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PRELIMINARY RESULTS OF GROWTH CHARACTERISTICS OF BUFFALOGRASS, BLUE GRAMA, AND WESTERN WHEATGRASS, AND METHODOLOGY FOR TRANSLOCATION STUDIES USING ^{14}C AS A TRACER

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ABSTRACT

The temperature response of various growth characteristics of buffalograss (*Buchloe dactyloides* [Nutt.] Engelm), blue grama (*Bouteloua gracilis* [H.K.B.] Lag. ex Steud.), and western wheatgrass (*Agropyron smithii* Rydb.) was determined under controlled conditions. Buffalograss and blue grama had highest growth rates in the 32.2/26.7°C day/night temperature regime, while western wheatgrass grew best in the 15.6/10.0°C and 10.0/4.4°C temperature regimes.

Methodology for studying carbohydrate translocation to belowground parts of buffalograss, blue grama, and western wheatgrass was developed using ^{14}C as a tracer. Methods for administering $^{14}CO_2$ and sampling the plant material, including sample preparation for radioactive analysis, was developed. These procedures and experimental data are discussed.

INTRODUCTION

Primary objectives were two-fold: first, to grow buffalograss (*Buchloe dactyloides* [Nutt.] Engelm), blue grama (*Bouteloua gracilis* [H.K.B.] Lag. ex Steud.), and western wheatgrass (*Agropyron smithii* Rydb.) under controlled environmental conditions to familiarize the investigators with the growth habits of these grasses, to determine growth responses to temperature, and to obtain preliminary chemical composition data of individual plant parts as influenced by temperature; and second, to establish procedures for administering, sampling, and analyzing ^{14}C as a tracer in carbohydrate translocation experiments in growth chamber and field experiments.

MATERIALS AND METHODS

Growth Characteristics

Seed obtained from the Longmont Seed Company, Longmont, Colorado, was germinated in sand flats in the greenhouse. Origin of the seed was obtained from the following areas: buffalograss, 1968 crop from Lubbock, Texas; blue grama, 1968 crop from Eads, Colorado; and western wheatgrass from Newell, South Dakota.

Uniform seedlings approximately 5 cm tall were transplanted into plastic pots (three seedlings/pot with 11 cm diameter by 14.6 cm deep with drainage) that contained greenhouse soil of high fertility. A completely random design with six replications with day/night temperature regimes of 37.8/32.2, 32.2/26.7, 26.7/21.1, and 21.1/15.6°C for buffalograss and blue grama and 32.2/26.7, 26.7/21.1, 21.1/15.6, 15.6/10.0 and 10.0/4.4°C for western wheatgrass was established over time in growth chambers. Plants were exposed to 1800 f.c. from cool-white florescent lamps and incandescent bulbs for an 18-hour

light period. All pots received adequate water and 50 kg/ha N as ammonium nitrate at two weeks and again at six weeks after the start of the experiment.

Blue grama and buffalograss were harvested when 50% of the emerged inflorescences of blue grama were at anthesis. Since western wheatgrass did not produce inflorescences, date of harvest was more arbitrary. Western wheatgrass was harvested at the approximate time when 15 leaves had emerged on the main plant culm. Days of growth, plant height, and number of tillers per pot were recorded for each species. Buffalograss and blue grama were cut to leave a 3 cm stem base, western wheatgrass was cut to leave a 5 cm stem base, and roots were washed free of soil.

Plants were separated into parts and data taken as follows: buffalograss; number of stolons, stolon nodes, and total stolon length per plant were determined. Main plant green leaves, stolon green leaves, total plant dry leaves, and total plant sheaths were separated. Stolons were cut into three equal lengths and handled separately. These plant parts plus stem bases and roots were dried at 70°C, weighed, and held for chemical analysis. For blue grama, the number of flowering culms and heads was recorded. Heads, green leaves, green sheaths, dry leaves, stems, stem bases, and roots were separated, dried at 70°C, weighed, and held for chemical analysis. For western wheatgrass, the number of leaves per pot was recorded. Green leaves, green sheaths, dry leaves, stems, stem bases, roots, and rhizomes were separated, dried at 70°C, weighed, and held for chemical analysis.

Sampling was begun at 9:00 AM and continued until completed. The time evolved from sampling to drying for any pot did not exceed 45 minutes. Harvest time for western wheatgrass was 15 to 30 minutes.

Radioactive Tracer Studies

Tracer introduction. Plexiglass feeding chambers were constructed to hold potted plants or to be placed over field plots. The closed system (Fig. 1) included a circulation pump, a $^{14}CO_2$ generating flask, a $^{14}CO_2$ trapping flask, and a heat exchanger. The preliminary experiments also included a second air pump to circulate a portion of the main gas streams through an infra-red gas analyzer. This was done to monitor CO_2 uptake during feeding and trapping.

Approximately 500 µc of radioactive sodium carbonate (specific activity ca 55 µc/m mole) was introduced into the acid containing $^{14}CO_2$ generator. After approximately five minutes of active CO_2 uptake via photosynthesis, the gas stream was routed through the 1 N sodium hydroxide trap for an additional five minutes. A 10 ml gas sample was taken from the chamber and injected through a serum cap into a 50 ml erlynmeyer flask containing 1 ml NCS solubilizer to determine residual $^{14}CO_2$ in the feeding system. Plants were removed from the chamber and allowed to continue active photosynthesis in the growth chamber. Aliquots were drawn from the NaOH trap and from the generator flask to determine their ^{14}C activity.

Plant sampling. Buffalograss, blue grama, and western wheatgrass plants grown in the greenhouse (28/21°C mean day/night temperatures) were given $^{14}CO_2$ and subsequently sampled at 3, 9, and 24 hours. Harvest plants were separated into leaves, stems, and stem bases and immediately frozen in dry ice. Roots were washed free of soil, separated into upper and lower portions and frozen in dry ice. A field trial was conducted at the Pawnee Site U.S. IBP Grassland Biome to compare greenhouse and field procedures.

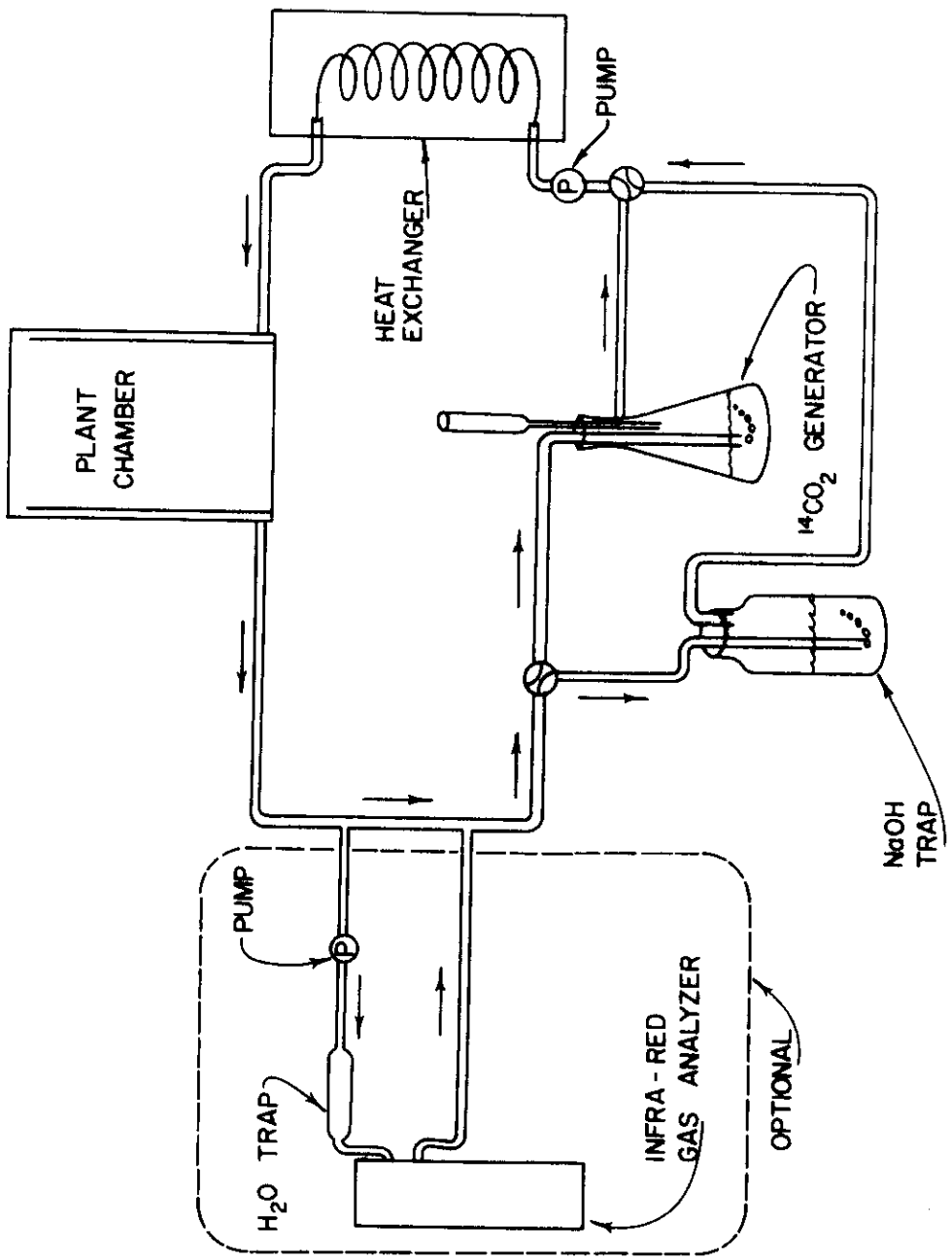

Fig. 1. Diagram of closed feeding system for administering $^{14}CO_2$ to plants in the growth chamber or field.

Blue grama was given $^{14}CO_2$ in the closed system (Fig. 1) at 7:15 MST. Samples were taken at 3, 9, and 24 hours after feeding. Plants were clipped to leave a 5 cm stem base. A root core, 7.6 cm in diameter and 10 cm deep, was taken. Stem base and herbage tissure was frozen immmediately in dry ice. Roots were washed free of soil and frozen in dry ice. All samples were freeze-dried, ground in a ball mill, and held for radioactive analysis.

Analysis. Several procedures for analyzing radioactivity of plant tissue were tested. These included tissue solubilizers (Nuclear Chicago Bulletin 16), wet combustion of 50-100 mg samples (Jeffay and Alvarez 1961), Schöniger dry combustion of up to 100 mg samples (Schöniger 1955), in-vial dry combustion of less than 4 mg samples (Gupta 1966), in-vial wet combustion of up to 25 mg sample (Shimshi 1969), and suspension of up to 25 mg samples in thixotropic gel (Eastin 1970). Samples were counted in a toluene based cocktail containing 5 g/l PPO (2, 5-diphenyloxazole) and 0.3 g/l dimethyl POPOP (2, 2^1-p-phenylenebis [4-methyl-5-phenyloxazole]). Procedures involving generation and subsequent trapping of CO_2 in ethanolamine also included addition of appropriate amounts of ethylene glycol monomethyl ether before scintillation counting (Jeffay and Alvarez 1961).

Liquid aliquots from the NaOH trap and the $^{14}CO_2$ generator were dissolved in ethanol and NCS (tradename for Rohn and Haas), respectively, and counted with the toluene cocktail. The CO_2 in the residual gas sample was trapped in NCS and counted with toluene cocktail. Other chemicals for solubilizing aqueous and alkaline samples were tested.

Quench curves. A chemical quench curve was constructed by adding varying amounts of chloroform to the toluene cocktail containing a toluene radioactive standard. The results were used to construct a graph of percent

counting efficiency vs. external standard ratio of the scintillation counter. The equation of this curve will be determined and used to correct sample counts per minute (cpm) to disintigration per minute (dpm). A single quench curve is required for most types of chemical quenching. Additional curves will be required if the procedures include variable mass quenching (self absorption) and color quenching.

RESULTS AND DISCUSSION

Growth Characteristics

Replication means with associated standard errors for several growth parameters of buffalograss, blue grama, and western wheatgrass grown under various temperature regimes are presented in Tables 1 through 6. Data that have been adjusted for length of growing period is most meaningful. Fig. 2 shows the temperature response curve of total dry matter production of the three grass species under the conditions of the experiment. Buffalograss and blue grama had an optimum growth rate at the 32.2/26.7°C temperature regime, while western wheatgrass exhibited an optimum growth rate between 15.6/10.0 and 10.0/4.4°C temperature regimes. Optimum growth rates of buffalograss and blue grama occurred over a rather narrow temperature range, while that of western wheatgrass occurred over a wide temperature range (nearly 11°C).

Most of the data for the additional parameters indicated trends that would be expected, given the total dry matter temperature response curves (Fig. 2). However, there are several interesting deviations.

Buffalograss. (Tables 1 and 2). Stolon internode length of buffalograss was not affected by temperature except in the 37.8/32.2°C regime where

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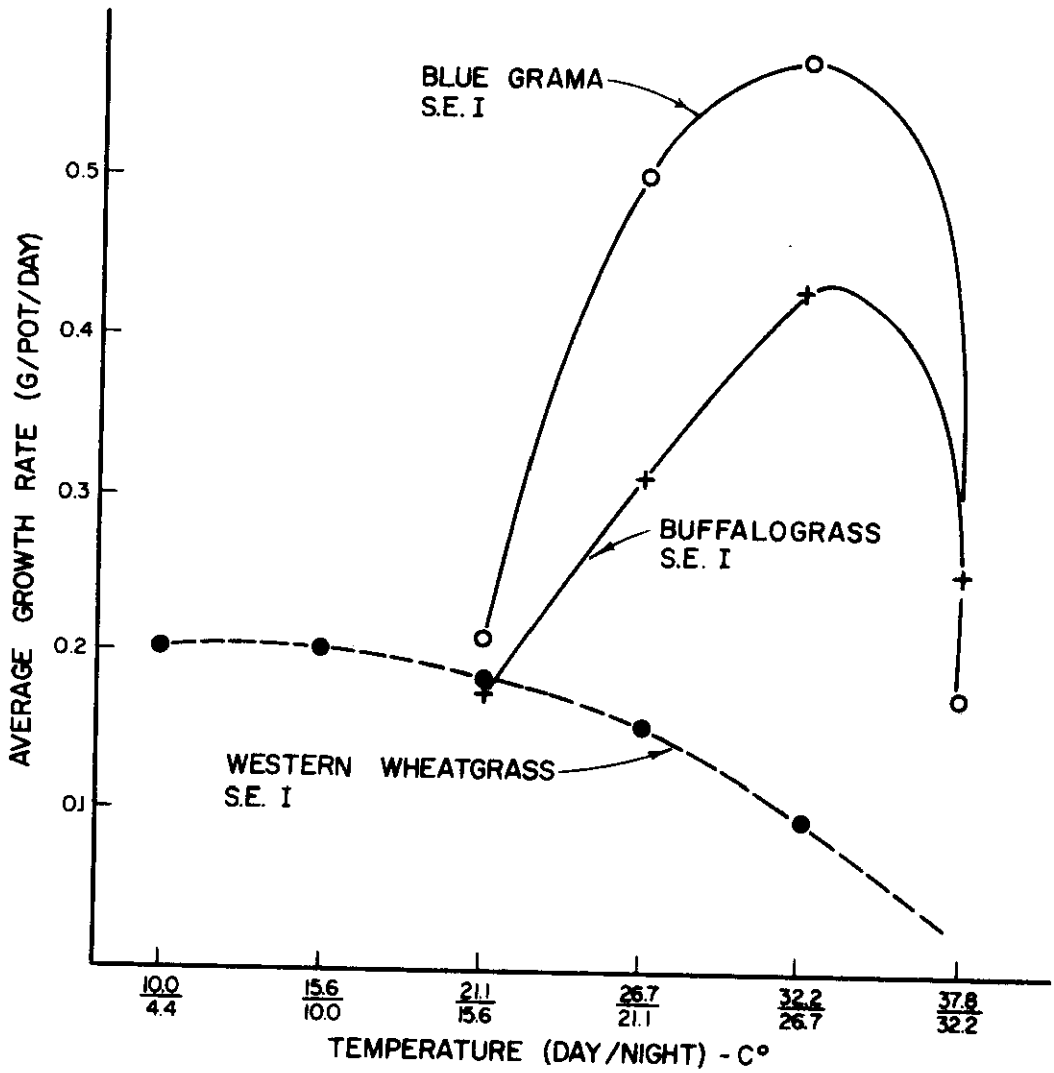

Fig. 2. Effect of temperature on the growth of buffalograss, blue grama, and western wheatgrass.

Table 1. Values (unadjusted for length of growth period) obtained for various parameters of buffalograss grown at four temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C				S.E.	"F" Test
		21.1/15.6	26.7/21.1	32.2/26.7	37.8/32.2		
Growth period	days	85	63	55	64	--	--
Plant ht.	cm	19.1	20.0	17.1	25.4	1.0	**
No. tillers	no./pot	16.1	61.5	88.2	38.2	3.4	**
Stolons/plant	no.	28.2	36.7	38.3	22.5	4.9	ns
Stolon nodes/ plant	no.	46.2	87.0	84.3	52.2	13.9	ns
Stolon length/ plant	m	2.83	5.27	4.97	2.24	0.21	**
Avg stolon internode length	cm	6.10	6.45	6.20	4.25	0.44	**
Main shoot green leaf wt	g/pot	1.32	1.25	1.90	1.48	0.30	ns
Stolon shoot green leaf wt	g/pot	1.59	2.64	3.54	1.94	0.23	**
Stolon wt	g/pot	4.57	7.32	7.95	2.50	0.63	**
Total plant stem plus sheath wt	g/pot	0.97	2.12	3.09	1.31	0.15	**
Dry leaf wt	g/pot	1.32	2.02	2.51	1.64	0.27	*
Stem base wt	g/pot	2.49	1.76	1.94	1.54	0.17	**
Total shoot wt	g/pot	12.27	17.12	20.93	12.92	1.13	**
Root wt	g/pot	2.85	2.39	2.54	3.16	0.28	ns
Total plant wt	g/pot	15.12	19.51	23.48	16.08	1.24	**

Table 2. Values (adjusted for length of growth period) obtained for various growth parameters of buffalograss grown at four temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C				S.E.	"F" Test
		21.1/15.6	26.7/21.1	32.2/26.7	37.8/32.2		
Tillers/pot	no./week	1.33	6.83	11.22	4.17	0.39	**
Stolon length/ pot	cm/week	28.3	58.5	63.3	24.5	7.4	**
Stolon wt/pot	g/week	0.376	0.814	1.012	0.274	0.064	**
Stem base wt/pot	g/week	0.205	0.196	0.248	0.168	0.015	*
Dry leaf wt/pot	g/week	0.109	0.224	0.320	0.179	0.031	**
Shoot wt/pot	g/week	1.01	1.90	2.66	1.41	0.12	**
Root wt/pot	g/week	0.235	0.266	0.323	0.346	0.031	ns
Total plant wt/pot	g/day	0.178	0.310	0.427	0.251	0.018	**
Total green leaf wt to sheath wt ratio	--	3.25	1.91	1.78	2.63	0.07	**
Dry leaf wt to green leaf wt ratio	--	0.448	0.518	0.500	0.481	0.024	ns
Shoot/root ratio	--	4.34	7.68	8.91	4.23	0.81	**
/S		.2	.13	.1	.24/		

internodes were significantly shorter than at cooler temperatures. Root weights increased as temperature increased, even to the 37.8/32.2°C regime. Unpublished data obtained previously suggests that this may be due to reduced soil temperature compared to air temperature caused by water evaporation from soil. Therefore, the temperature of the root zone was probably lower than that reported for air temperature.

Blue grama. (Tables 3 and 4). Although optimum growth occurred at 32.2/26.7°C (Fig. 2), maximum tiller production occurred at 21.1/15.6°C. Leaf-stem ratio was lowest at the temperature for optimum growth rate, suggesting that the dry matter production temperature response curve reflects the effect of temperature on morphological development--in this case, stem development. Unlike buffalograss, the shoot-root ratio was unaffected by temperature.

Western wheatgrass. (Tables 5 and 6). Tiller production was much higher at the high temperature regime compared with cooler temperatures. This result is contrary to the temperature response of tiller production by most cool-season grasses.

Radioactive Tracer Studies

The method of tracer introduction and plant sampling was formulated and tested by the trial and error method without conducting definite experiments. These procedures will be modified as conditions and experience suggest.

Analysis of radioactivity. NCS solubilizer obtained from the Nuclear Chicago Bulletin 16 (Division of Nuclear Chicago Corporation 1965), was tested for its ability to "solubilize" plant tissue for scintillation counting. Table 7 shows the external standard ratio obtained from various sample sizes of root, stem base, and leaf material. Samples were incubated

Table 3. Values (unadjusted for length of growth period) obtained for various parameters of blue grama grown at four temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C				S.E.	"F" Test
		21.1/15.6	26.7/21.1	32.2/26.7	37.8/32.2		
Growth period	days	86	62	52	66	--	--
Plant ht	cm	38.5	55.7	42.8	36.9	1.8	**
No. tillers	no./pot	350	169	146	103	5.9	**
No. flowering culms	no./pot	2.0	18.0	13.6	1.8	3.3	**
No. heads	no./pot	3.2	34.4	19.8	5.0	5.4	**
Head wt	g/pot	0.044	0.866	0.466	0.068	0.158	**
Green leaf wt	g/pot	4.99	7.43	7.86	3.28	0.62	**
Green sheath wt	g/pot	1.06	2.59	2.38	0.94	0.23	**
Dry leaf wt	g/pot	1.91	3.21	1.89	1.00	0.23	**
Stem wt	g/pot	0.45	2.29	2.40	0.69	0.43	**
Stem base wt	g/pot	4.47	7.84	8.19	2.35	0.60	**
Total shoot wt	g/pot	12.9	24.2	23.2	8.3	1.49	**
Root wt	g/pot	5.15	9.76	8.50	2.71	1.05	**
Total plant wt	g/pot	18.1	34.0	31.7	11.0	2.1	**

Table 4. Values (adjusted for length of growth period) obtained for various growth parameters of blue grama grown at four temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C				S.E.	"F" Test
		21.1/15.6	26.7/21.1	32.2/26.7	37.8/32.2		
No. tillers/pot	no./week	28.5	19.2	19.2	10.9	1.8	**
Flowering culms/ 10 tillers	no.	0.06	1.08	0.90	0.18	0.19	**
Heads/flowering culms	no.	1.77	1.92	1.54	1.14	0.12	ns
Wt/10 heads	g	0.12	0.24	0.27	0.05	0.03	**
Green leaf wt/stem wt	--	18.64	7.66	3.94	6.60	4.07	ns
Dry leaf wt/pot	g/week	0.15	0.36	0.25	0.11	0.03	**
Stem base wt/pot	g/week	0.36	0.88	1.10	0.25	0.07	**
Shoot wt/pot	g/week	1.05	2.74	3.12	0.88	0.18	**
Root wt/pot	g/week	0.42	1.10	1.14	0.29	0.13	**
Total plant wt/pot	g/day	0.210	0.548	0.609	0.167	0.037	**
Green leaf wt/sheath wt	--	5.17	2.84	3.84	3.57	0.71	ns
Dry leaf wt/ green leaf wt	--	0.41	0.45	0.24	0.31	0.05	*
Shoot wt/root wt	--	2.61	2.52	3.09	3.16	0.33	ns

5/8 .35 .40 .32 .32

Table 5. Values (unadjusted for length of growth period) obtained for various growth parameters of western wheatgrass grown at five temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C					S.E.	"F" Test
		10.0/4.4	15.6/10.0	21.1/15.6	26.7/21.1	32.2/26.7		
Growth period	days	84	73	80	63	58	--	--
Plant ht	cm	58.4	48.3	47.0	41.7	39.5	1.8	**
No. tillers	no./pot	16.2	24.3	12.0	9.4	26.4	3.2	**
Leaves/pot	no.	43.7	96.5	115.6	95.2	89.6	10.2	**
Green leaf wt	g/pot	3.78	4.50	4.75	3.31	2.55	0.31	**
Dry leaf wt	g/pot	0.94	0.38	0.24	0.29	0.09	0.03	**
Sheath wt	g/pot	2.67	1.89	0.92	0.56	0.61	0.16	**
Stem wt	g/pot	1.38	0.58	1.32	0.78	0.13	0.24	**
Stem base wt	g/pot	2.12	1.80	1.89	1.16	1.02	0.19	**
Total shoot wt	g/pot	10.89	9.20	9.12	6.11	4.38	0.75	**
Root wt	g/pot	4.29	4.27	3.96	1.62	1.36	0.40	**
Rhizome wt	g/pot	2.44	1.78	1.81	1.10	0.39	0.28	**
Total plant wt	g/pot	17.62	15.20	14.89	8.83	6.13	1.11	**

Table 6. Values (adjusted for length of growth period) obtained for various growth parameters of western wheatgrass grown at five temperature regimes in the growth chamber.

Parameter	Units	Temperature Regimes - °C					S.E.	"F" Test
		10.0/4.4	15.6/10.0	21.1/15.6	26.7/21.1	32.2/26.7		
Tillers/pot	no./week	1.35	2.33	1.05	1.04	3.19	0.38	**
Stem base wt/pot	g/week	0.18	0.17	0.16	0.13	0.12	0.02	ns
Shoot wt/pot	g/week	0.91	0.88	0.80	0.68	0.53	0.07	*
Dry leaf wt/week	g/week	0.08	0.04	0.02	0.03	0.01	0.01	**
Root wt/pot	g/week	0.36	0.41	0.35	0.18	0.16	0.04	**
Rhizome wt/pot	g/week	0.20	0.17	0.16	0.12	0.05	0.02	**
Root + rhizome wt/pot	g/week	0.56	0.58	0.50	0.30	0.21	0.05	**
Total plant wt/pot	g/day	0.210	0.208	0.186	0.140	0.106	0.015	**
Green leaf wt/ sheath wt	--	1.44	2.42	6.03	6.95	4.23	0.79	**
Green leaf wt/ stem wt	--	3.04	8.90	2.57	4.44	27.52	3.05	**
Dry leaf wt/ green leaf wt	--	0.25	0.09	0.05	0.09	0.03	0.02	**
Root wt/ rhizome wt	--	2.04	2.70	2.19	1.94	5.93	1.32	ns
Shoot wt/root + rhizome wt	--	1.65	1.57	1.57	2.71	2.63	0.36	ns

R/S

Table 7. The effect of sample type and size on the NCS digestion and subsequent counting efficiency as indicated by external standard ratio.

Sample		
Type	Size	External Standard Ratio
Root tissue	50 mg	.237
	75 mg	.024
	100 mg	.011
	125 mg	.001
Stem base tissue	50 mg	.063
	75 mg	.003
	100 mg	.000
	125 mg	.000
Leaf tissue	50 mg	.000
	75 mg	.000
	100 mg	.000
	125 mg	.000

at 50°C with 1 ml NCS for 12 hours and then counted in a toluene cocktail. In addition to getting incomplete digestion, color quenching was so severe that this method cannot be used for radioactive analysis of our plant samples.

Table 8 shows external standard ratios obtained using an in-vial dry oxidation technique (Gupta 1966). Sample size did not affect counting efficiency. However, samples must be kept below 4.0 mg to get adequate burning because of the low amount of oxygen available in the vial. Use of a Schöniger combustion flask would allow combustion of larger samples because of the larger flask volume.

The in-vial methods of Eastin (1970) and Shimshi (1969) are still being tested. It is hoped that an in-vial technique will prove satisfactory for radioactive analysis of all plant tissue because of its simplicity and short time requirements.

Quench curve. Fig. 3 shows the quench curve obtained for chemical quenching with the Beckman LS 150 Liquid Scintillation Counter. The equation of the curve will be determined and used to correct cpm data obtained from tracer samples.

Time of sampling. All the harvested plant material from the growth chamber and field preliminary experiments are not analyzed, but some information is available. Table 9 shows the results of radioactive analysis of blue grama given $^{14}CO_2$ in the field. Activity was observed in the roots three hours after feeding, with a peak in activity after nine hours. The decline in root activity after 24 hours may be due to translocation outside the sampled root core zone or due to losses from root and root-feeding microorganism respiration (personal communication with F. Warren Bourg, Department of Soil Science, University of Sasketchewan).

Table 8. The effect of sample size on in-vial oxidation of root tissue by burning and subsequent counting efficiency as indicated by external standard ratio.

Sample Size (mg)	External Standard Ratio
0.9	.576
1.7	.577
2.1	.581
3.4	.581
4.2	.563
5.4	.566
6.2	.541

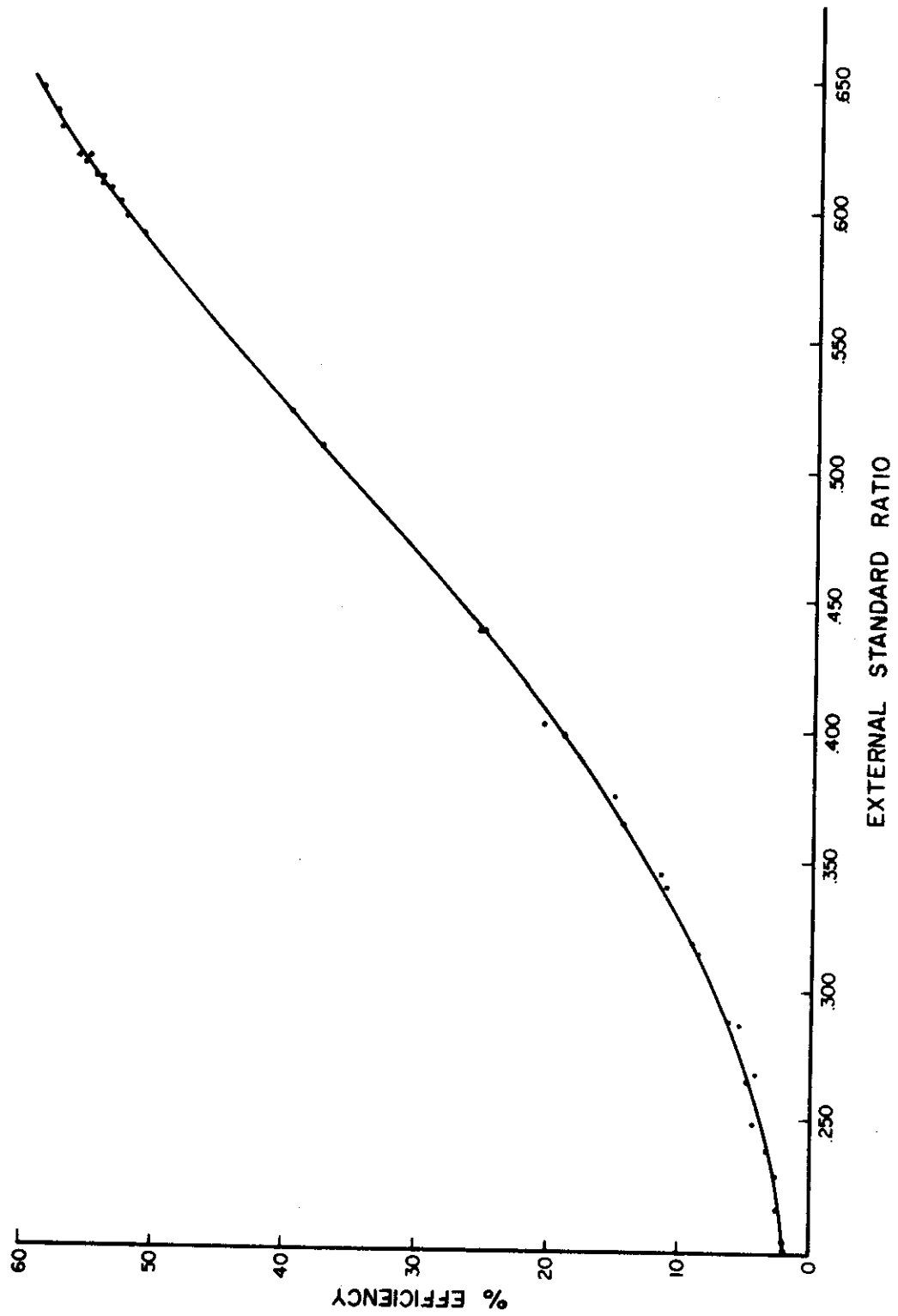

Fig. 3. Counting Efficiency Curve for ^{14}C on a Beckman LS 150 Liquid Scintillation Counter.

Table 9. Results of tracer analysis of blue grama after pulse feeding $^{14}CO_2$ (10^6 dpm).[1/]

	3 hr	9 hr	24 hr
Activity released	18.57	18.57	18.57
Activity trapped	0.39	3.18	0.38
Activity flushed	1.79	0.58	0.58
Activity fixed by plant (by subtraction)	16.39	14.81	17.61
Activity measured in roots (dpm/g)	73,235	146,110	72,223

[1/] Blue grama within exclosure containing a micro-watershed in heavy grazed pasture. Plants were fed on 20 August 1970, 9:15 AM MST.

Translocation calculations. Calculations of amounts of carbohydrate translocation are based on: (i) accumulation of radioactivity in the roots, and (ii) specific activity of carbohydrate in the translocation stream. The accumulation of radioactivity in the root zone will be determined by measuring the activity of roots at two successive time periods. These figure will be used to calculate the accumulation rate of radioactivity.

The specific activity of carbohydrate (sucrose) in the translocation stream will be measured by a combination of thin-layer chromatography and scintillation techniques. Sucrose will be extracted from the lower stem base or upper root sections and separated on cellulose acetate sheets according to the method of Vomhoff and Tucker (1965). The sucrose spots will be cut out, placed in a test tube, and the sucrose eluted. An aliquote will be analyzed for sucrose content by the ferricyanide procedure of Furuholmen et al. (1964). A second aliquote will be counted in the scintillation counter. These data will be used to determine sucrose specific activity. The amount of sucrose translocation to the roots sampled is the total accumulated activity in the roots divided by the specific activity of translocated sucrose.

Additional Research Studies

Methodology. A time course study is being conducted with the three grasses to determine the rate of sucrose labeling in the stem base and upper root sections. These data are needed to verify that large sucrose pools are not isolated from the translocation stream and therefore invalidate the proposed method of determining sucrose specific activity.

Experiments will be conducted in the greenhouse and field to determine the extent of radioactivity losses from root and micro-organism respiration.

Growth characteristics. Studies similar to those reported here are in progress using plants from the IBP site and from other North-South locations across the Great Plains. These data will be compared with the temperature response data reported in this report.

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APPENDIX I

FIELD DATA

Temperature Response of Grasses

Data on temperature response of grasses collected in 1970 is Grassland Biome data set A2U00AL. An explanation of the data formats and a listing of the data follow. The data format is different for each of the three grasses studied--blue grama, buffalograss, and western wheatgrass.

Card Type I Blue Grama

Column	Contents
1	Species, 1 digit code
3	Temperature day/night °F, 1 digit code
5	Replicate
7-8	Number days growth total
10-12	Plant height per replicate (cm)
14-16	Number of tillers per replicate
18-21	Stubble weight (g)
23-26	Root weight (g)
28-31	Shoot weight (g)
33-36	Total weight (g)
38-40	Stem weight (g)
42-44	Green sheath weight (g)
46-49	Green leaf weight (g)
51-53	Dry leaf weight (g)
55-57	Head weight (g)
59-60	Number of heads per replicate
62-63	Number of flowering culms
65-67	Total

Card Type II Buffalograss

Columns	Contents
1-36	Same as type I card
38-41	Total stolon weight (g)
43-45	Main tiller green leaf weight (g)
47-49	Stolon green leaf weight (g)
51-53	Green sheath weight (g)
55-57	Dry leaf weight (g)
59-60	Mean number stolons per plant
62-64	Mean number stolon nodes per plant
66-69	Mean stolon length per plant (cm)
71-73	Mean distance between nodes on stolons (cm)
75-77	Mean weight gain per day (g/day)

Card Type III Western Wheatgrass

Columns	Contents
1-36	Same as type I card
38-40	Number of leaves per pot
42-44	Green leaf weight (g)
46-48	Sheath weight (g)
50-52	Stem weight (g)
54-56	Dry leaf weight (g)
58-60	Rhizome weight (g)
62-64	Mean weight gain per day (g/day)

Codes Employed in the Data

Species

1 Blue Grama

2 Buffalograss

3 Western Wheatgrass

Temperature, Day/Night °F

1 50/40

2 60/50

3 70/60

4 80/70

5 90/80

6 100/90

+++ DATA +++

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          1         2         3         4         5         6         7
1234567890123456789012345678901234567890123456789012345678901234567890123456789

2 5 1 55 166  94  181  267 2511 2778  919 462 367 304 278 46 108  682  63 505
2 5 2 55 163  99  248  354 1990 2344  795 202 262 252 231 32  59  340  57 426
2 5 3 55 183  72  155  269 1836 2105  718 146 343 280 194 24  54  352  66 383
2 5 4 55 180  94  218  141 1956 2097  672 128 389 359 190 36  52  464  90 381
2 5 5 55 190  81  152  202 2040 2242  758 117 454 356 203 39  97  410  42 408
2 5 6 55 146  89  214  291 2228 2519  909  85 307 302 411 53 136  735  54 458
2 4 1 63 213  77  192  224 1176 1400  394 139 207 126 118 20  39  272  70 222
2 4 2 63 237  66  148  392 1814 2206  761 174 317 238 176 32  64  528  82 350
2 4 3 63 143  47  136  136 1404 1540  625  62 233 234 114 33  71  358  50 244
2 4 4 63 203  62  224  231 1889 2120  791 151 284 234 205 32  75  508  68 336
2 4 5 63 197  59  212  230 2072 2302  944  94 309 202 311 71 192  958  50 365
2 4 6 63 207  58  147  221 1918 2139  879 132 236 238 286 32  81  537  67 340
2 3 1 85 207  10  227  308 1342 1650  550 118 176 123 148 25  56  330  59 194
2 3 2 85 210  13  244  224 1210 1434  474 132 141  98 121 19  36  225  63 169
2 3 3 85 167  16  221  372 1445 1817  575 115 252 120 162 45  76  432  57 214
2   4 85 213  24  301  297 1697 1994  725 132 224 131 184 45  66  460  69 234
2 3 5 85 153  18  283  229  889 1118  229 168  70  58  81 15  16  107  65 132
2 3 6 85 197  16  221  280  780 1060  189 126  96  50  98 20  27  144  53 125
2 6 1 64 263  36  120  276 1389 1665  315 151 149 138 201 24  60  295  48 260
2 6 2 64 293  38  190  343 1236 1579  197 169 188 121 174 23  53  188  36 247
2 6 3 64 240  34  158  350 1129 1479  192 158 191 125 113 19  40  175  43 231
2 6 4 64 213  52  170  380 1411 1791  263 177 193 148 197 25  62  247  40 280
2 6 5 64 273  29  138  222 1291 1511  280 115 236 125 115 22  52  224  42 236
2 6 6 64 246  40  147  327 1298 1625  254 121 210 131 181 22  46  215  46 254
1 5 1 52 417 142  830 1479 2371 3850  216 251  810 230  34 19 13 740
1 5 2 52 387 149  610  539 1715 2254  141 204  647 104  09  4  2 433
1 5 3 52 413 129  848  828 2559 3387  448 286  735 170  72 26 17 651
1 5 4 52 460 174  995  926 2706 3632  254 330  806 220 101 46 33 698
1 5 5 52 463 138  811  480 2242 2722  142 120  933 219  17  4  3 523
1 4 1 62 577 170  796  874 2359 3233  206 241  712 365  39 22 10 521
1 4 2 62 620 110  522  828 1708 2536  306 178  422 226  54 33 16 409
1 4 3 62 513 155  724  914 2266 3180  204 258  722 306  52 22 14 513
1 4 4 62 476 171  807 1346 2714 4060   38 298 1060 403 108 39 22 655
1 4 5 62 597 243 1072  916 3068 3984  390 320  800 306 180 56 28 643
1 3 1 86 403 393  422  520 1296 1816   37 130  444 260  03  2  1 211
1 3 2 86 413 300  336  718 1190 1908   92 107  419 226  10  6  4 222
1 3 3 86 343 437  618  530 1624 2154   66 138  667 129  06  4  3 250
1 3 4 86 377 290  359  410 1153 1563   16 105  535 136  02  2  1 182
1 3 5 86 387 330  501  396 1199 1595   13  50  430 204  01  2  1 185
1 6 1 66 417 100  232  262  878 1140  151 106  263 106  20 19  7 173
1 6 2 66 363  95  224  352  784 1136   42  96  317  96  09  6  2 172
```

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1	6	4	66	350	79	229	248	816	1064	30	79	366	112	00	0	0	161
1	6	5	66	320	114	262	276	853	1129	60	86	344	101	00	0	0	171
3	5	1	58	400	10	47	77	240	357	140	40	10	40	45	062	03	
3	5	3	58	407	16	69	87	350	442	219	52	07	05	84	076	03	
3	5	4	58	390	48	175	196	640	888	365	81	08	52	144	153	11	
3	5	5	58	423	36	152	231	671	973	368	94	35	71	115	168	25	
3	5	6	58	353	22	67	89	290	406	181	36	05	27	60	070	01	
3	4	1	63	443	13	144	19	662	781	342	84	86	100	115	124	06	
3	4	2	63	470	6	100	329	609	1026	344	48	106	88	69	163	11	
3	4	3	63	383	12	146	215	704	971	348	84	62	52	111	154	64	
3	4	4	63	357	7	92	124	512	782	282	26	54	146	82	124	58	
3	4	6	63	430	9	96	124	567	853	341	40	82	162	99	135	08	
3	3	2	80	503	10	118	324	627	1101	415	89	0	150	82	138	05	
3	3	3	80	460	12	186	498	1036	1758	512	112	208	224	119	220	18	
3	3	4	80	450	16	240	424	1047	1631	540	90	132	160	147	204	45	
3	3	5	80	510	10	228	434	1161	1799	534	136	224	204	115	225	39	
3	3	6	80	427	12	174	300	690	1156	374	34	96	166	115	144	12	
3	2	1	73	520	19	177	331	861	1263	429	184	42	71	79	173	29	
3	2	2	73	490	26	192	486	983	1675	426	226	65	206	99	229	74	
3	2	3	73	500	27	149	274	863	1340	404	194	56	230	99	184	33	
3	2	4	73	503	20	180	528	946	1650	451	200	73	176	94	226	42	
3	2	5	73	407	31	228	424	996	1624	539	182	30	204	122	222	17	
3	2	6	73	480	23	152	520	871	1571	448	150	85	180	86	215	36	
3	1	1	84	650	14	178	383	1068	1662	367	229	213	211	41	198	81	
3	1	2	84	533	15	221	357	938	1466	328	211	106	171	51	174	72	
3		3	84	583	17	233	480	1310	2094	415	324	206	304	42	249	132	
3	1	4	84	530	19	280	522	1340	2001	470	349	122	139	49	238	119	
3	1	5	84	573	17	180	427	1000	1861	401	249	96	434	43	222	74	
3	1	6	84	633	15	179	406	879	1488	290	238	86	203	36	177	86	