Green Needlegrass and Blue Grama Seedling Growth in Controlled Environments

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Abstract

Green needlegrass [*Stipa viridula* Trin.] and blue grama [*Bouteloua gracilis* (H.B.K.) Lag.] possess the C₃ and C₄ photosynthetic pathways, respectively. Objectives of this study were to compare growth analysis traits of green needlegrass and blue grama and to determine the effects of 2 temperature regimes on seedling growth characteristics of both species. Seedlings of 2 accessions each of green needlegrass and blue grama were grown in growth chambers under 20° C day-15° C night (20-15° C) and 25° C day-20° C night (25-20° C) temperature regimes (with a 15-hr photoperiod). Beginning 2 weeks after planting, seedlings were harvested twice a week for 3 weeks. Growth analysis traits were calculated with data obtained from each harvest using exponential regression equations. Net assimilation rates of blue grama were higher than those of green needlegrass at both temperatures. Seedling growth of blue grama was more rapid at 25-20° than at 20-15° C, while seedling growth of green needlegrass did not differ between temperatures. Blue grama seedlings exhibited higher relative growth rates than green needlegrass seedlings at 25-20° C but not at 20-15° C. Green needlegrass accessions differed for relative growth rates at 25-20° C, which indicated the possibility of selecting for rapid seedling growth.

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Research was supported in part by the U.S. Department of Energy under Contract No. DE-AS02-76EV04018.

Manuscript accepted October 29, 1984.

Amount of leaf area present and net assimilation rate (NAR) govern the rate of seedling growth. Growth analysis provides a means of determining the relative importance of leaf area and net assimilation rate in seedling growth rates (Patterson et al. 1978).

Potter and Jones (1977) found plant growth to be more closely correlated with leaf area partitioning coefficient (LAP) than with NAR. LAP indicates that extent of partitioning of daily assimilation into new leaf area. Relative leaf area expansion rate (K_a) is the product of NAR and LAP. Various investigators have reported high correlations between relative leaf area expansion rates and growth (Potter and Jones 1977, Patterson et al. 1978, Townsend and Wilson 1981).

Green needlegrass (*Stipa viridula* Trin.) possesses the C₃ photosynthetic pathway (Waller and Lewis 1979). Blue grama [*Bouteloua gracilis* (H.B.K.) Lag.], a common associate of green needlegrass throughout much of its range, possesses the C₄ pathway. Growth rates of blue grama and green needlegrass would be expected to differ because of the physiological dissimilarities between the 2 species. Objectives of this study were to use growth analysis traits to (1) explain differences in seedling growth rates between blue grama and green needlegrass and (2) determine the influence of temperature on seedling growth of each species. Growth analysis can be used in screening plant materials for traits that enhance seedling vigor (Townsend and Wilson 1978, 1981). A third objective of the study was to determine the influence of accession on seedling growth of each species.

Materials and Methods

Seeds of Lodorm and accession SD-93 green needlegrass were obtained from the USDA Soil Conservation Service Plant Materials Center at Bismarck, N. Dak. Lodorm is a variety of green needlegrass with reduced seed dormancy (Larson and Carter 1970). Blue grama accessions were PM-K-1482 and PM-K-1483. Accession PM-K-1482 is a synthetic blend of 6 blue grama accessions that originated in Nebraska and northern Kansas. Accession PM-K-1483 is a synthetic blend of 12 blue grama accessions from southern Kansas and northwestern Texas.

Seeds were planted in plastic pots (15 cm diameter by 15 cm deep) filled with autoclaved sandy loam soil. Thirty seeds were placed in each pot and covered with 2 mm of soil. Soil in each pot was watered to approximately field capacity. Seeds were then covered with a 2-cm layer of dry soil.

The experiment was conducted in 2 sequential phases: (1) a 14-day emergence and initial seedling growth phase and (2) a 21-day temperature treatment phase. In the first phase pots were placed in a growth chamber at a 20-15°C temperature regime with a 15-hr photoperiod. Photosynthetic photon flux density (PPFD) was 400 $\mu\text{E m}^{-2}\text{s}^{-1}$. Pots were subirrigated to retain the dry surface layer of soil. Soil below the dry layer was maintained at about field capacity.

In the second phase of the experiment, pots were removed from the first growth chamber and seedlings were thinned to the 5 largest in each pot. A layer of gravel was placed on the soil surface of each pot to reduce evaporation. Pots were then placed in 1 of 2 growth chambers adjusted for temperature regimes of 20-15°C and 25-20°C. PPFD was 450 $\mu\text{E m}^{-2}\text{s}^{-1}$ during the 15-hr photoperiod. Pots were surface irrigated when necessary during the second phase of the experiment to maintain the soil at field capacity.

Seedlings were harvested immediately before the temperature treatment phase and then twice a week for 3 weeks. A total of 7 harvests were made. All data were based on averages of the 5 seedlings in each pot. Each harvest consisted of first counting tillers then excising excerted leaf blades. Leaf blade area was measured with an automatic leaf area meter having a sensitivity of 0.1 mm. Soil was removed from each pot, placed on a screen, and carefully washed from seedling roots with a fine spray of water. Seedlings were then taken to the laboratory and placed on moist blotter paper. Seminal and adventitious roots were excised with a razor blade and counted. Leaf blades, roots, and other seedling parts were oven-dried at 60°C for 24 hours and then weighed.

Data obtained from the 7 harvests were used to calculate the following seedling growth traits: net assimilation rate (NAR), leaf area partitioning coefficient (LAP), relative leaf area expansion rate (K_a), relative growth rate (K_w), relative rate of shoot development (K_s), and relative rate of root development per shoot (K_r). Definitions and units of measurement for each growth trait are given in Table 1.

Seedling growth traits were calculated using exponential regression equations described by Potter and Jones (1977) of the form:

$$W = W_0 e^{K_w t}$$

where W is total seedling weight (mg) at any given time, W_0 is initial seedling weight (mg), K_w is relative growth rate, and t is time (days). The value of t in calculations of NAR and LAP was 14 since LAP and NAR were estimated 14 days after the start of the 2 temperature treatments. Values for each seedling growth trait were calculated for each temperature regime, accession, and replication to allow statistical comparisons.

A randomized complete-block design with a factorial arrangement of treatments was used in the experiment. Factors were temperature regimes, accessions, and harvest dates. Five replications were conducted sequentially over time and data were analyzed by analysis of variance and polynomial regression. Simple correlation coefficients (r) and linear regression coefficients (b)

Table 1. Definitions and units of measurement of traits used to describe seedling growth of 2 accessions each of green needlegrass and blue grama.¹

Trait	Definition ²	Units
Net assimilation rate (NAR) ³	dW/dt	mg day^{-1}
	A	cm^2
Leaf area partitioning coefficient (LAP) ³	dA/dt	$\text{cm}^2 \text{ day}^{-1}$
	dW/dt	mg day^{-1}
Relative leaf area expansion rate (K_a)	dA/dt	$\text{cm}^2 \text{ day}^{-1}$
	A	cm^2
Relative growth rate (K_w)	dW/dt	mg day^{-1}
	W	mg
Relative rate of shoot development (K_s)	dS/dt	No. shoots day^{-1}
	S	No. shoots
Relative rate of root development (K_r)	dR/dt	No. roots day^{-1}
	S	No. shoots

¹Adapted from Townsend and Wilson (1981).

²The following abbreviations were used: A, leaf area; S, number of shoots (prior to treatment); W, total seedling weight; dW/dt , rate of change in seedling weight; dA/dt , rate of change in leaf area; dS/dt , rate of change in the number of shoots; dR/dt , rate of change in the number of roots.

³LAP and NAR were estimated on the fifth harvest date (14 days after the beginning of temperature treatments).

were calculated to examine relationships between seedling growth traits. All differences were significant at the 0.05 level of probability unless otherwise stated.

Results

Leaf Blade Area and Total Seedling Weight

Green needlegrass seedlings developed greater leaf blade area than blue grama seedlings under the 20-15°C temperature regime (Table 2). Leaf blade area of green needlegrass seedlings did not differ between temperature treatments while that of blue grama was greater under the warmer temperature regime than under the cooler regime. Lodorm green needlegrass seedlings had greater leaf blade area than SD-93 green needlegrass seedlings under both temperature regimes. Seedlings of accession PM-K-1483 blue grama developed greater leaf blade area than PM-K-1482 blue grama seedlings under the 25-20°C temperature regime but not under the 20-15°C regime.

Leaf blade area of green needlegrass seedlings was more than double that of blue grama seedlings on the first harvest date (Table 2). Leaf blade area on the first harvest date was highly correlated ($r=0.81$, $p<0.01$) with caryopsis weight. Weight of Lodorm and SD-93 green needlegrass caryopses averaged 1.2 and 1.5 mg, respectively, compared to 0.4 mg for caryopses of the blue grama accessions. Differences in leaf blade area between species were probably associated with differences in caryopsis weight. Townsend and Wilson (1981) found that seed weight of cicer milkvetch [*Astragalus cicer* L.] was positively associated with initial leaf area and initial seedling weight, but net assimilation rate, leaf area partitioning coefficient, relative leaf area expansion rate, and relative growth rate were entirely independent of seed weight.

Total weight of green needlegrass seedlings was 28% higher than that of blue grama seedlings on the final harvest date under the 20-15°C temperature regime, but total weight of green needlegrass seedlings was 32% lower than that of blue grama seedlings at 25-20°C on the final harvest date (Table 2). Total weight of green needlegrass seedlings did not differ between temperature treatments, while blue grama seedlings developed greater total weight at 25-20°C than at 20-15°C. Lodorm green needlegrass seedlings accumulated more dry matter than SD-93 seedlings. Seedlings of accession PM-K-1483 blue grama developed greater total seedling weight than PM-K-1482 blue grama seedlings under the 25-20°C

Table 2. Leaf blade area (cm²) and total seedling weight (mg) of 2 accessions each of green needlegrass and blue grama grown under 2 different temperature regimes.

Species	Accession and days after planting	Leaf blade area ¹ (cm ²)		Total seedling weight ¹ (mg)	
		20-15°C	25-20°C	20-15°C	25-20°C
Green needlegrass	Lodorm				
	14	1.7	1.5	18	16
	18	2.3	3.0	24	28
	21	5.1	5.9	51	60
	25	8.5	11.2	99	118
	28	16.9	17.7	180	220
	32	23.1	26.8	326	374
	35	35.7	34.5	501	533
	$\bar{x} \pm 95\%$	13.3 $\pm$ 1.2	14.4 $\pm$ 1.2	171 $\pm$ 24	193 $\pm$ 24
	Confidence interval				
	SD-93				
	14	1.2	1.7	15	19
	18	2.4	2.9	28	31
	21	4.6	5.3	51	58
	25	8.7	9.1	97	109
	28	14.9	15.0	180	187
	32	19.6	20.9	257	284
	35	29.9	31.6	409	462
	$\bar{x} \pm 95\%$	11.6 $\pm$ 1.2	12.4 $\pm$ 1.2	148 $\pm$ 24	164 $\pm$ 24
	Confidence interval				
Blue Grama	PM-K-1482				
	14	0.6	0.5	15	13
	18	0.9	1.4	24	29
	21	1.6	2.8	39	54
	25	3.7	7.3	80	127
	28	6.3	11.8	128	216
	32	9.4	20.8	200	378
	35	16.9	31.5	349	691
	$\bar{x} \pm 95\%$	5.6 $\pm$ 1.2	10.9 $\pm$ 1.2	119 $\pm$ 24	215 $\pm$ 24
	Confidence Interval				
	PM-K-1483				
	14	0.5	0.6	13	16
	18	1.1	1.5	25	31
	21	2.3	3.4	47	62
	25	3.7	7.9	77	136
	28	6.9	13.6	141	237
	32	11.5	26.3	239	463
	35	18.0	35.0	362	774
$\bar{x} \pm 95\%$	6.3 $\pm$ 1.2	12.6 $\pm$ 1.2	129 $\pm$ 24	246 $\pm$ 24	
Confidence Interval					

¹Analysis of variance indicated significant ($p < 0.05$) main effects for temperatures, accessions, and planting dates. In addition, all 2-way and 3-way interactions were significant ($p < 0.05$). The pooled confidence interval is appropriate for differences between treatment combinations but not for identifying significantly different main effects and interaction means.

temperature regime but not under the 20-15°C regime.

Net Assimilation Rate (NAR) and Leaf Area Partitioning Coefficient (LAP)

Blue grama seedlings exhibited higher values for NAR than did green needlegrass seedlings (Table 3). NAR values were higher at 25-20°C than at 20-15°C. NAR did not differ between green needlegrass accessions or between blue grama accessions.

Green needlegrass seedlings had higher values for LAP than blue grama seedlings at both temperatures (Fig. 1). There was an interaction of accession with temperature for LAP. LAP of green needlegrass seedlings tended to be lower at 25-20°C, while LAP of blue grama seedlings tended to be higher at 25-20°C than at 20-15°C. No difference in LAP existed between green needlegrass accessions or between blue grama accessions.

Relative Leaf Area Expansion Rate (K_a) and Relative Growth Rate (K_w)

Blue grama seedlings had higher values for K_a than green needlegrass seedlings at 25-20°C but not at 20-15°C (Fig. 2). K_a of

green needlegrass seedlings did not differ between temperatures. Blue grama seedlings had 17% higher values for K_a under the 25-20°C temperature regime than under the 20-15°C regime. No difference existed between green needlegrass accessions or between blue grama accessions for K_a.

K_w of blue grama seedlings exceeded that of green needlegrass seedlings at 25-20°C (Fig. 3). Blue grama and green needlegrass had similar values for K_w at 20-15°C. K_w of green needlegrass did not differ between temperature treatments. Blue grama seedlings had higher values for K_w under the 25-20°C temperature regime than under the 20-15°C regime. Lodorm green needlegrass seedlings exhibited higher values for K_w than SD-93 green needlegrass seedlings at 25-20°C but not at 20-15°C. There was no difference in K_w between blue grama accessions.

Differences in growth rates between green needlegrass and blue grama seedlings can be explained by the relationship between K_w and K_a. K_w was more highly correlated with K_a than with NAR for all accessions except SD-93 green needlegrass (Table 4). There was no significant correlation between K_w and LAP. Thus, blue grama

Table 3. Net assimilation rate (NAR), relative rate of shoot development (K_s), and relative rate of root development per shoot (K_r) of 2 accessions each of green needlegrass and blue grama grown under 2 temperature regimes.

Species	Temperature and accession	Seedling growth trait ^{1,2,3,4}		
		NAR	K_s	K_r
Green needlegrass	20-15°C			
	Lodorm	2.04	0.06	0.27
	SD-93	1.98	0.06	0.33
	PM-K-1482	3.30	0.08	0.11
Blue grama	20-15°C			
	PM-K-1483	3.28	0.09	0.10
	25-20°C			
	Lodorm	2.19	0.07	0.31
Green needlegrass	25-20°C			
	SD-93	1.98	0.07	0.34
	PM-K-1482	3.76	0.09	0.13
	PM-K-1483	3.56	0.10	0.13
95% Confidence Interval		$\bar{x} \pm 0.34$	$\bar{x} \pm 0.01$	$\bar{x} \pm 0.03$

¹Values of R^2 for equations used to calculate these traits for each replication ranged from 0.83 to 0.99.

²Interactions of temperature treatments with accessions were not significant ($p > 0.05$) for NAR, K_s , and K_r . Main effects of temperature and accession were significant ($p < 0.05$) for NAR, K_s , and K_r .

³NAR in $\text{mg day}^{-1} \text{cm}^{-2}$; K_s in no. of shoots day^{-1} no. of shoots; K_r in no. of roots day^{-1} no. of shoots.

⁴The pooled confidence interval is appropriate for differences between treatment combinations but not for identifying significantly different main effects and interaction means.

Fig. 1. Temperature effects on leaf area partitioning coefficient (LAP) of 2 accessions each of green needlegrass and blue grama. The interaction of accession and temperature was significant ($p < 0.05$). The main effect of temperature was not significant ($p > 0.05$), and the main effect of accession was significant ($p < 0.05$). The pooled confidence intervals are appropriate for differences between treatment combinations but not for identifying significantly different main effects and interaction means.

Fig. 2. Temperature effects on relative leaf area expansion rate (K_a) of 2 accessions each of green needlegrass and blue grama. The interaction of accession and temperature was significant ($p < 0.05$). Main effects of temperature and accession were also significant ($p < 0.05$). The pooled confidence interval is appropriate for differences between treatment combinations but not for identifying significantly different main effects and interaction means.

seedlings had higher K_w values than green needlegrass at 25-20°C because K_a values of blue grama were higher than those of green needlegrass seedlings (Table 3). K_w values of the 2 species were similar under the 20-15°C temperature regimes because K_a values did not differ.

Differences between species in the effect of temperature on growth rates can also be explained by the relationship between K_w and K_a . K_w of green needlegrass seedlings did not differ between temperature treatments because K_a did not differ between temperatures (Table 3). Blue grama had a higher K_w at 25-20°C than at 20-15°C because K_a was highest at 25-20°C.

The higher K_w of Lodorm green needlegrass seedlings in comparison to SD-93 green needlegrass seedlings may have resulted in part from the greater leaf blade area of Lodorm. Also, K_a and NAR of Lodorm tended to be higher than that of SD-93, although neither trait differed significantly between the 2 accessions. Blue grama accessions did not exhibit differences in K_w because they did not differ in K_a , LAP, or NAR.

Relative Rate of Shoot Development (K_s) and Relative Rate of Root Development Per Shoot (K_r)

Blue grama seedlings exhibited a higher K_s than green needlegrass seedlings (Table 3). Blue grama seedlings had an average of 1.2 tillers on the first harvest date, when seedlings were 2 weeks old. Green needlegrass seedlings did not develop tillers until the third harvest date, when seedlings were 3 weeks old. Blue grama seedlings had an average of 15.6 tillers on the final harvest date at 25-20°C, compared to 2.6 for green needlegrass. K_s was more rapid at 25-20°C than at 20-15°C and did not differ between accessions for either species.

Green needlegrass seedlings had higher values for K_r than did blue grama seedlings under both temperature treatments (Table 3). The higher values of K_r for green needlegrass possibly resulted from the greater size of green needlegrass shoots and deeper

Fig. 3. Temperature effects on relative growth rate (K_w) of 2 accessions each of green needlegrass and blue grama. The interaction of accession and temperature was significant ($p < 0.05$). Main effects of temperature and accession were also significant ($p < 0.05$). The pooled confidence intervals are appropriate for differences between treatment combinations but not for identifying significantly different main effects and interaction means.

placement of the coleoptilar node, from which the adventitious roots develop, in the soil. Although K_r of green needlegrass was higher than that of blue grama, blue grama seedlings developed a greater total number of adventitious roots than green needlegrass seedlings because blue grama seedlings developed a greater total number of shoots than green needlegrass seedlings. Blue grama seedlings had an average of 22.4 adventitious roots per seedling on the final harvest date at 25-20°C, compared to 12.9 for green needlegrass seedlings. K_r was more rapid at 25-20°C than at 20-15°C. SD-93 green needlegrass seedlings had higher values for K_r than did Lodorm seedlings. Blue grama accessions did not differ in K_r .

Discussion

Growth rate differences between blue grama and green needlegrass would probably have been more pronounced at higher light intensities than those used in this study. The light intensity in the temperature treatment phase of the experiment was less than 25% of full sunlight. Green needlegrass was probably near light saturation, while blue grama was much below its light saturation level. Photosynthesis of most C_3 species is saturated at intensities of 25%

Table 4. Simple correlation coefficients (r) and linear regression coefficients (b) for the relationship between relative growth rate (K_w) and net assimilation rate (NAR), leaf area partitioning coefficient (LAP), and relative leaf area expansion rate (K_a) of 2 accessions each of green needlegrass and blue grama.

Bivariate relationship ¹ and coefficient	Accession			
	Lodorm	SD-93	PM-K-1482	PM-K-1483
K_w -NAR				
r	0.69*	0.78**	0.85**	0.75*
b	0.02	0.03	0.04	0.04
K_w -LAP				
r	-0.43	-0.52	-0.11	0.38
b	-0.46	-0.68	-0.56	1.54
K_w - K_a				
r	0.76*	0.68*	0.98**	0.96**
b	0.87	0.71	1.02	0.82

***Significant at the 0.05 and 0.01 levels, respectively.

¹The dependent variable is listed first for each growth characteristic relationship.

²Data represents the combined analysis of data from the 20-15°C and 25-20°C temperature treatments.

of full sunlight, while photosynthesis of C_4 species is generally not saturated at 100% of full sunlight (Moore 1977).

Potter and Jones (1977) reported that K_w was highly correlated with LAP for 7 of the 9 species they studied and that a low correlation between K_w and NAR existed for 5 species. NAR was a more important factor than LAP in growth of green needlegrass and blue grama seedlings under the conditions of our study. Potter and Jones (1977) found that temperature differences affected K_w and LAP but for 4 of the species they studied had little effect on NAR. Our results were interpreted to show that NAR was affected by temperature differences, especially for blue grama.

Results of this study were in agreement with results of other studies (Potter and Jones, 1977; Patterson et al. 1978; Townsend and Wilson 1981) in which high correlations between seedling growth and K_a were found. K_a appeared to be the major growth trait related to differences in growth rates between Lodorm green needlegrass and blue grama seedlings. The importance of K_a in seedling growth is supported by Townsend and Wilson (1981) who found that although NAR was significantly correlated with growth of cicer milkvetch seedlings, it tended to affect growth indirectly through its effect on K_a .

The highest NAR for green needlegrass and blue grama seedlings recorded under the conditions of this study was 2.2 and 3.8 mg dry matter $cm^{-2} day^{-1}$, respectively. The higher NAR of blue grama reflects the greater photosynthetic capacity of plants that photosynthesize by the C_4 pathway. Field studies by Wilson and Briske (1979) yielded lower NAR measurements than those recorded for plants grown in growth chambers in the present study. They reported rates of 2.6 to 3.0 mg biomass $cm^{-2} day^{-1}$ during the early summer for blue grama seedlings harvested from seeded stands at the Central Plains Experimental Range in northeastern Colorado.

Green needlegrass has been used extensively in the northern Great Plains in range seeding mixtures and in mined-land reclamation plantings (Thornburg 1982). Differences in relative growth rate and relative rate of root development per shoot between green needlegrass accessions indicate the possibility of selecting green needlegrass for rapid seedling growth and rapid root development. Slow growth during the seedling stage is one of the primary reasons for difficulty in grass establishment in semiarid regions (Vallentine 1980). Development of varieties with rapid seedling growth and root development in addition to low seed dormancy would enhance the value of green needlegrass for use in reseeding of deteriorated rangeland and in revegetation of strip mined and other drastically disturbed lands.

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