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Deer Responses to Repellent Stimuli

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Abstract Four repellents representing different modes of action (neophobia, irritation, conditioned aversion, and flavor modification) were tested with captive white-tailed deer in a series of two-choice tests. Two diets differing significantly in energy content were employed in choice tests so that incentive to consume repellent-treated diets varied according to which diet was treated. When the high-energy diet was treated with repellents, only blood (flavor modification) and capsaicin (irritation) proved highly effective. Rapid habituation to the odor of meat and bone meal (neophobia) presented in a sachet limited its effectiveness as a repellent under conditions with a high feeding motivation. Thiram, a stimulus used to condition aversions, was not strongly avoided in these trials, that included only limited exposures to the repellent. These data support previous studies indicating that habituation to odor limits the effectiveness of repellents that are not applied directly to food, while topically-applied irritants and animal-based products produce significant avoidance.

Keywords Aversion · Foraging behavior · Herbivore · *Odocoileus virginianus* · Wildlife damage management

Introduction

Damage to agricultural, horticultural, and forest resources by deer is a substantial economic problem (Wywiałowski 1998). In managed systems, deer browse damage may result in widespread tree losses as well as reduced future value via decreased growth and plant deformities (Nolte 1998). In natural systems, deer can impact ecosystem properties negatively (Cote et al. 2004) and threaten rare understory herbaceous species (Mcgraw and Furedi 2005). Fear of browse damage also may result in reduced purchases of susceptible tree and shrub species by homeowners (Lemieux et al. 2000). Potential economic impact has encouraged the timber industry to employ various methods to minimize ungulate damage to seedlings during reforestation. For example, in British Columbia, Canada, nearly one-third of the 9–12 million western redcedar (*Thuja plicata* Donn ex. D. Don) seedlings planted each year are protected with physical barriers at a cost of nearly \$5 (USD) per protected seedling to promote free-to-grow trees (Annette van Nuijenaus, Western Forest Products, Inc. personal communication, August 2006).

Chemical repellents also are frequently employed to deter browsing of trees and shrubs by deer in managed systems (Nolte and Wagner 2000). Herbivore repellents are thought to promote avoidance behavior by several different mechanisms or modes of action. These mechanisms differ in the consequences that result from interactions between herbivore and the repellent-treated food. Available data suggest that herbivore repellents promote avoidance via four mechanisms: 1) neophobia; 2) irritation; 3) conditioned

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aversion; and 4) flavor modification. Known repellent formulations employ these mechanisms singly or in combination.

At the most basic level, all repellents may deter herbivores by exploiting their fear of unfamiliar visual, olfactory, or taste cues (neophobia). However, repellents that rely on neophobia alone (there are no additional negative consequences associated with them) are subject to habituation and will not be avoided for extended periods (Nolte 1999). Visual and vapor repellents often rely on neophobia. Repellent stimuli disassociated from the food source (not applied directly to the food) can be referred to as “vapor repellents” as they are not ingested (thus, do not contribute to the flavor of the repellent stimuli) and are detectable at variable distances from the source.

Most contact repellents (applied directly to the plant) employ active ingredients that impart additional consequences beyond neophobia. One such mechanism is associated with activation of the trigeminal system. The consequence of peripheral (oral/nasal/ocular) contact with these repellents is pain. Among mammals, capsaicin is a well-known trigeminal irritant (Nolte and Wagner 2000). Another consequence of ingesting certain repellents is malaise. Repellent compounds that produce negative postingestive consequences (i.e., malaise or gastrointestinal distress) are avoided as a result of learning. This mechanism is often termed aversion learning or conditioned aversion (Burritt and Provenza 1989). The active ingredient required to produce the negative consequences is typically a toxin. Sensory cues of the repellent formulation (usually flavor) are associated with the negative consequences of toxin ingestion and are avoided at future encounters. In laboratory studies, lithium chloride often is used as the toxin to condition aversions (Riley and Tuck 1985). In formulated repellents, thiram (tetramethylthiuram disulfide) is a fungicide used to condition aversions (Nolte and Wagner 2000). Among other symptoms, chronic thiram exposure produces anemia and nausea (Maita et al. 1991).

Numerous compounds have been used to alter the flavor of treated plants without eliciting pain or malaise. One such strategy has been to employ compounds that impart bitter taste. In practice, repellents employing only bitter compounds are typically ineffective as deer repellents (Nolte and Wagner 2000) and there is some question about the reliability of bitter taste *per se* as a warning of toxicity (Glendinning 1994; Nolte et al. 1994b). Blood and egg are examples of ingredients that yield effective herbivore repellency when applied to plants without causing pain or malaise (Nolte and Wagner 2000). In recent years, hydrolyzed casein (HC) has been added to the list of stimuli that produce long-lived avoidance (Kimball and Nolte 2006). Repellent ingredients like blood, egg, and HC are non-toxic (i.e., unlikely to condition aversions) and

typically are not subject to habituation in repeated tests (i.e., unlikely to cause avoidance merely via neophobia).

The relative effectiveness of repellents that rely on any of these mechanisms may depend on the individual herbivore's motivation to consume the protected resource. For example, when alternative foods are available, shiny ribbons (a visual repellent with no consequence) may provide significant protection in localized areas. However, when alternative foods are scarce, repellents with actual consequences to the consumer may be required to reduce browsing effectively. Previous studies of herbivore repellents failed to account for feeding motivation. In this study, the incentive to consume test diets was manipulated by allowing captive deer to learn about two test diets that differed in energy content. A series of experiments then were conducted to compare the different mechanisms of deer repellency and evaluate repellent effectiveness when incentive to consume the treated diet was varied.

Methods and Materials

Subjects Ten hand-reared white-tailed deer (*Odocoileus virginianus*) were group housed in a large (ca 2 ha) outdoor pen except during individual bioassays. Shelter, water, and mineral block were available *ad libitum*. Basal diet was provided at varying intervals: *ad libitum* on days with no scheduled bioassays and overnight from 1600 h to 0800 h daily in advance of individual bioassays. Thus, subjects were restricted from basal diet for 6 h. For individual bioassays, deer were led into individual pens (sheltered stalls measuring approximately 5×3 m). Water was provided in the rear of the stalls, and access doors located at the head of each stall allowed for placement and removal of plastic feed containers (ca 50 cm diam and 15 cm deep). This study was approved by the National Wildlife Research Center's Institutional Animal Care and Use Committee (QA-1642) and conducted during the period of 24 March to 25 April 2009.

Diets Three different pelleted diets were used during the study, including a basal diet familiar to the subjects (Antler Max[®]; Purina Mills, St. Louis, MO, USA). All test subjects had several years experience with Antler Max[®] as their primary food source. Two test diets were formulated to differ in net energy while containing similar protein (Table 1; X-Cel Feeds, Tacoma, WA, USA). High energy (HE) and low energy (LE) test diets were distinctly flavored with citrus-anise-vanilla or maple-anise flavors, respectively, to facilitate easy discrimination during bioassays (Table 1). Animals learn about foods they eat by integrating flavor with the postingestive consequences of consuming that food (Provenza 1995a). Preferences (or aversions)

Table 1 Composition and nutritional content of the high energy (HE) and low energy (LE) test diets

Ingredient	High energy (HE)	Low energy (LE)
Barley	22%	15%
Corn grain, ground	35%	7%
Corn, distillers	9%	6%
Wheat mill run	0	15%
Alfalfa meal	6%	19%
Soybean hulls	0	14%
Beet pulp	13%	14%
Soybean meal	11%	7%
Minerals and vitamins	3%	2%
Dry dairy krave [®] flavor ^a	0.1%	0
Anise-maple flavor	0	0.1%
Crude protein	15.3%	15.3%
Non-structural carbohydrates	47.3%	21.3%
Relative feed value	430	152
Net energy gain	1017 Mcal	803 Mcal

^a Citrus-anise-vanilla and other natural flavors

based on flavor are formed such that these flavors are recognized readily at future encounters. Upon learning by the subjects, the distinct flavors were expected to be readily associated with the energy content of the food.

Repellents Test diets were treated with commercially-obtained repellents according to labeled use as specified by the manufacturers. Deerbusters[®] sachets (Trident Enterprises, Frederick, MD, USA) represented the neophobia mechanism. The irritation mechanism was represented by Miller's Hot Sauce[®] (Miller Chemical and Fertilizer Corp., Hanover, PA, USA). Chew-Nott[®] (Nott Products, Coram, NY, USA) that contained the fungicide thiram was the repellent chosen for conditioned aversion. The final mechanism, flavor modification, was represented by Plantskydd[®] (Tree World Plant Care Products Inc., St. Joseph, MO, USA), which contains blood meal.

Sachets similar to those marketed as Deerbusters[®] repellent, but containing only meat and bone meal were used as a vapor repellent (the repellent was not in contact with the diets). Unlike the usual commercial product, our experimental sachets did not contain capsaicin (irritant). As such, the sachets were suited perfectly for this study because their mode of action was limited to neophobia—largely owing to the fact that the meat and bone meal was not applied directly to the test diets. Sachets were attached to the inside of feed bowls by use of zip-ties passing through two holes drilled near the top edge of the bowl.

Two hundred and forty mL of Miller's Hot Sauce[®] (2.5% capsaicin) were mixed with 5 mL Tactic[®] (a latex-based sticker; Loveland Industries, Greeley, CO, USA) and 4.0 L tap water (resulting in a 0.14% capsaicin solution). A hand-held pump sprayer was used to treat test diets until the pellets were visibly coated, and were allowed to dry

overnight. Approximately 40 ml were used to treat 2 Kg of diet. Two additional contact repellents were similarly prepared according to label directions and applied directly to the test diets. Chew-Nott[®] (20% thiram) was mixed 1:1 with tap water prior to application and Plantskydd[®] was employed as the ready-to-use formulation (Tree World Plant Care Products Inc., St. Joseph, MO, USA) consisting of 16.7% dried porcine and/or bovine blood.

Pre-trial Experience with Test Diets For 2 wk prior to individual bioassays, either HE or LE test diets were provided *ad libitum* in group housing according to a predetermined schedule (Table 2). Pre-trial exposure was designed to promote association of energy content of the diets with their specific flavors. During group feeding, the two test diets were offered in separate 100-L feed bins. For individual bioassays, HE diet always was presented in a blue-colored bowl, and LE diet was presented in a black-colored bowl—regardless of presence or absence of repellent treatment.

Experiment 1: Diet Preference Subjects were led/herded into individual stalls and untreated test diets (HE and LE) were offered in a two-choice test for two consecutive days (days 16 and 17; Table 2). The right/left position of the diets was predetermined and alternated on the 2nd day. The 30 min bioassays commenced at 1400 h daily following a 6 h period of basal-diet restriction. Intake of each diet was determined by difference (mass immediately prior to and after the 30 min bioassay).

Experiment 2: Repellency and Feeding Incentive Experiment 2 was initiated the following day and similarly employed two-choice tests with HE and LE in 30 min trials. The purpose of this experiment was to offer a choice

Table 2 Pelleted diets offered *Ad libitum* to test subjects in group housing before, during, and in between experiments 1, 2, and 3 (HE = High energy diet; LE = Low energy diet; BOTH = both HE and LE; MAX = Antler max[®])

		Day1	Day 2	Day 3	Day 4	Day 5
		LE	LE	HE	HE	LE
Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
LE	HE	LE	HE	LE	HE	LE
Day 13	Day 14	Day 15	Day 16 Exp. 1	Day 17 Exp. 1	Day 18 Exp. 2	Day 19 Exp. 2
HE	Both	Both	Max	Max	Max	Max
Day 20 Exp. 2	Day 21 Exp. 2	Day 22 Exp. 2	Day 23 Exp. 2	Day 24 Exp. 2	Day 25 Exp. 2	Day 26
Max	Max	Max	Max	Max	Max	Both
Day 27	Day 28 Exp. 3	Day 29 Exp. 3	Day 30 Exp. 3	Day 31 Exp. 3	Day 32 Exp. 3	Day 33 Exp. 3
Both	Max	Max	Max	Max	Max	Max

of treated diet and untreated alternative, while also varying the incentive to consume the treated diet. One diet was treated with a single repellent treatment, while the other remained unadulterated (Table 3). For example, one subject was offered a choice of HE diet treated with blood and untreated LE in a two-choice test, while another subject was offered a choice of LE diet treated with blood and untreated HE (i.e., the opposite diet-treatment pair). Each comparison was repeated on consecutive days with the right/left position determined in advance and alternated on the 2nd day. Each subject was tested with all four repellent treatments in four of the eight possible combinations of diet (HE or LE) and repellent in a balanced incomplete block design. As a result, all possible treatment and diet combinations were replicated five times over the 8 d experiment (Table 3). Intake of each diet was determined by difference (pre- and post-bioassay mass).

Experiment 3: Pair-wise Repellent Comparison Following a 2 d intermission, experiment 3 consisted of two-choice tests conducted with HE-treated diets (Table 4). Each diet was treated with a single repellent, and the four different treatments were compared pair-wise such that comparisons

were repeated on consecutive days, and each subject was tested with three of the six possible comparisons (Table 5). As a result, all possible pair-wise comparisons were replicated five times over the 6 d experiment. Intake of each diet was determined by difference (pre- and post-bioassay mass).

Statistical Analyses Data from each experiment were analyzed separately. Preference scores (intake of one diet divided by the sum of both diets) from two-choice tests were analyzed by mixed model analyses of variance (ANOVA), and residual plots were generated to evaluate ANOVA assumptions. Outliers (defined as having studentized residuals greater than 3 or less than -3) were removed from the data set prior to all analyses. Subject was a random effect in all models. When necessary, the null hypothesis of indifference (defined as a preference score of 0.5) was tested by using the value 0.5 minus the preference score as the response in the model.

Day, position of the HE diet (right or left), and the interaction (day*position) were fixed effects in experiment 1. Diet preference was first evaluated by examining the distribution of mean HE preference scores (2 d averages for

Table 3 Low energy (LE) and high energy (HE) diets were offered in two-choice tests in experiment 2. One choice was treated with one of the repellent ingredients as indicated in parentheses (S = Sachet; T = Thiram; B = Blood; C = Capsaicin)

Subject	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
5	HE(S) vs. LE	HE(S) vs. LE	LE(T) vs. HE	LE(T) vs. HE	HE(B) vs. LE	HE(B) vs. LE	LE(B) vs. HE	LE(B) vs. HE
21	HE(B) vs. LE	HE(B) vs. LE	LE(C) vs. HE	LE(C) vs. HE	HE(T) vs. LE	HE(T) vs. LE	LE(S) vs. HE	LE(S) vs. HE
22	HE(T) vs. LE	HE(T) vs. LE	LE(B) vs. HE	LE(B) vs. HE	HE(S) vs. LE	HE(S) vs. LE	LE(C) vs. HE	LE(C) vs. HE
25	LE(B) vs. HE	LE(B) vs. HE	HE(S) vs. LE	HE(S) vs. LE	LE(C) vs. HE	LE(C) vs. HE	HE(T) vs. LE	HE(T) vs. LE
92	HE(C) vs. LE	HE(C) vs. LE	LE(S) vs. HE	LE(S) vs. HE	HE(B) vs. LE	HE(B) vs. LE	LE(T) vs. HE	LE(T) vs. HE
93	LE(S) vs. HE	LE(S) vs. HE	HE(C) vs. LE	HE(C) vs. LE	LE(T) vs. HE	LE(T) vs. HE	HE(B) vs. LE	HE(B) vs. LE
95	LE(C) vs. HE	LE(C) vs. HE	HE(T) vs. LE	HE(T) vs. LE	LE(B) vs. HE	LE(B) vs. HE	HE(S) vs. LE	HE(S) vs. LE
97	LE(T) vs. HE	LE(T) vs. HE	HE(B) vs. LE	HE(B) vs. LE	LE(S) vs. HE	LE(S) vs. HE	HE(C) vs. LE	HE(C) vs. LE
98	HE(S) vs. LE	HE(S) vs. LE	LE(B) vs. HE	LE(B) vs. HE	HE(B) vs. LE	HE(B) vs. LE	LE(T) vs. HE	LE(T) vs. HE
99	LE(C) vs. HE	LE(C) vs. HE	HE(T) vs. LE	HE(T) vs. LE	LE(S) vs. HE	LE(S) vs. HE	HE(B) vs. LE	HE(B) vs. LE

Table 4 Pair-wise comparisons of repellents in experiment 3 and the reference treatment chosen for calculation of preference score (intake of reference diet divided by total intake)

Comparison	Reference	Alternative
A	Blood	Thiram
B	Blood	Capsaicin
C	Capsaicin	Thiram
D	Sachet	Thiram
E	Sachet	Blood
F	Sachet	Capsaicin

each subject) using the Shapiro-Wilk test for normality (Proc Univariate; SAS 2002). The indifference response (0.5 minus HE preference score) was then subjected to *t*-test for the null hypothesis (mean=0) using the univariate procedure. Average total intake (sum of both diets) was determined for each subject, and the mean and standard error were calculated for later comparison with total intake during experiment 2.

Treatment preference scores were calculated for experiment 2 (treated diet intake divided by total intake). When total intake was zero (neither diet consumed), the preference score was considered a missing value. Fixed effects were: “protected” diet (either HE or LE receiving treatment); treatment (repellent); protect*treatment; position of the treated diet (right or left); protect*position; treatment*position; treatment*protect*position; and day. Separate ANOVA models also were produced for each level of protected diet (HE or LE) by using treatment, position, and treatment*position as fixed effects. Multiple comparisons of means were made by controlling the false discovery rate according to the procedures of Benjamini and Hochberg (1995). For HE diet protection, one *post-hoc* comparison of treatment*position was made for right and left positioning of the food container with sachet treatment. Total intake data also were subject to ANOVA with fixed effects: “protected” diet, treatment, protect*treatment, position, protect*position, treatment*position, treatment*protect*position, and day.

Four paired *t*-tests were conducted using data from experiment 1 and LE-protected diet data from experiment 2. Mean LE preference scores were calculated for each subject in experiment 1 (equal to 1—HE preference score as previously determined). Experiment 1 means were subtracted from experiment 2 preference scores according to subject. A *t*-test was conducted for each treatment using the univariate procedure in SAS. Each subject*day occurrence was considered a replicate for that treatment. False discovery rate for multiple comparisons was controlled by using the procedures of Benjamini and Hochberg (1995).

There were six pair-wise comparisons of repellents in experiment 3 (Table 3). Preference scores were calculated using one treatment as the reference (numerator) for all instances of that comparison. The indifference response (0.5 minus preference score) was calculated and subjected to ANOVA with comparison (Table 3), position of the reference treatment (right or left), comparison*position, and day the fixed effects. The null hypothesis (indifference response=0) was evaluated by *t*-test using the false discovery rate controlling procedure (Benjamini and Hochberg 1995).

Results

Experiment 1 Mean intake of the HE diet was 221±44 g and of the LE diet was 2.2±0.8 g. The resulting preference score (0.99) indicated a strong preference for HE diet ($P<0.001$). Preference scores were normally distributed ($P=0.625$) and not subject to day ($P=0.136$), position ($P=0.344$), or day*position ($P=0.852$) effects. Mean total intake for the ten subjects was 223±44 g.

Experiment 2 Preference scores were not subject to a day effect ($P=0.154$), but all other effects were highly significant ($P<0.001$). Inspection of treatment*protect*position means indicated that LE diet was avoided regardless of treatment or position, while avoidance of HE diets varied by treatment and position. Evaluation of protected LE diets alone confirmed that avoidance was not subject to treatment ($P=0.368$), position ($P=0.238$), or treatment*position ($P=0.587$). Treatment preference scores were less than 0.007 for all treatments (Fig. 1).

Paired *t*-tests indicated that two treatments reduced preference for LE diet in experiment 2 with respect to LE diet preference in experiment 1. Differences for capsaicin

Table 5 Assignment of pair-wise comparisons (Table 4) to the ten subjects in experiment 3. High energy (HE) diet was treated with test repellents and offered to the subjects in two-choice tests

Subject	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
5	D	D	B	B	C	C
21	E	E	C	C	D	D
22	D	D	F	F	A	A
25	B	B	D	D	E	E
92	A	A	F	F	C	C
93	C	C	E	E	F	F
95	B	B	C	C	E	E
97	F	F	A	A	B	B
98	A	A	D	D	B	B
99	E	E	A	A	F	F

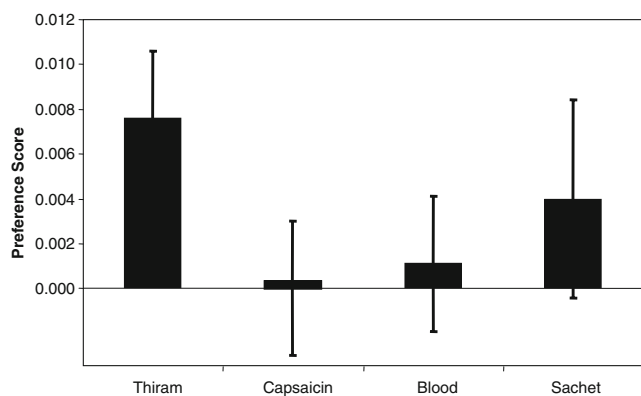


Fig. 1 LE diet preference scores (repellent-treated diet intake divided by total intake) did not differ significantly among repellent treatments ($P=0.368$). High energy (HE) diet was the alternative in 30 min two-choice tests. *Error bars* represent standard error

(-0.01 ; $P=0.007$) and blood (-0.009 ; $P=0.022$) reflected LE preference scores being reduced from approximately 0.01 in experiment 1 to nearly 0.0 in experiment 2 as a result of treating the LE diet with the test repellents (Fig. 2).

Conversely, avoidance of protected HE diets was subject to all fixed effects ($P<0.001$), including treatment*position. Thiram-treated HE diet was strongly preferred over the LE alternative, while both capsaicin and blood significantly reduced intake of the highly preferred HE diet regardless of position (Fig. 3). Interestingly, preference scores for HE diet presented on the left side with the sachet were higher than HE diet presented on the right side with the sachet ($P<0.001$) while being consumed equally with the LE alternative.

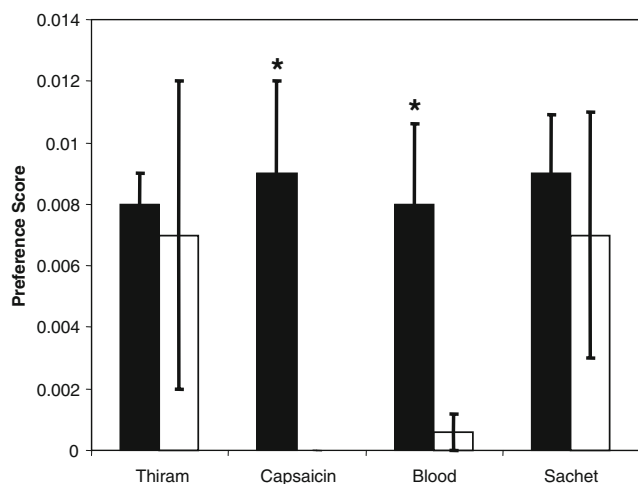


Fig. 2 Low energy (LE) preference scores (LE diet intake divided by total intake) from experiment 1 (pre-treatment intake in the absence of repellents) and experiment 2 (LE diets treated with repellent) in 30 min two-choice tests with high energy (HE) diet as the alternative. *Error bars* represent standard error. Paired *t*-tests indicated that preference scores differed significantly (*) between the experiments for capsaicin and blood ($\alpha=0.05$). Legend: ■ Pretreatment (Experiment 1), □ Repellent (Experiment 2)

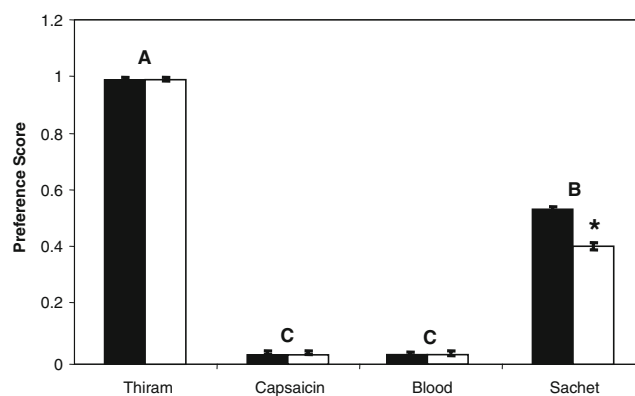


Fig. 3 Experiment 2 preference scores (repellent-treated diet intake divided by total intake) for high energy (HE) diets treated with the test repellents. *Error bars* represent standard error. Low energy (LE) diet was the alternative in 30 min two-choice tests. Letters indicate differences in preference scores due to repellent treatment (main effect). The asterisk indicates a position effect for one of the four treatments which led to the significant treatment*position interaction. Legend: ■ Treatment in left position, □ Treatment in right position

Total intake data was analyzed to determine if the odor of the test repellent influenced intake of both diets, not just the diet associated with the repellent. Total intake was impacted by diet protected ($P=0.001$), treatment ($P=0.003$), and day ($P=0.026$). Total intake was 203 ± 59.4 g when the LE diet was protected and 145 ± 59.3 g when the HE was treated with repellent. This indicates that when HE diet was treated with effective repellents, subjects did not compensate for reduced HE intake with increased LE consumption. Treatment ($P=0.003$) and day ($P=0.026$) also were significant.

Among the treatments, total intake when one of the diets was treated with thiram (223 ± 60.5 g) was not significantly different from capsaicin (189 ± 60.5 g; Fig. 4). At the same time, total intake when blood (132 ± 71 g), sachet (154 ± 60.5 g), or capsaicin treatments were applied to one of the

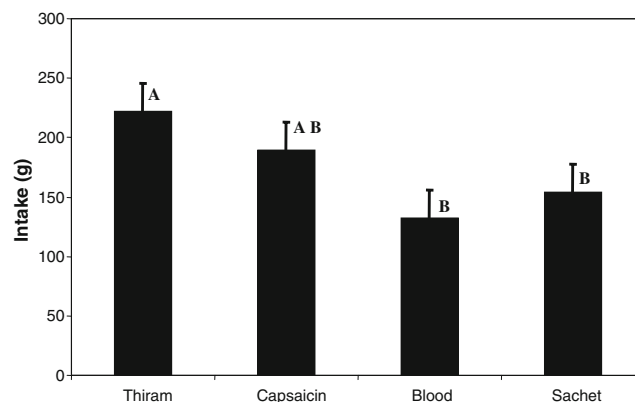


Fig. 4 Total intake data in experiment 2 of 30 min two-choice tests with either low energy (LE) or high energy (HE) diets treated with a repellent. Means marked with different letters are significantly different ($\alpha=0.05$) and *error bars* represent standard error

diets were not statistically different. By comparison, total intake of HE and LE diet in the absence of a repellent (experiment 1) was 223 ± 44 g.

Experiment 3 Treatment comparison was not subject to position ($P=0.485$), comparison*position ($P=0.751$), or day ($P=0.745$) effects. Further examination of the comparison effect ($P<0.001$) indicated that a preference for one of the repellents was evident for every two-choice comparisons except for capsaicin vs. blood (Fig. 5). Avoidance (repellency) of the treatments followed the order of greatest avoidance to least: blood = capsaicin > thiram > sachet.

Discussion

As intended, HE diet was significantly preferred by all subjects, by a factor of 99 to 1. By having a dramatic difference in preference for the two diets, subsequent experiments could be conducted in a manner that incorporated varying incentives to consume treated or alternative food items. Thus, experimental conditions modeled two extreme circumstances possible under field conditions: 1) the food source requiring protection from herbivory is highly desirable relative to the alternatives; and 2) alternative foods are more desirable than the food treated with repellents.

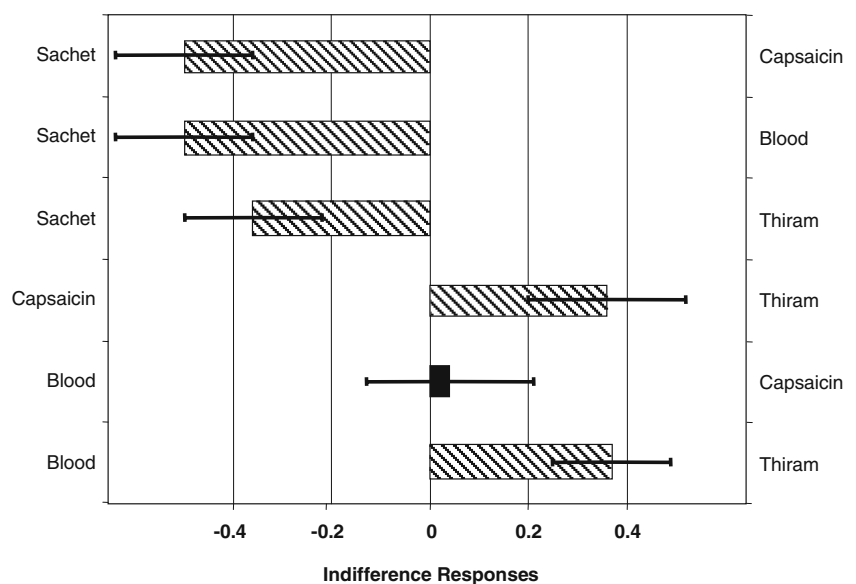
Deer strongly avoided repellent-treated LE diet when HE was available, regardless of repellent type (Fig. 1). Two repellent ingredients, capsaicin and blood, significantly reduced preference for the LE diet relative to untreated diets in experiment 1 (Fig. 2). Although statistically significant, the effect of capsaicin and blood avoidance was of no practical consequence, as LE preference scores

were merely reduced from approximately 0.01 in the absence of repellents (experiment 1) to 0.0 when LE diets were treated with blood or capsaicin (experiment 2). When highly preferred alternatives are available, the less preferred food is easily protected.

A wider range of repellent activities were revealed when the highly desirable HE diet was treated with the repellents and the alternative was the less desirable (LE diet). In experiment 2, capsaicin and blood were extremely effective repellents when applied to the HE diet (Fig. 3). Previous studies have also shown that blood is an effective repellent (Nolte and Wagner 2000; Wagner and Nolte 2001; Kimball et al. 2008) and that avoidance of capsaicin is concentration dependent (Andelt et al. 1994). In the current study, subjects strongly avoided the HE diet treated with blood or 0.14% capsaicin despite being motivated to select the HE diet over the LE alternative.

Thiram was ineffective as a repellent. It was not unexpected for the deer to consume thiram-treated HE diet during the first two exposures to the treatment in experiment 2. In previous studies with mule deer (*Odocoileus hemionus*) and elk (*Cervus elaphus nelsoni*), Andelt et al. (1991, 1992) reported similar responses to thiram-treated foods. With both species, consumption of the thiram-treated food in cafeteria-type tests decreased daily in 4 day and 5 day experiments, as it took repeated exposures for subjects to develop an aversion to the familiar food. When familiar foods are treated with a toxin that promotes conditioned aversion, multiple exposures to the toxin-food pair are required to produce an avoidance response (Kimball and Nolte 2005). This is particularly true when the treatment does not impart a distinct cue (e.g., taste, visual, and/or odor). Unlike blood and capsaicin that discolored the diets, thiram treatment was not visually evident as the maximum absorbance of thiram

Fig. 5 Indifference responses from experiment 3 (with standard error bars). Indifference is indicated as a score of zero and determined by subtracting the preference score (intake of one choice divided by total intake) from the value of 0.5. Diagonal pattern indicates an indifference score significantly different from zero ($\alpha=0.05$). The direction of the bar (positive or negative) indicates which of the two choices (right and left axes) was preferred in 30 min two-choice tests



(200–300 nm) is in the ultraviolet region (Talrose et al. 2009). Similar to many mammals, deer lack visual capability at ultraviolet wavelengths (Jacobs et al. 1994). Thiram is anecdotally reported to impart a bitter taste, but bitter taste is not a relevant cue to herbivores that forage in an environment replete with bitter plant stimuli (Nolte et al. 1994b).

The sachet was only moderately effective as a feeding deterrent in experiment 2 (Fig. 3). Several subjects were apprehensive about the sachet and limited consumption of diets placed in food bowls with attached sachets. However, there was tremendous variation among the subjects in response to the sachet treatment associated with HE diet. One subject ignored the sachet and readily consumed HE diet; one ate both diets; a few avoided the HE diet and consumed only LE diet; while others did not consume either diet. It was not evident why position (right or left presentation) of the sachet influenced repellency while position was not significant for the other repellents (Fig. 3). Odor may serve as a cue that can be associated with the palatability of foods via learning processes (Provenza 1995a). Learning about the odor of foods permits avoidance (or preference) at future encounters with that food on the basis of odor alone.

It is possible that the sachet was not the only treatment that served as a vapor repellent. Because volatiles were present throughout the test area (treatment odors were not confined to the treated diet), vapor repellents could be expected to influence intake of both diet choices. To test the effects of the treatments on intake of both choices, total intake data were analyzed for treatment and diet effects. Total intake data from experiment 2 suggests that non-volatile thiram had less influence on feeding from both food bowls than the more odiferous blood or sachet treatments (Fig. 4). Volatile components of blood treatment influenced not only intake of the treated diet, but also the alternative choice. Protein hydrolysis and lipid oxidation of animal-based stimuli (such as blood) produce volatile odors such as sulfides, aldehydes, and organic acids (Kamiya and Ose 1984). Sulfurous volatiles have been implicated in repellency of predator urine and egg (Nolte et al. 1994a; Lewison et al. 1995).

Volatile odors also may confer information regarding the surroundings. As such, certain odors such as blood, egg, meat, and bone meal have been thought to indicate danger from predators and have been called “fear” repellents (Nolte and Wagner 2000). However, behaviors such as approaches or head entries into feeders were unaffected by predator-based repellents (Pfister et al. 1990), and these repellents failed to exclude herbivores from treated locations as would be anticipated if predation were a consequence of foraging near the odor source (Belant et al. 1998; Nolte and Wagner 2000). Predator odors may provide cues regarding predator density and influence decisions about

where and when to forage, but do not influence intake during a feeding bout once the decision to forage has been made (Chabot et al. 1996).

Extinction (cessation of avoidance behavior) among repellents that do not produce pain or malaise is likely to occur when the treated food is highly desirable. When the basal ration was restricted, deer and elk increased daily consumption of food treated with egg product (Andelt et al. 1991, 1992). In a winter field study conducted when alternative foods were scarce, plants treated with an egg-based repellent and netting were not protected (Milunas et al. 1994). At the same time, reappearance of alternative food sources can result in recurrence of the avoidance behavior. In a previous study, addition of casein to a ground diet reduced its intake relative to the unadulterated diet (Kimball et al. 2005). When the casein diet was offered subsequently to deer in a single-choice experiment, avoidance was not observed. Yet, after readily consuming the casein diet for 8 days, significant avoidance of casein-treated diet was evident when offered again in the presence of the unadulterated diet. Therefore, the mechanism by which repellency is achieved with animal-based products is not neophobia.

Avoidance of blood and other animal-derived substances may be the result of an “evolutionary memory” (Provenza 1995b) that conveys information about potential sources of pathogens. Just as a wide variety of toxins have been associated with bitter taste to humans (Bachmanov and Beauchamp 2007), compounds indicative of pathogenic activity (e.g., certain proteins or peptides) may be distinctly identified by herbivores. Importantly, the food item must be “contaminated” with these compounds in order to affect intake. Animal products employed as vapor repellents may alert foraging herbivores about the potential of contamination, and reduce their intake until habituation occurs (e.g., sachet). However, significant repellent efficacy is achieved when the animal product is in contact with the food and the herbivore avoids the treated food thus avoiding potential pathogens (e.g., blood).

In contrast to experiment 2, fewer subjects avoided the sachet in experiment 3; while avoidance of thiram treated HE diet was more evident (Fig. 5). Although not specifically tested in this study, experience with the sachet during experiment 2 may have contributed to habituation to the odors. Similarly, experience with thiram-treated diets also may have facilitated learning about the postingestive effects of thiram. Animals can learn that novel cues are associated with positive consequences (or have no associated consequences) as well as they can learn about negative consequences (Provenza 1995b). These data suggest that learning was not a necessary component of blood and capsaicin avoidance. When highly desirable diets were treated with blood or capsaicin (experiment 2), as well as

in head-to-head comparisons (experiment 3), the flavor modification (blood) and irritation (capsaicin) mechanisms continued to demonstrate significant repellency (Fig. 5).

In field applications, existence of alternative forage options is not always evident. It may not be feasible to compare nutritional quality of the agriculture resource in need of protection with forage alternatives. However, it is clear that when valuable resources are at risk, effective tools are needed to minimize losses due to herbivory. The results of our study suggest that repellents that rely only on neophobia as the mode of action may be effective in field applications only when foraging options are plentiful. Furthermore, repellents that rely on conditioned aversion may not be useful. The deer in this study did not reduce intake upon initial offering of thiram-treated diets. Thiram may be an impractical repellent if numerous exposures are required to condition an aversion. Conversely, repellents with immediate consequences to the consumer (i.e., irritation and flavor modification) can be effective repellents, even when the treated resource is highly desirable in comparison to other foraging options.

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