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Kurt T. Smith
University of Wyoming

Jennifer S. Forbey
Boise State University

Jeffrey L. Black
University of Wyoming

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Kurt T. Smith[1,*], Jennifer S. Forbey[2], and Jeffrey L. Beck[1]

[1]Department of Ecosystem Science and Management, College of Agriculture and Natural Resources, University of Wyoming, Dept. 3354, 1000 E. University Ave., Laramie, Wyoming 82071, USA

[2]Department of Biological Sciences, Boise State University, Boise, Idaho 83725, USA

*Correspondence: Email: ksmith94@uwyo.edu; Phone: (570) 337-5321; Fax; (307) 766-6403

ABSTRACT

Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. w*yomingensis* 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 m x 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 how treatments influence other wildlife species in fragile sagebrush ecosystems.

KEYWORDS: crude protein, Greater sage-grouse (*Centrocercus urophasianus*), plant secondary metabolites (PSMs), *Artemisia tridentata wyomingensis*

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 not only provides critical vegetative cover for wildlife, but it is also the primary food source for greater sage-grouse (*Centrocercus urophasianus*; hereafter, 'sage-grouse') during late fall, winter, and spring (Connelly et al. 2000; Wallestad et al. 1975) and pygmy rabbits (*Brachylagus idahoensis*) during winter (Thines et al. 2004). Sagebrush may also comprise greater than 50% of the winter diets of pronghorn (*Antilocapra americana*) and mule deer (*Odocoileus hemionus*; Austin and Urness 1983; Mason 1952).

The loss of sagebrush through both natural and human-mediated disturbances is therefore linked to the loss of several species (Coates et al. 2016; Connelly et al. 2004). Specifically, sage-grouse have experienced long-term range-wide declines (Connelly and Braun 1997) and occur in less than 60% of their pre-settlement 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; Kirol et al. 2015; Smith et al. 2014). 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 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 understory 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 (Forbey et al. 2013; Kohl et al. 2015; Stirby et al. 1987). 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 (Frye et al. 2013; Remington and Braun 1985; Welch et al. 1988). 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) has 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 (Connelly et al. 2000; Wallestad et al. 1975; Thines et al. 2004) and the nutritional quality of sagebrush influences patch and plant use by these species in winter (Frye et al. 2013; Remington and Braun 1985; 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 km^2 (735,879 ac; Fig. 1), and was composed of approximately 81% Federal, 6.9% State, and 12.1% privately administered lands. Average annual 30-year normal precipitation and temperature were 26 cm and 6.1 °C, respectively (Prism Climate Group 2016). Elevation ranged from 1642 to 2499 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 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 2 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 un-grazed post treatment vegetation responses impossible without exclosures. Therefore, we installed 12, 30 x 60 m exclosures in mowed sites and 12, 30 x 80 m exclosures in tebuthiuron

155 treated sites during May 2014 to serve as controls for livestock grazing. Exclosures constructed
156 in tebuthiuron-treated areas were larger to account for potential herbicide leaching into the
157 untreated side. For each treatment type, exclosures were placed such that half of the exclosure
158 contained treated and the other half contained untreated sagebrush. The general design of these
159 exclosures was to exclude a 30 x 30 m (0.09 ha) area of untreated sagebrush with an adjoining 30
160 x 30 m area excluding livestock grazing in treated sagebrush. Treatments occurred in a mosaic
161 pattern across four general locations (two tebuthiuron and two mowing treatments). During
162 January and February 2014, 489 ha of sagebrush habitats were mowed to a height of 25.4 cm
163 across the two mowing treatment areas. Treatments were mowed at this height to be consistent
164 with previous mowing studies and to minimize soil disturbance (Davies et al. 2009; Pyke et al.
165 2014). Tebuthiuron application occurred in early May 2014. Contractors applied 0.22 kg/ha
166 active ingredient to 607 ha across the two study areas, anticipating a 50% kill rate of sagebrush.
167 Treatments occurred across less than 5% of each study to be consistent with WGFD guidelines
168 (WGFD 2011).

169 *Field Methods*

170 Prior to treatments, we randomly selected 18 Wyoming big sagebrush plants (with at least six
171 plants less than 25.4 cm) within the treated portion of each mowing exclosure to maximize the
172 likelihood of at least six plants surviving (assuming less than a 50% kill outcome in treatments)
173 and 12 plants (with at least six plants less than 25.4 cm) within the untreated portions of each of
174 the 12 exclosures. We collected 5–8 sprigs from each selected sagebrush plant within each
175 collection site by clipping the stems with pruning shears and minimizing damage to remaining
176 leaves and stems. Each plant was marked with a metal plant tag to allow for long term
177 monitoring of treatment effects on dietary quality of plants. Sagebrush samples were stored in a –

178 20 °C freezer. We were unable to sample at tebuthiuron exclosure locations prior to treatment
179 because these locations were not yet delineated during the pre-treatment sampling period.

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

189 *Laboratory Methods*

190 Of the original 18 plants sampled within the treated portions of exclosures, we only analyzed
191 those plants that survived through the post-treatment sampling periods. In addition, we ensured
192 that the sizes of plants sampled were similar between treatment and control plots. We found no
193 differences between new or repeatedly sampled shrubs collected during 2014 in the mowing
194 exclosures (control and mow) for any of the PSMs analyzed (ANOVA, $P > 0.05$); therefore, new
195 plants were selected for all analyses for 2015 mowing and tebuthiuron treatments. Six samples
196 from each exclosure were selected to create composite samples for each independent site,
197 treatment and exclosure. Composite samples were submerged in liquid nitrogen and sagebrush
198 leaves were removed from woody stems. We ground composite leaves into a coarse powder
199 using a mortar, pestle and liquid nitrogen until particles were ≤ 2 mm. Samples were then

allocated into headspace vials for gas chromatography (50 mg wet weight [ww]) and micro-centrifuge 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 hours and assessed using combustion method elemental analysis of nitrogen (Dairy One Forage Laboratories, Ithaca, New York). Monoterpenes of sagebrush were quantified (AUC/mg dry weight, dw) using headspace gas chromatography (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 x 250 µm x 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, California, USA). Peak areas were calculated by integrating chromatogram curves. Only compounds with peak areas greater than 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-minute 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 multi-mode plate reader (BioTek, Winooski, Vermont, USA) 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 to 80 µM). We used an adapted Folin-Ciocalteau 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 to 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-Ciocalteau reagent was added to each well, mixed and 80 µl of 700 mM (7.5%) sodium carbonate was added and mixed. Plates were allowed to incubate for 2 hours, and were then shaken on the plate reader for 60 seconds before reading. Color intensity was measured using a BioTek Synergy MX multi-mode 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 year, with exclosures (with year 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; Length 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 ($F_{1, 22} = 1.76$, $P = 0.198$), year ($F_{2, 44} = 1.65$, $P = 0.205$), or treatment by year 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: $F_{1,22} = 0.13$, $P = 0.722$; Treatment x Year: $F_{2,44} = 0.42$, $P = 0.662$). We detected a difference across years ($F_{2, 44} = 6.26$, $P = 0.004$), with lower monoterpene concentrations in 2013 compared to 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 years (Treatment: $F_{1,22} = 1.68$, $P = 0.209$; Year: F2,44 = 2.20, $P = 0.124$; Treatment x Year: $F_{2,44} = 0.49$, $P = 0.619$). 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 x Year: $F_{2,44}$ = 0.44, P = 0.645). However, coumarin concentrations differed across years ($F_{2,44}$ = 20.51, P < 0.001), with coumarins lower in 2015 compared to 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 x Year: $F_{2,44}$ = 0.22, P = 0.800), but found differences across years ($F_{2,44}$ = 15.1326, P < 0.001), with 2015 samples having lower total phenolic concentrations compared to 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 year ($F_{1,22}$ = 21.90, P < 0.001), but did not detect a significant treatment by year interaction ($F_{1,22}$ = 0.18, P = 0.677; Fig. 2). We found higher crude protein in tebuthiuron treatments compared to 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 to 2015 (*post hoc*, P < 0.001). For monoterpenes, we did not detect differences between treatment and controls ($F_{1,22}$ = 0.12, P = 0.735) across years ($F_{1,22}$ = 3.11, P = 0.092) or for the treatment x year interaction ($F_{1,22}$ = 0.032, P = 0.859). For 1,8-cineole, we did not detect differences between treatment and controls ($F_{1,22}$ = 0.00, P = 0.985) across years ($F_{1,22}$ = 2.47, P = 0.131) or for the treatment x year 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 x Year: $F_{1,22}$ = 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 to 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 x year 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 year ($F_{1,22} = 0.19$, P = 0.663) and the treatment by year interaction was not significant ($F_{1,22} = 1.20$, P = 0.28). Crude protein was greater at tebuthiuron treated exclosures compared to mowing exclosures during 2014 (*post hoc*, P = 0.003), but no differences were detected during 2015 (*post hoc*, 0.073; Fig. 3). We found no differences between treatments, years, or treatment x year 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 treatments resulted in sagebrush plants with greater leaf crude protein content compared to untreated controls. We did not collect pre-treatment information on herbicide treatments; nonetheless our results suggest that sagebrush plants treated with herbicide had greater leaf crude protein content compared to mowing treatments at least during the first year 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 (Beck et al. 2012; Davies et al. 2011b). Increases in CO_2, 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 pre-laying period prior to 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 years 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 is 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 approximately 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 to crude protein

356 levels alone (Frye et al. 2013). Based on these findings, it is unlikely that marginal increases in
357 crude protein as a result of herbicide treatments would improve winter habitat use or
358 reproductive success for sage-grouse.

359 **Implications**

360 The sagebrush ecosystem is among the most imperiled ecosystems in the United States (Davies
361 et al. 2011b) and loss and fragmentation of sagebrush habitats has been identified as a significant
362 threat for remaining sage-grouse populations (Knick et al. 2003). As such, managers should take
363 great caution when altering remaining sagebrush habitats. While some evidence suggests that
364 tebuthiuron treatments may improve breeding habitats for sage-grouse in mountain big sagebrush
365 communities (Dahlgren et al. 2006, 2015), our results provide evidence that treating Wyoming
366 big sagebrush communities to benefit sage-grouse may not significantly improve diet quality
367 relative to detrimental decreases in reduced cover associated with treatments. Specifically,
368 sagebrush communities recover slowly following disturbances (Baker 2011) and often do not
369 increase important forb and insect abundance for sage-grouse diets during the breeding season
370 (Davies et al. 2007, 2012; Fischer et al. 1996; Hess and Beck 2014; Nelle et al. 2000; Rhodes et
371 al. 2010). Treatments reduce sagebrush cover important for nesting and brood-rearing habitats
372 (Hagen et al. 2007) and provide only a slight increase in nutritional quality for winter diets
373 (Davies et al. 2009; this study). In addition, emphasis on improving habitats for sage-grouse does
374 not reflect the numerous wildlife species that rely on sagebrush communities yearlong. For
375 example, identifying how to maximize the availability of palatable sagebrush as well as
376 associated forbs could benefit other herbivores such as pronghorn, pygmy rabbits, mule deer and
377 elk (*Cervus elaphus*) that rely on sagebrush communities for forage. Further work is needed to
378 understand the relationship between 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 sagebrush occurring wildlife.

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Figure 2. Mean (± 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 ($P < 0.05$).

Figure 3. Mean paired differences (± 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 ($P < 0.05$).