

BIRD AVERSIVE PROPERTIES OF METHYL ANTHRANILATE, YUCCA, *Xanthoxylum*, AND THEIR MIXTURES

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Abstract—We tested the bird aversive properties of methyl anthranilate, yucca extracts, and *Xanthoxylum* spp. extracts in one- and two-bottle drinking assays that used European starlings (*Sturnus vulgaris*). In one- and two-bottle tests, methyl anthranilate proved to be the more potent stimulus in producing an avoidance response. Starlings avoided consuming *Xanthoxylum* and yucca only in the two-bottle tests. Previous studies showed that yucca was a good adjuvant in stabilizing lipophilic compounds in water. Starlings did not avoid binary mixtures of methyl anthranilate and yucca differently from what would be expected if they were only responding to the solution's methyl anthranilate content. However, yucca enhanced the aversive qualities of *Xanthoxylum*. The ability to identify mode of action for repellency and synergistic combinations of chemicals derived from natural products for use in repellent mixtures is an important aspect of the development of cost-effective, environmentally safe repellents for use in conflict resolution between humans and wildlife.

Key Words—Aversive properties, methyl anthranilate, yucca, birds, *Xanthoxylum*.

INTRODUCTION

Natural products are important sources for new agrochemicals. Moreover, surveys of natural products for avian repellents are appealing from a regulatory and environmental standpoint (Crocker, 1990; Crocker and Perry, 1990; Mason,

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1990; Mason and Clark, 1992, 1997) because natural products often pose low environmental risk owing to their specific biological action and because they have a lower potential for bioaccumulation relative to synthetic compounds (Secoy and Smith, 1983; Balandrin et al., 1985). Specifically, the US Environmental Protection Agency has given special consideration to natural product-based vertebrate repellents under the Federal Insecticide, Fungicide, and Rodenticide Act (40 CFR 160) that could potentially result in considerable economic savings during product registration (Fagerstone and Schafer, 1998). Nonetheless, of particular concern in using any naturally derived repellent compound is its expense and availability. Few compounds are currently approved for use in North America and Europe, and those that are tend to be expensive (Clark, 1998a). Besides efforts to identify effective compounds or source material, there is a need to identify methods that would reduce the amount of material used.

One area of research that directly bears upon the issue of expense and amount of material used is formulation chemistry. In many cases, the amount of active ingredient necessary for repellency in the field is higher than indicated from laboratory studies (Cummings et al., 1998). This is not surprising because application efficiency onto treated substrates and delivery efficiency to the target animal and its mediating sensory system is often optimal in the laboratory, but the efficiency is less so in the field. Besides developing more effective delivery tactics for a specific formulation, another area of productive research is the use of adjuvants to enhance the intrinsic aversive qualities of the active ingredient. There are indications that some repellents may be combined to yield synergisms between the mixture's components, yet more often than not, antagonisms are found to occur (Clark, 1998b, 1999).

In pilot studies, we found that yucca extracts possessed physicochemical properties that might prove useful in stabilizing formulations containing the bird repellent, methyl anthranilate (Stevens and Clark, 1998). Methyl anthranilate is a naturally occurring aromatic compound commonly used as a human flavoring whose bird-specific repellent attribute is mediated by chemoreceptive nociceptors of the trigeminal nerve (Clark, 1998c). Yucca extracts contain a variety of surfactants, including saponins. These extracts previously proved useful as adjuvants in forming stable aqueous emulsions with various lipophilic substances (Stevens and Clark, 1998). Two objectives of this study were to explore the bird aversive properties of yucca extracts and to determine how these extracts influence the aversive properties of methyl anthranilate, possibly by increasing access of methyl anthranilate to nociceptors distributed within the oral mucosal lining of a bird's mouth (Finger et al., 1990). A third objective was to evaluate the repellent properties of an additional plant extract derived from *Xanthoxylum* spp. that previously had been shown to be active against rodents (Bryant and Mezine, 1999). Because *Xanthoxylum* extracts also were lipophilic, as was the case for methyl anthranilate, a fourth objective was to determine whether yucca

extract could be used to stabilize a water-based *Xanthoxylum* formulation and whether yucca altered the aversive qualities of the *Xanthoxylum* extract.

METHODS AND MATERIALS

Animals. Adult European starlings (*Sturnus vulgaris*) were captured at cattle feedlots near Fort Collins, Colorado, by using modified crow traps. During quarantine, adaptation, and testing, starlings were individually housed and visually isolated in cages (61 × 36 × 41 cm) under a 12L : 12D cycle and given free access to food. Fresh fruit, bath water, and novel cat toys were provided once a week. The cat toys helped reduce spillage of food and fluid throughout testing by redirecting the starlings' inquisitive behavior. Starlings were chosen as test animals because previous experiments showed them to be good models of avian chemosensitivity (Clark and Shah, 1994). All procedures described complied with standards set forth by the USDA, National Wildlife Research Center's Institutional Animal Care and Use Committee.

Chemical Stimuli. Methyl anthranilate was obtained from Sigma-Aldrich (Milwaukee, Wisconsin). Aqueous yucca extracts were provided by New Waste Concepts, Inc. (Perryville, Ohio). *Xanthoxylum* fruits were obtained from Nepal and extracted according to methods established by Bryant and Mezine (1999).

Test Protocol. After a two-week adaptation period, 40 experimentally naive starlings were randomly assigned to one of eight groups, and water consumption was monitored every hour over a 4-hr period over the course of three days to ensure that consumption was within the normal range empirically established for starlings under test conditions in our laboratory. Birds deviating more than 2 standard deviation units from a seasonally adjusted mean were excluded from experiments (such birds were often hyperactive or sick). Similarity for total 4-hr water intake among groups was verified by using a fixed effect, repeated measures (on days) analysis of variance, and was a precondition for additional testing.

The tests consisted of standard one- and two-bottle drinking assays (Clark and Shah, 1994). Location of the drinking tubes was randomized (left vs. right) to eliminate side bias effects. For each test (all totaled, $N = 9$ tests), groups of starlings were randomly assigned to receive one of seven concentrations of a test solution, with an eighth group serving as the control, i.e., receiving tap water. Fluid intakes were monitored every hour (to check for spillage) for a total of 4 hr. At the end of the test, ad libitum food and water were made available to the birds, and posttest consumption was monitored over the next 20 hr to determine if the test materials had any carryover effects on normal feeding or drinking. In no case did any of the tests described here have an impact on food and water consumption after the tests (data and analysis not shown). After a three-day rest

and baseline water monitoring, birds were rerandomized, assigned to a new test group, and the testing process was repeated.

One- and two-bottle tests were used to evaluate the concentration response of starlings to simple solutions of either methyl anthranilate [MA_i], yucca extract [Y_i], or *Xanthoxylum* extract [X_i], where i was 0, 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, and 0.5% (ml/ml). Together these combinations combined for a total of six tests. Additionally, two-bottle tests were used to evaluate the concentration–response to binary mixtures of $MA_iY_{0.1\%}$ and $MA_iY_{0.01\%}$, where i was 0.5, 0.25, 0.1, 0.062, 0.025, 0.013, 0.008, and 0.001% (for a total of two tests with 45 birds, $N = 5/\text{group}$). Finally, a two-bottle test was used to evaluate the concentration–response to binary mixtures of X (0.5, 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005%) and yucca extract (0.1%) (for 1 test).

Analysis. Responses of starlings, expressed as preference scores (R), from the two-bottle tests were calculated as

$$R = t_i / (w + t_i) \quad (1)$$

where t_i is the amount of test solution consumed in a specified period of time and w is the amount of alternatively available fresh water consumed during that same time period. R of 1.0 was indicative of complete preference of the test fluid, a value of 0.5 indicated indifference (i.e., equal amounts of w and t_i were consumed), and a score of zero indicated complete avoidance of the test fluid. Concentration–response curves were calculated by using iterative procedures to fit the observations to a four parameter nonlinear function of the form

$$R = y_o + ax^b / (c^b + x^b), \quad (2)$$

where y_o and a were the constrained asymptotic maximum and minimum values for the preference score (1 and 0, respectively), b was the slope, c was the inflection point of the curve, and x was the concentration of the test solution (SigmaPlot, v 4.0) (SPSS, 1997).

Preference scores were analyzed in the context of a fixed effects, two-way repeated-measures analysis of variance, where concentration group ($N = 8$) was the between-measures factor and hour ($N = 4$) was the within, i.e., repeated, measures factor. Because not all effects were of biological interest, we addressed specific a priori questions by using contrasts. Thus, within each test two questions were of general interest: first, whether the different concentration groups expressed equal preferences (R); and second, whether the observed preference at a specific concentration of test fluid (w vs. t_i) was similar to the internal control (w vs. w), which itself was always statistically indistinguishable from $R = 0.5$ across all tests ($P > 0.05$). This effect was examined by using a simple contrast

that compared the relative preference of the test solution to the negative control ($H_o: R_{i>0\%} = R_{i=0\%}$) as a function of the cumulative relative intake during the preceding test period, thus controlling for hydration status when making comparisons on the concentration effect relative to the control condition (Statistica, v 4.3) (StatSoft, 1994).

Patterns of absolute fluid intake also were of interest and were analyzed in the context of a fixed effects, two-way repeated measures analysis of variance, where concentration group ($N = 8$) was the between-measures factor, hour was the within, i.e., repeated, measures factor. It was assumed that randomization for group assignment and washout period between tests eliminated carryover effects that might otherwise occur by the reuse of birds. The primary question of interest was whether the test substances affected total fluid intake of starlings, i.e., water balance. This question was addressed by comparing total fluid intake for treatment groups within a test. In cases of significance for this main effect, a Tukey's Honestly Significant Difference (THSD) post hoc test was used to isolate differences among the levels.

The nature of the effect owing to the possible interaction of agents in binary solutions was examined relative to three hypotheses. First, starlings may respond to the blend of the mixture's components. This is the default expectation of most so-called averaging models (Berenbaum, 1985). Formally stated, the response to individual agents and their mixtures is described as follows: $R_{[A],i}$ and $R_{[B],i}$ are the responses to agents A and B at concentration i . $R_{[AB],i}$ is the response to the mixture composed of agents A and B at concentration, i . The theoretical null condition of independence of agents in mixture at the receptor level for concentration, i , is defined as

$$R_{[AB],i}^o = (R_{[A],i} + R_{[B],i})/2 \quad (3)$$

and the interaction index for concentration, i , is defined as

$$I = R_{[AB],i}^o - R_{[AB],i} \quad (4)$$

where no interaction occurs between the agents when $I = 0$. When $I < 0$, the interacting agents are antagonistic. When $I > 0$, the interacting agents are synergistic (Clark, 1998b). It is also possible that the bird may be responding to only one or the other of the agents in a binary mixture owing to cognitive overshadowing or masking of one component by the other (Lawless and Stevens, 1989). In the case of these possible masking or overshadowing effects, the null condition is: $H_o: R_{[A],i} = R_{[AB],i}$ or $R_{[B],i} = R_{[AB],i}$.

RESULTS

Concentration-Response Relationships for Single-Source Solutions: One-Bottle Tests. Starlings decreased their fluid intake as a function of increased concentration of methyl anthranilate (Table 1, Figure 1A). Starlings also reduced their intake of *Xanthoxylum* extract as a function of increasing concentration, and this effect was amplified as a function of time (Table 1, Figure 1C). However, there were differences in the magnitude and pattern of avoidance for these two substances (Table 2). At lower concentrations (<0.1%), the starlings' response to the two solutions was about the same, $R_{0.05\%} = 0.45$ and 0.37 for *Xanthoxylum* and methyl anthranilate, respectively. However, at the highest concentrations tested, the maximum potency, i.e., avoidance ($p^* = R_{[MA],0.5\%}/R_{[X],0.5\%}$) was higher for methyl anthranilate ($R_{0.5\%} = 0.74$) relative to *Xanthoxylum* ($R_{0.5\%} = 0.46$) (Figure 2).

The tendency for starlings to avoid *Xanthoxylum* extract remained constant for concentrations greater than 0.02% (Figure 1C), suggesting that the active agents responsible for avoidance responding became saturated in aqueous solution or that the active ingredients saturated the starlings' physiological or behavioral capacity to respond. Overall, methyl anthranilate was about as potent a repellent to starlings as were *Xanthoxylum* extracts at concentrations of 0.1% or less (after adjusting for differences in baseline water intake for the two experiments, Figure 2). However, methyl anthranilate was approximately twice as potent as the *Xanthoxylum* extract at a concentration of 0.5%. *Yucca* extracts were not aversive to starlings in the context of a one-bottle drinking assay,

TABLE 1. SUMMARY STATISTICS FOR ABSOLUTE FLUID INTAKE FOR DRINKING BIOASSAYS

	F_G^a	P	F_H^b	P	$F_{G \times H}^c$	P
1-Bottle test, single source						
MA_i	8.63	<0.001	21.25	<0.001	1.01	0.459
Y_i	0.23	0.973	9.90	<0.001	1.04	0.424
T_i	2.05	0.080	16.54	<0.001	2.52	0.001
2-Bottle test, single source						
MA_i	0.97	0.467	19.43	<0.001	0.57	0.928
T_i	0.79	0.598	42.06	<0.001	1.31	0.189
Y_i	1.35	0.259	35.57	<0.001	1.12	0.345
2-Bottle test, mixture						
$MA_i Y_{0.01\%}$	0.80	0.594	55.75	<0.001	0.79	0.715
$MA_i Y_{0.1\%}$	1.60	0.171	30.56	<0.001	0.78	0.735
$T_i Y_{0.1\%}$	0.74	0.640	43.78	<0.001	2.29	0.003

^a F value for the main effect, concentration group, with $df = 7,32$.

^b F value and probability for the main effect, hour, with $df = 3,96$.

^c F value and probability for the interaction of concentration-group and hour, with $df = 21,96$.

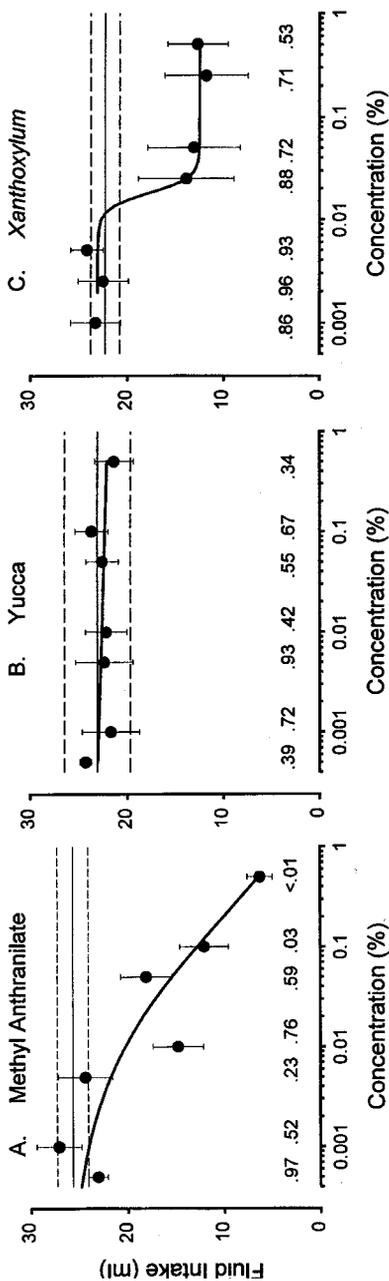


FIG. 1. The absolute concentration-response for starlings presented with single component aqueous solutions of methyl anthranilate, yucca extract, or *Xanthoxylum* extract in a one-bottle drinking assay. Symbols depict mean response (± 1 SEM) for each concentration-group tested ($N = 5$ /group). The parameter values used to define the curve are presented in Table 2. The horizontal solid line depicts the mean water intake for the control group, while the dashed lines depict ± 1 SEM. The numerical insets represent the probabilities for contrast comparisons between a given test solution and the control group.

TABLE 2. PARAMETERS AND STATISTICS FOR CONCENTRATION-RESPONSE CURVES

	R^2	$F_{3,4}$	P	a^a	b	c	y_0
1-Bottle test							
MA	85.3	7.71	0.039	-31.91 (45.41) ^a	0.50 (0.54)	0.191 (1.051)	26.18 (3.50)
<i>Xanthoxylum</i>	98.5	89.63	<0.001	-10.56 (0.80)	5.72 (9.24)	0.023 (0.005)	23.07 (0.46)
Yucca							
2-Bottle test, single source							
MA	96.1	32.51	0.003	-0.46 (0.07)	1.37 (0.72)	0.011 (0.001)	0.50 (0.05)
Yucca	62.1	15.53	0.012	-4.39 (7.92)	0.35 (0.35)	100.000 (100.005)	0.55 (0.06)
<i>Xanthoxylum</i>	92.4	16.12	0.011	-0.44 (0.10)	1.26 (0.69)	0.021 (0.001)	0.48 (0.04)
2-Bottle test, mixture							
MA Y _{1,0%}	98.6	121.90	<0.001	-0.52 (0.03)	1.03 (0.19)	0.001 (0.001)	0.57 (0.03)
MA Y _{0,1%}	97.6	67.26	<0.001	-0.41 (0.04)	1.33 (0.36)	0.003 (0.001)	0.45 (0.03)
T Y _{0,1%}	98.3	78.9	<0.001	-0.51 (0.06)	0.50 (0.05)	0.003 (0.001)	0.50 (0.03)

^aNumbers in parentheses are \pm SEM.^bParameter values (a , b , c , y_0) for the concentration response curves (see Methods).

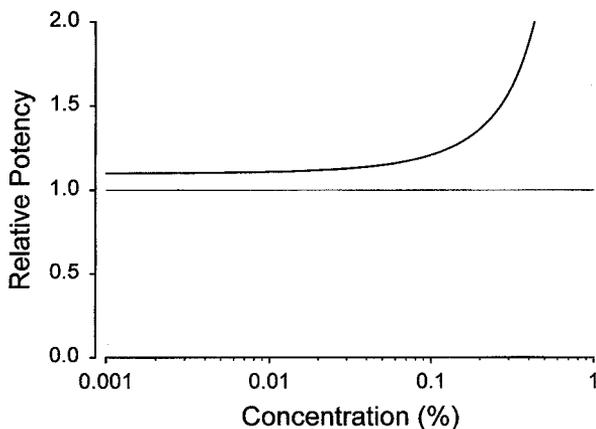


FIG. 2. The relative potency of methyl anthranilate to *Xanthoxylum* extract (MA_i/X_i) for the one-bottle, single-source drinking assays as a function of solution concentration.

regardless of the test concentration (Figure 1B). The significant time effect for yucca extracts (Table 3) indicated that, although not aversive, starlings decreased fluid intake consistent with volume limitations for gastric loading. That is to say, in the first hour, starlings consumed the greatest amount of fluid, with substantial reductions in intake for hours 2–4. However, this consumption pattern did not differ from that seen in the birds presented with fresh water. This same timed pattern of intake was observed for starlings presented with methyl anthranilate and *Xanthoxylum* extract. In these cases, the amount of fluid consumed in the first hour was below control (i.e., fresh water) levels, indicating that starlings showed a congenital avoidance of the treated fluids. The further reduction in fluid intake for hours 2–4 suggested that starlings formed a learned avoidance response as well.

Concentration-Response Relationships for Single Source Solutions, Two-Bottle Tests. Starlings consumed the same total fluid volume independent of test solution or concentration of the fluid (Table 1). Moreover, the starlings showed the same temporal pattern to fluid consumption as was seen in the first experiment. These observations suggested that starlings, if given an opportunity, maintained their hydration status and that the test solutions did not adversely affect short-term fluid intake.

Starlings decreased their relative fluid intake as a function of increasing concentration for all three test solutions (Table 3, Figure 3). This increased sensitivity to the aversive qualities of compounds in two bottle tests relative to one-bottle tests is a common occurrence. For methyl anthranilate, the increased potency of the two-bottle assay reflected a sensitization in both inflection and minimum

TABLE 3. SUMMARY STATISTICS FOR PREFERENCE SCORES FOR TWO-BOTTLE DRINKING BIOASSAYS

	F_{C-G}^a	P	F_H^b	P	$F_{C-G \times H}^c$	P
2-Bottle test, single source						
MA_i	4.03	0.003	4.93	0.003	1.20	0.273
Y_i	2.28	0.050	11.27	<0.001	0.77	0.751
T_i	5.30	<0.001	6.48	<0.001	0.82	0.688
Mixture						
$MA_iY_{0.01\%}$	5.93	<0.001	2.24	0.089	0.872	0.626
$MA_iY_{0.1\%}$	13.11	<0.001	4.43	0.006	1.25	0.227
$T_iY_{0.1\%}$	5.06	<0.001	6.65	<0.001	0.99	0.477

^a F value for the main effect, concentration-group, with $df = 7,32$.

^b F value and probability for the main effect, hour, with $df = 3,96$.

^c F value and probability for the interaction of concentration-group and hour, with $df = 21,96$.

asymptote of the concentration-response curves (cf. Figures 1A and 3A). For yucca and *Xanthoxylum* extracts, the increased potency seen in the two-bottle assay was not reflected as a sensitization in inflection, i.e., a leftward shift of the response curve; rather it was a sensitization of the minimum asymptote, i.e., a lowering of the response (cf. Figures 1B,C and 3B,C). Overall, methyl anthranilate had the highest relative potency, being three times as potent as *Xanthoxylum* at 0.03% and six times as potent as yucca at 0.07%. *Xanthoxylum* was relatively more potent than yucca, achieving maximal difference in effect (three times) at 0.2% (Figure 4).

Concentration-Response Relationships for Binary Mixtures, Two-Bottle Tests. Adding yucca extract did not substantially affect the relative preference of starlings for methyl anthranilate solutions (Table 3, Figure 5A,B). In both cases, where a moderately aversive concentration of yucca (0.1%) was added to methyl anthranilate solutions, and where a nonaversive concentration of yucca (0.01%) was added to methyl anthranilate solutions, the hypothesis that methyl anthranilate masked the effects of yucca for all concentrations tested was the most plausible explanation for the consumption patterns (Figure 5C,D). That is to say, the area under the curve was the smallest for H_0 : Mix = MA (Figure 5C,D). Starlings did not respond to the mixtures as if the salient cue was yucca alone or the average of the expected response to mathematical combinations of single component solutions of methyl anthranilate and yucca.

In contrast to the methyl anthranilate-yucca interaction in mixture, yucca enhanced the starlings' avoidance response to *Xanthoxylum* extract (Figure 6A). The interaction of yucca and *Xanthoxylum* appeared to be a true synergism for repellency because none of the three competing hypotheses, masking by yucca, masking by *Xanthoxylum*, or the averaging model, adequately explained the observed concentration-specific response of starlings.

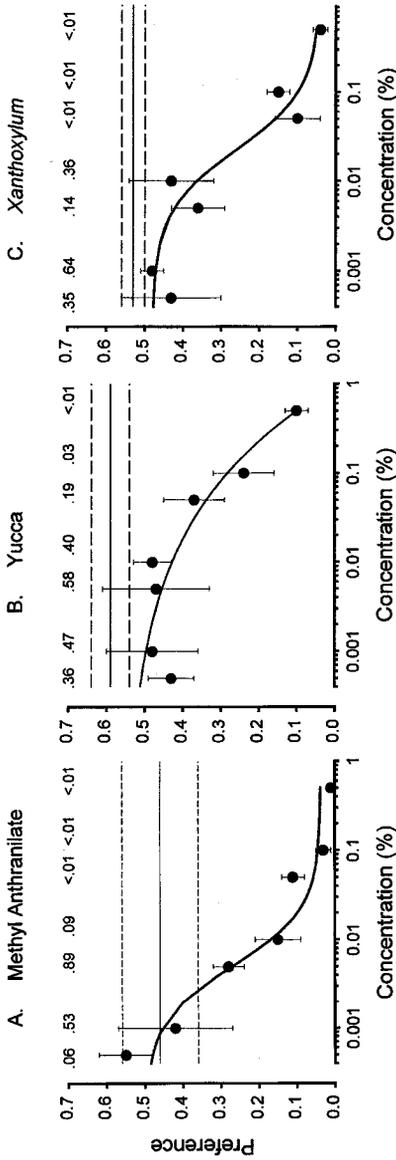


FIG. 3. The relative concentration-response (preference) for starlings presented with single-component aqueous solutions of methyl anthranilate, yucca extract, or *Xanthoxylum* extract in a two-bottle drinking assay. Symbols depict mean response (\pm SEM) for each concentration-group tested ($N = 5$ /group). The parameter values used to define the curve are presented in Table 2. The horizontal solid line depicts the mean water intake for the control group, while the dashed lines depict \pm SEM. The numerical insets represent the probabilities for contrast comparisons between a given test solution and the control group.

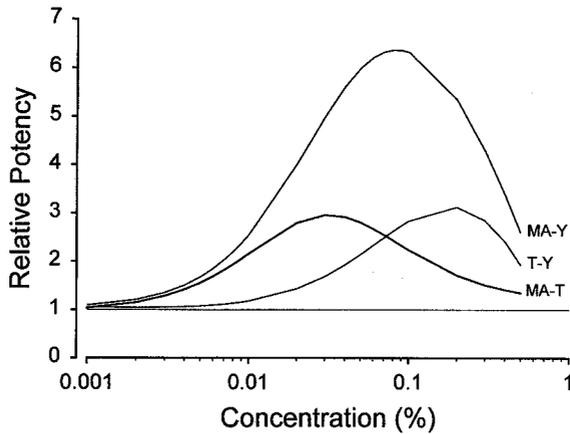


FIG. 4. The relative potency of methyl anthranilate to *Xanthoxylum* extract ($R_{[MA],i}/R_{[T],i}$) for the two-bottle, single source drinking assays as a function of solution concentration.

As was the case for the other experiments, starlings maintained their hydration status by consuming similar amounts of fluid irrespective of the concentration of test fluid presented to them, and starlings showed evidence of both congenital and learned avoidance of test mixtures (Table 1).

DISCUSSION

When mixed together, yucca and methyl anthranilate form a relatively stable emulsion. This trait alone may make the formulations of yucca and methyl anthranilate useful as a bird repellent agent (Nachtmann et al., 1997). However, it did not appear that yucca enhanced the penetrability of methyl anthranilate to the mediating receptors in starlings, as evidenced by the similar concentration–response profiles for a simple solution of methyl anthranilate and the mixture of methyl anthranilate and yucca. The lack of interaction in these drinking trials is consistent with experiments that tested the effects of methyl anthranilate and yucca formulations as repellent aerosols (Stevens and Clark, 1998). In contrast, yucca substantially enhanced the repellency of *Xanthoxylum* extract. This synergism is arguably the result of increased accessibility of the active molecules contained within *Xanthoxylum* to the mediating receptors. This accessibility may be attributable to increased solubility of *Xanthoxylum*'s components in the presence of yucca, or owing to the ability of yucca to enhance the penetration of *Xanthoxylum*'s components across the mucosal lining of the mouth. Further work on the constituent chemistry of both yucca and

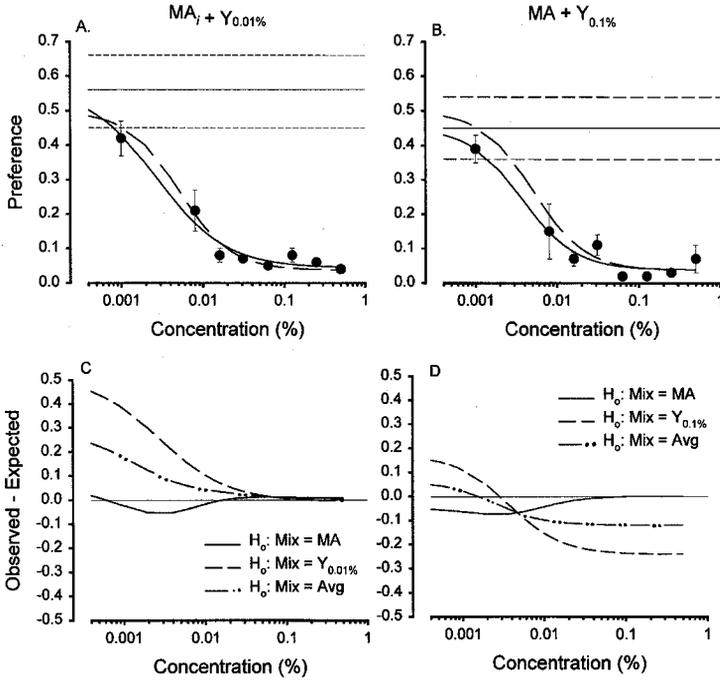


FIG. 5. The relative concentration–response for starlings presented with aqueous mixtures of methyl anthranilate (MA) and yucca extract (Y) (A, 0.01% yucca; B, 0.1% yucca) in a two-bottle, drinking assay. The symbols depict the mean response (\pm SEM) for each concentration-group tested ($N = 5/\text{group}$). For both tests (panels A and B) all the contrasts between the responses for a given concentration of test solution and the control were $P < 0.001$, except for the 0.001% concentrations, which did not differ from the control. The parameter values used to fit the solid curve to the observations are presented in Table 2. The dashed curve depicts the expected response of starlings to a simple solution of MA_i (Fig. 3A). The horizontal solid line depicts the mean water intake for the control group, while the horizontal dashed lines depict \pm SEM. The bottom panels depict the interaction index for the mixtures of methyl anthranilate and yucca (C: MA_iY_{0.01%}; D: MA_iY_{0.1%}) for three different hypotheses for how starlings might be responding to mixtures. Values for the interaction index were generated by subtracting the observed response ($R_{[AB],i}^o$) by the predicted response. In the first case, the predicted response was based on the assumption that starlings responded to the mixture as if only attending to the concentration of methyl anthranilate in the solution (solid curve, $R_{[A],i}$). In the second case, the predicted response was based on the assumption that starlings responded to the mixture as if only attending to the concentration of yucca in the solution (dashed curve, $R_{[B],i}$). In the third case, the predicted response was based on the assumption that starlings responded to the mixture based on the average concentration of the solution’s components (dot-dashed line, $R_{[AB],i}$). Hypotheses were ranked by integrating the area under the curves, with the smallest area representing the best fit.

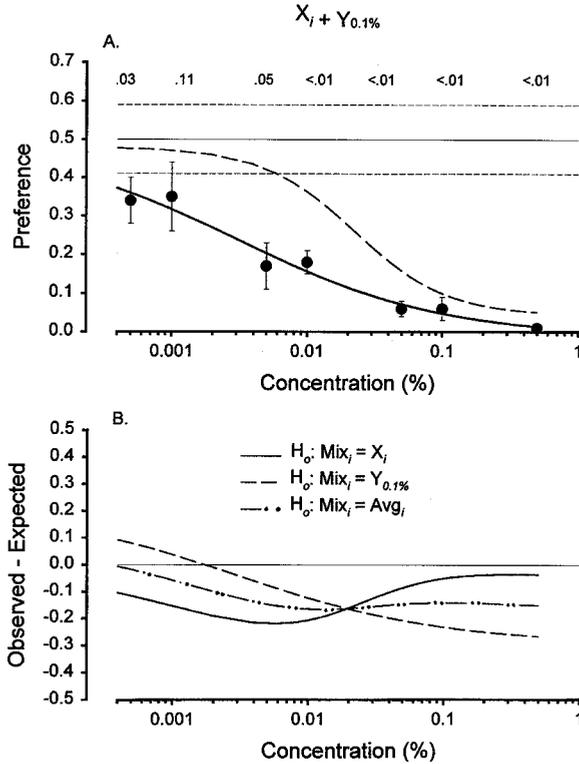


FIG. 6. The top panel (A) depicts the relative concentration–response for starlings presented with aqueous mixtures of *Xanthoxylum* (X) and yucca (Y) extracts in a two-bottle drinking assay. The symbols depict the mean response (\pm SEM) for each concentration–group tested ($N = 5/\text{group}$). The numerical insets represent the probabilities for contrast comparisons between a given test solution and the control group. The parameter values used to fit the solid curve to the observations are presented in Table 2. The dashed curve depicts the expected response of starlings to a simple solution of X_i (Figure 3C). The horizontal solid line depicts the mean water intake for the control group, while the horizontal dashed lines depict \pm SEM. The bottom panel (B) depicts the interaction index for the mixtures of *Xanthoxylum* and yucca. The interaction indices for the mixture were calculated for the three hypotheses by using the method outlined in Figure 5.

Xanthoxylum are needed to elucidate the mechanism(s) of synergism between these two extracts. Preliminary studies on *Xanthoxylum* indicated that at least one of several alkylamides is responsible for the stimulation of thermal nociceptors as well as nerve endings sensitive to touch (Bryant and Mezine, 1999). In humans, this experience produces sensation of tingling, cooling, and pain. The

saponins commonly contained within yucca extracts may act to increase the solubility of the volatile and nonvolatile alkylamides in *Xanthoxylum* and/or render them more likely to penetrate to the bird's receptor fields. It is also possible that yucca and *Xanthoxylum* may stimulate different chemosensitive receptors that, when centrally integrated, produce the synergistic behavioral avoidance of the test mixtures. Understanding the factors stimulating the different mediating receptor mechanisms or the central processes involved in perception of mixtures is important because one goal in the development of chemical repellents as tools for managing the behavior of wildlife is to reduce the amount of material needed to affect a response. To that end we have begun investigations that use cell culture techniques to assess the interaction of extracts and their components on isolated nociceptors responsible for mediating the behavioral avoidance responses (Bryant et al., unpublished data). A second goal is to draw focus to the constituents of natural plant products that might prove useful as repellents and adjuvants for formulations. Our combined efforts in isolation and identification of plant metabolites and their evaluation as repellents and adjuvants in cell culture and behavioral assays will bring us closer to our goals of identifying natural products that can be used in the nonlethal management of conflicts between wildlife and humans.

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