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PREPARATION AND ANALYSIS OF ALTERNATIVE
4-AP BAITs FOR BLACKBIRDS

by J. E. Davis, D. J. Cunningham and J. L. Cummings/1/

ABSTRACT

Red-winged blackbird (Agelaius phoeniceus) acceptance of corn as a bait for use in ripening sunflower has been questioned. This study demonstrated that sunflower meats, sunflower achenes, and pearled barley all have potential as alternative bait carriers for 4-AP. Each can be treated with 4-AP so that individual bait particles carry dosages equivalent to those of cracked corn in the registered product, Avitrol FC Corn Chops 99S. Treated baits produced a distress response in redwings in times ranging from 21.7 min (sunflower meats) to 64.5 min (sunflower achenes). The time to distress elicited by sunflower meats coated with 4-AP was similar to that obtained with cracked corn baits used in the commercial product. Simulated rainfall or contact with moist soil resulted in a loss of 4-AP from all treated baits. Sunflower achenes proved the most durable of the baits, retaining 75% of their original 4-AP content following 1/4 in of simulated rainfall.

INTRODUCTION

Blackbirds invade ripening sunflower and corn fields in flocks which can number in tens of thousands. Avitrol FC Corn Chops 99S is a commercial product used to disperse these flocks. It consists of chopped corn bait where one in 100 particles is treated with 3% 4-aminopyridine (4-AP). Distress behavior results from birds ingesting a treated particle, and the subsequent vocalizations and erratic flights are intended to frighten the remaining flock from the baited field. Success depends upon bait acceptance, two important aspects of which are probably a (1) fast response time to treated

birds, and (2) number of birds exhibiting distress behavior at the same time.

Avitrol FC Corn Chops 99S has given inconsistent results when tested in ripening sunflower (Guarino 1974 unpubl. rept. No. 45/2/, Besser and Cummings 1975 unpubl. rept. No. 59, Henne et al. 1979, Jaeger et al. 1983). Poor bait acceptance by the principal pest species, the Red-winged Blackbird (Agelaius Phoeniceus), is believed to be an important reason. Field observations and tests indicate that sunflower achenes or meats (Knittle et al. 1985 unpubl. rept. No. 202), millet (Besser 1981 unpubl. rept.), and pearled barley (Burst and Cunningham 1982 unpubl. rept. No. 230) are all more readily accepted by redwings than is cracked corn. However, more information is needed on the quantitative and qualitative chemical properties of these baits. This paper describes (1) the treatment of each of these four alternative baits with 4-AP, (2) the laboratory analysis of treated baits, (3) the bioassay results on caged redwings, and (4) bait durability with respect to moisture.

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METHODS AND MATERIALS

Bait Preparation

Sunflower and millet whole seed were soaked in a solution of 4-AP, the edible seed being impregnated through the hull in this manner. Sunflower achenes (200 g) were placed in a sealed container and soaked for 72 hr in a solution of 5.0 g 4-AP, 87.3 ml methanol, 2.7 ml water, and 5.0 ml HCl. After soaking, the achenes were drained and

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2. Unpublished reports cited here refer to Bird Damage Research Reports available upon request from the Section of Bird Damage Control, Denver Wildlife Research Center, Denver Federal Center, Denver, Colorado 80225.

air dried. The same procedures were used for treating millet, where a solution containing 20 g of 4-AP (HCl) dissolved in 20 ml of water was used as the soaking solution for 100 g millet.

Sunflower meats and pearled barley were surface coated, although it is likely that a small percentage of 4-AP was impregnated into the seed itself. One hundred g of each bait was placed separately in a pint jar, each jar containing 3.0 g 4-AP, 4.0 ml methanol, 2.0 ml water, and 3.0 ml of HCl. Jars were rotated until a thick film had formed around the walls. Each jar was briefly uncapped, allowing partial evaporation of solvents, resealed, and rotated to coat the seeds with 4-AP paste from the sides of the jar. This process of rotating and venting was repeated until no additional material could be removed from the jar walls. Baits were then removed and air dried.

Determining 4-AP Levels

Ten bait particles were randomly selected from each of the four bait types in order to sample 4-AP levels. For each bait type, five particles were analyzed individually and five as a composite. For sunflower achenes the hulls were removed and the meats inside analyzed. Baits were macerated with mortar and pestle and extracted with 2 ml (individual samples) or 10 ml (composite samples) of methanol. Each sample was extracted for 2 hours in a sealed test tube followed by 3 minutes of agitation on a vortex mixer. Five μ l were drawn from each extract and individually applied to a silica gel thin layer chromatography (TLC) plate in 6 mm diameter spots. A series of reference standards were applied to the same plate to bracket the unknown values where each spot in the series contained from 0.5 to 5.0 μ g of 4-AP (HCl) dissolved in methanol. The TLC plate was then placed in a chromatography tank containing toluene and ethyl acetate (1:1), and the solvent front allowed to rise 10 cm. The plate was removed, dried, and placed in a second tank containing methanol. The front was again allowed to move 10 cm. Following drying the plate was examined under

short wave UV light (254 nm) and each sample spot was visually matched to the density of the reference standard that it most closely resembled. Micrograms of 4-AP per microliter of sample were then averaged to determine the 4-AP content of the bait(s). Although the active ingredient on baits is actually 4-AP HCl, all values in this paper are reported as free-base equivalents. This was done to allow direct comparison to earlier studies in which 4-AP was used to treat baits.

Bioassay

Treated samples of each bait type were tested for distress inducement and toxicity on six individually caged male redwings that had fasted for 2 hours. Whole sunflower achenes were not tested; instead the hulls were removed and the impregnated meats tested. Each bird was dosed by force-feeding it a single weighed bait and it was then returned to its cage for observation. The length of time to first distress response and to death were recorded.

Moisture Resistance

Bait moisture resistance was tested with simulated rainfall and prolonged contact to moist soil. To simulate rainfall, water was passed through a linear arrangement of inverted T-jet conical spray tips and the flow rate was adjusted to provide 1/4 in of water in approximately 20 min. Bait samples were spread on a wire mesh rack to allow water to drain. After water exposure baits were air dried under ambient conditions and then analyzed for 4-AP. Conditions of moist soil and high humidity were simulated by placing a second bait sample on dampened soil in a tray covered by a sheet of plastic suspended 8 cm above the soil surface. Baits were observed daily for changes in their appearance and samples were removed at 24 hr intervals, air dried, and analyzed for 4-AP.

RESULTS AND CONCLUSIONS

4-AP Uptake

Analysis of each bait type indicated that 4-AP levels were within 2 to 16% of the 780 μ g 4-AP per particle mean

content of the commercial product (Table 1). Mean 4-AP levels in the different baits varied from 652 µg in sunflower achenes to 800 µg in pearled barley which is within the 600 to 900 µg dosage range necessary to adequately affect blackbirds with single baits (Cunningham et al. 1982, unpubl. rept. No. 245). With the exception of millet, the current registered limit of 3% 4-AP was not exceeded on any bait material. Millet is small, averaging 6 mg per particle, thus, it was necessary to exceed the 3% 4-AP concentration in order to provide enough chemical to yield an effective dose on a single bait. The high concentration of 4-AP on millet resulted in the baits absorbing moisture (4-AP HCl is hygroscopic) and they were almost impossible to dry. Analysis of sunflower meats yielded 4-AP levels ranging from 1.5 to 2.1% for baits receiving a 3.0% treatment. The discrepancy between the amount of 4-AP present in the treatment solution and that analyzed in sunflower meats is unexplained. Additional study is nec-

essary in order to determine if this represents a chemical degradation during treatment or a limitation in extraction efficiency.

Bioassay

The mean time to distress was significantly faster ($P < 0.05$, Kruskal-Willis Test) with sunflower meats than with either achenes or barley (Table 2). The time of 21.7 minutes for meats is similar to that for 4-AP treated cracked corn in Avitrol FC Corn Chops 99S. The difference in response times between treated meats and meats taken from treated achenes may stem from the treated meats being primarily surface coated making the 4-AP more readily absorbed by the bird. Because achene meats are impregnated, the 4-AP is less readily available and the uptake by the bird is slower. This does not explain the slow response time to 4-AP surface-coated barley. Millet did not receive a comparable bioassay in the laboratory, however, in the field, two female redwings each treated with a single

Table 1. Analysis of 4-AP Uptake by Different Baits.

	Weight (mg)	Mean particle		
		4-AP (µg)	4-AP (%)	4-AP dose/ ^{1/} (mg/kg)
Sunflower meats	46.4	718.6	1.5	10.3
Sunflower achenes	39.6(58.1)/ ^{2/}	652.2(1401.0)	1.6(2.4)	9.3(20.0)
Pearled barley	37.9	799.5	2.1	11.4
Proso millet	7.3	712.6	9.8	10.2
Commercial Product	-	780	-	-

1. Dosage based on ingestion by 70 gm bird.
2. Values outside parenthesis are from meat portion of achene, while values in parentheses represent the entire achene.

Table 2. Bioassay of Alternative 4-AP Baits on Redwings.

	Mean bird weight (g)	Mean/ ^{1/} dose (mg/kg)	Mean time first distress (min ± 1 SD)	Mean time death (min)
Sunflower meats	68.3	13.3	21.7(6.7)	49.8
Sunflower achenes/ ^{2/}	69.4	14.6	64.5(64.4)	101.5
Pearled barley	63.2	15.7	51.2(26.7)	82.5

1. Mean concentration of 4-AP of treatment batch multiplied by weight of bait.
2. Meat portion of achene used for bioassay.

millet bait distressed in 12 and 14 minutes (Cunningham pers. comm.).

Bait Durability

After 1/4 in of simulated precipitation, sunflower meats, achenes, and barley lost 68%, 25%, and 87%, respectively of their original 4-AP content. When exposed on moist soil, sunflower meats and barley lost 50% or more 4-AP at 24 hr, while it took 168 hr for the achenes to lose this much. Sunflower meats appeared to have physically decomposed and discolored at 4 to 5 days and achenes at 6 days. The hull of the sunflower achene, provides some protection against 4-AP loss due to moisture and bait deterioration. If meats and barley also prove to be desirable baits, methods are needed to protect them from 4-AP loss and deterioration.

Conclusions

(1) This study demonstrated that sunflower meats, sunflower achenes, and pearled barley all have the potential to be alternative bait carriers for 4-AP. Each can be treated with 4-AP so that individual bait particles

carry dosages equivalent to those of the cracked corn in the registered product, Avitrol FC Corn Chops 99S.

(2) Sunflower meats coated with 4-AP showed the most rapid distress response when given to redwings, and the times were similar to those obtained with the cracked corn baits in the commercial product.

(3) Sunflower achenes were the most durable of the baits with exposure to moisture.

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