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Capsicum oleoresin: development of an in-soil repellent for pocket gophers†

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Abstract: A pre- and post-monitoring study was conducted of the potential use of capsicum oleoresin as an in-soil repellent for northern pocket gophers (*Thomomys talpoides*). Pocket gophers were captured in irrigated alfalfa (*Medicago sativa* L), affixed with radio transmitters, and monitored daily for location. Six plots (4.87 × 4.87 m) each were randomly assigned to capsicum oleoresin and soybean oil treatments; these were set up based upon the centers of initial core areas of gophers. Mean (±SD) volumes of capsicum oleoresin and water and soybean oil and water mixtures (10 + 90 by volume) dispensed onto plots equaled 178.5 (±4.7) and 175.7 (±14.0) liters, respectively. Movements (m) of the radio-transmitted gophers from plot centers were computed for four daily readings (i.e., 0801–1000, 1101–1300, 1501–1700 and 1801–2000 h). Spectrophotometric analysis of soil samples from capsicum oleoresin plots validated the presence of capsicum on plots and the absence of capsicum on placebo- and off-plot locations. Analysis of variance for movement distances of gophers yielded a Date main effect [$F(11, 103) = 2.08$, $P \leq 0.03$] and a Date × Reading (time) interaction [$F(32, 299) = 3.21$, $P \leq 0.01$]. Results showed that gophers were located farther from plot centers for the 0801–1000 and 1501–1700 h telemetry readings for ≤3 days post-chemical application—a probable 'disturbance' effect rather than a chemical-induced avoidance. In a prior laboratory study, capsicum oleoresin and soil treatments of 1.5% w/w capsicum caused nearly a 50% decrease in soil contact time by gophers relative to placebo-exposed control animals. This implies that procedural variables warrant revision before abandoning this approach. The potential for soil insertion of repellents as a technique for expelling pocket gophers from territories and some methodological changes for future research of the technique are discussed.
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Keywords: alfalfa; capsicum oleoresin; pocket gopher; repellent, soil; *Thomomys talpoides*

1 INTRODUCTION

Pocket gophers (*Thomomys* spp., *Geomys* spp., *Cratogeomys* spp.) are small fossorial rodents that inhabit much of North America.[1,2] These rodents are highly territorial and dig extensive burrow systems.[2] Mounds of soil are produced as the gophers excavate new tunnels. 'Feeder' holes are produced at the soil surface after the animal closes (plugs) a tunnel opening that was used for above-ground foraging on plant material; these appear as 6–9 cm diameter, dirt-filled holes flush with the soil surface. The term 'pocket gopher' is derived from the animal's outward-folded, fur-lined cheek pouches (pockets) that are used to hold food during foraging bouts to and from food caches within the burrows.[2]

In reality, pocket gophers probably exert both beneficial and deleterious effects upon rangeland agriculture.[3] Their burrow-building, soil-excavating and mound-making (i.e., above-ground deposits of excavated soil) behaviors can decrease soil compaction and increase soil moisture retention; however, the excavated soil can reduce plant density and break harvest equipment.[4–6]

Traditional management of pocket gopher damage has relied on the use of rodenticide baits for population reduction. Strychnine alkaloid and diphacinone have been reported to provide between 60% and 90% efficacy,[7,8] but the rapid reinvasion by other gophers into existing tunnel systems typically requires repeated baiting in most situations.[9] Improved management methods for these rodents are needed.

Recently, a report showed that mixing 1.5% (w:w) capsicum oleoresin in soil during laboratory exposures reduced the soil contact time by northern pocket gophers.[10] The capsicum–soil-exposed gophers spent roughly 50% less time on the treated soil (i.e., moved to a clean, elevated platform) than animals exposed to placebo (moist) soil. In a later persistence study, capsicum–soil concentrations of 60.0, 30.0, 15.0 and 7.5 $g\,dm^{-3}$ were depleted of active ingredient (AI) rapidly during 14 consecutive days of 0.64 $ml\,cm^{-2}\,day^{-1}$ simulated rainfall, then stabilized

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and remained unchanged during an additional 14 days without water applications.[11]

Capsaicin (CAS No. 404-86-4) is a biochemical pesticide.[12] Capsicum oleoresin (CAS No. 8023-77-6) is made by distilling the powdered AI in a solvent and evaporating the solvent, then preparing a liquid using a 'carrier' (e.g., soybean oil). These derivatives of hot pepper plants are mammalian irritants—substances that depolarize sensory receptor cells and cause pain.[13] Both chemicals are considered 'safe'. Capsaicin is used medicinally as a 0.075% cream prescribed for patients with pain due to shingles.[14] Risks of dietary and dermal toxicity for target and non-target terrestrial species are minor—the Probable Oral Lethal Dose of capsaicin is $0.5–5\,g\,kg^{-1}$ and sensory irritation precludes prolonged ingestion.[15] The Environmental Protection Agency lists few restrictions for the use of capsicum oleoresin (i.e., capsaicin); a warning against runoff of products into watersheds is one of the few guidelines required on product use labels by registrants.[12]

This paper describes results of an initial field trial to assess the potential for in-soil applications of capsicum oleoresin to expel northern pocket gophers from established burrow systems in irrigated alfalfa. The null hypothesis was that the mean radio-telemetry location distances from plot centers would be equal for gophers on capsicum oleoresin and soybean oil (placebo) plots for both pre- and post-chemical application dates.

2 METHODS AND MATERIALS

2.1 Timeline and general procedures

The study was conducted between August 16 and October 25, 1999. Gophers were captured and affixed with transmitters between August 16 and 18. Core areas (95% utilization) of gophers were determined based on the August 16 and 20 telemetry data. Plots were selected and demarcated based on gopher core areas on August 21. Alfalfa was cut and removed on August 22. Plots were prepared for chemical applications (holes augured) on August 23. Soybean oil (placebo) and capsicum oleoresin applications occurred on separate days: August 24 and 25, 1999, respectively. Radio-telemetry locations of gophers occurred during the continuous 17-day period between August 16 and September 2, while two follow-up data recordings occurred on September 9 and October 25.

2.2 Study site

The study site was a 65 ha field located ~20 km northwest of Wellington, Colorado. The field was irrigated using an overhead rotating pivot system and had a mature stand of alfalfa (>6 years). Research plots (4.87 × 4.87 m) were located in a roughly 200 × 50 m area along the northern edge of the field; this area had dense pocket gopher signs (i.e., ~1 tunnel opening, mound, or feeder plug per $3\,m^2$).

Soil analysis revealed that the site consisted of a sandy loam soil (62% sand, 26% silt, and 12% clay), with pH 7.9 and 2.5% organic matter (Agvise Laboratories, Northwood, ND).

2.3 Pocket gophers

Pocket gophers (*Thomomys talpoides*) were live-trapped (Colorado License 99-TR621A2) at the site using custom one-way, hinged-door, Mason jar (0.95-liter) traps.[10] Traps were set and checked every 2–5 h between 0800 and 1900 h (daylight).

Within 6 h of capture, gophers were anesthetized using a 1.9-liter glass jar containing a cotton pad soaked in Metofane® (Mallinckrodt Veterinary, Inc., Mundelein, IL), each gopher having 2–3 min inhalation of the opened jar's atmosphere. A small-mammal radio transmitter (Holohil Systems, Ontario, Canada), a 1.5 × 1.0 × 0.7 cm battery pack (40–50 days projected life), and a ~10 cm long wire-whip antenna were attached snugly around each gopher's neck using shrink tubing-covered wire, leader sleeves and Duro Super Glue® (Lucite Corp, Newington, CT). The transmitter weight was <2% of mean gopher body weight. Upon recovery from anesthesia, each gopher was released at the original capture location within its unsealed tunnel.

A total of 12 gophers (four males and eight females) were studied in the final design: six per capsicum oleoresin and six per soybean oil (placebo) treatment condition. Random assignment of gophers to chemical applications yielded six female gophers having a mean (±SD) weight of 136.3 (±10.9) g inhabiting capsicum oleoresin plots and four male and two female gophers having a mean (±SD) weight of 126.7 (±27.7) g inhabiting soybean oil plots.

2.4 Application of capsicum oleoresin and soybean oil mixtures

Capsicum oleoresin was obtained as a dark-red, viscous liquid (Penta Manufacturing Co., Livingston, NJ; Mfg No. 03-09000; Lot Nos 46051 and 52577). The manufacturer reported that the material assayed at 1 000 039 Scoville units contained 4.92% capsinoids and had a soybean oil base.[16] Soybean oil (placebo) was obtained as food-grade oil from a commercial food products supplier.

To apply chemicals, the alfalfa was cut to a height of approximately 2.54 cm and removed from each plot. Each plot surface was lined into 256 (16 × 16) equal-sized grids—30.5 × 30.5 cm cells. The centers of alternate cells (checkerboard style) were then marked with a spot of non-toxic paint. Next, 128 holes, each approximately 15 cm in diameter by approximately 0.45 m deep, were dug at these spots using a gas-powered auger. Some auger holes intersected gopher tunnels.

The chemical application equipment was similar to that used by the pest control industry for injecting termiticides into soil along the foundations of buildings. This entailed a commercial pump and tank system powered by a gasoline engine (5 hp) and Hypro Roller Pump (Mdl. 7560N, Pest Control Supplies, Kansas City, MO). Chemical mixtures or water were pumped from a 378.5-liter polyethylene tank through a 30 m, 2.54 cm (OD) PVC hose to a 1.2 m long, 1.27 cm (OD) hollow stainless steel rod injector having a lever-controlled on/off handle (B&G Versatool, Plumsteadville, PA). Calibration trials showed that the gauge settings of 90, 150 and 210 psi (620, 1035 and 1450 kPa) pump pressure provided mean volume outputs of 5.1, 7.6, and 9.2 liters min^{-1}, respectively.

We originally sought to inject 170 liters of tank mixtures (10 + 90 by volume) per plot using the pump and tank system (i.e., analogous to termiticide applications). However, the compacted soil at the site led to use of the auger hole procedure (i.e., the injector rod could only be inserted 5–10 cm into the compacted soil), and the tank-mixing method produced precipitates from the soybean oil which led to the direct application of concentrates and flushing with water. Initially, two placebo applications (Plots 5p and 6p) involved dispensing tank mixtures of soybean oil and water (10 + 90 by volume) into auger holes and auger holes with tunnel intersects (Table 1). The remaining ten applications involved placing measured amounts of the soybean oil (placebo) or the capsicum oleoresin concentrate into each augured hole or augured hole–tunnel intersect, and subsequently adding prescribed amounts of water to the hole or tunnel. Calculations to divide the 170 liters equally based upon the unique augured hole/tunnel pattern that occurred per plot were computed prior to each plot application. In general, 43.5 ml of the soybean oil (placebo) or capsicum oleoresin concentrate was dispensed into each augured hole, with the remaining volume of concentrate (11–12 liters) dispensed equally among the number of augured hole–tunnel intersects encountered on specific plots (Table 1). Mean (±SD) volumes of capsicum oleoresin + water and soybean oil + water mixtures dispensed onto plots equaled 178.5 (±4.7) and 175.7 (±14.0) liters, respectively.

2.5 Radio-telemetry measurements

Radio-collared gophers were monitored for the nearest ground surface location four times daily. Locations were determined using a hand-held, three-element Yagi antenna and a portable radio receiver (Advanced Telemetry Systems, Isanti, MN). Geographic locations were then determined using a Global Positioning System (GPS) receiver (GeoExplorer®, Trimble Navigation, Sunnyvale, CA). The accuracy of GPS data was estimated by collecting routine readings for a stationary point (fence post) located ~50 m north of the field and assessing this variance.

For gopher core area and movement determinations, data were corrected via Pathfinder® software (Pathfinder®, Trimble Navigation, Sunnyvale, CA); these corrected locations were then imported into ArcView 3.2 Geographical Information System (GIS) software, ArcView Animal Movement Analysis Module Suite (ESRI, Redlands, CA). The 'grid extent parameter' was generated automatically by the software based on the scale of view, with the same scale used throughout all views. A least-square, cross-validation test was run using 95% contours to describe the area utilized by the gophers. The 'adaptive kernel' method was used to estimate home ranges and movement distances of gophers.[17]

2.6 Capsicum oleoresin detection in soil samples

A total of 37 core samples (15–45 cm depth) were obtained from four capsicum oleoresin plots, one soybean oil plot and several off-plot locations during the study. Some samples were arbitrarily removed from augured holes (chemical application) and others were randomly removed from non-augured spots on plots; the off-plot samples were obtained to detect possible capsaicin contamination and to quantify capsaicin residues on plots. Samples were emptied into clean, 3.8-liter plastic bags and stored in a dry, plastic bin in a trailer at the research site.

To detect the uniformity of chemical treatment at augured holes and possible migration of chemical treatments, on-plot samples were distinguished between those obtained at augured holes (i.e., points where chemicals were applied) and at non-augured holes (i.e., points where no chemicals were applied, but migration of chemical expected). Specifically, soil samples were obtained from capsicum oleoresin plots on August 25 (Plot 4e), 27 (Plot 2e), 29 (Plot 6e), plus September 2 (Plot 1e), respectively; soil samples were collected from a soybean oil plot (Plot 2p) on August 31. Soybean oil plot and off-plot samples were viewed to be control samples.

Soil analyses were performed using a spectrophotometric method.[11] For single-blind purposes, 25 g of the original soil sample was removed and placed into a clean, pre-labeled (numbered 1 to 37), 3.8-liter plastic bag. At the time of analysis, the analyst emptied the 25 g samples on to clean 12.7 × 12.7 cm plastic weigh boats and dried them at 42–48 °C for 24 h in a laboratory oven. Next, dual 10 g portions of the dried soil were placed into separate, pre-labeled 50 ml Falcon tubes. Reagent-grade methanol (50 ml) was added to each tube, and these mixtures were shaken by hand vigorously for 3 min and centrifuged at 3000 rpm for 3 min. The methanol/capsaicin extract was poured off into pre-labeled 250 ml Erlenmeyer flasks. Next, another 50 ml of methanol was added to the soil samples, which were again shaken and centrifuged for 3 min. The extract was added to the previous one in the respective flasks. A 1 ml aliquot of the extract was withdrawn and added to 14 ml of methanol. This process resulted in a 1:15 dilution ratio of the extract. Dual 1 ml aliquots of extract from the independent soil samples were subsequently analyzed for capsaicin content using a Hewlett Packard Model 8451-A Spectrophotometer.

Table 1. Chemical application data for soybean oil, capsicum oleoresin and water by plot

Chemical	Plot	Augered holes (no.)	Augered holes with tunnel intersects (no.)	Soybean oil or capsicum in augered holes (liters)	Water in augered holes (liters)	Soybean oil or capsicum in tunnel intersects (liters)	Water in tunnel intersects (liters)	Total volume (water + soybean oil or capsicum) (liter)	Chem. in mix (% v/v)
	1p (c)	117	11	5.1	50.9	12.1	126.2	194.3	8.8
	2p (3c)	107	21	4.6	46.5	12.6	127.8	191.5	9.0
	3p (8c)	115	13	5.0	50.0	11.7	100.0	166.7	11.1
	4p (9c)	118	10	5.1	51.3	11.0	104.3	171.7	9.4
	5p (10c*)	114	14		49.6*		109.8*	159.4*	10.0
	6p (12c*)	104	24		45.4*		125.3*	170.7*	10.0
Mean (±SD)		112.6 (±5.7)	15.5 (±5.7)	4.9 (±0.2)	49.0 (±2.4)	11.8 (±0.7)	115.6 (±12.3)	175.7 (±14.0)	9.7
Capsicum	1e (4e)	111	17	4.8	48.2	11.9	118.2	183.1	9.1
	2e (5e)	114	14	5.0	49.5	11.2	109.5	175.2	9.2
	3e (6e)	121	7	5.3	52.6	11.2	109.5	178.6	9.2
	4e (7e)	109	19	4.7	47.4	11.4	115.6	179.1	9.0
	5e (11e)	118	10	5.1	51.3	11.0	104.3	171.3	9.4
	6e (13e)	113	15	4.9	49.1	12.0	117.7	183.7	9.2
Mean (±SD)		114.3 (±4.5)	13.7 (±4.5)	5.0 (±0.2)	49.7 (±1.9)	11.4 (±0.5)	112.5 (±5.6)	178.5 (±4.7)	9.2

* For Plots 5p and 6p, soybean oil:water was dispensed as a 10:90 % v/v tank mixture using the pump and tank system.

An empty quartz cuvette was used as a reference for a cuvette of pure methanol. Methanol absorbance was scanned at wavelengths between 190 and 820 nm. This spectrum was obtained mainly as a check for potential contaminants, and the methanol sample was, in turn, used as a reference for the soil extracts. This procedure preceded every batch of soil sample extracts that were analyzed. The absorption maximum (λ_{max}) was recorded for each extracted sample at 282 nm.[18]

2.7 Data analyses

Mean spectrophotometric measurements (AU) obtained from dual aliquots of soil samples were graphed and described. Efficacy of the chemical applications to repel gophers from plots was assessed using the movement distance relative to center plot (m). A mixed-model analysis of variance (ANOVA) was computed using PROC MIXED software, with gophers (plots) considered a random effect and Dates and Readings (time-of-day) fixed effects and repeated measures;[19] significant sources of variance were further analyzed using least square means comparisons.[20] The design was viewed as a 2 (Chemical) ×12 (Date) ×4 (Readings—approximate time of day for telemetry), with gophers (Plots) nested within chemicals.[21] The 0.05 level of significance was used to test both ANOVA and *post hoc* means statistics.

3 RESULTS

3.1 Capsicum oleoresin detections in soil samples

The majority of AU readings for soil samples from augured holes ranged between +0.0304 and +0.0990 (Fig. 1).[13] All samples collected at non-augured hole spots on the capsicum-treated plots (i.e., >20 cm from a capsicum application) yielded mean AUs ≤+0.0052—sixfold less absorbance for these sampling points than for those of direct capsicum oleoresin application. Soil samples from the placebo plot (2p) and from off-plot locations were essentially capsaicin free, all Plot 2 (placebo) soil samples yielding mean AU values ≤+0.0091. Mean off-plot samples yielded the lowest AU values (≤+0.0042 AU).

3.2 Radio-telemetry locations and gopher movements

The ANOVA for movement distance yielded a Date main effect [F (11, 103) = 2.08, $P \leq 0.03$] and a Date × Reading interaction [F (32, 299) = 3.21, $P \leq 0.01$]. These effects demonstrated that the mean movement distances of gophers increased post-chemical application. The radio-telemetry data indicated that the dispensing of the capsicum oleoresin mixture onto plots caused no emigration of gophers from experimental plots. Rather, animals on the soybean oil plots displayed more frequent and farther off-plot movements than gophers on the capsicum oleoresin treated plots—an observation suggesting that,

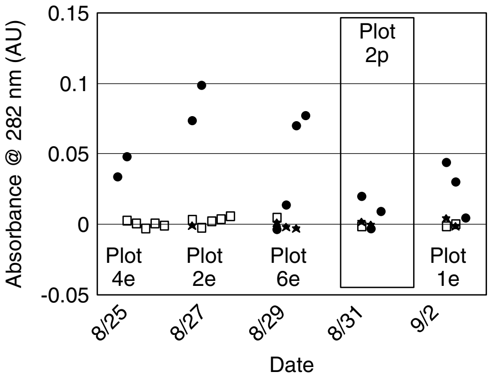

Figure 1. AU (282 nm capsaicin) values for soil samples collected from capsicum oleoresin (Plots 1e, 2e, 4e, and 6e), soybean oil (Plot 2p) and off-plot locations by date. Symbols depict three types of soil sample collection: (●) on-plot within an augured hole where chemical was applied; (□) on-plot but at spaces (grids) between augured holes where no chemical was applied; and (★) off-plot where no chemical was applied.

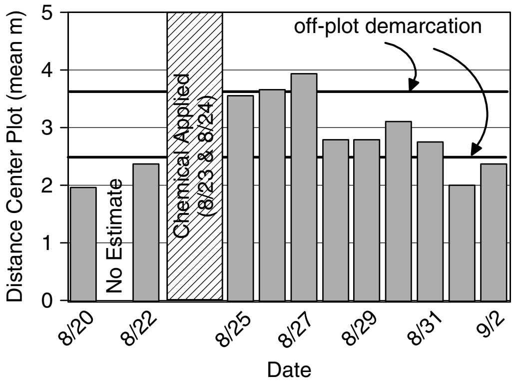

Figure 2. Date main effect for distance-from-center-plot variable (m).

rather than expelling gophers from burrows, perhaps capsicum inhibited gopher movements relative to soybean oil.

Regarding the Date main effect, mean movement distance (m) of gophers from center plots on the successive dates before chemical application were 1.97, 'non-estimated' and 2.38; while mean movement distances on successive dates after chemical application were 3.56, 3.66, 3.94, 2.80, 2.80, 3.11, 2.78, 2.00 and 2.37 m (SEs between 0.73 and 0.75), respectively (the 'non-estimated' value was attributed to a number of missing data due to satellite conversion on August 26; Fig. 2). Least squares mean comparisons showed that means for the first three dates after chemical applications were greater than for all other days, but not different from each other. Means >3.54 m also indicate that these locations were off-plot: a 2.50 m linear distance from plot center denoted the nearest plot edge and a 3.54 m linear distance from plot center denoted the plot corner (i.e. off-plot demarcation).

The Date × Reading interaction showed that mean movement distances of gophers also differed during the August 25–27 dates for the 0801–1000 h and 1500–1700 h telemetry readings on these days (Fig. 3). Mean (±SE) movements of gophers were

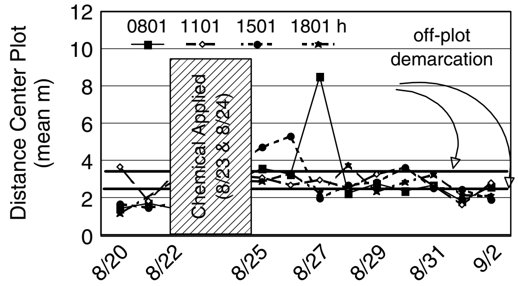

Figure 3. Date × Reading interaction for distance-from-center-plot variable (m).

4.74 (±0.95) and 5.31 (±0.98) m for the 1501–1700 h telemetry locations of August 25 and 26, respectively, with a 'spike' of 8.51 (±0.92) m from center plot recorded on August 25 for the 0801–1000 h reading of the day. *Post hoc* least squares mean tests confirmed that the initial reading for August 27 differed from all other readings, and that the readings for the afternoon session (1501–1700 h reading) on August 26 and 27 were not different from each other, but differed from all other mean movements from center plot.

4 DISCUSSION

The current findings show that gophers exposed to soil and tunnels soaked with either capsicum oleoresin or soybean oil mixtures increased forays away from chemically treated areas relative to pre-application dates. Nevertheless, these increased forays were pronounced for only the first three days after chemical application and were greatest during the 0800–0959 and 1500–1659 h measurement sessions. The null hypothesis was not rejected; no Chemical × Date interaction occurred. Whether the acute main effect for Date was due to chemical treatments, residual disturbances (i.e., noise and vibration from previous auger or chemical application methods) or a combination of these factors cannot be isolated.

It is possible that the application of an irritant into tunnels might actually impede, rather than increase, movements of gophers from plots. The lack of prolonged days involving gopher forays off plots could indicate that irritation from capsaicin residues inhibited movements of animals as they traversed treated tunnels. That is, gophers on capsicum oleoresin plots may have stayed in relatively capsicum-free nest or food cache chambers for the duration of our observations, whereas placebo-exposed gophers were seeking to find new oil-free tunnels or new areas in which to dig chambers. The extreme territoriality of pocket gophers would probably deter excursions of all gophers into new soils where agonistic encounters with other gophers would typically occur.[2,22]

Basically, these results are counter to prior laboratory data which indicated that soil mixtures ≥1.50 % w/w capsicum oleoresin caused a 46% reduction in soil contact time by pocket gophers relative to water-exposed controls.[10] Methodological differences between the laboratory and field tests offer prime explanations for the current lack of effects. Two obvious methodological factors involved in this technique are uniformity of capsicum distribution and sufficiency of capsicum concentration. First, spectrophotometric analyses of soil samples showed that modest capsaicin AU values were found only at points of capsicum oleoresin application on treated plots (i.e., augured holes)—values suggestive of capsicum residues between 0.25 and 0.75% w/w.[11] Little migration of capsicum oleoresin was evident within the soil on treated plots. Low adsorption and dispersion of capsicum within treated soils could account for the lack of a repellent effect. Second, the use of a w:w soil mixture in this field trial was intentionally avoided, and this could have precluded the establishment of a sufficient concentration of capsicum oleoresin on experimental plots. However, such a capsicum–soil mixture was deemed practically and economically prohibitive. Assuming that plots are three-dimensional (i.e., $4.87 \times 4.87 \times 4.87\,\text{m} = 115.5\,\text{m}^3$, including the ground surrounding tunnels, nest chambers, and food cache chambers), computations with soil weighing $1410\,\text{kg}\,\text{m}^{-3}$ for such a plot would have required the use of 2296–2541 liters (specific gravity 0.94–1.04) of capsicum oleoresin, i.e., $[(115.5\,\text{m}^3 \times 1410\,\text{kg}\,\text{m}^{-3}) \times 1.5\%] = (162\,857\,\text{kg} \times 0.015) = 2296–2541$ liters depending on specific gravity. At US $30.00/kg for capsicum oleoresin (Penta Manufacturing Co., Livingston, NJ), this experimental application would have been prohibitive (i.e., $68 880–76 230 per plot).

Despite the lack of direct chemical effects showing expulsion or sustained repellency of gophers from burrows in this field assessment, we contend that the development of a 'soil insertion' concept of irritants/repellents for fossorial rodents remains feasible. Successful research and development of this methodology probably hinges upon (1) the discovery of cheaper, more effective irritants and (2) the development of improved insertion and chemical distribution technologies for establishing sufficient, uniform concentrations of chemicals under diverse soil conditions.

We contend that research of specific, less pervasive soil insertion applications is needed. It seems reasonable that the use of even 1.5% w:w mixtures of capsicum oleoresin in soils or trenches surrounding buried cables could deter damage and be economically feasible, especially with high-cost military or security applications of buried cables. Treating burrows with chemical irritants could also deter reinvasion by pocket gophers following the traditional use of poisons in forestry situations where establishment of seedlings is sought.[22] These applications would greatly lower the expense and quantity of capsicum oleoresin required for wide-area lawn or crop treatments.

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