

## Response of European starlings to menthone derivatives: evidence for stereochemical differences in repellency

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The present experiments were designed to identify inexpensive substitutes for D-pulegone, an effective vertebrate repellent. In experiment 1, European starlings (*Sturnus vulgaris*) were presented with D-pulegone and 12 additional menthone derivatives in one-cup tests. No derivative was as effective as D-pulegone. In experiment 2, starlings were presented with DL-pulegone, D-pulegone and L-pulegone in one-cup tests. DL-pulegone was as aversive as D-pulegone, and both DL-pulegone and D-pulegone were more aversive than L-pulegone. More extensive evaluation of DL-pulegone as a vertebrate repellent appears warranted. Published by Elsevier Science Ltd

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Despite increasing demand, no general repellents are available for the control of vertebrate pests (Thomson, 1995). Those materials that are available either affect one taxonomic group more than others, or are difficult to use. For example, methyl anthranilate is an effective bird repellent (Cummings, Mason, Otis and Heisterberg, 1991; Mason, Adams and Clark, 1989), but the cost of formulated product is high ( $> \$45.00 \text{ kg}^{-1}$ ), and effectiveness against mammals is limited (Mason, Clark and Shah, 1992). Capsaicin is an effective mammal repellent, but it is aversive to humans, difficult to formulate (Nolte, Mason and Clark, 1993), and taxonomically specific. Even high concentrations of capsaicin ( $\geq 1000 \text{ ppm}$ ) are inoffensive to birds (Norman, Mason and Clark, 1992; Szolcsanyi, Sann and Pierau, 1986).

A promising source of non-specific repellents may be plant natural products that serve as omnibus defenses against herbivory (e.g. Jakubas, Shah, Mason and Norman, 1992). Such chemicals are readily available, and may be easy to commercialize since the US Environmental Protection Agency proposes to deregulate their use (Fed. Reg. 59[178], 47289-47292). D-pulegone is a volatile natural product distilled from mint (*Mentha* spp.; Duke, 1987). Although it is avoided by birds and mammals at low concentrations [ $\leq 1.0\% \text{ m/m}$  (mass/mass); Mason, 1990; Mason and Dolbeer, unpubl. data; Mastrota and Mench, 1995], D-pulegone is probably too expensive for many applica-

tions. The present experiments were designed to explore two plausible schemes for cost reduction. Experiment 1 tested whether other potentially less expensive menthone derivatives might substitute for D-pulegone. Experiment 2 evaluated whether less expensive racemic (DL) pulegone might be as aversive as D-pulegone alone.

### Materials and methods

#### Experiment 1

**Subjects.** Twenty adult European starlings (*Sturnus vulgaris*) were selected from the laboratory colony. Each bird was individually caged (cage dimensions:  $61 \times 36 \times 41 \text{ cm}$ ) under a 6/18 hour light/dark cycle. Prior to the experiment, all birds were permitted free access to Purina Flight Bird Conditioner (PFBC) and crushed oyster shell grit.

**Chemicals.** D-pulegone, thymol, D-menthol, DL-menthol, L-carveol, dihydrocarvone, isopulegol, isopulegol acetate, terpineol coeur, L-carvone, menthone, isomenthone and piperitone were obtained from International Flavors and Fragrances (Union Beach, NJ, USA). Each substance was mixed with PFBC to produce 0.5% m/m stimulus concentrations.

**Procedure.** Starlings were randomly assigned to four groups ( $n = 5$  per group). On the day following group assignment, a 5-day pre-treatment period began. On each day, PFBC was removed from cages within 1 h of light onset. Next, a cup containing 20 g of plain feed was placed in the front center of each cage. After 6 h,

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consumption was recorded. After testing and until light onset of the following day, birds had free access to unadulterated feed.

A 12-day treatment period followed pre-treatment. Each of the four groups was presented with a different randomly selected order of the 12 menthone derivatives. Only one derivative was presented each day. Procedures were similar to those followed during pre-treatment. For each trial, PFBC was removed from cages within 1 h of light onset, and birds were presented with stimulus foods in one-cup tests. After 6 h, consumption was recorded, and untreated PFBC was provided until light onset of the following day.

**Analysis.** Data were evaluated in a two-factor analysis of variance (ANOVA) with repeated measures over stimuli. The independent factor was groups. Mean pre-treatment consumption was included in the analysis as a level of the stimulus factor. Subsequent to the omnibus procedure, Tukey *post-hoc* tests were used to isolate differences ( $P < 0.05$ ) among means.

### Experiment 2

**Subjects.** Eighteen adult European Starlings were selected from the laboratory colony, individually caged, and maintained as previously described.

**Chemicals.** D-pulegone, L-pulegone, and DL-pulegone were mixed with PFBC to produce 0.5% m/m stimulus concentrations. The racemic mixture contained approximately equal amounts of each isomer.

**Procedure.** The starlings were assigned to three groups ( $n = 6$  per group). After 4 days of pre-treatment, each group was exposed to a stimulus food in 6 h, one-cup tests. On all four treatment days, group 1 was presented with D-pulegone, group 2 was exposed to L-pulegone, and group 3 was presented with the racemic mixture.

**Analysis.** Data were evaluated in a three-factor ANOVA with repeated measures over periods and

days. The independent factor was groups. Tukey tests were used to isolate differences among means.

## Results

### Experiment 1

There were differences ( $P < 0.0001$ ) among chemicals. D-pulegone decreased ( $P < 0.05$ ) consumption relative to pre-treatment or to any of the other menthone derivatives (Figure 1). Isomenthol also reduced ( $P < 0.05$ ) consumption relative to pre-treatment, isopulegol, dihydrocarvone, L-carveol, terpineol, thymol and racemic menthol.

### Experiment 2

There were differences among groups ( $P < 0.04$ ), and between periods ( $P < 0.00001$ ). The interaction between these terms was significant ( $P < 0.05$ ) and the analysis was interpreted in terms of that highest order effect. All three forms of pulegone reduced consumption relative to pre-treatment ( $P < 0.05$ , Figure 2). However, D-pulegone and the DL-pulegone were more aversive ( $P < 0.05$ ) than L-pulegone.

## Discussion and management implications

D-pulegone was more repellent than the other 12 menthone derivatives. Among these other stimuli, only isomenthol decreased consumption relative to pre-treatment; however, isomenthol was less effective than D-pulegone.

Avian responsiveness to chemical stimuli is eclectic and not readily predictable from standard physico-chemical parameters (Kare and Mason, 1986). This is especially true for natural products. Turpentine, for example, is repellent to insects (Duke, 1987), rodents (Janzen, 1978), deer (Connolly, Ellison, Fleming, Geng, Kepner, Longhurst, Oh and Russell, 1980), and some birds (e.g. brown-headed cowbirds, *Molothrus ater*; Mason and Bonwell, 1993). However, red-winged

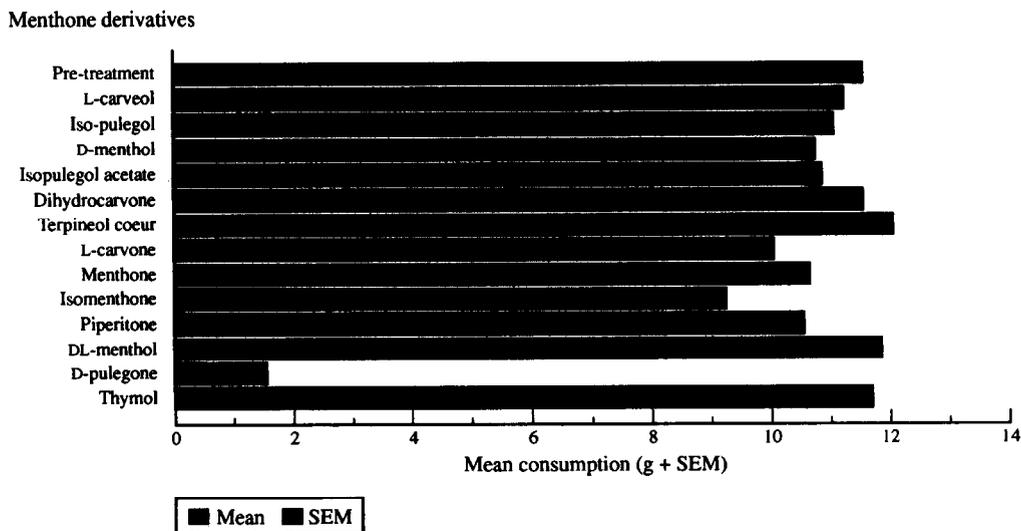


Figure 1. Mean consumption by European starlings of feed adulterated with D-pulegone and 12 other menthone derivatives in 6-h, one-cup tests – experiment 1. All substances were mixed with chow to produce 0.5% (m/m) concentrations

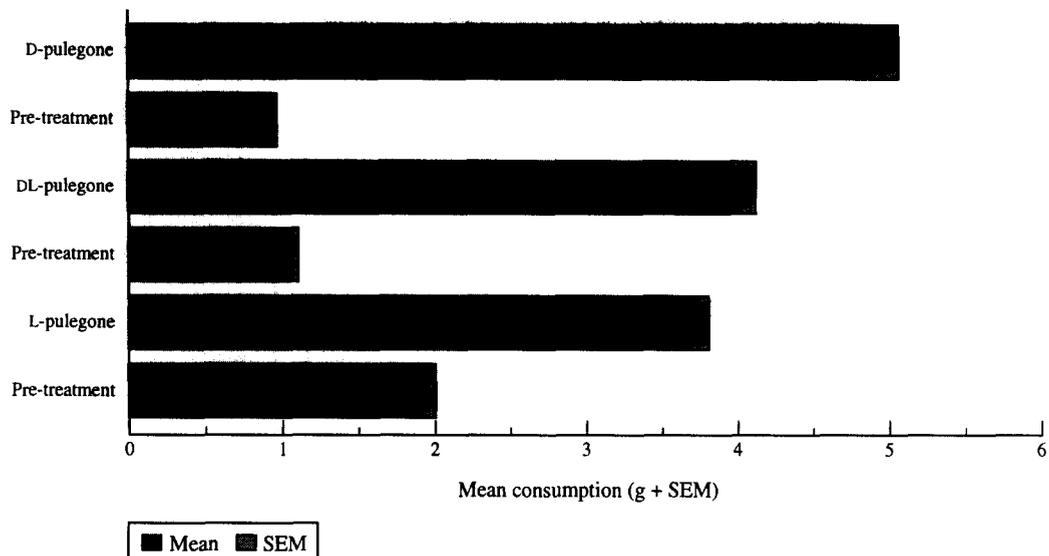


Figure 2. Mean consumption by European starlings of feed adulterated with D-pulegone, DL-pulegone and L-pulegone in 6-h, one-cup tests – experiment 2. Pre-treatment consumption by each group is also shown

blackbirds (*Agelaius phoeniceus*) and common grackles (*Quiscalus quisculus*) are indifferent to turpentine mixed into feed at concentrations  $\geq 5.0\%$  (m/m; Mason and Bonwell, 1993). No simple explanation for species and taxonomic differences can be offered, although overall chemosensitivity is probably important. There is evidence that cowbirds are more responsive to chemical stimuli than either red-wings or grackles (Clark and Mason, 1989; Mason, Avery, Glahn, Otis, Matteson and Nelms, 1991).

In experiment 2, there were no differences in the repellency of D-pulegone and DL-pulegone, and both were more aversive than L-pulegone. These results have two implications. One is that the relatively less expensive racemic mixture could serve as a practical substitute for the D isomer. The other is that stereochemistry is important for repellency. Isomeric effects could be mediated by either sensory or physiological processes, and the relative importance of these possibilities is a topic for further investigation. Regarding chemosensation, stereochemistry may influence irritation (e.g. stereochemistry affects trigeminal receptor response to stimulus applications; Silver, Walker and Kendall-Reed, in press). Also, the available evidence suggests that stereochemistry affects odor quality perception (Cain, 1978). Regarding physiological mechanisms, toxicity is directly related to the stereochemical details of natural products (Flippen-Anderson and Gilardi, 1985). At least in part, avoidance of pulegone is mediated by toxic effects (Mason, 1990).

Why the racemic mixture was as repellent as D-pulegone is unclear: half of the mixture was the less offensive L-isomer. Whatever the explanation, this result is consistent with the finding that intrasensory repellent mixtures are rarely more aversive than the most offensive component (Clark and Mason, unpublished). Plausible explanations include competition for receptor sites, competitive inhibition of neural response or some sort of interaction at a cognitive level. At present, there is no evidence that can be used to decide among these alternatives.

Our results have clear management implications. DL-pulegone is  $\geq 30\text{--}40\%$  less expensive than D-pulegone (D. DeRovira, Flavor Innovations, Rahway, NJ) but not less aversive. Because mint oil is on the list of compounds proposed by the US Environmental Protection Agency for exemption from FIFRA requirements, it is conceivable that its active constituents (pulegone) may be relatively inexpensive to commercialize. Additional testing, perhaps with free-ranging birds, appears warranted.

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