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# Efficacy of IGI<sup>®</sup> Carbon Dioxide Gas to Kill Ground Squirrels and Pocket Gophers in Underground Burrows

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**ABSTRACT:** Both ground squirrels and pocket gophers are significant pests in urban, rural, and agricultural settings within California. Various techniques are used to control these “pest rodents”; however, this presentation will explore the use of carbon dioxide (CO<sub>2</sub>) gas as a fumigant to control these rodents in their burrows. These studies were conducted to support efficacy submission requirements for the US Environmental Protection Agency (EPA) and selected state regulatory agencies in California, Washington, and Oregon. The Eliminator<sup>®</sup> System, Inert Gas Injection, LLC (IGI) was used for delivery of the carbon dioxide gas (cylinders) into underground burrows. Pre-treatment and post-treatment monitoring, visual counts, and burrow activity were used to determine the efficacy of the burrow fumigations. Carbon dioxide gas treatment of ground squirrel burrows resulted in 93.6-84.3% reduction in visual ground squirrels in the treatment plots 5-7 days after treatment, respectively, compared with untreated plots. CO<sub>2</sub> gas treatment of ground squirrel burrows resulted in 71.5 ± 4.3% to 67.8 ± 4.3% reduction of reopened ground squirrel burrows in the treatment plots 1 and 5 days after treatment, respectively. In the pocket gopher trial, there was no evidence of tunneling or mound building in the treated test plots for 4 days after the last CO<sub>2</sub> treatment, demonstrating 100% effect of the CO<sub>2</sub> treatments against gophers with the Eliminator<sup>®</sup> System.

**KEY WORDS:** burrowing rodents, carbon dioxide, CO<sub>2</sub>, efficacy, gopher, ground squirrel, inert gas, *Spermophilus* spp., *Thomomys* spp.

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## INTRODUCTION Ground Squirrels

Ground squirrels (*Spermophilus* spp.) are troublesome and serious pests to agriculture and public health throughout the United States. Ground squirrels live in a variety of habitats and populations are particularly high in grazed rangeland and in areas disturbed by humans such as road and ditch banks, levees, fence rows surrounding crops, orchards, pastures, around buildings, parks, schools and activity fields.

Ground squirrels are primarily herbivorous, and they usually forage close to their burrows. Their diet changes throughout the year and they are capable of causing damage to young shrubs, vines and trees by gnawing bark, girdling trunks, and feeding on many types of fruit and nut trees as well as ornamentals. Ground squirrels can also damage irrigation equipment and have been known to eat the eggs of ground nesting birds such as the California quail.

Ground squirrels live in a burrow system that has many chambers and can be 5-30 ft in length and extend 2-4 ft below the surface. There are often multiple openings connecting the tunnels and more than one squirrel can live in a burrow system. Ground squirrels live in colonies that can include several dozen animals in a complex of burrows. Burrowing systems can be very destructive presenting hazards to machinery, pedestrians, livestock, compromising ditches, roads, levees, buildings/ structures, and wildlife. Ground squirrels have been linked to several diseases such as Rocky Mountain spotted fever, rat bite fever, tularemia, Chagas' disease, audio-spiromycosis, and encephalomyocarditis. The disease they are most often associated with is sylvatic or bubonic plague.

Ground squirrels are active during the day, but have two seasonal periods of dormancy, one in the winter (hibernation) and one in the summer (estivation). Above ground activity is greatest during the breeding season from late winter into early summer and has a great effect on

control options.

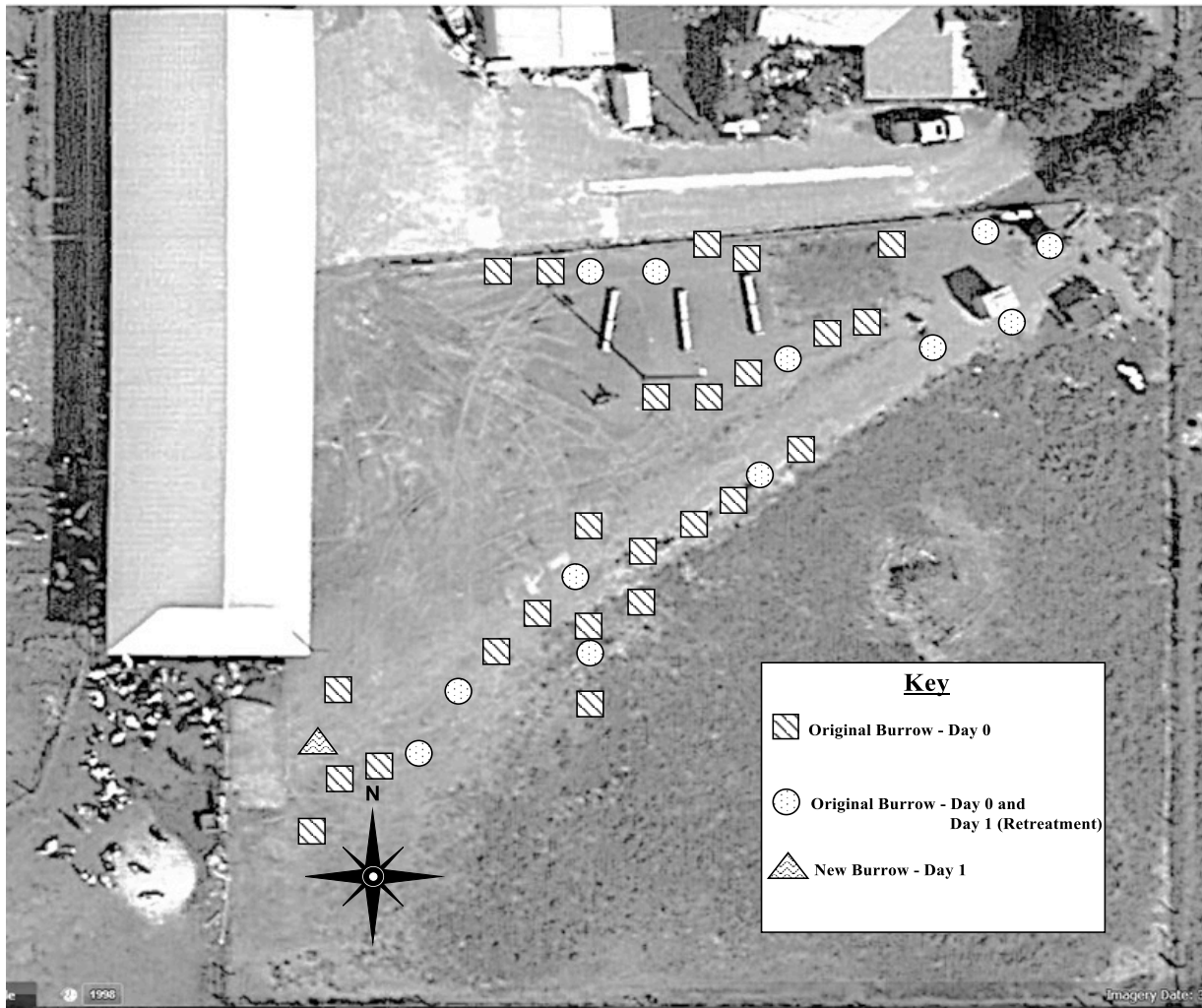
Ground squirrels are a non-game species most commonly considered a vertebrate pest; however, some ground squirrel species in the U.S. may be protected or live in areas with other protected (threatened or endangered) animals. Due to the many complexities of ground squirrel biology and ecology an integrated pest management (IPM) system is highly encouraged for the control of this very adaptable vertebrate pest. The use of a carbon dioxide (CO<sub>2</sub>) gas as a burrow fumigant applied with a unique injection system is an attractive option for controlling ground squirrels with minimum impact to non-target organisms and the environment (Salmon and Gorenzel 2010).

## Pocket Gophers

Pocket gophers (*Thomomys* spp.) are troublesome and serious pests to agriculture, horticulture and homeowners throughout the United States. These burrowing rodents create extensive tunnel systems and the characteristic mounds can be numerous from a single gopher. Gophers do not hibernate and are active year-round, their burrow systems can cover an area of 200 to 2,000 square feet. Gophers are solitary rodents except when females are caring for their young or during the breeding season. Gopher densities can reach very high numbers, up to 60 or more per acre in agricultural settings. Sexual maturity is reached in approximately one year and gophers can live up to three years, producing up to three litters per year, with 5-6 young per litter.

Pocket gophers feed on a wide variety of vegetation preferring herbaceous plants, shrubs, and trees, feeding on the roots and fleshy parts of the plants. They occasionally feed above ground, but only about a body length away from the tunnel opening. Gophers can cause damage to irrigation systems and mowing equipment with their mounds.

Pocket gophers are a non-game species most commonly considered a vertebrate pest. Due to the many complexities



**Figure 1. Site map of a single plot (replicate) for a ground squirrel field test using carbon dioxide gas as a burrow fumigant.**

of gopher biology and ecology an integrated pest management (IPM) system is highly encouraged for the control of this very adaptable vertebrate pest. The use of a carbon dioxide (CO<sub>2</sub>) gas as a burrow fumigant applied with a unique injection system is an attractive option for controlling pocket gophers with minimum impact to non-target organisms and the environment (Salmon and Baldwin 2009).

#### **Properties of Carbon Dioxide and Mode of Action**

Carbon dioxide has a density twice that of oxygen making it heavier than air and ideal as a burrow treatment for rodent pests. Because of its excellent water and lipid solubility, carbon dioxide is readily absorbed by vertebrates with CO<sub>2</sub> being more soluble in water than oxygen. CO<sub>2</sub> is exchanged more easily than oxygen in the lungs and undergoes chemical changes in the blood. Excess CO<sub>2</sub> inhaled by a vertebrate will diffuse rapidly into the blood, giving rise to acute CO<sub>2</sub> poisoning. The clearance of CO<sub>2</sub> from vertebrate tissues is almost 100% dependent on alveolar ventilation.

Because of excellent solubility and rapid absorption of

carbon dioxide in the vertebrate blood system the resulting toxicosis is two-fold. Hypercapnia is an excessive concentration of carbon dioxide in the bloodstream, typically caused by inadequate respiration. The second and concurrent condition is acidosis, which is too much acid (carbonic) in the body, a distinctly abnormal condition resulting from accumulation of acid which lowers the pH of blood and tissues. When the FiCO<sub>2</sub> (i.e., Fractional Concentration of Inspired CO<sub>2</sub>) is greater than 30%, the resulting symptoms are unconsciousness, heart failure, and death.

Both the AVMA and NIH consider CO<sub>2</sub> an acceptable method of euthanasia because of its rapid onset of action, safety, low cost and is readily available (AVMA Guidelines 2013). Many universities' institutional animal care and use committee (IACUC) protocols recommend that euthanasia of laboratory rodents be administered with compressed CO<sub>2</sub> in cylinders only and only after proper training, with the correct equipment and adequate CO<sub>2</sub> exposure times. The Center for Wildlife Management Website advises the use of CO<sub>2</sub> to euthanize wildlife including squirrels, skunks, racoons, prairie dogs, and racoons.

## METHODS

### Ground Squirrel Field Test

Initial visual assessment of the test site indicated a high level of ground squirrel infestation and a very extensive burrow system located in areas that were conducive to the study. The primary ground squirrel burrow systems were located on berms, ditch banks, heifer corrals, and feed bunks within the test site. There was no history of ground squirrel control measures on the test site property (Figure 1).

Pretreatment census activities occurred over a five (5) day period, three (3) days for visual counts and two (2) days for closed burrow evaluations. The visual count censuses were conducted during mid-morning hours on each of the three days by conducting three scans approximately 15 minutes apart. After the three scans the investigators conducted a “walk-through” count to assess the ground squirrel density and activity in each plot. Some squirrels were foraging in locations that were obstructed from view because of structures and vegetation blocking the view of the scans. Once the investigators entered the test plots the ground squirrels sounded the alert call and all squirrels rapidly returned to the burrows. The high scan and walk through counts for each test plot were recorded and a four-day lag period occurred prior to treatments to allow the ground squirrels to resume normal daily behavior.

All burrows within the test area of Plots #1, #3, and #5 were closed with surrounding soil or other available media and observed for reopening after two days (closed burrow evaluations). Any burrow that was opened back up was considered active and was included in the treatments.

The pretreatment Plots #2 and #3 were combined (now referred to as Plot #3) and buffer zones added between Plots #1, #3 and #4 due to the high numbers of ground squirrels and the density of the burrow openings within the ground squirrel colonies. Plots #5 and #6 had a natural buffer zone crossing the drainage ditch and the ground squirrel and burrow densities were lower in these two plots compare with Plots #1 through #4 on this irrigation berm.

### Treatments

Active burrows (holes) were identified and marked for each test plot using orange marker flags. Each active burrow was identified with a plot number, sequential hole number, and treatment date. CO<sub>2</sub> gas delivery rate was set at 25 liters per minute as indicated by the gauges attached to the CO<sub>2</sub> gas cylinder with a 3-minute application to each active burrow opening for a total of 75 liters of CO<sub>2</sub> per application. The probe of the Eliminator (IGI, LLC., Acampo, CA) was placed as far as possible into the burrow with the maximum length (33”) at the T-handle. Soil was placed at the opening of the burrow with the probe in place and lightly tamped down to seal the probe and minimize CO<sub>2</sub> escape from the entrance hole. The handle was depressed completely to allow flow of CO<sub>2</sub> into the burrow system for three minutes. Visual observations approximately one hour after CO<sub>2</sub> burrow treatments indicated no ground squirrel activity, i.e., tunneling to escape treatment, or above ground activity in the treatment plots.

After CO<sub>2</sub> was injected into the burrow, the probe was removed, and the soil gently tamped to prevent gas escape. A CO<sub>2</sub> meter (pSense Model AZ-0001. CO<sub>2</sub> Meter, Inc., Ormond Beach, FL) was used to determine CO<sub>2</sub> concentration ~12” downwind from the burrow opening after treatment. Air temperature and relative humidity were also recorded from the same device. Burrows (holes) that were reopened 24 hours after the initial treatment were retreated the following day using the same procedures as previously described. All application times and duration were recorded. Due to the high number of ground squirrel burrows to be treated, Plots #1 and #5 were treated on 8 June 2016 and Plot #3 was treated on 9 June 2016 with the corresponding retreatments 24 hours later (day 1).

### Post-treatment Assessments

Twenty-four hours after the initial CO<sub>2</sub> treatment all treated burrows were inspected for reopening and any previously inactive unopened burrows that may have been utilized and opened after CO<sub>2</sub> treatment were also inspected

**Table 1. Number of ground squirrels visually recorded (visual counts) pre and post treatment using gaseous carbon dioxide as a fumigant of their burrows (n = 3).**

Treatment Group	Plot #	Highest Scan Count	Individual Squirrels - Walk Through Counts			
			Day 4 Pretreatment	Day 5 13-Jun-16	Day 6 14-Jun-16	Day 7 15-Jun-16
CO <sub>2</sub>	1	14	14	0	2	4
	3	16	25	1	2	4
	5	9	2	1	1	1
	Σ	39	41	2	5	9
	% Reduction (±SE) Group Adjusted %			82.0 (16.0)	75.9 (13.1)	68.5 (9.9)
Untreated Control	4	7	16	10	13	22
	6	17	9	9	11	13
	Σ	24	25	19	24	35
	% Change (±SE)			18.8 (18.8)	-1.7 (20.5)	-41.0 (3.5)

and noted. Any reopened burrows were retreated, and another census repeated the following day. The number of open burrows was recorded, and the average was used to calculate the percent of burrows not reopened (efficacy).

Walk-through census counts of active ground squirrels were conducted on test day 5, 6 and 7, recording the total number of ground squirrels observed in each test plot. The number of ground squirrels per plot was recorded and the average was used to calculate the percent reduction from pretreatment counts. Open burrows were observed and recorded on test day 5 from each plot as well.

### Pocket Gopher Field Test

Initial visual assessment of the test site indicated a high level of pocket gopher infestation and a very extensive burrow system located in areas that were conducive to the study. There was no history of gopher control measures on the test site property. Pretreatment census activities occurred over a ten-day period to observe mound constructions. The visual mound count censuses were conducted on each of the pretreatment days by looking for new mounds and fresh dirt. Since gophers remain in their burrows no other census methods were employed.

### Treatments

Active mounds (burrows) were identified and marked for each test plot (~30 × 40 ft) using numbered orange marker flags. Each mound complex was identified with a plot number, sequential hole number and treatment date. CO<sub>2</sub> gas delivery rate was set at 25 liters per minute as indicated by the gauges attached to the CO<sub>2</sub> gas cylinder with a 3-minute application to each active burrow opening for a total of 75 liters of CO<sub>2</sub> per application. The probe of the Eliminator with a flexible hose extension was placed as far as possible into the burrow. If there were multiple mounds, then all mounds were individually opened and treated. Soil was placed at the opening of the mound with the probe in place and lightly tamped down to seal the probe and minimize CO<sub>2</sub> escape from the entrance hole. The handle was depressed completely to allow the flow of CO<sub>2</sub> into the burrow system for three minutes. The CO<sub>2</sub> meter was placed at each new gas injection site to determine if tunnels were connected (higher than ambient CO<sub>2</sub> concentrations) and the CO<sub>2</sub> was dispersing through the entire tunnel system.

After CO<sub>2</sub> was injected into the burrow the probe was removed and the soil gently tamped to prevent gas escape. A CO<sub>2</sub> meter (pSense Model AZ-0001. CO<sub>2</sub> Meter, Inc.,

Ormond Beach, FL) was used to determine CO<sub>2</sub> concentration ~12 inches downwind from the mound after treatment. Air temperature and relative humidity were also recorded from the same device.

Mound activity (fresh dirt) was evaluated starting 24 hours after the initial treatment and any fresh activity was retreated the following day using the same procedures as previously described. All application times and duration were recorded on the data capture forms.

### Post-treatment Assessments

Twenty-four hours after the initial CO<sub>2</sub> treatment, all treated mounds were inspected for activity and any treated mounds were inspected for activity and any previously inactive mounds that may have been utilized and opened after CO<sub>2</sub> treatment were also inspected and noted. Any new mounds were retreated, and another census repeated the following day. The number of active mounds was recorded, and the average was used to calculate the percent of mounds not reopened (efficacy). Walk-through census counts of active gopher mounds were conducted on test days 1 through 6, recording the total number of active mounds observed in each test plot. The number of active mounds per plot was recorded.

## RESULTS

### Ground Squirrel Field Test

Ground squirrel populations and burrow density were very high on the test site. During the visual counts the highest scans were between seven and seventeen visible animals at any single point in time during the midmorning scans. The pastures and surrounding areas provided ideal habitat for these animals including food and shelter. The burrow systems were very extensive in the test areas as well as all the buffer zones and areas outside of the test plots. After the initial visual assessments, three treatment plots (#1, #3, #5) and two untreated plots (#4, #6) – plus the buffer zones – were designated.

The pastures are flood irrigated and the majority of the burrows were located along the berms and ditches on the test site. Closer to the pole barn in Plot #1 the burrows were located in and around many of the features such as feed bunks, pavement edges, brush piles, irrigation water distribution tanks, and along the berm.

The closed burrow census revealed which burrows in the test site were active during the pretreatment phase of the study. Only reopened burrows were marked for treatment in Plots #1, #3 and #5. A large number of the burrow openings

**Table 2. Number of open ground squirrel burrows recorded (burrow counts) pre and post treatment using gaseous carbon dioxide as a fumigant of their burrows (n = 3).**

Treatment	Plot #	Open Burrows			Total Holes Treated
		Treatment Day 0	Retreatment Day 1	Observation Day 5	
CO <sub>2</sub>	1	36	13	15	37
	3	61	22	22	73
	5	47	11	14	54
	Σ	144	46	51	164
	% Reduction (±SE)	-	71.5 (4.3)	67.8 (4.3)	

remained closed after they were filled with soil and these were not treated on test day 0. The number of reopened burrows that were then treated on test day 0 were as follows: Plot #1 = 36 burrows, Plot #3 = 61 burrows, and Plot #5 = 47 burrows. Due to the large number of burrows present in each test plot, treatments were staggered over two days, Plots #1 and #5 were treated on one day, and Plot #3 was treated on the next day with retreatments conducted approximately 24 hours after initial treatment. Environmental data (temperature, relative humidity, and CO<sub>2</sub> concentrations) were recorded for each initial burrow treatment with the CO<sub>2</sub> meter. The total pretreatment scan and walk through ground squirrel counts were n = 39 and n = 41, respectively (Table 1). These two visual census methods were nearly identical for assessing ground squirrel activity on the test plots during the pretreatment phase, so all subsequent observations were made by doing walk through counts to allow for quicker assessments by less individuals.

The efficacy of CO<sub>2</sub> gas to control ground squirrels, i.e., average percent reduction of ground squirrels or evidence of activity was calculated by two methods, visual census counts and open burrow counts. Visual census percent reductions were conducted for each individual plot, with group means  $\pm$  standard error (SE) of  $82.0 \pm 16.0\%$  for day 5,  $75.9 \pm 13.1\%$  for day 6, and  $68.5 \pm 9.9\%$  for day 7 (Table 1). The visual census reductions were calculated for the combined group using the Henderson-Tilton formula (Henderson and Tilton 1955) and determined to be 93.6, 87.3, and 84.3% on test day 5, 6, and 7, respectively (Table 1). The average ground squirrel visual census in the untreated control plots demonstrated 18.8% reductions on test day 5; however, there was a 1.7% and 41.0% increase in ground squirrel counts on test days 6 and 7 (Table 1). This was not unexpected, since these colonies were very large and ground squirrels could roam freely in the untreated and buffer zones and over time move back into the treated plots.

Reduction of ground squirrel activity based on open burrow counts in the CO<sub>2</sub> treated plots shows a similar trend as the visual squirrel counts with  $71.5 \pm 4.3\%$  and  $67.8 \pm 4.3\%$  reductions on test days 1 and 5, respectively (Table 2).

### **Pocket Gopher Field Test**

Gophers were active for a week in each of the test plots prior to treatment. It was difficult to determine how many gophers were present in each plot since the mounds were not disturbed during the pretreatment phase of the study. Test Plots #1 and #2 had eleven and seven mounds, respectively, with four mounds being treated in Plot #1 and two mounds being treated in Plot #2. The untreated control Plot #3 had eight mounds that remained untreated for these evaluations. The four treated mounds in Plot #1 were chosen by their location and apparent connection to other mounds by what was deemed as a single active gopher per treated mound. The mound located at the end of the string of mounds was selected for treatment with the Eliminator. After treatment, the mounds were stomped down, and the CO<sub>2</sub> allowed to remain in the tunnel system. After 24 hours the mounds were re-inspected and one new mound was observed in Plot #1. This mound was treated as the initial mounds and resealed and observed for five additional days, with no new activity noted in Test Plots #1 or #2.

## **DISCUSSION**

Ground squirrels have extensive burrow systems and live in colonies making it extremely difficult to determine how many ground squirrels are present in an extensive habitat such as the one used for these evaluations. These burrows could have been opened from within or from the outside by other individuals in the colony that may not have been in the burrow at the time of treatment. It has also been reported that more than one ground squirrel may occupy a single burrow and that there are multiple entrances and escape burrows within the burrow system complicating control measures.

The treatment site maps for the three test plots illustrate this adaptive behavior where new burrows (escape burrows) were marked in yellow and were not present at the pretreatment census or during treatments. It has been reported that ground squirrels will dig the burrows with exits close to the surface and can open them quickly if threatened for escape. This result demonstrates the necessity for multiple CO<sub>2</sub> treatments over time, possibly combined with additional integrated pest management (IPM) strategies for heavy infestations like the ones encountered in this trial. Ground squirrel surveillance and monitoring is critical for the success of a CO<sub>2</sub> treatment program for burrowing rodents.

Treatments of pocket gophers with carbon dioxide proved very efficacious in controlling these pests. The layout of their tunnel systems, in combination with their behavior to naturally seal off any openings, makes using a heavier than air gas, such as CO<sub>2</sub>, a very effective way to fumigate with little chance for nontarget species or applicator exposure to the treatment. CO<sub>2</sub> around the mounds remained slightly above ambient levels after treatment with a range of 495 to 995 ppm. Using the CO<sub>2</sub> meter to check gas levels in the tunnels helped determine the tunnel structure and ensure all parts of the tunnel system were treated. The inert gas remained well within the tunnel system during treatments and was not detected in any significant levels outside the treated mound.

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