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Management and Conservation

Using Gas Chromatography to Determine Winter Diets of Greater Sage-Grouse in Utah

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ABSTRACT Sagebrush (*Artemisia* spp.) constitutes the majority (>99%) of sage-grouse (*Centrocercus* spp.) winter diets. Thus, identification and protection of important winter habitats is a conservation priority. However, not all sagebrush may be alike. More information is needed regarding sage-grouse sagebrush winter dietary preferences for application to management. The objective of our research was to determine if chemical analysis of fecal pellets could be used to characterize winter sage-grouse diets as a substitute for more invasive methods. We collected and analyzed fecal pellets and sagebrush samples from 29 different sage-grouse flock locations in northwestern and southcentral Utah. Using gas chromatography, we were able to identify crude terpene profiles that were unique to Wyoming sagebrush (*A. tridentata wyomingensis*) and black sagebrush (*A. nova*). We subsequently used the profiles to determine sagebrush composition of sage-grouse fecal pellets, thus reflecting sage-grouse winter diets. This technique provides managers with a tool to determine which species or subspecies of sagebrush may be important in the winter diets of sage-grouse populations. © 2011 The Wildlife Society.

KEY WORDS *Artemisia*, black sagebrush, *Centrocercus urophasianus*, gas chromatography, Utah, winter diet, Wyoming sagebrush.

Greater sage-grouse (*Centrocercus urophasianus*; hereafter sage-grouse) populations have been declining over the last 5 decades (Connelly et al. 2004, Garton et al. 2011). On 4 March 2010, the United States Fish and Wildlife Service (USFWS) designated greater sage-grouse as a candidate species for protection under the Endangered Species Act of 1973 (USFWS 2010). The USFWS cited continued loss and/or fragmentation of important sage-grouse habitats as a major conservation concern (USFWS 2010).

Sage-grouse are sagebrush (*Artemisia* spp.) obligates, thus sagebrush is the most dominant feature of sage-grouse winter habitats (Gullion 1966). Sagebrush constitutes >99% of their winter forage (Patterson 1952, Dalke et al. 1963, Gullion 1966, Wallestad et al. 1975) and is used exclusively for escape and protective cover (Eng and Schlandweiler 1972, Beck 1977, Hupp and Braun 1989, Robertson 1991, Battazzo 2007). Previous research reported that sage-grouse exhibit forage preferences for certain sagebrush species or subspecies during the winter (Remington and Braun 1985, Welch et al. 1989, Welch et al. 1991, Rosentreter 2005). Preferences may be explained by protein levels in sagebrush leaves (Remington and Braun 1985). Other studies suggested that factors such as availability and secondary compounds influenced sagebrush selection (Welch et al. 1989, 1991). Sage-grouse dietary preferences likely vary among populations (Remington and Braun 1985, Welch et al. 1991). Sage-grouse use a variety of sagebrush habitats across the population range, therefore it is imperative for managers to be able to identify what types of sagebrush are being used as forage as part of their winter habitat assessment (Dalke et al. 1963, Remington and Braun 1985, Welch et al. 1989, Connelly et al. 2004, Thacker 2010).

Previously, sage-grouse diets have been determined using crop analysis or observational methods (Wallestad et al. 1975, Remington and Braun 1985, Barnett and Crawford 1994, Gregg et al. 2008). Crop sampling is accurate but requires grouse to be harvested in order to examine crop contents. This sampling technique may not be a viable option in small or declining populations and may not be a widely accepted practice for a candidate species. Observational studies can be effective, but have logistical limitations. Approaching winter sage-grouse flocks without disturbing or influencing foraging behavior is difficult (Thacker 2010). Indirect observations (identifying evidence of herbivory) can be used but lacks specificity to quantify grouse abundance in a foraging area, time of foraging, or composition of the diet. Likewise, captive studies have limited applicability to populations at landscape levels, because observations are made in artificial settings (Welch et al. 1991). Given the limitations of these methods, a simple, reliable, and accurate way to classify sage-grouse winter diets is needed.

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Sagebrush contains a suite of secondary compounds called terpenoids, which can be used to identify sagebrush species or subspecies (Kelsey et al. 1976). Because wintering sage-grouse feed exclusively on sagebrush, we hypothesized that sage-grouse winter diets could be determined by identifying sagebrush terpene profiles in fecal pellets. Others have used gas chromatography (GC) to identify terpene profiles of sagebrush species in the ingesta of other sagebrush obligates (White et al. 1982), but we were not able to find an example where this technique was used on fecal pellets. Our objective was to determine if GC could be used to identify sagebrush terpene profiles in sage-grouse pellets to classify winter diets.

STUDY AREA

We conducted our study in 2 Utah sage-grouse populations. One study area was located in western Box Elder County in northwestern Utah; the other study area was on Parker Mountain in Wayne County in southcentral Utah. Sage-grouse winter habitat occurs between 1,500–1,600 m and 2,400–2,600 m in Box Elder County and on Parker Mountain, respectively (Dahlgren 2006, Thacker 2010). West Box Elder County wintering areas were characterized by Wyoming sagebrush (*A. tridentata wyomingensis*) flats with black sagebrush (*A. nova*) inclusions on shallow soils and ridge tops. Parker Mountain has large black sagebrush flats, with large patches of Wyoming sagebrush occurring in areas with deeper soils.

METHODS

Sage-Grouse Tracking

We used radio-marked sage-grouse to locate and identify winter flocks for sampling purposes (Thacker 2010). We trapped sage-grouse during the spring and summer using the spotlight method; we captured 35 grouse in Box Elder County and 45 at Parker Mountain (Giesen et al. 1982). We conducted all handling under protocols approved by the Utah State University Institutional Animal Care and Use Committee (permits #1194 and #942). We weighed and aged captured grouse and fitted them with 19-g Holohil Systems^{TM} necklace radio-collars (Holohil Systems Ltd., Carp, ON, Canada).

We located radio-marked grouse using Communications Specialists^{TM} receivers (Orange, CA) and 3 element yagi antennas. We located each marked individual once during the study period (15 Jan 2008–10 Mar 2008); we attempted to locate all radio-marked grouse that we detected. At each winter flock location, we flushed and counted grouse (flock size range = 5–100). After we flushed grouse, the boundaries of the flock feeding areas were identified by observing fresh tracks in the snow and evidence of browsing by sage-grouse (Remington and Braun 1985). We carefully approached flocks because some flocks flushed at distances greater than 300 m. The premature flushes made it difficult to get an exact flock location. Sometimes, we were able to spot grouse with binoculars from several hundred meters away, allowing us to mark the location of the flock prior to flushing.

Sample Collection

Once we identified the location, we categorized vegetation communities based on the dominant sagebrush species. We classified habitats into 3 categories: Wyoming sagebrush, black sagebrush, and mixed sagebrush. We defined mixed sagebrush communities as feeding areas where both Wyoming sagebrush and black sagebrush were present.

We collected fresh pellet piles at each flock location. Fresh pellets were damp, dark green or dark brown in color (Dahlgren et al. 2006). We defined a pellet pile as a cluster of more than 2 pellets. We assumed all pellets in a cluster were deposited by a single grouse. We chose to collect 10 samples per flock because this would allow us to collect an adequate number of samples to test the GC method. If a flock was smaller than 10 grouse, we collected pellet piles equal to the number of grouse observed in the flock. We collected pellet clusters by walking transects within the feeding area until the specified number of clusters were collected. Therefore, transect lengths varied at each flock location. We were careful to avoid double sampling by observing tracks to ensure the pellet clusters collected were from different individuals. We labeled and stored each pellet sample individually in a sealable plastic bag.

We also collected sagebrush samples from each flock site. We collected a sagebrush sample consisting of a single live branch from 10 different plants along a 50-m transect within the feeding area, by collecting sample every 5 m. We sampled the plant closest to each 5 m interval. At mixed sagebrush sites, we collected 5 samples of each species for a total of 10 samples. We collected 1 branch with abundant leaf material and if plants had few leaves, we collected 2 branches to ensure sufficient leaf material for extraction processes (approx. 500 mg of fresh leaf material). We individually bagged and labeled all samples in sealable plastic bags. We stored pellet and sagebrush samples in a soft sided cooler packed with snow or ice until we could transfer them to a freezer.

Chemical Analysis

All samples were processed by the United States Department of Agriculture (USDA) Poisonous Plant Research Lab in Logan, Utah. We stored samples (fecal and sagebrush) in a freezer at -20° C until processing. We removed sage-grouse pellets from the freezer and thawed them at room temperature until they were soft. We manually crushed and mixed each sample and placed a 100-mg subsample into a 10-mL screw cap test tube. We removed sagebrush samples from the freezer and all leaves were stripped from the stems by hand. We crushed leaves using a mortar and pestle and placed 100 mg of the sample into a 10-mL screw cap test tube.

The chemical extraction process was the same for pellets and sagebrush samples. We added dichloromethane (5 mL) to each 100-mg sample and mixed the sample by mechanical rotation (inverting the tubes) for 15 min to extract terpenes. We removed a 1-mL aliquot with a glass pipette and filtered it through anhydrous sodium sulfate into a 2-mL auto

sample vial. We analyzed the samples using GC with flame ionization detection (FID) using a gas chromatograph (GC-2010; Shimadzu, Kyoto, Japan) and an auto sampler (AOC 20; Shimadzu). We injected samples (1.5 μL) in a split mode (30:1 split ratio) with an injection port temperature of 250° C. The GC column was a DB-5 capillary column (30 m × 0.32 mm, 0.25 μm) using helium as the carrier gas at a flow rate of 2 mL/min. Detector (FID) temperature was 325° C. We set column temperature to 60° C for 1 min, increased to 160° C at 5° C/min and then held at 160° C for 1 min for a total analysis time of 22 min. We characterized the terpene profiles of the samples by the GC retention time and relative peak intensities of the resulting GC chromatogram (Fig. 1). We used a visual examination of the terpene retention time peaks to identify differences in terpene profiles of sagebrush species. We used crude terpene profiles from all flock locations to identify specific peaks that were present in all locations. We then used the identified peaks to determine the species of sagebrush in each pellet sample. We compared crude terpene profiles using visual pattern recognition. We did not attempt to quantify the amount or specific information about the types of terpenes found in the sagebrush or pellet samples.

RESULTS

Black and Wyoming sagebrush exhibited unique terpene profiles (Fig. 1). We used 2 marker peaks within the crude terpene profile to identify each species of sagebrush (4.95 min and 6.91 min for black sagebrush, and 7.78 min and 10.71 min for Wyoming sagebrush). The crude terpene profiles from the fecal pellets were similar in terms of their terpene profiles when compared to respective plant profiles (Fig. 1). Therefore, we could identify the sagebrush species contained in each fecal pellet or if a pellet sample contained both species of sagebrush.

We collected samples from 29 sage-grouse flocks (19 and 10 in Box Elder and Parker Mountain, respectively) for a total of 286 pellet samples (185 and 101 in Box Elder and Parker Mountain, respectively). We were able to identify terpene profiles in all of the pellet samples. In Box Elder, 72% ($n = 134$) of pellets contained only black sagebrush, 22% ($n = 41$) were mixed, and 5% ($n = 10$) contained only Wyoming sagebrush. Over half (61%, $n = 62$) of Parker Mountain pellets contained only black sagebrush, whereas 33% ($n = 33$) contained only Wyoming sagebrush and 6% ($n = 6$) were composed of both species of sagebrush. These results are presented to demonstrate the use of the method and have limited inference beyond the identification of sagebrush species from these pellets; therefore, we are unable to discuss diet composition, preference, or selection mechanisms.

DISCUSSION

The GC method will enable biologists to describe winter diet composition and selection without using destructive or observational techniques. We were only able to approach

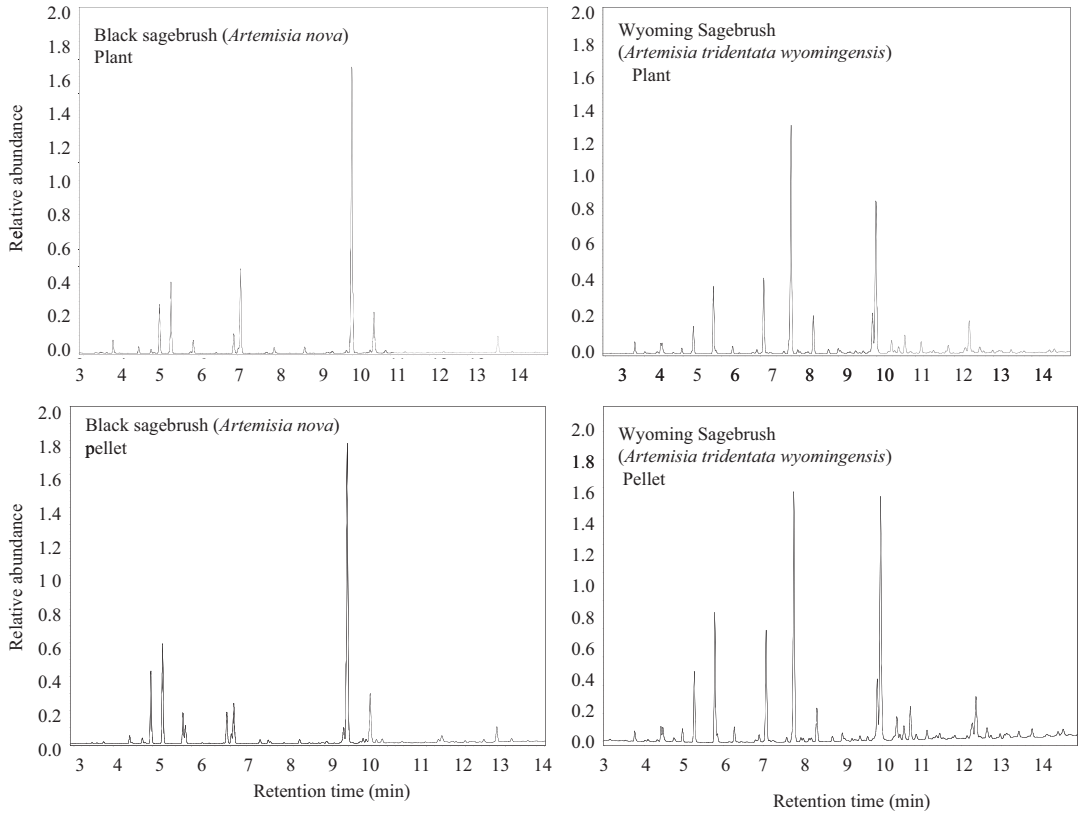

Figure 1. Terpene profiles from black sagebrush (*Artemisia nova*) and Wyoming sagebrush (*A. tridentata wyomingensis*) plants and sage-grouse (*Centrocercus urophasianus*) fecal pellets collected in West Box Elder County, Utah, 2008. These chromatograms show the similarities between plant and pellet profiles for black sagebrush and Wyoming sagebrush.

1 of the 29 flocks close enough to observe grouse foraging on specific plants using binoculars; thus, we would have to increase the amount of time spent in the field in order to collect reliable observational data. Remington and Braun (1985) relied heavily on evidence of herbivory at flock locations to determine sagebrush preferences. Indirect observation does not provide information such as diet heterogeneity among individuals and diet mixing. If relegated to observational methods in our study, we would not have been able to detect diet mixing by individual grouse. Crop sampling could detect diet mixing and variation between individuals, but crop sampling requires grouse to be harvested. Grouse harvest could limit the use of crop sampling as well as limiting the sample size because of its destructive nature. Theoretically, there is no limit to the number of samples that could be collected for GC analysis as samples can be readily collected in areas where grouse are present.

Limitations do exist with the GC method. If pellets contained both species of sagebrush, we were not able to determine the proportion of each species, only the presence or absence of each species. We also had no way of quantifying the amount of sagebrush eaten by grouse. Spring, fall, and summer diets contain other plants and insect material; therefore, diets during these periods are too complex to describe using GC analysis (Connelly et al. 2000). Quantification of false positives would require performing experiments using confined grouse and feeding them controlled diets. We conducted our study using wild birds in their natural environment; therefore, we could not document false positives. However, we are confident that false positives were low because grouse generally consumed the sagebrush type(s) that they were located in. Furthermore, the use of GC to distinguish sagebrush species using crude terpene profiles has been well documented (Kelsey et al. 1976), thus bolstering our confidence that false positives were low. Therefore, the GC method provides managers with a reliable tool for determining sage-grouse diets during the winter at broad spatial scales.

Our research was not designed to address causal factors in dietary selection or habitat selection. The results of the fecal analysis were presented to demonstrate the use of the method and have limited inference beyond the identification of sagebrush species from these pellets; therefore, we are unable to discuss diet composition, preference, or selection mechanisms. Further research is needed to understand winter diets and diet selection across the range of sage-grouse. The importance of understanding changes in winter diets due to various factors (i.e., climatic factors such as snow depth and temperature) are vital to understanding the importance of winter habitats and their long-term stability. Additionally, the GC method could be very important to help understand the impact of winter diet composition and nutrition on the reproductive success of sage-grouse hens. This could be important because winter diets may have an effect on the breeding success of sage-grouse hens (Gregg et al. 2008). All of these questions merit further research and the GC method will provide researchers and managers a valuable tool to address these questions.

MANAGEMENT IMPLICATIONS

The GC method should become an integral part of sage-grouse winter habitat assessments; this method will identify specific sage-grouse winter forages. Once important sagebrush species are identified managers can then determine abundance of preferred forages and plan management activities that will conserve and increase abundance and availability of important forage species. We also encourage the use of this method in future sage-grouse research; it will be essential in determining if winter diet composition influences reproductive success. The GC method will also help researchers better understand how winter diets are selected at a landscape scale. The GC method may also be used for any wildlife species, if they are consuming monotypic diets composed of plants containing secondary compounds.

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LITERATURE CITED

Barnett, K., and J. A. Crawford. 1994. Pre-laying nutrition of sage-grouse hens in Oregon. Journal of Range Management 47:114–118.

Battazzo, A. M. 2007. Winter survival and habitat use by female greater sage-grouse (*centrocercus urophasianus*) in south Philips County, Montana 2004–2006. Thesis, University of Montana, Missoula, USA.

Beck, T. D. 1977. Sage-grouse flock characteristics and habitat selection during winter. Journal of Wildlife Management 41:17–26.

Connelly, J. W., S. T. Knick, M. A. Schroeder, and S. J. Stiver. 2004. Conservation assessment of greater sage-grouse and sagebrush habitats. Western Association of Fish and Wildlife Agencies. Cheyenne, Wyoming, USA.

Connelly, J. W., M. A. Schroeder, A. R. Sands, and C. E. Braun. 2000. Guidelines to manage sage-grouse populations and their habitats. Wildlife Society Bulletin 28:967–985.

Dahlgren, D. K. 2006. Greater sage-grouse reproductive ecology and response to experimental management of mountain big sagebrush on Parker Mountain, Utah. Thesis, Utah State University Logan, USA.

Dahlgren, D. K., R. Chi, and T. A. Messmer. 2006. Greater sage-grouse response to sagebrush management in Utah. Wildlife Society Bulletin 34:975–985.

Dalke, P. D., D. B. Pyrah, D. C. Stanton, J. E. Crawford, and E. F. Schlatterer. 1963. Ecology, productivity, and management of sage-grouse in Idaho. Journal of Wildlife Management 27:811–841.

Eng, R. L., and P. Schlandweiler. 1972. Sage-grouse winter movements and habitat use in central Montana. Journal of Wildlife Management 36:141–146.

Garton, E. O., J. W. Connelly, C. A. Hagen, J. S. Harne, A. Moser, and M. A. Schroeder. 2011. Greater sage-grouse population dynamics and probability of persistence. Pages 293–382. *in* S. T. Knick and J. W.

Connelly, editors. Ecology and conservation of Greater sage-grouse: a landscape species and its habitats in avian biology series (Volume 38). University of California Press, Berkely, USA.
Giesen, K. M., T. J. Schoenberg, and C. E. Braun. 1982. Methods for trapping sage grouse in Colorado. Wildlife Society Bulletin 10: 224–231.
Gregg, M. A., J. K. Barnett, and J. A. Crawford. 2008. Temporal variation in diet and nutrition of preincubating Greater sage-grouse. Journal of Range Ecology and Management 61:535–542.
Gullion, G. W. 1966. A viewpoint concerning the significance of studies of game bird food habitats. Condor 68:372–376.
Hupp, J. W., and C. E. Braun. 1989. Topographic distribution of sage grouse foraging in winter. Journal of Wildlife Management 53:823–829.
Kelsey, R. G., M. S. Morris, and F. Shanfizadeh. 1976. The use of sesquiterpene lactones as taxonomic markers in the shrubby species of *Artemisia* (section tridentatae) in Montana. Journal of Range Management 29:502–505.
Patterson, R. L. 1952. The sage-grouse in the upper Green River Basin of Wyoming. Dissertation, University of Wyoming, Laramie, USA.
Remington, T. E., and C. E. Braun. 1985. Sage-grouse food selection in winter, North Park, Colorado. Journal of Wildlife Management 49:1055–1061.
Robertson, M. D. 1991. Winter ecology of migratory sage-grouse and associated effects of prescribed fire in southeastern Idaho. Thesis, University of Idaho Moscow, USA.
Rosentreter, R. 2005. Sagebrush identification, ecology, and palatability relative to sage-grouse. Pages 3–16 *in* N. L. Shaw, M. Pellant, and S. B. Monsen, compilers. Sage-grouse habitat restoration symposium proceedings. U.S. Department of Agriculture, Forest Service Proceedings, RMRS-P-38, Boise, Idaho, USA.
Thacker, E. T. 2010. Greater sage-grouse seasonal ecology and responses to habitat manipulations in northern, Utah. Dissertation, Utah State University, Logan, USA.
United States Fish and Wildlife Service [USFWS]. 2010. Endangered and threatened wildlife and plants: a 12 month finding for petitions to list the greater sage-grouse (*Centrocercus urophasianuas*) as threatened or endangered. Department of Interior, Fish and Wildlife Services. <http://www.fws.gov/mountain-pairie/species/birds/sagegrouse/FR03052010.pdf>. Accessed 23 Jul 2010.
Wallestad, R. O., J. G. Peterson, and R. L. Eng. 1975. Foods of adult sage-grouse in Central Montana. Journal of Wildlife Management 39:628–630.
Welch, B. L., J. C. Pederson, and R. L. Rodriguez. 1989. Monoterpenoid content of sage-grouse ingesta. Journal of Chemical Ecology 15:961–969.
Welch, B. L., F. J. Wagstaff, and J. A. Roberson. 1991. Preference of wintering sage-grouse for big sagebrush. Journal of Range Management 44:262–265.
White, S. M., B. L. Welch, and J. T. Flinders. 1982. Monoterpenoid content of pygmy rabbit stomach ingesta. Journal of Range Management 35:107–109.

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