September 2004

Increasing acceptance and efficacy of zinc phosphide rodenticide baits via modification of the carbohydrate profile

J. J. Johnston
USDA/APHIS/WS National Wildlife Research Center

D. L. Nolte
USDA-APHIS-Wildlife Services, Dale.L.Nolte@aphis.usda.gov

B. A. Kimball
USDA/APHIS/WS National Wildlife Research Center, bruce.kimball@ars.usda.gov

K. R. Perry
USDA/APHIS/WS National Wildlife Research Center

J. C. Hurley
USDA/APHIS/WS National Wildlife Research Center

Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Crop Protection 24 (2005) 381–385
Crop Protection
www.elsevier.com/locate/cropro

Increasing acceptance and efficacy of zinc phosphide rodenticide baits via modification of the carbohydrate profile

J.J. Johnston[a,*], D.L. Nolte[b], B.A. Kimball[a], K.R. Perry[b], J.C. Hurley[a]

[a]USDA/APHIS/Wildlife Services/National Wildlife Research Center, Chemistry Project, 4101 LaPorte Avenue, Fort Collins, CO 80521, USA
[b]USDA/APHIS/Wildlife Services/National Wildlife Research Center, Olympia Field Station, 9730B Lathrop Industrial DR. S.W, Olympia, WA 98512, USA

Received 9 March 2004; received in revised form 27 August 2004; accepted 9 September 2004

Abstract

Toxicant coated grain-based baits are widely used to control rodent pests throughout the world, but where alternative food sources are available, bait acceptance and efficacy are often less than optimal. In an attempt to develop baiting strategies to increase bait acceptance and efficacy of zinc phosphide-coated baits, a sugar-enhanced rolled oat based zinc phosphide bait was evaluated. With pre-baiting, 100 and 60% mortality was achieved for California and Belding's ground squirrels, respectively. A series of two-choice tests with a variety of potential bait matrices indicated that rolled oats was a desirable base matrix for both species but the lecithin sticker negatively impacted bait acceptance by Belding's ground squirrels.

Keywords: Squirrels; Rodent; Pest; Malt; Bait; Zinc phosphide; Sugar

1. Introduction

Zinc phosphide baits are used with limited success for the control of Belding's (*Spermophilus beldingi*) and California (*Spermophilus beecheyi*) ground squirrels in California, primarily due to low bait acceptance. These species are significant agricultural pests in the western United States and Canada (Marsh, 1994; Salmon et al., 2000; Bourne et al., 2002; O'brien, 2002; Whisson et al., 2000).

It was believed that increased effectiveness could be accomplished using existing active ingredients, if bait uptake could be increased by improving the attractiveness of the bait to pest species. As rodent preferences for sugars are well documented (Lund, 1988; Smith and Wilson, 1989; Spector and Smith, 1984; Howard et al., 1976), adding sugar (i.e. sucrose or glucose) to bait formulations would probably increase the palatability of cereal baits. An alternative approach to increase the sugar/nutritive content and palatability of grain baits was to substitute malted grains for the unmalted grains that are currently used to prepare baits; the centuries old technique of malting converts grain starches to sugars (MacLeod et al., 1953; Craver et al., 1996). While rolled oats are widely used as a base for zinc phosphide baits, malted rolled oats are not commercially available, so these were simulated by coating rolled oats with Ceresweet, flour prepared from enzymatically hydrolyzed oats. Two percent zinc phosphide baits on Ceresweet-coated rolled oats and its efficacy were compared with traditional rolled oat based bait by conducting no-choice tests with California ground squirrels and Belding's ground squirrels.

In an attempt to gain insight into the results of the no-choice tests, a series of two-choice tests were also conducted. Offerings included rolled oats, Ceresweet-coated rolled oats, lecithin-coated rolled oats, whole unmalted oats and whole malted oats.

*Corresponding author. Tel.: +1 970 266 6082; fax: +1 970 266 6089.
E-mail address: john.j.johnston@aphis.usda.gov (J.J. Johnston).

0261-2194/$ - see front matter
doi:10.1016/j.cropro.2004.09.006

2. Materials and methods

2.1. Test materials

Malted and control oats were obtained from Briess Malting (Milwaukee, WI). Ceresweet (enzymatically hydrolyzed oat flour) was obtained from Grain Millers (Bellevue, WA) and formulated onto rolled oats using lecithin (alcolec-s, American Lecithin Co., Woodside, NJ) as an adhesive. Toxic baits were coated with zinc phosphide (Bell Labs, Madison, WI) and lecithin to give final concentrations of 2% zinc phosphide and 5% lecithin. Chemical analyses confirmed that the zinc phosphide concentrations of the baits were within 10% of the target concentration (Mauldin et al., 1996). Formulated products were prepared in the NWRC formulation chemistry laboratory (Fort Collins, CO) and shipped to the NWRC Olympia, WA field station for use in the bioassay studies. Grains and baits were stored in a refrigerator (ca. 5 °C) until used in the experiment.

2.2. Test animals/bioassays

Belding's and California ground squirrels, trapped live in California, were transported to the NWRC Olympia, Washington field station and were quarantined for 2 weeks prior to being housed in the test system for the duration of the acclimation period. Test procedures were identical for both species. For each study, 12 of each species were used as test subjects in the ratio of 1:1 males to females. Animals were given free access to water and a rodent maintenance diet (Wayne Rodent BLOX 8604; Harlan Teklad Laboratory, Madison, WI, USA) in their nest chamber and offered rolled oats from 0900 to 1500 h in the goal boxes during the acclimation period. The following 4-day pre-treatment period differed only in that the goal boxes contained untreated whole oats. During the subsequent treatment period, different test substances were placed in each test box.

For two-choice tests, a "T" maze with a start box at the bottom of the "T" and goal boxes at the end of each "arm" was used. For no-choice tests, the goal box was directly connected to the nest box with a 1-m run (Table 1). Bait consumption and animal status were monitored at 2-h intervals for the first 6 h, then again at 12, 24, 48, and 72 h. Time to death was also noted for the toxicant study.

Table 1
Dimensions of one- and two-choice test systems

	Beldings ground squirrels	California ground squirrels
Nest chamber	45 × 30 × 30 cm box	35 × 50 × 30 cm box
Goal box (es)	25 × 26 × 10 cm box	35 × 50 × 30 cm box
Runs	1 m × 5 cm (l × dia)	1 m × 10 cm (l × dia)

2.2.1. Study 1

No-choice tests were conducted using 2% zinc phosphide baits and 5% lecithin on Ceresweet-coated rolled oats or uncoated rolled oats. Percent mortality was determined for each treatment group. Six animals with previous access to Ceresweet-coated rolled oat and six naïve animals received the Ceresweet-coated rolled oat baits. The other animals received the baits prepared with uncoated rolled oats.

2.2.2. Study 2

Three two-choice tests were conducted with each test subject: (1) whole malted oats vs. whole unmalted oats, (2) Ceresweet-coated rolled oats vs. unmalted whole oats and (3) malted whole oats vs. Ceresweet-coated rolled oats. The test order was counter-balanced among subjects. Each test was conducted for four consecutive days. To minimize habituation to the novel foods, there was a minimum of 1 week between tests with each test subject.

2.2.3. Study 3

Counter-balanced, four day, two-choice tests were conducted with each test subject: (1) uncoated rolled oats vs. whole unmalted oats and (2) lecithin coated rolled oats vs. Ceresweet (lecithin) coated rolled oats.

2.3. Statistical analyses

Mortality data (Study 1) were analyzed by producing a three-way contingency table and computing a measure of association (treatment vs. mortality) across species with the Cochran–Mantel–Haenszel statistic (CHM; SAS, 1989). Treatments were defined by the type of grain offered and rodent experience. Four treatments were designated as follows: Ceresweet-coated oats with no previous exposure (naïve); uncoated oats with naïve subjects; Ceresweet-coated with previous exposure (pre-exposed); and uncoated oats and pre-exposed subjects.

Preference scores were calculated for each subject (Studies 2 and 3). Preference scores were subjected to repeated measures analyses of variance (ANOVA) to assess species, day, and species*day effects. Species effects were analyzed using subject (nested in species) as the error term (Rice, 1989).

3. Results

3.1. Study 1

For at least one species, mortality was associated with treatment ($p = 0.0301$). Inspection of he cell frequencies

Table 2
Efficacy of steam rolled oat vs. Ceresweet-coated steam rolled oat based zinc phosphide baits

	California ground squirrels				Beldings ground squirrels			
	Regular		Ceresweet		Regular		Ceresweet	
	Pre-exposed	Naive	Pre-exposed	Naive	Pre-exposed	Naive	Pre-exposed	Naive
Died	2	2	6	1	3	1	3	1
Survived	4	3	0	4	3	6	2	5
Mortality (%)	33	40	100	20	50	14	60	17

suggested that increased mortality was produced by the use of Ceresweet-coated baits with pre-exposed subjects (Table 2).

3.2. Study 2

Three different two-choice preference tests were conducted with each species. As there were no significant species, day, or species*day effects, mean preference scores were calculated across all days for each subject. Unmalted whole oats were preferred by both species ($p = 0.0289$).

Comparison of malted whole oats and Ceresweet-coated oats yielded a significant species effect ($p = 0.0020$); preference scores from the two species were analyzed separately. For California ground squirrels, no preference was observed ($p = 0.1299$). Belding's ground squirrels significantly preferred malted oats vs. Ceresweet-coated oats ($p = 0.0027$).

As comparison of Ceresweet-coated rolled oats and unmalted whole oats demonstrated significant species ($p = 0.0004$) and day ($p = 0.0227$) effects, preference scores were examined separately for each species on each test day. California ground squirrels preferred the Ceresweet-coated rolled oats on day 4. Belding's ground squirrels strongly preferred whole oats on each test day (Fig. 1).

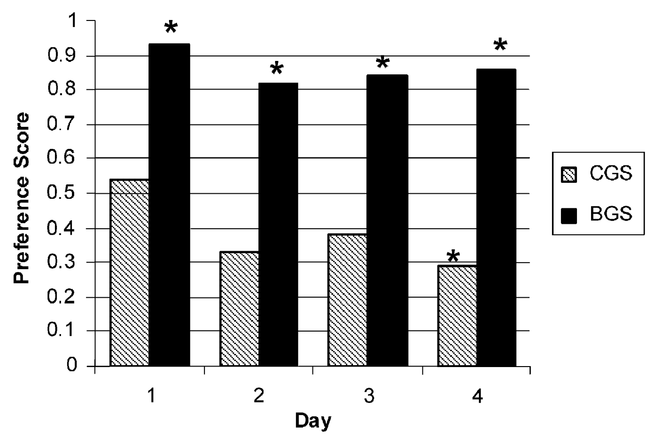

Fig. 1. Preference score is whole oats/total intake. Scores >0.5 indicate a preference for whole oats. Scores <0.5 indicate a preference for Ceresweet-coated rolled oats. * = scores that were significantly higher or lower than 0.5 ($\alpha = 0.05$) CGS = California ground squirrel; BGS = Belding's ground squirrel.

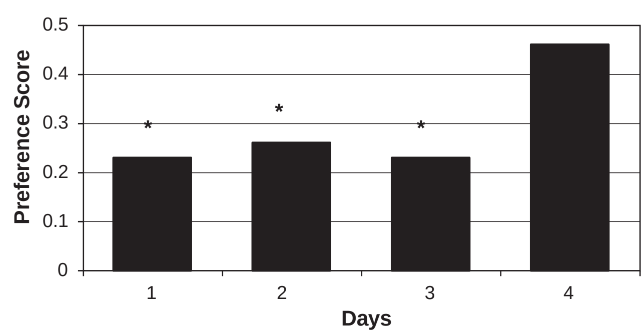

Fig. 2. Preference score is whole oats/total intake for both species. Scores >0.5 indicate a preference for whole oats. Scores <0.5 indicate a preference for Ceresweet-coated rolled oats. * = scores that were significantly higher or lower than 0.5 ($\alpha = 0.05$).

3.3. Study 3

Subjects did not demonstrate a preference when given the choice of Ceresweet-coated rolled oats and lecithin-treated (control) rolled oats ($p = 0.1024$). Both species strongly preferred rolled oats on days 1–3 of the test, but showed no preference on day 4 (Fig. 2).

4. Discussion

Study one was conducted to determine if the efficacy of zinc phosphide baits could be improved by using a grain with increased sugar content as the base. At least one of the treatments significantly impacted mortality; the most significant treatment effect (percent mortality) for both species was observed for the pre-exposed Ceresweet treatment groups. The highest level of efficacy was observed in the pre-tested Ceresweet treatment group (Table 2). This suggests that previous exposure (pre-baiting) with Ceresweet-coated rolled oats followed by baiting with toxicant containing Ceresweet-coated rolled oats increased efficacy for California ground squirrels. Pre-exposure also increased the efficacy of both zinc phosphide baits to Belding's ground squirrels. This suggests that the common practice of pre-baiting is valuable for increasing acceptance of oat-based zinc phosphide rodenticide baits.

For the required US Environmental Protection Agency registration of toxicant baits for the control of vertebrate pest species, a minimal target population reduction of 70% must be demonstrated. This requirement was observed for only pre-baited Ceresweet-coated zinc phosphide bait fed to California ground squirrels. The highest efficacy observed for Belding's ground squirrels was 60%, which indicates that both types of zinc phosphide bait are inadequate for the control of Belding's ground squirrels.

Studies 2 and 3 were conducted to gain insight into the bait matrix qualities preferred by each species. The aim of study two was to determine if ground squirrels exhibited a preference for grains with elevated sugar content.

Both species were offered a choice between two grains with elevated sugar content, Ceresweet-coated rolled oats and malted whole oats. California ground squirrels exhibited no significant preference for either sugar-enhanced matrix. Belding's ground squirrels showed a strong preference for the malted whole oats. Our initial interpretation of these results was that Belding's ground squirrels may have a preference for the physical and/or organoleptic qualities associated with malted whole oats (Hodge and Ossman, 1976). However, since the Ceresweet-coated rolled oats contain lecithin, these data also suggest that Belding's ground squirrels have a stronger avoidance of lecithin than do California ground squirrels.

California ground squirrel preferred Ceresweet-coated rolled oats to (unmalted) whole oats on day 4 (Fig. 1). Belding ground squirrels preferred whole oats on all four test days. These findings are consistent with the study one results. The highest efficacy was associated with Ceresweet-coated rolled oats and pre-exposed (pre-baited) California ground squirrels as: (1) Ceresweet-coated rolled oats are preferred more by California ground squirrels than by Belding's ground squirrels and (2) the California ground squirrel preference for Ceresweet-coated rolled oats increased with continued exposure. Belding's ground squirrels preferred the whole oat matrix over rolled oats, even though the Ceresweet-coated rolled oats were sugar enhanced. This suggests a Belding's ground squirrel preference for whole oats vs. rolled oats. Again, the presence of lecithin in the Ceresweet rolled oats may contribute to the apparent Belding's ground squirrel preference for whole oats.

Both ground squirrel species preferred whole oats to malted whole oats. The elevated sugar content in the malted whole oats may not have been sufficiently attractive to offset other malting induced effects such as textural changes and the development of roasted flavor and aroma. Thus, the California ground squirrel preference for Ceresweet-coated oats vs. malted whole oats observed in the first two-choice test presented for study 2 may be influenced by avoidance to malting associated changes in the oat grain.

The aim of study 3 was to isolate the effects of the lecithin sticker on ground squirrel preferences to develop a better understanding of the apparent preferences exhibited during studies 1 and 2. In study 3, both species strongly preferred rolled oats on days one through three of the four day test period (Fig. 2). Since Belding's ground squirrels preferred uncoated rolled oats to whole oats in this study, the consistent Belding's ground squirrel preference for whole oats over Ceresweet-coated oats observed in study 2 is probably due to an avoidance of the lecithin sticker.

When offered only lecithin containing choices (Ceresweet-coated rolled oats, lecithin coated rolled oats) no preference was noted for either species. Also, the addition of Ceresweet to rolled oats had no effect on acceptance. This suggests that lecithin may mask the sweetness of the Ceresweet. When forced to choose between two substances with lecithin, the animals appear not to be able to identify the sugar-enhanced matrix. In study 1, however, ground squirrels were offered a choice between Ceresweet-coated rolled oats (containing lecithin) and whole oats. For the first three days, California ground squirrels showed no preference for either bait. This pattern is consistent with the hypothesis that lecithin masked the sweetness of Ceresweet. On day 4, California ground squirrels preferred Ceresweet-coated rolled oats, possibly because California ground squirrels associated a positive post-ingestive feedback from Ceresweet (increased sugar and digestible carbohydrates) with the presence of lecithin and/or Ceresweet. Due to the constant lack of preference for lecithin containing choices, Belding's ground squirrels avoided the lecithin containing Ceresweet baits and could not subsequently associate any postingestive feedback with this matrix. The fact that the highest level of efficacy was noted for pre-exposed California ground squirrels is consistent with our hypothesis that California ground squirrels develop a preference for Ceresweet and lecithin coated baits with continued exposure. The results (Table 3) suggest that this learned response also pertains to Belding's ground squirrels; mortality was higher for pre-exposed than naive Belding's ground squirrels for the lecithin-coated zinc phosphide baits with and without Ceresweet.

California ground squirrels consistently preferred rolled oat matrices over whole oat matrices while Belding's ground squirrels preferred uncoated rolled oats to all other tested potential grain based bait bases, including (uncoated) unmalted and malted whole oats (Table 3). While no lecithin coated whole oats were tested, the data suggest that Belding's ground squirrels

Table 3
Preferences summary for studies 1 and 2

Species	Matrix preferences						
California ground squirrels	Rolled oats (Ceresweet, lecithin coated, uncoated)	>	Unmalted whole oats	>	Malted whole oats		
Belding's ground squirrels	Uncoated rolled oats	>	Unmalted whole oats	>	Malted whole oats	>	Lecithin-coated rolled oats (Ceresweet, lecithin coated)

would likely prefer lecithin coated rolled oats to lecithin coated unmalted or malted whole oats. Thus using whole oats as the grain base for the baits would not increase the efficacy of the baits for either species.

Overall, the higher levels of mortality associated with California ground squirrels are consistent with the observed Belding's ground squirrels preference for food sources that are free of lecithin.

The sugar enhanced (Ceresweet-coated) rolled oat based zinc phosphide baits show promise for the development of improved rodenticide baits for pest squirrel control. This study showed that the means by which sugar content is increased in a grain can influence acceptance; malting induced changes were associated with avoidance as unmalted whole oats were preferred over malted whole oats by both species. The enzymatic hydrolysis process used to produce the Ceresweet product did not display the roasted characteristics and the associated avoidance of the malted whole oats. Pre-baiting with Ceresweet-coated rolled oats was required to demonstrate acceptable efficacy (>70% mortality) with California ground squirrels. The choice of inert formulation adjuvants can influence bait acceptance as the presence of lecithin appeared to limit acceptance by Belding's ground squirrels. This finding suggests that future research aimed at identifying a less avoided sticker should be conducted. It is likely that the use of such a sticker would further increase the acceptance of Ceresweet-coated rodenticide baits and produce efficacious rodenticide bait for both California and Belding's ground squirrels.

References

Bourne, J.B., Roy, L.D., Hiltz, M., Merril, P.N., Hoffman, W., 2002. Strychnine baits to control Richardson's ground squirrels. In: Salmon, T.P., Crabb, A.C. (Eds.), Proceedings of the 20th Vertebral Pest Conference, Vol. 20. University of California, Davis, pp. 11–16.

Craver, R.K., Kimball, B.A., Johnston, J.J., Nolte, D.L., 1996. Carbohydrate characterization of malted and unmalted grains. 13th Rocky Mountain Regional ACS Meeting.

Hodge, J.E., Ossman, E.M., 1976. Carbohydrates. In: Fennema, O.R. (Ed.), Principles of food Science. Marcel Dekker, Inc., New York, pp. 41–138.

Howard, W.E., Marsh, R.E., Palmateer, S.D., 1976. Rat acceptance of different sugar concentrations in water baits. Int. Pest Control 11, 17.

Lund, M., 1988. Selection of baits and their distribution. In: Prakash, I. (Ed.), Rodent Pest Management. CRC Press, Boca Raton, Florida, pp. 261–268.

MacLeod, A.M., Travis, D.C., Wreay, D.G., 1953. Studies on the free sugars of the barley grain III. Changes in sugar content during malting. J. Inst. Brew. 59, 154–165.

Marsh, R.W., 1994. Current ground squirrel control practices in California. In: Salmon, T.P., Crabb, A.C. (Eds.), Proceedings of the 16th Vertebral Pest Conference, Vol. 16. University of California, Davis, pp. 61–65.

Mauldin, R.E., Goldade, D.A., Engeman, R.M., Goodall, M.J., Craver, R.K., Johnston, J.J., 1996. Determination of zinc phosphide residues in the California ground squirrel (*Spermophilus beecheyi*) by gas chromatography-flame photometric detection. J. Agric. Food Chem. 44, 189–194.

O'brien, J.M., 2002. Fresh cabbage bait for ground squirrel control. In: Salmon, T.P., Crabb, A.C. (Eds.), Proceedings of the 20th Vertebral Pest Conference, Vol. 20. University of California, Davis, pp. 7–10.

Rice, W.R., 1989. Analyzing table of statistical tests. Evolution 43, 223–225.

Salmon, T.P., Whisson, D.A., Gorenzel, W.P., 2000. Use of zinc phosphide for california ground squirrel control. In: Salmon, T.P., Crabb, A.C. (Eds.), Proceedings of the 19th Vertebral Pest Conference, Vol. 19. University of California, Davis, pp. 346–357.

SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, fourth ed., Vol. 1, Cary, NC: SAS Institute, Inc., p. 943.

Smith, C.S., Wilson, L.S., 1989. Study of a lifetime of sucrose intake by the Fisher-344 rat. Ann. New York Acad. Sci. 561, 291–306.

Spector, A.C., Smith, J.C., 1984. A detailed analysis of sucrose drinking in the rat. Physiology and Behavior 33, 127–136.

Whisson, D.A., Salmon, T.P., Gorenzel, W.P., 2000. Reduced risk anticoagulant baiting strategies for California ground squirrels. In: Salmon, T.P., Crabb, A.C. (Eds.), Proceedings of the 19th Vertebral Pest Conference, Vol. 19. University of California, Davis, pp. 362–364.