IMPORTANCE OF COLOR FOR METHIOCARB-INDUCED FOOD AVERSIONS IN RED-WINGED BLACKBIRDS

J. RUSSELL MASON, Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104
RUSSELL F. REIDINGER, JR., U.S. Fish and Wildlife Service, c/o Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

Abstract: Red-winged blackbirds (*Agelaius phoeniceus*) were intubated with methiocarb, lithium chloride (experimental groups), or propylene glycol (control groups) before or after eating colored oats. Only birds given toxic gavage after eating colored oats showed food-aversion learning. Blackbirds intubated with methiocarb after eating familiar bird chow paired with colored rectangles avoided chow only when it was paired with the appropriate color cue. Blackbirds intubated with methiocarb subsequently avoided, in decreasing order, (1) food and color paired with intubation, or color paired with intubation but unpaired food; (2) food paired with intubation but unpaired color; and (3) unpaired food and color. Birds intubated with propylene glycol showed no such differential behavior. Use of distinctive visual cues may improve the efficacy of methiocarb as a bird repellent, visual cues may be effective without actually coloring the crop (e.g., colored flags), and Batesian mimicry could serve as the conceptual basis for studies of methiocarb as a bird repellent.

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Blackbird damage to crops is a serious problem in some areas of the United States (Wakely and Mitchell 1981). At times, the problem can be economically severe and even disastrous for individual farmers (Dolbeer 1981, Woronecki et al. 1981). Paradoxically, the same blackbirds that depredate crops in late summer and early fall probably provide economic benefits for farmers at other locations and times of the year (Dolbeer 1980). The agricultural benefits, as well as the impracticality or social unacceptability of lethal control (Rogers 1978*a*), are arguments for nonlethal management of blackbirds when they are pests. Methiocarb (3,5-dimethyl-4-methylthiophenol methylcarbamate) is the most commonly used agent that acts as a bird repellent rather than a lethal toxin. The compound has been applied successfully to reduce damage to seeds (Hermann and Kolbe 1971) and sprouts (Porter 1977), and to crops such as blueberries (Bollengier et al. 1973, Stone et al. 1974) and cherries (Guarino and Stone 1973). However, more effective techniques are still needed for use of methiocarb with grain crops as evidenced by recent concerns regarding net economic benefits of some repellent applications (Dolbeer 1981).

Food-aversion learning (i.e., learned avoidance of a food associated with postingestional sickness) is commonly regarded as the theoretical basis of bird repellency (e.g., Rogers 1978*a*). Although that basis appears sound, the broader concept of Batesian mimicry may be more useful in that it (1) incorporates food-aversion learning as a fundamental mechanism by which a predator learns to avoid food, (2) incorporates other mechanisms such as observational learning (i.e., vicarious learning that occurs when 1 animal watches the activities of another [Wilson 1975:51]), and (3) is derived from evolutionary theory based on the population concept (Wickler 1968:31) and gives a basis for management of blackbirds at the population level rather than at the individual level.

Nonetheless, food-aversion learning is an important consideration in Batesian mimicry and the role of methiocarb as an

illness-inducing agent (i.e., an unconditioned stimulus) in food-aversion learning is unclear. For example, methiocarb has been demonstrated as the unconditioned stimulus in food-aversion learning with blackbirds only after repeated pairings with a food (Rogers 1978*b*), whereas food-aversion learning usually occurs after a single pairing of the food (i.e., the conditioned stimulus) with the unconditioned stimulus. Perhaps methiocarb is a weak unconditioned stimulus, or the conditioning was attempted with inappropriate (or nonsalient) conditioned stimuli. For bird species, color cues can be more salient than taste cues (e.g., Garcia et al. 1977), although relative importance of color and taste may be influenced by such factors as species (Wilcoxin et al. 1971, Brett et al. 1976, Wilcoxin 1977, Westbrook et al. 1980) and context (Gillette et al. 1980*a*).

We initiated a series of laboratory studies to provide behavioral information fundamental to improved field applications of methiocarb as a repellent for blackbirds. Our approach has been systematic, based on the concept of Batesian mimicry wherein palatable organisms (mimics) are avoided by predators because of their resemblance to (mimicry of) unpalatable organisms (models) that the predators avoid (Brower 1960). In the mimicry paradigm of bird repellency, the predator would be the bird, the model would be the portion of the crop treated with methiocarb, and the mimic would be the untreated portion of the crop.

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METHODS

Experiment 1

This experiment reassessed the ability of methiocarb to serve as an unconditioned stimulus in food-aversion learning with red-winged blackbirds. We compared methiocarb with lithium chloride (LiCl), the most commonly used unconditioned stimulus in laboratory studies of food-aversion learning (Riley and Clarke 1977). Further, we added colors to the food as distinctive cues to determine if such cues would encourage food-aversion learning after a single conditioned stimulus/unconditioned stimulus pairing in redwings as has been demonstrated for other bird species (Czaplicki et al. 1976, Martin et al. 1977, Logue 1980). The experiment was designed to both replicate and extend earlier work (Rogers 1974, 1978*b*) on methiocarb-induced food-aversion learning in redwings.

Thirty adult male red-winged blackbirds were decoy-trapped during July and August 1980 at Sandusky, Ohio. Birds were individually housed and adapted to laboratory conditions as described elsewhere (Mason and Reidinger 1982).

Steel-cut hulled oats were dyed yellow (Atlas Colors: FD&C Yellow #5) or green (Atlas Colors: Hercules Mint Green) by immersing them for 1 hour in 1 liter of water to which 1 g of dye had been added. The dyed oats were dried at 28 C for several days before use. In earlier work, we demonstrated that redwings did not initially prefer yellow- or green-dyed feed

(pastries, oats) (Mason and Reidinger 1981).

Following adaptation, the redwings were visually isolated from each other with pieces of cardboard (36 × 61 cm). On the next day, 9 birds each were given 20 g of yellow or 20 g of green oats during the 1st hour of light. When each of the birds had consumed at least 1 g of the distinctively colored oats, the birds were randomly assigned to 3 groups (6 birds/group with equal numbers of birds exposed to yellow and green oats in each group). The 1st group (methiocarb and seed, M+S) received an intubation of methiocarb (2 mg/kg body weight), the 2nd group (LiCl and seed, L+S) received an intubation of LiCl (2 mg/kg body weight), and the 3rd group (propylene glycol and seed, PG+S) received an intubation of propylene glycol (2 mg/kg body weight) to control for the effects of intubation. The remaining 12 birds were randomly assigned to 2 groups (6 birds/group) and given methiocarb (unpaired methiocarb, UpM) or LiCl (unpaired LiCl, UpLiCl) intubations (2 mg/kg body weight), but were not presented with oats. The UpM and UpLiCl groups served as controls for the effects of toxin-induced illness. In all cases, the birds were intubated within 10 minutes after feeding. The birds were intubated with LiCl or methiocarb rather than exposed to treated food to allow a more precise delivery of known amounts of repellents. Stock solutions of methiocarb and LiCl were prepared by dissolving 3 mg of either substance in 25 ml of warmed propylene glycol.

One day, and 1, 2, 4, and 8 weeks after treatments (Rogers 1974), the birds were given 2-choice preference tests between the 2 colors of oats. For each test, all food was removed from the cage and the birds were given 5 g of each color of oats for the 1st hour of light. The oats were presented in 2 food cups located 5 cm apart at the front of the cages. To reduce spillage, each food cup (7.5 cm diam) was placed within a larger cup (11.3 cm diam) that caught spillage from the smaller cup during most feeding activity (Rogers 1974). At the beginning of the 2nd hour of light, the oats were removed from the cages and consumption and spillage of each color of oats were measured. For the remaining hours of light on test days (and during the entire period of light on other days), the birds were left undisturbed and permitted free access to Purina Flight Bird Conditioner (PFBC) and apples.

We separately assessed measurements of consumption and spillage of oats during each preference test using 3-way analysis of variance with repeated measures on 2 factors. The independent factor was groups. The repeated factors were color of oats paired with intubation vs. the other color of oats and preference tests (1 day, 1, 2, 4, and 8 weeks). The Bonferroni t test (Games 1971) was used to identify differences among means ($P < 0.05$).

Experiment 2

In this experiment we controlled for the possibility of other than visual cues by dissociating the color cues from the food. Colored cards were used for the stimuli and the cards were attached to the far sides (from the birds) of the food dishes in such a manner that the cards were inaccessible to the birds. The same food, PFBC, was used with both colors so that visual, taste, and other stimuli (other than the colored cards) associated with the food were as identical as possible.

Sixteen redwings were randomly selected from the 30 used in Experiment 1 (colors used in Experiment 2 were different from those used in Experiment 1). The birds were housed under the same conditions as in Experiment 1.

The redwings were visually isolated from each other as described in Experiment 1. On the next day, during the 1st hour of light, each bird was given PFBC from a cup (7.5 cm diam) attached to the front of the cage. Behind the cup on the outside of the cage was a red ($N = 8$) or blue ($N = 8$) plastic rectangle (17 × 12.5 cm) that the bird could see but not contact. When each bird had consumed at least 1 g of food, all were randomly assigned to 2 groups (8 birds/group with equal numbers of birds exposed to red and blue rectangles in each group). The birds in the 1st group (methiocarb and PFBC, M+PFBC) were intubated with methiocarb (2 mg/kg body weight) whereas those in the 2nd group (propylene glycol and PFBC, PG+PFBC) were intubated only with propylene glycol (2 mg/kg body weight) as a control for the effects of gavage. The stock solution of methiocarb was prepared as described in Experiment 1 and all birds were intubated within 10 minutes after feeding.

One, 3, 6, and 9 days after intubation, the birds were given 2-choice preference tests during the 1st 2 hours of light. During the tests, each bird was presented with 2 cups, each containing 10 g of PFBC, attached 5 cm apart to the front of the cages. Attached to the back of 1 of the cups was the colored rectangle associated with the earlier intubation treatment. At the beginning of the 3rd hour, the food cups were removed and consumption was measured. Spillage was not measured because it was closely related to consumption in Experiment 1. For the remaining hours of light on the test days (and during the entire light period on other days), all birds were left undisturbed and permitted free access to PFBC and apples.

We used a 3-way ANOVA having factors analogous to those in Experiment 1 to assess the effects of these factors on consumption. Bonferroni t tests were again used to identify different means ($P < 0.05$).

Experiment 3

This experiment was designed to parametrically assess the interaction of food with color cues in the formation of methiocarb-induced food-aversion learning in redwings. Birds were presented with (1) a food-color combination associated with gavage, (2) a food associated with gavage but another color, (3) a color associated with gavage but another food, and (4) a food and color other than those associated with gavage.

Sixteen experimentally naive blackbirds were trapped and housed as described for Experiments 1 and 2. The birds were visually isolated as described in Experiment 1 and then randomly assigned to 4 groups ($N = 4$/group). During the 1st hour of light on the following day, birds in 2 groups were each given 20 g of steel-cut hulled oats in each of 2 cups (7.5 cm diam) attached 5 cm apart at the front of the cages. Birds in the other 2 groups were given 20 g of PFBC in each cup. A red (2 birds/group) or white (2 birds/group) plastic rectangle (conditioned stimulus, 17.5 × 12.5 cm) was attached to the back of 1 cup of each pair. The birds could see but not contact the rectangles. When all of the birds had consumed at least 1 g of food, birds in 2 groups (1 given oats, 1 given PFBC) were intubated with methiocarb (2 mg/kg body weight) within 10 minutes after feeding whereas birds in the other 2 groups were intubated with propylene glycol (2 mg/kg body weight).

During the 1st 2 hours of light on the 4 days immediately following intubation, the birds were given 2-choice preference tests. Each bird was presented with 2 food cups at the front of the cage. The cups contained oats during 2 tests and PFBC

for the other 2. The conditioned-stimulus color (CS color, 2 preference tests) or the other color (2 preference tests) was attached to the back of 1 of the cups in each pair during the test. Thus, each bird was presented with the 4 food-color combinations. The food cups were removed and consumption was measured at the beginning of the 3rd hour of light on each of the 4 days. For the remaining hours of light, all birds were left undisturbed and permitted free access to PFBC and apples.

We assessed measurements of consumption over the successive preference tests using a 3-way ANOVA with repeated measures on 2 of the factors. The independent factor was groups, and the repeated factors were food type and color cue. Different means were identified using Bonferroni t tests ($P < 0.05$).

RESULTS

Experiment 1

There were no differences ($P > 0.25$) between groups in consumption or spillage. However, within groups, the types of oats affected consumption ($F = 46.2$; df = 3,45; $P < 0.01$) and spillage ($F = 28.6$; df = 3,45; $P < 0.01$). Preference tests also affected consumption ($F = 21.2$; df = 4,60; $P < 0.005$). Consumption was correlated (Spearman's $r > 0.95$) with spillage for both the color of oats paired with intubation and the other color of oats.

Overall, groups M+S and LiCl+S ate less of the colored oats that had been paired with intubation ($\bar{x} = 1.1$ g for M+S and 0.5 g for LiCl+S) than of the other colored oats ($\bar{x} = 1.4$ g for M+S and 1.6 g for LiCl+S). For the LiCl+S group, this difference was evident for the 1st 4 preference tests; for the M+S group, the difference was evident for only the 1st 2 preference tests. Consumption and spillage were depressed ($P < 0.05$) for all groups on the day immediately following treatments (i.e., the 1st preference test) compared with the other preference tests. There were no other differences ($P > 0.25$) in consumption and spillage by the birds (Fig. 1).

Experiment 2

Preference tests ($F = 7.8$; df = 3,42; $P < 0.05$) and presence or absence of the colored rectangles with the food ($F = 5.8$; df = 1,14; $P < 0.05$) affected consumption. Birds intubated with methiocarb (group M+PFBC) ate less food from the cup with the rectangle attached ($\bar{x} = 0.74$ g) than from the other cup ($\bar{x} = 1.32$ g). Birds intubated with propylene glycol (group PG+PFBC) showed no such differences in consumption ($\bar{x} = 1.30$ and 1.34 g, respectively). Overall, both groups consumed the same total amount of food ($P > 0.25$) (Fig. 2). Aversions were strongest during the 1st preference test, weakened but still present during the 2nd and 3rd tests, and absent during the 4th test.

Experiment 3

Consumption was significantly affected by all 3 factors, i.e., groups ($F = 3.8$; df = 1,12; $P < 0.05$), food types ($F = 5.3$; df = 1,12; $P < 0.05$), and color cues ($F = 8.0$; df = 1,12; $P < 0.05$). The interactions between food type and groups ($F = 7.7$; df = 3,12; $P < 0.05$) and food type and color cues ($F = 4.7$; df = 3,12; $P < 0.05$) also affected consumption.

Birds intubated with methiocarb ate less food overall and less from the cup paired with color cues (CS or other cue) than did birds intubated with only propylene glycol (Bonferroni t tests, $P < 0.05$). Further, the methiocarb-intubated birds ate less ($P < 0.05$) from the cup paired with the CS color than from the cup paired with the other color. Red and white were

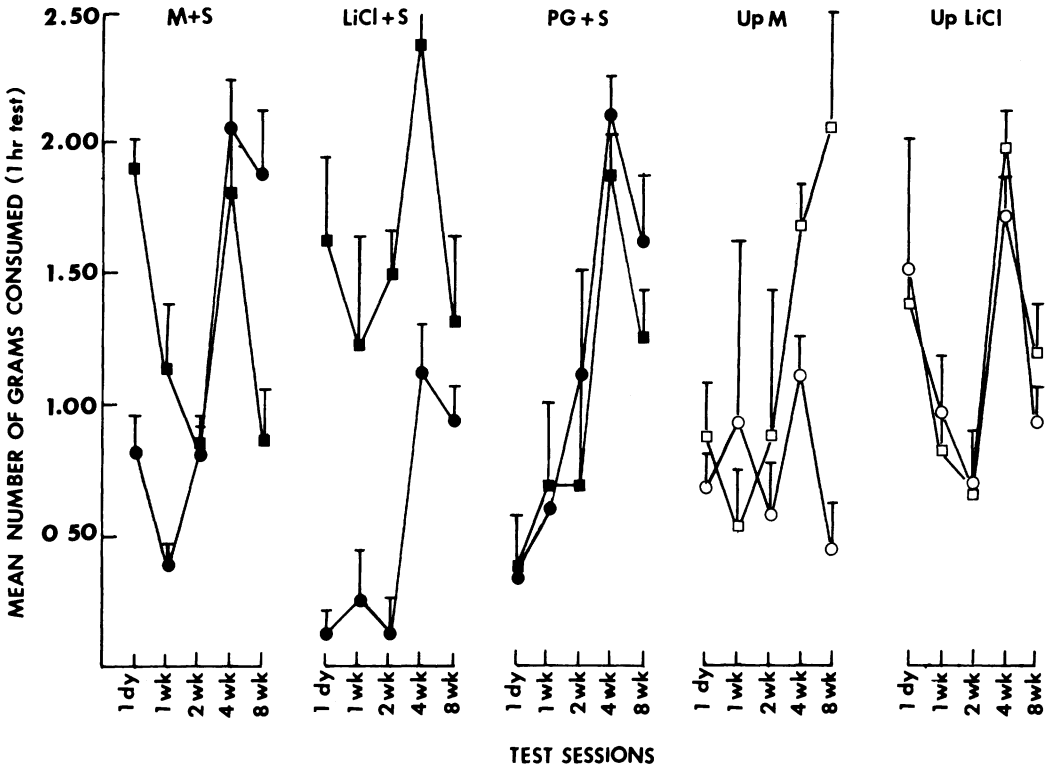

Fig. 1. Consumption of colored oats paired with gavage (closed circles) and another color of oats (closed squares) for birds intubated with methiocarb (M+S), lithium chloride (LiCl+S), or propylene glycol (PG+S). For birds given gavages with methiocarb (UpM) or lithium chloride (UpLiCl) not paired with colored oats, open circles represent consumption of green oats and open squares represent consumption of yellow oats. Vertical bars represent standard errors of the means.

equally effective conditioned stimuli, and birds intubated with only propylene glycol showed no such differential consumption. Methiocarb-intubated birds ate less of either food type (i.e., oats or PFBC) when it was associated with intubation, regardless of the color cue with which it was associated. In contrast, birds intubated with propylene glycol showed no such differential consumption. Avoidance of food by birds intubated with methiocarb was different depending on whether the food was oats or PFBC. For birds given PFBC, differences ($P < 0.05$) in avoidance occurred in the following order of decreasing strength: (1) food and color paired with intubation, or color cue paired with intubation but the other food type; (2) food paired with intubation of methiocarb but the other color cue; and (3) food type and color cues other than those paired with intubation. For birds given oats, avoidance occurred in a similar order of decreasing strength, except aversions for food and color cue paired with intubation were greater ($P < 0.05$) than aversions for the color cue paired with intubation but the other food type. When consumption by birds intubated with methiocarb was compared with consumption by birds intubated with propylene glycol, the only differences ($P < 0.05$) were be-

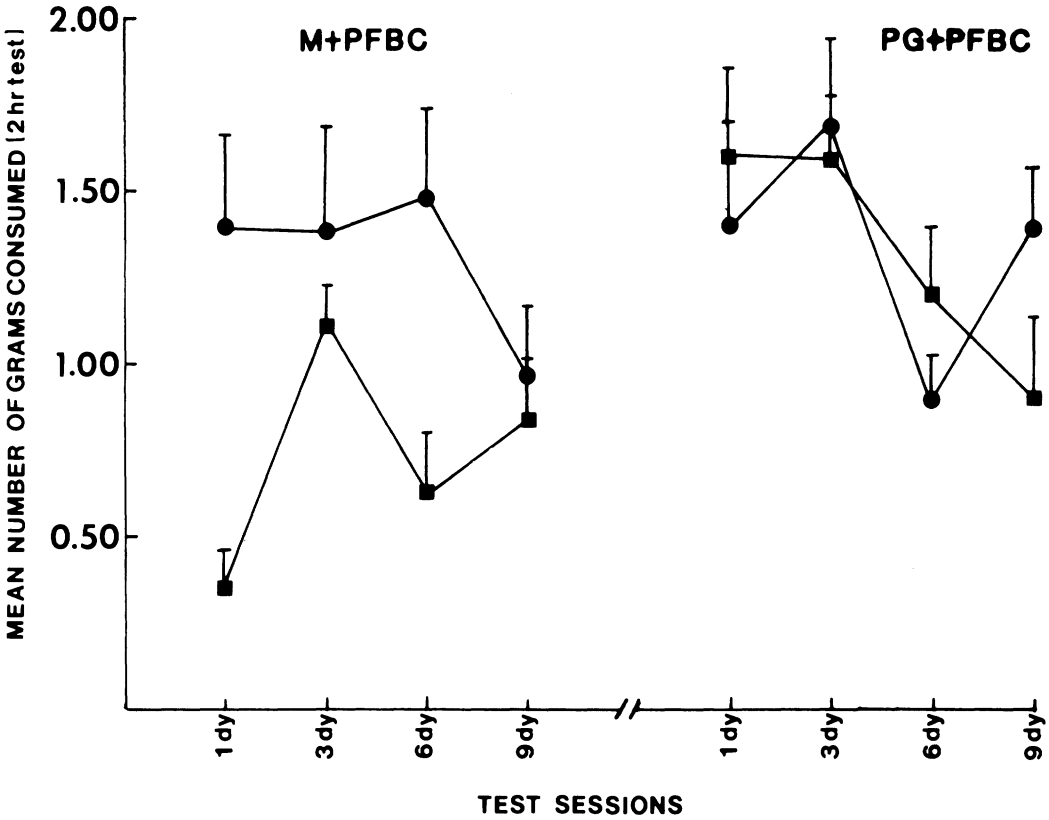

Fig. 2. Consumption of Purina Flight Bird Conditioner (PFBC) paired with the appropriately colored rectangle after gavage with methiocarb (M+PFBC, left panel) or propylene glycol (PG+PFBC, right panel). For both panels, squares represent consumption from dishes with colored rectangle present and circles represent consumption from dishes with colored rectangles absent. Vertical bars represent standard errors of the means.

tween (1) food and color paired with intubation and (2) color cue paired with intubation but the other food type (Fig. 3).

DISCUSSION

In Experiment 1, reduced consumption and spillage of foods paired with methiocarb support the findings of Rogers (1974, 1978*b*) that methiocarb can serve as an unconditioned stimulus in food-aversion learning in redwings. Further, our results extend the previous findings with redwings by showing that food-aversion learning can occur after a single pairing with methiocarb, if appropriate conditioned stimuli are also provided. We interpreted the results as consistent with the notion that addition of dyes, presumably due to their distinctive colors, was important for the food-aversion learning after a single pairing, and as discounting the notion that methiocarb is a weak unconditioned stimulus that requires multiple pairings to induce a conditioned flavor aversion. This interpretation is consistent with the findings from published studies on conditioned flavor aversions in other bird species (Czaplicki et al. 1976, Wilcoxin 1977).

In Experiment 1, LiCl appeared to be an even stronger unconditioned stimulus

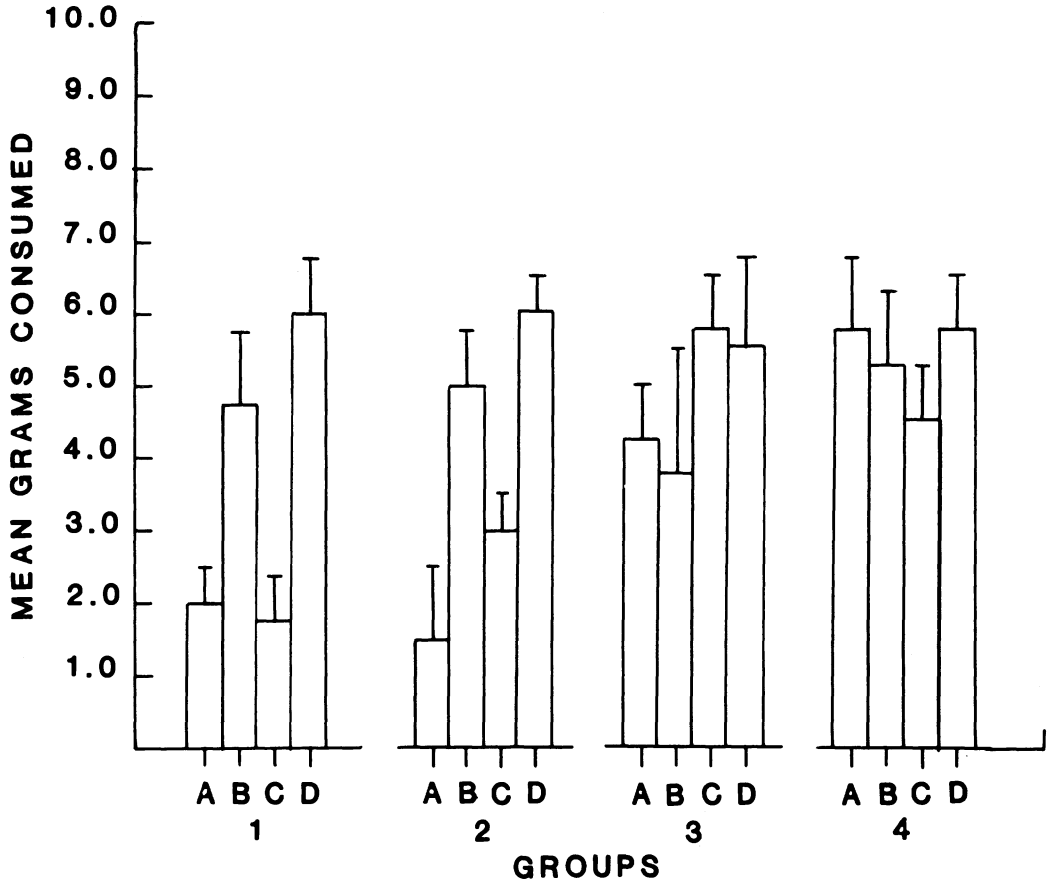

Fig. 3. Mean consumption by birds in the 4 groups during preference tests. Group 1 was intubated with methiocarb after consuming Purina Flight Bird Conditioner paired with the CS color. Group 2 was intubated with methiocarb after consuming rolled oats paired with the CS color. Groups 3 and 4 were intubated with propylene glycol after consuming Purina Flight Bird Conditioner or rolled oats, respectively, paired with the CS color. For each group: (A) represents consumption of food paired with the CS color and gavage; (B) represents consumption of food paired with gavage and the other (non-CS) color; (C) represents consumption of the food not associated with gavage but paired with the CS color; and (D) represents consumption of the food not associated with gavage and paired with the other (non-CS) color. Standard errors of the means are represented by capped vertical bars.

than methiocarb in that the aversion to the color of oats paired with LiCl-induced illness lasted longer (i.e., extinguished more slowly) than the aversion learned with methiocarb-induced illness. However, the concentrations of LiCl and methiocarb were arbitrarily set at 2 mg/kg of body weight for this experiment and it is likely (Nowlis 1974) that the strength of aversions was a function of the relative concentrations of the unconditioned stimuli. We concluded that both LiCl and methiocarb served as unconditioned stimuli in the food-aversion learning of redwings.

The results of Experiment 2 confirmed those from Experiment 1 in that red-winged blackbirds formed food aversions after a single pairing of a feeding experience with an intubation of methiocarb. In Experiment 2, however, the same food (unadulterated by dye) was available in either cup so that differences in food types

could not have influenced feeding preferences. In addition, PFBC was familiar to the birds and familiarity is known to decrease the likelihood of a material being associated with post-ingestional sickness (Elkins and Hobbs 1979), further decreasing the likelihood that food cues would have been used as conditioned stimuli by the birds. The only obvious, novel difference between the foods was therefore the colored rectangle attached to 1 dish but not the other. Treated birds avoided food in the dish with the colored rectangles and in the absence of other differentiating cues. We suggest that the redwings formed methiocarb-induced aversions based on visual cues (i.e., colored rectangles) alone. This interpretation is also consistent with findings from studies of other avian species (e.g., Brower 1960, Czaplicki et al. 1976, Wilcoxin 1977).

Data from Experiment 3 indicated that redwings can form reliable methiocarb-induced food aversions after a single pairing with a combination food and distinctive color. The results also confirmed findings from Experiment 2 in that the birds expressed conditioned flavor aversions based on the color-conditioned stimulus alone (i.e., when the color-conditioned stimulus was combined with the food type not present during intubation). However, at least for birds given unfamiliar food (oats), the conditioned aversions were best expressed when both the CS color and the food type that had been paired with intubation of methiocarb were presented. Our findings are consistent with those from studies on some other bird species. For example, Shumake et al. (1977) have reported that the strongest conditioned aversions learned by weaver finches (*Quelea quelea*) involved both color and taste cues. Likewise, others (Clarke et al. 1979, Gillette et al. 1980*b*) have reported that taste cues can act synergistically with color cues to increase their importance in food-aversion learning for other bird species.

The results from Experiment 3 are also consistent with the earlier finding of Rogers (1974, 1978*b*) that red-winged blackbirds failed to express aversions after a single pairing of methiocarb-induced illness toward a food–color combination when distinctive color-conditioned stimuli were absent (i.e., in Experiment 3, the preference tests wherein the food paired with intubation but the other color cue was used and wherein the food type and color cue other than those paired with intubation were used).

MANAGEMENT IMPLICATIONS

Although one must obviously be cautious in extrapolating findings from the laboratory to field situations, we believe that our findings raise some fundamental issues that warrant additional study in both laboratory and field settings, and that could lead to improved use of methiocarb as a bird repellent. First, based on the attention that blackbirds give to visual cues while feeding (e.g., Alcock 1970), and specifically during the formation of methiocarb-induced food-aversion learning (this study), we suggest that the addition of visual cues might improve the effectiveness of methiocarb as a bird repellent. As currently applied to crops, methiocarb is not distinctively colored. Addition of such cues might act synergistically with the cues for the food type (i.e., the crop) already available to the redwings to provide strong aversion learning after a single feeding experience. Second, based on the results of Experiments 2 and 3 wherein the redwings formed aversions when the color cue was contiguous with but not part of the food type, it might be possible to provide effective visual cues without actually coloring the crop (e.g., distinctively

colored flags might be used). Support for this idea is provided by a study in which perches coated with glue effectively repelled Philippine weavers (*Lonchura* spp.) from rice fields (Reidinger and Libay 1979). Although the glue itself remained effective (i.e., caught birds, who subsequently exhibited distress) for only 3 days, birds avoided fields having the perches for at least an additional 5 days. Apparently, the visual presence of the perches in the fields was an adequate cue for expression of the observationally learned avoidance in these species. Red-winged blackbirds, at least in the laboratory, also express food preferences (Mason and Reidinger 1981) and aversions (Mason and Reidinger 1982) as a function of observational learning. Finally, our results are consistent with the notion that methiocarb can serve as a toxin in the development of a model (i.e., a poisoned portion of a crop) for the potential application of the concept of Batesian mimicry to bird repellency.

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