated pest management program. In the studies we report, contact irritancy serves as a measure of deterrence and helps to identify compounds that may serve as effective protectants for premises. It



should be noted that limitations of this method are that individuals are only exposed to the treated surface for a 5-minute period and can only characterize a short-term response.

German cockroaches and house flies responded negatively to all solutions evaluated. These results demonstrate the efficiency of the assay and add support for catnip essential oil as an insect repellent. Specifically, cockroaches showed greatest avoidance of filter papers treated with the purified nepetalactone isomers, *Z,E* and *E,Z*, and house flies showed greatest avoidance of *Z,E*-nepetalactone. Both nepetalactone isomers were compared during trials on the German cockroach, and the result was a much higher percentage repellency from papers treated with *E,Z*-nepetalactone. These results raise the need for structure-activity relationship studies, since *Z,E*-nepetalactone and *E,Z*-nepetalactone are very similar compounds that only differ in orientation of groups across one bond on the molecule. Additional studies on the mode of action of deterrents are required before conclusions are drawn on how the minimal structural difference in *Z,E* and *E,Z*-nepetalactone cause significantly different responses from *B. germanica*.

Initial investigations of mosquito repellency with catnip and osage orange essential oil allowed us to directly compare with DEET, the current commercial standard, and further analysis helped identify differences in the activity of these compounds as insect repellents. At present, there is no one characteristic that fits all repellents or a single mechanism that explains how specific chemicals and blends act on insects. Studies have shown that an insect's response to the chemicals in the environment is dependent on their physiological and developmental state (*14*). The studies presented in this report focus on adult female mosquitoes and their responsiveness to various rates of catnip and Osage orange essential oil, elemol, and DEET over time. Results from mosquito repellency assay show that after 15-minutes, the northern house mosquito was most significantly repelled from the filter paper surfaces treated with catnip essential oil (100%). The percentage repellency values from the DEET and essential oil (100%). The percentage repellency values from the DEET and elemol treatments resulted in a lower range (81%-44%) than catnip essential oil, but showed higher contact repellency. Observations during the assay showed that individuals exposed to catnip essential oil moved further away from the treated surface than in the DEET and elemol treatments. Over time, this effect started to decrease with catnip essential oil as mosquitoes redistributed through the tube, eventually reaching a distribution similar to the control

Mosquitoes exposed to DEET and elemol settled far enough from the treated surface to achieve an adequate level of contact repellency. As time increased, individuals would continually reject the treated surface up to the end of the 180-minute period, unlike the catnip essential oil, which exhibited an initially high repellency response that decreased over time. DEET and elemol showed a longer duration of repellency compared to catnip essential oil, as is evidenced with higher significance in contact repellency. Additional studies are needed to better understand how these differences occur, including studies on the chemical volatilization, and interference with behavioral stages of mosquito host-finding and acceptance.

The second mosquito assay focused on quantifying the residual repellency of the northern house mosquito to aged filter papers of catnip essential oil, elemol and DEET. All 0.5% and 0.1% test solutions showed significant percentage repellency following application (i.e., with no ageing period). This repellency effect slowly decreased over time for both concentrations of catnip essential oil (0.5%, $P$=0.02, 0.1%, $P$=0.003). There was no significant loss in percentage repellency seen in the DEET and elemol treatment solutions, accounting for continual mosquito repellency over 3 hours from a treated surface. Olfactory repellency differs from contact repellency, and the method used here allows for some differentiation between the two types. The high initial repellency of catnip essential oil is not sustained over a 3-hour period, but elemol and DEET do show residual repellency to that time-point.

The series of experiments presented here give supporting evidence for catnip and Osage orange essential oils, elemol, and nepetalactone as effective insect repellents to common household pests and pests of human health. Investigations with mosquito behavioral responses in a static-air apparatus showed that catnip essential oil, and elemol can act as effective mosquito repellents from treated surfaces, but differ in residual efficacy. Further studies are currently underway to evaluate residual repellency effects of other natural products in Osage orange essential oil and examine differences in the mechanism of repellency.

## Acknowledgements

We appreciate the assistance from Dr. Wayne Rowley and Brad Tucker for providing the mosquitoes for the studies. We thank Dianna Wilkening, Theresa Redding, and Melinda Thede for their help with insect rearing and conducting bioassays. Contents of this paper were presented at the 226$^{th}$ National American Chemical Society Meeting, Anaheim, CA, March 28, to April 1, 2004.



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