

COMPENSATORY SHOOT GROWTH IN TREMBLING ASPEN (*POPULUS TREMULOIDES* MICHX.) IN RESPONSE TO SIMULATED BROWSING

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ABSTRACT: Moose (*Alces alces*) browsing influences plant growth and architecture. We sought to determine the impact of the timing of moose browsing on bud development and growth in aspen shoots in the subsequent spring through simulation by clipping aspen (*Populus tremuloides*) stems in the field in June, July, and August 2005 at the University of Northern British Columbia, Prince George, BC. To observe new leaf+shoot development in aspen over a 60-day period, the top meristems of both simulated browse treatments and unbrowsed controls were harvested in January 2006, and incubated in a growth chamber that simulated local springtime conditions. Total leaf+shoot biomass produced from stems was higher for June- and August-'browsed' stems relative to unbrowsed controls. Mean stem diameter was significantly higher and number of total buds significantly lower on clipped relative to unclipped stems. The number of buds that broke winter dormancy and became active in the growth chamber remained relatively constant for both clipped and unclipped aspen, but with fewer dormant buds on clipped stems than controls. Overall, our findings suggest that the mechanical effects of moose browsing on aspen stimulate the production of compensatory leaf+shoot biomass, and therefore potential browse.

ALCES VOL. 45: 101-108 (2009)

Key words: *Alces alces*, *Populus tremuloides*, browsing, herbivory, plant-animal interaction.

The nature and level of plant response to browsing by moose can vary (Bergström and Danell 1995). Response may be species dependent or may vary individually within a species as a result of differences in time of year or the amount of tissue removed (Rea and Gillingham 2001). The compensatory growth response of many plants browsed in winter (Danell et al. 1985) and the growing season (Bergström and Danell 1995, Gadd et al. 2001) is equal to the level of annual growth in unbrowsed plants of the same species. However, the degree of compensatory growth (e.g., location of meristems, number of dormant buds activated, shoot size, and length) varies in response to the degree of browsing damage; such variance can affect both plant productivity and quality of forage. For example, birch (*Betula pendula* and *B. pubescens*) produced larger shoots with larger and more chlorophyll-rich leaves following

browsing (Danell et al. 1985).

Almost all studies of plant response to herbivory have documented the overall effects of browsing damage to individual plant health and morphology, but few have investigated specifically how individual "plant units" respond. Honkanen and Haukioja (1994) speculated that individual plant units, such as branches or ramets, can act as semiautonomous units in that response to damage as an isolated unit would be similar to its response when attached to the parent tree.

In order to examine the compensatory response of aspen meristem units, we observed isolated meristems under incubation that were clipped in simulated browsing treatments during the previous growing season. We believed that the response to clipping damage would result in greater allocation of new biomass to stems as compared to undamaged branches, as found in a similar study by Stevens et

al. (2008). Prior to the simulated browsing treatments, we experimented by incubating different stem cuttings of different woody shrubs and trees at different times of the year to observe their growth response. We determined that branches of aspen that were clipped at different times during the previous growing season altered their tissue repair physiology in response to clipping (Carson et al. 2007). Here, we sought to determine whether the timing of simulated browsing would influence the compensatory growth response of aspen in the next growing season.

STUDY AREA

We conducted our study on an approximately 20 ha area located adjacent to the University of Northern British Columbia (UNBC) endowment lands near Prince George, B.C., Canada (lat 53° 53' N, long 122° 40' W). The topography was rolling at an elevation of 780 m above sea level. The climate is continental and characterized by seasonal extremes with cold winters and warm, moist summers. Mean annual precipitation is approximately 460 mm; snow fall averages approximately 200 cm and the mean annual temperature ranges 1.7-5 °C (Atmospheric Environment Service 1993). The study area was clear-cut approximately 15 years prior to the study. Young trembling aspen was the dominant tree species on site, while pioneering species such as shrub willows (*Salix* spp.), paper birch (*Betula papyrifera*), and alder (*Alnus* spp.) were also present. Moose and deer (*Odocoileus* spp.) are both native and foraged within the study area. Our observations indicated that most browsing of aspen was by moose (~1.5 moose/km²; Walker et al. 2006) at the time of this study.

METHODS

The simulated browsing (clipping) treatments imposed on aspen saplings (approximately 1-5m height) within the aspen-dominated stand (14,240 ± 5696 S. D. stems/ha) were described in Carson et al. (2007). Four

simulated browsing treatments (no-browse control and three growing-season clipping dates: 1 June, 16 July, and 30 August 2005) were imposed on 200 naturally growing aspen saplings. To approximate the mechanical effects of browsing, apical stems were clipped at 4.0 mm stem diameter proximal to the apical meristem, which is the average bite diameter of shoots browsed by moose in the study area (Carson et al. 2007).

The top 50 cm of winter-dormant stems from the aspen sapling crowns of control and simulated browsing aspens were harvested 7-14 January 2006. Approximately 5 aspen stems from within each treatment and control were collected on each of the 7 harvest dates for a total of 160 stems from the original 200. Forty of the individuals were damaged or killed by moose between the time of treatment and harvest (Carson et al. 2007). Immediately after removal, stems were placed in water buckets with their cut stem ends immersed in water to a depth of approximately 10 cm to reduce the effects of cavitation (Williamson and Millburn 1995). Harvested stems were then transported to the Enhanced Forestry Laboratory (EFL) at UNBC to record the extent of stem dieback resulting from the simulated browsing treatments imposed during the previous summer (Carson et al. 2007), and prepared for sprouting in an Environmental Growth Chamber (EGC; Model GCW 30, Chagrin Falls, Ohio, USA).

The necrotic (dieback) region below the point of summer clipping of each harvested stem segment was cut off at the terminus to eliminate unproductive and potentially phytopathogenic stem tissue. Harvested stems were reduced to a set mass of 12.0 ± 3.0 g by cutting from the stem bottom (harvest point) and were defined as “set weight stems.” Set weight stems were incubated in water baths within the controlled growth chamber for 45 days at a light and temperature regime that approximated the mean local climate in May, followed by 15 days at the mean climate conditions in

June (Meteonorm 4.0; Fig. 1). During the first 4 weeks, the daytime photosynthetically active radiation (PAR), air temperature, and relative humidity (RH) were set at 600 Watts m^{-2} , 15 °C, and 44% RH, respectively, over a 16-h photoperiod; a 17-h photoperiod at 650 Watts m^{-2} , 19 °C, and 48% RH was used in the last 2 weeks. Conditions at night were held constant during the full incubation period (0 Watts m^{-2} , 6 °C, and 87% RH). Water baths only contained plants from the same treatment to avoid possible effects due to water-mediated hormone transport between stems of different treatments. Baths were covered with white plastic and trays were painted white to prevent any light-induced temperature change to the medium (Fig. 1a). Stems were incubated in the growth chamber for 60 days (Fig. 1b). During incubation, stems were monitored for the time of bud burst and maximum growth time prior to leaf desiccation as a result of stem embolism and/or cavitation (Williamson and Millburn 1995). A data logger (HOBO Temp/External Channel Data Logger, Onset Computer Corporation, H08-002-02, Massachusetts, USA) was used to monitor light intensity, temperature, and RH throughout the incubation period.

After the 60-day growing period, set weight stems were harvested and separated into new growth (new leaf+shoot) and pre-existing stem. The number of active and dormant buds was recorded for each stem. Fresh weights for new growth and pre-existing stem were recorded, and then dried at 60 °C for 2 (leaf+shoot) or 6 (old stem) days to measure oven-dry weight.

Statistical Analyses

We used one-way analysis of variance for unequal sample sizes (ANOVA; Zar 1999) to compare differences between clipping treatments and controls; new growth and pre-existing stem mass, mean stem diameter normalized to set weight stem mass, and dormant and active buds normalized to set



Fig. 1. Harvested stem tops of aspen (12 ± 3.0 g) within an Environmental Growth Chamber at; a) initial and b) final stages of a 60-day incubation period to assess regrowth potential.

weight stem mass were compared. Tukey's honestly significant difference (HSD) test for unequal sample sizes (Zar 1999) was used for post-hoc comparisons among treatments. All ANOVAs were performed using Statistica (Version 6.0, Statsoft 2005, Tulsa, OK). We used linear regressions to determine the relationship between the number of active buds and dry leaf mass per stem unit. Regression equations were computed using Excel (Microsoft Office 2003).

RESULTS

Overall, significant differences in the ratio of leaf+shoot mass:total branch mass (new growth + pre-existing stem) were observed between the treatments and controls when examining the fresh weight of incubated stems (Table 1). Specifically, June and August

Table 1. Mean ratio of new growth (leaf+shoot) mass:total branch mass in clipped aspen stems and unclipped control stems after simulated browsing (clipping) at 3 different times during summer. Means in a row not sharing a common superscript indicate significant differences as determined by Tukey's HSD post-hoc tests.

	Month of simulated browsing								<i>F</i>	<i>P</i>
	June		July		August		Control			
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.		
	n = 31		n = 38		n = 34		n = 39			
	New growth:total branch ratio									
Fresh weight	0.088 ^{ab}	0.004	0.077 ^{cb}	0.004	0.090 ^a	0.002	0.075 ^{cd}	0.004	4.385	0.006
Dry weight	0.058	0.003	0.053	0.003	0.060	0.002	0.051	0.003	2.192	0.092

clipping trials had higher leaf+shoot mass to total branch mass when compared to controls. Also, the ratio of leaf+shoot mass to total branch mass for August-clipped stems was higher than that of July-clipped stems. No differences were found relative to dry weight of incubated stems, although June and August clipped stems were about 10% heavier than controls and approached statistical significance ($P = 0.092$).

The ratio of mean diameter normalized to the set weight stem mass was higher for treatments (~0.44 mm/g for all treatments) than controls (0.33 mm/g; $F(1,3) = 24.5$, $P < 0.001$). However, Tukey's HSD indicated

that only controls were different from treatments ($P < 0.001$). The ratio of dormant buds ($F(1,3) = 9.599$, $P < 0.001$) and total buds ($F(1,3) = 5.5015$, $P = 0.001$) normalized to set weight stem mass was not different among clipping treatments, but was higher for controls than for any clipping treatment (Fig. 2). We found no differences ($F(1,3) = 0.4436$, $P = 0.722$) in the ratio of active buds normalized to set weight stem mass between any clipping treatment or the control (Fig. 2). Weak relationships were detected between the number of active buds and dry leaf mass (Fig. 3); as the number of active buds increased, the dry leaf mass increased for all treatments

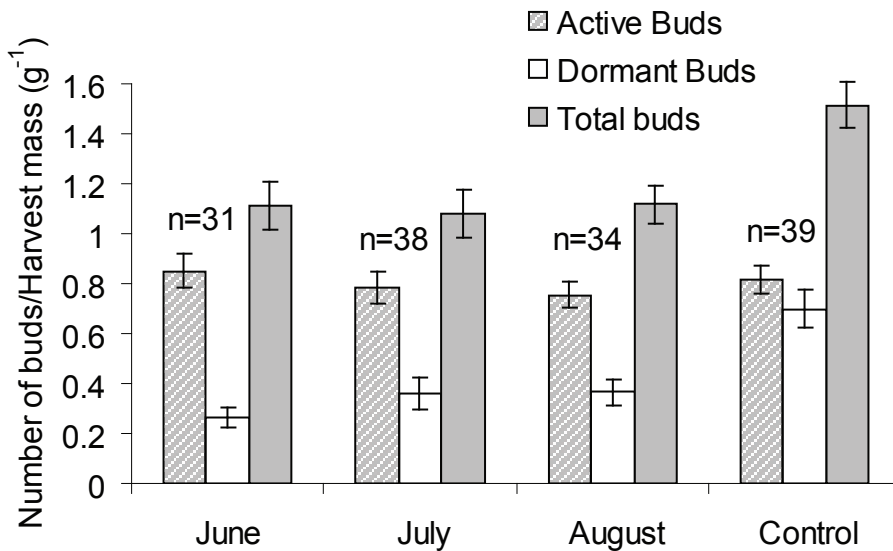


Fig. 2. The number of active buds and dormant buds normalized by the set weight stem mass (12 ± 3.0 g) prior to incubation for treatments after 60 days of incubation in a growth chamber. The numbers of total and dormant buds for all treatments were significantly different from the control ($P < 0.001$).

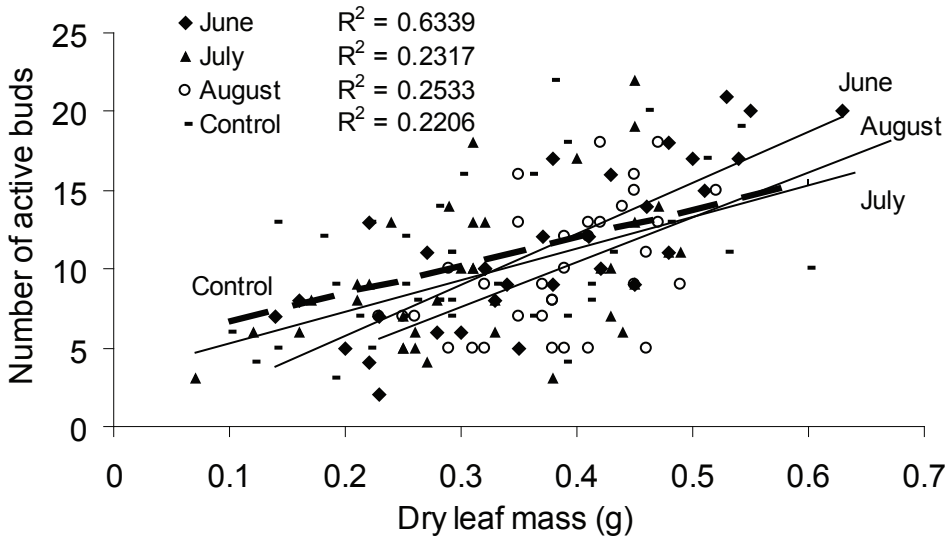


Fig. 3. Relationship between the number of active buds and the dry leaf mass per set weight stems (12 ± 3.0 g) for treatments and controls.

and the control (June: $Y = 32.349x - 0.6691$, $R^2 = 0.6339$; July: $Y = 20x + 3.2593$, $R^2 = 0.2317$; August: $Y = 28.507x - 0.9541$, $R^2 = 0.2533$; and Control: $Y = 17.704x + 4.8627$, $R^2 = 0.2206$).

DISCUSSION

Clipping stems to simulate browsing generally produces the same responses as natural browsing (Haukioja and Huss-Danell 1997), but the effects of clipping and natural browsing on plant morphology and productivity have not been adequately examined in aspen. Indeed, the question of whether browsing animals such as moose positively ‘cultivate’ their browse species is an open one. We found no evidence that season of simulated browsing on meristems affected the overall production of leaf and stem mass or influenced the proportion of active vs. dormant buds in the spring following clipping. However, we were able to demonstrate a significant effect of simulated browsing on these quantitative aspects of regrowth in aspen stem units when compared with unclipped controls. Given that young aspen is important browse for moose, and that aspen can rapidly grow beyond browsing height of moose, a positive feedback from

aspen browsing on forage availability is of more than academic interest.

Overall, our findings suggest that moose browsing can stimulate the production of more compensatory leaf+shoot biomass (potential browse) than is produced by unbrowsed stems. Although aspen is not a preferred browse species in our area, it is consumed frequently by moose in areas of northern BC and elsewhere (Renecker and Schwartz 1998), especially in the absence or low abundance of other preferred browse. Aspen has a high juvenile growth rate and productivity that combined with its ability to tolerate stress better than other tree species (Liefers et al. 2001), may explain the compensatory response we observed in response to clipping. Stevens et al. (2008) examined herbivory tolerance in aspen and found a positive correlation between tolerance and increased allocation of new biomass to stems under high nutrient conditions.

Because we clipped aspens on the main stem, a loss of apical meristem dominance may help explain the compensatory response we observed. According to the sink-source hypothesis, a change in the ability of meristems to compete with other plants and even other branches of the same plant for resources is the

primary way in which damage affects plants (Honkanen and Haukioja 1994). In this way, plant tissues (such as our aspen meristems) that have been damaged or removed by browsing (or clipping) are no longer available to photosynthesize and “sink” resources. This results in a reallocation of plant root resources to shoot production and plant compensation derived from axillary bud development (Pratt et al. 2005).

Simulated browsing treatments also had an effect on the mean diameter of winter-dormant stems (normalized to set weight stem mass), increasing mean diameter of such stems over unclipped controls. Clipping was conducted at a diameter pre-determined from bite marks of moose within the study area, so it was not surprising that unclipped stems with their intact leaders would have a lower mean diameter than stems damaged from browsing or clipping. Although this difference between the mean stem diameter was an artifact of the clipping treatment, the change in architecture (either by clipping or browsing) can have a direct effect on a tree's ability to compensate for tissue loss from browsing over time. Plants with larger mean diameters had a lower number of total buds, presumably affecting the plants capability for shoot production relative to smaller diameter shoots. Like our aspens, the mean shoot diameter of birch (*Betula* spp.) was shown to be higher on stems previously browsed by moose than on unbrowsed trees of the same age (Danell 1983).

While the number of active buds per gram of stem tissue was similar between treatment and control stems, the number of dormant buds was significantly less on clipped stems (Fig. 2). The reduction of dormant buds is likely related to the availability of total buds on clipped stems and their capacity to activate in response to tissue loss. For example, active buds represented 76.8% of total buds on stems clipped in June and only 53.6% of total buds on controls. Thus, stems clipped in June had approximately the same number of

active buds as controls despite a reduction in the total number of buds available. Therefore, it appears that aspen can compensate from a single summer browsing event during the following spring through the activation of dormant buds.

If we relate the number of active buds to the production of new leaf+shoot mass for both treatment and control individuals - we find some correlation (Fig. 3; we did not test differences between clippings, but illustrate individual trends for the sake of interest). Our results indicated a somewhat positive relationship between the number of active buds and production of leaf mass. For single browsing events, a stem's ability to maintain the required number of active buds to maximize growth does not seem to reduce plant productivity. It is possible that repeated browsing events on the same stems could eventually hamper the tree's ability to compensate for tissue losses and decrease new shoot production by reducing the availability of meristems. While not evaluated, this negative feedback on vertical growth could have other beneficial effects for the browser (e.g., shoots and leaves produced in the following year might remain within reach of moose).

When we compared the response of plant units and individual plants to damage from simulated browsing, we found similar responses. Clipped stems had significantly fewer mean buds per stem than the controls; similarly, Bergstrom and Danell (1987) found an overall reduction in the mean number of buds per tree on clipped individuals. As well, clipped individual stems in our experiment produced the same leaf+shoot biomass as unclipped stems. Defoliation of long shoots on individual birch (*Betula pendula*) during the summer resulted in lower leaf biomass on defoliated trees; however, total leaf biomass produced during the season was about the same on both treated and untreated individuals (Bergstrom and Danell 1995). Although we did not see a difference in the production

of new leaf+shoot biomass between clipping treatments, clipping at different times of the growing season can produce variable levels of biomass production as compared to unclipped stems. Thorne et al. (2005) found that the frequency of clipping alone had no significant effect on biomass, rather, it was specific combinations of seasonal clipping that produced the highest variation.

We suggest further investigation into the relationships among meristem availability, height-specific browse production, and aspen's ability to compensate for tissue loss, specifically with respect to the influence of varying intensity and frequency of browsing events. Related research has identified activation from bud dormancy as a basic component of compensatory response within plants (Tuomi et al. 1994), but as with our study, has been tested only within the scope of a single browsing event. Stevens et al. (2008) found that the response of aspen to herbivory was dependent on soil nutrient conditions; we presumed that soil conditions were reasonably consistent within our relatively small study site. A more detailed approach may be required to observe aspen response to repeated and variable levels of browsing intensity. Furthermore, the relationship between stem volume and number of buds should be studied over a variety of branch sizes to better understand the general characteristics governing morphometric responses and browse production in aspen stems, as well as stems of other browse species. Palatability and nutritional differences between compensatory growth of clipped aspen stems versus unclipped stems is also of interest. Moose are known to select for compensatory shoots that grow from plants that have been browsed or cut (Danell et al. 1985), and appear to select for shoots based on the season of cutting (Alpe et al. 1999). Presumably, nutritive quality varies depending upon the season of browsing (Rea and Gillingham 2001), however, such responses are unmeasured in aspen.

We did not find distinct differences in

shoot/leaf production between clipping treatments as we did between controls and clipped stems. However, we did not assess whether our clipped samples included only new (current year) or a combination of new and old growth. In retrospect, accounting for whether we clipped new or old growth might have helped us discern any effects associated with new and older growth, and possible interactions with time of clipping. We recommend that similar research account for the age of clipped growth as opposed to clipping indiscriminately at the diameter of an average bite.

ACKNOWLEDGMENTS

The authors would like to thank A. Kantakis and D. Hoekstra for their assistance with the clipping trials, and A. Skoblenick for volunteering his time during the harvest period. J. Orlowsky and S. Storch were of great assistance with the experimental design, and maintenance and operation of the growth chamber in the Enhanced Forestry Lab at UNBC. We are grateful to Dr. P. Jackson for providing modeled weather data specific to the study area.

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