JOURNAL OF ANIMAL SCIENCE
The Premier Journal and Leading Source of New Knowledge and Perspective in Animal Science

Effects of cis-²-ocimene, cis-sabinene hydrate, and monoterpene and sesquiterpene mixtures on alfalfa pellet intake by lambs

R. E. Estell, E. L. Fredrickson, D. M. Anderson and M. D. Remmenga

J Anim Sci 2008.86:1478-1484.
doi: 10.2527/jas.2007-0699 originally published online Feb 13, 2008;

Effects of *cis*-β-ocimene, *cis*-sabinene hydrate, and monoterpene and sesquiterpene mixtures on alfalfa pellet intake by lambs[1]

R. E. Estell,*[2] E. L. Fredrickson,* D. M. Anderson,* and M. D. Remmenga†

*USDA/ARS Jornada Experimental Range, Las Cruces, New Mexico 88003;
and †University Statistics Center, New Mexico State University, Las Cruces 88003

ABSTRACT: The transition of grasslands to shrub-dominated scrubland reduces livestock productivity and contributes to impoverished conditions for humans in arid and semiarid regions worldwide. Many shrubs that are increasing in dominance contain secondary compounds that deter browsing by herbivores. Knowledge concerning the effects of specific compounds in herbivore diets is limited but may provide useful insights into desertification. *Flourensia cernua* is a dominant shrub in the northern Chihuahuan Desert that contains an abundance of terpenes. Four experiments were conducted to determine the effects of terpenes on intake of alfalfa pellets by lambs. Two individual monoterpenes (*cis*-β-ocimene and *cis*-sabinene hydrate) were examined in Exp. 1 and 2, and mixtures of monoterpenes (borneol, camphene, camphor, 1,8-cineole, limonene, myrcene, and α-pinene) and sesquiterpenes (β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene) were examined in Exp. 3 and 4, respectively. Forty-five lambs (9 lambs/treatment) were individually fed treated alfalfa pellets for 20 min each morning for 5 d. Five treatments (0×, 0.5×, 1×, 2×, and 10×; multiples of the concentrations of the same terpenes in *F. cernua*) were applied to alfalfa pellets (637 g, DM basis) in an ethanol carrier. The experiments were preceded by a 10-d adaptation period of the lambs to untreated pellets. Except during the 20-min test, the lambs were maintained outdoors and fed untreated alfalfa pellets (total mean intake = 4.7% of BW, DM basis). Day × treatment interactions were detected ($P < 0.04$) in Exp. 1 and 4 because of a greater intake for 0× than for the other treatments on d 1 (Exp. 1) and a lower intake for the 10× treatment on d 1 and 2 (Exp. 4). A trend for decreased intake (g/kg of BW) as the concentration of the sesquiterpene mixture increased was observed in Exp. 3 ($P = 0.093$ for the linear contrast). Although there was a tendency for the sesquiterpene mixture to decrease intake, *cis*-β-ocimene, *cis*-sabinene hydrate, and the monoterpene mixture did not appear to affect intake by lambs. Thus, sesquiterpenes may exert antiherbivory properties under certain conditions that may contribute to shrub dominance with extended periods of livestock foraging.

Key words: diet selection, herbivory, intake, monoterpene, sesquiterpene, sheep

J. Anim. Sci. 2008. 86:1478–1484
doi:10.2527/jas.2007-0699

INTRODUCTION

Replacement of grasslands with shrub-dominated scrublands is a major concern for livestock producers in the western United States and in arid and semiarid regions worldwide. Shrubs that are increasing in dominance typically contain secondary chemicals and are often avoided by livestock even though they can be high in protein and other nutrients. Avoiding plants containing aversive chemicals is the first line of defense by herbivores against toxins and antinutritional compounds (Marsh et al., 2006), and diet selection is affected by both pre- and postingestive processes (Provenza, 1995; Pass and Foley, 2000). Preingestive (sensory) chemical cues that affect feeding behavior include both smell and taste (Elliott and Loudon, 1987; Provenza, 1995; Narjisse et al., 1997; Pass and Foley, 2000). Thus, perception of the aroma, taste, or both of secondary chemicals may affect intake independently of (or in concert with) postingestive feedbacks and learned responses.

Our initial studies with sheep and goats forced to browse tarbush (*Flourensia cernua* DC) in small paddocks revealed that animals exhibited differential se-

[1]Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA. The authors are grateful for the assistance of Alfredo Gonzalez, Will Hooper, and Yuan-Feng Wang.

[2]Corresponding author: restell@nmsu.edu

Received October 31, 2007.
Accepted February 9, 2008.

lectivity that was related to leaf surface epicuticular wax and individual mono- and sesquiterpene concentrations (Estell et al., 1994; 1998a). When crude tarbush extracts were applied to alfalfa pellets at concentrations representing those in tarbush, all 3 (hexane, ether, and ethanol) extracts significantly reduced intake of alfalfa pellets (by approximately 42 to 48%) compared with controls receiving only the ethanol carrier (Estell et al., 2001). In an attempt to understand the role of volatile phytochemicals in consumption of shrubs by livestock, we used tarbush as a shrub model and conducted a series of experiments designed to systematically evaluate the effects of individual compounds on intake of alfalfa pellets by sheep. Of the 23 volatile compounds examined (Estell et al., 1998b, 2000, 2002, 2005, 2007), only 4 compounds (camphor, α-pinene, camphene, and caryophyllene oxide) were related to intake when tested individually.

Our objectives were to determine the effects of 1) 2 untested monoterpenes and 2) mixtures of mono- and sesquiterpenes on intake by lambs. Our hypothesis was that intake would decrease as concentration of individual terpenes or mixtures applied to alfalfa pellets increased.

MATERIALS AND METHODS

All experiments were conducted according to USDA guidelines and were approved by the New Mexico State University Institutional Animal Care and Use Committee. Four experiments were conducted to examine the effects of 2 individual monoterpenes (*cis*-β-ocimene and *cis*-sabinene hydrate; Exp. 1 and 2) and a mixture of monoterpenes (borneol, camphene, camphor, 1,8-cineole, limonene, myrcene, and α-pinene; Exp. 3) and sesquiterpenes (β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene; Exp. 4) on intake of alfalfa pellets by lambs. Mean concentrations of terpenes on the leaf surface of tarbush in previous studies (Estell et al., 1994, 1998a; Tellez et al., 1997; R. E. Estell, unpublished data) were approximately 10, 10, 300, 100, 5, 50, 100, 100, 100, 100, 30, 5, and 25 µg/g of DM for *cis*-β-ocimene, *cis*-sabinene hydrate, borneol, camphene, camphor, 1,8-cineole, limonene, myrcene, α-pinene, β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene, respectively. These concentrations approximate the levels to which animals would be exposed when browsing tarbush. In each experiment, a terpene or terpene mixture was applied at 5 concentrations (treatments), with treatments being multiples (0×, 0.5×, 1×, 2×, or 10×) of the exposure concentration of that chemical or mixture. The rationale for selection of compounds for inclusion in each mixture was based on 2 criteria. Those related to tarbush intake (Estell et al., 1994, 1996a, 1998a), to alfalfa pellet intake when applied individually (Estell et al., 1998b, 2002), or both were included. Myrcene and 1,8-cineole were also included in the monoterpene mixture because they are present in large concentrations on the leaf surface of tarbush and have been related to intake in other mammals (Reichardt et al., 1985; Bray et al., 1991; Riddle et al., 1996; Vourc'h et al., 2002). A few terpenes present in substantial concentrations in tarbush were not included because they were not commercially available (e.g., artemisia alcohol, flourensadiol) or were prohibitively expensive (e.g., β-eudesmol).

Forty-five Rambouillet ewe lambs (approximately 6 mo of age, mean initial BW = 32.5 ± 1.2 kg) without previous browsing experience were randomly assigned to pen (n = 15) and group (n = 3). Pen and group assignment were constant across experiments. Before each experiment, the lambs were randomly assigned to 1 of 5 treatments (9 lambs per treatment, restricted to 3 lambs·treatment^{-1}·group^{-1}) using a randomized complete block design.

Experiments were 5 d in length, with a 2-d interval between experiments. Lambs were individually fed treated alfalfa pellets for 20 min each morning in an enclosed metabolism unit (1.22 × 2.44 m pens). Groups (blocks) were fed at 0800, 0830, and 0900 h, respectively. Two preliminary 5-d adaptation periods were conducted 1) to familiarize the lambs with the handling procedures and pen feeding, and 2) to determine the baseline intake of untreated alfalfa pellets during the 20-min interval. Before the adaptation periods, the lambs were adapted to alfalfa pellets for 5 d in a drylot pen. *Cis*-β-ocimene, *cis*-sabinene hydrate, the sesquiterpene mixture, and the monoterpene mixture were examined in Exp. 1 through 4, respectively. The order of individual terpenes was selected randomly in Exp. 1 and 2, and the order of mixtures was selected randomly in Exp. 3 and 4.

Alfalfa pellets (637 g, DM basis; ≥15% CP; 0.95 cm in diameter) were fed during the 20-min interval, and orts were recorded daily. Alfalfa pellet samples composited across experiments were ground in a Wiley mill (2-mm screen; Thomas Scientific, Swedesboro, NJ) and analyzed for DM (100°C for 24 h, DM = 93.72%). Lambs were weighed before the 0800-h feeding on d 5 each week (mean ADG = 203 ± 6.4 g/d) and maintained as 1 group, with free access to water and trace-mineralized salt (93 to 97% NaCl, 3 g/kg of Mn, 2.5 g/kg of Zn, 1.5 g/kg of Fe, 0.15 g/kg of Cu, 0.09 g/kg of I, 0.025 g/kg of Co, and 0.01 g/kg of Se) except during the 20-min feeding periods. Lambs were group-fed untreated alfalfa pellets at 1000 h (4.67% of BW, DM basis, adjusted weekly). The amount of untreated pellets fed at 1000 h on the test days was decreased by the total amount of treated pellets consumed during the 20-min period. The nutrient content of tarbush was similar to alfalfa, ranging across season from 60 to 65, 15 to 25, and 18 to 22% (DM basis) for IVDMD, CP, and NDF, respectively (Estell et al., 1996b).

Borneol (98%), camphene (95%), camphor (96%), 1,8-cineole (98%), limonene (97%), myrcene (90%), α-pinene (98%), β-caryophyllene (90%), and caryophyllene oxide (95%) were purchased from Aldrich (Milwaukee, WI), and *cis*-β-ocimene (70%), *cis*-sabinene hydrate (98%), α-humulene (98%), and α-copaene (90%) were obtained from Fluka (Milwaukee, WI). We were unable to obtain *cis*-β-ocimene with a high degree of purity (ours contained 25% limonene). Although not ideal, limonene was unrelated to intake by lambs in an earlier study (Estell et al., 1998b); thus, it was assumed that if an effect was observed it would be related to *cis*-β-ocimene.

Stock solutions in 100% ethanol (adjusted for the manufacturer's specified purity) were formulated to contain the appropriate amount of chemical(s) when applied to alfalfa pellets at 0.05 mL/g of DM. The stock solution and 5-, 10-, and 20-fold dilutions equated to the 10×, 2×, 1×, and 0.5× treatments, respectively, for each experiment. Control diets were sprayed with ethanol (0× treatment). Treatments were applied in an adjacent room and placed in the pens immediately before the 20-min test. The treatment application protocol and the rationale for various aspects of the design and conduct of the experiments (e.g., the amount of feed offered, the 20-min feeding interval, alfalfa pellets as treatment carrier, the volume of treatment applied, etc.) were based on pilot studies that have been described in detail previously (Estell et al., 1998b, 2000).

Volatile loss in the interval between application and feeding was estimated as described by Estell et al. (2002), except that recoveries were determined by using gas chromatography (Hewlett-Packard 5890 Series II) with a flame-ionization detector and a DB-5 capillary column (30 m, 0.25-mm i.d., 0.25 μm d.f., Agilent Technologies, Wilmington, DE). Conditions were injector temperature, 220°C; detector temperature, 260°C; carrier gas, helium (1 mL/min); split flow, 8 mL/min; and injection volume, 1 μL. Oven temperature varied with analyte: 75°C for *cis*-β-ocimene; 80°C for *cis*-sabinene hydrate; 150°C for the sesquiterpene mixture; and programmed [70°C isocratic (9 min), 2 min increase to 170°C (50°C/min), and 170°C isocratic (1 min)] for the monoterpene mixture. Concentrations of all terpenes were determined by using external standard curves.

Mean recovery (corrected for extraction efficiency) and CV (in parentheses) at 10, 20, and 30 min, respectively, were 82.1 (3.7), 78.9 (8.4), and 74.2 (7.0) for *cis*-β-ocimene; 93.9 (4.2), 93.3 (4.2), and 93.4 (9.2) for *cis*-sabinene hydrate; 99.7 (5.4), 99.4 (5.6), and 99.4 (4.8) for borneol; 78.6 (8.0), 57.4 (7.2), and 45.4 (7.5) for camphene; 92.6 (11.0), 92.6 (3.4), and 86.7 (5.6) for camphor; 98.2 (5.7), 84.6 (4.8), and 77.0 (3.1) for 1,8-cineole; 97.1 (9.8), 80.1 (4.6), and 71.9 (3.4) for limonene; 94.3 (9.8), 76.2 (5.9), and 67.6 (4.7) for myrcene; 82.9 (10.1), 62.7 (6.5), and 51.2 (6.8) for α-pinene; 101.3 (3.7), 100.4 (8.2), and 99.8 (4.5) for α-copaene, 100.1 (6.2), 99.9 (7.9), and 100.1 (7.8) for β-caryophyllene; 100.4 (5.0), 100.3 (7.6), and 100.4 (5.2) for caryophyllene oxide; and 99.4 (5.0), 99.6 (8.0), and 99.2 (5.3) for α-humulene.

Because the interval between treatment application and feeding was approximately 10 min, the 10-, 20-, and 30-min estimates approximated the beginning, midpoint, and end of the 20-min test period. In addition, untreated alfalfa pellets were analyzed to determine the background terpene concentrations. None of the compounds examined was present above the detection limits in the untreated alfalfa pellets.

For each experiment, daily intake during the 20-min interval was analyzed as a repeated measure using the MIXED procedure (SAS Institute Inc., Cary, NC). The model included group as a fixed block effect, treatment (terpene concentration), day, and day × treatment interaction. A first-order autoregressive, AR(1), covariance structure was used for the repeated measurements on individual animals across days. Linear and quadratic models were fit to intake across concentrations averaged over days, and across days averaged over concentration when the main effect of treatment or day was significant. Data from d 1 of Exp. 2 (*cis*-sabinene hydrate) were removed from the analysis because the lambs were inadvertently fed before the 20-min tests.

RESULTS AND DISCUSSION

Day × treatment interactions were detected for Exp. 1 ($P < 0.003$) and Exp. 4 ($P < 0.04$). Interactions were due to greater intake on d 1 for 0× than for the other treatments in Exp. 1 (*cis*-β-ocimene) and to lower intake for the 10× than for the other treatments on d 1 and 2 in Exp. 4 (monoterpene mixture; Figure 1). This pattern suggests the possibility of an initial adaptation to the taste of terpenes in these 2 experiments, although adaptation to odor cannot be ruled out, given that odor and taste both affect the preference of sheep and their effects are difficult to separate (Arnold et al., 1980). A day effect on intake ($P < 0.05$; both linear and quadratic contrasts) was detected in all 4 experiments, apparently because of the generally low intake at all concentrations (including controls; Figure 1) on d 1. This pattern of lower intake during the first day or 2 (regardless of treatment concentration) has been observed in 15 of 23 previous studies (Estell et al., 1998b, 2000, 2002, 2005, 2007). Neither individual nor mixtures of terpenes appeared to exert postingestive feedback effects, given the lack of day × treatment interactions in Exp. 2 and 3 and the nature of those interactions in Exp. 1 and 4 (treatment differences occurred on d 1). Intake of lambs consuming terpene-treated alfalfa pellets did not decrease over time in any experiment (Figure 1), in contrast to expectations if negative feedback was observed.

Neither *cis*-β-ocimene nor *cis*-sabinene hydrate (Exp. 1 and 2, respectively) affected intake of alfalfa pellets in this study (Table 1). Both compounds occur in low concentrations in tarbush; consequently, treatment levels used were low. Individual terpenes have been negatively related to intake of sagebrush (Bray

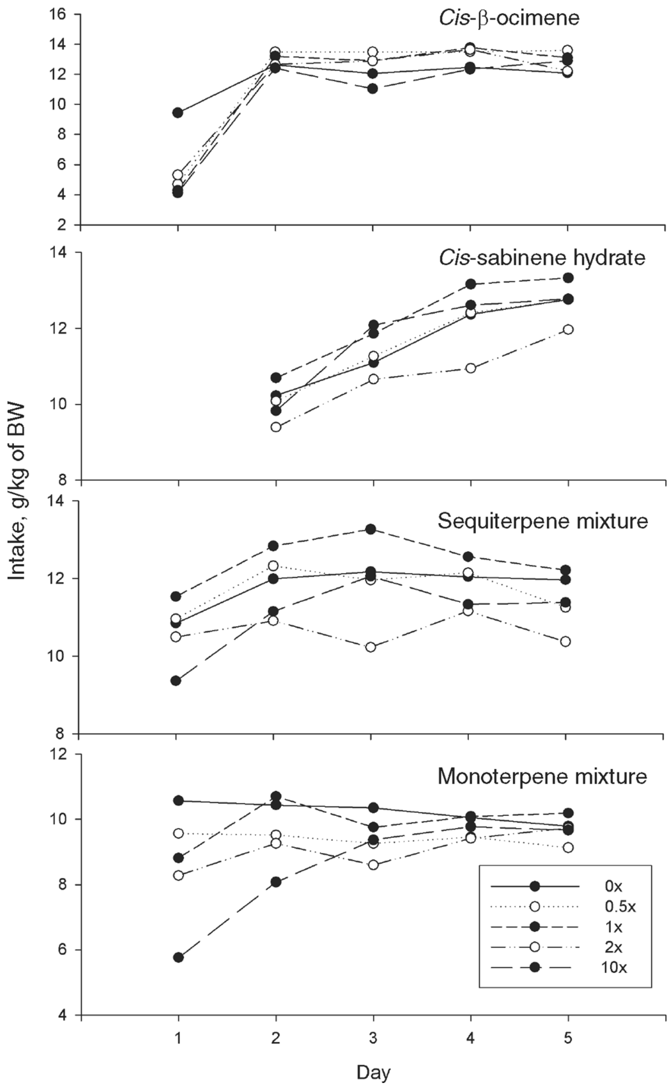

Figure 1. Mean DMI (g/kg of BW) of alfalfa pellets treated with individual or mixtures of terpenes by lambs during a 20-min interval for 5 consecutive days; n = 9 lambs/d for each concentration; SEM = 0.75, 0.60, 0.49, and 0.56 for *cis*-β-ocimene, *cis*-sabinene hydrate, sesquiterpene mixture (β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene), and monoterpene mixture (borneol, camphene, camphor, 1,8-cineole, limonene, myrcene, and α-pinene), respectively. Treatments (0×, 0.5×, 1×, 2×, and 10×) are multiples of the concentration of the specific compounds in tarbush. Linear and quadratic day effects ($P < 0.05$) pooled across treatments were observed for all 4 compounds. Day × treatment interactions ($P < 0.04$) were detected in Exp. 1 and 4.

et al., 1991), juniper (Riddle et al., 1996), tarbush (Estell et al., 1998a), and western red cedar (Vourc'h et al., 2002) by ruminants, but to our knowledge, neither *cis*-β-ocimene nor *cis*-sabinene hydrate have been examined previously with respect to mammalian herbivory.

Only 4 of the 25 individual volatile compounds examined (including *cis*-β-ocimene and *cis*-sabinene hydrate) affected intake of alfalfa pellets by sheep at levels present in tarbush. We hypothesized that the lack of intake response to specific compounds may be due to their cumulative or synergistic effects, or both. Total terpene concentration has been reported to be negatively related to intake by ruminants in other studies (Schwartz et al., 1980; Riddle et al., 1996). Ruminants browsing tarbush exhibited differential selectivity among plants that was related to the quantity of epicuticular wax on the leaf surface (Estell et al., 1994), and subgroups of terpenes on the leaf surface were identified that could distinguish among plants in high-, moderate-, and low-use categories as determined by livestock use (Estell et al., 1996a, 1998a). Moreover, the total essential oil yield from tarbush (approximately 1% of DM; Tellez et al., 1997) was substantially greater than the 10× treatment concentration for any chemical examined previously.

Effects of terpene mixtures on intake were examined in Exp. 3 and 4. A trend for a treatment effect on intake (g/kg of BW) was observed for the sesquiterpene mixture (Exp. 3; linear contrast; $P = 0.0926$), but no treatment effect was detected for the monoterpene mixture (Exp. 4; Table 1). This finding is particularly interesting because the sesquiterpene mixture contained fewer compounds in substantially lower concentrations than the monoterpene mixture. As mentioned earlier, the mixtures contained compounds present in tarbush that were related to intake in earlier studies or that are present in the greatest amounts on the leaf surface of tarbush, or both. Because multiple criteria were used to construct the mixtures, compounds were added that were not important in the subsets based on multivariate analysis (myrcene, camphene, and 1,8-cineole), or that did not produce an effect on intake when applied individually to alfalfa pellets (borneol, limonene, 1,8-cineole, and myrcene). The subset of variables that best distinguished among high-, moderate-, and low-defoliated plants contained limonene, camphor, borneol, α-copaene, β-caryophyllene, α-pinene, and caryophyllene oxide (Estell et al., 1996a), whereas the set that best distinguished between high- and low-use categories contained α-pinene, limonene, camphor, borneol, β-caryophyllene, and α-humulene (Estell et al., 1998a). Some of the compounds present in the largest amounts in tarbush are not commercially available or are prohibitively expensive and unfortunately could not be examined (e.g., artemisia alcohol, flourensadiol, β-eudesmol). In particular, flourensadiol concentration was greater on leaves of low-use than high-use plants (Estell et al., 1998a). With the exception of artemisia alcohol in the ethanol fraction, all 3 of these compounds were present in crude extracts (hexane, ether, and ethanol) that averted intake when applied to sheep diets (Estell et al., 2001; Tellez et al., 2001).

Although we hypothesized the sesquiterpene mixture would exhibit the strongest antiherbivory response, it is somewhat surprising that the monoter-

Table 1. Mean daily consumption (g/kg BW, DM basis, ± SEM) of alfalfa pellets treated with individual or mixtures of terpenes by lambs during a 20-min interval

Concentration[1]	Exp. 1[2]	Exp. 2[2]	Exp. 3[2,3]	Exp. 4[2]
0×	11.7 (0.75)	11.6 (0.60)	11.8 (0.49)	10.2 (0.56)
0.5×	11.7 (0.75)	11.6 (0.60)	11.7 (0.49)	9.4 (0.56)
1×	11.5 (0.75)	12.3 (0.60)	12.5 (0.49)	9.9 (0.56)
2×	11.3 (0.75)	10.7 (0.60)	10.6 (0.49)	9.0 (0.56)
10×	10.6 (0.75)	11.8 (0.60)	11.1 (0.49)	8.5 (0.56)

[1]Concentrations of volatile chemicals applied to alfalfa pellets were multiples (0×, 0.5×, 1×, 2×, or 10×) of the concentration of that compound in tarbush (1×); n = 9 lambs/concentration. *Cis*-β-ocimene, *cis*-sabinene hydrate, a mixture of sesquiterpenes (containing β-caryophyllene, caryophyllene oxide, α-copaene, and α-humulene), and a mixture of monoterpenes (containing borneol, camphene, camphor, 1,8-cineole, limonene, myrcene, and α-pinene) were examined in Exp. 1, 2, 3, and 4, respectively.

[2]Day × treatment interactions ($P < 0.04$) occurred in Exp. 1 and 4. A linear and quadratic day effect was observed in all 4 experiments ($P < 0.05$).

[3]A concentration effect ($P = 0.093$) was detected for Exp. 3.

pene mixture did not affect intake, given that 3 of the components of the mixture affected intake in previous studies when applied individually at the same concentrations (Estell et al., 1998b, 2002). Although no significant treatment effect was detected, it is noteworthy that intake decreased numerically in a linear fashion as treatment concentration increased for the monoterpene mixture (Exp. 4; Table 1). Even though a decrease of nearly 20% was observed, the high variability associated with the treatment means overshadowed any statistically detectable reduction in intake. Schwartz et al. (1980) fed mule deer pelleted diets containing various volatile oil fractions from juniper and found the lowest preference for pellets containing the oxygenated monoterpene fraction, intermediate for the hydrocarbon monoterpene fraction, and highest for the sesquiterpene fraction. However, concentrations applied were greater and the profiles for each fraction were more complex in that study. Although we did not differentiate between oxygenated and hydrocarbon monoterpenes, there were 3 oxygenated compounds (borneol, camphor, and 1,8-cineole) in the mixture.

Very few other data using terpene mixtures are available for comparison, and no other data on sesquiterpene mixtures exist to our knowledge. A few studies have examined the role of terpenes in diet selection by using plant fractions or mixtures of individual monoterpenes to simulate a partial profile of the volatile fraction of a particular shrub based on the predominant components at concentrations comparable to those found in the plant. Narjisse et al. (1997) examined the effects of the taste and odor of a monoterpene mixture similar to that of sagebrush (7 monoterpenes, including α-pinene, camphene, 1,8-cineole, and camphor) on preference for alfalfa pellets and found that sheep and goats appeared to discriminate against the mixture's odor and taste, respectively. Villalba et al. (2002) showed that a mixture of 6 monoterpenes at concentrations found in sagebrush (including 1,8-cineole, α-pinene, camphor, and camphene) depressed intake of high-energy diets by lambs. Similarly, Villalba and Provenza (2005) reported adverse effects on intake by lambs when fed a mixture of 4 terpenes at concentrations found in sagebrush (including 1,8-cineole and camphor).

Given the lack of a consistent response to the monoterpene mixture, cumulative or synergistic effects do not appear to explain the lack of effect of most individual compounds observed in previous studies. When applied individually, only 1 sesquiterpene (caryophyllene oxide) in the sesquiterpene mixture was related to intake previously (Estell et al., 2002). It is possible the trend for a treatment effect noted for the sesquiterpene treatment was due entirely to the presence of caryophyllene oxide in the mixture. For the compounds related to intake in previous studies with multivariate analyses (Estell et al., 1996a, 1998a), other explanations for inconsistencies are possible. For example, 1,8-cineole and limonene are both correlated strongly with various formylated phloroglucinol compounds in eucalyptus (Moore et al., 2004). Thus, these compounds may not be driving intake, but rather may simply be correlated with other compounds or classes of compounds that affect intake. In cases in which individual compounds have been applied to alfalfa pellets, the lack of effect is probably not related to content of other secondary compounds. Some of the compounds used in each mixture were unrelated to intake of alfalfa pellets by lambs when applied individually (1,8-cineole, borneol, myrcene, limonene, β-caryophyllene, α-copaene, and α-humulene) at concentrations present in tarbush.

Many of the individual components of the mixtures have been associated with intake or preference of various mammalian species (primarily those in the monoterpene mixture; very little work with sesquiterpenes is available). For example, cineole (Reichardt et al., 1985) and camphor (Sinclair et al., 1988) were negatively related to feeding by hares, and Bucyanayandi et al. (1990) observed less bark damage by voles on conifer species containing myrcene and limonene. Asaro et al. (2003) reported that fox squirrels avoided pine cones containing higher concentrations of myrcene, β-caryophyllene, and α-humulene, and Epple et al. (1996) reported that pocket gophers ate less in areas containing a commercial pine needle oil containing α-pinene and myrcene. Vourc'h et al. (2002) reported α-pinene, myrcene, and limonene to be repellent to deer, and Riddle et al. (1996), who examined correlations between terpenes and juniper intake by goats, found myrcene, α-pinene, and limonene to be among the terpenes negatively related to juniper consumption by goats. Bray et al. (1991) found 1,8-cineole to be repellent to deer at concentrations present in sagebrush. In addition, red deer exposed to the odor of α-pinene, borneol, or limonene rejected a pelleted diet (Elliott and Loudon, 1987). In other cases, specific terpenes in the monoterpene mixture were unrelated (or even positively related) to intake or preference in other studies. For example, Reichardt et al. (1985) found no relationship between α-pinene and hare preference, and Nolte et al. (1994) reported no relationship of limonene to guinea pig feeding preference. In addition, Riddle et al. (1996) reported camphene and camphor to be among the terpenes positively related to juniper intake by goats. These studies are not directly comparable because of differences in concentrations and combinations of chemicals, diet composition or plant species, intake, plane of nutrition, presence of other secondary compounds or classes of compounds, and physiological differences among animal species (e.g., detoxification mechanisms, pregastric microbial activity). Many studies indicating that terpenes are related to dietary preferences or intake examined animals on a low plane of nutrition, whereas secondary compounds are generally less aversive when animals are fed a high-quality diet (Villalba and Provenza, 2005), as in the present study.

Understanding the chemical mediation of livestock herbivory in complex foraging environments will provide insights into mechanisms for using shrubs as forage and possibly for shifting desert scrub to grasslands, thereby enhancing the long-term productivity of arid and semiarid environments. The 2 individual terpenes tested in this study were not related to intake of alfalfa pellets by lambs at the concentrations examined. A monoterpene mixture representative of the major components of the tarbush profile was also unrelated to intake; however, the sesquiterpene mixture tended to be related to intake. Thus, sesquiterpenes may exert antiherbivory properties under certain conditions.

LITERATURE CITED

Arnold, G. W., E. S. de Boer, and C. A. P. Boundy. 1980. The influence of odour and taste on food preferences and food intake of sheep. Aust. J. Agric. Res. 31:571–587.

Asaro, C., S. C. Loeb, and J. L. Hanula. 2003. Cone consumption by southeastern fox squirrels: A potential basis for clonal preferences in a loblolly and slash pine seed orchard. For. Ecol. Manage. 186:185–195.

Bray, R. O., C. L. Wambolt, and R. G. Kelsey. 1991. Influence of sagebrush terpenoids on mule deer preference. J. Chem. Ecol. 11:2053–2062.

Bucyanayandi, J. D., J. M. Bergeron, and H. Menard. 1990. Preference of meadow voles (*Microtus pennsylvanicus*) for conifer seedlings: Chemical components and nutritional quality of bark of damaged and undamaged trees. J. Chem. Ecol. 16:2569–2579.

Elliott, S., and A. Loudon. 1987. Effects of monoterpene odors on food selection by red deer calves (*Cervus elaphus*). J. Chem. Ecol. 13:1343–1349.

Epple, G., H. Niblick, S. Lewis, D. L. Nolte, D. L. Campbell, and J. R. Mason. 1996. Pine needle oil causes avoidance behaviors in pocket gopher *Geomys bursarius*. J. Chem. Ecol. 22:1013–1025.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 1996a. Tarbush leaf surface terpene profile in relation to mammalian herbivory. Pages 223–241 in Proc. Shrubland Ecosystem Dynamics in a Changing Climate, Gen. Tech. Rep. INT-GTR-338. USDA, Forest Service, Intermountain Res. Stn., Ogden, UT.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 1998a. Relationship of leaf surface terpene profile of tarbush with livestock herbivory. J. Chem. Ecol. 24:1–12.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 2000. Effects of individual terpenes on consumption of alfalfa pellets by sheep. J. Anim. Sci. 78:1636–1640.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 2002. Effects of four mono- and sesquiterpenes on the consumption of alfalfa pellets by sheep. J. Anim. Sci. 80:3301–3306.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, W. F. Mueller, and M. D. Remmenga. 1994. Relationship of tarbush leaf surface secondary chemistry to livestock herbivory. J. Range Manage. 47:424–428.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, and M. D. Remmenga. 2005. Effects of γ-terpinene, terpinolene, α-copaene, and α-terpinene on consumption of alfalfa pellets by sheep. J. Anim. Sci. 83:1967–1971.

Estell, R. E., E. L. Fredrickson, D. M. Anderson, and M. D. Remmenga. 2007. Effects of eugenol, α-terpineol, terpin-4-ol, and methyl eugenol on consumption of alfalfa pellets by sheep. Small Rumin. Res. 73:272–276.

Estell, R. E., E. L. Fredrickson, and K. M. Havstad. 1996b. Chemical composition of *Flourensia cernua* at four growth stages. Grass Forage Sci. 51:434–441.

Estell, R. E., E. L. Fredrickson, M. R. Tellez, K. M. Havstad, W. L. Shupe, D. M. Anderson, and M. D. Remmenga. 1998b. Effect of volatile compounds on consumption of alfalfa pellets by sheep. J. Anim. Sci. 76:228–233.

Estell, R. E., M. R. Tellez, E. L. Fredrickson, D. M. Anderson, K. M. Havstad, and M. D. Remmenga. 2001. Extracts of *Flourensia cernua* reduce consumption of alfalfa pellets by sheep. J. Chem. Ecol. 27:2275–2285.

Marsh, K. J., I. R. Wallis, R. L. Andrew, and W. J. Foley. 2006. The detoxification limitation hypothesis: Where did it come from and where is it going? J. Chem. Ecol. 32:1247–1266.

Moore, B. D., I. R. Wallis, J. P. Paul, J. J. Brophy, R. H. Willis, and W. J. Foley. 2004. Antiherbivore chemistry of *Eucalyptus*—Cues and deterrents for marsupial folivores. J. Chem. Ecol. 30:1743–1769.

Narjisse, H., J. C. Malechek, and J. D. Olsen. 1997. Influence of odor and taste of monoterpenoids on food selection by anosmic and intact sheep and goats. Small Rumin. Res. 23:109–115.

Nolte, D. L., J. R. Mason, and S. L. Lewis. 1994. Tolerance of bitter compounds by an herbivore, *Cavia porcellus*. J. Chem. Ecol. 20:303–308.

Pass, G. J., and W. J. Foley. 2000. Plant secondary metabolites as mammalian feeding deterrents: Separating the effects of taste of salicin from its post-ingestive consequences in the common brushtail possum (*Trichosurus vulpecula*). J. Comp. Physiol. B 170:185–192.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage. 48:2–17.

Reichardt, P. B., T. P. Clausen, and J. P. Bryant. 1985. Plant secondary metabolites as feeding deterrents to vertebrate herbivores. Pages 37–42 in Symp. Plant-Herbivore Interactions, Intermountain Res. Stn., Forest Service, USDA, Ogden, UT.

Riddle, R. R., C. A. Taylor Jr., M. M. Kothmann, and J. E. Huston. 1996. Volatile oil contents of ashe and redberry juniper and its relationship to preference by Angora and Spanish goats. J. Range Manage. 49:35–41.

Schwartz, C. C., W. L. Regelin, and J. G. Nagy. 1980. Deer preference for juniper forage and volatile oil treated foods. J. Wildl. Manage. 44:114–120.

Sinclair, A. R. E., M. K. Jogia, and R. J. Andersen. 1988. Camphor from juvenile white spruce as an antifeedant for snowshoe hares. J. Chem. Ecol. 14:1505–1514.

Tellez, M. R., R. E. Estell, E. L. Fredrickson, and K. M. Havstad. 1997. Essential oil of *Flourensia cernua* DC. J. Essential Oil Res. 9:619–624.

Tellez, M. R., R. E. Estell, E. L. Fredrickson, J. Powell, D. E. Wedge, K. K. Schrader, and M. Kobaisy. 2001. Extracts of *Flourensia cernua* (L): Volatile constituents and antifungal, antialgal, and antitermite bioactivities. J. Chem. Ecol. 27:2263–2273.

Villalba, J. J., and F. D. Provenza. 2005. Foraging in chemically diverse environments: Energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. J. Chem. Ecol. 31:123–138.

Villalba, J. J., F. D. Provenza, and J. P. Bryant. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: Benefits or detriments for plants? Oikos 97:282–292.

Vourc'h, G., M. De Garine-Wichatitsky, A. Labbe, D. Rosolowski, J. L. Martin, and H. Fritz. 2002. Monoterpene effect on feeding choice by deer. J. Chem. Ecol. 28:2411–2427.