Evaluating Norway rat response to attractant and repellent odors to improve rodenticide baiting effectiveness

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Abstract: Control of rodent infestations using acute-rodenticide baiting is frequently fraught with difficulties involving bait-shyness and neophobic effects. To simulate some of the parameters encountered in baiting situations, pre-weighed quantities of Environmental Protection Agency (EPA) standard challenge bait mixture were used as a highly palatable bait base to induce feeding without the need for food deprivation in Wistar-strain laboratory albino rats. Individual animals were tested for attractant effects of 3 odors (rat urine, preputial gland extract, and carbon disulfide) and a natural repellent odor (coyote urine) in an observation area constructed of clear plastic panels containing a central alley and 2 choice compartments. Three measures of rat preference -- the bait intake levels of challenge bait, arena compartment choice, and time expended in each compartment -- suggested that the carbon disulfide at 10 ppm had an attractant effect. None of the other tested odors produced consistent effects on these measures. A low level of 0.20% zinc phosphide (ZP) rodenticide added to the EPA challenge bait was then used to examine effects of the carbon disulfide attractant odor when compared to deionized (DI) water odor in separate groups of Wistar albino rats. This was accomplished by presenting the foods in containers that incorporated an odor-dispensing wick (filter paper) that surrounded the ZP baits. Consumption levels of EPA challenge bait were elevated in the presence of the carbon disulfide odor when compared with DI water odor during a 3-day pre-baiting period. The effect appeared in both males and females, indicating the potential for improved baiting efficacy. Although mortality was 11% higher when carbon disulfide was present as an odor additive to the ZP bait, the mg/kg dosages of ZP ingested were not significantly higher when compared to rats in the DI water group. Thus, the rate of bait feeding may have been increased by the addition of the attractant odor and this then led to increased mortality with no measurable change in rodenticide dosage consumed. This increase in efficacy, if confirmed in field tests, could indicate improved economic and safety features afforded by addition of a carbon disulfide odor ingredient to rodenticide baits.

Key words: attractant, baits, baitshyness, carbon disulfide, odor, rodenticide

Rodent control with baits and traps in agricultural settings is often ineffective for crop protection due to several factors. Among these factors are: bait acceptance problems (neophobia or taste rejection), sub-lethal dosing effects (taste or odor aversion, toxic symptom detection), social feeding habits, and dietary preferences (Berdoy and MacDonald 1991). Frequently, a lack of attraction to the baiting or trapping sites can be due to poor placement of baits or traps, or inadequate pre-baiting.

Although zinc phosphide (ZP) is reported to be an effective, acute rodenticide, numerous researchers have reported bait acceptance problems related to bitter taste and/or sub-lethal illness and subsequent conditioned aversion after rodents ingest minimal levels of bait (Sridhara 1983, Prakash and Ghosh 1992, Reidinger 1995). Bait-shyness effects induced through conditioned taste aversion can last more than a year, even with the ZP removed from the baits. In addition, the bitter taste of ZP sometimes makes it effective only when there is little or no alternative food available other than the rodenticide baits. In no-choice tests, ZP often produces 100% mortality, but in 2-choice tests, mortality levels range from 50-75% (i.e., frequently below the 70% efficacy level suggested for EPA rodenticide registration). Several researchers have also noted the need for an attractant to ZP baits when alternative foods are available, as is frequently the case in field applications.

Despite several published reports indicating many sources of putative attractive or repellent odors (Marsh 1988, Koehler et al. 1994, Mason et al. 1994, Mason 1997, Witmer et al. 1997), only a few laboratory and field studies have empirically evaluated their effects on improving either rodenticide baiting or trapping effectiveness (Burwash et al. 1998*a,b*). The present study was designed to assess the degree of improved baiting efficacy using ZP rodenticide in conjunction with a selected attractant (e.g. sulfur dioxide odor, preputial gland extract, or rat urine) and/or a repellent (predator urine odor) in laboratory tests with albino rats. Results were used for the planning of advanced validation studies to be conducted under field conditions with wild rats and other problem rodent species.

Materials and methods

Animals

Adult Wistar-strain albino rats (30 males - body weight range = 138.7 - 155.8 g ; 30 females - body weight range = 145.0 - 173.8 g) were purchased from Simonsen Laboratories, Inc. (Gilroy, California). Rats were housed in individual suspended cages with rodent food and water available ad libitum throughout the studies. Housing-room conditions consisted of a 12 hr reversed light cycle with the overhead fluorescent lights off at 0600 and on at 1800 MST. Room temperatures were controlled to range between 23 and 27 degrees C with relative humidity levels uncontrolled but low (i.e., typically <30%).

Arena tests

Two arenas (150 cm x 60 cm x 75 cm) for observing individual animals were constructed with clear plexiglass panels. A double-walled partition in the center of each arena contained openings allowing animals unrestricted access to both sides. Spill-proof food cups, each containing 40 g of EPA challenge bait (standard mixture of 5.0% corn oil, 5.0% powdered sugar, 65% ground corn, and 25% ground rolled oats), were attached with spring clips to the far walls of each arena. Animals were individually observed by videotape for the 60 min sessions with DI water presented as a placebo odor material in a 15 ml glass vial near a bait-station food cup on a randomly chosen arena side. Simultaneously, a test odor (preputial gland extract, rat urine, coyote urine, or carbon disulfide) was presented in a 15 ml glass vial near the second bait station food cup on the opposite side of the arena. The carbon disulfide solution was presented successively over 4 levels (0.1, 1.0, 10.0 and 20.0 ppm) to evaluate relative odor concentration effects in different groups of rats. Digital-video tape recordings were used to measure time events (e.g., seconds until initial bait contact, total time in choice compartment, average feeding bout time) and an electronic balance was used to weigh back bait cups so that mass of consumed bait could be calculated and used as the main indicator of attractant or repellent odor effects.

Rodenticide baiting tests

Animals were randomly selected (7 males; 7 females) for each of 4, 14-animal groups. Each group received either: a selected odor attractant (10 ppm carbon disulfide in DI water) placed adjacent to the EPA bait material during both a 3-day pre-bait phase (7 hr per day) and on the ZP bait day (24 hr), or the placebo odor (DI water) during the pre-bait and ZP bait day, or no pre-baiting, but with either carbon disulfide or DI water odors first presented on the ZP bait day. Odor materials were presented under the food cup covers using filter paper wicks saturated with 2 ml of each agent, so that the odors would disperse and surround the top and inside of the cups as the animals fed. Bait consumption levels for each animal were measured and used to calculate the individual mg/kg dosage of ZP ingested. These values formed the data sets for later analysis of effects. Animals surviving the baiting with the rodenticide were euthanized within 4 hr after exposure to minimize rodenticide-induced pain or stress as recommended by Dr. Al Dale, the attending veterinarian for the study.

Data analyses

For tests involving attractant or repellent materials in the arenas, intake of EPA bait for each animal was measured for the 60-min sessions. A one-way analysis of variance (ANOVA) was performed on the data sets to detect preference or repellent effects generated by the candidate odor. In cases where significant effects were observed, video tapes of the sessions were analyzed to measure the seconds of elapsed time until the animals initially fed in each side of the arena, the total time spent feeding at each food cup, and the total times spent in each arena side. For the pre-baited animals in the rodenticide baiting tests, bait consumption levels in the presence of the selected odor attractant (10 ppm carbon disulfide) and placebo (DI water) odor were tabulated on a daily basis. Treatment × day ANOVAs were then performed on these data sets. For the baiting day, the mg/kg ZP intakes were calculated for each animal based on 0.2 % active ingredient in EPA bait. Gender × treatment group ANOVAs for rodenticide dosages and consumption of bait materials were also performed on the data sets for each animal group.

Results

Arena tests

One of the tested odor materials, carbon disulfide at 10 ppm, consistently produced more EPA bait consumption compared to untreated EPA bait ($F_{1,15} = 18.34$, $P = 0.003$). The other tested materials produced no statistically-detected effects on feeding or rat movements in the arenas. The carbon disulfide attractant effect is shown in Table 1 in a comparison of 4 concentrations

Table 1. Consumption levels of EPA bait offered for 60 min in the observation test arenas.

Concentration (ppm)	Carbon disulfide odor-treated bait (g)	DI water odor-treated bait (g)
0.1	1.51 ± 0.31	2.19 ± 0.45
1.0	2.29 ± 0.36	2.06 ± 0.42
10*	3.43 ± 0.51	1.28 ± 0.21
20	1.02 ± 0.35	1.15 ± 0.38

* $P = 0.003$

with DI water as the diluent. Animals in this 10 ppm group also showed a mean of 29% less elapsed time until their first feeding and a mean of 29% more time in exploring on the odor-treated side of the arena. Coefficients of variation for the time measures were, however, relatively high (C. V.s = 0.52 and 0.73, respectively) so that statistical detections of effects were not obtained with these time analyses ($P > 0.25$, both measures) . A comparison between genders for EPA bait consumption levels during the 60 min trials detected a gender effect ($F_{1,10} = 7.56$, $P = 0.046$), with females feeding more than the males in the presence of carbon disulfide odor.

Pre-bait consumption

Table 2 shows the daily bait consumption levels for EPA bait with either 2 ml of 10 ppm carbon disulfide odor or DI water presented under the food cup covers on filter paper wicks. Two trends were apparent in the data sets: a neophobic response of the rats to both carbon disulfide and DI water (control) odors on day 1, and an increase in preference toward the carbon disulfide odor treated bait on days 2 and 3. Both odor treatment effects ($F_{1,52} = 7.75$, $P = 0.0099$) and a day effect ($F_{2,52} = 10.25$, $P = 0.0002$) were detected with the highest consumption levels of carbon disulfide treated bait occurring on days 2 and 3. Females preferred the carbon disulfide odor treated bait more than males based on the consumption levels for days 2 and 3 (female means = 54.7 and 61.9%; male means = 53.2 and 57.2%). However, females also showed more preference than the males for the DI water odor treated bait compared to male consumption levels (female means = 56.3 and 62.2%; male means = 40.7 and 47.9%) on the last 2 pre-bait days. These differences in preference, however, did not achieve statistical significance (P>0.25, both comparisons).

Table 2. Mean consumption levels of EPA bait offered for 7 hr per day during a 3-day pre-baiting period to compare placebo odor (DI water) versus treatment odor (10 ppm carbon disulfide).

	Treatment Groups (Females)					
	Placebo			Attractant		
Day	DI water (g)	No odor (g)	Preference (%)	Carbon disulfide (g)	No odor (g)	Preference (%)
1	4.76	7.06	38.3	6.02	11.72	34.4
2	6.46	5.78	56.3	9.15	7.83	54.7
3	7.47	4.36	62.2	10.17	6.95	61.9

	Treatment Groups (Males)					
	Placebo			Attractant		
Day	DI water (g)	No odor (g)	Preference (%)	Carbon disulfide (g)	No odor (g)	Preference (%)
1	5.13	11.56	31.6	7.40	11.24	38.7
2	9.04	9.23	47.9	11.99	8.66	57.2
3	7.84	10.70	40.7	11.11	10.02	53.2

Zinc phosphide bait consumption

Table 3 shows the mortality ratios and the mean mg/kg dosages of ZP rodenticide consumed during the 24-hr bait exposure in 2-choice test for each of the 4 groups. The increase in consumed dosage of ZP for the female pre-baited groups was pronounced (84%) when compared to female non-prebaited groups. This was reflected in a 2-fold increase in their mortality after baiting. The males showed no increase in ZP consumption when pre-baited, but their mortality increased from 0.0% to 57.1% when pre-baiting was combined with the carbon disulfide odor attractant. This could have resulted from an initial increase in their rate of consumption of ZP bait after pre-baiting.

Table 3. Rat mortality ratios (deaths/group n), percent mortalities, and mean zinc phosphide dosage consumed by the 4 treatment groups and 2 genders after the 24 hr ZP bait exposure.

Group[a]	Ratio	Percent	Dose (mg/kg)
Pre-bait CSM	4:7	57.1	19.63
Pre-bait DIM	1:7	14.3	15.99
Pre-bait CSF	5:7	71.4	36.05
Pre-bait DIF	6:7	85.7	37.14
No pre-bait CSM	0:7	0.0	13.89
No pre-bait DIM	0:7	0.0	20.75
No pre-bait CSF	2:7	28.6	19.13
No pre-bait DIF	3:7	42.9	20.37

[a] Abbreviations: CS = carbon disulfide; DI = deionized water; M = males; F = females \

Discussion

Observational data from the arenas supported and agreed with previous studies (Galef, Jr. et al. 1988, Mason et al. 1988, Mason, 1997) that have found increased food detection, acceptance, and consumption with carbon disulfide present at 10 ppm. This compound was also found as a natural component odor in the breath of rats and mice (Galef, Jr. et al. 1988, Mason et al., 1994). It is thought to act as a safety signal odor when rodents encounter new foods or foods in new places, thereby reducing their neophobic reactions and enhancing their food sampling, acceptance, and consumption levels. The other agents tested in the arena under non-food deprivation conditions did not produce significant effects on EPA bait consumption.

When present as an odor near a bait source in the arenas for Wistar rats, carbon disulfide affected the females more than the males. This agreed with previous findings (Bean et al. 1988, Mason et al. 1994). Dosages of ZP rodenticide consumed were also increased 82-88% in the female rat group given pre-baiting, but this effect was not detected in the male groups. Although mortality ratios were increased in the carbon disulfide (12:28) vs. the DI water (9:28) odor groups exposed to the 0.2% ZP baits, ingested mg/kg dosages were not significantly affected. This could have been an indication that the rats increased their initial rates of ingestion of the rodenticide bait in the presence of the attractant, generating increased oral toxicity, but confirmatory studies would be needed to detect and monitor the putative feeding rate changes. A more rapid consumption rate for ZP or anticoagulant rodenticide baits may lead to a lessened need to leave baits exposed to non-target species over extended time periods. The affects of the odor attractant baits should also be evaluated in non-target animals in future studies. Further research studies are also needed to confirm the attractant effects in wild rodent species in agricultural situations when a variety of alternate food sources and attractive odors may compete with the odor-treated baits.

Management implications

Male rats were shown to be most affected by carbon disulfide odor when the ZP baits were presented and this was reflected in their increased mortality ratios. The pre-baiting procedure per se had the most effect on females, and the presence of carbon disulfide odor did not lead to an additional increase in their mortality ratios. However, females did show the strongest initial attraction to carbon disulfide odor in the short term 60-min arena tests. At this point, reasons for the gender differences are essentially unknown. These observations, however, lead to predictions that the use of ZP baits will be more effective in reducing the number of males relative to the females, whereas with traps and tracking powers, a larger proportion of females may be affected since these management tools depend more on immediate responses of the animals. These effects would probably be amplified in those situations that generate neophobic responses. It may be possible to integrate the attractive properties of carbon disulfide with other rodenticides, traps, or tracking powders to optimize the efficacy of controlling both genders in a given situation. Repellents, if proven effective in field trials, may likewise produce more effects in one gender than in the other in a given situation.

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