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Short communication

Comparison of mixing methods and associated residual levels of zinc phosphide on cabbage bait for rodent management

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ABSTRACT

Zinc phosphide is a toxicant that is used extensively for rodent management throughout many parts of the world. Some rodent species, such as Belding's ground squirrels (*Urocitellus beldingi* Merriam, 1888), often avoid zinc phosphide grain baits, leaving green vegetation such as cabbage as the only viable carrier for rodenticides. However, to date, ambiguity has existed as to the most appropriate mixing strategy for zinc phosphide-coated cabbage baits, and it is unknown how rapidly zinc phosphide degrades on these green carriers. Following laboratory and field-enclosure trials, we detected no significant difference in mean zinc phosphide concentrations or variability in zinc phosphide concentrations between mechanical and hand mixing strategies. However, the use of a mechanical mixer was determined to be the more practical option given that it is quicker and requires less effort for mixing large quantities of bait, it minimized worker exposure to phosphine, and because it yielded mean concentrations that were closer to target values. Both the moisture content of cabbage and zinc phosphide concentrations diminished over time, resulting in a fairly minimal window of exposure for non-target wildlife. Field investigation of this exposure risk, as well as an assessment of efficacy of zinc phosphide-coated cabbage baits for Belding's ground squirrel management, are warranted.

1. Introduction

Rodenticides play a key role in managing many rodent pest species throughout the world (Eason et al., 2010). Zinc phosphide is a rodenticide that is used extensively in the United States, Australia, Asia, New Zealand, and other regions globally (Marsh, 1987; Eason et al., 2013). Zinc phosphide has several attributes that make it attractive for use including a short time from consumption to death, low secondary toxicity risk, and it is considered moderately humane (Marsh, 1987; Fisher et al., 2004; Eason et al., 2013). However, zinc phosphide has a distinctive odor and taste that sometimes leads to bait avoidance by target species, and if consumed in sublethal amounts, can lead to bait shyness, thereby reducing the likelihood of success of future applications due to a learned avoidance of associated baits (Marsh, 1987).

One potential strategy to overcome avoidance issues associated with zinc phosphide includes the use of a more palatable bait carrier. This is particularly important for certain species, such as Belding's ground squirrels (*Urocitellus beldingi*), which do not readily consume grain baits, but instead prefer green vegetation (O'Brien, 1978). Applicators generally prefer using grain or pelletized baits rather than green baits (e.g., cabbage leaves and artichoke bracts; O'Brien, 1978; Baldwin et al., 2016) given their longer-term stability and the fact that they can be premixed by certified mixing facilities. The use of green baits requires daily mixing at local sites, potentially leading to greater risk of phosphine exposure for the mixer if improperly trained, and could lead to lower efficacy or greater non-target risk if the resultant concentration is too low or too high. Furthermore, green baits are often highly palatable to non-target species, leading to concern for non-target poisoning from a zinc phosphide-coated green bait. However, green baits lose moisture, and subsequently, palatability, over time, and zinc phosphide can slowly degrade after exposure to moisture in the environment (Sterner and Ramey, 1995). This dynamic has ramifications both for efficacy of zinc phosphide over time, as well as to potential risks to non-target species; the quicker that green vegetation loses palatability and the quicker zinc phosphide breaks down in the environment, the less effective it might be for rodent control. However, this would also result in a concomitant reduction in risk to non-target species as well. Further exploration of this dynamic is needed to better understand potential benefits and risks of zinc phosphide-coated green baits for rodent control.

Belding's ground squirrels provide an interesting case study for testing this dynamic. Belding's ground squirrels cause extensive damage

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in alfalfa fields throughout much of northeastern California and southeastern Oregon (Sauer, 1976, 1984; Kalinowski and deCalesta, 1981; Whisson et al., 1999). Historically, Belding's ground squirrels were effectively controlled through the use of Compound 1080 (sodium monofluoroacetate) treated cabbage. However, in 1990, 1080 was deregistered for this use (Whisson et al., 2000). Alfalfa growers have been searching for a viable control option since that time.

Recently, a zinc phosphide-coated cabbage bait was registered for Belding's ground squirrel control in alfalfa and immediately adjacent non-crop areas in both Oregon during 2014 and California during 2015. The label allows for the mixing of the cabbage bait with vegetable oil and zinc phosphide either in a bucket or via a mechanical mixer. The use of a commercial-style mechanical mixer has proven effective at thoroughly mixing rodenticide-coated artichoke bract baits for vole control (Salmon and Lawrence, 2006; Baldwin et al., 2016), but there is some concern that hand mixing in a bucket may result in an uneven distribution of the active ingredient. If this did occur, the bait would be both less effective and potentially more hazardous to non-target species. A thorough comparison of these approaches is needed to ensure that proper concentrations of zinc phosphide are attainable using either of these mixing approaches. Therefore, we established a study to test the following objectives to better define the utility of zinc phosphide-coated cabbage as a potential tool for managing Belding's ground squirrels: 1.) determine if the concentration of zinc phosphide on cabbage differs between mechanical and hand mixing strategies, 2.) determine if the variance of zinc phosphide concentrations on cabbage differs between the two mixing strategies, and 3.) determine if zinc phosphide concentrations on cabbage diminish over a 72-h study period under field conditions.

2. Methods

2.1. Sample preparation

For all tests, we used zinc phosphide concentrate (CAS No. 1314-84-7; Lot 5-2016; 63.2% purity) that was purchased from the Pocatello Supply Depot (Pocatello, Idaho, USA), and cabbage and vegetable oil that were purchased from a local grocery story. At the onset of testing, we assayed the zinc phosphide for purity and found it to be 59.9% (SE = 0.05) pure. For all tests, we cut cabbage into 8–15 cm strips that were at least 1.3 cm in width. We mixed 4.5 kg of cabbage strips with 28.3 g of vegetable oil until the cabbage was fully coated. Finally, we thoroughly mixed 40 g of zinc phosphide concentrate to the cabbage-oil mixture until all cabbage appeared to be well coated. For hand mixing, we used 60.6-L plastic tubs. For mechanical mixing, we used a small cement mixer (ProForce 0.14 m^3 cement mixer, Midwest Air Technologies, Inc., Long Grove, Illinois, USA). All mixing protocols followed the label directions for this product (EPA Reg. No. 56228-6), and all components of this study were conducted at the National Wildlife Research Center in Fort Collins, Colorado, USA.

2.2. Comparison of mixing methods

To test for potential differences in zinc phosphide concentrations between hand and mechanical mixing strategies, we took 10 randomly selected 2–3 g samples of cabbage strips for each mixing method. Following Mauldin et al. (1996), zinc phosphide concentrations were determined for each sample by first hydrolyzing the zinc phosphide in a 40 mL solution of 30% sulfuric acid and water in a sealed 500-mL Erlenmeyer flask of known volume. The resultant phosphine gas was liberated into the headspace of the flask in this reaction. The headspace was then sampled and was injected into the gas chromatograph for detection by a flame photometric detector. Testing was initiated shortly after the mixing was completed. We were interested in if both variability and mean values differed between the two mixing strategies. We used an F-test to determine potential differences in variability and a two-sample *t*-test to assess potential differences in mean values (Zar, 1999).

2.3. Zinc phosphide field stability

We used an animal holding pen that was exposed to outside temperatures and humidity to assess zinc phosphide field stability. The pen included a covered roof and wire mesh walls. We placed 60.6-L plastic tubs that contained approximately 8 cm of top soil (Wetmore-Boyle-Rock outcrop complex, average pH estimated at 6.4; Soilweb, 2017) that was collected from the Colorado foot hills to serve as the substrate that the coated cabbage would rest on during the weathering period. The tubs were not covered during the experiment. We continuously monitored temperature and humidity using an EasyLog 21CFR data logger (Lascar Electronics Inc., Erie, Pennsylvania, USA) that was placed on top of the study substrate. The temperature varied from −2 to −7 °C and humidity varied from 50 to 89% during our study period.

We collected 5 sub-samples each at 0, 4, 12, 32, 48, and 72 h after preparation of the bait to test for zinc phosphide content. The testing procedure for residual zinc phosphide concentrations followed that outlined in section 2.2. To account for moisture loss during the sampling period, we pre-weighed samples that were placed in plastic weigh boats at the onset of the study. At each time point, we reweighed one of these samples to determine moisture loss. We used simple linear regression to determine if moisture loss changed over time (Zar, 1999). We then corrected observed zinc phosphide concentration data collected from the field stability trial so that all results were representative of wet weight. This was achieved by multiplying the observed zinc phosphide concentration of each sample by using the following equation:

$$ZP_{corr} = (ZP_{uncorr} \times \text{sample mass}) / (\text{sample mass} \times (1 + \%\ \text{moisture loss}))$$

where ZP_{corr} is the corrected wet weight concentration of zinc phosphide, ZP_{uncorr} is the uncorrected zinc phosphide concentration, sample mass is the mass of cabbage sampled, and % moisture loss is the percentage of moisture loss for the sample tested during the designated timeframe. We used analysis of covariance with mass of the sample used as the covariate in the analysis. We tested for differences in mean zinc phosphide concentrations between sampling periods using Fisher's least significant difference *post hoc* test (Zar, 1999).

3. Results

We observed no significant difference in mean concentrations (mechanical = 5170 μg/g, hand = 5910 μg/g; $t_{18} = -1.31$, $p = 0.207$) or associated variance (mechanical SE = 345, hand SE = 444; $F_{9,9} = 1.66$, $p = 0.230$) of zinc phosphide following mechanical and hand mixing strategies. However, mean values for mechanical mixing were substantially closer to target levels (mechanical and hand mixing were 101% and 115% of target concentration, respectively; Goldade and Abbo, 2017), so outdoor testing was conducted with cabbage bait mixed mechanically.

The untreated cabbage bait experienced steady, consistent moisture loss over the duration of the project ($F_{1,3} = 52.4$, $p = 0.005$, $r^2 = 0.95$; $\beta = 0.29$, SE = 0.04; Table 1). We also observed a significant reduction in zinc phosphide concentration over time ($F_{5,34} = 2.8$, $p = 0.042$); this difference was driven by a reduction 32- and 48-h post-mixing when compared to time zero (Fig. 1). No other substantial differences were noted, although overall zinc phosphide concentrations generally declined over time (Fig. 1).

4. Discussion

Although we did not observe a significant difference in zinc

Table 1
The loss of moisture from cabbage samples over a 72-h period.

Time (h)	Initial mass (g)	Residual mass (g)	Moisture loss (g)	Moisture loss (%)
4	47.03	45.75	1.28	2.72
12	55.08	52.65	2.43	4.41
32	63.11	59.13	3.98	6.31
48	57.76	49.78	7.98	13.82
72	61.63	47.75	13.88	22.52

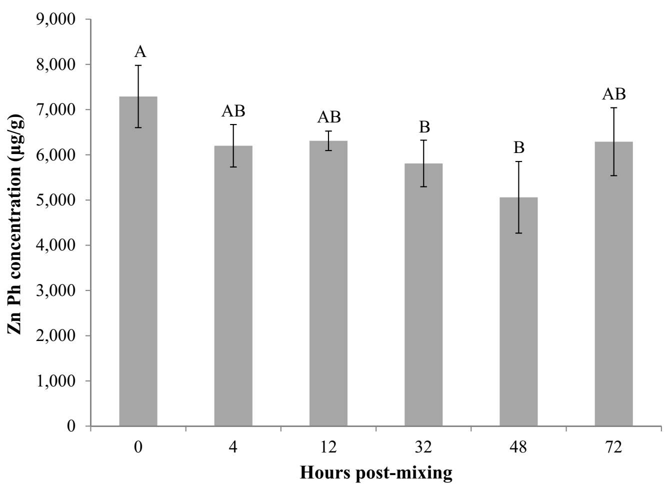

Fig. 1. Residual zinc phosphide concentrations on cabbage leaves at set intervals post-mixing. Time intervals that differed ($p < 0.05$) are noted by different letters (A and B).

phosphide concentrations or associated variance between mechanical and hand mixing methods, the resultant concentrations from mechanical mixing were much closer to the desired level and less variable than results observed through hand mixing. When mixing by hand, some cabbage frequently stuck to the sides of the container and may have impeded our ability to evenly coat zinc phosphide across all cabbage strips. We did not observe this same problem when using the mechanical mixer, as the constant tumbling within the drum appeared to coat the cabbage more completely. It bears noting that the use of a mechanical mixer is also far more practical for mixing large amounts of bait given the ease with how it can be mixed and the amount that can be mixed at a time. Hand mixing may be best suited for situations when small amounts of bait are needed.

As with some previous studies with grain baits (Sterner and Ramey, 1995), we noted a substantial reduction in zinc phosphide concentration over time (31% reduction after 48 h; Fig. 1). Various factors are believed to influence degradation of zinc phosphide including physical weathering such as rainfall and wind, soil and atmospheric moisture, and potentially soil pH, although the exact interaction of these factors likely varies across sites (Sterner and Ramey, 1995). Interestingly, we observed an increase in zinc phosphide concentrations 72-h post-mixing, but this increase was likely the result of the substantial variability we observed throughout the mixing process. There is no reason that zinc phosphide concentrations would increase over time.

The use of pelletized and paraffin baits has shown some promise at reducing zinc phosphide degradation due to weathering (Merson and Byers, 1985; Koehler et al., 1995), but these strategies are incompatible or impractical for use on green baits. Regardless, the primary factor limiting the longevity of green baits appears to be desiccation. Although temperatures were freezing at the time of this investigation, we noted substantial water loss of baits over our 72-h study period. Freezing temperatures are quite common during the early baiting season in northern California, and can occur throughout the entire baiting season. That said, during warmer temperatures, far more rapid moisture loss is likely (90% weight loss after three days; R.E. Marsh, University of California, Davis, unpublished data). This shriveling effect reduces palatability, thereby reducing the likelihood that both target and non-target species will consume the bait. This is beneficial for reducing non-target exposure given that baits are generally unpalatable fairly soon after application, yet the relatively short palatability window appears to be sufficient for effective management of ground squirrel populations (O'Brien, 2002; Balliette et al., 2006). Collectively, the slow degradation of zinc phosphide, combined with the fairly rapid reduction in palatability of cabbage bait, should result in a fairly minimal window of exposure to non-target wildlife. Field investigation of this exposure risk, as well as an assessment of efficacy of zinc phosphide-coated cabbage baits for Belding's ground squirrel management is warranted.

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