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Field Measurements of Photosynthesis and Leaf Growth Rates of Three Alpine Plant Species

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FIELD MEASUREMENTS OF PHOTOSYNTHESIS AND LEAF

GROWTH RATES OF THREE ALPINE PLANT SPECIES

by

Douglas A. Johnson

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Range Science

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ABSTRACT

Field Measurements of Photosynthesis and Leaf
Growth Rates of Three Alpine Plant Species

by

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Utah State University, 1973

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Leaf photosynthetic measurements using a portable $^{14}CO_2$ field system were carried out and correlative leaf relative growth rates, RGR, were determined at different leaf positions of three alpine plant species throughout the growing season. Initially there was a period of high leaf RGR associated with a period of increasing photosynthetic activity. Following this stage was a long period of no net change in length of the living leaf. During this period, photosynthetic activity generally increased to a maximum level and then decreased steadily. The final ontogenetic stage was a period of negative leaf RGR denoting leaf senescence which was associated with a marked decline in leaf CO_2 uptake. Ontogenetic timing of these alpine species is geared with the surge and decline of individual leaf photosynthetic activity so that one to several leaves operating at near maximal photosynthetic capacity are always maintained during the growing season for each plant. These findings are discussed in relation to their adaptive significance for these species.

(30 pages)

INTRODUCTION

Photosynthesis of individual leaves exhibits an increase after leaf emergence, a leveling off before or after full leaf expansion, and a decline with leaf senescence. This pattern has become apparent from field CO_2 uptake studies done for individual leaves of agricultural species (e.g., Beuerlein and Pendleton, 1971; Turner and Incoll, 1971). In alpine areas where the growing season is short and relatively severe field photosynthesis measurements of whole plants have been carried out (e.g., Scott and Billings, 1964; Moser, 1973). However, little or no field work has been done in alpine areas with photosynthesis of individual leaves. The present investigation with three major alpine tundra species was concerned with investigating the field photosynthetic response of individual leaves at several leaf positions and correlating this photosynthetic response with the seasonal progressions of leaf growth rate during the alpine growing season.

METHODS

Leaf photosynthetic measurements were determined using a portable $^{14}CO_2$ field system. The field system and procedure have been described in detail by Tieszen, et al. (in press). The field system consists of a plexiglass leaf chamber which is temperature controlled by a Peltier thermoelectric stage. The procedure involves exposing an intact leaf to a $^{14}CO_2$ air mixture, immediately cooling the leaf sample in the field following exposure, subsequent drying of the exposed leaf sample, combusting this sample, and radioactivity counting using liquid scintillation techniques.

According to studies by Ludwig and Canvin (1971) net $^{14}CO_2$ uptake in a leaf is maximum at exposure periods less than 30 sec. This initial period approximates what might be termed as gross CO_2 influx. After 30 sec, net CO_2 uptake decreases as the exposure time in $^{14}CO_2$ is extended and after 10 min approaches normal net CO_2 exchange rates. This decrease in $^{14}CO_2$ uptake is associated with $^{14}CO_2$ evolution from the leaf. Thus, with exposure times of 1 min used in this study, CO_2 uptake measured was between gross CO_2 uptake and net photosynthesis.

All CO_2 uptake determinations were carried out under a constant chamber temperature of 10.0 $\pm$.5°C which is representative of the mean daytime growing season temperatures at the study area. These were measured using a shielded copper-constantan fine wire thermocouple. Leaf temperatures were also monitored and were always within ± 1.0°C of chamber air temperature. Artificial irradiation was provided by a high intensity incandescent lamp and was monitored with a Lambda Co. Model LI-190SR quantum sensor. A constant intensity of 2700$\pm$200 microeinsteins$\cdot m^{-2} \cdot sec^{-1}$ in the 400-700 nm wavelength range was used for all determinations and is approximately equal to maximum midday solar radiation values. A period of five minutes was allowed for the leaf to attain a steady state gas exchange rate under these environmental conditions before $^{14}CO_2$ exposure. These temperature and radiation conditions were used consistently in this study in order to assess the combined influence of other factors such as leaf age and leaf position.

Leaf areas for Deschampsia caespitosa and Kobresia myosuroides were determined by direct measurement of leaf length and width and

are accurate to $\pm 5\%$. Area values for the highly dissected leaves of <u>Geum rossii</u> were estimated from regression relationships of leaf dry weight, leaf length and width, and leaf area which was determined by a modified optical planimeter (Caldwell and Moore, 1971). These basic relationships were determined at three times during the season.

For the leaf growth rate study, ten plants each of <u>Geum rossii</u> and <u>Kobresia myosuroides</u> in the <u>Kobresia</u> meadow site and ten plants each of <u>Deschampsia caespitosa</u> and <u>Geum rossii</u> in the <u>Deschampsia</u> meadow site were selected on 15 June. These individual plants were observed and measured every five days for leaf emergence, leaf elongation, and senescence throughout the course of the growing season. Leaf length was measured from the base of the plant to the most distal green portion of the outstretched leaf. Each leaf was individually measured and the same leaf was followed through emergence, expansion, and senescence.

From these individual leaf measurements, the mean leaf relative growth rate, RGR, was calculated by (Kvĕt, et al., 1971):

$$RGR = (\ln l_2 - \ln l_1)/(t_2 - t_1) \quad length \cdot length^{-1} \cdot time^{-1} \quad (1)$$

where l_2 and l_1 are the lengths at times t_2 and t_1 respectively.

In both the leaf photosynthetic and leaf RGR studies the same numerical identification of leaf position was used. The first green leaf present on the plant and thus the oldest living leaf on the plant was given the designation of #1 leaf, followed by the second green leaf to emerge as #2 leaf, with successive numbering up to the youngest leaf.

STUDY AREA

The area of the study was on Niwot Ridge (40° 04'N, 105° 36'N) at an elevation of 3476 m in the Front Range of the Colorado Rocky Mountains. The study sites were the U. S. Tundra Biome intensive sites, one a Kobresia community and the other a Deschampsia meadow. The Kobresia site has a southwest aspect and 5° slope, while the Deschampsia site has a southeast aspect and a 4° slope.

The two intensive study sites are located on a gently sloping saddle and have vegetation characterized by low perennial grasses, sedges, and herbs. The Kobresia site is a well-developed mesic meadow, whereas the Deschampsia site is an area with heavy snow melt water runoff. A further characterization of the study sites can be found in Fareed (1973). Plant nomenclature follows that of Weber (1967). The CO_2 uptake and leaf growth rate studies were initiated in June and continued through August, 1972.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the photosynthetic capacity and leaf RGR of Geum rossii, Deschampsia caespitosa, and Kobresia myosuroides at different leaf positions throughout the growing season. In Geum rossii all leaves arose from the current year's growth. The first leaf in Deschampsia caespitosa and Kobresia myosuroides was one which typically had initiated growth in the prior season, but which had not attained complete exsertion and development. Thus, during the second season of growth, leaf elongation ensued.

In all species studied, the greatest leaf RGR was exhibited by
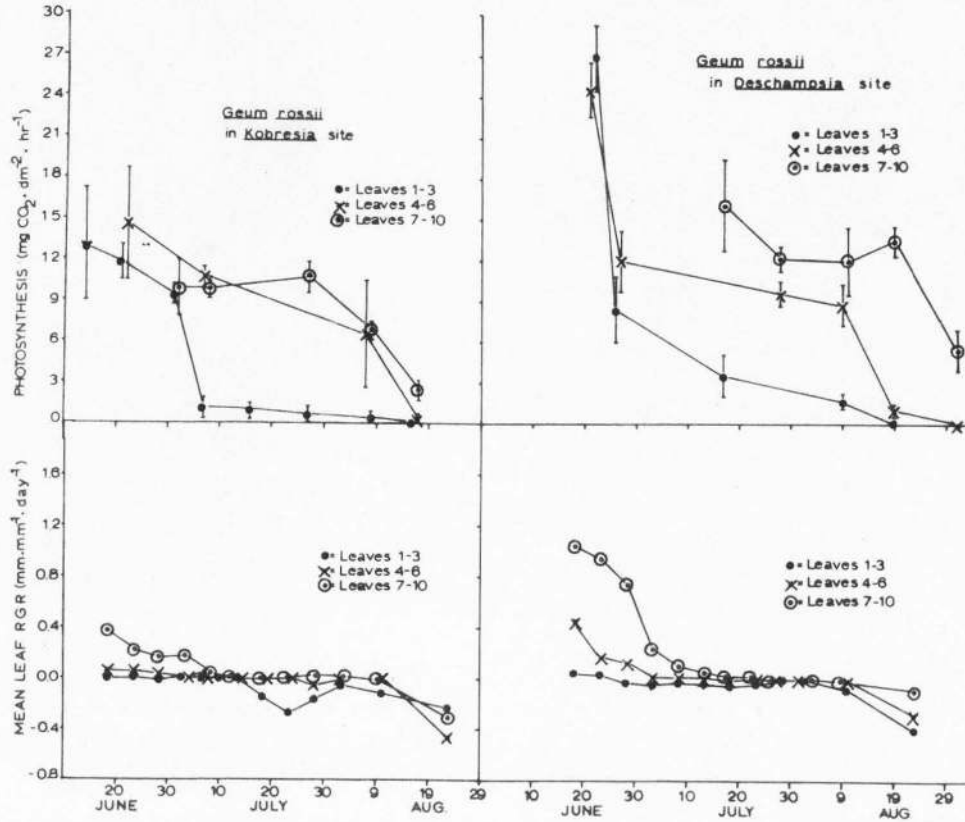
Figure 1. Leaf photosynthesis rates and leaf RGR of Geum rossii in the
Kobresia site and the Deschampsia site. Each point on the
photosynthesis figure is the mean of four to eight replicates
with the vertical bars representing + one standard error.
Each point on the leaf RGR figure is the mean of ten plants
with the standard errors ranging from 0.01 to 0.58. Negative
leaf RGR denotes leaf senescence.
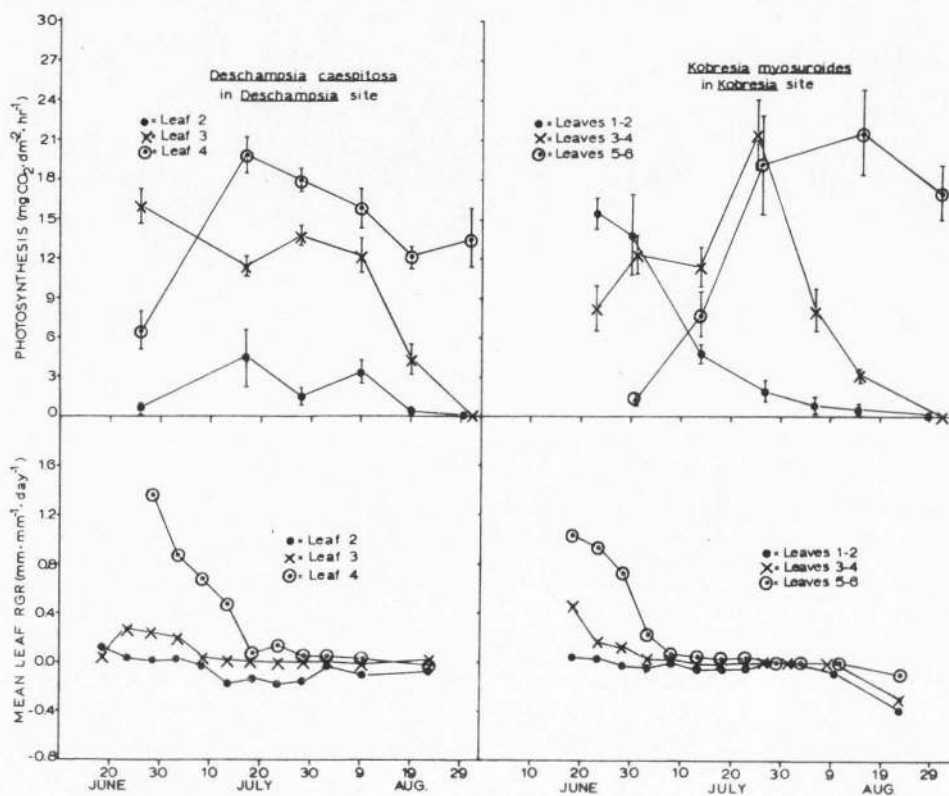
Figure 2. Leaf photosynthesis rates and leaf RGR of Kobresia myosuroides in the Kobresia site and Deschampsia caespitosa in the Deschampsia site. Each point on the photosynthesis figure is the mean of four to eight replicates with the vertical bars representing + one standard error. Each point on the leaf RGR figure is the mean of ten plants with the standard errors ranging from 0.01 to 0.94. Negative leaf RGR denotes leaf senescence.

the upper leaves. Presumably, leaves at lower positions on the plant exhibited the same pattern of growth but this took place prior to our first sampling date. In Deschampsia caespitosa and Kobresia myosuroides, this period of rapid growth may have taken place the previous growing season and the leaves may have been completing exsertion in this subsequent growing season. Standard errors of the leaf RGR means ranged from 0.01 to 0.94.

A similar ontogenetic pattern was exhibited in all species studied. Initially there was a period of high leaf RGR associated with a period of increasing photosynthetic activity. Following this stage was a long period of no net change in length of the living leaf. During this period, photosynthetic activity generally increased to a maximum level and then decreased steadily. The final leaf ontogenetic stage was a period of negative leaf RGR denoting leaf senescence which was associated with a marked decline in leaf CO_2 uptake rate. All species studied exhibited a decrease in photosynthetic rate prior to negative leaf RGR.

Growth in the Deschampsia meadow site was initiated during the beginning of June. Growth in the Kobresia stand site was initiated somewhat earlier in the season with substantial growth before the first sampling period. This earlier growth initiation in the Kobresia site may be attributed to the snow melt date of 22 May as compared with 9 June as the snow melt date in the Deschampsia site (Diane Ebert May, unpublished data).

This difference between sites is particularly apparent when photosynthetic and leaf relative growth rates in leaf positions #7-10

of _Geum rossii_ are compared. In the _Kobresia_ site leaves in these positions approached zero net leaf RGR near 8 July as contrasted to 18 July in the _Deschampsia_ site. In the _Kobresia_ site, _Geum rossii_ leaves in positions #7-10 declined earlier in their photosynthetic rates and exhibited less than 3 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ uptake by 17 August. In the _Deschampsia_ site comparable leaves exhibited rates of more than 13 mg $CO_2 \cdot dm^{-2} \cdot hr^{-1}$ on 19 August. Even by 31 August these leaves had not declined to the low levels of CO_2 uptake exhibited by 17 August in the _Kobresia_ site.

This earlier decline in leaf photosynthetic capacity and leaf RGR of _Geum rossii_ in the _Kobresia_ site may be due to differences in plant age. The same ontogenetic sequence took place in _Geum rossii_ in both the _Kobresia_ and _Deschampsia_ sites. However, due to the earlier snow melt date in the _Kobresia_ site, the ontogeny in the _Kobresia_ site was initiated earlier than in the _Deschampsia_ site. Thus, as _Geum rossii_ was completing its ontogenetic pattern in the _Kobresia_ site, _Geum rossii_ in the _Deschampsia_ site was exhibiting a time lag of more than ten days.

Differences in water stress may also account for this earlier decline in the _Kobresia_ site. Leaf water potentials for _Geum rossii_ as measured by the Scholander pressure bomb technique were on the order of -18 bars by 18 July in the _Kobresia_ site, whereas comparable leaf water potentials were not observed until 29 July in the _Deschampsia_ site. Leaf water potential measurements throughout the 1972 growing season ranged from -1 bar to less than -30 bars in _Deschampsia_ _caespitosa_, -1 bar to -20 bars in _Geum rossii_, and -7 bars to -29 bars in _Kobresia_ _myosuroides_. Leaf photosynthetic

capacities of <u>Geum rossii</u> and <u>Deschampsia caespitosa</u> have been shown to be significantly depressed with these decreases in leaf water potential for leaves of the same age (Johnson, et al., in press). In addition the number of leaves operating at near maximal efficiencies at any specific time may also be reduced.

Leaves in the lower positions exhibited their highest photosynthetic capacity in the early portion of the season and generally showed a steady decline throughout the rest of the season. However, as photosynthetic activity in the older senescing leaves declined, new leaves were emerging to assume the function of the primary photosynthetic structures (Fig. 3). Ontogenetic timing of these alpine species is geared with the surge and decline of individual leaf photosynthetic capacity so that one to several leaves operating at near maximal photosynthetic capacity are always maintained during the growing season of each plant. This generalized seasonal CO_2 uptake pattern has been shown for other species such as <u>Nicotiana sanderae</u> (Šesták and Čatský, 1962) and <u>Sorghum almum</u> and <u>Vigna luteola</u> (Ludlow and Wilson, 1971).

When leaves are first formed, they are developed near the top of the canopy and are well illuminated. As new leaves are produced these older leaves become increasingly shaded. Thus, the effects of leaf age on photosynthetic capacity are confounded with the effects of decreased light intensity. Osman and Milthorpe (1971) and Woledge (1971) indicate that there is a more marked decline in photosynthetic capacity with age under high radiation intensities than under low light conditions. Furthermore, Woledge (1971) indicated that the old leaves become saturated at low light intensities, whereas saturation
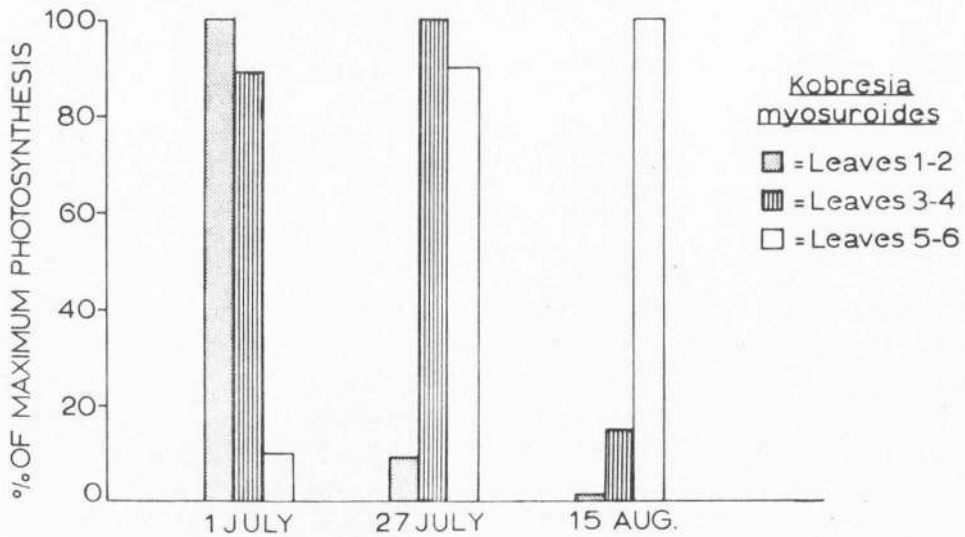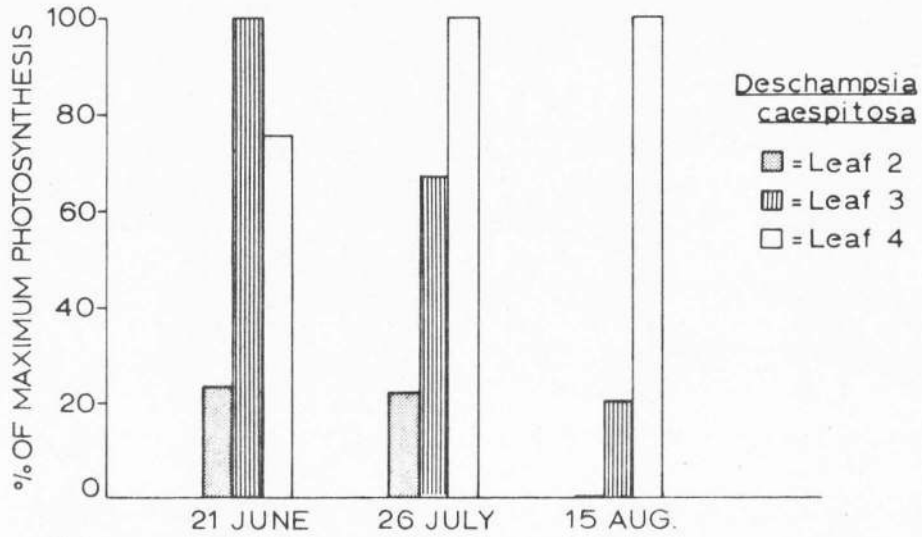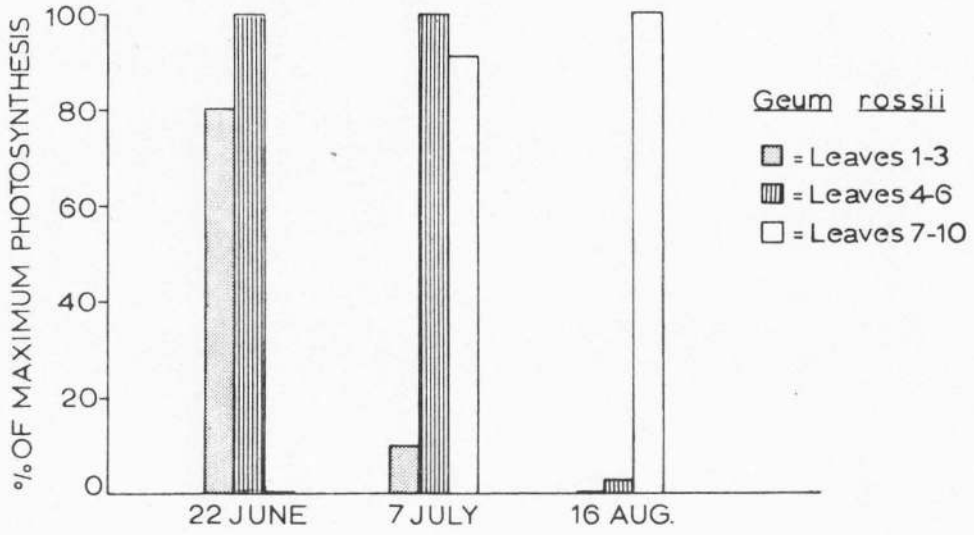
in the young leaves is not reached until higher light intensities.
Thus, under high light intensities the CO_2 uptake activity is
substantially higher in the younger leaves than in the older
leaves.

Having the older leaves in the position of low light intensity
may both prolong the photosynthetic contribution of the older leaves
and minimize the reduction in photosynthesis due to the aging of
leaves. With this strategy the leaves of the plant are arranged for
maximum photosynthesis at any one time of the season. The young
leaves with their peak photosynthetic capacities intercept the high
light intensities, whereas the older leaves with their declining
stages of photosynthesis receive lower light intensities. This
results in maximum photosynthetic efficiency in the upper, younger
leaves as well as prolonged photosynthetic efficiency in the lower,
older leaves. Since these older leaves are saturated at low light
intensities, shading would be of no immediate disadvantage for
these older leaves.

When the photosynthetic capacity of these older leaves reaches
extremely low levels, death of these leaves would represent a
conservation of energy and nutrients for the plant (Leopold, 1961;
Hopkinson, 1966; Leopold, 1967). Williams (1955) indicates that this
decline and eventual senescence of the older leaves permits a
reutilization of nutrients from these organs. Williams (1936) showed
that there was a net loss of nitrogen and phosphorus from the older
leaves into the upper, younger leaves. According to Hopkinson (1966)
these leaf nutrients in the older leaves can presumably be more

profitably used to produce photosynthetic surfaces at more favorably illuminated sites. Thus, this redistribution of nitrients results in the continuous rearrangement of the assimilating surfaces as to ensure the most efficient exploitation of the available light energy. When a leaf's photosynthetic contribution is no longer worth the price of its maintenance, the plant mobilizes as much useful material as possible before total leaf death takes place.

This change in leaf photosynthetic capacity with leaf age was also apparent in multiple regression analysis of the leaf photo-synthesis data. A leaf phenology index was a consistently significant independent variable and corresponded to the periods of positive leaf RGR, zero leaf RGR, and negative leaf RGR. In addition the leaf area/dry weight ratio was a consistently significant independent variable. A reciprocal function, specific leaf weight (SLW, dry weight/area), has been used as an indicator of internal leaf anatomy in crop species (e.g., Pearce, et al., 1969; Wolf and Blaser, 1972). Straley and Cooper (1972) propose that light intensity during and following leaf formation as well as light intensity within the canopy are principal factors influencing SLW for leaves. Thus, it was not surprising that both the leaf phenology index and the leaf area/dry weight ratio were consistently significant, since both these variables represent to some degree the internal metabolic activity of the leaf.

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APPENDIX A -- A Portable System for the Measurement of

Photosynthesis Using 14-Carbon Dioxide

1. Delivery System:

Air mixtures containing $^{14}CO_2$ are ordered from Matheson Gas Products and contain 300 ppm CO_2 and a specific activity of 1.5 mcurie·mmole^{-1}. With our flow rates and exposure periods, more than 400 analyses can be made from each bottle. Since absolute determinations of photosynthesis are critically dependent on accurate analyses of CO_2 concentrations and $^{14}CO_2$ specific activity, a routine procedure for calibrating all air mixtures is used. The cylinders are analyzed for total CO_2 concentration with a differential infrared gas analyzer, IRGA, with an accuracy of at least 1 ppm. A correction is not applied for the differential sensitivity of the infrared analyzer to $^{14}CO_2$.

The specific activity is determined by absorbing all CO_2 from a known volume of air and subsequent absolute counting. Air is bubbled from the lecture bottle at a known flow rate through a series of five absorbing vessels with the intakes in each vessel immersed under 1 ml of NCS. At the end of 30 seconds, the air flow is stopped, the NCS divided into 0.5 ml portions, 10 ml of counting solution added (270 ml methanol, 460 ml toluene, 5 g PPO, and 100 mg of POPOP), and the samples counted using the channels ratio method for quench correction.

Leaf Chamber with Temperature Control

Components of the temperature controlled delivery system are illustrated in Figure 1. The leaf chamber (Figure 2) is constructed of 1/4" plexiglass and consists of a hinged top and a bottom with a $^{14}CO_2$ labeled-air inlet, an unlabeled-air inlet and an air outlet. Polyurethane strips line the borders of both the top and bottom
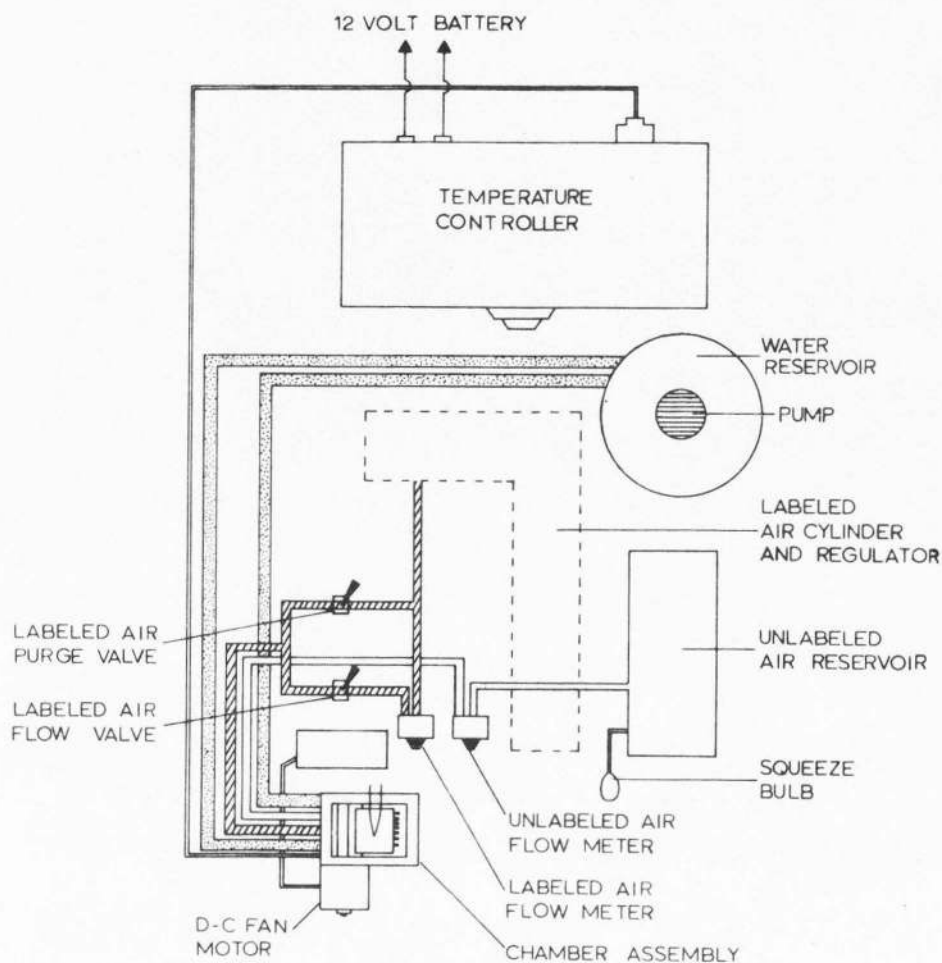
Figure 1. Schematic of system used to deliver prepared $^{14}CO_2$ air mixture
to the temperature controlled leaf chamber. The labeled air
cylinder and regulator are represented with dashed lines.
Board on which system is mounted is about 74 cm in length and
60 cm in width. Total weight of the field system is about
15 kg without the 12 volt battery.

TOP VIEW

D-C FAN MOTOR

WATER COOLING CONNECTIONS

LEAF

FIN PLATE COVER

ALUMNIUM BLOCK

D-C FAN MOTOR

OUTLET

TOP

POLYURETHANE STRIP

BOTTOM

FAN

HEAT EXCHANGER

INLET

SIDE VIEW

FRONT VIEW

Figure 2. Diagram of temperature controlled leaf chamber showing the thermoelectric stage, mica, aluminum fins, fin cover plate, "squirrel cage" fan, and plexiglass construction.

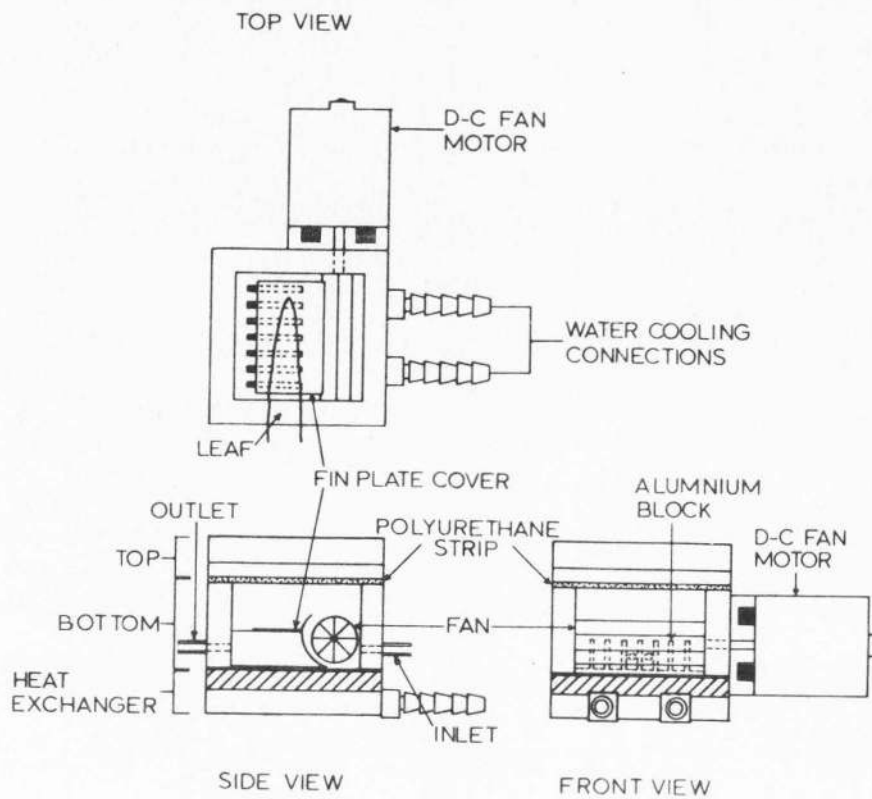chambers preventing leaf damage during exposure yet providing a
nearly airtight seal. This chamber is mounted on a Peltier thermo-
electric stage which acts as a heat exchanger for the chamber. Mounted
on the thermoelectric stage is an aluminum block with fins for
maximizing convective heat exchange between the thermoelectric stage
and the leaf chamber. A layer of mica separates the aluminum block
from the thermoelectric stage. This mica layer conducts heat
effectively to the aluminum block, and electrically insulates the
block from the thermoelectric stage. An aluminum cover plate is
mounted across the top of the aluminum fins. A circular "squirrel
cage" fan, milled from a teflon rod and driven by a dc motor
with rheostatic control, continually circulates air between the
aluminum fins and then across the plant leaf. This effectively
exchanges heat between the leaf and the Peltier heat exchanger,
minimizes boundary layer development around the leaf, and mixes
the chamber air.

The portion of the thermoelectric heat exchanger that is external
to the leaf chamber must usually be cooled. This is accomplished by
a water cooling system. The water cooling system has a dc operated
pump which circulates water from a one gallon insulated reservoir.
The reservoir is filled two-thirds full with ice and provides adequate
cooling for more than eight hours of operation. The cooling reservior
is linked to the thermoelectric module with 1/8" I. D. tygon tubing
wrapped with aluminum foil. The leaf chamber and thermoelectric
module are mounted on a flexible goose neck from a microphone stand.
The goose neck is fitted with a stake which is pushed into the ground.
This system allows for a great deal of maneuverability while at the

same time giving a firm and steady support during the exposure period.

The thermoelectric module is controlled by a bipolar proportional controller. The bipolar nature of the controller allows for both heating and cooling of the leaf chamber. The leaf chamber has a temperature control range from $0^{\circ}C$ to $50^{\circ}C$ and a stability of $\pm 5^{\circ}C$. The controller can be operated from a 12 volt dc source, has dimensions of 20 cm x 36 cm x 25 cm and a total weight of 11.5 kg.

Because of the relatively large chamber volume in this temperature-controlled leaf chamber, it was necessary to initially flush the leaf chamber rapidly with $^{14}CO_2$-labeled air. This rapid replacement of the leaf chamber air with $^{14}CO_2$-labeled air is of paramount importance if absolute values of photosynthesis are to be obtained. This is accomplished with two lever-operated toggle valves. One valve is placed immediately after the $^{14}CO_2$-labeled air flow meter and the other valve is placed in a by-pass around this flow meter. With sufficient pressure in the labeled-air line, the by-pass valve can be depressed and the leaf chamber purged with labeled air in less than three seconds.

When ambient air temperatures are not used, a period of equilibration is necessary for the leaf to attain a new steady-state temperature and photosynthetic rate. A 2-liter rubber air reservoir with an attached "squeeze bulb" for pressurizing this reservoir provides a constant flow of unlabeled air during this equilibration time. In order to reduce CO_2 diffusion the air lines were constructed of 1/8" copper tubing and fittings.

2. <u>Exposure of Plant</u>:

Use of this exposure system involves placing an intact leaf (or other plant part) across the lower portion of the chamber and tightly closing the chamber top. The unlabeled air reservoir is filled, the chamber is appropriately oriented, unlabeled air flow is initiated, the chamber fan and water pump are started, and the bipolar controller is adjusted to the desired temperature. After leaf temperature and photosynthetic equilibration, the unlabeled air stream is stopped, and the by-pass valve is opened for purging of the chamber with labeled air. Then the by-pass valve is closed and simultaneously the labeled-air flow valve is opened for the short exposure period.

3. <u>Sample Preparation</u>:

Following exposure, the labeled section is immediately cut, its area is measured, and it is placed in a vial on ice in a freezing chest until it can be returned to the laboratory for processing. The sample is then placed in a drying oven at $100^{\circ}C$ for 12 hours. The dry weight of the sample is taken and it is placed in a tightly capped storage or scintillation vial until combustion.

4. <u>Combustion</u>:

A dry in-vial combustion technique provides a rapid, convenient method of combustion and allows accurate determinations of $^{14}CO_2$ from exposed leaf samples. This procedure involves making a cup container from lens paper soaked in black ink and placing it into a coiled wire holder. The cup container in the wire holder is then placed into an empty scintillation vial. A dried sample is placed into the cup

container, the vial is momentarily flushed with a stream of pure oxygen, and the vial is immediately capped tightly with a #15 serum stopper cap. The sample in the coiled wire stand is then ignited with a focused light beam from a modified slide projector. The sample ignites and burns to completion in approximately 5 seconds. Maximum sample size for this technique is dependent upon caloric content of the material and is usually less than 10 mg dry weight.

5. Counting:

After a 5 minute cooling period, 0.5 ml of NCS are injected through the serum stopper cap with a glass syringe. After an absorption period of 6 hours (sufficient for complete absorption), the serum stopper cap is removed and 20 ml of scintillation grade counting solution are placed in the vial which is then closed with a cap containing a teflon liner. A combination of NCS and a toluene base scintillation liquid minimizes problems of chemiluminescence, remains stable for at least several days, and yields consistent counting efficiencies of 70-75%. The samples are counted in a liquid scintillation counter. The counting efficiency for each vial is determined by the channels ratio method and DPM are calculated. The calibration curve is established with a toluene base quenched standard set.

6. Calculations:

The absolute rates of CO_2 fixation (P) are calculated from the counts per minute (CPM), counting efficiency as a fraction (E), area in dm^2 or gram dry weight of the leaf sample (A), exposure time

in minutes (T), and the specific activity of the $^{14}CO_2$ air mixture (SA). The conversion equation can be expressed as:

$$P = \frac{(CPM)\ (44\ mg \cdot mMole^{-1}\ CO_2)}{(E)\ (T,min)\ (2.22 \times 10^9 DPM \cdot mc^{-1})\ (SA, mc \cdot mMole^{-1} CO_2)\ (A, dm^2)}$$

$$= \frac{(CPM)\ (1.19 \times 10^{-6})}{(E)\ (T)\ (SA)\ (A)} \qquad mg\ CO_2 \cdot dm^{-2} \cdot hr^{-1}$$

CPM = COUNTS PER MINUTE

E = EFFICIENCY AS A FRACTION, CPM/DPM

T = EXPOSURE TIME AS PART OF A MINUTE

SA = SPECIFIC ACTIVITY OF $^{14}CO_2$-AIR MIXTURE

A = AREA OF SAMPLE

In summary, the system described is inexpensive relative to most gas analysis systems, has the advantage of ease of use and complete portability, and provides data comparable to those obtained with conventional IRGA techniques. The method is recommended whenever portability is an essential prerequisite or where IRGA methods are impractical.

VITA

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