Catnip, *Nepeta cataria* (Lamiales: Lamiaceae)—A Closer Look: Seasonal Occurrence of Nepetalactone Isomers and Comparative Repellency of Three Terpenoids to Insects

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ABSTRACT Evidence of repellent properties in catnip, *Nepeta cataria* L., to flies and cockroaches was observed in preliminary studies. This study compared catnip essential oil from steam distillation and elemol, a major constituent of osage orange essential oil, to current commercial repellents. These comparative studies found both the catnip steam distillate and elemol to be as good, and in some cases better, at repelling house flies, *Musca domestica* L., and American cockroaches, *Periplaneta americana* L., than *N,N*-diethyl-*m*-toluamide (DEET) or citronellal. Both short-term and long-term repellency bioassays were used to assess repellency. Catnip essential oil showed greater repellency than DEET and citronellal in the short term. Extended repellency bioassays showed elemol to be more repellent than catnip steam distillate, citronellal, and DEET. Nepetalactone, the major constituent of catnip essential oil, is present as two isomers, and previous studies have shown the *E,Z*-nepetalactone [2-(2-hydroxy-1-methylethenyl)-5-methyl-cyclopentanecarboxylic acid delta lactone] isomer to be even more repellent to cockroaches than the dominant isomer, *Z,E*-nepetalactone. This study examined the seasonal variation of the two isomers, *Z,E*- and *E,Z*-nepetalactone, in catnip. Samples of fresh catnip mature leaves, immature leaves, and stems were steam-distilled separately, and isomer composition was analyzed using high-performance liquid chromatography and gas chromatography (GC). An analysis of variance (ANOVA) showed significant differences by week. The mature leaf essential oil samples were tested in a repellency bioassay and exhibited significant repellency to German cockroaches, *Blattella germanica* L. The catnip floral volatiles were sampled using solid phase microextraction, and analysis with GC/mass spectrometry showed the presence of *Z,E*-nepetalactone, *E,Z*-nepetalactone, and β-caryophyllene as the major constituents. Phytophagous insects and potential pollinators present on sampling dates were recorded.

KEY WORDS catnip, repellency, osage orange, nepetalactone, elemol

IN TODAY'S SOCIETY, THERE is a desire to reduce the use of synthetic pesticides. Several biological pesticides have high economic potential and currently make up a significant amount of the pesticide market (Hall and Menn 1998). These compounds are often specific to target species, and most have low toxicity to nontarget species. Biological pesticides can have other advantages, such as rapid biodegradation, activity at low application rates, quick knockdown, and the appeal of producing safer foods, as well as a cleaner environment (Mandava 1985).

One specific area of study, the botanical pesticides, provides new perspectives in insect–plant interactions. Because these chemicals come from natural sources, they have the potential to offer novel modes of action for use in pest management, as well as unlocking the structure–function relationships of biologically active compounds (Hall and Menn 1998).

Several botanical insect repellents have also been commercially successful. Citronellal (3,7-dimethyl-6-octenal) is one of the most popular natural compounds and was first used for controlling fleas and lice. It is now found in candles, incense, aroma-therapy, and many other commercial products. Citronella oil comes from *Cymbopogon nardus* L., a grass native to Southeast Asia. More recently, experiments have supported folklore about the repellent activity of the fruit of the osage orange tree or hedgeapple, *Maclura pomifera* (Raf.) Scheid (Moraceae) (Peterson et al. 2002a) and catnip, *Nepeta cataria* L. (Peterson et al. 2002b).

Catnip has historically been known as an insect repellent and also as a folk remedy. In the 1960s, it was reported to repel at least 13 families of insects (Eisner 1964). Also, chemicals present in catnip have been found within defensive secretions of the coconut stick insect, *Graeffea crouani* (Le Guillou), (Smith et al. 1979), and the lubber grasshopper, *Romalea guttata* (Houttuyn) (Snook et al. 1993), and in the sex attractant pheromones of the damson-hop aphid, *Pho-*

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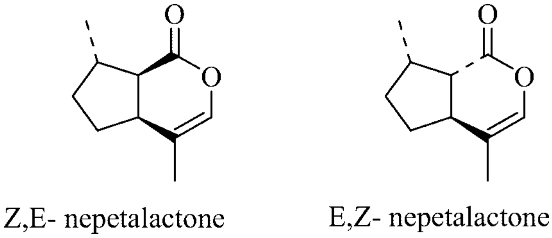

Fig. 1. *Z,E* and *E,Z* racemic nepetalactone isomers in catnip.

rodon humuli (Schrank) (Campbell, et. al. 1990), and black bean aphid, *Aphis fabae* (Scopoli) (Hardie et al. 1994). Specifically, much attention has focused on one of the active components of the essential oil, nepetalactone (5,6,7,7a-tetrahydro-4,7-dimethylcyclopenta [c]pyran-1-(4aH)-one) (McElvain et al. 1941).

Nepetalactone is an iridoid monoterpenoid that appears as two (or more) isomers in catnip, primarily as *Z,E*-nepetalactone and *E,Z* nepetalactone (Fig. 1) (Bates and Sigel 1963). In a recent study, Peterson et al. (2002b) found that both of the isomers repelled German cockroaches (*Blattella germanica*). However, the *E,Z*-nepetalactone was found to be more repellent than the *Z,E*-nepetalactone, even at a lower concentration.

In the past, the ratio of *Z,E*-nepetalactone:*E,Z*-nepetalactone has been identified as 17:3 (Peterson et al. 2002b). Research in the field of insect pheromones has indicated that many insects rely on specific ratios of compounds for attraction (Sorensen 1996). However, the field of insect repellency is still exploring various mechanisms and relationships to determine a physiological explanation for repellency.

In this study, we evaluated changes in the ratio of the two isomers within three locations of the plant throughout the spring-summer season and their relationship to *B. germanica* repellency. A short summary of the insects found on the collected samples is included, and an analysis of the floral volatiles was conducted, with observation of potential pollinators on collection day.

The other component of this study included short-term and extended repellency bioassays assessing differences between catnip essential oil from steam-distillation and elemol (a major component of osage orange essential oil) to some commercially available insect repellents.

Materials and Methods

Collection and Isolation of *Z,E*-nepetalactone and *E,Z*-nepetalactone. Aerial catnip biomass was sampled nine times, at 7-d intervals, from 22 May 2001 to 17 July 2001. Samples were from unsprayed, wild catnip stands found on the Iowa State University campus, Ames, IA. The majority was taken from established rootstocks bordering the north side of a railroad track on the north side of campus. The habitat was sunny and dry and surrounded by a variety of plant species, primarily mullein, *Verbascum thapsus* L., common ragweed, *Ambrosia artemisiifolia* L., bee balm, *Monarda didyma* L., crown vetch, *Coronilla varia* L., and common lambsquarters, *Chenopodium album* L. Each week's sample consisted of a mass collection of catnip that was clipped at the base of the stem. After collecting, plants were immediately taken to the laboratory and separated according to three plant parts: mature leaves, immature leaves, and stems. Leaf maturity was based on location of the plant and leaf size; leaves >4 cm in length were considered mature. Each plant part was steam distilled according to the method in Pavia et al. (1988). The steam distillate was extracted twice in half volumes of hexane, and the water layer was discarded. Hexane was removed using a rotary evaporator with a 635-mm Hg vacuum at 36°C. The essential oil was stored in a 4°C refrigerator until used for high-performance liquid chromatography (HPLC) analysis and repellency bioassays.

The concentration of each isomer within the extract was determined by diluting each essential oil in hexane, followed by analysis using HPLC coupled with UV detection. A Hewlett–Packard 1100 HPLC with a Pirkle Covalent phenylglycine Hi-Chrom preparative column (25 cm, 10 mm ID, 5-μm S5NH Modified Stereosorb; Regis, Morton Grove, IL) was used with a mobile phase of 19:1 hexane:ethyl acetate, at a 2.5-ml/min flow rate under the detection of a Spectroflow 757 UV detector at 254 nm. The ratio was calculated as *Z,E* concentration/*E,Z* concentration.

Samples were also analyzed by gas chromatography (GC) to confirm identification and ratio of the two nepetalactone isomers. A Varian 3700 model with a 15-m DB5 column and helium carrier with an FID detector was used. The injection temperature was 250°C, with an initial column temperature of 40°C, held for 2 min, ramped at 10°C/min, finishing at 150°C for 5 min.

An ANOVA was preformed on the resulting ratios of *Z,E:E,Z*-nepetalactone to determine significance of plant part and week (date collected). Data were compared statistically by using SAS (PROC GLM; SAS Institute 1982).

Heat of formation for the two nepetalactone isomers was calculated by CAChe Work System (Oxford Molecular 1999), using an AM1 basis set to optimize structures and energies of the molecules.

Short-Term Repellency Bioassays. Test solutions consisted of 5, 1, 0.5, and 0.1% concentrations (vol:vol) of each compound or essential oil. Compounds tested included *N,N*-diethyl-*m*-toluamide (DEET), citronellal, and steam-distilled catnip essential oil, all in acetone, as well as the samples of mature leaf catnip steam distillates (containing a known ratio of *Z,E:E,Z*-nepetalactone in hexane). DEET and citronellal (3,7-dimethyl-6-octenal) were obtained from Aldrich Chemicals (St. Louis, MO).

Insect behavioral response to the repellent compounds was determined using adult male German cockroaches (*B. germanica*), adult male American cockroaches (*Periplaneta americana*), and house flies (*Musca domestica*) of mixed sexes (Orlando regular strain). This experiment provides a measure of contact irritancy that is more applicable for use in the control of structure-invading pests. Insects were reared in colonies in the ISU Entomology/Toxicology Laboratory. One milliliter of test solution or solvent was applied to one-half of 12.5-cm-diameter round filter paper with an area of 61 cm^2 and allowed to dry. A solvent-only half-piece of filter paper (control) was placed in the remaining one-half of the 15-cm plastic petri dish after solvent evaporation. At the time of initiation, a single insect was introduced through a centered hole in the petri dish lid and covered with masking tape. Time the insect spent on treated and control filter paper out of 5 min (300 s) was recorded using two stopwatches. Ten replications for each concentration were performed. Percentage repellency was calculated with the following formula:

$$[(\text{Time on Untreated} - \text{Time on Treated})/300] \times 100$$

A random-number table determined the location of the treated filter paper in each replicate. Significant differences were determined by ANOVA, and the multiple comparison tests, including Dunnett and Tukey, were completed on SAS (PROC GLM; SAS Institute 1982). Details of this repellency bioassay have been reported earlier (Peterson et al. 2002b).

Collection of *Nepeta cataria* Floral Volatiles and Insect-Plant Ecological Survey. Flowers were collected between 1 July 2001 and 10 July 2001 from established catnip bordering the site described above. Once clipped, the cut stems of the plants were put in water and allowed to stabilize for 30 min. The floral test was conducted in bottomless french square bottles, sealed off with Parafilm (American National Can, Menasha, WI). A single catnip raceme was sealed in at the mouth of the french square bottle, and a Supelco SPME portable field sampler with a polydimethylsiloxane fiber was suspended above, projecting through a parafilm seal. Three sampling collections and a control were conducted. Before each sampling, the SPME field sampler was preconditioned for 30 min at 250°C in a GC/MS. The solid phase microextraction sampler was set up in the apparatus described and allowed to absorb for 30 min for three test replicates.

The samples were run using GC/MS. A Varian 3200 model with a DB 5-ms nonpolar 30-m column (0.25-mm ID, 0.25-mm film thickness) and a Finnigan TSQ 700 triple quadrupole mass spectrometer with electron impact at 70 eV was used.

β-Farnesene and β-caryophyllene were identified by comparing their GC retention times and mass spectra with those of synthetic standards from Sigma-Aldrich. α-Caryophyllene was tentatively identified by the match of its mass spectrum with an available spectrum in the Wiley 138K mass spectral database. *E,Z* and *Z,E* nepetalactone were identified by comparison of proton NMR spectra from a Varian VXR-300 Spectrometer to NMR data reported in previous literature (Eisenbraun et al. 1980).

The insect–plant ecological observations included the collection of phytophagous insects feeding on catnip at the time of plant tissue collection. Insect spec-

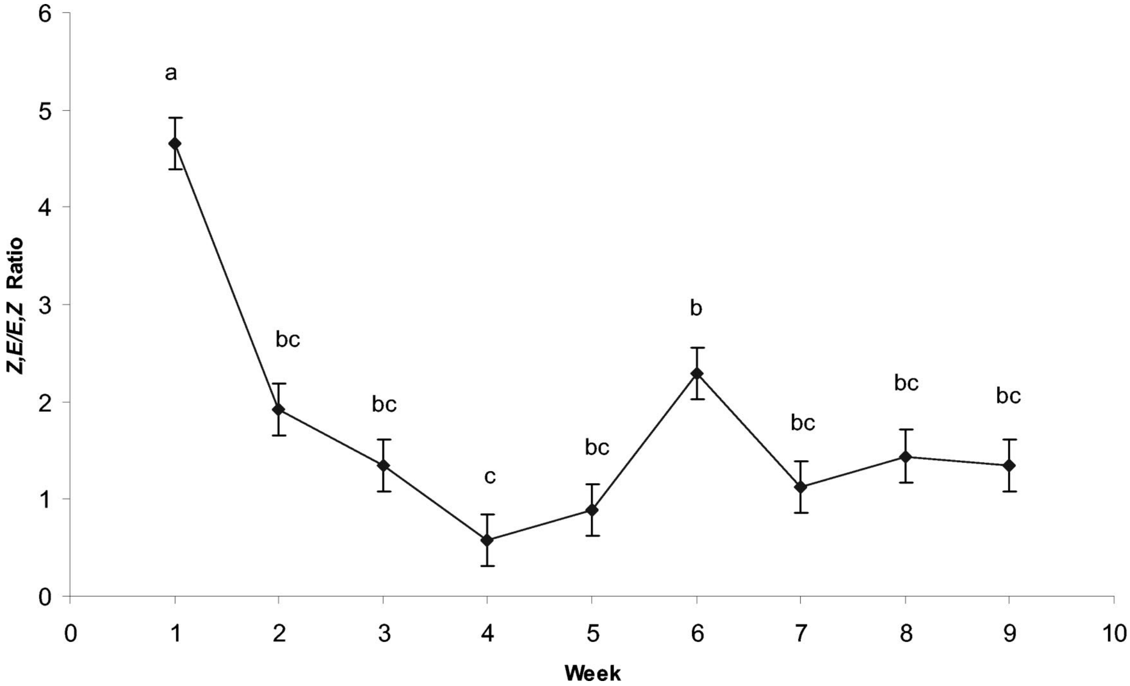

Fig. 2. Seasonal variation of the ratio of *Z,E:E,Z*-nepetalactone isomers in catnip essential oil distilled from leaves and stems as analyzed by gas chromatography with a flame ionization detector (GC-FID). Means that do not share the same letter are significantly different at α = 0.05.

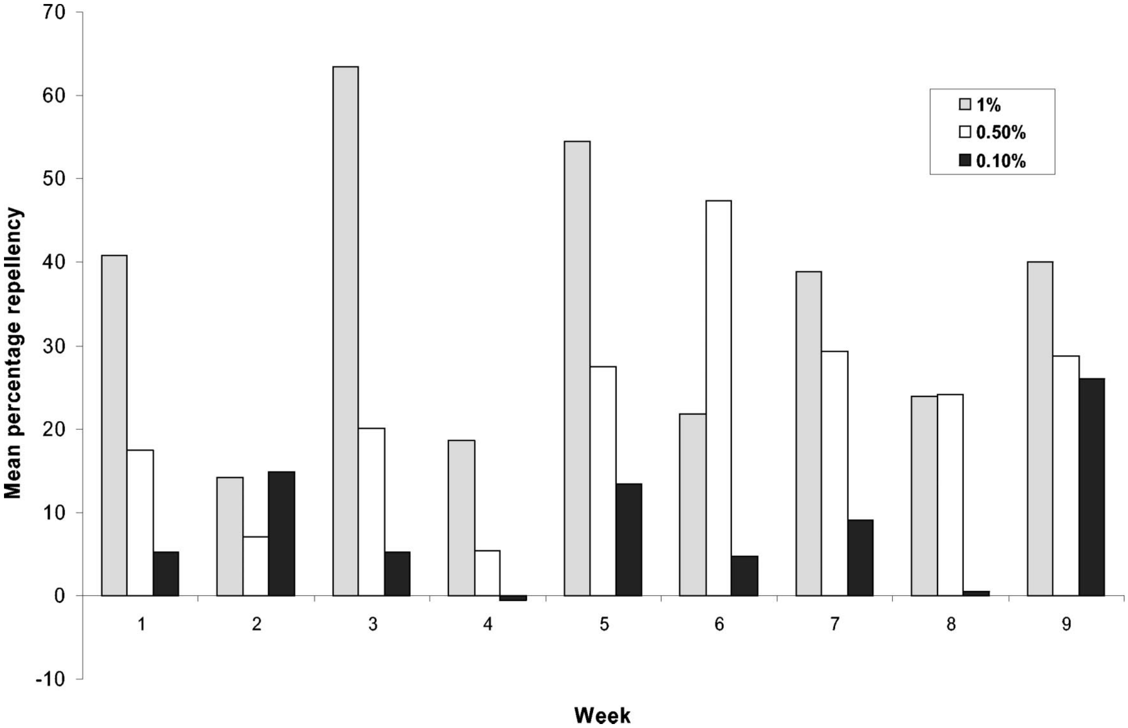

Fig. 3. Repellency of catnip mature leaf essential oil, obtained by steam distillation, to the adult male German cockroach, *Blattella germanica*, in the 5-min arena bioassay.

imens were taken to the laboratory, where they were placed in a growth chamber set at 12-h light:12-h dark at 30°C. Each day they were provided with fresh water and fresh catnip as food. At the end of the growing season, the survival of each insect was recorded. After week 5 of collection, the flowers had opened, and potential pollinators were recorded.

Extended Repellency Bioassay. Test solutions were made consisting of 5, 1, and 0.5% concentrations by volume for catnip steam distillate, DEET, citronellal, and elemol, which was obtained from Augustus Oils (Hampshire, UK). One-half of a 12.5-cm filter paper with an area of 61 cm^2 was treated with 1 ml of test solution and allowed to dry (30 s) before being placed in a 15-cm petri dish. The other half of the filter paper was treated only with solvent for control. One adult male American cockroach was placed in each petri dish and enclosed by a mesh, which eliminated any fumigation effects and allowed volatilization of the repellent under ambient laboratory conditions. Adult American cockroaches of mixed sexes were used for tests of the 1 and 0.5% concentrations of catnip essential oil. The location of the insect (presence on the treated or untreated filter paper) was recorded at seven time-points after initiation: 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, and 24 h. Ten replicates were done for each specific preparation.

Spearman rank correlation (Miller and Freund 1965) was used to examine the overall repellent response of cockroaches to the treated half of the test arena in this particular bioassay. Treatment and concentration differences were identified with a Cochran-Q test (Siegel 1956), assuming 100% repellency across all seven time-points to be a successful response. Pair-wise comparisons among treatments were completed using Fisher Exact tests (PROC FREQ; SAS Institute 1982).

Results

Seasonal Variation of Nepetalactone Isomers and Repellency. The ANOVA showed that the overall model design was statistically significant ($F = 16.11$; df = 10,16; $P < 0.0001$) with differences caused by week ($P < 0.0001$); no significance caused by plant part was found. The mean average values of the *Z,E:E,Z* nepetalactone ratio, sorted by week, are represented in Fig. 2. Overall, the mean ratio was 1.73.

With respect to percentage repellency, the individual catnip mature leaf distillates collected at different seasonal time-points differed in an ANOVA ($F = 3.42$; df = 27,252; $P < 0.0001$). Distilled catnip essential oil from weeks 3, 5, 6, 7, and 9 were found to significantly differ in percentage repellency compared with the control (Fig. 3). Direct comparisons between weekly concentrations showed significant differences in percentage repellency for weeks 2 and 3 at the 1% concentration and weeks 2 and 6 at the 0.5% concentration. The Tukey multiple comparison also showed significant differences in repellency between the 1, 0.5, and 0.1% concentrations of each mature leaf distillate yielding a dose response.

Table 1. GC-MS analyses of SPME collections of catnip flower, *Nepeta cataria*

	Z,E Nepetalactone	*E,Z* Nepetalactone	β-Caryophyllene	β-Farnesene	α-Caryophyllene
Mean	55%	32%	12%	0.9%	0.9%
SEM	3.5	3.5	1.2	0.06	0.06

Overall, the catnip essential oil was significantly repellent in all 1% concentrations. No clear relationship was found between the *Z,E*:*E,Z* ratio and the percentage repellency between weeks.

Collection of Floral Volatiles and Insect-Plant Ecological Observations. The floral volatile analysis detected the presence of three major compounds (Table 1). Of the released volatiles, the *Z,E*-nepetalactone made up 54.6%, *E,Z*-nepetalactone made up 31.9%, and β-caryophyllene made up 11.6%. β-Farnesene and α-caryophyllene were minor constituents.

Generalist phytophagous insects found on catnip during the collection included Aphididae, Noctuidae, Cicadellidae, and Tettigoniidae. Under laboratory conditions, only two loopers (Noctuidae) and one leafhopper (Cicadellidae) were able to complete development on a diet of only catnip. Insects families observed on the flowers included Tachinidae, Apidae, Nymphalidae, and Pieridae.

Comparative Repellency. Percentage repellency of catnip essential oil and citronellal to *P. americana* significantly differed from the control at all concentrations ($F = 4.02$; df = 19,180; $P < 0.0001$). DEET only differed from the control at the 5% dose. All three concentrations of catnip essential oil and citronellal were highly repellent (>80%). The 1 and 5% concentrations were more repellent than the 0.5% concentration, indicating a likely dose–response relationship (Fig. 4).

Similarly, percentage repellency of catnip essential oil and citronellal to *M. domestica* significantly differed at the 5 and 1% doses from the control. No DEET solutions differed from the control. The 1% concentration of catnip essential oil showed the highest percentage repellency value to *M. domestica* in short-term tests (Fig. 5). All concentrations of catnip essential oil and citronellal had >50% repellency. In general, these data do not show a clear dose–response relationship.

Catnip and Elemol Extended Repellency Bioassay. The Spearman rank correlation coefficient, r_s, equaled 0.321, with a critical r_s value of 0.714 ($\alpha = 0.05$), showing an overall repellent response to the compounds tested. The Cochran-Q test found significant differences among the four treatments ($Q = 16.33$; *Q-critical* = 16.227, $\alpha = 0.01$) and three concentrations ($Q = 17.87$; *Q-critical* = 13.82, $\alpha = 0.01$). The Fisher exact test of each treatment against the control showed significance at the 5% concentration. Elemol was the only treatment significantly different from the control at the 1 ($P = 0.0271$) and 0.5% ($P = 0.0027$)

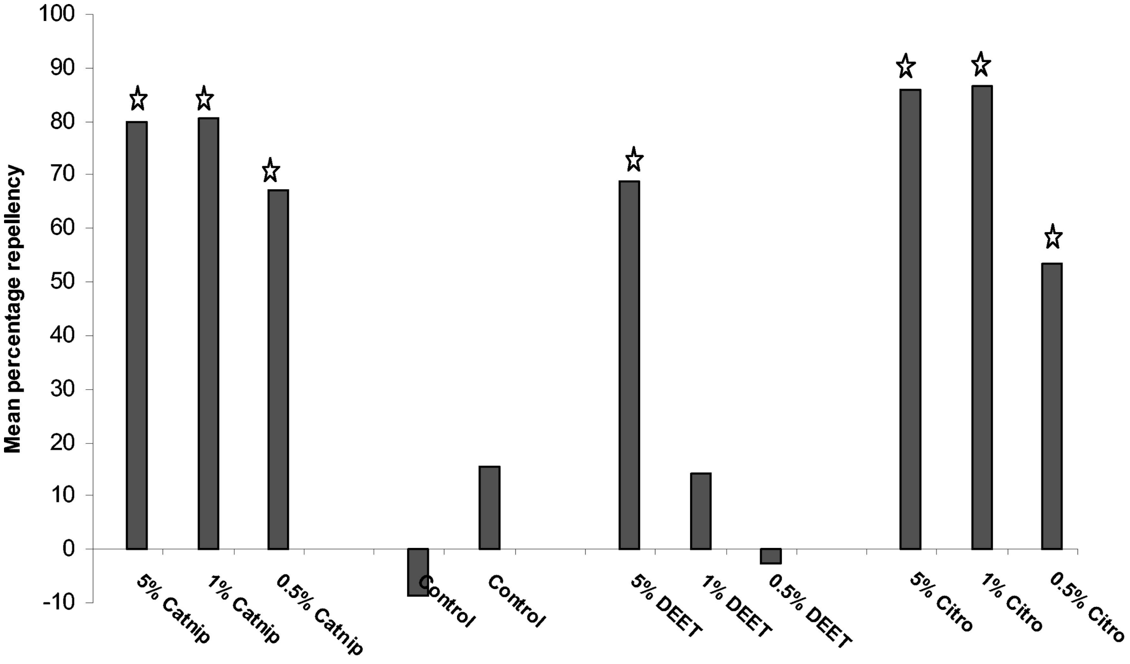

Fig. 4. Repellency of catnip essential oil, DEET, and citronellal to the adult American cockroach, *Periplaneta americana*, in the 5-min arena bioassay. Significantly different from acetone control at $\alpha = 0.05$.

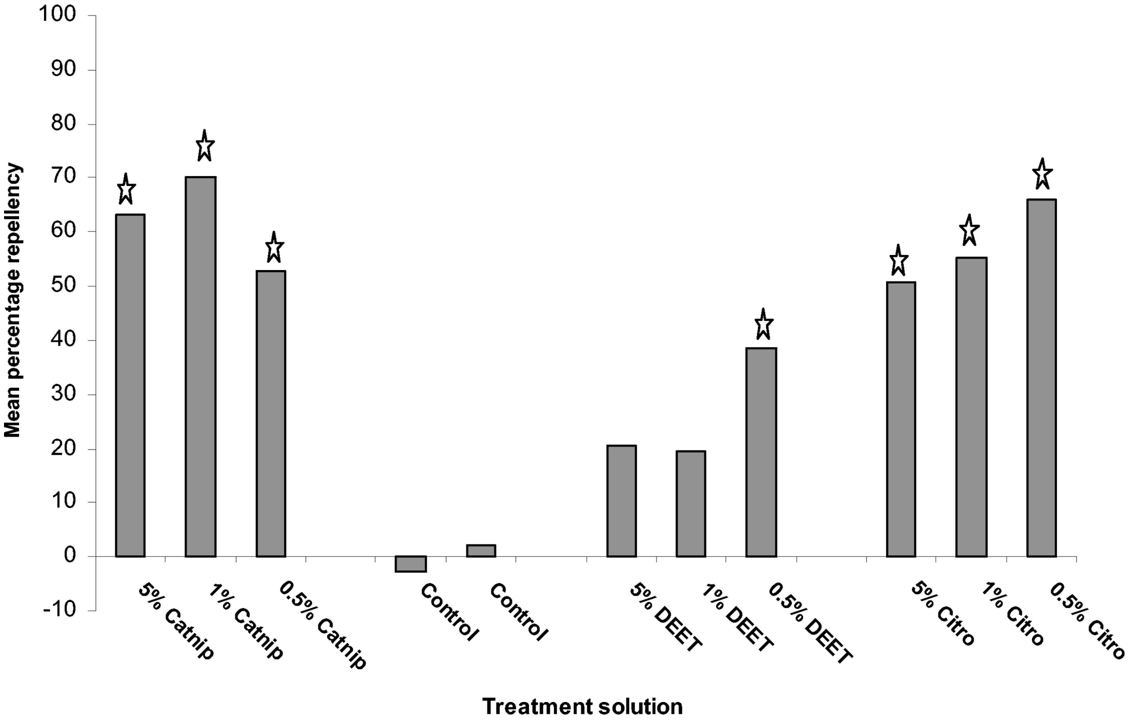

Fig. 5. Repellency of catnip essential oil, DEET, and citronellal to adult housefly, *Musca domestica*, in the 5-min arena bioassay. Significantly different from acetone control at $\alpha = 0.05$.

concentrations (Table 2). Pair-wise comparisons of the four treatments indicated that elemol was significantly more repellent than the other treatments, with the highest amount of 100% repellency over the seven time-points ($P < 0.02$). There were no significant differences found among the other essential oils.

Discussion

The seasonal variation of the two nepetalactone isomers was studied in the summer of 2001, and significant differences were found in the ratio of these two isomers over the 9 wk of sampling (Fig. 2). Critical time-points include the following: week 2, generalist insects were first seen feeding on the plant; week 4, floral buds were present but unopened; week 5, the flowers first opened; and week 9, flowers had senesced and ovaries were beginning to ripen.

Nepetalactone has previously been identified as a repellent to many insect species (Eisner 1964). The data from this study shows the ratio of *Z,E:E,Z* at week 4 was 0.58, the lowest of the season, and it showed the lowest repellency value in the short-term bioassay with *B. germanica* (Fig. 3). This raises additional questions about the ecological role of the repellent nepetalactone isomers toward insect herbivores, indicating that more quantitative studies of the nepetalactone present within the plant are needed. Because the data

Table 2. Extended repellency of DEET, citronellal, elemol, and catnip essential oil at various concentrations to the adult American cockroach, *P. americana*

Treatment solution	Percentage of cockroaches on untreated half of arena							Fisher exact test	
	15 min	30 min	1 h	2 h	4 h	6 h	24 h	Mean	*P* value
Control	50	60	40	40	60	60	40	50	—
5% DEET	90	90	90	90	90	90	90	90	0.0005
1% DEET	40	60	70	80	80	50	80	66	0.2477
0.5% DEET	80	70	80	80	70	60	50	70	0.1354
5% Citronellal	100	100	100	100	90	90	70	93	0.0095
1% Citronellal	90	90	60	70	80	50	40	69	0.3947
0.5% Citronellal	40	80	90	70	70	70	50	67	0.3947
5% Elemol	100	100	100	100	100	100	100	100	0.00005
1% Elemol	80	80	100	100	100	100	90	93	0.0271
0.5% Elemol	80	90	100	100	100	100	100	96	0.0027
5% Catnip essential oil	90	100	100	100	100	100	70	94	0.0271
1% Catnip essential oil	70	80	70	90	70	80	70	76	0.1354
0.5% Catnip essential oil	90	90	80	70	70	80	40	74	0.1354

presented did not show a significant relationship between the nepetalactone isomer ratio and time, it is difficult to make conclusions about how nepetalactone might be used by catnip for protection from injurious insects.

After week 4, the ratio increased again, reflecting a decrease in the amount of the more repellent isomer, *E,Z*. The thermodynamics of the compounds show a 5.7 kcal/mol difference in the heat of formation of the two molecules, with *E,Z* being the higher and *Z,E* being the lower. It is possible that there is an adjustment at the flowering stage for the attraction of pollinators. However, the composition of the floral volatiles still contained a 1.7 *Z,E:E,Z* ratio. This is still a low ratio and poses many questions of possible species-specific olfactory response to the flowers.

The individual repellency comparisons of these weekly distillations yielded little information. In Fig. 3, the dose response is shown from the Tukey analysis; 1, 0.5, and 0.1% concentrations significantly differed from each other. As apparent from Fig. 3, all 1% concentrations of mature-leaf essential oil were significantly repellent, which coincides with previous studies of catnip repellency (Peterson et al. 2002b).

The data provide insight into an optimal time-point for harvesting catnip material at maximum repellency to the German cockroach. This study indicates harvest should take place when the flower buds are first present until when they open. Additional studies are needed to identify other factors that influence insect repellency and how they vary throughout the season before determining a specific time-point for catnip collection.

There are many interesting questions still to be answered regarding the insect–plant interactions and chemical responses of the catnip plant. Areas for further investigation are quantitative repellency bioassays of the two nepetalactone isomers with German cockroaches, as well as new bioassays for repellency or attraction to insects recorded at the flowers (Tachinidae, Apidae, Nymphalidae, and Pieridae). There may be specificity between species chemoreceptors or the specificity of the ratio of the two isomers, and additional chemicals present might be necessary to elicit repellency and attraction responses. The mechanisms of insect repellency are poorly understood. Improvements in our knowledge of possible receptors affected by repellent compounds and their correlation to neurobehaviors will add greatly to our understanding of insect repellency.

Periplaneta americana and *M. domestica* showed an avoidance response to all of the botanical solutions tested in the catnip comparative repellency bioassay in at least one concentration. In tests with *M. domestica* and *P. americana*, insects showed a significantly higher avoidance to catnip essential oil at values lower than citronellal and DEET. These results indicate that catnip essential oil or components may have promise as commercially viable products.

In some cases, the lower concentrations of the solutions were more effective in the closed petri dish arena than the higher concentrations. The dispersion of the compound molecules probably reaches equilibrium faster at higher concentrations. If the compounds rapidly reach equilibrium in the air, the insects may have more difficulty detecting which side is treated versus untreated. It is also possible that the insects may become desensitized to the high levels of chemical in the petri dish and not be able to choose an untreated area. Overall, these tests show the effectiveness of catnip steam distillate and osage orange constituents as repellents and indicate they may be effective at low concentrations.

The catnip and elemol extended repellency experiment showed that, although all treatments tested were repellent, the current commercial repellents (DEET and citonellal) were not the most effective against the American cockroach. Elemol, a component of the fruit of the osage orange tree, provided the longest duration of repellency. This residual repellency may serve for improved protection of premises, materials, or animals from insect pests.

The results of these studies indicate that some compounds merit further investigation of botanicals as insect repellents, including catnip and osage orange components. Future studies should include various tests of repellency on additional insect species and further examination of elemol as a repellent. Distance and contact repellency on multiple mosquito species is currently in progress. More definitive testing on how effective nepetalactone and elemol are as mosquito repellents will need to be studied in a biting assay or field study.

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