



Original Research

Effects of Mowing and Tebuthiuron on the Nutritional Quality of Wyoming Big Sagebrush[☆]Kurt T. Smith^{a,*}, Jennifer S. Forbey^b, Jeffrey L. Beck^a^a Department of Ecosystem Science and Management, College of Agriculture and Natural Resources, University of Wyoming, Laramie, WY 82071, USA^b Department of Biological Sciences, Boise State University, Boise, ID 83725, USA

ARTICLE INFO

Article history:

Received 26 April 2017

Received in revised form 12 February 2018

Accepted 13 February 2018

Key Words:

Artemisia tridentata wyomingensis

crude protein

greater sage-grouse

(*Centrocercus urophasianus*)

plant secondary metabolites (PSMs)

ABSTRACT

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young) is the most abundant and widely distributed subspecies of big sagebrush and has been treated through chemical application, mechanical treatments, and prescribed burning in efforts thought to improve habitat conditions for species such as greater sage-grouse (*Centrocercus urophasianus*) and mule deer (*Odocoileus hemionus*). Although the response of structural attributes of sagebrush communities to treatments is well understood, there is a need to identify how treatments influence the quality of sagebrush as winter food for wildlife. Our purpose was to identify how mowing and tebuthiuron treatments influenced dietary quality of Wyoming big sagebrush in central Wyoming. Two study areas were mowed in January and February 2014, and tebuthiuron was applied in two study areas in May 2014. We constructed 6 exclosures in each of these four study areas (24 total), which encompassed 30 × 30 m areas of treated and untreated sagebrush within each exclosure. Samples of current annual growth were collected from 18 sagebrush plants from treated and 12 plants from control portions of mowing exclosures during November 2013–2015 and tebuthiuron exclosures during November 2014–2015. Samples were analyzed for crude protein and plant secondary metabolites known to influence dietary selection of sagebrush by sage-grouse and other sagebrush-occurring herbivores. Our results suggest mowing and tebuthiuron treatments may slightly increase crude protein concentrations directly after treatments without immediate changes in plant secondary metabolites. Slight increases in dietary quality of sagebrush following treatments coupled with potential trade-offs with loss of biomass associated with treatments corroborates previous research that treating Wyoming big sagebrush may have little benefit for sage-grouse and other sagebrush-dependent wildlife. Future work should evaluate not only how treatments influence sage-grouse habitat use and reproductive success but also how treatments influence other wildlife species in fragile sagebrush ecosystems.

© 2018 The Society for Range Management. Published by Elsevier Inc. All rights reserved.

Introduction

Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*) is the most widely distributed subspecies of big sagebrush and provides important cover and foraging resources for many wildlife species (Beck et al., 2012). Sagebrush provides critical vegetative cover for wildlife and is also the primary food source for greater sage-grouse (*Centrocercus urophasianus*; hereafter, “sage-grouse”) during late fall, winter, and spring (Wallestad et al., 1975; Connelly et al., 2000) and pygmy rabbits (*Brachylagus idahoensis*) during winter (Thines et al., 2004). Sagebrush

may also comprise > 50% of the winter diets of pronghorn (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*; Mason, 1952; Austin and Urness, 1983).

The loss of sagebrush through both natural and human-mediated disturbances is therefore linked to the loss of several species (Connelly et al., 2004; Coates et al., 2016). Specifically, sage-grouse have experienced long-term range-wide declines (Connelly and Braun, 1997) and occur in < 60% of their presettlement habitats (Schroeder et al., 2004). Declining sage-grouse populations are largely attributed to human-mediated loss and fragmentation of sagebrush habitats. Sage-grouse are a sagebrush obligate species that rely on a variety of sagebrush-dominated habitats for food and cover throughout the year (Connelly et al., 2004; Crawford et al., 2004). A developing body of research has coupled habitat selection and demographic rates to measure habitat quality and population-level consequences for sage-grouse (e.g., Aldridge and Boyce, 2007; Smith et al., 2014; Kirol et al., 2015). Unfortunately, these studies are not often conducive to long-term monitoring of wildlife following natural or management-directed changes in habitat quality across landscapes. In

[☆] This work was supported through funds allocated through Local Sage-grouse Working Groups by the Wyoming Sage-Grouse Conservation Fund of the Wyoming Game and Fish Dept (002032, 002202, and 002527); the Wyoming Wildlife and Natural Resource Trust (112413JJ01); and the National Science Foundation (DEB-1146194).

* Correspondence: Kurt T. Smith, Dept of Ecosystem Science and Management, College of Agriculture and Natural Resources, University of Wyoming, Dept 3354, 1000 E University Ave, Laramie, WY 82071, USA. Tel.: +1-570-337-5321; fax: +1-307-766-6403.

E-mail address: ksmith94@uwyo.edu (K.T. Smith).

addition, the majority of research has focused on defining sagebrush habitat quality for sage-grouse in terms of height and structural cover, with myriad studies demonstrating the importance of structure and cover for sage-grouse during different life stages (e.g., Hagen et al., 2007). However, because sagebrush comprises a substantial portion of sage-grouse diets, quality of sagebrush habitats should not be defined solely in terms of structural characteristics.

Habitat treatments in big sagebrush communities have been implemented with the intent of improving sage-grouse habitats by reducing competition between sagebrush overstory and herbaceous understorey to improve important foraging resources for sage-grouse during the reproductive period (Beck et al., 2012). Treatments often reduce the age structure and density of sagebrush communities, and younger age classes of Wyoming big sagebrush plants contain slightly higher levels of crude protein (Wambolt, 2004). In addition, reduction in sagebrush density likely alleviates competitive effects between individual plants, allowing greater resource acquisition of remaining unaltered plants (Casper and Jackson, 1997) and possibly greater nutritional quality. Plant secondary metabolites (PSMs) occur in high concentrations in sagebrush (Kelsey et al., 1982) and may have negative nutritional and energetic consequences for herbivores consuming sagebrush (Stirby et al., 1987; Forbey et al., 2013; Kohl et al., 2015). Mechanisms responsible for tolerating PSMs are relatively unknown for most wild vertebrate herbivores but likely include regulated absorption, rapid rates of detoxification, and molecular insensitivity to cellular toxicity (Sorensen and Dearing, 2006; Sorensen et al., 2006).

There is considerable evidence that nutritional and chemical quality of the diet is important to herbivores (Beckerton and Middleton, 1982, 1983; Jakubas et al., 1993a, b), including sage-grouse (Remington and Braun, 1985; Welch et al., 1988; Frye et al., 2013). Sage-grouse excrete PSMs from sagebrush unchanged (Thacker et al., 2012; Kohl et al., 2015), are less sensitive to enzyme inhibition by sagebrush PSMs (Kohl et al., 2015), and may rely on functional genes within the gut microbiome (Kohl et al., 2016). Frye et al. (2013) determined that sage-grouse selected black sagebrush (*A. nova* A. Nelson) with lower PSM concentrations over Wyoming big sagebrush in winter in southern Idaho. In addition, sage-grouse also selected individual plants within black sagebrush patches that were higher in nutrient concentrations and lower PSM concentrations than available plants (Frye et al., 2013). Dietary quality of sagebrush may have a significant impact on body condition as grouse enter the reproductive period. For example, ruffed grouse (*Bonasa umbellus*) consuming diets with higher crude protein had higher reproductive success (Beckerton and Middleton, 1982) and willow grouse (*Lagopus lagopus*) consuming diets with high digestibility had higher reproductive success (Brittas, 1988). In addition, ruffed grouse consuming winter diets higher in crude protein and lower chemical defenses had higher population densities (Beckerton and Middleton, 1982, 1983; Jakubas et al., 1993b).

Our specific objective was to evaluate how tebuthiuron application and mechanical removal of sagebrush through mowing influenced the dietary quality of Wyoming big sagebrush. Herbicide applications, mechanical treatments, and prescribed burning form the major types of treatments that have been applied in efforts to enhance wildlife habitats in Wyoming big sagebrush (Beck et al., 2009, 2012; Davies et al., 2009; Hess and Beck, 2012). Prescribed burning Wyoming big sagebrush to enhance habitat for sage-grouse is problematic in most instances because the shrub structure needed by sage-grouse for nesting, brood-rearing and winter habitat is lost for decades (Beck, 1977; Beck et al., 2009; Hess and Beck, 2012). In contrast, mechanical and herbicide treatments may be more suitable to treat sage-grouse habitat than burning because residual sagebrush remains on treated sites (Olson and Whitson, 2002) and shrub skeletons are left behind that sage-grouse may use for cover (Dahlgren et al., 2006). Only Davies et al. (2009) have investigated the influence of mowing on crude protein of sagebrush leaves, and no studies to our knowledge have evaluated the influence of herbicide treatment on dietary quality of

sagebrush. Further, increase in crude protein alone does not necessarily indicate an increase in nutritional quality as PSMs strongly influence selection by sage-grouse (Forbey et al., 2013). We thus evaluated how mowing and tebuthiuron applications influenced crude protein and PSMs in leaves of treated and untreated Wyoming big sagebrush plants. We focused on sagebrush because it is the primary food source for sage-grouse and pygmy rabbits for several consecutive months in winter (Wallestad et al., 1975; Connelly et al., 2000; Thines et al., 2004), and the nutritional quality of sagebrush influences patch and plant use by these species in winter (Remington and Braun, 1985; Frye et al., 2013; Ulappa et al., 2014). We used crude protein as a nutrient variable because it can affect herbivore foraging behavior and reproductive success (Mattson, 1980). We chose monoterpenes and polyphenolics (coumarins and total phenolics) because these classes of compounds exert deleterious effects (e.g., toxicity, increased energy expenditure, nutrient binding) on herbivores (Dearing et al., 2005) and occur in relatively high concentrations in sagebrush (Kelsey et al., 1982).

Methods

Study Area

Our study area included portions of Fremont and Natrona counties, Wyoming and encompassed ~3 098 km² (735 879 ac; Fig. 1). It was composed of approximately 81% federal, 6.9% state, and 12.1% privately administered lands. Average annual 30-yr normal precipitation and temperature were 26 cm and 6.1°C, respectively (Prism Climate Group, 2016). Elevation ranged from 1 642 to 2 499 m. The study area was dominated by Wyoming big sagebrush with smaller amounts of mountain big sagebrush (*A. t. Nutt. ssp. vaseyana* [Rydb.] Beetle), basin big sagebrush (*A. t. Nutt. ssp. tridentata*), silver sagebrush (*A. cana* Pursh), black sagebrush, and greasewood (*Sarcobatus vermiculatus* [Hook.] Torr.). Major land uses during the study included livestock grazing. Treatments consisted of mechanical mowing and aerially

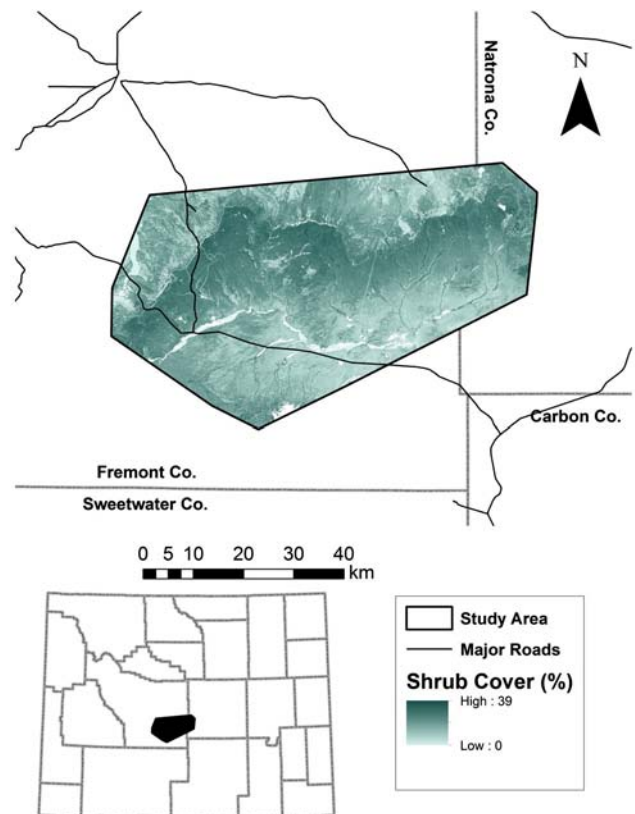


Figure 1. Study area location map, Fremont and Natrona Counties, Wyoming.

broadcasted tebuthiuron (Spike 20P, Dow AgroSciences, Indianapolis, IN) to Wyoming big sagebrush in early brood-rearing habitats during winter and spring 2014. We selected tebuthiuron because it is a translocated, soil-active herbicide that is partly selective (i.e., selective at low rates or nonselective at high rates). Thus, at low rates it leaves live sagebrush within the treated landscape (Olson and Whitson, 2002). Treatments followed guidelines of the Wyoming Game and Fish Department (WGFD) Protocols for Treating Sagebrush to be consistent with Wyoming Executive Order 2011-5; Greater Sage-Grouse Core Area Protection (WGFD, 2011). The only exception to the WGFD protocols was that instead of grazing rest for two growing seasons following treatments, we installed exclosures to measure post-treatment vegetative response in the absence of grazing. This was necessitated by the fact that only one allotment in the four treatment study areas had cross fencing and a rotational grazing system. The remaining treatment study areas occurred in areas with season-long continuous grazing, making evaluations of ungrazed post-treatment vegetation responses impossible without exclosures. Therefore, we installed twelve 30 × 60 m exclosures in mowed sites and twelve 30 × 80 m exclosures in tebuthiuron-treated sites during May 2014 to serve as controls for live-stock grazing. Exclosures constructed in tebuthiuron-treated areas were larger to account for potential herbicide leaching into the untreated side. For each treatment type, exclosures were placed such that half of them contained treated and the other half contained untreated sagebrush. The general design of these exclosures was to exclude a 30 × 30 m (0.09-ha) area of untreated sagebrush with an adjoining 30 × 30 m area excluding livestock grazing in treated sagebrush. Treatments occurred in a mosaic pattern across four general locations (two tebuthiuron and two mowing treatments). During January and February 2014, 489 ha of sagebrush habitats were mowed to a height of 25.4 cm across the two mowing treatment areas. Treatments were mowed at this height to be consistent with previous mowing studies and to minimize soil disturbance (Davies et al., 2009; Pyke et al., 2014). Tebuthiuron application occurred in early May 2014. Contractors applied 0.22 kg/ha active ingredient to 607 ha across the two study areas, anticipating a 50% kill rate of sagebrush. Treatments occurred across < 5% of each study to be consistent with WGFD guidelines (WGFD, 2011).

Field Methods

Before treatments, we randomly selected 18 Wyoming big sagebrush plants (with at least six plants < 25.4 cm) within the treated portion of each mowing exclosure to maximize the likelihood of at least six plants surviving (assuming < a 50% kill outcome in treatments) and 12 plants (with at least six plants < 25.4 cm) within the untreated portions of each of the 12 exclosures. We collected five to eight sprigs from each selected sagebrush plant within each collection site by clipping the stems with pruning shears and minimizing damage to remaining leaves and stems. Each plant was marked with a metal plant tag to allow for long-term monitoring of treatment effects on dietary quality of plants. Sagebrush samples were stored in a -20°C freezer. We were unable to sample at tebuthiuron exclosure locations before treatment because these locations were not yet delineated during the pretreatment sampling period.

During sampling following treatments (November 2014 and 2015), we collected vegetation from six previously sampled plants that survived treatment, plus an additional six plants in each treatment that were not sampled during the previous sampling period. Collection and analysis of new plants allowed us to account for effects of clipping on diet quality. Post-treatment sampling focused on collecting stems from plants containing new growth during the second winter season. Because we were unable to sample at tebuthiuron exclosure locations before treatment, 2014 sampling at tebuthiuron exclosures was consistent with pretreatment sampling at mowing exclosure locations (e.g., 18 plants within the treated portion and 12 plants within the untreated portions of each of the 12 exclosures).

Laboratory Methods

Of the original 18 plants sampled within the treated portions of exclosures, we only analyzed those plants that survived through the post-treatment sampling periods. In addition, we ensured that the sizes of plants sampled were similar between treatment and control plots. We found no differences between new or repeatedly sampled shrubs collected during 2014 in the mowing exclosures (control and mow) for any of the PSMs analyzed (analysis of variance, $P > 0.05$); therefore, new plants were selected for all analyses for 2015 mowing and tebuthiuron treatments. Six samples from each exclosure were selected to create composite samples for each independent site, treatment, and exclosure. Composite samples were submerged in liquid nitrogen, and sagebrush leaves were removed from woody stems. We ground composite leaves into a coarse powder using a mortar, pestle, and liquid nitrogen until particles were ≤ 2 mm. Samples were then allocated into headspace vials for gas chromatography (GC; 50 mg wet weight [ww]) and microcentrifuge tubes (100 mg ww) for chemical analysis of coumarins and total phenolics. For crude protein (% dry matter), a minimum of 1.7 g ww of coarsely ground sagebrush composites were dried for 48 h and assessed using combustion method elemental analysis of nitrogen (Dairy One Forage Laboratories, Ithaca, NY). Monoterpenes of sagebrush were quantified (AUC/mg dry weight, dw) using headspace GC using an Agilent 7694 Headspace Autosampler coupled with an Agilent 6890N gas chromatograph. One mL of headspace gas was injected into J and W DB-5 capillary columns (30 μm × 250 μm × 0.25 μm ; operating conditions: oven temperature at 100°C, loop temperature at 110°C, transfer line temperature at 120°C, vial equilibrium time of 20 min, a pressurization time of 0.20 min, a loop fill time of 0.50 min, a loop equilibrium time of 0.20 min, and an injection time of 0.50 min; operating conditions for GC: splitless injector at 250°C, flame ionization detector at 300°C, oven temperature at 40°C for 2 min, then increasing 3°C/min to 60°C, then increasing 5°C/min to 120°C, then increasing 20°C/min to 300°C, and held at 300°C for 7 min). The make-up gas was nitrogen, and the carrier gas was helium. The inlet pressure was 80 KPa with a flow rate of 1.0 mL/min. Volatile monoterpenes were identified by matching retention times to cocktails of known monoterpene composition and concentration. Retention times and peak areas were calculated using HP ChemStation version B.01.00 (Santa Clara, CA). Peak areas were calculated by integrating chromatogram curves. Only compounds with peak areas > 1% of the total area and present in at least 75% of samples were summed to calculate total monoterpenes used in the analysis. In addition, we included relative concentration of 1,8-cineole (AUC/mg dw) in analysis because this specific monoterpene is known to influence foraging behavior of herbivores (Bray et al., 1991; Shipley et al., 2012) including sage-grouse (Frye et al., 2013).

Coumarins (umol/g) and total phenolics (umol/g) of sagebrush were assessed using colorimetric assays. Composite leaves were extracted for two separate 3-min periods in 1.0 mL GC-grade methanol in a sonicating water bath and filtered through glass wool. For the coumarin assay, 50 μL subsamples were pipetted into a 96-well plate in triplicate. Color intensity was measured using a BioTek Synergy MX multimode plate reader (BioTek, Winooski, VT) at an absorbance of 350 nm excitation and 460 nm emission at room temperature. Scopoletin (number 5995-86-8, Acros Organics) diluted in methanol was used as a standard (0–80 μM). We used an adapted Folin-Ciocalteu assay to measure total phenolics (Ainsworth and Gillespie, 2007). Samples were diluted with methanol to fit within the standard curve of gallic acid (number 92-6-15, Acros Organics) diluted in methanol (0–580 μM). For each sample and standard, 20 μL of the dilution was pipetted in triplicate into 96 well plates. Next, 100 μL of 10% Folin-Ciocalteu reagent were added to each well and mixed, and 80 μL of 700 mM (7.5%) sodium carbonate were added and mixed. Plates were allowed to incubate for 2 hours and then shaken on the plate reader for 60 s before reading. Color intensity was measured using a BioTek Synergy MX multimode plate reader at an absorbance of 765 nm at room temperature.

Statistical Analysis

We assessed monoterpenes, 1,8-cineole, total phenolics, coumarins, and protein for correlations and found that no variables were correlated above ($|r| > 0.7$). We used linear mixed models (package nlme; Pinheiro et al., 2016) to test the response of sagebrush dietary quality (crude protein, total monoterpenes, 1,8-cineole, coumarins, and total phenolics) to mowing and tebuthiuron treatments. Fixed factors included treatment type and yr, with exclosures (with yr and type nested within exclosure) treated as a random effect. We performed separate models for each treatment type (mowing or tebuthiuron) and dietary response to compare differences between treatments and controls within exclosures. In addition, we assessed differences between mowing and tebuthiuron treatments during 2014 and 2015, where the response of paired control plots was subtracted from treatments. We used least square means with Tukey adjustments to assess post hoc differences

between treatment and controls or mowing and herbicide treatments across sampling years when main effects were significant (package lsmeans; Lenth, 2016). We removed any outliers from analysis and assessed normality of model residuals. We performed all statistical analyses in R statistical software (R version 3.2.4; R Core Team, 2016) and set statistical significance at $\alpha = 0.05$.

Results

Mowing Treatments

Comparison of mowing exclosures revealed no differences in crude protein between treatment and control ($F_{1, 22} = 1.76, P = 0.198$), yr ($F_{2, 44} = 1.65, P = 0.205$), or treatment-by-yr interaction ($F_{2, 44} = 2.31, P = 0.111$; Fig. 2). For total monoterpenes, we found no difference between treatment and controls at mowing exclosures (treatment:

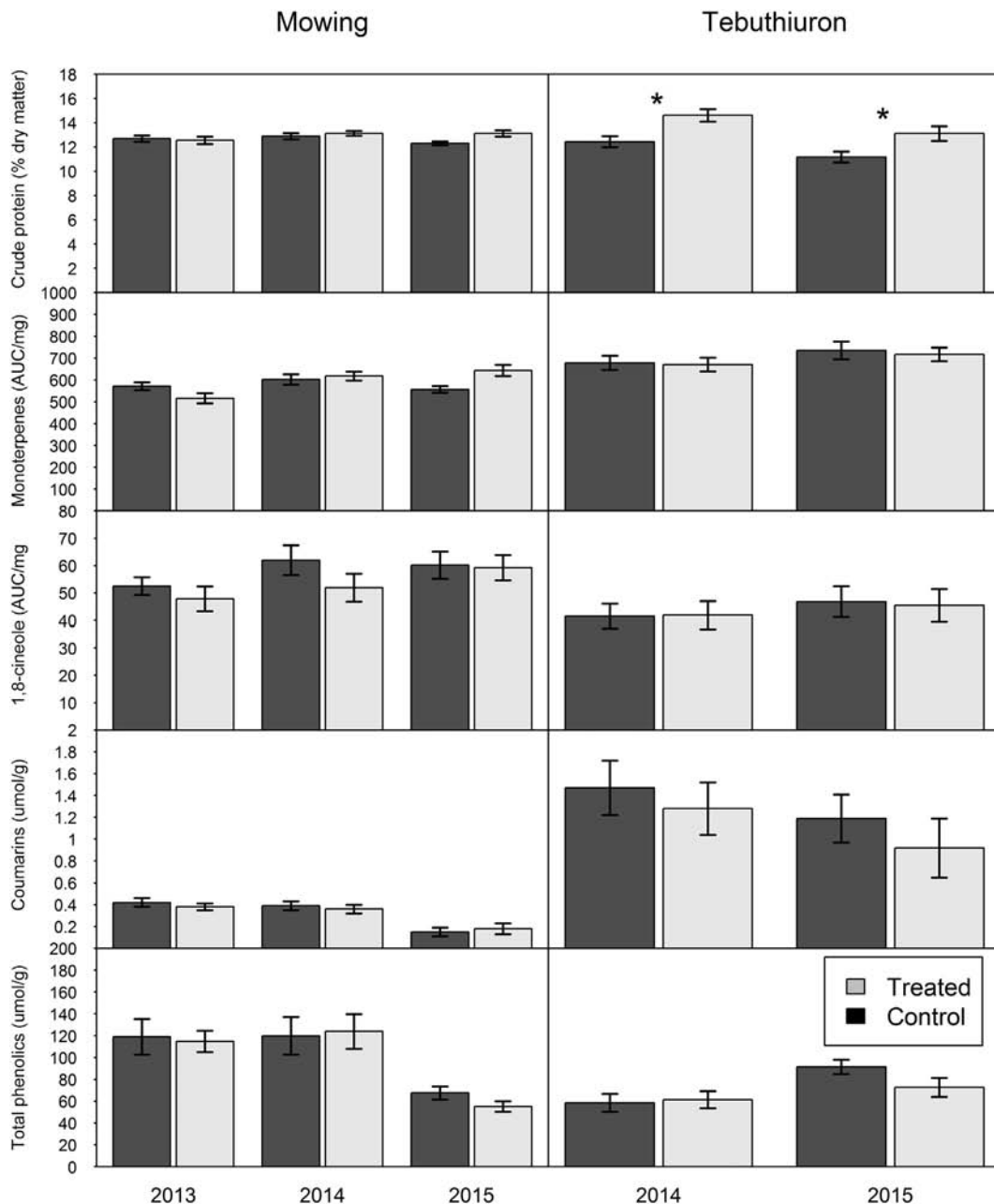


Figure 2. Mean (\pm SE) crude protein (% dry matter), monoterpenes (AUC/mg), 1,8-cineole (AUC/mg), coumarins (umol/g), and total phenolics (umol/g) of sagebrush leaves in mowing (2013–2015) and control (left column) and tebuthiuron (2014–2015) and control exclosures. Asterisks indicate yearly differences between treatment and control plots ($P < 0.05$).

$F_{1,22} = 0.13, P = 0.722$; treatment \times yr: $F_{2,44} = 0.42, P = 0.662$). We detected a difference across yr ($F_{2,44} = 6.26, P = 0.004$), with lower monoterpene concentrations in 2013 compared with 2014 (post hoc, $P = 0.05$) and 2015 (post hoc, $P = 0.003$). For 1,8-cineole, we found no difference between treatment and controls at mowing exclosures or across yr (treatment: $F_{1,22} = 1.68, P = 0.209$; yr: $F_{2,44} = 2.20, P = 0.124$; treatment \times yr: $F_{2,44} = 0.49, P = 0.619$). Similarly, we found no differences in coumarin concentrations between treatment and controls at mowing exclosures (treatment: $F_{1,22} = 0.19, P = 0.664$; treatment \times yr: $F_{2,44} = 0.44, P = 0.645$). However, coumarin concentrations differed across yr ($F_{2,44} = 20.51, P < 0.001$), with coumarins lower in 2015 compared with 2013 and 2014 (post hoc, $P < 0.001$). For total phenolics, we did not detect differences between treatment and controls (treatment: $F_{1,22} = 0.14, P = 0.707$; treatment \times yr: $F_{2,44} = 0.22, P = 0.800$) but found differences across yr ($F_{2,44} = 15.1326, P < 0.001$), with 2015 samples having lower total phenolic concentrations compared with 2013 and 2014 (post hoc, $P < 0.001$).

Tebuthiuron Treatments

We found differences in crude protein between herbicide and control treatments ($F_{1,22} = 9.78, P = 0.005$) and yr ($F_{1,22} = 21.90, P < 0.001$) but did not detect a significant treatment \times yr interaction ($F_{1,22} = 0.18, P = 0.677$; see Fig. 2). We found higher crude protein in tebuthiuron treatments compared with paired controls during 2014 (post hoc, $P = 0.006$) and 2015 (post hoc, $P = 0.014$), and overall crude protein levels were greater in 2014 compared with 2015 (post hoc, $P < 0.001$). For monoterpenes, we did not detect differences between treatments and controls ($F_{1,22} = 0.12, P = 0.735$) across yr ($F_{1,22} = 3.11, P = 0.092$) or for the treatment \times yr interaction ($F_{1,22} = 0.032, P = 0.859$). For 1,8-cineole, we did not detect differences between treatments and controls ($F_{1,22} = 0.00, P = 0.985$) across yr ($F_{1,22} = 2.47, P = 0.131$) or for the treatment \times yr interaction ($F_{1,22} = 0.00, P = 0.999$). We found no differences in coumarin concentrations between treatment and control in tebuthiuron exclosures (treatment: $F_{1,22} = 0.49, P = 0.490$; treatment \times yr: $F_{1,22} = 0.12, P = 0.734$). However, we did detect differences across years ($F_{1,22} = 7.35, P = 0.013$), with greater coumarin concentrations in 2014 compared with 2015. Similarly, we did not detect differences in concentrations of total phenolics between tebuthiuron and control treatments ($F_{1,22} = 0.79, P = 0.384$) or the treatment \times yr interaction ($F_{1,22} = 2.67, P = 0.116$), but total phenolics were greater in 2015 ($F_{1,22} = 11.22, P = 0.003$).

Comparison of Mowing and Tebuthiuron Treatments

Comparison between paired differences of treatment and controls indicated a difference in crude protein between tebuthiuron and mowing treatments ($F_{1,22} = 11.58, P = 0.003$; Fig. 3). There were no differences between yr ($F_{1,22} = 0.19, P = 0.663$), and the treatment \times yr interaction was not significant ($F_{1,22} = 1.20, P = 0.28$). Crude protein was greater at tebuthiuron-treated exclosures compared with mowing exclosures during 2014 (post hoc, $P = 0.003$), but no differences were detected during 2015 (post hoc, 0.073; see Fig. 3). We found no differences between treatments, years, or treatment \times yr interactions for monoterpenes, 1,8-cineole, coumarins, or total phenolics.

Discussion

Relatively little information exists about the effects of management practices on the dietary quality of shrubs. Although several studies have investigated how management practices influence diversity and composition of sagebrush communities (Davies et al., 2011a, 2012), only Davies et al. (2009) have investigated the influence of mowing on dietary quality of sagebrush and our study is the first to evaluate the influence of herbicide treatments on sagebrush dietary quality and of any treatment on secondary metabolites. We found that herbicide

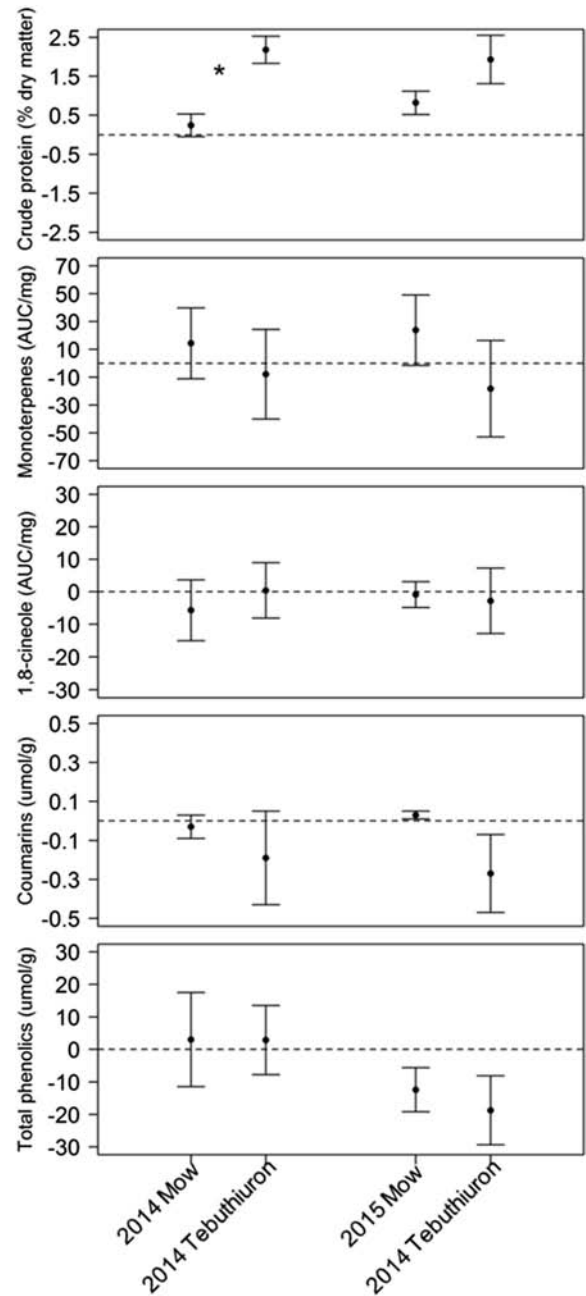


Figure 3. Mean paired differences (\pm SE) in crude protein (% dry matter), monoterpenes (AUC/mg), 1,8-cineole (AUC/mg), coumarins (umol/g), and total phenolics (umol/g) in sagebrush leaves collected at mowing and herbicide treatment exclosures, 2014–2015. Asterisks indicate yearly differences between treatment types ($P < 0.05$).

treatments resulted in sagebrush plants with greater leaf crude protein content compared with untreated controls. We did not collect pretreatment information on herbicide treatments; nonetheless, our results suggest that sagebrush plants treated with herbicide had greater leaf crude protein content compared with mowing treatments at least during the first yr following treatments. However, we did not detect differences in PSMs between treated and untreated plants, or between treatment types, but our results corroborate others that found annual variation in PSMs (Cedarleaf et al., 1983; Wilt and Miller, 1992).

Changes in the availability of quality food pose a threat to a variety of terrestrial species. Recent work from Idaho revealed strong evidence that the nutritional and chemical quality of sagebrush, not structural cover, explained habitat selection by sage-grouse (Frye et al., 2013) and pygmy rabbits (Ulappa et al., 2014). This research suggested that

wildlife managers should be concerned with preserving the dietary quality of sagebrush and should identify how management-driven changes to habitats influence the dietary quality of sagebrush, specifically in areas dominated by Wyoming big sagebrush. Wyoming big sagebrush communities have undergone significant changes due to invasion of non-native grass species, wildfire, and management practices that reduce sagebrush (Davies et al., 2011b; Beck et al., 2012). Increases in CO₂, drought, and temperatures associated with climate change are likely to reduce the dietary quality of remaining sagebrush (Bidart-Bouzat and Imeh-Nathaniel, 2008; Karban, 2011; Robinson et al., 2012). Future sage-grouse populations may experience both the loss of biomass and reduction in the dietary quality of existing sagebrush, which could be mitigated or exacerbated by management practices (Forbey et al., 2013).

Sage-grouse are reliant on sagebrush for food during winter (Wallestad et al., 1975), and treated sagebrush may be used by sage-grouse during this time for food, provided snow cover does not preclude access to remaining sagebrush canopy. Further, sagebrush is an important dietary component of female sage-grouse during the prelaying period before new forb growth (Connelly et al., 2000; Gregg et al., 2006). As such, treatments that increase crude protein or decrease PSMs in sagebrush may benefit sage-grouse populations. Davies et al. (2009) found slight increases in crude protein levels in treated Wyoming big sagebrush habitats up to 6 yr following mechanical treatments. Similarly, we detected slight increases in crude protein in tebuthiuron-treated sagebrush without detecting changes in PSMs in tebuthiuron or mowing treatments. Although the mechanisms for these changes are unknown for sagebrush, increased protein could be due to new vegetative growth. For example, crude protein increases in grasses and forbs following fires (Hess and Beck, 2014; Powell et al., 2018). Herbicides are known to alter nutritional quality of plants through changes in plant composition (Soper et al., 1993; Han and Twidwell, 2017) or changes in the soil microbiome (Lekberg et al., 2017), but these responses are not well described in shrubs. Regardless of mechanism, we agree with Davies et al. (2009) that minor increases in nutritional quality of treated sagebrush are unlikely to offset the negative impacts of long-term reduction in cover and density for sage-grouse and other wildlife. Tebuthiuron treatments do leave behind shrub skeletons that sage-grouse may use for cover (Dahlgren et al., 2006). If sufficient cover remains within herbicide treatments, increased palatability of sagebrush may improve habitat quality as a result of herbicide treatments. However, we did not assess how herbicide influences other forage species that may be particularly important to nesting sage-grouse (Gregg et al., 2008). Beckerton and Middleton (1982) found that captive female ruffed grouse fed diets with \approx 2% greater crude protein on a percentage dry matter basis had greater clutch sizes and mean egg weights but did not exhibit increased hatching success. An approximate 10% increase in crude protein in the diet of captive female ruffed grouse did increase hatching success, but clutch size, mean egg weight, and hatching success were similar to wild females (Beckerton and Middleton, 1982). In addition, we did not detect differences in PSM concentrations relative to mowing or herbicide treatments, which may better predict plant and patch selection by sage-grouse compared with crude protein levels alone (Frye et al., 2013). On the basis of these findings, it is unlikely that marginal increases in crude protein as a result of herbicide treatments would improve winter habitat use or reproductive success for sage-grouse.

Implications

The sagebrush ecosystem is among the most imperiled ecosystems in the United States (Davies et al., 2011b), and loss and fragmentation of sagebrush habitats has been identified as a significant threat for remaining sage-grouse populations (Knick et al., 2003). As such, managers should take great caution when altering remaining sagebrush habitats. Although some evidence suggests that tebuthiuron treatments

may improve breeding habitats for sage-grouse in mountain big sagebrush communities (Dahlgren et al., 2006, 2015), our results provide evidence that treating Wyoming big sagebrush communities to benefit sage-grouse may not significantly improve diet quality relative to detrimental decreases in reduced cover associated with treatments. Specifically, sagebrush communities recover slowly following disturbances (Baker, 2011) and often do not increase important forb and insect abundance for sage-grouse diets during the breeding season (Fischer et al., 1996; Nelle et al., 2000; Davies et al., 2007, 2012; Rhodes et al., 2010; Hess and Beck, 2014). Treatments reduce sagebrush cover important for nesting and brood-rearing habitats (Hagen et al., 2007) and provide only a slight increase in nutritional quality for winter diets (Davies et al., 2009). In addition, emphasis on improving habitats for sage-grouse does not reflect the numerous wildlife species that rely on sagebrush communities yearlong. For example, identifying how to maximize the availability of palatable sagebrush, as well as associated forbs, could benefit other herbivores such as pronghorn, pygmy rabbits, mule deer, and elk (*Cervus elaphus*) that rely on sagebrush communities for forage. Further work is needed to understand the relationships among management practices, changes in cover, diet, and reproductive success of sage-grouse, as well as how habitat management targeted at sage-grouse influences habitat quality for other wildlife that inhabits sagebrush.

Acknowledgments

We received logistical support from T. Christiansen, S. Harter, B. Parker, and D. Lemon from the Wyoming Game and Fish Department, along with many others from the Department who assisted with implementing sagebrush treatments. S. Oberlie and T. Vosburgh from the Bureau of Land Management–Lander Field Office also served as valuable liaisons conducting environmental assessments before treatment implementation. We thank C. Merriman, J. Peña, and N. Rizvic for their assistance with laboratory analysis. J. LeVan, K. Lawson, and K. Kauffman served as valuable field technicians. We thank Ag Flyers of Torrington, Wyoming for aerial tebuthiuron application and B & A Leasing of Greybull, Wyoming for erecting fence enclosures.

References

- Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols* 2, 875–877.
- Aldridge, C.L., Boyce, M.S., 2007. Linking occurrence and fitness to persistence: a habitat-based approach for endangered greater sage-grouse. *Ecological Applications* 17, 508–526.
- Austin, D.D., Urness, P.J., 1983. Overwinter forage selection by mule deer on a seeded big sagebrush-grass range. *Journal of Wildlife Management* 47, 1203–1207.
- Baker, W.L., 2011. Pre-Euro-American and recent fire in sagebrush ecosystems. In: Knick, S.T., Connelly, J.W. (Eds.), *Greater sage-grouse: ecology and conservation of a landscape species and its habitats*. *Studies in Avian Biology* 38. University of California Press, Berkeley, CA, USA, pp. 185–201.
- Beck, T.D.I., 1977. Sage grouse flock characteristics and habitat selection in winter. *Journal of Wildlife Management* 41, 18–26.
- Beck, J.L., Connelly, J.W., Reese, K.P., 2009. Recovery of greater sage-grouse habitat features in Wyoming big sagebrush following prescribed fire. *Restoration Ecology* 17, 393–403.
- Beck, J.L., Connelly, J.W., Wambolt, C.L., 2012. Consequences of treating Wyoming big sagebrush to enhance wildlife habitats. *Rangeland Ecology & Management* 65, 444–455.
- Beckerton, P.R., Middleton, A.L.A., 1982. Effects of dietary-protein levels on ruffed grouse reproduction. *Journal of Wildlife Management* 46, 569–579.
- Beckerton, P.R., Middleton, A.L.A., 1983. Effects of dietary-protein levels on body-weight, food-consumption, and nitrogen-balance in ruffed grouse. *Condor* 85, 53–60.
- Bidart-Bouzat, M.G., Imeh-Nathaniel, A., 2008. Global change effects on plant chemical defenses against insect herbivores. *Journal of Integrative Plant Biology* 50, 1339–1354.
- Bray, R.O., Wambolt, C.L., Kelsey, R.G., 1991. Influence of sagebrush terpenoids on mule deer preference. *Journal of Chemical Ecology* 17, 2053–2062.
- Brittas, R., 1988. Nutrition and reproduction of the willow grouse *Lagopus lagopus* in central Sweden. *Ornis Scandinavica* 19, 49–57.
- Casper, B.B., Jackson, R.B., 1997. Plant competition underground. *Annual Review of Ecology and Systematics* 28, 545–570.
- Cedarleaf, J.D., Welch, B.L., Brotherson, J.D., 1983. Seasonal variation of monoterpenoids in big sagebrush (*Artemisia tridentata*). *Journal of Range Management* 36, 492–494.

- Coates, P.S., Ricca, M.A., Prochazka, B.G., Brooks, M.L., Doherty, K.E., Kroger, T., Blomberg, E.J., Hagen, C.A., Casazza, M.L., 2016. Wildfire, climate, and invasive grass interactions negatively impact an indicator species by reshaping sagebrush ecosystems. *Proceedings of the National Academy of Sciences of the United States of America* 113, 12745–12750.
- Connelly, J.W., Braun, C.E., 1997. Long-term changes in sage-grouse *Centrocercus urophasianus* populations in western North America. *Wildlife Biology* 3, 229–234.
- Connelly, J.W., Knick, S.T., Schroeder, M.A., Stiver, S.J., 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. In: WAOFAW Agencies (Ed.). WAOFAW Agencies, Cheyenne, WY, USA 610 pp.
- Connelly, J.W., Schroeder, M.A., Sands, A.R., Braun, C.E., 2000. Guidelines to manage sage-grouse populations and their habitats. *Wildlife Society Bulletin* 28, 967–985.
- Crawford, J.A., Olson, R.A., West, N.E., Mosley, J.C., Schroeder, M.A., Whitson, T.D., Miller, R.F., Gregg, M.A., Boyd, C.S., 2004. Ecology and management of sage-grouse and sage-grouse habitat. *Journal of Range Management* 57, 2–19.
- Dahlgren, D.K., Chi, R., Messmer, T., 2006. Greater sage-grouse response to sagebrush management in Utah. *Wildlife Society Bulletin* 34, 975–985.
- Dahlgren, D., Larsen, R.T., Danvir, R., Wilson, G., Thacker, E.T., Black, T.A., Naugle, D.E., Connelly, J.W., Messmer, T.A., 2015. Greater sage-grouse and range management: insights from a 25 year case study in Utah and Wyoming. *Rangeland Ecology & Management* 68, 375–382.
- Davies, K.W., Bates, J.D., Johnson, D.D., Nafus, A.M., 2009. Influence of mowing *Artemisia tridentata* ssp. *wyomingensis* on winter habitat for wildlife. *Environmental Management* 44, 84–92.
- Davies, K.W., Bates, J.D., Miller, R.F., 2007. Short-term effects of burning Wyoming big sagebrush steppe in southeast Oregon. *Rangeland Ecology & Management* 60, 515–522.
- Davies, K.W., Bates, J.D., Nafus, A.M., 2011a. Are there benefits to mowing Wyoming big sagebrush plant communities? An evaluation in southeastern Oregon. *Environmental Management* 48, 539–546.
- Davies, K.W., Bates, J.D., Nafus, A.M., 2012. Mowing Wyoming big sagebrush communities with degraded herbaceous understories: has a threshold been crossed? *Rangeland Ecology and Management* 65, 498–505.
- Davies, K.W., Boyd, C.S., Beck, J.L., Bates, J.D., Svejcar, T.J., Gregg, M.A., 2011b. Saving the sagebrush sea: an ecosystem conservation plan for big sagebrush plant communities. *Biological Conservation* 144, 2573–2584.
- Dearing, M.D., Foley, W.J., McLean, S., 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. *Annual Review of Ecology, Evolution, and Systematics* 36, 169–189.
- Fischer, R.A., Reese, K.P., Connelly, J.W., 1996. An investigation on fire effects within xeric sage-grouse brood habitat. *Journal of Range Management* 49, 194–198.
- Forbey, J.S., Wiggins, N.L., Frye, G.G., Connelly, J.W., 2013. Hungry grouse in a warming world: emerging risks from plant chemical defenses and climate change. *Wildlife Biology* 19, 374–381.
- Frye, G.G., Connelly, J.W., Musil, D.D., Forbey, J.F., 2013. Phytochemistry predicts habitat selection by an avian herbivore at multiple spatial scales. *Ecology* 94, 308–314.
- Gregg, M.A., Barnett, J.K., Crawford, J.A., 2008. Temporal variation in diet and nutrition o preincubating greater sage-grouse. *Rangeland Ecology & Management* 61, 535–542.
- Gregg, M.A., Dunbar, M.R., Crawford, J.A., Pope, M.D., 2006. Total plasma protein and reneating by greater sage-grouse. *Journal of Wildlife Management* 70, 472–478.
- Hagen, C.A., Connelly, J.W., Schroeder, M.A., 2007. A meta-analysis of greater sage-grouse *Centrocercus urophasianus* nesting and brood-rearing habitats. *Wildlife Biology* 13, 42–50.
- Han, K.J., Twidwell, E.K., 2017. Herbage mass and nutritive value of Bermudagrass influenced by non-growing-season herbicide application. *Agronomy Journal* 109, 1024–1030.
- Hess, J.E., Beck, J.L., 2012. Burning and mowing Wyoming big sagebrush: do treated sites meet minimum guidelines for greater sage-grouse breeding habitats? *Wildlife Society Bulletin* 36, 85–93.
- Hess, J.E., Beck, J.L., 2014. Forb, insect, and soil response to burning and mowing Wyoming big sagebrush in greater sage-grouse breeding habitat. *Environmental Management* 53, 813–822.
- Jakubas, W.J., Karasov, W.H., Guglielmo, C.G., 1993a. Coniferyl benzoate in quaking aspen (*Populus tremuloides*)—its effect on energy and nitrogen digestion and retention in ruffed grouse (*Bonasa-umbellus*). *Physiological Zoology* 66, 580–601.
- Jakubas, W.J., Wentworth, B.C., Karasov, W.H., 1993b. Physiological behavioral-effects of coniferyl benzoate on avian reproduction. *Journal of Chemical Ecology* 19, 2353–2377.
- Karban, R., 2011. The ecology and evolution of induced resistance against herbivores. *Functional Ecology* 25, 339–347.
- Kelsey, R.G., Stephens, J.R., Shafizadeh, F., 1982. The chemical-constituents of sagebrush foliage and their isolation. *Journal of Range Management* 35, 617–622.
- Kirol, C.P., Beck, J.L., Huzurbazar, S.V., Holloran, M.J., Miller, S.N., 2015. Identifying greater sage-grouse source and sink habitats for conservation planning in an energy development landscape. *Ecological Applications* 25, 968–990.
- Knick, S.T., Dobkin, D.S., Rotenberry, J.T., Schroeder, M.A., Vander Haegen, W.M., van Riper, C.I.II., 2003. Teetering on the edge or too late? Conservation and research issues for avifauna of sagebrush habitats. *Condor* 105, 611–634.
- Kohl, K.D., Connelly, J.W., Dearing, M.D., Forbey, J.S., 2016. Microbial detoxification in the gut of a specialist avian herbivore, the greater sage-grouse. *FEMS Microbiology Letters* 363, fnw144.
- Kohl, K.D., Pitman, E., Robb, B.C., Connelly, J.W., Dearing, M.D., Forbey, J.S., 2015. Monoterpenes as inhibitors of digestive enzymes and counter-adaptations in a specialist avian herbivore. *Journal of Comparative Physiology B* 185, 425–435.
- Lekberg, Y., Wagner, V., Rummel, A., McLeod, M., Ramsey, P.W., 2017. Strong indirect herbicide effects on mycorrhizal associations through plant community shifts and secondary invasions. *Ecological Applications* 27, 2359–2368.
- Lenth, R.V., 2016. Least-squares means: The R package lsmeans. *Journal of Statistical Software* 69, 1–33.
- Mason, E., 1952. Food habits and measurements of Hart Mountain antelope. *Journal of Wildlife Management* 16, 387–389.
- Mattson, W.J., 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* 11, 119–161.
- Nelle, P.J., Reese, K.P., Connelly, J.W., 2000. Long-term effects of fire on sage grouse habitat. *Journal of Range Management* 53, 586–591.
- Olson, R.A., Whitson, T.D., 2002. Restoring structure in late successional sagebrush communities by thinning with tebuthiuron. *Restoration Ecology* 10, 146–155.
- Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. *nlme: Linear and nonlinear mixed effects models.* R package version 3.1-127. Available at: <http://CRAN.R-project.org/package=nlme>, Accessed date: 6 September 2016.
- Powell, J., Martin, B., Dreitz, V.J., Allred, B.W., 2018. Grazing preferences and vegetation feedbacks of the fire-grazing interaction in the northern Great Plains. *Rangeland Ecology & Management* 71, 45–52.
- PRISM Climate Group, 2016. PRISM Climate DATA. Available at: <http://www.prism.oregonstate.edu>, Accessed date: 13 July 2016.
- Pyke, D.A., Shaff, S.E., Lindgren, A.I., Schupp, E.W., Doescher, P.S., Chambers, J.C., Burham, J.S., Huso, M.M., 2014. Region-wide ecological responses of arid Wyoming big sagebrush communities to fuel treatments. *Rangeland Ecology & Management* 67, 455–467.
- R Core Team, 2016. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: <https://www.r-project.org>, Accessed date: 24 April 2016.
- Remington, T.E., Braun, C.E., 1985. Sage grouse food selection in winter, North Park, Colorado. *Journal of Wildlife Management* 49, 1055–1061.
- Rhodes, E.C., Bates, J.D., Sharp, R.N., Davies, K.W., 2010. Fire effects on cover and dietary resources of sage-grouse habitat. *Journal of Wildlife Management* 74, 755–764.
- Robinson, E.A., Ryan, G.D., Newman, J.A., 2012. A meta-analytical review of the effects of elevated CO₂ on plant arthropod interactions highlights the importance of interacting environmental and biological variables. *New Phytologist* 194, 321–336.
- Schroeder, M.A., Aldridge, C.L., Apa, A.D., Bohne, J.R., Braun, C.E., Bunnell, S.D., Connelly, J.W., Deibert, P.A., Gardner, S.C., Hiliard, M.A., Kobriger, G.D., McAdam, S.M., McCarthy, C.W., McCarthy, J.J., Mitchell, D.L., Rickerson, E.V., Stiver, S.J., 2004. Distribution of sage-grouse in North America. *Condor* 106, 363–376.
- ShIPLEY, L.A., Davis, E.M., Felicetti, L.A., McLean, S., Forbey, J.S., 2012. Mechanisms for eliminating monoterpenes in sagebrush by specialist and generalist rabbits. *Journal of Chemical Ecology* 38, 1178–1189.
- Smith, K.T., Kirol, C.P., Beck, J.L., Blomquist, F.C., 2014. Prioritizing winter habitat quality for greater sage-grouse in a landscape influenced by energy development. *Ecosphere* 5 article15.
- Soper, R.B., Lochmiller, R.L., Leslie, D.M., Engle, D.M., 1993. Nutritional quality of browse after brush management on cross timbers rangeland. *Journal of Range Management* 46, 399–410.
- Sorensen, J.S., Dearing, M.D., 2006. Efflux transporters as a novel herbivore countermechanism to plant chemical defenses. *Journal of Chemical Ecology* 32, 1181–1196.
- Sorensen, J.S., Skopec, M.M., Dearing, M.D., 2006. Application of pharmacological approaches to plant-mammal interactions. *Journal of Chemical Ecology* 32, 1229–1246.
- Stirby, K.D., Wambolt, C.L., Kelsey, R.G., Havstad, K.M., 1987. Crude terpenoid influence on in vitro digestibility of sagebrush. *Journal of Range Management* 40, 244–248.
- Thacker, E.T., Gardner, D.R., Messmer, T.A., Guttery, M.R., Dahlgren, D.K., 2012. Using gas chromatography to determine winter diets of greater sage-grouse in Utah. *Journal of Wildlife Management* 76, 588–592.
- Thines, N.J.S., Shipley, L.A., Saylor, R.D., 2004. Effects of cattle grazing on ecology and habitat of Columbia Basin pygmy rabbits (*Brachylagus idahoensis*). *Biological Conservation* 119, 525–534.
- Ulappa, A.C., Kelsey, R.G., Frye, G.G., Rachlow, J.L., Shipley, L.A., Bond, L., Pu, X., Forbey, J.S., 2014. Plant protein and secondary metabolites influence diet selection in a mammalian specialist herbivore. *Journal of Mammalogy* 95, 834–842.
- Wallestad, R.O., Peterson, J.G., Eng, R.L., 1975. Foods of adult sage grouse in central Montana. *Journal of Wildlife Management* 39, 628–630.
- Wambolt, C.L., 2004. Browsing and plant age relationships to winter protein and fiber of big sagebrush subspecies. *Journal of Range Management* 57, 620–623.
- Welch, B.L., Pederson, J.C., Rodriguez, R.L., 1988. Selection of big sagebrush by sage grouse. *Great Basin Naturalist* 48, 274–279.
- Wilt, F.M., Miller, G.C., 1992. Seasonal variation of coumarin and flavonoid concentrations in persistent leaves of Wyoming big sagebrush (*Artemisia tridentata* ssp. *wyomingensis*). *Biochemical Systematics and Ecology* 20, 53–67.
- Wyoming Game and Fish Department, 2011. Wyoming Game and Fish Department protocols for treating sagebrush to benefit sage-grouse. Available at: http://gf.state.wy.us/wildlife_management/sagegrouse/index.asp, Accessed date: 15 November 2012.