Behavioral Responses to Pine Needle Oil in the Northern Pocket Gopher

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ABSTRACT

For many herbivorous mammals, oils from conifers are feeding repellents. Our study investigated effects of pine needle oil on feeding and other behaviors of northern pocket gophers. In one-choice feeding trials pocket gophers were offered sweet potato from single feeding stations placed into each subject's home cage. Stations contained either a scent dispenser with pine needle oil or with mineral oil. Pine needle oil did not inhibit food retrieval under these conditions. Responses to pine needle oil and to a control odorant, d-pulegone, were also tested in mazes where subjects were offered choices between two goal boxes, each containing food associated with an odorant. To examine the possibility that pocket gophers avoid any unfamiliar odor because of neophobia, freshly caught animals were tested in a maze offering a choice between goals scented with d-pulegone and goals containing mineral oil. The behavior of the animals in the side of the maze containing pulegone did not differ from their behavior in the side containing mineral oil, indicating indifference to this stimulus. Pine needle oil, however, elicited aversive responses. When one goal box contained pine needle oil, the second mineral oil, subjects were less frequently located in the side of the maze containing pine scent and consumed less food there. Responses of northern pocket gophers to pine needle oil in mazes are similar to those of plains pocket gophers studied earlier under comparable conditions. Results obtained with both species suggest that pine needle oil may be an effective repellent for all species of pocket gophers.

KEY WORDS

Pocket gophers, avoidance behavior, repellents, plant secondary metabolites, pine needle oil
INTRODUCTION

Pocket gophers are among a number of fossorial rodents that damage agricultural and forest crops in the United States; therefore, effective repellents are needed to control these pests. Pocket gophers are generalist herbivores that forage mostly underground by excavating tunnels to reach food plants (Chase et al. 1982). Species of the genus Thomomys, in particular, interfere with conifer reforestation (Burton and Black 1978, Gottfried and Patton 1984, Radwan et al. 1982, and others) and are a persistent problem in orchards (Sullivan et al. 1987).

Semiochemicals that have ecological significance for a target species are prime candidates in the search for nonlethal repellents. Plants produce a great variety of secondary metabolites in defense against herbivores (reviews: Haslam 1988, Langenheim 1994) and thus represent potential repellent sources. Many of these compounds are toxic or interfere with metabolic processes if ingested by herbivores. As a result, herbivorous mammals avoid ingestion of some plants or parts of plants, and this avoidance is based on chemosensory detection of plant secondary metabolites.

Essential oils from conifers contain secondary metabolites that inhibit feeding in a number of herbivores, among them snowshoe hare (Lepus americanus) (Bell and Harestad 1987, Sinclair et al. 1988), Townsend vole (Microtus townsendii) (Bell and Harestad 1987), meadow vole (M. pennsylvanicus) (Roy and Bergeron 1990), prairie vole (M. ochrogaster) (Wager-Page et al., this volume, moose (Alces alces) (Sunnheim-Sjoberg 1992, Sunnheim-Sjoberg and Hamalainen 1992), red deer (Cervus elaphus) (Elliott and Loudon 1987), and mule deer (Odocoileus hemionus) (Schwartz et al. 1980).

Pocket gophers also appear to be sensitive to some conifer oils. Radwan et al. (1982) found that ponderosa pine (Pinus ponderosa) seedlings from some geographic areas are protected against attack by northern pocket gophers (Thomomys talpoides), while other seedlings are heavily damaged by the gophers. Different parts of the same seedling are also discriminated against during feeding. Preferences are negatively correlated to the α-pinene plus sabinene component of stem oils, but positively correlated to the total terpene yield of root oils.

Epple et al. 1996 found that plains pocket gophers (Geomys bursarius) avoided areas in a maze where the odor of pine needle oil was present and exhibited reduced feeding in these areas. Pine needle oil also reduced gopher gnawing of electrical cable. These findings suggest that pine needle oil or some of its constituents may be useful repellents for pocket gophers in general. Our study evaluates the effects of volatile cues from pine needle oil on behavior of the northern pocket gopher.

METHODS

Subjects

All pocket gophers were wild caught near Olympia, WA. Sixteen animals were shipped to the laboratory at the Monell Chemical Senses Center and held in captivity for 7 months before being used in the first study. At the Monell Center, the pocket gophers were individually housed.
in stainless steel cages (60 × 50 cm, 22 cm high), containing aspen chips, plastic pipe (15 cm long, inner diameter 8 cm), and cardboard boxes for nesting. Animals received a diet of Purina Guinea Pig Chow, Mazur Omnivore A pellets, and a mixture of rolled oats, sunflower seeds, and peanuts. This was supplemented with dandelion, kale, and crab apple branches.

Twelve pocket gophers were maintained at the Denver Wildlife Research Center's (DWRC) field station in Olympia. These animals had been in captivity for 2 weeks before being used in studies. They were housed in mouse cages (25 × 20 cm, 18 cm high), with polyester fiber as bedding material. The animals were fed deer chow.

**Stimulus Material**

Pine needle oil (brand: Siberian Pine Needle Oil) was purchased from the Penn Herb Co. Ltd., Philadelphia, PA. A partial analysis of the oil, performed in our laboratory, documented the presence of α-pinene, β-pinene and myrcine (see also Wager-Page et al., this volume). Light white mineral oil, used as a control odor, was obtained from the Lannett Co., Philadelphia, PA, and d-pulegone, used as a novel odor, was obtained from the Aldrich Chemical Company, Saint Louis, MO.

**One-Choice Feeding Trials**

The effect of pine needle oil odors on retrieval of preferred food was studied, using the 16 individuals maintained at the Monell Center. All tests were performed in the home cages of the subjects. Standard diet was available during trials.

Each trial consisted of a 1-hr period during which the pocket gophers had access to a single feeding station that contained 10 g of diced sweet potato and an odor dispenser with either pine needle oil or mineral oil. The amount of sweet potato remaining in the feeding station at the end of the trial was used as a measure of the effects of the odorants on food retrieval and consumption.

Each feeding station consisted of a plastic tube (25 cm long, inner diameter 8 cm) that was open at one end and sealed at the other end by inserting a 6-cm-deep stainless steel cup. The cup contained the sweet potato and the odor dispenser that was taped to the cup above the food. Tunnels were placed parallel to the front walls of the home cages and were kept in place with a clean brick.

Stimulus fluids (100 μl) were applied to filter paper (3 × 9 cm strips, folded into 3 × 3 cm pads). Pads were placed into plastic mesh capsules (Histoprep, Fisher Scientific, USA, 25 × 6 mm). These odor dispensers, which allowed the animals to smell but not to contact the stimuli, prevented contamination of the food with odorants.

One set of tubes and cups was used only with mineral oil and another set, only with pine needle oil. All testing equipment was washed in a commercial cage washer at 82 ºC and thoroughly rinsed with clear water between trials. Odor dispensers were discarded after each trial.

Only 8 individuals per day were tested, 4 receiving sweet potato from a feeding station containing pine needle oil and 4 from a feeding station containing mineral oil. Each subject was tested once with mineral oil and once with pine needle oil. Half of the pocket gophers received
mineral oil first, followed by pine needle oil several days later. This order was reversed for the other half. Results were evaluated by paired t-tests.

**Maze Trials**

*Maze A*

Responses to pine needle oil and to a novel control odor, d-pulegone, were tested in a maze. Animals maintained at the Olympia field station served as subjects. The maze consisted of a plastic start box (30 x 20 cm, 14 cm high), covered with a clear Plexiglas lid. The start box was connected to two clear plastic goal boxes (28 x 28 cm, 12 cm high) via tunnels made from translucent piping (inner diameter 5 cm). One 25 cm long tunnel exited from the center of the start box and led to a T-shaped piece from where the animals could enter a left or right tunnel system to reach either the left or the right goal box. Air was drawn into the maze through holes in the lids of the goal boxes and exhausted by connecting the start box to a fume hood via a hose, attached to an opening in the wall of the box.

Each goal box contained 150 ml of wood shavings, a Histoprep odor dispenser lined with filter paper and a small apple cube (2 cm). To achieve dim, uniform lighting in the entire testing area, the maze was mounted on a table and enclosed on three sides by sheets of plywood. A sheet of brown packing paper covered the top. Between trials, all equipment was hand-washed in hot water and dried with clean paper towels.

All trials were 5 min long. They were divided into 30 intervals of 10 sec, indicated by an audible timer signal. The location of the subject in the maze and selected behaviors were scored by an observer seated in front of the maze. At each 10-sec signal, the observer noted the location of the animal as being in the start box, the single exit tunnel, the left or right tunnel system, or the left or right goal box. Two behaviors, "enter the left\right goal box" and "eat apple in the left\right goal box," were recorded as 1–0 scores (Altmann and Wagner 1970) per 10-sec intervals. For each interval, the subject received a score of 1 for each behavior if the pattern was displayed, regardless of the actual number of occurrences. This method of recording provides rough estimates of the frequencies and durations of behaviors.

All subjects to be tested on the same day were transported from the animal room to the laboratory in their home cages, which had been placed into large plastic boxes and loosely covered with a lid. Animals remained there, with food and water available ad libitum, until the end of the testing day. Gophers were transferred to the maze by scooping them up into a wide-mouth plastic bottle and releasing them into the start box. Trials began with the first 10-sec interval after the subject left the start box.

Each subject was introduced into the maze for a 30-min period at least 1 day before being tested for the first time. No observations were made during the 30 min. To ensure that the animals had accepted the maze and did not exhibit side preferences, all subjects were then given one 5-min trial in the clean maze, with both goal boxes containing empty scent dispensers. Following these trials the pocket gophers were tested as described below. Each individual was tested no more than once a day.
Responses to pulegone: some rodents show neophobic responses to a variety of odorants that are not ecologically relevant to the species (Garbe et al. 1993, Kemble and Gibson 1992). Therefore, it was important to evaluate the tendency of pocket gophers to exhibit neophobia to any unfamiliar odor. Effects of d-pulegone, a strong minty scent that is repellent to some mammals and birds (Mason 1990), were evaluated in six animals that experienced this compound as the first experimental odor stimulus after they had been brought to the laboratory. Pulegone was chosen as a novel odor because tests with plains pocket gophers had shown that this species ignored the compound under conditions identical to those where individuals avoided pine needle oil (Epple et al., 1996).

Each subject was given 2 trials in the maze on 2 consecutive days. During each trial one goal box contained a dispenser with 250 μl pulegone, the other a dispenser with 250 μl mineral oil. One goal box was always used with pulegone; the other, with mineral oil. The left-right positions of goal boxes with pulegone and mineral oil were counterbalanced across subjects and replications. Results were analyzed by paired t-tests.

Responses to pine needle oil: 12 pocket gophers, including the animals previously tested with pulegone, were each given 2 trials during which one goal box contained an odor dispenser with 250 μl of pine needle oil and the second goal box contained a dispenser with 250 μl of mineral oil. Goal boxes were dedicated to use with pine needle or mineral oil, but their left-right positions were counterbalanced across subjects and trials. Results were analyzed by paired t-tests.

**Maze B**

Responses to pine needle oil were tested at the Monell Center, using 4 of the 16 pocket gophers that had served as subjects in the feeding trials. These maze trials were performed 19 months after the feeding trials at which point only 4 of the original 16 animals were alive.

The maze, described fully by Epple et al. (1996), differed in some minor details from Maze A. The main difference was the fact that, instead of entering a T-piece first, subjects could enter the left and a right tunnel system directly through exits in the left and right side of the start box. Each goal box contained a Histoprep odor dispenser and three cubes (2 cm) of sweet potato. The maze was located on the floor of an evenly-lit room to which the animals were taken for testing, one at a time. Testing procedures were identical to those used with maze A. Being in the start box, in the left or right tunnel system, or in the left or right goal box were the locations scored in Maze B.

Responses to pine needle oil: each animal received a one 5-min test in the unscented maze containing blank odor dispensers. This was followed on separate days by 2 tests during which one goal contained a dispenser with 100 μl of pine needle oil and the second goal, a dispenser with 100 μl of mineral oil. Since only four subjects were available, no statistical analysis was performed.
RESULTS

In one-choice feeding trials the pocket gophers took approximately the same amount of sweet potato from feeding stations with pine needle oil (6.9 ± 1.0 g) and from feeding stations with mineral oil (7.5 ± .9 g).

During tests given in Mazes A and B, the animals showed no differences in location or behavior scores obtained in the right or left sides of the mazes in the absence of any odor stimuli.

Pulegone had no significant effect on behavior in Maze A. Animals were located in tunnels and goals scented with pulegone as frequently as in those containing mineral oil. Entry and eating scores also did not differ (Figure 1).

When mazes offered choices between pine needle oil odor and mineral oil odor, the pocket gophers were less frequently located in the tunnels and goals scented with pine needle oil. They also ate less frequently in goals containing pine scent (Figure 2). These differences are statistically significant for results obtained with Maze A. Although no statistical analysis was performed on results obtained from the four individuals tested in Maze B, mean response levels exhibited in Maze A and Maze B were very similar (Figure 2).

DISCUSSION

The results of the one-choice feeding trials indicated that northern pocket gophers were indifferent to the odor of pine needle oil when it was associated with a single source of preferred food that was encountered in the home environment. However, in mazes where the study animals had a choice between food associated with pine needle oil or with a control odor, pine needle oil appeared to be an aversive stimulus that inhibited food caching and eating and caused area avoidance. These differences implicate the environmental context in which a repellent is encountered as one of the factors that determine responses of pocket gophers to that repellent. Factors other than environmental context, however, among them types of behaviors measured, motivation to obtain preferred food when only one source of it was available, or differences in odor concentrations inside feeding stations and mazes, may also have influenced responses.

Newly caught pocket gophers and animals tested after 19 months of captivity exhibited remarkably similar response levels when tested in mazes that differed slightly in design and that contained different stimulus concentrations (Maze A, 250 μl; Maze B, 100 μl; however, the actual vapor pressure of the pine needle oil components was not measured). In contrast to pine odor, pulegone odor did not affect the behavior of the pocket gophers, although this compound is aversive to some other mammals, among them woodchucks (Bean, et al., this volume). These results show that avoidance of pine needle oil by pocket gophers is not caused by neophobia.

The indifference of northern pocket gophers to pine needle oil encountered in the home cages is in strong contrast to findings obtained with plains pocket gophers that were tested in the same laboratory under identical conditions and during the same period (Epple et al., 1996). Based on available data, this difference between the two species cannot easily be explained, especially since it was only observed when odorants were encountered in the home cages. Both species responded to pine needle oil in mazes. Plains pocket gophers avoided pine needle oil odor when exposed to
FIGURE 1. Scores obtained in Maze A for location in tunnel systems and goal boxes (top) and for entering goal boxes and eating in goal boxes (bottom). Striped bars—pulegone-scented side. Open bars—mineral oil-scented side.

FIGURE 2. Scores obtained in Mazes A and B for location in tunnel systems and goal boxes (top) and for entering goal boxes and eating in goal boxes (bottom). Solid bars—pine needle oil scented side. Open bars—mineral oil-scented side. * - P = 0.03; ** - P = 0.02.
it in type B mazes, using methods identical to those used in the present study. Interestingly, laboratory-born plains pocket gophers that encountered pine needle oil for the first time avoided it to the same degree as wild-caught animals (Epple et al., 1996). It appears that, similar to innate avoidance of predator odors by some mammals (Dickman 1992, Müller-Schwarze 1972, and others), aversion to certain plant secondary metabolites may be innate.

Responses to pine needle oil are mediated by volatile cues in both pocket gopher species. In our studies with northern and with plains pocket gophers (Epple et al., 1996), stimuli were enclosed in plastic capsules that prevented direct contact. Volatile cues from the pine needle oil might have been detected by the olfactory or the trigeminal system that mediates irritation (Bryant, this volume). However, if irritation were a major sensory effect of pine needle oil, both species of pocket gophers should have responded to the stimulus in a similar way when it was encountered in the home cage. Wager-Page et al. (this volume) provide some experimental evidence that responses of prairie voles to pine needle oil do not involve the trigeminal system. Thus, it appears most likely that the aversive stimulus characteristics to which pocket gophers respond are mainly based on olfactory cues.

Secondary plant metabolites, such as terpenoids and phenolic compounds, are feeding deterrents for many mammalian herbivores (Iason and Palo 1991, Langenheim 1994, Reichardt et al. 1990, Roy and Bergeron 1990, Sinclair et al. 1988, Sunnerheim-Sjoberg and Hamalainen 1992, and others); and for some species, olfactory cues alone cause this effect. The odor of pine oil extracted from pulp waste inhibits feeding in snowshoe hares and Townsend voles (Bell and Harestad 1987). Snowshoe hares also avoid conifer seedlings to which odor dispensers with pinosylvin, a phenolic compound from Alaskan green alder (Alnus crispa) (Clausen et al. 1986) have been attached (Sullivan et al. 1992). The odors of crushed foliage from lodgepole pine (Pinus contorta), and Sitka spruce (Picea sitchensis), and of some of their monoterpene constituents, inhibit feeding in red deer calves (Elliott and Loudon 1987). Mule deer respond to the odors of juniper oil in selecting food (Schwartz et al. 1980).

Detection and avoidance of odors from secondary plant metabolites may be of adaptive advantage to herbivores, since it may prevent ingestion of even small amounts of plant parts that interfere with digestion (Freeland and Janzen 1974, Robbins et al. 1987a,b) or are energetically expensive to detoxify. Pocket gophers may avoid pine needle oil for these reasons. Some of its constituents, among them α-pinene, β-pinene and myrcene (Wager-Page et al., this volume) may act as feeding deterrents under natural conditions. These compounds are widespread in conifers (Radwan et al. 1982). Both species of pocket gophers used in the present study consume conifer parts during the winter (Burton and Black 1978), and Thomomys is a major factor in seedling mortality in pine and fir plantations (Gottfried and Patton 1984, Radwan et al. 1982). Indeed, recent studies indicate that α-pinene is as aversive to plains pocket gophers as is pine needle oil (Epple et al. 1996).
MANAGEMENT IMPLICATIONS

Sensory stimuli that elicit innate aversive responses without the need for conditioned aversions are promising candidate repellents. Pine needle oil exhibits some of these characteristics. Therefore, its use as a repellent for pocket gophers should be further explored, particularly under field conditions. Pine needle oil may be particularly useful for the protection of underground cable from gnawing by pocket gophers. Our studies have shown that impregnation with pine needle oil protects soil-embedded cable from gnawing by plains pocket gophers (Eppl et al., 1996). Given the aversive responses shown by northern pocket gophers in the present study, it is likely that this species will also avoid pine needle oil encountered on underground cable.

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LITERATURE CITED


