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# Seasonal marking efficacy of Dupont oil blue A dye on steam-rolled oat groat baits for valley pocket gophers

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## Abstract

We examined if Valley pocket gophers (*Thomomys bottae*) exhibited a seasonal preference for consuming steamed-rolled oat (SRO) groat bait treated with 1.6% Dupont oil blue A (fat-marking dye). Field tests were conducted in California alfalfa fields during winter, summer, and fall, and in walnut orchards during winter and summer. Five treatment units (TUs) were established each with ~60 burrows for each season and habitat studied during 1997–1999. SRO groat marker bait (4 g) was placed inside each active burrow. All bait sites were re-opened about 96 h later and examined for the presence (all or some) or absence of bait. The average bait site disturbance after 4 days of baiting was 78.2%. Traps were used to capture gophers up to 5 days after baiting. Valley pocket gophers ( $n=744$ ) were examined for the presence of blue dye in their cheek pouches, skin/fur, and fat. We expected that if gophers moved the bait, their cheek pouches and fur would be marked; if they consumed bait, their fat would be marked. In alfalfa, 54.2%, 46.8%, and 65.7%, of gophers were marked (trapping on days 5–9) by blue dye in one or more of their cheek pouches, skin, fur, or subcutaneous fat in the winter, summer, or fall, respectively. In orchards, 57.1% of gophers were marked in winter and 53.4% in summer. Of those that were marked, all (100%) had their fat dyed blue, followed by skin/fur (34.4% males: 43.7% females) and cheek pouches (5.7% males: 10.1% females). ANOVA results indicated no difference in seasonal marking efficacy in either alfalfa or orchards ( $F=3.59$ ,  $P=0.0598$  and  $F=0.12$ ,  $P=0.7384$ , respectively). The usefulness of 1.6% Dupont oil blue A dye as a marking agent for Valley pocket gophers was not demonstrated overall or for any season in either habitat. Therefore, a better marker for this gopher is needed. Some factors that may have influenced these results were discussed including: (1) baiting methodology (~66% of the bait sites were devoid of bait at the end of the study), (2) species specific dye properties, (3) bait acceptance (i.e. aversion to the dye), or (4) availability of alternative foods.

**Author Keywords:** Pocket gophers; Marker; Dupont oil blue A

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### Abstract

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### 1. Introduction

Pocket gophers (*Thomomys sp.*) cause major damage to irrigated alfalfa fields in California (Lee et al., 1990). During the late 1980s, the California Department of Food and Agriculture (CDFA) received reports from cooperators in the Sacramento Valley that strychnine alkaloid grain baits had become less effective for controlling this genus (J. Clark, CDFA, pers. commun.). Howard et al. (1968) and Marsh and Howard (1978) explained that some pocket gophers may not develop the typical bait/poison shyness that other rodents demonstrate after ingesting a sublethal dose of the pesticide, but they may develop an acquired physiological tolerance to strychnine after ingesting a series of sublethal doses (Lee et al., 1990). Consequently, CDFA sought to

improve their rodenticide formulations and baiting methodologies.

A variety of markers including dyes, inert particles, tetracycline, blood markers, and radio-isotopes have been used to study free-ranging mammals and birds to help understand their: (1) population dynamics, (2) non-target exposure to control techniques, and (3) bait acceptance (Savarie et al., 1992). Some markers used to evaluate bait acceptance include rhodamine B in black-tailed jackrabbits (*Lepus californicus*) (Evans and Griffith, 1973), dimethylchlorotetracycline in Norway rats (*Rattus norvegicus*) (Nass et al., 1971), and metallic flake particle markers in Norway rats (Fall and Johns, 1988). Linhart et al. (1993) and Creekmore et al. (1994) used Dupont oil blue A dye (DuPont Chemicals, Wilmington, DE, USA) to evaluate oral rabies baits in Indian mongooses (*Herpestes auro-punctatus*) and (*H. javanicus*), respectively. They found it was visible in both abdominal fat and lower intestines, and

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they concluded that it was an effective short-term marker (< 1 day).

In laboratory studies, northern pocket gophers (*T. talpoides*) were marked by DuPont oil blue A, and the blue dye was visible in cheek pouches and skin fur (externally) and in adipose tissues (internally) (P. Savarie, NWRC, pers. commun.). It seemed to demonstrate each gopher's propensity to move and/or ingest bait (bait acceptance). Four concentrations (0.2%, 0.4%, 0.8%, or 1.6%) were used on steam rolled oat (SRO) groat bait in a laboratory study to determine its marking effectiveness on pocket gophers. Only the 1.6% concentration effectively marked 100% ( $n = 13$ ) of the northern pocket gophers tested; consumption ranged from 0.03 to 4.07 g/day (G. Matschke et al., NWRC, unpublished Report No. 289, 1993). To further evaluate this marker on SRO groat bait, a larger laboratory study was conducted with the same species; consumption averaged 2.48 g/day ( $n = 38$ ), with intake ranging from 0.17 to 4.24 g. Dyed fat was detected in 36 of 38 (95%) of the gophers at 5 days after ingesting the marker (G. Matschke et al., NWRC, unpublished Report No. 317, 1994).

A field trial with 1.6% DuPont oil blue A showed that northern pocket gophers disturbed 32 of 37 (86.5%) the bait sites, but they consumed much less marker bait than placebo bait. Only 35% of 20 captured pocket gophers during the alfalfa growing season had blue-dyed fat (G. Matschke et al., NWRC, unpublished Report No. 310 Part I, 1994). It was hypothesized that the low consumption of marker bait was due to a seasonal preference for the growing alfalfa roots and tubers. Two field trials conducted in the late fall when alfalfa was dormant seemed to support this hypothesis, with increased marking rates of 87.5% and 96.0% (G. Matschke et al., NWRC, unpublished Report No. 310 Parts II and III, 1996, 1997, respectively).

In this field study, we investigated the addition of DuPont oil blue A to CDFA's non-toxic base bait to determine whether Valley pocket gophers exhibit a seasonal marking efficacy.

## 2. Materials and methods

### 2.1. Study sites

The two study sites (alfalfa and orchards) were located within a 10 mile radius of Visalia, Tulare County, California. Fifteen treatment units (TUs) were placed in flood-irrigated alfalfa (*Medicago sativa*) fields, and 10 TUs were placed in flood-irrigated walnut (*Juglans californica*) orchards (Table 1). In the latter habitat, most of the surface vegetation had been removed to reduce competition for nutrients and to increase harvest yields. Topography for all TUs was consistent, with each site having  $\leq 5\%$  slope. The average elevation was 101 m above sea level. Climatological data were obtained from the National Weather Service station located at Visalia, California. Temperature and precipitation

Table 1  
Description of treatment unit (TU) borrows baited for gophers by season in alfalfa and orchards

Season & crop	Year	TU	Size (ha)	Active burrows	Baiting date	Amount of bait (kg)
Winter alfalfa	1999	26	0.91	60	1/12	0.61
		27	0.98	60	1/17	0.88
		28	0.65	60	1/18	0.57
		29	0.46	60	1/22	0.97
		30	1.48	60	1/30	0.84
Summer alfalfa	1997	1	2.68	52	7/14	0.51
		2	5.70	46	7/16	0.48
		3	4.10	34	7/22	0.29
	1998	15	4.84	60	7/9	0.36
		16	10.51	60	7/9	0.41
Fall alfalfa	1998	20	10.44	60	9/23	0.32
		21	4.77	60	9/25	0.30
		22	9.47	60	9/30	0.32
		23	2.30	60	10/7	0.39
		24	4.80	60	10/9	0.39
Winter orchard	1998	6	2.20	60	1/24	0.57
		7	1.57	60	1/28	0.52
		8	1.57	60	2/15	0.34
		9	3.48	60	2/25	0.45
	1999	25	0.88	60	1/10	0.41
Summer orchard	1997	4	1.30	62	7/23	1.04
		5	2.00	45	7/24	0.57
	1998	17	2.20	60	7/13	0.42
		18	4.84	60	7/17	0.36
		19	3.18	60	7/18	0.34

varied depending on the month and year (24 h maximum range 3.9–39.4°C and minimum range 1.7–27.8°C; total precipitation ranged from 0–11.7 cm per month).

### 2.2. DuPont oil blue a baits

Two separate batches of SRO groat bait were used in this study during 1997–1999 because initial estimates of usage were not sufficient for the entire study. The first batch, weighing 11.5 kg and the second weighing 4.8 kg, were formulated on July 3, 1997 and on November 11, 1998, respectively. Both batches were mixed using the same proportions: (1) 97.15% steamed-rolled oat groats, (2) 1.60% DuPont oil blue A, and (3) 1.25% Alcolec S (lecithin binder). After mixing, the bait was placed in plastic bags and stored at  $-18^{\circ}\text{C}$  or colder until used.

### 2.3. Bait application

Valley pocket gopher burrows were baited during three seasons of the year (winter, summer, and fall) in alfalfa and two seasons (summer and winter) in orchards. The size and shape of each TU was dependent upon the number and distribution of gopher mounds. Our goal was to locate 60 fresh

mounds within each TU and establish 5 TUs per season. Each fresh mound was marked with a single colored wire flag numbered consecutively. Individual TUs were separated by a buffer of at least 30.5 m. In alfalfa fields, the majority of gopher activity was concentrated on the dirt berms constructed for irrigation. Likewise, gopher activity in orchards was found mainly on the berms.

The subterranean tunnel systems of gophers were located by probing near the center of each fresh fan-shaped mound. Some burrow openings contained a characteristic round earthen plug. Once a tunnel was found, each site was hand baited with 4 g of bait. If a gopher tunnel ran in more than one direction, each direction was baited. All bait was placed in a pile as far back in the tunnel as the measuring spoon would allow (~10 cm). Because pocket gophers never leave their burrows open for long, one piece of newspaper was immediately placed over each opening and covered with dirt to minimize human disturbance. Four days (96 h) after bait placement, all flagged sites were re-examined. The newspaper was carefully removed and each tunnel was re-opened and examined for bait site disturbance by recording all bait present (AP), some bait present (SP), or no bait present (NP). All bait was weighed pretreatment and posttreatment. Over 1400 bait sites were constructed in alfalfa (531 winter, 387 summer, and 499 fall) and 993 bait sites in orchards (516 winter and 477 summer).

Immediately after examining bait sites, traps (either Macabee traps, cinch traps, or Black Hole traps) were set in each of the re-opened tunnels. If a tunnel was plugged so tightly that it could not be re-opened, a new opening was prepared as near to the old one as possible and a trap placed there. Traps were checked morning and evening on each TU and left in place for a total of 5 days. Carcasses of all captured gophers were placed in individual plastic bags, appropriately labeled, and recorded. Bagged carcasses were frozen until examined for the presence of DuPont oil blue A dye. Six gophers were captured about 1 km away from the study area and served as controls.

Body weight and sex were recorded before each gopher was examined externally for the presence of blue coloration on the nose, feet, tail, ears, skin, and fur. Cheek pouches were also examined for blue coloration and presence of dyed bait. Then, each gopher was examined for blue coloration in the subcutaneous fat around its back, shoulders, hips, neck, tail, and vital organs including the heart.

Five TUs were established in alfalfa during winter, summer, and fall, and they averaged 0.90, 5.57, and 6.36 ha, respectively. Winter TUs were baited in January 1999 with an average of 0.77 kg of bait. Two summers were needed to establish the summer TUs, and they were baited in July 1997 (3 TUs) and 1998 (2 TUs) with an average of 0.41 kg of bait. Fall 1998 TUs were baited in September and October with an average of 0.34 kg of bait.

In orchards, five TUs were established in winter and summer. Winter TUs averaged 1.94 ha and were baited in January and February 1998 (4 TUs) and January 1999 (1 TU)

Table 2  
Valley pocket gopher bait site disturbance after four days of baiting for each treatment unit (TU)

Season & crop	Year	TU	All bait present	Some bait present & no bait present	Bait disturbance (%)
Winter alfalfa	1999	26	17	79	82.3
		27	13	94	87.9
		28	20	93	82.3
		29	26	82	75.9
		30	18	89	83.2
Summer alfalfa	1997	1	15	48	76.2
		2	14	46	76.7
		3	19	41	68.3
	1998	15	19	82	81.2
		16	14	89	86.4
Fall alfalfa	1998	20	15	84	84.8
		21	22	74	77.1
		22	20	77	79.4
		23	12	91	88.3
		24	12	85	87.6
Winter orchard	1998	6	20	90	81.8
		7	46	65	58.6
		8	29	69	70.4
		9	20	78	79.6
	1999	25	17	82	82.8
Summer orchard	1997	4	60	50	45.4
		5	27	46	63.0
	1998	17	14	84	85.7
		18	20	74	78.7
		19	15	87	85.3

with an average of 0.46 kg of bait. The summer TUs averaged 2.70 ha and were baited in July 1997 (2 TUs) and July 1998 (3 TUs) with an average of 0.55 kg of bait (Table 1).

### 3. Results

#### 3.1. Bait site disturbance

Bait site disturbance (i.e. percent of bait sites with all or some bait missing versus all bait present after 4 days of baiting) was 82.3, 79.1, and 83.5 for winter, summer, and fall alfalfa, respectively. Bait site disturbance in orchards was less in both winter (74.4) and summer (71.5) (Table 2). The average bait site disturbance was 78.2%.

#### 3.2. Seasonal marking

Fat was the best indicator of bait consumption using SRO groats treated with 1.6% DuPont oil blue A, because all of the marked animals had blue-dyed fat whereas only some of these were externally marked (cheek pouches < skin/fur). However, the overall percentage of marked gophers was only 56.6% of those captured. Regardless of habitat type,

Table 3  
Seasonal variation in gopher captures and dye patterns from treatment units (TU)<sup>a</sup>

Season & crop	Year	Sex	Total captures	No./percent marked	Dyed (No./percent of marked animals)		
					Cheek pouch	Skin fur	Fat
Winter alfalfa	1999	F <sup>1</sup>	134	75/56.0	15/20.0	41/54.7	75/100
		M <sup>2</sup>	69	35/50.7	3/8.6	11/31.4	35/100
		F & M	203	110/54.2	18/16.4	52/47.3	110/100
Summer alfalfa	1997	F	25	11/44.0	1/9.1	5/45.5	11/100
		M	6	5/83.3	0/0	1/20.0	5/100
		F & M	31	16/51.6	1/6.3	6/37.5	16/100
	1998	F	30	11/36.7	0/0	7/63.6	11/100
		M	16	9/56.3	0/0	5/55.6	9/100
		F & M	46	20/43.5	0/0	12/60	20/100
	97 & 98	F	55	22/40.0	1/4.6	12/54.6	22/100
F & M		77	36/46.8	1/2.8	18/50.0	36/100	
Fall alfalfa	1998	F	114	76/66.7	12/15.8	34/44.7	76/100
		M	55	35/63.6	1/2.9	16/45.7	35/100
		F & M	169	111/65.7	13/11.7	50/45.0	111/100
Winter orchards	1998	F	107	57/53.3	0/0	17/29.8	57/100
		M	70	44/62.9	4/9.1	10/22.7	44/100
		F & M	177	101/57.1	4/4.0	27/26.7	101/100
Summer orchards		F	92	48/52.2	3/6.3	23/47.9	48/100
		M	26	15/57.7	1/6.7	5/33.3	15/100
		F & M	118	63/53.4	4/6.4	28/44.4	63/100

<sup>a</sup>F<sup>1</sup> = Females and M<sup>2</sup> = Males.

51.0% of gophers were marked during summer, 65.7% during fall, and 55.6% during winter. In alfalfa, 54.2%, 46.8%, and 65.7% were marked during the winter, summer, or fall, respectively (Table 3). Of the gophers not marked, 87%, 66%, and 74% were taken from sites where all the bait had been removed during the winter, summer, or fall, respectively. Results from baiting in orchards indicated 57.1% of the gophers were marked during the winter and 53.4% during summer (Table 3). Of the gophers not marked, 71.0% were taken from sites devoid of all bait during winter and 80% during summer. ANOVA results indicated no difference in seasonal marking efficacy in either alfalfa or orchards ( $F = 3.59$ ,  $P = 0.0598$  and  $F = 0.12$ ,  $P = 0.7384$ , respectively).

### 3.3. Marking of gophers by sex

In alfalfa, 48.3% of the females and 50.0% of the males were marked; in orchards, 52.8% of the females and 61.5% of the males were marked. Of those that were marked, all (100%) had dyed fat, followed by skin/fur (43.7% females:34.4% males) and cheek pouches (10.1% females:5.7% males). Typically, females had blue-dyed fat around their bladder; males tended to have blue-dyed fat around their testicles.

### 3.4. Non-target trapping hazard

Only 3 non-target mammals were captured and none were marked. Two were California moles (*Scapanus latimanus*) that were captured in kill traps in different orchard TUs. The third animal was a long-tailed weasel (*Mustela frenata*) which was captured in alfalfa and released unharmed.

## 4. Discussion

Several factors may have led to these marking results. First, with our baiting methodology over 66% of the bait sites were devoid of the bait after 4 days. If these burrows held multiple pocket gophers, some of the gophers captured may not have come in contact with the bait. Multiple gopher captures occurred at 105 (4.4%) bait sites, with 90 (42.4%) gophers marked and 123 (57.7%) not marked. Therefore, having either more bait at each site or more bait sites would probably not have significantly increased the number of marked gophers.

Second, DuPont oil blue A may not have had the same marker characteristics with the Valley pocket gophers as it did with the northern pocket gopher. It may be more species specific than we had anticipated. In a laboratory report to evaluate the short-term longevity of the marker in fat in the northern pocket gopher at 2, 4, 7, and 14 days after

ingestion, 100%, 97%, 80%, and 55% were marked (each  $n = 20$ ), respectively (G. Matschke et al., NWRC, unpublished Report No. PS-108, 1999). By comparison in the current study, the 56.6% overall marker success at days 5–9 from the day of baiting was unexpected low, even with a high of 65.7% in fall alfalfa. Therefore, the use of other dyes may produce better marker results. For example, rhodamine B has been used to mark black-tailed jackrabbits for at least 6 weeks (Evans and Griffith, 1973).

Third, the marker dye must be palatable and well accepted. Information concerning this criterion is limited. Bait aversion to baits formulated with 1.6% DuPont oil blue A was observed in laboratory tests with the northern pocket gopher (Matschke et al., 1999, NWRC unpublished Report No. PS-108). They reported consumption of the marker bait was significantly lower than undyed bait ( $P = 0.0017$ ). In field work conducted later, they stated bait aversion to DuPont oil blue A may be overcome by the baiting methods used in the present study (Matschke et al., 1997, Part III, NWRC unpublished Report No. 310). Therefore, we expected similar marking results with the Valley pocket gopher to theirs with the northern pocket gopher. However, we believe our lower than expected marker results may have demonstrated a possible bait aversion to consumption by gophers versus the movement of bait as measured by bait site disturbance of ~80%. Before any additional field studies are conducted with this species, this question should be investigated. Also, we expected the external marking data from cheek pouches would support our results from the bait site disturbance data. It did not, probably because the use of external markers was more transitory than anticipated.

Fourth, did they prefer natural forage to the SRO marker baits? Some data were obtained about this factor by investigating the possible seasonal differences in the marking of gophers. We assumed that it was more plausible for gophers in summer or fall to prefer natural forage to baits and less likely for gophers in winter when alternative forage is dormant. Because there were no seasonal marking differences, we believe some factor other than a preference for natural forage is a more likely an explanation of these results.

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