REP 1991 - 021

ORTHO-AMINOACETOPHENONE REPELLENCY TO BIRDS: SIMILARITIES TO METHYL ANTHRANILATE

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Abstract: Methyl anthranilate is an effective bird repellent at concentrations ≥1.0% (g/g). Ortho-aminoacetophenone (OAP) has an odor similar to that of methyl anthranilate and is chemically (structurally) similar. Coincidentally, OAP is present in the scent gland secretions of mustelid species that prey on birds. For these reasons, we chose to test the bird repellency of this material and 3 isomers to European starlings (*Sturnus vulgaris*). Ortho-aminoacetophenone was repellent at concentrations ≤0.01% in both choice and no-choice feeding tests. The other structural isomers (meta-, para-, alpha-) were less effective. Chemically, the results suggest that hydrogen-bonded ring structure formation and basicity predict bird repellent activity. We speculate that methyl anthranilate and OAP are avoided because of perceptual and/or chemical similarities.

J. WILDL. MANAGE. 55(2):334–340

Dimethyl and methyl anthranilate are aversive to many avian species under laboratory conditions (Kare and Pick 1960, Mason et al. 1985, Kare and Mason 1986). Moreover, when anthranilates are used as practical bird repellents under field conditions, 1.0% concentrations reduce livestock feed losses due to consumption by starlings and also reduce the number of birds (mainly starlings) present at treated sites. Consumption by livestock is unaffected (Glahn et al. 1989).

Olfaction and nasal trigeminal chemoreception underlie the aversiveness of dimethyl and methyl anthranilate to birds (Mason et al. 1989), i.e., avoidance is based on odor quality and irritation. To humans, these chemicals have a grape-like or fruity odor and a slightly bitter, pungent taste (Furia and Bellanca 1975:346). Methyl anthranilate is a commonly used grape flavoring in human food preparations (Furia and Bellanca 1975:346) and is the chemical traditionally blamed for the "foxy" quality of red wines produced from *Vitis labrusca* grapes (Amerine and Singleton 1966:28, Broadbent 1970:94). The term "foxy" presumably is derived from the colloquial name of the Concorde grape (i.e., fox grape), and there are suggestions that wines from *V. labrusca* have an "animal-den" odor (Amerine and Singleton 1966:28).

Recent evidence shows that trace quantities of ortho-aminoacetophenone (OAP), and not methyl anthranilate, are responsible for the foxy quality of some wines (Acree et al. 1990). Ortho-aminoacetophenone has an odor similar to that of methyl anthranilate and is structurally similar, differing only in the substitution of a ketone for an ester group. Coincidentally, OAP is present in the scent gland secretions of mustelids that prey on birds (Acree et al. 1990). For these reasons, we chose to evaluate the bird repellency of OAP and 3 isomers. Our aim was to examine the various chemical structures in relation to avoidance exhibited by birds.

MATERIALS AND METHODS

Birds

Adult European starlings were decoy-trapped (U.S. Fish and Wildl. Serv. 1973) in the Philadelphia area and transported to the laboratory.

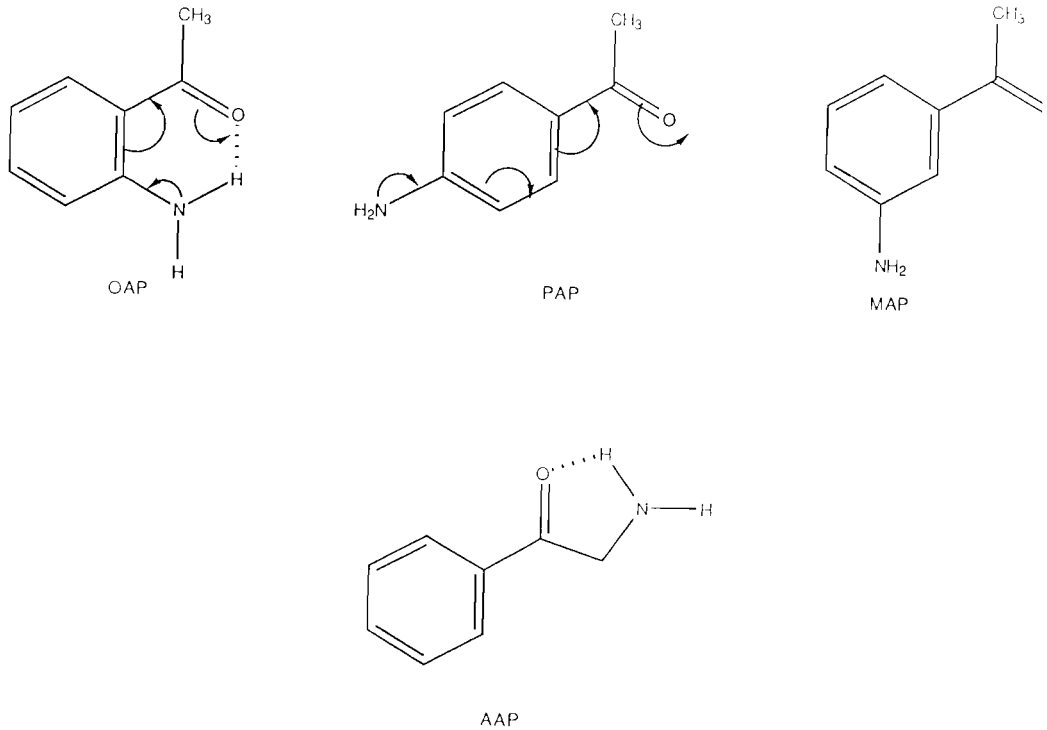

Fig. 1. Chemical structures of ortho-aminoacetophenone (OAP), para-aminoacetophenone (PAP), meta-aminoacetophenone (MAP), and alpha-aminoacetophenone (AAP). Dotted lines depict intramolecular hydrogen bonds. Curved arrows depict delocalization of lone pair of electrons on nitrogen of the amino function (i.e., resonance). On MAP, resonance is impossible, and inductive effects keep electron densities higher on the hetero-atoms oxygen (O) and nitrogen (N).

Upon arrival, the birds were individually caged (61 × 36 × 41 cm) under a 12:12 light:dark cycle with light onset at 0700 hours. Water was available ad libitum. Before experiments began, the birds were permitted free access to Purina Flight Bird Conditioner (feed; Purina Mills, St. Louis, Mo.) and crushed shell grit.

We chose to use starlings for 3 reasons. First, they show good chemical sensing abilities (Mason and Silver 1983, Clark and Mason 1987). Second, comparable data exist concerning the responses of starlings to other avian repellents (e.g., anthranilate derivatives [Mason et al. 1985, 1989; Glahn et al. 1989] and d-pulegone [Mason 1990]) and mammalian irritants (e.g., capsaicin, zingerone, gingerol, allyl isothiocyanate [Mason and Otis 1990]). Third, starlings are considered agricultural pests (Bailey 1966; Besser et al. 1967, 1968).

One week prior to each of the feeding trials described below, birds were subjected to a deprivation regime that involved removing feed and grit from the cages just before dark. Within 30 minutes of light on the next day, feed was returned to the cages.

Chemicals

Ortho-aminoacetophenone (OAP; CAS # 551-93-9), para-aminoacetophenone (PAP; CAS # 99-92-3), meta-aminoacetophenone (MAP; CAS # 99-03-6), and alpha-aminoacetophenone (AAP; CAS # 5468-37-1) were obtained from Sigma Chemical Company (St. Louis, Mo.). Each of these isomers has a phenyl ring with an electron-donating primary amino (NH_2) group and an electron-withdrawing acetyl group ($COCH_3$). They differ only in their substitution patterns on the phenyl ring (as depicted in their names) (Fig. 1). In AAP, the amino group is connected to the methyl of the acetyl group, whereas in the other 3 compounds, the amino group is connected to the phenyl ring. For feeding tests, each was mixed with Purina Flight Bird Conditioner to produce the following concentrations: 1.0, 0.5, 0.1, and 0.01% (g/g).

Procedures

Two-Cup Tests.—We followed the procedures detailed in Mason et al. (1989) for 2-cup avian repellency evaluations. For each isomer, 24 starlings were weighed and then assigned to

4 groups (n = 6/group) on the basis of mass. Specifically, the heaviest bird was assigned to the first group, the next heaviest to the second group, and so on. During the 4-day pretreatment period, all food was removed from the cages within 1 hour of light onset. Next, 2 cups, each containing 50 g of feed, were placed in the front center of each cage. Cups were bound together with a rubber band to reduce spilling, and consumption was assessed after 2 hours. After testing and until light onset of the following day, birds had free access to feed.

On the day following the last pretreatment session, a 4-day treatment period began. Within 1 hour of light onset, each group was given 2 cups. One cup contained 50 g of feed thoroughly mixed with a quantity of an aminoacetophenone isomer. The other cup contained 50 g of plain feed. Cups were bound together with a rubber band to prevent spilling, and cup positions were alternated daily. For each aminoacetophenone isomer, the 4 designated groups received feed containing 1.0, 0.5, 0.1, or 0.01% chemical, respectively. As in pretreatment, consumption was measured after 2 hours. At the end of the fourth treatment trial, all birds were reweighed to assess whether any change from pretreatment mass had occurred.

One-Cup Tests.—We followed the procedures detailed in Mason et al. (1989) for 1-cup avian repellency evaluations. After we completed 2-cup tests for each isomer, the 24 starlings were given a 4-day pretreatment period, identical in all respects to the 2-cup pretreatment period, except that each bird was presented with only 1 cup containing 50 g of feed. A 4-day treatment period immediately followed pretreatment, and on each day, each group was presented with 50-g samples of feed adulterated with a different amount of aminoacetophenone isomer (1.0, 0.5, 0.1, or 0.01% for groups 1–4, respectively). Consumption was recorded after 2 hours. Birds had free access to plain feed and water during the night. At the end of the fourth treatment trial, all birds were reweighed. The same birds were used in both 2-cup and 1-cup tests because it would have been difficult for us to conveniently trap the nearly 200 starlings that would have been required for use of naive birds in each test of each experiment. Second, we were interested in the possibility that birds might habituate to stimulus presentations. The use of experienced birds in 1-cup tests permitted an evaluation of this possibility.

Analysis

For 1-cup tests, consumption on pretreatment Day 4 and treatment Days 1–4 was examined in a 2-factor ANOVA with repeated measures over days. For 2-cup tests, pretreatment and treatment data were analyzed separately in 3-factor ANOVA's with repeated measures over days and cups. Because there were no pretreatment differences, the results of those analyses are not reported.

To determine which isomer was the most effective repellent, mean pretreatment and treatment consumption by each bird in each 1-cup test was computed, and these values were compared across chemicals in a 3-factor ANOVA with repeated measures between periods. The independent factors in this analysis were chemical (4 levels) and concentration (4 levels). We used Tukey post hoc tests (Winer 1962:198) to isolate significant differences among means ($P < 0.05$).

RESULTS

Ortho-Aminoacetophenone.—In 1-cup tests (Fig. 2), consumption differed among concentrations ($F = 3.5$; 3,20 df; $P < 0.03$) and across days ($F = 22.2$; 4,80 df; $P < 0.00001$). Post hoc tests showed that birds given 0.01% treated feed ate significantly more (4.3 ± 0.7 [SE] g) than birds presented with 0.5% treated feed (2.8 ± 0.4 g). However, neither of these groups differed from birds presented with 1.0% or 0.1% treated feed; mean consumption by these birds was 3.8 ± 0.7 and 3.4 ± 0.8 g, respectively. Among days, consumption was significantly higher on pretreatment Day 4 (6.4 ± 0.7 g) than on any treatment day (Days 1–4: 2.9 ± 0.9, 2.5 ± 0.5, 3.6 ± 0.7, and 2.4 ± 0.5 g, respectively). The lack of an interaction between concentrations and days indicated that all OAP concentrations reduced consumption during the treatment period relative to pretreatment Day 4.

Analysis of 2-cup tests (Fig. 2) showed that consumption of treated feed (1.1 ± 0.3 g) was significantly less ($F = 105.6$; 1,20 df; $P < 0.00001$) than consumption of untreated feed (4.0 ± 0.6 g). However, there was also a significant interaction between concentrations and cups ($F = 3.5$; 3,20 df; $P < 0.03$). Post hoc tests showed that consumption of 0.01% treated feed was significantly higher than that of any other treated feed concentration, but also significantly lower than consumption of untreated feed.

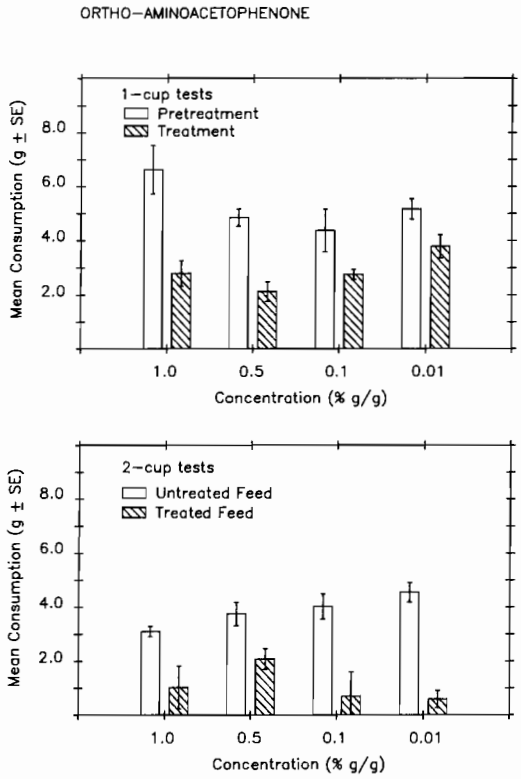

Fig. 2. Consumption of ortho-aminoacetophenone adulterated feed in 1-cup (top) and 2-cup (bottom) tests. Capped vertical lines represent standard errors of the means.

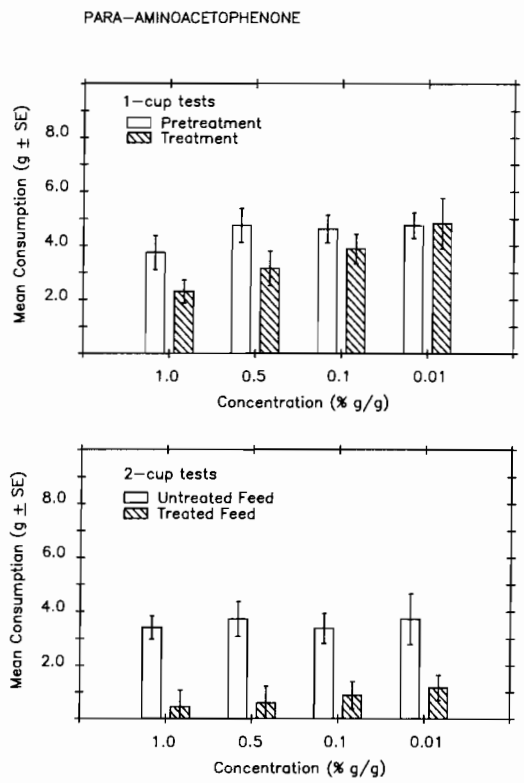

Fig. 3. Consumption of para-aminoacetophenone adulterated feed in 1-cup (top) and 2-cup (bottom) tests. Capped vertical lines represent standard errors of the means.

Para-Aminoacetophenone.—In 1-cup tests (Fig. 3), consumption differed among concentrations ($F = 3.1$; 3,20 df; $P < 0.05$) and across days ($F = 12.7$; 4,80 df; $P < 0.00001$). Post hoc tests showed that consumption of 1.0% treated feed (2.6 ± 0.5 g) was less, and that of 0.01% treated feed (4.9 ± 0.9 g) greater, than consumption of 0.5% (3.6 ± 0.6 g) or 0.1% (4.0 ± 0.6 g) feed. Over days, consumption on pretreatment Day 4 (4.6 ± 0.6 g) was significantly higher than consumption on treatment Days 1 and 2 (2.9 ± 0.5 and 2.9 ± 0.6 g, respectively), but not treatment Days 3 and 4 (3.9 ± 0.7 and 4.4 ± 0.7 g, respectively).

Analysis of 2-cup tests (Fig. 3) showed that consumption of treated feed (0.8 ± 0.1 g) was significantly less ($F = 90.34$; 1,20 df; $P < 0.00001$) than consumption of untreated (3.5 ± 0.6 g) feed. However, there was also a significant interaction between days and cups ($F = 4.7$; 3,60 df; $P < 0.006$). Consumption of treated feed was significantly higher on treatment Day 4 (2.7 ± 0.4 g) than on Days 1 (2.1 ± 0.3 g), 2 (1.7 ± 0.3 g), or 3 (2.0 ± 0.5 g). There were no differences in consumption of untreated feed across days.

Meta-Aminoacetophenone.—In 1-cup tests (Fig. 4), there were significant differences among days ($F = 3.3$; 4,80 df; $P < 0.01$), but not among concentrations ($P > 0.25$). Post hoc tests showed that consumption on pretreatment Day 4 (5.4 ± 0.5 g) did not differ from consumption on treatment Days 1–3 (4.5 ± 0.6, 4.8 ± 0.5, and 4.5 ± 0.4 g, respectively) but was significantly greater than consumption on treatment Day 4 (4.2 ± 0.5 g).

Analysis of 2-cup tests (Fig. 4) showed that consumption of treated feed (1.0 ± 0.2 g) was significantly less ($F = 185.5$; 1,20 df; $P < 0.00001$) than consumption of untreated feed (4.2 ± 0.4 g). Otherwise there were no significant differences ($P > 0.10$).

Alpha-Aminoacetophenone.—In 1-cup tests (Fig. 5), consumption differed among concen-

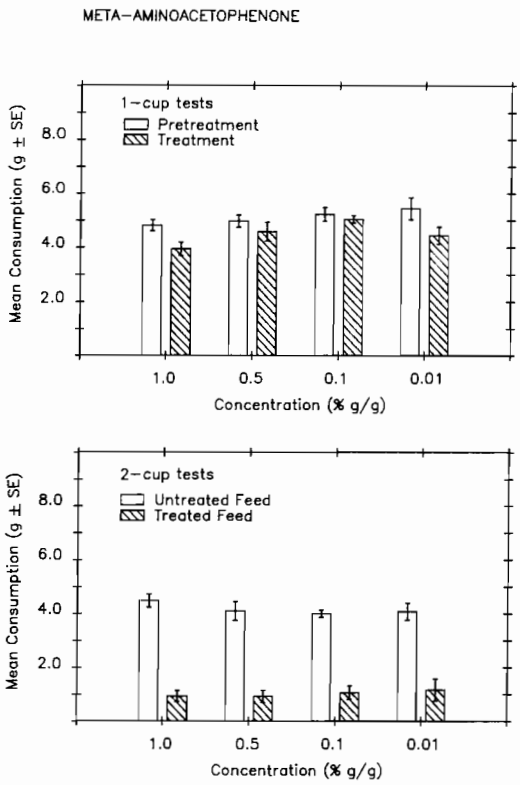

Fig. 4. Consumption of meta-aminoacetophenone adulterated feed in 1-cup (top) and 2-cup (bottom) tests. Capped vertical lines represent standard errors of the means.

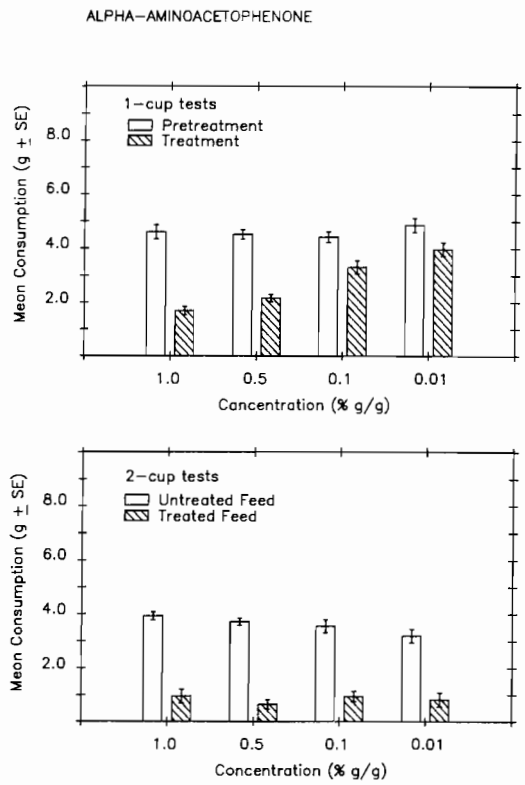

Fig. 5. Consumption of alpha-aminoacetophenone adulterated feed in 1-cup (top) and 2-cup (bottom) tests. Capped vertical lines represent standard errors of the means.

trations ($F = 6.2$; 3,20 df; $P < 0.004$), and across days ($F = 34.3$; 4,80 df; $P < 0.00001$). Post hoc tests showed that consumption increased as concentration decreased (1.0%, 2.2 ± 0.3 g; 0.5%, 2.6 ± 0.3 g; 0.1%, 3.5 ± 0.4 g; and 0.01%, 4.1 ± 0.5 g). Among days, consumption on pretreatment Day 4 (4.3 ± 0.4 g) was greater than on any treatment day (Days 1–4: 2.4 ± 0.3, 2.8 ± 0.4, 2.9 ± 0.4, and 3.0 ± 0.4 g, respectively).

There was also a significant interaction between concentrations and days ($F = 2.8$; 12,80 df; $P < 0.003$). Post hoc tests revealed that the difference between consumption on pretreatment Day 4 and on the treatment days became smaller as concentration decreased.

Analysis of 2-cup tests (Fig. 5) showed that consumption of treated feed (0.8 ± 0.3 g) was significantly less ($F = 381.9$; 1,20 df; $P < 0.00001$) than consumption of untreated feed (3.6 ± 0.2 g). Also, there was a significant difference among days ($F = 16.4$; 3,60 df; $P < 0.00001$); significantly less was eaten on Day 3 (1.9 ± 0.2 g) than on Day 2 (2.6 ± 0.3 g). Overall consumption did not differ significantly on either of these days from consumption on Day 1 (2.0 ± 0.3 g) or Day 4 (2.3 ± 0.2 g).

Overall Comparison of 1-Cup Test Results.—There were differences among aminoacetophenone isomers as a function of mean pretreatment and treatment consumption ($F = 9.2$; 3,80 df; $P < 0.0001$). Post hoc tests showed that OAP produced the largest pretreatment to treatment drop in consumption, followed by AAP, and then PAP (Fig. 6). Meta-aminoacetophenone produced the smallest pretreatment to treatment decrement in consumption.

DISCUSSION

All of the aminoacetophenone isomers elicited strong avoidance in 2-cup feeding tests. This result was not unexpected, given the sensitivity of 2-cup tests in detecting repellency. However, differences among isomers emerged in relatively less sensitive 1-cup trials. Ortho-aminoacetophenone produced the strongest avoidance, followed by AAP and PAP. Meta-aminoaceto-

phenone was least effective. When compared with methyl anthranilate, which is effective to concentrations of 0.3% in the laboratory and 1.0% in the field (Glahn et al. 1989, Mason et al. 1989), OAP is at least 1 order of magnitude more effective.

Chemically, the results suggest that intramolecular hydrogen bonding capacities are associated with effectiveness—both OAP and AAP have high capacities whereas PAP and MAP have none (Fig. 1). These latter compounds are only capable of weak intermolecular hydrogen bonding. (The same capacities can be used to explain the superior repellency of dimethyl and methyl anthranilate, relative to other anthranilates.) In addition, basicity (and/or electron donating ability of the amino group) is related to repellency. Both OAP and PAP have an electron-withdrawing acetyl group in resonance (i.e., the electrons are directly in conjugation with the double bonds or pi-cloud, resulting in higher delocalization [Fig. 1]) with an amino group, resulting in lower basicity of the latter. Conversely, for MAP, electron donation by resonance cannot occur. The amino group of this compound withdraws electrons by inductive effect; i.e., the electrons cannot delocalize, but the differences in the electronegativities of the connected atoms will partially pull the electrons on the more electronegative side of the connecting bond, keeping the basicity of the amino group at a higher level.

Ecologically, OAP may be a more effective repellent than MAP because it occurs naturally in mustelid scent gland secretions (Acree et al. 1990). On another level, the relatively greater effectiveness of OAP can be explained chemically. Ortho-aminoacetophenone remains in only 1 hydrogen-bonded ring-form structure, but methyl anthranilate can assume 2 different forms—one of which may be more active than the other. Perhaps the repellency of methyl anthranilate is diluted by the presence of the relatively ineffective form.

MANAGEMENT IMPLICATIONS

Few nonlethal chemicals (i.e., repellents) are available for the control of avian depredation and nuisance problems (Thomson 1989:115–148). Flavorings such as dimethyl and methyl anthranilate are used in human and animal foods and are potential sources of new repellents (Mason et al. 1985, Clark and Mason 1987, Glahn et al. 1989). We suggest that aminoacetophenone isomers also might be used in some bird damage control applications and that OAP shows the greatest promise. As with dimethyl and methyl anthranilate, it might be possible to incorporate low levels of OAP into livestock feeds as a bird repellent (Glahn et al. 1989). Alternatively, OAP could be used to deter crop depredation, or as a repellent additive to formulated agricultural chemicals or mammalian poisons to reduce accidental ingestion by birds.

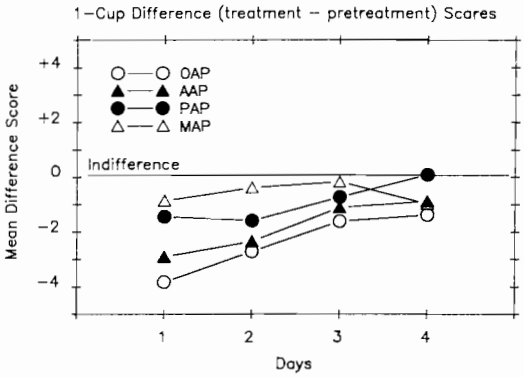

Fig. 6. Mean difference scores calculated by subtracting 1-cup pretreatment consumption from 1-cup treatment consumption. Negative scores indicate that birds ate more during pretreatment. OAP = ortho-aminoacetophenone, AAP = alpha-aminoacetophenone, PAP = para-aminoacetophenone, MAP = meta-aminoacetophenone.

Broadly, the most important management implications of this experiment may be that hydrogen-bonded ring structure formation and basicity might predict bird repellent activity. Although avian chemical repellents clearly are irritants (i.e., trigeminal stimuli [Mason et al. 1989]), it is equally clear that there are fundamental differences between irritants for mammals and those for birds (Mason and Otis 1990: 309–325). To date, no mammalian irritant has been shown to successfully repel birds. Even capsaicin, the pungent principle in *Capsicum* (jalepeno) peppers, is inoffensive to birds. While capsaicin concentrations as low as 20 ppm reliably repel mammals, concentrations as high as 20,000 ppm are readily accepted by parrots (*Amazona* spp.) (Mason and Reidinger 1983:20–39), pigeons (Szolcsanyi et al. 1986), and red-winged blackbirds (Mason and Maruniak 1983). The present experiment, to our knowledge, is one of only 2 attempts to examine the structure of avian irritants in relation to behavioral activity, and the first to suggest that specific molecular features, apart from volatility and lipophyllicity (Mason et al. 1989), are correlated

with effectiveness. Because no mammalian irritant can be assumed a priori to affect birds, we propose that empirical studies of structure–activity relationships are one of the most promising approaches to the development of new avian repellents.

LITERATURE CITED

ACREE, T. E., E. H. LAVIN, R. NISHIDA, AND S. WATANABE. 1990. The serendipitous discovery of ortho-amino acetophenone as the 'foxy' smelling component of Labruscana grapes. Chem. and Eng. News 9:80.

AMERINE, M. A., AND V. L. SINGLETON. 1966. Wine: an introduction for Americans. Univ. California Press, Los Angeles. 357pp.

BAILEY, E. P. 1966. Abundance and activity of starlings in winter in northern Utah. Condor 68:152–162.

BESSER, J. F., J. W. DEGRAZIO, AND J. L. GUARINO. 1968. Costs of wintering starlings and red-winged blackbirds at feedlots. J. Wildl. Manage. 32:179–180.

———, W. C. ROYALL, AND J. W. DEGRAZIO. 1967. Baiting starlings with DRC-1339 at a cattle feedlot. J. Wildl. Manage. 31:48–51.

BROADBENT, J. M. 1970. Wine tasting. Wine and Spirit Publ., Ltd., London. 106pp.

CLARK, L., AND J. R. MASON. 1987. Olfactory discrimination of plant volatiles by the European starling. Anim. Behav. 35:227–235.

FURIA, T. E., AND N. BELLANCA. 1975. Handbook of flavor ingredients. CRC Press, Cleveland, Oh. 771pp.

GLAHN, J. F., J. R. MASON, AND D. R. WOODS. 1989. Dimethyl anthranilate as a bird repellent in livestock feed. Wildl. Soc. Bull. 17:313–320.

KARE, M. R., AND J. R. MASON. 1986. The chemical senses in birds. Pages 59–74 *in* P. D. Sturkie, ed. Avian physiology. Springer-Verlag, New York, N.Y. 516pp.

———, AND H. L. PICK. 1960. The influence of the sense of taste on feed and fluid consumption. Poult. Sci. 39:697–706.

MASON, J. R. 1990. Evaluation of d-pulegone as an avian repellent. J. Wildl. Manage. 54:130–135.

———, M. A. ADAMS, AND L. CLARK. 1989. Anthranilate repellency to starlings: chemical correlates and sensory perception. J. Wildl. Manage. 53:55–64.

———, J. F. GLAHN, R. A. DOLBEER, AND R. F. REIDINGER. 1985. Field evaluation of dimethyl anthranilate as a bird repellent livestock feed additive. J. Wildl. Manage. 49:636–642.

———, AND J. A. MARUNIAK. 1983. Behavioral and physiological effects of capsaicin in red-winged blackbirds. Pharmacol. Biochem. Behav. 19:857–862.

———, AND D. L. OTIS. 1990. Chemical nociception in birds. Pages 309–322 *in* B. Green and J. R. Mason, eds. Chemical nociception in the nose and mouth. Marcel Dekker, New York, N.Y. 361pp.

———, AND R. F. REIDINGER, JR. 1983. Exploitable characteristics of neophobia and food aversions for improvements in rodent and bird control. Pages 20–39 *in* D. E. Kaukienen, ed. Vertebrate pest control and management materials. Am. Soc. for Testing and Materials, Philadelphia, Pa. 315pp.

———, AND W. L. SILVER. 1983. Conditioned odor aversions in starlings (*Sturnus vulgaris*) possibly mediated by nasotrigeminal cues. Brain Res. 269: 196–199.

SZOLCSANYI, J., H. SANN, AND F-K. PIERAU. 1986. Nociception in pigeons is not impaired by capsaicin. Pain 27:247–260.

THOMSON, W. T. 1989. Agricultural chemicals III—fumigants, growth regulators, repellents, and rodenticides. Thomson Publ., Fresno, Calif. 210pp.

U.S. FISH AND WILDLIFE SERVICE. 1973. Building and operating a decoy-trap for live capturing of cowbirds and other birds. U.S. Fish Wildl. Serv. Res. Publ. AC-211. 4pp.

WINER, B. J. 1962. Statistical principles in experimental design. McGraw-Hill Book Co., New York, N.Y. 907pp.

Received 21 August 1990.
Accepted 31 October 1990.
Associate Editor: Conover.