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EFFECTS OF CS_2-STARCH XANTHATE ON CONSUMPTION BY RATS

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ABSTRACT: We conducted a series of preliminary feeding trials with Norway rats *(Rattus norvegicus),* roof rats (/?. *rattus),* and Polynesian rats *(R. exulans)* to examine the effects of carbon disulfide (CS_2) on consumption of nontoxic foods. We formulated CS_2 at target concentrations of 10 ppm in deionized water, and of 50 ppm to 100,000 ppm in a starch xanthate matrix. However, we did not analyze actual concentrations of CS_2 in the test foods or measure its rate of volatilization, and thus cannot verify the levels of CSj the rats were exposed to. CS_2 diluted in water and applied directly to food had no apparent effect on consumption by any of the species. Formulations with 50 ppm CS2 in starch xanthate influenced food choice by Norway rats and roof rats in one test, but not in another. Concentrations ^ 10,000 ppm repelled Norway rats. CS_2-starch xanthate had little effect on consumption by Polynesian rats. Further testing is needed to develop effective formulations and delivery methods for utilizing CS_2 as a bait additive.

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INTRODUCTION

Norway rats *(Rattus norvegicus),* roof rats *(R. rattus),* and Polynesian rats *(R. exulans)* cause substantial economic losses in Hawaiian sugarcane fields (Pemberton 1925, Doty 1945, Hood et al. 1970, Tobin et al. 1990) and macadamia nut orchards (Fellows 1982, Tobin 1990). Zinc phosphide is the only rodenticide registered for use in these two crops. However, the results of operational control programs using a variety of zinc phosphide bait formulations have been inconsistent (Pank et al. 1973, Fellows et al. 1982, Sugihara et al. submitted). Bait shyness resulting from sublethal consumption of bait may reduce the efficacy of zinc phosphide baits (Shepherd and Inglis 1993).

Interactions with conspecifics influence food selection by rats (Galef and Wigmore 1983, Posadas-Andrews and Roper 1983, Strupp and Levitsky 1984, Galef et al. 1985, Galef and Stein 1985, Galef 1990), and may also interfere with the development of food aversions (e.g. bait shyness) (Galef 1989, Galef et al. 1990). These effects are mediated largely by olfactory cues (Galef and Wigmore 1983), with rat-produced odors (semiochemicals) being particularly potent (Galef et al. 1985, Galef and Stein 1985, Galef 1990). Carbon disulfide (CS_2) is a volatile component of rat breath (Galef et al. 1988) that has enhanced the attractiveness of food and feeding sites to captive rodents (Bean et al. 1988, Galef et al. 1988, Mason et al. 1988). Incorporating this biologically meaningful cue into rodenticide baits may increase their attractiveness, and thus enhance consumption and reduce bait shyness (Bean et al. 1988, Mason et al. 1988). We conducted a series of laboratory tests to evaluate the effects of CS_2 applied in a starch xanthate matrix on consumption of nontoxic foods by three species of Hawaiian rats.

METHODS

Norway, roof, and Polynesian rats were captured in and around sugarcane fields and forested areas near Hilo, Hawaii, and quarantined at the Denver Wildlife Research Center's Hawaii Field Station for a minimum of 14 days before testing. Rats were maintained in individual stainless steel cages (1 8 x 1 8 x 3 6 cm) with *ad libitum* access to Rodent Laboratory Chow #5001* (Purina Mills, Inc.) and water (reference to commercial products for identification does not imply endorsement by the authors or the U. S. Department of Agriculture). Animals were reweighed, randomly assigned to treatment groups by weight and sex, and transferred to another room for testing. Only healthy appearing Norway rats and roof rats weighing S: 90 g and Polynesian rats weighing ^ 35 g were used. Both quarantine and test rooms were maintained at about 25°C (range 23-26°C) with a 12 hour light: 12 hour dark cycle.

Unless otherwise specified, the same procedures were used in all tests. CS_2 was applied to test food less than two hours before the food was weighed and presented to the animals. For test 1, CS_2 was reduced in strength to 10 ppm by serial dilution in deionized water. For all remaining tests, CS_2 was incorporated into a corn starch xanthate matrix to slow volatilization. Just prior to each trial, we mixed the appropriate amount of CS_2-starch xanthate with the test food for 10-15 min using an electric mixer. We did not verify the actual concentrations of CS_2 in the foods, or monitor changes in concentrations of CS_2 during the trials.

Test foods were weighed into bowls just prior to offering them to the test animals. Norway rats and roof rats were offered 20 g of their assigned test food/animal/trial, and Polynesian rats were offered 10 g of test food/animal/trial. We calculated daily consumption by adjusting the amount of test food offered for moisture gain or loss (based on changes in the weight of three samples of each test food that were exposed in the test room throughout each trial), and subtracting the weight of uneaten and spilled food. Water (all tests) and rodent laboratory chow (single choice tests 1-3) were available *ad libitum* throughout testing. We performed ANOVAs and Duncan's Multiple Range Tests to compare treatments (SAS Institute, Inc. 1988).

Test 1—We offered five rats/sex/species cracked corn treated with 10 drops of dilute (10 ppm) aqueous CS_2, and five other rats/sex/species cracked corn treated with 10 drops of deionized water during a single 14 hour trial.

The CS_2 treatment was prepared by diluting 0.01 % CS_2 in 100 ml deionized water, and then adding 0.1 ml of this solution to 0.9 ml deionized water. A syringe was used to apply 10 drops of the appropriate treatment (10 ppm CS_2 solution or deionized water) to each bowl of cracked corn. Consumption was not adjusted for moisture gain or loss.

Test 2-We offered 0, 100, 1,000, and 10,000 ppm CS_2-starch xanthate-treated oat groats to separate groups of three rats/species during two 20-hour trials.

Test 3--We offered oat groats treated with CS_2-starch xanthate at 0, 1,000, 10,000, and 100,000 ppm to separate groups of five rats (2-3/sex)/species during a single 18-hour trial.

Test 4—Rats were acclimated to the test food by replacing their standard maintenance diet with an *ad libitum* supply of untreated ground rodent laboratory chow for five days. Rats were then fasted for 20 hours before each of four 1-hour trials. During the test, we offered 12 rats of each species (sex ratios balanced as much as possible) two bowls of cinnamon-flavored ground laboratory chow (Purina Rodent Laboratory Chow #5001 with 1 % Schilling Ground Cinnamon* by weight). The chow in one bowl was treated with 50 ppm CS_2-starch xanthate, and the chow in the other bowl was dyed blue with Schilling Food Color* and acetone. Untreated ground rodent laboratory chow was available to the rats *ad libitum* between each trial and the next fast. All animals received the CS_2-chow in the rear of their cages during trials 1 and 3, and in the front of their cages during trials 2 and 4.

Test 5—We conducted two-choice trials to evaluate the effect of CS_2-starch xanthate (50 ppm) on the consumption of two novel foods: oat groats and hulled red wheat. Six 16-hour trials were conducted on alternate days. Five rats (2-3/sex)/species were offered untreated oats vs untreated wheat throughout the study, and five rats/species were offered untreated oats vs CS_2-treated wheat during trials 1-3 (phase 1) and CS_2-treated oats vs untreated wheat during trials 4-6 (phase 2). Rats were fasted eight hours prior to all trials except the first, when they were fasted for 22.5 hours. Rodent laboratory chow was returned to the cages between the end of each trial and the beginning of the next fast. The bowl positions of the tow treatments (front and rear of the cage) were alternated between animals. In successive trials, the positions of the treatments were reversed. Effects were evaluated based on the proportion of total consumption that was oats.

RESULTS

Test 1—Ten drops of 10 ppm CS_2 solution had little effect on consumption of cracked corn by any of the three species (Norway rats: $F = 1.19$; $df = 1,18$; $P = 0.2890$; roof rats: $F = 0.53$; $df = 1,18$; $P = 0.4772$; Polynesian rats: $F = 0.01$; $df = 1,18$; $P = 0.9419$) (Table 1).

Table 1. Consumption of CS_2-treated foods by three species of rats during single-choice tests. The length of each trial was 14 h during test 1, 20 h during test 2, and 18 hour during test 3.

Test	Bait	CS_2 (ppm)	No. Trials	N	Mean consumption (g) (± SE)		
					Norway	Roof	Polynesian
1	Cracked corn	0	1	10	3.5 (1.4)	4.7 (1.2)	1.8 (0.4)
		10	1	10	5.8 (1.5)	3.8 (0.6)	1.9 (0.6)
2	Oat groats	0	2	3	12.4 (0.8)	10.9 (1.5)	3.8 (0.8)
		100	2	3	9.8 (1.7)	9.8 (0.8)	3.8 (0.6)
		1,000	2	3	12.5 (0.4)	10.8 (1.5)	5.1 (0.3)
		10,000	2	3	6.4 (2.7)	9.0 (0.7)	4.3 (1.0)
3	Oat groats	0	1	5	6.3 (2.5)	3.6 (2.1)	1.9 (0.9)
		1,000	1	5	4.5 (2.2)	3.6 (1.6)	2.0 (1.0)
		10,000	1	5	0.2 (0.0)	6.3 (1.6)	1.6 (0.9)
		100,000	1	5	1.1 (0.7)	5.1 (1.8)	2.7 (1.5)

Test 2--CS-,-starch xanthate at 0, 100, 1,000, or 10,000 ppm had little effect on consumption of oat groats by roof rats (F =0.50; df = 3,16; P = 0.6904) and Polynesian rats (F = 0.73; df = 3,16; P = 0.5483). However, Norway rats receiving 10,000 ppm CS_2-treated oats consumed 48 % less than rats in the control group (F = 2.57; df = 3,16; P = 0.0903), suggesting that high concentrations of CS_2 are repellent to this species (Table 1). Consumption varied little between trials for Norway rats (F = 0.61; df = 1,16; P = 0.4456) and roof rats (F = 0.50; df = 1,16; P = 0.4897). Polynesian rats ate less during the first trial (F = 3.85; df = 1,16; P = 0.0674).

Test 3--CS_7-starch xanthate (0, 1,000, 10,000, or 100,000 ppm) had little effect on consumption of oat groats by roof rats (F = 0.52; df = 3,16; P = 0.6737) and Polynesian rats (F = 0.19; df = 3,16; P = 0.9039) (Table 1). Norway rats offered the two higher CS_2 concentrations ate 97% (10,000 ppm) and 83% (100,000 ppm) less than those in the control group (F = 2.72; df = 3,16; P = 0.0789).

Test 4—Total consumption of CS_2-treated chow and dyed chow increased over the course of the four 1-hour trials for each of the three species (Norway rats: F = 5.77; df = 3,44; P = 0.0020; roof rats: F = 20.28; df = 3,44; P = 0.0001; Polynesian rats: F = 12.22; df = 3,44; P = 0.0001) (Figure 1). This suggests an attenuation of neophobia to the novel cinnamon-flavored ground laboratory chow.

For each species, the proportion of the diet comprising CS_2-chow varied as the position of the CS_2-treatment alternated from trial to trial; rats ate more of whichever treatment was presented in the rear of the cage (Figure 1). This is supported by a significant interaction between treatment and trial (Norway rats: F^^o,, = 14.00; df = 3,88; P = 0.0001; roof rats: F^^ = 33.57; df = 3,88; P = 0.0001; Polynesian rats: F^^ = 12.90; df = 3,88; P = 0.0001). CS_2-starch xanthate had no apparent effect on consumption as the proportion of consumption that was CS_2-chow averaged 0.52 for Norway rats, 0.45 for roof rats, and 0.45 for Polynesian rats.

Test 5—Total consumption of oats and wheat was similar in both groups of Norway rats (F = 0.05; df = 1,8; P = 0.8325), roof rats (F = 0.01; df = 1,8; P = 0.9218), and Polynesian rats (F = 2.52; df = 1,8; P = 0.1514) (Fig. 2). There was an interaction between the effects of trial and phase for Norway rats (F^^don = 11.76; df = 2,16; P = 0.0007) and roof rats (F^^^ = 5.48; df = 2,16; P = 0.0154).

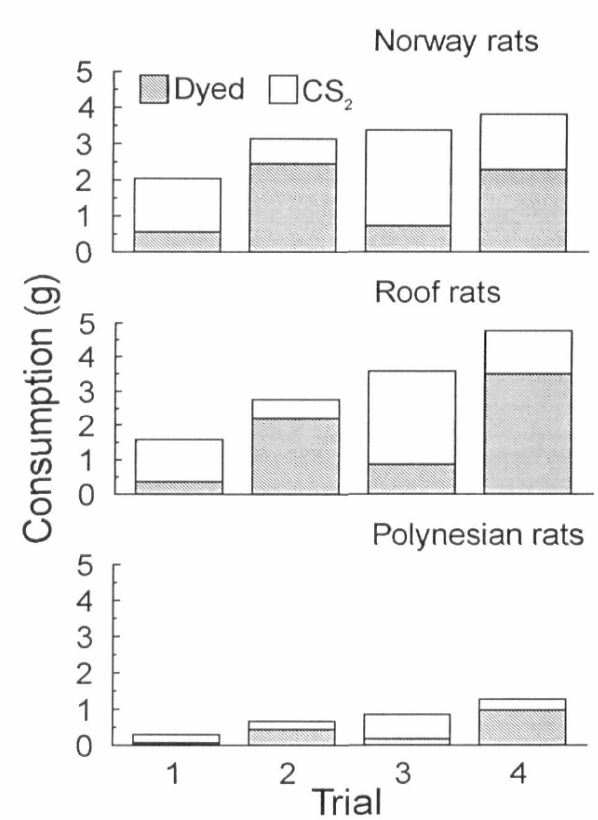

Figure 1. Mean consumption of dyed and CS_2-treated (50 ppm) cinnamon-flavored ground laboratory chow by 12 rats of each species during 1-hour feed trials. All animals received CS_2-chow in the rear of their cages and dyed chow in the front of their cages during trials 1 and 3. The bowl positions were reversed during trials 2 and 4.

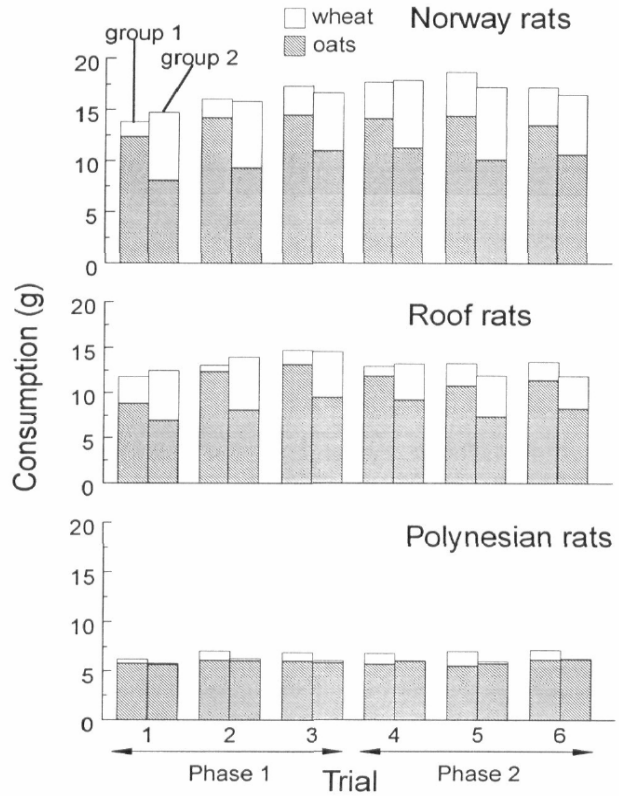

Figure 2. Mean consumption of wheat and oats by rats during 16-hour feeding trials. The five rats in group 1 received untreated grains throughout the test. The five rats in group 2 received untreated oats and CS_2-treated oats (50 ppm) and untreated wheat during phase 2.

Consumption increased during phase 1 and leveled off during phase 2, suggesting an initial neophobia to these novel foods.

All three species consumed more oats than wheat throughout the test (Figure 2). Norway rats ($F = 2.93$; $df = 1,8$; $P = 0.1253$) and roof rats ($F = 4.69$; $df = 1,8$; $P = 0.0623$) ate more wheat when it was treated with CS_2-starch xanthate. Polynesian rats that received CS_2-treated wheat consistently ate less of this grain than did rats in the control group, although this difference was statistically marginal ($F = 1.76$, $df = 1$, $P = 0.2211$). Once established, food preferences for all three species persisted for the remainder of the test.

DISCUSSION

Free-ranging rats that are surrounded by familiar foods and odors probably perceive rodenticide baits as novel food and consume them cautiously. This can lead to sublethal consumption and subsequent bait shyness. By enhancing initial bait consumption, CS_2 might improve the efficacy of operational baiting with rodenticides.

Previous studies indicate that solutions of 10 ppm CS_2 enhanced consumption of bait by laboratory house mice (dropped directly on food, Bean et al. 1988) and wild Norway rats (in a vial adjacent to bait, Mason et al. 1988). When we applied 10 drops of a 10 ppm-CS_2-deionized water solution to cracked corn, we saw no evidence of enhanced feeding by any of the 3 species of rats in our study. In the remaining tests, we formulated test foods with 50-100,000 ppm CS_2 in a starch xanthate matrix. In no-choice tests, CS_2-starch xanthate had little effect on consumption by roof rats and Polynesian rats, but appeared to repel Norway rats at high concentrations. CS_2-starch xanthate had no apparent effect on consumption by rats offered a choice between a novel-flavored food treated with dye and the same food treated with CS_2-starch xanthate. In another test, Norway rats and roof rats increased their consumption of wheat relative to oats when the former was treated with CS_2-starch xanthate. CS_2-starch xanthate had little effect on consumption by Polynesian rats in any of the tests.

CS_2 volatilizes rapidly, and any initial effects on consumption during our tests may have been obscured by later feeding. Bean et al. (1988) offered house mice CS_2-treated food for only 20 minutes, and Mason et al.(1988) placed CS_2 in solution adjacent to bait in a vial with a wick during overnight trials with wild Norway rats. Also, both Bean (1988) and Mason (1988) used bait enclosures that presumably concentrated the CS_2 odor. We incorporated CS_2 in a starch xanthate matrix to slow volatilization. However, we did not analyze the actual concentrations of CS_2 in the foods and did not monitor the rate of volatilization. Thus, we cannot verify what levels of CS_2 the rats were exposed to. Future testing to evaluate CS_2 as a bait additive should determine the actual concentration of CS_2 in the baits as well as the rate of volatilization.

SUMMARY

We conducted a series of preliminary feeding trials with Norway, roof, and Polynesian rats to examine the effects of various concentrations of CS_2-starch xanthate on consumption of nontoxic foods. CS_2-starch xanthate enhanced consumption in only one test, and only for Norway rats and roof rats. High concentrations of CS_2-starch xanthate repelled Norway rats. None of the concentrations altered consumption by Polynesian rats. Further testing is needed to develop an effective formulation and delivery method for utilizing CS_2 as a bait additive.

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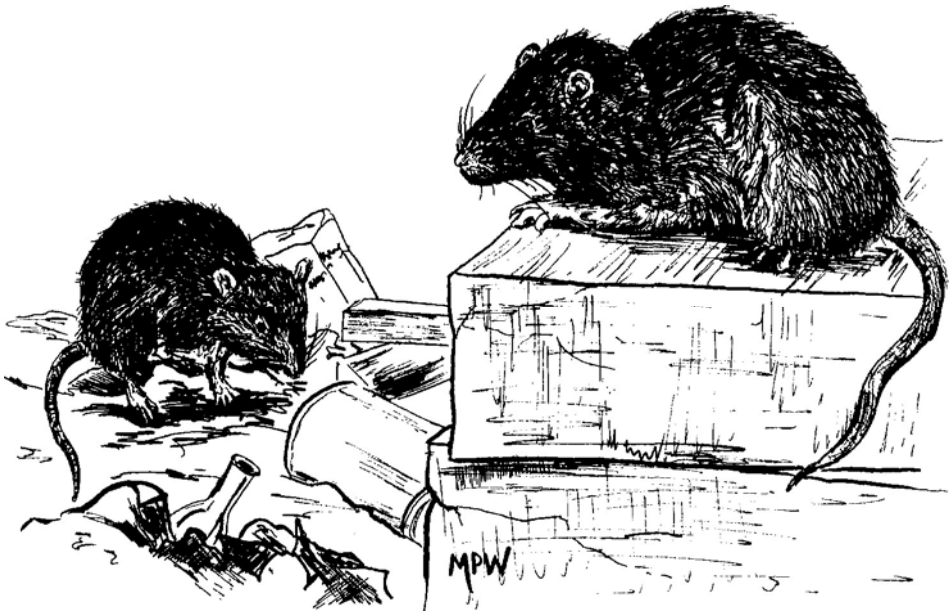
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