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GROWTH OF *BOUTELOUA GRACILIS* IN A BIOSYNTHESIS CHAMBER:

I. FACILITIES AND METHODS FOR GROWING UNIFORMLY ^{14}C-LABELED BLUE GRAMA GRASS.

II. DYNAMICS OF CO_2 FLUXES WITHIN THE SYSTEM.

III. USE OF MATERIAL IN DECOMPOSITION STUDIES.

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ABSTRACT

Blue grama grass sods, 10 cm in depth, having a total surface area of 0.62 m^2 were grown in a closed system to which $^{14}CO_2$ of 0.3 μCi/mmole specific activity was added. Uniformly labeled top regrowth was collected in three successive harvests. Measurements of soil and plant respiration and net CO_2 assimilation (photosynthesis) were made. Proposed use of the material produced within the biosynthesis chamber environment in a study to estimate field decomposition rates is discussed.

GROWTH OF *BOUTELOUA GRACILIS* IN A BIOSYNTHESIS CHAMBER: I. FACILITIES AND METHODS FOR GROWING UNIFORMLY ^{14}C-LABELED BLUE GRAMA GRASS

INTRODUCTION

Blue grama grass (*Bouteloua gracilis*), uniformly ^{14}C-labeled, was needed for studies of decomposition rates at the Pawnee Site. Production of labeled plant material requires a special growth chamber in which plants can be grown for extended periods of time in an atmosphere containing carbon dioxide labeled with Carbon-14. Several biosynthesis chambers have been constructed specifically for growing ^{14}C-labeled plants (Smith, Allison, and Mullins 1962). This paper describes a growth chamber of a simple design which proved suitable for growing *B. gracilis* in a controlled atmosphere for labeling purposes.

FACILITIES

The biosynthesis chamber was a 0.50 mm thick polyvinyl tent (dimensions, 0.6 × 0.6 × 1.2 m) placed within a standard growth chamber (1.1 × 2.5 × 2.5 m) which provided control of light and temperature. Temperature within the biosynthesis chamber was maintained at approximately 30°C by operating the external growth chamber at 21°C. Light intensity within the biosynthesis chamber as measured by an Eppley pyranometer (180° measurement of both direct and indirect radiation) could be varied from .052 g cal cm^{-2} min^{-1} to .246 g cal cm^{-2} min^{-1} (old tubes) and from .091 cal cm^{-2} min^{-1} to .349 cal cm^{-2} min^{-1} (new tubes installed July 16) depending upon the number of cool white fluorescent tube banks and incandescent bulbs used. A ceiling exhaust fan was connected to a duct of the chamber to maintain a reduced air pressure inside the chamber and continually exhaust air from the chamber to the out-of-doors through a roof vent.

The biosynthesis chamber was equipped with sealed gloves to allow manipulation of materials inside the tent; access ports allowed attachment of sampling and return lines. The carbon dioxide concentration within the closed system of the biosynthesis chamber (tent, traps, and sample return lines) was continuously monitored in a closed loop sampling line passing through a Beckman Infrared Gas Analyzer calibrated to read from 300-900 ppm CO_2. A condenser trap cooled by tap water removed excess water vapor from the sample line. A small electric fan (synchronous motor, no brushes) provided mixing of the atmosphere with a breeze sufficient to cause gentle waving of grass blades on plants growing within the biosynthesis chamber. Continuous rapid air movement was essential to mix added carbon dioxide as well as to reduce temperature gradients within the tent (Fig. 1).

The CO_2 concentration within the biosynthesis chamber was continuously recorded on a Varian Strip Chart recorder connected to the IR Analyzer. When the CO_2 concentration within the system dropped to 300 ppm, a low limit switch on the recorder tripped a timer mechanism which released a preset volume of one molar Na_2CO_3 into a generating flask containing concentrated sulfuric acid. The CO_2 produced was swept from the generating flask through a water trap into the tent by a continuous flow of air circulated from the tent by a small pump. A water trap was placed in the inlet line to minimize flow of acid fumes and aerosols from the generator into the tent atmosphere. To compensate for the volume of CO_2 added to the closed system, a pressure regulator was attached to an outlet port on the biosynthesis chamber. The gaseous efflux was passed through an Indicarb filter to remove all $^{14}CO_2$ (Fig. 2).

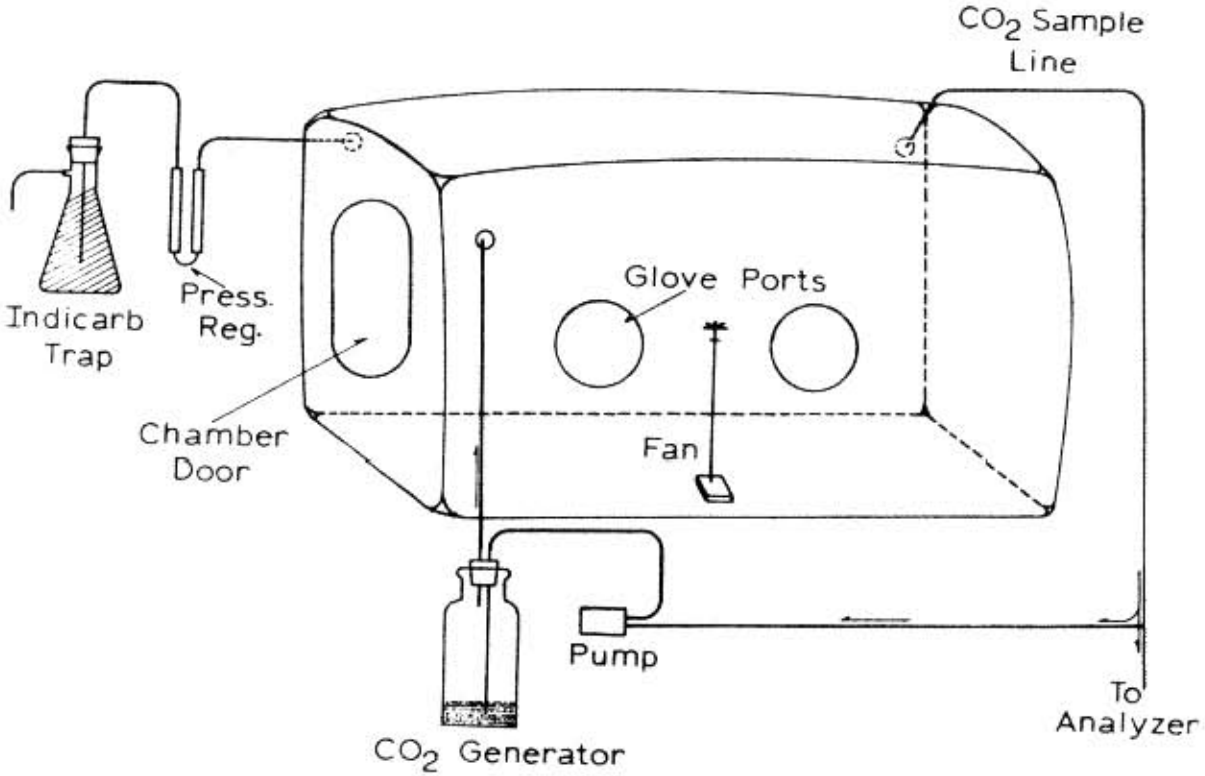


Fig. 1. General diagram of the biosynthesis chamber showing primary access ports, sample lines, and pressure regulator.

Fig. 2. Functional groups of the biosynthesis chamber.

A. Na_2CO_3 reservoir and metering system: A large reserve of Na_2CO_3 was securely stored in a 20-liter capacity container (1). Indicarb filters were attached to mineral oil traps to prevent passage of CO_2 from the system into the atmosphere or from the atmosphere into the system (2a, 2b). The 2-liter Na_2CO_3 reservoir (3) from which Na_2CO_3 is metered directly into the generator (B1) may be filled as required by closing clamp 5b, opening 5a, attaching a squeeze bulb (4), by-passing 2b with connector 8, and placing the Na_2CO_3 solution (1) under pressure. Flow is the result of pressure and gravity. When not refilling 3, clamp 5a is closed to insure that no leakage of the main reserve of Na_2CO_3 occurs; 5b is opened to allow pressure equalization. A photographic timer (6) (Singer Industrial Timer Corporation, Model P59) can be set to open the solenoid (7) for 0 - 60 seconds to allow flow of Na_2CO_3 into the generating vessel (B1).

B. The CO_2 generating system: Na_2CO_3 was metered into a 4-liter carboy (1) containing approximately 500 ml concentrated H_2SO_4. Air was pumped (2) from the tent and returned through the CO_2 generating vessel. CO_2 evolved within the vessel was swept into a 1000-ml flask (3) where it was bubbled through water to remove aerosol impurities. The CO_2 was then swept into the biosynthesis chamber (C1).

C. The atmosphere within the biosynthesis chamber (1) was pumped (3) through a gas flow regulator (4), through the IR Analyzer (5), (Beckman Infrared Analyzer, Model IR 15A) through a Beckman O_2 analyzer, Model D (6), and returned to the chamber. A water cooled condenser (2) was placed in the sample line to remove the condensation prior to the air sample passing through the IR Analyzer.

D. A strip recorder (1) (Varian Associates Graphic Recorder, Model G-10) was connected to the IR Analyzer. The analyzer and recorder were calibrated to record 300-900 ppm CO_2. The concentration of CO_2 recorded at the low limit on the chart was 300 ppm. At 300 ppm the timer was activated by a microswitch attached at the low limit of the recorder. The timer opened the solenoid for a preset time to allow flow of Na_2CO_3 into the generating vessel.

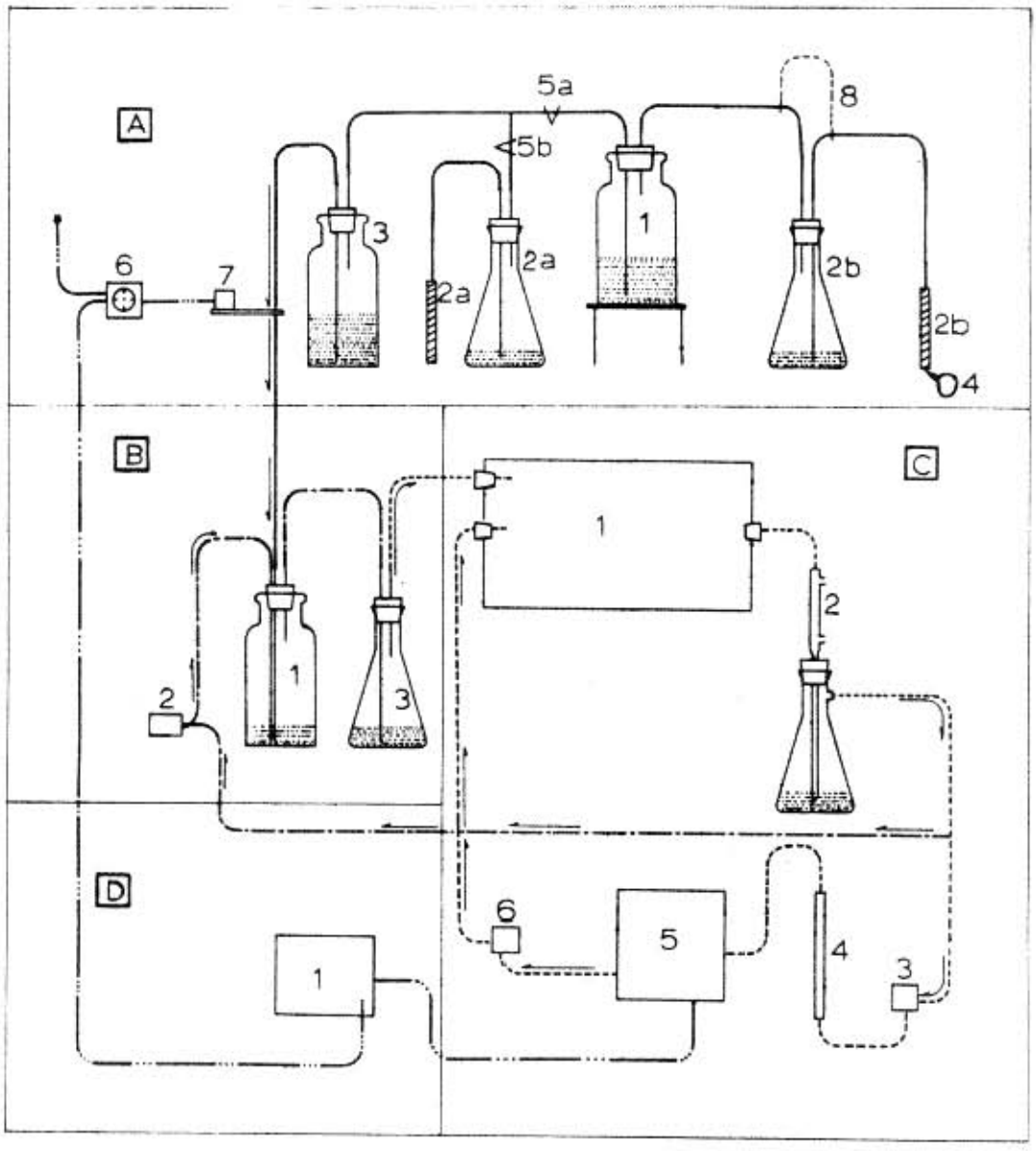


Fig. 2.

RADIATION HAZARD

Environmental

The maximum permissible concentration, M.P.C., is 1.0×10^{-6} μCi/ml (State of Colorado Rules and Regulations Pertaining to Radiation Control, January 1, 1968). The worst leakage situation visualized would be if the tent contained all 5 mCi of ^{14}C to be furnished a given crop as CO_2 and a leakage rate of 1% per day out of the tent. Capacity of the exhaust fan to the outside is 250 cfm or 7,000 liters per minute. The resultant activity level in the exhaust air would be 4×10^{-9} μCi/ml. The safety factor is approximately 500.

Occupation Hazard to Personnel

The M.P.C. occupational for ^{14}C CO_2 is 5×10^{-5} μCi/ml. The worst possible situation visualized is rupture of the tent containing 5 mCi of CO_2 inside the growth chamber, failure of the exhaust fan, and 30-minute exposure of the personnel during a one-week period. Average activity level during this time period would be 9×10^{-6} μCi/ml. The safety factor is twofold.

Proposed levels of ^{14}C should not exceed a total of 20 μCi in the tent at a given time. This is an activity level of 3.6×10^{-5} μCi/ml within the tent.

Precautionary Procedures

Procedures to be followed to minimize environmental contamination and personnel hazards are as follows:

(i) Leak tests of tent and chamber using freon and sensitive freon detectors prior to setting up equipment.

(ii) A dry run of all equipment and operational procedures with unlabeled material.

(iii) Monitoring of ^{14}C activity in the growth chamber.

METHODS

Dormant blue grama grass sods, 10 cm thick, were brought into the greenhouse from the Pawnee Site on 27 April 1970. Sods weighing 7 kg each were contained in rectangular plastic pans measuring 25 × 30 × 13 cm. Nutrient solutions were first applied on 29 April 1970 (Table 1), and sods were placed in a standard growth chamber having continuous lighting with average light intensity of .246 g cal cm^{-2} min^{-1} and a temperature of 30°C. Because of the rapid rate of CO_2 evolution from the sods at this time, they were not placed within the closed biosynthesis chamber until 25 June 1970. On 25 June the stems and leaf sheaths of the sods were clipped to within 5 cm of the soil surface, and the sods were placed within the biosynthesis chamber. The temperature of the external chamber was lowered to 21°C to maintain a temperature of approximately 30°C within the biosynthesis chamber. Unlabeled Na_2CO_3 was metered into the system to check the mechanics and equipment. The plants were grown under continuous 24-hour light conditions to avoid the high CO_2 concentration buildup due to respiration during a dark period.

Prior to harvesting the plants, the CO_2 generating equipment was turned off, and plants were allowed to lower the CO_2 to the compensation point. The door of the biosynthesis chamber was then removed, and the remaining traces of CO_2 from the biosynthesis chamber were exhausted through the roof vent by the exhaust fan. Plants were then harvested.

Table 1. Nutrient additions[a/] to individual sods.

Application Date	Milliequivalents							Fe Versenate (ml)	Trace Element (ml)
	K	Ca	Mg	NH_4	NO_3	SO_4	H_2PO_4		
29 April	12	16	8		36				
13 May	12	16	8		28	8			
16 May	12	16	8		28	8			
20 May	12	16	8		28	8			
26 June	16	8	4		14	4	10		
13 July	30				30				
21 July	6	8	4		14	4			
5 August				12			12	10	10
13 August	13	16	8		28	8	1	5	5
Total	113	96	48	12	206	40	23	15	15

[a/] 206 me NO_3/sod = 375 kg nitrogen/hectare.

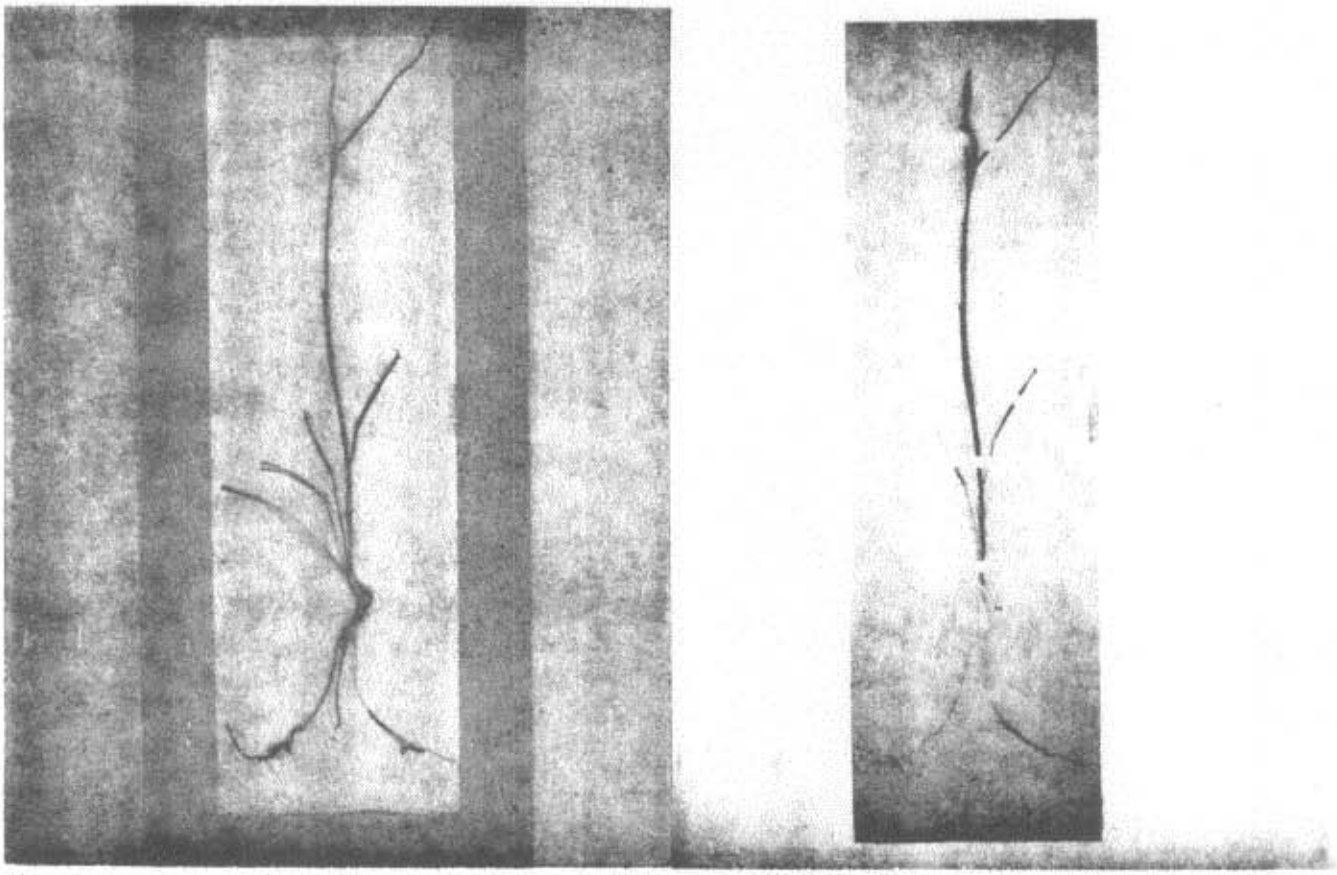
On 7 July 1970, the biosynthesis chamber was opened and serial plant growth harvested to within 5 cm of the soil surface. The system was again sealed, and ^{14}C-labeled Na_2CO_3 was substituted for the unlabeled solution previously used.

An autoradiograph of an entire plant was made at the time of the first labeled harvest (20 July 1970). Distribution of the labeling within the new plant growth after the growth period of 12 days in the labeled atmosphere can be seen by comparing a photograph (Fig. 3a) with the autoradiograph of the plant (Fig. 3b).

Three successive harvests of ^{14}C-labeled plant material were made during the growing period 8 July to 12 September 1970. The quantity of material harvested and the specific activity of the material is presented in Table 2. A minimum activity of 5.0 μCi/g dry matter was requested for the decomposition studies (see Section III). Based on the assumption that the plant dry matter is 50% carbon by weight, the specific activity of the CO_2 added into the plant environment was adjusted to produce plant matter that would have an activity of 10 μCi/gdm if all CO_2 incorporated into the plant matter came from the labeled source. The activity of the successive crops increased as the unlabeled source of CO_2 within the biosynthesis system (respiration from unlabeled plant and soil organic matter) diminished. The distribution of the added ^{14}C label among the various components of the biosynthesis system is presented in Table 3.

SUMMARY

Advantages of this system are: (i) no special construction of facilities is required; system may be realized by using existing or readily obtainable



3a 3b

Fig. 3a. Photograph of an entire blue grama grass plant from the first-labeled crop.

Fig. 3b. Autoradiograph of the same plant showing distribution of the ^{14}C label.

Table 2. Isotope dilution by unlabeled CO_2 within the system.

Specific Activity of Labeled CO_2 Added to the System Through the Generator (μCi/gram carbon)	Specific Activity of Plant Top Regrowth[a/] (μCi/gram carbon)	Isotope Dilution by Unlabeled CO_2 Evolved From Within the Biosynthesis Chamber (%)
25.00	1st crop, 11.84	52.6
25.00	2nd crop, 14.72	41.1
25.00	3rd crop, 14.50	42.0

a/ Carbon content of the plant material was assumed to be 50%.

Table 3. Distribution of the added ^{14}C among the components of the biosynthesis system.

Component	Activity (μCi/gdm)	Dry Matter (Grams)	Total Activity	μCi added
Top Regrowth				
Crop 1	5.92	90	532	1926
Crop 2	7.36	143	1053	3075
Crop 3	7.25	130	943	2670
Total Roots	a/	a/	a/	a/
Total Soil	a/	a/	a/	a/
Total Residual (Stems, etc.)	a/	a/	a/	a/
TOTALS			a/	7671

a/ Analyses not completed at time of report.

multipurpose facilities, equipment, and material; (ii) possibility of radiation hazard due to equipment breakdown or malfunction was well within safety limits of the Atomic Energy Commission and Colorado standards; and (iii) plant material may be grown continuously in the system for an extended time period resulting in uniform incorporation of the label.

GROWTH OF *BOUTELOUA GRACILIS* IN A BIOSYNTHESIS CHAMBER:

II. DYNAMICS OF CO_2 FLUXES WITHIN THE SYSTEM

INTRODUCTION

Bouteloua gracilis is of tropical origin. In the species belonging to the tropical tribe Chloridoideae tested by Downes and Hesketh (1968), including *B. curtipendula*, net assimilation of CO_2 was not enhanced more than 8% (average 0%) in a low O_2 environment. Lack of enhancement indicates presence of the C-4 pathway; plants which contain this pathway do not display loss of CO_2 through photorespiration. Other species from varying altitudes and latitudes were tested, and all species within a genera consistently had the C-4 pathway. *Bouteloua gracilis* was not tested, but can be expected to be similar to *B. curtipendula* in having the C-4 pathway.

In a closed system, the rate of photosynthesis of plants having no loss of CO_2 through photorespiration may be calculated by adding the rate of soil and plant dark respiration (increase in CO_2 concentration during a dark period) to the rate of net CO_2 assimilation (decrease in CO_2 concentration during a light period). Net CO_2 assimilation for a given time period is correlated to plant growth during that period. Net increase in plant material may be predicted from the total CO_2 added to the closed system duuring the growth period.

METHODS AND RESULTS

Soil plus plant respiration and net CO_2 assimilation were measured in the biosynthesis chamber. There are three ways of arriving at net CO_2 assimilation within the biosynthesis chamber during a given time period: (i) measure the total moles of Na_2CO_3 added to the system through the reaction vessel to maintain a reference CO_2 concentration; (ii) measure the rate of decline of CO_2 concentration with the IR Analyzer over a 10-60 minute time period; and (iii) measure the net oxygen produced within the biosynthesis chamber

during the period. In this system, the most precise method is measurement of the amount of Na_2CO_3 added.

The volume of CO_2 added to the chamber and the rate of addition were calculated from the number of times the CO_2 generator was triggered during a given time period (Fig. 4). Each time the Na_2CO_3 metering system was triggered, a preset volume of Na_2CO_3 was released into the CO_2 generator.

Direct measurement of net CO_2 assimilation was also obtained from the chart record of the rate of decrease of CO_2 concentration over a given time period. The rate of dark respiration (soil and plant) was similarly obtained by turning off the lights in the chamber and measuring the increase in CO_2 concentration for a given time period (Fig. 5). Respiration rates determined by IR analyzer measurements of increase of CO_2 within the biosynthesis system during a dark period and soil and leaf sample respiration measurements determined using the Gilson Respirometer were very similar (Table 4). Total CO_2 assimilation within the system was calculated by adding the net CO_2 assimilation rate (decline in CO_2 in chamber with lights on) to the dark respiration rate (Fig. 6). Blue grama belongs to a subgenus known to have the ^{14}C photosynthetic pathway, and it is assumed to have little or no loss of CO_2 through photorespiration. Representative total assimilation and respiration curves were obtained from the chart record on 2 July from sods within the biosynthesis chamber after seven days of top regrowth. Rates are expressed on a sod area basis (Fig. 6). Total assimilation was calculated by adding respiration and net assimilation rates.

Oxygen concentration within the biosynthesis chamber was recorded daily. The O_2 concentration measurement was obtained with a Beckman paramagnetic O_2 analyzer in the chamber atmosphere sample line. For each mole of CO_2 assimilated within the biosynthesis chamber, an equal volume of O_2 is evolved. To

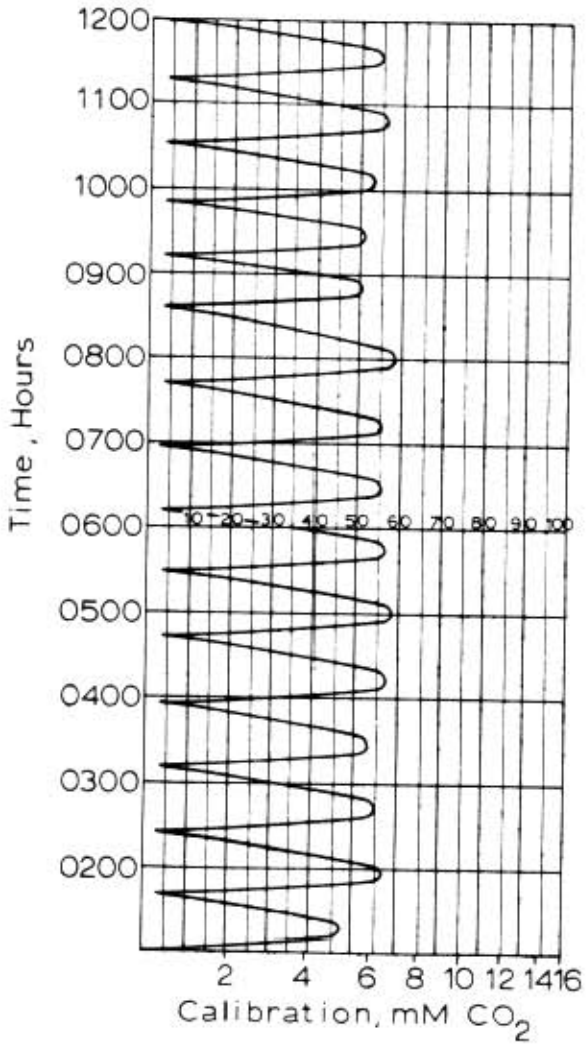


Fig. 4. Representative CO_2 strip chart record. The Na_2CO_3 metering system was triggered at the low end of the scale (0). CO_2 concentration within the system steadily increased as CO_2 was evolved from the generating system. The peak of the curves represents the point in time where the rate of CO_2 evolution from the generator was equal to the rate of net CO_2 assimilation.

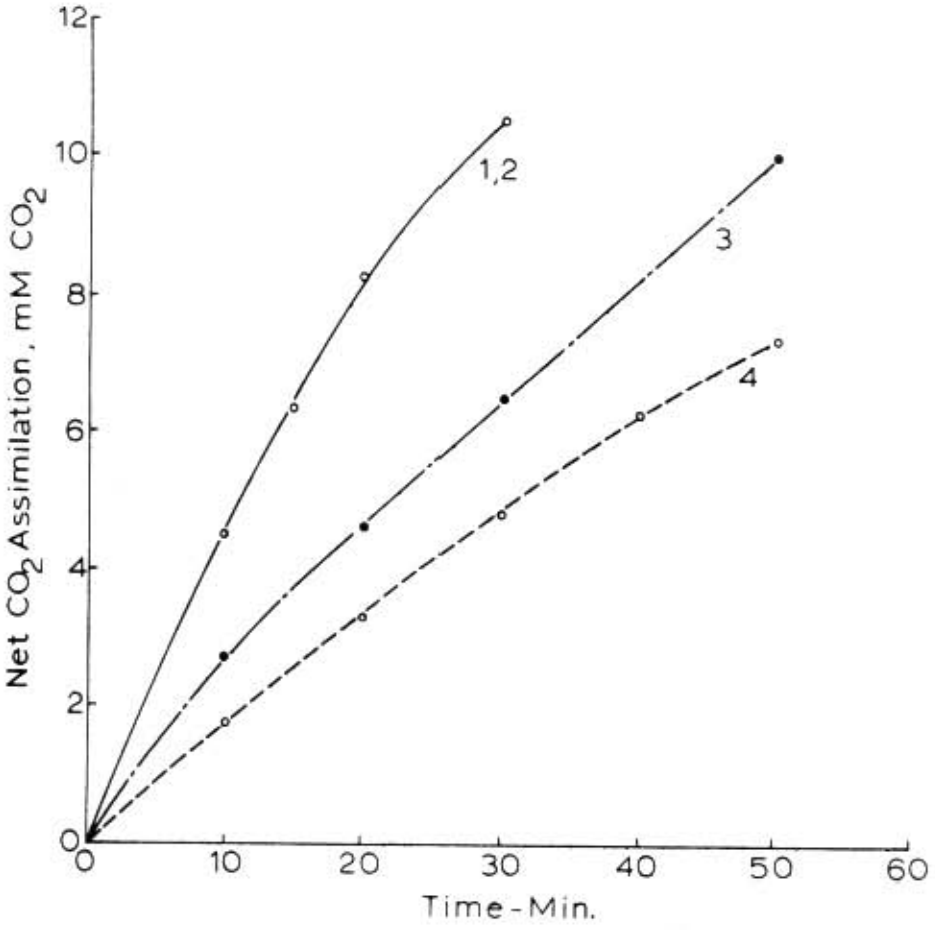


Fig. 5a. Effect of light quantity and light source on rate of net assimilation. Curves showing rate of net CO_2 assimilation at four different total light radiations (measured by an Eppley pyranometer). (1) 22 fluorescent tubes supplemented by six each 100-watt incandescent bulbs resulting in a measurable radiation of 0.246 gram cal cm^{-2} min^{-1}; (2) 22 fluorescent tubes with a measurable radiation energy of 0.142 gram cal cm^{-2} min^{-1}; (3) 14 fluorescent tubes supplemented by six each 100-watt incandescent bulbs yielding a measurable radiation of 0.194 gram cal cm^{-2} min^{-1}; and (4) 14 fluorescent tubes with a measurable radiation of 0.091 gram cal cm^{-2} min^{-1}.

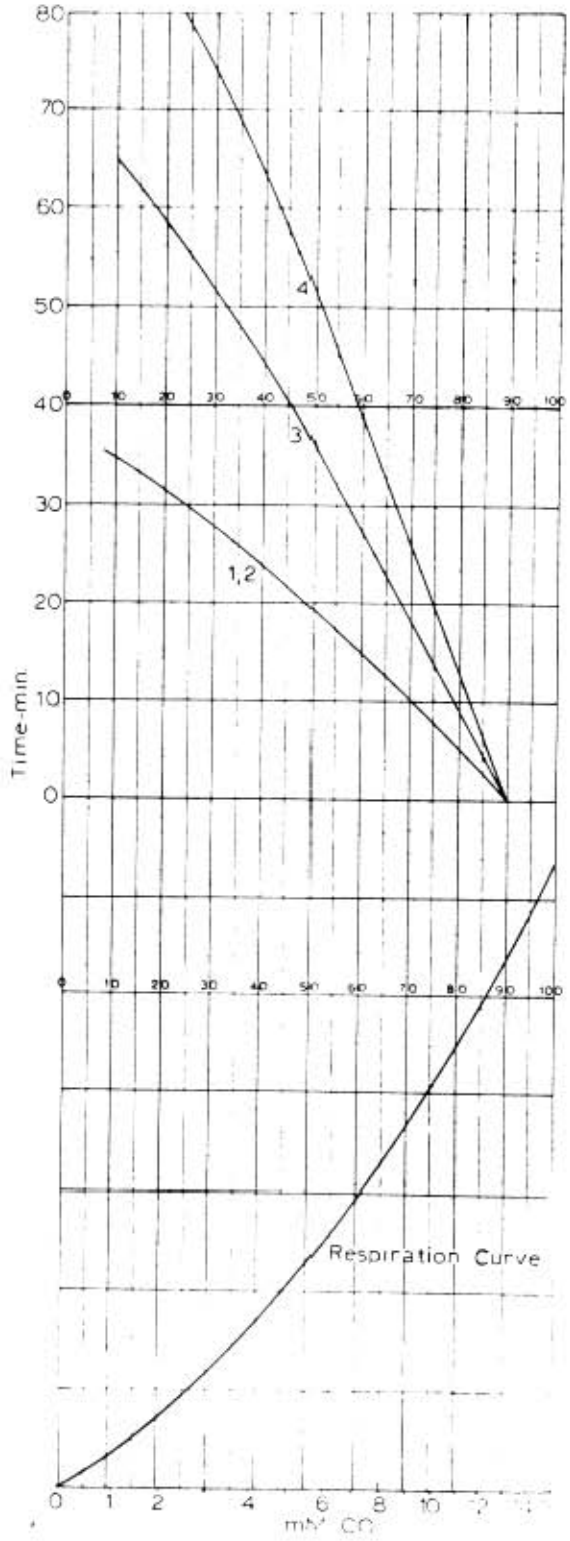


Fig. 5b. Effect of light quantity and light source on rate of net assimilation. Strip recorded data from which the curves in Fig. 5a were derived. Curves are for same light source and quantity as respective curves in 5a.

Table 4. Decline in dark respiration within the biosynthesis system from the beginning of the regrowth period of crop 3.

Date	mmole CO_2 hr^{-1} (IR Analyzer)	Gilson Respirometer[a/]
20 July	22.9	22.7
21 July	21.7	
29 July	14.5	
30 July	13.9	

a/ A comparable respiration rate was obtained using the Gilson respirometer. Respiration rate of leaf material was added to that obtained for soil from which all visible roots had been removed. Respiration rate of leaves = 5.19 mmole CO_2 hr^{-1}/chamber (2.07 µmole CO_2/hr/g leaf). Respiration rate of soil = 17.5 mmole CO_2 hr^{-1}/mass of soil in chamber (.313 µmole CO_2 $hr^{-1}g^{-1}$ soil).

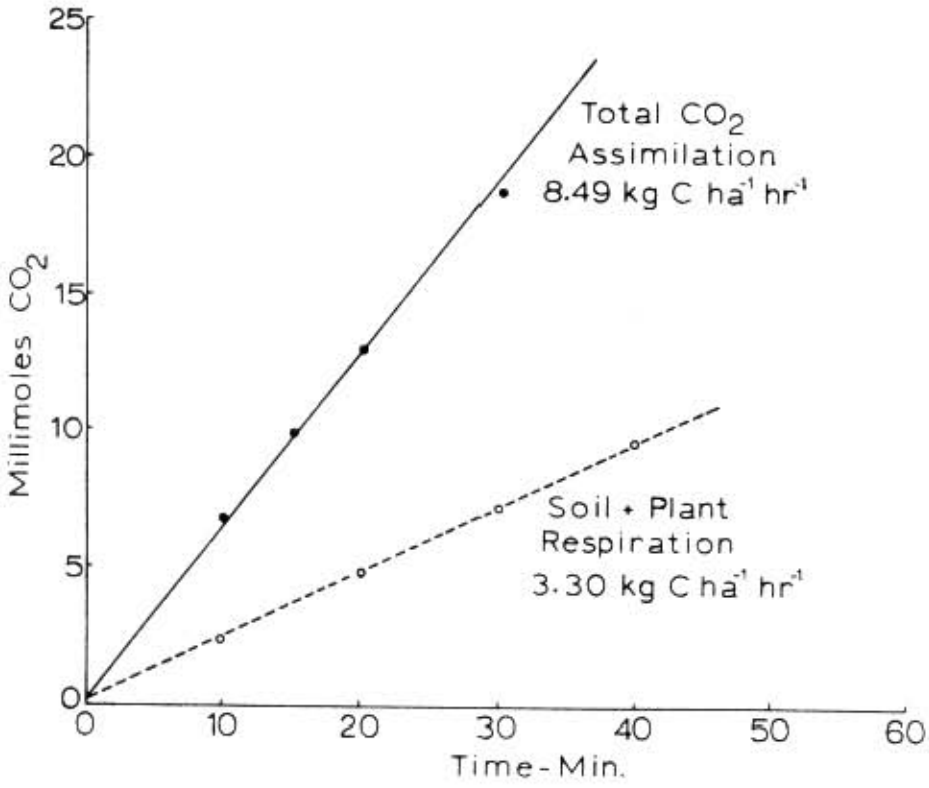


Fig. 6. Representative total CO_2 assimilation and dark respiration curves for blue grama grass sods having seven days top regrowth. Rates were recorded on 2 July 1970 within the biosynthesis chamber.

compensate for the volume of CO_2 added into the system through the reaction vessel, a pressure regulator was attached to an exit port of the chamber. Therefore, total O_2 produced was estimated from the change in O_2 pressure within the chamber plus a correction for oxygen loss through the pressure regulator. The volume of O_2 lost through the pressure regulator was calculated by multiplying the increase in O_2 concentration in the system during that period by the respective mean O_2 concentration. Estimates of total O_2 production were in satisfactory agreement with amounts of CO_2 added (see Table 5).

The quantity of CO_2 added to the system was used to estimate the regrowth of stems and leaf sheaths at any given time (Table 6). The relationship of top regrowth to CO_2 added remained constant (60% of expected net growth) during the entire growing season. It should be emphasized that CO_2 evolved within the system from respiration was also available for top regrowth. However, the amount of CO_2 from respiration (Table 4) steadily decreased during the growth period. The constant proportions of net CO_2 assimilated appearing in top growth, in the face of declining system respiration rates in successive growth periods, indicates reduced translocation of assimilated CO_2 into belowground components. Further study of these relationships may provide better estimates of relative top to root production in blue grama.

The net CO_2 assimilation rates within the biosynthesis chamber increased linearly during a 12-day growing period (Fig. 7b). However, the rate of net CO_2 assimilation over a 24-day period reached a maximum at approximately 14 days, after which it began to decline, probably due to maturity and senescence of the plants (Fig. 7a). Increase in oxygen concentration was measured to study the effect of oxygen concentration on net CO_2 assimilation. Plants

Table 5. Relationship of O_2 evolved within the biosynthesis system to the volume of CO_2 added through the CO_2 generator.

Days Regrowth	Liters CO_2 Added (Cumulative)[a/]	Liters O_2 Evolved		
		O_2 Within Tent[b/]	Bleedoff[c/]	Total O_2 Evolved
0	000.0	000.0	00.0	000.0
8	62.7	55.9	12.5	68.4
9	76.7	68.5	3.4	71.9
10	90.6	74.0	1.5	75.5
11	104.0	86.6	3.7	90.3
12	120.6	98.2	3.6	101.8
13	136.8	110.7	4.0	114.7
14	152.9	122.5	4.0	126.5

[a/] Liters CO_2 added = moles Na_2CO_3 added to generator × 29.7 liters $mole^{-1}$ (635 mm, 30°C).

[b/] O_2 within tent = (partial pressure O_2 × estimated volume of tent) starting O_2 concentration.

[c/] Bleedoff = Change in liters of O_2 during period × mean O_2 concentration during that period.

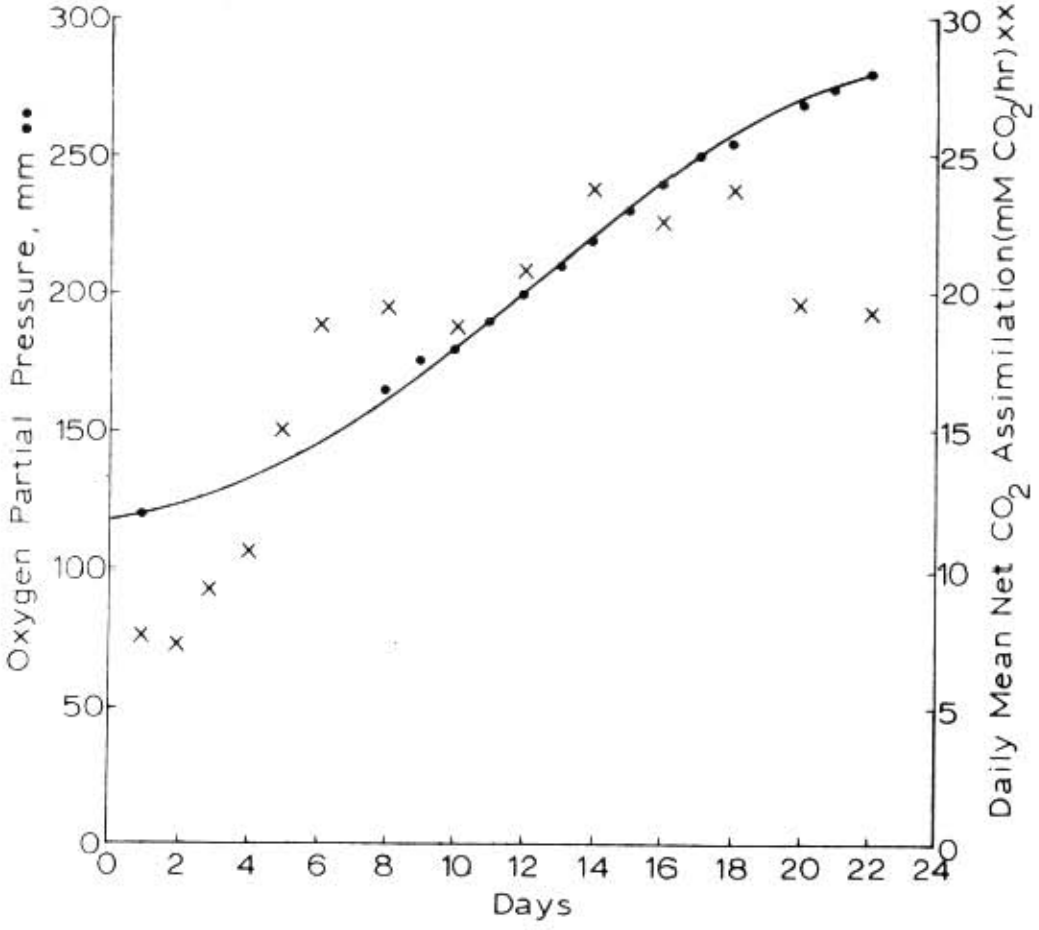
Table 6. Net CO_2 assimilation related to actual quantity of top growth harvested.

Crop	Grams Carbon Added[a/]	Expected Plant Growth (gdm)[b/]	Plant Growth Harvested (gdm)[c/]	% of Expected Growth Harvested
1	77	154	90	58.4
2	120	240	143	59.6
3	107	214	130	60.7

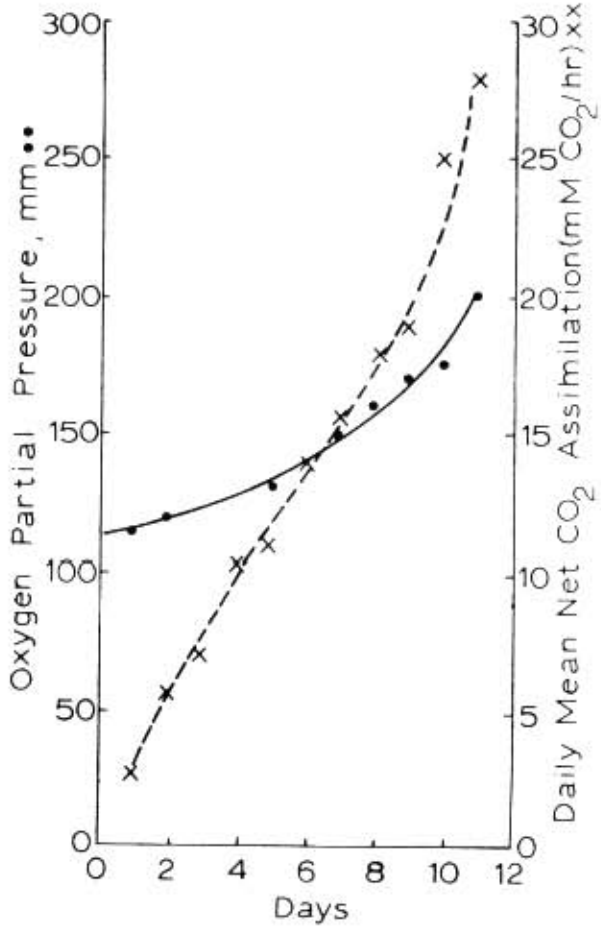
a/ Grams carbon added = moles Na_2CO_3 added through generator × 12 g C $mole^{-1}$.

b/ Expected plant growth = grams C added × 2 (plant was assumed to be 50% carbon on a dry weight basis).

c/ Plant growth harvested = regrowth during the period of leaf and stem (regrowth was clipped to within 5 cm of soil surface prior to growth period and at termination of growth period).



7a



7b

Fig. 7. Relationship of increase in oxygen concentration to rate of net CO_2 assimilation.
a. Data recorded daily during growth period of second crop.
b. Data recorded daily during the first 14 days of the growth period of third crop.

having the C-3 pathway of photosynthesis and photorespiration demonstrate a decreasing net CO_2 assimilation with increasing oxygen concentration. Blue grama grass had no decrease of net CO_2 assimilation with increasing O_2 concentration over the range studied, supporting the assumption of C-4 pathway in this species.

A survey of the effect of varying light intensity and quality on net CO_2 assimilation is shown in Fig. 5. Net CO_2 assimilation by sods which had been in the chamber under constant light and temperature for seven days was determined at different light intensities. Four different light intensities were obtained by varying the number of fluorescent light banks used in the presence or absence of supplemental incandescent light. Total light intensities, as measured by the Eppley pyranometer, ranged from .091 to .246 cal cm^{-2} min^{-1}. Net CO_2 assimilation rates cannot be correlated with total light intensity as measured by the Eppley pyranometer. For interpretation of the results, it would be necessary to have more detailed measurements of light quality, as well as measurements of the possible leaf temperature changes under different light conditions.

DISCUSSION

Helpful discussions of environmental measurements and their importance in evaluating net CO_2 assimilation are presented in the report of the Proceedings of the Copenhagen Symposium on the Function of Terrestrial Ecosystems at the Primary Production Level. The statement of Eckhardt (1968) is particularly appropriate: "By enclosing a parcel of vegetation or a plant organ in a chamber which delimits the volume of air to be analyzed, one necessarily modifies these energy and mass fluxes. These modifications can

to some extent be compensated for by adequate air-conditioning, but true reproduction of natural conditions is impossible. In order to obtain a correct determination of photosynthesis by this procedure, it is essential, therefore, not only to create in the plant chamber conditions approximating those of the natural environment, but also to evaluate the influence of the residual environmental deviations on the plant's metabolism."

Valuable recommendations for instrumentation to evaluate photosynthetically active radiation (PAR) are presented in the papers of Setlik (1968), McCree (1968), and Baumgartner (1968). The spectral range of the light radiation may have a large effect on the temperatures of the plants. The ratio of leaf temperature to air temperature may vary widely under different climatic conditions, as explained in the discussions following Niciporovic's (1968) presentation by Gates and Montieth.

A number of factors control the light energy conversion efficiency of the plant: temperature, light, nutrient supply, genetic differences among strains, top root ratio, and leaf shape (Wassink 1968).

SUMMARY

Measurements of soil plus plant respiration and net CO_2 assimilation by blue grama grass sod cultures were made while growing ^{14}C-labeled plants in the biosynthesis chamber. Because of the special cultural conditions under which these plants were grown, the measurements have limited value for interpretation of respiration and CO_2 assimilation rates under field conditions. However, the results indicate the potential use of the biosynthesis chamber as a research tool for investigation of these processes under more carefully controlled environmental conditions.

GROWTH OF *BOUTELOUA GRACILIS* IN A BIOSYNTHESIS CHAMBER: III. USE OF MATERIAL IN DECOMPOSITION STUDIES

Limitations in using blue grama grass grown in the biosynthesis chamber in decomposition studies to estimate the rate of decomposition of the native plant material on the Pawnee Site must be emphasized. Plant material grown in the chamber with nutrient amended soil, confined root mass, relatively high moisture conditions, lower light intensity, and harvested prior to maturity may be of a different chemical and structural composition than blue grama grown on the Pawnee Site.

There are three main factors affecting type of plant material harvested: (i) species, (ii) environment, and (iii) physiological age of the material. Although blue grama grass is the predominant species on the Pawnee Site, other species also prevail. Ojima and Isawa (1968) reported large species differences in storage carbohydrates and the component sugars of hemicellulose in the aerial parts (leaf sheaths and stems) of grasses. Members of the subfamily, Festucoidea, were fructosan-storing species. Species classified under the subfamily, Eragrostoidea, and to which *B. gracilis* belongs (Gould 1968), accumulated starch rather than fructosan. The hemicellulose of Eragrostoidea contained a higher proportion of glucose relative to xylose than did the Festucoidea grasses. In addition, environmental and cultural conditions under which a given plant species is grown have a major influence on the mineral and organic composition of the material. If the supply of nitrogen compounds to an actively growing vegetative meristem is abundant relative to the supply of carbohydrates, a large quantity of protoplasm will be formed relative to the amount of cell-wall material constructed. The resulting cells are usually large and thin-walled, contain an abundance of protoplasm, and are more readily disintegrated (Meyer, Anderson, and Bohning 1960). Other factors are operating in addition to nitrogen. Maturation is accompanied

by lignification and related alterations; the content of nitrogen, proteins, and water-soluble substances falls, and the proportions of cellulose, lignin, and hemicelluloses rises. A large part of the resistance associated with aging probably is a consequence of the abundance of lignin (Alexander 1961). Major factors affecting plant decomposition in a given soil are reported to be: C:N ratio of the plant residue, age of the plant at the termination of growth, and the degree of physical disintegration of the material (Alexander 1961).

Comparison of chemical and physical analyses of sample material with plant material produced on site in the field should greatly aid in interpreting decomposition data.

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