5-2017

The Influence of Aspen Chemistry and the Nutritional Context on Aspen Herbivory

Kristen Y. Heroy
Utah State University

Recommended Citation

Heroy, Kristen Y., "The Influence of Aspen Chemistry and the Nutritional Context on Aspen Herbivory" (2017). *All Graduate Theses and Dissertations*. 6479.
https://digitalcommons.usu.edu/etd/6479

THE INFLUENCE OF ASPEN CHEMISTRY AND THE NUTRITIONAL CONTEXT ON ASPEN HERBIVORY

by

Kristen Y. Heroy

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Wildlife Ecology

Approved:

Juan J. Villalba, Ph.D. Major Professor	Samuel B. St.Clair, Ph.D. Committee Member
Paul C. Rogers, Ph.D. Committee Member	Johan du Toit, Ph.D. Committee Member
Eric Thacker, Ph.D. Committee Member	Jeffery O. Hall, DVM, Ph.D. Committee Member

Mark R. McLellan, Ph.D.
Vice President for Research and
Dean of the School of Graduate Studies

UTAH STATE UNIVERSITY
Logan, Utah

2017

ABSTRACT

The Influence of Aspen Chemistry and the Nutritional Context on Aspen Herbivory

by

Kristen Y. Heroy, Doctor of Philosophy

Utah State University, 2017

Major Professor: Dr. Juan J. Villalba
Department: Wildland Resources

Herbivory is one major force accelerating aspen decline in North America, but it is unclear why herbivores prefer certain aspen stands over others, or over other plant species in the understory. In this dissertation, I determined the influence of nutrients and plant secondary compounds (PSC), physiological state, chemical composition, and prior experience on aspen preference by sheep in controlled pen experiments. In addition, I explored the relationship between herbivory, regeneration, recruitment, and other landscape elements for specific aspen stands within Wolf Creek Ranch in northern Utah using biomass and chemical composition of the understory and chemical defenses of juvenile aspen trees (i.e., the foodscape). Aspen intake was enhanced when lamb diets contained a high crude protein to energy ratio or when the basal diet contained a low density of energy. Intake was depressed as concentrations of PG (phenolic glycosides) increased in aspen leaves or when lambs were fed a high energy to protein ratio. The effects of nutrients on aspen intake were greater when phenolic glycosides in aspen were present at low concentrations. However, when given a choice between aspen leaves of high or low PG content, lamb preference depended more on aspen nutrient and mineral availability, or on prior diet, than on defense chemistry. On the landscape, I found that stands at low elevations with low abundance of nutrients in the understory are more likely to experience less regeneration and recruitment than those growing within nutrient-rich sites. Aspen browsing was negatively correlated with PG content in aspen stands, and elk presence (measured via fecal pellets) was negatively correlated with abundance of understory protein.

In conclusion, aspen herbivory appears to be controlled by the interplay between types and amounts of nutrients offered by the landscape and the chemical composition of aspen stands. A clear assessment of these variables on the landscape, i.e., the foodscape, will aid in the development of novel management strategies aimed at providing nutrients (e.g., through supplements, introduced forages) at strategic locations in order to reduce aspen herbivory within at-risk aspen stands.

(174 pages)

PUBLIC ABSTRACT

The Influence of Aspen Chemistry and the Nutritional Context on Aspen Herbivory

Kristen Y. Heroy

Consumption of aspen by herbivores is one major force causing aspen decline in North America. In this Dissertation, I aimed to determine why herbivores prefer browsing on certain aspen stands over others, and why they prefer consuming aspen that contains chemical defenses over understory forages like grasses, forbs, and shrubs. I explored the influence of nutrients and chemical defenses within aspen on aspen intake and preference by lambs in pen experiments. I also explored drivers of aspen preference on the landscape by looking at relationships between aspen herbivory, indicators of aspen health, amount of nutrients available in the understory, and chemical defenses in aspen leaves.

I found that as dietary protein and energy increased or decreased, respectively, lambs increased the amount of aspen consumed, and these effects were greater when chemical defenses in aspen leaves occurred at low concentrations. In addition, when lambs were presented with a choice between aspen stands of contrasting concentrations of chemical defenses (high vs. low), other nutrients in aspen leaves (e.g., minerals, protein), or prior experience that lambs had with high-protein rations were more important at driving aspen preference than chemical defense content. On the landscape, aspen health was better at high elevations where amounts of crude protein and grasses were greater than at lower elevations. A negative association was found between aspen herbivory and concentration of chemical defenses, and between elk presence (measured by fecal pellets) and the amount of protein in the understory.

These results suggest that nutrients in the understory interact with chemical defenses of aspen trees to influence aspen use by herbivores. This knowledge could be used by managers to modify the distribution of nutrients in the landscape (e.g., via supplementation, seeding programs, fire) in relation to the nutritional composition of the understory and aspen trees such that browsing is minimized in at-risk aspen stands.

ACKNOWLEDGMENTS

Dr. Juan Villalba, thank you for allowing me this opportunity to complete my degree, and for putting up with me throughout this entire learning process. Your patience has been more than admirable–thank you. I would also like to thank my committee members, Drs. Johan du Toit, Jeff Hall, Paul Rogers, Sam St. Clair, and Eric Thacker for their support and guidance throughout these past few years.

I give special thanks to my family. Dad and Gommie- although I wish you were here in body, I know you’re still here in spirit. Your love and guidance are and will always be apparent no matter how long you’ve been gone. Mom, thank you for your continued support all these years. I know I put all of you through hell for quite a while, but without you, I wouldn’t be alive today to write this. I really can’t thank you enough. I could not have done it without you. I love you. Finally, I would like to thank my kids- Allie and Ben- you two have been my rocks throughout this entire process. Thank you for being so wonderful.

Kristen Y. Heroy

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Aspen (*Populus tremuloides* Mich.) is the most widely distributed tree species in North America, ranging from the east to the west coast and from Arizona to Alaska (Little 1971). As a keystone species, aspen significantly contributes to biological diversity in sometimes otherwise relatively low-diversity landscapes (Kay 1997), providing significant habitat for vascular plants (Chong et al. 2001), wildlife (DeByle 1985), and insects (Jones et al. 1985; Chong et al. 2001), while contributing to improved water retention in watersheds (Mueggler 1985; Bartos and Campbell 1998; Lamalfa and Ryle 2008).

Despite its successful establishment across diverse landscapes and environmental conditions, some scientists report that aspen is declining throughout the Intermountain West (Bartos and Campbell 1998; Worrall et al. 2008). Climate change, fire suppression, and browsing by both livestock and wildlife have been suggested as causes for this decline (Kay 1997; White et al. 1998; Bartos and Campbell 1998; Bartos 2001). Within the multiple variables that influence aspen decline, ungulate herbivory emerges as a significant causative force with the potential to be controlled through management (Seager et al. 2013; Rogers et al. 2014). For instance, in many parks and wildlife refuges, Rocky Mountain elk (*Cervus elaphus* L.) experience low hunting pressure. Elk congregate in these areas in high densities, leading to significant increases in aspen herbivory (White et al. 2003). In addition to browsing by wildlife, cattle and sheep also browse on aspen trees and suckers (Dockrill et al. 2004). Concerns over aspen herbivory are currently causing considerable conflicts between federal land managers, hunters, environmental groups, and ranchers (e.g., Monroe Mountain Working Group).

Herbivory is a powerful disturbance that influences the evolution of all plants and plant communities (Stephen and Krebs 1986; Palo and Robbins 1991). Repeated loss of foliage and damage to meristematic tissues due to herbivory can negatively impact aspen suckers through the next growing season, especially if resources such as water and nutrients are limited (Erbilgin et al. 2014). Damage is compounded when cattle grazing coincides with wildlife herbivory (Kay and Bartos 2000), opening avenues for other plant species to invade (Vavra et al. 2007). In addition, chronic herbivory may decrease the amount of successful sucker recruitment leading to “missing age classes” in the aspen stand (Bartos et al. 1994; Ripple and Larsen 2000). With fewer young trees surviving, stand resilience decreases. Without intervention, the loss of successful sucker recruitment can lead to stand death (Hessl 2002).

Aspen suckers and juvenile trees are more nutritious than slower growing mature trees and therefore are more appealing to herbivores (Cebrian and Duarte 1994; Cook 2002). As the growing season progresses, understory plants start to senesce and available nutrition declines. During this period, many ungulates switch to aspen as their primary forage source causing significant damage to suckers and juvenile trees (Bartos and Campbell 1998; Dockrill et al. 2004). Because individual aspen trees are short-lived, intense herbivory can lead to stand collapse (Hessl 2002; Rogers and Mittanck 2014).

Compounding the problems caused by foliage herbivory, large herbivores such as elk and moose will eat the bark of the remaining aspen trees when understory and other food resources are scarce, such as in the depths of winter or in cases of overbrowsing, when animal densities are high (DeByle 1985). Additional bark damage is incurred when elk and other mammals rub their antlers on aspen trees to remove velvet before the rut (Hinds and Krebill 1975; DeByle 1985; Johnston and Naiman 1990). Damage to the trunks of aspen predisposes trees to fungal infections as well as to premature death through damage of the vascular tissues under the bark (Hart and Hart 2001; White et al. 2003; Packard 1942; Walters et al. 1982).

Being rhizomatous and having fast turnover of trees, aspen stands thrive in the presence of fire (Bartos and Mueggler 1981; Brown and DeByle 1989). Fire helps to remove other above ground plants, giving aspen competitive advantage over other trees. The survival of underground aspen roots is dependent on the amount of flammable ground cover and therefore on the length, severity, and temperature of the fire (Dimitrakopoulous and Martin 1990). After a number of highly destructive fires following a slew of poor management decisions, a crusade began in the late 1800's that labeled all fires as bad for people and the land—a sentiment which still remains popular today (Dombeck et al. 2003). Fire suppression efforts have only grown since the late 1800's. The spread of humans into fire-prone areas increases the amount of resistance to allowing these fires to run their course. Without proper fire management, aspen can struggle to compete with the surrounding vegetation.

Uses for aspen

Evidence of browsing pressure on trembling aspen and accompanying understory in western North America are not only present, but also ecologically far reaching (Rogers and Mittanck 2014). There are multiple reasons to maintain healthy aspen stands, from aesthetics, economics, proper functioning of ecosystems, and diversity (Mitton and Grant 1996; Seager et al. 2013). The aesthetic draw of aspen is essential for some tourism locales, bringing in money essential for their economy. One example in Colorado, where aspen is central to the state's aesthetic beauty, visitors to Colorado National Parks in 2012 brought $14.7 billion to the state (Cullinane et al. 2014). The pulp and paper industry also relies heavily on aspen for a large portion of their economy (Mitton and Grant 1996; Perala and Carpenter 1985; Youngquist and Spelter 1990). Aspen is also used for a wide range of other applications such as pellet fuels, matchsticks, tongue depressors, and many others because of the ease of working with the straight-grained soft wood and because of its ability to hold glue and paint (Perala and Carpenter 1985; Youngquist and Spelter 1990).

Aspen trees provide productivity and structural diversity to both large and small flora and fauna (Reynolds 1969; Debyle 1985; Turchi et al. 1995; Hollenbeck and Ripple 2007; McCullough et al. 2012). Understory diversity is aided with the presence of healthy sprouting aspen stands, which also lead to increased animal diversity (Krzic et al. 2003; Royo et al. 2010; Debyle 1985; Bailey and Witham 2002).

Aspen herbivory

Aspen herbivory appears to be a function of the nutrients available in aspen tissues relative to those present in the landscape (Holeski et al. 2016), and of plant defense chemicals which impose barriers to the abilities of insect (e.g., Holeski et al. 2009) and mammalian (Wooley et al. 2008) herbivores to utilize aspen trees. A lack of understanding regarding the interactive effects of defense chemicals and nutrients from aspen and surrounding understory on aspen herbivory makes implementing management strategies aimed at abating this process difficult to attain. In order to devise such strategies, critical research is needed to explore the impact of aspen chemistry and nutrition on intake and preference by mammalian herbivores.

Aspen and chemical defenses

Defensive strategies employed by plants to reduce herbivory involve the production of chemical defenses or plant secondary compounds (PSC) (Palo and Robbins 1991). Aspen has evolved a chemical defense system of carbon based secondary compounds—such as phenolic glycosides (PG) and condensed tannins (CT)—which are reliable indicators of total secondary compounds contained in the plant (Clausen et al. 1989; Wooley et al. 2008). Condensed tannins produced within aspen leaves and bark may aid to deter ungulate browsing (Hagerman et al. 1992). Some studies have shown that PG content of aspen may reduce consumption of the defended plant (Bailey et al. 2007; Wooley et al. 2008; Villalba et al. 2014). However, less is known about the influence of PG than CT on mammalian herbivores (Lindroth and St. Clair 2013), especially interactions between PG content of aspen and other nutrients on the landscape.

Young aspen trees and suckers are more susceptible to herbivory have as much as 25% of their total leaf weight comprised of these PSC (Seager et al. 2013). As young ramets grow above the browse line (to >2 m) and are generally safe from herbivory, the amount of phenolic glycosides in the foliar tissue decreases (Donaldson and Lindroth 2007; Smith et al. 2011). Captive elk tend to prefer aspen with lower PG concentrations, suggesting that high concentrations of these phenolic compounds may decrease herbivory (Wooley et al. 2008). The ability to successfully deter herbivory may allow these stands to survive times of high browsing pressure (Lindroth 2001). However, secondary compounds are not always effective at deterring herbivory of young aspen trees, as browsing may occur regardless of the concentrations of defense chemistry in the clone or stand (Hessl 2002; Lindroth and St. Clair 2013), particularly where animal populations are very high.

Tannins cause digestive upset and reduce the amount of available proteins and minerals in the diet of animals without tannin-binding salivary proteins (Frutos et al. 2004; Robbins et al. 1987a; Kumar and Singh 1984; Kibon and Ørskov 1993). Browsers, or concentrate feeders such as deer, produce tannin-binding salivary proteins in order to counteract some of the negative effects of dietary tannins on consumers' physiological processes (Shimada 2006). In fact, aspen tannins may be ineffective against browsing ungulates because of the presence of such salivary proteins (Erwin et al. 2001). Because they do not routinely consume tannin-containing forage and because it is metabolically expensive to produce these proteins, grazers do not typically produce tannin-binding salivary proteins. Intermediate feeders (e.g., goats, elk, sheep) fall somewhere in between grazers and browsers, and have a plastic response to tannin consumption. When these animals do consume tannin-containing forages, over time they are able to produce these proteins (Robbins et al. 1987b; Robbins et al. 1991). Because salivary proteins are not always needed, intermediate feeders reduce the costs of producing tannin-binding salivary proteins by employing this plastic strategy (McArthur et al. 1995; Bennick 2002). Grazers, like cattle, have a less successful strategy than sheep, elk, and other intermediate feeders. When present however, tannin-binding salivary proteins in cattle have less than half the affinity for tannins than do salivary proteins in deer (Shimada 2006). Grazers, therefore, will show less preference for forages containing tannins than would browsers or intermediate feeders. Sometimes however, tannins can be beneficial like in the case of bypass proteins; certain types of condensed tannins may also enhance protein nutrition by increasing the supply of dietary essential and branched-chain amino acids reaching the small intestine (Reed 1995; Foley et al. 1999)

Phenolic glycosides deter insect herbivory, but much less information is available regarding their effects on ungulates (Hagerman et al. 1992; Hemming and Lindroth 1995). Reduced intake rates, fecundity, and growth have been recorded in herbivorous insects (Lindroth and Hwang 1996; Osier et al. 2000; Osier and Lindroth 2001; 2004; and 2006). Two studies conducted with elk and aspen suggest that elk prefer aspen with a low concentration of PG over aspen with high concentrations of PG (Bailey et al. 2007; Wooley et al. 2008). Wooley et al. (2008) concluded elk consumed 30% less aspen when PG concentrations in the plant were high (>20%) than when PG concentrations were below 15%. They also concluded that relatively high concentrations of PG in aspen (>20%) will only discourage aspen consumption if animal density is low or if other forage options are available in the landscape. In addition, Villalba et al. (2014) found that sheep increase consumption of aspen as PG concentration declines.

Aspen and complementarity among food resources

Plant secondary compounds may interact with nutrients present in aspen and in the aspen understory to influence foraging by mammalian herbivores (Provenza and Villalba 2006). Certain nutrients and PSC within the surrounding vegetation and aspen foliage may interact in the gastrointestinal tract of an herbivore leading to synergisms or antagonisms among food resources. For instance, synergy among chemically defended forages occurs when ingestion of these forages results in a greater negative effect than the effects incurred by consuming similar amounts of each forage in isolation (Hay et al. 1994; Hay 1996). Examples of synergism can be seen when inhibitors of detoxification pathways like piperonyl bytoxide, present in commercially available pesticides, are ingested in close temporal proximity to plant secondary compounds (Herrera and Pellmyr 2009).

Antagonism occurs when negative postingestive effects of secondary compound-containing forages are attenuated when ingested with other forages. This is seen when proteins bind to tannins and reduce the negative postingestive effects of tannins on an animal's physiological processes (Hay et al. 1994; Hay 1996). Plant secondary compounds within nearby forages may also be complementary or non-complementary. Complementary PSC are those which are detoxified by different systems—i.e. one compound is detoxified by the liver and the other is detoxified within the digestive tract (Cheeke 1998; McLean and Duncan 2006). Ingestion of complementary toxins allows a ruminant to consume an amount of forage from each PSC family, which is only limited by the animal's ability to metabolize toxins through each system individually. Non-complementary PSC are those which are detoxified by the same system. Ingestion of non-complementary toxins limits the total amount of forage an animal can ingest because it constrains the quantity of toxins which can be handled by one detoxification system. In this way, preference for or against a particular forage depends on context–the availability of other forages in the plant community.

Biochemical context and aspen preference

The diversity of biological chemicals in the landscape, as discussed above, influences food choice, and can be understood as the biochemical context in which a certain plant is ingested by an herbivore. Temporal and spatial distribution of plants, as well as types and concentration of nutrients and defenses in those plants are all key variables of the biochemical context that shape intake and preference by mammalian herbivores (Baraza et al. 2005; Villalba et al. 2006). A nutritious plant growing with neighbors of lower nutritional quality will experience greater herbivory than the same plant growing with neighbors of greater nutritional value. Thus, the context where a plant is growing influences foraging preference (Bergman et al. 2005; Atsatt and O'Dowd 1976; Tahvanainen and Root 1972). In contrast, the Repellent Plant Hypothesis describes the effect of a desirable plant growing among less desirable species (Atsatt and O'Dowd 1976; Barbosa et al. 2009; Wahl and Hay 1995). For instance, if a group of highly nutritious plants are growing with defended neighbors within a patch, the nutritious plants are less likely to be browsed given that those defended neighbors may discourage herbivores from visiting the patch (Augner et al. 1991).

The temporal dimension also influences the biochemical context because plants change in chemical composition throughout the growing season. Thus, as the year progresses, the context changes, and the acceptability of different forages in the landscape changes as a consequence. Herbivores form strong preferences for foods which contain required concentrations and proportions of nutrients (Villalba and Provenza 1999), and aspen suckers growing in patches can represent a concentrated source of nutrients that attract wild (White et al. 2003) and domestic (Jones et al. 2011) ungulates—particularly in a senescent understory during late summer and early fall—thus suppressing aspen regeneration (Bailey et al. 2007).

Prior experience and aspen preference

Prior experience with various forages and PSC-containing species drives forage choices. Animals begin learning *in utero* and continue after birth when they consume milk that contains flavors of foods their mother is ingesting. After weaning, animals will continue to prefer those flavors they experienced when they received milk from their mothers (Galef and Sherry 1973; Nolte and Provenza 1992; Nolte et al. 1991). If an animal does consume a forage that causes digestive upset—caused by a physiological feedback system triggered by the stimulation of the emetic system within the midbrain and brainstem—an aversion to the food may be formed (Garcia 1989; Mitchelson 1992). Aversions can be formed up to eight hours after the food is consumed and may last years after the initial experience (Burritt and Provenza 1991). If there is no alternative available other than the food that caused the aversion, aversions last less time than if there were other forages available with shorter extinction times as the nutritional quality of the alternative food decreases (Kimbal et al. 2002). Taste plays an important role in mediating aversions because animals are more likely to associate nausea with taste than other stimulation such as audiovisual stimulation (Garcia and Koelling 1966). When few desirable food alternatives are available within a grazing area, such as during the dry season or in cases of high animal densities, ruminants will consume PSC-containing forage (Papachristou et al. 2007; Genin and Pijoan 1993; Mellado et al. 2003). However, because PSC-containing forage causes food aversions, animals may be less likely to consume the PSC-containing forage during periods when other options are available (Baraza et al. 2005; Provenza et al. 2003). Social facilitation can reduce the time required to extinguish a food aversion, which can otherwise last for years (Ralphs 1997). Social facilitation is the "performance of a pattern of behavior already in an individual's repertoire, as a consequence of the performance of the same behavior by other individuals." (Hinde 1970). Therefore, if one animal (or animals) in a group learned that aspen stands provide required nutrients at certain times of the year, other individuals within the group will learn from that model.

Nutritional state and aspen preference

Provenza (1995) referred to the ability of an animal to determine what forages they need to consume as nutritional wisdom. The interplay between the needs of the animal (the physiological state) and the nutritional make-up of the plant leads to the development of food preferences (Provenza 1996; Provenza and Villalba 2006), as physiological need dictates which foods ought to be preferred (i.e., those providing the required nutrients) or avoided (i.e., those providing an excess or deficit of nutrients or toxins). These concepts are apparent when animals have recently consumed food high in protein, as they will seek out foods high in energy, and vice-versa (Villalba and Provenza 1999; Emmik 2007). When an animal consumes enough of a nutrient, any excess of that nutrient can cause digestive upset leading to avoidance of that nutrient (Villalba and Provenza 1999), also referred to as nutrient-specific satiety (Villalba and Provenza 2007).

Animals are also able to distinguish their nutritional needs after consuming a toxic food. Sheep will choose a food with a higher protein to energy ratio after consuming a food containing tannins therefore reducing the toxic effects incurred from consuming the toxic food (Villalba et al. 2002a; Villalba et al. 2002b). This same concept can be applied when an animal has consumed forages with required ratios and concentrations of protein to energy. They will improve their nutritional state and experience an appropriate plane of nutrition. Animals on an appropriate plane of nutrition are able to consume greater amounts of PSC-containing forages than when they experience a lower plane of nutrition because detoxification and elimination of toxins require energy and protein (Illius and

Jessop 1995; 1996; Baraza et al. 2005).

Hypothesis, predictions, and objectives

Given the aforementioned ability of herbivores to discriminate their nutritional needs and the potential impact of the biochemical context on aspen preference, *I hypothesized that nutrients in aspen and surrounding vegetation interact with plant secondary compounds to influence aspen use by herbivores.* Thus, *I predicted that:*

(1) Supplementary nutrients (e.g., carbohydrates, protein) enhance, whereas aspen chemical defenses (e.g., phenolic glycosides or condensed tannins) and forage alternatives constrain aspen intake and preference by herbivores.

(2) An animal's physiological state, as well as its experiences with ingesting nutrients and chemical defenses, influence aspen intake and preference.

(3) The availability of nutrients and chemical defenses in the landscape (e.g., the foodscape) reduce aspen use by herbivores.

Different studies were designed to test these predictions in controlled (Chapters 2 and 3) and field (Chapter 4) settings:

Objective 1 (Chapter 2): Determine aspen intake and preference by sheep in response to the provision of supplementary nutrients or plant secondary compounds

I fed different supplements (e.g., high in protein, high in energy, high in plant secondary compounds) to sheep and then determined the animals' capacity to ingest aspen with different concentration of chemical defenses (e.g., phenolic glycosides). During testing, aspen leaves were offered as the sole feed or in a choice with other forages (Utah pea, smooth bromegrass) commonly present in an aspen understory (Test of Prediction 1).

Objective 2 (Chapter 3): Explore the influence of the nutritional state and prior experience on aspen intake and preference by sheep

I determined the capacity of sheep to consume aspen leaves with different concentration of phenolic glycosides (high or low) when their nutritional state was skewed by offering rations with high concentrations protein or energy. I also explored the influence of the chemical composition of aspen leaves (and the impact of prior feeding experiences) on aspen preference by offering choices of aspen leaves with high or low concentration of phenolic glycosides (Test of Prediction 2).

Objective 3 (Chapter 4): Assess the influence of nutrients and plant secondary compounds in the landscape (the foodscape) on aspen herbivory and on indicators of aspen resilience

I characterized the chemical and nutritional composition of different aspen stands and understories across a gradient of aspen use by elk in order to determine whether aspen herbivory is influenced by secondary chemistry and/or the nutrients offered by the landscape (e.g., the foodscape) (Test of Prediction 3).

Objective 4 (Chapter 5): Summary

I integrated the findings obtained in controlled and field experiments with the aim of providing the basis for the development of innovative management strategies aimed at mitigating aspen use by mammalian herbivores in at-risk stands.

References

Atsatt PR, O’Dowd DJ (1976) Plant defense Guilds. Science 193(4247):24-29

Augner M, Fagerström T, Tuomi J (1991) Competition, defense and games between plants. Beh Ecol Sociobiol 29(4):231-234

Bailey J, Witham TG (2002) Interactions among fire, aspen, and elk affect insect diversity: reversal of a community response. Ecology 83, 1701-1712

Bailey JK, Schweitzer JA, Rehill BJ, Irschick DJ, Whitham TG, Lindroth RL (2007) Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. Biol Invasions 9:715–722

Baraza E, Villalba JJ, Provenza FD (2005) Nutritional context influences preferences of lambs for foods with plant secondary metabolites. Appl An Beh Sci 92(4):293-305

Barbosa P, Hines J, Kaplan I, Szendrei Z (2009) Associational Resistance and Associational Susceptibility: Having Right or Wrong Neighbors. Annual Rev Ecol Evol Sys 40(1): 1-20

Bartos DL, Brown JK, Booth BD (1994) Twelve years biomass response in aspen communities following fire. J Range Manage 47:79–83

Bartos DL (2001) Landscape dynamics of aspen and conifer forests. In: Shepperd WD, Binkley D, Bartos DL, Stohlgren TJ (eds) Sustaining aspen in Western landscapes: symposium proceedings; Grand Junction, CO. Proceedings RMRS-P-18. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station pp 5-14

Bartos DL, Campbell RB Jr (1998) Decline of quaking aspen in the interior West-examples from Utah. Rangelands 20(1):17-24

Bartos DL, Mueggler WF (1981) Early succession in aspen communities following fire in western Wyoming. J Range Manage 34(4): 315-318

Bennick A (2002) Interaction of plant polyphenols with salivary proteins. Crit Rev Oral Biol Med 13(2):184-196

Bergman M, Iason GR, Hester AJ (2005) Feeding patterns by roe deer and rabbits on pine, willow and birch in relation to spatial arrangement. Oikos 109(3): 513–520

Brown JK, DeByle NV (1989) Effects of prescribed fire on biomass and plant succession in western aspen. USDA Forest Serv Res Pap INT-412

Burritt EA, Provenza FD (1991) Ability of lambs to learn with a delay between food ingestion and consequences given meals containing novel and familiar foods. Appl Anim Behav Sci 32:179–189

Cebrian J, Duarte CM (1994) The dependence of herbivory on growth rate in natural plant communities. Func Ecol 8:518–525

Cheeke PR (1998) Natural toxicants in feeds, 2nd Edn, Interstate publishers, Inc., Danville, Il

Chong GW, Simonson SE, Stohlgren TJ, Kalkhan MA (2001) Biodiversity: Aspen stands have the lead, but will nonnative species take over? In: Shepperd WD, Binkley D, Bartos DL, Stohlgren TJ, Eskey LG (Compilers). Symposium Proceedings RMRS-P-18 on Sustaining Aspen in Western Landscapes, Grand Junction, CO, 13–15 June 2000. USDA, Forest Service, Rocky Mountain Research Station, Fort Collins, CO, pp 261–271

Clausen TP, Reichardt PB, Bryant JP, Werner RA, Post K, Frisby K (1989) Chemical model for short-term induction in quaking aspen (*Populus tremuloides*) foliage against herbivores. J Chem Ecol 15:2335

Cook JG (2002) Nutrition and food. In: Toweill DE, Thomas JW (eds) North American Elk: Ecology and Management. Stackpole Books, Harrisburg, PA, pp 259–349

Cullinane Thomas C, Huber C, Koontz L (2014) 2013 National Park visitor spending effects: Economic contributions to local communities, states, and the nation. Natural Resource Report NPS/NRSS/EQD/NRR—2014/824. National Park Service, Fort Collins, Colorado

DeByle NV (1985) Wildlife. In: DeByle NV, Winokur RP (eds) Aspen: Ecology and Management in the Western United States. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp. 135–152, GTR-RM-119

Dimitrakopoulous AP, Martin RE (1990) Measuring and modelling soil-temperature profiles during simulated wildland fire conditions. Proceedings of the International Conference on Forest Fire Research, Coimbra, Portugal, pp 21-115

Dockrill CWM, Blenis PV, Bailey AW, King JR (2004) Effect of summer cattle grazing on aspen stem injury, mortality and growth. The Forestry Chronicle 80: 257-261

Dombeck MP, Williams JE, Wood CA (2003) Wildfire policy and public lands: integrating scientific understanding with social concerns across landscapes. Conserv Biol 18(4):883-889

Donaldson JR, Lindroth RL (2007) Genetics, environment, and their interaction determine efficacy of chemical defense in trembling aspen. Ecology 88:729–739

Emmik DL (2007) Foraging behavior of dairy cattle on pastures. Ph.D. Thesis. Logan, UT: Utah State University

Erbilgin N, Galvez DA, Zhang B, Najar A (2014) Resource availability and repeated defoliation mediate compensatory growth in trembling aspen (*Populus tremuloides*) seedlings. PeerJ 2:e491 https://dx.doi.org/10.7717/peerj.491

Erwin EA, Turner MG, Lindroth RL, Romme WH (2001) Secondary plant compounds in seedling and mature aspen (*Populus tremuloides*) in Yellowstone National Park, Wyoming. The American Midland Naturalist 145(2):299-308

Foley WJ, Iason GR, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Jung HG and Fahey GC Jr. (eds) Nutritional ecology of herbivores. Proc Vth Int Symp Nutr Herb Am Soc Anim Sci, Il, pp 130–209

Frutos P, Hervás G, Giráldez FJ, Mantecón AR (2004) Review. Tannins and ruminant nutrition. Spanish Journal of Agricultural Research 2(2):191-202

Galef BG Jr, Sherry DF (1973) Mother's milk: a medium for transmission of cues reflecting the flavor of mother's diet. J Comp Physiol Psychol 83: 374–378

Garcia J (1989) Food for Tolman: Cognition and cathexis in concert. In: Archer T and Nilsson L (eds) Aversion, Avoidance and Anxiety. Earlbaum, Hillsdale, NJ, pp 45-85

Garcia J, Koelling RA (1966) Relation of cue to consequence in avoidance learning. Psychonomic Science 4:123-124

Genin D, Pijoan AP (1993) Seasonality of goat diet and plant acceptabilities in the coastal scrub of Baja California. Small Rum Res 10:1–11

Hagerman AE, Robbins CT,WeerasuriyaY, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. J Range Manage 45:57–62

Hart JH, Hart DL (2001) Interaction Among Cervids, Fungi, and Aspen in Northwest Wyoming. USDA Forest Proceddings RMRS 18:197-205

Hay ME (1996) Defensive synergisms? Reply to Pennings. Ecology 77:1950-1953

Hay ME, Kappel QE, Fenical W (1994) Synergisms in plant defense against herbivores: interactions of chemistry, calcification, and plant quality. Ecology 75:1714-1727

Hemming JD, Lindroth RL (1995) Intraspecific variation in aspen phytochemistry: effects on performance of gypsy moths and forest tent caterpillars. Oecologia 103:79–88

Herrera CM, Pellmyr O (2009) Plant animal interactions: an evolutionary approach. John Wiley & Sons

Hessl A (2002) Aspen, elk, and fire: the effects of human institutions on ecosystem processes. BioScience 52:1011–1022

Hinde RA (1970) Animal Behaviour. A Synthesis of Ethology and Comparative Psychology. McGraw-Hill, New York

Hinds TE, Krebill RG (1975) Wounds and canker diseases on western aspen. US Dep Agric For Pest Leafl 152

Holeski LM, Vogelzang A, Stanosz G, Lindroth RL (2009) Incidence of Venturia shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype. Biochem Syst Ecol 37:139–145

Holeski LM, McKenzie SC, Kruger EL, Couture JJ, Rubert-Nason K, Lindroth RL (2016) Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. J Ecol 104:850-863

Hollenbeck JP, Ripple WJ (2007) Aspen and conifer heterogeneity effects on bird diversity in the northern Yellowstone ecosystem. West North Am Naturalist 67:92-101

Illius AW, Jessop NS (1995) Modeling metabolic costs of allelochemical ingestion by foraging herbivores. J Chem Ecol 21, 693–719

Illius AW, Jessop NS (1996) Metabolic constraints on voluntary intake in ruminants. J Anim Sci 74: 3052–3062

Johnston CA, Naiman RJ (1990) Browse Selection by Beaver Effects on Riparian Forest Composition. Can J For Res 20(7):1036-1043

Jones JR, DeByle NV, Bowers DM (1985) Insects and other invertebrates. In: DeByle NV, Winokur RP (eds) Aspen: Ecology and Management in the Western United States. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp 107–114, GTR-RM-119

Jones BE, Lile DF, Tate KW (2011) Cattle selection for aspen and meadow vegetation: implications for restoration. Range Ecol Manage 64:625–632

Kay CE (1997) Is aspen doomed? J Forest 95(5):4–11

Kay CE, Bartos DL (2000) Ungulate herbivory on Utah aspen. Assessment of long-term exclosures. J Range Manage 53:145–153

Kibon A, Ørskov ER (1993) The use of degradation characteristics of browse plants to predict intake and digestibility by goats. Anim Prod 57:241–247

Kimball BA, Provenza FD, Burritt EA (2002) Importance of alternative foods on the persistence of flavor aversions: Implications for applied flavor avoidance learning. Appl Anim Behav Sci 76:249–258

Kumar R, Singh M (1984) Tannins: their adverse role in ruminant nutrition. J Agric Food Chem 32:447–453

Krzic M, Newman RF, Broersma K (2003) Plant species diversity and soil quality in harvested and grazed boreal aspen stands of northeastern British Columbia. For Ecol Manage 182:315–325

LaMalfa E, Ryle R (2008) Differential Snowpack Accumulation and Water Dynamics in Aspen and Conifer Communities: Implications for Water Yield and Ecosystem Function. Ecosystems 11(4):569-581

Lindroth RL (2001) Adaptations of quaking aspen for defense against damage by herbivores and related environmental agents. In: Shepperd, W.D., Binkley DB, Bartos DL, Stohlgren TJ, Eskew LG (eds) Sustaining Aspen in Western Landscapes: Symposium Proceedings. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. Fort Collins, pp 273-284

Lindroth RL, Hwang SY (1996) Clonal variation in foliar chemistry of quaking aspen (*Populus tremuloides* Michx.). Biochem Syst Ecol 24:357–364

Lindroth RL, St.Clair SB (2013) Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. For Ecol Manage 299:14–21

Little EL Jr (1971) Atlas of United States trees, vol. 1. Conifers and important hardwoods. USDA Forest Service Miscellaneous Publication 1146, Washington, DC, 9:202

McArthur C, Sanson GD, Beal AM (1995) Salivary proline-rich proteins in mammals: roles in oral homeostasis and counteracting dietary tannin. J Chem Ecol 21:663-691

McCullough SA, O'Geen AT, Whiting ML, Sarr DA, Tate KW (2012) Quantifying the consequences of conifer succession in aspen stands: decline in a biodiversity-supporting community. Environ Monit Assess 8:1–14

McLean S, Duncan AJ (2006) Pharmacological perspectives on the detoxification of plant secondary metabolites: implications for ingestive behavior of herbivores. J Chem Ecol 32: 1213-1228

Mellado M, Valdez R, Lara LM, Lopez R (2003) Stocking rate effects on goats: a research observation. J Range Manage 56:167–173

Mitchelson F (1992) Pharmacological agents affecting emesis: A review (Part I). Drugs 43:295

Mitton JB, Grant MC (1996) Genetic variation and the natural history of quaking aspen. Bioscience 46(1): 25-31

Mueggler WF (1985) Resources and uses. In: DeByle NV, Winokur RP (Eds) Aspen: Ecology and Management in the Western United States. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp 129–188, GTR-RM-119

Nolte DL, Provenza FD (1992) Food preferences in lambs after exposure to flavors in solid foods. Appl Anim Behav Sci 32:337–347

Nolte DL, Provenza FD, Callan RL, Panter KE (1991) Food preferences in lambs after exposure to flavors in utero. Appl Anim Behav Sci 32(4):381-389

Osier TL, Lindroth RL (2001) Effects of genotype, nutrient availability, and defoliation on aspen phytochemistry and insect performance. J Chem Ecol 27: 1289–1313

Osier TL, Lindroth RL (2004) Long-term effects of defoliation on quaking aspen in relation to genotype and nutrient availability: plant growth, phytochemistry and insect performance. Oecologia 139:55-65

Osier TL, Lindroth RL (2006) Genotype and environment deter- mine allocation to and costs of resistance in quaking aspen. Oecologia 148:293–303

Osier TL, Hwang SY, Lindroth RL (2000) Within- and be- tween-year variation in early season phytochemistry of quaking aspen (*Populus tremuloides* Michx.) clones. Biochem System Ecol 28:197–208

Packard FM (1942) Wildlife and aspen in Rocky Mountain National Park. Ecology 23:478–482

Palo RT, Robbins CT (1991) Plant Defenses against mammalian herbivory. CRC Press

Papachristou T, Dziba L, Villalba JJ, Provenza FD (2007) Patterns of Diet Mixing by Sheep Offered Foods Varying in Nutrients and Plant Secondary Compounds. Appl Anim Beh Sci 108(1-2):68-80

Perala DA, Carpenter EM (1985) Aspen—An American Wood. US Dept of Agriculture FS 217

Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J Range Manage 48:2-17

Provenza FD (1996) Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J Anim Sci 74:2010-2020

Provenza FD, Villalba JJ, Bryant JP (2003) Foraging by herbivores: linking the biochemical diversity of plants to herbivore culture and landscape diversity. In: Bissonette JA, Storch I (eds) Landscape Ecology and Resource Management: Linking Theory with Practice, Island Press, New York

Provenza FD, Villalba JJ (2006) Foraging in domestic herbivores: Linking the internal and external milieu. In: Bels VL (ed) Feeding in Domestic Vertebrates: From Structure to function. CABI Publ, Oxfordshire, UK, pp 210-240

Ralphs MH (1997) Persistence of aversions to larkspur in naive and native cattle. J Range Manage 50:367–370

Reed J.D (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. J Anim Sci 73:1516-1528

Reynolds HG (1969) Aspen grove use by deer, elk, and cattle in southeastern coniferous forests. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Research Note RM-138

Ripple WJ, Larsen EJ (2000) Historic aspen recruitment, elk, and wolves in northern Yellowstone Park, USA. Biol Conserv 95:361–370

Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW (1987a) Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68:98-107

Robbins CT, Mole S, Hagerman AE, Hanley TA (1987b) Role of tannins in defending plants against ruminants: reduction in dry matter digestion? Ecology 68:1606-1615

Robbins CT, Hagerman AE, Austin PJ, McArthur C, Hanley TA (1991) Variation in Mammalian Physiological Responses to a Condensed Tannin and Its Ecological Implications. J Mammal 72:480–486

Rogers PC, Mittanck CM (2014) Herbivory strains resilience in drought-prone aspen landscapes of the western United States. J Veg Sci 25(2): 457–469

Royo AA, Collins R, Adams MB, Kirschbaum C, Carson WP (2010) Pervasive interactions between ungulate browsers and disturbance regimes promote temperate forest herbaceous diversity. Ecology 91:93–105

Rogers PC, Landhäusser SM, Pinno BD, Ryel RJ (2014) A Functional Framework for

Improved Management of Western North American Aspen (*Populus tremuloides* Michx.). For Sci 60(2):345-359

Seager ST, Eisenberg C, St.Clair SB (2013) Patterns and consequences of ungulate herbivory on aspen in western North America. For Ecol Manage 299:81–90

Shimada T (2006) Salivary Proteins as a Defense Against Dietary Tannins. J Chem Ecol 32:1149

Smith EA, Collette SB, Boynton TA, Lillrose T, Stevens MR, Bekker MF, Eggett D, St.Clair SB (2011) Developmental contributions to phenotypic variation in functional leaf traits within quaking aspen clones. Tree Physiol 31:68–77

Stephens DW, Krebs JR (1986) Foraging Theory. Princeton University Press, Princeton, NJ

Tahvanainen JO, Root RB (1972) The influence of vegetational diversity on the population ecology of a specialized herbivore, Phyllotreta cruciferae (Coleoptera: Chrysomelidae). Oecologia 10(4):321-346

Turchi GM, Kennedy PL, Urban D, Hein D (1995) Bird species richness in relation to isolation of aspen habitats. Wilson Bulletin 107(3): 463–474

Vavra M, Parks CG, Wisdom MJ (2007) Biodiversity, exotic plant species, and herbivory: the good, the bad, and the ungulate. For Ecol Manage 246:66–72

Villalba JJ, Provenza FD, Banner RE (2002a) Influence of macronutrients and polyethylene glycol on intake of a quebracho tannin diet by sheep and goats. J Anim Sci 80:3154–3164

Villalba JJ, Provenza FD, Bryant JP (2002b) Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? Oikos 97:282–292

Villalba JJ, Provenza FD, Shaw R (2006) Sheep self-medicate when challenged with illness-inducing foods. Anim Behav 71:1131–1139

Villalba JJ, Provenza FD (1999) Effects of food structure and nutritional quality and animal nutritional state on intake behaviour and food preferences of sheep. Appl Anim Behav Sci 63: 145-163

Villalba JJ, Provenza FD (2007) Self-medication and homeostatic behaviour in herbivores: Learning about the benefits of nature's pharmacy. Animal 1:1360–1370

Villalba JJ, Burritt EA, St.Clair SB (2014) Aspen (*Populus tremuloides* Michx.) Intake and Preference by Mammalian Herbivores: the role of plant secondary compounds and nutritional context. J Chem Ecol DOI 10.1007/s10886-014-0507-0

Wahl M, Hay ME (1995) Associational resistance and shared doom: effects of epibiosis on herbivory. Oecologia 102:329–40

Walters JW, Hinds TE, Johnson DW, Beatty J (1982) Effects of partial cutting on diseases, mortality, and regeneration of Rocky Mountain aspen stands. RM-RP-240, U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO

White CA, Olmsted CE, Kay CE (1998) Aspen, elk, and fire in the Rocky Mountain national parks of North America. Wild Soc Bul 26:449–462

White CA, Feller MC, Bayley S (2003) Predation risk and the functional response of elk–aspen herbivory. Forest Ecol and Manage 181: 77–97

Wooley SC, Walker S, Vernon J, Lindroth RL (2008) Aspen decline, aspen chemistry, and elk herbivory: are they linked? Aspen chemical ecology can inform the discussion of aspen decline in the West. Rangelands 30(1):17-21

Worrall JJ, Egeland L, Eager T, Mask RA, Johnson EW, Kemp PA, Shepperd WD (2008) Rapid mortality of *Populus tremuloides* in southwestern Colorado, USA. Forest Ecol Manag 255:686–696

Youngquist JA, Spelter H (1990) Aspen wood products utilization: impact of the Lake States composites industry. In Proceedings, Aspen Symposium, Duluth Minnesota, RD Adams (ed), USDA For Serv NC-GTR-140, pp91-102

CHAPTER 2

NUTRIENTS AND SECONDARY COMPOUNDS INFLUENCE TREMBLING ASPEN (*Populus tremuloides*) INTAKE BY SHEEP[1i]

Abstract- Nutrients and plant secondary compounds in aspen (*Populus tremuloides*) may interact with nutrients in the surrounding vegetation to influence aspen use by herbivores. Thus, this study aimed to determine aspen intake and preference by sheep in response to supplementary nutrients or plant secondary compounds (PSC) present in aspen trees. Thirty-two lambs were randomly assigned to one of four molasses-based supplements (N=8) during three experiments. The supplements were as follows: (1) high-protein (60% canola meal), (2) a PSC (6% quebracho tannins), (3) 25% aspen bark, and (4) control (100% molasses). Supplements were fed from 0700 to 0900, then lambs were fed fresh aspen leaves collected from stands containing high (Experiment 1, 2) or low (Experiment 3) concentrations of phenolic glycosides (PG). In Experiment 2, lambs were simultaneously offered aspen, a forb (*Lathyrus pauciflorus*), and a grass (*Bromus inermis*) collected from the aspen understory. Animals supplemented with high protein or tannins showed greater intake of aspen leaves than animals supplemented with bark or the control diet ($P<0.05$), likely because some condensed tannins have a positive effect on protein nutrition and protein aids in PSC detoxification. Animals supplemented with bark showed the lowest aspen intake, suggesting PSC in bark and aspen leaves had additive inhibitory effects on intake. In summary, these results suggest that not only the concentration but also the types and proportions of nutrients and chemical defenses available in the plant community influence aspen use by herbivores.

---

[1] Kristen Y. Heroy, Samuel B. St. Clair, Elizabeth A. Burritt, and Juan J. Villalba

INTRODUCTION

Large ungulates make foraging choices in a hierarchical fashion, from plant communities at the landscape scale, to feeding stations at the plant community scale, and individual plants at the bite scale (Senft et al. 1987). Within the plant community, these decisions are influenced by the spatial and temporal distribution of nutrients and plant secondary compounds (PSC), i.e., the biochemical context, which changes the probability of plant damage. Preference for a particular plant depends not only on its intrinsic (e.g., nutritional, toxicological) properties, but also on the biochemical context in which that plant is consumed (Baraza et al. 2005; Villalba and Provenza 2005). This is because nutrients and PSC in the community create a surrounding chemical matrix that may attract or deter herbivores from consuming a particular plant (Villalba et al. 2002; Baraza et al. 2006). For instance, nutritious and palatable plants gain “associational protection” by growing close to unpalatable neighbors (e.g., Atsatt and O’Dowd 1976; Baraza et al. 2006). Conversely, unpalatable toxin-containing plants may experience greater herbivory or “associational susceptibility” when growing near palatable species (Hjältén et al. 1993; Rautio et al. 2008). The latter effect may be due to supplementary energy and protein provided by palatable plants since additional amounts of carbohydrates and protein are needed for detoxification processes (Illius and Jessop 1995; 1996; Villalba and Provenza 2002). In addition, certain combinations of energy and protein may be more efficient than others in assisting detoxification and improving the ability of herbivores to consume PSC-containing plants (Villalba and Provenza 2002). On the other hand, antagonisms among different PSC due to overlaps in detoxification pathways reduce intake of specific PSC-containing foods (Burritt and Provenza 2000; Marsh et al. 2006). Intake of PSC-containing foods may also be constrained by the high availability of nutritious and less defended forages, favoring the competitive ability of defended plants and consequently the continued persistence of plant defenses (Leimar and Tuomi 1998; Rautio et al. 2012). Collectively, interactions among nutrients and PSC at the plant community level lead to complementary or antagonistic relationships among food resources that either enhance or constrain herbivory.

Quaking aspen (*Populus tremuloides*) communities represent an ideal model to explore the influence of the biochemical context on preference by ungulates because aspen trees growing in patches represent a concentrated source of nutrients that attract both wild (White et al. 2003) and domestic (Jones et al. 2011) ungulates. In addition, it has been suggested that aspen browsing increases when the concentration of nutrients within aspen leaves is greater than the concentration of nutrients present in the surrounding plant community, especially at the end of summer and during fall when plants in the understory begin to senesce (Jones et al. 2011; Villalba et al. 2014). Repeated loss of foliage and damage to meristematic tissues due to herbivory can impact aspen suckers through the next growing season, particularly if resources are limited (Erbilgin et al. 2014), thus suppressing aspen regeneration (Bailey et al. 2007).

Plants produce PSC to reduce herbivory (Palo and Robbins 1991), and PSC found in aspen tissues—condensed tannins (CT) and phenolic glycosides (PG)—deter ungulate browsing (Wooley et al. 2008; Lindroth and St. Clair 2013; Villalba et al. 2014). Phenolic glycosides negatively impact the performance of many generalist herbivores (Boeckler et al. 2011) and CT cause digestive upset and readily bind to dietary proteins and some minerals, reducing the amount of available protein in the diet (Frutos et al. 2004; Robbins et al. 1987). However, some tannins can form tannin-protein complexes that can increase the supply of essential and branched-chain amino acids available to the herbivore in the upper small intestine (Hagerman et al. 1992; Reed 1995; Foley et al. 1999), enhancing detoxification and reducing their negative post-ingestive effects (Illius and Jessop 1995; 1996). Thus, interactions among nutrients and PSC in aspen communities may lead to complementary (e.g., the positive effects of nutrients on PSC detoxification) or antagonistic relationships (e.g., overlaps in detoxification pathways, high availability of palatable neighbors) that, respectively, enhance or constrain aspen intake.

We hypothesized that nutrients and PSC in aspen interact with nutrients in the surrounding vegetation to influence aspen use by herbivores. Thus, we predicted that: (i) supplementary nutrients (e.g., protein) enhance, whereas (ii) aspen chemical defenses (e.g., phenolic glycosides or condensed tannins) or (iii) availability of nutritive forage alternatives (e.g., grasses and forbs growing in an aspen understory) constrain aspen intake and preference by sheep. In order to test these predictions, we provided protein and PSC (PG in aspen bark and condensed tannins) inputs via supplementation to sheep in order to explore the impact of nutrients and PSC on intake of aspen leaves with either high (Experiment 1) or low (Experiment 3) concentrations of PG, because aspen occurs with a wide genetically based variation in concentration of defenses (test of predictions i and ii). Since aspen communities are diverse, we then provided nutritive forage alternatives during a choice test to determine the influence of palatable plants in the community and the provision of the aforementioned protein and PSC inputs on aspen preference by sheep (Experiment 2; test of prediction iii).

METHODS AND MATERIALS

General Protocol

Three experiments were conducted to explore the influence of the chemical context on aspen use by sheep. Groups of mixed-gendered lambs (4 months of age, 32±1.2 kg of body mass at the beginning of the study) were supplemented with protein, energy, aspen bark, or tannins before being offered aspen leaves. Once per month, aspen bark was harvested from fallen aspen trees with trunk bark separation in the same location as aspen leaf harvest (see Table 2-1 for nutritional and defense chemical analysis of the aspen bark). Animals were then fed a basal diet of chopped tall fescue (*Festuca arundinacea*) hay (1 to 3 cm particle size) at a rate of 2.2% of body weight (BW) to meet minimum maintenance requirements (NRC 1985). Aspen tissue used to feed sheep contained either relatively high (Experiments 1 and 2) or relatively low (Experiment 3) concentrations of PG. During Experiment 2, animals received a simultaneous offer of aspen leaves and two forages commonly found in the aspen understory: Utah sweet pea (*Lathyrus pauciflorus*), and smooth bromegrass (*Bromus inermis*).

Study Site

Pen trials were conducted at the Utah State University Green Canyon Ecology Center, Logan, UT using 32 crossbred lambs according to the Utah State University Animal Care and Use Committee guidelines (approval # IACUC-2238). Plant material for the study was collected from four locations within the Uintah/Wasatch/Cache National Forest in northern Utah, USA. All aspen leaves were harvested from suckers below the browse line (less than 2 m tall). No more than two aspen branches were harvested from any one tree.

Table 2-1 Nutritional composition and concentration of plant secondary compounds (% dry matter) in aspen bark and nutritional composition of supplements and tall fescue hay

	CP[a]	ADF[b]	NDF[c]	TDN[d]	Trem[e]	Sal[f]	PG[g]	CT[h]		
Bark only	2.7	52.6	63.9	42.5	0.494	0.511	0.252	0.25		
Supplements	CP[a]	ADF[b]	NDF[c]	TDN[d]	DE[mn]					
High protein[i]	28.4±0.2	21.6±0.1	28.1±0.2	71.6±0.1	3.11					
Tannin[j]	9.8±0.3	23.6±0.1	31.5±0.1	69.2±0.1	2.97					
Bark[kq]	8.0±0.04	31.2±0.2	40.6±0.5	62.1±0.3	2.37					
Control[l]	9.8±0.1	24.1±0.3	32.8±0.7	68.6±0.3	3.16					
Supplements-Minerals	Ash	Ca	Cu	Fe	K	Mg	Mn	Na	P	Zn
High protein	10.2±0.1	1.1±0	0.8±0	0.6±0	2.6±0	0.1±0	0.02±0	0.005±0	0.005±	0.001±0
Tannin	11.9±0.2	0.9±0	0.2±0	0.4±0	4.1±0.1	0.2±0	0.03±0	0.002±0	0.003±0	0.001±0
Bark	10.4± 0.2	1.2±0	0.2±0	0.3±0	3.5±0.1	0.1±0	0.03±0	0.002±0	0.005±	0.001±0
Control	11.8±0.4	1.0±0	0.2±0	0.4±0	4.3±0	0.1±0	0.03±0	0.002±0	0.004±0	0.002±0
Tall Fescue[p]	CP[a]	ADF[b]	NDF[c]	TDN[d]						
	14.9±0.2	32.6±0.4	45.7±0.2	ND[o]						

[a] Crude Protein
[b] Acid detergent fiber
[c] Neutral detergent fiber
[d] Total digestible nutrients
[e] Tremulacin
[f] Salicortin
[g] Total Phenolic Glycosides (Trem + Sal)
[h] Condensed Tannins
[i] High protein supplement (60% Canola meal + 40% molasses)
[j] Condensed tannin supplement (6% quebracho tannins + 94% molasses)
[k] Aspen bark supplement (25% aspen bark + 75% molasses)
[l] Control supplement (100% molasses)
[m] Digestible energy expressed as Mcal per kg of feed
[n] Calculated values from NRC (1985)
[o] Not determined
[p] Values from Hamilton et al. (2015)
[q] Calculated from Bark Only and C supplement analysis

Familiarization Period

Lambs naïve to aspen were housed as a group in an outdoor pen (18.4 m×10 m) built under cover prior to Experiment 1. Lambs were introduced to increasing amounts of mature aspen leaves collected on-site every day for a period of 2 weeks. From days 1 to 3, lambs received four 1 m aspen branches with their leaves in an attempt to initiate interest in aspen leaves attached to the branch. From days 4 to 7, lambs received three 1 m branches in addition to 500 g of aspen leaves placed in a trough. From days 7 through 14, lambs received 2 kg of aspen leaves in the trough without additional branches.

Every morning one hour before aspen was placed in the pen, a supplement (59.5% barley, 35.7% soybean meal, 4.7% beet pulp) was offered to all lambs at a rate of 262.5 g/animal/day (for a total of 8.4 kg of supplement offered to the entire group) in a metal trough adjacent to the trough used for aspen offers. The purpose of feeding this supplement was to familiarize lambs to the ingredients of the supplements used during the experiments. A basal diet of alfalfa pellets was offered in *ad libitum* amounts in a third trough. During familiarization, all animals were introduced to nipple drinkers secured to the north side of the pen, which provided water to lambs. Until all lambs were drinking from the nipple drinkers, a 56.8-liter tub remained in the pen to ensure *ad libitum* access to water. Culinary water, salt, and trace mineral blocks were available in *ad libitum* amounts throughout the study.

After 2 weeks, lambs were weighed and transferred to individual pens (1.5 m x 2 m) built under a protective roof. Prior to the first experiment, all lambs were dewormed via subcutaneous administration of ivermectin (0.2 mg/kg BW). Lambs were allowed an adjustment period of three days in their pens, during which time they were offered *ad* *libitum* amounts of alfalfa pellets.

Every morning at 0900 following the three-day adjustment period to individual pens, all lambs received freshly harvested aspen leaves collected from a stand located near Tony Grove lake (N 41° 53'35.58" W 111° 36'16.11") with a concentration of 13.2% PG. This stand was chosen based on a previous aspen feeding study conducted in the same region (Villalba et al. 2014). The first day lambs received 125 g of aspen leaves (on an as-fed basis). On ensuing days, aspen offers were increased for lambs when refusals for those animals fell below 50 g during the previous day. Refusals were collected at 1100. Individual aspen intake was determined during five consecutive days following the pen adjustment period by subtracting the amounts of aspen refused from those offered each day. Immediately after aspen refusals were collected, a basal diet of alfalfa pellets was offered to all lambs in *ad libitum* amounts until 1800, when alfalfa pellets were removed from all feeders and no other feed was offered until the following day.

Experiment 1—High Content of PG

Preliminary Period (11 June 2014 to 30 June 2014). During a 10-day pretrial period, lambs were familiarized with feeds and the experimental conditions of the study. Lambs were randomly assigned to one of four treatments (8 lambs per group), blocked by aspen intake assessed during the familiarization period to ensure similar intakes of aspen across all treatments. Each treatment was assigned one of four molasses-based supplements: (1) high-protein (60% canola meal; 28.4% crude protein [CP]; 3.11 Mcal/kg digestible energy [DE]), (2) condensed tannins (6% quebracho tannins; 9.8% CP; 2.97 Mcal/kg DE), (3) aspen bark (25% bark; 8.0% CP; 2.37 Mcal/kg DE), and (4) control (100% molasses; 9.8% CP; 3.16 Mcal/kg DE) (see Table 2-1 for chemical composition of the supplements). All animals remained in their assigned treatment groups throughout this and subsequent studies. These treatments were chosen to compare the effects of high concentrations of protein, or two types of PSC (one containing only tannins, the other containing the same plant secondary compounds found in aspen leaves—both PG and tannins), relative to a control treatment (100% molasses molasses) on aspen intake. The control group was fed molasses to keep lamb diets isoenergetic. The amounts of supplements offered (ranging from 300 to 400 g) were such that they all provided the same amounts of calories to lambs in all treatments on a daily basis (0.95 Mcal/day/lamb).

The high protein, condensed tannin, and bark supplements were offered each morning at 0700 for 2 hours, which provided enough time for lambs to eat the entire offered supplement. Subsequently, aspen leaves collected each morning from Tony Grove (see Table 2-2 and 2-3 for nutrition and defense chemistry) were fed to all lambs from 0900 until 1100, when refusals were collected and intakes were determined. Aspen leaves were initially fed in amounts based on each lambs' intake during the familiarization period. On ensuing days, aspen offers were increased for lambs when refusals for those animals fell below 50 g during the previous day. After collecting aspen refusals, lambs were fed a basal diet of tall fescue hay at a rate of 2.2% BW, and control animals were fed their molasses-based supplement along with their basal diet. The control supplement was offered with the basal diet after 1100 to reduce the likelihood of interaction with the aspen eaten in the morning. Refusals were collected and weighed at 1700 and no other feed was offered until the following morning.

Testing Period (1 July 2014 to 10 July 2014). The protocol for the testing period was as described above, but the amounts of supplement offered on a daily basis were increased to 1.42 Mcal/lamb/day (ranging from 450 g to 600 g of supplement/lamb/day). The study was conducted during 10 consecutive days.

Experiment 2—Forage Preference (14 July 2014 to 23 July 2014)

All animals received alfalfa pellets in *ad libitum* amounts for four days after Experiment 1 had ended. Lambs were then weighed again (11 July 2014) for accurate estimations of food intake per Kg of metabolic BW. Average lambs mass was 34±1.2 kg.

Condensed tannin, aspen bark, and protein supplements were offered at a rate of 1.42 Mcal/day/lamb at 0700 for 2 hours. Refusals were collected and intakes were determined. At 0900, lambs were offered a choice of three buckets containing *ad libitum* amounts of: aspen leaves, Utah sweet pea, and smooth bromegrass, all collected in the aspen understory at the Tony Grove location (see Table 2-2 and 2-3 for nutritional and defense chemical analysis). Additional amounts (50 g) of any plant species were added to

Table 2-2 Nutritional composition (% dry matter) of offered aspen leaves, Utah pea, and smooth bromegrass

Experiment 1[ac] – Aspen leaves, High PG				
	Crude protein	ADF[f]	NDF[g]	TDN[h]
	12.46 ± 0.36	16.96 ± 0.95	20.50 ± 0.79	80.48 ± 1.01
Experiment 2[e] – Aspen leaves, Utah pea and smooth brome grass				
	Crude protein	ADF	NDF	TDN
Aspen	12.62 ± 0.89	17.42±0.59	20.72±0.52	80.02±0.63
Utah Pea	24.70 ± 1.92	33.54±1.10	42.2±0.96	62.82±1.18
Bromegrass	11.28 ± 0.43	34.70±0.64	58.46±1.09	63.54±0.71
Experiment 3[bd] – Aspen leaves, Low PG				
	Crude protein	ADF	NDF	TDN
	10.60 ± 0.32	19.86 ± 1.02	22.20 ± 1.13	77.44 ± 1.09

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] July 1 to July 10, 2014
[d] August 11 to August 20, 2014
[e] July 14 to July 23, 2014
[f] Acid detergent fiber
[g] Neutral detergent fiber
[h] Total digestible nutrients

Table 2-3 Concentration of plant secondary compounds (% dry matter) within offered aspen leaves

Experiment 1[ac] – Aspen leaves, High PG			
Tremulacin	Salicortin	Total PG	Condensed tannins
12.26±1.26	0.89±0.05	13.20±1.31	3.09±0.18
Experiment 2[d] – Aspen leaves, High PG			
Tremulacin	Salicortin	Total PG	Condensed tannins
10.75±1.25	0.78±0.07	11.53±1.32	3.25±0.23
Experiment 3[be] – Aspen leaves, Low PG			
Tremulacin	Salicortin	Total PG	Condensed tannins
7.23±0.86	0.69±0.07	7.92±0.87	3.29±0.46

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] July 1 to July 10, 2014
[d] July 14 to July 23, 2014
[e] August 11 to August 20, 2014

the buckets when the amounts refused by an animal were below 50 g during the previous day. Utah sweet pea and smooth bromegrass were selected because these species commonly grow in the aspen understory in amounts sufficient to conduct a feeding trial with sheep. Forage refusals were collected at 1100, at which time aspen, Utah sweet pea, and smooth bromegrass intakes were determined. Subsequently, all lambs were offered tall fescue hay at a rate of 2.2% BW. Control animals were offered their molasses-based supplement along with their basal diet as described for Experiment 1. Refusals were collected at 1700 and no other food was offered until the next day at 0700. The study was conducted during 10 consecutive days.

Experiment 3—Low Content of PG (11 Aug 2014 to 20 Aug 2014)

Fully expanded leaves from aspen trees were collected from different locations on July 28 and 29, 2014 to identify aspen clones with low concentrations of PG relative to the PG concentration in the stand used in Experiment 1 and 2. A low PG stand was desired so that aspen intake could be statistically compared between this experiment and Experiment 1 and 2 in which leaves contained higher concentrations of PG. The stands used in Experiment 3 were Franklin Basin #1 (N 41° 55.826’ W 111° 33.810’) and Sink Hollow (N 41° 55.016’ W 111° 28.846’) (see Table 2-2 and 2-3 for nutrition and defense chemistry analysis).

The protocol for this experiment was as described for Experiment 1, with the amounts of supplement offered ranging from 450 g to 600 g of supplement/lamb/day, which delivered a total of 1.42 Mcal/day/lamb. The study was conducted during 10 consecutive days: 7 days using aspen from Franklin Basin #1, and 3 days using leaves from Sink Hollow. The change in location was due to a lack of sufficient leaf material in Franklin Basin #1. All animals were again weighed at the end of Experiment 3 (21 Aug 2014). Final animal mass was 39±1.3 kg.

Chemical Analyses

Five composite samples of aspen leaves from each experiment were placed in plastic bags and transported to a freezer where they were kept at -20 °C. They were subsequently freeze dried, ground in a Wiley mill with a 1 mm screen, and analyzed for dry matter (Method 930.15; AOAC 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al. 1991), and crude protein (CP) (Method 990.03; AOAC 2000). Total digestible nutrients (TDN) were calculated from CP and fiber based on equations from Weiss et al. (1992) as an estimate of digestible energy of the samples (NRC 1985, Swift 1957).

During each day of the study, representative offered and refused samples of aspen leaves (Experiments 1, 2, and 3), Utah sweet pea, and smooth bromegrass (Experiment 2) were placed in paper bags and dried in a forced-air oven at 60 °C for 48 hours to estimate dry matter content so intake values could be expressed on a dry matter basis. Oven-dried samples of supplements, smooth bromegrass, and Utah pea (due to the lack of temperature-sensitive PSC) were ground in a Wiley mill with a 1 mm screen and analyzed for dry matter, NDF, ADF, and CP as described before. Tall fescue hay nutrition was as the same as cited in Hamilton et al. (2015), and is reported in Table 2-1. Minerals were also analyzed for the supplements using a nitric acid digestion procedure and determined using inductively coupled plasma emission spectrometry (ICP) according to procedures described in Villalba et al. (2008).

Phenolic glycosides were extracted from 40 mg of freeze dried leaf material in 1 ml of methanol. The samples were vortexed on high for 5 minutes and centrifuged at 16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes. This procedure was repeated a second time, and the extracts were pooled to yield 2 ml of crude extract. Phenolic glycosides (salicortin and tremulacin) were quantified using high performance liquid chromatography (Agilent 1100 Series, Santa Clara, CA, USA) with a Luna 2, C18 column (150x4.6 mm, 5 μm) at a flow rate of 1 ml/min. Compound peaks were detected at 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves (Lindroth et al. 1993).

Condensed tannins were extracted from approximately 50 mg of freeze-dried leaf tissue with 1 ml of a 70% acetone-10 mM ascorbic acid solution. Samples were vortexed on high for 20 minutes at 4 °C followed by centrifugation at 16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes, and the extraction was then repeated. Condensed tannin concentrations were measured spectrophotometrically (SpectraMax Plus 384, MDS, Toronto, Canada) using the acid butanol method (Porter et al. 1986) standardized with purified condensed tannins isolated from aspen leaves (Hagerman and Butler 1980).

Statistical Analyses

The effects of supplement on aspen intake over time were assessed using a one-way analysis of variance in a randomized complete block design with repeated measures. Utah pea and smooth bromegrass intake were analyzed in the same way as aspen intake in Experiment 2 (*Forage Preference*). Comparisons of nutrient concentrations between feed types (Utah pea, smooth bromegrass, aspen, and supplements) within experiments were analyzed using a one-way analysis of variance in a randomized complete block design with repeated measures. The effects of experiment and supplement on aspen intake was determined using a two-way analysis of variance in a randomized complete block design with repeated measures. Blocks were groups of four lambs with similar aspen intake during the familiarization period. A lamb was the experimental unit to which a supplement was randomly assigned. Repeated measures on lambs over time were modeled with a compound symmetry covariance structure. Calculations were made using the MIXED procedure in the SAS System (SAS Inst., Inc., Cary, NC, USA; Version 9.4 for Windows).

Aspen intake for all experiments, expressed as dry matter intake (DMI) and DMI per unit of metabolic body weight (MBW), was calculated to standardize intake by unit of body weight (BW) (using the equation $MBW=DMI \div [BW^{0.75}]$). Intake of tall fescue hay was expressed on an as-fed basis given the low content of moisture in this feed ($< 5\%$).

RESULTS

Experiment 1—High Content of PG

Preliminary Period. Aspen Intake. Aspen intake was greater for lambs that received the condensed tannin supplement than for those that received the bark (*P*=0.001), high protein (*P*=0.006), or control (*P*=0.009) supplements (treatment effect *P*=0.005). Aspen intake fluctuated across days (day effect *P*<0.001), but there was no treatment by day interaction (*P*>0.05).

Preliminary Period. Tall Fescue and Supplement Intake. Tall fescue intake was greater for those lambs in the high protein and tannin treatments than for those in the bark or control treatments (treatment effect *P*<0.001; treatment × day *P*>0.05) (see Table 2-4 for intake values). Tall fescue intake increased from days 2 through day 7 (starting from an

Table 2-4 Average tall fescue hay intake (DM basis)

Preliminary period[c] – Aspen leaves, High PG[a]	High protein[h]	Tannin[i]	Bark[j]	Control[k]	Overall[l]
Tall Fescue[g]	758.1±18.5	728.4±26.32	661.7±20.3	671.6±29.0	704.9±13.3
Experiment 1[d] – Aspen leaves, High PG[a]	High protein	Tannin	Bark	Control	Overall
Tall Fescue	788.4±31.9	759.9±23.5	613.7±23.8	702.9±35.9	716.2±17.7
Experiment 2[e] – Aspen leaves[a], Utah pea and smooth brome grass	High protein	Tannin	Bark	Control	Overall
Tall Fescue	746.8±37.5	709.5±37.2	563.5±30.0	554.9±28.6	643.7±21.2
Experiment 3[f] – Aspen leaves, Low PG[b]	High protein	Tannin	Bark	Control	Overall
Tall Fescue	838.6±18.2	799.1±17.3	710.9±27.0	696.5±28.7	761.3±14.7

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] June 11 to June 30, 2014
[d] July 1 to July 10, 2014
[e] July 14 to July 23, 2014
[f] August 11 to August 20, 2014
[g] Expressed as grams of intake on an as fed basis
[h] High protein supplement
[i] Tannin and molasses supplement
[j] Aspen bark and molasses supplement
[k] Control (molasses only) supplement
[l] Average intake across days and treatments

average intake of 659.2±31.2 g on day 2 to an average intake of 728±33.3 g on day 7), with a small decline on day 8 (674.1±22.7 g) and a subsequent increase on days 9 and 10 (832.3 ±20.9 g) (day effect $P<0.001$). On average, animals consumed 97.7% of their supplement during the pretrial period of Experiment 1.

Testing Period. Aspen Intake. Aspen intake was lower for lambs in the bark treatment than for those in the high protein ($P<0.001$), control ($P<0.001$), or tannin ($P<0.001$) treatments (Fig. 2-1; treatment effect $P<0.001$; treatment × day $P>0.05$). Average aspen intake across all treatments declined from day 1 to day 3, and then slowly increased until the end of the experiment (day effect $P<0.001$; Fig. 2-2).

Testing Period. Tall Fescue and Supplement Intake. Tall fescue intake was lower for

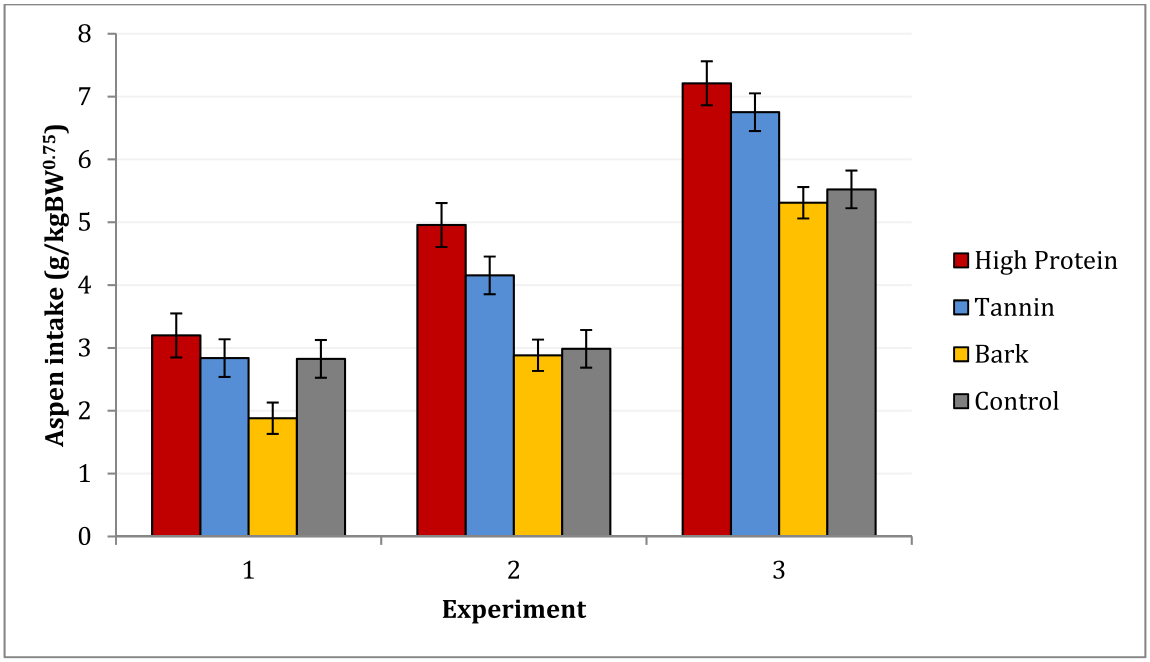

Fig. 2-1 Average aspen intake by sheep under four treatments during three experiments: Experiment 1—High content of PG in aspen; Experiment 2—Choice of aspen, smooth bromegrass, and Utah pea; Experiment 3—Low content of PG in aspen. Lambs received molasses-based supplements containing: high protein, tannins, bark, or a control supplement. Bars (with SEMs) are means for intake values recorded during 10 consecutive days for 8 lambs/treatment.

lambs in the bark treatment than for those in the high protein (*P*<0.001), control (*P*=0.001), and tannin (*P*<0.001) treatments (see Table 2-4 for intake values). The lambs in the control treatment ate less tall fescue than those in the high protein (*P*=0.002) and tannin (*P*=0.035) treatments (treatment effect *P*<0.001). Tall fescue intake was variable across days (day effect *P*<0.001), but there was no treatment by day interaction (*P*>0.05). On average, animals consumed 97.7% of their supplement during the testing period of Experiment 1.

Experiment 2—Forage Preference

Aspen Intake. When averaged across days, lambs ate the most aspen when supplemented

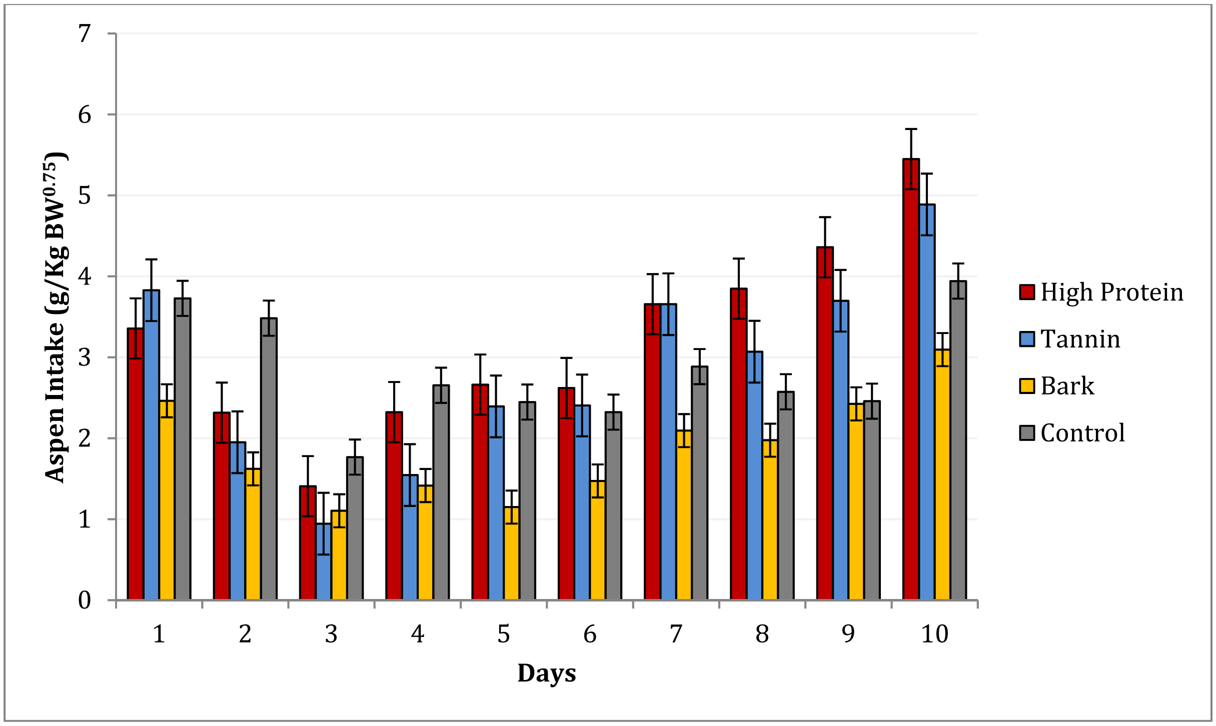

Fig. 2-2 Daily intake of aspen leaves by 4 groups of lambs during Experiment 1—High content of PG in aspen. Lambs received molasses-based supplements containing: high protein, tannins, bark, or a control supplement. Bars (with SEMs) are means for intake values recorded during 10 consecutive days for 8 lambs/treatment.

with high protein, followed by tannin, then control and bark (treatment effect $P<0.001$; Fig. 2-1). Aspen intake was variable across days (day effect $P<0.001$), but there was no treatment by day interaction ($P>0.05$; Fig. 2-3).

Utah Pea Intake. Lambs supplemented with tannin ate more Utah sweet pea than lambs supplemented with bark, whereas lambs supplemented with high protein and control showed the lowest intake of Utah sweet pea (treatment effect $P<0.001$; Fig. 2-4). Lambs supplemented with tannin ate more Utah pea than the rest of the groups during days 2 and 3 and more than those in the control treatment during days 3 and 5, whereas those in the high protein supplement had lower intakes of Utah sweet pea than those in the tannin

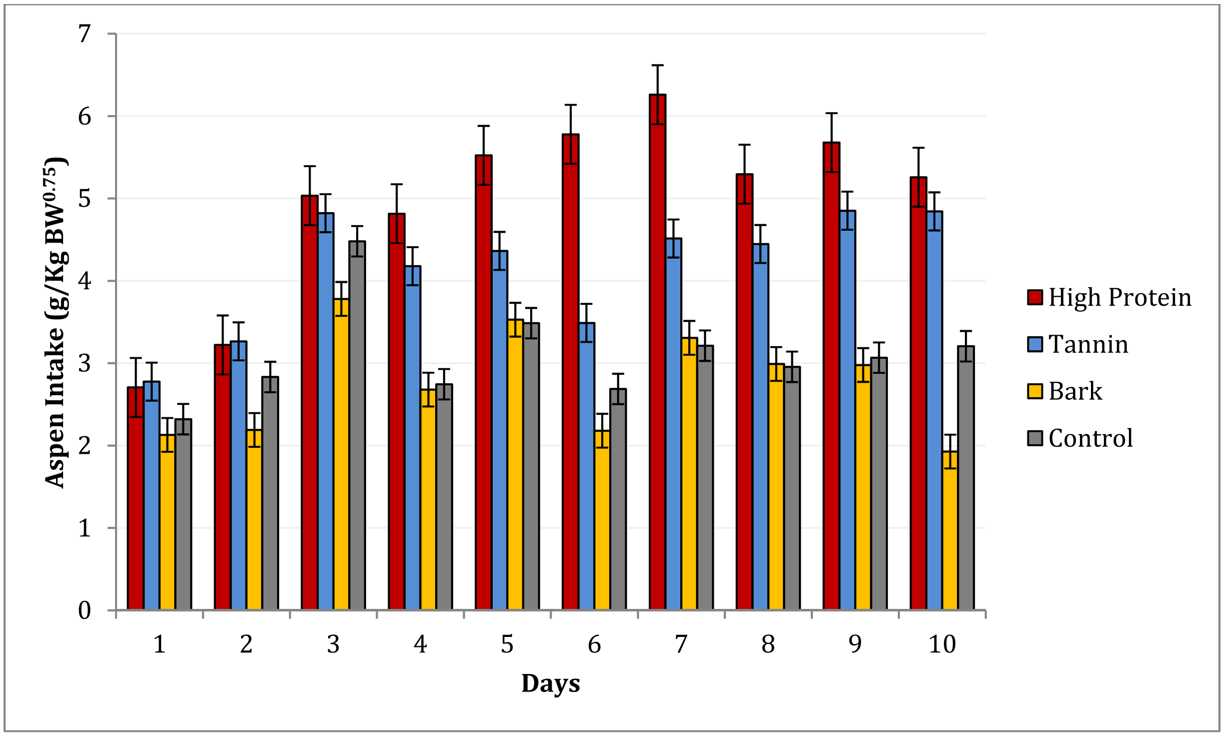

Fig. 2-3 Daily intake of aspen leaves by 4 groups of lambs during Experiment 2—High content of PG in aspen with choice of Utah sweet pea and smooth bromegrass. Lambs received molasses-based supplements containing: high protein, tannins, bark, or a control supplement. Bars (with SEMs) are means for intake values recorded during 10 consecutive days for 8 lambs/treatment.

(days 3 and 10) and control treatments (days 5 and 10) (treatment effect P<0.001; treatment × day P<0.001). Averaged across treatments, Utah sweet pea intake was also variable across days (day effect P<0.001; Fig. 2-4).

Smooth Brome Intake. When averaged across days, lambs preferred smooth bromegrass to aspen and Utah sweet pea (*P*<0.001; Fig. 2-4). Lambs in the bark treatment ate the least amount of smooth bromegrass during day 5 and lower amounts than lambs in the high protein treatment on day 10. Lambs in the high protein treatment consumed more smooth bromegrass than animals in the tannin treatment on days 3 and 10 and less than those in the control treatment on days 5 and 10. Lambs in the control treatment ate more than those in the tannin treatment on day 3, but the opposite pattern was observed on day 5 (treatment × day interaction *P*<0.001). Smooth bromegrass intake was also variable

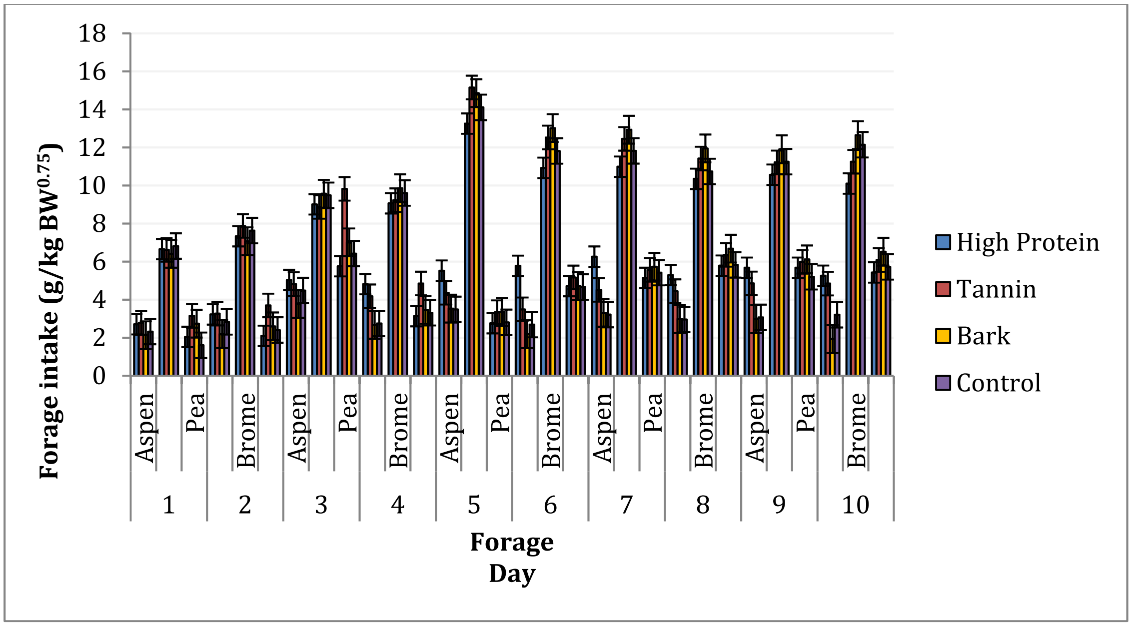

Fig. 2-4 Daily intake of aspen leaves, Utah sweet pea, and smooth bromegrass by 4 groups of lambs during Experiment 2—High content of PG in aspen. Lambs received molasses-based supplements containing: high protein, tannins, bark, or a control supplement. Bars (with SEMs) are means for intake values recorded during 10 consecutive days for 8 lambs/treatment.

across days (day effect *P*<0.001; Fig. 2-4).

Tall Fescue and Supplement Intake. Tall fescue intake was greater for lambs in the high protein treatment than for those in the bark (*P*<0.001) and control (*P*<0.001) treatments (Table 2-4). Fescue intake was greater for lambs in the tannin treatment than for those in the bark (*P*<0.001) and control (*P*<0.001) treatments (treatment effect *P*<0.001). Tall fescue intake was variable across days (day effect P<0.001), but there was no treatment by day interaction (*P*>0.05). On average, animals consumed 97.7% of their supplement during Experiment 2.

Experiment 3—Low Content of PG

Aspen Intake. When averaged across days, lambs in the high protein and condensed tannin treatment consumed more aspen than those in the control or bark treatments

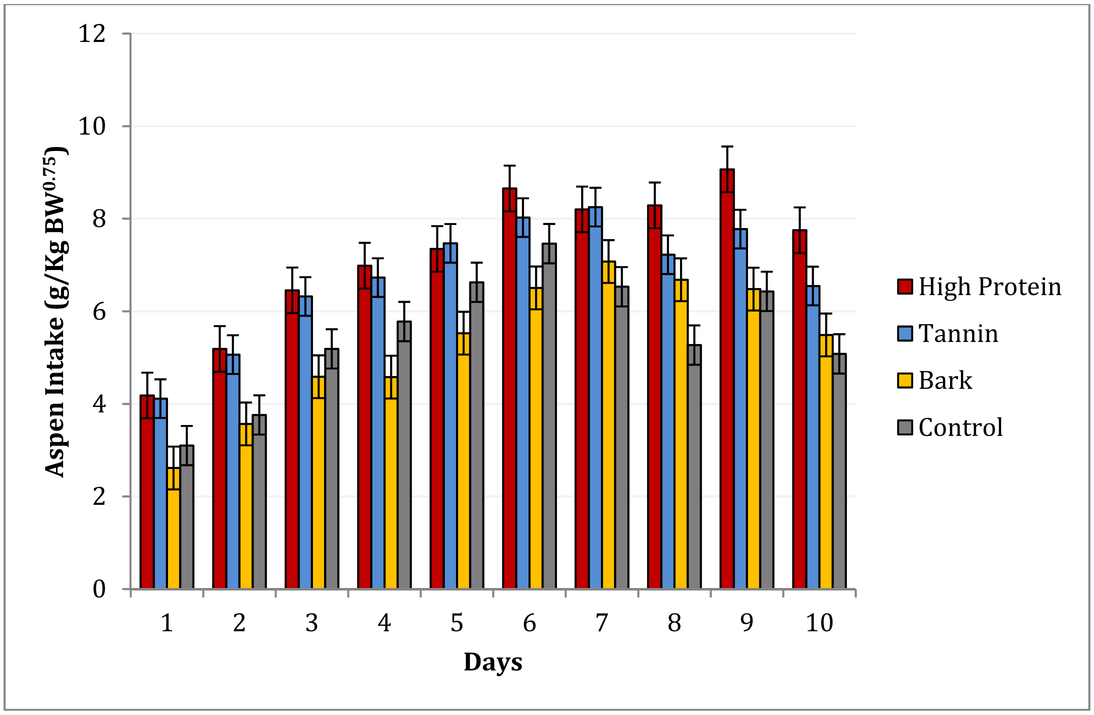

Fig. 2-5 Daily intake of aspen leaves by 4 groups of lambs during Experiment 3— Low content of PG in aspen. Lambs received molasses-based supplements containing: high protein, tannins, bark, or a control supplement. Bars (with SEMs) are means for intake values recorded during 10 consecutive days for 8 lambs/treatment.

(treatment effect *P*<0.001; Fig. 2-1). Aspen intake was variable across days (day effect P<0.001), but there was no treatment by day interaction (P>0.05; Fig. 2-5).

Tall Fescue and Supplement Intake. Tall fescue intake was greater for lambs in the high protein than for those in the control (P<0.001) and bark (P<0.001) treatments (see Table 2-4 for intake values). Intake was also greater for lambs in the tannin than for those in the control (P<0.001) or bark (P<0.001) treatments (treatment effect *P*<0.001). Tall fescue intake was variable across days (day effect *P*<0.001), but there was no treatment by day interaction (*P*>0.05). On average, animals consumed 96.2% of their supplement during Experiment 3.

Aspen Intake across Experiments

Averaged across days, aspen intake was greater in Experiment 3 (*Low Content of PG*) than in Experiment 2 (*Forage Preference*), and greater in Experiment 2 than in Experiment 1 (*High Content of PG*) for all treatments (treatment × experiment and experiment effect *P*<0.001), except for the control treatment, where aspen intake did not differ between Experiments 1 and 2 (*P*>0.05; see Fig. 2-1 and 2-4).

Nutritional analyses

Crude protein (CP) and fiber content of aspen leaves was consistent across experiments (*P*>0.05; see Table 2-2). In Experiment 2, Utah sweet pea contained twice the protein content of aspen or smooth bromegrass (*P*<0.001). In contrast, fiber content was greater in smooth bromegrass than the other two species (*P*<0.001). Tall fescue contained greater concentrations of CP than smooth bromegrass, but less than Utah sweet pea (*P*<0.05), and had a lower fiber content than smooth bromegrass, but more than aspen (*P*>0.05), and Utah sweet pea contained comparable concentrations of ADF ($P>0.05$) but slightly less NDF than tall fescue ($P=0.023$).

The high protein supplement contained the greatest concentrations of CP and TDN ($P<0.05$), and lowest concentrations of ADF and NDF than the other supplements ($P<0.05$; Table 2-1). The bark supplement contained the greatest concentrations of ADF and NDF ($P<0.05$), and the lowest concentrations of CP, TDN, and DE compared to other supplements ($P<0.05$). The bark component of the bark supplement contained high concentrations of fiber and low concentrations of CP relative to aspen leaves. The tannin supplement had a similar concentration of CP as the control ($P>0.05$), and slightly greater concentrations of ADF and NDF than the high protein supplement ($P<0.05$). The control supplement contained the greatest concentration of DE ($P<0.05$), followed by high protein, tannin, and bark supplements. Phosphorous, Mg, and Mn content was highest in the high protein supplement ($P<0.05$), Na concentrations were highest in the control supplement, and K was greatest in the control and tannin supplements ($P<0.05$; see Table 2-1).

Plant secondary compounds

Aspen PG content was greater in Experiments 1 (*High content of PG*) and 2 (*Forage Preference*) than in Experiment 3 (*Low content of PG*) ($P<0.05$; see Table 2-3). Phenolic glycoside concentrations were lower in aspen bark than in aspen leaves in all experiments ($P<0.05$, see Tables 2-1 and 2-3). Condensed tannin content in aspen leaves remained relatively constant across experiments ($P>0.05$), with lower values in aspen bark than in leaves ($P<0.05$).

DISCUSSION

The value of any food to an herbivore is not solely dependent upon its intrinsic characteristics but on the chemical context–i.e., the kinds and numbers of foods available and consumed in the environment and each animals' unique experiences with those foods (Baraza et al. 2005; Villalba et al. 2015). Nutrients interact with other nutrients and plant secondary compounds (PSC), and PSC interact with other PSC in the diet to modify herbivores' foraging preferences (Provenza and Villalba 2006; MacAdam and Villalba 2015).

Nutrient supplementation and aspen intake

We hypothesized that nutrients and PSC in aspen interact with nutrients in the surrounding vegetation to influence aspen use by herbivores. Thus, we predicted that protein inputs would enhance aspen intake by sheep. Consistent with this prediction, sheep offered the protein-based supplement ingested more aspen than sheep in the control or aspen bark treatments. The positive effects of protein on aspen intake can be explained by the positive influence of protein on PSC detoxification by mammalian herbivores (Villalba and Provenza 2002; Villalba and Provenza 2005). Plant secondary compounds impose nutritional costs to herbivores because the process of detoxification requires nutrients such as protein and glucose that otherwise would be available for maintenance and production (Illius and Jessop 1995; 1996). Thus, ingestion of appropriate amounts of protein may enable herbivores to ingest more PSC-containing foods (Villalba and Provenza 2002; Villalba and Provenza 2005). Consequently, we suggest that the increase in aspen intake observed in sheep supplemented with protein in this study was mediated by a reduction in the negative post-ingestive impacts of phenolic glycosides (PG) and condensed tannins present in aspen leaves. High foliar concentrations of phenolic glycosides in aspen have been implicated in insect (Lindroth and Hwang 1996) and mammalian herbivore deterrence (Bailey et al. 2007; Wooley et al. 2008), and condensed tannins are digestibility reducers with the potential to inhibit forage fermentation in ruminants (Robbins et al. 1991).

While the mechanisms of phenolic glycoside detoxification by herbivores are still unclear, it is known that the metabolism of phenolic glycosides depends on enzymatic processes that require protein for detoxification to occur (Boeckler et al. 2011). Likewise, terpenes require protein for detoxification and sheep supplemented with protein eat more sagebrush (*Artemisia tridentata*; a terpene-containing shrub) (Villalba et al. 2002), or terpene-containing rations (Villalba and Provenza 2005) than animals that received supplements with a lower protein content. Nevertheless, the impact of protein supplementation on aspen intake was evident when the concentration of phenolic glycosides in aspen was low (Experiment 3; in the range of 7-8%) or when animals had alternative forages in a choice test (Experiment 2). When lambs were offered aspen leaves with high concentrations of phenolic glycosides without alternatives during Experiment 1, the concentration of these plant secondary compounds (in the range of 13% PG) were likely above the threshold for protein to be effective at attenuating the negative post-ingestive effects of aspen.

Condensed tannins bind to proteins with great affinity, which reduces their bioavailability (Hagerman et al. 1992), and this process may also explain the positive effects of protein supplementation on consumption of tannin-containing aspen leaves, i.e., inactivation of foliar condensed tannins through binding with proteins in the rumen.

Minerals may have also played a role in mediating increased aspen intake by lambs. The tannin-containing supplement contained high concentration of K, whereas the high-protein supplement showed low concentrations of K but high concentrations of Mg, minerals which have been identified as having strong positive relationships with aspen preference by deer (Holeski et al. 2016). Thus, high-protein and tannin supplements might have also enhanced aspen utilization via the provision of these key minerals to the diet. However, minerals were not assessed in aspen leaves for this particular study. Mineral analyses in aspen leaves collected from the same region in a different study (see Chapter 3) show average concentrations of 0.7% for K and 0.2% for Mg in aspen leaves, suggesting that additional inputs of these minerals could have enhanced aspen use by sheep.

Chemical defenses and aspen intake

In this study, we also predicted that aspen chemical defenses would constrain aspen intake and preference by sheep. Consistent with this notion, sheep consumed less aspen when the concentration of PG was in the range of 11-13% (Experiments 1 and 2) than when the concentration of PG was in the range of 7-8% (Experiment 3). This suggests that exposure to PG promoted negative post-ingestive consequences in lambs, which led to a reduction in the acceptability of aspen leaves.

Aspen leaves of young ramets that are more susceptible to browse pressure can contain upwards of 25% (dry weight) of phenolic glycosides (Donaldson et al. 2006). Thus, the high concentrations of PG in Experiments 1 and 2 represented approximately 50% of the maximum concentration that can be found in young aspen suckers that have not escaped ungulate herbivory through vertical growth. Yet, prior research suggests that concentrations of PG in the range reported for this study can deter ungulate herbivory, contributing to the persistence of undamaged genotypes during periods of high browse pressure (Lindroth and St Clair 2013; Seager et al. 2013).

An alternative explanation for greater consumption of aspen leaves with a lower content of chemical defense (Experiment 3) involves compensatory intake. Less defended aspen leaves contained lower concentrations of needed nutrients (e.g., leaves were lower in protein content than leaves from Experiments 1 and 2) which may have prompted lambs to produce a compensatory increase in aspen intake, particularly as growing lambs require greater concentrations of crude protein in their diets than adults (NRC 1985).

Adding bark to the supplement fed to sheep reduced aspen use by sheep relative to the control when the concentration of phenolic glycosides in aspen leaves was high (in Experiment 1—*High content of PG* and Experiment 2—*Forage Preference*) despite the low concentration of phenolic glycosides observed in bark relative to aspen leaves. It is likely that aspen bark increased the negative post-ingestive effects of phenolics glycosides in aspen leaves through an additive effect that lead to an antagonistic relationship between aspen leaves and bark, as both shared the same chemical defenses. When herbivores consume chemical defenses that are metabolized by overlapping detoxification pathways, i.e., those that share the same enzymes or co-substrates during the detoxification process, they ingest less food due to saturation of the process than when chemical defenses are metabolized by non-overlapping pathways (Freeland and Janzen 1974; Marsh et al. 2006).

In contrast to bark supplementation, lambs supplemented with condensed tannins ate more aspen compared to control animals during Experiments 2 and 3. Certain types of condensed tannins may enhance protein nutrition by binding with proteins and reducing their bioavailability as they travel through the rumen. Those proteins are then released in the abomasum and upper intestine where pH values decline, increasing the supply of dietary essential and branched-chain amino acids available to the herbivore in the upper small intestine (Hagerman et al. 1992; Reed 1995; Foley et al. 1999). Thus, condensed tannin supplementation likely provided additional dietary proteins to the animal that may have increased PSC detoxification, which in turn favored aspen intake.

Availability of forage alternatives and aspen intake

Finally, we predicted that availability of nutritious forage alternatives (e.g., grasses and forbs growing in an aspen understory) would constrain aspen intake and preference by sheep. Nevertheless, when the concentration of PG was similar (in the range of 11-13%) for Experiments 1 and 2, intake of aspen was greater in Experiment 2 (when animals had a choice of aspen, smooth bromegrass and Utah pea) than when no alternatives were available (Experiment 1—*High Content of PG*). It is likely that the nutrients available during the forage preference test allowed for a greater consumption of aspen leaves, either through the positive effects of nutrients on detoxification of PSC as discussed above–or through nutrient balancing. Often, an herbivore cannot meet its nutrient requirements from a single plant or plant part and must select foods with nutrients that complement each other (Westoby 1978). Herbivores maintain a balance of energy to protein in their bodies that meets their nutritional needs, and in the process, they recognize different internal states and discriminate among different nutrients (Provenza 1995; Provenza and Villalba 2006). Sugars (e.g., non-structural carbohydrates) can have sizable effects on aspen preference by herbivores (Holeski et al. 2016) and is likely that sheep increased intake of aspen leaves to balance the excess of protein ingested with Utah pea during the choice tests with soluble carbohydrates. Alternatively, it is possible that the increased intake of aspen over the testing periods for Experiments 1, 2, and 3 include adaptation of rumen microbes to chemical defenses in aspen (Freeland and Janzen 1974; Odenyo and Osuji 1998; Cardozo et al. 2004).

During choice tests, lambs preferred smooth bromegrass to Utah sweet pea and aspen leaves, even though Utah sweet pea had the greatest content of CP out of all forages available in the choice test, a nutrient preferred by herbivores (Provenza and Villalba 2006). This is also in agreement with recent studies conducted with deer showing low preference for forages containing high concentrations of nitrogen (Holeski et al. 2016). It is likely that the high concentration of protein in Utah sweet pea overloaded the animals' deaminating systems, leading to high concentrations of ammonia in the rumen which can be toxic (Harper 1974). This excess can explain a reduction in preference for Utah sweet pea, particularly in animals that had already consumed a protein load like those that received the high-protein supplement. In fact, lambs supplemented with high protein showed some of the lowest intakes of Utah sweet pea. Given pre-loads of protein, lambs avoid foods high in protein and prefer flavors previously paired with energy, during ensuing meals (Villalba and Provenza 1999). In contrast, animals supplemented with condensed tannins showed greater preference for Utah sweet pea likely due to the binding capacity of protein with tannins and the aforementioned positive effects of condensed tannins on protein utilization (Hagerman et al. 1992; Reed 1995; Foley et al. 1999).

Lambs also preferred smooth bromegrass to aspen leaves, even when aspen leaves had greater concentrations of TDN and less fiber, a structural constituent of forages that lowers food intake and the nutritional quality of food (Van Soest 1994). When presented with a wide variety of alternatives, herbivores will choose less-defended or non-defended forages over defended ones, particularly if these forages are highly available and easy to handle (Bryant and Kuropat 1980). Thus, it is suggested that the negative effects of phenolic glycosides in aspen were responsible for this preference pattern. In fact, intake of aspen by sheep was greater for animals supplemented with high protein or tannins during choice tests, chemicals that–as discussed above–attenuate the negative post-ingestive impacts induced by phenolic glycosides.

This study isolated key variables from the biochemical context in controlled conditions to explore their influence on aspen intake by sheep. Extrapolation of these findings to the landscape should consider additional intervening variables such as the abundance and diversity of forage alternatives. Animals in this study had a limited number of forages available, and during two experiments aspen was offered without alternatives during testing. Additional interacting variables may influence aspen intake under natural conditions such as the presence of predators and time constraints for searching and handling food. Lambs in our experiments were offered their supplement, followed by aspen leaves and then their basal diet for set amounts of time on a fixed schedule.

Conclusions

The information presented in this study suggests that the biochemical context–nutrients and plant secondary compounds in the target plant and in the plant community–

influences the extent to which herbivores are willing to consume a target plant (in this case, aspen leaves). Some chemicals may enhance (e.g., through synergism) or depress (e.g., through antagonism) forage consumption depending on the types and proportions of nutrients and chemical defenses ingested with the diet. Thus, once the chemical composition of aspen and the nutritional composition of the associated plant community are characterized, innovative management strategies like targeted supplementation may aid at mitigating aspen dieback triggered by herbivory. For instance, if a reduction in the consumption of the target plant like aspen is desired (e.g., stands at risk of becoming over-browsed), then it may be possible to provide foods or forages with PSC that antagonize aspen use, i.e., with PSC that are metabolized by overlapping detoxification pathways, promoting associational protection for at-risk stands. In contrast, low abundance of forages with high protein content (e.g., forbs) in the aspen understory may provide "supplementary protein" to consumers, which will use aspen for the bulk of their diet with the potential to enhance aspen herbivory and further contribute to aspen dieback through associational susceptibility.

REFERENCES

AOAC (2000) Official methods of analysis, 17th edn. Association of official analytical chemists. Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484

Atsatt PR, DJ O'Dowd (1976) Plant defense guilds. Science 193(4247):24-29

Bailey JK, Schweitzer JA, Rehill BJ, Irschick DJ, Whitham TG, Lindroth RL (2007) Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. Biol Invasions 9:715–722

Baraza E, Villalba JJ, Provenza, FD (2005) Nutritional context influences preferences of lambs for foods with plant secondary metabolites. Appl Anim Behave Sci 92:

293–305

Baraza E, Zamora R, Hódar JA (2006) Conditional outcomes in plant–herbivore interactions: neighbours matter. Oikos 113:148-56

Boeckler GA, Gershenzon J, Unsicker SB (2011) Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. Phytochemistry 72:1497-1509

Bryant JP, Kuropat PJ (1980) Selection of winter forage by subarctic browsing vertebrates: the role of plant chemistry. Ann Rev Ecol Sys 11(1):261-85

Burritt EA, Provenza FD (2000). Role of toxins in intake of varied diets by sheep. J Chem Ecol 26(8): 1991-2005

Cardozo PW, Calsamiglia S, Ferret A, Kamel C (2004) Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture. J Anim Sci 82(11):3230-3236

Donaldson JR, Stevens MT, Barnhill HR, Lindroth RL (2006) Age-related shifts in leaf chemistry of clonal aspen (*Populus tremuloides*). J Chem Ecol 32:1415-1429

Erbilgin N, Galvez DA, Zhang B, Najar A (2014) Resource availability and repeated defoliation mediate compensatory growth in trembling aspen (*Populus tremuloides*) seedlings. Peer J 2:e491 https://dx.doi.org/10.7717/peerj.491

Foley WJ, Iason GR, McArthur C (1999) Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? In: Jung HG and Fahey GC Jr. (eds) Nutritional ecology of herbivores. Proc Vth Int Symp Nutr Herb Am Soc Anim Sci, Il, pp 130–209

Freeland WJ, Janzen DH (1974) Strategies in herbivory by mammals: The role of plant secondary compounds. Am Nat 108:269-288

Frutos P, Hervás G, Giráldez FJ, Mantecón AR (2004) Review. Tannins and ruminant nutrition. Span J Agric Res 2(2):191-202

Hagerman AE, Butler L (1980) Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28:947–952

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. J Range Manag 45:57–62

Hamilton T, Burritt EA, Villalba JJ (2015) Assessing the impact of supplements, food aversions, andsilica on medusahead (*Taeniatherum caput-medusae* (L.) Nevski) use by sheep. Small Ruminant Res 124:45-54

Harper AE (1974) Amino acid excess. In: White PL and Fletcher DC (eds) Nutrients in Processed Foods—Proteins. Acton, MA

Hjältén J, Danell K, Lundberg P (1993) Herbivore avoidance by association: vole and hare utilization of woody plants. Oikos 1:125-31

Holeski LM, McKenzie SC, Kruger EL, Couture JJ, Rubert-Nason K, Lindroth RL (2016) Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. J Ecol 104:850-863

Illius AW, Jessop NS (1995) Modeling metabolic costs of allelochemical ingestion by foraging herbivores. J Chem Ecol 21:693–719

Illius AW, Jessop NS (1996) Metabolic constraints on voluntary intake in ruminants. J Anim Sci 74:3052–3062

Jones BE, Lile DF, Tate KW (2011) Cattle selection for aspen and meadow vegetation: implications for restoration. Rangeland Ecol Manag 64:625–632

Leimar O, Tuomi J (1998) Synergistic selection and graded traits. Evol Ecol 12:59–71

Lindroth RL, Hwang SY (1996) Diversity, redundancy, and multiplicity in chemical defense systems of aspen. In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, pp 25–56

Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO2: productivity, phytochemistry, and insect performance. Ecology 74:763–777

Lindroth RL, St. Clair SB (2013) Adaptations of quaking aspen (Populus tremuloides Michx.) for defense against herbivores. Forest Ecol Manag 299**:**14–21

MacAdam JW, Villalba JJ (2015) Beneficial effects of temperate forage legumes that contain condensed tannins. Agriculture 20:475-91

Marsh KJ, Wallis IR, McLean S, Sorensen JS, Foley WJ (2006) Conflicting demands on detoxification pathways influence how common brushtail possums choose their diets. Ecology 87(8):2103-12

NRC (1985) Nutrient requirements of sheep, 6th edn. National Academy Press, Washington

Odenyo AA, Osuji PO (1998) Tannin-tolerant ruminal bacteria from East African ruminants. Can J Microbiol 44:904–909

Palo RT, Robbins CT (1991) Plant Defenses against mammalian herbivory. CRC Press, Boca Raton, Florida

Porter LJ, Hrstich LN, Chan BC (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223–230

Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J Range Manag 48:2-17

Provenza FD, Villalba JJ (2006) Foraging in domestic herbivores: Linking the internal and external milieu. In: Bels VL (ed) Feeding in Domestic Vertebrates: From Structure to function. CABI Publ, Oxfordshire, UK pp 210-240

Rautio P, Bergvall UA, Tuomi J, Kesti K, Leimar O (2012) Food selection by herbivores and neighbourhood effects in the evolution of plant defences. Annales Zoologici Fennici 49(1–2): 45-57

Rautio P, Kesti K, Bergvall UA, Tuomi J, Leimar O (2008) Spatial scales of foraging in fallow deer: implications for associational effects in plant defences. Oecologica 34(1):12-20

Reed JD (1995) Nutritional toxicology of tannins and related polyphenols in forage legumes. J Anim Sci 73:1516-1528

Robbins CT, Hagerman AE, Austin PJ, McArthur C, Hanley TA (1991) Variation in Mammalian Physiological Responses to a Condensed Tannin and Its Ecological Implications. J Mammal 72:480–486

Robbins CT, Hanley TA, Hagerman AE, Hjeljord O, Baker DL, Schwartz CC, Mautz WW (1987) Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68:98-107

Seager ST, Eisenberg C, St. Clair SB (2013) Patterns and consequences of ungulate herbivory on aspen in western North America. Forest Ecol Manag 299:81–90

Senft RL, Coughenour MB, Bailey DW, Rittenhouse LR, Sala OE, Swift DM (1987) Large herbivore foraging and ecological hierarchies. Bioscience 37:789-799

Swift RW (1957) The caloric value of TDN. J Anim Sci 16:753–756

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597

Van Soest PJ (1994) Nutritional Ecology of the Ruminant, 2nd ed. Cornell University

Press, Ithaca, NY

Villalba JJ, Burritt EA, St. Clair SB (2014) Aspen (*Populus tremuloides* Michx.) Intake and Preference by Mammalian Herbivores: the role of plant secondary compounds and nutritional context. J Chem Ecol 40(10):1135-1145

Villalba JJ, Provenza FD (1999) Nutrient-specific preferences by lambs conditioned with intraruminal infusions of starch, casein, and water. J Anim Sci 77(2):378-87

Villalba JJ, Provenza FD (2002) Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: Benefits or detriments for plants? Oikos 97(2):282-292

Villalba JJ, Provenza FD (2005) Foraging in chemically diverse environments: Energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. J Chem Ecol 31(1):123-38

Villalba JJ, Provenza FD, Banner RE (2002) Influence of macronutrients and polyethylene glycol on intake of a quebracho tannin diet by sheep and goats. J Anim Sci 80:3154-3164

Villalba JJ, Provenza FD, Catanese F, Distel RA (2015) Understanding and manipulating diet choice in grazing animals. Anim Prod Sci 55:261-271

Villalba JJ, Provenza FD, Hall JO (2008) Learned appitites for calcium, phosphorus, and sodium in sheep. J Anim Sci 86(3):738-747

Weiss WP, Conrad HR, Pierre NRS (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim Feed Sci Technol 39:95–110

Westoby M (1978) What are the biological bases of varied diets? Am Nat 112:627–631

White CA, Feller MC, Bayley S (2003) Predation risk and the functional response of elk-aspen herbivory. Forest Ecol Manag 181:77–97

Wooley SC, Walker S, Vernon J, Lindroth RL (2008) Aspen Decline, Aspen Chemistry, and Elk Herbivory: Are They Linked? Rangelands 30(1):17-21

CHAPTER 3

NUTRITIONAL STATE AND SECONDARY COMPOUNDS INFLUENCE TREMBLING ASPEN (*Populus tremuloides*) INTAKE BY SHEEP[2]

Abstract

The foraging ecology of mammalian herbivores is determined by plant secondary compounds (PSC) that defend plants against herbivory, by nutrients that are required for maintenance, growth, and reproduction, and by the interaction between these variables. In this study, we explored the influence of an herbivore's nutritional state on intake of aspen (*Populus tremuloides*) leaves with contrasting concentrations of phenolic glycosides (PG). Thirty-two lambs were randomly assigned to two groups (N=16). Each group received aspen leaves with either a high (21.2%) or low (16.3%) concentration of PG throughout the study. In Experiment 1, half of the animals within each group received rations with a high (22.2% crude protein [CP]; 3.13 Mcals/kg) or low (11.9% CP; 3.13 Mcals/kg) concentration of CP. During Experiment 2, half of the animals within each group received rations with a high (3.45 Mcals/kg; 16.1% CP) or low (2.52 Mcals/kg; 18.5% CP) concentration of energy. During Experiment 3, all lambs received a simultaneous offer of aspen leaves containing high (20.6%) and low (14.8%) concentrations of PG. Aspen intake was greater when animals received the high-protein or the low-energy rations ($P<0.05$), and when they received aspen leaves with low concentrations of PG ($P=0.009$). During Experiment 3 (choice of high or low PG aspen), aspen intake was not affected by concentrations of PG, but intake increased when

---

[2] Kristen Y. Heroy, Samuel B. St. Clair, Elizabeth A. Burritt, Susan L. Durham, and Juan J. Villalba

nutrients were present in aspen in a combination of low concentrations of Cu, P, K, S, crude protein, and an intermediate concentration of condensed tannins. In summary, nutritional state and aspen's chemical composition modulated the extent to which aspen was consumed by sheep. Herbivores grazing plant communities with high concentrations of protein or low concentrations of energy may be more likely to consume greater amounts of aspen than those animals grazing forages with low concentrations of protein or high concentrations of energy.

Introduction

The foraging ecology of mammalian herbivores is determined by plant secondary compounds (PSC) that defend plants against herbivory (Freeland and Janzen 1974, Foley et al. 1999, Dearing et al. 2000) and by nutrients that are required for maintenance, growth, and reproduction (Van Soest 1994, Robbins 2012). These variables do not act in isolation, as nutrients interact with other nutrients and with PSC, while animals forage in chemically diverse landscapes (Provenza et al. 2003, Illius and Jessop 1995, 1996). For instance, plant secondary compounds restrict food intake (Freeland and Janzen 1974, Palo and Robbins 1991), compromise energy budgets (Sorensen et al. 2005), and disrupt acid-base homeostasis (Foley et al. 1995) in herbivores, causing loss of sodium, body protein, and glucose during the detoxification and elimination processes (Dearing et al. 2001, Illius and Jessop 1995, 1996). Given that plant defenses induce nutrient losses while constraining food intake, it follows that an herbivores' nutritional state plays a fundamental role in the mitigation of the costs associated with processing and eliminating of PSC when ingesting defended plants (Villalba and Provenza 2002, Sorensen et al. 2005).

Aspen (*Populus tremuloides*) communities provide an excellent system to explore the influence of an herbivore's nutritional state on preference for a defended plant as aspen trees produce a suite of phenolic compounds such as simple phenolic glycosides (PG), which reduce feeding, growth, and survival of insect herbivores and deter feeding by mammalian herbivores (reviewed by Lindroth and St Clair 2013, Villalba et al. 2014). In addition, aspen shows substantial genetically based variation in phytochemical traits (Lindroth and Hwang 1996), which provides a diverse range of PSC concentrations to consumers. Young and defended aspen trees growing in patches represent a concentrated source of nutrients that attract both wild (White et al. 2003) and domestic (Jones et al. 2011) ungulates. Overbrowsing by ungulates is one outcome of this process, and a major cause of poor aspen stand regeneration in some areas of North America (Romme et al. 1995) and Eurasia (Myking et al. 2011). From the previous analysis, it follows that variations in nutritional state–induced by diets with different concentration of nutrients ingested across the landscape–and variability in aspen's phytochemical traits emerging from genetic variation, may act in concert to influence the extent to which herbivores browse on young aspen trees. This process, in turn, may have a significant impact on the likelihood of aspen regeneration and recruitment in aspen-dominated landscapes.

In addition to nutritional state, an herbivore's experiences with chemically defended foods may influence the extent to which those foods are incorporated into their diet. Prior experience with defended foods shape an herbivore's physiological abilities to metabolize toxins through changes in the diversity and population structure of the gut microbiome (Kohl et al. 2014) or increments in the production of tissue enzymes that detoxify plant toxins (i.e., cytochrome P450s) (Delgoda and Westlake 2004).

Collectively, prior exposure to plant defenses may induce adaptive responses in herbivores that promote tolerance. On the other hand, negative post-ingestive consequences induced by PSC may lead to the development of food aversions that overwhelm the animals' adaptive responses, which enhances food avoidance during subsequent encounters with the defended target plant (Garcia 1989, Mitchelson 1992, Provenza 1995, 1996).

We hypothesized that nutrients and PSC in aspen tissues interact with nutrients in the surrounding vegetation to influence aspen use by herbivores. Thus, we predicted that: (i) herbivores under a nutritional state induced by diets high in protein or energy content will be more likely to consume aspen than those animals ingesting diets with low concentrations of protein or energy because protein and energy are known to aid detoxification processes. Alternatively (ii), the reverse could be expected if the benefits of accruing energy or protein from aspen tissues in energy- or protein-deficient animals are more consequential than the costs incurred by consuming PSC. Thus, this prediction suggests that the concentration of chemical defenses in aspen modulates the influence of the nutritional state on aspen intake. Finally, (iii) herbivores previously exposed to aspen stands with high concentrations of chemical defenses would show greater preferences for such stands than animals previously exposed to aspen with lower concentrations of phytochemicals because prior experience with high concentrations of chemical defenses improves the efficiency of detoxification. Thus, we modified the nutritional state of sheep by providing basal diets of contrasting protein (Experiment 1) or energy (Experiment 2) content while animals ingested aspen leaves with either high or low concentrations of PG (test of predictions i and ii). Finally, we determined the influence of prior experience with consuming aspen on intake of and preference for aspen stands that varied in chemical composition (Experiment 3; test of prediction iii).

Materials and methods

Study Site

Experiments were conducted at the Utah State University Green Canyon Ecology Center, Logan, UT, USA using 32 crossbred lambs of both sexes (4 months of age; 22±1.3 kg of body mass at the beginning of the study) housed in individual pens according to the Utah State University Animal Care and Use Committee guidelines (approval # IACUC-2238). In addition to wildlife, domestic herbivores including sheep browse aspen (Smith et al. 1972, Beck and Peek 2005). This study used sheep, a mesoherbivore that consumes browse and grass, in controlled conditions as a model system to allow a rigorous analysis of the influence of nutritional state and plant secondary compounds on aspen utilization by ruminant herbivores.

Plant material for the study was collected from ten locations within the Uintah/Wasatch/Cache National Forest, Utah, USA. All aspen leaves were harvested from suckers below the browse line (less than 2 m tall) with no evidence of browsing. No more than two aspen branches were harvested from any one tree.

During the *Familiarization Period*, aspen leaves were harvested from Turner Campground (N 41° 53.127’ W 111° 33.921’), and aspen used for the *Intake Assessment* was harvested from Franklin Basin #1 (N 41° 56.730’ W 111° 34.636’). The aspen used in Experiment 1 was harvested from Franklin Basin #2 (N 41° 56.253’ W 111° 34.202’; high phenolic glycoside [PG] aspen [HPG]) and Franklin Basin #3 (N 41° 55.826’ W

111° 33.810’; low PG aspen [LPG]), and Experiment 2 aspen was harvested from Franklin Basin #4 (N 41° 57.579’ W 111° 35.471’; HPG) and Sink Hollow (N 41° 55.016’ W 111° 28.846’; LPG). Aspen used in Experiment 3 was harvested from Temple Fork Rd (N 41° 47.440’ W 111° 33.911’; HPG) and Beaver Recreation area (N 41° 59.010’ W 111° 31.883’; LPG).

General Protocol

We conducted two experiments (Experiments 1 and 2) to explore the influence of the nutritional state and concentration of aspen chemical defenses on aspen intake by sheep. This was achieved in a 2 x 2 factorial design where we influenced the nutritional state of 32 lambs by providing isoenergetic total mixed rations (TMR) with high (N=16) or low (N=16) concentrations of protein (Experiment 1), or rations of similar concentrations of protein with high (N=16) or low (N=16) concentrations of energy (Experiment 2). Half the lambs in each ration arrangement received aspen leaves with either high or low concentrations of phenolic glycosides. Thus, Experiments 1 and 2 tested how selection for leaf material with low and high PG varied depending on the lambs’ nutritional state (i.e., protein and energy). Each lamb received aspen leaves with either high or low concentrations of phenolic glycosides throughout both experiments, although after analyzing leaf samples we found the stands chosen during Experiment 2 (HPG: 18.9%; LPG: 17.5%) were much less contrasting than during Experiment 1 (HPG: 23.4%; LPG: 15.1%). Subsequently, lambs received a simultaneous offer of aspen leaves from stands containing high or low concentrations of phenolic glycosides (Experiment 3). Thus, Experiment 3 tested for the effects of PG concentration and prior experience with exposure to different concentrations of PG on aspen preference. Aspen intake for all experiments was expressed as dry matter intake (DMI) and as DMI per unit of metabolic body weight (MBW), using the equation: intake/unit of MBW = DMI ÷ ($BW^{0.75}$).

Familiarization period

Lambs naïve to aspen were housed as a group in a covered outdoor pen (15.7 m × 14.6 m) prior to Experiment 1 and were familiarized with increasing amounts of freshly harvested mature aspen leaves containing 20.8% of PG obtained from Turner Campground every day for 2 weeks. All stands were chosen from samples analyzed from fully expanded aspen leaves sampled at different locations on July 28 and 29, 2014 to identify stands with high and low concentrations of PG. From days 1 to 3, lambs received 1 kg of aspen branches with their leaves in an attempt to initiate interest in aspen leaves attached to the branch. From days 4 to 7, they received 2 kg of aspen leaves in a trough, which was increased to 3 kg of leaves from days 8 to 14. Aspen was offered at 0900 each morning.

On day 3, animals were randomly assigned to one of two pens (N=16) blocked by body mass. Each outdoor covered pen measured 57.4 m^2 and the average lamb mass in pen 1 and 2 was 20.0±2.0 kg and 21.6±1.7 kg, respectively. Once in their pens, in addition to aspen, lambs were introduced to their respective rations. Lambs in pens 1 and 2 received a ration with low (LP) or high (HP) concentrations of CP, respectively (see Table 3-1 for nutritional composition of each ration). The protein content of HP was slightly greater than what is typically seen in the aspen understory at the beginning of summer, but the protein content in LP was similar to what is found by the end of summer in mid-September (DeByle et al. 1989). Digestibility values of the rations (estimated by

Table 3-1. Nutritional analyses (% dry matter of total ration) and DE of the rations fed during Experiments 1 (ration with high or low protein) and 2 (ration with high or low energy).

Experiment 1[a] – High- and Low-Protein Ration							
High protein (HP)	Percent[i]	DE[c]	CP[d]	CP[e]	ADF[f]	NDF[g]	TDN[h]
Beet Pulp	35	1.19	3.36				
Molasses	6.8	0.215	0.6596				
SBM[i]	22	0.825	10.494				
Alfalfa	36	0.9	5.22				
Urea	0.2	0	0.562				
Total	100	3.13	20.296	22.2±0.93	26.106±0.47	36.15±0.79	66.64±0.49
Low protein (LP)	Percent[i]	DE[c]	CP[d]	CP[e]	ADF[f]	NDF[g]	TDN[h]
Beet Pulp	40	1.36	3.84				
Molasses	40.8	1.289	3.9576				
SBM[i]	0.1	0.00375	0.0477				
Alfalfa	19	0.475	2.755				
Urea	0.1	0	0.1405				
Total	100	3.13	10.741	11.87±0.07	26.599±0.09	39.136±0.03	66.13±0.09
Experiment 2[b] – High- and Low-Energy Ration							
High energy (HE)	Percent[i]	DE[c]	CP[d]	CP[e]	ADF[f]	NDF[g]	TDN[h]
Wheat bran	4	0.112	0.608				
Barley	47	1.8236	6.345				
SBM	3	0.1125	1.431				
Alfalfa	15	0.375	2.175				
Beet pulp	30	1.02	2.88				
Grape pomace	1	0.0109	0				
Total	100	3.45	13.439	16.09±0.51	19.433±0.59	31.906±0.54	73.514±0.61
Low energy (LE)	Percent[i]	DE[c]	CP[d]	CP[e]	ADF[f]	NDF[g]	TDN[h]
Wheat bran	40	1.12	6.08				
Barley	2	0.0776	0.27				
SBM	3	0.1125	1.431				
Alfalfa	35	0.875	5.075				
Beet pulp	5	0.17	0.48				
Grape pomace	15	0.1635	0				
Total	100	2.52	13.336	18.47±0.11	29.817±0.11	43.67±0.04	62.819±0.11

[a] June 3 to June 12, 2015
[b] June 25 to July 4, 2015
[c] Digestible energy (Mcal per kg of dry matter) (NRC 1985)
[d] Crude protein (NRC 1985)
[e] Crude protein (Method 990.03 AOAC 2000)
[f] Acid detergent fiber (Van Soest et al. 1991)
[g] Neutral detergent fiber (Van Soest et al. 1991)
[h] Total digestible nutrients
[i] Percent of ration
[j] Soy bean meal

total digestible nutrients in the current study) were slightly greater than those values reported in aspen understories by DeByle et al. (1989).

Each morning at 1000, during days 3 to 14 of the familiarization period, and one hour after aspen leaves were offered, 16 kg of the assigned ration was provided to lambs in each pen at a rate of 3.1 Mcal DE/animal/day, for a total of 1 kg of TMR/animal/day.

All animals were introduced to nipple drinkers secured to the north side of each pen. Until all lambs were drinking from the nipple drinkers, a 56.8 liter tub remained in the pen to ensure *ad libitum* access to water. Culinary water, salt, and trace mineral blocks were available in *ad libitum* amounts throughout the study.

After receiving their respective TMR for 1 week in group pens, lambs were weighed and transferred to adjacent individual pens, measuring 1.5 m x 2 m, built under a protective roof. Prior to the first experiment, all lambs were dewormed with ivermectin (0.2 mg/kg BW). Lambs were allowed an adjustment period to the individual pens for three days before the intake assessment began, during which time they were offered their respective TMR at an increased rate of 3.5 Mcal DE/animal/day, to total 1.1 kg/animal/day. The *Familiarization Period* was conducted from May 14 to May 25, 2015.

Preliminary Intake Assessment

Aspen leaves were harvested from a new stand containing 17.8% of PG (Franklin Basin #1). The change in location was due to a lack of sufficient leaf material in Turner Campground. During the first day of exposure, lambs received 50 g of aspen leaves (on an as fed basis) at 0900 for 2 hours. On ensuing days, aspen offers were increased for lambs by 50 g when refusals for those animals were recorded below 50 g during the previous day. Refusals were collected at 1100 and then lambs received their respective rations until 1800 when refusals were removed from all feeders and no other feed was offered until the following day. Individual aspen intake was determined by subtracting the amounts of aspen refused from those offered each day. After four days of exposure, the amount of TMR offered was reduced to 2.504 Mcal DE/animal/day, or 177.6 and 95.2 g CP/lamb/day for lambs fed HP and LP, respectively (for a total of 800 g TMR/animal/day) because some animals were showing signs of bloat. All lambs were weighed on May 26, one week prior to Experiment 1, and average lambs mass was 25.6±1.3 kg.

Before Experiment 1 began, half of the lambs from each TMR were randomly assigned to receive aspen leaves from a stand with either high (HPG; N=16) or low (LPG; N=16) concentrations of PG. Thus, lambs were assigned to two different aspen stands (HPG and LPG) and two different TMR diets (LP and HP) (8 lambs/treatment group) in a 2 x 2 factorial design. Lambs were blocked by aspen intake determined during the last 3 days of the *Intake Assessment Period* to ensure similar initial aspen intakes across treatment groups. All animals remained in their assigned HPG or LPG treatments throughout both of the experiments that follow. The *Preliminary Intake Assessment* period was conducted from May 25 to June 2, 2015.

Experiment 1—Rations with high or low protein on aspen intake

Fresh aspen leaves were collected from both the HPG and LPG locations every morning (see Tables 3-2 and 3-3 for nutritional and defense chemistry analyses). The protocol was as described for the *Preliminary Intake Assessment* period. The amount of aspen offered on day 1 was determined by the average aspen intake for each lamb during

Table 3-2. Nutritional analyses (% dry matter) of the high and low PG aspen leaves fed during Experiments 1 (ration with high or low protein), 2 (ration with high or low energy), and 3 (choice of high- and low-PG aspen)

Experiment 1–High- and Low-Protein Ration[c]

	CP[i]		ADF[f]		NDF[g]		TDN[h]	
	High PG[a]	Low PG[b]	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 1[c]	19.86±0.51	18.86±1.11	11.89±0.36	15.88±0.56	15.55±0.51	20.76±0.77	74.42±0.29	71.24±0.47

Experiment 2–High- and Low-Energy Ration[d]

	CP		ADF		NDF		TDN	
	High PG	Low PG	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 2[d]	15.27±0.24	15.81±0.59	17.13±0.76	18.07±0.64	22.65±0.6 0	22.75±0.66	70.13±0.60	69.41±0.5

Experiment 3–Choice of High- and Low-PG Aspen[e]

	CP		ADF		NDF		TDN	
	High PG	Low PG	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 3[e]	13.20±0.41	16.27±0.11	18.88±0.93	17.95±0.43	23.77±0.84	22.48±0.40	68.68±0.74	69.52±0.34

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] June 3 to June 12, 2015
[d] June 25 to July 4, 2015
[e] July 13 to July 18, 2015
[f] Acid detergent fiber
[g] Neutral detergent fiber
[h] Total digestible nutrients
[i] Crude Protein

Table 3-3. Concentration of plant secondary compounds (% dry matter) within high and low PG aspen leaves fed during Experiments 1 (ration with high or low protein), 2 (ration with high or low energy), and 3 (choice of high- and low-PG aspen)

Experiment 1 –High- and Low-Protein Ration [c]

	Tremulacin		Salicortin		Total PG		Condensed tannins	
	High PG[a]	Low PG[b]	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 1[c]	11.34±0.47	6.94±0.19	12.09±0.74	8.18±0.37	23.43±1.13	15.12±0.44	4.62±0.99	7.92±2.24

Experiment 2 –High- and Low-Energy Ration [d]

	Tremulacin		Salicortin		Total PG		Condensed tannins	
	High PG	Low PG	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 2[d]	9.31±0.74	7.08±0.47	9.64±0.81	10.39±1.21	18.94±1.41	17.46±1.63	2.63±0.29	2.36±0.38

Experiment 3 –High- and Low-PG aspen [e]

	Tremulacin		Salicortin		Total PG		Condensed tannins	
	High PG	Low PG	High PG	Low PG	High PG	Low PG	High PG	Low PG
Exp. 3[e]	9.8±0.42	6.84±0.38	10.82±0.73	7.97±0.66	20.62±1.11	14.81±1.03	5.54±0.71	2.31±0.30

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] June 3 to June 12, 2015
[d] June 25 to July 4, 2015
[e] July 13 to July 18, 2015

the last three days of the *Preliminary Intake Assessment* period. Experiment 1 was designed as a 2-way factorial in a split-plot design with repeated measures so that both levels of TMR were crossed with both levels of phenolic glycoside concentration. Experiment 1 was conducted from June 3 to June 12, 2015.

Experiment 2—Rations with high or low energy on aspen intake

Familiarization Period

All animals were kept in their assigned high (HPG; N=16) or low (LPG; N=16) PG aspen groups from Experiment 1, but they were re-randomized into each aspen group to receive a new total mixed ration (TMR) with a high (HE) or low (LE) content of digestible energy (see Table 3-1 for nutritional analyses). The protein offered in the high- and low-energy rations was similar to what animals may encounter in the aspen understory during mid-summer, and digestibility of the HE ration was greater than what may be encountered in the aspen understory. On the other hand, the digestibility of the LE ration was similar to what could be found in the understory of aspen communities in early to mid-summer (DeByle et al. 1989). Randomization was done by blocking lambs by average aspen intake for the last three days of Experiment 1 (rations with high- and low-protein) to ensure similar initial intake of aspen across all treatments. Half of the lambs from each aspen PG group were randomly assigned to HE and the other half to LE, so 8 subjects were in each PG-TMR configuration.

All lambs received alfalfa pellets in *ad libitum* amounts for a four-day washout period post-Experiment 1 so all lambs were on an even plane of nutrition to reduce the likelihood of carry-over effects from rations fed during Experiment 1 into Experiment 2.

On day 5, animals began to transition to their respective high- or low-energy TMR. In order to minimize the likelihood of any gastrointestinal problems associated with the transition to new diets, all lambs received their respective rations in incremental amounts over the course of a period of 9 days, starting with a diet composed of 80% alfalfa pellets (640 g) and 20% TMR (160 g), and ending with a diet composed of only TMR (800 g; 2.52 Mcal DE/lamb/day for LE and 3.45 Mcal DE/lamb/day for HE). Experiment 2 began after all lambs received 100% of their respective rations for four consecutive days. All lambs were weighed on June 23, two days before Experiment 2 began (average lamb body mass 28.3±1.2 kg). The *Familiarization Period* was conducted from June 12 to June 24, 2015.

Testing period

Every morning fresh aspen leaves were obtained from the new HPG and LPG stand locations (see Tables 3-2 and 3-3 for nutritional and defense chemical analyses). The change in location from Experiment 1 was due to a lack of sufficient leaf material within those stands. Experiment 2 was designed as a 4-factor split-plot design. The protocol for Experiment 2 was as described for Experiment 1, but lambs received their respective TMR rations at a rate of 2.5% BW (a total of 708 g for the average lamb body weight of 28.3 kg) which delivered a total of 113.91 and 130.77 g CP/lamb/day and 2.443 and 1.784 Mcal DE/lamb/day for lambs fed HE and LE, respectively. The rate of 2.5% BW was calculated from the average intake of TMR during Experiment 1. Final animal body mass was 30.6±1.3 kg at the end of Experiment 2. Animals were allowed a washout period of eight days after Experiment 2 in which they received alfalfa pellets in *ad libitum* amounts and no aspen leaves. The *Testing Period* was conducted from June 25 to

July 4, 2015.

Experiment 3—Choice

Freshly harvested aspen leaves from novel high- and low-PG stands were obtained each morning, and all lambs were offered two buckets side by side simultaneously, secured to the front each pen at 0900 for 30 minutes. Aspen offers for day 1 were calculated from average individual intakes in the previous two experiments on a scale from 100 g to 300 g. Those lambs with the lowest and highest average aspen intake received 100 g and 300 g, respectively, of HPG or LPG leaves. Buckets were monitored constantly for spillage and amount of remaining aspen. Any spillage that could be identified as originating from one bucket or the other was picked up and placed back into the appropriate bucket. If either bucket contained 20 g or less of aspen leaves, both buckets were removed. Immediately after all aspen refusals were collected, a basal diet of alfalfa pellets was offered to all lambs in *ad libitum* amounts from 0930 until 1800, when alfalfa pellets were removed from all feeders and no other feed was offered until the following day. This experiment was designed as a 5-factor (1-Previous exposure to aspen leaves with high or low concentration of PG [during Experiments 1 and 2], 2-Previous exposure to ration in Experiment 1 [Experiment 1 TMR], 3-Previous exposure to ration in Experiment 2 [Experiment 2 TMR], 4-Concentration of PG in aspen leaves fed during Experiment 3, and 5-lamb) split-split-plot design with repeated measures. Experiment 3 was conducted from July 13 to July 18, 2015.

Chemical analyses

Samples of offered aspen leaves (collected every other day for Experiments 1 and

2, and every day during Experiment 3) were placed in plastic bags and transported to a freezer where they were kept at −20 °C. They were subsequently freeze dried, ground in a Wiley mill with a 1 mm screen, and analyzed for dry matter (Method 930.15; AOAC 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al. 1991), and crude protein (CP) (Method 990.03; AOAC 2000). Total digestible nutrients (TDN) were calculated from CP and fiber based on equations from Weiss et al. (1992) as an estimate of digestible energy of the samples (NRC 1985, Swift 1957).

During each day of the study, representative offered and refused samples of aspen leaves and TMR diets were placed in paper bags and dried in a forced-air oven at 60 °C for 48 hours or were freeze-dried for defense chemistry analysis to estimate dry matter content, in order to express intake values on a dry matter basis. Oven-dried TMR samples were used to determine NDF, ADF, and CP as described before.

Phenolic glycosides were extracted from 40 mg of freeze-dried leaf material in 1 ml of methanol. The samples were vortexed on high for 5 minutes and centrifuged at 16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes. This procedure was repeated a second time, and the extracts were pooled to yield 2 ml of crude extract. Phenolic glycosides (salicortin and tremulacin) were quantified using high performance liquid chromatography (Agilent 1100 Series, Santa Clara, CA, USA) with a Luna 2, C18 column (150x4.6 mm, 5 μm) at a flow rate of 1 ml/min. Compound peaks were detected at 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves (Lindroth et al. 1993).

Condensed tannins were extracted from 50 mg of freeze-dried leaf tissue with 1 ml of a 70 % acetone-10 mM ascorbic acid solution. Samples were vortexed on high for

20 minutes at 4 °C followed by centrifugation at 16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes, and the extraction was then repeated. Condensed tannin concentrations were measured spectrophotometrically (SpectraMax Plus 384, MDS, Toronto, Canada) using the acid butanol method (Porter et al. 1986) standardized with purified condensed tannins isolated from aspen leaves (Hagerman and Butler 1980).

For Experiment 3, mineral analyses were conducted by the Utah State University Analytical Laboratory in Logan, UT on dried aspen samples collected during each day. Dried and ground plant samples were digested in a digestion block for 1 hour at 95 ºC with 8 mL of 70% HNO_3. Once removed, 4 mL 30% H_2O_2 was added and tubes containing individual samples were placed back into the digestion block for 30 minutes. The addition and heating with H_2O_2 was repeated two additional times. Tubes were cooled and distilled water was added until the final volume totaled 25 mL. Mineral analysis was then completed on these samples using a Thermo Electron iCAP ICP (inductively-coupled plasma spectrophotometer).

Statistical analyses

Experiment 1—Rations with high or low protein on aspen intake

Blocks (the whole plot) were groups of 4 lambs with a similar body mass during the *Familiarization Period*. All lambs within each block were randomly assigned the same TMR (the whole plot factor). Two randomly chosen lambs (the subplot unit) within each block were assigned to each level of aspen PG concentration (HPG or LPG; the subplot factor). Repeated measures on pairs of lambs over time were modeled with a first-order autoregressive covariance structure, chosen based on the Akaike information criterion. Mean intake over two lambs within each pair was computed for each day and subsequently used as the response variable in the statistical analysis. Pairwise comparisons among means were made as needed and were adjusted for family-wise Type I error rate using the Tukey method. Model assumptions of normality and homogeneity of variance were assessed visually using random effect estimates and residuals. Calculations were made using the MIXED procedure in SAS/STAT 14.1 in the SAS System for Windows 9.4 TS1M3 (SAS Institute Inc., Cary, North Carolina, USA).

Experiment 2—Rations with high or low energy on aspen intake

Because sample sizes for combinations of aspen PG group, TMR offered during Experiment 1, and TMR offered during Experiment 2 were too small (between 2 and 6) to allow for an appropriate estimation of high-order interactions, we computed mean intake over days. Using the mean as the response variable, we examined fixed-effects models with different levels of complexity. We chose the model with only main effects based on AICc. Subsequently, the effects of aspen PG group, Experiment 1 TMR, and Experiment 2 TMR on intake of aspen over time were assessed with an analysis of variance with aspen PG group, Experiment 1 TMR, Experiment 2 TMR, and the interaction of each of these variables with day as fixed effects factors. Whole plot units were lambs, randomly assigned to one of three whole plot factors (aspen PG group, Experiment 1 TMR, and Experiment 2 TMR). Repeated measures on each lamb were subplot units, associated with the subplot factor, day. Covariances among repeated measures were modeled using a first-order autoregressive covariance structure, chosen based on AICc.

Experiment 3—Choice

Lambs' ingestive responses during choice tests were analyzed to investigate the influence of prior experience and chemical composition on aspen intake and preference. Non-metric multidimensional scaling (NMDS) was also applied to identify important variables and to serve as an exploratory analysis of relationships between mineral and non-mineral constituents with preference for aspen. High-PG aspen preference ratio (HPR) was used as a proportional measure of preference using the equation: HPR = (HPG leaves consumed as measured by MBW) ÷ (Total aspen leaves consumed as measured by MBW). A P-value of 0.05 or less was considered significant for all three experiments.

Intake

We examined models in the same fashion as described for Experiment 2, but including "stand consumed" as a fixed-effects factor. Diet components were sub-subplot units, associated with the sub-subplot factor "stand consumed."

Preference

We selected a base model for preference with only main effects based on the AICc criterion, using a generalized linear mixed model with a beta distribution. Subsequently, the effects of prior experience to aspen and to rations in Experiments 1 and 2 on preference for aspen leaves with high content of PG (HPR) over time were assessed in a split-plot design. Whole plot units were lambs, randomly assigned to a level of each of three whole-plot factors (prior experience to aspen and to rations in Experiments 1 and 2). Repeated measures on each lamb were subplot units, associated with the subplot factor, day. Results reported here are based on a compound symmetry structure for repeated measures. Calculations for Experiments 2 and 3 were made using the GLIMMIX procedure in SAS/STAT 14.1 in the SAS System for Windows 9.4 TS1M3 (SAS Institute Inc., Cary, North Carolina, USA).

Nonmetric multidimensional scaling (NMDS) ordination

Joint assessment of relationships between mineral and non-mineral forage constituents and intake were explored using ordinations of forage constituents with subsequent fitting of a smooth response surface (a topographical surface) of intake values over the ordination space. We used nonmetric multidimensional scaling with Bray-Curtis dissimilarity as implemented in the metaMDS and ordisurf functions in the vegan package Version 2.4-1 (Oksanen et al. 2016b) in R Version 3.3.1 using RStudio (R Core Team 2016, RStudio Team 2015). Initially, an ordination was completed using the full set of mineral and non-mineral forage constituents. Subsequently, a subset of these forage constituents was chosen using r^2 values produced by environment fit using the envfit function. Vector length and r^2 values produced by the envfit function are proportional to each other and represent the importance or non-importance of each variable as a predictor, and therefore were used as measures of variable importance (the greater the r^2, the greater the importance) (Oksanen et al. 2016a). Any forage constituent with r^2>0.4 was retained in the ordination. The value 0.4 was chosen because it marked a natural separation in r^2 values for these data. We completed the analysis of relationships using ordination of this subset of forage constituents with an overlaid intake response surface.

Comparison of nutrients across experiments

Comparisons of nutrient concentrations within feed types (aspen and supplements) across all three experiments were analyzed using a one-way analysis of variance for each nutrient.

Results

Experiment 1—Rations with high or low protein on aspen intake

Averaged across days, aspen intake was greater when animals received the high-protein ration (HP) than when they received the low-protein ration (LP) (ration effect P=0.020; Fig. 3-1a). Aspen intake was also greater for animals that received leaves from the aspen stands with a low concentration of PG (LPG) than for those that received aspen leaves with a high content of PG (HPG) (aspen PG effect P=0.009; Fig. 3-1a).

Aspen intake fluctuated across days and differences between treatments became more evident after day 4 (day effect P<0.001). For lambs in the HP treatment, aspen intake increased from day 1 to day 6, and then it remained fairly constant from days 6 to 10. For the LP treatments, aspen intake fluctuated throughout the 10-day period (ration × day interaction P<0.001; Figure 3-1b). For animals that received LPG, average aspen intake increased from day 1 to day 5, and then it remained fairly constant from days 5 to 10. For lambs that received HPG, average aspen intake increased from days 1 to 3, decreased from day 3 to day 4, and then it remained steady from days 4 through 10 (aspen PG concentration × day interaction P<0.001; Fig. 3-1c).

Experiment 2—Rations with high or low energy on aspen intake

Averaged across days, aspen intake was greater when animals received the low-

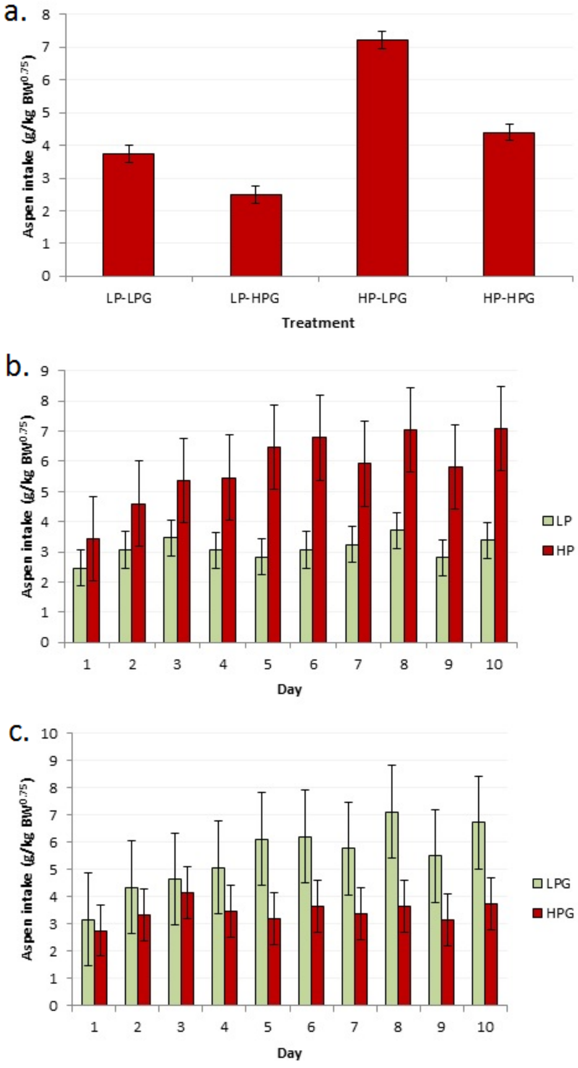

Figure 3-1. Average intake of aspen leaves by groups of lambs offered a ration of either low (LP) or high (HP) protein content and aspen leaves of either low (LPG) or high (HPG) concentrations of phenolic glycosides during Experiment 1: (**a.**) Average intake of aspen leaves by the four treatment combinations (8 lambs/treatment), (**b.**) average daily intake of aspen leaves by lambs offered the LP and HP rations (16 lambs/treatment), and (**c.**) average daily intake of aspen leaves by lambs offered LPG or HPG aspen leaves (16 lambs/treatment). Bars (with SEMs) are means for intake values recorded during ten days.

energy ration (LE) than when they received the high-energy ration (HE) (P=0.001; Fig. 3-2a). However, concentration of PG did not affect aspen intake (aspen PG concentration effect P>0.05). Intake steadily increased from day 1 to day 10 regardless of PG

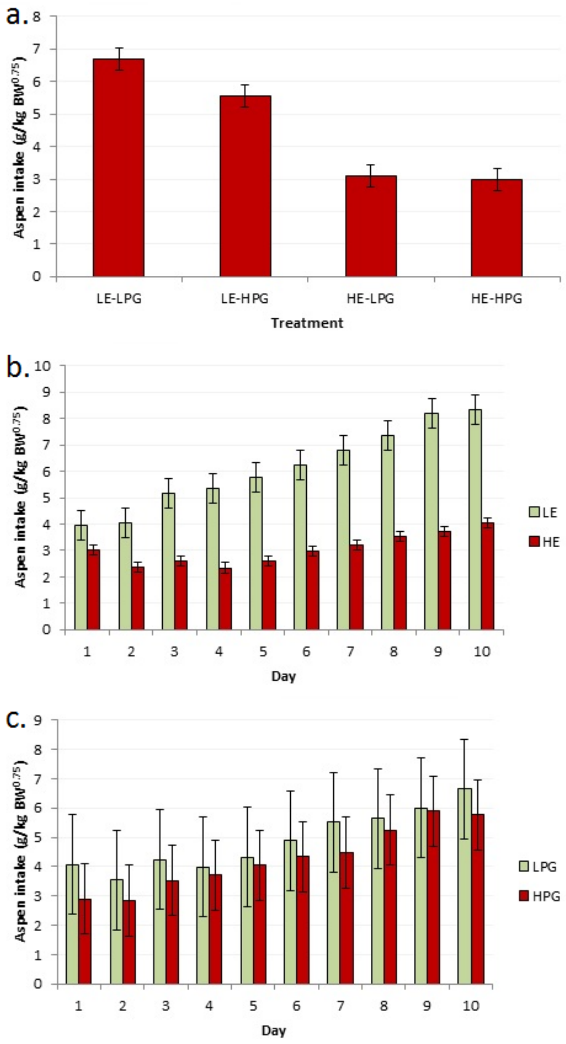

Figure 3-2. Average intake of aspen leaves by groups of lambs offered a ration of either low (LE) or high (HE) energy content and aspen leaves of either low (LPG) or high (HPG) concentrations of phenolic glycosides during Experiment 2: (**a.**) Average intake of aspen leaves by the four treatment combinations (8 lambs/treatment), (**b.**) average daily intake of aspen leaves by lambs offered the LE or HE ration (16 lambs/treatment), and (**c.**) average daily intake of aspen leaves by lambs offered LPG or HPG aspen leaves (16 lambs/treatment). Bars (with SEMs) are means for intake values recorded during ten days.

concentration (day effect $P<0.001$).

Aspen intake for lambs in the LE treatments increased throughout the 10 days. Aspen intake in the HE treatments oscillated from day 1 to day 5, then it slowly increased from day 5 through day10 (ration × day interaction P=0.004; Fig. 3-2b). Average aspen intake for animals that received LPG was steady from day 1 to day 4, intake then increased through day 10. Average aspen intake for animals that received HPG was similar on days 1 and 2, intake then increased from days 3 to 9, and it finally dropped on day 10 (aspen PG × day interaction P=0.058; Fig. 3-2c).

A *post hoc* analysis showed that animals that consumed the high-protein ration in Experiment 1 and then the low-energy ration in Experiment 2, ingested more aspen (2.2±0.16 g/kg $BW^{0.75}$) than animals in the other three treatments (1.7±0.15 g/kg $BW^{0.75}$) (Experiment 1 TMR × Experiment 2 TMR interaction P=0.009).

Experiment 3—Choice

Intake

Average aspen intake was greater for leaves with high (HPG) than for leaves with low (LPG) concentrations of PG (PG effect; P=0.005; Fig. 3-3a). Averaged across days and PG concentrations, aspen intake increased from day 1 (1.19±0.1 g/kg $BW^{0.75}$) through day 6 (2.6±0.1 g/kg $BW^{0.75}$) (day effect P<0.001). No differences in intake were detected for the main effects of prior experience with HPG or LPG (see Fig. 3-3b), or prior exposure to rations in Experiments 1 and 2 (see Fig. 3-3c), or their interactions with day (P>0.05).

Preference

Lambs that were exposed to HPG during Experiments 1 and 2 avoided HPG on day 1 (P<0.001) but they preferred HPG on day 3 (P=0.010). Those lambs that were exposed to LPG aspen during Experiments 1 or 2 only avoided HPG on day 1 (P=0.020)

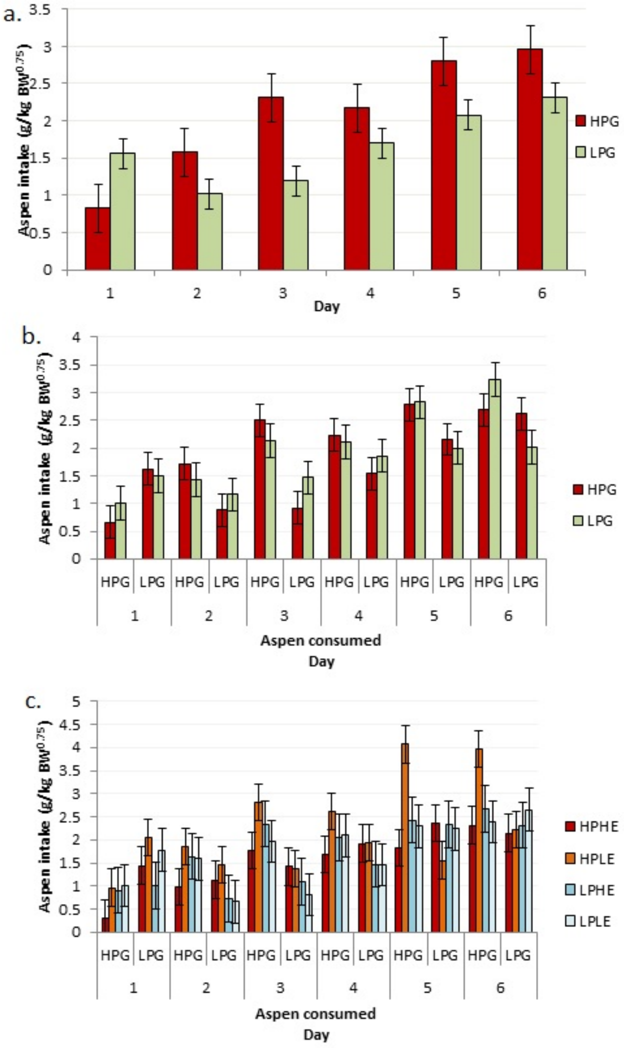

Figure 3-3. Intake of aspen leaves collected from stands containing high (HPG) or low (LPG) concentration of phenolic glycosides by sheep during Experiment 3 (**a.**) for all lambs presented with a simultaneous choice of HPG and LPG aspen, (**b.**) for lambs preconditioned with HPG and LPG aspen, and (**c.**) for lambs preconditioned with high protein then high energy rations (HPHE), high protein then low energy rations (HPLE), low protein then high energy rations (LPHE), and low protein then low energy rations (LPLE). Bars (with SEMs) are means for intake values recorded during six consecutive days for (**a.**) 32 lambs, (**b.**) 16 lambs per aspen PG group, and (**c.**) 6 lambs for HPHE and LPLE and 10 lambs for HPLE and LPHE groups.

(prior exposure to aspen PG × day interaction P=0.050). The main effects of prior exposure to different concentrations of PG and prior exposure to rations in Experiments 1 or 2 did not affect preference (P>0.05).

Nonmetric multidimensional scaling (NMDS) ordination

Two convergent solutions were found after 20 runs using metaMDS. Scaling was automatically applied by the metaMDS command (centering, PC rotation, half-change scaling). Expanded scores were based on Wisconsin transformations, as set by metaMDS in vegan. Stress type was 1, and two dimensions were chosen by metaMDS (stress value = 2.6%), which was not improved significantly by increasing to three dimensions.

The fitted environmental (topographical) surfaces in Fig. 3-4 show that aspen intake was the greatest when nutrients were present in aspen in a combination of low percent Cu, P, K, S, and CP, and medium concentrations of condensed tannins. Intake was not affected by percent B, Ba, Ca, Si, Mg, PG (tremulacin and salicortin), or ADF (see Fig. 3-4).

High-PG (HPG) and low-PG (LPG) aspen leaves were of contrasting chemical composition, as shown by the non-overlapping polygons depicting the nutrients contained in both stands on the fitted environmental surface (Fig. 3-4). Percent B, Mg, tannin, and PG concentrations (tremulacin and salicortin) were greater in HPG than in LPG leaves. Percent Ba, S, K, Cu, P, and CP were greater in in LPG than HPG leaves. Percent Ca was slightly greater in LPG than in HPG aspen leaves, whereas percent ADF and Si were similar in HPG and LPG aspen leaves.

Nutritional analyses

Rations

For Experiment 1, the high-protein ration (HP) had greater concentration of CP than the low-protein ration (LP) (P=0.008; Table 3-1). Content of total digestible

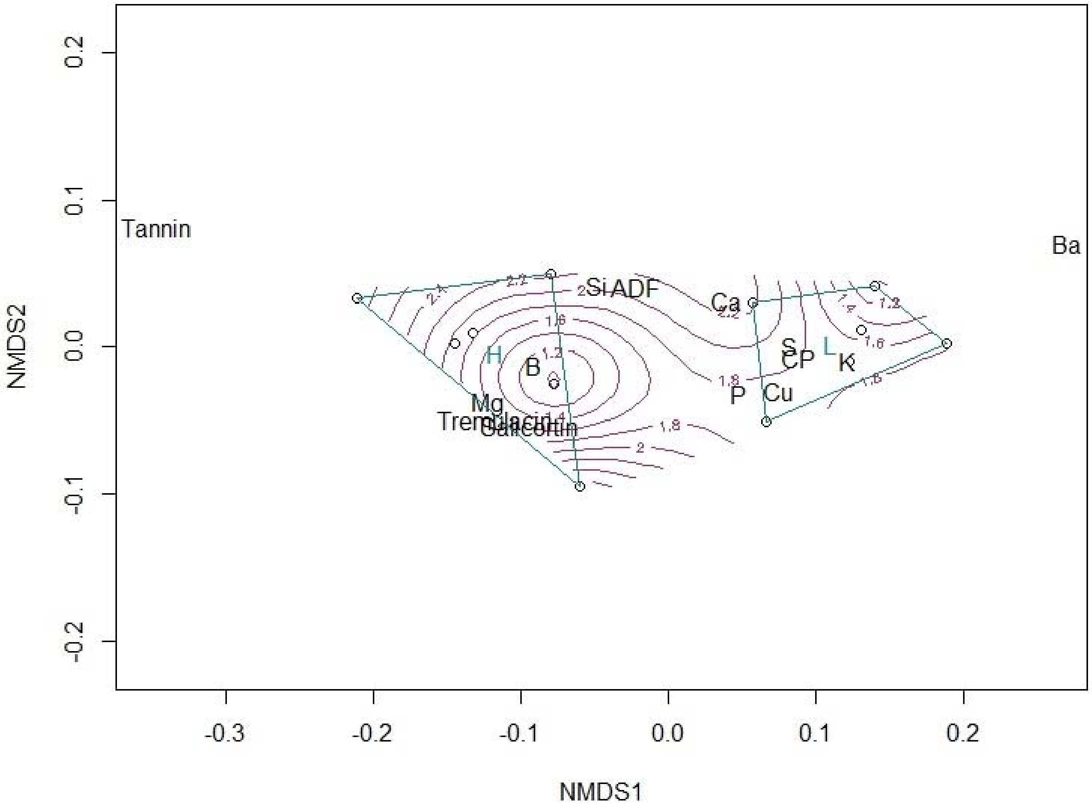

Figure 3-4. Organization of nutrients contained within aspen leaves (expressed as percent dry matter) in a Nonmetric Multidimensional Scaling (NMDS) ordination plot with an overlaid response surface representing aspen intake (maroon topographic lines; expressed as grams per kg of BW$^{0.75}$) for aspen tested during Experiment 3 (Choice of High- and Low-PG Aspen) with the first two axes. The stress value is 0.026 (type 1). The two cyan polygons on the plot surface depicts the differences between the nutrients contained within the high (H) and low (L) PG aspen (a total of twelve samples [one per day from each of the high and low-PG aspen] was used in testing, represented by the open circles on the plot surface). The following thirteen nutrients included in the plot were tested for their influence on both high and low PG aspen intake: B, Ba, Ca, Cu, K, Mg, P, S, Si, ADF, CP, tremulacin, and salicortin.

nutrients (TDN) and fiber (ADF and NDF) was similar (P>0.05) between rations. For Experiment 2, the content of TDN in the high-energy (HE) ration was much greater than the content of TDN in the low-energy (LE) ration (P=0.003). Moreover, LE contained greater concentrations of fiber (P<0.05), consistent with the lower quality of this ration.

Aspen leaves

Crude protein (CP) content was similar between the LPG and HPG stands used in Experiments 1 and 2. The LPG stand used in Experiment 3 contained similar CP concentrations as the LPG stands used in Experiments 1 and 2 (P>0.05) (see Table 3-2 for nutritional analyses for aspen). However, the HPG stand used in Experiment 3 contained less CP than the LPG stand used in Experiment 3 (P<0.001), or than the HPG stands used in Experiments 1 and 2 (P<0.05). Fiber content was greater for LPG than for HPG in Experiment 1 (P<0.05), with similar values between HPG and LPG for Experiments 2 and 3 (P>0.05). Nutritional quality of leaves in Experiments 2 and 3 was generally lower (lower content of CP and greater content of fiber; P< 0.05) than in Experiment 1. For aspen leaves tested during Experiment 3, content of B, Cd, Mg, and Ni was greater in HPG than in LPG leaves and concentrations of Ba, Cu, K, Mn, S, and Sr were greater in LPG than in HPG leaves (*P*<0.05; Table 3-4).

Plant Secondary Compounds

Phenolic glycosides

Total PG concentration in Experiments 1 and 3 was greater in HPG than in LPG aspen leaves (P<0.002; Table 3-3). However, total PG concentration in Experiment 2 was similar between HPG and LPG aspen stands (P>0.05). The HPG aspen stand contained

Table 3-4. Mineral concentrations in the high- and low-PG aspen stands used in Experiment 3 (choice of high- and low-PG aspen) (N=28)

Exp. 3[c]	Al[d]	As[d*]	B[d]	Ba[d]	Ca[e]	Cd[d]	Co[d]	Cr[d]	Cu[d]	Fe[d]	K[e]	Mg[e]
High PG	48.3±10.6	0.1±0	34.0±1.0	23.0±1.5	1.0±0	0.7±0.1	0.3±0.1	0.4±0	4.1±0.2	78.6±11.2	0.6±0	0.2±0
Low PG	24.3±4.2	0.1±0	25.2±1.1	50.6±3.5	1.1±0.1	0.5±0.1	0.2±0	0.5±0.2	4.7±0.2	54.4±4.6	0.8±0	0.2±0

Exp. 3[c]	Mn[d]	Mo[d]	Na[d]	Ni[d]	P[e]	Pb[d]	S[e]	Se[d*]	Si[d]	Sr[d]	Zn[d]
High PG	38.2±4.7	0.1±0	22.3±2.0	1.0±0.7	0.2±0	0.1±0	0.1±0	<	728±31.6	31.6±3.1	72.7±3.4
Low PG	52.9±2.2	0.1±0	26.8±8.5	0.4±0.1	0.2±0	0.2±0	0.2±0	<	644.7±26.6	48.2±4.5	72.7±11.2

[a] Aspen stand with high concentrations of phenolic glycosides
[b] Aspen stand with low concentrations of phenolic glycosides
[c] July 13 to July 18, 2015
[d] Shown as ppm
[e] Shown as % DM
[*] Values shown as "<" were present in amounts less than detectable limits

greater concentrations of PG in Experiment 1 than in Experiment 2 (P=0.024), and the LPG aspen stands for Experiments 1 and 2 contained similar concentrations of total PG (P>0.05). The total concentration of PG in HPG and LPG aspen stands was similar between Experiments 2 and 3 (P>0.05).

Condensed tannins

Concentrations of CT were similar between stands in Experiments 1 and 2 (P>0.05). However, CT concentration was greater in the HPG stand than in the LPG stand for Experiment 3 (P=0.001). Both the HPG and LPG aspen stands contained greater concentrations of CT in Experiment 1 than in Experiment 2 (P≤0.05), and the HPG stand in Experiment 3 contained more CT than in Experiment 2 (P=0.004), while the content of CT in LPG did not differ between Experiments 2 and 3 (P>0.05).

Discussion

Variations in foraging preferences by herbivores across temporal and spatial scales impinge significant consequences on plant communities by, for instance, selectively removing plant tissues that alter plant competition and coexistence across heterogeneous landscapes (e.g., Bryant and Kuropat 1980, Rosenthal and Kotanen 1994, Erbilgin et al. 2014). Food preference is variable because it is influenced by an herbivore’s physiological condition, a food’s chemical characteristics, and an herbivore’s prior experiences with the food (Provenza 1995, Provenza et al. 2003). The present study was an attempt to understand aspen use by ungulates in this context by modifying the physiological state of sheep using basal diets of different protein or energy content while animals ingested aspen leaves with either high or low concentrations of PG. Additionally, the study explored the influence of prior experience with aspen consumption on preference for aspen stands that varied in nutrient and phytochemical concentration.

Nutritional State and Aspen Intake

Our study shows that protein and energy availability, as well as the concentration of PSC in aspen affected aspen intake. Intakes of aspen leaves were the highest when the basal diet was high in protein and when the concentration of PG in aspen leaves was low, suggesting an incremental benefit of dietary protein on an animal's ability to consume aspen as concentrations of PG in the plant declined. These results are consistent with previous research suggesting that the ability to ingest PSC by ungulate herbivores depends on nutrient intake (Illius and Jessop 1995, Baraza et al. 2005, Villalba and Provenza 2005). Detoxification processes deplete the body of protein and glucose, and thus, adequate amounts of nutrients are needed to better cope with PSC ingestion. The mechanisms of phenolic glycoside detoxification in mammalian herbivores depend on enzymatic processes, which require protein in order for detoxification to occur (Boeckler et al. 2011). Consistent with this, when sheep were supplemented with foods high in protein, intake of terpenes (detoxified by enzymatic processes) in shrubs (Villalba et al. 2002b) and rations (Villalba and Provenza 2005) was enhanced relative to intake of terpene-containing foods by control animals. Supplemental macronutrients also increase intake of foods that contain alkaloids (Villalba et al. 2011) and tannins (Villalba et al. 2002a). Results from Chapter 2 are also consistent with the present findings. Lambs supplemented with a high-protein food showed greater aspen intake than control animals. Finally, even when animals were re-randomized when offered rations with high or low energy content, in order to eliminate any carry-over effects from the previous rations, the positive effects of protein on aspen intake were still observed when animals were offered rations high or low in energy in an ensuing experiment. It is likely that lambs receiving the high-protein ration learned a positive association between aspen intake and the provision of adequate amounts of protein to their bodies, which carried over to the ensuing experiment; in fact, such association has been shown to condition preferences for unpalatable foods in sheep (Freidin et al. 2011). Nevertheless, the enhanced ingestion of aspen due to a high-protein basal ration was only observed to carry over to the ensuing experiment when the ration was low in energy, suggesting that the energy content of the diet still played a major role on the lambs' ability to ingest aspen.

We also hypothesized that animals in an energy- or protein-deficient state may consume more aspen if the benefits of accruing energy or protein from aspen in energy or protein-deficient animals outweighed the cost of PSC detoxification. Our results show that when lambs were calorie-restricted in the low-energy basal diet, they ate more aspen than when they ingested an energy-dense basal diet. This pattern suggests that the benefits of ingesting aspen when animals were energy restricted outweighed the costs incurred by consuming a defended plant. Consistent with this notion, previous findings show that energy-dense non-structural carbohydrates (sugars and starch) in aspen tissues have strong positive relationships with preference by white-tailed deer (Holeski et al. 2016). Changes in the nutritional needs of herbivores can interact with a plant's nutritional composition to influence preference (Provenza 1995, Foley et al. 1999, Burney and Jacobs 2013). Protein and energy-dense non-structural carbohydrates are key constituents of ungulate foods (Robbins 1983, Burney and Jacobs 2013), and energy-restricted sheep display strong preferences for foods that provide starch (Villalba and

Provenza 1997). The ongoing need for calories by sheep in the present study (an equivalent of approximately 1,000 g of starch per day; NRC 1985), is much greater (in terms of mass/day) than needs for protein (an equivalent of approximately 220 g of CP per day; NRC 1985). This may help explain why energy-restricted lambs ate more aspen than protein-restricted lambs in the previous experiment. The ingestion of aspen leaves could be more consequential in accruing starch and soluble carbohydrates present in aspen tissues (Holeski et al. 2016) which are needed in greater absolute quantities than protein. On the other hand, satiety induced by calories in animals consuming the energy-dense diet likely reduced appetite for less desirable–and defended–forages like aspen. Moreover, given pre-loads of starch, lambs reduce their preference for starch in ensuing meals (Villalba and Provenza 1999), so it is possible that the amount of energy consumed with high-energy diets reduced the need to consume starch and soluble-carbohydrate-containing aspen leaves.

Protein intake is also under tighter control than energy intake (Webster 1993). Nitrogen recycling and the negative effects of excess ammonia on an animal's physiological processes and tissues could account for such effects (Provenza 1995). Animals maintain a balance of energy to protein in their diets that meets their nutritional needs (Provenza et al. 2003), so it is likely that lambs receiving a high-protein basal diet increased intake of aspen–given the presence of starch and soluble carbohydrates in aspen leaves–to reduce the high protein to energy ratio in their diets incurred by consuming a high-protein basal diet. The aforementioned positive effects of protein on PSC detoxification may have also favored an increase in aspen intake.

Wildlife such as elk consume greater amounts of aspen in the early fall when other plant material is senescing. It has been hypothesized that such increases are due to the animal's energy requirements and the high nutritional content of aspen relative to other understory species (Jones et al. 2005, Beck et al. 2006). This explanation is consistent with the observation that a low-energy basal diet primes animals to consume greater amounts of aspen. On the other hand, an understory with high concentrations of protein (like the high-protein ration) may help explain why herbivores browse on defended aspen stands regardless of PSC content in aspen leaves (Holeski et al. 2016).

Plant defenses and aspen intake

We predicted that the concentration of chemical defenses in aspen modulates the influence of the nutritional state on aspen intake. Thus, while sheep received different basal diets to modify their nutritional state, they were offered aspen leaves with high or low concentration of phenolic glycosides. It was expected that animals would consume greater amounts of aspen leaves with lower content of PG because high foliar concentrations of PG in aspen have been implicated in insect (Lindroth and Hwang 1996) and mammalian herbivore deterrence (Bailey et al. 2007, Wooley et al. 2008, Villalba et al. 2014). Plant secondary compounds typically restrict plant biomass loss to herbivores, limiting the amount of PSC-containing foods that can be ingested (Palo and Robbins 1991, Tuomi et al. 1994, Foley et al. 1999). Results from the experiment in which lambs received rations with high or low protein content suggest that PG in aspen had a negative impact on aspen intake by lambs. Aspen intake was greater for animals that received aspen leaves with low concentrations of PG than for those subjects that were offered aspen leaves containing greater content of PG. In contrast, when differences in PG concentration between two aspen stands were less pronounced during the following experiment, no differences in aspen intake were detected between groups of animals exposed to leaves from either stand.

Condensed tannin concentrations in aspen fed during the first experiment (when lambs received rations with a high or low protein content) were greater in aspen leaves with low concentrations of PG. Yet, lambs consumed greater amounts of these leaves than those containing lower concentrations of tannins. Thus, it appears that concentrations of condensed tannin were not involved in modulating aspen intake as it has been shown in previous studies (Villalba et al. 2014). Similarly, condensed tannins in aspen do not appear to affect elk preference (Wooley et al. 2008), another mesoherbivore that prefers graminoids like sheep (Beck et al. 1996).

During the last experiment in which lambs were offered a choice between aspen stands with a high or low concentrations of PG showed that aspen chemical defenses may not always be the main cause underlying deterrence; on the contrary, results show that lambs consumed more aspen leaves collected from stands containing greater concentrations of PG than leaves with lower content of PG. In contrast to this general pattern of preference, the opposite outcome was observed during the first day of testing. It is likely that lambs were still not familiar with the chemical composition of both stands at the beginning of the experiment, as aspen leaves were collected from new locations.

Concentrations of condensed tannins were also greater in aspen stands with greater concentrations of PG, also suggesting that PSC did not constrain intake or preference by sheep. Consistent with these findings, a recent study exploring aspen preferences by white tailed deer showed that chemical defenses had little impact on browse deterrence, and that nutrients had greater impact than chemical defenses on aspen preference (Holeski et al. 2016).

Plant nutrients and aspen intake

The chemical composition of aspen leaves in the aspen choice experiment was contrasting, as shown by the non-overlapping polygons depicted in the NMDS ordination for the chemical composition of both aspen stands. As described above, the ordination shows that neither concentrations of PG nor tannins constrained aspen intake by sheep. On the contrary, medium concentrations of condensed tannins appeared to favor aspen preference, which is consistent with the potential nutritional benefits that some types of tannins provide to herbivores (i.e., enhanced protein availability for the herbivore; Waghorn 2008).

The NMDS ordination also suggested no effects of fiber content on aspen intake, but a negative relationship between aspen intake and CP concentration. Likewise, Holeski et al. (2016) found negative relationships between aspen preference by deer and nitrogen content in aspen trees. During the aspen choice experiment, lambs were fed a basal diet of alfalfa pellets, which contain high concentrations of CP relative to other forages (Frame et al. 1998), possibly reducing preference for aspen stands with a high content of CP. Herbivores satiate on nutrients consumed too frequently or in large amounts. For instance, given a high-protein meal, lambs reduce their preference for flavors previously paired with nitrogen during the ensuing meals (Villalba and Provenza 1999). Likewise, aspen stands in the Holeski et al. (2016) study were located in close proximity to alfalfa and clover crops and thus deer had ample protein available from these forages, which may explain the negative relationship between browse preference and CP content. Moreover, because lambs ate a basal diet with high concentrations of CP in our study (i.e., alfalfa pellets), it is likely that protein aided in detoxification of PG present in aspen (Illius and Jessop 1995; see sections above), which in turn reduced the magnitude to which PG constrained intake.

Nutrient concentrations of high- and low-PG stands were not consistent across days and thus each nutrient appeared to carry a certain weight that may either enhance or reduce aspen intake on a case-by-case basis. For instance, the minerals that had the greatest effect on aspen intake during the aspen choice experiment were those shown in the ordination plot depicted in Fig. 3-8. Low amounts of Cu, P, K, S, CP, and medium concentrations of condensed tannins enhanced aspen intake by lambs. Holeski et al. (2016) showed that concentrations of Ca, Mg, K, S, and Fe had a strong positive relationship with aspen preference by deer.

Because of the fingerprint appearance of the fitted smooth environmental surface (the topographical surface) that represents intake in the ordination, none of the nutrients that lie outside of the center of the ordination had a linear relationship with intake (see Fig. 3-8). This was supported when each nutrient was plotted against intake in univariate plots (data not shown). Both desirable and undesirable nutrients may co-occur at various concentrations and ratios–typically unbalanced–within different forages. Under these conditions, lambs may quit eating an unbalanced food as they satisfy their requirements of the nutrient in highest concentration, but without satisfying requirements for nutrients occurring in lower concentrations, a phenomenon known as incidental restriction (Raubenheimer 1992). It is likely that incidental restriction reduced the intake of the aspen stands with high concentration of Cu, P, K, S, and CP in this study.

Prior experience and aspen intake

We predicted that sheep previously exposed to aspen stands with high concentrations of defenses would show greater preference for such stands than animals previously exposed to aspen with lower concentrations of phytochemicals because prior experience improves the efficiency of detoxification processes (Delgoda and Westlake 2004, Kohl et al. 2014). We controlled for the lambs' previous experience with high or low concentrations of PG by consistently offering aspen stands with high or low concentrations of PG during the first two experiments. Under these conditions, prior experience with aspen stands of contrasting PG concentrations, or with diets of different nutritional composition, did not influence the pattern of preference for aspen leaves containing high or low concentrations of PG. Thus, decisions made by lambs seemed to be based on the intrinsic chemical characteristics of aspen leaves offered during choice tests rather than on the previous experience that lambs had with aspen stands of contrasting PG content.

Aspen intake and extrapolation to the landscape scale

This study explored the influence of the lambs' nutritional state and prior feeding experiences on aspen intake under controlled conditions. Extrapolation of these findings to the landscape should consider additional intervening variables such as the abundance and diversity of forage alternatives typically observed in aspen communities. For instance, when abundant dietary protein is available at the landscape scale, lambs may be less likely to eat aspen because of the presence of abundant preferred forages (i.e, forbs, young grasses). However, when high-protein forages are restricted, then such forages may act as "protein supplements" which may enhance the herbivores' ability to ingest defended aspen trees in order to satisfy the bulk of their diet and incorporate other nutrients such as sugars and starch from aspen tissues. Our study also assumed minimal concentrations of defenses in the surrounding plant community, but additional PSC may also be present in aspen-dominated communities with understory species containing different types and concentrations of PSC. Predators may further influence foraging behavior and thus the likelihood of aspen browsing by ungulates (Brown et al. 1999, Nersesian et al. 2011). Finally, the results found with sheep may vary from other species of ungulates with different foraging (e.g., variations in food searching and handling times) and nutritional ecologies (e.g., variations in tolerance to PSC).

Conclusions

In summary, our results suggest that intake of defended plants, like aspen, depends on the interplay between the intrinsic properties of the target plant and the nutritional state of the animal. Landscapes that provide diets with high concentrations of protein or low concentrations of energy are predicted to increase herbivores' ability to browse on young aspen trees relative to landscapes offering diets with low concentration of protein and high concentrations of energy. In addition, diets with high content of protein are predicted to attenuate the negative impacts that phenolic glycosides typically impinge on aspen intake. Nevertheless, our results also suggest that the reason underlying aspen preference by herbivores is not straightforward and is most likely multidimensional. When sheep were offered a choice between aspen stands of different nutrient and phytochemical concentration, neither phenolic glycoside concentration or condensed tannin content constrained aspen preference. On the contrary, animals preferred aspen stands with a high content of phenolic glycosides and condensed tannins.

Preferences appeared to be based on the interplay among the intrinsic nutritional characteristics of aspen leaves rather than on previous experience with aspen stands of contrasting phenolic glycoside concentrations or with diets of different nutritional composition. A comprehensive exploration of the chemical composition of aspen stands, their surrounding vegetation, and the physiological state of consumers may lead to the development of management plans geared at reducing herbivory in at risk stands.

References

AOAC. 2000. Official methods of analysis, 17th edn. –Association of official analytical chemists. Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT 06484.

Bailey, J. K. et al. 2007. Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. –Biol. Invasions 9:715–722.

Baraza, E. et al. 2005. Nutritional context influences preferences of lambs for foods with plant secondary metabolites. –Appl. Anim. Behave Sci. 92**:**293–305.

Beck, J. L. and Peek, J. M. 2005. Diet Composition, Forage Selection, and Potential for Forage Competition Among Elk, Deer, and Livestock on Aspen–Sagebrush Summer Range. –Rangeland Ecol. Manage. 58(2):135-147.

Beck, J. L. et al. 1996. Elk and domestic sheep interactions in a North-Central Utah Aspen Ecosystem. –Res. Pap. A 13.78:INT-RP-491. Ogden, UT: U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station.

Beck, J. L. et al. 2006. Estimates of elk summer range nutritional carrying capacity constrained by probabilities of habitat selection. –J. Wildlife Manage. 70(1):283-294.

Boeckler, G. A. et al. 2011. Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. –Phytochemistry 72:1497-1509.

Bryant, J. P. and Kuropat, P. J. 1980. Selection of winter forage by subarctic browsing vertebrates: The role of plant chemistry. –Annu. Rev. Ecol. Syst. 11:261-285.

Brown, J. S. et al. 1999. The ecology of fear: optimal foraging, game theory, and trophic interactions. –J. Mammal. 80:385–399.

Burney, O. T. and Jacobs, D. F. 2013. Ungulate herbivory of boreal and temperate forest regeneration in relation to seedling mineral nutrition and secondary metabolites. - New Forests 44:753–768.

Dearing, M. D., et al. 2000. Diet breadth of mammalian herbivores: nutrient versus detoxification constraints. –Oecologia 123(3):397-405.

Dearing, M. D., et al. 2001. Plant Secondary Compounds as Diuretics: An Overlooked Consequence 1. –American Zoologist 41(4):890-901.

DeByle, N. V. et al. 1989. Forage quality in burned and unburned aspen communities. – Res. Pap. INT-404. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station.

Delgoda, R. and Westlake, A. C. G. 2004. Herbal Interactions Involving Cytochrome P450 Enzymes. –Toxicological Reviews 23(4):239-249.

Erbilgin, N. et al. 2014. Resource availability and repeated defoliation mediate compensatory growth in trembling aspen (*Populus tremuloides*) seedlings. –Peer J 2:e491.

Foley, W. J. et al. 1995. Consequences of biotransformation of plant secondary metabolites on acid-base metabolism in mammals—A final common pathway? –J. Chem. Ecol. 21(6):721-743.

Foley, W. J. et al. 1999. Role of plant secondary metabolites in the nutritional ecology of mammalian herbivores: how far have we come in 25 years? –In: H.G. Jung and G.C. Fahey, Jr. (eds) Nutritional ecology of herbivores. Proc. Vth. Int. Symp. Nutr. Herb. Am. Soc. Anim. Sci., Il, pp. 130–209.

Frame, J. et al. 1998. Temperate legume forages. –CAB International, Wallingford, UK.

Freeland, W. J. and Janzen D. H. 1974. Strategies in herbivory by mammals: The role of plant secondary compounds. –Am. Nat. 108:269-288.

Freidin, E. et al. 2011. Mechanisms of intake induction of a low-nutritious food in sheep (*Ovis aries*). –Behavioural Processes 87:246-252.

Garcia, J. 1989. Food for Tolman: Cognition and cathexis in concert. – In: T. Archer and L. Nilsson (eds.). Aversion, Avoidance and Anxiety. Earlbaum, Hillsdale, NJ. pp. 45–85.

Hagerman, A. E. and Butler, L. 1980. Condensed tannin purification and characterization of tannin-associated proteins. –J. Agric. Food Chem. 28:947–952.

Holeski, L. M. et al. 2016. Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. –J. Ecol. 104:850–863.

Illius, A. W. and Jessop, N. S. 1995. Modeling metabolic costs of allelochemical ingestion by foraging herbivores. –J. Chem. Ecol. 21:693–719.

Illius, A. W. and Jessop, N. S. 1996. Metabolic constraints on voluntary intake in ruminants. –J. Anim. Sci. 74:3052–3062.

Jones, B. E. et al. 2005. Removal of Encroaching Conifers to Regenerate Degraded Aspen Stands in the Sierra Nevada. –Restor. Ecol. 13(2):373-379.

Jones, B. E. et al. 2011. Cattle selection for aspen and meadow vegetation: implications for restoration. –Range Ecol. Manage. 64:625–632.

Kohl, K. D. et al. 2014. Gut microbes of mammalian herbivores facilitate intake of plant toxins. –Ecology Letters 17(10):1238-1246.

Lindroth, R. L. and Hwang, S. Y. 1996. Diversity, redundancy, and multiplicity in chemical defense systems of aspen. –In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, pp 25–56.

Lindroth, R. L. and St. Clair, S. B. 2013. Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. –For. Ecol. Manage. 299:14-21.

Lindroth, R. L. et al. 1993. Responses of deciduous trees to elevated atmospheric CO2: productivity, phytochemistry, and insect performance. –Ecology 74:763–777.

Mitchelson, F. 1992. Pharmacological agents affecting emesis: A review (Part I). -Drugs 43:295.

Myking, T. et al. 2011. Life history strategies of aspen (*Populus tremula* L.) and browsing effects: a literature review. –Forestry 84(1):61-71.

Nersesian, C. L. et al. 2011. Titrating the cost of plant toxins against predators: determining the tipping point for foraging herbivores. –J. Anim. Ecol. 80:753-760.

NRC. 1985. Nutrient requirements of sheep, 6th edn. –National Academy Press.

Oksanen, J. F. et al. 2016a. Vegan: Community Ecology Package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan. Accessed 12/01/2016.

Oksanen, J. F. et al. 2016b. Vegan tutorial. https://cran.r-project.org/web/packages/vegan/vegan.pdf . Accessed 12/26/2016.

Palo, R. T. and Robbins, C. T. 1991. Plant Defenses against mammalian herbivory. – CRC Press.

Porter, L. J. et al. 1986. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. –Phytochemistry 25:223–230.

Provenza, F. D. 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. –J. Range Manage. 48:2-17.

Provenza, F. D. 1996 Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. –J. Anim. Sci. 74:2010-2020.

Provenza, F. D. et al. 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. –Small Ruminant Research 49:257-274.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Accessed 05/01/2016

Raubenheimer, D. 1992. Tannic Acid, Protein, and Digestible Carbohydrate: Dietary Imbalance and Nutritional Compensation in Locusts. –Ecology 73(3):1012-1027.

Robbins, C. T. 1983. Wildlife Feeding and Nutrition. –Academic Press, New York, NY.

Robbins, C. 2012. Wildlife feeding and nutrition. –Academic Press, New York, NY.

Romme, W. H. et al. 1995. Aspen, Elk, and Fire in Northern Yellowstone Park. –Ecology 76(7):2097-2106.

Rosenthal, J. P. and Kotanen, P. M. 1994. Terrestrial plant tolerance to herbivory. –Trends Ecol. Evol. 9:145-148.

RStudio Team. 2015. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/. Accessed 04/01/2016

Smith, A. D. et al. 1972. The effects of deer and domestic livestock on aspen regeneration in Utah. Utah Div. Wildl. Resour. Publ. No. 72-1.

Sorensen, J. S. et al. 2005. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. –Ecology 86(1):125-139.

Swift, R. W. 1957. The caloric value of TDN. –J. Anim. Sci. 16:753–756.

Tuomi, J. et al. 1994 A dilemma of plant defences: Is it really worth killing the herbivore? –J. Theor. Biol. 170:427–430.

Van Soest, P. J. 1994. Nutritional Ecology of the Ruminant. –Cornell University Press.

Van Soest, P. J. et al. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. –J. Dairy. Sci. 74:3583–3597.

Villalba, J. J. and Provenza, F. D. 1997. Preference for wheat straw by lambs conditioned with intraruminal infusions of starch. –Br. J. Nutr. 77(2):287-297.

Villalba, J. J. and Provenza, F. D. 1999. Nutrient-specific preferences by lambs conditioned with intraruminal infusions of starch, casein, and water. –J. Anim. Sci. 77(2):378-87.

Villalba, J. J. and Provenza, F. D. 2002. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: Benefits or detriments for plants? –Oikos 97(2):282-292.

Villalba, J. J. and Provenza, F. D. 2005. Foraging in chemically diverse environments: Energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. –J. Chem. Ecol. 31(1):123-38.

Villalba, J. J. et al. 2002a. Influence of macronutrients and polyethylene glycol on intake of a quebracho tannin diet by sheep and goats. –J. Anim. Sci. 80:3154–3164.

Villalba, J. J. et al. 2002b. Consequences of the interaction between nutrients and plant secondary metabolites on herbivore selectivity: benefits or detriments for plants? -Oikos 97:282–292.

Villalba, J. J. et al. 2011. Preference for diverse pastures by sheep in response to intraruminal administrations of tannins, saponins, and alkaloids. –Grass Forage Sci. 66:224-236.

Villalba, J. J. et al. 2014. Aspen (Populus tremuloides Michx.) Intake and Preference by Mammalian Herbivores: The Role of Plant Secondary Compounds and Nutritional Context. –J. Chem. Ecol. 40(10):1135-1145.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. –An. Feed Sci. Tech. 147(1-3):116-139.

Webster, A. J. F. 1993. Energy partitioning, tissue growth and appetite control. –Proc. Nutr. Soc. 52:69-76.

Weiss, W. P. et al. 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. –Anim. Feed. Sci. Technol. 39:95–110.

White, C. A. et al. 2003. Predation risk and the functional response of elk–aspen herbivory. –Forest Ecol. Manage. 181: 77–97.

Wooley, S. C. et al. 2008. Aspen Decline, Aspen Chemistry, and Elk Herbivory: Are They Linked? –Rangelands 30(1):17-21.

CHAPTER 4

THE INFLUENCE OF THE FOODSCAPE ON QUAKING ASPEN USE BY UNGULATES AND STAND CONDITION[3]

Abstract In order to study the effects of herbivory on plant communities, we determined whether the types and concentrations of chemicals present in different aspen (*Populus tremuloides* Michx.) stands and understories, i.e., the foodscape, are associated with aspen use by elk and with aspen regeneration and recruitment. Transects were established in aspen stands with high, medium, and low regeneration levels (N=5 sites/regeneration level; ranging from 2,331 m to 2,724 m in elevation) in Wolf Creek Ranch in northern Utah. Using non-metric multidimensional scaling (NMDS) ordination and regression analyses, we examined the relationships between aspen regeneration, recruitment, elk presence, browsing, and other landscape elements with the foodscape (e.g., biomass and chemical composition of the understory and chemical defenses of juvenile aspen trees). Elk presence, as measured by pellet counts, was negatively correlated with understory crude protein biomass, and aspen regeneration tended to be greater in stands with understories comprised of high crude protein biomass. Recruitment also tended to increase in stands with high levels of protein biomass. Concentrations of chemical defenses negatively influenced percent of browsed aspen. Our findings suggest that

---

[3] Kristen Y. Heroy, Samuel B. St. Clair, Paul C. Rogers, and Juan J. Villalba

Author Contributions: JJV, SBS, PCR, and KYH originally formulated the idea, JJV and KYH developed methodology, PCR developed plot framework and managed baseline mensuration, data collection, and analysis, SBS generated defense chemical analyses, KYH conducted fieldwork, generated nutritional analyses, developed the statistical models, performed statistical analyses, and wrote the manuscript.

foodscapes of lower nutrient content–occurring at lower elevations under drier climatic conditions–are more likely to foster aspen stands with lower levels of regeneration and recruitment with greater levels of elk presence than those growing at nutrient-rich sites. Thus, a novel management approach for enhancing aspen regeneration and recruitment through reductions in aspen use by ungulates may entail increasing the amount of nutrients and chemical defenses in the foodscape.

Introduction

Landscapes offer herbivores a diversity of types and concentrations of chemicals (i.e., the foodscape) packaged inside an array of forage species distributed across different temporal and spatial scales (Atsatt and O'Dowd 1976; Villalba and Provenza 2005; Baraza et al. 2006; Searle et al. 2007; see Chapters 2 and 3). In turn, foraging decisions by herbivores are influenced by the heterogeneous distribution of chemicals in time and space, relative to the type of animal and its history with the foodscape (Bailey et al. 1996; Perez-Barberia et al. 2004; Provenza and Villalba 2006; MacFarlane and Coulson 2007; Taillon and Cote 2007). In addition to the distribution of chemicals, foraging choices are driven by other biotic (e.g., perceived likelihood of predation, human presence, hunting, co-grazing) and physiographic (e.g., elevation, climate, slope) factors, which further influence animal movement and grazing patterns across plant communities (Senft et al. 1987; Smith 1988; Bailey et al. 1996).

Aspen (*Populus tremuloides* Michx.) communities represent an ideal study system to explore the influence of the foodscape on foraging decisions by herbivores because they provide a wide variety of plant diversity to consumers (Kay 1997; White et al. 2003; Jones et al. 2011), and because aspen trees show substantial genetically-based variation in phytochemical traits that influence foraging behavior (Lindroth and Hwang 1996). Despite this diversity and presence of chemical defenses, repeated foliage removal and damage to meristematic tissues from herbivory continue to impact aspen trees to the point of representing a major cause of poor aspen regeneration in some areas of North America (Romme et al. 1995) and Eurasia (Myking et al. 2011).

Herbivores are sensitive to changes in the nutritional quality of plants in a community; they modify their dietary breadth as well as the amounts and proportions of ingested plant parts and species in order to meet their nutritional needs (e.g., Shaw et al. 2006; Provenza et al. 2003). This is why wild and domestic ungulates typically prefer aspen in the fall, when the average nutritional quality offered by the understory drops below that present in aspen tissues (Beck et al. 2006; Jones et al. 2011; Villalba et al. 2014). Additionally, studies with sheep have revealed that aspen intake is dependent on the types of feed an animal has recently consumed (see Chapter 2), as well as on the animals' nutritional state (see Chapter 3). For instance, ingesting foods containing high concentrations of protein enhances aspen intake, especially if plant defenses in aspen are present in low concentrations (Chapters 2 and 3). On the other hand, because aspen is a good source of starch, energy-restricted sheep consume greater amounts of aspen leaves than control (i.e., non-restricted) animals (see Chapter 3).

Herbivores also respond to plant secondary compounds (PSC) by reducing the amount of PSC-containing plants that they consume (Provenza 1996), a process regulated by the complementarities and antagonisms occurring across different detoxification pathways and the availability of nutrients needed for detoxification processes (Freeland and Janzen 1974; Provenza 1996; Illius and Jessop 1996). Aspen chemical defenses (phenolic glycosides and condensed tannins) have been shown to deter ungulate browsing, but when ungulate numbers increase above a certain threshold, the capacity of these defenses to deter browsing to a level that effectively restricts tissue loss to herbivores gets compromised (reviewed by Lindroth and St. Clair 2013). Consistent with this idea, a recent study conducted at the same location where the present study was carried out reports that a majority of the aspen stands assessed were not recruiting new stems at sufficient levels to replace overstory trees (Rogers et al. 2015). This response was likely a consequence of elk numbers exceeding the carrying capacity desired by managers for the region (Rogers et al. 2015), which was estimated to be below one animal km^{-2} (Runyon et al. 2014; Durham and Marlow 2010). Nevertheless, Rogers et al. (2015) did not determine the types and amounts of nutrients provided by the surrounding understory or the chemical composition of aspen trees in that region.

Collectively, it follows that chemicals present in aspen, as well as those offered by the surrounding vegetation, shape herbivores' decisions on how much aspen will be incorporated into their diet. Thus, identifying the concentration of different nutrients and PSC across the landscape, i.e. the geospatial variation in the quality of food or "foodscape," is critical for understanding herbivores' preferences in diverse plant communities like those observed in aspen-dominated landscapes (Searle et al. 2007; Marsh et al. 2014). The objective of this study was to characterize the chemical composition of different aspen and accompanying understory communities across a gradient of aspen recruitment in order to determine whether the types and concentrations of nutrients and PSC in the landscape (i.e., the foodscape) are associated with aspen use by elk and with aspen regeneration and recruitment. We hypothesized that nutrients in juvenile aspen and the surrounding vegetation interact with plant secondary compounds to influence aspen use by herbivores. Thus, we predicted that (i) as nutritional biomass in the understory increased (i.e., greater amounts of crude protein), aspen use would decrease and recruitment (number of stems reaching > 2 m in height) and regeneration (number of stems growing to ≤ 2 m in height) would increase because herbivores would prefer an understory with greater amounts and concentrations of nutrients over defended aspen tissue. Additionally, we predicted that (ii) as defense content in aspen stands increased, aspen use would decrease because phytochemicals constrain food intake. If our predictions are true, aspen in areas with high understory biomass would experience less browsing, especially if they contained high concentrations of defense compounds. However, if the surrounding understory contains low understory biomass, then aspen herbivory would be less constrained by such defenses and aspen intake would increase because those animals would be more willing to consume defended foliage in order to meet nutritional requirements. This means that stands with low understory biomass may be more at risk of succumbing to herbivory pressure and would need more intensive management than stands with greater understory biomass.

Materials and methods

Study site

Wolf Creek Ranch (WCR) is located east of Park City, UT, USA (N 40° 30.6365’ W 111° 14.673’), and is situated on a 5,382 hectare private parcel of land, with approximately 2,333 hectares (~43% of the property) covered by aspen forests that consist of a stable aspen community-topped plateau that borders public land to the east and private land on all other sides (Rogers et al. 2014; Rogers et al. 2015). Loamy soils dominate WCR, and surface soils primarily overlay Keetley volcanic tuffs and resemble those soils found in forested areas within this region (Rogers et al. 2015). Although most of the aspen within WCR are found between 1,950 and 2,443 m of elevation, the property ranges from 1,950 to 2,750 m of elevation. The average precipitation at WCR is 694 mm (measured from 1987 to 2012 using the nearest rain gauge; SNOTEL #330), most of which occurs in the form of snow during the winter season, and with mid-summer being the driest period of the year (Rogers et al. 2015).

Because elevation is variable within WCR, aspen phenology, morphology, and community composition varies markedly across the property (Abraham 2013). Sites at lower elevations tend to be drier and contain aspen and conifer forests among areas of mountain big sagebrush (*Artemisia tridentata* ssp. *vaseyana* Rydb.) or bigtooth maple (*Acer grandidentatum* Nutt.) and Gambel oak (*Quercus gambelii* Nutt.). Wetter sites at higher elevations are dominated by stable aspen stands (single-species stands with little to no competition with conifers; also called "pure" aspen stands) (Harniss and Harper 1982; Shepperd 1990; Rogers et al. 2014) with some conifer cover (mainly Douglas-fir [*Pseudotsuga menziesii* Franco], subalpine fir [*Abies lasiocarpa* Nutt.], and white fir [*Abies concolor Lindl.* ex Hildebr.]) on north- and east-facing slopes (Rogers et al. 2015).

Herbivores within WCR are primarily mule deer (*Odocoileus hemionus* Raf.), rocky mountain elk (*Cervus elaphus* L.), and sheep (*Ovis* spp.), although moose are occasionally spotted in the area. Elk numbers were estimated to be moderate-to-high for the habitat found in WCR. Deer numbers are not well known on the property (Rogers et al. 2015). Hunting is not typically permitted on WCR, but a small number of guided elk hunting permits were issued in 2013. Hunting is allowed on adjacent National Forest and private properties to the west, north, and east of WCR. This proximity of hunted lands to privately restricted lands increases elk numbers seasonally as animals flea to safer zones. Property managers in WCR allow 3,000 sheep to graze for two weeks each year in June and six to seven weeks in October and November. Although sheep herders are instructed to keep sheep out of aspen stands to reduce aspen browsing, browsing sometimes occurs (Rogers et al. 2015).

Preceding study

In a preceding study completed by Rogers et al. (2015), the authors identified fifty random sample points from an overlaid grid and aspen cover layer using a GIS program. Seven of the plots were eliminated because aspen cover was less than 50% tree cover. Within the forty-three remaining locations, a 1-ha monitoring plot was established within each location. Within each plot, forest structure, tree composition, regeneration, recruitment, landscape elements, percent of browsed aspen, and herbivore use was measured. Tree diameters and heights were converted to estimates or classifications to accommodate non-expert field technicians. The data were collected by trained citizen scientists during June and July of 2012.

Measurements within 1-ha monitoring plots were completed within two 2 m x 30 m belt transects oriented perpendicular to each other at cardinal directions to capture differences in terrain. Aspen regeneration (number of stems < 2 m tall), recruitment (number of stems ≥ 2 m and ≤ 6 m tall), and mature canopy trees (trees > 6m tall) were determined within transects at each location. Average canopy height was estimated for the tallest layer of trees using a Biltmore stick. In addition, the number of distinct fecal piles within the transects were counted (Bunnefeld et al. 2006), and separated by species for mule deer, elk, and sheep. Fecal piles that could not be positively identified were not counted, and the frequency of these incidences was not noted. Mean values from variables measured within transects were assumed to represent the surrounding 1 ha area and were extrapolated from the area of the transects (120 m^2) to 1 ha values (x 83.33) (Rogers et al. 2015).

Rogers et al. (2015) found that 46% of the stands analyzed were not self-replacing and 19% were marginally self-replacing using regeneration standards provided in O'Brien et al. (2010). Using browse thresholds for regeneration sustainability presented in Jones et al. (2005), 72% of the stands sampled did not reach the recruitment threshold for long-term sustainability of the stand. The majority of counted fecal pellet piles within the entire 43 sites sampled corresponded to elk (96 elk fecal piles, 8 deer fecal piles, 0 sheep fecal piles), and populations were estimated to occur in a density of 7.8 elk km^{-2}. Previous studies concluded that elk presence of < 1 elk km^{-2} was ideal for successful stand-replacing recruitment (Runyon et al. 2014; Durham and Marlow 2010). Rogers et al. (2015) also found there was a negative relationship between elk presence (estimated via pellet counts) and aspen regeneration and recruitment. The same areas with high elk pellets also had poor regeneration, recruitment, and stand conditions. Elk presence did not show a relationship with slope however, in agreement with Rogers and Mittanck (2014). Hill aspect had a positive relationship with recruitment and a negative relationship with elk presence. Elk seemed to prefer drier aspects and browse impacts were greater in these areas, or fecal pellets were easier to find in the less densely covered understory.

Foodscape Assessment

Fifteen locations were chosen from the forty-three locations studied by Rogers et al. (2015). We chose fifteen stands because of sampling logistics and because five stands of each treatment was expected to provide enough power to detect differences across sites. Five high, medium, and low recruitment TPA (recruitment as a percentage of live mature aspen trees per acre) sites, were chosen to be surveyed and sampled, ranging in elevation from 2,331 m to 2,724 m (see Fig. 4-1 for locations of aspen stands sampled). The cut-offs for high, medium, and low recruitment TPA were developed by Rogers et al.

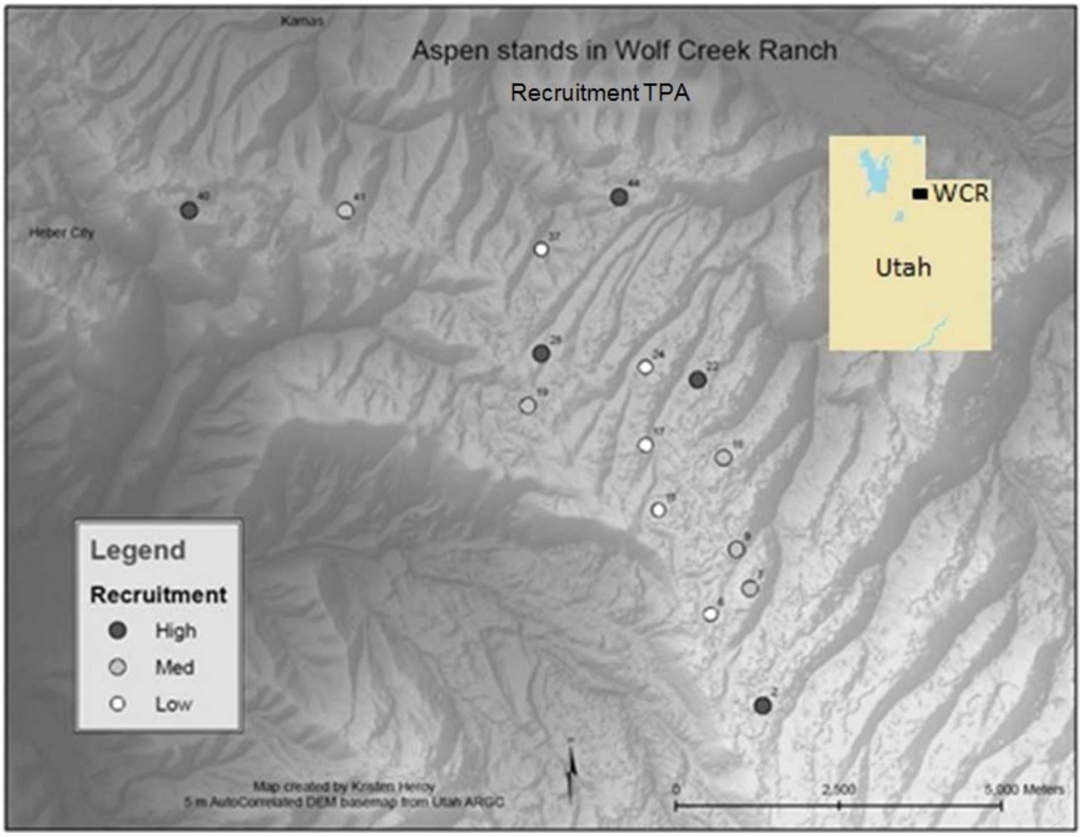

Fig 4-1 Locations of high, medium, and low regeneration aspen stands (five of each regeneration level to total fifteen stands) sampled during the study at the Wolf Creek Ranch (WCR). The location of WCR within Utah is shown in the inlaid map of Utah in the upper right corner.

(2015) based on the ability of the aspen stand to replace itself over time under varying browse levels. Stands were selected so that one stand from each recruitment TPA level was located within a distance of 1.5 km of each other in order to minimize variation in environmental conditions across the stands. Factors that disqualified sites were slopes greater than 20° (given constraints with site access), sites that were completely defoliated by aspen blight, and sites that were less than 100 m from a paved road or human structure.

Measurements within 1 ha monitoring plots were completed within two 1 m x 30 m belt transects oriented perpendicular to each other at cardinal directions to capture terrain variations according to the methods of Rogers et al. (2015). Forage samples were taken every 5 m on alternating sides of the belt transect using a 0.1 m^2 quadrat sampling square, so that twelve samples were taken for each site and placed in separate paper bags. Sampling occurred during 6 consecutive days from 24-Aug-2015 to 29-Aug-2015, since browsing ungulates appear to consume greater amounts of aspen in the early fall (St. Clair et al. 2015).

To assess shrub density and abundance, the length and width of all shrubs within the 1 m x 30 m belts were recorded (Rittenhouse and Sneva 1977; Uresk et al. 1977). In addition, a reference branch was chosen from a shrub of the same species that lay outside of the transects, which was used to estimate the leaf biomass of the shrubs within the lanes, using the reference unit method (Andrew et al. 1979). Briefly, leaf biomass was estimated by holding up the reference branch to the shrub in the 1 m x 30 m lane and approximating how many reference branches fit inside the shrub in the lane. The reference branch leaf biomass–later measured in the lab–was then multiplied by this number in order to estimate the leaf biomass on each shrub (Andrew et al. 1979). Reference branches were replaced at least once per day and leaves were stripped off the branch and placed into an individual paper bag, or sooner if leaves began to dry out because the reference branch leaves had to be intact for accurate estimations of dry matter. Mean values of variables measured within transects and quadrats were extrapolated to represent the surrounding 1 ha area. Shrub leaf weight was extrapolated from the area of the transects (60 m^2) to 1 ha values (× 166.66).

In order to determine food type biomass, weights of all twelve clip samples were summed, then divided by 1.2 m^2 to determine average weight (kg) of samples in 1 m^2, and then converted to kg ha^{-1} (× 10,000). All forage weights were expressed as kg DM ha^{-1}. The nutritional constituent biomass (i.e., the amount of nutrients available per unit of area) was calculated by the product of the forage biomass and the concentration of nutrients in the forages (e.g., i.e., kg crude protein ha^{-1}, kg fiber ha^{-1}).

Aspen leaf samples were taken from each site from trees with an approximate maximum height of 2 to 2.5 m, when possible, by stripping leaves from no more than two branches per aspen tree and placing them into paper bags. The range of 2 to 2.5 m was chosen because trees at or below this height are below the browse line and consequently used by large ungulates like elk (DeByle 1985). A minimum of 25 g of leaves were harvested from each stand by collecting leaves from each tree within a 30 m radius of the center of the transect. If a site did not contain any aspen trees between 2 to 2.5 m within the 30 m radius, then trees closest in height to 2 to 2.5 m were used. Stand number and tree height for the stands that did not contain any aspen trees within the selected height range were: stand 9 (high regeneration stand; ~3 m in height), stand 6 (medium regeneration; < 1 m in height), and stand 7 (low regeneration stand; ~3 m in height).

We utilized information gathered by Rogers et al. (2015) (e.g., recruitment stems ha^{-1}, regeneration stems ha^{-1}, recruitment TPA, live aspen stems ha^{-1}, percent aspen cover, canopy height, percent of aspen browsed, elevation, slope, and aspect) from the fifteen sampled stands to determine their relationship with the foodscape (i.e., understory food type biomass, understory nutrient constituent biomass, aspen defense chemistry) assessed in the present study (see Table 4-1 for variables assessed in Rogers et al. [2015] and variables assessed in the current study).

Table 4-1 Variables used in the study.

Variables assessed by Rogers et al. 2015:
- Regeneration stems ha^{-1}
- Recruitment stems ha^{-1}
- Recruitment TPA percentage
- Landscape (physiographic) elements
  - Elevation[f]
  - Slope
  - Aspect
- Percent browsed aspen
- Fecal pellet counts
- Percent aspen canopy cover
- Canopy height

Variables assessed during the current study:
- Aspen leaf chemistry[g]
  - CP[a], ADF[b], NDF[c], TDN[d], Tremulacin, Salicortin, Total PG, Condensed tannins
- Understory food type biomass[e]
  - Grass, Forb, Dead material, Shrubs
- Nutrients within each understory food type[e]
  - CP[a], ADF[b], NDF[c], Hemicellulose, TDN[d]
- Total understory nutrients within each site[e]
  - CP[a], ADF[b], NDF[c], Hemicellulose, TDN[d]
- Total understory biomass within each site[e]

[a] Crude protein
[b] Acid detergent fiber
[c] Neutral detergent fiber
[d] Total digestible nutrients
[e] Kg ha^{-1} on a dry matter basis
[f] Meters
[g] Percent of dry matter

Forage analyses

All understory, shrub, and aspen leaf samples were stored at -20 °C within 60 minutes of sample collection. Frozen samples were transported in coolers to Utah State University in Logan, UT and stored in a freezer upon arrival. All aspen and understory samples were kept at -20 °C until they were freeze-dried. Samples were weighed before and after freeze-drying in order to determine dry matter content.

Forage separation

After drying, each forage sample obtained from the quadrats was separated into three food types. The food types consisted of grasses, forbs, and dead understory. Food types from each bag were weighed to determine the amount of forage within each sampled quadrat, and then added to get the total dry matter harvested from all twelve quadrat squares for each stand.

Chemical analyses

After separation into food types, a composite food type sample for each stand was ground in a Wiley Mill with a 1 mm screen, and analyzed for dry matter content (Method 930.15; AOAC 2000), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al. 1991), and crude protein (CP) (Method 990.03; AOAC 2000). Total digestible nutrients (TDN) were calculated from CP and fiber using equations from Weiss et al. (1992) as an estimate of digestible energy of the samples (NRC 1985, Swift 1957). The amount of hemicellulose was determined by subtracting ADF from NDF.

Phenolic glycosides were extracted from 40 mg of freeze-dried leaf material in 1 ml of methanol. The samples were vortexed on high for 5 minutes and centrifuged at

16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes. This procedure was repeated a second time, and the extracts were pooled to yield 2 ml of crude extract. Phenolic glycosides (salicortin and tremulacin) were quantified using high performance liquid chromatography (Agilent 1100 Series, Santa Clara, CA, USA) with a Luna 2, C18 column (150 x 4.6 mm, 5 μm) at a flow rate of 1 ml/min. Compound peaks were detected at 280 nm using purified salicortin and tremulacin standards isolated from aspen leaves (Lindroth et al. 1993).

Condensed tannins were extracted from approximately 50 mg of freeze-dried leaf tissue with 1 ml of a 70% acetone-10 mM ascorbic acid solution. Samples were vortexed on high for 20 minutes at 4 °C followed by centrifugation at 16,000 G for 2 minutes. Supernatants were removed and placed in separate micro-centrifuge tubes, and the extraction was then repeated. Condensed tannin concentrations were measured spectrophotometrically (SpectraMax Plus 384, MDS, Toronto, Canada) using the acid butanol method (Porter et al. 1986) standardized with purified condensed tannins isolated from aspen leaves (Hagerman and Butler 1980). Defense content of the understory forage samples was not assessed given the minimal to nil content of chemical defenses in grasses and dead plant material and uncertainties about the type of chemical defenses present in forbs.

Statistical analyses

Multivariate analysis–Non-metric multidimensional scaling (NMDS) ordination

An exploratory joint assessment of relationships between the foodscape (understory nutritional constituent biomass, understory food type biomass, aspen defense chemistry [tremulacin, salicortin, total PG, condensed tannins]) and aspen browsing indicators (percent browsed aspen, fecal pellets), indicators of aspen resilience (recruitment stems ha^{-1}, recruitment TPA, regeneration stems ha^{-1}, live aspen stems ha^{-1}), biotic (canopy height, percent aspen cover), or physiographic conditions (elevation, slope, aspect) was conducted using nonmetric multidimensional scaling (NMDS) ordinations. Separate ordinations were completed for each foodscape group (understory nutritional constituent biomass, understory food type biomass, and aspen defense chemistry) with subsequent fitting of smooth response surfaces of aspen browsing indicators, aspen resilience indicators, biotic factors, and physiographic conditions over each individual ordination.

We used NMDS scaling with Bray-Curtis dissimilarity as implemented by the metaMDS and ordisurf functions in the vegan package Version 2.4-1 (Oksanen et al. 2016a) in R Version 3.3.1 using RStudio (R Core Team 2013, RStudio Team 2015). Scaling was automatically applied by the metaMDS command (centering, PC rotation, half-change scaling). Expanded scores for food type biomass and nutritional constituent biomass were based on Wisconsin and square root transformations, and Wisconsin transformations for aspen defense chemistry, as set by metaMDS. Percent stress, the overall measure of quality of fit of the ordination to the data, and the percentage of variation not explained by all dimensions in the ordination, was calculated using the metaMDS command in the vegan package (Oksanen et al. 2016b). The command envfit with 1,000 permutations was used to obtain r^2 and P-values for all aspen browsing indicators, aspen resilience indicators, biotic, and physiographic variables on each foodscape group ordination.

Univariate correlation analysis

Univariate correlations were conducted after completing the multivariate analysis because using the vegan package in R does not generate r^2 and *P*-values for each variable within the foodscape groups to further explore relationships between foodscapes and indicators of aspen browsing, aspen resilience, and other biotic and physiographic conditions assessed. Multivariate analyses (i.e., from the NMDS ordination analyses) with resulting *P*-values of 0.24 or lower were included in univariate regressions with foodscape variables (i.e., food type biomass, nutritional biomass constituents, and aspen defense chemical constituents). A *P*-value of 0.24 was chosen as the cut-off to include all relationships that were considered a trend at the decimal point ($P < 0.2$). Thus, those variables included in the univariate analysis were aspect, canopy height, percent aspen cover, elevation, percent browsed aspen, regeneration stems ha^{-1}, recruitment stems ha^{-1}, and recruitment TPA. Fecal pellets and aspen regeneration were also included in the univariate analyses despite yielding *P*-values > 0.24 in the ordination because they are important indicators of herbivory and stand health because fecal pellets were the main measure of stand visitation available to us, and regeneration is a measure of how successfully a stand establishes new shoots.

We used the xyplot command for regressions using the lattice package Version 0.20-33 (Sarkar 2008) in R Version 3.3.1 using RStudio (R Core Team 2013, RStudio Team 2015). A significant correlation was defined as any variable with a *P*-value of 0.1 or less, and trends were defined as any variable with a *P*-value of 0.2 or less.

Results

Multivariate NMDS ordination analyses

Two convergent solutions were found after 20 runs using metaMDS analyses for understory food type biomass ha^{-1}, total understory nutritional constituent biomass ha^{-1}, and aspen defense chemistry. Two dimensions (k=2) were selected by the metaMDS function for understory nutrient biomass ha^{-1}, understory food type biomass ha^{-1}, and aspen defense chemistry (NMDS stress value=1.9%, 5.8%, and 1.5%, respectively). Explanations of the variability in the data were not improved by increasing the ordinations to 3 dimensions (k=3; NMDS stress reduced to 0.7%, 2.4%, and 0.77% for understory nutrient biomass ha^{-1}, understory food type biomass ha^{-1}, and aspen defense chemistry, respectively).

Relationship of the foodscape with aspen browsing

Fecal pellets

We found non-significant relationships between understory nutrient constituent biomass ha^{-1} (r^2=0.199, P=0.255), food type biomass (r^2=0.039, P=0.771), and aspen defense chemistry (r^2=0.119, P=0.472) with fecal pellets.

Percent browsed aspen

There was no relationship between food type biomass and percent of browsed aspen (r2=0.010, P=0.931). However, the analysis showed a trend between percent of browsed aspen and both understory nutrient constituent biomass ha^{-1} (r^2=0.228, P=0.239) and aspen defense chemistry (r^2=0.219, P=0.233). The ordination shows that areas with high percentages of aspen browsing have high concentrations of understory hemicellulose, low concentrations of ADF, and an intermediate amount of understory NDF, TDN, and CP biomass ha^{-1} (plot not shown). The ordination also suggests that areas with a high percent of browsed aspen have greater concentrations of tremulacin and tannins, but lower concentrations of salicortin (Fig. 4-2).

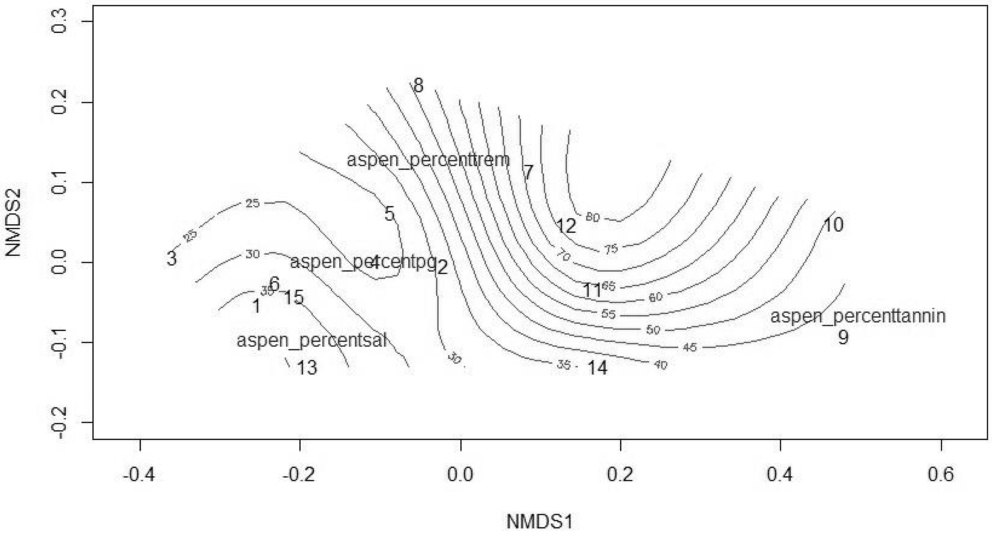

Fig 4-2 Organization of aspen defense chemistry characteristics (expressed as grams in 100 g of dry matter; dark grey lettering) in a nonmetric multidimensional scaling (NMDS) ordination showing the first two dimensions. Aspen defense characteristics were measured in aspen leaves sampled from each stand (fifteen values [stands] for each of the four defense characteristics). Defense characteristics that appear in the ordination are as follows: aspen_percenttrem (percent tremulacin), aspen_percentsal (percent salicortin), aspen_percentpg (percent total PG), and aspen_percenttannin (percent condensed tannins). An overlaid response surface was placed over the ordination surface representing a gradient of percent browsed aspen in each of the fifteen stands (light grey topographical surface, with each topographical line labeled with its value ranging from 25 to 80%). The stress value was 1.5%. Stand numbers (1 through 15) appear on the surface in black lettering.

Relationships of the foodscape with indicators of stand resilience

Recruitment stems ha^{-1}

We also found a correlation between aspen defense chemistry and recruitment stems ha^{-1} (r^2=0.418, P=0.037). High-recruitment aspen stands contained high concentrations of salicortin, low concentrations of tremulacin, and intermediate concentrations of total phenolic glycosides and condensed tannins within aspen leaves (plot not shown). There was no relationship between understory nutritional biomass (r^2=0. 099, P=0.519) or understory food type biomass and recruitment stems ha^{-1} (r^2=0.009, P=0.951).

Recruitment TPA

We found a trend between aspen defense chemistry and recruitment TPA (r^2=0.253, P=0.186), but no relationship between recruitment TPA and understory food type biomass (r^2=0.012, P=0.930) or understory nutritional constituent biomass (r^2=0.184, P=0.262). Stands with high recruitment TPA contained high concentrations of salicortin, high-to-intermediate concentrations of total PG, intermediate concentrations of tremulacin, and low concentrations of condensed tannins within aspen leaves (plot not shown).

Regeneration stems ha^{-1}

There were non-significant relationships between regeneration and the foodscapes (food type biomass [r^2=0.186, P=0.288], understory nutrient biomass [r^2=0.053, P=0.732], or aspen defense chemistry [r^2=0.106, P=0.523]).

Relationship of the foodscape with biotic factors

Canopy height

The analysis showed a correlation between canopy height and understory food type biomass ha^{-1} (r^2=0.588, P=0.008; Fig. 4-3). The ordination suggests stands with high canopy heights have low understory shrub and dead biomass ha^{-1}, intermediate amounts of understory grass biomass ha^{-1}, and high understory forb biomass ha^{-1}. There was no relationship between aspen defense chemistry (r^2=0.151, P=0.387) or understory nutrient

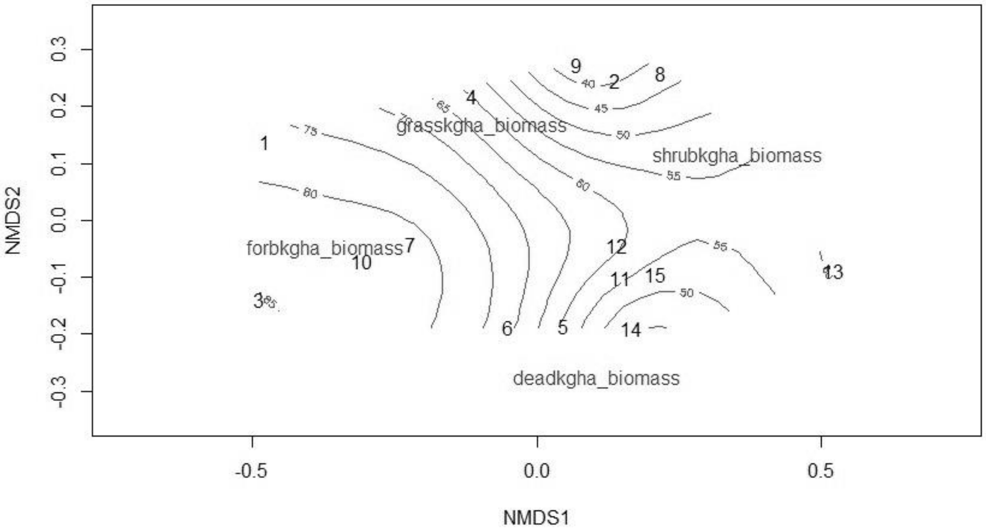

Fig 4-3 Organization of understory food type biomass (expressed as kg hectare^{-1}; dark grey lettering) in a nonmetric multidimensional scaling (NMDS) ordination showing the first two dimensions. Understory food type biomass was measured in each aspen stand (fifteen values [stands] for each of the four understory food types). Understory food types that appear in the ordination are as follows: grasskgha_biomass (grass biomass), forbkgha_biomass (forb biomass), deadkgha_biomass (dead material biomass), and shrubkgha_biomass (shrub biomass). An overlaid response surface was placed over the ordination surface representing a gradient of canopy heights (expressed as meters) in each of the fifteen stands (represented by the light grey topographical surface, with each topographical line labeled with its value ranging from 40 to 80 meters). The stress value was 5.8%. Stand numbers (1 through 15) appear on the surface in black lettering.

biomass (r^2=0.001, P=0.993) and canopy height.

Percent aspen cover

There was a trend between percent aspen cover and both understory food type biomass (r^2=0.224, P=0.209) and understory nutrient constituent biomass (r^2=0.219, P=0.226), but no relationship with aspen defense chemistry (r^2=0.106, P=0.520). Stands with a high percent of aspen cover had high understory forb biomass ha^{-1}, low understory shrub biomass ha^{-1}, and intermediate amounts of understory dead and grass biomass ha^{-1}. The ordination also suggested that these stands had high understory TDN, high-to-intermediate understory ADF and CP, intermediate amounts of NDF, and low hemicellulose biomass ha^{-1} (plots now shown).

Relationship of the foodscape with physiographic conditions

Aspect

We found a trend between aspect and aspen defense chemistry (r^2=0.256, P=0.166). No relationships were found between aspect and understory food type biomass (r^2=0.059, P=0.702) or understory nutrient biomass (r^2=0.056, P=0.677). The NMDS ordination suggests stands on wetter north to northeast aspects (aspect=0.65 to 0.8) contained greater tannin and salicortin content in aspen leaves, and lower tremulacin content than in stands growing on drier southwest aspects. In addition, aspen PG was greater on northwest and southeast aspects (aspect=0.6; plot not shown).

Elevation

There was a correlation between elevation and understory food type biomass ha^{-1}

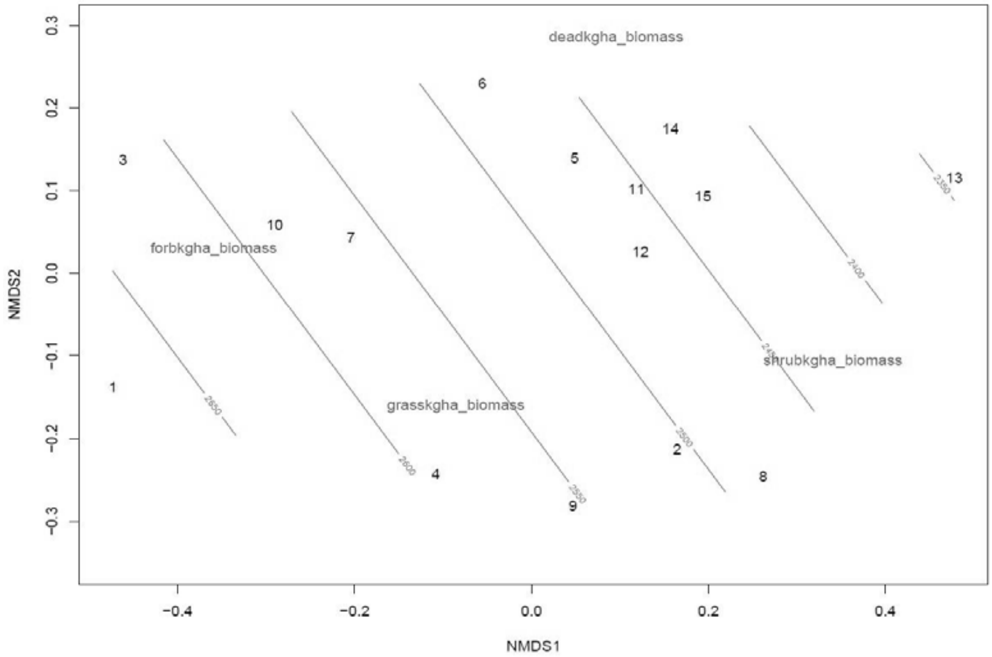

Fig 4-4 Organization of understory food type biomass (expressed as kg hectare^{-1}; dark grey lettering) in a nonmetric multidimensional scaling (NMDS) ordination showing the first two dimensions. Understory food type biomass was measured in each aspen stand (fifteen values [stands] for each of the four understory food types). Understory food types that appear in the ordination are as follows: grasskgha_biomass (grass biomass), forbkgha_biomass (forb biomass), deadkgha_biomass (dead material biomass), and shrubkgha_biomass (shrub biomass). An overlaid response surface was placed over the ordination surface representing a gradient of elevation (expressed as meters) in each of the fifteen stands (represented by the light grey topographical surface, with each topographical line labeled with its value ranging from 2350 to 2650 meters). The stress value was 5.8%. Stand numbers (1 through 15) appear on the surface in black lettering.

(r^2=0.626, P=0.006) and a trend between elevation and understory nutrient constituent biomass ha^{-1} (r^2=0.331, P=0.081). There was no relationship found between elevation and aspen defense chemistry (r^2=0.185, P=0.310). The ordination shows that at high elevations, understory forb biomass ha^{-1} was high while shrub biomass ha^{-1} was low, and understory dead and grass biomass ha^{-1} was greatest at intermediate elevations (around 2575 m; see Fig. 4-4). At high elevations, understory fiber biomass ha^{-1} was found in

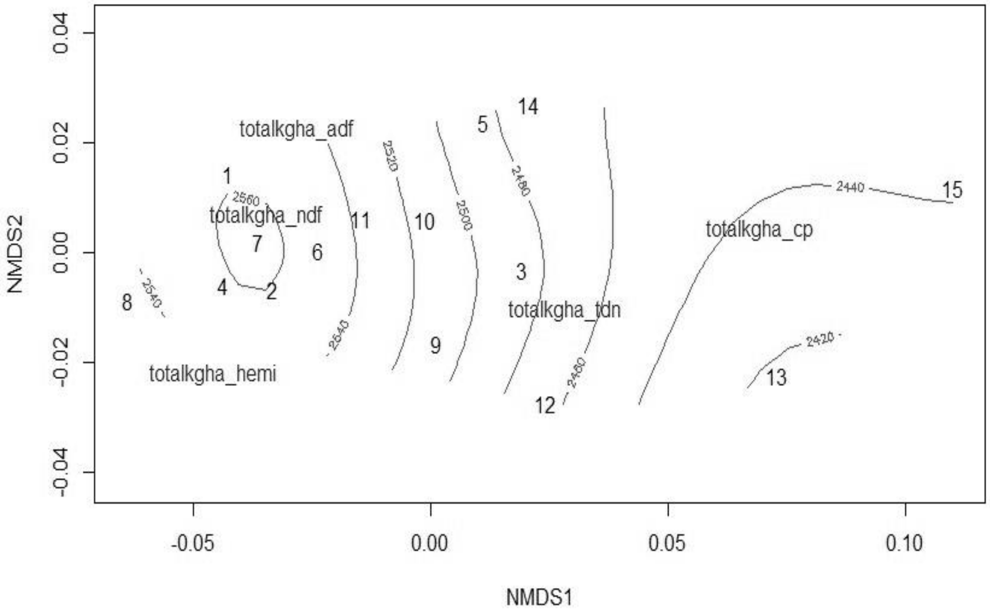

Fig 4-5 Organization of understory nutrient constituent biomass (expressed as kg hectare^{-1}; dark grey lettering) in a nonmetric multidimensional scaling (NMDS) ordination showing the first two dimensions. Understory nutrient constituent biomass was measured in each aspen stand (fifteen values [stands] for each of the five understory nutrient constituents). Understory food types that appear in the ordination are as follows: totalkgha_adf (ADF [acid detergent fiber] biomass), totalkgha_ndf (NDF [neutral detergent fiber] biomass), totalkgha_hemi (hemicellulose biomass), totalkgha_cp (CP [crude protein] biomass), and totalkgha_tdn (TDN [total digestible nutrients] biomass). An overlaid response surface was placed over the ordination surface representing a gradient of elevation (expressed as meters) in each of the fifteen stands (represented by the light grey topographical surface, with each topographical line labeled with its value ranging from 2420 to 2540 meters). The stress value was 1.9%. Stand numbers (1 through 15) appear on the surface in black lettering.

high concentrations and understory CP biomass ha^{-1} was found in low concentrations, and understory TDN biomass ha^{-1} was greatest at intermediate elevations (around 2470 m; see Fig. 4-5).

Univariate regression analyses

Relationship of the foodscape with aspen browsing

Fecal pellets

We found a negative correlation between understory CP biomass ha^{-1} and fecal pellets, and a negative trend between understory TDN biomass ha^{-1} and fecal pellets. In addition, there was a positive trend between tremulacin content in aspen and fecal pellet counts (see Table 4-2 for r^2 and P-values).

Percent browsed aspen

There was no relationship between percent browsed aspen and individual variables pertaining to understory nutrient constituent biomass ha^{-1} (CP, ADF, NDF, hemicellulose, TDN), but there were negative correlations between percent browsed aspen and both salicortin and total PG content in aspen leaves (see Table 4-2 for r^2 and P-values).

Relationships of the foodscape with indicators of stand resilience

Recruitment stems ha^{-1}

There were no relationships between stand recruitment and aspen chemical defenses (see Table 4-4 for r^2 and P-values), but there were positive trends between understory grass biomass, CP biomass, and TDN biomass with recruitment (see Table 4-2 for r^2 and P-values).

Table 4-2 P-values and r^2 values from univariate regression analyses conducted between the foodscape and indicators of aspen resilience, aspen browsing, and other biotic and abiotic factors assessed at the Wolf Creek ranch. Significant relationships are in bold.

Food type biomass[e]	Grass		Forb		Dead		Shrub			
	r^2	P-value	r^2	P-Value	r^2	P-value	r^2	P-Value		
Aspect	0.001	0.940	0.001	0.922	<0.001	0.944	0.041	0.470		
Canopy Height[f]	<0.001	0.970	**0.327**	**0.026**	0.016	0.653	**0.471**	**0.005**		
Percent aspen cover	0.003	0.857	0.107	0.233	0.015	0.667	0.101	0.248		
Elevation[f]	**0.307**	**0.032**	0.202	0.093	**0.239**	**0.064**	**0.390**	**0.013**		
Fecal pellets ha^{-1}	0.044	0.451	0.035	0.506	0.007	0.770	0.051	0.418		
Percent browsed aspen	0.007	0.762	0.005	0.799	<0.001	0.996	0.002	0.874		
Recruitment stems ha^{-1}	0.120	0.206	<0.001	0.944	0.012	0.696	0.085	0.291		
Recruitment TPA	0.069	0.344	0.006	0.782	0.011	0.708	0.016	0.651		
Regeneration stems ha^{-1}	0.005	0.797	0.116	0.215	0.002	0.869	0.042	0.464		
Nutrient constituent biomass[e]	CP[a]		ADF[b]		NDF[c]		Hemicellulose		TDN[d]	
	r^2	P-value	r^2	P-Value	r^2	P-Value	r^2	P-value	r^2	P-Value
Aspect	0.056	0.396	0.014	0.670	0.012	0.703	0.0070	0.767	0.033	0.518
Canopy Height[f]	0.027	0.560	0.015	0.663	0.013	0.689	0.0086	0.742	0.012	0.704
Percent aspen cover	0.040	0.472	0.002	0.874	0.074	0.327	<0.001	0.991	0.006	0.782
Elevation[f]	**0.160**	**0.140**	**0.240**	**0.064**	**0.215**	**0.057**	**0.2645**	**0.050**	**0.170**	**0.126**
Fecal pellets ha^{-1}	**0.207**	**0.089**	0.081	0.305	0.074	0.327	0.0606	0.377	**0.126**	**0.195**
Percent browsed aspen	0.002	0.879	0.003	0.858	0.006	0.781	0.0165	0.648	0.006	0.792
Recruitment stems ha^{-1}	**0.179**	**0.116**	0.118	0.211	0.109	0.229	0.0917	0.273	**0.153**	**0.149**
Recruitment TPA	0.061	0.374	0.038	0.488	0.033	0.514	0.025	0.570	0.034	0.509
Regeneration stems ha^{-1}	0.113	0.221	0.039	0.479	0.038	0.484	0.0357	0.500	0.057	0.393
Aspen defense chemistry[g]	Tremulacin		Salicortin		Total PG		Condensed Tannin			
	r^2	P-value	r^2	P-value	r^2	P-value	r^2	P-value		
Aspect	0.015	0.661	0.016	0.653	0.017	0.641	0.076	0.320		
Canopy Height[f]	**0.215**	**0.082**	0.100	0.252	**0.148**	**0.157**	0.047	0.437		
Percent aspen cover	0.013	0.690	0.037	0.493	0.029	0.541	0.002	0.876		
Elevation[f]	**0.446**	**0.007**	**0.368**	**0.017**	**0.432**	**0.008**	0.046	0.443		
Fecal pellets ha^{-1}	**0.133**	**0.182**	0.035	0.504	0.068	0.347	<0.001	0.947		
Percent browsed aspen	0.102	0.246	**0.223**	**0.076**	**0.192**	**0.103**	0.018	0.639		
Recruitment stems ha^{-1}	0.052	0.416	0.032	0.522	0.002	0.891	0.043	0.456		
Recruitment TPA	0.004	0.813	**0.131**	**0.184**	0.073	0.330	0.090	0.278		
Regeneration stems ha^{-1}	0.011	0.710	0.050	0.424	0.036	0.498	0.081	0.303		

[a] Crude protein
[b] Acid detergent fiber
[c] Neutral detergent fiber
[d] Total digestible nutrients
[e] Kg ha^{-1} on a dry matter basis
[f] Meters
[g] Percent dry matter basis

Recruitment TPA

There was a positive trend between recruitment TPA and salicortin, but the other defense chemicals (tremulacin, total PG, and tannins) did not show a relationship with recruitment TPA (see Table 4-2 for r^2 and P-values).

Regeneration stems ha^{-1}

We found non-significant relationships between regeneration and the foodscape, except for a positive trend between regeneration and understory CP biomass (see Table 4-2 for r^2 and P-values).

Relationship of the foodscape with biotic factors

Canopy height

There was a positive correlation between canopy height and understory forb biomass ha^{-1}, and a negative correlation between canopy height and understory shrub biomass ha^{-1}. We also found a positive correlation between tremulacin content in aspen leaves and aspen canopy height, and a positive trend between total PG content in aspen leaves and aspen canopy height (see Table 4-2 for r^2 and P-values).

Percent aspen cover

There was no significant relationships between the individual variables within understory food type biomass or understory nutrient biomass and percent aspen cover (see Table 4-2 for r^2 and P-values).

Relationship of the foodscape with physiographic conditions

Aspect

No relationships between individual variables from aspen defense chemistry (salicortin, tremulacin, total PG, tannins) and aspect were found (see Table 4-2 for r^2 and P-values).

Elevation

A positive correlation was found between elevation and both understory grass and forb biomass ha^{-1}, and a negative correlation between elevation and both understory dead and shrub biomass ha^{-1}. A positive correlation was also found between elevation and understory ADF, NDF, hemicellulose biomass ha^{-1}, and tremulacin, salicortin, and total PG content in aspen leaves. Additionally, a positive trend was found between elevation and understory TDN and CP biomass ha^{-1} (see Table 4-2 for r^2 and P-values).

Nutritional analyses

Crude protein (CP) content was similar between aspen leaves collected from all fifteen stands, as well as shrub leaves and forbs collected from the understory of high recruitment TPA stands (Table 4-3). In general, CP concentration was low in grasses and dead plant material collected from the understory, particularly for high and medium recruitment TPA sites (P>0.05), and were lower than CP content of forbs in medium and low recruitment TPA stands (P<0.05). Acid (ADF) and neutral (NDF) detergent fiber content was low in aspen and shrub leaves, with high concentrations in dead plant material and grasses. Total digestible nutrient (TDN) concentration was the greatest in

Table 4-3 Nutritional analyses (% dry matter) of aspen leaves and understory samples collected from different aspen stands at Wolf Creek Ranch showing different levels of aspen recruitment TPA.

High recruitment TPA aspen stands- # 1[b], 9[a], 11[e], 13[e], 15[d]

	Crude protein	ADF[f]	NDF[g]	TDN[h]
Grasses	11.5 ± 2.1	38.11 ± 1.39	61.89 ± 1.72	56.17 ± 1.82
Forbs	13.71±1.4	30.9±1.42	42.56±2.55	59.22 ± 1.16
Dead	11.11±0.54	44.64±1.01	64.71±1.04	51.01 ± 0.73
Aspen	14.73±0.31	18.39±1.52	26.26±1.65	69.12 ± 1.08

Medium recruitment TPA aspen stands- # 3[d], 4[a], 6[d], 8[e], 14[c]

	Crude protein	ADF	NDF	TDN
Grasses	8.89 ± 0.48	40.05 ± 0.57	65.76 ± 1.54	53.74 ± 0.56
Forbs	11.87±1.33	32.68±3.9	42.57±3.21	57.76 ± 3.12
Dead	8.88±0.59	45.53±0.91	65.32±2.54	49.52 ± 0.75
Aspen	14.53±0.8	17.43±0.58	24.96±2.23	69.86 ± 0.39

Low recruitment TPA aspen stands- # 2[b], 5[d], 7[c], 10[c], 12[c]

	Crude protein	ADF	NDF	TDN
Grasses	8.97 ± 0.08	40.78 ± 0.72	66.03 ± 0.29	53.21 ± 0.58
Forbs	12.26±0.41	30.33±2.94	42.21±2.99	59.62 ± 2.30
Dead	11.56±0.21	41.76±0.61	61.57±1.37	53.38 ± 0.46
Aspen	14.84±0.58	20.29±1.19	29.21±1.49	67.62 ± 0.85

Composite leaf samples from all stands sampled

	Crude protein	ADF	NDF	TDN
Shrub	13.24	17.71	27.80	69.60

[a] August 25, 2015
[b] August 26, 2015
[c] August 27, 2015
[d] August 28, 2015
[e] August 29, 2015
[f] Acid detergent fiber
[g] Neutral detergent fiber
[h] Total digestible nutrients

Table 4-4 Plant secondary compounds (% dry matter) of aspen leaves at Wolf Creek Ranch across stands with different levels of recruitment TPA.

High recruitment TPA aspen stands

	Tremulacin[f]	Salicortin[f]	Total PG[f]	Condensed tannins
Aspen[g]	5.13 ± 0.99	8.33 ± 1.81	13.47 ± 2.71	1.59 ±0.56
Aspen[h]	5.04 ± 0.75	7.73 ± 1.52	12.77 ± 2.18	2.42 ± 2.04

Medium recruitment TPA aspen stands

	Tremulacin	Salicortin	Total PG	Condensed tannins
Aspen[g]	6.15 ± 1.72	8.55 ± 2.95	14.7 ± 4.62	1.68 ± 0.92
Aspen[h]	6.33 ± 1.3	9.08 ± 2.29	15.41 ± 3.55	1.55 ± 1.41

Low recruitment TPA aspen stands

	Tremulacin	Salicortin	Total PG	Condensed tannins
Aspen[g]	6.04 ± 0.9	6.05 ± 1.38	12.09 ± 2.03	2.97 ± 1.16
Aspen[h]	5.59 ± 0.84	5.5 ± 1.2	11.09 ± 1.89	2.66 ± 1.87

[a] August 25, 2015
[b] August 26, 2015
[c] August 27, 2015
[d] August 28, 2015
[e] August 29, 2015
[f] Percent of dry sample weight
[g] Excluding stands that did not contain 2 m trees for sampling
[h] Including stands that did not contain 2 m trees for sampling

aspen and shrub leaves. Concentration of TDN was lowest in dead plant material for all recruitment TPA levels, and lowest in grasses for low recruitment TPA sites ($P<0.05$).

Plant secondary compound analyses

Total concentration of phenolic glycosides (PG) and condensed tannins were similar in high, medium, and low recruitment TPA stands, before and after excluding stands that did not contain trees between 2 to 2.5 meters in height (i.e., stand 22 [high recruitment TPA], 16 [medium recruitment TPA], and 17 [low recruitment TPA]) (see Table 4-4).

Discussion

Previous research suggests that nutrients and plant secondary compounds (PSC) influence aspen use by ungulates (Wooley et al. 2008; Villalba et al. 2014; Holeski et al. 2016; see Chapters 2, 3, and 4). However, little work has been completed on the interplay between the chemicals present on the landscape and aspen stand health and browsing by ungulates. Here we document relationships of stand resilience indicators (regeneration, recruitment, recruitment TPA), aspen browsing indicators (fecal pellets, percent browsed aspen), biotic characteristics of the stand (canopy height, aspen canopy cover), and physiographic conditions (elevation) with the foodscape (understory food type biomass, nutritional constituent biomass of the understory, and aspen defense chemistry).

Nutritional constituent biomass

We predicted that as understory nutritional biomass at the sampled sites increased (e.g., greater crude protein content, lower fiber content, greater TDN content), aspen use by ungulates would decline, and consequently recruitment and regeneration would increase because ungulates would prefer a higher quality and abundant understory to less nutritious and defended aspen tissues. Consistent with this notion, understory crude protein biomass yielded positive relationships with aspen recruitment and regeneration, suggesting lower browsing pressure in stands with foodscapes that provided greater amounts and concentrations of crude protein. Although protein is typically considered a highly desirable nutrient often utilized as a proxy for estimating and understanding diet quality of ungulates (Owen-Smith 1988; Steuer et al. 2014), a negative relationship was found between understory crude protein biomass and fecal pellets, an indicator of elk presence. In addition, the NMDS analysis suggested that areas with high percentages of aspen browsing have intermediate, instead of high, amounts of understory TDN and CP biomass. These results are in agreement with previous studies showing negative relationships between nitrogen and browse preference by ungulates (Berteaux et al. 1998; Holeski et al. 2016). It is likely that the protein needs of mature elk were below those offered by the nitrogen-rich landscape (Mould and Robbins 1981) or that elk had more than an adequate protein supply from other feeding areas, thus reducing their number of visits to aspen understory sites with high CP content. In fact, excess CP cause food aversions in ruminants (Provenza 1995), which could explain this pattern for animals with relatively low CP requirements in nutrient-rich landscapes. Alternatively, elk prefer graminoids (Beck et al. 1996; Jenkins and Wright 1987) and graminoids contain less crude protein than forbs, so those areas which contained more forbs (and thus more CP) were less likely to be visited by elk. In addition, animals learn foraging behaviors and patterns as well as locations of preferred feeding sites from their mother via social facilitation (Hinde 1970). Animals tend to utilize areas that contain forages that fit their nutritional needs, and because adult elk have a lower protein nutritional requirement than calves (Jelinski and Fisher 1991), adults may forage in areas with lower protein content and still meet their CP requirements. Subsequently, this behavior may be passed onto calves, leading to a greater use of areas with lower protein content in the understory.

In addition to protein, sites with high understory nutrient biomass (e.g., TDN) tended to be positively associated with aspen recruitment and negatively associated with fecal pellet counts. Collectively, sites with a nutrient-rich understory (e.g., TDN and CP) tended to have less elk visitation and greater recruitment and regeneration than sites with lower nutrient content. Thus, we can speculate that elk may have utilized more aspen at sites where understories offered a lower nutrient supply, a substitution process that incorporates more aspen in the diet as abundance of nutrients in the understory declines. This is consistent with findings from Chapter 3, where a restriction of energy in the diet prompted sheep to increase consumption of aspen leaves.

In addition to the prior analysis, it is important to indicate that several other reasons–external to the foodscape–could have accounted for the pattern observed in this study regarding indicators of aspen resilience at nutrient-rich sites. For instance, increased aspen recruitment and regeneration in stands with an understory of high nutrient biomass could simply reflect site-specific factors such as favorable soil conditions for plant growth, which may be unrelated to browsing.

In contrast to the relationships found in this study, recent pen experiments with sheep cite that aspen use increases with greater concentrations of protein in the diet (see Chapters 2 and 3). Because sheep and elk are both mesoherbivores and grazer-intermediate feeders, diet composition is similar and can be compared between the two species (Hoffman 1988; Beck et al. 1996). However, in Chapter 3 sheep did not have a choice among different forage alternatives, as they only received aspen leaves and subsequently a nitrogen-rich basal ration. Additionally, in Chapter 2, animals received a limited choice of forages in addition to their supplement. In contrast, mature elk with low nitrogen requirements foraging in a diverse landscape have several feed alternatives available and they may select forages and feeding sites with lower nitrogen content than the nitrogen-rich supplements and rations offered in the studies described in Chapters 2 and 3. Thus, the availability of choices in the foodscape may lead to different foraging responses by ungulates (as was seen in the present landscape scale study) than when no alternatives are present in a controlled pen study.

Understory food type biomass

We also predicted that understory biomass would be inversely related to aspen browsing because if nutrient biomass at these sites was above the threshold required to meet nutritional needs, then animals did not need to seek extra nutrients from aspen leaves and consequently aspen use would decline. We found a significant effect of elevation on understory biomass, and previous studies show elevation is positively correlated with moisture (Hamon 1971; Meerveld and McDonnell 2005). Higher elevation sites have greater soil moisture than those at lower elevations and aspen and forbs tend to thrive in areas of high moisture (Latva-Karjanmaa et al. 2006; Turner et al. 2003; Harte and Shaw 1995), and grasses and forbs senesce when temperatures increase and less moisture is available to the plants. Shrubs establish in warm and dry climates (Harte and Shaw 1995), and therefore thrive at lower elevations. These patterns are in agreement with findings from the current study, with positive associations between elevation and understory forb and grass biomass, and between elevation and both TDN and CP abundance, with negative correlations between elevation and shrub biomass. Thus, through the growth and establishment of different food types at various elevations on the landscape, elevation affected the quality of the foodscape (e.g., food type biomass and therefore concentration and amount of TDN and CP), which in turn may have influenced elk foraging sites, as well as aspen recruitment and regeneration. For instance, elk may have utilized more aspen at sites where understories offered lower biomass (e.g., shrubs at lower elevation), a selection process with negative impacts on aspen recruitment and regeneration. Alternatively, it is possible that sites at higher elevation, due to water availability, may be simply more resilient–i.e., able to replace browsed stems at a high enough rate to not experience growth limitation through compensatory growth.

Defense content in aspen

Lastly, we predicted that as defense content in aspen stands increased, aspen use would decrease because phytochemicals constrain intake. In our study, the concentration of specific PSC like tremulacin tended to be positively correlated with pellet counts (from the univariate analysis) and with percentage of browsed aspen (from the NMDS analysis), and recruitment TPA was positively associated with salicortin content in aspen for both univariate and multivariate analyses. These patterns appear counterintuitive because at similar or equal concentrations, tremulacin is more toxic than salicortin (Lindroth et al. 1988), and previous studies have found aspen chemical defenses negatively impact aspen browsing by ungulates (e.g., Bailey et al. 2007; Wooley et al. 2008; Villalba et al. 2014; see Chapter 2 and 3). Consistent with this notion, salicortin and total PG concentrations were negatively correlated with percent of browsed aspen. These relationships emerged despite the fact that the content of PG in aspen leaves sampled in the current study was below the threshold described by Wooley et al. (2008) for reducing aspen browsing by elk. However, no clear patterns were observed regarding the relationship between aspen regeneration or recruitment stems ha^{-1} and aspen chemical defenses. Only recruitment stems ha^{-1} was positively associated with salicortin content in aspen leaves, but negatively associated with tremulacin concentration. It is likely that because elk numbers exceed recommended stocking rates on the WCR property (Rogers et al. 2015), the influence of chemical defenses on aspen regeneration or recruitment stems ha^{-1} was less relevant than in situations when animals are present at lower densities (Lindroth and St. Clair 2013), as also mentioned by Rogers et al. (2015). In other words, it may be that elk overwhelm aspen defense chemical capacity at WCR because chemical defenses only satiate the detoxification capabilities of herbivores at a critical threshold of plant abundance relative to the number of consumers. Below this threshold (e.g. when animal numbers increase), local extinction is more likely as a species becomes less abundant and thus detoxification capabilities of the larger group of animals are not satiated (Provenza et al. 2003).

Although no explanations for the relationship between aspen defense content and canopy height emerged from the current study, possible explanations may be found in current understandings of the relationship between canopy height and soil microclimate or total available moisture. As canopy height increases, the amount of light that reaches the understory is reduced (Martens et al. 2000). Understory light environments affect microclimate (e.g., solar radiation, soil and leaf temperature, soil moisture) (Breshears et al. 1997; 1998), and increased light intensity can increase soil temperature and soil evaporation rates (Breshears et al. 1998), which can influence plant establishment and growth (Floyd 1983; Padien and Lajtha 1992; Martens et al. 1997). Alternatively, the relationship between canopy height and soil moisture may be due to bottom-up effects instead of top-down effects—meaning that soil microclimate may drive canopy height differences instead of canopy height driving soil microclimate differences. Because we did not measure soil microclimates, we cannot conclude in which direction the effect occurs. In either case, increased light intensity has been shown to increase defense chemical content within aspen stands (Calder et al. 2011; Lindroth and St. Clair 2013; Wan et al. 2014; Randriamanana et al. 2015), but our findings suggest the opposite–that increased canopy height (shading) may have increased aspen PG content in the sampled juvenile aspen trees. Such changes may be in response to other variables that affect PG content such as temperature, soil moisture, or soil nutrients that were not assessed in the current study (Lindroth and St. Clair 2013; Randriamanana et al. 2015).

Conclusion

Results from this study suggest that the foodscape had an effect–either from direct effects or through physiographic conditions such as elevation–on aspen herbivory and stand condition. Aspen regeneration stems ha^{-1} and recruitment stems ha^{-1} were positively correlated with understory CP biomass, a variable from the foodscape which was in turn positively associated with elevation. The abundance of forbs and grasses at higher elevation sites, as observed in the multivariate and univariate relationships, helped to explain the distribution of CP biomass across the foodscape. In contrast, stands with low CP and TDN concentrations in the understory (e.g., those observed at lower elevations) appeared more likely to experience low aspen regeneration and recruitment and greater elk presence than those growing at nutrient-rich sites. Moreover, aspen stands at lower elevations may be more at risk of succumbing to overbrowsing, because aspen in those areas are more likely to be stressed from lack of moisture (Latva-Karjanmaa et al. 2006; Turner et al. 2003; Rogers and Mittanck 2014). Novel management approaches for enhancing aspen condition may entail improving the nutrient content (or increasing the concentration of chemical defenses) of the foodscape at these lower elevation sites either through supplementation or revegetation programs. Although improving the nutrient content of the foodscape may attract more herbivores to aspen-dominated communities, aspen use may decline because preference will be directed towards more abundant and greater quality resources (e.g., forages, foods) and not towards lower-quality and defended aspen leaves. Nevertheless, further research needs to focus on the trade-offs between offering supplementary feeds or forages and the impacts of herbivory at the plant community level. For instance, when elk density is high, adding nutrients to the foodscape may increase herbivory pressure on aspen suckers because even when intake of aspen per head may be low, the combined browsing by the herd will lead to a substantial removal of aspen biomass from the community. The concept of foodscape and foraging by ungulates developed in this study could be used to explore other relationships, on a wider range of landscapes–like browsing and mineral content of aspen trees and understories–to address concerns of overbrowsing in aspen-dominated communities.

References

Abraham PJ (2013) Wolf Creek Ranch Forest Stewardship Plan. Utah Division of

Forestry, Fire and State Lands. Heber City, UT

Andrew MH, Noble IR, Lange RT (1979) A non-destructive method for estimating the weight of forage on shrubs. Australian Rangeland Journal 1:225-231

AOAC (2000) Official methods of analysis, 17th edn. Association of official analytical chemists. Perkin Elmer 5300 DV ICP. Perkin Elmer, 710 Bridgeport Avenue, Shelton, CT

Atsatt PR, O'Dowd DJ (1976) Plant defense guilds. Science 193(4247):24-29

Bailey DW, Gross JE, Laca EA, Rittenhouse LR, Coughenour MB, Swift DM, Sims PL (1996) Mechanisms that result in large herbivore grazing distribution patterns. J Range Manage 49:386-400

Bailey JK, Schweitzer JA, Rehill BJ, Irschick DJ, Whitham TG, Lindroth RL (2007) Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. Biol Invasions 9:715-722

Baraza E, Zamora R, Hódar JA (2006) Conditional outcomes in plant–herbivore interactions: neighbours matter. Oikos 113:148-56

Beck JL, Flinders JT, Nelson DR, Clyde CL, Smith HD, Hardin PJ (1996) Elk and domestic sheep interactions in a North-Central Utah Aspen Ecosystem. U.S. Dept. of Agriculture, Forest Service, Intermountain Research Station Research Paper INT-RP-491

Beck JL, Peek JM, Strand EK (2006) Estimates of elk summer range nutritional carrying capacity constrained by probabilities of habitat selection. J Wildlife Manage 70(1):283-294

Berteaux D, Crete M, Huot J, Maltais J, Ouellet JP (1998) Food choice by white- tailed deer in relation to protein and energy content of the diet: a field experiment. Oecologia 115:84-92

Breshears DD, Rich PM, Barnes FJ, Campbell K (1997) Overstory imposed heterogeneity in solar radiation and soil moisture in a semiarid woodland. Ecol Appl 7:1201-1215

Breshears DD, Nyhan JW, Heil CE, Wilcox BP (1998) Effects of woody plants on microclimate in a semiarid woodland: soil temperature and evaporation in canopy and intercanopy patches. Int J Plant Sci 153:425-433

Bunnefeld N, Linnell JDC, Odden J, van Duijn MAJ, Anderson R (2006) Risk taking by Eurasian lynx (*Lynx lynx*) in a human-dominated landscape: effects of sex and reproductive status. Journal of Zoology 270:31-39

Calder WJ, Horn KJ, St. Clair SB (2011) Conifer expansion reduces the competitive ability and herbivore defense of aspen by modifying light environment and soil chemistry. Tree physiology 31(6): 582-591

DeByle NV (1985) Animal impacts. In: DeByle NV, Winoker RP (eds) Aspen: Ecology and management in the western United States. GTR-RM-119. USDA, Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp 115-123

Durham DA, Marlow CB (2010) Aspen response to prescribed fire under managed cattle grazing and low elk densities in southwest Montana. Northwest Science 84:141-150

Floyd ME (1983) Dioecy in five *Pinus edulis* populations in the Southwestern United States. Am Midl Nat 110:405–411

Freeland WJ, Janzen DH (1974) Strategies in Herbivory by Mammals: The Role of Plant Secondary Compounds. Am Nat 108(961):269-289

Hagerman A, Butler L (1980) Condensed tannin purification and characterization of tannin-associated proteins. J Agric Food Chem 28:947–952

Hamon WR (1971) Reynolds Creek, Idaho. Agricultural Research Service Precipitation Facilities and Related Studies, U.S. Dept. Agric ARS 41-176 pp 117

Harniss RO, Harper KT (1982) Tree dynamics in seral and stable aspen stands of central Utah. INT-RP-297, USDA, Forest Service, Intermountain Forest and Range Experiment Station, Ogden, Utah

Harte J, Shaw R (1995) Shifting Dominance Within a Montane Vegetation Community: Results of a Climate-Warming Experiment. Science 267(5199):876-880

Hofmann RR (1988) Anatomy of the gastro-intestinal tract. In: Church DC (ed) The Ruminant Animal: Digestive Physiology and Nutrition. Prentice-Hall, New Jersey, pp 14-43

Hinde RA (1970) Animal Behaviour. A Synthesis of Ethology and Comparative Psychology. McGraw-Hill, New York

Holeski LM, McKenzie SC, Kruger EL, Couture JJ, Rubert-Nason K, Lindroth RL (2016) Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. J Ecol 104:850–863

Illius AW, Jessop NS (1996) Metabolic constraints on voluntary intake in ruminants. J

Anim Sci 74:3052–3062

Jelinski DE, Fisher LJ (1991) Spatial Variability in the Nutrient Composition of *Populus tremuloides*: Clone-to-Clone Differences and Implications for Cervids. Oecologia 88:116-124

Jenkins KJ, Wright RG (1987) Dietary niche relationships among cervids relative to winter snowpack in northwest Montana. Can J Zoo 65(6):1397-1401 DOI:10.1139/z87-220

Jones BE, Burton D, Tate KW (2005) Effectiveness monitoring of aspen regeneration on managed rangelands. R5-EM-TP-004, USDA, Forest Service, Pacific Southwest Region, Vallejo, California

Jones BE, Lile DF, Tate KW (2011) Cattle selection for aspen and meadow vegetation: implications for restoration. Range Ecol Manage 64:625–632

Kay CE (1997) Is aspen doomed? J Forest 95(5): 4–11

Latva-Karjanmaa T, Suvanto L, Leinonen K, Rita H (2006) Sexual Reproduction of European Aspen (*Populus tremula* L.) at Prescribed Burned Site: The Effects of Moisture Conditions. New For 31(3):545-558

Lindroth RL, Hwang SY (1996) Diversity, redundancy, and multiplicity in chemical defense systems of aspen. In: Romeo JT, Saunders JA, Barbosa P (eds) Phytochemical diversity and redundancy in ecological interactions. Plenum Press, New York, pp 25–56

Lindroth RL, St. Clair SB (2013) Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. For Ecol and Manage 299:14-21

Lindroth RL, Scriber JM, Hsia MTS (1988) Chemical ecology of the tiger swallowtail: mediation of host use by phenolic glycosides. Ecology 69:814–822

Lindroth RL, Kinney KK, Platz CL (1993) Responses of deciduous trees to elevated atmospheric CO2: productivity, phytochemistry, and insect performance. Ecology 74:763-777

MacFarlane AM, Coulson G (2007) Sexual segregation in western grey kangaroos: testing alternative evolutionary hypotheses. Journal of Zoology 273: 220–228.

Marsh KJ, Moore BD, Wallis IR, Foley WJ (2014) Feeding rates of a mammalian browser confirm the predictions of a ‘foodscape’ model of its habitat. Oecologia 174:873-882

Martens SN, Breshears DD, Meyer CW, Barnes FJ (1997) Scales of above- and below-ground competition in a semiarid woodland as detected from spatial pattern. J Veg Sci 8:655-664

Martens SN, Breshears DD, Meyer CW (2000) Spatial distributions of understory light along the grassland/forest continuum: effects of cover, height, and spatial pattern of tree canopies. Ecol Model 126:79-93

Meerveld HJT, McDonnell JJ (2005) On the interrelations between topography, soil depth, soil moisture, transpiration rates and species distribution at the hillslope scale. Adv Water Resour 29:293-310

Mould ED, Robbins CT (1981) Nitrogen metabolism in elk. J Wildl Manage 1:323-34

Myking T, Bøhler F, Austrheim G, Solberg EJ (2011) Life history strategies of aspen (*Populus tremula* L.) and browsing effects: a literature review. Forestry 84(1):61-71

NRC (1985) Nutrient requirements of sheep, 6th edn. National Academy Press, Washington

O'Brien M, Rogers PC, Mueller K, MacWhorter R, Rowley A, Hopkins B, Christensen B, Dremann P (2010) Guidelines for aspen restoration on the National Forests in Utah. Western Aspen Alliance, Utah State University, Logan, Utah

Oksanen JF, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, SzoecsE, Wagner H (2016a). Vegan: Community Ecology Package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan. Accessed 12/01/2016.

Oksanen JF, Blanchet G, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Henry M, Stevens H, SzoecsE, Wagner H (2016b) Vegan tutorial. https://cran.r-project.org/web/packages/vegan/vegan.pdf . Accessed 12/26/2016.

Owen-Smith N (1988) Megaherbivores. Cambridge University Press, Cambridge

Padien DJ, Lajtha K (1992) Plant spatial pattern and nutrient distribution in pinyon-juniper woodlands along an elevational gradient in northern New Mexico. Int J Plant Sci 153:425-433

Perez-Barberia FJ, Walker DM, Gordon IJ (2004) Sex differences in feeding behaviour at feeding station scale in Soay sheep (*Ovies aries*). Behaviour 141: 999–1020

Porter LJ, Hrstich LN, Chan BC (1986) The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25:223-230

Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. Rangeland Ecology and Management 48:2-17

Provenza FD (1996) Acquired aversions as the basis for varied diets of ruminants foraging on rangelands. J Anim Sci 74(8):2010-2020 doi:10.2527/1996.7482010x

Provenza FD, Villalba JJ, Bryant JP (2003) Foraging by herbivores: Linking the biochemical diversity of plants to herbivore culture and landscape diversity. In: Bissonette JA, Storch I (eds) Landscape Ecology and Resource Management. Linking Theory with Practice. Island Press, Washington, D.C. USA pp 387-421

Provenza FD, Villalba JJ (2006) Foraging in domestic herbivores: Linking the internal and external milieu. In: Bels VL (ed) Feeding in Domestic Vertebrates: From Structure to function. CABI Publ, Oxfordshire, UK pp 210-240

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/. Accessed 05/01/2016

Randriamanana TR, Labola A, Julkunen-Tiitto R (2015) Interactive effects of supplemental UV-B and temperature in European aspen seedlings: Implications of growth, leaf traits, phenolic defense and associated organisms. Plant Physiol Biochem 93:84-93 DOI: 10.1016/j.plaphy.2015.03.001

Rittenhouse LR, Sneva FA (1977) A technique for estimating big sagebrush production. J Range Manage 30:68-70

Rogers, PC, Landhäusser SM, Pinno BD, Ryel RJ. 2014. A Functional Framework for Improved Management of Western North American Aspen (*Populus tremuloides* Michx.). Forest Science 60:345-359

Rogers PC, Jones A, Catlin J, Shuler J, Morris A, Kuhns M (2015) Quaking Aspen in the Residential-Wildland Interface: Elk Herbivory Hinders Forest Conservation. Nat Area J 35(3):416-427 DOI: http://dx.doi.org/10.3375/043.035.0305

Rogers PC, Mittanck CM (2014) Herbivory strains resilience in drought-prone aspen landscapes of the western United States. J Veg Sci 25(2):457-469 DOI: 10.1111/jvs.12099

Romme WH, Turner MG, Wallace LL, Walker JS (1995) Aspen, Elk, and Fire in Northern Yellowstone Park. Ecology 76(7):2097-2106

RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/. Accessed 04/01/2016

Runyon MJ, Tyers DB, Sowell BF, Gower CN (2014) Aspen Restoration Using Beaver on the Northern Yellowstone Winter Range under Reduced Ungulate Herbivory. Restor Ecol 22(4):555-561 DOI: 10.1111/rec.12105

Sarkar D (2008) Lattice: Multivariate Data Visualization with R. Springer, New York. ISBN 978-0-387-75968-5

Searle KR, Hobbs NT, Gordon IJ (2007) It's the "Foodscape", not the landscape: Using foraging behavior to make functional assessments of landscape condition. Isr J Ecol Evol 53:297-316

Senft RL, Coughenour MB, Bailey DW, Rittenhouse LR, Sala OE, Swift DM (1987) Large herbivore foraging and ecological hierarchies. BioScience 37:789-799

Shaw RA, Villalba JJ, Provenza FD (2006) Influence of stock density and rate and temporal patterns of forage allocation on the diet mixing behavior of sheep grazing sagebrush steppe. Appl Anim Beh Sci 100(3-4):207-218 http://dx.doi.org/10.1016/j.applanim.2005.12.002

Shepperd WD (1990) A classification of quaking aspen in the central Rocky Mountains based on growth and stand characteristics. Western Journal of Applied Forestry 5:69-75

Smith MS (1988) Modelling: three approaches to predicting how herbivore impact is distributed in rangelands. New Mexico State Univ. Agr Exp Sta Res Rep pp 628

St. Clair SB, Rogers PC, Kuhns MR (2015) Managing Ungulate Browsing for Sustainable Aspen. Western Aspen Alliance Briefs #2. Aspen Bibliography. Paper 7313 http://digitalcommons.usu.edu/aspen_bib/7313; https://extension.usu.edu/rangelands/files/uploads/Aspen/StClair-Aspen_Herbivory.pdf

Steuer P, Südekum KH, Tütken T, Müller DW, Kaandorp J, Bucher M, Clauss M, Hummel J (2014) Does body mass convey a digestive advantage for large herbivores? Funct Ecol 28:1127-1134

Swift RW (1957) The caloric value of TDN. J Anim Sci 16:753-756

Taillon J, Cote SD (2007) Social rank and winter forage quality affect aggressiveness in white-tailed deer fawns. Animal Behaviour 74: 265–275.

Turner MG, Romme WH, Reed RA, Tuskan GA (2003) Post-fire aspen seedling recruitment across the Yellowstone (USA) Landscape. Landscape Ecology 18:127-140

Uresk DW, Gilbert RO, Rickard WH (1977) Sampling big sagebrush for phytomass. J Range Manage 30:311-314

Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583-3597

Villalba JJ, Burritt EA, St. Clair SB (2014) Aspen (*Populus tremuloides* Michx.) Intake and Preference by Mammalian Herbivores: the role of plant secondary compounds and nutritional context. J Chem Ecol 40(10):1135-45 DOI 10.1007/s10886-014-0507-0

Villalba JJ, Provenza FD (2005) Foraging in chemically diverse environments: Energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. J Chem Ecol 31(1):123-38

Wan HY, Rhodes AC, St. Clair SB (2014) Fire severity alters plant regeneration patterns and defense against herbivores in mixed aspen forests. Oikos 123(12):1479-1488. DOI: 10.1111/oik.01521

Weiss WP, Conrad HR, Pierre NRS (1992) A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim Feed Sci Technol 39:95-110

White CA, Feller MC, Bayley S (2003) Predation risk and the functional response of elk-aspen herbivory. Forest Ecol Manage 181:77-97

Wooley SC, Walker S, Vernon J, Lindroth RL (2008) Aspen Decline, Aspen Chemistry, and Elk Herbivory: Are They Linked? Rangelands 30(1):17-21

CHAPTER 5

SUMMARY AND FUTURE DIRECTIONS

Some scientists have reported that over the last 200 years, the land area covered by aspen has been declining in the Intermountain West, causing a reduction in biological diversity (Kay 1997), habitat for vascular plants (Chong et al. 2001), wildlife (DeByle 1985), insects (Jones et al. 1985; Chong et al. 2001), and water retention in watersheds (Mueggler 1989; Bartos and Campbell 1998; LaMalfa and Ryle 2008). Herbivory by both wild and domestic ungulates are significant causative forces for this decline that can possibly be controlled or modified through management (Kay 1997; White et al. 1998; Bartos and Campbell 1998; Bartos 2001).

Herbivores develop preferences for foods that offer the balance and concentrations of nutrients necessary for maintenance, growth, and reproduction (Provenza 1995; Provenza and Villalba 2006). Aspen offers more nutrients than slower growing trees or shrubs in the surrounding plant community (Cebrian and Duarte 1994; Cook 2002), particularly during late summer and early fall when other plant species in the understory begin to senesce (Bartos and Campbell 1998; Dockrill et al. 2004). Aspen herbivory appears to be a function of the nutrients available in aspen tissues relative to those present in the landscape (Holeski et al. 2016), and of plant defense chemicals which impose barriers to the abilities of insect (Holeski et al. 2009) and mammalian (Wooley et al. 2008) herbivores to utilize aspen trees. A lack of understanding regarding the interactive effects of defense chemicals and nutrients from aspen and the surrounding understory on aspen herbivory makes implementing management strategies aimed at abating this process difficult. My Dissertation was an attempt to fill this knowledge gap by exploring some of these interactions using controlled pen feeding studies (Chapters 2 and 3) and during an exploratory landscape experiment (Chapter 4).

My Dissertation shows that preference for or against aspen is a complex process emerging from interactions among different variables such as: supplemental nutrients and plant secondary compounds (Chapter 2), nutritional state (Chapter 3), foraging history (Chapter 3), defense chemistry in aspen (Chapters 2 and 3), and understory functional group and nutritional biomass (i.e., the foodscape; Chapter 4).

Sheep offered supplements or a basal diet high in protein were able to consume greater amounts of aspen leaves than those animals that received supplements low in protein, with plant secondary compounds (PSC; e.g., bark), or than sheep exposed to a low plane of protein nutrition (Chapters 2 and 3). These results are in agreement with models proposed by Illius and Jessop (1995; 1996) stating that protein aids in detoxification of PSC by mammalian herbivores. Ingesting appropriate amounts of protein increases the threshold of PSC tolerance, enabling lambs to ingest more PSC-containing foods like aspen leaves (Villalba and Provenza 2005).

Although the exact mechanisms of phenolic glycoside (PG) detoxification and metabolism is unknown, PG like other compounds such as terpenes, do require protein for detoxification (Boeckler et al. 2011). When protein is fed in addition to a terpene-containing food, intake of the terpene containing food is enhanced (Villalba et al. 2002; Villalba and Provenza 2005). Condensed tannins bind to proteins with great affinity, which reduces their bioavailability (Hagerman et al. 1992), and this process may also explain the positive effects of protein supplementation on consumption of tannin-containing aspen leaves, (i.e., inactivation of foliar condensed tannins by binding with protein in the rumen).

In addition to protein, PSC had an influence on aspen intake by sheep. Aspen intake was greater when aspen leaves contained low concentrations of phenolic glycosides (PG) (Chapters 2 and 3), in agreement with previous findings (Bailey et al. 2007; Wooley et al. 2008; Villalba et al. 2014). These responses appeared to occur in a dose-dependent manner as differences in intake between treatments were less pronounced when differences in defense chemistry between aspen stands were small (Chapter 3). In addition, when other forages were offered concurrently with aspen, aspen intake declined relative to when aspen was offered as a single feed (Chapter 2). This response was likely due to the ability of herbivores to balance different nutritional needs (Provenza 1995; Provenza et al. 2003) while reducing the negative post-ingestive impacts of PSC-containing forages. This mechanism was also suggested when animals had a choice between leaves from stands with high and low content of PG (Experiment 3 of Chapter 3). Thus, the availability of alternatives in the landscape has a significant impact on the capacity of herbivores to ingest chemically defended plant tissues like aspen leaves. During spring and summer, there is an abundance of alternative forages available, which may decrease preference for aspen (as reported in Chapter 2), but depending on the nutrient makeup and protein to energy ratios in the understory, aspen intake may also be enhanced (Chapter 2 and 3). For instance, lambs consuming rations with low energy content display a much greater capacity to consume aspen than lambs fed rations of greater energy density (Chapter 3).

It has been suggested that aspen use is a consequence of the greater concentration of nutrients in aspen suckers relative to the available understory and surrounding plant community (Jones et al. 2005; Beck et al. 2006). Findings from this Dissertation bring a deeper understanding of the relationship between the nutritional composition of the understory and the chemical composition of aspen leaves; intake does not only depend on nutrients but also on the types and ratios of nutrients present in the surrounding plant community. High protein to energy ratios in the understory enhance aspen intake regardless of the concentration of PSC in aspen tissues (Chapter 3). However, if the understory contains adequate or excessive concentrations of energy (i.e., those that meet or exceed the animal's requirements), the herbivore may be less likely to consume aspen, especially if those aspen stands contain high concentration of chemical defenses (i.e., ≥ 15% of PG).

My results also suggest that in addition to protein or energy, preference for aspen depends on the mineral content of aspen leaves and of recently consumed forages, and that aspen defense chemicals may not always be the main determinant of preference by ungulates–meaning that preference is multidimensional (Chapter 3). These results are in agreement with recent findings on aspen preference by wild ungulates foraging in diverse landscapes (Holeski et al. 2016). In Experiment 3 of Chapter 3, lambs selected greater amounts of leaves collected from aspen stands containing high concentrations of PG than of leaves with lower concentrations of PG on most days during testing. This highlights the multidimensional nature of aspen preference by herbivores and the weak role that chemical defenses may play on this variable under circumstances when concentrations of PG in different aspen stands are in the range of 15 to 20%. Nevertheless, the basal diet of animals described in Chapter 3 was high in protein (e.g., alfalfa pellets), suggesting that the lambs' ability to detoxify aspen PSC was enhanced, likely minimizing the negative post-ingestive effects incurred by ingesting greater proportions of aspen leaves with greater concentration of defenses. The protein intake in this experiment was above amounts that animals may have available in the wild at the end of summer, when the nutritional quality of the understory declines. Therefore, during this time of the year, wild and domestic herbivores may not be able to detoxify PSC as effectively as those animals studied earlier in Chapter 3 with diets of high protein content (Lindroth and St.Clair 2013; see Experiment 1 of Chapter 3). Thus, under different environmental conditions and constraints (e.g., a diet low in protein, a broader range of concentration of PG across stands), chemical defenses may be more relevant than other variables (i.e., minerals) at underlying aspen preference by ungulates.

Experiment 3 of Chapter 3 was designed so that two groups of lambs received the same amount of exposure to aspen stands with either high or low concentration of PG. Under these conditions, a majority of the lambs still preferred aspen leaves with high concentrations of PG. Therefore, reasons other than prior experience were driving the choice of high-PG aspen leaves. I found that nitrogen and mineral content in aspen leaves, as well as the basal diet lambs received during exposure to aspen, were more consequential than prior experience at modifying preference for aspen stands with contrasting concentrations of chemical defenses (Experiment 3 of Chapter 3). Additionally, prior experience with a high protein food increased aspen intake by lambs that subsequently received a low energy ration, most likely because the beneficial effects of protein on detoxification carried over from the previous diet (Chapter 3).

Findings in Chapter 4 suggest that on the landscape, elk presence was negatively correlated with understory crude protein biomass, which is in line with prior studies that reported a negative relationship between nitrogen content and browse preference by deer (Berteaux et al. 1998; Holeski et al. 2016). In turn, aspen regeneration was greater in high-elevation stands with understories comprised of high crude protein biomass. These findings appear to be at odds with results from my previous chapters suggesting that protein enhances aspen intake, potentially accelerating the dieback of aspen communities (e.g., Chapters 2 and 3). However, during those studies, sheep did not have a choice among a wide variety of forage alternatives as they only received aspen leaves and a nitrogen-rich feed during each day. In contrast, mature elk with low nitrogen requirements foraging in a diverse landscape had multiple forage alternatives available, and they could have selected plants and feeding sites with lower nitrogen content than the nitrogen-rich feeds offered in Chapters 2 and 3. Thus, the availability of choices in the landscape may lead to different foraging responses by ungulates relative to when no alternatives are present or when alternatives are limited. For instance, when protein-rich resources are limited in the landscape (e.g., forbs) and animals need to devote more time and effort to gathering small quantities of such forages, the process could be interpreted as “nitrogen supplementation” given that animals ingest restricted amounts of a high-quality feed. Under these conditions, animals may need to increase intake of aspen–with protein aiding in this process–in order to make up for the bulk of their diet. In contrast, when high-quality forages are abundant and highly accessible and energy requirements are met by these forages, herbivores may reduce their preference for aspen trees and spread the foraging load more evenly across all forages in the landscape, as shown in Chapter 2 when lambs ate less aspen when presented in a choice with Utah pea and smooth bromegrass.

My research also shows that at lower elevations, where conditions are drier (Hamon 1971; Meerveld and McDonnell 2005) and forb and grass understory biomass is low, the nutrients available to elk are limited. As stated above, lack of energy in the diets of ungulates increases aspen intake (Chapter 3). Additionally, because low elevations are drier (Hamon 1971; Meerveld and McDonnell 2005) and aspen thrive better in moist environments (Latva-Karjanmaa et al. 2006; Turner et al. 2003), aspen at lower elevations under drier conditions are already stressed and more likely to succumb to browsing pressure. As the climate continues to warm, aspen at low elevations will likely continue to become increasingly more stressed and more prone to herbivory. Novel management approaches for enhancing aspen condition may entail improving the nutrient content of the foodscape at these lower elevation sites. Results from Chapter 3 suggest that sheep grazing an understory with high protein to energy ratios are more prone to consuming greater amounts of aspen than those animals grazing understories of greater energy and lower protein content. Integrating this information with Chapter 4, it appears that the best strategy to enhance the forage quality of the foodscape would involve amending the understory at lower elevations with forages or feeds with high energy/protein ratios.

Future research needs to be conducted on the landscape using supplements, introduced forages, or food plots to determine whether the antagonistic and complementary relationships observed in this Dissertation between nutrients and aspen apply to more complex environments at larger scales. New science and management should consider whether foraging distribution and aspen preference by wild and domestic ungulates could be modified through such interventions, which delivery method(s) and timing are more efficient, and at what distance from aspen stands new food alternatives need to be placed for maximum effect. For instance, questions to be asked include: How many supplementary feed stations relative to the number of animals present in a certain landscape are needed in order to reduce aspen herbivory? How large do these sites need to be, or how much feed/forages need to be placed in each station relative to the stand size? What is the ideal food to be offered relative to the nutritional quality of the foodscape? Will wildlife accept feeding stations with supplements as willingly as forages because of their heightened neophobia (Mangus 2011)? What is the best type and spatial distribution of introduced forages (e.g., how far from aspen stands) to be offered in order to influence patch selection with the aim of ameliorating aspen use by ungulates? Does placement of food on the landscape affect growth or establishment of other flora or fauna? When and for how long should the food be distributed or (re)seeded? How often should feeders be checked and refilled? What is the benefit of patch burning (i.e., increasing the nutritional quality of the foodscape) at altering herbivores' feed and foraging location preferences away from aspen stands? What is the most cost-effective solution in various locales with various intensities of herbivory? Concerns include the proposed stations acting as bait stations during hunting season, logistics of placing and checking on feeder stations as necessary, and costs associated with initial assessment and upkeep. Identifying biomass and the nutritional characteristics of the understory across a gradient of aspen regeneration (as described in Chapter 4), elk density, stand elevation, and climate may aid both private and public land managers to identify at-risk areas and to implement management tactics at those sites.

References

Bailey JK, Schweitzer JA, Rehill BJ, Irschick DJ, Whitham TG, Lindroth RL (2007) Rapid shifts in the chemical composition of aspen forests: an introduced herbivore as an agent of natural selection. Biol Invasions 9:715–722

Bartos DL (2001) Landscape dynamics of aspen and conifer forests. In: Shepperd WD, Binkley D, Bartos DL, Stohlgren TJ (eds) Sustaining aspen in Western landscapes: symposium proceedings; 2000 June 13-15; Grand Junction, CO. Proceedings RMRS-P-18. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp 5-14

Bartos DL, Campbell RB Jr (1998) Decline of quaking aspen in the interior West-examples from Utah. Rangelands 20(1):17-24

Beck JL, Peek JM, Strand EK (2006) Estimates of elk summer range nutritional carrying capacity constrained by probabilities of habitat selection. J Wildl Manage 70(1):283-294

Berteaux D, Crete M, Huot J, Maltais J, Ouellet JP (1998) Food choice by white- tailed deer in relation to protein and energy content of the diet: a field experiment. Oecologia 115:84-92

Boeckler GA, Gershenzon J, Unsicker SB (2011) Phenolic glycosides of the Salicaceae and their role as anti-herbivore defenses. Phytochemistry 72:1497-1509

Cebrian J, Duarte CM (1994) The dependence of herbivory on growth rate in natural plant communities. Func Ecol 8:518–525

Chong GW, Simonson SE, Stohlgren TJ, Kalkhan MA (2001) Biodiversity: Aspen stands have the lead, but will nonnative species take over? In: Shepperd WD, Binkley D, Bartos DL, Stohlgren TJ, Eskey LG (Compilers) Symposium Proceedings RMRS-P-18 on Sustaining Aspen in Western Landscapes, Grand Junction, CO, 13–15 June 2000. USDA, Forest Service, Rocky Mountain Research Station, Fort Collins, CO, pp 261–271

Cook JG (2002) Nutrition and food. In: Toweill DE, Thomas JW (eds) North American Elk: Ecology and Management, Stackpole Books, Harrisburg, PA, pp 259–349

DeByle NV (1985) Wildlife. In: DeByle NV, Winokur RP (eds) Aspen: Ecology and Management in the Western United States. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp. 135–152, GTR-RM-119.

Dockrill CWM, Blenis PV, Bailey AW, King JR (2004) Effect of summer cattle grazing on aspen stem injury, mortality and growth. The Forestry Chronicle 80: 257-261

Hagerman AE, Robbins CT, Weerasuriya Y, Wilson TC, McArthur C (1992) Tannin chemistry in relation to digestion. J Range Manage 45:57–62

Hamon WR (1971) Reynolds Creek, Idaho. Agricultural Research Service Precipitation Facilities and Related Studies, US Dept Agric ARS 41-176, pp 117

Holeski LM, McKenzie SC, Kruger EL, Couture JJ, Rubert-Nason K, Lindroth RL (2016) Phytochemical traits underlie genotypic variation in susceptibility of quaking aspen (*Populus tremuloides*) to browsing by a keystone forest ungulate. J Ecol 104:850-863

Holeski LM, Vogelzang A, Stanosz G, Lindroth RL (2009) Incidence of Venturia shoot blight in aspen (*Populus tremuloides* Michx.) varies with tree chemistry and genotype. Biochem Syst Ecol 37:139–145

Illius AW, Jessop NS (1995) Modeling metabolic costs of allelochemical ingestion by foraging herbivores. J Chem Ecol 21, 693–719

Illius AW, Jessop NS (1996) Metabolic constraints on voluntary intake in ruminants. J Anim Sci 74: 3052–3062

Jones JR, DeByle NV, Bowers DM (1985) Insects and other invertebrates. In: DeByle NV, Winokur RP (eds) Aspen: Ecology and Management in the Western United States. USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, Fort Collins, CO, pp 107–114, GTR-RM-119

Jones BE, Burton D, Tate KW (2005) Effectiveness monitoring of aspen regeneration on managed rangelands. R5-EM-TP-004, USDA, Forest Service, Pacific Southwest Region, Vallejo, CA

Kay CE (1997) Is aspen doomed? Journal of Forestry 95(5):4–11

LaMalfa E, Ryle R (2008) Differential Snowpack Accumulation and Water Dynamics in Aspen and Conifer Communities: Implications for Water Yield and Ecosystem Function. Ecosystems 11(4):569-581

Latva-Karjanmaa T, Suvanto L, Leinonen K, Rita H (2006) Sexual Reproduction of European Aspen (*Populus tremula* L.) at Prescribed Burned Site: The Effects of Moisture Conditions. New For 31(3):545-558

Lindroth RL, St. Clair SB (2013) Adaptations of quaking aspen (*Populus tremuloides* Michx.) for defense against herbivores. Forest Ecol Manage 299:14–21

Mangus D (2011) Reducing reliance on supplemental winter feeding in elk (*Cervus canadensis*): an applied management experiment at Deseret Land and Livestock

Ranch, Utah. Thesis, Utah State University, Logan, USA

Meerveld HJT, McDonnell JJ (2005) On the interrelations between topography, soil depth, soil moisture, transpiration rates and species distribution at the hillslope scale. Adv Water Resour 29:293-310

Mueggler WF (1989) Age distribution and reproduction of Intermountain aspen stands. West J Appl For 4:41-45

Provenza FD (1995) Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J Range Manage 48:2-17

Provenza FD, Villalba JJ (2006) Foraging in domestic herbivores: Linking the internal and external milieu. In: Bels VL (ed) Feeding in Domestic Vertebrates: From Structure to function. CABI Publ, Oxfordshire, UK, pp 210-240

Provenza FD, Villalba JJ, Bryant JP (2003) Foraging by herbivores: linking the biochemical diversity of plants to herbivore culture and landscape diversity. In: Bissonette JA, Storch I (eds) Landscape Ecology and Resource Management: Linking Theory with Practice, Island Press, New York pp 387-421

Turner MG, Romme WH, Reed RA, Tuskan GA (2003) Post-fire aspen seedling recruitment across the Yellowstone (USA) Landscape. Landscape Ecology 18:127-140

Villalba JJ, Provenza FD, Banner RE (2002) Influence of macronutrients and polyethylene glycol on intake of a quebracho tannin diet by sheep and goats. J Anim Sci 80:3154–3164

Villalba JJ, Burritt EA, St.Clair SB (2014) Aspen (*Populus tremuloides* Michx.) Intake and Preference by Mammalian Herbivores: the role of plant secondary compounds and nutritional context. J Chem Ecol DOI 10.1007/s10886-014-0507-0

Villalba JJ, Provenza FD (2005) Foraging in chemically diverse environments: Energy, protein, and alternative foods influence ingestion of plant secondary metabolites by lambs. J Chem Ecol 31(1):123-38

White CA, Kay CE, Feller MC (1998) Aspen forest communities: a key indicator of ecological integrity in the Rocky Mountains. International Conference on Science and the Management of Protected Areas 3:506–517

Wooley SC, Walker S, Vernon J, Lindroth RL (2008) Aspen Decline, Aspen Chemistry, and Elk Herbivory: Are They Linked? Rangelands 30(1):17-21

APPENDICES

APPENDIX - PERMISSION TO USE LETTERS

3/21/2017

Kristen Heroy
Utah State University
5210 Old Main Hill
Wildland Resources Department
Logan, UT 84322

Dear Beth Burritt:

I am in the process of preparing my (dissertation) in the Wildland Resources department at Utah State University. I hope to complete my degree program in Wildlife Ecology.

I am requesting your permission to include the Chapters 2 through 4: Nutrients and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep, Nutritional state and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep, and Dietary nutrients influence aspen (*Populus tremuloides*) preference by lambs (*Ovis aries*) when presented with a choice of aspen papers.

I will include acknowledgments and/or appropriate citations to your work as shown and copyright and reprint rights information in a special appendix. The bibliographic citation will appear at the end of the manuscript as shown. Please advise me of any changes you require.

Please indicate your approval of this request by signing in the space provided, attaching any other form or instruction necessary to confirm permission. If you have any questions, please don't hesitate to contact me at any time.

Thank you for you cooperation,
Kristen Heroy
702-675-5572

I hereby give permission to Kristen Heroy to use the following material in his/her thesis/dissertation:

Nutrients and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep, Nutritional state and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep, and Dietary nutrients influence aspen (*Populus tremuloides*) preference by lambs (*Ovis aries*) when presented with a choice of aspen

Signed: Beth Burritt

3/21/2017

Kristen Heroy
Utah State University
5210 Old Main Hill
Wildland Resources Department
Logan, UT 84322

Dear Susan Durham:

I am in the process of preparing my dissertation in the Wildland Resources department at Utah State University. I hope to complete my degree program in Wildlife Ecology.

I am requesting your permission to include the Chapter 4/ Dietary nutrients influence aspen (*Populus tremuloides*) preference by lambs (*Ovis aries*) when presented with a choice of aspen paper.

I will include acknowledgments and/or appropriate citations to your work as shown and copyright and reprint rights information in a special appendix. The bibliographic citation will appear at the end of the manuscript as shown. Please advise me of any changes you require.

Please indicate your approval of this request by signing in the space provided, attaching any other form or instruction necessary to confirm permission. If you have any questions, please don't hesitate to contact me at any time.

Thank you for you cooperation,

Kristen Heroy
702-675-5572

I hereby give permission to Kristen Heroy to use the following material in her dissertation:

Dietary nutrients influence aspen (*Populus tremuloides*) preference by lambs (*Ovis aries*) when presented with a choice of aspen

Signed: Susan Durham

CURRICULUM VITAE

Kristen Y. Heroy, Ph.D. Candidate

650 S 100 E #A202
Logan, UT 84321
www.researchgate.net/profile/Kristen_Heroy
kristen.heroy@aggiemail.usu.edu
Mobile: 702-675-5572

Education

Jan 2014 – May 2017 **Utah State University**
Doctor of Philosophy, Wildlife Ecology
Logan, United States

Jan 2010 – Dec 2013 **Angelo State University**
Master of Science, Animal Science
San Angelo, United States

Aug 2004 – May 2008 **University of Delaware**
Bachelor of Science, Animal Science
Newark, United States

Research Experience

Jan 2014 – present **Research Assistant**
Utah State University, Department of Wildland Resources
Logan, United States

Project focus: My current project identifies the nutritional and secondary chemical thresholds that drive preference for or against aspen (Populus tremuloides) using sheep in pen trials. Using information on preference gained from the pen trials coupled with primary and secondary chemistry of aspen leaves, nutrition of surrounding understory, and animal use via pellet counts I will create a spatio-temporal herbivory risk-map for aspen stands on a small-scale area near Kamas, UT in Wolf Creek Ranch.

Jan 2010 – Jan 2011 **Research Assistant**
Angelo State University, Department of Agriculture
San Angelo, United States

Project focus: Effects of preconditioning and protein supplementation on forages containing plant secondary compounds.

Jan 2008 – May 2008 **Undergraduate research assistant**
University of Delaware, Department of Animal and Food Sciences
Newark, United States

Project focus: Broiler house depopulation, comparing CO2, Ar, and foam.

Teaching Experience

Jan 2016-present **Teaching Assistant**
Introductory Biology Labs; Teaching 3 sections of 30 students each week
Utah State University, Logan, UT

Awards & Grants

Feb 2017 **Scholarship**
Scholarship: Elizabeth Bullock Harderlie Scholarship

Jun 2015 **Award**
USU Ecology Center Graduate Award, research funding

Jan 2014 **Assistantship**
USU Wildland Department

Skills & Activities

Skills/Areas of expertise	Animal Science, Natural Resource and Range Management, Ruminant Nutrition, Plant Secondary Metabolism, Aspen (Populus tremuloides), GIS
Languages	English; Beginner/intermediate French
Scientific Memberships	Utah Chapter of the Wildlife Society Society for Range Management
Interests	Clubs and volunteer experience: Utah State University Wildlife Society Club, 2015-2017 Eco-lunch coordinator, Utah State University, 2016-2017 Wildland Department Seminar committee member, 2016-2017 LGBT student mentor 2015-present

Toastmasters International, 2016-present
Member of the American Brittany Club, volunteer at hunt tests and
field trials, 2013-present
Volunteer with National Brittany Rescue, 2014-present
Member of NRA, 2010-present

Hobbies:
Snowboarding
Hiking
Shooting- Target, trap, skeet
Archery
Fly fishing
Drawing

Conference Proceedings

Kristen Y. Heroy, Beth A. Burritt, Samuel B. St. Clair, Juan J. Villalba, Nutritional state influences trembling aspen (*Populus tremuloides)* intake by sheep. Annual Society for Range Management, St. George, UT, 2/2017

Kristen Y. Heroy, Beth A. Burritt, Samuel B. St. Clair, Juan J. Villalba. Nutrients and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep. Annual Society for Range Management, Corpus Christi, TX; 2/2016.

Kristen Y. Heroy, Juan J. Villalba: *Presentation: Aspen: a young life nipped in the bud*. Front Range Ecology Symposium, Colorado State University; 02/2015

Publications

Heroy KY, St. Clair SB, Burritt BA, Villalba JJ. 2017. Nutrients and secondary compounds influence trembling aspen (*Populus tremuloides*) intake by sheep. J Chem Ecol, *Submitted*.

Heroy KY, St. Clair SB, Rogers PC, Villalba JJ. 2017. The influence of the foodscape on quaking aspen use by ungulates and stand condition. Oecologia, *Submitted*.
