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MONOTERPENE EFFECT ON FEEDING CHOICE BY DEER

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Abstract—A previous study showed that Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) consumption was negatively correlated with monoterpene content in western redcedar (*Thuja plicata*). To test whether these monoterpenes were deterrent to Sitka black-tailed deer, we performed feeding choice experiments with four hydrocarbon (sabinene, myrcene, α-pinene, and $d + l$-limonene) and one oxygenated (α,β-thujone) monoterpene solution at their highest natural concentration in western redcedar foliage. To test whether deer response was species specific, we ran similar experiments on European roe deer (*Capreolus capreolus*) and rusa deer (*Cervus timorensis russa*). In all experiments, monoterpenes were repellent. Solutions with α,β-thujone, the major monoterpene in redcedar leaves, were the most repellent of the solutions tested. We then analyzed how black-tailed and roe deer responded to (1) an increase in concentration of the monoterpenes with the weakest repellent effects (hydrocarbon monoterpenes) and (2) a decrease in concentration of the monoterpene with strongest effect (α,β-thujone). Repellency tended to increase with concentration for hydrocarbon monoterpenes, but remained strong for α,β-thujone. As wild deer regularly feed on plants containing monoterpenes, this raises the question as to how the animals deal with these molecules.

Key Words—Monoterpenes, deer, cervids, feeding choice, herbivory.

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INTRODUCTION

Monoterpenes, 10-carbon compounds biosynthesized from two isoprene (2-methyl-1,3-butadiene) units, are widely found as secondary metabolites in plants and are produced by nearly all conifers (Banthorpe and Charlwood, 1980; Bohlmann and Croteau, 1999; Trapp and Croteau, 2001). Numerous studies have shown that higher monoterpene concentrations in plants correlate with lower consumption by ungulate herbivores and, therefore, likely play an important role in the plant–herbivore interaction (Connolly et al., 1980; Personius et al., 1987; Duncan et al., 1994; Riddle et al., 1996; Estell et al., 1998a; Vourc'h et al., 2001). However, other studies have failed to find this correlation (Welch et al., 1983; Behan and Welch, 1985). To prove that monoterpenes are the cause of the deterrence of a plant, one needs to test their effects experimentally. We know of only three studies that have done so, using ungulate herbivore food-choice assays. The first, by Elliot and Loudon (1987), tested the effect of monoterpene odors on captive red deer calves (*Cervus elaphus*). The other two, by Estell et al. (1998b, 2000), tested the effect of monoterpenes on domestic sheep. These studies found that some monoterpenes are indeed deterrent for these particular species. Nevertheless, the possibility exists that different ungulate species vary in response to different feeding deterrents (Oh et al., 1967; Zahorik and Houpt, 1991). Furthermore, exposure to certain compounds can trigger learning processes (Provenza, 1996; Spalinger et al., 1997; Tixier et al., 1998) or adaptations of the rumen flora (Oh et al., 1967) with the consequence that the reaction of the animal will depend on whether it had already encountered a compound in its diet or not.

Vourc'h et al. (2001, 2002) have shown that Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) prefer western redcedar (*Thuja plicata* Dann ex D. Don) leaves that have low monoterpene concentrations. Based on this, the purpose of this study was to test directly the deterrent effect of western redcedar monoterpenes on Sitka black-tailed deer, independently of the plant material the compounds are found in. To explore whether our results applied to other conifer foraging deer species, we ran a second set of experiments with European roe deer (*Capreolus capreolus*), a temperate deer species with similar body size to Sitka black-tailed deer. Finally, we tested the effect of these compounds on a tropical deer species with little dependence on conifers as a food source, the rusa deer (*Cervus timorensis russa*).

METHODS AND MATERIALS

Monoterpenes. Two oxygenated monoterpene isomers (α,β-thujone) and four hydrocarbon monoterpenes (sabinene, myrcene, α-pinene and $d + l$-limonene) have been identified by Vourc'h et al. (2001) in western redcedar leaves and were tested in feeding choice experiments. Monoterpenes were obtained from

Aldrich Chemical (Milwaukee, Wisconsin, USA) except for sabinene, which was obtained from R. C. Treatt (Bury St. Edmunds, Suffolk, England). For α,β-thujone, sabinene, and myrcene, only solutions of technical-grade purity could be obtained. The thujone solution was extracted from western redcedar leaf and contained 65% α,β-thujone (67% β-thujone and 33% α-thujone at equilibrium), 15% fenchone, 10% bornyl acetate, 3% camphor, and a mixture of other minor terpenes. The sabinene solution contained 70% sabinene, 20% β-pinene, 4% α-pinene (tested as pure solution in our experiment), and a mixture of other minor terpenes. The myrcene solution contained 75% myrcene and other minor terpenes. Although these impurities may introduce some noise in the analysis of the effect of the main compounds, they all consist of compounds that occur commonly in most terpene-producing plants and are, for the most part, characterized by low biological activity (T. P. Clausen, personal communication).

We first tested these solutions on Sitka black-tailed deer, roe deer, and rusa deer (with a simplified protocol), with the concentration corresponding, for each monoterpene, to the mean concentration found in young western redceder foliage (Vourc'h et al., 2002). Trees of this age have the highest natural monoterpene concentration observed in western redcedars (Vourc'h et al., 2001, 2002). We defined this concentration as *in vivo* redcedar concentration.

Second, to analyze the effect on monoterpene identity, we tested these solutions at different concentrations, with Sitka black-tailed deer and roe deer. On the basis of the experiment with monoterpenes at *in vivo* redcedar concentration on Sitka black-tailed deer, we separated the solutions into two groups: (1) strongly repellent, meaning deer ate food containing monoterpenes in less than 50% of the trials, and (2) weakly repellent, meaning food containing monoterpenes was eaten in more than 50% of the trials. We then tested whether the strongly repellent solutions retained activity at lower concentrations, defining lower concentration as the minimum concentration of each monoterpene found in young western redcedar foliage (Vourc'h et al., 2002). We tested the second group of solutions at higher concentrations, defining it as the concentration of the most concentrated monoterpene in redcedar foliage, i.e., α,β-thujone. We refer to these two levels as "manipulated concentrations" throughout the remainder of the paper.

The amount of monoterpene solution (MonoSol) to be used in the experiments was calculated by taking into account the concentration that we wanted to achieve (MonoConc), the purity of the manufactured monoterpene solution (MonoPurity), the density of the compound (MonoDensity), and the dry weight of the food substrate (SubstrateDW):

$$\text{MonoSol (ml)} = \frac{\dfrac{\text{MonoConc (mg/g)}}{1000} \times \text{SubstrateDW (g)}}{\text{MonoDensity (g/ml)} \times \dfrac{\text{MonoPurity (\%)}}{100}}$$

This amount was put into 5 ml solvent (85% ethanol and 15% methanol) per 100 g of fresh substrate to form the monoterpene solution. The control solution was made of 5 ml solvent per 100 g of fresh substrate.

Black-Tailed Deer on Haida Gwaii. Feeding choice experiments with Sitka black-tailed deer involved wild deer living on Haida Gwaii (Queen Charlotte Islands, British Columbia, Canada), where western redcedar is a preferred food for that species (Pojar et al., 1980; Coates et al., 1985). To our knowledge, this is the first study testing the effect of secondary metabolites on wild ungulates. Sitka black-tailed deer were introduced from the nearby mainland to Haida Gwaii near the beginning of the 20th century (Carl and Guiguet, 1972). At that time, there was no other large herbivores on the archipelago (Cowan, 1989). Five male and four female free ranging black-tailed deer of Haida Gwaii were used at three different sites: one "natural site" (Vertical Point), and two logging camps (Beattie Anchorage and Eden Lake). One female and two males were 1–2 years old; the others were mature adults. At Vertical Point, deer got used to human presence within a few days. In logging camps, deer were already used to being close to humans.

Black-tailed deer were habituated to eating apples with the solvent for a few days before proceeding to the experiments. For each trial, we prepared two bowls with 100 g of fresh cut apples each. One bowl was sprayed with a monoterpene solution and one with the control solution. We then presented the pair of bowls to one individual deer and let it eat until it moved away. Because we did not stop the trial until the deer moved away, the deer could eat off either bowl. We weighed the amount of food left in each bowl to calculate the amount of food eaten. No weight changes in the food substrate occurred because of the short duration of each trial (3–30 min). Our protocol was to run three to five trials per deer per monoterpene solution at a given concentration. The order of monoterpenes was randomized per deer for a given concentration, starting with the *in vivo* redcedar concentration. No more than three trials per deer and per day were run. The experiment lasted three weeks.

Roe Deer in France. We ran experiments with roe deer raised in captivity at the Centre d'Etudes Biologiques de Chizé (France). Roe deer eat a wide range of plant species from all major taxonomic groups including fungi, ferns, shrubs, deciduous trees, and conifers (Duncan et al., 1998). During the experiment, roe deer had access to a small forest patch with understory and a grassy area. They also had *ad libitum* access to pellets (Caprilat: 18% protein, 3% fat, 8.7% cellulose, 7.2% ash) and water.

The captive roe deer were very sensitive to human presence and could not be kept in individual pens. Therefore, we could not work with individual deer and had to run the experiments in four pens with three to five deer in each (pen 1: three females; pen 2: four females and one male; pen 3: two females and one male; and pen 4: two females and one male). Pens ranged from a 450 m^2 to 1500 m^2 pasture and 2000 m^2 forest. For a given trial in a pen, we presented as many pairs of

monoterpene and control bowls as there were deer in the pen. Each monoterpene bowl contained 100 g of apples with the same monoterpene solution and the control bowl contained 100 g of apples with solvent similar to the experiments with Sitka black-tailed deer (discussed above). We recorded the number of deer that took part in each trial as well as the number of pairs of bowls that were used. After a 2-hr trial, the total amount of control or monoterpene apples remaining in the bowls was weighed for each pen. Because the apple masses could change during the 2 hr that the trials lasted, we put a bowl with 100 g of apples outside each pen and weighed the bowl again at the end of each experiment. The amount of apple consumed was then calculated using the equation:

$$[(H_i \times O_f / O_i) - H_f]$$

where H_i and H_f were the initial and final masses of apples exposed to herbivory, and O_i and O_f the initial and final masses of apples outside the pens (Cronin and Hay, 1996). Our protocol was to run three to five trials per monoterpene solution per pen per concentration at a rate of two trials per day (one in the morning and one in the evening) in each pen. We randomized the order of monoterpene that were tested for a given pen, starting with the *in vivo* redcedar concentration. This experiment took place over a period of two months.

Rusa Deer in New Caledonia. Rusa deer were introduced to New Caledonia from the Indonesian island of Java in the 1870s (Chardonnet, 1988). Before the colonization of the island by Europeans in 1845, there were no large herbivores (Gargominy et al., 1996). Four domesticated rusa deer (a subadult male and a 1- or 2-year-old female, a 2- or 3-year-old stag, and a hind with her young) were available for experiments at the research Station of Port-Laguerre. Before the trials, the deer were raised in paddocks with cultivated grass (*Brachiaria mutica*, *Brachiaria decubems*, and *Pennisetum prurpureum*) and trees (*Calliandra calothrysus* and *Leucaena leucocephala*). During the trials, a similar diet was cut and provided daily to the animals in their cages, where they had *ad libitum* access to water.

For each trial, we presented a pair of monoterpene and control bowls to individual deer as in the experiments with Sitka black-tailed deer. The food substrate was altered to 20 g of fresh bread in each bowl. We tested monoterpenes at *in vivo* concentrations. Assays with myrcene had to be canceled because the quality of the chemical sample deteriorated. Three trials were run per day per deer using the same monoterpene solution. The order of the monoterpenes tested was randomized per deer. The experiment lasted three weeks.

Statistical Analyses. Data for each deer species were analyzed separately. For black-tailed and rusa deer, the statistic unit was a one-deer trial with a single monoterpene at a given concentration. For roe deer, the unit was a one-pen trial with one monoterpene at a given concentration. First, we calculated the frequency of zero values per monoterpene solution at each concentration, i.e., the frequency

of control or monoterpene bowls not being eaten. Then, for each trial, we calculated a log ratio of the proportion of food eaten for each option according to the method described in Elston et al. (1996):

$$\text{Log}\frac{\left(\frac{\text{g of treated food eaten}}{\text{g of treated food presented}}\right)}{\left(\frac{\text{g of control food eaten}}{\text{g of control food presented}}\right)} = \text{Log}\frac{(\text{g of treated food eaten})}{(\text{g of treated food eaten})}$$

For each trial, the sum of the proportions for each option must be 1, i.e., the data are compositional. Therefore, the log ratio transformation was the most appropriate, as it led to analyses in which the unit-sum constraint was automatically satisfied (Elston et al., 1996). If the proportion of food eaten for each option was equal, the log ratio was zero; if deer ate more control food, the log ratio was negative. To deal with the problem of 0 values, i.e., when either the control or treated food was not eaten, we replaced all the 0 values by 0.1 (Elston et al., 1996). To check the robustness of the replacement, we conducted the same analyses with values of 0.5, 1, and 2 instead of zeros, 0.5 g and 1 g being the smallest amount of bread and apple eaten, respectively. The results were consistent.

For black-tailed and roe deer, we tested the effect of concentration and monoterpene identity (= solution effect) by mixed analysis of variance of the log ratios data using the procedure MIXED in SAS (1999). The factors of interest (i.e., solution, concentration, and solution $\times$ concentration) were declared as fixed effects, whereas additional variables assumed to impact the variability of the data were declared as random effects: for black-tailed deer, deer identity and its interaction with fixed effects, as well as the rank of trial nested in deer, for roe deer, pen number and its interaction with the fixed effects, as well as the rank of trial nested in pen, the number of deer taking part in the trial in each pen, and the number of pairs of bowls from which the food was eaten at each trial. The same procedure was used for rusa deer except that the concentration effect in the mixed analysis of variance was not considered. Therefore, the fixed effect was the solution effect, and random effects were deer identity and its interactions with the solution effect, as well as the rank of the trial nested in deer. The variances of the random effects parameters became the covariance parameters. The selected structure was fitted to the data using the method of restricted maximum likelihood (REML). A likelihood-based scheme was used to infer type III F statistics to test the significance of the fixed effects (SAS, 1999). Mean differences were tested by Lsmeans (SAS, 1999). Lsmeans were also used to test whether the log ratios for each monoterpene at each concentration were significantly negative, i.e., whether the monoterpenes had a repellent effect.

RESULTS

All three species of deer smelled the food before tasting it.

Black-Tailed Deer in Haida Gwaii. In all but two of the 10 treatments, each deer was tested at least three time (Table 1). Deer tried the apples in bowls with α,β-thujone at *in vivo* redcedar concentration in only 17% of the trials. For the other monoterpenes, deer tested the bowls with monoterpene solutions in more than 50% of the trials and ate apples in 98% of the control bowls (Figure 1). The only three instances in which deer did not touch control bowls occurred in trials with α-pinene at *in vivo* redcedar concentration (i.e., in 10% of trials for this solution). The percentage of apples actually consumed in bowls with α,β-thujone was less than 2%. It varied between 40 and 70% for the other monoterpenes, whereas the percentage of apples consumed in control bowls was over 80% on average (Figure 2). Figure 3 shows a representative example of a trial for thujone. Because deer ate apples from bowls with α,β-thujone in less than 50% of the trials, α,β-thujone was tested at a diluted manipulated concentration. The other four monoterpenes solutions (sabinene, myrcene, α-pinene, and $d + l$-limonene) were all tasted by deer in more than 50% of the trials. We tested them at one elevated manipulated concentration.

TABLE 1. EFFECTS OF MONOTERPENE SOLUTIONS[a]

	Proportion of pens									
	Thujone		Sabinene		Myrcene		α-Pinene		$d + l$-Limonene	
	In vivo	Manip.	In vivo	Manip.	In vivo	Manip.	In vivo	Manip.	In vivo	Manip.
Sitka black-tailed deer [deer (N) with 3–5 trials/total deer (N) tested]	7/9 a[b]	9/9	9/9	5/9 b	9/9	9/9	9/9	9/9	9/9	9/9
Roe deer [pens (N) with 3–5 trials/total pens (N) tested]	4/5 c	2/2	4/5 c	2/2	5/5	2/2	4/5 c	2/2	5/5	2/2
Rusa deer [deer (N) with 3–5 trials/total deer (N) tested]	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4

[a] The number of trials was at least 3 and at most 5 in each test. Concentrations of compounds were at *in vivo* levels and at manipulated higher (thujone) or diluted (other compounds) levels.
[b] a, 2 deer with 1 trial only; b, 4 deer with 1 trial only; c, 1 pen with 2 trials only.

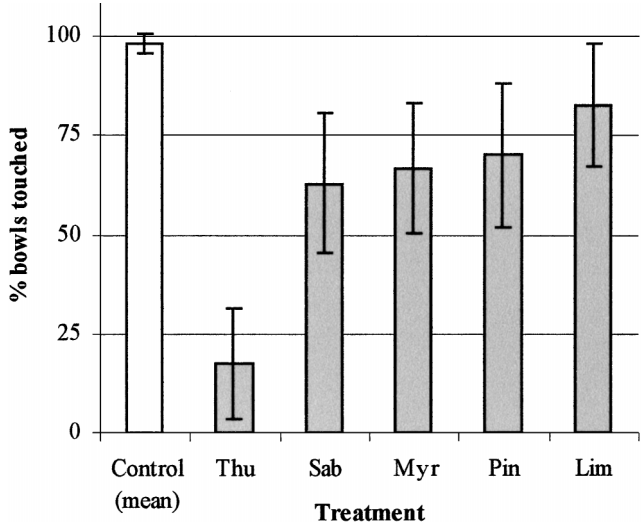

FIG. 1. Percent of bowls with apples that were touched by Sitka black-tailed deer for trials with monoterpene solutions at *in vivo* redcedar concentration or with a control solution. The percentage of control bowls touched is an average over all trials. Vertical bars are 5% confidence intervals bars. Thu = α,β-thujone, Sab = sabinene, Myr = mycrene, Pin = α-pinene, and Lim = $d + l$-limonene.

Deer tasted the content of bowls with the α,β-thujone solution at diluted concentration in 28% of the trials and consumed 12% of the apples. They tasted the content of bowls with elevated concentration of the other monoterpenes in 54–68% of trials, consuming 25% (pinene) to 35% (sabinene) of the apples (Figure 4). Deer tasted the apples in all control bowls, consuming 80–97% of the apples.

For each solution and at both concentrations, the repellent effect was significant, i.e., the mean of the log ratio was significantly smaller than zero ($P < 0.05$ for all cases). Mixed analysis of variance showed an effect of solution ($F_{4,32} = 10.07$, $P < 0.001$), concentration ($F_{1,8} = 11.43$, $P = 0.009$), and the solution $\times$ concentration interaction ($F_{4,32} = 2.99$, $P = 0.033$). The solution effect was significant at *in vivo* redcedar concentration ($F_{4,32} = 9.72$, $P < 0.001$), and at manipulated concentrations ($F_{4,32} = 3.62$, $P = 0.015$). α,β-Thujone solutions were more repellent than the other solutions at both concentrations ($P < 0.05$) (Figures 2 and 4). The repellent effect was greater at the manipulated concentration than at the *in vivo* redcedar concentration for α-pinene and for $d + l$-limonene ($P < 0.05$), but not for the other compounds.

Roe Deer in France. A total of 10 of 14 roe deer took part in the trials: all three deer in pen 1, three of five deer in pen 2, and two of three in pens 3 and 4. Deer in pens 3 and 4 were shy and only took part in the first set of experiments

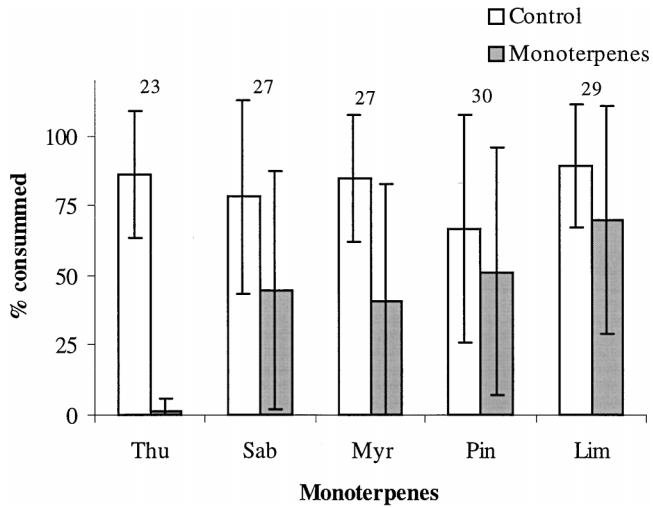

FIG. 2. Average percent of apples consumed by Sitka black-tailed deer in trials with different monoterpene solutions at *in vivo* redcedar concentration or with a control solution. Number of trials per deer varied from 3 to 5 (with some exceptions, see Table 1). Numbers above bars are number of trials. Vertical bars are standard deviations, abbreviations as in Figure 1. All differences in consumption between control and monoterpene bowls are significant ($P < 0.05$).

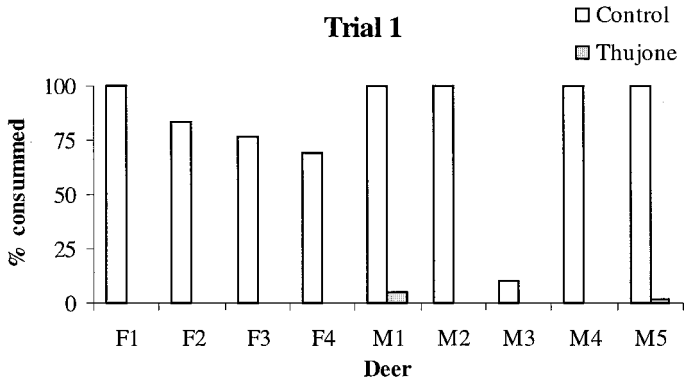

FIG. 3. Representative example of effect of α,β-thujone on Sitka black-tailed-deer consumption. Thujone = bowl of apple with α,β-thujone solution at *in vivo* redcedar concentration. Control = bowl of apple with control solution. F1 to F4 = females 1–4, M1–M5 = males 1–5.

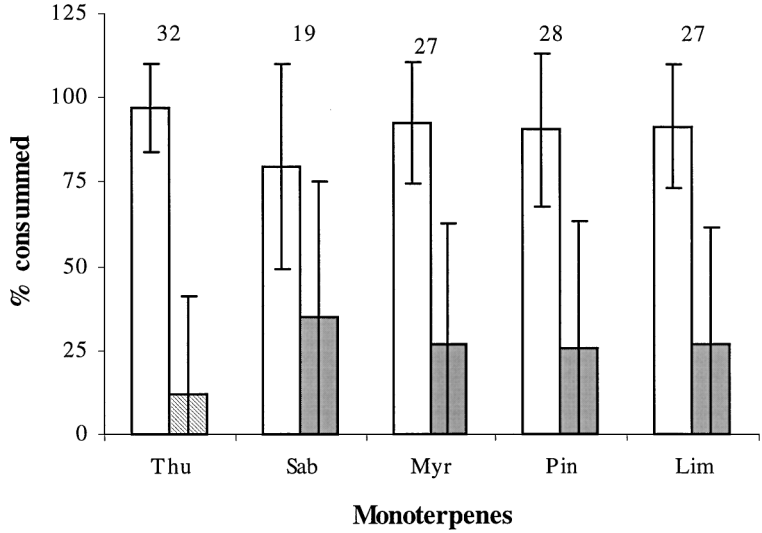

FIG. 4. Average percent of apples consumed by Sitka black-tailed deer in trials with different monoterpene solutions at manipulated concentrations. Hatched bars = monoterpene tested at lower than *in vivo* concentration; shaded bars = monoterpenes tested at higher than *in vivo* concenterations; open bars = control solutions. Vertical bars are standard deviations. Numbers above bars are number of trials. Abbreviations as in Figure 1. All differences in consumption between control and monoterpene bowls are significant ($P < 0.05$).

(*in vivo* concentrations). Except for three cases, at least three trials were run for each of the pens tested (Table 1).

Deer ate apples from the control bowl in each trial except once for the α-pinene, myrcene, and $d + l$-limonene trials at *in vivo* redcedar concentration. At *in vivo* concentrations, food with α,β-thujone was tested in 40% of the trials, that with sabinene in 94%, myrcene in 88%, α-pinene in 62%, and $d + l$-limonene in 65%. Deer tested 62% of bowls with α,β-thujone at diluted manipulated concentration. At elevated manipulated concentration, deer tested 63% of the bowls with sabinene, myrcene, and α-pinene, and 75% of the bowls with $d + l$-limonene. In the trials at *in vivo* concentrations, deer ate less than 2% of the apples with α,β-thujone solution, ate between 19 and 27% of the apples with the other monoterpene solutions, and about 50% of the apples in the control bowls (Figure 5a). In the trials at manipulated concentrations, deer ate about 13% of the apples in the bowls with α,β-thujone at diluted concentration and 9–20% of the apples in trials with the other monoterpenes at elevated concentrations (Figure 5b). In these experiments, deer ate 68–82% of the apples in the control bowls. Figure 6 shows, as an example, the results of the trials for sabinene.

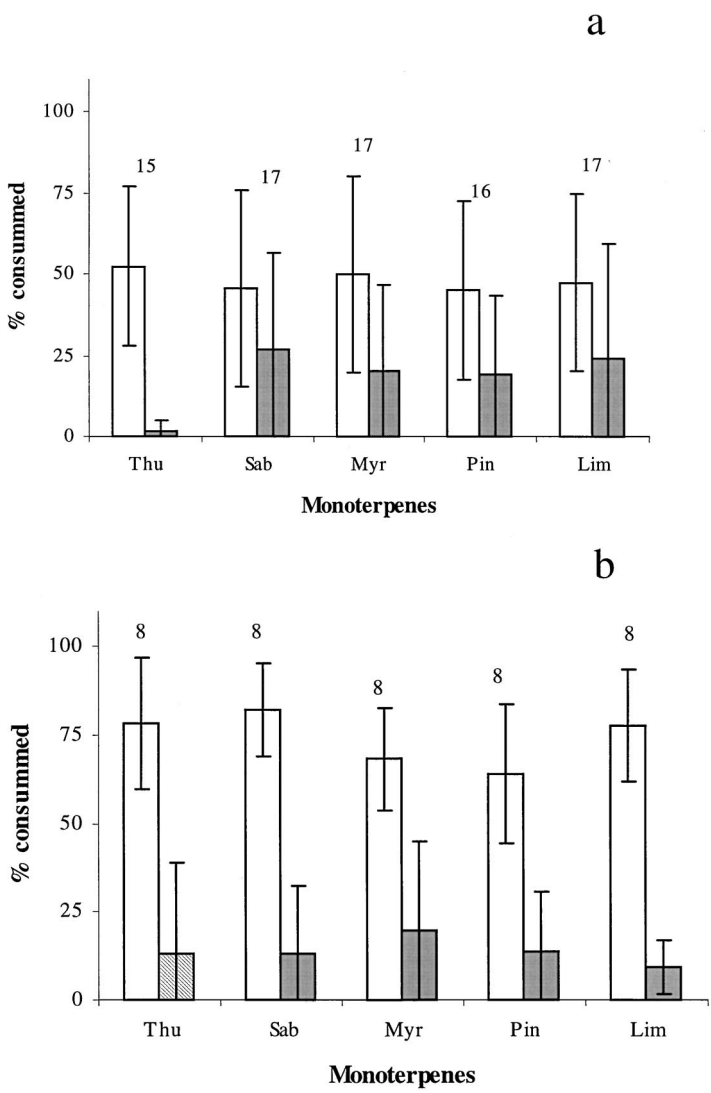

FIG. 5. Average percent of apples consumed by roe deer in trials with different monoterpene solutions at *in vivo* redcedar concentrations (a) and at manipulated concentrations (b). Vertical bars are standard deviations. Numbers above bars are number of trials pooled for all pens. (a) Shaded bares = monoterpene solutions, open bars = control solutions. (b) Hatched = monoterpenes tested at lower than *in vivo* concentration; shaded = monoterpenes tested at higher than *in vivo* concentration; open bars = control solutions. Abbreviations as in Figure 1. All differences in consumption between control and monoterpene bowls are significant ($P < 0.05$), except for sabinene at *in vivo* concentration.

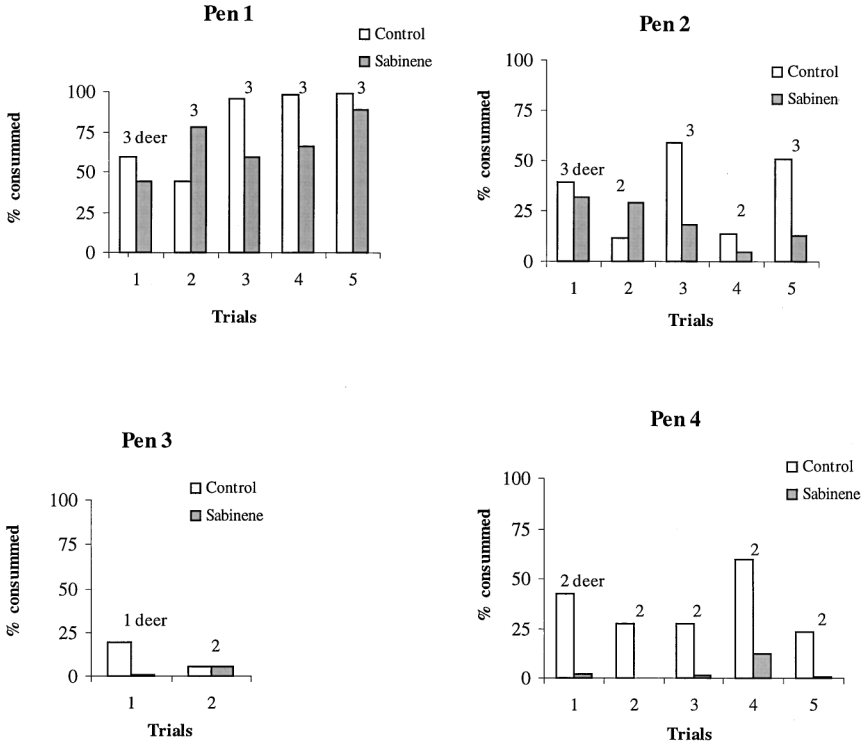

FIG. 6. Example of weak response to sabinene by roe deer from different pens. Deer had access to bowls with apples plus a control solution (control) or with apples plus a sabinene solution at *in vivo* redcedar concentration (sabinene). Numbers above bars are number of deer taking part in the trial in each pen.

The mixed analysis of variance showed no significant effect of the interaction term solution × concentration; therefore, we tested the single effects without the interaction. The solution effect was not significant ($F_{4,8} = 1.84$, $P = 0.207$), nor was the concentration effect ($F_{1,1} = 1.99$, $P = 0.393$). Even though α,β-thujone tended to be more repellent than other solutions at *in vivo* redcedar concentration (Figure 5), the solution effect was not significant either at *in vivo* ($F_{4,12} = 2.25$, $P = 0.124$) or at manipulated concentrations ($F_{4,4} = 0.05$, $P = 0.993$).

For each monoterpene at each concentration, the repellent effect was significant ($P < 0.05$ for all cases), except for sabinene at *in vivo* redcedar concentration ($P = 0.746$).

Rusa Deer in New Caledonia. Three trials were run per monoterpene solution and per deer. Of the bowls with α,β-thujone, sabinene, $d + l$-limonene, and α-pinene, 12%, 74%, 75%, and 100%, respectively, were touched. Note that myrcene

was not tested. All control bowls were touched, and over 95% of their bread was eaten. The percentage of bread eaten in bowls with the α,β-thujone solution at *in vivo* concentration was 2% and was 47%, 88%, and 57%, respectively, for sabinene, α-pinene, and $d + l$-limonene.

The mixed analysis of variance showed a significant effect of solution ($F_{3,9} = 15.05$, $P < 0.001$) at *in vivo* redcedar concentration, the only concentration used. The repellent effect was significant for α,β-thujone, sabinene, and $d + l$-limonene ($P < 0.010$ in each case), but not for α-pinene ($P = 0.315$).

DISCUSSION

Monoterpenes Are Repellent to Deer. The results of this study show that monoterpenes are feeding deterrents to deer. These results confirm the correlation observed between monoterpene content and western redcedar foliage choice by Sitka black-tailed deer (Vourc'h et al., 2001, 2002). The deterrent effect was strongest for the thujone solution, oxygenated monoterpene isomers, extracted from western redcedar leaves. This contrasted with the lower effect of the rest of the monoterpenes tested, which were all hydrocarbon monoterpenes. There was no difference between the repellent effect of these hydrocarbon monoterpenes. A stronger repellency of oxygenated monoterpenes has been suggested by Oh et al. (1967) and Schwartz et al. (1980) but has not always been confirmed (Estell et al., 2000). Diluting the concentration of α,β-thujone did not decrease its repellency. Increasing the concentration of hydrocarbon monoterpenes did increase their repellency for Sitka black-tailed deer. The same trend was observed for roe deer, but was not significant (deer from two of four pens did not take part in the trials on manipulated concentrations, reducing the power of the statistical tests).

Variation among Deer. Despite differences in the experimental protocols, we observed a remarkable homogeneity in the response pattern across species, suggesting a broad effect of monoterpenes on deer food choice. The experiments also revealed some variation in the response of the three deer species studied. At *in vivo* redcedar concentrations, sabinene and α-pinene were not repellent to roe and rusa deer, respectively, but were to Sitka black-tailed deer. A repellent effect of α-pinene on sheep was also observed by Estell et al. (1998b) and on red deer by Elliott and Loudon (1987) at concentrations similar to those in our study. At a lower concentration, however, Estell et al. (2000) did not find a deterrent effect of sabinene on sheep. Finally, Sitka black-tailed deer, which include western redceder in their regular diet, responded more strongly to α,β-thujone than roe deer, which are usually not exposed to redcedar.

Signal Effect and Consequences. Our observations of deer first smelling their food before eating or avoiding it are consistent with the findings of Elliott and London (1987) that indicate it is the odor of monoterpenes that deter red deer. However, for a plant defense to be effective, a signal has to be associated with the

negative consequences of eating the plant (Lawler et al., 1999). Indeed, if there is no physiological consequence associated with a signal, deer will ultimately learn that the food is palatable (Tixier et al., 1998; Villalba and Provenza, 2000). The nature of these negative postingestive effects of monoterpenes is still under investigation. Studies on small mammals (Millet et al., 1981; Hiroi et al., 1995; Höld et al., 2000) have suggested that it could be neurotoxicity. *In vitro* experiments by Nagy et al. (1964), Oh et al. (1967, 1968), and Schwartz et al. (1980) suggested that monoterpenes could inhibit rumen microbial fermentation, but studies based on rumen inocula (Welch and Pederson, 1981; Cluff et al., 1982) suggest that monoterpenes in the rumen might not reach concentrations high enough to affect rumen microorganisms. Further work is needed to fully understand the effect of monoterpenes on cervid digestion.

Monoterpene Effects on Foraging in the Wild. The influence of monoterpenes on deer foraging in the wild is another area where additional work is needed. Indeed, the strong repellent effect of monoterpenes we observed seems to be at odds with the regular consumption (and even the preference) of western redcedar by Sitka black-tailed deer (Pojar et al., 1980; Coates et al., 1985) and with the habit of roe deer to forage on various monoterpene-containing conifers (Duncan et al., 1998). This discrepancy could result from deer in the wild not being able to access more palatable food or from monoterpenes in conifer leaves being less detectable than monoterpenes sprayed on food substrate. It also suggests that deer can deal with monoterpenes once they are ingested, either through detoxification and/or by regulating their toxin intake according to food flavor, toxin, nutrient, and dietary experience (Provenza, 1995, 1996).

For tropical rusa deer, the context is different, as they live on New Caledonia where conifers are present, but whose contribution to deer diet is unknown. Rusa deer may encounter a much more extensive assortment of plants and could have developed a rumen flora and feeding behavior adapted to a more varied array of plant secondary metabolites including monoterpenes (Oh et al., 1967).

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