Is Diet Selection by Greater Sage-Grouse Influenced by Biomass Availability or Toxins?

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Abstract

Foraging herbivores must meet nutritional requirements by not only finding enough plant biomass to consume, but also finding plants with high protein content and low concentrations of potentially toxic plant secondary metabolites (PSMs). Greater sage-grouse (Centrocercus urophasianus; hereafter, sage-grouse) are sagebrush obligate herbivores that consume relatively high concentrations of PSMs. To meet their nutritional needs and avoid ingesting high amounts of PSMs, sage-grouse may select species of sagebrush for food that have lower concentrations of PSMs than a more abundant species with higher concentration of PSMs. Diet selection by sage-grouse may also be driven by chemical factors at finer scales once a species is selected. For example, different morphotypes of sagebrush (identified by leaf morphology and plant structure) within a patch have different chemical profiles that may influence selection at a patch scale. Our objective was to determine how diet selection is influenced by available biomass and chemical characteristics of morphotypes within a foraging patch, and whether sage-grouse select specific morphotypes of sagebrush to maximize biomass consumed per bite or minimize toxin consumed per bite. For each sagebrush morphotype, we determined density of plants within a patch and available biomass which we calculated as plant volume. We then measured biomass and monoterpene concentrations of the leaves per bite. Our results showed that browsing is not proportional to biomass availability, but that sage-grouse selected sagebrush morphotypes that minimized toxin intake per bite. Our research aims to understand plant-herbivore interactions and how sage-grouse select and use habitats at different spatial scales.

Introduction

Foraging herbivores obtain protein from plants to use for activities necessary for survival like growth and reproduction. The Greater sage-grouse (*Centrocercus urophasianus*, hereafter sage-grouse) is a specialist avian herbivore that consumes up to 100% sagebrush during the winter months (Patterson, 1952), which produce a wide variety of plant secondary metabolites (PSMs) (Kelsey et al., 1982). Sage-grouse select patches and individual plants within a patch with high protein and low toxin concentrations (Remington and Braun, 1985; Frye et al., 2013). High quality diets may improve reproductive success (Beckerton and Middleton, 1983; DeGabriel et al., 2009). Within a landscape, not all plants have the same level of protein because protein content varies between different sagebrush species (*Artemisia spp.*) and forbs (Barnett and Crawford, 1994; Gregg et al., 2008). Similarly, concentrations of PSMs, including monoterpenes vary among species and among individual plants (Kelsey et al., 1982). Adult sage-grouse consume forbs and insects during nesting, and juveniles consume primarily forbs and insects during their growth period (Gregg et al., 2008). These alternative food sources are high in protein, and sage-grouse select the highest protein foods available in any season (Gregg et al., 2006). Sagebrush habitats have been declining in quality and quantity for several decades, which has coincided with simultaneous declines in sage-grouse (Schroeder et al., 2004; Garton et al., 2011) and sage-grouse rely on the sagebrush habitat for cover.

Plants often have physical and chemical defenses as a mechanism to deter foraging herbivores. Sagebrush produces PSMs, which are defensive chemicals generally toxic to herbivores and therefore influence habitat and diet selection (DeGabriel et al., 2009; Frye et al., 2013; Ulappa et al., 2014). PSMs, such as monoterpenes, can inhibit digestive enzymes, and some compounds can only be excreted by conjugation with protein, which requires animals to consume enough protein both for metabolism and for their own body maintenance (Kohl et al., 2015). Metabolism and excretion of compounds is energetically expensive and may result in altered energy budgets

(Sorensen et al., 2005c) which is likely to be fitness-relevant and is perhaps an evolutionary consequence of plant-herbivore interactions (Boyle et al., 2000; Mclean and Duncan, 2006; Sorensen et al., 2006). Sagebrush structure and chemistry vary at different spatial and temporal scales and may influence foraging selection (Kelsey et al., 1982; Wiggins et al., 2006; Frye et al., 2013). Sage-grouse forage selectively at the patch and landscape scale by selecting specific sagebrush species and plants within a species that are high in protein and low in monoterpene concentrations (Frye et al., 2013).

Herbivores forage selectively to avoid toxins and to maximize protein intake (DeGabriel et al., 2009; Frye et al., 2013; Ulappa et al., 2014). Herbivores also sometimes select plants that are low in toxins but may not have the highest protein content of available plants (Guglielmo and Karasov, 1996; McArthur et al., 2014; Marsh et al., 2006), which suggests there may be a trade-off for some herbivores where animals must select between high protein or low toxins, as available plants may not have both characteristics. Plant toxicity and protein content influence diet selection, but consumption of plant matter is limited because increasing intake of plant matter may not compensate for low nutrients (Sedinger, 1997), and herbivores may not be able to increase consumption of plants with high toxin concentrations (Wiggins et al., 2003). Some herbivores reduce their meal size or bite intake as a strategy to limit toxin intake (Wiggins et al., 2003; Sorensen et al., 2005a; Sorensen et al., 2005b). Sage-grouse exhibit diet selection at the landscape and patch scale (Frye et al., 2013) and may exhibit selection at the leaf scale to maximize nutrient intake while minimizing PSMs.

This study investigates how sage-grouse forage at the fine scales (within a patch, and at the bite scale) on three different sagebrush morphotypes (*A. tridentata wyomingnesis,* medium *A. arbuscula,* and small *A. arbuscula*) to determine if diet selection is influenced by food availability (leaf biomass) or food toxicity. We evaluated if sage-grouse maximize biomass consumption or minimize toxin intake per bite. We hypothesized that sage-grouse select specific sagebrush species or morphotype with the lowest toxin risk.

Methods

Study site

The study site was in Raft River, Idaho, USA (42° 35'N, 113° 14'W) in Cassia County. We flushed radio marked sage-grouse during the winter of January 2015. Wyoming big sagebrush (*A. tridentata wyomingensis*) was the dominant shrub across the landscape, followed by low sagebrush (*A. arbuscula*), native grasses, and juniper. However, at sites used by sage-grouse, low sagebrush was more abundant than Wyoming big sagebrush. Elevation ranged from 1,380 m to 2,140 m and the average annual precipitation was 33 cm. Temperatures ranged from 4 to 5° C and snow depth was about 5 cm during the time that transects were conducted.

Field methods

Sage-grouse were trapped by Idaho Fish and Game using standard trapping and marking techniques (Giesen et al., 1982; Wakkinen et al., 1992) during spring 2012 and 2013. Birds were flushed using radio-telemetry in January 2015. Foraging patches were identified at flush sites using fresh tracks, pellets, and bite marks on sagebrush plants. Bite marks were also used to identify browsed plants and quantify intensity of use by sage-grouse. Sagebrush morphotypes were defined as structural classes of each species, with small ($h < 15$ cm), medium ($55 > h > 15$ cm), and large ($h \geq 56$ cm) structural classes (Figure 1). Sagebrush species were identified in the field using morphological characteristics and verified in the lab using monoterpene profiles (Thacker et al., 2012). We conducted line-intercept transects (10 m transects extending in each cardinal direction) to calculate percent cover of each morphotype (Canfield, 1941), and conducted density counts on a 1 m belt transect around the transect lines. Transects originated from the center of the foraging patch. Sage-grouse bite marks were counted on each sagebrush plant within 1 m of transect, and we classified bite marks as fresh (determined by bright green meristematic tissue along the leaf margin) or old (brown or dark green leaf edges). For each plant, we used the height (cm), length (cm) and width (cm) to calculate the volume (cm^3) of the plant using the equation for an ellipsoid to best approximate plant volume (Messina et al., 2002). We collected plant samples from three browsed and three non-browsed plants of each morphotype of sagebrush. Browsed plants had at least ten fresh bite marks by sage-grouse and non-browsed plants had no more than one fresh bite mark. The plant samples were kept on ice in coolers during fieldwork and transferred to a -20° C freezer in the laboratory.

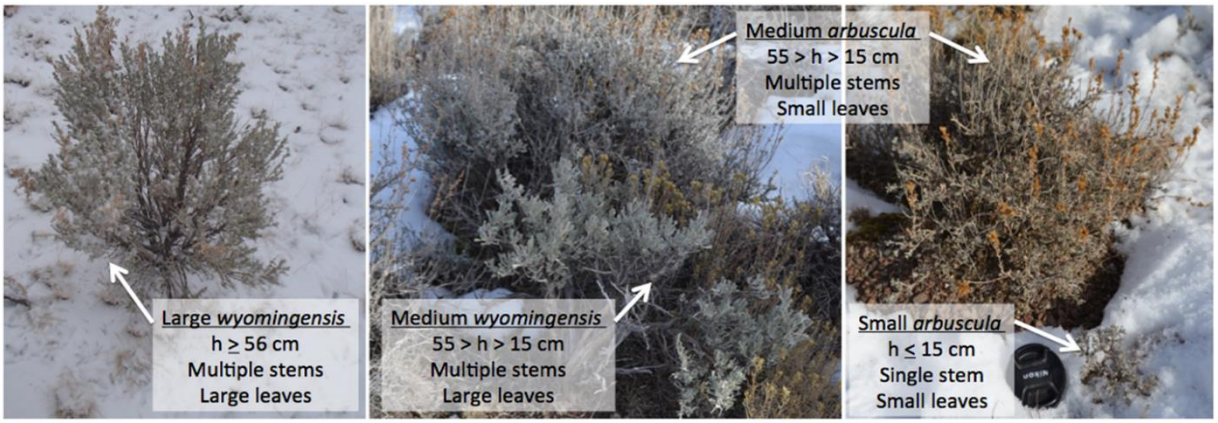

Figure 1. Morphotypes of sagebrush found at the Raft River site, Cassia County, Idaho, USA. Photo by Marcella Fremgen

Laboratory methods

Because sage-grouse do not eat stems, we removed leaves from woody biomass for chemical analysis. Leaves were removed by dipping samples into liquid nitrogen and using forceps to brush leaves off the stems into a separate container. Samples were ground using a mortar and pestle in liquid nitrogen into a particle size of about 2 mm, and weighed into separate vials for analysis. All samples were stored at -20° C.

We used headspace gas chromatography to detect monoterpenes in leaf samples, using a gas chromatograph (Agilent 6890N) with a headspace auto-sampler (Hewlett Packard HP7694). After grinding, we measured a 100 mg subsample of ground leaf into a 20 ml gas chromatography headspace vial. A cocktail of monoterpene standards was used to generate reference retention times (min) to identify compounds from samples. Not all compounds were identified, and unknown compounds were labeled based on retention times. Peak areas (area under the curve, AUC) and retention times were calculated using HP ChemStation version B.01.00 (Santa Clara, California, USA).

A leaf clipping experiment was performed from three sagebrush morphotypes: *A. t. wyomingensis* (n = 20), medium *A. arbuscula* (n = 20) and small *A. arbuscula* (n = 20). We clipped 10 "bites" from each individual sagebrush morphotype to mimic sage-grouse bite marks, and we measured the weight (g) of the total leaf biomass for the clipped leaves. We determined the average weight (g) per "bite" of each morphotype to compare the bite sizes for the calculation of monoterpenes per bite.

Statistical methods

All statistical analysis used JMP Pro 11.0 (SAS Institute Inc. 2013) and R version 3.2.0 (R Foundation for Statistical Computing 2015). The average volume per plant for each sagebrush morphotype was calculated from the methods of Messina et al., 2002. The leaf biomass (g) of Wyoming big sagebrush, medium low sagebrush, and small low sagebrush morphotypes were compared using ANOVA, and for significantly different ANOVAs we compared all pairs using a Tukey's HSD test. The total monoterpene concentration (AUC/ 100 µg dry weight, DW) and individual monoterpene concentrations were determined from gas chromatographs. The average monoterpenes per bite were calculated from the total monoterpenes (AUC/g dry mass) times the average clipped bite size (g) for each sagebrush morphotype to determine toxicity per bite.

Results

Sage-grouse selected medium plants of *A. arbuscula* for browsing over small *A. arbuscula* and *A. t. wyomingensis* within a patch (Figure 2; Chi-squared: $\chi 2 = 285.12$, P = < 2.2e-16). The proportion of non-browsed plants was higher than browsed which shows that sage-grouse are selectively foraging within their habitat. Small *A. arbuscula* had less biomass than medium *A. arbuscula* which had less biomass per shrub (cm^3) compared to *A. t. wyomingensis* (Figure 3; ANOVA: $F_{2,4330} = 572.8$, $P < 0.0001$).

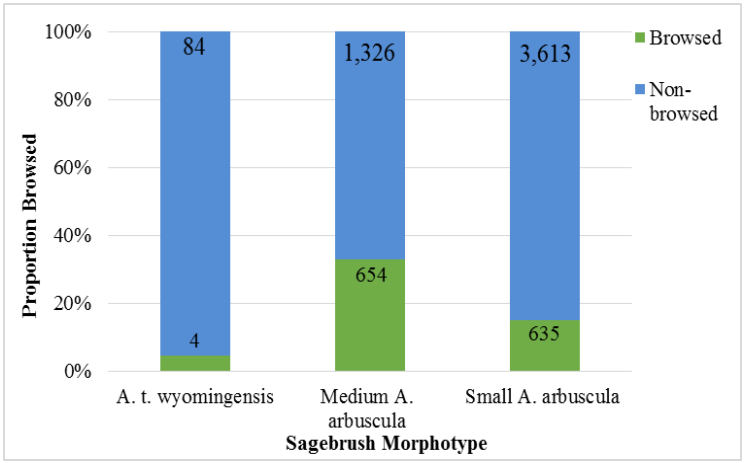

Figure 2. Proportion of *A. t. wyomingensis* shrubs (5% of available) and *A. arbuscula* shrubs (15-33% of available) within a used patch that were browsed. (Chi-squared = 285.12, df = 2, p-value < 2.2e-16).

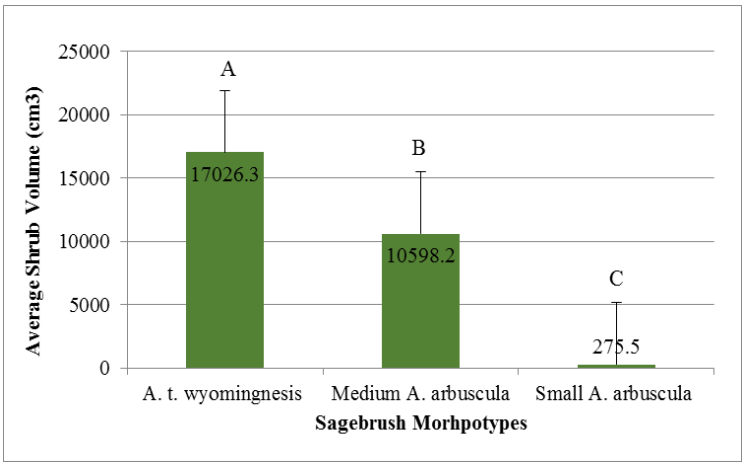

Figure 3. Average shrub volume of each sagebrush morphotype within used patches ($F_{2,4330}$=572.8, p <0.0001, different letters = significant difference)

For the clipping experiment of leaf biomass, *A. arbuscula* has less leaf biomass per bite (g) compared to *A. t. wyomingensis* (Figure 4; ANOVA: $F_{2,4330} = 572.8$, $P < 0.0001$). Leaf biomass for small and medium *A. arbuscula* did not differ from one another ($P = 0.9077$). The toxicity per bite for sagebrush species was significantly different for *A. arbuscula* and *A. t. wyomingensis* (Figure 5; ANOVA: $F_{2,4330} = 572.8$, $P < 0.0001$) but small and medium *A. arbuscula* did not differ from one another ($P = 1.0$).

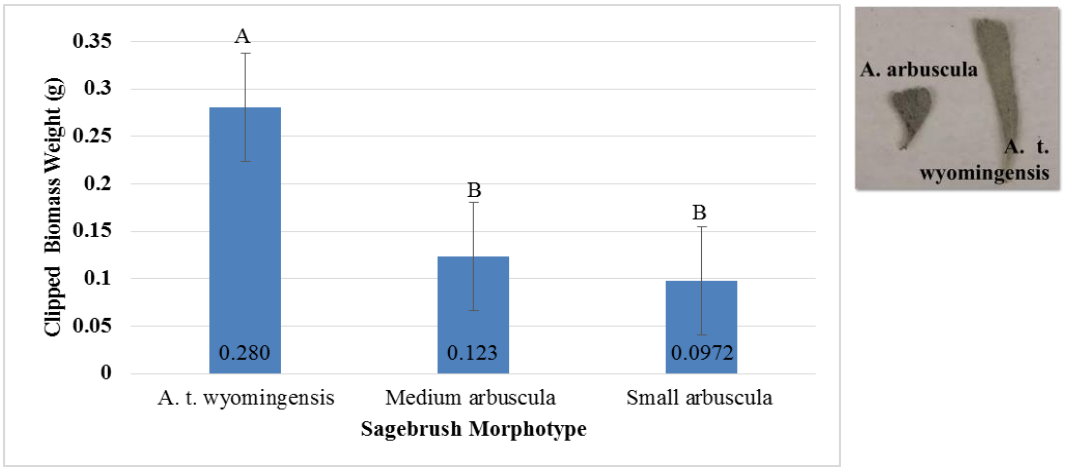

Figure 4. Average biomass (g) of leaves per "bite" (clipped weight) for each sagebrush morphotype ($F_{2,4330}$=572.8, p <0.0001, different letters = significant difference between groups)

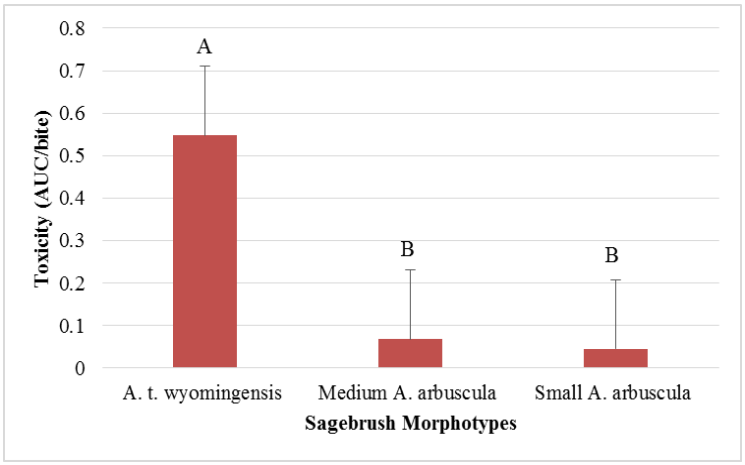

Figure 5. Average toxin per bite (AUC/bite) for each sagebrush morphotype from total area under the curve (F2,4330=572.8, p <0.0001, different letters = significant difference between groups)

Discussion

Our results suggest that diet selection of *A. arbuscula* is driven by toxins at the plant and leaf scale. Herbivores typically select plants that are high in protein (Gregg et al., 2008; Frye et al., 2013; Ulappa et al., 2014). In contrast, protein content was higher in *A. t. wyomingensis* than in *A. arbuscula* at our study site (results not shown). However, in another study with sage-grouse we compared the nutritional and chemical quality at the bite scale and found that diet selection was still driven by plant toxins (unpublished data). Our results suggest that sage-grouse foraged on plants that were smaller in shrub volume and leaf biomass because those plants had lower toxin concentrations. When examining the different sagebrush morphotypes in the field, *A. t. wyomingensis* had fewer bites per plant, but small and medium *A. arbuscula* had more bites per plant. *A. t. wyomingensis* has large shrub volume and leaf biomass per bite, but is the most toxic morphotype (highest concentration of monoterpenes) and fewer bites of this plant could be a strategy to limit toxin intake. Increased toxin consumption is energetically costly to process and to metabolize (Sorensen et al., 2005b; Sorensen et al., 2005c) and reducing the bite size is a strategy herbivores may employ to reduce toxicity (Torregrossa and Dearing, 2012).

Diet selection in winter increases our ecological understanding of how sage-grouse use their habitat at different spatial scales (patch, plant, and bite) and browsing may influence sagebrush morphotypes across a

landscape. Larger shrub volume did not influence browsing at the species level because sage-grouse selected *A. arbuscula* which has relatively low biomass. However, shrub volume for a particular sagebrush morphotype influenced foraging where sage-grouse selected medium over small *A. arbuscula*. Plant chemistry affects habitat selection at the patch and landscape scale (Wiggins et al., 2006; Frye et al., 2013), but there are few studies documenting diet selection at smaller spatial scales. Diet selection at the bite scale adds further insight about selective foraging which is important for herbivore foraging dynamics and in an avian specialist, the sage-grouse. The fine scale level of diet selection is unknown and bite scale selection may translate to diet selection at the landscape scale. Understanding multi-scale habitat selection can give insights how plant toxicity drives diet selection for sage-grouse. At the species level, sage-grouse foraged on sagebrush plants to minimize toxin intake by consuming *A. arbuscula* rather than *A. t. wyomingensis*, but at the morphotype level, sage-grouse selected *A. arbuscula* plants based on the shrub volume to increase biomass consumption. In addition, understanding the age of sagebrush plants along with morphotypes should be considered in future studies to know if the toxicity level increases with age.

In a changing climate, understanding sagebrush morphotypes at multiple spatial scales can increase our knowledge to protect habitats from destruction. By prioritizing high quality resources and habitats, managers may be able to decrease population declines for sensitive species such as sage-grouse, because declines in sage-grouse populations are associated with habitat fragmentation and degradation (Connelly et al., 2000; Schroeder et al., 2004; Aldridge et al., 2008). Biomass availability and toxicity of sagebrush species and morphotypes should be taken into consideration in habitat prioritization for conservation of sage-grouse.

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