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SOME MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF DENSITY AND DENSITY-WATER STRESS IN *BOUTELOUA CURTIPENDULA* AND *BOUTELOUA GRACILIS*

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SOME MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF DENSITY AND DENSITY-WATER STRESS IN *BOUTELOUA CURTIPENDULA* AND *BOUTELOUA GRACILIS*

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ABSTRACT

Bouteloua curtipendula and *B. gracilis* were grown in mono- and mixed cultures under imposed density and density-water stress to assist in the understanding of the operating mechanisms whereby *B. curtipendula* replaces *B. gracilis* under moist conditions and yet the situation is reversed under drought.

Growth responses, leaf diffusive resistance and osmotic potential, soluble carbohydrates, starch, amino acids and protein were measured in 60 day old seedlings from treatments of low (8 plants per pot), medium (32) and high densities (64) with two water regimes, adequate and stressed. Stress was imposed by withholding water approximately 12 days prior to harvest.

B. gracilis was suppressed by *B. curtipendula* in high density mixed cultures, but attained a significantly higher root-shoot ratio in high density monocultures which may have accounted for continued transpiration at lower soil water levels. In addition, under water stress, *B. gracilis* had a greater desiccation-resistance and soluble carbohydrate accumulation, two conditions correlated with drought resistance.

SOME MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF DENSITY AND DENSITY-WATER STRESS IN *BOUTELOUA CURTIPENDULA* AND *BOUTELOUA GRACILIS*

CHAPTER I

INTRODUCTION

Clements, Weaver and Hanson (1929) define the beginning of competition when the supply of a single necessary factor falls below the combined demands of the plants. This factor may be water, nutrients, light, oxygen, carbon dioxide and at times agents of pollination (Free 1968). To achieve a competitive situation and assess plant response, the supply of one or more of the above factors can be controlled or the demands made on a given area changed by altering plant density. This experiment may result, for example, in water stress and density stress respectively.

Density stress may result in the growth of some individuals at the expense of others. In sunflowers, Kuroiwa (1960) showed that dominant plants continued growth at a constant rate while suppressed individuals demonstrated a decreased growth rate which was in part due to shading. Stern (1965) found the same pattern of dominance and suppression in subterranean clover swards.

Working with Bouteloua curtipendula (Michx.) Torr., side-oats grama, Risser (1970) noted a hierarchy of exploitation such that as density increased, the average weight of the individual decreased with fewer larger plants and many smaller plants. Harper (1967) states that density stress on plant populations is threefold: (i) to elicit a plastic response from the individuals so they adjust to share limiting resources, (ii) to increase mortality, and (iii) to exaggerate differentials within the populations and encourage a hierarchy of exploitation.

Mixed cultures such as forbs and grasses (Dwyer 1958), legumes and grasses (DeWit, Tow and Ennik 1966), perennials and annuals (Litav and Seligman 1969, 1970) have also been examined to determine competitive responses in plant populations. Blaser and Brady (1950) found that when the potassium content in the soil was at a limiting level, potassium uptake occurred earlier in the season for grass in a grass-Ladino clover association which reduced the production of the Ladino clover. To examine the relationship between light and nutrients in mixed cultures of Lolium perenne L. (perennial ryegrass) and Phalaris tuberosa L., Donald (1958) used panels as substrate dividers to isolate shoot competition, root competition and full competition. While field data showed Phalaris to be suppressed by Lolium, experiments with a reduction of nitrogen reduced Phalaris weight at harvest by 75% and thereby reduced its capacity to utilize available light. Under light competition without root competition, sugar production was reduced and Phalaris had a reduced capacity to utilize available nitrogen. These experiments illustrate the importance of the interrelationships of metabolism under various competitive situations. Competition in mono- and mixed culture systems among herbaceous grassland plants has been reviewed by Risser (1969).

The present study concerns the effects of density stress, and density-water stress in monocultures and mixed cultures of two naturally occurring associated grassland species, *Bouteloua curtipendula*, side-oats grama, and *B. gracilis*, blue grama.

Bouteloua curtipendula ranges from the southern plains of Missouri and Texas to Wisconsin, North Dakota and Montana and is the largest and most widely distributed of the grama grasses. It is a perennial warm season grass which only rarely forms sod and under favorable conditions the vegetative shoots can achieve a height of 45 to 60 cm (Weaver and Albertson 1956). Plants develop late in the spring and may remain green late in the growing season.

Bouteloua gracilis is a warm season short grass which extends from Texas and Arizona to Alberta and Saskatchewan. This species achieves a maximum vegetative height of about 15 cm. It grows as a bunch grass in the southern portion of its range but may form a sod farther north (Weaver 1954).

The ecological amplitude of *B. curtipendula* ranges from xeric to mesic conditions, however, under drought conditions in a mixed prairie it may be replaced by *B. gracilis* (Weaver and Albertson 1956), especially at the junction between mixed and short grass prairies, where these two occur together as climax species. Bouteloua curtipendula is a mixed grass which extends into the short grass, especially on deeper soils, and B. gracilis extends into the mixed prairie on shallow soils. The depth of rooting is similar in both species, and both are drought resistant.

After the "Great Drought" of 1934, B. gracilis and B. curtipendula were found in areas previously occupied by the more mesic bluestem, Indian grass and Kentucky bluegrass (Weaver and Albertson 1936). Due to its prolific seeding habit and propagation by rhizomes, B. curtipendula had spread rapidly into both lowland and upland areas whereas B. gracilis had extended its distribution primarily in upland sites. Seedling counts indicated an abundance of both B. curtipendula and B. gracilis (Weaver and Albertson 1939). Work done by Mueller and Weaver (1942) on soil and atmospheric drought resistance of prairie grass seedling substantiated field observations made during the drought. B. gracilis was ranked first followed by Buchloe dactyloides, Bouteloua hirsuta, Sporobolus asper and Bouteloua curtipendula which was fifth among the fifteen species included in the study.

As water becomes less available to plants during water stress or drought conditions, a number of physiological as well as morphological responses may become evident. Among the early physiological responses to water reduction is the inhibition of leaf enlargement (Boyer 1970a, Acevedo, Hsiao and Henderson 1971) and stomatal closure. These responses may decrease water lost by transpiration and CO_2 movement by gas exchange, causing a reduction in photosynthesis (Troughton 1969, Boyer 1970b). Although photosynthesis is reduced, soluble sugars accumulate within the leaf either due to a reduction and/or a delay in translocation (Wardlaw 1969) or as a result of hydrolysis of starch to sugar (Stewart 1971).

Kemble and MacPherson (1954) found protein degradation to α-amino acid occurring in wilted perennial rye. The amount was less than would be expected from protein breakdown in all amino acids except proline which was present in amounts higher than could be accounted for by protein breakdown. Proline has also been found to increase under stress in Bermuda grass (Barnett and Naylor 1966). In creosote bush, proline, phenylalanine and glutamic acid accounted for the greatest portion of the doubling of soluble amino acids under drought (Saunier, Hull and Ehrenreich 1968). Stewart, Morris and Thompson (1966) suggest proline functions as a storage form for nitrogen and in its conversion to glutamic acid a good energy source.

Bouteloua curtipendula and *B. gracilis* are taxonomically closely related, but respond differently to ecological conditions, especially in terms of moisture regime. The design of the experiments described in this paper was to examine morphological and physiological responses of the two species to imposed density and water stress. The results of the studies will assist in the understanding of the operating mechanisms whereby *Bouteloua curtipendula* replaces *B. gracilis* under moist conditions and yet the situation is reversed under drought conditions. No attempt was made to eliminate the factor of possible chemical inhibition.

CHAPTER II

Methods and Materials

Seeds of *Bouteloua curtipendula*, Butte variety, and *B. gracilis* obtained in Kansas were germinated in petri dishes on quartz sand soaked in Hoaglands solution. After the emergence of the first leaf, the seedlings were planted in 8 inch plastic pots containing greenhouse soil. Monocultures of each species and mixed cultures containing an equal number of both species were established at low, medium, and high density levels of 8, 32 and 64 per pot corresponding approximately to 250, 1,000 and 2,000 plants per sq m respectively. Six pots were used for each treatment. Cultures were planted in a circular design with each plant being equidistant from the adjacent two plants and from the edge of the pot so that in mixed cultures no two plants of the same species occurred side by side. The plants were grown for approximately 60 days in a growth chamber under 1,000 ft-c with a 16 hour photoperiod at 27° C and 8 hour dark period at 20° C. Since not all plants survived, at twenty days, four pots were selected for each treatment and transplants made from the remaining two pots to re-establish density levels.

Water stress was applied by withholding water after 48 days in two of the four pots of each density treatment and soil water potential was measured daily thereafter using soil psychrometers (Zollinger, Campbell and Taylor 1966) which had been embedded at a depth of 80 mm in the soil. A summary of experimental design is found in Figure 1.

The influences of competition and water stress on growth was followed by morphological measurements consisting of plant height, length of longest leaf, width of longest leaf, length of second longest leaf, width of second longest leaf and leaf number. These measurements made at ages of twenty, forty, and fifty days and at harvest, approximately sixty days.

Leaf diffusive resistance of five plants per species per pot from all treatments was measured on the youngest fully developed leaf using a Model L1-60 diffusive resistance meter (Lambda Instrument Co., Inc. 1972) equipped with a horizontal rectangular moisture sensor modified with a 2mm x 20mm slit for narrow leaves. This instrument is designed to estimate transpiration and stomatal aperture of leaves over a short interval of time (van Bavel, Nakayama and Ehrler 1965, Kanemasu, Thurtell and Tanner 1969). Measurements were taken at 24 hour intervals on the same leaf until transpiration was significantly reduced in the water stressed plants. Leaf water potential of the leaves used for transpiration was determined immediately after the last leaf diffusive resistance measurement by thermocouple psychrometry with a Model C-51 sample chamber (Wescor, Inc. 1971). The narrow leaves of these two species necessitated the use of leaf segments instead of leaf discs. A 5 minute equilibration time and a 15 second thermocouple cooling time were used.

The plants were removed by carefully washing away the soil and then separating into roots and shoots. The roots were oven dried at 80^{o} C for 48 hours to determine dry weights. A subsample (25%) of the shoot material was oven dried under the same conditions to obtain plant water content at harvest. The remaining shoot material was fixed in boiling 80% (v/v) ethanol for biochemical analysis.

Plant extraction followed a modified method of Nickell (1972) (Figure 2). The shoot material was ground in a Virtis grinder and thoroughly extracted with 80% ethanol. TCA (5%) was added to the pellet for 24 hours followed by a protein extraction using 0.1N NaOH. Starch was then extracted using 60% perchloric acid. Soluble carbohydrates and digested starch, amino acids and proteins were quantified colorimetrically using anthrone (Yemm and Willis 1954), ninhydrin (Yemm and Cocking 1955) and Folin phenol (Lowry, Rosebrough, Farr and Randall 1951) respectively.

Figure 1.--Flow diagram of experimental design.

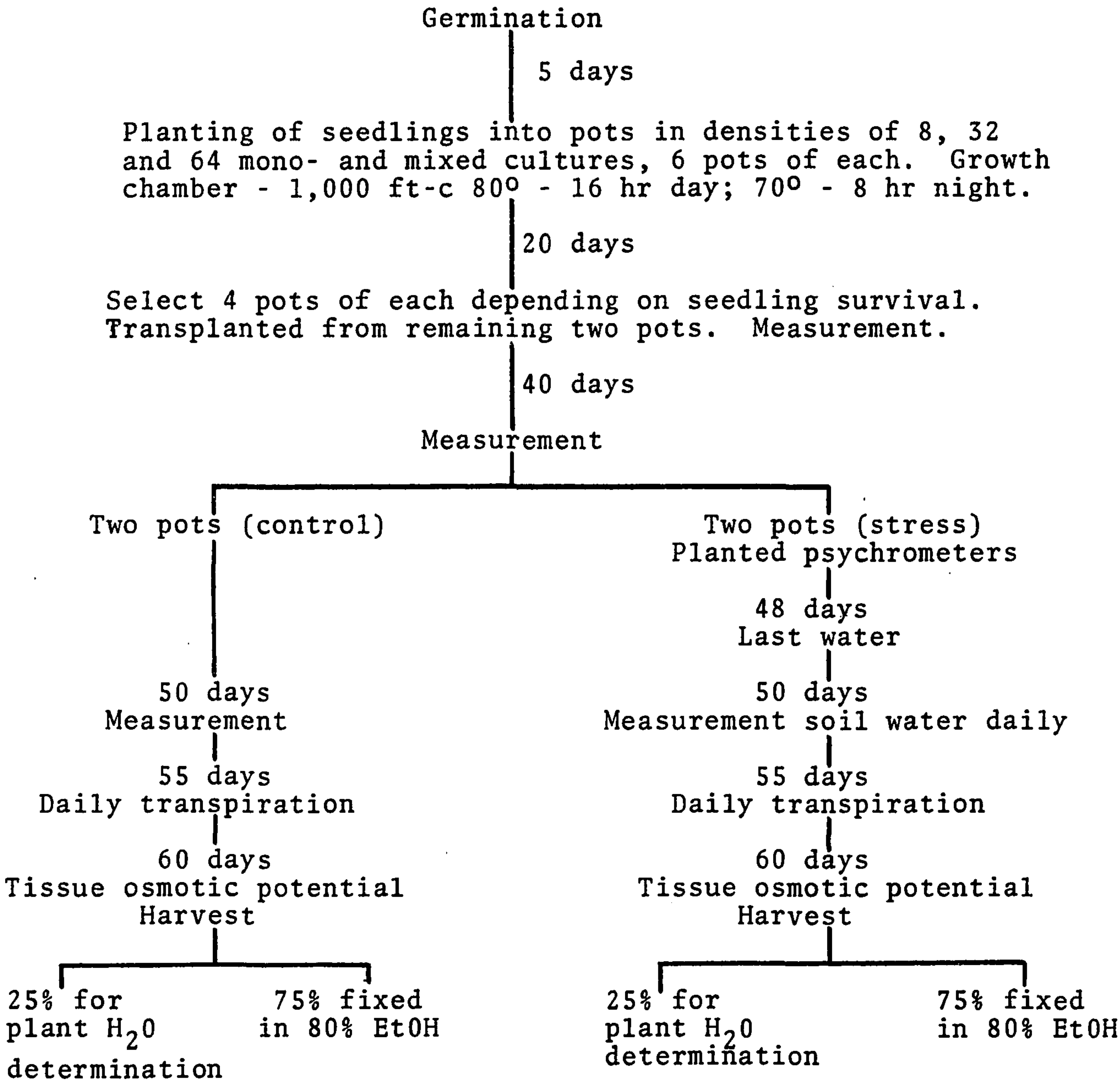
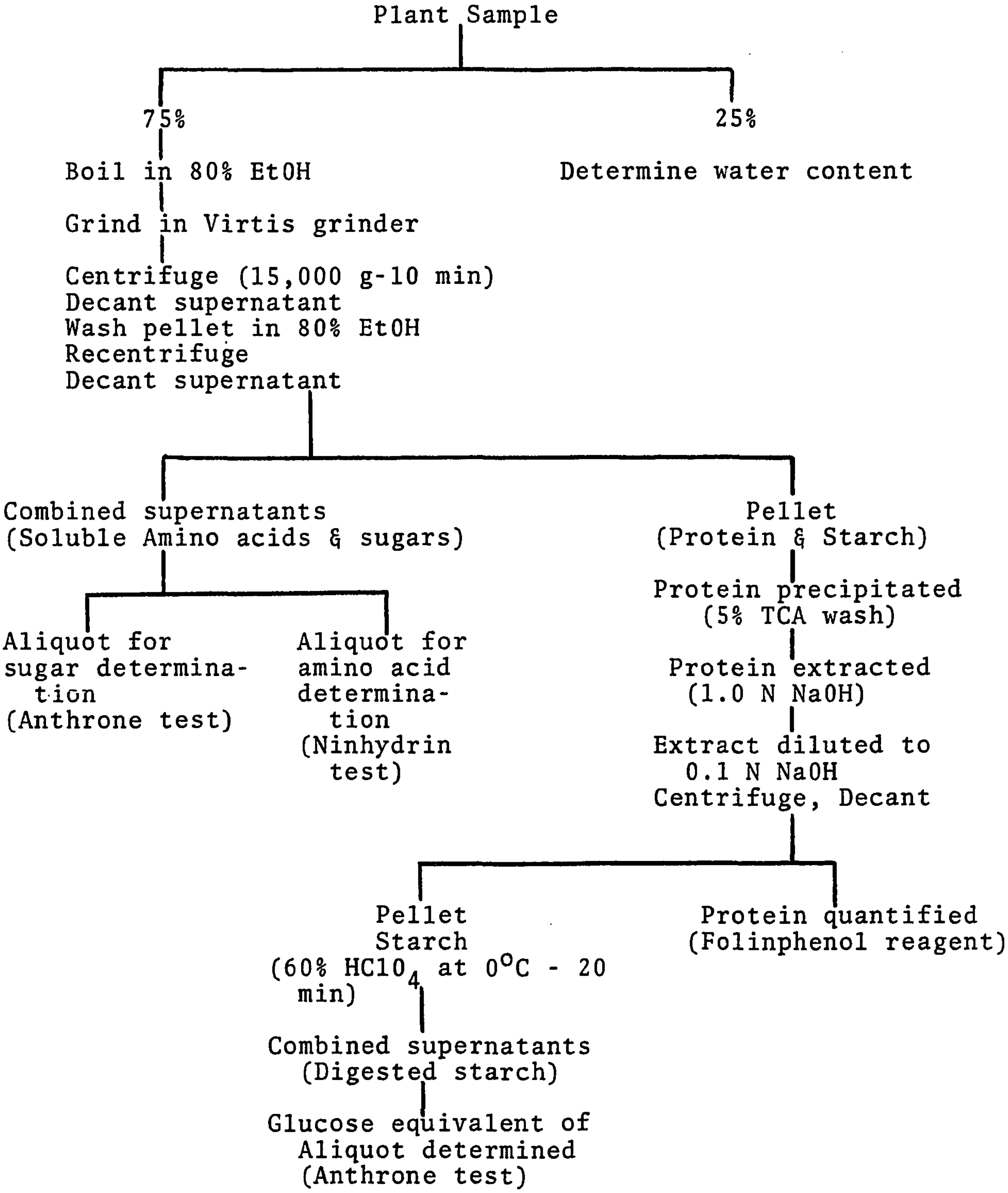

Germination
5 days
Planting of seedlings into pots in densities of 8, 32 and 64 mono- and mixed cultures, 6 pots of each. Growth chamber - 1,000 ft-c 80° - 16 hr day; 70° - 8 hr night.
20 days
Select 4 pots of each depending on seedling survival. Transplanted from remaining two pots. Measurement.
40 days
Measurement
Two pots (control)
50 days
Measurement
55 days
Daily transpiration
60 days
Tissue osmotic potential
Harvest
25% for plant H_2O determination
75% fixed in 80% EtOH
Two pots (stress)
Planted psychrometers
48 days
Last water
50 days
Measurement soil water daily
55 days
Daily transpiration
60 days
Tissue osmotic potential
Harvest
25% for plant H_2O determination
75% fixed in 80% EtOH

Figure 2.--Flow diagram of experimental design.


Plant Sample
75%
25%
Boil in 80% EtOH
Determine water content
Grind in Virtis grinder
Centrifuge (15,000 g-10 min)
Decant supernatant
Wash pellet in 80% EtOH
Recentrifuge
Decant supernatant
Combined supernatants
(Soluble Amino acids & sugars)
Pellet
(Protein & Starch)
Aliquot for sugar determination (Anthrone test)
Aliquot for amino acid determination (Ninhydrin test)
Protein precipitated (5% TCA wash)
Protein extracted (1.0 N NaOH)
Extract diluted to 0.1 N NaOH
Centrifuge, Decant
Pellet
Starch
(60% HClO4 at 0°C - 20 min)
Protein quantified
(Folinphenol reagent)
Combined supernatants
(Digested starch)
Glucose equivalent of Aliquot determined
(Anthrone test)

CHAPTER III

RESULTS AND DISCUSSION

Morphological Response

The influence of density stress and combined density-water stress on the growth rate and development in mono- and mixed cultures of the two species system used is illustrated in Figures 3 and 4 for *Bouteloua curtipendula* and Figures 5 and 6 for *B. gracilis*. Because plant height was highly correlated with other measured morphological parameters, (longest leaf, width of the longest leaf, and number of leaves) this characteristic was selected and used as the diagnostic response to the various treatments.

In the control condition (no water stress) plant height was significantly reduced as density increased. The heights of the low density, (8 plants per pot) were significantly greater than the medium density plants (32) which in turn were significantly higher than high density plants (64). This reduction occurred in both species in mono- as well as mixed cultures. The growth rate was greatest at the low density level with the lag phase occurring prior to the twenty day measurement. The higher densities demonstrated a longer lag phase with a gradual increase in growth rate at approximately forty days but never achieved a rate equal to the low density.

The pots which had water withheld approximately two days

Figure 3.--Growth rate of _Bouteloua curtipendula_ in monocultures under density stress and density, water stress combined. (+ = low density (8), watered; O = medium density (32), watered; △ = high density (64), watered; ✦ = low density (8), water stress; ● = medium density (32), water stress; ▲ = high density (64), water stress).

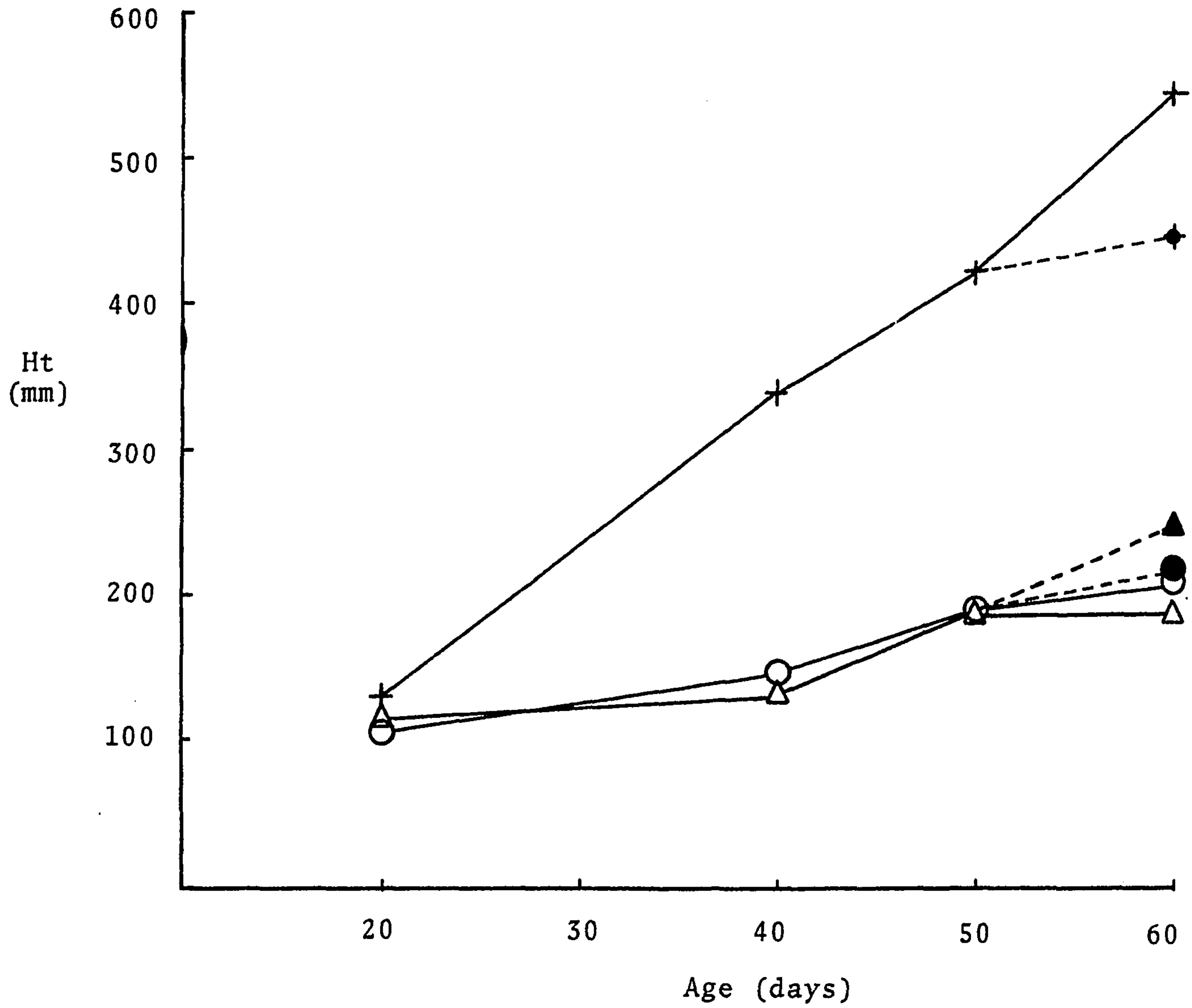

600
500
400
Ht
(mm)
300
200
100
20
30
40
50
60
Age (days)

Figure 4.--Growth rate of <u>Bouteloua curtipendula</u> in mixed cultures under density stress and density, water stress combined. (+ = low denisty (8), watered; ○ = medium density (32), watered; △ = high density (64), watered; -◆- = low density (8), water stress; ● = medium density (32), water stress; ▲ = high density (64), water stress).

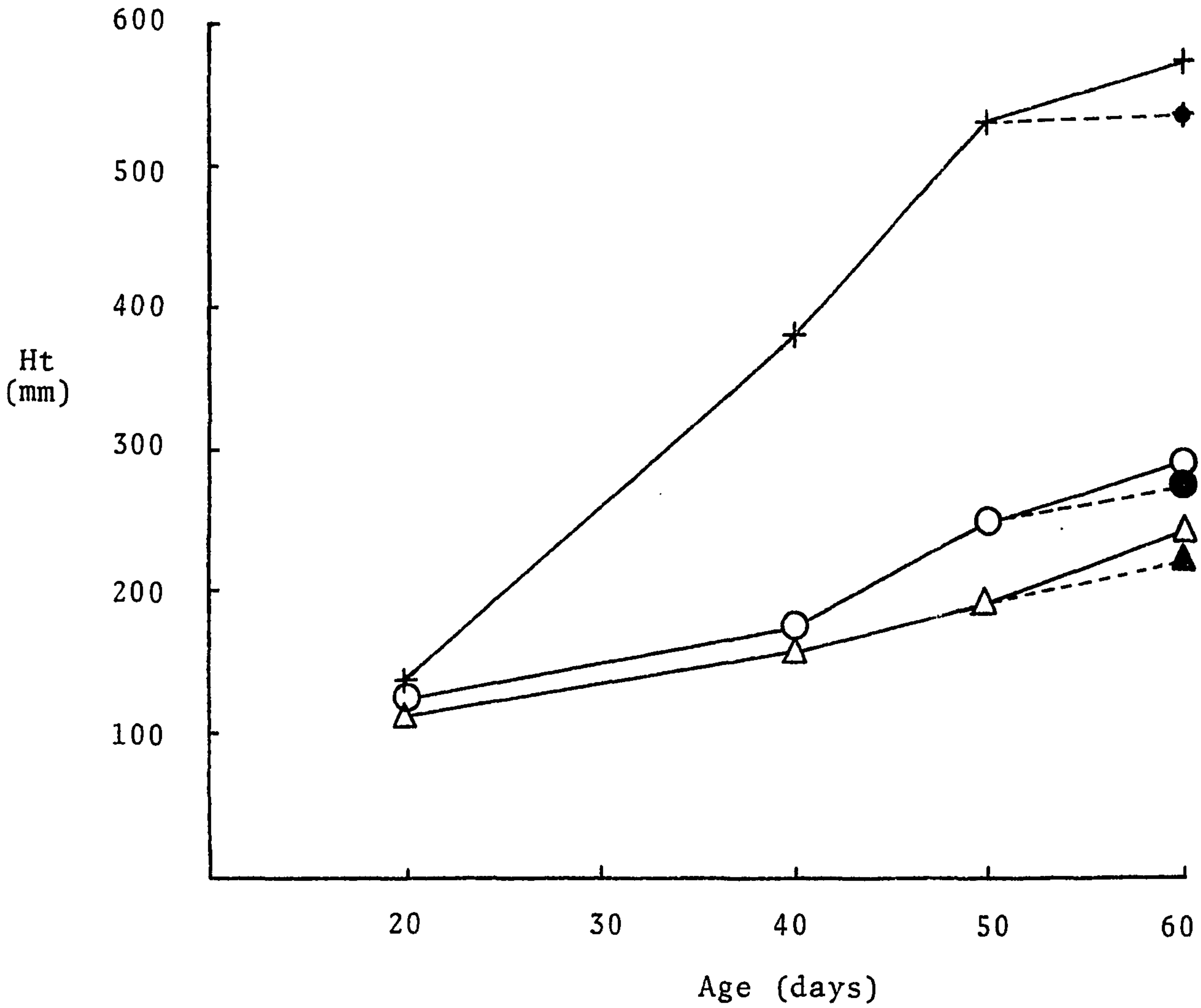

600
500
400
Ht
(mm)
300
200
100
20
30
40
50
60
Age (days)

Figure 5.--Growth rate of _Bouteloua gracilis_ in monocultures under density stress and density, water stress combined. (+ = low density (8), watered; O = medium density (32), watered; △ = high density (64), watered; ✦ = low density (8), water stress; ● = medium density (32), water stress; ▲ = high density (64), water stress).

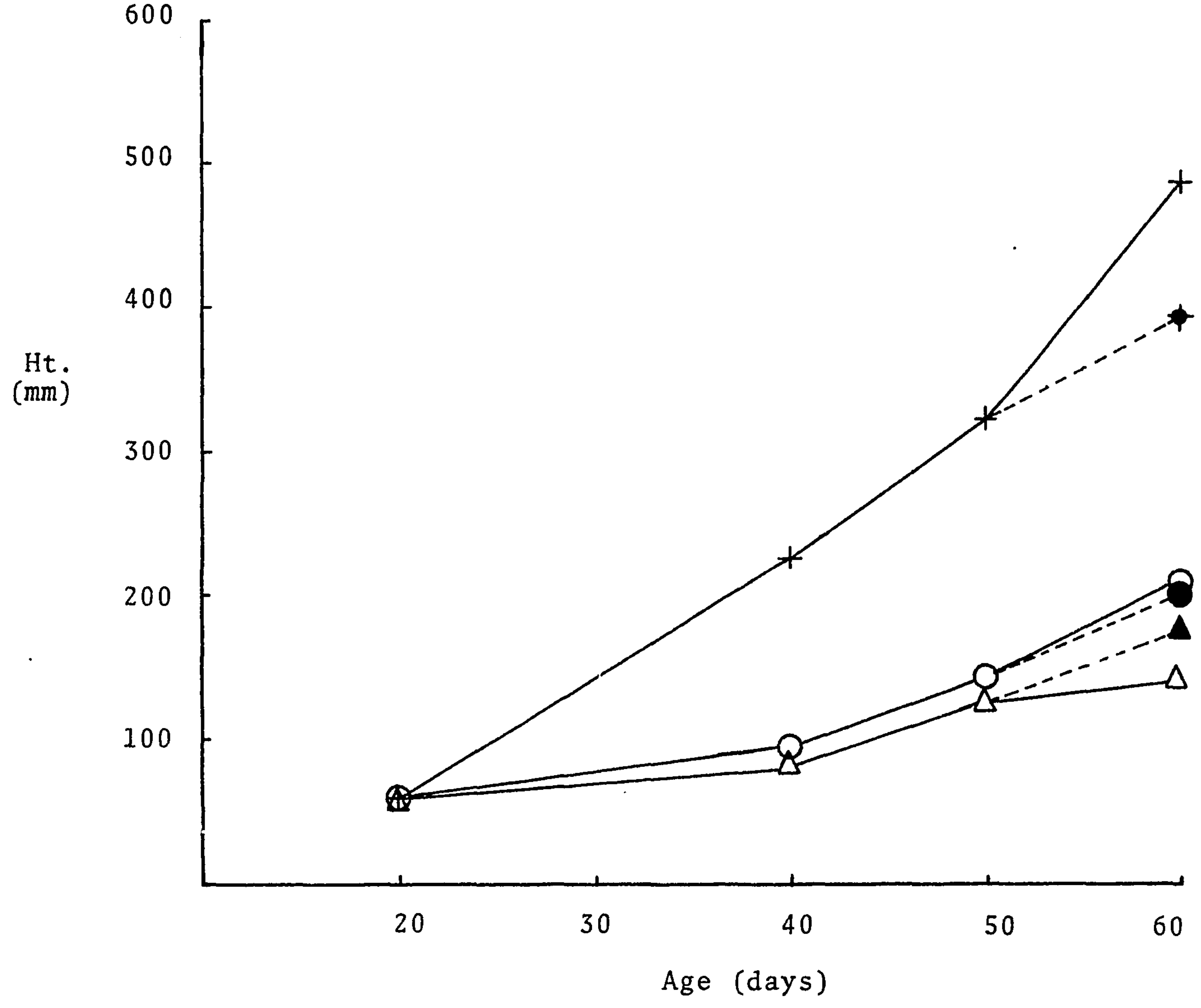
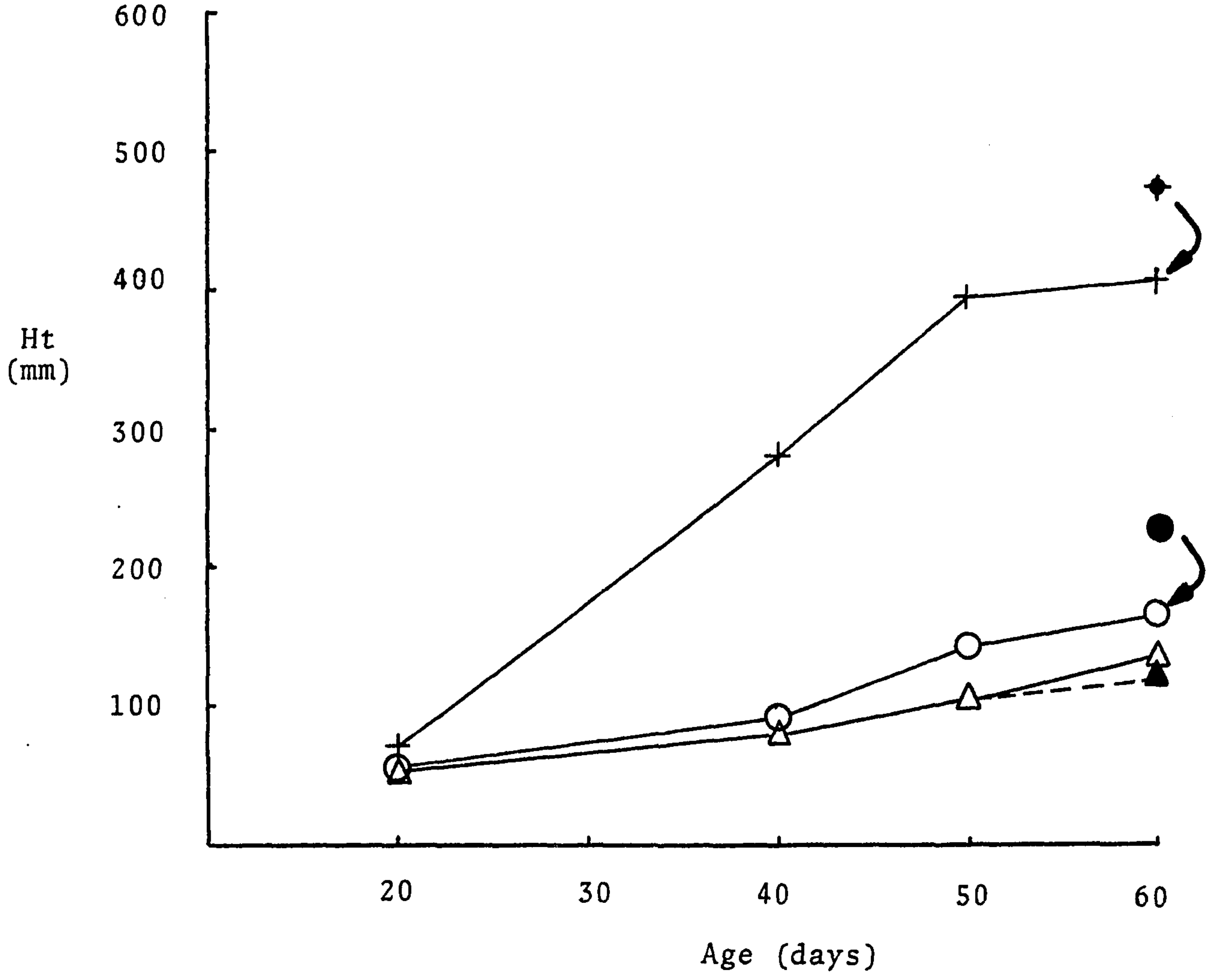

600
500
400
300
200
100
Ht.
(mm)
20
30
40
50
60
Age (days)

Figure 6.--Growth rate of *Bouteloua gracilis* in mixed cultures under density stress and density, water stress combined. (+ = low density (8), watered; = medium density (32), watered; △ = high density (64), watered; ✚ = low density (8), water stress; = medium density (32), water stress; ▲ = high density (64), water stress).


600
500
400
300
200
100
Ht
(mm)
20
30
40
50
60
Age (days)

prior to the fifty day measurement showed varied results in the growth rate between fifty and sixty days and in final height. A significant reduction occurred in the growth rates and final heights between controls and stressed plants in monocultures of both species at the low density level (Table 1). This same comparison within all treatments of medium density showed no significant difference. The only other treatment with a significant reduction under water stress was in B. gracilis in the high density mixed culture. One treatment, the high density monocultures of B. curtipendula, showed a significant increase in plant height over controls when water was withheld.

The influence of one species on the other can be determined in part by comparing the heights of each species in mixed cultures to the heights obtained in monocultures. This analysis at the lowest density level showed no significant change in B. gracilis but a significant increase in the stressed treatment in B. curtipendula. At the medium and high density levels, B. curtipendula had a final height 137.9% and 127.5% of the control monocultures respectively. B. gracilis in these same pots obtained heights 79.4% and 83% of the control monocultures. This same pattern was evident in the stressed pots of medium densities, and in the high densities both species showed a reduction in the mixed association.

The length of the longest leaf illustrated responses identical to the height responses in the various treatments and similar significant differences. Leaf width and leaf number follow the same trends, both generally being reduced as density increased in

TABLE 1

Final morphological measurements (60 days) of *Bouteloua curtipendula* (BOCU) and *Bouteloua gracilis* (BOGR) from mono- and mixed cultures of different densities; low, medium and high and different water regimes: control (watered) and stressed. Values are averages expressed in mm.

Species and Density	Water Treatment	Height		Longest Leaf		Width Longest Leaf		Number of Leaves	
		Mono-	Mixed	Mono-	Mixed	Mono-	Mixed	Mono-	Mixed
BOCU 8	control	546.4[a]	575.7	422.7[a]	404.3	4.8[a]	4.8[a]	5.0[a]	5.5[a]
BOCU 8	stressed	447.3[b]	537.7	362.9[b]	429.1	2.1[b]	3.4	3.4[b]	4.1
BOGR 8	control	486.6[a]	406.7	391.2[a]	326.4	1.8[a]	1.7[a]	5.4[a]	5.1[a]
BOGR 8	stressed	394.1	406.2	323.3	328.8	1.0	1.1	4.1	4.3
BOCU 32	control	213.3[b]	292.9	176.4[b]	239.4	1.5[b]	2.2[a]	2.7[ab]	4.0[a]
BOCU 32	stressed	219.6[b]	276.9	185.2[b]	230.3	1.5[b]	1.9	3.4	3.5
BOGR 32	control	210.0[b]	166.7	172.0[b]	134.6	0.9[b]	0.8	4.4[b]	3.4[a]
BOGR 32	stressed	203.2[b]	165.8	164.2[b]	137.5	1.0[b]	0.8	4.6[b]	3.0
BOCU 64	control	191.9[ab]	244.7	164.9[ab]	205.3	1.3[ab]	1.7	2.9[b]	3.6[a]
BOCU 64	stressed	250.3[b]	225.8	208.9	202.1	1.4[b]	1.6	2.9	3.0
BOGR 64	control	164.3[b]	136.4[a]	126.1	111.2[a]	0.8	0.7[a]	3.7[b]	3.3[a]
BOGR 64	stressed	176.6[b]	122.6	145.7[b]	99.9	0.9[b]	0.6	3.6[b]	2.7

[a]Significant at the 0.05 level between water treatments within either mono- or mixed cultures.

[b]Significant at the 0.05 level between mono- and mixed cultures of the same water treatment.

monocultures and both increasing in *B. curtipendula* and decreasing in *B. gracilis* in mixed cultures as compared to monocultures.

Water stress, based on morphological responses in this experiment, had its greatest effect on the plants having the most rapid growth rate. This was evident in the monocultures with *B. curtipendula* exhibiting an 18% reduction in height under water stress at the low density and *B. gracilis* a 19% reduction. This reduction was not so true in the mixed cultures at this density level, but the growth rate in the mixed controls was greatly reduced between the fifty and sixty day measurement as compared to the monocultures. The higher two densities, which demonstrated a reduced rate of growth during the time of water stress, showed no great additional morphological affects.

Response of the two species when grown in mixed cultures illustrates the increase or success of one species at the expense or suppression of the other as mentioned by Harper (1967). *B. curtipendula* was generally taller, with longer wider and more leaves in the mixed culture compared to the monoculture, whereas the opposite occurred in *B. gracilis*.

Production

One measure of plant success over a given period of time under different treatments is the amount of biomass produced. Shoot dry weight expressed on a per plant basis showed responses to the various treatments similar to those discussed under height responses (Tables 2 and 3). An increase in density caused a reduction in biomass in both mono- and mixed cultures, watered or

TABLE 2

Shoot dry weight of *Bouteloua curtipendula* (BOCU) from mono- and mixed cultures of low (8), medium (32) and high (64) densities with two water regimes: control (watered) and stressed. Values are expressed gm dry wt. per plant and per pot.

Species and Density	Water Treatment	Shoot dry wt. in gm on a per plant basis				Shoot dry wt. in gm on a per pot basis	
		Monocultures		Mixed		Pure	Mixed
		Replicate Values	Treatment Mean	Replicate Values	Treatment Mean		
BOCU 8	Control I	0.2566	0.2516[a]	0.1013	0.2670	1.7964	0.4051
	Control II	0.2467		0.4328		1.7267	1.5147
	Stressed I	0.1898	0.1799[b]	0.3999	0.3596	1.5180	1.3996
	Stressed II	0.1700		0.3193		1.3596	1.2772
BOCU 32	Control I	0.2336	0.2564[a]	0.1880	0.1620	6.3069	3.0078
	Control II	0.2793		0.1359		8.9370	2.1749
	Stressed I	0.1790	0.1678	0.1361	0.1440	5.3704	2.1771
	Stressed II	0.1566		0.1519		4.2294	2.4298
BOCU 64	Control I	0.0251	0.0275	0.0924	0.0707	1.3037	2.5881
	Control II	0.0299		0.0490		1.7053	1.3222
	Stressed I	0.0481	0.0385	0.0605	0.0518	2.5998	1.8769
	Stressed II	0.0292		0.0432		1.6934	1.2526

[a]Significant at the 0.05 level between water treatments within either pure or mixed cultures.

[b]Significant at the 0.05 level between pure and mixed cultures of the same water regime.

TABLE 3

Shoot dry weight of Bouteloua gracilis (BOGR) from mono- and mixed cultures of low (8), medium (32), and high (64) densities with two water regimes: control (watered) and stressed. Values are expressed gm dry wt. per plant and per pot.

Species and Density	Water Treatment	Shoot dry wt. in gm on a per plant basis				Shoot dry wt. in gm on a per pot basis	
		Monocultures		Mixed		Pure	Mixed
		Replicate Values	Treatment Mean	Replicate Values	Treatment Mean		
BOGR 8	Control I	0.1379	0.1367[a]	0.0907	0.1234	1.1028	0.3627
	Control II	0.1355		0.1561		1.0840	0.5464
	Stressed I	0.0732	0.0737	0.0822	0.0742	0.5856	0.3286
	Stressed II	0.0742		0.0663		0.5939	0.2650
BOGR 32	Control I	0.0233	0.0578	0.0523	0.0506	0.7442	0.6794
	Control II	0.0923		0.0489		2.5842	0.6841
	Stressed I	0.0268	0.0340	0.0372	0.0545	0.7495	0.5944
	Stressed II	0.0411		0.0718		1.1092	0.8614
BOGR 64	Control I	0.0270	0.0263	0.0231	0.0176	1.7281	0.5300
	Control II	0.0256		0.0121		1.4826	0.3018
	Stressed I	0.0171	0.0225	0.0102	0.0100	1.0254	0.2042
	Stressed II	0.0280		0.0097		1.7331	0.3014

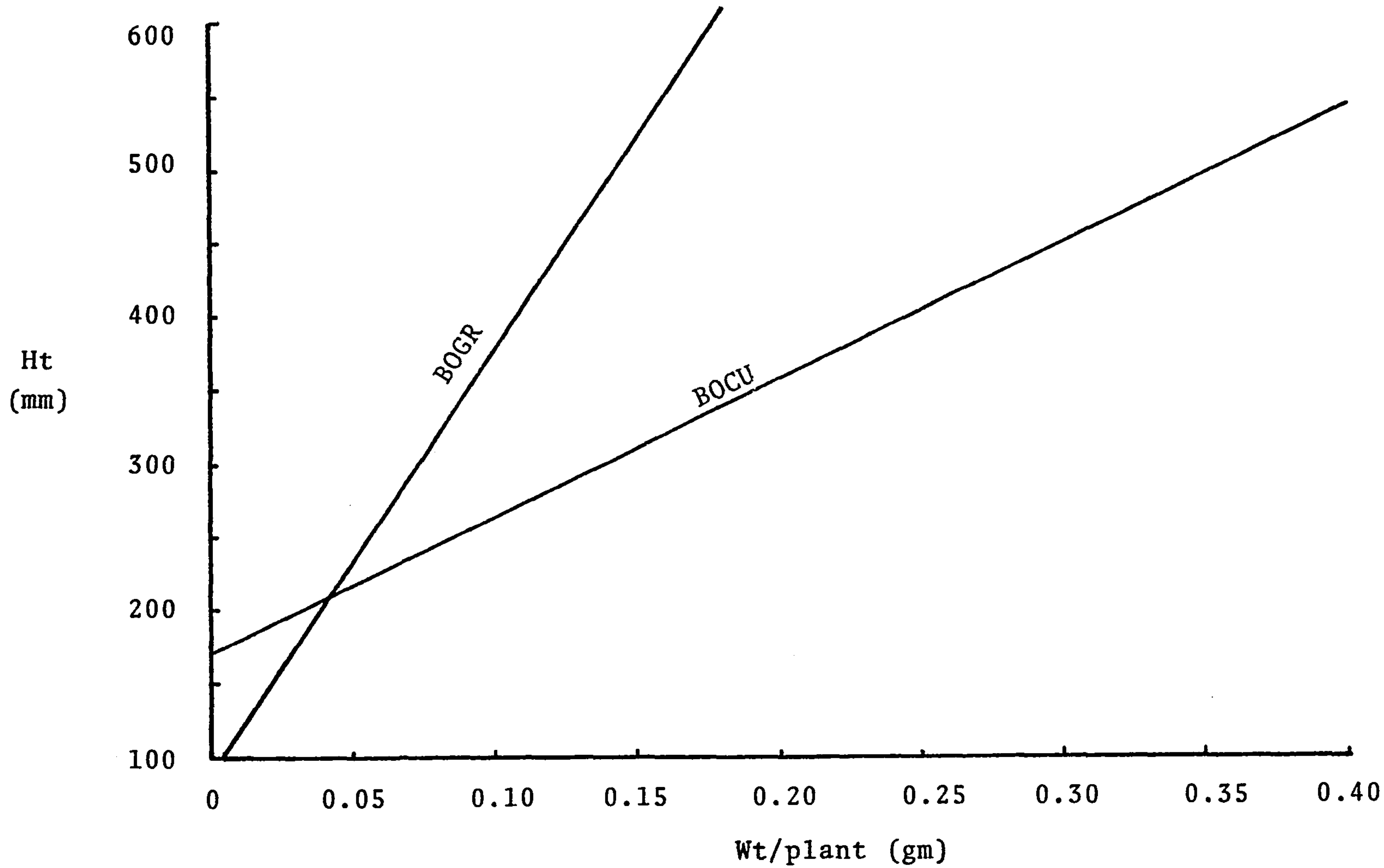
[a]Significant at the 0.05 level between water treatments within either pure or mixed cultures.

stressed. The high density mixed cultures of control and stress treatments compared to monocultures, showed a biomass increase in *B. curtipendula* and a decrease in *B. gracilis*. Dry weight per plant in the high density monocultures of *B. curtipendula* increased under water stress compared to controls. A response which corresponds to the height measurements. The relatively small sample size of the various treatments may limit the possibility of showing significant differences between the various treatments. However, average dry weight per plant was significantly correlated ($p = 0.01$) to the average height in all treatments (Figure 7; BOCU $r = 0.738$, BOGR $r = 0.902$).

If the amount of biomass obtained in these pot cultures is placed on a gm/m^2 basis, then there are 55, 238 and 47 gm/m^2 of *B. curtipendula* and 34, 52 and 50 gm/m^2 of *B. gracilis* in monoculture control low, medium, and high density respectively. The only mixed culture where the combined weights of both species exceeded the monoculture at a given density level was the high density achieving 74 gm/m^2 or, 147.7% of the monoculture of *B. gracilis*. It is of interest to note that the biomass per m^2 of *B. gracilis* at the highest density was higher than *B. curtipendula*, but not significantly so, and demonstrated only a slight reduction from the medium to high density.

The relationship of aboveground biomass to belowground biomass can be depicted as a ratio of root material to shoot material and expressed as $^R/S$. Root/shoot ratios have been shown to increase under conditions of decreased available soil water (Struik and Bray 1970). In pot cultures, less water will be

Figure 7.--Average height per plant versus average weight per plant of *Bouteloua curtipendula* (BOCU) and *B. gracilis* (BOGR) from all treatments.


Ht
(mm)
600
500
400
300
200
100
0
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
Wt/plant (gm)
BOGR
BOCU

available in the same volume of soil as the amount of root biomass is increased such as increasing density, or by withholding water. The $^R/S$ ratio in B. curtipendula increased under water stress in both low and medium densities when compared to controls, but no difference occurred at the high density level (Table 4). B. gracilis showed an increase in the ratio at all levels of the stressed treatments as compared to the controls and also as density increased. The ratios for both species were similar at the low and medium densities when compared to results from the same water treatments, but the ratios in both water treatments at the high density of B. curtipendula were significantly lower than B. gracilis. Ratios by species were not included from mixed cultures because of the difficulty in separating the intermingling roots.

These ratios were much higher than the 0.24 for Andropogon scoparius, and 0.29 for Bouteloua gracilis obtained in pot cultures (Weaver and Zink 1946). Seedlings were also used but adequate moisture was continually maintained, and the duration of the experiment was twice as long in the work of Weaver and Zink. Seedling density was approximately equal to 8 plants per pot in B. gracilis and 3 plants per pot in Andropogon scoparius when equated to the density used in this study. Struik and Bray (1970) found a higher relative belowground production in a droughty portion (33%) of a field of Zea mays than in a non-droughty portion (13%).

Transpiration

As an estimate of transpiration and stomatal aperture, leaf

TABLE 4

Root/shoot ratios of <u>Bouteloua curtipendula</u> (BOCU) and <u>Bouteloua gracilis</u> (BOGR) from mono- cultures of low (8), medium (32) and high (64) densities exposed to adequate water (control) and water stress (ratio obtained by dividing root dry weight per plant by shoot dry weight).

Density	Water Treatment	BOCU Root/shoot per Replicate	BOCU Treatment Mean	BOGR Root/shoot per Replicate	BOGR Treatment Mean
8	Control I	0.351	0.406	0.381	0.417
	Control II	0.461		0.453	
	Stressed I	0.496	0.607	0.348	0.514
	Stressed II	0.718		0.679	
32	Control I	0.828	0.718[a]	0.757	0.704[a]
	Control II	0.608		0.651	
	Stressed I	1.307	1.274	1.789	1.575
	Stressed II	1.240		1.159	
64	Control I	0.647	0.557	0.757	1.049
	Control II	0.467		1.340	
	Stressed I	0.578	0.539	1.459	1.511
	Stressed II	0.499		1.563	

[a]Significant at the 0.05 level between water treatments within the same density level.

diffusive resistance was measured on plants inside the growth chamber at approximately the same time each day. One replicate of a stressed treatment was measured, followed by a control replicate of the same treatment, so that any two replicates of the same treatment was measured within approximately a 30 minute time period.

With one exception leaf diffusive resistance of control monocultures remained stable with a trend towards lowering during the time period of this experiment (Table 5). The exception was the high density B. curtipendula which showed a 233% increase in the average final reading over the average initial reading and was significantly higher than the two lower density levels of this species. Similarly B. gracilis in the high density exhibited high initial readings and considerable variation among leaves, but over the time period of these experiments the resistance decreased significantly resulting in final reading lower than initial.

The overall comparison of these two species under control monocultures resulted in the greatest differences occurring at the high density level. The average shoot dry weight per plant for these two treatments was not significantly different, but a difference was evident in the amount of root biomass. B. gracilis with a $^{R}/S$ ratio greater than B. curtipendula (1.049 to 0.557) had a combination of less aboveground biomass with a lower leaf area for water loss in relation to the amount of water absorbing biomass in the soil, namely, the roots. This could indicate a susceptibility for water lost via transpiration to exceed water

TABLE 5

Initial and final diffusive resistance in mono- and mixed cultures of <u>Bouteloua curtipendula</u> (BOCU) and <u>B. gracilis</u> (BOGR)

Species	Density	Control				Stress			
		Monocultures		Mixed		Monocultures		Mixed	
		I_0	Final	I_0	Final	I_0	Final	I_0	Final
BOCU	8	30.4±21.0	27.5±5.6	19.4±5.6	23.2±7.7		49.6±26.2		44.3±5.4
BOCU	32	21.7±6.7	17.6±3.3	21.2±4.4	27.7±5.6	24.2±4.9	188.8±35.3	25.6±5.4	85.9±34.7
BOCU	64	42.0±5.7	97.9±24.2	50.4±26.4	94.1±57.2	42.8±12.2	225.1±60.0	63.8±18.1	174.2±62.8
BOGR	8	25.4±4.9	23.7±4.8	35.7±9.9	27.8±5.6		43.6±7.8		51.4±9.6
BOGR	32	50.5±10.6	35.0±7.7	40.6±4.2	27.2±5.0	49.2±12.1	77.6±20.0	76.4±24.5	174.4±49.3
BOGR	64	70.8±27.3	39.8±10.4	123.8±57.3	140 6±42.8	75.7±21.3	158.0±51.1	188.0±64.3	233.6±66.1

Note: Values are means with 95% confidence limits of 10 measurements on the youngest fully developed leaf. (Values expressed as; cm sec^{-1}; initial = first measurement taken; final = measurement where stressed plants were significantly higher than controls at p = 0.10 level.)

uptake in *B. curtipendula* resulting in a leaf water deficit and thereby stimulate stomatal closure. The $^{R}/S$ ratio and the general yellowing appearance of the leaves in *B. curtipendula* may well account for these contrasting results at the high density level between these two species.

B. curtipendula in the mixed control cultures exhibited similar resistance measurements at all densities with a higher variation between leaves at the high density. *B. gracilis* however had very high diffusive resistance reading in the high density level both in the initial and final stages compared to the monocultures. This in part can be explained by leaf width within this species, particularly in the high density mixed cultures. The width of the opening in the humidity sensor is 2 mm. The widths of the leaves in *B. gracilis* occasionally exceeded 2 mm but the majority of leaves measured for transpiration fell below this width in the higher densities. In the monocultures of *B. gracilis*, 23 measurements taken on leaves ranging from 1.2 to 1.9 mm in width showed no significant correlation between diffusive resistance and leaf width. Measurements of resistance from mixed cultures with leaf widths ranging from 0.5 to 1.9 show a correlation of $r = 0.86$, ($p = 0.01$). Therefore, this increase of leaf diffusive resistance in *B. gracilis* mixed culture high density was caused in part by the reduction in leaf width in the mixed culture, high density treatment rather than an indication of stomatal closure.

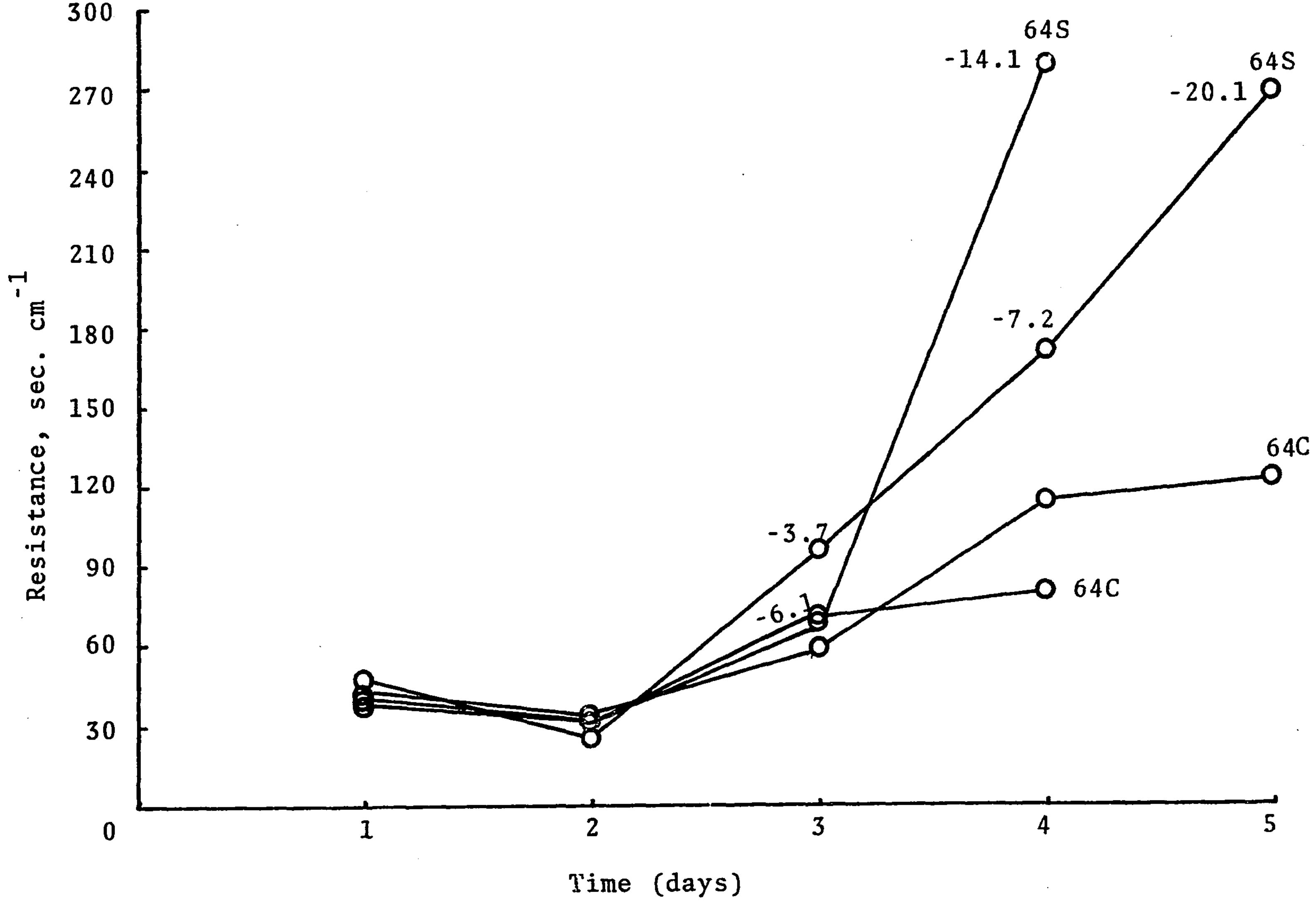
A measurement of leaf diffusive resistance prior to the beginning of a decrease in soil water was not obtained in the mono- and mixed stressed pots at the low density level (Table 5).

The initial measurements taken in the medium and high densities of *B. curtipendula* in both mono- and mixed cultures were not significantly different from the comparable measurements in the control pots. *B. gracilis* also was not significantly different in the monocultures, but the initial measurement in the mixed cultures of both medium and high densities were much higher than the mixed controls, probably due in part to a reduction in leaf width.

The final leaf diffusive resistance from the plants in the stressed pots was determined as the point at which the stressed leaves had reduced transpiration significantly from the watered controls. These values do not necessarily mean that a higher diffusive resistance could not have been obtained. In some instances the variation in the data made it difficult to determine the point where a significant difference had occurred, thus additional readings were made and in these cases, higher readings usually resulted.

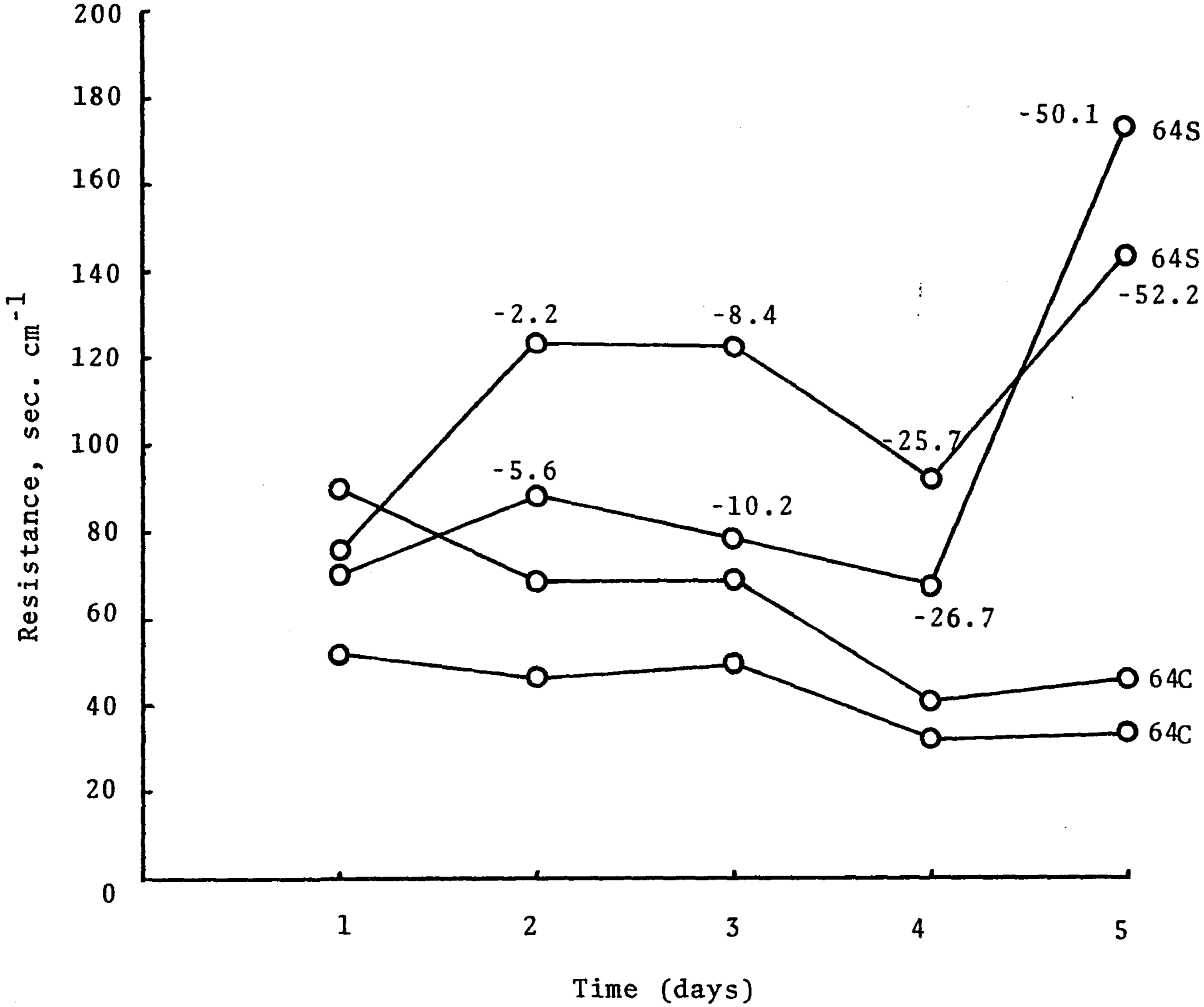
Figures 8 and 9 illustrate the leaf diffusive resistance measurements of stress and control plotted against time at the high density level for *Bouteloua curtipendula* and *B. gracilis* respectively. The soil water level in bars at each measurement for the stressed pots is noted on each graph. The resistance measurements in *B. curtipendula* began to increase beyond a soil water level of -6.1 bars and was significantly reduced at -14.1 bars. It would appear from Figure 9 that *B. gracilis* had reduced transpiration as soon as soil water began to lower but the variation between leaves was high, indicating reduction of transpiration in some leaves but not in others. Reduction in transpiration in this treatment came somewhere between -26.7 and -50.1 bars.

Figure 8.--Leaf diffusive resistance from *Bouteloua curtipendula* high density (64) of control and stressed plants. 64S = stressed, 64C = control. Numbers labeled at data points are soil water in bars at time of resistance measurement. (Resistance values are averages of 5 measurements.)



300
270
240
210
180
150
120
90
60
30
0
Resistance, sec. cm^{-1}
1
2
3
4
5
Time (days)
64S
-14.1
64S
-20.1
-7.2
64C
-3.7
-6.1
64C

Figure 9.--Leaf diffusive resistance from *Bouteloua gracilis* high density (64) of control and stressed plants. 64S = stressed, 64C = control. Numbers labeled at data points are soil water in bars at time of resistance measurement. (Resistance values are averages of 5 measurements.)


Resistance, sec. cm^{-1}
200
180
160
140
120
100
80
60
40
20
0
1
2
3
4
5
Time (days)
-50.1
64S
64S
-52.2
-2.2
-8.4
-25.7
-5.6
-10.2
-26.7
64C
64C

This same analysis was applied to all treatments to obtain the soil water level at which transpiration was significantly reduced (Table 6). An interesting comparison exists between the two species in the monocultures at the high density level where B. gracilis continued to maintain transpiration at lower soil water levels than B. curtipendula. B. gracilis mixed high densities failed to show a significant reduction because initial readings were so high. The data also show the low density B. gracilis monocultures had a reduced transpiration rate at a fairly high water level in comparison to all other treatments. The results from this particular treatment are questionable since leaf diffusive resistance was followed beyond a soil water level of -50 bars with no greater reduction in resistance occurring.

The continuation of some transpiration measurements down to very low soil water levels caused some tissue osmotic potential measurements taken at harvest to exceed the limits of the instrument used in this study. Tissue osmotic potentials were plotted against percent plant water at harvest for both species to determine if one species could maintain a higher plant water level at low tissue osmotic potentials than the other. One presumed characteristic of drought resistant plants as compared to plants with lesser drought resistance is a desiccation-resistance; to have a small decrease in water content for a given decrease in water potential (Weatherly and Slatyer 1957; Conner and Turnstall 1968). Sanchez-Diaz and Kramer (1971) examining corn and the more drought resistant sorghum under water stress, found a large decrease in water content in corn leaves accompanied by a small

TABLE 6

Soil water in bars from stressed mono- and mixed cultures of *Bouteloua curtipendula* (BOCU) and *B. gracilis* (BOGR), X = soil water content in bars when leaf diffusive resistance from plants in the stressed pots became significantly different from controls.

Species	Density	Soil water when transpiration was significantly reduced (bars)	
		Monocultures	Mixed
BOCU	8	-31.1 < x < -24.6	-50.0 < x < -24.0
BOCU	32	-34.7 < x < -26.6	-50.0 < x < -32.6
BOCU	64	-14.1 < x < -6.1	-14.7 < x < -8.7
BOGR	8	-8.1 < x < -3.3	-50.0 < x < -24.0
BOGR	32	-42.8 < x < -18.1	-50.0 < x < -32.6
BOGR	64	-50.0 < x < -26.7	

NOTE: The highest reading in bars equals the measurement taken 24 hours prior to reduction in leaf diffusive resistance. The lowest reading occurred on the day of the significant reduction in leaf diffusive resistance.

decrease in water potential as compared to sorghum. *B. gracilis* appears to have this same, but slight advantage over *B. curtipendula* (Figure 10). The greatest deviation in this data occurs near the -50 bar level which is the limits of the sample chamber psychrometer. The differences between these relatively drought resistant two species were not as great as those between corn and sorghum.

Biochemical Analysis

Soluble carbohydrates increased significantly under water stress in both mono- and mixed cultures of both species (Table 7). The greatest increases occurred in the low density *B. gracilis* mono- and mixed and in the high density monocultures where amounts reached over 325% of controls. It is difficult to make comparisons from this table as to the effects of density and species-association under the water stress regimes. Not all treatments were stressed to the same degree which could account for the differing amounts of soluble carbohydrates accumulated under stress. A comparison of water stressed plants can be made by plotting amounts present against percent plant water at harvest (Figure 11). The data points include controls in addition to stressed plants. *B. gracilis* had a higher soluble carbohydrate content at a given water content than *B. curtipendula* and the rate of accumulation was greater. The data from mixed cultures showed no significant correlations.

Levitt (1972) points out that the role of sugar accumulation in drought tolerance had not been proven even through a correlation exists between sugar accumulation and water stress. The role of increased cell sap has been given two explanations by

Figure 10.--Tissue osmotic potential of *Bouteloua curtipendula* (○) and *B. gracilis* (●) plotted against % plant water at harvest. Each tissue osmotic potential is an average of 5 measurements. (BOCU, r = -0.925, p = 0.01, y = 74.975 + 0.671x; BOGR, r = 0.87, p = 0.01, y = 80.938 + 0.631x).

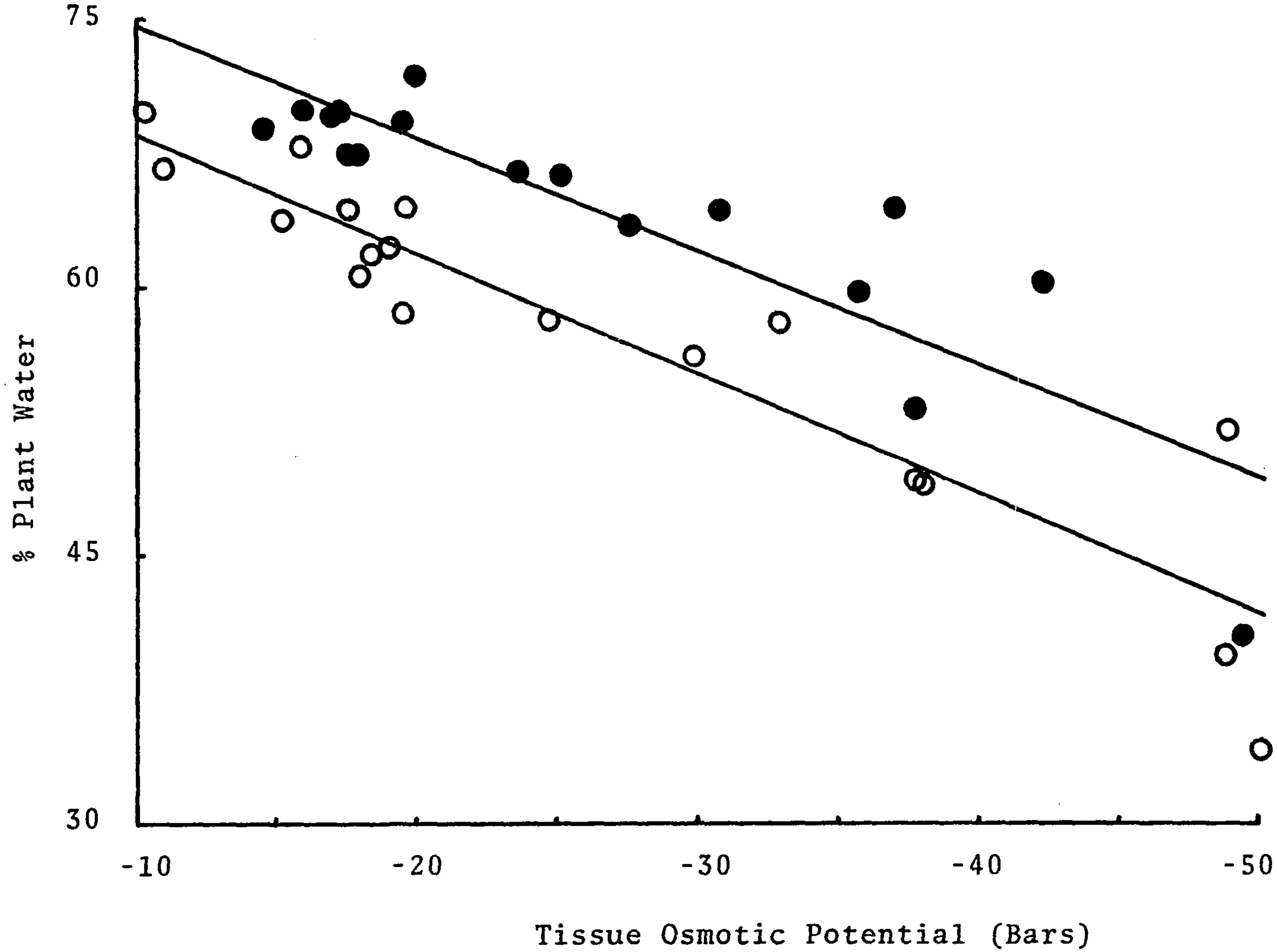
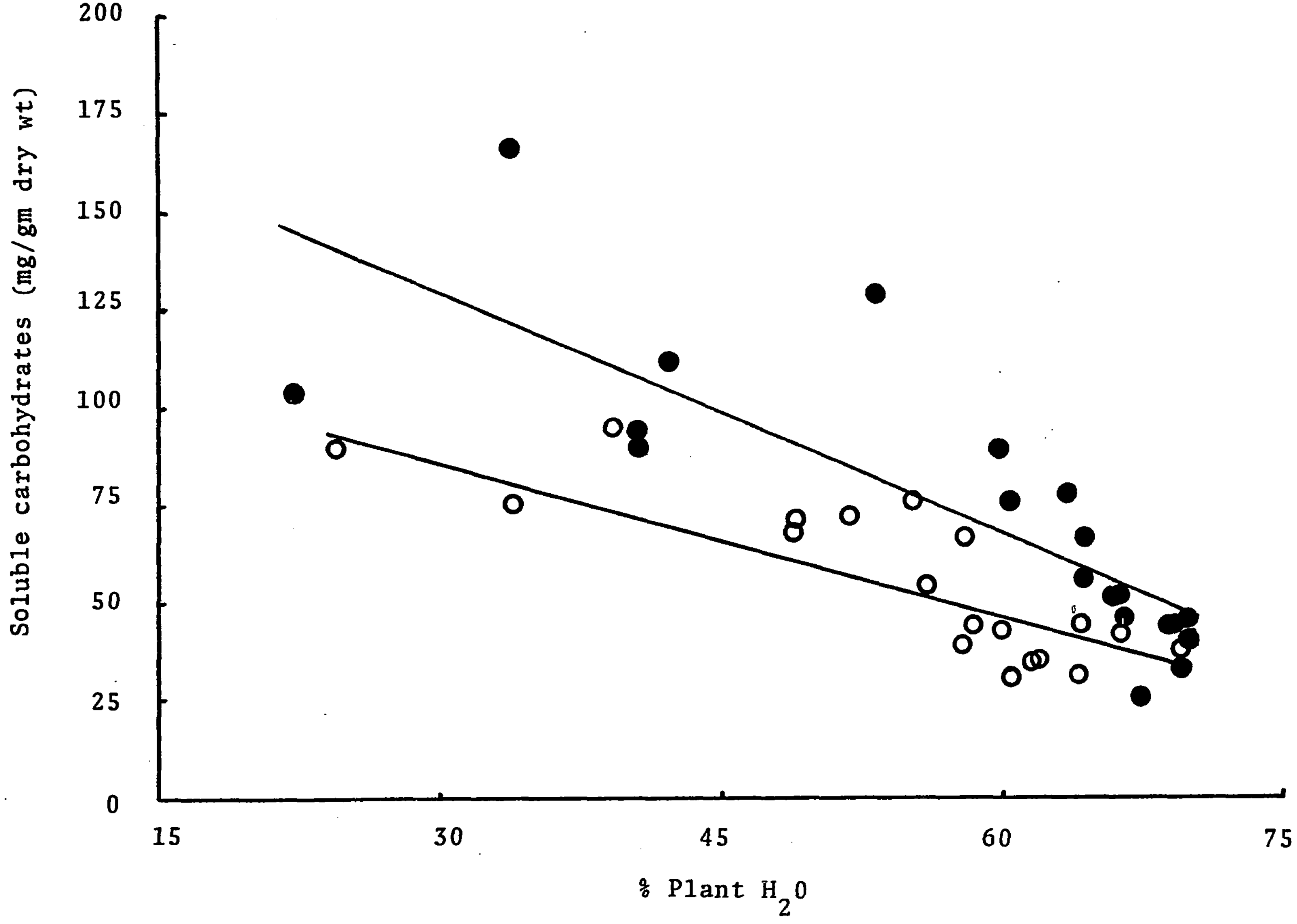

75
60
45
30
% Plant Water
-10
-20
-30
-40
-50
Tissue Osmotic Potential (Bars)

TABLE 7

Soluble carbohydrates equated to a glucose equivalent in mono- and mixed cultures of *Bouteloua curtipendula* (BOCU) and *B. gracilis* (BOGR) of watered (control) and stressed plants. (Values are averages based on two replicates expressed as mg/gm dry wt.)

		Monoculture			Mixed		
Species	Density	Control	Stress	% of Control	Control	Stress	% of Control
BOCU	8	39.87	85.33	214.0	45.28	107.07	236.5
BOCU	32	44.29	87.85	198.4	37.58	74.99	199.5
BOCU	64	30.79	69.79	226.7	32.22	83.67	259.7
BOGR	8	45.14	147.94	327.7	51.27	172.59	336.6
BOGR	32	42.21	99.23	325.1	46.66	94.40	202.3
BOGR	64	29.33	101.13	344.8	43.20	82.84	191.8

Figure 11.--Soluble carbohydrates from stress and control plants in monocultures of *Bouteloua curtipendula* (○) and *B. gracilis* (●) plotted against % plant water at harvest. (BOCU r = -0.83, p = 0.01, y = 125.62 - 1.32x; BOGR r = -0.81, p = 0.01, y = 191.21 - 2.05x).


Soluble carbohydrates (mg/gm dry wt)
200
175
150
125
100
75
50
25
0
15
30
45
60
75
% Plant H_2O

Maximov (1929): 1) the accumulation of substances which might protect the protoplasm from coagulation and desiccation, and 2) in spite of an increasing water deficit, the high concentration might prevent visible wilting for a long time.

In corn (Maranville and Paulsen 1970) and bean leaves (Stewart 1971) sucrose was the sugar which increased under water stress. Stewart found that the amount of starch lost in the excised bean leaves accounted for the gain in free sugars. Sosebee and Wiebe (1971) found that translocation of photosynthate in crested wheatgrass (*Agropyron cristatum*) and cultivated barley (*Hordeum vulgare*) was decreased under water stress. This would also result in an increase in free sugars or starch in the leaf tissue.

As density increased in both mono- and mixed cultures, the starch content in *B. curtipendula* decreased under stress at a diminishing rate (Table 8). This may be attributed to the length and degree of water stress which lessened as density increased. The change in starch content under stress in *B. curtipendula* may account for the increase in soluble carbohydrates. *B. gracilis* exhibited little change in starch content under water stress. Total carbohydrates, obtained by adding the amount of soluble carbohydrates to the amount of starch present showed little change in *B. curtipendula*, but an increase occurred in *B. gracilis*. Some general factors which could account for this difference are: 1) continued photosynthesis in both species with a reduction in translocation in *B. gracilis*, 2) starch hydrolysis exceeding starch synthesis in *B. curtipendula*, 3) a higher

TABLE 8

Starch expressed as a glucose equivalent from mono- and mixed cultures of *Bouteloua curtipendula* (BOCU) and *B. gracilis* (BOGR). (Values are averages based on two replicates expressed as mg/gm dry wt.)

Species	Density	Monoculture		% of	Mixed		% of
		Control	Stress	Control	Control	Stress	Control
BOCU	8	273.2	174.1	63.7	251.7	152.7	60.7
BOCU	32	113.7	80.1	70.4	255.9	153.4	59.9
BOCU	64	324.8	304.9	93.9	335.3	272.3	81.2
BOGR	8	172.9	210.6	121.8	227.4	274.3	91.2
BOGR	32	260.2	253.9	97.6	275.7	274.4	99.5
BOGR	64	222.2	227.2	102.3	407.4	379.8	93.2

respiration rate in *B. curtipendula*, and/or 4) photosynthesis maintained at a greater rate in *B. gracilis*. Gas exchange of forty day old seedlings of these two species at 4,000 ft-c showed that *B. gracilis* had a higher rate of photosynthesis and a lower rate of respiration than *B. curtipendula* (Risser and Johnson 1973). Additional experiments as to the effects of water stress on the gas exchange rates in these two species and enzymology work need to be examined before the significance of these factors can be evaluated.

Although the differences were not significantly different when compared to controls, the soluble amino acids from the stressed plants increased in *B. gracilis* and decreased in *B. curtipendula* as density increased (Table 9). Possible sources for this increase include proteolysis and/or *de novo* synthesis (Kemble and MacPherson 1954, Barnett and Naylor 1966, Thompson, Stewart and Morris 1966). In these studies the amino acid, proline, accumulated in amounts greater than could be accounted for by proteolysis. It has been suggested that proline operates as a non-toxic nitrogen and energy storage compound. The individual components of the amino acid fraction were not analyzed in this study so it is not possible to say which amino acids or acid may be increasing as a result of water stress. The protein data from the plant tissue was variable and not significant to account for any direct relationship between protein breakdown and amino acid buildup. The Folin phenol protein assay is affected by the presence of phenols (Lowry *et al*. 1951) and phenolics such as chlorogenic acids and isochlorogenic acids have been found to increase

TABLE 9

Soluble amino acids in mono- and mixed cultures of *Bouteloua curtipendula* (BOCU) and *B. gracilis* (BOGR) of watered (control) and stressed plants. (Values are averages based on two replicates, expressed as mg/gm dry wt.)

		Monoculture		% of	Mixed		% of
Species	Density	Control	Stress	Control	Control	Stress	Control
BOCU	8	0.41	0.75	182.9	0.45	0.91	202.2
BOCU	32	0.32	0.58	181.3	0.30	0.55	183.3
BOCU	64	0.35	0.43	122.9	0.31	0.47	151.6
BOGR	8	0.77	0.77	100.0	1.08	1.18	109.3
BOGR	32	0.79	0.81	102.0	0.69	0.89	129.0
BOGR	64	0.69	0.84	121.7	0.54	1.02	188.9

under water stress (del Moral 1972). Water extraction of protein with and without polyclar AT (General Aniline and Film Corporation Dyestuffs and Chemical Division, 436 Hudson Street, New York, New York) which precipitates phenols, resulted in the protein values from tissue in the absence of polyclar AT to be 15% to 19% lower than when polyclar AT was present.

CHAPTER IV

SUMMARY

An increase in density resulted in a reduction in height, leaf length, width and number in watered monocultures of *Bouteloua curtipendula* and *B. gracilis*. The mixed cultures exhibited the same response with an added effect of the suppression of *B. gracilis* at high densities.

Water stress, based on morphological responses had its greatest effect on the low density cultures having the most rapid growth rate. The higher two densities showed no additional morphological affects.

The root/shoot ratio in monocultures of *B. gracilis* showed an increase at all levels of the stressed treatments as compared to the controls and also as density increased. *B. curtipendula* R/S ratio increased under water stress in monocultures of both low and medium densities when compared to controls, but no difference occurred at the high density level. *B. gracilis* at the high density level maintained transpiration at lower soil water levels than *B. curtipendula*. The combination of more aboveground biomass with greater leaf area for water loss in relation to the amount of water absorbing belowground biomass in *B. curtipendula* at the high density level could indicate a susceptibility in water lost via transpiration to exceed water uptake stimulating stomatal closure. *B. gracilis* also exhibited a slightly greater desiccation-resistance as evidenced by its ability to have a small decrease in water content for a given decrease in water potential.

Biochemical analysis of water stressed plants indicated a significant increase of soluble carbohydrates in mono- and mixed cultures of both species. *B. gracilis* had a higher soluble carbohydrate content at a given water content in monocultures than *B. curtipendula*. Starch content decreased in *B. curtipendula* but exhibited little change in *B. gracilis*. The soluble amino acids from water stressed plants increased in *B. gracilis* and decreased in *B. curtipendula* as density increased.

The replacement of *B. gracilis* by *B. curtipendula* under moist field conditions may operate similarly to the suppression effect *B. curtipendula* had over *B. gracilis* in the high density mixed cultures. *B. curtipendula* being taller with wider leaves may cause a shading effect resulting in light competition. However, since shoot and root competition were not kept separate, possible nutrient deficiencies due to uptake rates can not be eliminated.

Based primarily on root-shoot ratios, desiccation-resistance and carbohydrate accumulation, *B. gracilis* had slight advantages over *B. curtipendula* under water stress in monocultures. These factors under drought conditions would allow a greater percentage of *B. gracilis* to survive and eventually replace the lesser drought resistant *B. curtipendula*.

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