Research Note

Monoterpene Production in Redberry Juniper Foliage Following Fire

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Abstract

Prescribed fire is commonly used to initiate redberry juniper (*Juniperus pinchotti* Sudw.) suppression, and herbivory by goats presents a potentially effective mechanism to prolong the efficacy of the reclamation treatment. Monoterpenes in redberry juniper leaves serve as a barrier to effective herbivory, but fire has the potential to reduce this barrier through reversion of aboveground growth to juvenile tissue. Traditional optimal defense theory predicts that because of the assumed fitness value of vegetative regrowth, plant secondary chemicals will be higher in this tissue than mature growth. This study was designed to measure the monoterpene concentration and composition from redberry juniper foliage sampled from 3 different ages of plant tissue. Prescribed fire was used to create 3- and 11-month regrowth juniper foliage, and mature growth of juniper was sampled as a control. Total monoterpene levels were lowest in the 3-month regrowth ($P = 0.018$). Monoterpene concentration and composition was similar for the 11-month and mature foliage. Concentration of terpinen-4-ol ($P = 0.001$) and alpha-terpineol ($P = 0.007$), identified as particularly aversive monoterpenoids to goats, was lowest in the 3-month regrowth but increased to levels found in mature leaves by 11 months of age. There was a trend in changes in composition of total oil as relative concentrations of monoterpene hydrocarbons (α-pinene, β-pinene/sabinene) decreased and monoterpene alcohols and oxygenated monoterpenes increased. These results identify a short period of time following a burn during which monoterpene levels in regrowth are low. This suggests a period of vulnerability in plant biochemical defenses that has the potential to be utilized by strategic herbivory by goats for more effective juniper management.

Resumen

El fuego prescrito es comunmente usado para iniciar la supresión del “redberry juniper” (*Juniperus pinchotti* Sudw.) y la herbivoría de las cabras se presenta como un mecanismo potencialmente efectivo para prolongar la eficacia del tratamiento de mejoramiento. Los monoterpenos de las hojas del “redberry juniper” sirven como una barrera a la herbivoría, pero el fuego tiene el potencial de reducir esta barrera a travès de la reversión del crecimiento aéreo a tejido juvenil. La téoria tradicional de defensa óptima predice que debido al valor de aptitud asumido en el rebrote vegetativo, los compuestos químicos secundarios serán más altos en este tejido que en el maduro. Este estudio se diseño para medir la concentración y composición de monoterpenos del follaje del “redberry juniper” en tres edades del tejido de la planta. El fuego prescrito se utilizó para crear follaje de reborte de “redberry juniper” de 3 y 11 meses de edad, mientras que el follaje maduro fue muestrado como tratamiento control. Los niveles totales más bajos de monoterpenos se presentaron en el rebrote de 3 meses ($P = 0.018$). La concentración y composición de monoterpenos fue similar en el rebrote de 11 meses y el follaje maduro. La concentracion más baja de terpineno-4-ol ($P = 0.001$) y el alfa-terpineol ($P = 0.007$), identificados como monoterpenoides particularmente aversivos para las cabras, se presentó en el rebrote de 3 meses, pero a los 11 meses de edad del rebrote se incrementó a los niveles encontrados en el follaje maduro. Hubo una tendencia en los cambios de la composición del aceite total porque la concentración de hidrocarburos monoterpénicos (α-pineno, β-pineno/sabineno) decreció y la de alcohol monoterpenoides y monoterpenoides oxigenados se incrementó. Estos resultados identifican un periodo corto de tiempo después de la quema en el cual los niveles de monoterpenos del rebrote son bajos. Esto sugiere un periodo de vulnerabilidad en las defensas bioquímicas de la planta que tiene el potencial para ser utilizado con herbivoría estrátegica con cabras para un manejo más efectivo del “Redberry juniper.”

Key Words: *Juniperus pinchotti*, terpenes, glands, prescribed burning, goats

INTRODUCTION

Juniper expansion in the western and southwestern United States has become a deleterious occurrence that threatens other vegetation ecosystems through a steady encroachment and ultimate domination (Ansley and Rasmussen 2005). The invasion and increase of redberry juniper (*Juniperus pinchotii* Sudw.) into areas previously dominated by herbaceous vegetation has been a problem in west Texas since the development of the livestock industry. Redberry juniper infestations increased by about 60% (1.5 million ha) during the period 1948–1982 in a 65-county region in northwestern Texas (Ansley et al. 1995). The factors that have contributed to the increased juniper include fire suppression, climate change, and overgrazing by livestock (Smeins 1983; Miller and Tausch 2001; Ansley and Rasmussen 2005).

Redberry juniper suppression with the purpose of increasing herbaceous production is a goal of many resource managers; however, juniper management is usually expensive and economic feasibility can be variable for most techniques (Whitson et al.

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Manuscript received 12 December 2004; manuscript accepted 14 October 2006.

1984). Suppression and management of juniper-dominated rangelands requires an understanding of the causes of the problem as well as the implementation of cost-effective methods to shift the vegetative complex in a desired successional direction.

Properly implemented, prescribed fire has the potential to be an effective low-cost sustainable control method (Steuter and Britton 1983; Ueckert et al. 2001). The successful application of fire can easily kill ashe juniper (*Juniperus asheii* Buchholz*);* however, redberry juniper has a belowground crown that resprouts following top-kill of the plant. An analysis of sustainability of juniper control indicates that conversion of juniper woodlands back to grasslands will not only require initial reclamation treatments, but also maintenance control practices (Dye et al. 1995; Ueckert et al. 2001).

Herbivory by goats can potentially fill this niche and positively impact plant communities through selectively targeting seedling recruitment (Taylor and Fuhlendorf 2003; Taylor 2004). Juniper intake by goats is limited, however, by the presence of monoterpenes (Pritz et al. 1997; Riddle et al. 1999). Monoterpenes are chemicals synthesized via the mevalonic acid pathway in plants with a clearly defined ecological defensive role as feeding deterrents in a variety of mammals and insects (Gershenzon and Croteau 1991). Negative postingestive consequences experienced by large ungulates following consumption of high levels of monoterpenes include rumen microbial inhibition (Oh et al. 1967; Schwartz et al. 1980), hepatic pathogenesis (Pritz 1997; Bisson 2001; Straka 2004), and feeding cessation (Dziba et al. 2006). To overcome this barrier to effective juniper management with goats, our efforts are directed toward identifying a physiological state in the target plant where concentration of the aversive monoterpenes is lowest and subsequent susceptibility to herbivory is highest.

Traditional theories on plant resource allocation (e.g., the carbon–nutrient balance) predict that redberry juniper regrowth, as a rapidly growing disturbance-adapted woody plant, will exhibit a well-developed juvenile resistance syndrome (Bryant and Kuropat 1980). Recently, however, these nonadaptive, supply-side theories have come under fire (Hamilton et al. 2001), predicating the need for a re-evaluation of adaptive theories of chemical defense such as optimal defense theory (Foley and Moore 2005). In order to avoid group generalizations regarding plant secondary metabolites and the effect of herbivory on plant fitness (Foley and Moore 2005) an evaluation of species-specific patterns of monoterpene production following fire is required. Thus, the objective of this study was to determine monoterpenoid composition and concentration in 3-month, 11-month, and mature redberry juniper foliage. The null hypothesis was that monoterpene levels in redberry juniper regrowth from trees sampled 3 months and 11 months following top-kill with fire would be similar to monoterpene levels in mature unburned trees.

MATERIALS AND METHODS

Study Site

The study was conducted on the Texas A&M University Agricultural Experiment Station (TAES) located 45 km southeast of Sonora, Texas (lat 31.14°N; long 100.19°W). The 1402-ha research station was established in 1916 in Sutton and Edwards counties within the Edwards Plateau (Hatch et al. 1990) as a setting for sheep and goat research. The elevation of the research station is about 632 m. The region is dominated by rangelands composed of mixtures of grasses, forbs, and woody species. The vegetation is a mosaic of juniper and oak mottes interspaced with mid- and shortgrasses. For a complete description of the climate, soils, and vegetation at the research station see Smeins et al. (1976).

Experimental Design

Juniper samples were collected in December 1993 from a 63-ha pasture that had received 2 respective burn treatments. One 5-ha site was burned in January 1993, the other 5-ha site in September 1993. Juniper samples were collected in the same pasture and on the same date to reduce variability due to site differences and seasonal variation (Riddle et al. 1996). Samples were randomly collected from 0800 to 0900 hours by hand-clipping nonburned plants, 3-month-old resprouts, and 11-month regrowth. Regrowth collection was scheduled during December in order to minimize any metabolic variability, which increases during periods of active growth in the spring and summer (Adams 1970). The control treatment, representing mature growth of foliage, was randomly sampled from nonburned trees in the same pasture. Ten trees per treatment were sampled for the control and 11-month regrowth. Only 5 trees were sampled for the 3-month regrowth, because they were the only trees that had an adequate amount of leaf material to perform the chemical analyses.

Fifty 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 in order to halt leaf-tissue metabolism and monoterpene volatilization.

In December 1993 15 g of 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 8 h to ensure maximum recovery of monoterpenes (Owens et al. 1998). Five microliters of tetradecane were added to condensate as an internal standard.

Aliquots (1.2 μL) were analyzed for monoterpene composition and concentration with the use of an HP5790 gas chromatograph equipped with a splitless capillary injection port, HP-1 column (25 m × 0.32 mm × 0.52 μm film thickness), and flame ionization detector (FID). Helium carrier gas was maintained at a flow rate of 6.5 $mL \cdot min^{-1}$, injection port temperature was 280°C, and the detector temperature was 280°C. Monoterpene separation was achieved with the following temperature and time program: initial temperature of 40° for 2 minutes, a 7°C per-minute increase to 150°C and then a 15°C per-minute increase to 280°C. Monoterpenes were identified by comparison to known retention times of commercially available standards (Sigma-Aldrich) and peak areas measured with an HP3394 integrator. Sabinene and beta-pinene did not separate adequately for accurate identification (Maarse and Kepner 1970; Riddle et al. 1996), and were therefore combined for analysis. Monoterpenes were expressed on a fresh weight basis to reflect the actual concentrations found in living plants.

Samples of juniper leaves were also photographed at standard magnification with the use of an Olympus automatic camera.

Analysis of variance was conducted with the general linear model univariate analysis option of SAS (SAS 1987) due to the unequal number of samples per treatment. Trees were considered the experimental unit, regrowth periods represented the fixed treatment effects, and individual and total amount of monoterpenes considered dependent variables. Means found to be significant ($P < 0.05$) were compared using Duncan's multiple-range test.

RESULTS

Total monoterpene concentration averaged 5.1, 10.1, and 12.2 $\mu g \cdot mg^{-1}$ for 3- and 11-month regrowth and mature juniper, respectively (Table 1). The age treatments differed in total monoterpene levels ($P = 0.018$), with the 3-month regrowth lower in monoterpenoid concentration than the 11-month or mature juniper foliage. Monoterpene concentration from mature growth juniper was comparable to other data collected from the same population of trees (Owens et al. 1998).

Eighteen different monoterpenes were identified (Table 1). Tricyclene ($P = 0.018$), camphene ($P = 0.010$), myrcene ($P = 0.005$), cymene ($P = 0.004$), limonene ($P = 0.002$), γ-terpinene ($P = 0.002$), terpinolene ($P = 0.002$), fenchyl alcohol ($P = 0.003$), camphor ($P = 0.033$), citronellal ($P = 0.059$), borneol ($P = 0.058$), terpinen-4-ol ($P = 0.000$), α-terpineol ($P = 0.007$), and carvone ($P = 0.004$) all increased with maturity. One of the predominant compounds, alpha-pinene, declined with maturity ($P = 0.001$).

The increase in monoterpenes with maturity has particular significance for herbivory. Consumption of the more mature regrowth (11 months) by goats effectively increases the dose of monoterpenes and the subsequent likelihood of negative post-ingestive consequnces. Dziba (2006) provided evidence of an inverse relationship between intraruminal dosage of the monoterpene cineole and feeding behavior in sheep. Previous research on the Texas A&M University Research Station has identified β-pinene/sabinene, myrcene, and alpha-terpineol as particularly aversive monoterpenoids to goats (Riddle et al. 1996). Increasing concentrations of these compounds in particular suggest an increased defensive barrier to herbivory as time period for regrowth increases.

As well as a quantitative change over time (amount of total oils per fresh weight of leaf tissue), there was also general trend where the oil composition (relative concentration of individual oils in total oil) changed. There were higher relative concentrations (individual oil in total oil) of the monoterpene hydrocarbons (i.e., alpha-pinene and beta-pinene), and lower levels of oxygenated monoterpenes (i.e., citronellol, borneol, terpinen-4-ol, and terpineol) and the carbonyl compound camphor in the 3-month regrowth, with this trend reversed in the 11-month regrowth and mature growth.

DISCUSSION

These analytical results and visual observations of redberry juniper leaf gland development evince a short-term ontogenetic driven pattern of tissue chemical defense. Resource allocation to growth of aboveground leaf and stem expansion is consistent with a demand-side approach (Lerdau et al. 1994) to the growth-differentiation balance hypothesis (GDBH) first proposed by Loomis in 1932. A general principle of the GDBH is the distinction between growth of new tissue (i.e., primary metabolism) and differentiation, which is defined as the production of specialized tissue, organs, and compounds that are not dedicated to resource capture (i.e., secondary metabolism; Lerdau et al. 1994). Although the original GDBH is a source-driven model that does not consider need or demand for growth or differentiation products, when plants encounter changes in demand (such as phenologically driven increases in leaf and stem biomass) growth should be favored over differentiation, independently of changes in resource availability (Lerdau et al. 1994). Presumably the need to produce aboveground tissue capable of photosynthesis following top-kill of redberry juniper represents a classic demand scenario.

Although the traditional juvenile reversion scenario (Kozlowski 1971) predicts that juvenile tissue is more heavily defended, Bryant et al. (1991) provides 3 alternatives to resource allocation during juvenile reversion, the first two resulting in higher levels of chemical defenses and the third in lower defense levels and increased palatability. Recently Pavia et al. (2002) proposed the necessity for elasticity analysis as a tool to predict intraplant variation in defenses. The authors caution against assumptions about differences in fitness values among plant parts based on general reasoning and suggest that a stronger focus on variation in life histories among species and populations could lead to a better understanding of intraplant defense levels in modular organisms.

Table 1. Monoterpene concentrations[1] (μg oil $\cdot$ mg^{-1} juniper fresh weight) in redberry juniper regrowth from trees top-killed by a September burn (3-month regrowth) and a January burn (11-month regrowth), and from mature unburned trees. Duncan multiple means comparison ($\alpha = 0.05$).

Monoterpene	3-month regrowth	11-month regrowth	mature growth
Tricyclene	0.011 ± 0.013a	0.079 ± 0.042b	0.081 ± 0.024b
α-pinene	1.296 ± 0.360a	0.620 ± 0.272b	0.377 ± 0.117c
Camphene	0.014 ± 0.010a	0.080 ± 0.039b	0.082 ± 0.023b
β-pinene/sabinene	2.161 ± 0.985a	3.940 ± 0.681b	4.722 ± 1.656b
Myrcene	0.126 ± 0.047a	0.316 ± 0.059b	0.352 ± 0.076b
Cymene	0.087 ± 0.025a	0.258 ± 0.080b	0.327 ± 0.066b
Limonene	0.360 ± 0.084a	0.795 ± 0.282b	1.000 ± 0.150c
γ-terpinene	0.190 ± 0.048a	0.540 ± 0.149b	0.683 ± 0.147c
Terpinolene	0.098 ± 0.026a	0.227 ± 0.047b	0.284 ± 0.064c
Linalool	0.000 ± 0.000a	0.001 ± 0.000a	0.010 ± 0.022a
Fenchyl alcohol	0.023 ± 0.006a	0.008 ± 0.026a	0.202 ± 0.043a
Camphor	0.022 ± 0.013a	0.491 ± 0.236a	1.080 ± 0.626b
Citronellal	0.033 ± 0.033a	0.170 ± 0.136b	0.236 ± 0.106b
Borneol	0.012 ± 0.015a	0.132 ± 0.120b	0.147 ± 0.089b
Terpinen-4-ol	0.387 ± 0.086a	1.073 ± 0.221b	1.491 ± 0.333c
Terpineol	0.040 ± 0.014a	0.074 ± 0.035b	0.113 ± 0.023c
Carvone	0.088 ± 0.065a	0.187 ± 0.101b	0.305 ± 0.159c
Bornyl acetate	0.147 ± 0.181a	1.203 ± 0.895b	0.844 ± 0.480ab
Total oils	5.095 ± 1.871a	10.194 ± 1.963b	12.155 ± 2.434b

[1]Means in the same row with the same lowercase letters are not different ($P = 0.05$).

In this vein, variation between plant species in defensive compound chemistry and vascular structure may directly influence defense allocation through availability for mechanical translocation to vulnerable plant tissue. Tannins are water soluble and readily translocated through the phloem of vascular plants (Macleod and Pridham 1965). In cases where severe pruning of a mature woody plant by mammals occurred, the average defense of regrowth by tannins increased (Bryant 1981; Bryant et al. 1983; Provenza et al. 1990; Bryant et al. 1991). However, terpenoids accumulate in extracellular compartments, are remote from the cytoplasm, and their general lipophilicity makes them unavailable for translocation throughout the plant tissue (Fahn 1979). In addition, monoterpenes also require specialized storage structures to prevent autocytotoxicity (Lerdau et al. 1994), a problem not encountered with phenolic tannins. In fact, the literature supports the ontogenic development of monoterpene-producing structures as a prerequisite for monoterpene production in a wide variety of genera (Maarse and Kepner 1970; Zavarin et al. 1971; Leach and Whiffen 1989; Brun et al. 1991; King et al. 2004).

Among conifers differences are also seen in constitutive and wound-induced monoterpene biosynthesis among species (Lewinsohn et al. 1991). Monoterpene-producing glands are a distinct group of highly specialized cells that discharge substances to either the exterior (exotropic) or into special intercellular cavities (endotropic) (Schnepf 1974). Plant species of the conifer (*Coniferopsida*) class exhibit both types of glands and the anatomical organization of these secretory structures is an important criterion in the systematics of conifers (Lewinsohn et al. 1991). Members of the pine (*Pinus*) family are examples of an endotropic glandular system where oil secretions collect in internal resin ducts (Schnepf 1974) and in response to wounding rely primarily on the mobilization of preformed resin (cyclic monoterpenes and diterpene resin acids) to the wound site via the extensive network of resin ducts (Lewinsohn et al. 1991). In contrast, a salient anatomical characteristic of the cypress (*Cupressaceae*) family is no resin ducts (Young and Watson 1969) and the trees and shrubs of the genus *Juniperus* produce a single large oil gland in each leaf (Corell and Johnston 1970; Tatro et al. 1973).

Although this study did not directly investigate the metabolic turnover of monoterpenes (Powell and Adams 1973; Adams and Hagerman 1976), comparison of changes in oil composition at different stages of regrowth warrant further investigation in this area. Decrease of the relative concentration of α-pinene and β-pinene/sabinene could be attributed to changes in overall monoterpenoid concentration as other biogenic pathways are initiated adding more metabolites to the pool (Russell and Southwell 2002), or could be due to these compounds being utilized as precursor substrates for synthesis of other monoterpenes. Reviews of biosynthetic pathways suggest the possibility of pinene derived via the pinene synthase pathway (Croteau 1987) used as precursors for terpene class monoterpenes (Russell and Southwell 2002). A study investigating changes in relative composition of oils in *Melaleuca alternifolia* seedlings (Russell and Southwell 2002) reported similar results where high levels of terpinolene, α-pinene, β-pinene were present in early stages of growth, and fell as seedling development continued, accompanied by increases in terpinen-4-ol levels.

Another possible contributer to changes in overall oil composition is monoterpene volatility and emission. α-pinene and β-pinene are highly volatile compared to the other monoterpenes (National Library of Medicine 2004) and also those that decrease in relative concentration with respect to other monoterpenes in older leaf tissue. Currently, little is known about the mechanism of release of synthesized volatile compounds from plant tissues, but in general the rate of release is a function of the physical properties of the chemical itself (volatility) and the properties of cellular membranes through which the compound has to diffuse (Dudareva et al. 2004). Monoterpenes with low vapor pressures and volatilities were present in higher concentrations in 11-month and mature growth redberry juniper (Table 1). Loss of the more volatile oils over the growing season may also be influenced by the higher temperatures experienced during the summer months at the research site.

The change in relative composition during maturation, of these classes of monoterpenes, is consistent with research with other juniper species (Adams and Hagerman 1976), and with Douglas fir (Maarse and Kepner 1970). The changes in oil makeup over time bode poorly for rumen microbial activity. In vitro digestion trials involving fractionated oils indicated the monoterpene hydrocarbons were the least inhibitory fraction (Schwartz et al. 1980) or even stimulatory to rumen bacteria (Oh et al. 1967), whereas oxygenated monoterpenes were the most inhibitory to rumen microbial fermentation. (Oh et al. 1967, Schwartz et al. 1980). Therefore, the composition of monoterpenes changes from a composition with a potentially favorable effect on rumen fermentation at 3 months to a less favorable composition at the 11-month and mature-growth time periods sampled.

Whether these changes in monoterpene levels translated directly into reduced palatability was not tested in this study, but results from a previous preference trial provide evidence that growth stage of juniper seedlings can influence palatability. In a previous study on the Texas A&M University Research Station, a total of 16 cafeteria trials (4 goats per trial) were conducted to quantify goat use on 4 different plant species. For each trial 4 1-quart pots each of redberry juniper seedlings, Ashe juniper seedlings, and live oak and Ashe juniper with mature foliage, respectively, were offered for 5 minutes. Biting rate was measured and liveoak was preferred over other species ($P < 0.05$) and redberry and Ashe juniper seedlings were preferred over Ashe juniper with mature foliage ($P < 0.05$) (C. A. Taylor, personal communication, 2006).

MANAGEMENT IMPLICATIONS

Management of redberry juniper is a complicated and costly issue that many landowners must address. The use of prescribed fire as an initial treatment is effective (Steuter and Britton 1983) but longevity of this treatment requires consistent utilization of follow-up treatments. Prescriptive herbivory has the potential to effectively provide this follow-up treatment if barriers to herbivory represented by the secondary defensive chemicals (monoterpenes) can be overcome. Burning, by removing the above ground growth of redberry juniper and initiating immature growth, has the potential to shift the juniper biomass from a heavily defended form to a vulnerable form for a short period of time. Thus a potential "window of susceptibility" to herbivory may be created, where redberry juniper is more susceptible to herbivory, based on previous work. Management decisions should take into account this period of vulnerability of poorly defended shoot regrowth and insert goats into burned pastures soon after the redberry juniper initiates new growth, before the 11-month time period when gland development and monoterpenes reach defensive levels comparible to those found in mature tissue.

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