

FINAL REPORT

for

Vertebrate Pest Control Research Advisory Committee

STUDY TITLE:

Assessing efficacy of zinc phosphide-coated cabbage for Belding's ground squirrel control.

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EXECUTIVE SUMMARY

Ground squirrels cause extensive damage to agricultural systems throughout the world. Effective management relies on an integrated pest management (IPM) approach. Rodenticides are often included in an IPM program, but they must be both efficacious and have minimal impact on nontarget species. A zinc phosphide-coated green bait may meet these requirements, but has yet to be thoroughly tested against ground squirrel species. We established a study in northeastern California to test zinc phosphide-coated cabbage as a management tool for Belding's ground squirrels (*Urocitellus beldingi*). We specifically addressed factors that would influence efficacy of a baiting program, as well as potential exposure risk to nontarget species. Specific details for our sampling methodology and findings include:

1. We tested the efficacy of zinc phosphide-coated cabbage across 12 sites in Butte Valley, 4 sites in the Klamath Basin, and 6 sites in south-central Modoc County (around the Alturas area) during late winter-early spring 2016 and 2017. Efficacy was determined through ground squirrel counts.
2. We tested for differences in efficacy based on location (western vs. eastern portions of the study area), the presence of prebaiting, and the density of Belding's ground squirrels at the start of the baiting process.
3. We determined the impact that time of bait application, time since bait was applied, and the location of bait application (burrow vs. interstitial spaces) had on untreated cabbage consumption by nontarget species through the use of remote-triggered cameras. We also assessed consumption of treated cabbage through the use of these same cameras.
4. We found that prebaiting was an important application strategy, resulting in an 18% increase in efficacy. Efficacy increased as Belding's ground squirrel density increased, suggesting that the use of zinc phosphide-coated cabbage would be most appropriate in areas with moderate to high ground squirrel numbers. Efficacy was also greater in western portions of the study area, although the cause of this variability is unknown.
5. Belding's ground squirrels fed most heavily on cabbage during mid-morning and late afternoon; bait applications shortly before these time periods would likely increase bait consumption while minimizing residual bait for nocturnal nontarget species.
6. We observed a significant impact of both the duration following cabbage application and the day since application on bait consumption; consumption was greatest shortly after application, and again at the onset of the following morning.
7. Bait uptake was greatest around burrow entrances, although bait consumption still occurred in interstitial spaces between burrows suggesting that broadcast applications could be successful.
8. Only California kangaroo rats were documented feeding on untreated cabbage during monitoring trials. The duration following cabbage application influenced bait consumption by California kangaroo rats, with about a 4-fold reduction in consumption 18 hours post-application. However, time of night did not influence cabbage consumption.
9. As with Belding's ground squirrels, cabbage consumption by California kangaroo rats was greater around burrow sites than at non-burrow locations.

10. We did not observe a single incidence of consumption of treated bait by any nontarget species (including California kangaroo rats), although we did observe 3 common ravens (*Corvus corax*) and 1 black-billed magpie (*Pica hudsonia*) poking at bait with their beaks. Any potential concerns of bird consumption of treated bait would be alleviated with a bird hazing program before and following bait application.

Collectively, these results suggest that zinc phosphide-coated cabbage can be an efficacious tool for managing Belding's ground squirrel species in some settings, but there will be limitations on where and how it can be used effectively. It also appears to pose low risk to nontarget species present in our study area, although this could vary depending on the species present in a given location. Although effective in some settings, the use of a zinc phosphide-coated green bait should only be one part of an IPM strategy for managing Belding's ground squirrels.

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INTRODUCTION

Many ground squirrel species cause extensive damage to agricultural systems throughout the world. For example, African striped ground squirrel (*Xerus erythropus*) foraging results in a 9.7% loss in planted seeds and seedlings in maize (*Zea mays*) crops in Kenya (Key, 1990), Richardson's ground squirrels (*Urocitellus richardsonii*) have been estimated to cause a 24% reduction in alfalfa production (Johnson-Nistler et al., 2005), while California ground squirrel (*Otospermophilus* spp.) damage has been estimated at a revenue loss of 8.7% annually in nut orchards (Baldwin et al., 2014b). A variety of tools are available to help minimize agricultural losses to ground squirrels. Nonlethal tools, such as habitat modification, show some potential in mitigating crop losses in some settings (Gilson and Salmon, 1990; McGrann et al., 2014). However, in many situations, a reduction in ground squirrel numbers via lethal removal is needed given the ineffectiveness of many nonlethal options for these species (Marsh, 1994). Common examples of lethal tools include trapping, shooting, burrow fumigation, and rodenticide baiting. All can be effective in some settings, but practicality of use and impact on nontarget species are always a concern. Ground squirrel management programs that result in a substantial reduction in crop damage while posing minimal risk to nontarget wildlife are the ultimate goal, but can be challenging to develop.

Belding's ground squirrels (*Urocitellus beldingi*) provide an interesting model for developing such a strategy given the substantial damage they can cause to agricultural production (losses in annual alfalfa [*Medicago sativa*] production ranging from 17–66%; Kalinowski and deCalesta, 1981; Sauer, 1976, 1984; Whisson et al., 1999), as well as the limited effective options available to manage this species (Baldwin and Quinn, 2012; Whisson et al., 2000). Historically, Belding's ground squirrel damage was effectively reduced through population reductions associated with the application of sodium fluoroacetate-coated green baits such as cabbage and dandelion greens. However, the use of sodium fluoroacetate was banned in 1990 given concerns associated with nontarget poisoning, eliminating the primary management tool for Belding's ground squirrels. Alternative rodenticides have been tested, but none have proven consistently effective (Whisson et al., 2000). Burrow fumigants, such as aluminum phosphide, gas cartridges, and pressurized exhaust, are all effective (Baldwin and Quinn, 2012; Orloff, 2012), but are often too costly or time consuming to use in fields with high ground squirrel densities (Whisson et al., 2000; Wright, 1982). Likewise, trapping is generally deemed too time consuming and costly over large areas for moderate to high density ground squirrel populations. As such, recreational shooting has been the primary method for reducing Belding's ground squirrel populations across agricultural fields (Whisson et al., 2000). However, shooting has not led to satisfactory control of Belding's ground squirrels, and it poses a substantial risk for lead poisoning given availability of thousands of carcasses across the landscape which are readily scavenged by raptors and corvids that frequent the area (Herring et al., 2016; Knopper et al., 2006). An alternative approach that provides greater efficacy and lower nontarget risk was highly desirable.

One potential option recently introduced is a zinc phosphide-coated cabbage bait, which was approved for use in Oregon and California in 2014 and 2015, respectively. Zinc phosphide has several desirable attributes as a rodenticide including high efficacy when consumed, short time from consumption to death, very low secondary toxicity risk to nontarget species, and lack of environmental persistence (Eason et al., 2013; Marsh, 1987). Furthermore, cabbage as a carrier desiccates quickly, and zinc phosphide begins to degrade rapidly once exposed to the

environment, which also further reduces secondary exposure risk (Baldwin et al., 2018). That said, rodents sometimes avoid zinc phosphide baits given an unattractive odor and taste, and through a learned avoidance through sublethal exposure (Horak et al., 2018; Jacob et al., 2010; Marsh, 1987). Green baits such as cabbage can often overcome these concerns by providing a preferred food source for target species (Alsager, 1972; Baldwin et al., 2016; Sullins and Verts, 1978; White, 1972), although past experience has not always yielded positive results against ground squirrel species (e.g. O'Brien, 1978, 2002). Substantial variability in efficacy has often been the expectation when using zinc phosphide baits (Marsh, 1987; Salmon et al., 2000).

A better understanding of the factors driving this variability in efficacy is needed to more effectively utilize this product for managing ground squirrel species. One strategy for increasing efficacy is prebaiting with a nontoxic version of the rodenticide carrier (Marsh, 1987, 1994; Nelson et al., 2012). This allows the ground squirrel to become accustomed to consuming the bait, theoretically increasing the odds that the bait will be consumed once it is coated with the toxicant. Likewise, the density of ground squirrels in a given area may impact the efficacy of a rodenticide. Where high-density ground squirrel populations are found, vegetation is sparse because of ground squirrel foraging, potentially leading to greater bait acceptance given fewer food options. When alternative food resources are abundant, bait acceptance is likely to go down unless that food source is highly preferred (Brown and Roy, 1943; Matschke et al., 1982; White, 1972), which can be challenging when the current crop is highly preferred (e.g. alfalfa). Occasionally, regional differences in bait acceptance are also noted. For example, Belding's ground squirrels have often exhibited substantial differences in bait acceptance across their range in California (Sauer, 1976; White, 1972). The driver for this regional variability is unknown, but may still be a limiting factor in baiting programs.

A better understanding of foraging patterns of ground squirrels and how this relates to bait consumption could also help to increase the efficacy of management programs. For example, knowing peak times of foraging would allow applicators to target those timeframes to increase the likelihood for bait consumption. Also, how long does the bait remain attractive to the ground squirrel once applied on the landscape? Does efficacy associated with bait application vary depending on if the bait is applied around burrow entrances or spread throughout a field? Understanding these factors could allow land managers to more effectively target bait applications to attain management goals.

Any effective management strategy must by definition be highly efficacious, but risk to nontarget species should also be minimized to the greatest extent possible. One of the primary benefits of zinc phosphide is that it has a very low risk of secondary exposure (Eason et al., 2013; Marsh, 1987). That said, zinc phosphide is highly toxic to all vertebrates that might consume it directly, so care must be taken to minimize nontarget consumption of treated bait (Hood, 1972). To that end, it's important to know what nontarget species are present in application areas and how likely they are to consume bait that is applied. This information can then be used to develop methods to eliminate nontarget bait consumption through a variety of strategies including the timing of bait application and the implementation of deterrents to reduce the likelihood of bait consumption by nontarget species. Therefore, we established a study to address all of these questions and concerns. Specifically, our objectives were to: 1) determine the efficacy of zinc phosphide-coated cabbage bait for Belding's ground squirrels and assess

whether this is impacted by prebaiting, ground squirrel density, and region of application, 2) determine the daily foraging patterns for Belding's ground squirrels to identify potential periods to target for bait application, 3) determine if foraging activity is greatest around burrow entrances, 4) determine how the time since bait application impacts foraging activity, 5) identify nontarget species that consume bait, and 6) determine how often and during what time of day they consume bait to develop mitigation strategies if needed. Collectively, this information will be used to propose an integrated pest management (IPM) strategy that should allow for effective management of Belding's ground squirrels while posing minimal risk to nontarget species.

STUDY AREA

All field sites were located in alfalfa fields in Butte Valley in northeastern Siskiyou County, California, the Klamath Basin in northwestern Modoc County, California, or in the south-central portion of Modoc County, near Alturas, California (Fig. 1). Trials were conducted from early March through April during 2016 and 2017. Weather patterns during this timeframe were generally cold (-3.1 to 18° C), with moisture in the form of snow or rain occurring intermittently throughout the study period (61–74 mm and 68–74 mm of total precipitation for March–April 2016 and 2017, respectively). Alfalfa was dormant during the early portion of each year, although some minimal growth had initiated toward the end of each study period.

MATERIALS AND METHODS

Treatment efficacy

We selected 2 sites each in the Butte Valley, Klamath Basin, and Alturas areas for efficacy studies during 2016. In 2017, we identified 10 sites in the Butte Valley, 2 sites in the Klamath Basin, and 4 sites in the Alturas area for additional investigation. For analytical purposes, all locations in the Alturas area were considered the eastern portion of our study area, while the Butte Valley and Klamath Basin locations were defined as the western portion of our study area. We flagged a 0.4 ha core monitoring plot (64 m \times 64 m) within each one of these field sites to allow us to document ground squirrel numbers before and after treatment. Buffer zones were extended for at least 107 m on all sides of the monitoring plots to reduce the likelihood that a ground squirrel could reinvade into the monitoring plot area before the completion of the trials. However, in practice, all or almost all of each field was treated with zinc phosphide-coated cabbage, thereby further reducing any potential impact of reinvasion following bait application. When possible, plots were located in separate fields to maintain independence. In three fields, we included two plots per field. However, these plots were separated by at least 295 m which is more than double the distance used in a similar efficacy study on chlorophacinone-coated cabbage (minimum distance = 137 m; Baroch et al., 1996), thereby maintaining independence.

Following many past studies (e.g. Baldwin et al., 2017; Fagerstone, 1984; Salmon et al., 2007), we used ground squirrel counts before and after treatment to determine efficacy of bait applications. At each site, individual ground squirrels were counted 5 times during morning and 5 times during the afternoon, with each count in morning and afternoon sessions separated by 5 minutes. These counts were conducted for three days for a total of 30 counts per site. Ground squirrels were only recorded if they were observed within the 0.4-ha monitoring plot (i.e. we did not record individuals within buffer zones), and care was taken not to double-count ground squirrels during each unique count. All observations were made with binoculars from a blind



Figure 1. Three study areas in northern California used for testing the efficacy of zinc phosphide-coated cabbage bait for Belding's ground squirrel control. For analyses, the Butte Valley and Klamath Basin sites were combined to represent the western portion of the study area, while the Alturas site represented the east.

(usually vehicle) from the same spot located >30 m outside the census area. Counts were again conducted 3–10 days following completion of bait application in the exact same manner as that conducted before bait application. We then compared the maximum counts before and after treatment using the following equation:

$$\text{Efficacy (\%)} = \left[\frac{(\text{pretreatment} - \text{posttreatment})}{\text{pretreatment}} \right] \times 100$$

where pretreatment and posttreatment equal the number of observed ground squirrels before and after treatment.

Activity patterns

We used remote-triggered cameras (Scoutguard[®] SG550, HCO Outdoor Products, Norcross, Georgia, USA; Bushnell NatureView HD Max, Bushnell Outdoor Products, Overland Park, Kansas, USA) focused on untreated cabbage bait to determine bait consumption patterns of Belding's ground squirrels across all 6 sites during the 2016 season. Cabbage bait (4-5 pieces) was staked to the ground using wire stakes to increase the opportunity for recording feeding activity. We used 20 cameras per site, with 10 cameras focused on bait around burrow entrances, and 10 cameras focused on bait in non-burrow sites to allow us to ascertain the impact of bait location on cabbage consumption by ground squirrels. We set cameras to record 10-second videos upon activation to allow us a better chance (as compared to using photographs) to identify the species and activity of the target animal (i.e. bait consumption vs. simple presence in the detection zone at the time of the triggering). We only included videos in analyses that showed cabbage consumption given our interest in bait uptake. We also only included videos in analyses that were separated by ≥ 5 minutes to reduce the impact of repeat visits to a site (Baldwin et al., 2014a).

We applied bait once per day for 4 days to allow us to determine if consumption activity increased over time (meant to mimic the effect of prebaiting). The time of bait application varied depending on personnel availability and other field activities. Likewise, not all cameras were functional at a given time due to camera malfunctions. To account for this variability, we relativized the data across all sites and timeframes by developing a "consumption index" that represented the number of consumption events across all functional cameras per monitored timeframe (additional details provided in the Analysis section). Camera monitoring activity concluded several days before toxic bait application.

Bait application

All chopping and mixing of cabbage occurred offsite at a designated mixing area for each landowner or professional pest control company. Cabbage was chopped to approximately 5.1–15.2 cm strips of cabbage, approximately 1.3 cm in width. Mixing followed label specifications. In summary, 4.5 kg of cabbage were mixed with 29.6–59.1 cc of vegetable oil in a mechanical tumbling-type mixer until evenly coated. Then 32.3 g of zinc phosphide concentrate (63%) were added and thoroughly tumbled until the entire batch was evenly mixed. A related lab investigation showed this strategy to be a highly accurate approach for mixing bait, with resultant mean concentrations only 1% above target values ($\bar{x} = 5,170 \mu\text{g/g}$ of zinc phosphide; Baldwin et al., 2018).

All bait applications for this study were performed by local landowners or a local professional pest control company. We prebaited half of the sites in both 2016 and 2017; prebaited and non-prebaited sites were evenly split between the eastern (i.e. 3 of each) and western portions (i.e. 7 of each) of our study area. The prebaiting process involved applying 2-4 pieces of untreated cabbage around each burrow entrance within the designated study area. One to 6 days later, we applied zinc phosphide-coated cabbage in the same manner. We followed the same process for non-prebaited sites, except that no prebaiting occurred.

In order to identify nontarget consumption of zinc phosphide-coated cabbage bait, we placed 20 remote-triggered cameras throughout the same 6 sites we used to assess activity patterns during the 2016 season. The process was the same as that used to assess activity patterns except that bait was not staked down, and bait was applied only once. However, due to a number of nonfunctional cameras, error in setting up cameras, and rapid consumption of bait by ground squirrels, only 86 cameras were considered for analysis. We recorded the number of events of bait consumption by nontarget species across these camera locations. All aspects of this project were approved by the University of California, Davis' Institutional Animal Care and Use Committee (protocol no. 18924).

Data analysis

We used multiple linear regression to determine the potential impact of prebaiting (yes or no), regional location (west vs. east), and ground squirrel density before treatment (note this is not a true measure of density, but rather the maximum number of ground squirrels documented during our pretreatment ground squirrel counts) on observed efficacy for a given field (Zar, 1999). Mean efficacy was recorded for each regional and prebaiting combination to provide insight into actual utility of a zinc phosphide-coated cabbage baiting program.

To account for differing numbers of functional cameras and hours of activity across days of bait application (days = 1–4), field sites ($n = 6$), and bait application locations (burrow vs. non-burrow sites), we developed a consumption index as follows:

$$\text{Consumption index} = \# \text{ of videos} / (\# \text{ of functional cameras} \times \# \text{ of minutes cameras were functional})$$

where # = number. These index values were determined on a daily basis (days = 1–4) and were separated by whether or not the bait was applied at a burrow location or non-burrow location. We analyzed the consumption index for Belding's ground squirrels as a three-factor analysis-of-variance with site as the blocking effect that received all combinations of the number of bait applications (1–4), bait location (at burrow or between burrows), and time of day (categorized within 1-hr intervals; Zar, 1999). If a model resulted in a $P < 0.05$, we used Fisher's least significant difference *post hoc* test to determine which categories were different ($\alpha = 0.05$ for *post hoc* test). We only analyzed time intervals of 07:00–18:00 given that no ground squirrels were observed in any videos outside this timeframe.

We also used multiple linear regression (Zar, 1999) to determine the impact of the day since application (Day 1 or 2) and time since application (minutes; parameter was divided into 60-min categories for analysis) on the consumption index value derived for each "time since application" category (range 60–1,800 minutes). We included day as a parameter given that ground squirrels become inactive during the night; as such, initiation of activity the following day could yield

different feeding activity. Day 1 was defined as the day when cabbage was initially placed or replenished, while Day 2 was the subsequent day up until the time that the cabbage bait was again replenished or completely removed. We included data from all locations and for all bait application events ($n = 4$ per site) in this analysis to avoid small sample sizes for a specific timeframe. Even so, some time-interval categories were poorly represented. As such, we eliminated any time-interval category if it did not have a combined 50 camera days (i.e. number of functional camera days for that hourly interval) for that 60-minute interval.

We were also interested in bait consumption by non-target species at these camera sites. As such, we recorded all bait consumption events at remote-triggered camera sites in the same manner as that used for Belding's ground squirrels. Although a variety of wildlife were documented in the area (e.g. black-tailed jackrabbit [*Lepus californicus*], coyote [*Canis latrans*], European starling [*Sturnus vulgaris*], black-billed magpie [*Pica hudsonia*]), only California kangaroo rats (*Dipodomys californicus*) were documented feeding on untreated cabbage during monitoring trials. We used this video data from California kangaroo rats to calculate consumption indices for this species. Consumption indices were analyzed in the same manner as those developed for Belding's ground squirrels with the exception that visitations were only recorded during the nighttime. As such, we only analyzed time intervals for 18:00–06:00 to correspond to their periods of activity. Likewise, because California kangaroo rats were only active during the nighttime, there was no need to include the day since application in our regression analyses to determine the impact of bait freshness on bait consumption (i.e. there was no bimodal distribution of activity as we observed for Belding's ground squirrels). Therefore, we used a simple linear regression to assess the potential impact of time since bait application on California kangaroo rat consumption (Zar, 1999).

RESULTS

Belding's ground squirrels

Prebaiting, region of study area, and ground squirrel density all impacted efficacy ($F_{3,16} = 19.2$, $P < 0.001$, $R^2 = 0.78$; Fig. 2). Efficacy was greatest in the western portion of the study area ($F_{1,16} = 13.4$, $P = 0.002$), at sites that were prebaited ($F_{1,16} = 11.1$, $P = 0.004$), and when ground squirrel densities were high ($F_{1,16} = 18.4$, $P < 0.001$; Fig. 2). Only prebaited sites in the western portion of the study area exhibited mean efficacy values $>70\%$ (Table 1).

The number of bait applications ($F_{3,444} = 7.5$, $P < 0.001$), the location of bait application ($F_{1,444} = 8.6$, $P = 0.004$), and the time of day ($F_{10,444} = 3.3$, $P < 0.001$) influenced bait consumption. We observed a difference in the timing of feeding activity throughout the day, with feeding more common during mid-morning and early afternoon than during early morning or late afternoon (Fig. 3). Feeding activity increased for each additional bait application event indicating a learned response for consuming cabbage bait (Fig. 4). Feeding activity was greater at burrows (\bar{x} consumption index = 0.167, SE = 0.019) than at non-burrow sites (\bar{x} consumption index = 0.097, SE = 0.017), although feeding at non-burrow sites was non-trivial.

Table 1. The number of Belding's ground squirrels on study plots before (GS's before) and after (GS's after) application of zinc phosphide-coated cabbage bait, as well as the resultant efficacy of these treatments, for prebaited and non-prebaited sites in both western and eastern portions of the study area (Siskiyou and Modoc Counties, California) during 2016 and 2017. Mean efficacy values are provided for comparison.

Prebaited	Area	Year	GS's before	GS's after	Efficacy	
Yes	West 1	2016	7	2	71%	
	West 2	2016	56	5	91%	
	West 5	2017	40	7	83%	
	West 6	2017	12	3	75%	
	West 7	2017	17	3	82%	
	West 8	2017	17	8	53%	
	West 9	2017	62	7	89%	
					<i>Mean:</i>	78%
		East 2	2016	30	8	73%
		East 4	2017	10	8	20%
		East 5	2017	18	12	33%
					<i>Mean:</i>	42%
	No	West 3	2016	53	20	62%
		West 4	2016	64	12	81%
West 10		2017	14	7	50%	
West 11		2017	24	10	58%	
West 12		2017	58	6	90%	
West 13		2017	17	10	41%	
West 14		2017	19	12	37%	
					<i>Mean:</i>	60%
		East 1	2016	28	18	36%
		East 3	2017	8	6	25%
		East 6	2017	16	11	31%
				<i>Mean:</i>	31%	

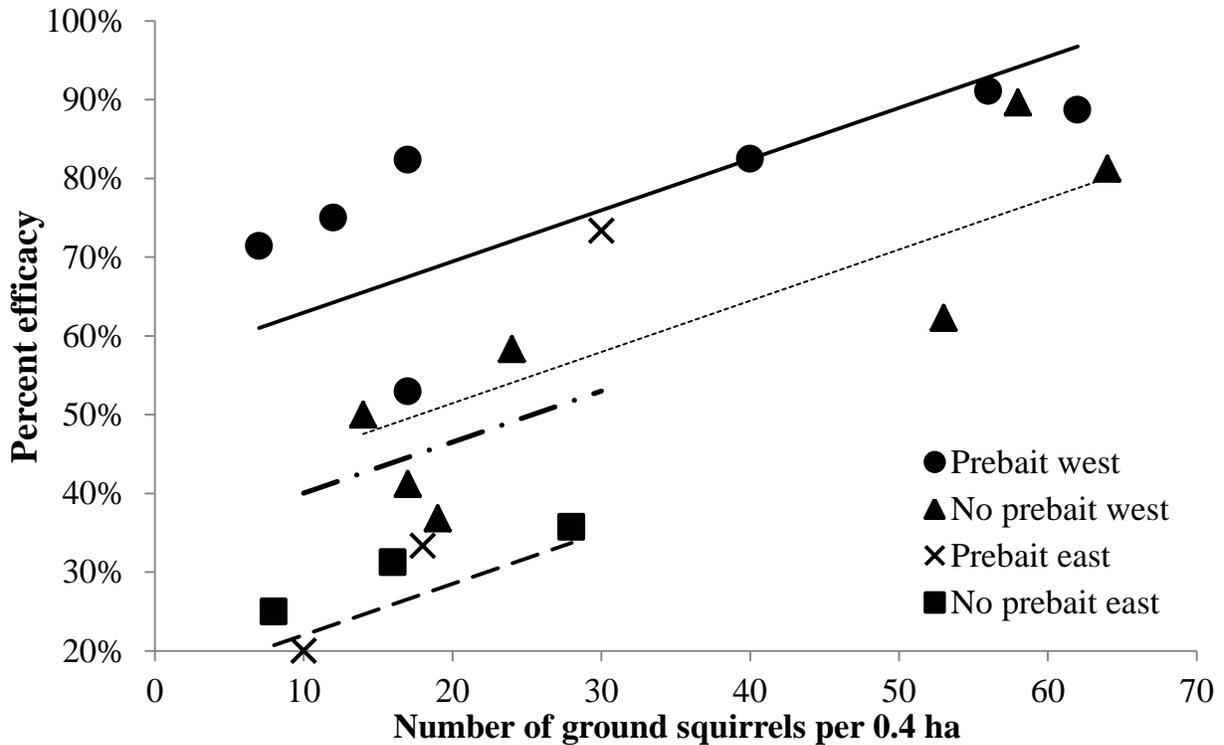


Figure 2. Modeled percent efficacy of zinc phosphide-coated cabbage bait for Belding's ground squirrel control in alfalfa fields in Siskiyou and Modoc Counties, California during late winter-early spring 2016 and 2017. Efficacy was dependent on whether or not a site was prebaited, whether sites were found in the western or eastern portions of the study area, and what the initial number of ground squirrels was in treatment plots: $\text{efficacy} = 15.5 [\text{SE} = 6.36] + (18.00 [\text{SE} = 5.40] \times \text{prebait status} [\text{prebait} = 1, \text{no prebait} = 0]) + (0.65 [\text{SE} = 0.15] \times \text{initial number of ground squirrels}) + (22.94 [\text{SE} = 6.27] \times \text{site} [\text{west} = 1, \text{east} = 0])$. Corresponding regression lines are as follows: solid line = prebait west, dotted line = no prebait west, dash-dot line = prebait east, and dashed line = no prebait east.

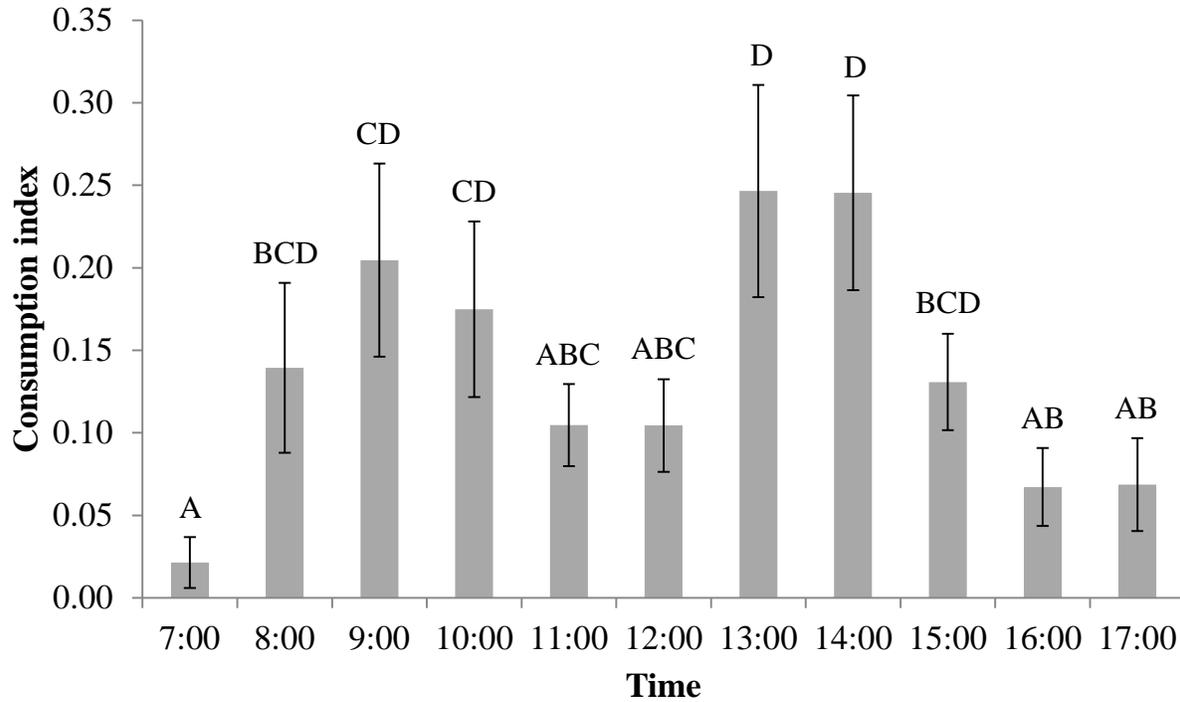


Figure 3. Belding's ground squirrel consumption of cabbage bait throughout their daily activity period across six field sites in Siskiyou and Modoc Counties, California, during late winter-early spring 2016. The consumption index was calculated by dividing the number of videos documenting cabbage consumption (determined hourly) by the total number of cameras that were functional at a site for a given hourly category. Mean and standard error bars are provided. Differences in mean values are denoted by different letters ($P \leq 0.05$).

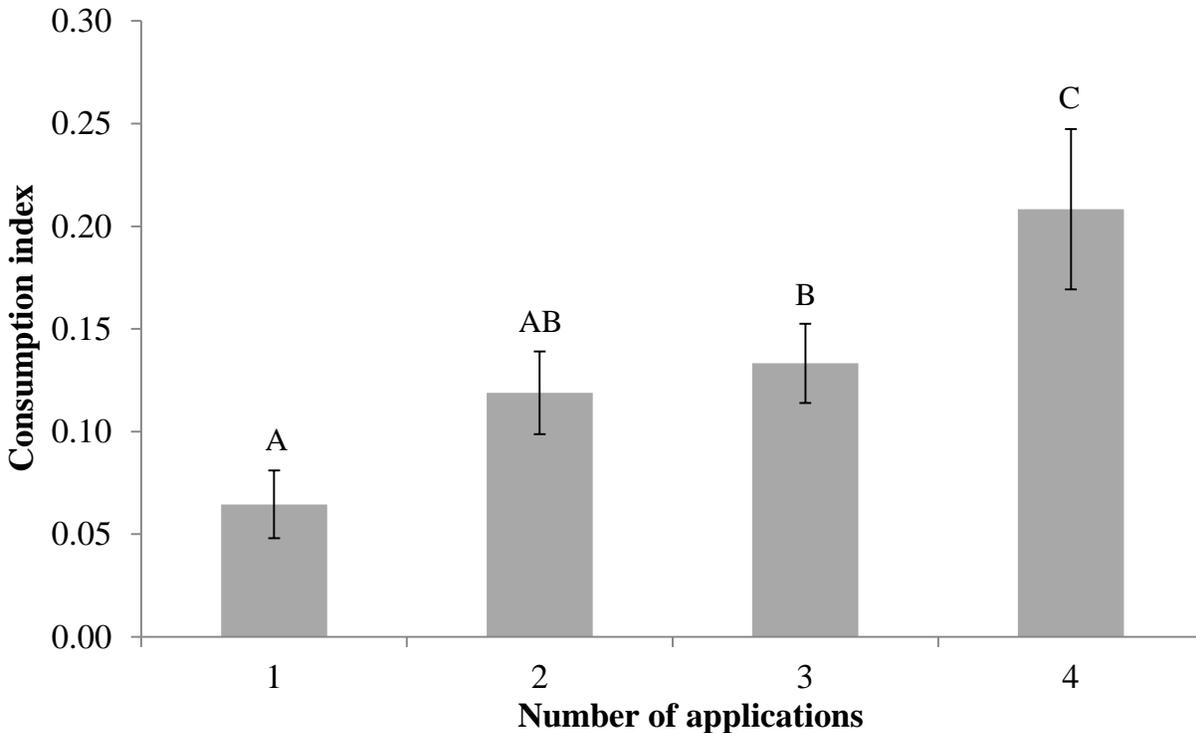


Figure 4. Belding's ground squirrel consumption of cabbage bait following four consecutive daily applications across six field sites in Siskiyou and Modoc Counties, California, during late winter-early spring 2016. The consumption index was calculated by dividing the number of videos documenting cabbage consumption (determined hourly) by the total number of cameras that were functional at a site for a given hourly category. Mean and standard error bars are provided. Differences in mean values are denoted by different letters ($P \leq 0.05$).

We observed a significant impact ($F_{2,18} = 7.0$, $P = 0.006$, $R^2 = 0.44$) of both the duration following cabbage application ($F_{1,18} = 13.5$, $P = 0.002$; $\beta = -0.00018$, $SE = 0.000049$) and the day since application on bait consumption ($F_{1,18} = 9.8$, $P = 0.006$; $\beta = 0.196$, $SE = 0.062$); consumption was greatest shortly after application, and again at the onset of the following morning (Fig. 5).

Non-target exposure

Only California kangaroo rats were documented feeding on untreated cabbage during monitoring trials ($n = 138$ feeding events). One site had no feeding by kangaroo rats (Tulelake 2), while we recorded only three feeding events from kangaroo rats at a second site (Alturas 1); these sites were excluded from analyses given extremely low or nonexistent feeding activity. The number of bait applications ($F_{3,365} = 7.8$, $P < 0.001$) and location of bait applications ($F_{1,365} = 22.7$, $P < 0.001$) influenced bait consumption by kangaroo rats, but the time of day did not ($F_{11,365} = 1.4$, $P = 0.192$). Feeding activity was 3–4 fold greater following the third and fourth applications when compared to the first two applications (Fig. 6), and cabbage consumption was 4 times more frequent around burrow sites ($\bar{x} = 0.088$, $SE = 0.014$) than at non-burrow locations ($\bar{x} = 0.021$, $SE = 0.005$). The duration following cabbage application influenced bait consumption by

California kangaroo rats ($F_{1,14} = 8.7$, $P = 0.011$, $R^2 = 0.38$; $\beta = -0.000075$, $SE = 0.000026$), with about a 4-fold reduction in consumption 18 hours post-application (Fig. 7). We did not observe a single incidence of consumption of treated bait by any nontarget species, although we did observe 3 common ravens (*Corvus corax*) and 1 black-billed magpie (*Pica hudsonia*) poking at bait with their beaks.

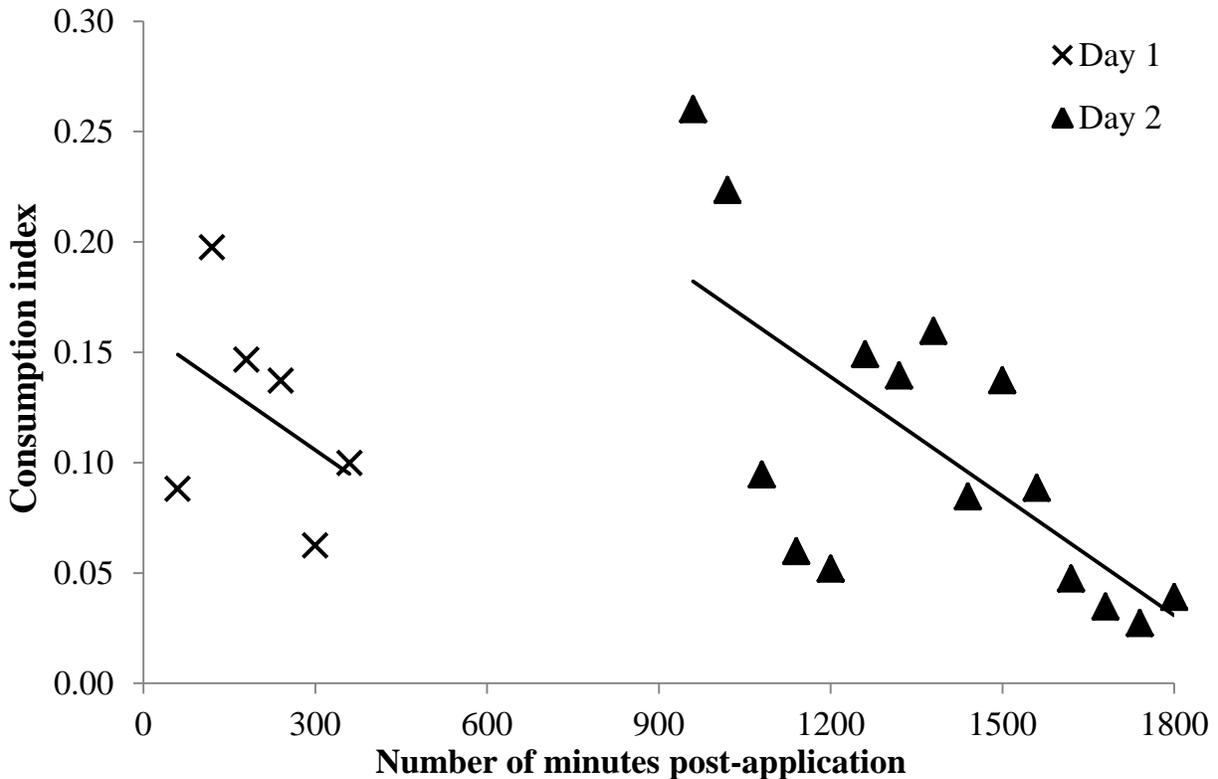


Figure 5. The relationship between consumption index values (number of videos of Belding's ground squirrels consuming bait per number of camera days for a given timeframe following bait application; see Methods section for additional detail) derived from Belding's ground squirrel visits to remote-triggered cameras baited with non-toxic cabbage, and the time interval following bait application, across 6 alfalfa fields in Siskiyou and Modoc Counties, California during late winter-early spring 2016. Data are provided for the day of application (Day 1) and the day following application (Day 2) for each bait application event ($n = 4$) across all study sites.

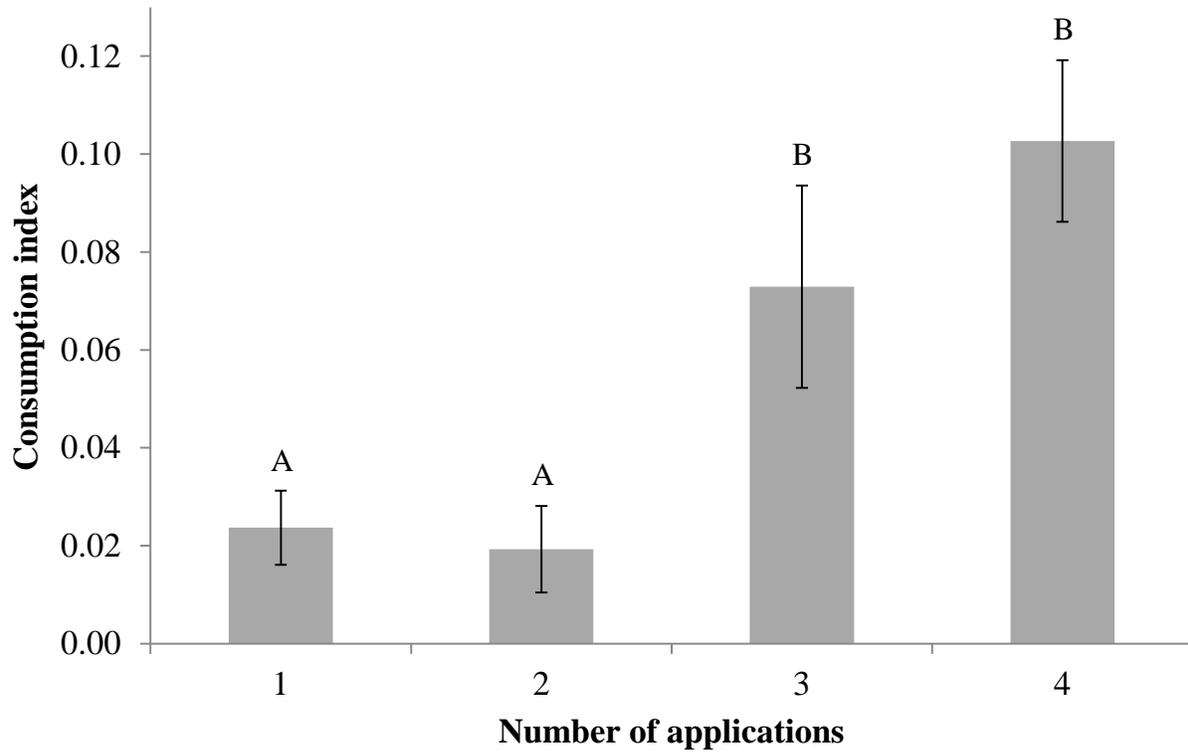


Figure 6. California kangaroo rat consumption of cabbage bait following four consecutive daily applications across six field sites in Siskiyou and Modoc Counties, California, during late winter-early spring 2016. The consumption index was calculated by dividing the number of videos documenting cabbage consumption (determined hourly) by the total number of cameras that were functional at a site for a given hourly category. Mean and standard error bars are provided. Differences in mean values are denoted by different letters ($P \leq 0.05$).

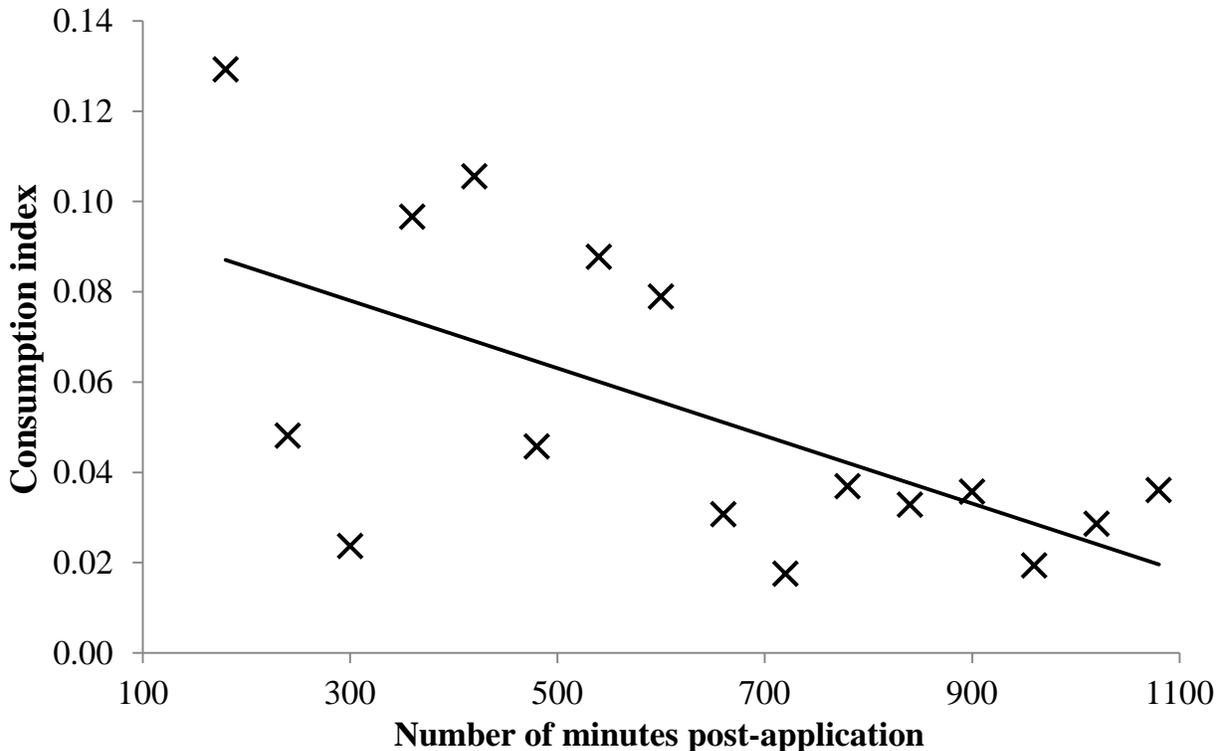


Figure 7. The relationship between consumption index values (number of videos of California kangaroo rats consuming bait per number of camera days for a given timeframe following bait application; see Methods section for additional detail) derived from California kangaroo rat visits to remote-triggered cameras baited with non-toxic cabbage, and the time interval following bait application, across 6 alfalfa fields in Siskiyou and Modoc Counties, California during late winter-early spring 2016.

DISCUSSION

Managing Belding's ground squirrels in agricultural areas has been challenging given a lack of efficacious, practical tools. The use of zinc phosphide-coated cabbage appears to be an effective option in western portions of our study site (Butte Valley and Klamath Basin), but efficacy was generally low at our eastern study site (Alturas area). Reasons why are unclear, although efficacy of grain and green baits has historically been less in this area (Sauer, 1976; White, 1972). The likely explanation is poor bait acceptance given that the development of tolerance or resistance to zinc phosphide has never been documented, nor has zinc phosphide been used extensively in these areas historically. Zinc phosphide has a distinctive odor and taste that rodents often avoid (Marsh, 1987). One strategy to potentially circumvent this limitation would be to microencapsulate the toxicant in a thin protein shell. Research continues in that direction (Horak et al., 2018), and could greatly increase the efficacy of zinc phosphide baits if proven effective. However, following the current bait application strategy, zinc phosphide-coated cabbage does not appear to be effective in all areas.

Prebaiting has long been proposed as a tool to increase efficacy for zinc phosphide baits (Hood, 1972; Marsh, 1987, 1994; Sterner, 1999). Prebaiting certainly had a substantial impact on

efficacy in this study, with prebaited sites experiencing an 18% increase in efficacy when compared to non-prebaited sites (Fig. 2). Ground squirrels continued to increase daily consumption of cabbage bait across a 4-day period (Fig. 4), further reinforcing the benefit of prebaiting. It is possible that with one or two additional prebaiting periods, efficacy could increase further, but prebaiting is an added time constraint and cost (up to 80% increase in cost; Marsh, 1987). Further investigation is needed to determine if additional prebait applications would be cost effective, and if additional prebaiting sessions might increase efficacy in eastern study sites to a level that would be considered acceptable to local producers. At a minimum, at least one prebaiting period appears to be necessary if a >70% reduction in ground squirrel numbers is desired, which is the threshold often used to consider an approach efficacious (Schneider, 1982).

Density of ground squirrels also had a substantial impact on efficacy across all study sites, with an estimated increase in efficacy of 6.5% for each additional 10 ground squirrels per 0.4 ha area (Fig. 2). Optimal foraging theory plays a role in bait acceptance, and subsequently efficacy, for zinc phosphide baits, as rodents are far more likely to consume baits when alternative food sources are scarce (Sterner, 1994). This is regularly observed with other ground squirrel species where bait application generally needs to occur before vegetation green-up or after vegetative senescence to maximize bait consumption (Matschke et al., 1982; Nelson et al., 2012; Sullins and Sullivan, 1995). For western study sites that were prebaited, all but one of the locations exhibited efficacy >70% (Fig. 2). Therefore, when prebaiting, density may be less of a factor. However, it appears to be a more important factor for non-prebaited sites; only high density sites exceeded a 70% reduction in ground squirrel numbers in the absence of prebaiting (Fig. 2). When dealing with low-density ground squirrel populations, alternative management tools may need to be considered.

In addition to prebaiting, targeting the time for bait application could also increase the efficacy of management programs while minimizing nontarget risks. Applying bait early in the day could potentially benefit baiting programs by ensuring that ground squirrels have access to the bait during their two peak daily foraging periods (Fig. 3). This would ensure access to the bait when it is the most fresh, which is considered an important factor for green baits (O'Brien, 2002). That said, bait cannot only be applied in early morning if large areas need to be treated. It is beneficial to know that when bait is applied later in the day, ground squirrels will still consume the cabbage at a high rate the following morning (Fig. 5).

It bears noting that a substantial removal of bait before nightfall should greatly reduce impact to nocturnal species such as California kangaroo rats. Furthermore, cabbage is known to desiccate quickly, and the zinc phosphide on the cabbage begins to break down immediately once it is applied (Baldwin et al., 2018; O'Brien, 2002). Therefore, zinc phosphide-coated cabbage bait may be mostly unpalatable by the evening following application. Although we did not officially test for consumption of zinc phosphide-coated cabbage by nontarget species given rapid bait consumption by ground squirrels and occasional issues with camera malfunctions, it is important to note that we never did observe a single incidence of zinc phosphide-coated cabbage consumption by California kangaroo rats (we only observed consumption of untreated cabbage), supporting the premise that a lowering of availability and palatability should reduce nontarget impacts. Furthermore, although we did not conduct official carcass searches across study plots,

we did regularly walk the study sites while engaged in other activities and did not identify a single nontarget mortality across any of our treatment plots following application.

Limiting bait application to areas likely to maximize bait consumption by target species could also reduce nontarget risk by eliminating residual bait on the landscape. We observed greater bait consumption around burrow entrances as opposed to locations between burrows for both Belding's ground squirrels and California kangaroo rats. Current application protocols require bait be spread around burrow entrances. This appears to be an appropriate strategy for maximizing efficacy but appears to have little impact on reducing consumption by California kangaroo rats.

Although bait consumption by Belding's ground squirrels was greater around burrow entrances, consumption was still fairly high in between burrows. Interestingly, consumption by nontarget species was quite low in these same interstitial areas. This suggests that a broadcast application of cabbage bait could yield substantial efficacy without a concomitant increase in nontarget risk. Historically, rodenticide-treated green baits have often been applied via broadcast approaches for Belding's ground squirrels (Kalinowski and deCalesta, 1981; Wright, 1982). Broadcast applications are generally quicker and more economical, and as such, may be worth further investigation to better quantify positive and negative attributes associated with this application strategy.

Although we never observed any wildlife feeding on treated cabbage bait, we did observe 3 ravens and 1 black-billed magpie poking at treated cabbage bait with their beaks following application; none were confirmed to consume cabbage. This suggests only marginal risk to bird species, but if such species are present during bait application, the implementation of a hazing program should be implemented to deter these nontarget bird species from baiting sites.

MANAGEMENT IMPLICATIONS

The use of zinc phosphide-coated cabbage was an effective tool for reducing Belding's ground squirrel numbers in many settings during this study. However, effective management programs do not rely on any single tool, but rather focus on an integrated approach that incorporates many management strategies (Baldwin et al., 2014b; Sterner, 2008). For example, when dealing with high-density ground squirrel populations, zinc phosphide-coated cabbage may be a preferred approach given the substantial cost and labor associated with other strategies (Baldwin and Quinn, 2012; Whisson et al., 2000; Wright, 1982). However, even in these settings, application of this bait may not reduce ground squirrel populations to the level desired by local land managers. A follow up approach that incorporates burrow fumigation or shooting could further reduce ground squirrel populations, perhaps to the desired level. If ground squirrels are subsequently eliminated from a crop field, exclusionary fencing could be implemented to slow or keep ground squirrels from reinvading from adjacent properties (Whisson et al., 2000). This could reduce long-term treatment costs and nontarget risk through less usage of a toxicant. The implementation of an IPM strategy for ground squirrel species is particularly important when using zinc phosphide given the potential for bait avoidance to develop when using this toxicant. Microencapsulation of zinc phosphide could help circumvent this limitation, but to date, microencapsulated zinc phosphide has yet to be thoroughly tested in a field setting (Horak et al., 2018).

This study showcases the utility of a green bait as a carrier for zinc phosphide. Belding's ground squirrels are not the only ground squirrel species for which acceptance of grain baits are challenging (e.g. Piute ground squirrel [*Urocitellus mollis*] in Utah, Nelson et al., 2012; Richardson's ground squirrel in Nevada, O'Brien, 2002). This baiting strategy has the potential to assist agricultural producers in mitigating ground squirrel damage in other regions and is worthy of further investigation in these regions and for these species.

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