EFFECTS OF ESSENTIAL OILS ON THE FEEDING CHOICE BY MOOSE

Sabine Edlich and Caroline Stolter

Department of Animal Ecology and Conservation, Biocenter Grindel, Martin-Luther-King Platz 3, 20146 Hamburg, Germany.

ABSTRACT: Moose (*Alces alces*) browse on coniferous tree species to different extents during winter; for example, Norway spruce (*Picea abies*) is avoided, Scots pine (*Pinus sylvestris*) is preferred, with juniper (*Juniperus communis*) of intermediate use. Conifers contain essential oils that may act as feeding deterrents, thereby reducing food intake by herbivores. Because essential oils are volatile, our objectives were to determine if 1) odour plays a role in the food choice by moose, 2) whether single monoterpenes act as feeding deterrents, and 3) if this might be a mechanism used to discriminate against unpalatable plants. The essential oils of Norway spruce and juniper and 2 monoterpenes (limonene and camphene) predominant in the essential oil of Norway spruce were tested for their potential as deterrents in feeding trials. Deterrence was assessed in food choice experiments by measuring the time spent feeding on food treated with the different odours associated with these compounds. There was no statistical evidence that food treated with the essential oils of spruce and juniper and single monoterpenes from Norway spruce were avoided by moose. However, our data indicate that the essential oil of Norway spruce probably has a negative effect on moose foraging because of the large absolute difference in feeding time between treatments and that overall, odour had a significant effect on feeding time. Because our experimental design may have influenced the results, we suggest research approaches to better measure deterrence effects.

Key words: *Alces alces*, conifers, essential oil, feeding choice, feeding time, monoterpenes, moose, odour.

Plants have evolved direct and indirect plant defence mechanisms for protection against pathogens and herbivores (Rosenthal and Janzen 1979, Dicke and Vet 1999). Defence mechanisms can be mechanical (e.g., burning hair, thorns, spikes, wax coatings) or chemical such as plant secondary metabolites (PSM; Fox 1981, Karban and Myers 1989). The largest groups of PSMs are phenols, terpenoids, and alkaloids; their effectiveness as feeding deterrents is due to their toxicity (post-absorptive effect), inhibition of food digestion (post-ingestive effect), and deterrence through smell or taste (pre-ingestive effect) (Bryant et al. 1991, Gershenzhorn and Dudareva 2007, Stolter et al. 2009). However, it is important to recognize that PSMs are common in the plant kingdom and part of the natural diets of many herbivores.

Conifers form vast forests distributed widely in the northern hemisphere, with many playing important economic roles in the wood industry, including production of resin and essential oils (Kubeczka and Schultze 1987). Foraging by large herbivores can cause substantial damage to coniferous forests including direct destruction of trees, especially in monocultures and young stands (Sjöberg and Danell 2001, Edenius et al. 2002). Conifers have a high diversity of PSMs that presumably deter feeding by mammalian herbivores (e.g., Bryant et al. 1991, Eppe et al. 1996).
Specifically, essential oils of conifers have a wide variety of monoterpenes with some acting as deterrents to snowshoe hares (Lepus americanus; Sinclair et al. 1988), red deer calves (Cervus elaphus; Elliott and Loudon 1987), and moose (Alces alces; Sunnerheim-Sjöberg 1992). Danell et al. (1990) found a negative correlation between consumption and concentration of the terpenoid pinifolic acid in Scots pine (Pinus sylvestris); a similar observation was made by Sunnerheim-Sjöberg (1992) with a different monoterpene [(-)-angelicoidenol-2-O-β-D-glucopyranoside] in Scots pine.

The reluctance of herbivores to feed on monoterpenes might relate to inhibition of microbial activity in the digestive system (post-ingestive effect). For example, Oh et al. (1967) studied the essential oils of Douglas-fir needles (Pseudotsuga menziesii) and found that oxygenated monoterpenes decreased microbial activity in the rumens of sheep (Ovis aries) and deer (Odocoileus hemionus columbianus). Therefore, avoidance of monoterpenes, hence avoidance of some coniferous tree species, might be learned from negative, post-ingestive effects. Furthermore, monoterpenes are characterized by their highly distinctive odour, and because of their volatility, monoterpenes and consequently essential oils might also deter animals prior to ingestion.

Moose forage on leaves, shoots, and twigs of lignified plants including twigs, needles, and bark of conifers in winter; their food selection can be influenced by PSMs (Danell et al. 1990, Stolter et al. 2005, Stolter 2008). Among conifers, moose prefer Scots pine which is known for low concentration, but high diversity in phenolic compounds compared to other coniferous trees (Stolter et al. 2010). In contrast, Norway spruce (Picea abies) that is common throughout Europe, is avoided and used only when food resources are scarce. Other conifers like common juniper (Juniperus communis) vary in utilization among habitats (Hörnberg 2001, Månsson et al. 2007, pers. observ., C. Stolter). Because monoterpenes of the essential oils of conifers are volatile, their odour might be one cue in forage selection by moose. However, the role of volatile monoterpenes and essential oils in forage selection is little explored, although smell appears important in food choice (Levin 1976, Bryant et al. 1991).

We investigated whether the essential oils of Norway spruce and common juniper, and specific monoterpenes influence forage selection of moose. Specifically, we wanted to determine if 1) odour plays a role in forage selection by moose, 2) whether single monoterpenes act as feeding deterrents, and 3) if this might be a mechanism to discriminate against unpalatable plants. Based on the assumption that an animal theoretically maximizes its net calorie intake per feeding time (Emlen 1966) and that diet optimization is influenced by nutritional value of food (e.g., positive effects of nutrients and negative effects of PSMs; Freeland and Janzen 1974, Pulliam 1975), we used feeding time to investigate the possible differences in deterrent effects.

**METHODS**

**Choice of Essential Oils**

We assumed our captive moose would have similar forage selection as wild moose that prefer Scots pine, typically reject Norway spruce, and have intermediate use of common juniper (Hörnberg 2001, Månsson et al. 2007, pers. observ. by authors). We tested and verified this assumption in a pilot study when we fed captive moose twigs of the 3 species (Edlich 2009); our findings were in accordance with previous studies. Consequently, we used 6 substances in our experiments: essential oils of Norway spruce and common juniper (because we assumed that these odours might be deterrent) and the monoterpenes limonene, camphene, borneol, and eucalyptol. These monoterpenes were chosen because they are predominant in the essential oils of Norway spruce, but rare in Scots pine and common
juniper (Edlich 2009). However, due to restrictions of the zoo, we removed the experiments for borneol and eucalyptol; thus, only 4 experiments are presented here. Because it was not possible to extract enough essential oils from Norway spruce and common juniper for the experiments, we substituted essential oils purchased commercially.

To examine differences in the terpenoid composition of the essential oils of plant samples, and to examine if the commercial oils (spruce oil, juniper oil; Shandiin, Hamburg, Germany) could be used for our experiments, we compared the chemical profiles of the commercial oils with the essential oils extracted by distillation from plant material collected during winter 2008-2009 in Lower Saxony, Germany (53°09’02”N, 9°54’44”E). We sampled up to 5 individual trees per species by clipping the first 4-8 cm of several branches; those samples were combined into a single composite sample per species. Plant material was frozen in plastic bags at -20° C until distillation. Oils were extracted by steam distillation as described by Pfannkuche (2000). About 50 g of frozen needles were distilled for 3 h and the extracted essential oil was analysed by gas chromatography linked with a mass spectrometer (Shimadzu GC-MS QP 2010S). The chemical profile of the distillate oils matched that of the commercial oils.

Experiments

Feeding trials were used to test the deterrent effect of odour with 3 female and 1 male moose housed together in a 12-ha enclosure in Wildpark Lüneburger Heide, Lower Saxony, Germany; all had previously eaten Scots pine and Norway spruce as part of their winter diet. They had access to 4 feeding troughs placed adjacently (~1 m apart) of which only 3 were used. Typically, moose used the feeding troughs in the same arrangement; often 2 moose (usually a cow and her yearling) fed together at 1 trough. Because they fed voluntarily, not all animals participated in each experiment (1-h feeding trial); therefore, experiments were repeated 10 times for each odour.

Toward the typical feeding times, we placed 2 plastic boxes (40 x 33.5 x 8.5 cm) with a known weight of food pellets in each of the 3 troughs (Wildkraftfutter Sommer für Wiederkäuer, Nösenberger Pferdefutter; Brackel, Germany). One of the boxes was perforated and underneath had an unreachable pad of cotton wool soaked with 5 µl of an essential oil or monoterpene; this low concentration was used to mimic the odour of a non-damaged tree. The plastic boxes were cleaned with 2-propanol and equipped with an unused pad after each trial. To prevent preference for a specific box, the positions of the boxes were changed randomly. Moose were allowed to acclimate to the feeding protocol and boxes for 2 days, after which the daily feeding routine consisted of 2 daily feeding times: 1000-1100 and 1500-1600 hr during which we carried out the 1-h experiments.

The experiments consisted of 4, 10-day periods partitioned into 2, 5-day periods separated by a 2 day break. In each 10-day period there were 10, 1-h feeding experiments of the 4 treatments (spruce, juniper, limonene, or camphene). Treatments differed between the morning and afternoon feedings. Because consumption of the food pellets was nearly complete in each trial, we used feeding time to assess deterrence. Further, because 2 moose often fed together at 1 trough, we were unable to measure the amount of food consumed by an individual moose. Therefore, a video camera (Sony Full HD Camcorder) was installed at each of the 3 used troughs to record consumption time and identify moose. Water and branches of deciduous trees were available ad libitum throughout the experiments.

Statistical Analysis

Statistics were performed with PASW 18 (SPSS 2010, IBM Cooperation). The Wilcoxon-Test was used to test for differences
in feeding time between the boxes with and without odour. We calculated a general linear model (GLM) for repeated measurements to test for differences among the different odour experiments. Before using this statistical approach, we tested against violation of sphericity using the Maucleys-Test. We included the differences in feeding time between the boxes with odour and without odour as a dependent variable. To gain a balanced design, we included only the first 4, 1-h measurements of each animal; because not every animal participated in every experiment, 4 days was the maximum participation of 1 moose per treatment. Further, by using only the first 4 measurements, we presumably minimized the possibility of habituation influencing the results. We used each treatment and individual moose as within-subject effects. We tested for differences between the treatment with essential oil of spruce and the other treatments using within-subject contrasts; this was also done to test for differences between animals (P-values are Bonferroni-corrected).

**RESULTS**

**Comparison between feeding boxes with and without odour**

We tested for differences between the boxes with and without odour for each treatment separately. The absolute mean feeding time was higher for boxes with odour of the essential oil of Norway spruce (80%) and common juniper (19%); the opposite occurred for limonene (13% lower) and camphene (4% lower) (Table 1, Fig. 1). Although no treatment was statistically different (all P > 0.05; Table 1), there was a strong tendency with Norway spruce.

**Comparison of different odours**

Because of our repeated measurement design, we conducted further analyses with a GLM for repeated measurements using the difference between boxes with and without odour (time feeding on the box with odour – time feeding on the box without odour). Again, we restricted our data to the first 4, 1-h experiments of each moose, and calculated the mean differences within a treatment (Fig. 2). Odour was a significant (P = 0.003) inner-subject factor (Table 2). The contrasts (within subject-contrasts) between spruce vs. limonene (P = 0.010) and spruce vs. camphene (P = 0.017) were also significant (Table 2);

<table>
<thead>
<tr>
<th>Odour</th>
<th>Box without odour</th>
<th>Box with odour</th>
<th>Mean</th>
<th>SE</th>
<th>Mean</th>
<th>SE</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce</td>
<td>212.94</td>
<td>385.50</td>
<td>22.28</td>
<td>28.61</td>
<td>-1.82</td>
<td>0.068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Juniper</td>
<td>298.00</td>
<td>355.25</td>
<td>71.41</td>
<td>79.29</td>
<td>-0.73</td>
<td>0.465</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limonene</td>
<td>269.88</td>
<td>236.13</td>
<td>44.15</td>
<td>48.97</td>
<td>-1.10</td>
<td>0.273</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camphene</td>
<td>292.63</td>
<td>282.06</td>
<td>79.26</td>
<td>78.65</td>
<td>-0.37</td>
<td>0.715</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Mean feeding time [s] of moose (n = 4) at the boxes with and without odour. Repeated measurements (4, 1-h experiments) were pooled for each animal before statistical analyses. We used Wilcoxon-test to test for significant differences.
though not significant, the spruce vs. juniper contrast showed a strong tendency ($P = 0.086$; Table 2). Moose were not significant in within-subject contrasts (Table 2).

**DISCUSSION**

Most PSMs are not acutely toxic (e.g., phenols and terpenes) but have negative effects at certain concentrations (Bryant et al. 1983, 1991, McArthur et al. 1991, McIntosh et al. 2003, Stolter et al. 2005). Therefore, many animals have evolved mechanisms to detect these compounds and regulate their intake (Dearing et al. 2005), which presumably reflects the variable use of coniferous species by herbivores (Hansson et al. 1986, Roy and Bergeron 1990, Eppele et al. 1996). Given their volatility, monoterpenes are detected through chemical sensory perception like smell (Chapman and Blaney 1979), and because the essential oil of each coniferous species has characteristic composition and concentrations of volatile monoterpenes, species-specific odours result (e.g., Norway spruce vs. Scots pine). Because Norway spruce is not a preferred browse of moose and used only when forage is limited, our aim was to determine whether its essential oil or one of its monoterpenes has a deterrent effect on moose.

We found no statistical difference between the feeding time spent on treated and untreated samples. Further, none of our treatments with single components acted as an absolute deterrent indicating that there was no strong individual effect of the PSMs on food consumption. However, because all food was consumed in each trial, it is possible that our ability to measure deterrence was masked by the experimental protocol. In contrast to a similar study with red deer (Elliott and Loudon 1987), we found no difference in the feeding time between boxes with or without odour for all treatments. However, we found a strong tendency (80% difference, Table 1) that moose fed longer on boxes treated with

### Table 2. Results of a general linear model (GLM) for repeated measurements ($n = 4$, 1-h experiments) using the difference in feeding time between the boxes with and without odour as a dependent variable. Odour (essential oil of Norway spruce and common juniper, limonene, and camphene) and individual moose ($n = 4$ animals) were used as within-subject effects. We tested for differences between the spruce treatment and the other treatments and for differences between the animals using within-subject contrasts ($P$-values are Bonferroni corrected).

<table>
<thead>
<tr>
<th></th>
<th>$df$</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odour</td>
<td>(3/9)</td>
<td>10.02</td>
<td>0.003</td>
</tr>
<tr>
<td>Moose</td>
<td>(1.07/3.20)</td>
<td>1.60</td>
<td>0.295</td>
</tr>
<tr>
<td>Interaction</td>
<td>(2.48/7.44)</td>
<td>1.12</td>
<td>0.390</td>
</tr>
</tbody>
</table>

### Table 2. Results of a general linear model (GLM) for repeated measurements ($n = 4$, 1-h experiments) using the difference in feeding time between the boxes with and without odour as a dependent variable. Odour (essential oil of Norway spruce and common juniper, limonene, and camphene) and individual moose ($n = 4$ animals) were used as within-subject effects. We tested for differences between the spruce treatment and the other treatments and for differences between the animals using within-subject contrasts ($P$-values are Bonferroni corrected).

<table>
<thead>
<tr>
<th></th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spruce/Juniper</td>
<td>6.39</td>
<td>0.086</td>
</tr>
<tr>
<td>Spruce/Limonene</td>
<td>34.51</td>
<td>0.010</td>
</tr>
<tr>
<td>Spruce/Camphene</td>
<td>23.07</td>
<td>0.017</td>
</tr>
<tr>
<td>Moose 1 / 2</td>
<td>0.02</td>
<td>0.906</td>
</tr>
<tr>
<td>Moose 2 / 3</td>
<td>5.52</td>
<td>0.100</td>
</tr>
<tr>
<td>Moose 3 / 4</td>
<td>2.32</td>
<td>0.225</td>
</tr>
</tbody>
</table>
the essential oil of Norway spruce indicating its possible negative effect on consumption. We suggest that moose fed longer on the box with odour due to deterrence because animals should maximize their food intake (Emlen 1966); slower feeding might indicate that animals are more cautious or avoid a particular food (e.g., post-ingestive effect). The GLM supported this idea because odour had a significant effect on feeding time between the boxes, and comparative testing showed that these differences were pronounced between the essential oil of Norway spruce and both monoterpenes, but not between the essential oils of both conifers (Table 2). Interestingly, an opposite result was found for limonene; moose fed longer on the box without odour resulting in a pronounced negative difference (Fig. 2), suggesting that limonene had no or positive influence on foraging.

Elliott and Loudon (1987) tested the odour of essential oils of sitka spruce (Picea sitchensis) and lodgepole pine (Pinus contorta) and selected monoterpenes on red deer by measuring mass of food consumed rather than time spent feeding. They found a deterrent effect for the essential oil of lodgepole pine on male deer, but not for sitka spruce or with female deer (but see Duncan et al. 1994, 2001); in contrast to our results, the monoterpene limonene acted as a feeding deterrent for female red deer. These differences might relate to experimental design, specifically, by measuring food intake and using higher concentrations of monoterpenes given that the effects of PSMs are dose-dependent (Harborne 1991). Our objective was to determine if consumption of the plant species was deterred by odour, not to measure the concentration limit where monoterpenes might act as a deterrent. Furthermore, the use of plant material instead of essential oils (our experiment) might be more “natural” experimentation, although cutting plant material causes an increase in odour due to the damage of resin ducts. Assuming that animal experience might also influence such studies, we note that our moose had previously fed on Norway spruce and may have been “olfactory-adapted.” Because all animals were housed together during the experiment, individual feeding might have been affected by dominance, although we found no significant effect with individual moose (Table 2).

The odours of essential oils function to signal chemical composition of a plant to an animal, which in the case of Norway spruce is determined by its variety of terpenoids and high concentration of specific phenolics (Stolter et al. 2010). These compounds might (in addition to terpenoids) have negative influences on digestion (Stolter et al. 2009). However, our results did not indicate that a strong deterrent effect exists because no treatment acted as an absolute deterrent. Interestingly, we found differences between the influence of monoterpenes and essential oils.

Chemical defence mechanisms are complex, and essential oils are a combination of individual components (i.e., specific monoterpenes) that can act synergistically to provide greater toxicity or deterrence than the equivalent amount of a single substance (Gershenzon and Dudareva 2007). In his assessment of the environmental effectiveness of terpenoids, Harborne (1991) showed that the concentration and universally dependent dose were important. Herbivores could not “smell out” certain monoterpenes from an essential oil, but if a monoterpene was predominant or overbalanced the concentration, the essential oil might provide an avoidance or deterrence effect. Because the study moose were not deterred by the treatments, we assume that smell alone probably plays a minor role in the relative use and avoidance of Norway spruce; taste and texture (Chapmann and Blaney 1979) and/or the concentrations of other compounds (e.g., phenols) are likely important.

Conducting experiments with captive moose is logistically difficult and has degrees of compromise because of limited animal number and size of treatment groups. Ac-
knowledging such limitations, future research concerning the effects of PSMs on food use and choice by moose might test treatments on individual moose, examine effects at different concentrations of PSMs, use mass of food consumed in combination with feeding time as indicators of preference, control for common experience with PSMs of experimental moose, and consider the influences of sight and taste.

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