

EVALUATION OF D-PULEGONE AS AN AVIAN REPELLENT

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Abstract: Despite increasing demand, few chemical repellents are available for control of avian depredation and nuisance problems. New potential repellents may include flavorings that are used in human and animal foods and are offensive to birds. D-pulegone, a mint flavor, may represent a useful repellent; it is used at low (<1%) concentrations as a food additive. I assessed the repellency of d-pulegone in 1-cup and 2-cup feeding tests with European starlings (*Sturnus vulgaris*). Concentrations as low as 0.01% (g/g) significantly reduced consumption by birds in both 1-cup and 2-cup tests. Further evaluation of d-pulegone and similar compounds (e.g., mangone) appears warranted.

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Despite increasing demand, few nonlethal chemicals (i.e., repellents) are available for the control of avian depredation and nuisance problems (Thomson 1989:115-148). Among those substances registered as repellents, only methiocarb appears useful. However, the cost of methiocarb is prohibitively high (\$54.00/kg) for use in many agricultural situations. I question whether the registration of this substance on food crops will be continued (D. L. Otis, Denver Wildl. Res. Cent., Denver, Colo., pers. commun.).

Some flavorings, such as dimethyl and methyl anthranilate (odor and/or taste materials) that are used in human and animal foods are offensive to birds and are potential sources of new repellents (Mason et al. 1985, Mason and Clark 1987, Glahn et al. 1990). D-pulegone may rep-

resent another useful flavor repellent; it is the active flavor principal in pennyroyal species (e.g., *Mentha pulegium*, *M. longifolia*) and, at low concentrations (<1%), is used as a mint additive in human food preparations. Recent evidence suggests that d-pulegone repels dogs (Mason et al. 1989c) and at high concentrations is an effective insecticide (Duke 1987:223-225). Because the physiological basis for the insecticidal properties of d-pulegone are similar to the physiological basis for methiocarb repellency to birds (i.e., reversible inhibition of acetylcholinesterase synthesis, Ryan and Byrne [1988]), I tested whether food treated with d-pulegone was aversive to European starlings.

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METHODS

Subjects.—Thirty adult male European starlings were trapped using decoys (U.S. Fish Wildl. Serv. 1973) during February 1989 and transported to the laboratory. Each bird was banded and individually caged (cage dimensions: 61 × 36 × 41 cm) under a 6/18 hour light/dark cycle. During the 2-week period prior to the beginning of testing, all birds were permitted free access to Purina Flight Bird Conditioner (PFBC) and crushed shell grit.

Chemical.—D-pulegone was obtained as a liquid from International Fragrances and Flavors (Union Beach, N.J., CAS No. 89-82-7) in the purest form (≥90%) commercially available.

Two-Cup Tests.—The procedures detailed in Mason et al. (1989b) for 2-cup avian repellency evaluations were followed. Briefly, 15 European starlings were randomly selected, weighed, and then assigned to 3 groups ($n = 5/\text{group}$) 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 5-day pretreatment period, all food was removed from the cages within 1 hour of light onset. Next, 2 cups, each containing 50 g of PFBC, were placed in the front center of each cage. Cups were bound together with a rubber band to reduce spillage, and consumption was assessed after 2 and 6 hours. After testing and until light onset of the following day, birds had free access to feed.

On the day immediately following the last pretreatment day, the 5-day treatment period began. Within 1 hour of light onset, each group was given 2 cups. One contained 50 g of PFBC thoroughly mixed with different quantities of d-pulegone. The other contained 50 g of plain PFBC. Cups were bound together with a rubber band, and cup positions were alternated daily. Groups 1–3 received PFBC containing 1.0% d-pulegone (g/g), PFBC containing 0.1% d-pulegone, or PFBC containing 0.01% d-pulegone, respectively. As in pretreatment, consumption was measured after 2 and 6 hours. Spillage reflected consumption and is not reported. At the end of the fifth treatment trial,

all birds were reweighed to assess whether any change from pretreatment mass had occurred.

Results were analyzed in 2 ways using parametric statistics. First, mean pretreatment and treatment consumption were assessed in a 3-factor ANOVA with repeated measures on the second (period) and third (cups) factors. Next, treatment period preference ratios for d-pulegone were calculated by dividing the mean pulegone consumption of each bird by the bird's total mean consumption. Mean preference ratios were assessed in a 1-factor independent measures ANOVA. Tukey post hoc tests (Winer 1962:198) were used to isolate significant differences among means ($P < 0.05$).

One-Cup Tests.—The procedures detailed in Mason et al. (1989b) for 1-cup avian repellency evaluations were followed. Briefly, the remaining 15 naive starlings were individually caged, maintained, and assigned to 3 groups ($n = 5/\text{group}$), as described above. On the day following group assignment, a 5-day pretreatment period began, identical in all respects to the 2-cup pretreatment period, except that each bird was presented with only 1 cup containing 50 g of PFBC. A 5-day treatment period immediately followed pretreatment, and during treatment each group was presented with 50 g samples of PFBC adulterated with a different amount of d-pulegone (1.0%, 0.1%, and 0.01% for Groups 1–3, respectively). Consumption was recorded at 2 and 6 hours. As in the 2-cup tests, spillage reflected consumption. Birds had free access to plain PFBC and water during the night. At the end of the fifth treatment trial, all birds were reweighed.

A 2-factor ANOVA with repeated measures on the second factor (periods) was used to assess 1-cup mean consumption. Tukey post hoc tests were used to isolate significant differences among means ($P < 0.05$).

RESULTS

Two-Cup Tests.—Measurements after 2 and 6 hours revealed the same pattern of results, so only the 6-hour data are reported. Analysis of mean consumption showed significant differences among concentrations, between periods, and between cups (Table 1). Also, interactions between concentrations and periods, and between periods and cups were significant. Final-

Table 1. Three-factor ANOVA used to examine mean pretreatment and treatment consumption in 2-cup tests. The independent factor was concentration (1.0%, 0.1%, and 0.01% d-pulegone), whereas the repeated factors were periods and cups.

Source	SS	df	MS	F	P
Between groups					
Concentration	21.67	2	10.83	8.4	0.0056
Error	15.50	12	1.29		
Within groups					
Period	9.70	1	9.70	33.53	0.0002
(Concentration)(period)	11.46	2	5.73	19.81	0.0003
Error	3.47	12	0.29		
Cup	90.65	1	90.65	21.54	0.0008
(Concentration)(cup)	20.75	2	10.37	2.46	0.1260
Error	50.51	12	4.21		
(Period)(cup)	123.75	1	123.75	26.56	0.0004
(Concentration)(period)(cup)	65.82	2	32.91	7.06	0.0095
Error	55.90	12	4.66		
Total	469.20	59			

ly, the 3-way interaction among concentrations, periods, and cups was significant.

Post hoc examination of the main effect for concentration showed that overall mean consumption (i.e., consumption collapsed over periods and cups) by birds given either 0.1% (3.9 ± 0.04 [SE]) or 0.01% d-pulegone (4.07 ± 0.02) was significantly lower ($P = 0.01$) than consumption by birds presented with 1.0% d-pulegone (5.3 ± 0.1). Regarding the main effect for periods, overall consumption during treatment was slightly, but significantly ($P = 0.01$), higher (4.8 ± 0.4) than during pretreatment (4.0 ± 0.11). Regarding the main effect for cups, overall consumption from the cup assigned to the d-pulegone treatment was significantly less ($P = 0.01$) (3.2 ± 0.2 vs. 5.6 ± 0.5). This difference reflected large reductions in consumption of pulegone-treated feed during the treatment period.

Post hoc examination of the interactions between concentrations and periods, and between periods and cups revealed the following patterns. First, although consumption by birds given 0.1% d-pulegone was significantly higher ($P = 0.01$) during the treatment period (4.9 ± 0.3) than during pretreatment (3.2 ± 0.2), birds presented with either 1.0% or 0.01% d-pulegone ate approximately equal amounts during both periods (1.0%: 5.4 ± 0.11 vs. 5.0 ± 0.6 , 0.01%: 3.3 ± 0.7 vs. 4.4 ± 0.6 , pretreatment vs. treatment, respectively). Second, regarding the interaction between periods and cups, consumption from the d-pulegone cup was significantly lower (P

$= 0.01$) (2.1 ± 0.9) than consumption from either cup pretreatment (left cup: 4.2 ± 0.25 , right cup: 3.8 ± 0.7) or from the plain PFBC cup during treatment (7.4 ± 0.68). Consumption from the plain PFBC cup during treatment was significantly higher ($P = 0.01$) than consumption from any other cup in either period.

Examination of the interaction among concentrations, periods, and cups showed that all concentrations of d-pulegone significantly reduced ($P = 0.01$) consumption relative to plain PFBC and that the greater the d-pulegone concentration, the larger these effects (Fig. 1).

Analysis of preference ratios, calculated on the basis of treatment period consumption, revealed significant differences among d-pulegone concentrations (Table 2). Post hoc tests showed that preference ratios for 1.0% d-pulegone were significantly lower ($P = 0.01$) than those for 0.1% d-pulegone, and both 1.0% and 0.1% d-pulegone produced ratios that were lower than those for 0.01% d-pulegone (Fig. 2).

Comparison of pretreatment and posttreatment mass of birds indicated no changes had occurred. The pretreatment mean mass of birds given 1.0%, 0.1%, and 0.01% pulegone were 75.1 ± 3.7 g, 76.6 ± 1.7 g, and 78.1 ± 2.1 g, respectively. On the fifth treatment day, the mean masses in these groups were 78.5 ± 3.4 g, 75.4 ± 2.5 g, and 76.1 ± 1.2 g, respectively.

One-Cup Tests.—As in 2-cup tests, measurements after 2 and 6 hours revealed the same pattern of results; only the 6-hour data are reported. Analysis of mean consumption showed

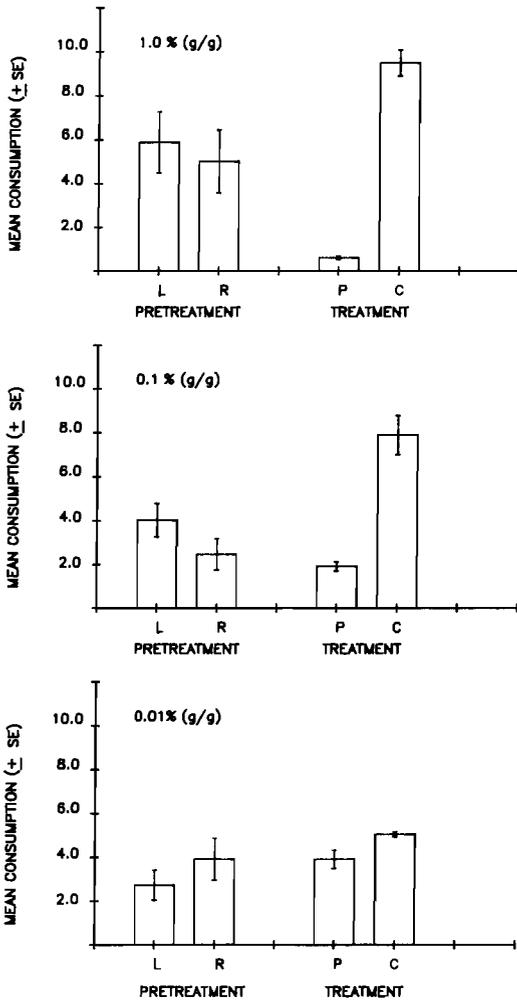


Fig. 1. Mean consumption by starlings of d-pulegone adulterated and plain Purina Flight Bird Conditioner in 2-cup, 6-hour tests. Capped vertical bars represent standard errors. L = left cup, R = right cup, P = pulegone cup, C = control cup.

no differences among concentrations, but differences between periods were significant (Table 3). The lack of a concentration by period interaction indicated that all concentrations were equally effective in reducing consumption (Fig. 3). As in 2-cup tests, the mean mass of birds did not change over the course of testing. The pretreatment mean masses of birds given 1.0%, 0.1%, and 0.01% pulegone were 76.2 ± 2.7 g, 75.3 ± 1.8 g, and 77.2 ± 3.2 g, respectively. On the fifth treatment day, the mean masses in these groups were 75.8 ± 3.3 g, 76.2 ± 3.5 g, and 75.1 ± 3.7 g, respectively.

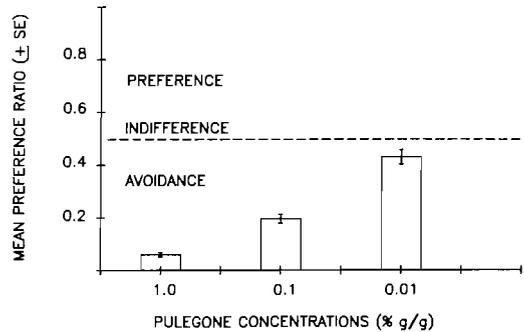


Fig. 2. Mean preference ratios exhibited by starlings for d-pulegone adulterated Purina Flight Bird Conditioner in 2-cup, 6-hour tests. Ratios were calculated by dividing consumption of d-pulegone-adulterated feed by total treatment consumption (d-pulegone and plain feed consumption). A ratio of zero indicates complete rejection of d-pulegone, whereas ratios of 1.0 and 0.5 indicate complete preference or indifference, respectively. Capped vertical bars represent standard errors.

DISCUSSION AND MANAGEMENT IMPLICATIONS

Despite increasing demand, few chemical repellents are available for control of avian depredation and nuisance problems. D-pulegone may represent an important addition to the materials used for these purposes. Concentrations of d-pulegone between 0.01 and 1.0% repel starlings in both 2-cup and 1-cup tests. Because even 0.01% d-pulegone is aversive (particularly in 1-cup tests), concentrations below 0.01% might elicit avoidance. Accordingly, d-pulegone appears to be at least 10 times more effective against starlings than methyl and dimethyl anthranilate that act via odor, taste, or trigeminal stimulation (Mason et al. 1989a). Besides being effective, d-pulegone appears relatively safe because the red-winged blackbird (*Agelaius phoeniceus*) LD₅₀ for d-pulegone is >316 mg/kg compared to the methiocarb LD₅₀ of 3-7 mg/kg (Schafer et al. 1983). Finally, d-pulegone is relatively inexpensive; in bulk quantities the price ranges between 18 and \$32/kg (D. DeRovira, Flavor Dynamics, Inc., Somerset, N.J., pers. commun.).

Table 2. Single-factor independent measures ANOVA of mean preference ratios calculated on the basis of the mean treatment consumption by each bird in 2-cup tests.

Source	SS	df	MS	F	P
Concentration	0.35	2	0.17	117.27	0.00001
Error	0.02	12	0.001		
Total	0.37	14			

Table 3. Two-factor ANOVA used to examine mean pretreatment and treatment consumption in 1-cup tests. The independent factor was concentration (1.0%, 0.1%, and 0.01% d-pulegone), whereas the repeated factor was periods.

Source	SS	df	MS	F	P
Between groups					
Concentration	0.46	2	0.23	0.2	
Error	14.13	12	1.18		
Within groups					
Period	51.48	1	51.48	54.10	0.0001
(Concentration)(period)	1.28	2	0.64	0.67	
Error	11.42	12	0.95		
Total	78.77	29			

Whether or not d-pulegone has the same bird-specific qualities as various anthranilate derivatives such as dimethyl or methyl anthranilate (Mason et al. 1985, 1989a) remains unclear. However, d-pulegone may repel both birds and mammals because the concentrations used in my experiments are significantly repellent to dogs (Mason et al. 1989c) and rats (J. G. Miller, Univ. Miss., pers. commun.). Further testing of d-pulegone with other species appears warranted.

In addition to more extensive comparative testing of d-pulegone, it may be worthwhile to examine a variety of compounds that contain d-pulegone. For example, mangone (d-pulegone mercaptan), although considerably more expensive than d-pulegone (\$164/kg, D. De-Rovira, Flavor Dynamics, Inc., Somerset, N.J., pers. commun.), is much more odorous, with a human olfactory detection threshold in the range of parts per billion (M. Rabin, Int. Flavors and Fragrances, pers. commun.). Given my assump-

tion that the repellency of pulegone is mediated in part by olfaction, if mangone is repellent to birds, then it may also be more aversive at considerably lower concentrations than d-pulegone.

Although I believe that volatility as an irritating sensory cue plays a role in d-pulegone avoidance, d-pulegone repellency might also be mediated by gastrointestinal malaise. D-pulegone is a reversible cholinesterase inhibitor (Duke 1987:223–225, Ryan and Byrne 1988) and therefore might elicit conditioned food avoidance learning via mechanisms similar to those that promote methiocarb-induced food avoidance. However, observation of the birds in my experiments provided no obvious evidence of malaise or changes in body mass, and in 2-cup tests, the highest d-pulegone consumption occurred on the first treatment day. Perhaps sufficient material was ingested on that day to condition avoidance responses, contributing to the low levels of consumption observed on subsequent treatment days. Experiments in which birds are force fed d-pulegone as an unconditioned stimulus following ingestion of a distinctive food will be necessary to resolve this issue.

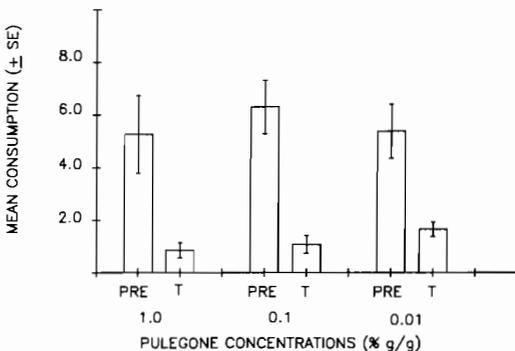


Fig. 3. Mean consumption by starlings of d-pulegone adulterated Purina Flight Bird Conditioner in 1-cup, 6-hour tests. Capped vertical bars represent standard errors. Pre = pretreatment, T = treatment.

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