2006. Advances in vertebrate pest management. Vol. IV. C.J. Feare and D. P. Cowan, editors. Filander Verlag, Furth, Federal Republic of Germany.

Bruce A. Kimball[1,2] & Dale L. Nolte[3]

Animal tissue-based herbivore repellents: scary odours or altered palatability?

Abstract

Contact herbivore repellents have historically been classified as irritants, taste modifiers, flavour aversion agents, or fear inducers. Irritants cause pain by acting on the trigeminal system. Taste modifiers and flavour aversion agents alter the palatability of the food. Malodorous volatiles released from animal-based repellents (e.g. egg, blood, urine) are thought to invoke a "fear response" through their chemosensory association with predators.

We offered western redcedar (Thuja plicata) seedlings treated with gelatin, milk casein, egg albumen, and soy protein to captive black-tailed deer (Odocoileus hemionus) and monitored herbivory for 20 days. Deer-Away Big Game Repellent® powder (BGR-P) and the agricultural sticker used to adhere treatments to the seedlings (Tactic®; control) were also included in the experiment. In each pen, twelve seedlings of each treatment were planted in 4 × 3 grids with 1 m tree spacings. Treatment plots were separated by a minimum of 3 m and the design was replicated in 8 pens, each containing three adult deer. Treatments were also analysed by purge and trap gas chromatography for volatiles and acid hydrolysates were analysed for methionine, cysteine and cystine by liquid chromatography.

After 20 days exposure to the subjects, all treatments reduced herbivory relative to the control. Among treatments, the mean number of bites sustained by the seedlings varied according to: BGR-P < casein = albumen < soy < gelatin. Chromatographic analyses of the treatments revealed that only BGR-P emitted volatile compounds in detectable quantities (aliphatic aldehydes). We propose that the protein fraction of animal-based repellents deters herbivory by altering palatability of the treated food and that avoidance is correlated with the methionine content of the protein. Contrary to current theory, sulphurous volatiles are not responsible for avoidance of animal-based repellents. However, avoidance may be further enhanced by addition of odourants (such as aldehydes) to the protein-based repellent.

[1] Correspondence to: USDA/NWRC, 4101 LaPorte Avenue, Fort Collins, CO, USA 80521. Email: bruce.a.kimball@aphis.usda.gov

[2] Graduate Degree Program in Ecology, Colorado State University, Fort Collins, CO, USA 80523.

[3] USDA/NWRC, 9730-B Lathrop Industrial Drive, Olympia, WA, USA 98512.

1 Introduction

A number of commercially-available products are employed to deter browsing of trees and shrubs by deer. These products contain a broad range of putative active ingredients. The majority of these products are contact repellents, i.e. they are topically applied directly onto the plants to be defended. Among contact repellents, four different modes of action have been proposed (Nolte & Wagner 2000). These are: flavour aversion learning (FAL), taste modification, chemical irritation and "fear".

The first two of these mechanisms, namely FAL and taste modification, are similar in that they alter the palatability of the resource being protected. Palatability is defined as the interrelationship between flavour and post-ingestive feedback. Physiological condition of the animal, the food's chemical characteristics, and the herbivore's experiences with the food also affect this relationship between flavour and feedback (Provenza 1996). In FAL, a toxin that produces emesis is used to produce negative consequences for the consumer. Mammals that ingest foods treated with the toxin form aversions to the treatment via associative learning (Provenza 1995). It is necessary to apply the toxin formulation to every plant because the negative consequences are associated with the treatment, not with plants in general. Repeated exposures to the repellent may be required for aversions to form (Nolte & Wagner 2000).

Another way to impact the palatability of a food is to alter the taste/ odour (flavour) of the food. Bitter agents, e.g. denatonium benzoate, are employed in several repellent products to alter the palatability of treated plants. Denatonium benzoate affects the taste of the food to which it is applied, but does not produce negative post-ingestive consequences in the consumer. A number of studies have demonstrated that products which rely on bitter taste as the sole mode of action are not effective repellents (Nolte & Wagner 2000).

Repellents that incorporate irritants, e.g. capsaicin, may deter herbivory by causing sensory pain in the eyes, nose, and mouth of the herbivore. Chemical irritation is a concentration-dependant effect and high concentrations of the irritant are frequently required to deter herbivory. Low concentrations of capsaicin, such as those found in many commercial repellents, do not effectively deter deer browsing (Andelt *et al.* 1994). However, concentrations approaching those found in food-grade hot sauces can reduce deer browsing.

Previous studies of repellents that employ predator odours (e.g. urine, scat, and gland secretions) and odours resulting from protein degradation (e.g. egg and blood products) have suggested that they probably function by the same mechanism (Lewison *et al.* 1995; Mason 1998; Nolte & Wagner 2000). The mode of action for repellents employing animal products as active ingredients has been termed "fear". The anthropomorphic designation arises from the notion that volatiles emitted by animal-based repellents are perceived by herbivores as indicators of predator activity (Nolte & Wagner 2000). Because volatile sulphur-containing compounds have been identified as ubiquitous components of predator odours, they are considered to be the mediators of fear-induced avoidance. In fact, predator urines shown to be repellent to herbivores can be rendered ineffectual by removal of sulphur-containing compounds by mercury precipitation (Nolte *et al.* 1994).

The behaviours exhibited by deer when they encounter so-called fear-inducing repellents have rarely been studied. Indirect measures of behaviour (e.g. intake of a treated food) have typically been measured. However, it is important to emphasize that these repellents are most effective as contact repellents (i. e. they must be applied to the plant). Repellents of this type generally fail to exclude herbivores from treated locations (i.e. they do not serve as area repellents; see Belant *et al.* 1998, Nolte & Wagner 2000). In fact, while food intake is typically reduced, behaviours such as approaches or head entries into feeders are unaffected by predator-based repellents (Pfister *et al.* 1990).

We recently observed goat interactions with BGR-P treated seedlings and noted that while they detected the treatment from a distance of several metres by olfaction, each individual approached the treated seedlings and made close contact with the treatment (pers. observ.). When only a portion of the seedling was treated with BGR-P, goats readily consumed the untreated portions of the same seedling. Furthermore, when browsing partially-treated seedlings, goats were observed to clip treated branches and spit them out.

Such observations suggest that olfaction is an important aspect of avoidance of animal-based repellents, but that the behaviour appears feeding related and not a consequence of predation-induced anxiety. There has been some debate as to whether predator avoidance or altered palatability is the actual mechanism of animal-based repellents (Chabot *et al.* 1996). The research described here is the first in a series of experiments designed to test our hypothesis that avoidance is mediated by the palatability of proteins present in animal-based repellents.

2 Methods

Treatments

Baker's soy flour was provided by Archer Daniels Midland Company (Decatur, IL, USA). Gelatins, Type A (acid hydrolyzed) and Type B (base hydrolyzed) porcine collagen, were provided by PB Leiner (Jericho, NY, USA). Egg albumen was provided by Belovo Inc. (Pinehurst, NC, USA). Milk casein was purchased from American Casein Co. (Burlington, NJ, USA). Tactic® (Loveland Industries, Greeley, CO, USA) was obtained for use as the agricultural sticker to adhere the protein treatments to the seedlings. Deer-Away Big Game Repellent® powder (BGR-P; IntAgra, Inc., Minneapolis, MN, USA) was employed as a positive control.

Bioassay

The experiment was conducted in eight 0.125 ha pens. Seven plots were created in each pen by transplanting western redcedar seedlings (c. 60 cm) in 3 × 4 patterns such that trees (12) within a plot were spaced 1 m apart and plots were separated by a minimum of 3 m. The plots were generally situated along an axis extending through the centre of each pen (three plots on one side, four on the other) to allow the sprinkler system located on this axis to water adequately all trees in the experiment. The transplanted trees were heavily watered for approximately 12 h before treatments were applied and as needed following the application.

Treatments were randomly applied to the plots within each pen. All twelve trees within a plot were treated identically. First, individual trees were wetted with a 0.054 % (v/v) Tactic® solution in tap water by use of a tank-type sprayer. The trees were "dusted" by hand with the treatments. Care was taken to ensure that the mass of treatment substance was similar among trees within a plot as well as among treatments, but no device was specifically employed for this purpose. Trees in the control plots were treated with the Tactic® solution only. Although BGR-P is formulated with a sticker agent and normally requires only the use of water for its application, the Tactic® solution was also used for BGR-P treatments to maintain consistency among treatments.

Test subjects, 3 per pen, were placed in the pens approximately 3 h post-application. During the 20-day test period, subjects were permanently housed in the test pens. No movement among pens was permitted during the experiment. Subjects had *ad libitum* access to pelleted deer feed and water throughout the bioassay. Shelter was provided by permanent 5 × 3 m covered structures in each pen.

The number of bites taken from each tree was measured 12 times during the 20-day experiment (days 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, and 20). The maximum number of bites available from seedlings of this size was previously demonstrated to be 25 (Nolte 1998). A small number of trees (46 out of 672) were pulled from the ground by the deer before the maximum number of bites was attained. The actual number of bites recorded for these trees were included in the data set until the day of removal. Following removal from the ground by the subjects, these trees were recorded as missing data points. Individual trees that had the maximum number of bites recorded prior to being pulled by the deer were recorded as having sustained 25 bites at each successive measurement day.

The experiment was conducted during the period 20 June – 10 July 2003. The procedures were reviewed and approved by the Institutional Animal Care and Use Committee of the National Wildlife Research Center.

Statistical analyses

A Kaplan-Meier survival analysis was performed to compare survivability distribution functions among treatments, using the Wilcoxon test of equality (PROC LIFETEST; SAS 1999). "Failure" was defined as the first experimental day when 25 bites were measured on the individual tree. Trees that survived to the end of the experiment (did not meet definition of failure) were censored. Another non-parametric test was used to evaluate the cumulative bite data at the end of the experiment (day 20 data). Bite data were rank-transformed by treatment (1 = low; 7 = high) and a Kruskal-Wallis test was performed where treatment was the lone factor and rank the response (Iman 1982). Multiple comparisons of the means were achieved by Tukey's least significant difference.

Headspace analyses

For analyses of volatiles generated by the treatments, 250 µl of Tactic® solution was placed in a 19 x 150 mm culture tube (approx. volume 30 ml) and the tube was manually rotated to coat the inside of the tube evenly with the solution. Quickly, 150 mg of the treatment material was then added to the tube such that the treatment material was evenly distributed throughout the tube. One tube was prepared for each treatment as well as one control (Tactic® solution only). Tubes were maintained on their sides for six to twelve days prior to analysis. All samples were subjected to one analysis by purge and trap gas chromatography except for the BGR-P sample which was analysed twice for quality control purposes.

Sample tubes were purged for 6 hours with helium on a Tekmar 3000 Purge and Trap Concentrator (Cincinnati, OH, USA). Volatiles were adsorbed onto a Carbopack B/Carboxen 1000 and 1001 trap (Supelco Trap K; Bellefonte, PA, USA) and desorbed at a temperature of 225 °C onto the gas chromatograph (GC). The GC (HP5890; Agilent Technologies, Avondale, PA, USA) was equipped with a HP 5973 mass selective detector (Agilent Technologies) and a fused silica capillary column (30 m × 0.25 mm 5% phenyl-methylpolysiloxane; 25 µm film thickness; J&W Scientific, Folsom, CA, USA). The detector was operated in the scan mode (m/z = 33 to 500).

Amino acid analyses

A semi-quantitative method was developed for the analyses of the sulphur-containing amino acids methionine, cysteine and cystine by high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS). A Hewlett-Packard 1100 HPLC (Agilent Technologies, Palo Alto, CA, USA) was equipped with a 150 mm × 2.0 mm octadecyl silane column (Aquasil® C18; Keystone Scientific, Bellefonte, PA, USA) and attached to an ion-trap tandem MS equipped with an electrospray ionization interface (Thermo Finnigan Corp., Sane Jose, CA, USA). The HPLC mobile phase consisted of 1% acetic acid in water:methanol (95:5) at a flow of 0.3 ml/min. Detection of the amino acids was achieved by MS-MS where the parent ions (M+H+) were isolated in the trap and subjected to collision-induced dissociation. The responses obtained from the resultant base peaks were used for the chromatographic output.

Treatment samples (50 mg) were extracted with a 50:50 mixture of ethanol:water and the extracts analysed for free amino acids by HPLC-MS-MS. Samples (5 mg) were also subjected to acid hydrolysis at 105 °C with 4M trifluoroacetic acid in vacuum-sealed ampules for 20 h. A portion of each hydrolysate was diluted in 50:50 ethanol:water and analysed by HPLC-MS-MS. The relative quantities of methionine and cysteine detected in the samples were compared among the treatments by normalising values to soy protein.

3 Results

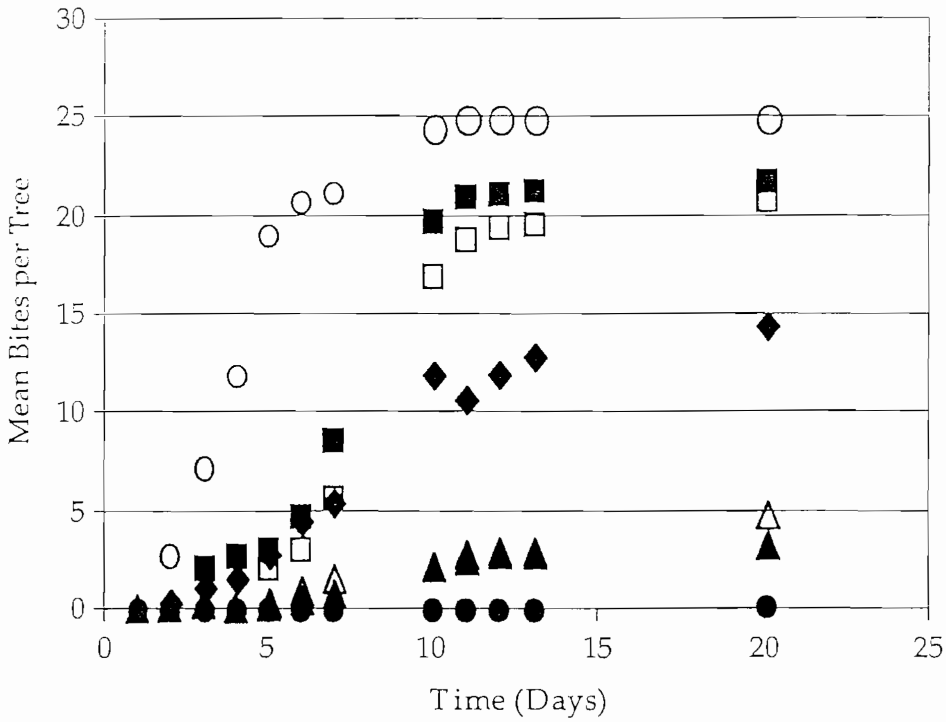
Bioassay

Bite data demonstrate that all control trees sustained the maximum of 25 bites by day 11 (Figure 1). By definition, a total 25 bites to an individual tree was considered a complete loss of the seedling. Several of the treatments appreciably deterred herbivory over the period of the experiment. In particular, BGR-P, casein and albumen drastically minimised deer browsing. Wilcoxon test of Kaplan-Meier failure-time data indicated that the survival distribution functions differed significantly among the treatments ($\chi^2_6 = 617$, $p < 0.0001$), because some treatments prevented failure (i. e. complete loss) of individual trees. Survival distributions at the end of the experiment ranged from 1.00 to 0.00 (Table 1).

At the end of the experiment, the mean number of cumulative bites sustained by the seedlings ranged from a low of 0.094 for BGR-P-treated trees to 25.0 for control trees (Table 2). Kruskal-Wallis rank-sum test of rank-transformed cumulative bite data (day 20) indicated that the number of bites to seedlings were significantly impacted by the treatments ($F_{6,49} = 45.46$, $p < 0.0001$). Multiple comparisons revealed the following order of bites by treatment: BGR-P < casein = albumen < soy < type A collagen = type B collagen = control. The majority of bites sustained by trees treated with BGR-P, casein, and albumen were located in just one of the eight test pens and exclusion of these data indicate that casein is similar to BGR-P in repellency (Table 2).

Headspace analyses

Gas chromatographic analyses of the headspace samples indicated that most treatment materials did not produce volatile compounds in detect-



Legend: O Control, ■ Type A, □ Type B, ◆ Soy, △ Albumen, ▲ Casein, ● BGR-P

Figure 1: *Mean deer bites on seedlings (max = 25) treated with proteins, sticker (control), or Deer-Away Big Game Repellent®. Twelve seedlings per treatment were placed in eight pens with three deer per pen. The decrease in cumulative bites observed for the soy treatment on day 11 is the result of seedlings being regarded as missing values after the deer pulled them from the ground*

able quantities. The lone exception was BGR-P, which yielded the aliphatic aldehydes hexanal, heptanal, octanal and nonanal. The BGR-P sample was analysed seven and twelve days after it was prepared. The resulting chromatograms were nearly identical from these two analyses, indicating that the volatile profile of BGR-P was consistent over the period the analyses of treatment materials were conducted.

Amino acid analyses

All treatments were void of sulphur-containing amino acids in the free form. No cystine was observed in any of the hydrolysates, and none of the amino acids of interest were observed from hydrolysis of BGR-P. Methionine was observed in soy, casein, and albumen hydrolysates in increasing abundance with levels in casein and albumen about five times greater than that of soy (Table 3). Cysteine was observed in most hydrolysates.

Table 1: *Survival distributions at the end of the experiment for the six putative repellent treatments, plus control, applied to western redcedar seedlings. "Failed Trees" were defined as trees that sustained the maximum number of bites (25) from captive deer.*

Treatment	Total	Failed	Survival
BGR-P	96	0	1.00
Casein	95	9	0.905
Albumen	96	17	0.823
Soy	90	50	0.444
Type B Collagen	85	65	0.235
Type A Collagen	78	65	0.167
Control	83	83	0.00

Table 2: *Cumulative deer bites sustained by western redcedar seedlings after exposure to captive deer for 20 days. Mean bites were determined from 12 seedlings in each of eight test pens. Least-square means were calculated to account for missing data (pulling of seedlings by deer prior to termination of the experiment)*

Treatment	Mean Bites		Comparisons[1]
BGR-P	0.094	(0.00)[2]	A (A)[3]
Casein	3.54	(0.57)	B (A)
Albumen	4.95	(2.14)	B (B)
Soy	15.0	(13.6)	C (C)
Type B Collagen	20.9	(20.4)	D (D)
Type A Collagen	22.4	(22.1)	D (D)
Control	25.0	(25.0)	E (E)

[1] Means followed by different letters are significantly different
[2] Mean bites when data from one pen (out of eight) are excluded
[3] Comparisons of means when data from one pen are excluded

Table 3: *Relative concentrations (unitless quantity) of methionine and cysteine in treatment materials*

Treatment	Methionine	Cysteine
BGR-P	0	0
Casein	5.4	1.6
Albumen	7.2	1.4
Soy	1.0	1.0
Type B Collagen	0	2.4
Type A Collagen	0	2.6

4 Discussion

While all treatments applied to western redcedar reduced deer herbivory relative to the control, three of the treatments had noteworthy impacts on seedling survivability over the course of the three-week bioassay (Tables 1 and 2). Deer browsing was greatly reduced by the application of BGR-P, casein, and albumen. Among these treatments, BGR-P (36% egg solids) was the most effective repellent. BGR-P was included in this experiment as a positive control because many previous studies have demonstrated similar efficacy (Engeman *et al.* 1995; Lemieux *et al.* 2000; Nolte 1998; Nolte & Wagner 2000; Witmer *et al.* 1997). Previous studies have also demonstrated the efficacy of whole chicken egg as a contact repellent (Andelt *et al.* 1991; Lutz & Swanson 1997). Thus, it was not surprising that egg albumen was efficacious. However, we were surprised that a plant-derived protein, soy, was moderately avoided. To our knowledge, this is the first demonstration of casein as an effective herbivore repellent.

In addition to the bioassay experiment, we also conducted chemical analyses of the treatment materials. We anticipated that chemical differences among the treatments would prove valuable for post-hoc explanations of the bioassay data. Analyses of the volatile components generated by the treatment materials indicated that only BGR-P produced volatile odours in detectable quantities. The BGR-P patent claims that repellency is achieved by the generation of aliphatic aldehydes by a "repellent-producing ingredient (which undergoes) auto-oxidation to an aliphatic aldehyde" (US Patent #4065577). Lipids present in egg yolk are the "repellent-producing ingredients" in BGR-P that yield aliphatic aldehydes.

The short-chain aliphatic aldehydes observed in BGR-P (hexanal, heptanal, octanal, and nonanal) are known invertebrate repellents (Douglas *et al.* 2001) and common ingredients in predator attractants (Scrivner & Howard 1984). However, these same volatiles are present in significant quantities in the forehead secretions of male white-tailed deer (Gassett *et al.* 1997). Given the conflicting roles these aldehydes play in ecological systems, it is possible that their presence in BGR-P has no ecological significance. Rather, they may merely serve as olfactory cues. Aversions to odours alone are transitory, while the combination of odour and taste can result in persistent aversions (Provenza *et al.* 2000). Furthermore, addition of an odorant increases the salience of protein (Brot *et al.* 1987).

The role of sulphur volatiles in avoidance of predator odours was established by removal of sulphur-containing volatiles from urine by precipitation with mercuric chloride (Nolte *et al.* 1994). Avoidance of other animal-based repellents is similarly assumed to be mediated by sulphur-containing volatiles, such as hydrogen sulfide, dimethyl sulfide, dimethyl disulfide, etc., produced from decomposition of the proteins. However, inspection of the chromatograms generated by purge and trap analyses revealed no sulphur-containing volatiles. These results are not consistent with findings that sulphur volatiles are responsible for the repellency of animal-based repellents (Lewison *et al.* 1995; Nolte *et al.* 1994). Observation of aldehydes resulting from auto-oxidation of lipids in the BGR-P treatment suggests that the instrumental technique employed for analyses of volatiles was capable of detecting odorants present in very small quantities.

In addition to precipitation of sulphurous odours, treatment of predator urine with mercuric chloride (as described in Nolte *et al.* 1994) would have similarly removed methionine and cysteine-containing proteins. Therefore, these amino acids (and proteins that contain them) were the focus of our investigation. Avoidance among purified proteins (i.e. casein = albumen > soy > gelatins; Table 2) was highly correlated with relative methionine content of these same proteins (Table 3). The methionine results may explain why soy protein was moderately avoided by deer – more so than the gelatins but not to the same extent as casein and albumen – even though it was not of animal origin (Table 2). Chemical analyses demonstrated that methionine was incorporated into the proteins themselves and not present in free form (i. e. only detectable after hydrolysis).

Because BGR-P is composed of egg products (among other ingredients), it should also contain methionine in quantities relative to the amount of albumen present in the formulation. It is not clear if our inability to detect methionine in BGR-P was a reflection of low albumen content or interference of hydrolysis by other ingredients in the formulation. Regardless, the chemical data indicate that avoidance among protein treatments was a function of methionine-containing protein content and avoidance of BGR-P was related to the presence of aliphatic aldehydes.

Mode of action for protein avoidance

Herbivores will avoid nutritious foods when they eat them to excess and aversions are formed (Provenza 1995). For instance, negative post-ingestive consequences resulted when lambs were infused with 75 g of casein (Arsenos & Kyriazakis 1999). Conversely, infusion of 15 g casein fashioned positive consequences in lambs. Given our delivery system and the minimal intake of the treatments, it is highly unlikely that sufficient protein was consumed to produce negative consequences. Therefore, avoidance of protein-treated seedlings was probably not a product of FAL. Nor is there evidence that proteins are mammalian irritants – which are characterized by acidity, an electron-poor phenyl ring, and a lipophilic side chain (Mason *et al.* 1991). Further, differences in repellency of the proteins we tested can not be attributed to the presence or absence of volatile sulphur or fatty acid compounds as has been suggested (Mason 1998; Nolte *et al.* 1994).

We propose that feeding anxiety exhibited by deer in this experiment is an attribute of neophobia. Food neophobia is characterized by initial avoidance followed by attenuation after considerable experience with the food (i. e. with repeated exposures animals learn that novel foods are safe). Foods are avoided for no other apparent reason than they are "new" or "strange". Domestic livestock commonly exhibit neophobia when feeds are supplemented with proteins (Bowman & Sowell 1997). Neophobia is a common strategy employed by mammals to avoid toxicosis from ingestion of unfamiliar foods (Launchbaugh & Provenza 1994).

Observing that lambs avoided beneficial foods with flavour additives, Augner *et al.* (1998) proposed the following rule of thumb for herbivore encounters with novel foods: "given a choice, avoid food with strong flavours". All the protein treatments offered in this experiment were novel to the test subjects. We propose that the proteins imparted strong and/or unusual flavours and the presence of methionine accentuated the differences among the proteins. These qualities of the proteins combined with the wary nature of deer conspire to generate repellency by neophobia. Because herbivores forage in protein-poor environments and rarely ingest animal-derived proteins, such proteins are out of the ordinary.

While novel flavours may deter mammalian herbivory, all flavours are not equal. For example, neophobia to denatonium benzoate-treated seedlings is quickly attenuated because herbivores commonly encounter bitter tasting foods (Nolte 1998). Conversely, proteins may play a special role in flavour-feedback interactions. When comparing sucrose, casein, saline, and vanilla solutions, rats were far more likely to associate the casein solution with negative consequences versus the other solutions (Kalat & Rozin 1970). Bernstein *et al.* (1984) similarly demonstrated that conditioned aversions arise more readily to proteins than to carbohydrates. The salient nature of proteins may be a function of proteins having a stronger flavour relative to other stimuli or they are more unusual to the animals.

Attenuation of neophobia is a function of experience with the food and strongly influenced by the motivation of the herbivore (Domjan 1977). Nutritional state and access to alternative foods are important components of motivation. Herbivores are likely to consume significant amounts of novel foods when offered without alternatives while avoiding these same novel foods when offered a choice of foods (Arsenos & Kyriazakis 2001). It is probable that neophobia to the treatments in this experiment (particularly BGR-P and casein) was not attenuated because a nutritionally adequate basal diet was available *ad libitum* and the subjects were not motivated to "test" the strange flavours. However, in different contexts, deer are likely to consume BGR-P treated foods. Deer that were moderately food deprived consumed significant quantities of BGR-P treated food pellets (Andelt *et al.* 1991). Furthermore, non-deprived deer consumed increasing quantities of BGR-P treated food pellets over the course of four days – indicating that they were learning that BGR-P ingestion did not have any post-ingestive consequences. In another study demonstrating the effect of herbivore motivation, a liquid BGR application to ornamental trees in early winter significantly reduced herbivory. However, a late winter application (when browse options are more limiting) did not minimize browsing (Milunas *et al.* 1994).

Finally, the novelty of animal-based repellents may be amplified by the presence of volatiles – such as BGR-P which produces volatile aldehydes from the egg lipids. These results are consistent with our hypothesis that avoidance of foods treated with animal-based proteins is mediated by changes in palatability. However, further experiments are necessary to resolve the specific roles of taste and odour.

Acknowledgements

The authors thank Connie Jo Bingham, Deborah Stalman, and Julie Harper for their assistance with the bioassay and Rick Engeman and Russ Mason for their assistance with the manuscript. Mention of specific products does not constitute endorsement by the United States Department of Agriculture. A portion of this research was funded by USDA CSREES IFAFS Program Code 14.1: Alternative Natural Resource Management Practices for Private Lands - Grant # 2001-52103-11215.

References

Andelt, W. F.; Burnham, K. P.; Manning, J. A. (1991): Relative effectiveness of repellents for reducing mule deer damage. *Journal of Wildlife Management* 55: 341–347

Andelt, W. F.; Burnham, K. P.; Baker, D. L. (1994): Effectiveness of capsaicin and bitrex repellents for deterring browsing by captive deer. *Journal of Wildlife Management* 58: 330–334

Arsenos, G.; Kyriazakis, I. (1999): The continuum between preferences and aversions for flavoured foods in sheep conditioned by administration of casein doses. *Animal Science* 68: 605–616

Arsenos, G.; Kyriazakis, I. (2001): Does previous protein feeding affect the response of sheep towards foods that differ in their rumen availability, but not content, of nitrogen? *Physiology & Behavior* 72: 533–541

Augner, M.; Provenza, F. D.; Villalba, J. J. (1998): A rule of thumb in mammalian herbivores? *Animal Behaviour* 56: 337–345

Belant, J. L.; Seamans, T. W.; Tyson, L. A. (1998): Predator urines as chemical barriers to white-tailed deer. *Proceedings of the 18th Vertebrate Pest Conference* 18: 359–362

Bernstein, I. L.; Goehler, L. E.; Fenner, D. P. (1984): Learned aversions to proteins in rats on a dietary self-selection regimen. *Behavioral Neuroscience* 98: 1065–1072

Brot, M. D.; Braget, D. J.; Bernstein, I. L. (1987): Flavor, not postingestive, cues contribute to the salience of proteins as targets in aversion conditioning. *Behavioral Neuroscience* 101: 683–689

Bowman, J.G.P.; Sowell, B.F. (1997): Delivery method and supplement consumption by grazing ruminants: a review. *Journal of Animal Science* 75: 543–550

Chabot, D.; Gagnon, P.; Dixon, E.A. (1996): Effect of predator odors on heart rate and metabolic rate of wapiti. *Journal of Chemical Ecology* 22: 839–868

Domjan, M. (1977): Attenuation and enhancement of neophobia for edible substances. In: *Learning mechanisms in food selection* (eds. L.M. Barker, M.R. Best and M. Domjan). pp. 151–179. Baylor University Press, Waco, TX, USA

Douglas III, H.D.; Co, J.E.; Jones, T.H.; Conner, W.E. (2001): Heteropteran chemical repellents identified in the citrus odor of a seabird (crested auklet: *Aethia cristatella*): evolutionary convergence in chemical ecology. *Naturwissenschaften* 88: 330–332

Engeman, R.M.; Campbell, D.L.; Nolte, D.; Witmer, G.W. (1995): Some recent research results on non-lethal means of reducing animal damage to reforestation projects in the western United States. *Proceedings of the 10th Australian Vertebrate Pest Control Conference* 10: 150–154

Gassett, J.W.; Wiesler, D.P.; Baker, A.G.; Osborn, D.A.; Miller, K.V.; Marchinton, R.L.; Novotny, M. (1997): Volatile compounds from the forehead region of male white-tailed deer (*Odocoileus virginianus*). *Journal of Chemical Ecology* 23: 569–578

Iman, R.L. (1982): Some aspects of the rank transform in analysis of variance problems. *Proceedings of the SAS Users Group International* 7: 676–680

Kalat, J.W.; Rozin, P. (1970): "Salience": a factor which can override temporal contiguity in taste-aversion learning. *Journal of Comparative and Physiological Psychology* 71: 192–197

Launchbaugh, K.L.; Provenza, F.D. (1994): The effect of flavor concentration and toxin dose on the formation and generalization of flavor aversions in lambs. *Journal of Animal Science* 72: 10–13

Lemieux, N.C.; Maynard, B.K.; Johnson, W.A. (2000): Evaluation of commercial deer repellents on ornamentals in nurseries. *Journal of Environmental Horticulture* 18: 5–8

Lewison, R.; Bean, N.J.; Aronov, E.V.; McConnell Jr., J.E.; Mason, J.R. (1995): Similarities between big game repellent and predator urine repellency to white-tailed deer: the importance of sulfur and fatty acids. *Proceedings of the Eastern Wildlife Damage Control Conference* 6: 145–148

Lutz, J.A.; Swanson, B.T. (1997): Reducing deer damage to woody and herbaceous plants. In: *Repellents in wildlife management: proceedings of the second DWRC Special Symposium* (ed. J.R. Mason), pp. 231–240. National Wildlife Research Center, Fort Collins, CO, USA

Mason, J.R.; Bean, N.J.; Shah, P.S.; Clark, L. (1991): Taxon-specific differences in responsiveness to capsaicin and several analogues: correlates between chemical structure and behavioral aversiveness. *Journal of Chemical Ecology* 17: 2539–2551

Mason, J.R. (1998): Mammal repellents: options and considerations for development. *Proceedings of the 18th Vertebrate Pest Conference* 18: 325–329

Milunas, M.C.; Rhoads, A.F.; Mason, J.R. (1994): Effectiveness of odour repellents for protecting shrubs from browsing by white-tailed deer. *Crop Protection* 13: 393–397

Nolte, D.L. (1998): Efficacy of selected repellents to deter deer browsing on conifer seedlings. *International Biodeterioration & Biodegradation* 42: 101–107

Nolte, D.L.; Mason, J.R.; Epple, G.; Aronov, E.; Campbell, D.L. (1994): Why are predator urines aversive to prey? *Journal of Chemical Ecology* 20: 1505–1516

Nolte, D.L.; Wagner, K.K.; (2000): Comparing the efficacy of delivery systems and active ingredients of deer repellents. *Proceedings of the 19th Vertebrate Pest Conference* 19: 93–100

Pfister, J.A.; Muller-Schwarze, D.; Balph, D.F. (1990): Effects of predator fecal odors on feed selection by sheep and cattle. *Journal of Chemical Ecology* 16: 573–583

Provenza, F.D. (1995): Postingestive feedback as an elementary determinant of food preference and intake in ruminants. *Journal of Range Management* 48: 2–17

Provenza, F.D. (1996): A functional explanation for palatability. *Proceeding of the International Rangeland Congress* 5: 123–125

Provenza, F.D.; Kimball, B.A.; Villalba, J.J. (2000): Roles of odor, taste, and toxicity in the food preferences of lambs: implications for mimicry in plants. *Oikos* 88: 424–432

SAS (1999): SAS Proprietary Software Release 8.2. Cary, NC, USA

Scrivner, J.H.; Howard, W.E. (1984): Aldehyde volatiles for use as coyote attractants. *Proceedings of the 11th Vertebrate Pest Conference* 11: 157–160

Sullivan, T.P.; Nordstrom, L.O.; Sullivan, D.S. (1985): Use of predator odors as repellents to reduce feeding damage by herbivores. II. black-tailed deer. *Journal of Chemical Ecology* 11: 921–935

U.S. Patent #4065577. Method for using a ruminant repellent comprising aliphatic aldehydes.

Witmer, G.W.; Saylor, R.D.; Pipas, M.J. (1997): Repellent trials to reduce reforestation damage by pocket gophers, deer, and elk. In: *Repellents in wildlife management: proceedings of the second DWRC Special Symposium* (ed. J.R. Mason), pp. 321–332. National Wildlife Research Center, Fort Collins, CO, USA