Roger W. Bullard,[1] *Edward W. Schafer, Jr.,*[1] *and Richard L. Bruggers*[1]

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Tests of the Enhancement of Avian Repellent Chemicals with Sensory Cues

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ABSTRACT: Spray applications of nonlethal repellents are often effective means of protecting maturing agricultural crops from bird damage. Repellents are generally classified as either primary, in which case the animal reacts to the noxious effects of the chemical alone, or secondary, in which case conditioned aversion responses are induced. Secondary repellents more reliably resist high bird depredation of these crops over time. Unfortunately, most pest bird species are not able to detect some secondary repellents at levels which induce conditioned aversion. Thus, higher levels must be used than are actually needed for crop protection. One alternative to this dilemma has been the incorporation of an inexpensive sensory cue (for example, calcium carbonate) into the formulation to replace some of the repellent and reduce crop protection costs. Methiocarb [3,5-dimethyl-4-(methylthio)-phenol methylcarbamate (Mesurol)], currently the most effective avian repellent for field crops, has been the model chemical for testing the action of secondary repellents at the Denver Wildlife Research Center. This paper presents a general review of laboratory and semifield tests used to evaluate various methiocarb-cue (olfactory, tactile, and visual) formulations on both quelea (*Quelea quelea*), a destructive African pest, and red-winged blackbirds (*Agelaius phoeniceus*), a problem species in the United States. Single-choice and paired-preference test methods used on wheat, rice, millet, sorghum, and sunflower in individual cages and group enclosures and conducted under conditions of varying palatability in the alternate food are described. Appropriately designed tests to answer specific questions will lead to further advancements in effectively using chemical repellent-cue combinations for protecting crops from bird pests.

KEY WORDS: vertebrate pest control, birds, repellents, repellent enhancement, sensory cues, preference test methods, alternate foods

Developing chemical repellents to prevent vertebrate pest damage to agriculture and silviculture crops has been a research priority of many laborato-

[1]Research chemists, and research biologist, respectively, U.S. Fish and Wildlife Service, Denver, Colo. 80225.

ries for years. A repellent has been defined by Rogers [1] as a "compound or combination of compounds that, when added to a food source, acts through the taste system to produce a marked decrease in the utilization of that food by the target species." He separated repellents into primary repellents, with which the animal reacts to the taste of the repellent alone, and secondary repellents, with which the taste of the repellent becomes a cue to later adverse effects.

Many primary repellents have been tested for protecting agricultural crops from bird damage, but none has produced reliable field results. Naturally occurring tannins in bird-resistant sorghum seeds have probably been the most effective. However, even the most effective varieties may be consumed if bird pressure (high populations or scarce alternative food, or both) is high [2]. Topically applied primary repellents have been even less successful. Although Curb[2] has provided some protection under field conditions [3], wattle tannin was not effective against quelea (*Quelea quelea*) when applied topically to cereal grain crops [4]. In studies with red-winged blackbirds (*Agelaius phoeniceus*) by Rogers [5], and black-capped chickadees (*Parus atricopellus*) by Alcock [6], only secondary repellents showed promise in high bird pressure situations. Apparently, only toxic, emetic, or nauseating effects of a chemical are sufficient stimuli to control crop damage when bird pressure is high.

Because of the limitations with primary repellents, the failure to exploit the secondary repellent concept, and the time and cost involved in registering new products, only one avian repellent, methiocarb, is widely used. Unfortunately, the chemical is too expensive for foliar use on many low value crops because of the large quantities required for protection. Several studies at the Denver Wildlife Research Center (DWRC) on quelea have indicated that methiocarb caused aversion in birds at levels below which the birds could make taste discriminations [7]. Inexpensive chemicals that could be recognized by the tactile, visual, or olfactory senses (wattle tannin, blue dye, and peppermint oil, respectively) were added to formulations, making them effective at lower methiocarb concentrations and thus lower application costs. This paper describes a wide variety of these tests that we used to study cue enhancement of the repellent activity of methiocarb formulations; these tests may be equally applicable to other secondary repellents.

General Procedures

Several basic considerations apply regardless of the test methodology used. Wild captured birds should be acclimatized for at least four weeks in large aviary cages (>20 m^3) before being tested, the photoperiod should be maintained at a constant light-to-dark schedule, and the test birds should be allowed to adapt to any change in the test cages for at least one to two days on their

[2]Synergized aluminum ammonium sulfate manufactured by Sphere Laboratories Ltd., London, Great Britain.

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normal ration before the food is changed. Also, the birds should be preadapted to any new food base [*8*], which is accomplished by mixing the new food item into the normal ration in increasing amounts over three or four days.

For individual cage tests on quelea at DWRC, the birds are transferred stepwise from the aviary into 53- by 25- by 31-cm communal cages for at least two weeks and then placed in individual 15- by 23- by 15-cm cages for testing [*8*]. Some gregarious birds like quelea, when tested individually, appear to exhibit less stress when caged next to a bird that they can see [*8*]. With other species (for example, redwings), confinement in small individual cages induces considerable stress and may influence the results of preference tests [*9*]. Enclosure studies with groups of birds thus may help alleviate stress and better approximate normal flock behavior.

Single-Choice Tests

Single-choice tests provide the most rigorous assessment of repellency. The treated food is the only source of nourishment for the test period (usually 18 to 24 h). Because of these rigorous requirements, the Denver Wildlife Research Center and others have used the single-choice R_{50} measurement (the concentration that will repel 50% of the test birds from a food source) as the basic index of avian repellent activity of candidate chemicals [*10, 11*]. The repellent qualities of methiocarb were initially recognized through this test. However, this paradigm apparently is not satisfactory for measuring cue enhancement of repellent activity. The food consumption in tests of methiocarb-cue (oleoresins and food colors) combinations was equivalent to that observed from earlier tests with methiocarb alone. The birds simply reacted to the total formulation; the food source and additives were of little influence.

Two-Choice Preference Tests—General

Two-choice preference tests involve the presentation of two food items, under identical conditions, to caged (individual or group) birds. Experience has taught us that the food items should not be placed more than one fourth the width of the cage apart or in a manner that other factors (such as location of perches, water, or doors) could influence the birds' choice. The two food items should be rotated at least daily, and an even number of trials should be conducted (that is, 2, 4, 6, and so on). Shumake et al [*12*] have developed a device that mechanically rotates foods at 1-h, 15-min, or 5-min intervals.

Two-choice preference results can be expressed in a number of different ways, but we usually expressed it as

$$\text{percent preference} = \frac{\text{treated consumption (in grams)}}{\text{treated + control consumption (in grams)}} \times 100$$

We normally analyze our data with a paired t-test to determine the difference between the mean daily consumption of the two foods and with a two-factor analysis of variance (AOV) when several treatments are each tested against a common control. If differences are significant, Duncan's multiple range test [*13*] is used to separate treatment means.

Two-Choice Preference Tests—Equally Preferred Alternate Food

Individual Cage

This paradigm is often used after the single-choice R_{50} method to obtain increased definition of the repellent properties of a chemical [*8*]. Chemicals are initially surface-coated at 0.01 to 0.1% on hulled proso millet seed and tested against the untreated seed on quelea. Only the most active primary repellents will show significant differences between treatment and control ($P \leq 0.05$). Usually at least three of the six test birds have a stereotyped left- or right-handed position preference that a primary repellent at 0.01% will not change. Secondary repellents can be recognized through observing the consumption of treated and untreated food during a six-day test period (Table 1).

It has been our experience with quelea that if a candidate repellent tested at 0.01% elicits nearly complete avoidance for the entire test period, it possibly has secondary repellent properties and produces an adequate sensory stimulus to be detected at low levels. Some plant extracts fall into this category (such as *Agarum caudatum* and *Veronia gigantea* tested at our laboratory). Birds display highly erratic responses after the first day to secondary repellents which are not detectable at low levels because aversion has been associated with the position of the food rather than its taste. There will generally be an overall decrease in total food consumption for at least the second and third test days. For example, in one study the secondary repellent methiocarb without a cue exhibited these characteristics at 0.008% (Table 1). However, when calcium carbonate cue was added, the birds readily avoided the treated food. Thus, by conducting tests using a series of concentrations (Table 1), one can determine the level (0.008% in this example) at which a secondary repellent induces aversion.

Laboratory Enclosure

Quelea—The laboratory enclosure is used to determine group responses to repellent treatments on cereal grain heads [*8*]. In one study five quelea were housed in a 2.5- by 2.4- by 2.2-m screened cage. A hexagonally shaped test apparatus (with 63.5-cm sides), positioned in the center of the cage, served as a simulated "field" for the placement of sorghum heads. Three methiocarb-treated sorghum heads were separated from three untreated heads by a 26-cm

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TABLE 1—*Mean percent preference*[a] *of* Quelea quelea *for methiocarb-calcium-carbonate*[b]*-coated wheat seeds in a two-choice test against untreated wheat seeds (individual cage tests, six birds per treatment).*

Treatment Formulation[c]									
Methiocarb, %	$CaCO_3$, %	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Overall Mean	*P* Value (*t*-Test)
0.0015	2	56.63	47.26	47.32	56.79	55.92	63.74	54.76	0.302
0.008	2	21.28	21.52	21.17	29.48	22.95	31.47	26.37	0.001
0.016	2	22.74	17.22	9.70	7.52	0.00	7.00	11.09	0.0002
0.024	2	19.94	15.05	0.32	0.46	0.00	1.06	5.06	0.00002
0.008	0	53.76	20.08	56.24	48.61	69.17	36.36	48.94	0.126

[a]The percent preference is the percentage by weight of the treated wheat seeds in relation to the total wheat seeds consumed (treated food consumed + control food consumed = 100 %).

[b]Calcium carbonate used as a cue to enhance the repellent properties of methiocarb formulations.

[c]Respective component or components surface coated on soft winter wheat seeds and tested in paired preference against untreated control wheat seeds.

high by 120-cm wide board, and the positions were alternated daily for six days. The results of a series of methiocarb, cue, and methiocarb-cue studies [*7*] closely resemble the results from the individual cage tests, in that the heads treated with methiocarb and a cue were consistently avoided. The cue-only treatments produced no significant avoidance, and the methiocarb-only-treatment was intermediate, with preference vacillating from day to day.

Red-winged Blackbirds—We conducted another study to determine the level of methiocarb that would reliably repel redwings from ripening sunflower seeds. (It is difficult to apply enough chemical to sunflower seeds in the immature head to induce the conditioned-avoidance response in birds [*14*].) Immediately before the test, we applied each treatment using a calibrated spray table to fresh sunflower heads, and the excess moisture was removed with fans. Then two treated heads, two untreated heads, and a feeder containing Purina Layena, were placed in 1.2- by 1.2- by 1.8-m enclosures (four replications) containing four birds each. Damage (in square centimetres) was read by template [*15*] at 21 h. One-tailed paired *t*-tests of the data ($P \leq 0.05$) indicated that 8.4 kg/ha was the lowest level that would elicit avoidance (Table 2). It would have been desirable to have used more replications, as indicated by the 2.8-kg/ha results. The enhancement effect of cues is illustrated by the percent preference for the 5.6-kg/ha and 16.8-kg/ha treatments compared with and without cue. Damage to the methiocarb-cue-treated heads was lower in both tests. An equation regressing preference on kilograms per hectare of methiocarb was significant ($P = 0.00002$); however, there was no significant correlation between the total damage and the alternate food consumption. This method appeared to be more appropriate than the less-preferred alternate-food method discussed in the section on laboratory enclosures using sunflowers.

TABLE 2—*Paired preference (equally preferred alternate food) enclosure test of methiocarb-cue protection from red-winged blackbird damage to sunflower heads.*

Methiocarb, kg/ha	$CaCO_3$,[a] kg/ha	Alternative[b] Food, g	21-h Damage, cm²		Percent[c] Preference	*P* Value (Paired *t*-Test)
			Treated	Control		
16.8	56.1	19.0	85	722	10.5	0.015
8.4	56.1	79.0	230	650	26.1	0.003
5.6	56.1	23.0	305	745	29.0	0.091
2.8	56.1	69.0	355	560	38.8	0.002
0.0	56.1	38.6	400	410	49.4	0.470
16.8	0	13.0	140	488	22.3	0.012
5.6	0	31.0	410	385	51.6	0.575

[a]Calcium carbonate used as a cue to enhance the repellent properties of methiocarb formulations.

[b]Purina Layena poultry mash provided in the poultry feeder in the center of the cage.

[c]The percent preference is the percentage in square centimetres of damage to the treated sunflower heads compared with the total damage (treated sunflower damage + untreated sunflower damage = 100%).

Two-Choice Preference Tests—Less Preferred Alternate Food

By altering the palatability of the alternate food, one has another means of investigating the repellent properties of a chemical. Rogers [5] used this less-preferred alternate food approach in his early investigations of the secondary repellent properties of methiocarb. This method involves treating a preferred food and choosing a less preferred food as the alternate food. Thus, one can "titrate" the activity of a secondary repellent by manipulating the acceptability of the alternate food or by prescreening for birds that have distinct preferences for one food over another.

Individual Cage

Garrison [16] preselected quelea that preferred hulled proso millet (≥70%) to Martin X sorghum in two-choice preference tests. These birds were then tested on a decreasing series (3.33×) of methiocarb-millet formulations against the sorghum (Table 3). The 0.0013% concentration elicited some change in preference, and the 0.0045% level caused nearly a complete switch to the nonpreferred sorghum. Garrison used this method to obtain an R_{50} index of 0.0015% which is ten times less than had been obtained earlier on quelea given no choice [10].

The same procedure was used to test wattle tannin (a primary repellent) on prescreened quelea [4]. Untreated millet was significantly preferred ($P \leq 0.05$) over 0.2% coated millet, but, with sorghum as the alternate food, tannin levels as high as 0.68% tannin did not induce repellency.

TABLE 3—*Consumption of methiocarb-treated millet and nontreated sorghum grains by caged quelea (individual cage tests, two choices with less-preferred alternate food).*[a]

Number of Birds	Sex	Treatment Concentration	Total Food Consumption, g	
			Millet	Sorghum
3	♂	0.015	1.0	20.2
3	♀		1.2	24.0
3	♂	0.0045	1.7	29.0
3	♀		3.1	32.4
3	♂	0.0013	23.5[b]	11.9
3	♀		23.9[c]	10.4
3	♂	0.00039	30.0	5.9
3	♀		34.7	3.8

[a]Courtesy of M. V. Garrison of the Denver Wildlife Research Center.
[b]One male consumed more nontreated sorghum than treated millet.
[c]One female consumed more nontreated sorghum than treated millet.

Laboratory Enclosure

This paradigm was tested as an alternative to the equally preferred alternate food method discussed earlier for determining the level of methiocarb that repels redwings from sunflowers. Two treated heads and Purina Layena poultry mash were placed in the enclosures (four replications) containing four birds each. Sunflower head damage and alternate food consumption at 21 h were measured (Table 4). The most obvious effect was methiocarb related. Twice as much damage occurred with untreated heads as with either 5.6- or 11.2-kg/ha treatment and 4.67 times as much damage with the 16.8-kg/ha treatment. Alternate food consumption had little measurable influence except possibly for the untreated head tests where no conditioned aversion occurred. Birds ate much higher quantities of sunflower, in this test, leaving less stomach capacity for the less preferred alternate food. An equation regressing square centimetres damage on kilograms per hectare of methiocarb was significant ($P = 0.01$), but it is obvious that this method is less sensitive than the equally preferred alternate food method for measuring small differences in methiocarb activity and consequently is less useful for the intended objective of detecting the lowest effective methiocarb concentration.

Field Enclosure

Methiocarb-cue studies on quelea have been conducted in enclosure studies of standing crops in Africa [7]. A 1- by 1- by 2-m (2-m^3) screen cage was placed over stands of sorghum or millet. Ten quelea were placed in each cage after the chemical application. Millet and sorghum grain (less palatable than the immature crop seed) were available as alternative food in containers suspended

TABLE 4—*Paired preference (less preferred alternate food) enclosure test of methiocarb-cue protection from red-winged blackbird damage to sunflowers.*

Methiocarb, kg/ha	$CaCO_3$,[a] kg/ha	Sunflower Damage, cm^2	Alternate Food Consumed, g
0	0	1714	58.0
0	56.1	1081	37.5
0	112.2	1388	85.0
5.6	0	470	44.3
5.6	56.1	751	128.0
5.6	112.2	863	109.0
11.2	0	485	58.0
11.2	56.1	602	111.0
11.2	112.2	914	92.0
16.8	0	275	84.0
16.8	56.1	340	81.0
16.8	112.2	280	101.0

[a]Calcium carbonate used as a cue to enhance the repellent properties of the methiocarb formulation.

[b]Purina Layena provided in the poultry feeder in the center of the cage.

from the cage tops at head height. In one study, three replications of 0.5% methiocarb plus 1% tannin were applied to both millet and sorghum plants in the dough stage. An identical set of cages was sprayed with 1.0% methiocarb, and a third set was left untreated. A significant treatment effect was detected for both cereals ($P \leq 0.001$; Kruskal-Wallis test) in that untreated sorghum and millet received significantly greater damage than either of the two treatment groups. Also, for both cereals there were no differences in damage between the two treatments. Similar results were later obtained in identical enclosure studies on wheat [7].

Method Combinations

Shumake et al [17] combined some of these principles in a more sophisticated study of the relative importance of taste versus visual feeding cues that could become associated with methiocarb exposure. Groups of quelea were preexposed to the specific stimuli in two-choice preference tests (that is, blue versus green; sweet versus sour) during the first week. During Week 2, the birds in each group were exposed every other day to 0.018% seeds treated with the color or taste that had been preferred during Week 1. The groups were then given three 18-h choice periods identical to the preexposure period each week. The changes in paired-preference patterns, influences of differences in

Discussion

The choice of a test method for assessing specific properties of a repellent is extremely important. If it is a rigorous test of overall food protection potential, then the single-choice R_{50} index method is probably appropriate. If one wishes to define the mode of action of the repellent, then the equally preferred alternate food paradigm is appropriate. However, concentrations must be kept low (ten times lower than the single-choice method) so that relative activity can be compared among chemicals. Subsequent tests with repellent-cue combinations would further clarify these differences.

The choice of conditions for using the less-preferred alternate food paradigm is more difficult to establish but can be extremely important in determining a specific chemical concentration that elicits aversion. One must carefully choose the alternate food to generate the appropriate preference differential for the intended objective. Shumake et al [*17*] only intended to establish a methiocarb level that would readily induce conditioning and therefore used the single-choice R_{50} method. However, Garrison [*16*] wanted to know the lowest level at which methiocarb elicits conditioned aversion, used slightly less preferred sorghum as the alternate food, and obtained a tenfold lower R_{50}. A wider preference differential (sunflower heads versus poultry mash) did not have as much sensitivity for detecting aversion as did the equally preferred alternate food method.

With the tremendous costs involved in registering new crop protection chemicals, it is unlikely that many new repellents will be developed. It is imperative that we find ways to make those currently available more effective. This may be through innovative application methods [*1*] or with additives such as sensory cues. As we learn more about the behavioral, physiological, and ecological aspects of a pest species, and begin to ask the proper questions and design the appropriate tests to answer them, further advancements toward effectively using repellents to protect crops will be made.

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