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Variable Palatability of Quaking Aspen for Large Ungulate Herbivores

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Variable Palatability of Quaking Aspen (*Populus tremuloides*) for Large Ungulate Herbivores

Submitted by

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A thesis submitted to the faculty of
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Master of Science

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ABSTRACT

Variable Palatability of Quaking Aspen (*Populus tremuloides*) for Large Ungulate Herbivores

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Master of Science

Aspen is a key resource in the Rocky Mountain Region for wildlife forage and habitat, lumber products, scenery, and plays important roles in fire ecology and hydrological processes. There is evidence of aspen decline over much of the Intermountain West for approximately 100 years. In Dixie and Fishlake National Forests, UT, aspen distribution has decreased by nearly half. Causes of this decline are not well understood, although wildlife browsing by ungulates has been implicated as playing a major role. The objective of this research was to examine what soil or plant factors might be involved in wildlife browse choice in aspen. Twenty-two pairs of moderately and intensively browsed sites were studied to identify factors related to browse preferences over two field seasons. In the summer of 2008, sites were sampled in June, July, and August, and in the summer of 2009 sites were sampled in August only. Soils were analyzed for pH, EC, total nitrogen and carbon, and mineral nutrients. Leaf tissue samples were analyzed for defense chemical (tannin and phenolic glycoside) concentrations, mineral nutrients (via acid digestion), acid-detergent fiber, water content, carbon:nitrogen ratio, and non-structural carbohydrate (sugar) concentration. No significant difference in phenolic glycoside concentrations between moderately and intensively browsed sites was found. Tannins were highest in sites with intensive levels of browsing. Iron was significantly higher and zinc lower in intensively than moderately browsed sites. Leaf moisture was also significantly lower in intensively browsed sites. In the absence of differences in phenolic glycosides, ungulates may be selecting browse sites based on iron requirements. Seasonal changes in the studied factors could be identified in 2008. Over the course of the summer, we found significant decreases in nitrogen, phosphorus, potassium, sulfur, zinc, iron, copper, phenolic glycosides, and moisture concentration. Seasonal increases in calcium, sodium, tannins, sugars, acid-detergent fiber, and carbon:nitrogen ratios were observed. The need for large ungulates to obtain specific nutrientsr may indicate that aspen is in higher demand as a forage at different times of the year, particularly in areas with forages low in these nutrients. Our data suggest that aspen high in iron may be at risk since other factors explaining browsing choice were not significantly different in our study. This information can help identify clones that are at risk and direct resources where and when they are needed most.

Keywords: aspen, aspen decline, browse pressure, defense chemistry, foraging, ungulates, nutrients, *Populus tremuloides*, Rocky Mountains, Utah

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RH: Nielson et al.• Seasonal Variation in Aspen Palatability I

Variable Palatability of Aspen for Large Ungulates Part I: Moisture, Fiber, Carbon:Nitrogen Ratio, and Defense Chemicals

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ABSTRACT This is the first in a two-part series on aspen palatability. Aspen is a key resource in the Rocky Mountain Region for wildlife forage and habitat, lumber products, and scenery. Aspen plays an important role in fire and hydrological processes. Aspen has been in decline over much of the west for approximately 100 years. The causes for this decline are not well understood, although wildlife browsing by ungulates has been implicated as playing a major role. We studied 22 pairs of intensively and moderately browsed sites to identify factors related to browse preferences in August 2008 and 2009 in an effort to identify or eliminate factors that are resulting in ungulates choosing to forage on one aspen clone while largely ignoring another in very close proximity. In addition, sites were sampled in June and July during 2008 to document seasonal changes in defense compounds and indicators of digestibility. We analyzed leaf tissue samples for tannins and phenolic glycosides (PGs) (defense compounds), and protein, fiber, sugar, moisture, and carbon (C). Carbon concentrations, and the C:N ratio were statistically, but not practically, higher and moisture and fiber were lower in sites with intensive browsing. Thus, it is unlikely that these differences play a role in impacting browsing preference for large ungulates since neither a high C:N ratio nor high tannins would lead to increased palatability. These leaf chemistry differences are likely an effect (stress response) of intensive browsing rather than being a factor of ungulates preferentially choosing one aspen clone over another as a forage source. Sugar and PGs were not different as a function of browse pressure. In addition, we found that tannin, fiber, C and C:N ratios increased over the season, while protein, PGs and moisture decreased. Sugar concentration remained unchanged. The findings of this study eliminate several potential factors that might drive preferential browsing by ungulates.

KEY WORDS aspen, aspen decline, browse pressure, defense chemistry, foraging, ungulates, nutrients, *Populus tremuloides*, Rocky Mountains, Utah.

Aspen plays integral roles in high plateau and mountain plant communities of the Intermountain West. Aspen is an important forage source for large ungulates as well as small mammals that eat regenerating aspen suckers and the chlorophyll-producing bark during winter months (Jones and DeByle, 1985 *b*; DeByle, 1985). Aspen's wind-blocking abilities make it an important source of cover for wildlife (Jones and DeByle 1985 *a*). The lush undergrowth provides shelter for ground-nesting birds and small mammals as well as forage for small and large species (Jones and DeByle, 1985 *a*, DeByle 1985, Costello 1944). Aspen also have beautiful colors in fall and stimulate local economies as people travel the mountain highways, hiking trails, and off-roading trails to see foliage. Aspen is also a source of wood products (Koepke 1976, Mackes and Lynch 2001).

Aspen reproduce both sexually and asexually. For seedlings to successfully establish from sexual reproduction, prolonged cool, moist conditions are needed. These conditions are relatively rare in most of the Western U.S., so aspen rely heavily on asexual reproduction in the form of sprouts from the roots (Barnes 1966, MacDonough 1985, Mitton and Grant 1996). Gradually, shade-tolerant species replace aspen in most areas unless there is a large disturbance, such as an avalanche or forest fire (St.Clair et al. 2010). Aspen is renewed and quickly revegetates an area immediately following fire burns (Jones and DeByle 1985 *c*).

Unfortunately, this important resource is declining in much of the western United States. Aspen decline has been reported from Canada south through Arizona and New Mexico and has been identified as an issue of “top concern” in the Western U.S. (Hogg et al. 2008, Bartos and Campbell 1998, St.Clair et al. 2010). Many factors have been implicated in driving aspen decline. These include disease, fire suppression, and climate change, and wildlife browsing (St.Clair et al. 2010). Even when fires burn through an aspen stand, wildlife browsing may inhibit regrowth of aspen (Halofsky et al., 2008). In areas where aspen is established, over-browsing from wildlife can heavily damage aspen stands and prevent proper replenishment when older trees in the canopy die (Kay 1997, Kay and Bartos 2000). Aspen provide relatively greater forage than conifers and, therefore, are preferred by ungulates (Canon et al. 1987, Kay 1997). Ungulate populations, particularly elk, have increased dramatically compared to historic numbers (Halofsky et al. 2008, Ripple and Bestcha 2004). As aspen stands decline for a variety of possible reasons and ungulate populations increase, it is reasonable to expect browsing pressure to increase proportionally. Eventually, this increased browsing pressure becomes so great that a “tipping point” is reached, where browsing exceeds regeneration and clones eventually die off (Canon et al. 1987, Kay 1997).

Aspen have relatively high within-species genetic diversity and many nutritional and morphological characteristics of aspen are genetically controlled (Lindroth and Hwang 1996, Madritch et al. 2006, Donaldson and Lindroth 2007, Lindroth et al. 2007). Levels of the two main defense chemicals in aspen (phenolic glycosides and condensed tannins) are mainly under genetic control, although condensed tannins are also influenced by environmental factors (Osier and Lindroth 2001, Stevens et al. 2007). Tannins reduce digestibility by binding digestive proteins, while phenolic glycosides are toxic and have bitter taste (Hagerman and Butler 1980, Wooley et al. 2008). Because of asexual reproduction, patches of genetically identical trees (ramets) develop that are connected by a common root system and are genetically distinct from nearby clones. This creates a situation of differential browsing opportunities within this species.

Preferential browsing has been observed among elk (*Cervus canadensis),* moose *(Alces alces),* and deer (*Odocoileus* spp.), which will select certain forages or mineral sources in a greater proportion than what is present in the habitat (Hill 1946, Belovsky 1978, Alldredge et al. 2002, Ceacero et al. 2010 *a*, *b*). However, not much is known about what drives preferential browsing within the forage species.

Seasonal changes in browse quality can also influence herbivore foraging patterns. Typically, the nutritional value in aspen communities remains high until the end of September, where the forage quality of many plant species declines at the end of summer to early September (Franzmann et al. 1976, Alldredge et al. 2002). Study of seasonal palatability changes and nutrient value of aspen may alert managers in areas of high-risk aspen to which clones are most susceptible to overbrowsing and provide a key to protecting declining stands.

The purpose of this study was to examine factors of palatability that may contribute to preferential browsing that is observed in Fishlake National Forest, UT, USA and to identify seasonal changes in those factors.

STUDY AREA

Fishlake National Forest occupies approximately 6,000 km^2 in South-central Utah. The study sites were located in the Tidwell Slopes area of the Fremont River Ranger District, Sevier County, UT, USA, with elevations between 2,000 and 3,000 meters. Sites were between 38.630 and 38.760° N latitude and 111.570 and 111.480 W longitude. This area of Fishlake National Forest contained aspen associated with Douglas fir (*Pseudotsuga menziesii*), pine (*Pinus* spp.), white fir (*Abies concolor*), and subalpine fir (*Abies lasciocarpa*) (O'Brien and Waters 1998), with adjacent sagebrush (*Artemisia tridentata*) and mixed grass and forb communities (Campbell and O'Brien 2002).

Precipitation varies from 20 to 100 cm annually (Campbell and O'Brien 2002), with about two-thirds of precipitation from winter snowfall, and the rest from summer thunderstorms (Stam et al. 2008). During the growing season (mid-May through late August), temperatures average 27ºC during the day and 4ºC at night (Alexander 1965). In winter, daytime temperatures drop to as low as -30ºC with heavy snowpack from November through May (Alexander 1965). Glacial moraine, landslide, and alluvial deposits, as well as old lava flows can be found in the region (Alexander 1965). Soils tended to be acidic loams with prevalence of stones. Salts are not a problem in higher plateaus in this area, and there is good drainage through intermittent streams draining into the Sevier River.

There were large, generally healthy populations of elk and mule deer (*Odocoileus. hemionus*) that browse aspen. In addition, domesticated livestock also browse aspen and were present in large numbers during the growing season as a function of permitted grazing. Other prominent mammal species which may feed on and interact with aspen ecosystems included: moose, deer mice (*Peromyscus* spp.), shrew (*Sorex* spp.), squirrel (*Spermophilus* spp.), northern pocket gopher (*Thomomys talpoides*), snowshoe hare (*Lepus americanus*), marmot (*Marmota flaviventer*), chipmunk (*Tamias* spp.), skunk (*Mephitis mephitis*), porcupine (*Erethizon dorsatum*), badger (*Taxidea taxus*), bobcat (*Lynx rufus*), cougar (*Puma concolor*), coyote (*Canis latrans*), red fox (*Vulpes vulpes*), and black bear (*Ursus americanus*). This area is also home to eagles (*Aquila chrysaetos*, *Haliaeetus leucocephalus*), other predatory birds, and a wide variety of song, migratory, and other birds.

METHODS

Clonal Pair Identification

In the Tidwell slopes area of Fishlake National Forest, we observed several instances of differentially browsed aspen clones found immediately adjacent to each other appearing to have nearly identical environmental and soil conditions, creating natural pairs of differentially browsed study sites. Each pair consisted of an intensely browsed clone and a separate moderately browsed clone within 50-100 m of one another and with similar elevation, slope, aspect, and soil type. We identified a clone as "intensely browsed" if it: 1) had > 90% of the estimated leaf area above a browse height of 2 m and 2) showed physical evidence of browsing on > 90% of ramets below the 2 m browse height. We identified a clone as "moderately browsed" if it: 1) had < 50% of the estimated leaf area above a browse height of 2 m and 2) showed physical evidence of browsing on less than 20% of ramets below the 2 m browse height. We studied twenty-two such pairs in a multiple measurement paired t-test experimental design.

Field Data Collection

In the summer of 2008, we took field measurements and collected samples from 12 site pairs during August 5-7. In the summer of 2009, we collected similar data and samples from 10 different site-pairs during August 21-22. We measured slope, aspect, elevation, and latitude/longitude coordinates for each site and statistically evaluated each to verify that there was not a significant difference between intensely vs. moderately browsed sites for slope (P = 0.585), aspect (P = 0.910), and elevation (P = 0.899).

In addition to our initial visual estimations of browsing pressure that we used to identify appropriate sites, we confirmed these visual assessments by estimating browse pressure at each site during each visit by calculating the number of twigs browsed in each stand. We did this by randomly selecting five ramets of two meters or less in height and counting the number of twigs browsed, total twigs available for browsing, and converting to a percentage of twigs browsed. Percent browse difference averaged 95% (range of 20 to 150%) higher in intensely browsed than in moderately browsed sites. We also estimated density of each clone by the wandering quarter method (Catana 1963). The average density of intensely browsed sites (little regeneration) was 2,770 trees ha^{-1} and the average density of moderately browsed sites (normal regeneration) was 15,873 trees ha^{-1}. These measurements included all age classes of aspen in the stands.

We collected leaf tissue samples by clipping five leaves from 20 randomly selected ramets per stand (Donaldson et al. 2006, Erwin et al. 2001) between ground level and two meters height (approximate ungulate browsing height). We clipped the top 1 cm of each leaf into one bag to be freeze-dried for defense chemical analysis, and we put the rest of the leaf into a larger bag for other analyses. In this way, we obtained two sets of samples that were representative of the whole clone. We determined initial mass of leaf samples by placing samples on a balance. These measurements were recorded to determine gravimetric moisture concentration later in the laboratory. We kept all leaf samples on ice during transport and short-term storage.

Genetic Analysis

We performed a genetic analysis using microsatellite (SSR) markers on leaf samples to show that adjacent clones were indeed separate individuals. We extracted DNA using the method of Sambrook et al. (1989), with modifications from Todd and Vodkin (1996). We analyzed DNA samples for purity and quantified samples with a NanoDrop ND-1000 spectrophotometer (NanoDrop, Wilmington, DE, USA). We then diluted samples with double deionized water to approximately 0.02-0.05 g L^{-1}.

We amplified six simple sequence repeat (SSR) loci using polymerase chain reactions (PCR) on a DNA Engine Dyad Peltier Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA). The six SSR markers we used (WPMS15, WPMS14, WPMS20, GCPM970-1, PMGC2571, and PMGC433) were previously developed by Smulders et al. (2001) and Mock et al. (2008). Each 10.0 µL reaction well contained 7.76 µL dd H_2O, 1µL 10x PCR buffer with $MgCl_2$ (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.4 µL dNTPs (2mM), 0.1 µL primers, 0.14 µL*Taq* Polymerase (Sigma-Aldrich, Inc., St. Louis, MO, USA), 0.1 µl (0.01 µM) IRD700 or IRD800 Dye-labeled M13 primer (CACGACGTTGTAAAACGAC) (Biomers.net, Ulm, Germany), and 0.5 µL template DNA. Reactions were heated in a thermocycler to 92º C for 5 min; subjected to nine cycles of 92º C for 15s, 59º C for 15s (dropping by 1º C each cycle to 50º C) and 72º C for 30s; and then subjected to 20 cycles of 92º C for 15s, 50º C for 15s, and 72º C for 30s; with a final extension stage at 72º C for 3 min (Cole, 2005). We viewed PCR products on a Li-Cor 4300 DNA Analyzer (Li-Cor, Lincoln, NE, USA) using M13 tailed primers on a 6.5% polyacrylamide gel according to Oetting et al. (1995).

Phytochemical Analysis

We freeze-dried the leaves previously set aside for defense chemical analysis overnight and ground samples using a mixer mill (Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA). We stored freeze-dried samples at -80°C until analysis was conducted.

We extracted condensed tannins with an acetone-ascorbic acid solution according to the method described by St. Clair et al. (2009). We determined condensed tannin concentrations with the Butanol-HCl method (Porter et al. 1986), obtaining readings with a spectrophotometer (SpectraMax Plus 384, MDS, Toronto, Canada). We created a standard curve from purified condensed tannin standard isolated from aspen leaves (Hagerman and Butler 1980).

We extracted phenolic glycosides from leaf samples using the methanol extraction described by St. Clair et al. (2009). We quantified phenolic glycoside concentrations using high performance liquid chromatography (HPLC, Agilent 1100 Series, Santa Clara, CA, USA) with 1ml min^{-1} flow rate and a Luna 2, C18 column (150 × 4.6 mm, 5.0 µm). We detected desired compound peaks with a UV lamp at 280 nm wavelength and compared results with a standard curve of purified salicortin and tremulacin (phenolic glycosides) standards isolated from aspen leaves (Lindroth et al. 1993, St. Clair et al. 2009).

We oven-dried the remaining leaves at 65° C and determined the mass to find gravimetric moisture concentration using the formula ((fresh weight − dry weight)/fresh weight)*100. We determined acid-detergent fiber (ADF) concentrations by the method described by Vogel et al. (1999), using an Ankom fiber analyzer for refluxing (Ankom Technology, Macedon, NY). We determined N and C concentration via combustion method using a nitrogen analyzer (TrueSpec CN, Leco, St. Joseph, MI, USA) and then calculated the C:N ratio. We estimated the protein concentration based on the assumption that the proteins were an average of 16% N (Tew 1970). We evaluated total non-structural carbohydrates (sugars) using the method described by DaSilveira et al. (1978), a calorimetric procedure with an amylase enzyme digestion.

Statistical Analysis

A mixed models analysis of variance (blocking by paired sites) was performed on all data with a *P* value criteria of 0.05 being used to determine significance. Analyses were performed using SAS computational analysis software 9.2 (SAS Institute, Cary, NC).

RESULTS

Genetics

Although in close proximity to one another, each sampled pair of aspen stands were isolated from each other and visually appeared to be distinct clones and, therefore, not considered to be at risk for being genetically identical. Nevertheless, we conducted DNA analysis to confirm that pairs were distinct from each other. The six sets of primers showed that sites within each of the pairs were genetically distinct, with the exception of three pairs that were inconclusive due to desiccation during DNA analysis. These three pairs were included in the analyses discussed below based on their unique visual characteristics and distinct geographical locations indicating that they were almost certainly different clones.

Phytochemical Analysis

Tannin concentration was more than twice as high in intensely browsed sites as compared to sites with only moderate browsing pressure (Table 1). Although the experimental design was not set up to evaluate the effect of year and the sites were analyzed independent of year, note that the tannin concentrations were much higher in 2009 than 2008 (average 6.25% and 1.33%, respectively). However, the magnitude of the difference between intensely and moderately browsed sites was similar in both years. Leaf tannin concentration was also significantly impacted on a seasonal basis (Table 2). When averaged across paired sites, leaf tannin concentration almost doubled in concentration from June to July and then dropped significantly in August, although the end of summer tannin concentration was still significantly greater than the concentration in June (Table 2 and Fig. 1).Phenolic glycoside concentration was not related to browsing intensity (Table 1). However, seasonal changes of phenolic glycoside were highly significant (Table 2) with concentrations unchanged from June to July and dropping significantly in August (Fig. 1).

Leaf moisture concentration was significantly less (4%) in intensively browsed sites compared to moderately browse sites (Table 1) and dropped significantly from June through August, although less dramatically than the other digestibility factors (Fig. 2). Protein concentration was higher (7%) in moderately browsed sites (Table 1). Seasonally, protein levels were highest in June, dropped 33% in July and remained constant in August (Table 2, Fig. 1). Carbon (C) concentration remained relatively similar throughout the season (Table 2), but the N concentration varied significantly with time (data not shown, Nielson et al., 2011). Therefore, the C:N ratio increased dramatically in July and then leveled off (Table 2, Fig. 2). The seasonal change in C concentration was statistically but not biologically, significant. When comparing intensely and moderately browsed sites, C:N ratio also was significantly, but not biologically, higher in intensely browsed sites (Table 1). Acid-detergent fiber (ADF) concentration followed the same pattern as tannins (Table 2, Fig 1), with concentrations increasing significantly from June to July, then dropping substantially in August (Table 2, Fig. 1). There was no significant relationship with browse intensity (Table 1). Non-structural carbohydrates (sugars) were not related to browse intensity (Table 1). Seasonally, sugar concentration remained constant from June to July and then showed a non-significant increase from July to August (Table 2, Fig. 1).

DISCUSSION

Large ungulates choosing to feed on one clone rather than another is a unique piece of the puzzle in understanding the role of wildlife in aspen decline. In some cases, clones are browsed so intensely that they eventually disappear (Kay 1997) while other clones seem to have a trait(s) that make them less desirable to ungulates and allow regeneration to continue normally. Changes in palatability factors over time may also contribute to the level of browsing pressure on a clone. Clones with higher sugar, protein, or moisture concentration might be increasingly appealing to ungulates, while those with high defense chemical or fiber concentration would be expected to be less desirable.

Although we observed higher tannin levels in intensely browsed sites and tannins increased from June to August (Table 1), the fact that the tannin levels were higher in the more intensively browsed sites virtually eliminates that possibility. In addition, tannin level probably does not affect deer or elk browsing levels anyway. In elk feeding trials, tannin concentrations had no effect on elk feeding preferences (Wooley et al 2008). Hagerman and Robbins (1993) found that moose, deer (O*docoileus spp.)*, black bear (*Ursos americanus)*, and beaver (*Castor canadensis)* all have salivary proteins that can bind condensed tannin. Animals that are primarily grazers, such as sheep and cows, do not have these proteins and consequently tannins are very effective in deterring herbivory in these cases. They also found that the more generalist an herbivore, the more types of tannin are bound by salivary proteins. Hagerman and Robbins (1993) indicated that a generalist herbivore, such as elk, most likely has these tannin-binding salivary proteins. Tannins did not have any noticeable effect on digestibility forage for white-tailed deer in Mississippi (Jones et al. 2010). Therefore, it is unlikely that high tannin concentration would have impacted the deer and elk browsing on aspen in this study. It is possible that the cattle present in our study area may have been impacted; however, the fact that the tannin levels were higher in the more intensively browsed sites virtually eliminates that possibility. In addition, the cattle seemed to largely ignore the aspen in lieu of grass and forbs during the times we were present at the study sites.

Age of the ramets in our study may play a role in the observed tannin level differences. In the sites that were intensely browsed, some stunted growth of trees was observed. Tannins increase in aspen as trees age (Erwin et al. 2001, Donaldson et al. 2006). Our sampling method of choosing random trees below two meters high was designed to minimize this effect. If intense browsing pressure and the resultant stunted growth has been reoccurring over an extended period of time, this ontogenic effect may be more pronounced and the reason for the differences in tannin concentration that we observed. Further study on the ages of individual trees is needed to document this effect.

On the other hand, phenolic glycosides have been shown to play a role in elk preferences. In feeding trials, elk consumed a third less aspen when phenolic glycoside concentrations were over 20% compared to concentrations less than 15% (Wooley et al. 2008). In another study, elk selectively removed trees with lower phenolic glycoside concentrations after removal of an exclosure (Bailey et al. 2007). These studies indicate that phenolic glycosides probably do behave as a defense against elk herbivory. However, in our study, the phenolic glycosides at most sites were relatively low and differences between intensive and moderate browsing sites were not significant.

The decrease in phenolics concentration later in the summer may mean aspen are more vulnerable and susceptible to browsing pressure toward the end of the growing season. This decrease in phenolics coincides with declines in nutritional value of many plant communities while aspen remains high in nutritional value through late September (Franzmann et al. 1976). This may compound the risk from high browsing pressure for aspen clones that are particularly low in phenolic glycosides.

As opposed to avoiding aspen clones due to defense chemistry, ungulates may be choosing browsing sites based on digestibility factors. For example, plant moisture may be a factor impacting whether or not ungulates choose to browse on one clone versus another. Additional moisture concentration in forage would add more essential water to the diet of browsers. However, in this study, water concentration was lower in intensely browsed sites and not a likely contributor to preferential browsing (i.e. it would be expected that browsers would preferentially choose more rather than less succulent tissue).

Protein concentration is another factor that could be involved in forage choice. For example, deer will preferentially feed on plants with higher levels of protein (Lindroth 1989). Ruffed grouse select aspen buds based on protein levels and defense chemical concentrations (Jakubus and Gullion 1991). Nitrogen is not only used as a protein component, but helps rumen bacteria digest more efficiently (Christianson and Creel 2009). Additionally, increased nitrogen intake decreases mass loss in winter (Christianson and Creel 2009) and aids in reproductive success of some animals (Jakubus and Gullion 1991). We did see a difference in protein concentration, but it was 1.2% (7% relatively) higher in moderately browsed sites. This is the opposite of what is expected if ungulates are selecting for protein concentration in aspen clones. The finding of differential protein in our study may be an effect rather than a cause of browsing differences, indicating a reduced ability of the plant to make and store protein as a result of stress.

Across the growing season, we did see fluctuations in protein level. Protein levels were highest in June, dropped by 33% in July and remained at that level thereafter. If there are insufficient levels of protein in forages of this area, aspen may be in higher demand as a protein source at the beginning of the season. This is particularly important when taking into account physiological factors. Elk need the most protein during the third trimester of pregnancy (Jelinski and Fisher 1991), so they may be seeking forages with higher levels of protein in the early growing season. For maintenance, elk need about 5-7% protein in their diets, while for growth they need 12-16% (Jelinski and Fisher 1991). Elk that are actively growing early in the year, such as calves or animals trying to recover weight lost during winter, may be seeking out high-protein forages. This overlaps with the period when our study sites showed highest levels of protein (averaging 23.3%).

Sugars are another possible factor in palatability. Some mammal species will select certain sugars in their diet or select for higher levels of sugars (Herrera M. 1999, Tixier et al. 2009). We postulated that ungulates may be selecting sites with higher levels of sugars, but sugars were at similar levels in intensely browsed and moderately browsed sites (Table 1). There were also no significant differences in sugars over the season (Table 2). Thus, sugars probably do not play a factor in aspen decline in this area.

Acid-detergent fiber (ADF) and carbon:nitrogen (C:N) ratios were used in this study as general indicators of overall digestibility. Generally, as ADF, C, and C:N ratios increase, overall digestibility decreases (Jelinski and Fisher 1991). We did not find any significant differences between intensely browsed and moderately browsed aspen for

ADF. The significant differences in C concentration and C:N ratio were small, of no practical value, and in the opposite direction expected if ungulates were selecting browsing material based on this parameter (C concentration and C:N ratio were higher rather than lower in the intensively browsed clones).

Over the growing season, ADF and C:N ratio increase by 26.5% and 53%, respectively, reflecting a significant decrease in digestibility from June to August. Allredge et al. (2002) also found a similar trend in digestibility of several forb, graminoid, and shrub species. Since other forage sources are also decreasing in digestibility from June to August, the relative differences in digestibility between aspen and other forage sources are expected to remain fairly constant until early fall. Therefore, these particular factors are probably not contributing to intense browse levels of aspen.

MANAGEMENT IMPLICATIONS

For managers of areas with declining aspen and high elk and deer populations, this information may provide valuable insights on differential browsing. First, tannins, moisture content, non-structural carbohydrates, acid-detergent fiber, and C:N ratio appear not to be related to browse preferences. This should help direct research and resources towards other factors that may play more significant roles. Second, our data provide a valuable baseline comparison to evaluate other clones for levels of defense chemicals and digestibility factors.

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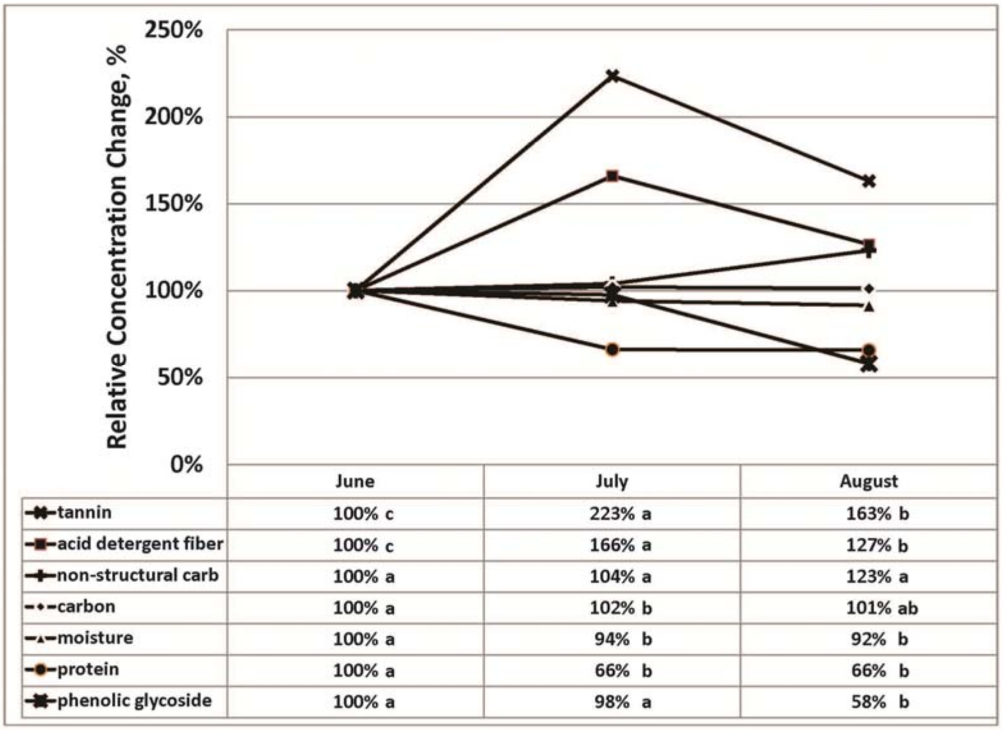

	June	July	August
tannin	100% c	223% a	163% b
acid detergent fiber	100% c	166% a	127% b
non-structural carb	100% a	104% a	123% a
carbon	100% a	102% b	101% ab
moisture	100% a	94% b	92% b
protein	100% a	66% b	66% b
phenolic glycoside	100% a	98% a	58% b

Figure 1. Seasonal changes in aspen leaf chemistry measured over the growing season for 12 paired sites (each pair consisting of one site with intense and one site with moderate browsing pressure) in the summer of 2008 in Fishlake National Forest, UT, USA. Percentages in each row are not significantly different from one another for those months with the same letter following the percentage. No comparison should be inferred across chemicals (columns). Means Separated by Duncan Waller test.

Table 1. Aspen leaf moisture, protein, acid detergent fiber (ADF), non-structural carbohydrates (sugar), carbon, carbon:nitrogen ratio (C:N), tannin, and phenolic glycosides (PG) for moderately and intensively browsed aspen sites sampled during August 2008 and 2009 in Fishlake National Forest, UT, USA.

	Browse Pressure				*P-Value*
	Intense	Moderate	Intense	Moderate	
Leaf Parameter	----- Range, % -----		----- Mean, % -----		
moisture	47.4-69.7	49.1-71.1	57.6	59.9	***<0.001***
protein [a, b]	11.0-18.6	11.6-18.9	17.4	18.6	***0.008***
ADF [a]	15.6-30.0	15.2-36.0	20.5	21.4	*0.355*
sugar [a, b]	3.0-6.7	1.9-20.1	4.9	5.1	*0.684*
carbon [a, b]	49.1-50.7	49.2-50.4	50.0	49.7	***0.031***
C:N ratio [a, b]	16.8-28.2	16.6-27.1	19.0	18.1	***0.018***
tannin [a]	0.2-12.4	0.1-9.7	4.8	2.2	***<0.001***
PG [a]	5.4-29.4	5.6-28.4	12.5	16.7	*0.964*

[a] Concentrations are on a dry weight basis.

[b] Data represents 24 sites in 2008 only.

Table 2. Aspen leaf moisture, protein, acid detergent fiber (ADF), non-structural carbohydrates (sugar), carbon, carbon:nitrogen ratio (C:N), tannin, and phenolic glycosides (PG) for three sampling dates during 2008 in Fishlake National Forest, UT, USA.

	Sampling Date			*P-Value*
	June	July	August	
Leaf Parameter	--------------- Concentration, % ---------------			
moisture	70.4	66.2	64.4	***<0.001***
protein [a]	23.3	15.4	15.3	***<0.001***
ADF [a]	17.3	28.6	21.8	***<0.001***
sugar [a]	4.6	4.7	5.6	*0.312*
carbon [a]	49.3	50.3	49.9	***<0.001***
C:N ratio [a]	13.7	21.0	21.0	***<0.001***
tannin [a]	0.8	1.8	1.3	***<0.001***
PG [a]	20.2	19.7	11.7	***<0.001***

[a] Concentrations are on a dry weight basis.

Part 2: Mineral Nutrients

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RH: Nielson et al.• Variable Aspen Palatability

Variable Palatability of Aspen for Large Ungulates Part 2: Mineral Nutrients

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ABSTRACT This is the second of a two-part series on palatability as related to aspen decline in the Intermountain West. Aspen has been in decline over much of this region for approximately the last century. This is a major concern because of aspen's value as a resource in providing cover and forage for wildlife species, playing important roles in fire and hydrologic processes, and supplying lumber products. Many factors play a role in aspen decline, but wildlife browsing has been implicated as one of the major factors. The purpose of this study was to identify possible factors of palatability of aspen for large ungulate herbivores and identify seasonal changes in these palatability factors. We studied 12 pairs of aspen stands (one intensely and one moderately browsed stand making up each pair) in Fishlake National Forest, UT, USA in June, July, and August 2008 and 10 pairs in August 2009. In this phase of the study, we analyzed soil and leaf tissue for nutrient concentration and soils for pH, EC, compaction, depth, and moisture. We also determined Normalized Differenced Vegetative Index (NDVI), based on red and near-infrared light absorption, with this index as an overall indicator of plant health. Leaf iron (Fe) concentrations were significantly higher in intensively browsed than moderately browsed sites, while zinc (Zn) and nitrogen (N) concentrations were lower. All other leaf nutrient concentrations, as well as NDVI and all soil parameters were not significantly different. In terms of changes from June through August (seasonal changes), calcium (Ca) and sodium (Na) increased from June to August, while N, phosphorus (P), potassium (K), sulfur (S), Fe, Zn, and copper (Cu) all decreased. Magnesium (Mg) and manganese (Mn) remained unchanged. In the absence of differences in effective defense chemicals (phenolic glycosides), these data suggest that ungulates may be selecting browse sites based on Fe requirements. Information on factors of aspen palatability and changes in those factors may help influence management decisions by identifying high-risk clones. Using this information, resources can be directed to the most critical areas.

KEY WORDS aspen, aspen decline, browse pressure, defense chemistry, foraging, ungulates, nutrients, *Populus tremuloides*, Rocky Mountains, Utah.

Aspen plays integral roles in high plateau and mountain plant communities of the U.S. Intermountain West as a source of forage and shelter for wildlife, lumber products, biodiversity, and scenery (Costello 1944, Koepke 1976, DeByle 1985, Mackes and Lynch 2001). Successful sexual reproduction in Rocky Mountain aspen is rare because seedlings need cool, moist conditions which are not often found in this area. Thus, aspen in the Rocky Mountians depend heavily on asexual reproduction where suckers initiate from roots (Barnes 1966, MacDonough 1985, Mitton and Grant 1996). Gradually, shade-tolerant species replace aspen in most areas unless there is a large disturbance, such as an avalanche or forest fire (St.Clair et al. 2010). After a disturbance, aspen revegetates the area through sucker growth (Jones and DeByle 1985).

Unfortunately, aspen is in decline over much of the Western U.S. and has been so over about the last century (Bartos and Campbell 1998, Kay 2001, St. Clair et al. 2010). Many factors have been implicated in driving aspen decline. These include disease, fire suppression, climate change, and, particularly, wildlife browsing (St. Clair et al. 2010).

Preferential browsing has been observed among elk (*Cervus canadensis),* moose *(Alces alces),* and deer (*Odocoileus* spp.), which will select certain forages or mineral sources in a greater proportion than what is present in the habitat (Hill 1946, Belovsky 1978, Alldredge et al. 2002, Ceacero 2010 *a, b*), but not as much is known about preferential browsing on different clones of the same species. In this second part of our series, we examine variations in mineral nutrient concentrations in preferentially browsed sites and the seasonal variations in those minerals.

The nutrient concentration of forage can influence feeding decisions. For example, moose choose forage specifically based on sodium concentration (Belovsky 1978). Elephants will go to great lengths to find sodium-rich soils and water when sodium is low in forages (Holdo et al. 2002). Studies in Europe found that red deer

(*Cervus elephus*) discriminate among sodium, cobalt, zinc, calcium, potassium, iron, magnesium, and phosphorus (Ceacero et al. 2010 *a,b*). Nutrients in aspen may play a role in the observed preferential browsing.

Seasonal changes in browse quality can also influence herbivore foraging patterns. For example, in Yellowstone National Park, seasonal migrations of ungulate herds were directly associated with nutrient concentration of available forage (Frank 1998). The nutritional value in aspen communities remains high until the end of September, where nutritional value of other forage sources typically declines at the end of summer to early September (Franzmann et al. 1976, Alldredge et al. 2002). Alldredge et al. (2002) showed that nutritional factors of several common elk forage species vary greatly throughout the year and elk must be selective in what they consume and when in order to obtain necessary nutrients.

Study of mineral nutrients and seasonal changes in those nutrients may alert managers in areas of high-risk aspen to which clones are most susceptible to overbrowsing and provide a key to protecting declining stands. The purpose of this study was to identify mineral nutrients that are factors of palatability for large ungulates and also examine seasonal trends in mineral nutrient concentration.

STUDY AREA

Fishlake National Forest occupies approximately 6,000 km^2 in South-central Utah. The study sites were located in the Tidwell Slopes area of the Fremont River Ranger District, Sevier County, UT, USA, as described in Nielson et al (2011). This area of Fishlake National Forest contained aspen associated with many other plant and animal species as described in Nielson et al (2011).

METHODS

Clonal Pair Identification

We visually identified intensely and moderately browsed aspen clones in the Tidwell slopes area of Fishlake National Forest. We selected paired intensely and moderately browsed sites with near identical elevation, slope, aspect, and soil type and within 50 to 100 m of each other. We identified clones as intensely or moderately browsed as described by Nielson et al (2011). We studied twenty-two such pairs in a multiple measurement paired t-test experimental design.

Field Data Collection

In the summer of 2008, we took field measurements and collected samples from 12 site pairs during August 5-7. In the summer of 2009, we collected similar data and samples from 10 different site-pairs during August 21-22. We measured slope, aspect, elevation, and latitude/longitude coordinates for each site and statistically evaluated each to verify that there was not a significant difference for slope, aspect, or elevation between intensely vs. moderately browsed sites (Nielson et al. 2011).

We took soil samples representative of each area with a 5 cm diameter soil auger, taking subsamples in a crisscross pattern along transects through each site. We recorded initial mass of soil samples on a balance in order to determine gravimetric moisture concentration in the laboratory. We measured soil compaction by taking penetrometer (FieldScout SC900, Spectrum Technologies, Inc., Plainfield, IL, USA) readings in 10-cm depth increments to a depth of 40 cm or until barriers were reached that prevented further penetration (Amacher and O'Neill 2004). We also recorded depth of penetrable soil up to 40 cm.

We measured red and near-infrared light absorption in both the understory and the lower tree canopy with a hand-held optical sensor with independent light source (GreenSeeker, NTech Industries Inc., Ukiah, CA, USA). We then calculated red Normalized Differenced Vegetative Index (NDVI) from these values and used that as an indicator of overall plant health (Wang et al. 2004).

We collected leaf tissue samples by clipping five leaves from 20 randomly selected ramets per stand (Donaldson et al. 2006, Erwin et al. 2001) between ground level and two m (approximate elk browsing height). We kept all leaf samples on ice during transport and short-term storage.

Soil Analysis

Soil analysis was performed in cooperation with the Brigham Young University Soil Testing Laboratory using the methods outlined by Miller and Amacher (2003). We dried a portion of each of the soil samples in a forced air oven at 105 °C to determine soil moisture gravimetrically using the formula ((wet weight − dry weight)/dry weight)*100. We air dried another portion of soil for chemical analysis. We measured soil pH and electrical conductivity (EC) from a saturated extract. We analyzed total nitrogen (N) and carbon (C) by combustion (TrueSpec CN, Leco, St. Joseph, MI, USA). All samples were acidic pH and none showed visual carbon dioxide (CO_2) effervescence from carbonates upon addition of hydrochloric acid and, therefore, we assumed that inorganic C was insignificant and no separation analysis for inorganic/organic C was necessary (therefore, total C is approximately equal to organic C in our study). We estimated plant-available phosphorus (P) and potassium (K) by sodium bicarbonate extraction; calcium (Ca), magnesium (Mg), and sodium (Na) by strontium chloride extraction; sulfur (S) by potassium chloride extraction; and zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) by DTPA extraction. We determined the concentrations of each of these inorganic elements by Inductively Coupled Plasma spectroscopy (ICP, IRIS Intrepid II XSP, Thermoelectron Corporation, Waltham, MA, USA).

Phytochemical Analysis

We oven-dried leaves at 65° C and ground samples using a mixer mill (Wiley Mill, Thomas Scientific, Swedesboro, NJ, USA). We determined P, K, Ca, Mg, Na, S, Zn, Fe, Mn, and Cu concentrations with a nitric-perchloric acid digestion (Johnson and Ulrich 1959) and ICP determination. We determined N concentration via combustion method using a nitrogen analyzer (TrueSpec CN, Leco, St. Joseph, MI, USA).

Statistical Analysis

A mixed models analysis of variance (blocking by paired sites) was performed on all data with a *P* value criteria of 0.05 being used to determine significance. Analyses were performed using SAS computational analysis software 9.2 (SAS Institute, Cary, NC).

RESULTS

When we compared the moderately versus intensely browsed sites, leaf N, Fe and Zn concentrations were significantly impacted (Table 1). Leaf Fe concentrations were nearly 20% higher in intensively browsed sites, while leaf Zn concentrations were an average of 24% lower. No differences were measured between browsing levels for leaf P, K, Ca, Mg, Na, S, Mn, or Cu concentrations (Table 1).

The interaction between browse pressure and time was not significant and, therefore, the data was combined for analysis and presentation. With the exception of Ca, Mg, Na, and Mn, mineral concentrations in leaves dropped significantly over time regardless of browse pressure (Table 2, Fig. 1) with the drop in P concentration (46% as much P in August as June) relatively greater than the rest (Fig. 1). In contrast, the Ca concentration increased in a general linear trend from June through August and Na remained constant from June through July, but then increased significantly (by 35%) from July to August (Table 2, Fig. 2). The Mg and Mn concentrations remained unchanged over the course of the season (Table 2). There were no significant differences in any soil parameter or NDVI between sites (Table 3).

DISCUSSION

An understanding of factors effecting forage choice of large ungulates may be an important key in understanding and trying to reverse aspen decline. In some cases, clones are browsed so intensely that they eventually disappear (Kay 1997) while other clones seem to have a trait(s) that make them less desirable to ungulates and allow regeneration to continue normally. Changes in palatability factors over time may also contribute to the level of browsing pressure on a clone. Clones with higher mineral nutrient levels might be increasingly appealing to ungulates, while those with lower nutrient levels would be expected to be less desirable.

When comparing intensely and moderately browsed sites, observing lower Zn concentrations while Fe is at higher levels is not a surprise, as these are competing ions that are often antagonistic with and impact each other (Safaya 1976, Hansen et al. 2006, Barben et al. 2010). Normal concentrations are approximately 46-232 mg kg^{-1} Zn and 31-151 mg kg^{-1} Fe (Tew 1970, Bartos and Johnston 1978, Alban 1985, Jelinski and Fisher 1991,Wang et al. 1995, Mills and Jones 1996, Chen et al. 1998, and Liang and Chang 2004.). Although the average concentrations of leaf Fe and Zn in our study fell within the range of what has been reported in the literature, some sites had concentrations that fell out of the norm (Table 1). We found Zn concentrations as low 19 mg kg^{-1}, which is lower than the previously reported low concentration of 46 mg kg^{-1} (Table 1). The intensively browsed sites in our study had statistically significantly higher concentration of Fe and lower Zn than clones that ungulates were choosing to avoid. Ungulates may sense a relatively higher Fe concentration in leaf tissue and, therefore, choose to browse preferentially on clones adapted to taking up more Fe from the soil (Ceacero et al. 2010 *a, b*). This is likely a genetic difference and not a soil difference, as the soils in this study showed no differences in soil pH or extractable Fe concentration when comparing paired sites (Table 3).

Iron is an important essential nutrient for animals that is needed for formation of Fe proteins (eg. hemoglobin), co-factor binding, enzymes, etc (Miller et al. 1991). Deficiency of Fe in the diet can result in Fe-deficiency anemia, which can result in a variety of health problems in mammals, especially infants (Miller et al. 1991). Many animals are known to be able to sense the presence of various minerals in their forage choices and choose those that have what they need for best health. For example, red deer (*Cervus elephus*) in Europe can discriminate between specific minerals in their diet according to their physiology, with cows, calves, and bulls selecting for different nutrients. Female calves and heavier adult females both selected higher levels of Fe than other adults and calves (Caecero et al. 2010*a, b*). Although the magnitude of the Fe concentration difference between intensely and moderately browsed sites is low in our study, the fact that there was a statistically significant difference may be a clue as to why ungulates were choosing to browse one clone over another. The fact that Zn was lower in intensively browsed sites is likely not impacting browsing choice, but rather a secondary effect of the antagonism between Zn and Fe uptake.

The ability of animals to distinguish between nutrients in their diet and make foraging decisions accordingly has been described and predicted by models. Accepted optimal foraging models dictate that feeding decisions are primarily nutritional based, modified by avoidance of defense chemicals (Belovsky and Schmitz 1994). Previously we showed no significant differences in phenolic glycosides between intensely browsed and moderately browsed sites (Nielson et al. 2011). In the absence of significant differences in effective defense chemicals (phenolic glycosides) at our study sites, we may be seeing foraging decisions that are based almost solely on Fe nutrition, although our study only suggests this as a possibility and needs to be followed up with feeding trials. In this case, it appears the elk in this area are able to utilize the maximum nutrient intake strategy predicted by optimal foraging theory (Belovsky and Schmitz 1994).

The increase in Ca from June to August is consistent with other findings (Tew 1970, McColl 1980, and Alban 1985). Calcium is critical for healthy bone and antler development (Johnson et al. 2007). Estimated Ca requirements for elk are 1.6-5.8 g kg^{-1}(Alldredge et al. 2002), so the aspen in this study (with a range of 5.1-19.2, Table 1) provide sufficient Ca to meet dietary needs. Other forage plants also meet Ca requirements with 2.5-14.3 g kg^{-1}(Alldredge et al. 2002), but the aspen in this study are at the higher end of this range and exceed the calcium concentration of some other forages. Calcium is a particularly important nutrient in late summer and early fall for late-lactating cow elk (Franzmann et al. 1976, Ceacero et al. 2010 *a,b*). With high Ca levels in relation to other browse choices, and decreased chemical defenses later in the summer, this nutrient could play a significant role in browse pressure and risk for aspen damage.

Sodium is also a very important nutrient for browsing animals. Sodium can be a limiting nutrient as it is required for osmotic homeostasis, buffering of body fluids, nerve transmission, reproduction, hair formation, lactation, growth, and maintenance of body weight and appetite (Belovsky and Jordan 1981). The increase in Na concentrations seen in the current study was consistent with the findings of Tew (1970). Alldredge et al. (2002) found that all forage species studied contained well-below the estimated requirement for elk (0.6 – 1.0 g Na kg^{-1}). Plants in that study contained higher levels of Na than the aspen in this study, but because Na is so important to physiological functions and because it may be a limiting nutrient, any Na source is likely in demand for elk and deer.

Copper and P are two minerals (in addition to Ca) that are critical to healthy bone and antler development (Johnson et al. 2007). In red deer (*Cervus elephus)* of Europe, Cu deficiency caused emaciation and wasting away even in the presence of plentiful volumes of available feed (Handeland et al 2008). Estimated Cu requirements for elk are 4 – 10 mg kg^{-1} (Alldredge et al. 2002), so the aspen in this study meet these requirements. Aspen are about average in Cu concentration and it is generally easy for elk to meet this requirement (Alldredge et al. 2002), so aspen are probably not at high risk for intense browse as a result of Cu demand.

Tew (1970) and Alban (1985) also found that P decreases through the season, although our study found higher values of P concentration compared to Tew's values. Aspen are about average in P concentration compared to other forages and it is generally easy for elk to get the estimated requirements of (Alldredge et al. 2002). It seems unlikely that P would be in high enough demand from aspen to cause intense browsing pressure.

The decrease in K concentration was consistent with the findings of Tew in aspen in Utah (1970), with comparable nutrient levels, but in contrast to the report of McColl (1980) who showed an increase in K concentration in Minnesota aspen over the season. These differences in K concentrations over time could be an example of Eastern and Western aspen. The aspen samples in this study were generally lower in K levels than forages studied by Alldredge et al. (2002). Demand for this nutrient probably does not pose a serious risk for high browse pressure on aspen. Levels of S, Fe, and Zn in these aspen samples are about equivalent to other forages and elk can easily obtain levels of these nutrients to meet estimated requirements (Alldredge et al. 2002). Although these nutrients play roles in forming amino acids and as coenzymes, among other roles, there is probably not a high risk of demand for these nutrients from aspen, even at higher levels earlier in the season, as they can be obtained from other forage sources as well.

MANAGEMENT IMPLICATIONS

The methods and findings of this study may help identify high risk aspen clones. In the absence of significant differences in phenolic glycosides, clones with higher Fe levels (18% or more) relative to other nearby aspen clones may be an indicator of risk. Managers trying to use exclosures or transplanting should be aware of these findings. We observed that clones not prone to heavy grazing were not benefited by exclosure, but those clones that were preferentially selected for browsing were definitely assisted in regeneration when exclosures were present (B.G. Hopkins, unpublished data 2008). The level of risk may also vary with time of year. Mineral licks tailored to the local needs may ameliorate seasonal damage on aspen clones, but further work needs to be done to verify this theory.

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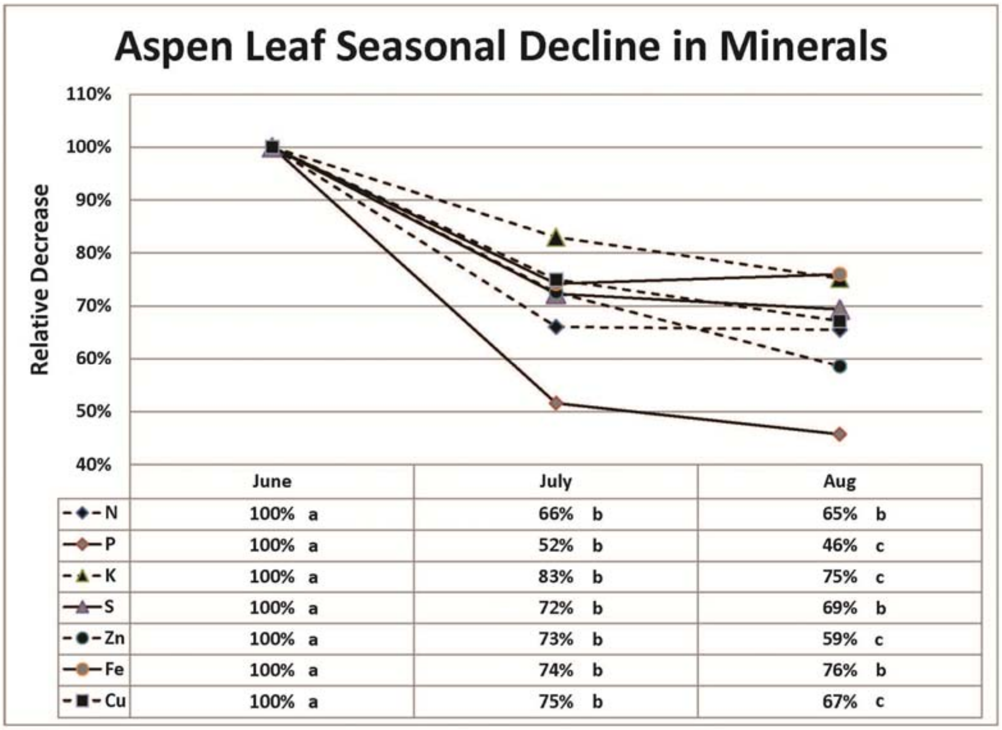
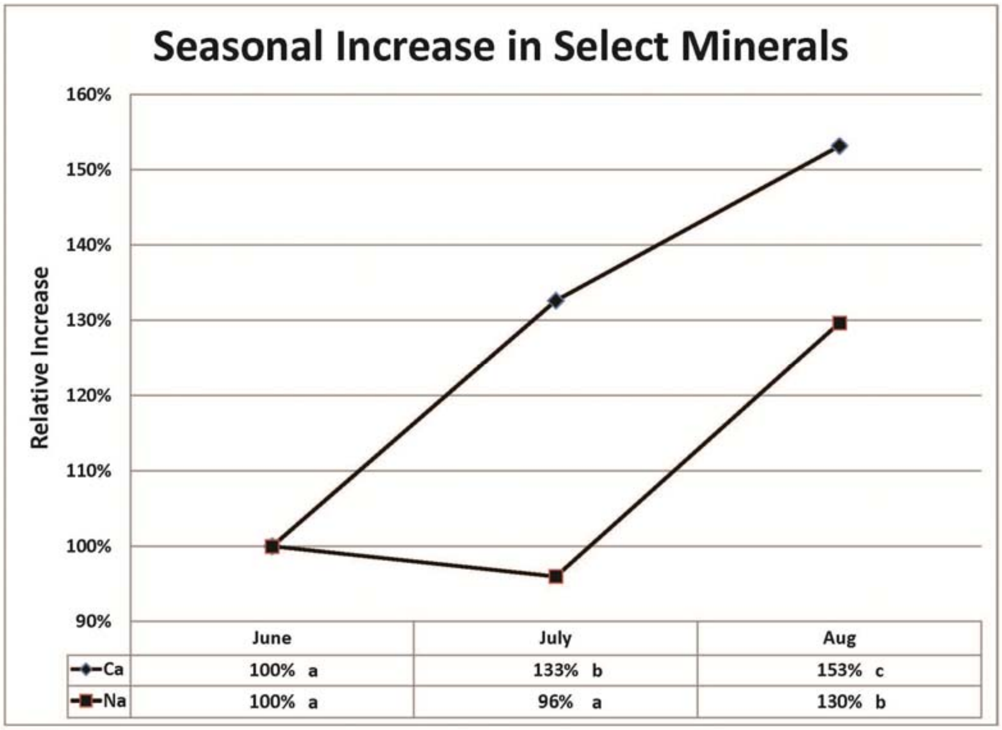


	June	July	Aug
◆ N	100% a	66% b	65% b
◆ P	100% a	52% b	46% c
▲ K	100% a	83% b	75% c
▲ S	100% a	72% b	69% b
● Zn	100% a	73% b	59% c
● Fe	100% a	74% b	76% b
■ Cu	100% a	75% b	67% c

Figure 1. Aspen leaf mineral concentrations for those elements measured and showing a significant relative decline over the course of the growing season for 12 paired sites in 2008 from Fishlake National Forest, UT, USA. Each pair consisted of a moderately and an intensively browsed (by ungulates) clone. Percentages shown are combined across browse treatments (no significant interaction with time). Percentages in each row are not significantly different from one another for those months with the same letter following the percentage. No comparison should be inferred across minerals (columns).



	June	July	Aug
◆ Ca	100% a	133% b	153% c
■ Na	100% a	96% a	130% b

Figure 2. Concentrations of aspen leaf minerals that were measuresd and showed a significant increase over the growing season for 12 paired sites (one with intense and one with moderate browsing pressure) in the summer of 2008 in Fishlake National Forest, UT, USA. Percentages shown were combined across browse treatments and had no significant interaction with time. Percentages in each row are not significantly different from one another for those months with the same letter following the percentage. No comparison should be inferred across minerals (columns).

Table 1. Aspen leaf mineral nutrient concentrations (dry weight basis) for previously reported studies as compared to the current study with moderately and intensively browsed aspen sites sampled during August 2008 and 2009 in Fishlake National Forest, UT, USA.

	Browse Pressure					*P-Value*
	Reported [a]	Intense	Moderate	Intense	Moderate	
	--------------- Range, g kg^{-1}		---------------	-- Mean, g kg^{-1} --		
Ca	3.2-41.8	5.1-19.2	7.4-18.8	11.6	12.4	*0.251*
K	1.5-25.9	5.9-31.1	6.9-26.7	13.5	16.0	*0.147*
Mg	0.3-3.9	1.9-4.9	2.2-5.5	3.2	3.2	*0.808*
N	15.7-50.0	17.6-29.8	18.5-30.3	27.9	29.7	***0.008***
P	1.3-4.7	1.3-4.4	1.6-3.3	2.10	2.30	*0.179*
S	3.8-4.2	0.80-2.8	1.3-2.0	1.6	1.6	*0.727*
	--------------- Range, mg kg^{-1}		---------------	-- Mean, mg kg^{-1} --		
Cu	8-17	4-14	5-11	8.2	8.5	*0.479*
Fe	31-151	43-224	67-156	111	94	***0.042***
Mn	15-660	28-85	31-89	50	57	*0.118*
Na	20-1300	27-86	22-85	46	43	*0.455*
Zn	46-232	19-117	28-248	56	74	***0.080***

[a] Adapted from Tew 1970, Bartos and Johnston 1978, Alban 1985, Jelinski and Fisher 1991,Wang et al. 1995, Mills and Jones 1996, Chen et al. 1998, and Liang and Chang 2004.

Table 2. Aspen leaf mineral nutrient concentrations (dry weight basis) for moderately and intensively browsed aspen sites sampled during 2008 in Fishlake National Forest, UT, USA.

	June	July	August	*P-Value*
	------------g kg^{-1}------------			
Ca	6.5	8.6	10.0	***<0.001***
K	19.2	15.9	14.4	***<0.001***
Mg	2.9	2.7	2.7	*0.130*
N	37.3	24.6	24.4	***<0.001***
P	4.9	2.5	2.2	***<0.001***
S	2.3	1.7	1.6	***<0.001***
	------------mg kg^{-1}------------			
Cu	101	76	68	***<0.001***
Fe	124	92	94	***0.018***
Mn	45	43	46	*0.516*
Na	36	35	47	***0.001***
Zn	85	62	50	***<0.001***

Table 3. Average of soil parameters and NDVI from moderately and intensively browsed aspen sites sampled during 2008 and 2009 in Fishlake National Forest, UT, USA.

Parameter	Intensely Browsed	Moderately Browsed	*P-value*
Aspen NDVI	0.86	0.82	*0.825*
Understory NDVI	0.67	0.74	*0.256*
volumetric H_2O, %	16.71	16.34	*0.251*
depth, cm	8.7	7.9	*0.554*
resistance 10 cm, MPa	1.96	1.96	*0.898*
resistance 20 cm, MPa	1.71	1.72	*0.888*
resistance 30 cm, MPa	1.53	1.86	*0.091*
pH	5.4	5.5	*0.454*
salts (EC), dS m^{-1}	0.20	0.20	*0.975*
C, g kg^{-1}	49.6	46.8	*0.666*
N, g kg^{-1}	3.3	3.1	*0.502*
P, g kg^{-1}	0.044	0.041	*0.616*
K, g kg^{-1}	0.54	0.60	*0.259*
Ca, g kg^{-1}	0.36	0.35	*0.627*
Mg, g kg^{-1}	0.076	0.076	*0.967*
Na, g kg^{-1}	0.087	0.071	*0.453*
S, g kg^{-1}	0.041	0.041	*0.911*
Zn, mg kg^{-1}	3.3	3.2	*0.786*
Fe, mg kg^{-1}	240	240	*0.895*
Mn, mg kg^{-1}	50.9	52.0	*0.912*
Cu, mg kg^{-1}	1.9	2.0	*0.367*

APPENDIX A

LITERATURE REVIEW:
INTRODUCTION TO ASEPN ECOLOGY AND DECLINE

Review of Literature: Palatability of Quaking Aspen (*Populus tremuloides)* for Large Ungulates

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Aspen (*Populus tremuloides* Michx.) is a valuable resource in the Rocky Mountains, providing habitat, forage, lumber, water conservation, and aesthetic value. Aspen is the most widely distributed North American tree species, growing in a wide range of habitats in the U.S. and Canada. In the Western U.S. aspen is confined to high plateaus and mountains due to temperature and moisture requirements. Aspen requires a minimum of 40 cm precipitation and a relatively cool temperature regime (up to 32°C summer daytime temperature) to thrive (Jones1985 *a, b*).

VALUABLE RESOURCE

Aspen is valued as a forage species and provides cover for wildlife, leading to higher density and greater species diversity than in neighboring conifer communities (DeByle 1985). Aspen stands have higher diversity, population density, reproducing females, and juvenile mammals (with normal numbers of males) than associated conifer systems (Oaten and Larsen 2008). Aspen stands provide increased shelter for wildlife, reducing wind by up to 80% compared to adjacent open areas and providing cover from predators (Jones and DeByle 1985 *a*). In addition, undergrowth is plentiful compared to adjacent conifer systems (Mueggler 1985). In an aspen understory, it is common to find 10 to 15 graminoid, 20 to 40 forb, and a variety of shrub species within 10 m^2 (Costello 1944). In Fishlake National Forest, undergrowth production ranges from 700-850 kg ha^{-1} dry matter (Mueggler and Bartos 1977).

Because of the dense undergrowth, aspen stands provide important habitat for ground-nesting birds—providing shelter and food (DeByle 1985). Ruffed grouse, for example, nest in the understory and eat the aspen buds as a substantial part of their winter diet (Jakubus and Gullion 1991). Many migratory bird species, such as swallows, sparrows, finches, wrens, warblers and buntings use aspen stands as they migrate through an area (Hollenbeck and Ripple 2007). At least 55 species of wild mammals, ranging from shrew to bison, use aspen habitat, and elk avidly seek out aspen from several possible browse choices (DeByle 1985). Aspen has moderate to high palatability ratings for white-tailed deer in the Black Hills of South Dakota, meaning there is a higher proportion in the diet than the proportion available in the habitat (Hill 1946). In winter, small mammals stay under the snow and feed on the nutritious, chlorophyll-producing bark (Jones and DeByle 1985 *b*, DeByle 1985). Many insect species also use aspen as a food source (Jones et al. 1985 *a*). Aspen also help support a prey base for several types of predators, including raptors and large mammals (DeByle 1985).

In addition to forage and cover for wildlife, aspen provides useful wood products. Furthermore, the fibrous wood is well-suited for animal bedding, pulps for fine papers and fiberboard, and raw lumber products for house siding, furniture, veneer, plywood, and cabinetry (Mackes and Lynch 2001). Excelsior or wood-wool is a popular aspen product that is used for packaging, padding, cover mats for reseeding, and cooling pads for swamp coolers (Koepke 1976).

Aspen is highly valued for its contribution to beautiful landscape scenery, as is manifest each fall when locals and tourists alike take to mountain roads to view the colorful aspen foliage. In landscaping, aspen provides shade, visual and noise barriers, space definition, and layering effects (Johnson et al. 1985).

Aspen fill many other important ecological roles. In some areas of the interior west it is the only major hardwood (DeByle and Winokur 1985). Aspen leaves decay rapidly and quickly return nutrients to the soil, acting as "effective nutrient pumps" (Jones and DeByle 1985 *c*). Decomposition is aided by invertebrate populations, which are more dense under aspen than conifers (Jones and DeByle 1985 *c*). The genetic diversity of aspen is correlated with diversity in microbial activity, levels of below-ground nutrients, and many other ecosystem processes (Madritch et al. 2009).

Aspen play important roles in fire ecology. Fires may drop out of the crowns of conifers and down to the ground when they reach an aspen stand. As a result, fires are less intense in terms of heat and speed while burning through aspen. When fires burn through parts of a stand, they typically remove organic debris, partially clear overstory cover, allow soil warming, and kill roots of shallow, competing vegetation. This in turn creates an ideal environment for aspen sucker growth. According to Jones and DeByle (1985d), aspen are fire-dependent and will be replaced by conifers in the entire Interior West without periodic burning. Regardless of the validity of this opinion,, fire has been shown to play a significant role in the health of aspen ecosystems.

Sites dominated by aspen are more water efficient than conifer stands, using 8 to 18 cm less than conifers annually (DeByle 1985 *b*). For every 400 hectares that convert from aspen to mixed-conifer, 300,000-600,000 m^3 of water are transpired rather than being available for water flow or understory plant use (Bartos and Campbell 1998). In addition to lower water usage, the soil under aspen has an average of 4% more organic matter and significantly higher water holding capacity than the lower-biomass-producing conifer stands (DeByle 1985 *b)*. The increased ground cover and soil water holding capacity in aspen stands reduce water runoff amount and rate, thus decreasing erosion (DeByle 1985 *b*). In addition, late snowpack in aspen systems, as compared to adjacent open areas, provides prolonged water supply for the understory vegetation (Shepperd and Jones 1985).

REPRODUCTION

Aspen reproduces both sexually and asexually. Favorable conditions for seedling establishment (namely prolonged moist, cool conditions) occur very rarely in the Rocky Mountain region (Mitton and Grant 1996). In recent history, such conditions occurred after the 1988 fires of Yellowstone National Park, which were followed by several wet years (Romme et al. 2005). These favorable conditions did not last long and 25% of the seedlings established after the 1988 fires died. Those that lived did not appear to flourish, many less than 2 m tall by the year 2000 (Romme et al. 2005). Aspen seed production is prolific, but seedlings are very sensitive to high temperature and low moisture and, therefore, aspen depend almost entirely on asexual reproduction (via suckering) in arid and semi-arid regions (Barnes 1966, MacDonough 1985, Mitton and Grant 1996). A single individual of sexual origin establishes and reproduces clonally by sending up suckers from the roots to perpetuate the stand (Jones and DeByle 1985). A few of the clones in Utah and Colorado may be those that established in the Pliocene era (~1 million years prior to present day) and grew vegetatively until the present (Barnes 1975). One such aspen clone, touted as the world's largest living organism, is the Pando clone of Fishlake National Forest, UT, USA, which covers approximately 43 hectares and has about 47,000 ramets (Grant et el. 1992).

Suckers can be produced from any section of aspen root, except those that are newly formed (Schier et al. 1985 *a*). Sucker growth is partially enhanced due to alteration of the hormonal balance between shoots and roots when the apical meristem is removed by fire, cutting, or other damage (Jones and DeByle 1985, Schier et al. 1985 *a*). Suppression of sucker growth occurs due to shade intolerance and hormonal suppression (apical dominance) (Jones and DeByle 1985, Schier et al. 1985 *a*).

Aspen have "remarkable genetic diversity" across clones (Mitton and Grant 1996). When phenotypic factors with strong genetic control were compared between populations, significant differences were found, indicating a genetic basis for the phenotypic variation (Barnes 1975). One study confirmed clonal variation of starch, nitrogen, and both types of defense chemicals (Lindroth and Hwang 1996). Many chemical and nutritional traits of aspen, especially defense chemicals, vary more with genotype than with environmental factors (Madritch et al. 2006, Donaldson and Lindroth 2007, Lindroth et al. 2007).

ASPEN DECLINE

Decline of this species has been identified as a top concern in the western United States (Bartos and Campbell 1998, Figs. 1 and 2). Aspen has been declining in Yellowstone for at least 80 years (Kay 2001b). In Utah, aspen has decreased by about half over the last 125 years (Bartos and Campbell 1998). In Fishlake National Forest, aspen decreased from 125,000 to 56,000 hectares, only 8,000 of which are assumed to be properly functioning and not at risk of disappearance (Bartos and Campbell 1998). Causes of decline have been attributed to many factors such as disease, over-browsing, and changes in the fire cycle.

Although aspen are susceptible to many diseases, there are relatively few instances of clones being killed or seriously injured by diseases. Outbreaks of fungal diseases are problematic in certain locations (Packard 1942, Hinds 1985). Foliage diseases tend to be of local significance, with individual stems being killed while the overall clone survives. Viral infections tend to be rare. Wood decay (trunk, root, and butt rots) is more prevalent in older stands, but usually does not enter the stand until there is a high percentage of old trees, i.e. as a secondary effect. Perennial cankers may be a problem because they will continue to enlarge and girdle and kill individual trees, but, again, the clone generally survives. Other cankers may hinder growth, but never girdle and kill trees (Hinds 1985).

Another factor that is considered a main cause of aspen decline is change in the fire cycle. Frequent fires remove competing conifers, kill older aspen, clear debris, and stimulate suckering. Human land use and fire control policy has altered the number, severity, and length of intervals between fires; tending towards infrequent, high temperature fires that are more damaging to the ecosystem (White et al. 1998).

Even when fires stimulate suckering, over-browsing by both wildlife and livestock can inhibit or prevent stand regeneration (Kay and Bartos 2000). As conifers crowd out aspen, browsers are crowded into smaller and smaller areas, thus increasing the relative pressure on aspen regeneration. Kay (1997) suggests that Native Americans set fires to manage forests and kept elk populations low, allowing aspen to grow successfully. In Jackson Hole Wyoming, burned stands of aspen did not regenerate successfully under moderate to high elk population (Kay 2001a). Trees that are browsed by elk are significantly more likely to die than trees that are not browsed by elk. In one study, nearly 84% of the aspen browsed by elk died as a result of the browsing (Bailey et al. 2007). When Rocky Mountain parks (such as Yellowstone) were established in the mid-1800s, most aspen and willow stands were young and there was evidence of frequent fires with minimal evidence of browsing. The stands that were mature showed no sign of elk bark stripping, indicating low elk densities at that time. Elk culling had ceased in all Rocky Mountain parks by 1970 and elk populations have risen since then, causing increasing difficulties in aspen regeneration (White et al. 1998).

In Arizona, aspen clones regenerate successfully only when in exclosures (fenced areas that excludes browsers), which cost \$2,300-\$3,800 km^{-1} to erect (Rolf 2001). Over 30 kilometers of fence had been put up by the year 2000. The Arizona Game and Fish Department increased elk permits by 400% to try and decrease the damage to aspen and other vegetation outside exclosures. Even after over 70% of the trees were greater than twelve feet tall, the damage to the trees upon fence removal (girdling, breakage, and resulting infections) was so extensive that the clones nearly disappeared in just three years (Rolf 2001).

Hot Sauce® deer repellant protects aspen from elk and other browsers, but is prohibitively expensive for the land area that needs to be protected and only provides protection for about five weeks during the growing season (Baker et al. 1999). Less expensive and more effective ways to protect regenerating aspen are needed. The Forest Service recommends research on wildlife and livestock use in order to reverse the trend of aspen decline with the adage “take action now, make action large, and take action often” in aspen research and restoration. (Bartos and Campbell 1998).

PALATABILITY FACTORS

There are many factors that affect palatability of various feedstuffs. One major determinant of browse quality is nutrient concentration. This may include non-structural carbohydrates (sugars), protein and mineral nutrients. Deer preferentially feed on plants with higher levels of protein (Lindroth 1989). Ruffed grouse select aspen buds based on protein and defense chemical concentrations (Jakubus and Gullion 1991). Nitrogen is not only used as a protein component, but helps rumen bacteria digest more efficiently. Increased nitrogen intake decreases mass loss in winter (Christianson 2009). Moose will consume different amounts of plants based on nutritional value (Belovsky 1981). Also, sugars play a role in the diet of some mammals. For example, fruit bats select specific types of sugars, preferring sucrose over glucose and fructose (Herrera M. 1999).

Macro- and micro mineral nutrients are essential for many physiological processes. These nutrients may be used for structural components, such as calcium in bones, or enzyme cofactors, such as iron in hemoglobin, as well as myriad other ways (Lindroth 1989). Moose have been shown to select plants based on the limiting nutrient sodium (Belovsky 1981). If elephants cannot get sufficient sodium from their diets, they will seek out sodium-rich water and soils (Holdo et al. 2002). Red deer (*Cervus elephus*) of Europe are able to discriminate between forages based on mineral concentration and will consume differing amounts of minerals based on their physiological state, i.e., cows, calves, and sexes consume different ratios. (Caecero et al. 2010 *a, b*). In Yellowstone National Park, seasonal migrations of ungulate herds were shown to be directly associated with nutrient concentration of available forage (Frank 1998).

A third determinant of palatability is overall digestibility. The carbon to nitrogen (C:N) ratio of a plant is one indicator of digestibility. A high C:N ratio indicates an excess of C, much of which is indigestible and tied up in structural molecules

(Schoenecker et al. 2004). A low C:N ratio indicates ample N compared to C, with the resulting tissue being more succulent and more palatable (Scheonecker et al. 2004). Acid-detergent fiber mimics animal digestion of plant materials and is used as an indicator of digestibility and browse quality, with relatively higher fiber being less palatable and digestible (Robbins 1987, Alpe et al. 1999).

The third major determinant of palatability is defense chemistry, which may be the determining factor of palatability in some cases. The main defense chemicals in aspen are tannins and phenolic glycosides. Tannins reduce digestibility by binding digestive proteins, while phenolic glycosides are toxic and bitter (Hagerman and Butler 1993, Wooley et al. 2008). Avoidance of phenolic glycosides was the most important factor in a study of porcupine food choice (Diner et al. 2009). In a study in Arizona, levels of tannins and phenolic glycosides of aspen trees in an exclosure were measured. The exclosure was removed, elk were allowed to browse at the site, and defense chemistry levels of trees were re-assessed. The trees that remained after elk browsing had a higher average concentration of phenolic glycosides (Bailey et al. 2007). This suggests that variation in levels of defense chemicals are a factor in palatability of aspen for elk, and that elk select against higher levels of defense chemicals. Phenolic glycosides and tannins affect the performance of defoliating insects, such as gypsy moths and tent caterpillars (Osier and Lindroth 2001, Donaldson and Lindroth 2007). Cooper and Owen-Smith (1985) found that several species of mammalian herbivores in Africa will reject plants as a food source if they contain high levels of condensed tannins. Production of these secondary metabolites is directly linked to soil nutrient availability, with plants able to produce significantly higher levels of defense chemicals when nutrient availability is high

(Osier and Lindroth 2001, Lindroth 2007).

The high level of genetic diversity in aspen plays a significant role with regard to defense chemicals. Genotypes differ significantly in phenolic glycoside and tannin concentrations. Not only do chemical defenses (resistance) vary with genotype, but response to defoliation, or ability to recover (tolerance), also varies with genotype. Patterns of genetic variation for tolerance and resistance tend to be consistent across soil nutrient conditions (Stevens et al. 2007). Osier and Lindroth (2001) examined genotype, leaf nutrient quality, and defoliation level in a controlled study. Genotype was the only significant factor correlated with the amount of phenolic glycosides, accounting for 93% of the variation observed. However, nearly all other research on genetic influence on defense chemicals and nutritional value has been done in the Eastern U.S. under conditions of defoliation, and will likely be different in the Western U.S. under severe browsing pressure.

A study of factors of palatability for large ungulates in the Intermountain West could have several management applications. Knowledge of factors of palatability may provide for more cost-effective management and restoration methods to be developed. There are some areas with ungulate populations where there is no difference in the growth of suckers inside and outside the exclosure (Fig. 3). The clone appears to be naturally avoided. In some cases, aspen seem to be “pushed out” by conifers, but in some cases it seems that grazing is the predominant negative impact (Fig. 4). Whether wildlife or cattle are the primary cause of damage to aspen is a heated debate , but evidence shows that both contribute, with effect sometimes variable by location (Figs. 5-7). Information from the current study may lead to accurate identification of at-risk clones, so that money and labor may be directed to critical areas. Also, such a study may allow for better selection of good clones for transplanting. This would foster higher survival in areas with high levels of wildlife where other clones have an inability to establish. A study including soil factors may provide some basis for soil manipulation, such as fertilization, to which aspen respond well (Jones and Shepperd 1985).

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Figure1. A declining aspen stand in Fishlake National Forest, UT, USA, in the Spring of 2007. There is a lack of regeneration to replace the older, decadent trees due to extensive browse pressure of suckers.

Figure 2. An aspen stand in Fishlake National Forest, UT, USA spring 2007, with normal regeneration. There is a layer of regenerating young trees that expand the clone and replace older trees.

Figure 3. An ineffective aspen exclosure in Fishlake National Forest, UT, USA, spring 2007, in which regeneration is roughly equivalent on both sides of the fence and the clone appears to be naturally avoided by browsers.

Figure 4. Browse pressure in an aspen stand without significant conifer encroachment. Note the near absence of young ramets. The ramet shown in the foreground is partially protected by a log, but shows significant browsing that is preventing it from growing normally.

Figure 5. Cattle exclosure showing substantial regeneration in the absence of cattle grazing (wildlife are able to cross the fence and are seemingly not a problem for aspen regrowth in this instance).

Figure 6. Cattle exclosure showing substantial regeneration in the absence of cattle grazing (wildlife are able to cross the fence and are seemingly not a problem for aspen regrowth in this instance).

Figure 7. Three-way exclosure in Fishlake National Forest showing little aspen regeneration in the foreground and in the cattle exclosure (left), but substantial regeneration in the adjacent wildlife exclosure (right). It is apparent that wildlife are the prevalent cause of lack of aspen regeneration in this instance.

APPENDIX B

GPS coordinates for study sites

Site	UTMs	
A1	12S 0457750	4278084
A2	12S 0457579	4278013
B1	12S 0453413	4283706
B2	12S 0453557	4283676
C1	12S 0451860	4283604
C2	12S 0451810	4283595
D1	12S 0451593	4283501
D2	12S 0451619	4283552
E1	12S 0449366	4282980
E2	12S 0449293	4282891
F1	12S 0452811	4288178
F2	12S 0452964	4288072
G1	12S 0452446	4288765
G2	12S 0452502	4288736
H1	12S 0452843	4289889
H2	12S 0452804	4290078
I1	12S 0451226	4286891
I2	12S 0451291	4286922
J1	12S 0451214	4286671
J2	12S 0451306	4286600
K1	12S 0451013	4286521
K2	12S 0450961	4286474
L1	12S 0450606	4287547
L2	12S 0450638	4287546
M1	0458401	4278112
M2	0458354	4278104
N1	0458487	4278132
N2	0458451	4278186
O1	0456141	4278095
O2	0456159	4278101
P1	0456217	4278234
P2	0456227	4278190
Q1	0456301	4277995
Q2	0456282	4277972
R1	0456325	4277955
R2	0455161	4277666
S1	0454867	4277695
S2	0454895	4277654
T1	0455809	4277901
T2	0455766	4277903
U1	0456124	4278463
U2	0456178	4278449
V1	0455125	4278920
V2	0455103	4278975