2-17-1994

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Dale L. Nolte
USD A/APHIS/ADC, Denver Wildlife Research Center, Dale.L.Nolte@aphis.usda.gov

Dan L. Campbell
USD A/APHIS/ADC, Denver Wildlife Research Center

J. Russell Mason
USD A/APHIS/APHIS, Denver Wildlife Research Center, c/o Monell Chemical Senses Center

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DALE L. NOLTE, and **DAN L. CAMPBELL,** USD A/APHIS/ADC, Denver Wildlife Research Center, 1835 Black Lake Blvd. SW, Olympia, Washington 98512.

J. RUSSELL MASON, USD A/APHIS/APHIS, Denver Wildlife Research Center, c/o Monell Chemical Senses Center, 3500 Market Street, Philadelphia, Pennsylvania 19103.

ABSTRACT: Economic losses caused by herbivores and other species that inflict damage by browsing or gnawing are substantial. Because lethal approaches to damage reduction are not always practical or desirable, there is an increase in interest in the development of alternative, non-lethal technologies. Repellents may provide a feasible alternative. Here, we present recent studies of three repellent types: 1) anthranilate derivatives (e.g., methyl anthranilate), 2) predator scents (e.g., coyote urine), and 3) bittering agents (e.g., denatonium saccharide). Anthranilate derivatives and predator odors both appear to be promising repellents. Avoidance of the former substances is based on irritant volatiles, and anthranilates may be especially beneficial when the aim is to prevent gnawing damage. Predator odors may be most applicable for protection of vegetation. The effectiveness of these substances appear to be based on the presence of highly volatile, light molecular weight sulfur compounds. Unlike anthranilates or predator odors, bitter substances are largely ineffective as repellents for herbivores.

Proc. 16th Vertebr. Pest Conf. (W.S. Halverson& A.C. Crabb, eds.) Published at Univ. of Calif., Davis. 1994.

INTRODUCTION

Human and wildlife interactions are becoming more frequent, often with detrimental effects to both human resources and to wildlife. Though limited data are available that document losses (Salmon 1988), it is generally accepted that vertebrates cause considerable economic harm (Conover, unpublished data). Although a number of lethal strategies are available to reduce agricultural damage these methods are not always practical or desirable. Non-lethal strategies are being investigated as alternatives or adjuvants.

One possible non-lethal approach is the use of repellents. Repellents deter damage by decreasing a plant's desirability to the foraging animal. Deterrence can be achieved through a conditioned aversion or through an unlearned initial avoidance (Mason and Clark 1992).

Conditioned food aversions occur when ingestion of novel foods is paired with nausea (Garcia 1989). Thus, any flavor paired with gastrointestinal distress can become an effective repellent. Efficacy of repellents based on conditioned aversions can be limited, however, because individuals need to be trained to avoid these stimuli and because the stimuli must be novel to form a strong aversion. Damage that occurs during training or subsequent sampling may be extensive. This can be especially problematic if the damage is inflicted by a transitory or a migrating species (i.e., deer moving from summer to winter ranges).

Repellents that elicit initial avoidance are generally either irritants (e.g., capsaicin) or those that evoke a "fear" response (e.g., predator scents). This type of repellent is especially promising because no training is needed to elicit avoidance behavior. Unfortunately, few compounds have been identified that induce innate avoidances. Those that are available are either broadly offensive to all mammals (Meehan 1988) or show considerable inter- and intraspecific variability in effectiveness. Here, we present studies of three categories of repellents: 1) irritating acetophenone or anthranilate derivatives (e.g., methyl anthranilate), 2) fear-inducing predator scents (e.g., coyote *[Canis latrans]* urine); and 3) bittering agents that have been assumed to serve as taste irritants (e.g., denatonium saccharide).

ACETOPHENONE OR ANTHRANILATE DERIVATIVES

Dimethyl and methyl anthranilate (MA) are aversive to many avian species under laboratory conditions (Kare and Pick 1960, Kare and Mason 1986). Field tests also indicate that these substances inhibit foraging damage by birds to crops (Askam 1992) and at livestock feeders (Mason et al. 1985, Glahn et al. 1989), and they can be effective at alleviating hazards imposed by the mere presence of birds (e.g., airports) (Vogt 1992). They also can be used to relieve non-target hazards that granular pesticides and treated seeds pose to birds (Mason et al. 1993). Ortho-aminoacetophenone (OAP) with similar olfactory properties (to humans) and chemical structure as MA also repels birds (Mason et al. 1991). Potential of these substances as mammalian repellents, however, has been largely ignored.

We tested the efficacy of five of these substances to inhibit water intake by mice *(Mus musculus)* (Nolte et al. 1993b). Water deprived mice were offered water treated with 1.0% concentrations (w/v) of MA, OAP, 2-amino-4',5'-methoxyacetophenone (AMAP), 2-methoxy-acetophenone (MAP) and veratryl amine (VA) in single choice tests. After adaptation to an 18 h water deprivation schedule mice were given 3 h access to water in 10 ml syringes fitted with sipper tubes on each of four pretreatment days. At the end of each 3 h period, ingestion was measured and the mice were permitted an additional 3 h ad libitum access to water. Water tubes were then removed from their cages, and animals were deprived until the following day.

A four-day treatment period immediately followed pretreatment. Treatment period sessions were similar to pretreatment sessions, except that five groups of mice

were presented with their respective compounds in aqueous emulsions during the initial 3 h period. A sixth group was presented plain water as an additional control. All five chemicals substantially reduced intake relative to pretreatment levels (Figure 1), although mice showed signs of habituating to MA, MAP, and VA (i.e., animals ingested more of these substances on the last day than on the first day of treatment). Intake of these chemicals on the last day of the treatment period, however, was still substantially below levels of water drunk during the pretreatment period. Decreased intake of AMAP over time suggests that the increased avoidance of this compound involved learning. Its effectiveness may depend partly on sensory factors and partly on food avoidance conditioning based on post-ingestional malaise.

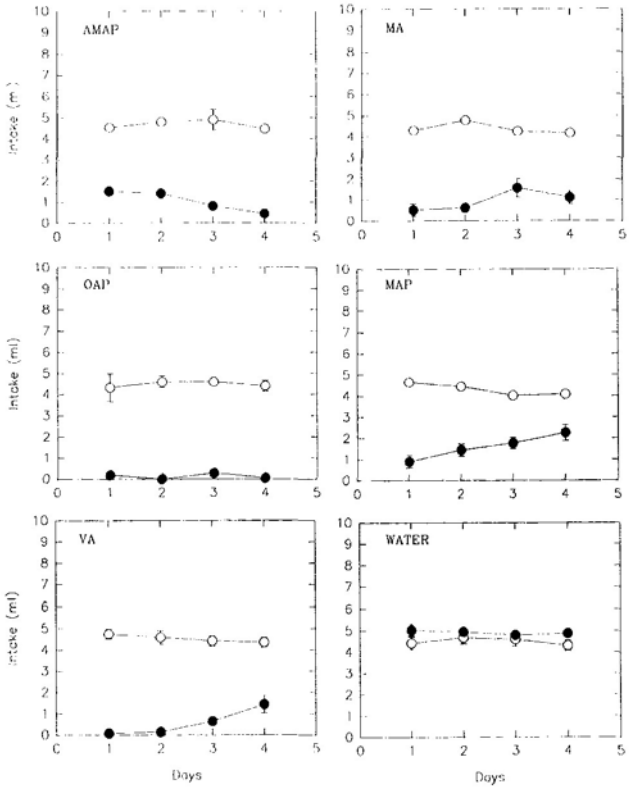


Figure 1. Intake by mice of water during a four-day pretreatment period (open circles) and subsequent intake of 2-amino-4'5'-methoxyacetophenone (AMAP, methyl anthranilate (MA), ortho-aminoacetophenone (OAP), 2-methoxy-acetophenone (MAP), veratryl amine (VA) and water (control) during a four-day treatment period (solid circles) (Nolte et al. 1993b).

OAP virtually eliminated ingestion and was the most aversive compound. Subsequent tests indicated that OAP concentrations as low as 0.25% still significantly repel deprived mice (Nolte et al 1993c). This result is consistent with evidence showing that OAP is superior to MA as a bird repellent (Mason et al. 1991).

Subsequent trials revealed a similar avoidance response of these compounds by other rodents (unpublished data). Deer mice, *(Peromyscus maniculatus)* spiny mice *(Acomys cahirinus),* and house mice avoided all five compounds in single choice tests as described above, except concentrations were reduced to 0.5% (w/v). As before, OAP was the most aversive of the five compounds tested. Prairie voles *(Microtus ochrogaster)* also ingested significantly less water treated with OAP than MA or AMAP (0.5% w/v), though all three substances reduced intake relative to pretreatment levels. Deprived rats *(Rattus rattus)* virtually stopped drinking water treated with 0.5% concentrations of OAP and guinea pigs *(Cavia porcellus)* restrict their intake of water and food when treated with OAP (1.0% w/v).

Not all mammals, however, avoid OAP. Mountain beaver *(Aplodontia rufa)* demonstrated a slight avoidance of OAP in tests with Douglas-fir seedlings *(Pseudotsuga menziesii),* but ingested similar amounts of OAP treated and untreated salal leaves *(Galtheria shallon)* (Nolte et al. 1993a). Deer appear to be indifferent to OAP. Apples, Douglas-fir, and western red cedar *{Thuja plicata)* seedlings treated with OAP (1.0% w/v) are taken as readily as their untreated cohorts (unpublished data). These results are not necessarily surprising since MA is palatable to humans and livestock (Furia and Bellanca 1975, Glahn et al. 1989).

Though not avoided by all mammals, OAP and perhaps MA may serve as effective rodent repellents. Although phytotoxicity may restrict their use on vegetation these substances could be used as additives to granular agricultural chemicals to reduce the hazards that these substances present to birds and rodents. In addition, they might be used to treat seeds or as livestock feed additives. These compounds might also be incorporated into packaging, fabrics, and plastics to prevent damage to electrical cables, containers and other storage or structural products.

PREDATOR SCENTS

Predator odors are generally aversive to potential prey species (Epple et al. 1993). Avoidance appears to be mediated, at least in part, by urinary constituents which are not species specific. Such compounds may constitute a generalized meat eater cue (Epple et al. 1993, Abbott et al. 1990), or a predator "Leitmotif" (Stoddart 1980). Although the chemical nature of this "Leitmotif" remains obscure, one possibility is that it features odoriferous constituents which reflect the diet composition of the predator. Such odors might include sulfur-containing metabolites of protein digestion (Mason et al. 1994). We conducted a series of experiments to assess whether diet manipulations would affect the repellency of a predator urine to several potential prey species, and to investigate the contribution of sulfurous compounds to its repellency (Nolte et al. 1994b).

First we investigated whether rodents differentiated between food associated with urine collected from coyotes maintained exclusively on a diet of cantaloupe (FU) and food associated with urine collected from coyotes fed minced meat (MU). Mountain beaver, deer mice, house mice, and guinea pigs were presented the choice of ingesting a preferred food from a bowl scented with MU

or the same food from a bowl scented with FU. Urine samples (1 ml) were pipetted onto pieces of absorbent paper placed inside perforated plastic containers. These odorized containers were then placed inside the respective food dishes. All species ingested more food from bowls containing FU than they did from bowls scented with MU (Figure 2).

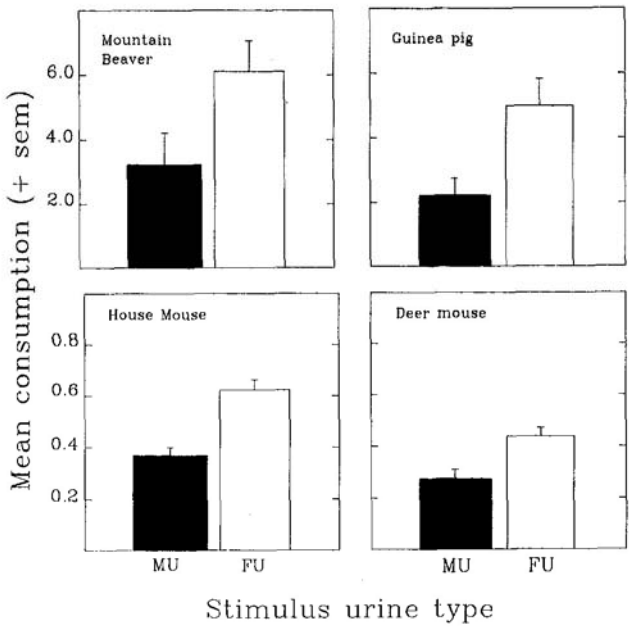


Figure 2. Intake by mountain beavers, guinea pigs, house mice, and deer mice of food (number of apple cubes, grams of guinea pig cow, grams of sunflower seeds, and grams of sunflower seeds, respectively) associated with urine collected either from coyotes on fruit diet (FU) or from coyotes fed meat (MU) (Nolle et al. 1994b).

Differences in responses between FU and MU samples may have reflected dilution effects. Cantaloupe-fed coyotes urinated more than coyotes that ate meat. To control for this possibility, FU and MU samples were lyophilized and then rehydrated to a common concentration. Procedures were similar, except we tested only deer mice and choices were between FU and a control (water scented bowl) or between MU and a control. Mice ate relatively more food from the FU scented bowl than from the MU scented bowl.

The above experiments indicated that meat in a predator's diet enhanced the repellency of its urine. Next, we investigated whether sulfur compounds, by-products of meat digestion, contributed to the repellency of predator urine. Tests were similar, except mountain beaver were given a choice of bowls scented with either MU and a control or between bowls scented with MU minus its sulfur components (SR) and a control. SR was prepared by precipitating MU with mercuric chloride (Golovnya et al. 1972). Sulfur-free urine samples from meat fed coyotes were less offensive to mountain beaver than non-precipitated MU.

These results are consistent with the hypothesis that sulfurous odors are important for the repellency of predator scents. Sulfur constituents also occur in a number of other effective herbivore repellents. At least some herbivores restrict their intake of food treated with thiram (Campbell and Evans 1989), Big Game Repellent-Powder (BGR-P) (Andelt et al. 1992, Andelt et al. 1991, Campbell and Evans 1988), starling powder (Campbell and Farley 1990) and garlic (Nolte and Provenza 1992). Volatile sulfur constituents are common to each of these substances. Thiram is tetramethylthiuram disulfide and dimethyl disulfide is a volatile component of fermented egg used in BGR-P (Bullard et al. 1978). Starling powder is degraded meat which emits sulfurous odors (Mason et al. 1994) and the odor of garlic is also primarily composed of sulfur compounds (Block and Aslam 1988).

BITTERING AGENTS

Many naturally occurring bitter compounds are toxic and bitter substances are generally regarded as unpalatable (Garcia and Hankins, 1975). Rejection of bitter substances, however, is not universal. A priori rejection of bitter substances may be maladaptive for an herbivore (Jacobs 1978). Bitter substances may occur so widely in plants including many which are not toxic that bitter per se is a poor discriminative cue (Jacobs et al. 1978).

We conducted an experiment to test the hypothesis that herbivores do not avoid "bitter" per se (Nolte et al. 1994a). Single-choice trials were used to evaluate guinea pig acceptance of bitter compounds from several structural classes: peptides (denatonium benzoate, denatonium saccharide), flavorones (naringin), carbohydrates (sucrose octaacetate), alkaloids (quinine), terpines (limonene), tannins (quebracho), and amino acids (L-phenylamine) (Belitz and Wieser 1985; Maga 1990).

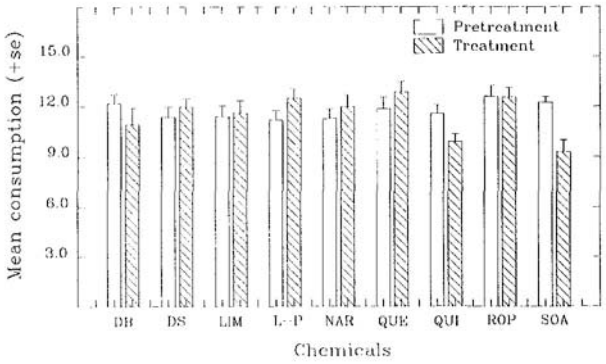
Briefly, guinea pigs were given three weeks to adapt to an 18 h food deprivation schedule for four consecutive days, followed by three days free access to food before being returned to the deprivation schedule. This schedule was maintained throughout the study. Adaptation was followed immediately by a four-day pretreatment period. On each pretreatment day, ether-treated pellets were presented at 0900, and food intake between 0900 and 1200 was determined. Animals were then given three hours to feed ad libitum.

Treatment procedures were identical to those described for pretreatment, except that guinea pigs were given a bitter food during the 3 h measurement period. Bitter compounds were first mixed with ether and then added to foods at a concentration of 1.0% (w/w). A counter-balanced schedule was followed to determine the intake of all nine bitter foods by each guinea pig. Each treatment session was preceded by a pretreatment session.

Guinea pigs were generally indifferent to the bitter tastants evaluated in this experiment (Figure 3). Only quinine (QUI) and sucrose octaacetate (SOA) reduce feeding relative to pretreatment levels. Intake of SOA pellets on the first treatment day was about 50% of that ingested during the pretreatment period. On subsequent days, however, guinea pigs increased their intake until on

day 4 it was similar to pretreatment levels. Conversely, guinea pigs initially accepted QUI and only on the last treatment day did their intake decline below that of pretreatment levels. Although animals were moderately food-deprived, these data, together with the high bitter tastant concentrations tested, support the hypothesis that herbivores do not reject bitter tastants a priori.

Figure 3. Mean ingestion by guinea pigs during pretreatment



and treatment period. Abbreviations: DB = denatonium benzoate, DS = denatomium saccharide, LIM = limonene, L-P = L-phenylalanine, NAR = naringin, QUE = quebracho, QUI = quinine, ROP = Po-Pel, SOA = sucrose octaacetate (Nolte et al 1994a).

These data suggest that bitter substances that fail to induce gastrointestinal malaise are largely ineffective as repellents for herbivores. Guinea pigs are indifferent to concentrations of denatonium benzoate and denatonium saccharide that are three orders of magnitude higher than concentration perceived by humans as intensely bitter (Mason personal observation). These denatonium compounds, however, are the principle active ingredient at concentrations substantially less than 1% in some commercial repellents. Efficacy tests to determine whether these products inhibit foraging by herbivores provide further support to the hypothesis that herbivores do not avoid bitter per se. Mule deer *(Odocoileus hemionus)* (Andelt et al. 1994, Andelt et al. 1991), elk *(Cervus elapus)* (Andelt et al. 1992), mountain beaver, pocket gopher *(Thomomys talpoides),* and black-tailed deer *{Odocoileus hemionus)* (unpublished data) have all failed to differentiate between forage treated with these products and untreated plants.

SUMMARY

Repellents can be an effective tool to alleviate the negative aspects of human and wildlife interactions. Forage selection, however, is relative and depends on the options available. An animal may select one food over another either because it is attracted to the first or because it is avoiding the alternative (Galef 1985). Therefore, the efficacy of repellents will be greater when palatable alternatives are available. It would be unreasonable to expect repellents to deter deprived animals from a desirable forage.

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