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Redberry Juniper Consumption Does Not Adversely Affect Reproduction of Meat Goats

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ABSTRACT

Goat browsing can slow the encroachment of juniper (*Juniperus pinchottii* and *Juniperus asheii*) onto rangelands, but potential detrimental effects of monoterpenoids on reproduction are unknown. We determined whether redberry juniper consumption by pregnant goats caused abortions or reduced offspring neonatal viability. Pregnant Boer-cross nannies (n = 19) were randomly divided into four treatments, three treatments fed redberry juniper 1 h daily for 22 d during one of the three trimesters and a control group fed alfalfa pellets throughout gestation at 2% BW to meet maintenance requirements. In a pasture trial, pregnant nannies (n = 20) were placed on juniper dominated rangeland throughout gestation; juniper preference was monitored once monthly via bite count surveys and fecal NIR analysis. In both trials, birth date and weight, offspring number, sex, and vigor scores were recorded at parturition. Kids were weighed on days 14 and 28 postpartum. No abortions occurred as a result of redberry juniper consumption and no differences ($P > 0.05$) were observed in offspring number, vigor scores, or weight. Predicted juniper in goat diets on pasture was similar. Producers can use goats as a management tool for slowing juniper encroachment onto rangelands without causing abortions or reducing neonatal viability.

KEY WORDS: abortifacient, aversive feedback, *Juniperus*, monoterpenes

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INTRODUCTION

Redberry (*Juniperus pinchotii* Sudw.) and ashe (*Juniperus asheii* Buch.) juniper continue to invade rangelands and become a problematic plant for ranchers in west central Texas (Ansley et al., 1995; Smeins et al., 1997). These two species of juniper were originally limited to rocky outcrops and steep canyons; however, with the suppression of wildfires, the density and location of juniper has increased. Several methods including the use of herbicides (spraying), prescribed burning, and mechanical removal (grubbing, chaining, root plowing) are utilized to aid in the management of these unwanted species (Stueter and Wright 1983; Ueckert et al., 1994; Johnson et al., 1999). However, with rising fuel, labor, and herbicide costs, many ranchers are looking for alternative management strategies. Preliminary evidence reveals that goats can slow the encroachment of juniper onto rangelands by browsing seedlings and immature plants (Taylor and Fuhlendorf 2003; Taylor 2004).

Monoterpenoids, a class of terpenes containing two isoprene units, found in juniper are known to cause aversive postingestive feedback (Riddle et al., 1996; Pritz et al., 1997) thereby limiting intake. During winter months when alternative forage availability and nutrient quality is limited, juniper consumption can range from 22-29% of the diet (Campbell et al., 2007a). Juniper intake and corresponding vegetation management of juniper can be further increased by preconditioning goats in a pen situation at weaning (Dietz et al., 2010).

Although extensive research has gone into conditioning goats to consume juniper (Bisson et al., 2001; Ellis et al., 2005; Dunson et al., 2007), little research has investigated the potential detrimental effects of juniper on goat reproduction. Evergreen species can inflict embryonic damage, resulting in abortion and birth defects (Panter et al., 1992). Ingestion of ponderosa pine (*Pinus ponderosa* Laws.) causes abortions in cattle due to acetyl isocupressic acid (ICA) which is converted to isocupressic acid in the rumen (Gardner et al., 1998). Trace levels (< 2 %) of ICA are present in lodgepole pine (*Pinus contorta* Dougl. ex. Loud.), common juniper (*Juniperus communis* L.), and Rocky Mountain juniper (*Juniperus scopulorum* Sarg.) (Gardner et al., 1998). Juniperus species have also been suspected to cause abortions in livestock. For instance, Johnson et al. (1976) discovered that feeding one-seeded juniper (*Juniperus osteosperma* Torr.) to sheep in the second and early third trimester caused abortions.

In west central Texas, concentrations of monoterpenoids in juniper are highest in the winter and spring (Owens et al., 1998); this timeframe directly coincides with the kidding season of most goat producers in west Texas. Because many producers use goat browsing to manage juniper, the potential effects on reproduction must be examined. Accordingly, this study determined if juniper consumption by pregnant goats caused abortions or reduced neonatal viability, and if so, in what trimester.

MATERIALS AND METHODS

Pen Feeding Trial. Forty-six female Boer-cross goats (25 yearling and 21 mixed age) with an average weight of 54.1 kg (± 4.9) were exposed to Boer billies for 3 mo for natural breeding. Conception dates were determined using ultrasonographic imagery (Classic Medical, Palm Scan PSM-3.5-S) at two week intervals throughout the breeding season.

Once pregnancy was confirmed, goats were allocated into treatments based on date of conception. Treatment 1 consisted of nannies fed redberry juniper in the first trimester of pregnancy; Treatment 2 consisted of nannies fed redberry juniper during the second trimester of pregnancy, and Treatment 3 consisted of nannies fed redberry juniper during the third trimester of pregnancy. Treatment 4 nannies served as the control and were not fed juniper anytime during pregnancy. At two week intervals throughout gestation, ultrasonographic imagery was used to monitor fetal development and health. Less than optimum body condition scores for pregnancy maintenance in yearling nannies and intense heat during breeding resulted in low conception rates and ultimately a variation in the number of goats per treatment.

In June 2006, redberry juniper leaves were stripped from plants located on the Texas Agrilife Research Center, Sonora, TX and stored at 4°C until feeding. During the first trimester, 10 randomly selected, pregnant Boer-cross female goats (Treatment 1) were placed in individual pens and fed redberry juniper at 0800 hours *ad libitum* for 1 h daily for 22 d at the Angelo State University Management, Instruction, and Research (MIR) Center, San Angelo, TX (31° N; 100° W). Individual pens (1 m X 1.5 m) were elevated with expanded metal floors to allow for removal of excreta. All excreta were removed from beneath pens at weekly intervals. Procedures in Treatments 2 and 3 were conducted exactly as Treatment 1 during the second and third trimesters, with 8 and 5 pregnant nannies, respectively. The remaining 5 pregnant nannies (Treatment 4) were fed only alfalfa pellets at 2% BW throughout the feeding trial.

Nannies in Treatments 1-3 were fed 50 g (as fed basis) of redberry juniper on Day 1 of the feeding trial, and amount of juniper fed was increased to ad libitum levels based on intake of individual goats throughout the remainder of feeding. At the conclusion of feeding each day, refusals were collected and weighed. To meet daily maintenance and pregnancy requirements, all goats were fed alfalfa pellets at 2% BW each day following juniper feeding and throughout the remainder of the feeding trial (NRC, 1981). Pregnancy was verified using ultrasonographic imagery at two week intervals throughout gestation. All goats were given free access to a calcium/phosphorous mineral supplement with trace minerals and fresh water throughout pregnancy.

At the conclusion of the feeding trial and just prior to parturition, all pregnant nannies (Treatments 1-4) were placed in kidding pens located on the Angelo State University MIR Center. In order to avoid pregnancy toxemia, the nannies were placed on a balanced ration based on NRC requirements for late-term pregnancy (Table 1). Each nanny was monitored daily, and at parturition, data including birth date, number of offspring, birth weight, and offspring sex was collected. Vigor scores were also assigned during the first 8 h of life based on the following scale: 0 = dead; 1 = extremely weak, no attempt to get up or stand; 2 = struggles to get up, delay in nursing; 3 = slow to get up, slow to nurse; 4 = strong but slow to nurse; and 5 = strong, nurses rapidly. Body weights of kids were collected again on days 14 and 28 postpartum.

Pasture Trial. Twenty-five mixed age Boer-cross female goats were exposed to Boer billies for three months for natural mating. At two week intervals throughout the breeding season, pregnancy rate was evaluated using ultrasonographic imagery to determine approximate conception dates. At the conclusion of breeding, 20 pregnant nannies were transported to the Texas Agrilife Research Center, Sonora, TX (30° N; 100° W) and placed in a 16.2 ha pasture on juniper dominated (20.3% canopy cover) rangeland for four months (Dietz et al., 2010). All goats were given free access to a calcium/phosphorous mineral supplement with trace minerals and fresh water during the grazing portion of the study.

To monitor juniper preference, monthly bite count surveys were conducted. Each goat was observed individually for a period of 10 min once a month during optimum grazing times in the afternoon. During this time, bite frequency and bite type was identified and recorded as either herbaceous, juniper, or other browse. Fecal samples were also obtained from each goat to evaluate predicted juniper percentage in diets using the fecal near infrared reflectance spectroscopy (NIRS) procedure described by Walker et al. (2007). During each monthly observation period, nannies were scanned using ultrasonographic imagery to ensure pregnancy.

After four months on pasture, and just prior to parturition, the nannies were transported back to the Angelo State University MIR Center for kidding. In order to avoid pregnancy toxemia, the nannies were placed on a balanced ration based on NRC requirements for late-term pregnancy (Table 1). Each nanny was monitored daily, and at parturition data including birth date, number of offspring, birth weight, and offspring sex was collected. Vigor scores were also assigned during the first 8 h of life based on the previously mentioned scale. Body weights of kids were collected again on days 14 and 28 postpartum.

To quantify that adequate herbaceous forage was available for goats throughout the trial, clip samples were obtained during each monthly observation period. Ten 1/3 m^2 quadrats, randomly located throughout the pasture, were clipped and bagged by herbaceous species; samples were dried in a forced-air oven at 60°C for 48 h and weighed to determine dry matter forage availability.

Table 1. Ingredients and nutritional value of balanced feed ration fed to pregnant nannies immediately prior to kidding in both experiments.

Ingredients	Percent (%) of Diet DM[a]
Alfalfa, Dehydrated	56.9
Cane Molasses	2.8
Corn	37.9
Premix[b]	2.4
Avg. Daily Feed Intake Per Head	1.4 kg
Digestible Energy	3.0 Mcal/kg
Total Digestible Nutrients	68.90%
Crude Protein	15.50%

[a]All percentages based on 1 ton (909.1 kg).

[b]Angelo State University Premix: Lasalocid 1158 g/ton, Calcium 19.0%, Salt 19.0%, Magnesium 1.4%, Zinc 2095 mg/kg, Manganese 1015 mg/kg, Iodine 0.02 mg/kg, Copper 1.0 mg/kg, Selenium 3.9 mg/kg, Vitamin A 18,364 IU/kg, Vitamin D3 13,400 IU, Vitamin E 280 IU.

In order to evaluate juniper monoterpene concentration and composition, 50 grams of leaf and small stem tissue was collected by hand clipping from the apical portion of sprouts from each tree and immediately placed in liquid nitrogen to halt physiological activity and prevent volatilization. Leaf tissue was then stored at -50°C. Samples were randomly collected from three redberry and three ashe plants during each observation period.

In August 2007, 15 g of previously frozen leaf material from each plant was individually combined with 150 ml of distilled water in a modified Clevenger (1928) type distillation apparatus. Hexane (5 ml) was used as a solvent for the distillate in the condensation tube, and the distillation time period was eight hours to ensure maximum recovery of monoterpenes (Owens et al. 1998; Campbell et al., 2007b). Five microliters of tetradecane were added to condensate as an internal standard.

The chromatographic system consisted of a Clarus 500 GC (Perkin Elmer, Shelton, CT) equipped with an FID. The analytical column was a 30 m x 0.25 mm Rtx-5, 0.25 um (Restek, Bellefonte, PA). One microliter splitless injections (split time of 0.1 min) were made under the following conditions: the injection temperature was 200°C and the detector was 300°C. The initial oven temperature of 40°C was held for 0.5 min. The first oven ramp took the oven to 110°C at a rate of 5°C/min (0-min hold time). The final ramp of 20°C/min took the oven to its final temperature of 300°C (0-min hold time). The total run time was 23 min. The carrier gas was helium delivered at a constant 39 cm/s by employing electronic pressure control. The detector gasses were hydrogen (45.0 ml/min), and air (450 ml/min). Analytical procedure followed a modification of the procedure described in Kimball et al. (2004).

Detector responses were evaluated for each analyte over the range of 0.25-1.0 ug/ml. For each compound three hexane solutions with concentrations in the range of interest were injected into the GC in triplicate. Linear regression analyses were conducted and external standard calibrations were used by comparing detector responses of the analytes from the sample extracts to responses from commercially available standards (Acros Organics, NJ).

Intake data from the pen feeding trial was analyzed using repeated measures analysis of variance with goats (replications) nested within treatments (trimesters) and day of collection. Differences between treatments for birth weight, 14 d weight, and 28 d weight were analyzed using analysis of variance. Number of offspring born per nanny and sex were included in the analysis to determine their influence on weights of kids. Vigor scores and the average number of births were compared among treatments using a Chi-square test. For the pasture trial, bite count data, NIRS estimates of forage preference, and forage availability were compared among sampling dates using analysis of variance. Mean birth weight, 14 d weight, 28 d weight, number of births, and vigor scores were not analyzed in the pasture trial because of a lack of a treatment effect. Means were separated using Least Significant Difference when $P \leq 0.05$ in both experiments. Data were analyzed using the statistical package JMP (SAS, 2007).

RESULTS AND DISCUSSION

Pen Feeding Trial. During the breeding portion of this trial, intense heat and drought-like conditions reduced conception rates in mature nannies. Also, yearling nannies obtained for this study just prior to breeding were not in optimum body condition for breeding and pregnancy maintenance. Both of these factors resulted in a variation in number of nannies per treatment in the pen feeding trial. Of the 46 nannies exposed to billies, 28 nannies became pregnant and were divided into 4 treatments for the feeding trial. A combination of heat stress and yearling nannies' inability to maintain pregnancy resulted in early termination of pregnancy in 5 nannies in Treatment 1 and 4 nannies in Treatment 2 prior to feeding juniper. Therefore, 19 of the 46 exposed nannies gave birth to 34 kids resulting in a 74% kidding percentage. Overall kidding percentage in both trials suffered due to environmental conditions and was not caused by juniper consumption.

Average daily juniper intake was similar (P = 0.09) among treatments during the pen-feeding phase of this study. Nannies in Treatment 1 consumed 1.1 ±0.3 g kg^{-1} of juniper per day. Treatment 2 nannies consumed 0.6 ±0.3 g kg^{-1} of juniper per day, and nannies in Treatment 3 consumed 0.5 ±0.4 g $kg^{-1}$of juniper per day. Juniper intake during Treatment 1 gradually increased through d 13 of feeding followed by a repeated pattern of decreased and increased intake throughout the remainder of the trial (Fig. 1). Goats in Treatments 2 and 3 maintained similar patterns of consumption through d 20 when Treatment 2 began to decrease intake and Treatment 3 increased juniper intake. No significant differences were observed in the treatment by day interaction. Goats can be preconditioned in a pen-fed situation to consume higher levels of juniper once released onto pasture (Dietz et al., 2010). Avoidance occurs because of monoterpenoids contained in juniper associated with aversive postingestive feedback (Riddle et al., 1996; Pritz et al. 1997). Goats in the pen feeding trial, although offered juniper for a longer period of time (22 d), tended to consume less juniper than goats in previous studies.

Monoterpenoid levels tend to be higher in the winter and spring (Owens et al. 1998). However, seasonal rainfall variations can effect monoterpenoid concentrations (Riddle et al. 1996). Gas chromatograph analysis of redberry juniper fed to goats revealed higher concentrations of monoterpenes inversely correlated to intake than that of a study conducted by Dietz et al. (2009). The severity of the drought-like conditions which occurred during the summer feeding trial may have played a role in elevating monoterpenoid levels in the juniper resulting in reduced overall intake. Also, r^2 values for intake may have been arbitrarily higher than Dietz et al. (2009) because of low sample size in this study.

Birth weights, 14 d weights, and 28 d weights were similar ($P \geq 0.05$) among treatments (Table 2). The number of single and multiple births were also similar among treatments (Table 3). Number of kids born per nanny had no apparent effect ($P > 0.05$) on birth weight, 14 d weight, or 28 d weight. Also, vigor scores were similar among treatments (Table 3).

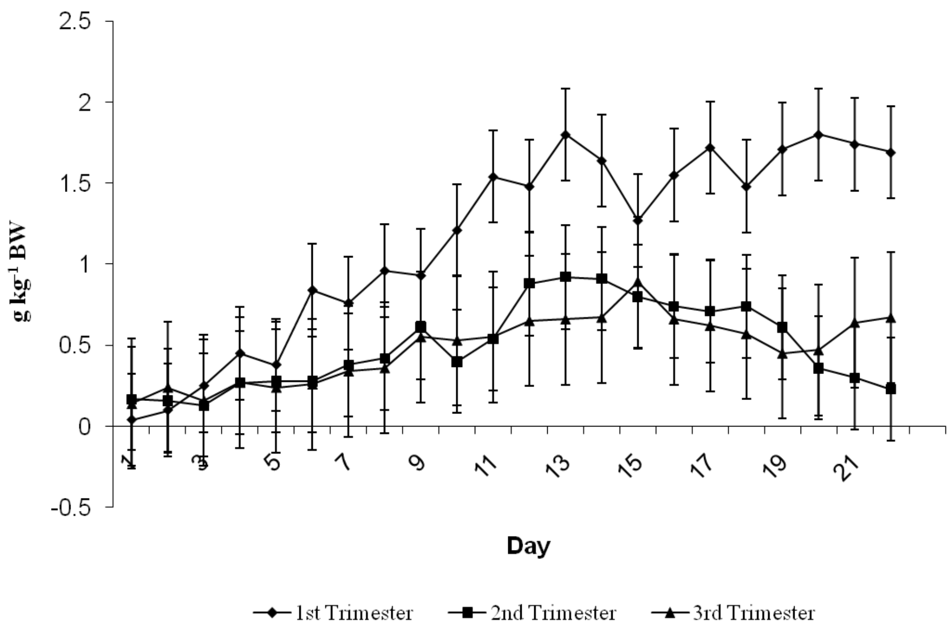

Figure 1. Redberry juniper intake for nannies throughout pregnancy.
*Average daily juniper intake for nannies fed redberry juniper *ad libitum* 1 hour daily for 22 d during each trimester of gestation in the pen feeding trial (P = 0.09).

Table 2. Birth weights (BW), 14 d weights (14W), and 28 d weights (28W) of all kids born to nannies in the pen feeding trial fed redberry juniper *ad libitum* 1 hour daily for 22 d during each trimester of gestation.

Trimester	Goats/Treatment	BW (kg)	SEM	14W (kg)	SEM	28W (kg)	SEM
1st	10	3.7	0.2	5.2	0.8	7.6	1.2
2nd	8	3.1	0.2	4.6	0.8	6.9	1.2
3rd	5	3.3	0.1	3.2	0.7	4.3	1.0
Control[a]	5	3.6	0.2	5.9	0.8	9.4	1.1

[a]Nannies in control received only alfalfa pellets at 2% BW throughout the feeding trial.

Pasture Trial. Goats were released onto pasture 20 July 2006, at a moderate stocking rate based on year-round grazing (1 animal unit year / 8.1 ha). Differences between goat forage preferences throughout gestation were observed and recorded (Table 4). During the August observation period, goats tended to prefer other shrubs (algerita, liveoak, persimmon, lotebush) over the other two forage classifications. Herbaceous forage was preferred during the September observation period, and juniper was the preferred forage class during the October observation. Herbaceous production during October increased as goat preference shifted from herbaceous forage to juniper.

Table 3. Number of kids born as singles, twins, or triplets and vigor scores of all nannies fed redberry juniper *ad libitum* 1 hour daily for 22 d during each trimester of gestation.

	Trimester			
Number born	1st	2nd	3rd	Control[a]
Single	0	0	1	1
Twins	6	4	0	10
Triplets	3	6	12	0
Vigor Score				
0	1	1	2	0
1	0	0	1	0
2	0	0	0	0
3	0	2	0	0
4	0	1	1	0
5	8	6	9	11

[a]Nannies in control received only alfalfa pellets at 2% BW throughout the feeding trial.

Table 4. Total herbaceous production (kg ha^{-1}) and preference of herbaceous vegetation, juniper, or other shrubs (percent of total bites) for pregnant nannies during 3 months of observation on juniper dominated rangeland.

	August	September	October	SEM
Herbaceous Production	1066.5	659.46	1459.2	309.04
Preference				
Herbaceous	6.8	53.7	33.9	1.2
Juniper	17.3	43.7	66.2	1.4
Other Shrubs	76.0	0.0	0.0	0.6

Average available herbaceous forage remained relatively constant throughout the trial with the exception of September where a decrease in kg ha^{-1} of forage produced was observed (Table 4). This decline may be a result of lack of significant rainfall along with greater preference for herbaceous forage over any other forage class during this time as noted during visual observation.

According to bite count estimates, juniper consumption steadily increased as the fall of the year approached. During gestation, monthly juniper samples were taken to evaluate monoterpene concentration and composition. Gas chromatograph analysis shows total monoterpene concentrations for both ashe and redberry juniper were highest during the July and August collection periods (Tables 5, 6). A significant difference was observed in sabinene/b-pinene, cymene, and terpeneol concentrations in ashe juniper and borneol, g terpinene, and terpinen4ol concentrations in redberry juniper over the four month collection period (Tables 5, 6). Juniper intake was inversely correlated to sabinene/b-pinene (r^2 = 0.96) concentrations in ashe juniper and a terpinolene (r^2 = 0.97), terpineol (r^2 = 0.99), and total monoterpene (r^2 = 0.96) concentrations in redberry juniper. Conversely, it was positively correlated to borneol (r^2 = 0.79) concentration in redberry juniper. Gas chromatograph analysis revealed lower levels of total monoterpene concentrations in ashe and redberry juniper during September and October. These results may explain increased juniper consumption and agree with the observation that lower monoterpene concentrations exist in juniper in west central Texas during the fall (Owens et al., 1998).

Fecal NIRS data obtained from samples collected revealed similar ($P > 0.05$) amounts of predicted juniper in goat diets between observation periods.

Table 5. Average monoterpene levels (mg oil g^{-1} leaf material) in ashe juniper collected during pasture trial.

Monoterpene	Month				
	July	August	September	October	SEM
alpha-pinene	0.20	0.17	0.14	0.14	0.03
Camphene	0.21	0.17	0.14	0.33	0.05
Sabinene/b-pinene	0.02	0.02	0.02	0.001	0.002
Myrcene	0.14	0.11	0.12	0.29	0.08
Cymene	0.13	0.17	0.10	0.07	0.02
Limonene	0.91	0.95	0.72	0.69	0.19
g terpinene	0.08	0.07	0.08	0.09	0.03
a terpinolene	0.07	0.07	0.07	0.07	0.02
Linalool	0.01	0.01	0.01	0.02	0.006
Fenchyl Alcohol	0.05	0.09	0.04	0.05	0.02
Camphor	5.32	5.86	4.73	3.67	0.85
a-citronellol	0.20	0.20	0.35	0.11	0.08
Borneol	0.40	0.46	0.25	0.12	0.09
Terpinen4ol	0.01	0.02	0.01	0.02	0.003
Terpineol	0.01	0.01	0.02	0.05	0.002
Carvone	0.06	0.07	0.05	0.03	0.01
Bornyl Acetate	1.88	1.70	1.64	1.24	0.36
Total	10.12	10.63	8.83	7.33	1.64

Twenty-five nannies were exposed to billies and 18 nannies gave birth to 34 kids resulting in a 136% kidding percentage. Data collected on birth weights, 14 d weights, and 28 d weights of all kids born to nannies in the pasture trial did not reveal any apparent differences. All weights were similar to those weights of kids in the pen feeding trial through the duration of the trial.

Based on the results from this study, monoterpenoids found in redberry and ashe juniper do not appear to have a negative effect on goat reproduction. No differences in neonatal vigor scores were observed in kids from any treatment group in either trial. Likewise, all kids from both trials, no matter how many siblings, maintained similar growth patterns based on birth, 14 d, and 28 d weights throughout the duration of the study. These results indicate that juniper intake does not inhibit offspring growth and development. Unlike results from Johnson et al. (1976) where abortions occurred in sheep fed juniper (1 lb of plant per day) via stomach pump in the second and third trimesters, no incidences of abortions or teratogenic effects (birth defects) were observed in this study. Goats may metabolize monoterpenes found in juniper better than sheep and are therefore able to avoid toxin-induced abortions.

Table 6. Average monoterpene levels (mg oil g^{-1} leaf material) in redberry juniper collected during pasture trial.

Monoterpene	Month				
	July	August	September	October	SEM
alpha-pinene	0.40	0.37	0.31	0.32	0.06
Camphene	0.08	0.10	0.09	0.11	0.02
Sabinene/b-pinene	3.98	3.32	2.77	2.80	0.70
Myrcene	1.00	0.95	0.76	0.81	0.14
Cymene	0.01	0.01	0.01	0.01	0.002
Limonene	0.92	0.98	0.79	0.74	0.13
g terpinene	1.08	0.95	0.75	0.62	0.09
a terpinolene	0.44	0.39	0.31	0.29	0.04
Linalool	0.04	0.03	0.03	0.04	0.01
Fenchyl Alcohol	0.03	0.03	0.03	0.03	0.0006
Camphor	1.24	1.35	1.29	1.21	0.23
a-citronellol	0.07	0.12	0.14	0.07	0.05
Borneol	0.01	0.03	0.03	0.05	0.005
Terpinen4ol	2.77	2.39	1.91	1.53	0.24
Terpineol	0.12	0.11	0.09	0.08	0.008
Citronellol	0.09	0.08	0.09	0.01	0.06
Bornyl Acetate	0.31	0.28	0.29	0.56	0.20
Total	12.58	11.48	9.70	9.26	1.51

CONCLUSION

Collectively, results of previous studies along with the results of this study indicate that goats should continue to be used as a cost-effective method of juniper management (Taylor and Fuhlendorf, 2003; Ellis et al., 2005; Dietz et al., 2010). Producers can implement strategies to increase juniper consumption by preconditioning yearling replacement nannies for 14 d at weaning along with concentrating the main breeding herd in juniper-dominated pastures without fear of a reduction in kidding percentage or lighter kid weaning weights.

Monoterpenoid concentrations in juniper are typically highest during the early spring and winter when most browse and herbaceous plants are dormant. This timeframe directly coincides with the breeding seasons of most goat ranchers in west central Texas. Concentrating goats into juniper-dominated pastures during this time period will increase browsing pressure on these plants and optimize potential management of juniper.

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