

# Utility of visual counts for determining efficacy of management tools for California ground squirrels

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**Abstract:** Visual counts are frequently used to assess efficacy of management tools for ground squirrels (*Marmotini*), but the effectiveness of this approach has not been assessed for many ground squirrel species including California ground squirrels (*Otospermophilus* spp.). As such, we used visual counts of California ground squirrels to determine the efficacy of diphacinone-treated oat groat applications in rangelands in central California, USA, and compared those results to efficacy values derived from the use of radio-collared ground squirrels in the same plots. We also used location data of radio-collared ground squirrels to explore the size of buffer zone needed around census plots to provide an accurate assessment of efficacy when using visual counts. We did not observe a difference in efficacy associated with the 2 monitoring strategies, indicating that visual counts are an effective monitoring tool for ground squirrels. We observed low efficacy in 2 treatment plots, likely due to low usage of those plots by ground squirrels. Increasing the size of buffer zones would increase the usage of treatment areas by the target population and would help to minimize reinvasion by adjacent ground squirrel populations, which could bias efficacy values low. We suggest a minimum of a 61-m buffer surrounding census plots. Increasing to 66 m or more would further benefit efficacy assessments, but increased size of the buffer zone must be balanced with greater costs and regulatory constraints.

**Key words:** California, California ground squirrel, diphacinone, efficacy, *Otospermophilus* spp., rangeland, telemetry, visual counts

CALIFORNIA ground squirrels (*Otospermophilus* spp.) cause extensive damage in many agricultural commodities including rangelands (Marsh 1998, Fleming et al. 2013, Baldwin et al. 2014). Many tools are used to manage ground squirrels including habitat modification, rodenticides, burrow fumigants, trapping, and shooting (Salmon and Schmidt 1984, Marsh 1994, Baldwin et al. 2014). Development of new tools requires methods to assess the effectiveness of those tools. Measuring changes in animal numbers (hereafter, efficacy) is one of the primary methods for assessing the effectiveness of management tools. This can be done in a variety of ways including assessing population size (i.e., mark-recapture approaches) and the use of indices that reflect population size (Stroud 1981, Engeman 2005). Indices are often the pre-

ferred tool for efficacy assessments given that they can be quicker and easier to employ, and they have less onerous assumptions to be met. That said, indices must be sensitive to changes in population size to be effective (see Engeman 2005 for detailed discussion on indices). A common indexing approach for ground squirrels is visual counts. Fagerstone (1984) provided an early assessment on the utility of visual counts for tracking population size of Richardson's ground squirrels (*Urocyon richardsonii*); she found this approach to be effective. Visual counts have subsequently been used extensively to assess the efficacy of various management tools for a variety of ground squirrel species (e.g., Whisson et al. 1999, Salmon et al. 2007, Nelson et al. 2012, Baldwin et al. 2017), although it has not been officially verified for

other species. Such an assessment would provide guidance as to the validity of this approach for other ground squirrel species.

One potential problem with visual counts is that some of the target population may move in or out of the study area between the pre- and post-treatment counts. This is not a problem with management tools that reduce populations within 1–2 days (e.g., burrow fumigants and acute toxicants). However, first-generation anticoagulant rodenticides such as diphacinone require an extended timeframe to reduce population size (Marsh 1994). This timeframe can vary depending on the application strategy used, as bait stations that deliver anticoagulant baits sometimes take longer to reduce populations than do broadcast applications or spot treatments given a neophobic response of some individuals to bait stations (Whisson and Salmon 2009). Regardless, it takes 2 weeks and sometimes longer to reduce a ground squirrel population with anticoagulant rodenticides; during that period, adjacent ground squirrels may reinvade treatment areas (Alsager 1972, Fagerstone et al. 1981), thereby confounding assessments of efficacy derived from visual counts. Increasing the size of buffer zones around visual-count plots can minimize the risk of reinvasion, but the necessary width of this buffer zone is unknown (Stroud 1982). Creation of buffer zones sufficiently sized to minimize ground squirrel reinvasion would increase the utility of visual counts as a monitoring approach.

Radio-telemetry is also used to track efficacy of various management approaches. This approach monitors survival of radio-collared individuals and determines efficacy based on the ratio of mortalities versus uncensored individuals (Fagerstone et al. 1981). The use of radio-telemetry is considered a more sensitive approach for assessing efficacy given direct knowledge of mortality for a subset of the population (Fagerstone et al. 1981). However, this approach is more costly and invasive given the need to capture and deploy transmitters on individuals, so it is not used as widely as other less-invasive approaches. That said, radio-telemetry provides movement data that are useful in establishing protocols for management practices as well as better defining the size and spacing of treatment areas for efficacy assessments.

Therefore, comparing efficacy values estimated from visual counts to those derived from radio-telemetry should provide a good test of the applicability of visual counts for monitoring changes in population size and should provide information on plot size needed to determine the efficacy of a management tool. Specifically, our goals for this project were to: (1) compare visual counts and radio-telemetry as methods of assessing the efficacy of management tools for California ground squirrels, and (2) determine the appropriate size of buffer zones for assessing the efficacy of management tools for California ground squirrels. This information will greatly assist researchers, regulatory agencies, and land managers on how to monitor this common agricultural pest.

## Study area

We conducted this study in seasonally grazed rangelands in west-central California, USA, in Stanislaus and San Joaquin counties. Cattle (*Bos taurus*) grazing occurred from October to March, which coincided with the timeframe when most precipitation fell in this region ( $\bar{x}$  = 25.4–30.5 cm annually). Annual temperatures for the area ranged from 4–35°C. Soils were similar throughout and consisted of Zacharias gravelly clay loam and Carbona clay loam. A small portion of the study area was comprised of Stomar clay loam that exhibited up to an 8% slope. Plant composition was primarily non-native annual grasses and forbs, including *Hordeum murinum*, *Bromus madritensis*, *Bromus diandrus*, *Bromus hordeaceus*, *Avena fatua*, *Medicago polymorpha*, and *Erodium* spp. Forage production on our study sites ranged from 479 kg/ha to 2,697 kg/ha, with a mean of 1,636 kg/ha (Becchetti et al. 2016; T. A. Becchetti, University of California, unpublished data).

## Methods

In summer 2018, we visually surveyed the study area for ground squirrels and associated burrow systems and established 4 64 × 64-m census plots (0.4 ha) in areas that had abundant ground squirrel activity. Similar to past studies (e.g., Baldwin et al. 2017), we surrounded interior census plots by a 61-m buffer on all sides; combined census plots and buffer zones (3.4 ha) served as our treatment plots for rodenticide bait application, although visual counts

of ground squirrels only occurred within census plots. This process was repeated within new plots in autumn 2018 and new plots in summer and autumn 2019, respectively. The edge of buffer zones for plots were located a minimum of 87 m from one another within a given season ( $\bar{x}$  minimum distance = 418 m) to minimize the likelihood that any ground squirrels would move from 1 plot to another. These distances appeared to be sufficient to maintain independence, as only once did we document a radio-collared ground squirrel in a treatment plot other than where it was captured. Each season, we randomly assigned the 4 plots to 1 of 3 bait application strategies (bait station, broadcast, or spot treatment) or as a control as part of a separate study addressing the impact of these application strategies on residual levels of anticoagulants in ground squirrel carcasses (Baldwin et al. 2021). We used these same plots and ground squirrels for the current study.

### Capture, collaring, and radio-tracking

We trapped ground squirrels using 20–25 Tomahawk cage traps (combination of  $13 \times 13 \times 46$  cm and  $15 \times 15 \times 61$  cm traps; Tomahawk Live Trap, Hazelhurst, Wisconsin, USA) distributed throughout each censusing plot. We focused collaring efforts on the census plots to reduce the likelihood that a ground squirrel would move off the treatment area given that we anticipated that the diameter of ground squirrel home ranges would be less than the width of the buffer zone ( $\bar{x}$  diameter of home range = 20–34 m; Boellstorff and Owings 1995). We initially tied traps open and prebaited traps with oat groats for 1–2 days, and then activated the traps for capture. We operated traps from early morning until 1100 hours to reduce potential problems with heat exposure. Traps were checked every hour. Upon capture, trapped ground squirrels were moved to a shaded location for processing, and we dusted all captured ground squirrels with a 0.25% permethrin dust (Hi-Yield Garden, Pet & Livestock Dust, Voluntary Purchasing Groups, Inc., Bonham, Texas, USA) to remove ectoparasites. We sexed and weighed captured ground squirrels to ensure that the very high frequency transmitter did not constitute >5% of their body weight (Eagle et al. 1984). We used a cloth handling cone as described by Koprowski (2002) to allow

us to collar captured ground squirrels around the neck via a cable tie (Model M1535, weight = 14 g; Advanced Telemetry Systems, Isanti, Minnesota, USA). We retrofitted all transmitters with a mortality switch that would trigger after 12 hours of inactivity. Captured ground squirrels were then taken back to the site of capture and released. We radio-collared 7 ground squirrels in each of the plots during both summer and autumn 2018. In summer and autumn 2019, we collared 8 individuals in each treatment plot and 4 in the control plots to increase treatment sample sizes for a separate study (see Baldwin et al. 2021). This kept the total number of collared ground squirrels consistent across all sampling periods ( $n = 28$ ). We did not initiate bait application until several days after the end of collaring activities ( $\bar{x} = 8.6$  days,  $SE = 0.2$ ) to allow the ground squirrels time to adjust to wearing the collar and to allow time to complete visual counts.

Upon release, we tracked squirrels every 1–2 days pre-treatment and daily post-treatment. To identify locations, we walked to where the ground squirrel was located as determined from daily telemetry assessments, and we documented if the ground squirrel was observed. If a mortality was observed above ground, we noted this and removed the ground squirrel carcass. Occasionally, we could not locate a ground squirrel during normal telemetry scans. If a ground squirrel was not found, we searched a 500-m radius around the treatment plot. If we still could not find it, we recorded it as missing for that day. We recorded all locations with a hand-held Global Positioning System (GPS) unit, and we plotted all locations in ArcMap 10.7 (Environmental Systems Research Institute, Redlands, California) to allow for a comparison of each ground squirrel's location data to their respective treatment plot. We also used a Student's *t*-test ( $\alpha = 0.05$ ; Zar 1999) to assess potential differences in the proportion of ground squirrel locations observed within treatment plots for ground squirrels that survived versus those that succumbed to diphacinone exposure, as access to bait could influence the efficacy of the application strategy.

### Visual counts

We conducted visual counts of ground squirrels upon completion of collaring activities ( $\bar{x} =$

4.4 days post-collaring, SE = 0.2). Our protocol followed the general approach originally outlined by Fagerstone (1984) and subsequently modified for use in numerous ground squirrel studies (e.g., Salmon et al. 2000, 2007; Baldwin et al. 2017). This approach was comprised of 5 counts at 5-minute intervals, with all counts occurring from a fixed location within a vehicle approximately 5–15 m outside the census area. Following Salmon et al. (2000), we waited 15 minutes after our arrival onsite to initiate counts to allow ground squirrels to resume normal activities. We conducted counts once in the morning (0710–1107 hours) and once in the evening (1600–1848 hours) to coincide with periods of high ground squirrel activity (Fitch 1948). Counts occurred across 3 consecutive days for a total of 30 counts per plot. We used the maximum number of ground squirrels counted in each plot in subsequent analyses. These counts occurred before bait application and at the end of the bait application period (between 14 and 19 days post-application depending on the year and season) to allow for comparison of numbers before and after treatment. We determined efficacy of the 3 different bait application strategies for each season using:

$$\text{Efficacy (\%)} = \frac{[(\text{pre-treatment} - \text{post-treatment}) / \text{pre-treatment}] \times 100}{1}$$

where pre-treatment and post-treatment equal the maximum number of ground squirrels observed before and after treatment. Natural changes in population size can influence visual counts as well. Therefore, we applied a correction factor for all bait application approaches in a given season if we observed a >30% change in maximum ground squirrel counts from the pre-treatment to the post-treatment survey period in the control plot. The correction factor for this study was calculated following O'Connell and Clark (1992):

$$\text{Post-treat expected GS bait} = (\text{pre-treat GS bait} \times \text{post-treat GS control}) / \text{pre-treat GS control}$$

$$\text{Percent adjusted efficacy} = [1 - (\text{post-treat GS bait} / \text{post-treat expected GS bait})] \times 100$$

where post-treat = post-treatment survey, pre-treat = pre-treatment survey, GS = maximum

number of ground squirrels, and bait = bait application strategy. Following U.S. Environmental Protection Agency (EPA) standards, we considered population reductions of  $\geq 70\%$  efficacious (Schneider 1982).

### Bait application

We initiated bait application the day following the completion of pre-treatment ground squirrel counts for each trial period. For spot treatments, we identified all active burrow entrances within the treatment area. Following label specifications, we applied 37 g of Rodent Bait Diphacinone Treated Grain (0.005%; California Department of Food and Agriculture, Sacramento, California) in a 3.7–4.6-m<sup>2</sup> area around the burrow entrance. We identified active burrow entrances by the presence of new footprints, fresh fecal pellets, scrapings, or clear openings (i.e., were devoid of leaf litter, spider webs, and overgrown vegetation). We noted the initial date of bait application as day 0. Following the label specification, we again applied bait in the same manner on day 4 to ensure adequate exposure to diphacinone (Whisson and Salmon 2002).

For bait stations, we used inverted T-shaped bait stations that were constructed of 10-cm polyvinyl chloride pipe. These stations were 1.2 m in length and 0.9 m in height. We cut endcaps in half and glued them onto both horizontal ends of the bait station to keep ground squirrels from kicking bait out onto the ground. We placed an endcap on the vertical arm of the station to eliminate access to bait from the top. We attached all bait stations to metal T-posts that were staked into the ground. We spaced all bait stations in an 8 × 8 grid structure with all stations 23 m apart (Baroch 1996); the bait stations covered the entire treatment plot. We applied 0.9 kg of Rodent Bait Diphacinone Treated Grain (0.005%) to each bait station on day 0. We checked bait stations at least every 3 days to ensure that they maintained a constant bait supply. If we determined that additional bait was needed, we documented the amount that was added. We continued to add bait to the bait stations until bait was no longer removed by ground squirrels.

For broadcast applications, we used a seed spreader (Solo 421-S, Newport News, Virginia, USA) that was calibrated to discharge bait at a rate of 11.4 kg/ha. To allow for efficient appli-

**Table 1.** The proportion of radio-collared California ground squirrels (*Otospermophilus* spp.) that died (Mortality) following application of diphacinone-treated oats (0.005% unless otherwise noted) following 3 application strategies, as well as a concomitant control plot, in rangelands in central California, USA, during summer and autumn 2018–2019. Efficacy (Eff) was defined as the ratio between the number of mortalities divided by the number of uncensored individuals. We censored individuals for a variety of reasons including a dropped collar, transmitter failure, and unknown fate or causes of mortality. Combined (Comb) values are provided across treatment types for comparative purposes.

	Bait station			Spot treatment			Broadcast			Control		
	Censored	Mortality	Eff (%)	Censored	Mortality	Eff (%)	Censored	Mortality	Eff (%)	Censored	Mortality	Eff (%)
Trial 1	2	5/5	100	2	5/5	100	3	0/4	0	0	0/7	0
Trial 2	1	6/6	100	5	2/2	100	3	3/4	75 <sup>a</sup>	0	0/7	0
Trial 3	2	6/6	100	2	6/6	100	1	0/7	0 <sup>a</sup>	0	0/4	0
Trial 4	4	3/4	75	2	6/6	100	4	4/4	100 <sup>a</sup>	0	0/4	0
Comb	9	20/21	95	11	19/19	100	11	7/19	37	0	0/22	0

<sup>a</sup>These broadcast treatments were applied using 0.01% diphacinone-treated oats.

**Table 2.** The maximum number of California ground squirrels (*Otospermophilus* spp.) observed through visual counts before (Pre) and after (Post) application of diphacinone-treated grain (0.005% unless otherwise noted), as well as the associated unadjusted efficacy (Eff) for 4 trial periods across 3 different bait application strategies and control plots in rangelands in central California, USA, during summer and autumn 2018–2019. Adjusted efficacy (A eff) is provided for trial 1 given a substantial reduction in ground squirrels in the control plot during that period.

	Trial 1			Trial 2			Trial 3			Trial 4			
	Pre	Post	Eff (%)	Pre	Post	Eff (%)	Pre	Post	Eff (%)	Pre	Post	Eff (%)	
Bait station	18	3	83	60	15	0	100	11	2	82	12	0	100
Spot treatment	15	2	87	68	11	2	82	11	1	91	9	3	67
Broadcast	17	6	65	14	7	2	71 <sup>a</sup>	11	11	0 <sup>a</sup>	11	2	82 <sup>a</sup>
Control	17	7	59	17	17	15	12	10	11	-10	7	6	14

<sup>a</sup>These broadcast treatments were applied using 0.01% diphacinone-treated oats.

cation of bait, we flagged transects that intersected active burrow systems. We applied bait along these transects on day 0 and day 4 to ensure required access to bait (Whisson and Salmon 2002). We initially used the Rodent Bait Diphacinone Treated Grain (0.005%) to allow us to most directly compare results across the 3 different application strategies. However, we observed no mortalities following the initial trial period for broadcast applications in summer 2018. At the time of this study, the label-specified concentration of diphacinone for broadcast applications was 0.01%. Therefore, we defaulted back to this label-specified rate (Rodent Bait Diphacinone Treated Grain [0.01%]; California Department of Food and Agriculture, Sacramento, California) for the remaining 3 trial periods.

### Fate of ground squirrels

We anticipated a variety of outcomes for radio-collared ground squirrels including lost signals, dropped collars, mortality from diphacinone exposure, unknown causes of mortality, and survivors. As such, we defined the specific fate of each ground squirrel, but for the purposes of this study, we placed all ground squirrels into 3 categories: (1) mortality from diphacinone exposure, (2) survival, and (3) censored individuals (all ground squirrels that did not fit into the first 2 categories). If we observed a dead ground squirrel above ground, we dusted it with a 0.25% permethrin dust, recorded the location with a hand-held GPS unit, and collected the carcass. For below-ground mortalities, we dug the ground squirrel up to document mortality, dusted it with a 0.25% permethrin dust, recorded the location, and collected the ground squirrel.

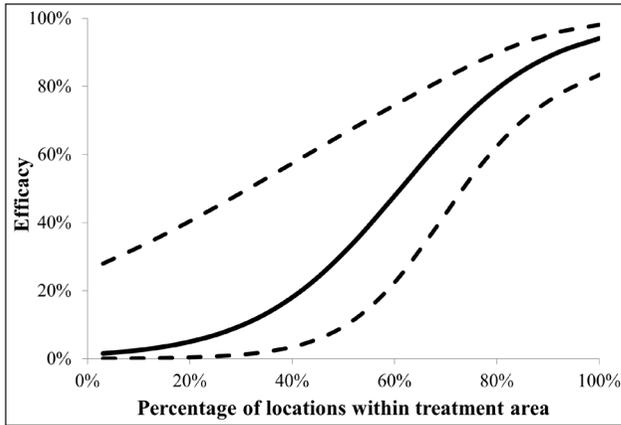
We determined efficacy for each plot by dividing the number of radio-collared ground squirrels that died from diphacinone exposure by the number of uncensored ground squirrels for that particular plot. We compared efficacy values derived from visual counts and radio-collared individuals for each bait application method and season using a general linear mixed-effect model with bait application strategy and season as random “subject” terms nested within these monitoring strategies (Zar 1999). We also used logistic regression to model the relationship between efficacy

(binary response included survival or mortality for each individual) and the proportion of locations found within treatment plots for each individual (Hosmer and Lemeshow 2000). The model was validated using the area under curve (AUC) approach, with AUC scores  $<0.7$  = uninformative,  $0.7-0.9$  = good, and  $>0.9$  = very good (Swets 1988). All aspects of this project were approved by the University of California, Davis’s Institutional Animal Care and Use Committee (protocol no. 20025).

### Results

We censored a large number of radio-collared ground squirrels for a variety of reasons including dropped collar = 13, lost signal = 9, unknown fate = 6, and unknown cause of mortality = 3. This left 81 ground squirrels for inclusion in efficacy assessments (Table 1). We observed 100% efficacy from spot treatments across all trial periods (Table 1). For bait stations, we observed 75–100% efficacy collectively. The 1 survivor was a ground squirrel that was located within the treatment area only once out of 33 locations. We did not observe a single mortality during summer trials in broadcast application plots. In contrast, we observed 75–100% efficacy in broadcast plots during autumn, with the sole survivor located only 3 times within the treatment area out of 25 total locations during the trial period. We observed no mortality events in control plots during any trial period (Table 1). The low efficacy we observed for broadcast plots during summer may have been driven by low usage of treatment areas, as the proportion of locations documented in broadcast plots was lower for ground squirrel survivors ( $\bar{x} = 54\%$ ,  $SE = 7$ ) than for mortalities ( $\bar{x} = 87\%$ ,  $SE = 10$ ;  $t_{16} = -2.4$ ,  $P = 0.029$ ).

Based on visual counts, we observed a reduction in numbers of ground squirrels within the control plot during the first trial period, so we adjusted efficacy for treatment types within that trial period accordingly (Table 2). We did not observe substantive changes in ground squirrel numbers in control plots during any other trial period, so we did not adjust efficacy values for those periods. All efficacy values exceeded 70% for bait station and spot treatment plots during trial periods 2–4, except for spot treatments during trial period 4, where efficacy was close to the desired threshold (67%; Table 2). We observed adjusted efficacy



**Figure 1.** Relationship between the percentage of California ground squirrel (*Otospermophilus* spp.) locations found within treatment areas and efficacy (derived from radio-collared individuals) associated with consumption of diphacinone-treated grain. Dashed lines represent 95% confidence intervals.

values under 70% during trial period 1 for both bait station and spot treatment plots. However, efficacy was well above this threshold if using the unadjusted rates (Table 2). We observed low efficacy for broadcast plots during trial periods 1 and 3 and high efficacy during trial periods 2 and 4 (Table 2). We did not observe a difference in efficacy values between visual counts ( $\bar{x} = 68\%$ ,  $SE = 9$ ) and telemetry estimates ( $\bar{x} = 79\%$ ,  $SE = 11$ ;  $F_{1,2} = 1.5$ ,  $P = 0.345$ ), although in general, efficacy from radio-collared individuals was higher.

We observed a strong relationship between the number of ground squirrel locations within bait application areas and efficacy ( $\chi^2 = 12.1$ ,  $P < 0.001$ ;  $\beta = 0.071$ ,  $SE = 0.020$ ). The accuracy of the model was very good ( $AUC = 0.92$ ) and indicated that efficacy was higher when a greater proportion of locations were found within treatment areas. Expected efficacy met the 70% U.S. EPA threshold when the percentage of ground squirrel locations within the treatment area surpassed 73% (Figure 1). Trial periods 1 ( $\bar{x} = 63\%$ ) and 3 ( $\bar{x} = 53\%$ ) for broadcast plots were substantially below this proportion, suggesting this played a role in their low observed efficacy.

## Discussion

We did not observe a difference in efficacy between visual counts and radio-telemetry, corroborating the findings of Fagerstone (1984) with Richardson's ground squirrels. Visual

counts are widely used to assess efficacy of various management strategies for ground squirrels (e.g., Whisson et al. 1999, Salmon et al. 2007, Nelson et al. 2012, Baldwin et al. 2017), as the approach is far less costly, time consuming, and invasive than radio-telemetry. This is particularly important for the registration of new pesticides (e.g., rodenticides, burrow fumigants, repellents, and chemosterilants), as multiple indexing tools are usually required by the U.S. EPA for their registration (Schneider 1982). Even if radio-telemetry is used, an additional strategy such as visual counts will be needed to register these products. Our findings indicate that visual counts can be effectively used to monitor California ground squirrel populations.

Although visual counts effectively tracked changes in ground squirrel numbers, radio-telemetry generally indicated greater efficacy values. For example, we observed equivalent or higher efficacy in 10 out of 12 plots with radio-telemetry data. This difference may be driven by reinvasion of adjacent ground squirrel populations into treated areas, as ground squirrels will often quickly reinvade depopulated sites (Stroud 1982, Salmon et al. 1987). We attempted to minimize this effect by conducting counts soon after bait application. We could not reduce this time further given the extended length of time required for first-generation anticoagulants such as diphacinone to lead to mortality (often 4–13 days or more; Clark 1978, Hindmarch and Elliott 2018). Such reinvasion would be most impactful on broadcast and spot treatments given that bait stations continued to supply bait throughout the duration of the project, and in fact, we observed greater efficacy associated with bait stations (bait station  $\bar{x} = 86\%$ , spot treatment  $\bar{x} = 77\%$ , broadcast  $\bar{x} = 42\%$ ). In short, visual counts do appear to effectively track efficacy of management tools, but they may provide somewhat conservative estimates when compared to results from radio-telemetry. Sample sizes for our telemetry results were limited given the number of ground squirrels that were censored. Therefore, a more robust assessment comparing these 2 monitoring strategies would be valuable in further elucidating

potential differences between them.

Although limited reinvasion by adjacent ground squirrel populations may marginally lower efficacy estimates, the biggest concern with ground squirrel counts may stem from the potential for ground squirrels to move out of application plots during the trial period. Such movements were most notable in broadcast plots, as the number of locations within treatment areas was much lower for broadcast plots (68%) than for spot treatments (93%) or bait stations (89%). This reduced use of diphacinone-treated areas seemed to influence efficacy of the rodenticide bait, as ground squirrels that succumbed to diphacinone were located substantially more often in treatment areas than survivors (87% vs. 54%, respectively). Interestingly, many ground squirrels also vacated the control plot during the summer 2018 trial period as well, with an average of only 50% of locations found within the treatment area during this timeframe. Given this substantial reduction of ground squirrels within the control plot, we adjusted our estimates of efficacy in all 3 treatment plots accordingly. However, these adjusted values may be overly conservative for the bait station and spot treatment plots given that radio-telemetry data indicated that ground squirrels were frequently found within the treatment areas (83% and 94%, respectively), reinforcing the idea that combining radio-telemetry data with another indexing tool will likely provide an improved assessment of efficacy. When such location data are unavailable, researchers and practitioners will likely need to rely on the use of adjusted efficacy values to counteract the potential for natural reductions in animal numbers at treatment sites.

One method to minimize the impact that ground squirrel movement patterns have on efficacy assessments would be to increase the size of buffer zones. However, plot size is often constrained by a number of factors. For example, treatment plots must be separated by some minimum distance to maintain independence. If multiple management tools are tested, then fields of sufficient size and an abundance of ground squirrels will be needed to incorporate all replicates. This becomes increasingly challenging as the size of treatment plots increase. Not only does it become more challenging to find appropriate field sites as plot sizes increase, but it also becomes more costly and logistically

challenging to treat large areas. It also bears noting that for pesticide testing, the U.S. EPA generally limits the area where an unregistered pesticide can be tested to 4.05 ha (U.S. EPA 2020). It is important to fit plots within this area constraint. For our study, we determined that we met the U.S. EPA threshold of 70% efficacy if 73% of ground squirrel locations occurred within the treatment area. We surpassed 73% for all spot treatment and bait station plots but were substantially below it for broadcast plots during summer 2018 and 2019. Increasing the size of buffers from 61–66 m would have allowed us to surpass this 73% threshold for the broadcast plot in summer 2018 and would have only increased the treatment area from 3.4–3.8 ha. However, in summer 2019, we would have had to increase the buffer zone to 96 m to surpass the 73% threshold, which would have come close to doubling the treatment area (3.4–6.5 ha). The treatment area for the broadcast plot in summer 2019 was unique in that it was located close to a farm with large alfalfa (*Medicago sativa*) haystacks (distance of 75 m from the closest edge of the buffer zone). Unexpectedly, the ground squirrels were frequently located in these haystacks, substantially reducing the use of the treatment area. Other complex habitat features may be equally attractive to ground squirrels. The presence of such features should be considered when establishing study plots, and investigation into the impact that these habitat features have on ground squirrel movement patterns is worthy of future investigation. Nonetheless, if we exclude this outlier plot, then an addition of 5 m to the edge of each buffer zone should increase the utility of our study design while minimizing additional costs and logistical concerns. At present, we recommend a minimum of a 61-m buffer zone for similar ground squirrel efficacy studies, and marginally increasing the treatment area may yield more robust results.

## Acknowledgments

We thank local ranchers and staff for allowing us to work on their ranch. We also appreciate D. Bucklin, University of California Division of Agriculture and Natural Resources, for support with GIS. R. Tebo with the Minnesota Department of Natural Resources provided assistance on collaring ground squirrels. This work was supported by the Vertebrate Pest Control

Research Advisory Committee of the California Department of Food and Agriculture (grant number 17-0218-000-SA) and the University of California's Division of Agriculture and Natural Resources. Comments provided by Michael Guttery, HWI associate editor, and 2 anonymous reviewers greatly improved an earlier version of our paper.

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