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D.D. Briske · T.W. Boutton · Z. Wang

Contribution of flexible allocation priorities to herbivory tolerance in C_4 perennial grasses: an evaluation with ^{13}C labeling

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Abstract The ability of plants to rapidly replace photosynthetic tissues following defoliation represents a resistance strategy referred to as herbivory tolerance. Rapid reprioritization of carbon allocation to regrowing shoots at the expense of roots following defoliation is a widely documented tolerance mechanism. An experiment was conducted in a controlled environment to test the hypothesis that herbivory-sensitive perennial grasses display less flexibility in reprioritizing carbon allocation in response to defoliation than do grasses possessing greater herbivory tolerance. An equivalent proportion of shoot biomass (60% dry weight) was removed from two C_4 perennial grasses recognized as herbivory-sensitive, *Andropogon gerardii* and *Schizachyrium scoparium*, and two C_4 perennial grasses recognized as herbivory-tolerant, *Aristida purpurea* and *Bouteloua rigidiseta*. Both defoliated and undefoliated plants were exposed to $^{13}CO_2$ for 30 min, five plants per species were harvested at 6, 72 and 168 h following labeling, and biomass was analyzed by isotope ratio mass spectrometry. The tallgrass, *A. gerardii*, exhibited inflexible allocation priorities while the shortgrass, *B. rigidiseta*, exhibited flexible allocation priorities in response to defoliation which corresponded with their initial designations as herbivory-sensitive and herbivory-tolerant species, respectively. *A. gerardii* had the greatest percentage and concentration of ^{13}C within roots and lowest percentage of ^{13}C within regrowth of the four species evaluated. In contrast, *B. rigidiseta* had a greater percentage of ^{13}C within regrowth than did *A. gerardii*, the greatest percentage of ^{13}C within new leaves of defoliated plants, and the lowest concentration of ^{13}C within roots following defoliation. Although both midgrasses, *S. scoparium* and *A. purpurea*, demonstrated flexible allocation priorities in response to defoliation, they were counter to those stated in the initial hypothesis. The concentration of ^{13}C within new leaves of *S. scoparium* increased in response to a single defoliation while the percentage and concentration of ^{13}C within roots was reduced. *A. purpurea* was the only species in which the percentage of ^{13}C within new leaves decreased while the percentage of ^{13}C within roots increased following defoliation. The most plausible alternative hypothesis to explain the inconsistency between the demonstrated responsiveness of allocation priorities to defoliation and the recognized herbivory resistance of *S. scoparium* and *A. purpurea* is that the relative ability of these species to avoid herbivory may make an equal or greater contribution to their overall herbivory resistance than does herbivory tolerance. Selective herbivory may contribute to *S. scoparium*'s designation as a herbivory-sensitive species even though it possesses flexible allocation priorities in response to defoliation. Alternatively, the recognized herbivory resistance of *A. purpurea* may be a consequence of infrequent and/or lenient herbivory associated with the expression of avoidance mechanisms, rather than the expression of tolerance mechanisms. A greater understanding of the relative contribution of tolerance and avoidance strategies of herbivory resistance are required to accurately interpret how herbivory influences plant function, competitive interactions, and species abundance in grazed communities.

Key words C_4 perennial grasses · Carbon allocation · ^{13}C labeling · Grazing resistance · Herbivory tolerance

D.D. Briske (✉) · T.W. Boutton · Z. Wang
Department of Rangeland Ecology and Management,
Texas A&M University, College Station TX 77843-2126,
Fax: (409) 845-6430, e-mail: DBRISKE@RLEM.TAMU.EDU

Introduction

The ability of plants to rapidly replace photosynthetic tissues following defoliation represents a resistance strategy referred to as herbivory tolerance (Briske 1991; Rosenthal and Kotanen 1994). Herbivory tolerance is conferred by a number of physiological processes capable of promoting rapid canopy re-establishment (e.g., Caldwell et al. 1981; Hodgkinson et al. 1989). These physiological processes are often interpreted as compensatory mechanisms when their rate following defoliation exceeds the

pre-defoliation rate (Belsky 1986). Rapid canopy re-establishment enables plants to resume pre-defoliation rates of whole-plant photosynthesis more quickly and attain a competitive advantage over plants that are less tolerant of herbivory (Caldwell et al. 1981, 1987; Briske and Richards 1994).

Defoliation reduces the availability of photosynthetic carbon to a greater extent than it does carbon demand (Thornley 1972; Wilson 1988). This creates an imbalance among sources and sinks to modify whole-plant allocation priorities (Briske and Richards 1994, 1995). Carbon allocation is known to play a central role in integrating plant responses to various stresses by supplying substrate for growth and by providing a means of communication among various organs within plants (Geiger and Servaites 1991). Reprioritization of carbon allocation occurs in response to defoliation because the strength of shoot sinks, established by the rate and magnitude of leaf and tiller growth from remaining and/or rapidly activated meristems, exceeds the strength of root sinks (Caldwell et al. 1981; Briske and Richards 1994, 1995). Therefore, the capability of plants to rapidly reprioritize carbon allocation between roots and shoots in response to defoliation may be an important determinant of herbivory tolerance.

Carbon allocation to regrowing shoots at the expense of roots is a consistently documented response to defoliation in numerous plant species (Ryle and Powell 1975; Detling et al. 1979; Danckwerts and Gordon 1987; Briske and Richards 1994). Although the specific signals which detect and restore source-sink imbalances in plants are largely unknown (Geiger 1979; Wardlaw 1990; Geiger and Servaites 1991), whole-plant allocation priorities are assumed to be sink- rather than source-driven (Bucher et al. 1987; Ho 1988). However, minimal insight into the physiological mechanisms and environmental signals regulating growth among various meristematic regions limits our ability to anticipate and interpret defoliation-induced modifications in allocation priorities (e.g., Mueller and Richards 1986; Olson and Richards 1988; Murphy and Briske 1992, 1994). Consequently, our current understanding of defoliation-induced shifts in allocation priorities is largely correlative, rather than causal (Wardlaw 1990).

An experiment was conducted in a controlled environment to test the hypothesis that herbivory-sensitive perennial grasses display less flexibility in reprioritizing carbon allocation in response to defoliation than do grasses possessing greater herbivory tolerance. Research protocol involved selection of two herbivory-sensitive and two herbivory-tolerant C_4 perennial grasses from the southern true prairie of North America. An equivalent proportion of shoot biomass was removed from one-half of the plants in a single defoliation to minimize the confounding effects of disproportionate defoliation intensities based on architectural differences among species. Both defoliated and undefoliated plants were labeled with $^{13}CO_2$ and then harvested at 6, 72 and 168 h following defoliation. This protocol was designed to evaluate rapid, short-term allocation responses among specific plant tissues similar to that conducted for several forage grasses (e.g., Ryle and Powell 1975, 1976). Greater insight into the processes determining the relative expression of herbivory tolerance among plants will increase our understanding of the mechanism(s) contributing to herbivore-induced shifts in competitive interactions and species replacement within plant communities.

Methods

The experiment was conducted in a controlled environment chamber on the campus of Texas A&M University. Four C_4 perennial grasses native to the southern true prairie were selected on the basis of their relative abundance and recognized resistance to grazing by domestic herbivores. Two of the species, *Aristida purpurea* Nutt. var. *longiseta* (Steud.) Vasey, a midgrass, and *Bouteloua rigidiseta* (Steud.) Hitchc. var. *rigidiseta*, a shortgrass, frequently increase in relative abundance in response to intensive grazing by domestic herbivores (Dyksterhuis 1946; Launchbaugh 1955). The other two species, *Schizachyrium scoparium* (Michx.) Nash var. *frequens* (Hubb.), also a midgrass, and *Andropogon gerardii* Vitman var. *gerardii*, a tallgrass, frequently decrease in relative abundance in response to intensive grazing by domestic herbivores.

Seed of *A. gerardii* and *S. scoparium* were obtained from the Soil Conservation Service, Plant Materials Center in Knox City, Texas while seed of the other two species was collected from native populations in south Texas (La Copita Research Area near Alice, Tex.). Seed were germinated on saturated paper blotters in covered plastic trays within a germinator set for a day/night temperature regime of 30°C/22°C and a photosynthetic photon flux density (PPFD) of 46 μmol m^{-2} s^{-1}. Seedlings of comparable size and morphology were planted in plastic tubes (8 cm diameter ×50 cm length) containing fritted clay on 23 October 1993. Distilled water (100 ml) was added to the containers every other day and 50 ml of half-strength Hoaglands' solution was provided weekly. Tubes containing seedlings were placed in an environmental chamber set for a 30°/23°C day/night temperature regime and a mean PPFD of 550 μmol $m^{-2}s^{-1}$ at tube height (approximately 30% of ambient maximum) during a 12-h photoperiod. PPFD was provided by a combination of metal halide and high pressure sodium lamps (12 400-W lamps of each type).

Plants were grown for 3 months prior to defoliation and ^{13}C labeling. Fifteen plants for each of the four species were randomly assigned to a control group and a defoliation treatment. To ensure that comparable defoliation intensities were imposed among species of various heights, height-weight relations were established for each species. These relationships were constructed for each species by dividing individual tillers of five plants into 5 cm increments, weighing each increment, and then regressing tiller height against tiller weight on an incremental basis. These relationships enabled us to uniformly remove 60% of the shoot weight from each of the species with hand clippers.

Immediately following the imposition of defoliation, both defoliated and control plants of all species were placed into a sealed plexiglass chamber (122 cm×91 cm×61 cm) located within the environmental chamber. Then 500 ml of 99 atom% $^{13}CO_2$ was injected into the chamber and circulated with two, 15-cm fans during a 30-min labeling period. Air temperatures within the chamber were measured with two shaded thermocouples located at tube height. Air temperatures within the labeling chamber exceeded ambient air temperatures within the environmental chamber by only 1–3°C during the labeling period.

Five defoliated and five control plants for each species were randomly harvested at 6, 72 and 168 h after labeling. All plants were separated into immature (expanding) blades, mature blades, sheaths (including tiller crowns), culms (when present), and roots. Regrowth was estimated by determining the dry mass of immature leaf tissue produced above defoliation height between harvest

times. *Bouteloua* was the only species to initiate a small number of reproductive culms during the experiment, but they were excluded from data presentation to provide a more direct comparison of allocation priorities with the other three species. Exclusion of culms did not modify either the percentage or concentration expression of ^{13}C data for this species. All plant tissues were dried at 60°C for 48 h, weighed to the nearest 0.1 mg, and ground to pass a 40-mesh screen.

Plant tissues were combusted at 850°C in sealed, evacuated quartz tubes containing an excess of CuO; the CO_2 was separated from other combustion products and purified by cryogenic distillation (Boutton 1991). The $^{13}C/^{12}C$ ratio of the CO_2 was determined on a dual-inlet, triple-collector gas isotope ratio mass spectrometer (VG-903; Middlewich, UK), and the results expressed as $\delta^{13}C_{PDB}$ (‰) values. Overall precision (± 1 SD on 5 replicates of the same sample) ranged from 0.1‰ for samples at natural abundance, to 2.0‰ for samples with $\delta^{13}C_{PDB}$ values greater than 100‰. The carbon concentration of each tissue sample was determined by combustion/gas chromatography (precision ± 0.01%) using a Carlo Erba NA-1500 (Fisons Instruments, Inc., Danvers, Mass.).

All $\delta^{13}C_{PDB}$ values were converted to atom percent excess and then expressed as relative distribution (RD) and relative enrichment (RE) of ^{13}C excess (see Appendix 1 for expressions). RD describes the amount of ^{13}C excess within a specific tissue category expressed as a percentage of the total ^{13}C excess with in the plant. RE describes the concentration of ^{13}C excess per tissue category relative to the mean concentation within the entire plant. RE also standardizes the variation in plant mass within and between species and is interpreted as an index of relative sink strength (Chester and Oechel 1986, Welker et al. 1987). Dry mass, percent total C, and ^{13}C atom percent excess values used to compute RD and RE are presented for each of the four species by tissue category, harvest times, and defoliation treatment in Appendices 2 and 3. Pre-dose (background) $\delta^{13}C$ values were determined by tissue for five plants of each species just prior to labeling. Pre-dose $\delta^{13}C$ values were -16.1 ± 0.3, −14.4 ± 0.5, −16.4 ± 0.3 and −14.6 ± 0.4‰ for *Aristida*, *Andropogon*, *Bouteloua*, and *Schizachyrium*, respectively. These values are consistent with those reported for numerous C_4 grasses (O'Leary 1988).

RD, RE, atom percent excess, percentage carbon, and tissue dry mass were subjected to analysis of variance within a factorial treatment structure. Species (4), treatment (2), and harvest time (3) were main-plot effects and tissues within plants (5) were sub-plot effects. Data were presented as both RD and RE values to indicate whole-plant allocation priorities and relative sink strength by tissue category among species in response to defoliation.

Results

Defoliation produced a significant treatment response within all tissues for RE, but only within new leaves for RD. However, the species × treatment (defoliation) interaction was significant for all tissues except sheaths for RE and old leaves for RD. Therefore, RD and RE means were presented and interpreted in the context of the species×treatment interaction. Both RE and RD means varied significantly with harvest time for all tissue categories except sheaths for RE and new leaves for RD. A species × time interaction was significant for only RE within new leaves.

Relative distribution of ^{13}C

Defoliation reduced RD within new leaves of *Aristida* by 45% compared to nondefoliated *Aristida* plants ($P<0.001$), but RD within new leaves was unaffected in the other three species (Fig. 1). In defoliated plants, new leaves of *Bouteloua* had a significantly ($P<0.05$) greater RD than the other three species. In nondefoliated plants, *Aristida* and *Bouteloua* had significantly ($P<0.001$) greater RD within new leaves than did either *Andropogon* or *Schizachyrium*. RD within new leaves did not vary significantly with harvest time in any species.

Neither treatment nor species or their interaction was significant ($P>0.10$) for RD within old leaves (Fig. 1). However, RD in this tissue category decreased 56% between the first and latter two harvest times ($P<0.001$).

Defoliation increased RD within sheaths of *Aristida* by 36% compared to nondefoliated *Aristida* plants ($P<0.01$), but RD within sheaths was unaffected in the other three species (Fig. 1). In defoliated plants, sheaths of *Aristida*, *Bouteloua*, and *Schizachyrium* had significantly ($P<0.001$) greater RD than did sheaths of *Andropogon* and RD within sheaths of *Schizachyrium* was significantly ($P<0.001$) greater than in those of *Aristida*. In nondefoliated plants, *Bouteloua* and *Schizachyrium* had significantly ($P<0.001$) greater RD within sheaths than did *Aristida* and *Andropogon*. RD in this tissue category showed a modest, but significant ($P<0.05$) decrease, at

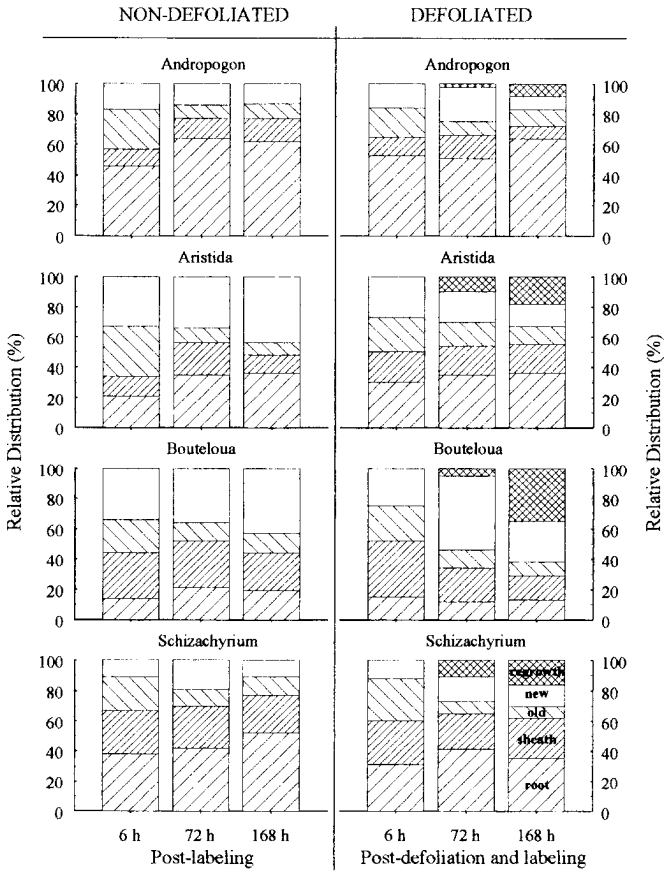

Fig. 1 Relative distribution of ^{13}C excess within five tissue categories of four perennial grasses at 6, 72, and 168 h following exposure to $^{13}CO_2$. One–half of the plants within each species were defoliated just prior to labeling

Table 1 Mean relative enrichment (RE+SE) for five tissues in four perennial grasses harvested at 6, 72, and 168 h after exposure to $^{13}CO_2$. One–half of the plants in each species were defoliated just prior to $^{13}CO_2$ labeling. See Appendix 1 for computation of RE; N = 5.

Species	Time of harvest					
Tissue	6 h		72 h		168 h	
	Undefoliated	Defoliated	Undefoliated	Defoliated	Undefoliated	Defoliated
Andropogon						
Regrowth	–	–	–	4.31±2.96	–	3.63±0.61
New leaf	3.44±0.45[1]	5.27±0.34	2.68±0.58	8.54±2.40	1.97±0.21	2.05±0.47
Old leaf	1.46±0.15	2.43±0.57	0.45±0.13	0.80±0.15	0.52±0.05	1.17±0.17
Sheath	1.27±0.20	1.27±0.14	1.40±0.09	1.37±0.29	1.32±0.20	0.86±0.08
Root	0.70±0.05	0.67±0.06	0.96±0.04	0.77±0.07	1.03±0.02	0.86±0.02
Schizachyrium						
Regrowth	–	–	–	5.70±1.28	–	4.19±0.65
New leaf	2.42±0.34	3.42±0.83	2.81±0.48	5.00±0.10	1.96±0.25	1.19±0.24
Old leaf	1.11±0.15	4.01±0.69	0.57±0.03	2.25±1.07	0.63±0.14	1.16±0.10
Sheath	1.04±0.06	1.08±0.10	1.09±0.05	0.82±0.09	0.94±0.02	0.81±0.03
Root	0.78±0.07	0.52±0.03	0.88±0.04	0.65±0.11	1.04±0.02	0.73±0.06
Aristida						
Regrowth	–	–	–	2.75±0.37	–	3.59±0.65
New leaf	2.69±0.19	3.38±0.87	2.42±0.22	2.55±0.17	1.72±0.14	1.19±0.25
Old leaf	1.65±0.17	2.84±0.52	0.54±0.03	2.23±1.57	0.60±0.16	1.05±0.27
Sheath	0.70±0.17	1.01±0.18	1.19±0.21	0.82±0.09	1.17±0.15	1.00±0.15
Root	0.43±0.06	0.55±0.21	0.73±0.05	0.79±0.12	0.69±0.16	0.77±0.08
Bouteloua						
Regrowth	–	–	–	1.10±0.25	–	3.18±0.35
New leaf	1.70±0.04	1.96±0.12	2.39±0.23	3.08±0.22	2.16±0.18	1.04±0.27
Old leaf	0.92±0.07	1.61±0.10	0.45±0.04	0.79±0.06	0.46±0.07	0.74±0.10
Sheath	0.99±0.04	1.07±0.18	1.16±0.09	0.70±0.14	1.14±0.20	0.58±0.13
Root	0.58±0.05	0.44±0.04	0.73±0.08	0.40±0.05	0.61±0.10	0.38±0.08

the final harvest time compared to the two earlier harvests.

Defoliation increased RD within roots of *Aristida* 29% ($P<0.05$), but reduced RD 18% within roots of *Schizachyrium* ($P = 0.075$) compared to nondefoliated plants (Fig. 1). In defoliated plants, *Andropogon* had the greatest and *Bouteloua* had the least RD within roots ($P<0.001$), while RD within roots of *Schizachyrium* and *Aristida* did not differ significantly ($P>0.10$). In nondefoliated plants, RD within roots differed significantly ($P = 0.01$) among all species with the following ranking: *Andropogon* > *Schizachyrium* > *Aristida* > *Bouteloua*. RD within this tissue category was approximately 30% higher at the latter two harvest times ($P<0.002$) than at the initial harvest.

RD within regrowth was significantly ($P<0.02$) lower in *Andropogon* than in the other three species (Fig. 1). RD within regrowth, averaged for all species, increased 175% between the second and third harvest times ($P<0.001$).

Relative enrichment of ^{13}C

Defoliation significantly ($P<0.01$) increased RE within new leaves of both *Andropogon* and *Schizachyrium* compared to nondefoliated plants (Table 1). In defoliated plants, RE within new leaves of *Andropogon* was significantly ($P<0.05$) greater than in all other species and RE within new leaves of *Schizachyrium* was greater than in *Aristida*. In nondefoliated plants, RE within new leaves did not differ among species ($P<0.10$). Large RE increases within new leaves of *Schizachyrium* at the final harvest and in *Andropogon* at the latter two harvests produced a significant ($P<0.001$) species × time interaction.

Defoliation significantly ($P<0.05$) increased RE within old leaves of *Aristida* (120%), *Bouteloua* (50%) and *Schizachyrium* (220%), but not *Andropogon* (Table 1). In defoliated plants, RE within old leaves of *Aristida* and *Schizachyrium* was significantly ($P<0.01$) greater than in the other two species. In nondefoliated plants, RE within old leaves did not differ significantly ($P>0.10$) among species. RE within old leaves decreased significantly ($P<0.001$) at the two latter harvest times compared to the initial harvest.

Defoliation significantly ($P<0.01$) reduced RE within sheaths of *Schizachyrium* compared to nondefoliated plants, while RE values for the other species were unaffected (Table 1). In both nondefoliated and defoliated plants, RE within sheaths was significantly ($P = 0.05$) greater in *Andropogon* than in the other three species. RE within sheaths did not differ significantly ($P>0.10$) among harvest times.

Defoliation significantly ($P = 0.05$) reduced RE within roots of *Andropogon*, *Bouteloua* and *Schizachyrium*, but not *Aristida*, compared to nondefoliated plants (Table 1). In defoliated plants, RE within roots was significantly greater in *Andropogon* than in those of *Bouteloua* and *Schizachyrium* and RE within roots of *Aristida* was greater than in those of *Bouteloua*. In nondefoliated

plants, RE within roots was significantly greater for *Andropogon* and *Schizachyrium* than in those of *Aristida* and *Bouteloua*. RE within this tissue category was significantly ($P<0.001$) lower at the second and third harvest times compared to the initial harvest.

RE within regrowth of *Andropogon* and *Schizachyrium* was greater than in *Bouteloua* ($P = 0.10$ and 0.007, respectively). RE within regrowth, averaged for all species, did not differ significantly ($P>0.10$) between harvest times.

Discussion

Data derived from this short-term ^{13}C labeling experiment do not falsify the hypothesis that herbivory-sensitive perennial grasses display less flexibility in reprioritizing carbon allocation in response to defoliation than do herbivory-tolerant grasses. However, species designated as herbivory-sensitive and herbivory-tolerant responded inconsistently, indicating either that flexible allocation priorities are not a prerequisite for herbivory tolerance or that the sensitivity of these species to herbivory has not been interpreted correctly. One of the two herbivory-sensitive, *Andropogon*, and herbivory-tolerant species, *Bouteloua*, conformed to the initial hypothesis, but the two remaining species did not.

Andropogon exhibited a limited ability to modify allocation priorities in response to defoliation. It had the greatest percentage of ^{13}C within roots of defoliated plants, lowest percentage of ^{13}C within regrowth of all species, and maintained a higher concentration of ^{13}C within roots following defoliation than did *Schizachyrium*. With the exception of comparable ^{13}C concentrations within new leaves and regrowth to those of *Schizachyrium*, allocation priorities of *Andropogon* were consistent with those of a herbivory-sensitive species. The other species designated as herbivory-sensitive, *Schizachyrium*, demonstrated flexible allocation priorities in response to defoliation. Both the percentage and concentration of ^{13}C within roots decreased and the concentration within new leaves increased in response to a single defoliation. These allocation priorities are counter to those of a herbivory-sensitive species according to the initial hypothesis.

In the two species designated as herbivory-tolerant, *Aristida* displayed the most responsive allocation priorities to defoliation. However, defoliation increased carbon allocation to roots, rather than to shoots as anticipated. *Aristida* was the only species in which the percentage of ^{13}C allocated to new leaves decreased while the percentage allocated to roots increased in response to defoliation. Similarly, the concentration of ^{13}C within roots decreased in all species except *Aristida* following defoliation. However, neither the percentage nor concentration of ^{13}C within regrowth differed significantly between *Schizachyrium* and *Aristida*. The other herbivory-tolerant species, *Bouteloua*, also displayed flexible allocation priorities in response to defoliation. This species had the highest percentage of ^{13}C in new leaves of defoliated plants, a similar percentage of ^{13}C within regrowth compared to *Schizachyrium* and *Aristida*, and the lowest concentration of ^{13}C within roots of defoliated plants. Consequently, of the two species recognized as herbivory-tolerant, only *Bouteloua* conformed to the initial hypothesis while *Aristida* did not.

The limited ability of *Andropogon* to reprioritize carbon allocation in response to defoliation may have partially been determined by its specific life history strategy and stage of phenological development. *Andropogon* plants developed the fewest number of tillers prior to defoliation of the four species investigated. This may have presented a meristematic limitation for rapid leaf replacement and may explain why *Andropogon* allocated the smallest percentage of ^{13}C to regrowth. However, the concentration of ^{13}C within regrowth of *Andropogon* was equal to that of *Schizachyrium* and was greater than that in *Bouteloua* indicating that defoliation did increase the sink strength of shoots, even though the total amount of ^{13}C within regrowth was limited by total tissue mass. Tissues with the most rapid growth rate, rather than size *per se*, are often most competitive as carbon sinks within plants (Wardlaw 1990). Species-specific and environmentally induced architectural variation among grass species have previously been demonstrated to modify allocation priorities by influencing the magnitude and seasonality of tiller initiation and reproductive development (Ryle 1970; Ryle and Powell 1972; Barta 1976; Fick and Moser 1978; Lauer and Simmons 1985).

Defoliation-induced allocation priorities in *Bouteloua* and *Andropogon* were consistent with the allocation priorities documented in a herbivory-tolerant and a herbivory-sensitive perennial grass investigated by Caldwell et al. (1981) and Richards (1984). In fact, the allocation priorities of *Schizachyrium* and *Aristida* are also consistent with their findings, if these two species are redefined as herbivory-tolerant and herbivory-sensitive, respectively. The herbivory-sensitive species, *Pseudoroegneria spicata*, was found to possess a limited capability to shift allocation priorities from roots to shoots in response to defoliation (Caldwell et al. 1981; Richards 1984). It is generally assumed that *P. spicata* experienced limited herbivory throughout its evolutionary history (Mack and Thompson 1982) while the more herbivory-tolerant *Agropyron desertorum*, which was introduced into the United States from the Eurasian steppes at the turn of this century, evolved with more intensive herbivory (Caldwell et al. 1981). Therefore, the inability of *P. spicata* to rapidly reprioritize carbon allocation in response to defoliation appears to be well correlated with its limited herbivory tolerance. Species in the southern true prairie are assumed to have evolved with intensive herbivory which may have functioned as a selective agent contributing to the evolution of greater herbivory resistance (Mack and Thompson 1982; Milchunas et al. 1988). However, the short-term allocation responses evaluated in this investigation do not comprise a direct comparison with the long-term growth and biomass partitioning re-

sponses documented by Caldwell et al. (1981) and Richards (1984).

The absence of a defoliation-induced shift in carbon allocation priorities in the herbivory-tolerant grass, *Cenchrus ciliaris*, investigated by Hodgkinson et al. (1989), also contradicts the hypothesis of flexible allocation priorities in herbivory-tolerant species. Constant carbon allocation priorities may have partially resulted from the development of a decumbent canopy architecture in response to frequent defoliation. Consequently, a portion of the herbivory resistance displayed by *C. ciliaris* may have resulted from the ability to avoid defoliation by developing substantial leaf area below defoliation height (Hodgkinson et al. 1989). Development of a decumbent canopy architecture may have prevented removal of sufficient leaf area to induce an imbalance between root and shoot sinks, or sufficient leaf area may have remained to allow continued carbon allocation to the root system. It is even less clear why frequent defoliation increased carbon allocation to roots in the herbivory-sensitive *Themeda triandra* (Hodgkinson et al. 1989), although this allocation response is consistent with that of a herbivory-sensitive species. A similar response was induced by a single defoliation in *Aristida* in this investigation. The adaptive value of increasing carbon allocation to roots following defoliation is difficult to interpret within the context of herbivory tolerance, but it may be associated with compensatory rates of nutrient absorption (e.g., Polley and Detling 1988; Black et al. 1994). Increases in specific root respiration rate and nutrient absorption capacity in response to defoliation have been most frequently observed in graminoids grown in nutrient-limited conditions (e.g., Chapin and Slack 1979; McNaughton and Chapin 1985).

Allocation priorities among the four native, C_4 perennial grasses varied considerably in the absence of defoliation and did not always conform to those reported for forage grasses (Fig. 1). Allocation priorities in undefoliated forage grasses are often greatest in immature blades followed by leaf sheaths, roots, and mature blades (Williams 1964; Ryle and Powell 1975, 1976; Muldoon and Pearson 1979; Danckwerts and Gordon 1987). In this investigation, the two largest grasses, *Andropogon* and *Schizachyrium*, allocated the greatest percentage of ^{13}C to roots and the least to new leaves. Old and new leaves were comparable sinks in *Schizachyrium* and *Andropogon*, new leaves were the strongest sink in *Bouteloua*, and roots were comparable sinks to new leaves in *Aristida*. Large interspecific variation in allocation priorities is not suprising given the range of growth forms represented within these four species (i.e., tall-, mid-, and shortgrass species).

The stable isotope, ^{13}C, effectively quantified the allocation priorities of both undefoliated and defoliated plants. However, allocation priorities within plants were very likely affected by the presence of constant growth conditions and high resource availability within the environmental chamber and the use of young, containerized plants. Resource availability has been demonstrated to modify allocation priorities (Wardlaw 1968; Chung and Trlica 1980; Prud'homme et al. 1993), but there is no reason to assume that high resource availability would have induced disproportionate allocation priorities among species. It is also possible that the use of containerized plants may have modified root:shoot ratios and the magnitude of carbon allocation between these organs (e.g., Robbins and Pharr 1988; Thomas and Strain 1991). Many of these experimental constraints could be minimized, if it were possible to efficiently retrieve the entire root systems of mature plants in the field for isotopic analysis.

Our experimental data do not falsify the hypothesis that herbivory tolerance among four perennial grasses from the southern true prairie is associated with the capacity to rapidly reprioritize carbon allocation from roots to shoots in response to defoliation. The tallgrass, *Andropogon*, and the shortgrass, *Bouteloua*, conformed to their respective designations as herbivory-sensitive and herbivory-tolerant. Although both midgrasses, *Schizachyrium* and *Aristida*, demonstrated flexible allocation priorities in response to defoliation, they were counter to those presented in the initial hypothesis. If it is assumed that flexible carbon allocation is essential for effective herbivory tolerance, then an alternative hypothesis is required to explain the inconsistency between the observed allocation priorities and the recognized herbivory resistance of these two midgrasses. The most plausible alternative hypothesis is that the relative ability of these species to avoid herbivory may be a more important determinant of herbivory resistance than is herbivory tolerance (Atkinson 1986; Brown and Stuth 1993; Anderson and Briske 1995). *Schizachyrium* appears to be selectively grazed by domestic herbivores in response to a relatively limited expression of avoidance mechanisms while *Aristida* is grazed infrequently and/or leniently in response to a greater expression of avoidance mechanisms (Heitschmidt et al. 1990; Anderson and Briske 1995). This pattern of selective herbivory would confer a competitive advantage on *Aristida*, even if *Schizachyrium* was the more tolerant species to herbivory. A greater understanding of the relative contribution of the tolerance and avoidance strategies of herbivory resistance are required to accurately interpret how herbivory influences plant function, competitive interactions, and species abundance in grazed communities.

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Appendix 1 Expressions required to compute mg excess ^{13}C, relative distribution of excess ^{13}C, and relative enrichment of excess ^{13}C. R represents the absolute ratio ($^{13}C/^{12}C$) and F represents the fractional abundance [$^{13}C/(^{13}C + ^{12}C)$]. These expressions were used to quantify the flexibility of allocation priorities following defoliation in C_4 perennial grasses. Expressions were derived from Boutton (1991). The absolute $^{13}C/^{12}C$ for R_{PDB} is 0.0112372.

a. $\delta\,^{13}C_{PDB} = \left[\frac{R_{sample}}{R_{PDB}} - 1\right] \times 1000$

b. $R_{sample} = \left[\frac{\delta^{13}C_{PDB}}{1000} + 1\right] \times R_{PDB}$

c. $F = \left[\frac{R_{sample}}{R_{sample} + 1}\right]$

d. Atom% excess $= (F_{post\text{-}dose} - F_{pre\text{-}dose}) \times 100$

e. mg C = tissue dry mass x% C

f. mg excess ^{13}C = mg of C in tissue × atom% excess

g. Relative distribution $= \frac{\text{mg excess } ^{13}C \text{ in tissue}}{\text{mg excess } ^{13}C \text{ in plant}} \times 100$

h. Relative enrichment $= \frac{\text{mg excess } ^{13}C/\text{g tissue in dry mass}}{\text{mg excess } ^{13}C/\text{g plant in dry mass}}$

Appendix 2 Mean dry mass (*DM*, mg + SE) and mean percent carbon (+SE) for five tissues in four perennial grasses harvested at 6, 72, and 168 h after exposure to $^{13}CO_2$. Half of the plants in each species were defoliated just prior to $^{13}CO_2$ labeling

Species		Time of harvest					
Tissue		6 h		72 h		168 h	
		Undefoliated	Defoliated	Undefoliated	Defoliated	Undefoliated	Defoliated
Andropogon							
Regrowth	DM	–	–	–	7.0±2.3	–	17.9±4.9
	%C	–	–	–	47.0±1.3	–	49.9±0.6
New leaf	DM[a]	50.9±14.1	28.9±5.7	57.7±13.5	19.4±7.6	95.3±37.5	37.5±12.4
	%C[b]	47.6±0.4	47.7±0.2	47.7±0.3	48.6±0.8	41.3±0.4	48.6±0.4
Old leaf	DM	177.2±35.0	76.8±20.9	191.4±33.3	53.9±12.5	303.4±100.7	100.2±31.6
	%C	45.0±0.7	44.1±0.8	45.1±0.4	45.7±0.8	42.8±1.6	46.7±0.2
Sheath	DM	88.6±20.0	90.4±21.5	98.0±19.4	66.6±20.0	186.5±60.0	103.9±31.5
	%C	43.3±0.4	43.1±0.6	44.8±0.5	45.2±0.5	44.6±0.1	45.9±0.3
Root	DM	631.0±116.0	760.7±158.3	694.8±134.0	401.3±135.8	901.0±290.5	771.9±259.8
	%C	45.1±1.3	47.2±0.5	46.4±1.3	49.1±0.6	47.4±0.4	48.3±0.8
Schizachyrium							
Regrowth	DM	–	–	–	26.6±9.0	–	58.6±15.4
	%C	–	–	–	49.9±1.3	–	46.3±0.4
New leaf	DM	73.6±20.9	44.6±11.0	86.5±9.5	50.0±10.9	88.3±30.1	119.9±42.4
	%C	50.4±0.3	49.6±0.3	50.0±0.2	50.1±0.3	46.6±0.7	45.7±0.2
Old leaf	DM	356.1±142.5	122.1±53.4	264.9±64.9	94.3±29.9	265.6±83.4	116.4±30.6
	%C	48.2±0.4	49.3±0.6	46.2±0.4	48.6±0.5	43.4±0.6	43.7±0.5
Sheath	DM	470.5±176.3	394.7±131.5	383.2±114.2	500.9±136.9	423.4±141.0	536.9±145.2
	%C	49.2±0.3	50.1±0.4	47.4±0.4	48.6±0.5	44.4±0.4	43.5±0.2
Root	DM	902.4±347.6	753.8±132.3	709.4±228.0	1058.3±255.4	807.2±265.0	772.1±163.5
	%C	51.2±0.6	52.4±0.3	49.5±0.5	51.0±0.5	45.8±0.5	46.4±0.8
Aristida							
Regrowth	DM	–	–	–	7.6±3.9	–	11.5±3.7
	%C	–	–	–	–	–	46.6±–
New leaf	DM	34.6±2.1	21.6±4.9	34.3±9.4	17.4±4.3	58.1±10.1	31.4±7.1
	%C	45.7±0.3	45.5±0.3	48.8±1.0	45.9±0.3	43.9±0.1	42.2±0.3
Old leaf	DM	58.9±9.9	22.9±5.7	44.1±8.4	22.9±5.2	47.7±10.8	24.2±2.4
	%C	44.2±0.5	44.6±0.2	46.0±0.7	44.9±0.9	42.1±0.5	39.5±0.3
Sheath	DM	51.6±6.5	54.5±8.5	44.1±9.0	50.5±15.9	38.1±8.8	46.2±3.5
	%C	35.7±7.8	43.7±0.3	43.0±0.4	44.0±0.4	40.8±0.5	38.4±0.3
Root	DM	143.6±23.1	152.5±30.5	112.4±20.5	129.1±54.4	171.8±56.4	114.1±19.2
	%C	45.7±2.0	48.6±0.4	48.2±0.4	48.7±0.4	44.5±0.6	42.0±0.3
Bouteloua							
Regrowth	DM	–	–	–	14.8±4.6	–	37.8±9.9
	%C	–	–	–	44.0±0.8	–	45.0±0.4
New leaf	DM	77.1±18.0	60.9±7.6	131.9±21.1	50.2±8.9	127.4±34.0	46.3±10.4
	%C	44.6±0.3	45.5±0.7	48.0±0.3	47.8±0.1	47.3±0.2	45.5±0.2
Old leaf	DM	99.8±29.8	73.3±15.3	229.0±36.9	48.9±10.8	189.3±71.1	53.4±25.8
	%C	40.5±1.2	44.3±1.0	40.7±0.9	42.6±0.6	40.7±0.8	39.4±1.2
Sheath	DW	125.8±30.5	185.5±34.8	226.9±35.2	105.5±36.5	163.3±52.3	107.3±40.4
	%C	44.6±0.3	46.7±0.3	43.9±0.7	45.1±0.3	43.5±0.4	43.0±0.2
Root	DM	102.4±29.4	226.0±86.8	240.1±22.0	106.8±32.6	214.1±88.6	148.6±72.5
	%C	50.7±0.3	50.0±1.0	44.7±2.9	49.6±1.9	48.5±0.6	46.7±1.5

[a] Dry mass of tissues was significantly different ($P<0.05$) for all species, harvest times and defoliation treatment with the exception of harvest time for roots.

[b] Percentage carbon of tissues was significantly different ($P<0.05$) for all species, harvest times and defoliation treatment with the exception of harvest time for regrowth

Appendix 3 Mean atom percent excess (APE ±SE) for five tissues in four perennial grasses harvested at 6, 72, and 168 h after exposure to $^{13}CO_2$. Half of the plants in each species were defoliated just prior to $^{13}CO_2$ labeling

Species	Time of harvest					
Tissue	6 h,		72 h		168 h	
	Undefoliated	Defoliated	Undefoliated	Defoliated	Undefoliated	Defoliated
Andropogon						
Regrowth	–	–	–	0.104±0.033	–	0.129±0.010
New leaf	0.312±0.013[a]	0.220±0.021	0.226±0.059	0.237±0.033	0.168±0.016	0.088±0.020
Old leaf	0.138±0.009	0.112±0.030	0.040±0.009	0.028±0.003	0.045±0.008	0.049±0.008
Sheath	0.129±0.022	0.057±0.005	0.119±0.011	0.046±0.010	0.102±0.009	0.038±0.005
Root	0.068±0.009	0.029±0.004	0.081±0.011	0.025±0.006	0.079±0.011	0.036±0.004
Schizachyrium						
Regrowth	–	–	–	0.117±0.029	–	0.089±0.018
New leaf	0.208±0.040	0.093±0.025	0.174±0.028	0.106±0.014	0.102±0.013	0.040±0.005
Old leaf	0.096±0.011	0.086±0.005	0.038±0.003	0.039±0.011	0.038±0.012	0.026±0.004
Sheath	0.090±0.011	0.025±0.004	0.073±0.009	0.018±0.004	0.053±0.004	0.018±0.002
Root	0.063±0.005	0.013±0.003	0.055±0.005	0.014±0.003	0.056±0.004	0.014±0.001
Aristida						
Regrowth	–	–	–	0.123±0.034	–	0.113±0.022
New leaf	0.234±0.021	0.175±0.040	0.214±0.022	0.142±0.011	0.158±0.019	0.037±0.008
Old leaf	0.144±0.009	0.131±0.020	0.051±0.006	0.089±0.065	0.047±0.012	0.032±0.009
Sheath	0.086±0.016	0.048±0.008	0.126±0.027	0.037±0.010	0.090±0.011	0.032±0.004
Root	0.039±0.008	0.035±0.022	0.070±0.012	0.031±0.007	0.058±0.013	0.023±0.001
Bouteloua						
Regrowth	–	–	–	0.047±0.007	–	0.129±0.014
New leaf	0.212±0.022	0.101±0.011	0.191±0.008	0.128±0.010	0.157±0.007	0.078±0.008
Old leaf	0.125±0.009	0.084±0.007	0.043±0.003	0.037±0.002	0.039±0.005	0.036±0.007
Sheath	0.124±0.013	0.054±0.010	0.103±0.009	0.031±0.007	0.092±0.018	0.026±0.006
Root	0.063±0.006	0.021±0.002	0.065±0.010	0.016±0.002	0.045±0.008	0.016±0.004

[a] APE for new leaf, old leaf, sheaths and roots was significantly different ($P<0.05$) for all species, harvest times and defoliation treatment with the exception of harvest time for roots. APE for regrowth did not differ significantly ($P>0.05$) for species or harvest time

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