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AS AFFECTED BY GENOTYPE AND SINK-SOURCE  
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PHYSIOLOGICAL BASIS FOR VARIATION OF NET PHOTOSYNTHESIS  
IN OAT (AVENA SPP.) LEAVES AS AFFECTED BY GENOTYPE  
AND SINK-SOURCE RATIOS

by

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## INTRODUCTION

The maximum economic yield which can be obtained with a crop depends on many factors. Although many physiological processes have been extensively studied and the influence of environmental factors on these processes has been investigated, the physiological basis for differences in yield between various genotypes of the same species, grown under optimal environmental conditions, is still somewhat obscure. It would seem clear, however, that the maximum amount of dry matter produced would depend ultimately on the carbon balance of the plant. Thus, the net photosynthetic efficiency would seem to be one of the most important physiological factors affecting the economic yield of many agronomic crops.

Recently attention has been devoted toward increasing yields by isolating genotypes with high photosynthetic efficiencies. Differences in net photosynthetic rate of species have been reported, and it is known that species with high net photosynthetic rates, such as corn, sugar cane, and sunflower are high dry matter producers. More recently genotypes within the same species have been reported to have different net photosynthetic rates. Again it appears that greater net photosynthetic rates are often associated with improved varieties having a high economic yield (Ojima et al., 1968).

Although many factors can affect yield Izhar and Wallace (1967a) state that the net carbon dioxide ( $\text{CO}_2$ ) exchange of individual leaves constitutes the most basic component of the photosynthetic efficiency of an intact plant. Therefore, by selecting for plants with highly ef-

efficient single leaves, one could probably also simultaneously select for photosynthetically efficient whole plants, provided the components for each are not negatively correlated.

The maximum net photosynthetic rate which can be obtained depends largely on the light and dark reactions, the CO<sub>2</sub> diffusion process, and other factors such as enhanced respiration in the light. Although voluminous literature is available on all of these processes, it is still unclear which process or processes are responsible for the variation in net photosynthetic rates of different genotypes of the same species. There is now evidence that the capacity of the plant to store the products of photosynthesis at some location (i.e., the sink) or the translocation of assimilated products out of photosynthetically active tissue (i.e., the source) may have some controlling influence on the net photosynthetic rate. It has been suggested by Hartt (1963) that the upper limit of crop yield might be raised by increasing the rate of translocation, which in some instances may automatically increase production by increasing photosynthesis. She has suggested a breeding approach for sugar cane based on factors favoring translocation such as size or number of vascular bundles, or strength of attachment of the leaf to the stem. It is postulated that perhaps the sink-source ratio could also influence the photosynthetic rate in a similar manner, and perhaps, this ratio could be genetically modified by either increasing the sink size or number of sinks.

Plants are more efficient converters of energy than animals, and because the world population is rapidly increasing, it would seem that an approach towards increasing crop yields, based upon increased

photosynthetic efficiencies, would certainly be justified. Hence, this study was conducted with the objectives being fourfold. The objectives of the experiment were:

1. To determine whether or not differences in net photosynthesis occurred in different oat genotypes.
2. To study the relationship between net photosynthesis and specific leaf weight.
3. To study the physiological basis for variation of photosynthetic rate.
4. To investigate how the net photosynthetic rate and carbohydrate partitioning patterns in oats were influenced by the sink-source ratio.

A separate experiment was designed to answer the first three objectives, and the results are discussed in Part I. A second experiment was conducted to investigate the fourth objective, and the results of this experiment are presented in Part II.

PART I. THE SCREENING EXPERIMENT

## LITERATURE REVIEW

Gaastra (1962) and Bierhuizen and Slatyer (1964) have stated that photosynthesis is influenced by three main processes: a photochemical process involving the utilization of light energy for photosynthesis, a diffusion process associated with the transport of  $\text{CO}_2$  to the photosynthetic reaction site, and biochemical processes involved with the fixation and chemical reduction of  $\text{CO}_2$ . The magnitude of the three processes can be influenced by many factors, and the interaction of such factors will ultimately affect the photosynthetic rate.

## Radiant Energy and Photosynthesis

In 1779 the Dutch physician Jan Ingenhousz discovered the necessity of light for plants to purify "bad air", a light requiring process now known as photosynthesis (Rabinowitch, 1948). Since this discovery much has been learned about the role of light in photosynthesis but the photochemical reactions involved are still not well understood.

The light response curve

The relationship between photosynthesis and radiant energy can best be defined by a hyperbolic shaped curve. At low light flux densities the relationship is essentially linear but as light flux density is increased, photosynthetic rate increases less rapidly until light saturation is reached in many species. There are four major features of the light response curve which need further explanation: the linear portion,

the curvilinear portion, the light saturation point, and the light compensation point.

The linear portion      The initial slope of the light response curve is believed to be affected by two factors; chlorophyll concentration and efficiency of light energy conversion per unit chlorophyll. The initial slope of the light response curve is generally accepted as indicating the maximum efficiency of light energy conversion (Gaastra, 1959).

Gabrielsen (1948) recognized the chlorophyll factor was a weak light factor and that at higher light flux densities the photochemical process became confounded with other processes. By use of weak light experiments he found that chlorophyll (a+b) contents of most leaves exceeded the value (4-5 mg.dm<sup>-2</sup> leaf area) required for maximum photosynthesis. Only in young leaves and in leaves of Sambucus canadensis aurea were chlorophyll concentrations below that needed for maximum photosynthesis.

Although it is generally accepted that chlorophyll content is generally not limiting for photosynthesis, there is evidence that concentration is influenced by the light regime under which the leaf develops. Friend (1961) showed chlorophyll content of Marquis wheat increased linearly with daylength under high light conditions (1,750 foot-candles) and the effect could not be duplicated by supplying an equal amount of radiation by extending the daylength with low light

(30-40 foot-candles). Previous work indicated greater chlorophyll accumulation under the highest light flux densities used (2,500 foot-candles), and it was concluded that the rate of chlorophyll formation follows the rate of an autocatalytic reaction (Friend, 1960). Logan and Krotkov (1969), however, reported a decrease in chlorophyll content of leaves of sugar maple seedlings (Acer saccharum) grown in 100 percent full sunlight compared with that of seedlings grown under various shading treatments. It has been observed by Björkman and Holmgren (1963) that chlorophyll contents are lower in populations of Solidago virgaurea L. from exposed habitats than those adapted to shaded habitats when both populations were grown under high light. Although plants from shaded habitats contained more chlorophyll, they observed that in some plants grown under high light conditions chloroplast destruction was pronounced. This finding has also been reported by Loach (1969) in shade tolerant species of trees grown in open conditions. According to Salisbury and Ross (1969), this phenomenon is known to occur under high light and is greater in the presence of oxygen. This oxidation of cellular components, including chlorophyll, is commonly referred to as photo-oxidation or solarization and it is believed that carotenoids may serve a protective function to prevent such an oxidation (Salisbury and Ross, 1969).

The distribution of chloroplasts in the leaf and the amount of light received and absorbed by the abaxial and adaxial surfaces may affect the net photosynthetic rate, but work by Moss (1964) has shown

that if this is a critical factor it is important only in dicotyledonous leaves which have more chloroplasts in palisade than spongy mesophyll tissue. A hyperbolic transformation indicated that radiation supplied to the abaxial surface of dicotyledonous leaves affected the photosynthetic rate more at low light flux densities because a significant amount was absorbed by chlorophyll-free structures. Under high light flux densities the transformation indicated that the maximum net photosynthetic rate was the same regardless of the direction from which the light was supplied. In monocotyledonous leaves, having a uniform distribution of chloroplasts, the photosynthetic response was the same regardless of the side of the leaf illuminated and light flux density.

The extent and rate of chloroplast flattening is also light dependent (Nobel et al., 1969). As the light level is increased the chloroplasts of peas (Pisum sativum L.) were found to flatten more and the lag period for the response was reduced. They postulate that decreases in chloroplast thickness may be related to a concomitant increase in photosynthetic efficiency.

From the above work it appears chlorophyll content is not generally limiting for photosynthesis even though content may be affected by the light regime. Distribution does not appear to be a critical factor, especially at higher light flux densities, and as yet little is known as to how the chloroplast flattening response is related to photosynthesis.

It would also appear that chlorophyll efficiency is not significantly different between species. Work by Hesketh (1963) with

nine species has shown no differences in chlorophyll efficiency, though large differences in assimilatory capacity existed. This conclusion was made from a hyperbolic transformation, however, and there was departure of the data from the transformation lines at light flux densities less than  $.25 \text{ ly}\cdot\text{min}^{-1}$ . But this departure was attributed to the questionable estimation of true photosynthesis at low light flux. This was taken as being equal to the sum of net photosynthesis and dark respiration, and the lack of fit at these low light levels can perhaps now be explained. Recent evidence suggests that respiration rates occurring in light may exceed those in dark (Lake, 1967a) and may be related to light flux. The consistent departure of the data in one direction from the lines would tend to support such a hypothesis, except for maize, a species which showed a low respiration rate in light. Again chlorophyll content did not explain species differences.

Gaastra (1959) observed that efficiency of light energy conversion was of the same order of magnitude for sugarbeet, turnip, cucumber, and tomato which were found to have energy conversion efficiencies of 13.1, 17.4, 12.2 and 12.8 percent, respectively. Bierhuizen and Slatyer (1964) reported that under weak light conditions and  $\text{CO}_2$  saturation the efficiency of energy conversion for cotton was 17.0 percent. Work by Dornhoff (1969) has shown the efficiency of energy conversion under weak light conditions in soybeans to be between 12.0 and 13.0 percent. Thus, it can be concluded that the efficiency of light energy conversion by chlorophyll for most species is similar and cannot explain large differences in  $\text{CO}_2$  assimilatory capacity under normal light flux densities.

The curvilinear portion      The curvilinear portion of the light response curve is approached as the photochemical process becomes more dominantly influenced by limiting factors such as CO<sub>2</sub> or temperature. At this point diffusion processes and biochemical reactions become limiting to photosynthesis. This is why Gabrielsen (1948) stated that, if the chlorophyll factor was to be studied, its full effect could only be observed in weak light where the photosynthetic rate is directly proportional to light. Gaastra (1959) found the photosynthetic rate of a cucumber leaf could be increased, depending upon whether or not diffusion or biochemical processes were limiting, by supplying either more CO<sub>2</sub> or heat to the leaf. Under high light and CO<sub>2</sub> conditions, increasing leaf temperatures from 20 to 30°C had the largest effect on photosynthesis, because temperature dependent biochemical reactions were most limiting. Under only high light and normal CO<sub>2</sub>, the assimilation rate could be increased at either temperature by increasing the CO<sub>2</sub> concentration, because the diffusion process was limiting. Moss et al. (1961) observed that increasing the CO<sub>2</sub> concentration enhanced the assimilation rate of corn, especially under high light conditions. It has been reported that soybean (Glycine max (L.) Merr.) varieties Hark and Chippewa-64 both become light saturated at about 21,530 lux at normal CO<sub>2</sub> concentrations (300 ppm), but when the CO<sub>2</sub> concentration is increased, light saturation does not occur even at light flux densities of 75,350 lux (Brun and Cooper, 1967). Perhaps, the low level of light needed for saturation at normal CO<sub>2</sub> concentrations can be explained by the fact that their soybeans were grown in growth chambers with lower

light flux densities than occur naturally. Dornhoff (1969) found higher light saturation levels for soybeans tested under normal atmospheric CO<sub>2</sub> conditions, but his plants were grown under natural solar radiation conditions.

The light saturation point      The leaf is said to be light saturated when no measurable increase in photosynthesis is attained by increasing illumination. The light flux density at which light saturation is reached is not necessarily the same for all species. Hesketh and Musgrave (1962) found net photosynthesis of single corn leaves did not show light saturation with increasing illumination up to at least 10,000 foot-candles. In another study Hesketh and Moss (1963) found light saturation not to occur in sugar cane, or sunflower; but nine other species examined showed light saturation. Warren-Wilson (1967) reported that assimilation rates of sunflower plants rose linearly with radiation, suggesting a very high light saturation level.

The light conditions under which a plant develops apparently influence the light saturation point. Burnside and Bohning (1957) observed that species adapted to high light, but grown under shaded conditions, became light saturated at lower light flux densities. The same plants grown under normal high light conditions became light saturated at higher light flux densities. When populations of Solidago virgaurea L. were grown at high light flux densities leaves from populations adapted to exposed habitats became saturated at higher light flux densities than those of populations normally occurring only in shaded

habitats (Björkman and Holmgren, 1963). When the same populations were grown under lower light flux density conditions the differences in light saturation points were not as pronounced.

The light compensation point      The light compensation point is the light flux density at which photosynthesis compensates for respiration, so that the net gas exchange is zero. Hesketh (1963) has observed that of the species now believed to show a light stimulated respiratory process, those exhibiting higher rates of net photosynthesis generally have higher light compensation points. Work with 30 species of woodland shrubs by Sparling (1967) has shown that shade tolerant species are often characterized by a lower light compensation point and a greater initial light-response slope.

Of economic interest, perhaps, is the fact that, as CO<sub>2</sub> concentration is elevated, the light compensation point is decreased. Heath et al. (1967) have stated that with lettuce or other crops growing under glass in weak light one of the beneficial effects of CO<sub>2</sub> enrichment of atmospheric air might, in part, be due to a reduction of the light compensation point.

#### Adaptation to light and shade effects

From the work of Björkman and Holmgren (1963) and Sparling (1967) it can be concluded that populations or species adapted to shaded habitats tend to show lower maximum net photosynthetic rates than those adapted to exposed habitats. Their results indicate, however, that

shade adapted plants often have a greater photosynthetic advantage under weak light conditions because the initial slope is greater and the light compensation point lower.

Photosynthetic properties of leaves may be influenced by light conditions under which the leaf develops. Kumura (1968) found that if the soybean variety Shirobana-Sai-No 1 was given a period of shade prior to testing, photosynthetic capacity at high light flux densities decreased, but upon cessation of shading the photosynthetic capacity showed a degree of recovery depending upon the age of the leaf. Photosynthetic capacity at low light flux densities was increased by shade treatments and respiratory activity in the dark was reduced. Pearce and Lee (1969) observed similar results in alfalfa (Medicago sativa L.). Net photosynthetic rates measured under a light flux density of 48 klux were higher when leaves developed under high light conditions (32-43 klux), as opposed to low (13-14 klux) light.

Seasonal changes in net photosynthesis have been found to exist and it is believed this is related to the amount of available sunlight (Hesketh, 1968). Seasonal changes occurring in Helianthus annuus L. and Gossypium hirsutum L. were presumably due to differences in photosynthetic potential inside the leaf as opposed to changes in diffusion processes.

Plant development under various light conditions may influence photosynthetic rate, but differences appear unexplainable on the basis of chlorophyll content or efficiency of light energy conversion per unit chlorophyll.

## The CO<sub>2</sub> Diffusion Process

Rates of photosynthesis may be limited by capacity of diffusion processes when other conditions for photosynthesis are optimal. Gaastra (1959) recognized that rates of diffusion of CO<sub>2</sub> from external air towards the chloroplast (CO<sub>2</sub> fixation site) depended upon the dimensions of the diffusion path through the laminar air layer, the stomata and intercellular spaces, and mesophyll cells. Qualitatively, much was known concerning the role of stomata in the diffusion process, but it was not until Gaastra (1959) developed procedures for measuring photosynthesis, transpiration, and leaf temperature simultaneously that quantitative values could be ascribed to stomatal as well as laminar and mesophyll diffusion resistance.

For convenience the diffusion pathways have been separated into three portions: (1) the laminar or boundary layer resistance ( $r_a$ ), (2) the stomatal and substomatal resistance ( $r_s$ ), and (3) the mesophyll resistance ( $r_m$ ). Sometimes a cuticular resistance ( $r_c$ ) is included, but Freeland (1948) and Holmgren et al. (1965) have shown this resistance to be extremely high and of little significance under most conditions.

### Factors affecting diffusion resistances

The boundary layer resistance ( $r_a$ ) is affected by the size and shape of the leaf, nature of the leaf surface, and wind velocity over the leaf (Gaastra, 1962). Both Waggoner and Zelitch (1965) and Baker and Myhre (1969) have reported narrower leaves to have lower  $r_a$  values than wide leaves. Gaastra (1962) and Avery (1966) have observed that

turbulence exerts its greatest effect on assimilation at low wind speeds, and studies by Warren-Wilson and Wadsworth (1958) have shown similar results. However, they found that if the wind speed was raised beyond an optimum ( $30 \text{ cm. sec}^{-1}$  for Brassica napus) leaf drying and other harmful effects became increasingly important.

Stomatal resistance ( $r_s$ ), which is primarily a function of stomatal aperture, is dependent upon light flux density,  $\text{CO}_2$  concentration, and moisture status of the leaf. Generally, as the light flux density falls the stomata tend to close, hence,  $r_s$  increases (Gaastra, 1962). Holmgren et al. (1965) have also shown  $r_s$  to increase in Acer platanoides as a function of leaf age, and Tieszen (1969) found  $r_s$  in Deschampsia caespitosa to decrease as the light regime under which the plants developed increased.

Mesophyll resistance ( $r_m$ ) is, unfortunately, a function of light flux density or any other factor which controls photosynthesis (Moss, 1968a). This is because  $r_m$  is not strictly a physical resistance, but is affected by biochemical processes. Gaastra (1962) reported that reduced moisture status may increase  $r_m$ , but that, generally, it was not possible to separate moisture effects on  $r_s$  from those on  $r_m$ . Troughton (1969) attempted to eliminate effects of moisture on  $r_s$  by measuring  $r_m$  in an oxygen-free atmosphere. (It was found that in oxygen-free air the stomates remained open at low relative leaf water contents.) Not until the leaf water content dropped below 75 percent did the mesophyll resistance increase in cotton (Gossypium hirsutum L.).

Troughton and Slatyer (1969) have reported that  $r_m$  values for cotton in normal air are about 25 percent higher than in oxygen-free air. There is evidence the  $r_m$  decreases under high light flux densities and Bierhuizen and Slatyer (1964) have suggested this phenomenon may indicate an increase in cell permeability under high light. An equation, developed by Brown (1969), predicts reduction in  $r_m$  as the external  $CO_2$  concentration increases.

#### Diffusion resistances as a limiting factor

It was observed by Gaastra (1959) that large variations in  $r_m$  occurred between species showing different net photosynthetic rates; consequently, he suggested that mesophyll resistance seemed to be an important yield limiting factor in crop plants. Hesketh (1963) also suggested that differences in diffusion resistances, or alternatively, kinetics of dark reactions might explain differences in net photosynthetic rates of various species.

The boundary layer resistance ( $r_a$ ) is small compared with the total diffusion resistance for  $CO_2$ . For this reason, breeding attempts in cotton (Gossypium hirsutum L.), with the intent of reducing  $r_a$  by employing the mutant character of "okra" leaves, have failed (Baker and Myhre, 1969). The deeply lobed "okra" leaves were found to have a lower  $r_a$ , but the depression of  $r_a$  was so small that photosynthesis was not significantly increased. Lower  $r_a$  values have been reported by Stevenson (1969) in normal soybean (Glycine max (L.) Merr.) leaves as compared to an isogenic line with dense pubescence.

Both the stomatal and mesophyll resistance terms are larger than the laminar resistance. Under high light flux densities, when the rate of the CO<sub>2</sub> diffusion process limits photosynthesis,  $r_m$  is from 1 to 4 times greater than  $r_s$  (Gaastra, 1959). Differences between species in  $r_s$  and  $r_m$  are known to exist.

El-Sharkawy and Hesketh (1965) examined 8 species and found  $r_s$  ranged from 1.1 sec.cm<sup>-1</sup> in Thespesia populnea L. to 3.6 sec.cm<sup>-1</sup> in tobacco (Nicotiana tabacum L.). Mesophyll resistance ranged from 1.0 sec.cm<sup>-1</sup> in corn (Zea mays L.) to 7.3 sec.cm<sup>-1</sup> in Hibiscus tiliaceus L. Similarly, Holmgren et al. (1965) noted that differences in net photosynthesis between species were related to differences in both  $r_s$  and  $r_m$ . The variation of  $r_s$  within a given species was greater than that of  $r_m$ .

Differences in  $r_s$  and  $r_m$  within the same species have been found to exist. Working with ecotypes of Solidago virgaurea L. from exposed and shaded habitats, Holmgren (1968) observed that  $r_s$  was reduced in plants adapted to exposed habitats when grown under high irradiation, whereas  $r_s$  was not affected in shade adapted ecotypes grown under either high or low light flux densities. The higher net photosynthetic rates in clones from exposed habitats grown under high light flux densities was attributed to a lower  $r_m$ . Work by Dornhoff and Shibles (1970) has shown that variation in net photosynthetic rates of soybean (Glycine max. (L.) Merr.) genotypes could be largely explained on the basis of differences in  $r_s$  and  $r_m$ .

Thus, it can be concluded that differences in diffusion resistances do occur both between species and within species. Mesophyll and stomatal

resistances are much larger than laminar resistance, and these appear to affect the photosynthetic process more.

### Theory of resistance equations

Most resistance models are based on the assumption that steady state conditions exist; i.e., constant light, temperature, and CO<sub>2</sub> conditions. All models and modifications of these have been founded on various assumptions.

The classical resistance model Gaastra (1959) proposed that under steady state conditions the sum of the resistances ( $r_a + r_s + r_m$ )CO<sub>2</sub> encountered in the passage of CO<sub>2</sub> from the atmosphere to the CO<sub>2</sub> fixation site could be expressed by the following equation:

$$(\Sigma r)_{CO_2} = (r_a + r_s + r_m)_{CO_2} = \frac{[CO_2]_a - [CO_2]_{chl}}{P} \quad (1)$$

where  $[CO_2]_a$  represents the CO<sub>2</sub> concentration of the external air and  $[CO_2]_{chl}$  represents that in the chloroplasts. The net photosynthetic rate (P) and  $[CO_2]_a$  can be determined experimentally. Gaastra (1959) assumed that under conditions of CO<sub>2</sub> limitation  $[CO_2]_{chl}$  is zero, however, he recognized the validity of this assumption could be questioned. The assumption that  $[CO_2]_{chl}$  is zero was based on the relation between net photosynthesis and  $[CO_2]_a$ , which remains linear at CO<sub>2</sub> concentrations very close to zero. Photosynthesis is assumed to be light saturated.

Accordingly, the resistance of water vapor passage from the surface of the mesophyll cells to the external atmosphere could be expressed by:

$$(\Sigma r)_{H_2O} = (r_s + r_a)_{H_2O} = \frac{[H_2O]_{int} - [H_2O]_a}{T} \quad (2)$$

where  $T$  represents the transpiration rate and  $[H_2O]_{int}$  and  $[H_2O]_a$  correspond to the saturated water vapor concentration at the measured leaf temperature of photosynthesis and the water vapor concentration in the external air, respectively. It is assumed that the intercellular air spaces of the leaf are saturated with water vapor. That this might be a valid assumption was indicated by the fact that Gaastra (1959) observed no decrease in transpiration rates of leaves upon exposing them to high light flux densities for long periods. This indicated to him a low mesophyll resistance for water, so that the intercellular spaces were essentially saturated with water vapor. Slatyer (1967) has calculated that error introduced into the  $([H_2O]_{int} - [H_2O]_a)$  term by assuming saturation in the internal portion of the leaf will generally not exceed 8 percent for a range of leaf water potentials of from 0 to -50 bars, provided air humidity values are less than 50 percent.

The external air resistance  $(r_a)_{H_2O}$  can not be derived from transpiration measurements because the water vapor concentration at the leaf surface is not known; however, it can be approximated by measuring the rate of evaporation from a piece of moist blotting paper the same size of the leaf, and exposed to the same experimental conditions. Thus, Gaastra (1959) calculated  $(r_a)_{H_2O}$  by the following method:

$$(r_a)_{H_2O} = \frac{[H_2O]_{sur} - [H_2O]_a}{E} \quad (3)$$

where  $E$  is the evaporation rate from the exposed surface of a wet blotter. The blotter surface is assumed to be saturated.

Gaastra (1959) assumed that stomatal and boundary layer resistances for  $\text{CO}_2$  transfer are analogous to the resistances for water vapor transfer and were related by the ratio of their diffusion coefficients for water vapor ( $D_{\text{H}_2\text{O}}$ ) and carbon dioxide ( $D_{\text{CO}_2}$ ) in air. Stomatal and laminar resistances to  $\text{CO}_2$  transfer could then be calculated from the following equations:

$$(r_s)_{\text{CO}_2} = \frac{D_{\text{H}_2\text{O}}}{D_{\text{CO}_2}}((r_a+r_s)_{\text{H}_2\text{O}} - (r_a)_{\text{H}_2\text{O}}) \quad (4)$$

$$(r_a)_{\text{CO}_2} = \frac{D_{\text{H}_2\text{O}}}{D_{\text{CO}_2}}(r_a)_{\text{H}_2\text{O}} \quad (5)$$

Gaastra (1959) used values of .14 and .24  $\text{cm}^2 \cdot \text{sec}^{-1}$  for  $D_{\text{CO}_2}$  and  $D_{\text{H}_2\text{O}}$ , respectively. Gale and Poljakoff-Mayber (1968), however, have quoted values of .165 and .258  $\text{cm}^2 \cdot \text{sec}^{-1}$  for the same diffusion coefficients. Use of these values alters the ratio of  $D_{\text{H}_2\text{O}}/D_{\text{CO}_2}$  by about 9 percent.

Gaastra (1959) then calculated mesophyll resistance from the following equation:

$$(r_m)_{\text{CO}_2} = (\Sigma r)_{\text{CO}_2} - (r_s+r_a)_{\text{CO}_2} \quad (6)$$

Although Gaastra (1959) stated the  $r_m$  was the resistance of diffusion of  $\text{CO}_2$  in the dissolved state through the mesophyll cells it has been emphasized by Moss (1968a) that  $r_m$  is not purely a physical resistance since, because it is calculated as a residual quantity, it is dependent on light or other factors which affect photosynthesis.

Gaastra (1959) found photosynthesis to be affected little by

stomatal regulation under  $\text{CO}_2$  limiting conditions because  $r_m$  was usually high compared to  $r_s$ . Species differences in net photosynthesis were believed due to variation in  $r_m$ .

Modifications of the classical model Since the development of resistance equations various modifications of them have emerged. Most modifications have attempted to eliminate errors involved in the assumption of a value for  $[\text{CO}_2]_{\text{chl}}$ , the  $\text{CO}_2$  concentration in the chloroplasts.

It has been reported that  $\text{CO}_2$  compensation points (the  $\text{CO}_2$  concentration at which photosynthesis equals respiration) of most plants generally fall between 60 and 70 ppm and this value is believed to reflect the  $\text{CO}_2$  concentration inside the leaf (Heath and Orchard, 1957; Moss, 1962b). Moss (1968a) calculated  $r_m$  values assuming zero  $\text{CO}_2$  concentration at the chloroplast; however, he believed this was not representative of the  $\text{CO}_2$  concentration at the chloroplast but that it was true at the end of the dark reactions -- i.e., the partial pressure of  $\text{CO}_2$  above sugar is zero.

Working with cotton (Gossypium hirsutum L.) Bierhuizen and Slatyer (1964) observed that  $r_m$  appeared to be overestimated at low external  $\text{CO}_2$  concentrations, but declined as the  $\text{CO}_2$  level in the external air increased. This occurred only when calculations were based on the assumption that  $[\text{CO}_2]_{\text{chl}}$  was equal to zero. No change in  $r_m$  was found upon increasing the  $\text{CO}_2$  concentration of the external air when the  $\text{CO}_2$  concentration at the chloroplast site was estimated as being equal to

the  $\text{CO}_2$  compensation point ( $\Gamma$ ). They believe  $\Gamma$  more accurately reflects the  $\text{CO}_2$  concentration at the chloroplast site.

Holmgren et al. (1965) have used the following equation to compute  $r_m$ :

$$(r_m)_{\text{CO}_2} = (\Delta[\text{CO}_2]_a / \Delta P) - (r_a + r_s)_{\text{CO}_2} \quad (7)$$

where  $\Delta P$  is the change in net photosynthesis (before any change in stomatal aperture occurs) when the  $\text{CO}_2$  concentration of the external air is changed by the amount  $\Delta[\text{CO}_2]_a$ . This method eliminates errors involved in the assumption of a value for  $[\text{CO}_2]_{\text{chl}}$  because the slope is used over the linear portion of the  $\text{CO}_2$  response curve to calculate  $r_m$ . However, the equation makes the implicit assumption that  $[\text{CO}_2]_{\text{chl}}$  is equal to  $\Gamma$ .

Direct estimates of  $r_m$  under conditions where the quantity  $(r_a + r_s)_{\text{CO}_2}$  is equal to zero have been made by Whiteman and Koller (1968). They found, that under various  $\text{CO}_2$  and temperature treatments, and saturating radiation, linear regressions of  $(r_a + r_s)_{\text{H}_2\text{O}}$  on net photosynthesis were highly significant for sunflower leaves (Helianthus annuus L.) at each  $\text{CO}_2$  level. Extrapolations of these lines to the point where  $(r_a + r_s)_{\text{H}_2\text{O}}$  was equal to zero gave estimates of maximum net photosynthetic rates ( $P_{\text{max}}$ ). Again the  $\text{CO}_2$  concentration at the chloroplast site was assumed to equal  $\Gamma$ . At the point where  $(r_a + r_s)_{\text{H}_2\text{O}}$  equals zero,  $(r_a + r_s)_{\text{CO}_2}$  is also equal to zero. Therefore  $r_m$  could be calculated from the following equation:

$$(r_m)_{CO_2} = \frac{(CO_2)_a - \Gamma}{P_{max}} \quad (8)$$

This method of computing  $r_m$  has practical advantage over that of Holmgren et al. (1965), in that it is not necessary to hold  $(r_a+r_s)_{CO_2}$  constant (Lake, 1967b). Both modifications have been criticized, however, on the basis that they may overestimate  $r_m$ . Lake (1967a) has stated that the  $CO_2$  compensation point can be defined by the following equation:

$$\Gamma = \beta R(r_m)_{CO_2} \quad (9)$$

where  $R$  is the flux density (dimensions,  $ML^{-2}T^{-1}$ ,  $M$  = amount,  $L$  = length,  $T$  = time) of respiratory  $CO_2$  production,  $(r_m)_{CO_2}$  is the mesophyll resistance (dimensions,  $L^{-1}T$ ), and  $\beta$  is a non-dimensional constant. If  $R$  (i.e., light respiration) increases as photosynthesis increases,  $\Gamma$  will underestimate the value of  $R(r_m)_{CO_2}$  appropriate to normal air, and Equation 8 will overestimate the mesophyll resistance. Equation 7 of Holmgren et al. (1965) also is believed by Lake (1967b) to make the implicit assumption that respiratory  $CO_2$  production in the light does not depend upon the photosynthetic rate.

Gaastra's (1959) model did not separate resistances to diffusion of respiratory  $CO_2$  from  $CO_2$  utilized in photosynthesis. An electrical resistance analogue proposed by Lake (1967a) accounts for all the resistance factors; however, his model also accounts for respiration from cells with and without chloroplasts (Figure 1). Therefore, Lake's (1967a) model seems to be more complete. Referring to Figure 1,

$$r_a = r_{ts}, \quad r_s = r_{sw}, \quad \text{and } \Sigma r = r_{tc}.$$

A mathematical model has been developed by Brown (1969) which suggests that the  $\text{CO}_2$  concentration at the chloroplast site varies as a function of the atmospheric  $\text{CO}_2$  concentration. Photosynthesis is assumed to be described by a simple first order reaction:

$$P = KI[\text{CO}_2]_{\text{chl}} \quad (10)$$

where  $K$  is the capacity of the acceptor site to fix  $\text{CO}_2$ , and  $I$  is the incident radiation. The term  $[\text{CO}_2]_{\text{chl}}$  again represents the  $\text{CO}_2$  concentration at the chloroplast site. Under steady state conditions Fick's law of diffusion is applicable and  $P$  is described by:

$$P = D([\text{CO}_2]_{\text{a}} - [\text{CO}_2]_{\text{chl}}) \quad (11)$$

where  $D = 1/(\Sigma r)_{\text{CO}_2}$ . Combining Equations 10 and 11 by elimination of  $(\text{CO}_2)_{\text{chl}}$  yields a hyperbolic equation:

$$P = \frac{D[\text{CO}_2]_{\text{a}}}{\frac{D}{KI} + 1} \quad (12)$$

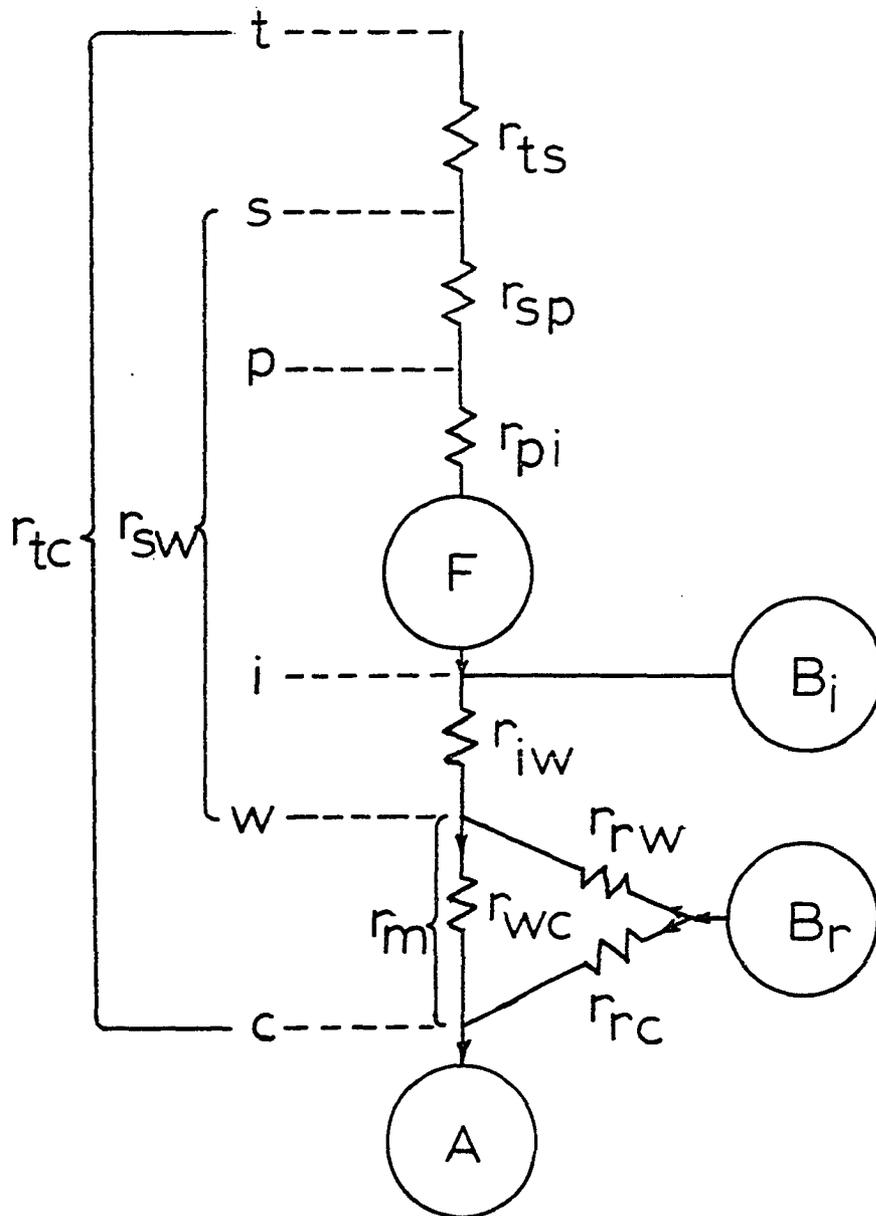
but the equation was believed clearly wrong by Brown (1969), because it called for zero net  $\text{CO}_2$  exchange in the dark -- if  $P$  is interpreted as net photosynthesis. Since respiratory and photosynthetic fluxes of  $\text{CO}_2$  cannot be separated, the net rate of photosynthesis ( $N$ ), defined as  $P - R$  where  $R$  equals respiration, is substituted for  $P$ . Equation 11 then becomes:

$$N = D([\text{CO}_2]_{\text{a}} - [\text{CO}_2]_{\text{chl}}) \quad (13)$$

and by combining Equation 13 with Equation 10 by elimination of

Figure 1. The CO<sub>2</sub> resistance model proposed by Lake (1967a).

t = CO<sub>2</sub> transducer; s = leaf surface; p = beneath stomatal pore; i = intercellular spaces; w = walls of mesophyll cells; c = chloroplasts; d = surface of cells respiring through photosynthetic cells; r = resistance between respective subscripts; F = net CO<sub>2</sub> flux into the leaf; B<sub>i</sub> = nonphotosynthetic cells which release CO<sub>2</sub> into the intercellular spaces; B<sub>r</sub> = respiratory CO<sub>2</sub> which must pass through the photosynthetic cells before escaping the leaf; A = CO<sub>2</sub> utilized in photosynthesis.



$(CO_2)_{chl}$  the following equation was derived:

$$N = \frac{K[CO_2]_a I - R}{\frac{K}{D} I + 1} \quad (14)$$

This model offers the advantage of allowing the net photosynthetic rate to be zero at light flux densities other than zero. Although other assumptions concerning stomata control at low light levels are involved, Brown (1969) found that by use of Equation 14 he could solve simultaneously for three groups of unknowns ( $K[CO_2]_a$ , R and K/D) using the values of N versus I obtained by other researchers. Since no direct solution was apparent, an iterative procedure of minimizing least squares was used. Once  $K[CO_2]_a$ , R, and K/D were determined,  $[CO_2]_a$  was used to solve K, which in turn was used to obtain D from which  $(\Sigma r)_{CO_2}$  could be calculated. K was substituted into the rate Equation 10 to obtain  $[CO_2]_{chl}$ , which was solved as a function of I. His results indicate the average value of  $[CO_2]_{chl}$  for corn at full sunlight was approximately 85 ppm. For photosynthetically less efficient species the  $[CO_2]_{chl}$  averaged 59 ppm. In no case was the  $[CO_2]_{chl}$  near zero, as might be expected from early work of Moss (1962b) and Meidner (1964), who both reported equilibrium  $CO_2$  concentrations near zero in maize. Respiration rates in light were calculated to be nearly twice that of those in the dark, which agrees with work by Lake (1967a). The capacity of the acceptor site for  $CO_2$  was variable. Mesophyll resistance was found to decrease as  $(CO_2)_a$  increased. This conflicts with results of Bierhuizen and Slatyer (1964), who found  $r_m$  to vary as a function of I, but Brown (1969) concluded that  $r_m$  could

vary as a function of either  $I$  or  $[CO_2]_a$ , depending upon which is assumed a variable.

Unfortunately, Holmgren et al. (1965) made the implicit assumption that  $[CO_2]_{chl}$  did not vary as a function of  $[CO_2]_a$ , but Brown's (1969) model indicates that it may, hence, he has proposed the following modification of their equation:

$$(\Sigma r)_{CO_2} = \frac{([CO_2]_a - [CO_2]_{chl}) - ([CO_2]_a - [CO_2]_{chl})}{P} \quad (15)$$

More recently Holmgren (1968) stated that the  $CO_2$  concentration at the "sink" of  $CO_2$  transfer was stable as long as the rate of  $CO_2$  transfer was linearly related to  $[CO_2]_a$  (i.e., rate of carboxylation and conditions of  $CO_2$  transfer between the sites of light respiration and photosynthesis are stable). The value of  $[CO_2]_{chl}$  was the extrapolated  $CO_2$  compensation concentration, which was based on extrapolation from the linear part of the curve. Holmgren (1968) found that the extrapolated  $[CO_2]_{chl}$  was between 42 and 48 ppm which, in spite of the modification suggested by Brown (1969), agrees quite well with Brown's results.

It has been shown by Gale and Poljakoff-Mayber (1968) that parallel pathways for diffusion through upper and lower leaf surfaces exist. In most leaves stomatal distribution is asymmetrical and error may be incurred when resistances are computed in series from measurements of photosynthesis and transpiration taken on the entire leaf. They found that this error could be reduced by obtaining simultaneous measurements

of photosynthesis and transpiration via two leaf surfaces. Resistance analogues for water vapor and CO<sub>2</sub> are represented by the following equations:

$$(r_a+r_s)_{H_2O} = \frac{(r_{su}+r_{au})_{H_2O}(r_{sl}+r_{al})_{H_2O}}{(r_{su}+r_{au}+r_{sl}+r_{al})_{H_2O}} \quad (16)$$

$$(\Sigma r)_{CO_2} = \frac{(r_{mu}+r_{su}+r_{au})_{CO_2}(r_{ml}+r_{sl}+r_{al})_{CO_2}}{(r_{mu}+r_{su}+r_{au}+r_{ml}+r_{sl}+r_{al})_{CO_2}} \quad (17)$$

where u and l represent the upper and lower leaf surfaces, respectively.

It is important that measurement of photosynthesis and transpiration be taken simultaneously on both surfaces of the leaf as Bertsch and Domes (1969) have found that quantities of CO<sub>2</sub> transported through the two surfaces of the leaf are not independent of each other (i.e., if CO<sub>2</sub> uptake is possible through only one surface of an amphistomatic leaf, the flux on that side will increase even though environmental conditions are the same as when the flux of CO<sub>2</sub> through both leaf surfaces is measured simultaneously).

#### "Photorespiration" and CO<sub>2</sub> Reassimilation

Accurate quantitative measurements of net photosynthesis are relatively easy to obtain. However, measurements of true photosynthetic rates are more difficult because there is now evidence that respiration rates in light may not necessarily be the same as those occurring in darkness. Measurements of true photosynthesis based on the assumption that true photosynthesis is equal to the dark respiration rate plus

the net photosynthetic rate may therefore be in error (Krotkov et al., 1958).

Evidence for a light stimulated respiratory process was first noted by Decker (1955). He observed that, when leaves of several species were placed in a closed system in light and allowed to reduce the CO<sub>2</sub> concentration down to the CO<sub>2</sub> compensation point ( $\Gamma$ ) and were then transferred to darkness, respiration was initially very high but decreased rapidly until a steady state of dark respiration was reached. This post-illumination "CO<sub>2</sub>-burst" has since been confirmed (Tregunna et al., 1966; Forrester et al., 1966a; Moss, 1966). The "CO<sub>2</sub>-burst" is thought to represent the overshoot of a greater CO<sub>2</sub> evolution (than dark respiration) which occurs in light, but which is usually not observed because of its short duration. Such evolution during illumination from leaves of certain species has been termed "photorespiration" (Tregunna et al., 1966).

#### Characteristics of photorespiring and non-photorespiring plants

Plants which appear to lack the photorespiratory process often have the capacity to photosynthesize at high rates. Hence, these plants have been termed "efficient" plants. Such plants are often tropical grass species. It has been observed that Atriplex nummularia, a species not showing photorespiration, has a low photosynthetic rate, and Hofstra and Hesketh (1969) state this is evidence that species lacking photorespiration are not necessarily always "efficient". Plants showing photorespiration usually have a low photosynthetic capacity and have

been termed "non-efficient" plants. Several differences between these two groups of plants have been reported. The two types of plants differ with respect to the "CO<sub>2</sub>-burst", the CO<sub>2</sub> compensation point, efflux rates of CO<sub>2</sub> from the leaf into CO<sub>2</sub>-free air in light, optimum temperatures, and response to oxygen.

The "CO<sub>2</sub>-burst"      The "CO<sub>2</sub>-burst" is present in species showing photorespiration. The size of the burst has been shown to increase with increasing light flux density, and it has been observed that the response actually consists of two bursts, the first being larger than the second (Tregunna et al., 1961). Holmgren and Jarvis (1967) have reported three "CO<sub>2</sub>-bursts" in Rumex acetosa L.; however, the third burst was not well defined. The magnitude of the burst is amplified as the oxygen content of the air is increased (Tregunna et al., 1966; Forrester et al., 1966a; Björkman, 1966).

Although the "CO<sub>2</sub>-burst" is generally absent in species lacking photorespiration, studies by El-Sharkawy et al. (1967) have shown the burst to be present in giant pigweed (Amaranthus edulis Speg.), an "efficient" species. There is research, however, which indicates that the post-illumination "CO<sub>2</sub>-burst" which occurs in giant pigweed is not related to photorespiration (Jolliffe et al., 1969).

The CO<sub>2</sub> compensation point ( $\Gamma$ )      Both Moss (1962b) and Hesketh (1963) observed that corn (Zea mays L.) showed a CO<sub>2</sub> compensation point near zero, a factor originally thought characteristic only of species lacking photorespiration. Data now indicate a more rapid uptake of

oxygen in light in corn leaves, although little  $\text{CO}_2$  is released to the external atmosphere which suggests a photorespiratory process (Jackson and Volk, 1968). In those species which release  $\text{CO}_2$  to the external atmosphere, the  $\text{CO}_2$  compensation point ( $\Gamma$ ) is affected by temperature, oxygen concentration, and moisture deficit.

Heath and Orchard (1957) found  $\Gamma$  increased nearly linearly as the temperature was raised from 10 to 35°C. Zelitch (1966b) reported that, in most plants showing photorespiration,  $\Gamma$  was at least 60 ppm at 25°C but at 35°C it was approximately doubled. In "efficient" species  $\Gamma$  remained near zero at both 25 and 35°C.

A linear increase of  $\Gamma$  has been reported as oxygen content is raised (Forrester et al., 1966a; Tregunna et al., 1966). Meidner (1964) observed that  $\Gamma$  increased in corn leaves subjected to a moisture deficit, but more recent work seems to show the increase in  $\Gamma$  is a result of reduced  $\text{CO}_2$  uptake by photosynthesis rather than increased photorespiration.  $\text{CO}_2$  evolution into  $\text{CO}_2$ -free air in light does not increase in stressed leaves (Meidner, 1967). Neither Meidner (1967) nor Bull (1969) have found evidence of increased photorespiration in leaves of corn (Zea mays L.) and sugar cane (Saccharum officinarum L.) when a moisture deficit was imposed on the plant. They found that exposing such leaves to different oxygen concentrations had little effect on  $\Gamma$  or the net photosynthetic rate.

Efflux of  $\text{CO}_2$  from the leaf into  $\text{CO}_2$ -free air in light      Species  
lacking photorespiration do not evolve  $\text{CO}_2$  into  $\text{CO}_2$ -free air in light.

El-Sharkawy et al. (1967) observed no leakage from leaves of "efficient" species, but found rates of leakage of from 1.7 to 3.1 mg CO<sub>2</sub>.dm<sup>-2</sup>.hr<sup>-1</sup> in "non-efficient" species.

The efflux of CO<sub>2</sub> into CO<sub>2</sub>-free air is dependent on irradiance. Holmgren and Jarvis (1967) observed that the minimum CO<sub>2</sub> efflux rate in Rumex acetosa L. at very low ambient CO<sub>2</sub> concentrations occurred at a light flux density of 2·10<sup>3</sup> erg·cm<sup>-2</sup>.sec<sup>-1</sup>. They believe that under these weak light conditions, photosynthetic uptake of CO<sub>2</sub> is initiated, dark CO<sub>2</sub> production is inhibited, and light CO<sub>2</sub> production occurs, but is not as rapid as that occurring at higher light flux densities. Light flux densities beyond 3·10<sup>4</sup> erg·cm<sup>-2</sup>.sec<sup>-1</sup> had little effect on the rate of CO<sub>2</sub> efflux into CO<sub>2</sub>-free air.

The rate of CO<sub>2</sub> efflux does increase with temperature. However, the Q<sub>10</sub> of CO<sub>2</sub> efflux into CO<sub>2</sub>-free air has been shown to be lower in light than in darkness (Holmgren and Jarvis, 1967; Hew et al., 1969). Low oxygen concentrations reduce the rate of CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light in photorespiring plants (Akita and Miyasaka, 1969).

Optimum temperatures Murata and Iyama (1963b), Murata et al. (1965) and El-Sharkawy and Hesketh (1964) have shown temperature optima for photosynthesis in "efficient" species to be in the range of 30 to 40°C. "Non-efficient" species tend to show optimum temperatures for photosynthesis in the 10 to 25°C range and a broad optimum temperature response is usually observed. In oxygen-free air, however, photosynthetic rates of "non-efficient" species are increased and temperature

response curves for photosynthesis take on characteristics similar to those of the "efficient" species (Akita and Miyasaka, 1969).

Hofstra and Hesketh (1969) found that, in photorespiring plants, maximum rates of photosynthesis and photorespiration (as measured by CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light) occurred at about the same temperature, whereas maximum dark respiration rates occurred at temperatures about 10°C higher. At low temperatures photorespiration rates were higher than dark respiration rates, but above 40°C the reverse occurred. Hew et al. (1969) have reported similar findings.

Oxygen effects High oxygen concentrations depress net photosynthetic rate, and two components are believed involved. One is a stimulation of photorespiration and the other is thought to be a direct inhibition effect of oxygen on photosynthesis (Forrester et al., 1966b; Tregunna et al., 1966). Björkman (1966) found the degree of photosynthetic inhibition independent of light flux density, rapidly produced, and fully reversible; Akita and Miyasaka (1969) found when experiments in oxygen-free air were conducted for longer than six hours, that photosynthetic measurements in rice (Oryza sativa L.) and wheat (Triticum aestivum L.) became "unstable". Experiments conducted on corn (Zea mays L.), have shown the direct effect of oxygen on photosynthesis not to be fully reversible (Forrester et al., 1966b).

Leonard and Bidwell (1969) found that if photosynthesizing barley (Hordeum) leaves were exposed to either high or low CO<sub>2</sub> concentrations in the absence of oxygen, production of 3-PGA (3-phosphoglycerate) and

alanine increased but there was little change in other Calvin cycle intermediates. They suggest oxygen exerts an inhibitory effect on the carboxylation of ribulose diphosphate to 3-PGA, the reaction catalyzed by carboxydismutase.

Working with rates of CO<sub>2</sub> efflux from leaves of photorespiring plants, Hew et al. (1969) concluded that the decrease in net photosynthesis between 20 and 30°C is due primarily to an increase in CO<sub>2</sub> evolution in light. At these temperatures only small direct effects of oxygen on photosynthesis were believed involved at normal atmospheric oxygen concentrations.

Other characteristics "Efficient" species differ from "non-efficient" species in other ways. They have unusual chlorophyllous parenchymatous bundle sheath cells, low levels of carbonic anhydrase, and different initial carboxylation reactions. These differences are discussed in the next section.

#### Mechanisms of photorespiration

Because photorespiration responds differently than dark respiration to light, oxygen, and temperature, it has been concluded by many researchers that CO<sub>2</sub> produced in light occurs by a different metabolic pathway than dark CO<sub>2</sub> production (Forrester et al., 1966a; Moss, 1966; Holmgren and Jarvis, 1967; Hew and Krotkov, 1967).

Moss (1966) observed that upon illumination of photorespiring leaves in a CO<sub>2</sub>-free atmosphere, CO<sub>2</sub> evolution decreased to a minimum, which he referred to as the "dip", then gradually increased and

eventually exceeded "steady state" dark respiration rates until an "illumination plateau" was reached. He interpreted the "dip" as indicating the production of a substrate for light respiration. That this substrate is rapidly metabolized in darkness was indicated by the "CO<sub>2</sub> surge" when the leaf was darkened.

Zelitch (1966a) proposes that the primary substrate functioning in photorespiration is glycolate and he believes it is oxidized by glycolate oxidase to form formic acid, CO<sub>2</sub>, and water. When an inhibitor of glycolate oxidase ( $\alpha$ -hydroxy-2-pyridimethane-sulfonic acid) was added to tobacco (a "non-efficient" species) leaf discs, photosynthesis was enhanced at 35°C but unaffected at 25°C, whereas the photosynthetic rate of corn (an "efficient" species) leaf discs was unaffected at either temperature by the inhibitor. Further work has confirmed that glycolate is rapidly metabolized in light and that it is metabolized more rapidly by tobacco leaves than by corn leaves (Moss, 1967). It has been calculated that, on the basis of glycolate accumulation in tobacco photorespiration from glycolate as a substrate may be at least twofold dark respiration (Moss, 1968a). Estimates of light versus dark respiration rates by Lake (1967a) and Brown (1969) show differences of this order of magnitude. In spite of the large light respiration potential from glycolate metabolism, Moss (1968a) has stated that differences in potential respiration rates are not large enough to account for differences in photosynthesis between maize and tobacco on the assumption that differences in photosynthesis are due to internal recycling of CO<sub>2</sub> in tobacco.

Conflicting reports on glycolate oxidase activity exist. Fock and Krotkov (1969) have observed that maximal rates of photorespiration and glycolate oxidase activity correspond. However, Curtis et al. (1969) found glycolate oxidase activity was from 2 to 10 times greater than ability of the leaf to fix CO<sub>2</sub>. No association was found between glycolate oxidase activity and photosynthesis, indicating to them that breeding programs designed to lower glycolate oxidase activity -- to slow photorespiration and thereby increase photosynthesis -- would meet with little success unless a deficient glycolate oxidase genotype could be isolated.

It has been noted by Tolbert (1969) and Kisaki and Tolbert (1969) that photorespiration is related to peroxisomal metabolism. Leaf peroxisomes are small granular micro-bodies in the cell and are characterized by a single membrane. They lack lamellae but they contain specific enzymes, two of which are glycolate oxidase and glutamate-glyoxylate amino transferase. Glycolate is a major end product of photosynthesis and is believed to be the substrate for peroxisomal metabolism. In the peroxisomes, glycolate is oxidized to glyoxylate by glycolate oxidase, and the glyoxylate is converted to glycine by glutamate-glyoxylate amino transferase. Further metabolism of glycine does not occur in the peroxisomes. Since experiments showed peroxisomes unable to oxidize glycolate or glyoxylate to CO<sub>2</sub>, whereas chloroplasts could slowly oxidize glyoxylate, Kisaki and Tolbert (1969) reasoned that excess glyoxylate not used in glycine synthesis could return to the chloroplasts to be reduced to glycolate or oxidized. They state that

oxidation of glycolate in chloroplasts would account for part of the  $\text{CO}_2$  lost during photorespiration.

Tregunna (1966) has evidence that corn lacks the coenzyme, flavin mononucleotide, required for glycolate oxidation. This may explain the presence or absence of photorespiration in certain species. On the other hand, El-Sharkawy et al. (1967) state that maize leaves have been shown to have a glycolic acid oxidase system of good activity without an exogenous supply of flavin mononucleotide.

The second mechanism advanced to explain photorespiration deals with efficiency of  $\text{CO}_2$  reassimilation rather than glycolate metabolism. El-Sharkawy et al. (1967) have conducted experiments which indicate that species with high photosynthetic rates are more efficient in re-assimilating endogenously produced  $\text{CO}_2$ , whereas less efficient species can re-assimilate only a part of the endogenously produced  $\text{CO}_2$ . All species produce endogenous  $\text{CO}_2$ , and the resistance between the source of  $\text{CO}_2$  production and site of utilization (chloroplasts) determines the efficiency of recycling. Lake (1967a), who has included such a resistance term in his diffusion model (refer to Figure 1), has stated that in tropical grass leaves most of the respiratory  $\text{CO}_2$  produced in light is trapped by the chloroplasts before it escapes from the leaf. That some species may re-assimilate endogenously produced  $\text{CO}_2$  more efficiently than other species, is perhaps supported by the research of Jackson and Volk (1968), who have shown a light stimulated respiratory process to be present in corn. Hofstra and Hesketh (1969) have

suggested that high oxygen concentrations might reduce the activity of carbonic anhydrase (an enzyme catalyzing the hydration of CO<sub>2</sub> of the cell) or the permeability of the cellular membranes to CO<sub>2</sub>, thereby affecting reassimilation.

#### Species differences in photorespiration

Photorespiration is of interest to physiologists because less carbon is lost in non-photorespiring "efficient" species, such as corn (Zea mays L.), sugar cane (Saccharum officinarum L.), and sorghum (Sorghum vulgare Pers.), all of which are known to be high dry-matter producers (Moss, 1966; Forrester et al., 1966b). Work by Black et al. (1969) and Bull (1969) also has shown the water requirement (g of water to produce 1 g of dry-matter) to be 30 to 50 percent lower in "efficient" species compared to "non-efficient" species. Tregunna et al. (1969) have noted that plants lacking photorespiration often tend to be "pioneers" in hot dry areas. They state that convergent evolution may have occurred, and so the mechanism of preventing the loss of CO<sub>2</sub> during photosynthesis may not be the same in all plant groups.

Measurements of CO<sub>2</sub> compensation points ( $\Gamma$ ) have been used successfully by many researchers engaged in the search for "efficient" species (Tregunna et al., 1966; Forrester et al., 1966b; Krenzer and Moss, 1969; Black et al., 1969). Moss (1968b) has even been successful in detecting "efficient" species on the basis of atrazine tolerance. Many tropical grasses are resistant to atrazine.

It has been found that photorespiration is not confined to tropical grasses of the Gramineae. Studies by Tregunna and Downton (1969) and Black et al. (1969) have shown zero CO<sub>2</sub> compensation points in different species of pigweeds (Amaranthus spp.), Russian thistle (Salsola kali L.), kochia (Kochia scoparis (L.) Roth), and common purslane (Portulaca oleracea L.). Generally, taxonomic groupings classify species quite well as to absence or presence of low CO<sub>2</sub> compensation points. However, differences have been reported in the genera of Atriplex, Panicum, Cyperus, and Euphorbia (Krenzer and Moss, 1969; Tregunna and Downton, 1969; Black et al., 1969; Tregunna et al., 1969).

Attempts to isolate "efficient" genotypes within a given species based upon differences in photorespiration, have not been successful as to date. It has been discovered that, when a mixture of "efficient" and "non-efficient" plants are grown together in a closed system, the CO<sub>2</sub> concentration will be reduced by the "efficient" plants to a point below that necessary for survival of the "non-efficient" plants. This results in death of the "non-efficient" seedlings after 5 to 8 days (Menz et al., 1969). Large numbers of genotypes can be screened with this method, and even though "non-efficient" seedlings will eventually die, if variations in photorespiration exist the time required for a seedling to die might be expected to vary and the difference could be used as an estimate of different degrees of photorespiration. A screening experiment involving 2,458 genotypes of soybeans (Glycine max (L.) Merr.) grown in mixture with corn (Zea mays L.), failed to detect any "efficient" soybean genotypes (Carnell et al., 1969). None were

found to survive, and a random sample of those which died first and last showed only small differences in  $\Gamma$ . Work by Curtis et al. (1969) and Dornhoff and Shibles (1970) has shown  $\text{CO}_2$  compensation point to be relatively constant between soybean genotypes; however, significant differences in  $\Gamma$  have been reported in corn--a low  $\text{CO}_2$  compensation point species--and furthermore,  $\Gamma$  was highly correlated with rate of net photosynthesis (Heichel and Musgrave, 1969b).

### $\text{CO}_2$ Fixation Pathways

If differences in biochemical pathways or enzymatic activities exist, these could, perhaps, explain some of the variation in photosynthetic rate. Such differences could be reflected in differences in the mesophyll resistance term of diffusion resistance equations. Evidence now indicates that  $\text{CO}_2$  fixation pathways differ between species, and enzymatic activities vary within the same species.

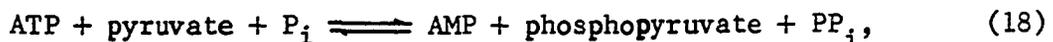
#### $\text{CO}_2$ fixation pathways

In the past the Calvin cycle has been considered the principle pathway for  $\text{CO}_2$  uptake; however, there is now evidence of a new major  $\text{CO}_2$  fixation pathway in some tropically derived grass species.

The Hatch-Slack or four-carbon pathway Working with sugar cane (Saccharum officinarum L.) Kortschak et al. (1965) observed that after a short exposure (15 sec) to  $^{14}\text{CO}_2$  the radioactivity in 3-PGA (3-phosphoglycerate) was never over 34 percent of the total radioactivity

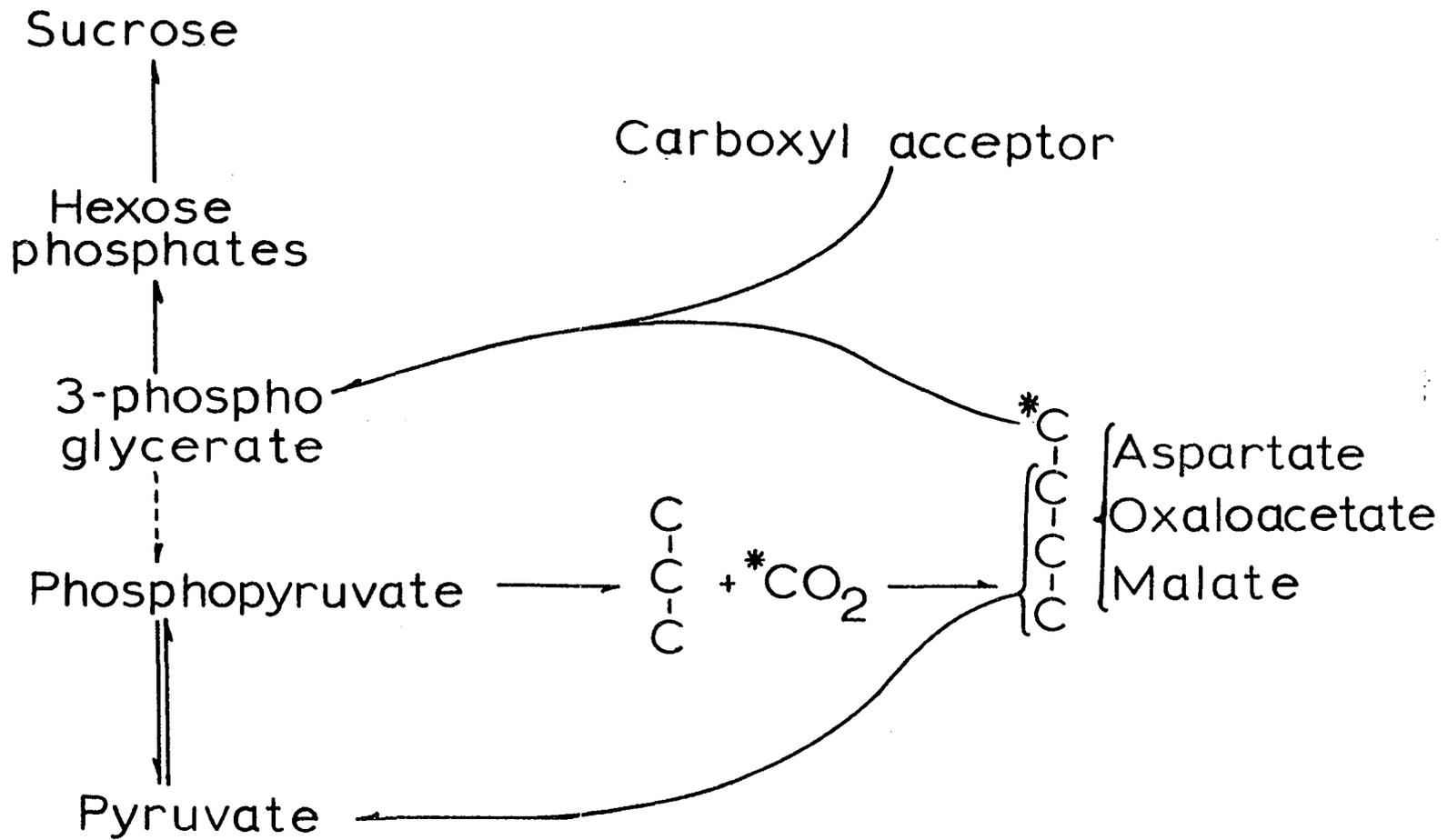
incorporated, but in soybeans (Glycine max (L.) Merr.) 3-PGA accounted for over 80 percent of the total radioactivity. In sugar cane, much initial radioactivity appeared in 4-carbon acids (malate and aspartate), suggesting a CO<sub>2</sub> fixation process different from the Calvin cycle. Evidence that a different pathway exists has since been confirmed by Hatch and Slack (1966). Different initial carboxylation reactions are involved than in the Calvin cycle, but it is suggested that subsequent dark reactions are identical. The proposed pathway is shown in Figure 2.

In their investigations, Hatch and Slack (1966) found that, after a 1 second exposure of sugar cane leaves to <sup>14</sup>CO<sub>2</sub>, as much as 93 percent of the radioactivity was located in oxaloacetate, malate, and aspartate. All three are easily interconvertible. Uptake of CO<sub>2</sub> by this pathway (as shown by labelled CO<sub>2</sub> studies) is believed to involve the carboxylation of phosphopyruvate (the CO<sub>2</sub> addition compound) followed by the transfer of the labelled fourth carbon of the oxaloacetate thus formed to an acceptor, yielding 3-PGA and pyruvate. To complete the cycle, the pyruvate has been reported to be reconverted back to phosphopyruvate by the following reaction:



which is catalyzed by pyruvate, P<sub>i</sub>-dikinase (Hatch and Slack, 1968; Hatch and Slack, 1969). The enzyme has been found only in tropically derived grass species. Further studies conducted in leaves of maize and Amaranthus palmeri S. Wats., both of which have the four-carbon pathway, have shown activity of pyruvate, P<sub>i</sub>-dikinase to fall upon

Figure 2. The proposed Hatch-Slack or four-carbon pathway. The broken arrow indicates a minor pathway. Pyruvate,  $P_i$ -dikinase converts pyruvate to phosphopyruvate and phosphopyruvate carboxylase acts as the carboxylating enzyme. Ribulose diphosphate has been suggested as the carboxyl acceptor which accepts the labelled fourth carbon from one of the four carbon acids (After Hatch and Slack, 1966).



transferring illuminated plants to darkness. Illumination of dark treated plants results in an immediate increase in activity of the enzyme, the final activity being dependent on the level of incident light (Hatch and Slack, 1969).

The carboxylating enzyme in plants with this pathway is phosphopyruvate carboxylase. Its activity is about 60 times greater in sugar cane, maize, and sorghum than in wheat, oats, and silver-beet (Beta vulgaris L.). The former three plants have the four-carbon pathway (Slack and Hatch, 1967).

Cellular localization of CO<sub>2</sub> fixation pathways Moss and

Rasmussen (1969) observed that after 2 minute exposures of photosynthesizing leaves of maize to <sup>14</sup>CO<sub>2</sub> there was a localization of isotopic activity in the parenchyma around the vascular bundle. In sugar beet (Beta vulgaris L.) leaves similarly exposed to <sup>14</sup>CO<sub>2</sub>, activity was generally evenly distributed in the mesophyll cells. This suggested to them that bundle sheath cells of maize contained a unique capacity to incorporate CO<sub>2</sub> and possibly specialized chloroplasts.

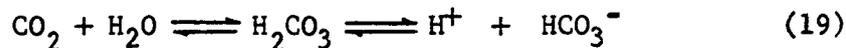
Attempts have now been successful in separating chloroplasts in the mesophyll from those of the parenchymatous bundle sheath in maize and (Amaranthus palmeri S. Wats.) by density fractionation (Slack, 1969). The results suggest that Calvin cycle enzymes (ribulose diphosphate carboxylase and phosphoribulokinase) are localized mainly in parenchymatous bundle sheath chloroplasts while Hatch-Slack or four-carbon pathway

enzymes (puruvate,  $P_i$ -dikinase and adenylate kinase) are restricted mainly to mesophyll chloroplasts.

#### Variation in enzyme amounts and activities

There is now evidence that enzyme activities and amounts vary, depending upon which biosynthetic pathway exists in the plant. Environmental conditions may also influence enzyme activities.

Amount and activity of carbonic anhydrase In a review paper, Nielsen (1960) has stated that, in terrestrial plants, free  $CO_2$  is exclusively assimilated, but the carboxylation process does possibly use bicarbonate ( $HCO_3^-$ ) ions. The presence of carbonic anhydrase in the plant cells makes such an arrangement possible. This enzyme catalyzes the hydration of  $CO_2$  by the following reaction:



This enzyme has been shown to occur in terrestrial plants, and because rates of  $CO_2$  uptake exceed uncatalyzed rates of  $CO_2$  hydration during photosynthesis, at high light flux densities, the role of carbonic anhydrase appears linked with photosynthesis.

The enzyme appears somewhat adaptive. Nelson et al. (1969) found carbonic anhydrase levels in Chlamydomonas reinhardtii were 20-fold higher when the algae were grown on .03 percent  $CO_2$  as compared to 1 percent  $CO_2$ . This work strengthens the hypothesis of Reed and Graham (1968), who found that Chlorella grown in 5 percent  $CO_2$  initially exhibited low rates of photosynthesis when transferred to low  $CO_2$

concentrations. Since differences in activities of Calvin cycle enzymes did not account for this effect, they reasoned initial sub-normal photosynthetic rates were correlated with low levels of carbonic anhydrase, which were found to increase markedly during adaptation to low  $\text{CO}_2$  concentrations. Srugoenyte and Shpokene (1969) observed that carbonic anhydrase activity was reduced 20.7 percent in oats and 26.2 percent in bean leaves after being shaded five days.

The level of carbonic anhydrase seems to depend on whether  $\text{CO}_2$  is fixed via the Calvin cycle or Hatch-Slack pathway. Everson and Slack (1968) found carbonic anhydrase level in "efficient" plants with the four-carbon pathway was one-fifth to one-tenth of that in species with larger amounts of Calvin cycle enzymes. In "efficient" species, the enzyme is found in the cytoplasm, whereas in "non-efficient" species, the enzyme is found in the chloroplasts. They noted phosphopyruvate carboxylase has a low  $k_m$  for  $\text{HCO}_3^-$  and would be expected to be a more efficient "scavenger" for  $\text{HCO}_3^-$  than ribulose diphosphate carboxylase. They suggest that low levels of carbonic anhydrase and the high affinity of phosphopyruvate carboxylase for  $\text{HCO}_3^-$  would act against the loss of  $\text{HCO}_3^-$  produced in photorespiration.

Amount and activity of ribulose diphosphate carboxylase (carboxydismutase) There is now evidence to support the contention that photosynthetic rates are dependent upon the activity of ribulose diphosphate carboxylase. Björkman (1968b) observed that the fraction of incident light absorbed by Solidago virgaurea L. leaves could not

explain differences in photosynthetic efficiencies between sun and shade adapted ecotypes grown in moderately strong light. Enzyme extracts were prepared, and it was found clones native to exposed habitats, which showed higher photosynthetic rates, contained considerably higher activities of carboxydismutase than clones from shaded habitats. The correlation of photosynthesis, expressed on the basis of soluble protein, versus the carboxydismutase activity was .96, and it was suggested the capacity to produce the enzyme (carboxydismutase) was genetically determined. Carboxydismutase activity has also been shown to differ in plant species from habitats with greatly contrasting light flux densities (Björkman, 1968a). Plants from exposed habitats were found to show both higher photosynthetic rates per unit leaf area and carboxydismutase activities when expressed on the basis of soluble protein, total chlorophyll, or fresh weight of leaf tissue per unit leaf area, than those species from shaded habitats.

Evidence of genetic control of carboxylating enzyme activity has been noted by Sarkissian (1962). He observed that carboxylation activity in a single  $F_1$  hybrid barley line exceeded that of the parents by at least 1.5 times. This indicated that the physiological basis of heterosis might be associated with certain key enzymes involved in photosynthetic carboxylation. He did not specify which enzymes were involved.

Miyazaki and Tatemichi (1968) found that differences in net photosynthetic rates of two ripening tobacco varieties, Hicks and Bright Yellow, were correlated with protein nitrogen content of the leaf,

and they found, with increasing leaf age, both photosynthesis and protein nitrogen content decreased. Photosynthesis, respiration, and nitrogen contents were higher in Hicks than in Bright Yellow, but photosynthesis per protein nitrogen content was lower in Hicks. Differences in photosynthetic rates between the two varieties were believed to originate from differences in protein levels. Similarly, in sun and shade populations of Solidago virgaurea L., Holmgren (1968) observed (when leaves damaged by high light in shade adapted plants were excluded from the analysis) a significant correlation ( $r = .81$ ) between leaf nitrogen and mesophyll conductance ( $1/r_m$ ). The correlation between leaf dry weight nitrogen content per leaf area was very high ( $r = .97$ ).

Enzymes are proteins and it is interesting to speculate on how protein levels might be related to enzymatic activities, and, ultimately, to photosynthesis. It appears species differences and differences within species can be explained, partially, on the basis of different enzyme contents and activities.

#### Leaf Anatomy in Relation to Photosynthesis

Species with differing photosynthetic rates often have different leaf anatomical characteristics. These anatomical characteristics may be associated with photorespiration, the  $CO_2$  diffusion process, or other internal factors.

### Chlorophyllous parenchymatous bundle sheath cells

Plants possessing the four-carbon carboxylation pathway have a well organized parenchymatous bundle sheath, the cells of which contain high concentrations of chloroplasts which accumulate large amounts of starch (Downton and Tregunna, 1968). In Calvin cycle species, normal chloroplasts are found only in mesophyll cells, and smaller chloroplasts occur in the ill-defined parenchymatous bundle sheath cells. It has now been observed that, in four-carbon pathway species, large mitochondria also occur in the chlorophyllous parenchymatous bundle sheath cells, and the chloroplasts are not ultrastructurally identical with those in mesophyll cells of the leaf (Bisalputra et al., 1969; Downton et al., 1969). The mechanism by which dimorphic chloroplasts develop in the same plant leaf remains obscure, but evidence indicates that cell and organelle differentiation parallel each other (Laetsch and Price, 1969).

Cytological evidence indicates that chloroplasts of the bundle sheath cells are more active than those of surrounding mesophyll tissue, and it has been postulated such chloroplasts may be responsible for biochemical and physiological differences between leaves of tropical and temperate grasses (Bisalputra et al., 1969). It has been shown that species can be clearly classified into "efficient" and "non-efficient" species on the basis of bundle sheath cell morphology. In a screening experiment of 100 species, Akita et al. (1969) found that without exception "efficient" species contained chlorophyllous paren-

chymatous bundle sheath cells. No intermediate types were observed.

#### Leaf thickness and density as related to photosynthesis

Net photosynthetic rates within a given species are often positively correlated with either leaf thickness, leaf dry weight per unit area, or leaf fresh weight per unit area. The physiological basis for these relationships are still unclear.

Correlation of net photosynthesis with leaf anatomical characteristics Pieters (1960) observed that, under light and CO<sub>2</sub> saturation conditions, net photosynthetic rates of sycamore (Acer pseudo-platanus L.) leaves, grown under different light flux densities, (100, 80, and 50 percent normal sunlight) were positively correlated with leaf thickness. Shade leaves showed lower photosynthetic rates and were thinner than sun leaves. Irvine (1967) found variations in rates of photosynthesis in 10 varieties of sugar cane (Saccharum officinarum L.) were significantly correlated with leaf thickness ( $r = .77$ ), but no correlation was found between photosynthesis and leaf fresh weight per unit area. Photosynthetic rates in ryegrass (Lolium) have also been shown to increase as leaf thickness increases (Wilson and Cooper, 1967).

In Marquis spring wheat (Triticum aestivum L.) a close relationship between leaf thickness, as measured by a micrometer, and leaf dry weight per unit area was observed (Friend et al., 1965). From this it would appear dry weight per unit area could also be used to estimate leaf thickness in some species. Evidence seems to support a positive

relationship between net photosynthesis and the leaf dry weight per unit area, sometimes referred to as specific leaf weight (SLW) or density-thickness (Pearce et al., 1969; Dornhoff and Shibles, 1970). In some instances positive correlations between net photosynthesis and leaf fresh weight per unit area have also been shown.

Although differences in photosynthetic rates can usually be associated with leaf dry weight or fresh weight per unit area, the relationship does not always occur. Barua (1964) was unsuccessful in relating differences in photosynthetic rates of four ecotypes of tea leaves (Camellia sinensis L.) to the area-fresh weight and area-dry weight ratios. Heichel and Musgrave (1969a) found dry weight of lamina per unit area was not significantly correlated ( $r = .05$ ) with photosynthetic differences between inbred, hybrid, and open-pollinated varieties of corn. However, fresh weight per unit area of lamina was significantly correlated with mean varietal photosynthetic rates ( $r = .53$ ). Using the data of Willstätter and Stoll, McClendon (1962) found a similar positive correlation between photosynthesis and fresh weight per unit leaf area in 23 species.

Pearce et al. (1969) observed that SLW and net photosynthetic rates of 13 alfalfa clones (Medicago sativa L.) were positively correlated, and Dornhoff and Shibles (1970) found net photosynthesis of 20 soybean varieties to be positively correlated with both the dry and fresh leaf weight per unit area ( $r = .71$  and  $r = .61$ , respectively).

Indirect evidence of a correlation between photosynthesis and leaf dry weight per unit area has been shown by Hayashi (1968). He observed

that net assimilation rate of rice varieties (Oryza sativa L.) diverged as the light flux density increased. There was a close linear, negative correlation between the regression coefficient of the net assimilation rate versus light and the specific leaf area (leaf area per unit leaf weight).

Factors affecting anatomical characteristics Hayashi (1968)

concluded that differential response of rice varieties to differing light flux densities was determined by genetic factors affecting the net assimilation rates. Barnes et al. (1969) have shown genotypic differences in the SLW of alfalfa, and they have suggested procedures for determining the SLW that could be routinely used in breeding programs to select for increased photosynthetic efficiency.

Although anatomical characteristics of a leaf are genetically controlled, environmental conditions influence expression of such characteristics. In Marquis spring wheat it has been observed that maximum leaf thickness and leaf dry weight per unit area occur under high light and cool temperature conditions (Friend et al., 1962; Friend et al., 1965). Pearce and Lee (1969) noted that SLW and net photosynthetic rate of alfalfa changed with changes in light flux density at all stages of maturity (1, 3, and 5 weeks after leaf unfolding). Leaves last exposed to high light (32 to 43 klux) showed a higher SLW and net photosynthetic rate than those exposed to low light (13 to 14 klux). Dornhoff and Shibles (1970) observed higher density-thickness of soybean leaves produced later in the season as opposed to earlier. Thicker

leaves have been observed in tomatoes (Lycopersicon spp.) grown under high atmospheric CO<sub>2</sub> concentrations (Madsen, 1968). Fresh weight per unit area also increased, and it was believed this was due to an increase in cell volume rather than cell number.

Mechanism of increased photosynthetic rates in thick and dense

leaves It appears CO<sub>2</sub> diffusive resistances are related to differences in leaf anatomy. Holmgren (1968) has stated that of leaf factors which influence transfer of CO<sub>2</sub> from ambient air to reaction sites in chloroplasts, mesophyll resistance ( $r_m$ ) might be most affected by internal leaf structure. In leaves of sun ecotypes, and shade ecotypes of Solidago virgaurea L. not damaged by high levels of radiation, he observed a good correlation ( $r = .82$ ) between leaf dry weight per unit area and mesophyll conductance ( $1/r_m$ ). Holmgren (1968) believed an increase in the ratio of internal surface area with increases in leaf dry weight and leaf thickness resulted in more parallel pathways for CO<sub>2</sub> transfer. Such a relationship was indicated by an increase in the number of palisade layers and palisade cell length induced by high irradiance. Such changes of internal anatomy are characteristic of sun leaves (Pieters, 1960).

Studies by Wilson and Cooper (1967) indicated photosynthetic efficiency in ryegrass (Lolium) depended upon either number of mesophyll cells per unit external surface area or leaf thickness, and these two factors could interact to determine the net photosynthetic rate. A greater number of smaller mesophyll cells in a leaf of a given thickness would presumably promote transfer of CO<sub>2</sub> to chloroplasts sites because

of a larger ratio of internal surface area to external surface area. On the other hand, thicker leaves with the same number of mesophyll cells, in spite of a larger mean size, would be expected to show greater photosynthetic rates, because the volume of intercellular spaces, which facilitate transport of  $\text{CO}_2$ , would increase. Irvine (1967), however, found no correlation in sugar cane between leaf porosity and leaf thickness. Wilson and Cooper (1967) have experimental evidence that cell size and leaf thickness may be independent and they suggest maximizing photosynthetic rate by producing plants with thick leaves, containing many small mesophyll cells. El-Sharkawy and Hesketh (1965) similarly observed that species with small diameter palisade mesophyll cells showed greater maximum net photosynthetic rates than species with larger diameter palisade mesophyll cells. Tropical grasses having high net photosynthetic rates were found to have a larger ratio of internal cell surface to cell volume. Friend (1966), who attempted to relate thicker leaves in Marquis wheat to greater photosynthetic rates, noted thicker lamina were characterized by thicker mesophyll cells with a higher degree of lobing.

Contrary to the hypothesis of Wilson and Cooper (1967), Hesketh (1963) and El-Sharkawy and Hesketh (1965) stated  $\text{CO}_2$  diffusion resistances would be expected to increase with increasing leaf thickness because of longer  $\text{CO}_2$  diffusion path lengths. They showed indications of an inverse relationship between leaf thickness and photosynthetic rates of varying species but little significance was given to this because

large differences in photosynthetic rates between species could not be explained on this basis.

Loach (1969) found that, when five tree species, differing in shade tolerance, were grown in 100, 44, and 17 percent full daylight, there was a highly significant correlation between maximum photosynthetic rate and volume of mesophyll cells per unit leaf area, but a poor correlation between net photosynthesis and exposed surface area of mesophyll cells per unit cell volume. He concluded biochemical factors, rather than physical diffusion factors, limited the photosynthetic rate in the mesophyll. Heichel and Musgrave (1969a) have suggested the correlation they obtained between lamina fresh weight per unit area and net photosynthesis was due to a greater quantity of photosynthetic enzymes in thicker leaves. They also believed thicker leaves should show greater mesophyll resistance. Pearce and Lee (1969) speculate that changes in photosynthesis and SLW of plants grown under different light flux densities are most likely associated with light absorption and chemical characteristics within the cells already present in the leaf because morphological features, such as cell and stomata number, would not be expected to change after full leaf expansion.

#### Variation in Net Photosynthesis

Differences in net photosynthetic rates have been found among genera, among species, among ecotypes of the same species, and among varieties or strains of a given species. Differing net photosynthetic

rates were first detected among genera, but with the advent of better analytical equipment and refinement of techniques, net photosynthetic rates have been shown to differ in closely related genotypes of the same species.

#### Variation of net photosynthesis among genera

Differences in net photosynthetic rates were first observed among genera. This is not surprising since greater genetic diversity would be expected among genera than among closely related genotypes within a given species.

Gaastra (1959) was one of the pioneer workers to investigate physiological factors responsible for generic differences in net photosynthesis. He emphasized the need for control of environmental factors to obtain repeatable results, and demonstrated how these factors could interact to influence the measurement of net photosynthesis. The need for high light flux densities and air flow rate, normal, limiting CO<sub>2</sub> concentrations, and optimal temperature conditions was stressed. His results indicated net photosynthesis differed among genera. Sugarbeet and turnip showed higher maximum net photosynthetic rates than cucumber and spinach; however, the net photosynthetic rates of cucumber and spinach were greater than those of tomato.

Hesketh and Moss (1963) reported large differences in photosynthetic rates among genera tested under both natural and artificial light conditions. Corn (Zea mays L.) was observed to have a very high net

photosynthetic rate. In a study conducted to determine why photosynthetic rates of genera varied, Hesketh (1963) observed net photosynthetic rates ranging from 31 to 64 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup> in corn. The net photosynthetic rate of sugar cane (Saccharum sp.) was 42 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup> and sunflower (Helianthus annuus L.) showed a net photosynthetic rate of 37 to 43 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>. However, maple (Acer saccharum Marsh.) and oak (Quercus rubra L.) were observed to have very low net photosynthetic rates, ranging from 5 to 12 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>. Other genera tested (castorbean, Ricinis communis L.; tobacco, Nicotiana tabacum L.; orchardgrass, Dactylis glomerata L.; and red clover, Trifolium pratense L.) showed intermediate net photosynthetic rates, ranging from 18 to 34 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>. Independent studies have confirmed these results (El-Sharkawy and Hesketh, 1965; Holmgren et al., 1965; El-Sharkawy et al., 1967).

That generic differences in net photosynthetic rates exist is now well established. Tropical grasses and other genera lacking the photorespiratory process or having the four-carbon CO<sub>2</sub> fixation pathway often appear to have high net photosynthetic rates. Only recently have differences in net photosynthetic rate among species been reported.

#### Variation among species

Photorespiration and specific CO<sub>2</sub> fixation pathways appear to be confined to specific genera. Therefore, smaller differences might be expected to occur among species of a given genus. Evidence from work with 26 species of cotton (Gossypium spp.) has suggested higher net photosynthetic rates in tetraploid species (AD genomes) than in species

with the A genome or in many species of the D genome (El-Sharkawy et al., 1965). The average photosynthetic rates of AD genome species were similar to those of the B, C, and E genome species. Differences in rate of dry matter production were shown to be related more to cotyledon size, low temperature tolerance, and rate of leaf area development than to differences in leaf photosynthetic rates. Bjurman (1959) noted higher net photosynthetic rates in diploid than tetraploid Ribes satigrum. Although the diploid leaves showed slightly lower chlorophyll contents, they had about 45 percent more stomata than the tetraploid species, and it was believed this may have been important in explaining why photosynthetic differences occurred. Stomatal resistances were not measured.

Significant photosynthetic differences among species have been reported in oats (Avena spp.). However, the variability of measurements within any given species was large (Criswell, 1968). Rodionov (1963) has reported lower rates of  $^{14}\text{CO}_2$  fixation in Lycopersicon peruvianum than L. hirsutum (tomato).

#### Variation among ecotypes

Discovery of differences in net photosynthetic rates within the same species was at first confined to widely-differing ecotypes, rather than closely related genotypes. Milner and Hiesey (1964) found net photosynthetic rates of six climatic races of Mimulus cardinalis Dougl. differed in response to light and temperature. Maximum net photosynthetic rates did not differ significantly, but at high and low

temperatures the temperature response curves for photosynthesis were found to diverge. Short-season races showed the steepest drop in photosynthetic efficiency at the two temperature extremes, whereas long-season races were found to have higher net photosynthetic rates at either high or low temperature extremes. It has been observed in arctic and alpine populations of Oxyria digyna Hill. that plants of northern populations have higher net photosynthetic rates at lower temperatures than do plants of southern alpine populations (Mooney and Billings, 1961). High elevation, low latitude plants attained photosynthetic light saturation at a higher light flux density than low elevation, high latitude plants. Duncan and Hesketh (1968) conducted an experiment with 22 races of maize and one selection of teosinte (Euchlaena mexicana Schrad.) grown and tested at temperatures ranging from 15 to 36°C. High altitude races showed lower photosynthetic rates at high temperatures than low altitude races.

Ecotypes of Solidago vigaurea L. have also been shown to have different photosynthetic rates (Björkman and Holmgren, 1963). Plants from shaded habitats showed lower net photosynthetic rates when populations from both shaded and exposed habitats were grown under high light flux densities; however, when grown under low light conditions, the populations from shaded habitats showed higher net photosynthetic rates. Barua (1964) found tea leaves (Camellia sinensis L.) from four sources varied in their photosynthetic response to light flux density. It was concluded that some ecotypes were intrinsically umbrophilic (shade

lovers) and performed better in weak light than did others, which were believed to be heliophilic (sun lovers) and to perform better at high light flux densities. Attempts to explain why photosynthetic rates of the tea ecotypes differed were unsuccessful, as neither lamina thickness or chlorophyll concentration could explain differences.

Seasonal variation in CO<sub>2</sub> absorption has been reported in tomatoes (Lycopersicon spp.) of differing geographic and ecological origins (Rodionov, 1963). Early-maturing varieties were found to show higher <sup>14</sup>CO<sub>2</sub> absorption rates than late-maturing varieties during the first half of the vegetative period, the pattern being reversed in the last half of the vegetative period.

Differences in Hill reaction activity have been observed in ecological races of cattail (Typha lotifolia L.) by McNaughton (1967). He suggested differences in Hill activity might reflect photochemical differences, which he suggested would explain variations in productivity of cattail.

#### Variation among strains and varieties

Only recently have differences in net photosynthetic rates been reported among varieties or closely related strains. Muramoto et al. (1965) were unable to detect differences in net photosynthetic rates between many cotton (Gossypium) varieties and several hybrids, and variability within any one variety was considerable. Differences in rates of dry matter production were shown to be a function of leaf area development. Previous work by Shibles and MacDonald (1962) had also shown that differences in seedling vigor of Viking and Empire birdsfoot

trefoil (Lotus corniculatus L.) could be attributed to differential rates of leaf area development, rather than differences in photosynthetic capacities of cotyledons and leaves. Schultz (1964) was unsuccessful in showing varietal differences in assimilation rates of sugarbeet (Beta vulgaris L.). In spite of these reports, there is now considerable evidence that differences in photosynthetic rates of closely related genotypes do occur.

Many experiments have been conducted on corn (Zea mays L.). Hesketh and Moss (1963) observed that there was a trend for corn variety NE913 to show slightly higher net photosynthetic rates than Conn. 870, but differences were not significant. Since then significant differences have been reported.

Hybrid vigor in maize has also been associated with increased net photosynthetic rates. Fousová and Avratovščuková (1967) observed heterosis for net photosynthetic rates in an  $F_1$  corn hybrid. Photosynthesis declined in the  $F_2$  generation, and, when the data was analyzed, the  $F_1$  found to have a greater yield per unit area of leaf than the  $F_2$ . Rahmankulov (1967) has reported greater growth rates and leaf areas in maize hybrids. Most hybrid combinations were also found to show greater rates of photosynthesis and lower respiration rates than their parental lines. Heichel and Musgrave (1969a) observed differences in mean photosynthetic rates of 100 to 200 percent among inbred, hybrid, and open-pollinated corn varieties. Photosynthetic heterosis was apparent in single crosses derived from inbreds of divergent ancestry.

Heterosis for net photosynthesis in corn hybrids has not always been observed. Heichel and Musgrave (1969a) did not observe significant heterosis in single-cross progeny of closely related inbreds, and Voříšek and Hudeová (1970) noted photosynthetic rates in single-cross maize hybrids intermediate from those of the parental lines.

Differential rates of cyclic photophosphorylation have been reported in maize, and in most instances activities from chloroplasts of  $F_1$  hybrids were intermediate from those of their respective inbred parents (Miflin and Hageman, 1966).

Photosynthetic rates have been shown to vary in sugar cane varieties (Saccharum spp.) from 34.4 to 86.4  $\text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$  (Irvine, 1967). The parent variety F. 36-819 showed a photosynthetic rate significantly higher than all other parent varieties, and hybrid combinations of Saccharum officinarum L. were observed to have lower net photosynthetic rates than interspecific hybrids.

Photosynthetic rates have been observed to vary in small grains as well as tropical grasses. Osada (1964) observed that late-maturing rice varieties (Oryza sativa L.) showed lower photosynthetic rates than shorter, earlier-maturing varieties, but varietal differences were not always consistent. Further work has shown relatively stable varietal differences in both young and mature japonica rice varieties (Osada and Murata, 1965). Hayashi (1968) has reported differences in net assimilation rates of rice varieties grown under various light flux densities.

Varietal differences for the photosynthetic process have also been

shown in wheat (*Triticum aestivum* L.). Apel and Lehmann (1967) made five crosses between ten different varieties of winter wheat. One cross (Wysokolitewska Szlrywnostoma (upper Lithuanian stiff strawed) X *Lutescens* 17) showed a photosynthetic rate considerably greater than either of the parental lines. Other hybrids showed intermediate rates or heterosis, and one showed a photosynthetic rate below that of either parent. It was concluded the heterosis effect for photosynthesis was polygenecally determined and unpredictable. Nátr (1964) reported differences in photosynthetic rates of four varieties of winter wheat. The highest yielding variety (Hadmerslebener VIII) had the highest photosynthetic rate and greatest number of vascular bundles in the main culm. Stoy (1965) did not show significant differences in photosynthetic rates in three varieties of spring wheat but was successful in showing differences in the duration of photosynthetic activity during the grain filling period. Sybanbekov (1967) observed higher rates of photosynthesis and transpiration in ears of the awned wheat variety Kazakhstan 126 than in the awnless variety Albedum 43, while Babulo, a short awned variety, showed intermediate rates. Photosynthetic rate did not differ significantly in leaves but transpiration rates differed in the same manner as those of ears. Varietal differences in photosynthesis in the ear and flag leaf of wheat have also been reported by Lupton (1964).

Thorne (1963) noted that photosynthetic rates of leaves of Proctor and Plumage Archer barley (*Hordeum*) were similar both before and after

ear emergence; however, the rates of photosynthesis she reports appear quite low. Working with barley, Kleese (1966) has been successful in demonstrating genetic variation in rates of photophosphorylation and Sarkissian (1962) has observed enhanced carboxylation rates in  $F_1$  barley hybrids.

Considerable effort has been devoted toward isolating photosynthetically efficient soybean genotypes (Glycine max (L.) Merr.). Brun and Cooper (1967) found the photosynthetic rate of the variety Hark to exceed that of Chippewa-64 in 45 out of 48 light- $CO_2$  concentration combinations. Since this experiment was conducted, several researchers have reported differing net photosynthetic rates on various soybean genotypes or strains. Ojima and Kawashima (1968) reported varietal differences which varied  $\pm 20$  percent against the mean varietal photosynthetic rate ( $26.7 \text{ mg } CO_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ ), and the coefficient of variation of photosynthesis among plants of different varieties varied as much as 5 to 25 percent. Further work showed the frequency of occurrence of improved soybean varieties with high photosynthetic rates increased when parent varieties had high photosynthetic activities (Ojima et al., 1968).

Dreger et al. (1969) have reported the soybean variety Hark to show higher photosynthetic rates than either Chippewa-64 or A-100. Curtis et al. (1969) measured net photosynthetic rates of 36 soybean varieties grown in flats, and they found photosynthetic rates of different genotypes ranged from  $24.0 \text{ mg } CO_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$  for the variety Richland, to  $12.0 \text{ mg } CO_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$  for the variety Patterson. Significant

varietal differences in soybeans have also been reported by Dornhoff and Shibles (1970). They observed the most efficient photosynthesizing variety (Corsoy) showed a photosynthetic rate of  $43.4 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ , whereas the genotype with the lowest photosynthetic rate (PI 85.019) showed a rate of only  $29.4 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ .

Genetic variation in the photosynthetic ability of the garden bean (Phaseolus vulgaris L.) has been reported by Izhar and Wallace (1967a). Photosynthetic rate of the variety Michelite-62 significantly exceeded that of the closely related variety Monroe by about 9 percent, and it exceeded varieties Perry Marrow, Steuben, and Red Kidney by 22, 35, and 31 percent, respectively.  $F_1$  populations from reciprocal crosses of Michelite-62 and Red Kidney had photosynthetic rates intermediate and significantly different from that of each parent. Variation of photosynthetic rates among  $F_1$  individuals was low and equal to the variance of the parental lines. The rates of the  $F_1$  means were significantly lower than the midparent value, suggesting the low rate of Red Kidney was dominant over the high rate of the variety Michelite-62. When the  $F_2$  generation was examined, variance in photosynthetic rates was significantly larger than that of either the  $F_1$  populations or the parental lines, which indicated genetic segregation for photosynthesis. Photosynthetic variations in the  $F_2$  generation were shown to fit a normal curve, and there was a relatively high frequency of plants with photosynthetic rates comparable to those of the parental variety, Michelite-62. The authors suggest this evidence indicates photosynthetic capacity is a quantitatively inherited trait controlled by a small number of genes.

Varietal differences have been reported in other crops. Higher photosynthetic rates have been reported in the tobacco (Nicotiana tabacum L.) variety Hicks than in Bright Yellow (Miyazaki and Tatemichi, 1968). Pearce et al. (1969) have also shown varietal differences in photosynthetic rates of alfalfa (Medicago sativa L.).

It now seems clear that there are differences in photosynthetic capacities, even among closely related genotypes. This holds much hope for future crop improvement through breeding and selection for higher net photosynthetic rates.

## METHODS AND MATERIALS

The methods and materials used in the screening experiment described in Part I are applicable to the sink-source experiment, which is discussed in Part II of this dissertation. Reference will be made to some of the sections described below as they apply to the second experiment.

## Genotypes

Plant material with great genetic diversity was sought, because it was reasoned that such material ought to provide the greatest opportunity for detecting differences in photosynthetic rates. The 20 genotypes (purified strains) used in the screening experiment in 1968 and 1969 are presented in Table 1. With the assistance of Dr. K. J. Frey, oat breeder, material differing in (1) ploidy level, (2) species (within hexaploids), (3) origin, and (4) productivity within Corn Belt varieties was chosen.

The diploid genotypes, Saia and A. brevis, were tall late-maturing genotypes characterized by small flag leaves and dark seeds. Tetraploid genotypes used were Glabrota and P.I. 193958. The A. fatua genotypes consisted of two late-maturing crop introductions, and A. sterilis genotypes used were two recent genetically purified plant introductions from Israel. Little is known about the A. sterilis genotypes except that they have a small, helical flag leaf and develop a short flat canopy. A-465 and Curt were the A. byzantina genotypes used. A-465

Table 1. Experimental grouping of genotypes used and dates of panicle emergence (the genotypes were planted on April 16 and April 21, in 1968 and 1969, respectively)

Comparison	Genotype	Days until panicle emergence	
		1968	1969
Diploids	Saia	68	72
	<u>A. brevis</u>	71	73
Tetraploids	Glabrota	70	74
	P.I. 193958	72	77
Hexaploids			
<u>A. fatua</u>	C.I. 1779	76	81
	C.I. 2528	76	75
<u>A. sterilis</u>	P.I. 296234	72	78
	P.I. 292546	74	77
<u>A. byzantina</u>	A-465	58	56
	Curt	62	64
<u>A. sativa</u>	Clintland-64	56	63
	Marion	55	62
Cool climate	Record	82	81
	Bingham	73	71
Red oat region	Victorgrain	74	70
	Appler	84	78
Low productivity	Richland	59	63
	Goodfield	57	63
High productivity	Garland	56	61
	Burnett	55	60

and Curt were the A. byzantina genotypes used. A-465 is an Australian introduction which is quite short and very early-maturing. This genotype has long drooping flag leaves, and the spikelets have exceptionally large outer glumes. Curt is a variety which was developed in

California. Both genotypes are adapted to warm climatic conditions. Clintland-64 and Marion are Midwestern, A. sativa varieties which are well adapted to the area where the experiment was conducted. Bingham and Record are well adapted for spring oat production in cool regions, whereas Victorgrain and Appler are red oat varieties developed for winter oat production in the southeastern states. Richland and Goodfield are two Midwestern varieties which have consistently yielded poorly in Iowa oat variety performance tests. The Midwestern developed varieties Garland and Burnett, however, have produced high yields in Iowa oat variety performance tests.

#### Management of the Material

The plant material was grown out-of-doors in pots, placed between greenhouse wings at Ames, Iowa, in both 1968 and 1969. A pot experiment was used because the plants had to be moved into the laboratory to test attached flag leaves. The oat genotypes were planted on April 16 and April 22 in 1968 and 1969, respectively. In 1968 three pots of each genotype were grown, whereas in 1969 only two field replications were used. Ten seeds of a given genotype were planted in each pot in 1968, and after two weeks growth, the population was reduced to five plants per pot. Twenty-four seeds of each genotype were planted in 1969, but the population was reduced to 18 plants per pot at the end of two weeks. A total of 60 and 40 pots were used in 1968 and 1969, respectively, excluding border pots. Both years the pots were arranged at random.

Six-inch clay pots, placed in three east-west plastic lined trenches, were used in 1968. Straw was packed in the trenches around the pots to prevent desiccation, because it was anticipated loss of soil moisture through the porous clay pots might become a problem. The straw quickly deteriorated, however, and appeared to be of little benefit late in the season when the plants were using the most moisture. Examination of the root system at the end of the 1968 season indicated that a majority of the plant root proliferation was towards the outside of the pots, and it was believed the plants may have become "pot-bound".

Much better growth was achieved in 1969 by growing the plants in plastic "waste-paper-baskets" which had dimensions of 27 x 19 x 29 cm. These were placed on black plastic sheets in three north-south rows. The plastic "waste-paper baskets" held a much larger volume of soil than did the clay pots (11.3 kg versus 1.9 kg), and examination of the root system at the end of the 1969 season showed roots to be well distributed throughout the soil. A hole was drilled in the bottom of each "waste-paper basket" for drainage.

Both years the oat plants were grown in a 2:1:1 mixture of soil, sand, and peat, respectively. In 1968 the soil was sterilized for 48 hours; however, because of the larger volume of soil used in 1969, the soil was not sterilized. A cement mixer facilitated mixing the soil, sand, and peat in 1969.

A commercial fertilizer with a 6-10-4 analysis was used in 1968 to insure adequate fertility conditions. In 1968, the equivalent of approximately 285 lb N, 210 lb P, and 160 lb K/A was applied in split

applications. The first application of fertilizer was made when oat plants were in the four leaf stage, and the second application was made about two weeks prior to panicle emergence of most of the genotypes. In 1969 phosphorus and potassium were applied in the form of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  and  $\text{K}_2\text{SO}_4$  at a rate of 80 lb P and 120 lb K/A. Since leaching is not a problem with these nutrients, they were mixed into the soil at the beginning of the experiment. Nitrogen, however, is more readily leached. For this reason, nitrogen in the form of  $\text{NH}_4\text{NO}_3$  was applied biweekly during the vegetative period and weekly during the reproductive period in 20 ml of distilled water. Nitrogen was applied at a rate of 17 ppm by weight of soil in the pots (approximately 34 lb N/A, assuming two million pounds of soil in an acre six inch layer of soil). A total of 272 lb N/A was applied in 1969.

Plant material was watered with tap water in both 1968 and 1969. Watering was generally done towards evening when daily tests were completed.

Zineb was used to prevent rust diseases, and Malathion was used to control insect infestations. In 1968 a few plants showed symptoms of yellow-dwarf (stunted growth and leathery leaves), a viral disease. The pathogen of the disease is transmitted by aphids. These plants were not used in the experiment. Early in the 1968 season, some rabbit damage was noted on a few plants in two pots, but applications of Chaperone (a rabbit and deer repellent) to the young plants (3-4 leaf growth stage) and erection of a snow-fence around the experimental site prevented further damage. DuPont monofilament garden netting was used to completely cover the entire

plot area in 1969. This precautionary measure was taken to prevent alteration of sink-source ratios (Part II) which might result from bird damage (i.e. damage to spikelets). Measurements taken on a clear day with a Weston foot-candle meter showed the netting reduced the light flux density by only about 7.5 percent.

#### Experimental Apparatus

The apparatus used to measure  $\text{CO}_2$  exchange and transpiration rates simultaneously was an "open-end" system similar to that used by Gaastra (1959). Analytical measurements of the  $\text{CO}_2$  and water vapor content of the air before and after it had passed through a chamber containing illuminated plant material were used to calculate  $\text{CO}_2$  exchange and transpiration rates. Air temperature and flow rates through the chamber were measured.

A dual system, utilizing two leaf chambers, was used. When measurements were being taken on plant material in one leaf chamber, the plant material which had been tested was removed from the second chamber, and fresh material to be tested was inserted and allowed to adjust to experimental conditions. Environmental conditions in the two chambers were identical for the most part, except that the material being induced in the second chamber received atmospheric air (approximately 320 ppm  $\text{CO}_2$ ), whereas the plant material being tested in the first chamber received air of various desired  $\text{CO}_2$  concentrations. The air supply entering the chamber could be switched by means of three-way stopcocks.

Consequently, the material previously induced and ready to be tested did not have to be removed and placed in a separate test chamber. Each of the chambers served both as an induction and test chamber.

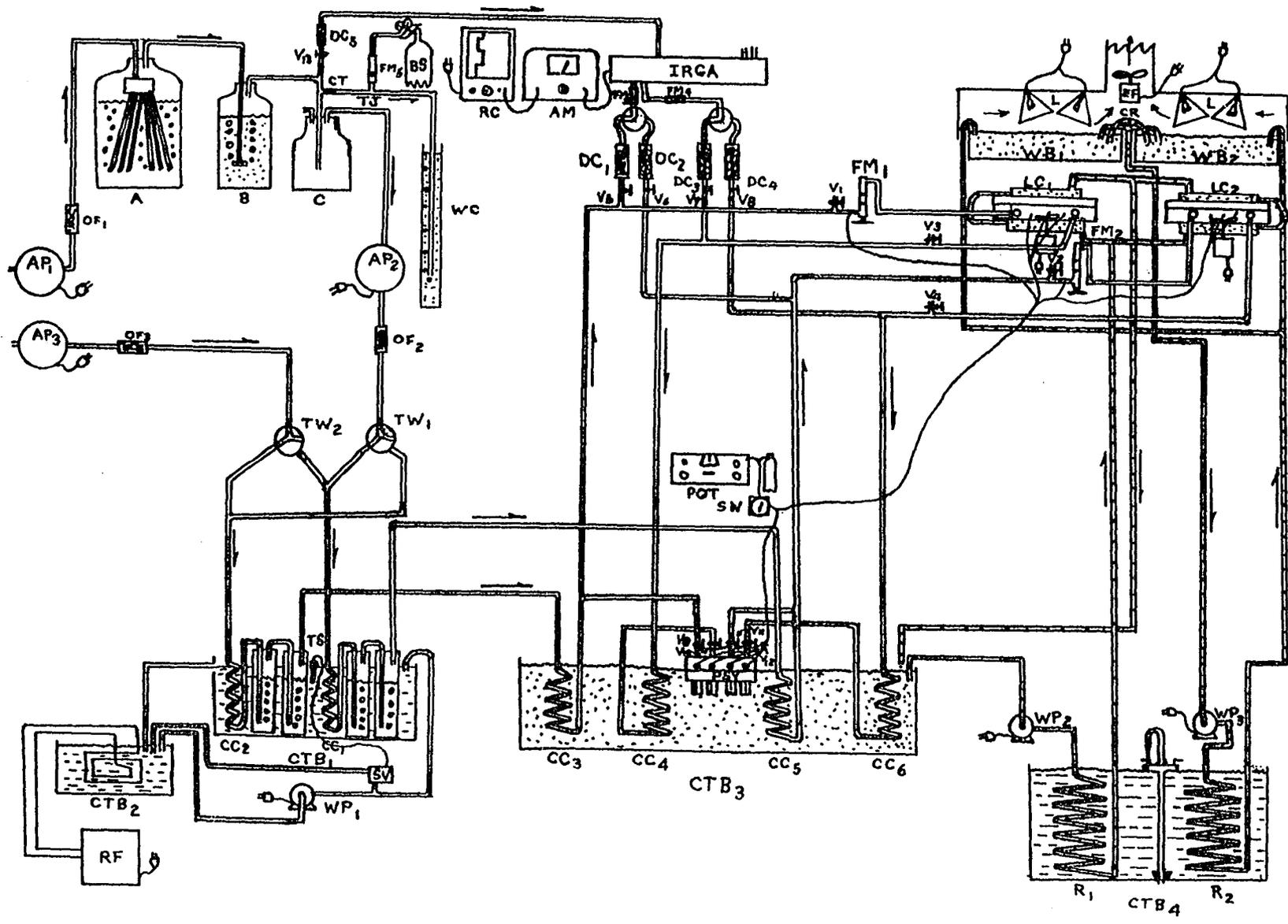
#### The gas circuit

The gas circuit consisted of various components connected in series by either 9.5 mm or 4.8 mm diameter tygon or copper tubing, respectively. The gas circuit can best be described by following the air stream through the system which is shown diagrammatically in Figure 3.

The CO<sub>2</sub> metering device      Air containing a desired CO<sub>2</sub> concentration was prepared by a method similar to that used by Gaastra (1959) and Stoy (1965). This procedure involved the removal of CO<sub>2</sub> from atmospheric air and adding a given amount of 100 percent CO<sub>2</sub> back into a CO<sub>2</sub>-free air stream to achieve an air mixture with a desired CO<sub>2</sub> concentration.

Atmospheric air was drawn through a tygon tube, extending outside the laboratory, by an air pump (AP<sub>1</sub>). All air pumps were Gast rotary vane pumps. The air stream next passed through an oil filter (OF<sub>1</sub>), composed of loose cotton in a glass tube, to remove traces of oil which passed through the vanes of the pump. The air then passed through six gas dispersion tubes placed in a polyethylene, CO<sub>2</sub>-absorbing jug (A) which contained 181 of 6 N KOH. In 1968 glass columns containing soda lime were used to remove the CO<sub>2</sub>; however, these

Figure 3. Diagram of apparatus. See text for description. A = CO<sub>2</sub> absorbing jug; B = distilled water jug; C = mixing bottle; CT = capillary tube; TJ = tee junction; BS = CO<sub>2</sub> supply; WC = water column; AP<sub>1</sub>, AP<sub>2</sub>, and AP<sub>3</sub> = air pumps; OF<sub>1</sub>, OF<sub>2</sub>, and OF<sub>3</sub> = oil filters; TW<sub>1</sub> and TW<sub>2</sub> = three-way stopcocks; CC<sub>1</sub>, CC<sub>2</sub>, CC<sub>3</sub>, CC<sub>4</sub>, CC<sub>5</sub>, and CC<sub>6</sub> = copper coils; V<sub>1</sub>, V<sub>2</sub>, V<sub>3</sub>, V<sub>4</sub>, V<sub>5</sub>, V<sub>6</sub>, V<sub>7</sub>, V<sub>8</sub>, V<sub>9</sub>, V<sub>10</sub>, V<sub>11</sub>, V<sub>12</sub>, and V<sub>13</sub> = valves; FM<sub>1</sub>, FM<sub>2</sub>, FM<sub>3</sub>, FM<sub>4</sub>, and FM<sub>5</sub> = flowmeters; CTB<sub>1</sub> and CTB<sub>2</sub> = ethylene glycol baths; CTB<sub>3</sub> = distilled water bath; CTB<sub>4</sub> = tap water bath; WB<sub>1</sub> and WB<sub>2</sub> = infrared absorbing distilled water baths; LC<sub>1</sub> and LC<sub>2</sub> = leaf chambers; L = lamps; EF = exhaust fan; DC<sub>1</sub>, DC<sub>2</sub>, DC<sub>3</sub>, DC<sub>4</sub>, and DC<sub>5</sub> = drying columns; WP<sub>1</sub>, WP<sub>2</sub>, and WP<sub>3</sub> = liquid transfer pumps; R<sub>1</sub> and R<sub>2</sub> = radiators; TS = thermostat; SV = solenoids; RF = refrigeration unit; PSY = psychrometer; POT = potentiometer; SW = multiple switch; CR = crossover tube; IRGA = CO<sub>2</sub> analyzer; AM = amplifier; RC = recorder.



required daily maintenance and the 6 N KOH was found to remove the CO<sub>2</sub> equally well. Once prepared, a flask of KOH effectively removed the CO<sub>2</sub> from the air throughout the duration of the test period with essentially no maintenance. Polyethylene gas dispersion tubes, however, were found to be more satisfactory than glass dispersion tubes (constructed of sintered glass), because the KOH eventually etched away the glass tubes.

The CO<sub>2</sub>-free air was then forced through a jug of distilled water (B) to remove traces of KOH which may have been carried over in the air stream. Tests with litmus paper gave a basic test, indicating the distilled water was necessary to neutralize the CO<sub>2</sub>-free air stream.

The desired CO<sub>2</sub> concentration in the stream was obtained by adding a given amount of 100 percent CO<sub>2</sub> to the CO<sub>2</sub>-free air through a fine capillary tube (CT). The amount of CO<sub>2</sub> added to the CO<sub>2</sub>-free air could be regulated by varying the amount of CO<sub>2</sub> forced through the capillary tube. This was accomplished by passing a slight excess of 100 percent CO<sub>2</sub> from a bottled source (BS) through a tee junction (TJ) to a small vertical glass tube, the open end of which could be immersed at various heights in a larger diameter, 4 foot long glass tube supporting a water column. By sliding the small tube up and down inside the larger tube, containing the water, the pressure on the back side of the capillary tube was altered. This pressure controlled the amount of CO<sub>2</sub> that passed through the orifice of the capillary tube.

The capillary tube was constructed by "heat-drawing" commercially-available capillary tubing down to the point where essentially no visible

orifice remained. The needle-like tip was then broken off, a fraction of an inch at a time, until the orifice size was such that nearly the full height of the water column had to be used to achieve  $\text{CO}_2$  concentrations ranging from 0 to 450 ppm.

From the capillary the air mixture then passed to a small mixing bottle (C), which was open to the external atmosphere. A slight positive pressure in the gas circuit at this point prevented entrance of extraneous air into the system. A second air pump ( $\text{AP}_2$ ) withdrew most of the air from the mixing bottle and passed the air through a second oil filter ( $\text{OF}_2$ ), before the air arrived at a three-way stopcock ( $\text{TW}_1$ ). This three-way stopcock was connected in parallel with another three-way stopcock ( $\text{TW}_2$ ). The second stopcock was supplied with atmospheric air by a third pump ( $\text{AP}_3$ ), which pumped air to the "induction chamber". The air supplied to the chamber containing the test material then passed through a series of bottles and copper coils, placed in constant temperature baths, to control temperature and humidity of the air.

The balance of the gas circuit      The remainder of the gas circuit used in 1968 differed slightly from that used in 1969. This was because an absolute type  $\text{CO}_2$  analyzer was used in 1968, and alternate samples of air, withdrawn from the air stream before and after it had passed over the leaf, were monitored separately. The  $\text{CO}_2$  analyzer was converted to a differential type analyzer in 1969, which necessitated altering the gas circuit to allow simultaneous passage of leaf chamber inlet and egress air samples through the  $\text{CO}_2$  analyzer. The gas circuit used in

1968 was the same as that described by Dornhoff (1969); consequently, only modifications of the system made in 1969 will be described.

After the air stream had passed through the constant temperature baths, it then passed through a flowmeter ( $FM_1$  or  $FM_2$  depending upon which chamber contained the test material). Flowmeters were the Matheson 620 BBV type with dual float tubes (type R-6-15-A) having a readability of approximately  $2.4 \text{ l}\cdot\text{hr}^{-1}$ . Flow rates were approximately  $110 \text{ l}\cdot\text{hr}^{-1}$  in 1968 and  $200 \text{ l}\cdot\text{hr}^{-1}$  in 1969. Air samples were withdrawn from the air stream both before and after the air had passed through the "test chamber". Because water absorbs infrared radiation, water vapor was removed before sample air streams entered the infrared  $CO_2$  analyzer by passing the air through glass, drying columns ( $DC_1$  and  $DC_2$  or  $DC_3$  and  $DC_4$ ), which contained indicating anhydrous  $CaSO_4$  (Drierite). Flow rates of the sample air streams were adjusted by valves  $V_5$  and  $V_7$  or  $V_6$  and  $V_8$  to insure equal flow rates of  $34.0 \text{ l}\cdot\text{hr}^{-1}$  through both cells of the  $CO_2$  analyzer. Air lines, leading to the  $CO_2$  analyzer from the drying columns, could be easily switched so  $CO_2$  differentials could be measured on either chamber containing the material being tested. The response time of the apparatus to changes in  $CO_2$  concentrations was rapid.

Water vapor differentials were psychrometrically analyzed by measuring the water vapor content of the air before and after passing through the chamber. Pinch clamps were used to regulate flow rates through the psychrometer.

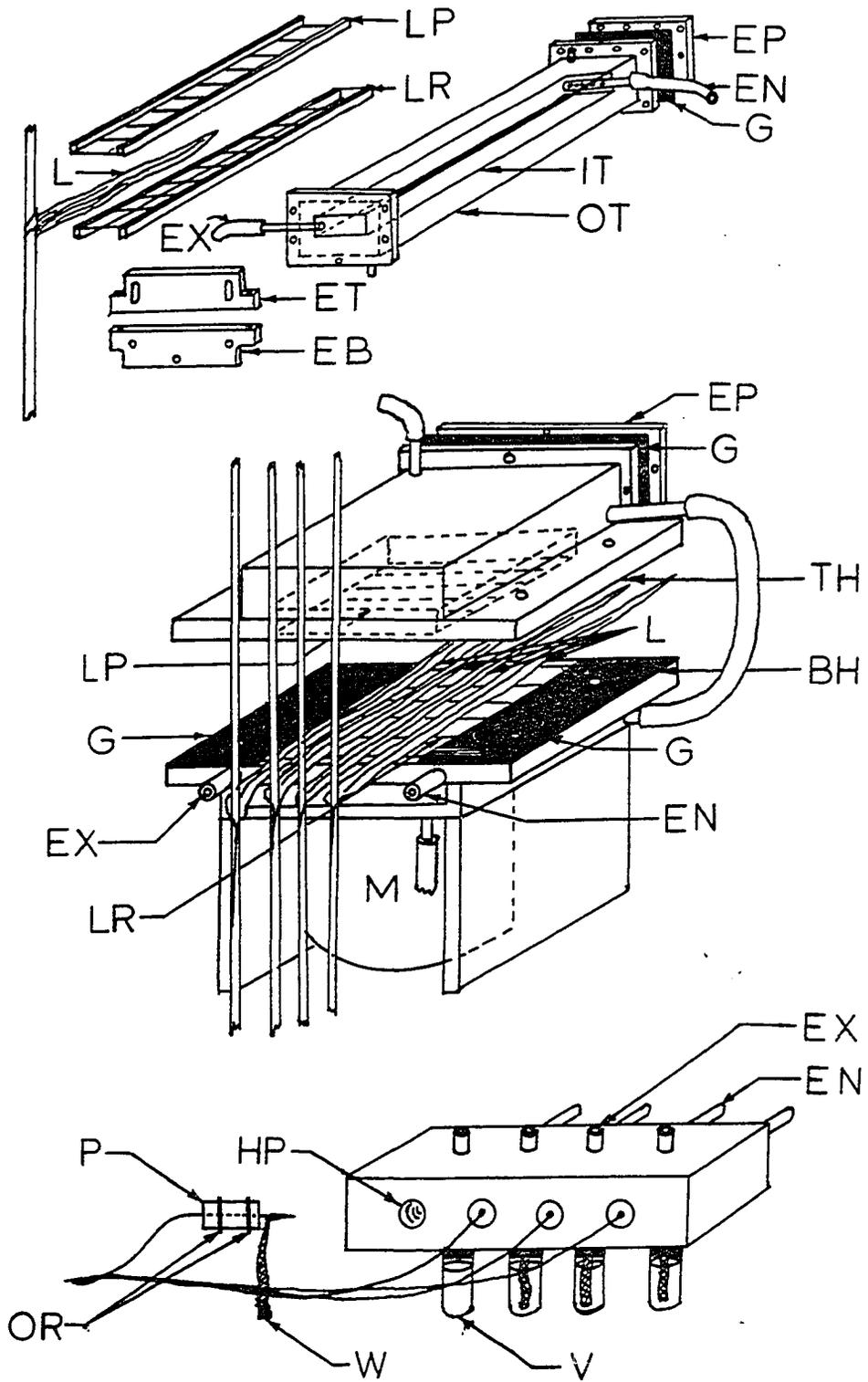
### The leaf chambers

Leaf chambers used in 1968 and 1969 were both constructed from acrylic plastic (Plexiglass) and were cooled by external water jackets. The 1968 chambers were of rectangular tube design, whereas the 1969 chambers were of "sandwich" design (two halves which bolted together, one half on each side of the leaves). A mixing fan was used in 1969 to provide turbulent air transport.

The 1968 leaf chambers Leaf chambers used in 1968 held one complete flag leaf blade. A schematic drawing of a 1968 leaf chamber is shown in Figure 4. The internal dimensions of the chamber were 40.0 X 3.0 X 1.5 cm. The internal rectangular tube (IT), which contained the leaf, was surrounded by an outer, larger, rectangular tube (OT) which served as a water jacket. Distilled water was circulated through the 1.5 cm thick water jacket to provide adequate temperature control. One end plate (EP) could be unbolted and removed to facilitate cleaning of the water jacket. Neoprene gasket material (G) between the tubular body of the water jacket and the end plate prevented water leaks.

The leaf could be inserted and removed through an opening measuring 3.0 X 1.5 cm in one end of the chamber. A leaf rack (LR) and press (LP), utilizing nylon filaments, were used to support and hold the leaf flat in the center (vertical) of the chamber. Once the leaf (L) and leaf-rack press combination were slid into the chamber, two split end pieces (ET and EB) could be bolted in place, one on each side of the leaf. Permagun, a nontoxic compound, was used to seal between the leaf lamina and the split end pieces.

Figure 4. 1968 leaf chamber (top), 1969 leaf chamber (middle), differential psychrometer (bottom). See text for description. IT = inner tube; OT = outer tube; EN = air inlet; EX = air outlet; EP = removable end plate; LP = leaf press; LR = leaf rack; ET = top of split end plate; EB = bottom of split end plate; TH = top half; BH = bottom half; M = fan motor; L = flag leaf lamina; HP = hole for thermocouple plug; P = thermocouple plug; OR = O-ring; W = wick; V = vial.



Air entered the chamber through a tube (EN) with five 1.5 mm holes drilled in the side to allow uniform air distribution inside the chamber. After the air had passed over the leaf, it was exhausted through a second tube (EX). Tests conducted with pieces of filter paper, which had been soaked in a cobalt chloride solution and dried, indicated uniform air distribution in the chamber. When high humidity air was passed through the chamber, the paper changed color (blue — pink). The color front progressed uniformly down the length of the chamber.

The 1969 leaf chambers      The chambers constructed and used in 1969 (Figure 4) were similar to those used by Stoy (1965), except they held portions of only four flag leaves. Each of the two leaf chambers had a small externally-mounted motor with a 6.5 cm internally-mounted fan to provide turbulence. The internal dimensions of each of the chambers were 8.0 X 8.0 X 2.5 cm. Each chamber consisted of two halves which could be bolted together. The midportions of flag leaves were inserted between the halves, and Permagun was used as a sealing compound between the leaf lamina and the chamber halves. The leaves were supported above the fan in each chamber by nylon filaments drawn across the top part of the bottom chamber half (BH). A leaf press of similar construction across the bottom part of the top half (TH) of the chamber held the leaves flat.

The top half of each chamber was constructed with a water jacket 2.5 cm thick, and the bottom half had a water jacket 0.3 cm thick, through which cooled, distilled water passed. A plate on the upper

section of the chamber could be removed to clean the larger water jacket; however, no provisions were made to allow cleaning of the bottom water jacket.

Air entered the chamber through a tube (EN), joined to a manifold with eight 1.0 mm holes equally spaced and drilled at an angle such that incoming air would be directed into the fan. The outlet manifold (EX) was identical, except larger 1.5 mm holes were used. Smoke tests showed almost immediate dispersal of the air entering the chamber, and tests with pieces of cobalt chloride treated filter paper showed uniform color transformations upon exposure to humid air.

The leaf chambers were mounted 25.0 cm below the light source in 1968 and 1969, respectively. Excluding the water in the cooling jackets, the light passed through 6.2 mm of acrylic plastic before striking the leaf surface in both 1968 and 1969.

#### Humidity control

The relative humidity of the air entering the chambers was maintained relatively constant by a method similar to that used by Gaastra (1959) and Nevins and Loomis (1970). The air for each chamber was passed through 100 cm of 4.8 mm diameter copper coils (CC<sub>1</sub> or CC<sub>2</sub>), submerged in an ethylene glycol constant low-temperature bath (CTB<sub>1</sub>). The air for each chamber was then forced through two sintered glass gas dispersion tubes, connected in series and extended into bottles of distilled water. The bottles were partially submerged in the ethylene glycol bath. Dispersing the air through the distilled water brought

the saturation vapor of the air to a value determined by the temperature of the distilled water in the bottles, the temperature of which was controlled by the temperature of the ethylene glycol solution in the bath. The bath was maintained at temperatures of 11.0 and 6.3°C in 1968 and 1969, respectively. The air was then passed through copper coils (CC<sub>3</sub> or CC<sub>5</sub>), immersed in a warmer water bath (CTB<sub>3</sub>), to depress the relative humidity of the air stream before it entered the chambers.

The ethylene glycol bath temperature was regulated by a Fenwal thermostat (TS) connected to two solenoid valves (SV), through which chilled ethylene glycol was pumped when the bath temperature was too high. The cold incoming coolant replaced slightly warmer coolant in the bath, which was allowed to flow back to a refrigerated storage bath (CTB<sub>2</sub>) through an overflow line. When the solenoids were normally closed, the ethylene glycol coolant did not enter the humidity bath, and it was recirculated directly back to the storage bath through a bypass line. Relatively stable humidity conditions could be maintained with respect to the air supplied to the chambers; however, relative humidity of air in the chambers varied as a function of the leaf area and transpiration rate of the plant material being tested.

#### The differential psychrometer

Transpiration rates were measured with a differential psychrometer similar to that described by Slatyer and Bierhuizen (1964). The psychrometer allowed dry and wet bulb measurements to be taken on air before

and after it had passed over the leaf. Psychrometric equations were used to compute transpiration rates.

The psychrometer (Figure 4) was constructed from a Plexiglass block having dimensions of 4.8 X 7.9 X 23.9 cm. Four air-inlet (EN) and four outlet (EX) holes, 4.76 mm in diameter, were drilled into the block. Holes (HP) 12.7 mm in diameter, drilled opposite the inlet holes, accommodated plastic "plugs" (P) surrounded by two neoprene O-rings (OR). Wet bulb thermocouples, constructed of No. 30 copper-constantan wire, were cemented into holes drilled through the "plugs". The tip of the thermocouples extended into the air inlet holes. The thermocouples could be inspected by removing the "plugs". Cotton thread was used to wrap the wet bulb thermocouples in 1968, and a very fine muslin material was used in 1969. The muslin seemed to carry water up the wicks (W) better than did thread. The wick extended into a vial containing distilled water. Vials (V) were attached to the bottom of the psychrometer, which was partially submerged in a constant temperature water bath.

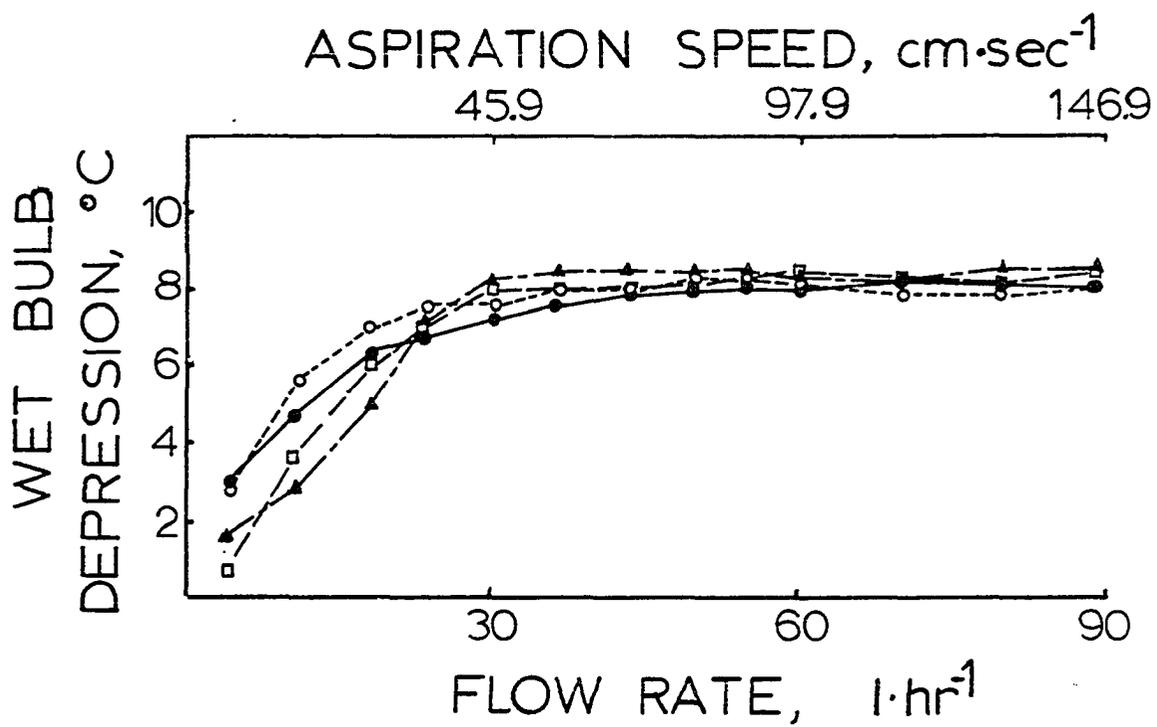
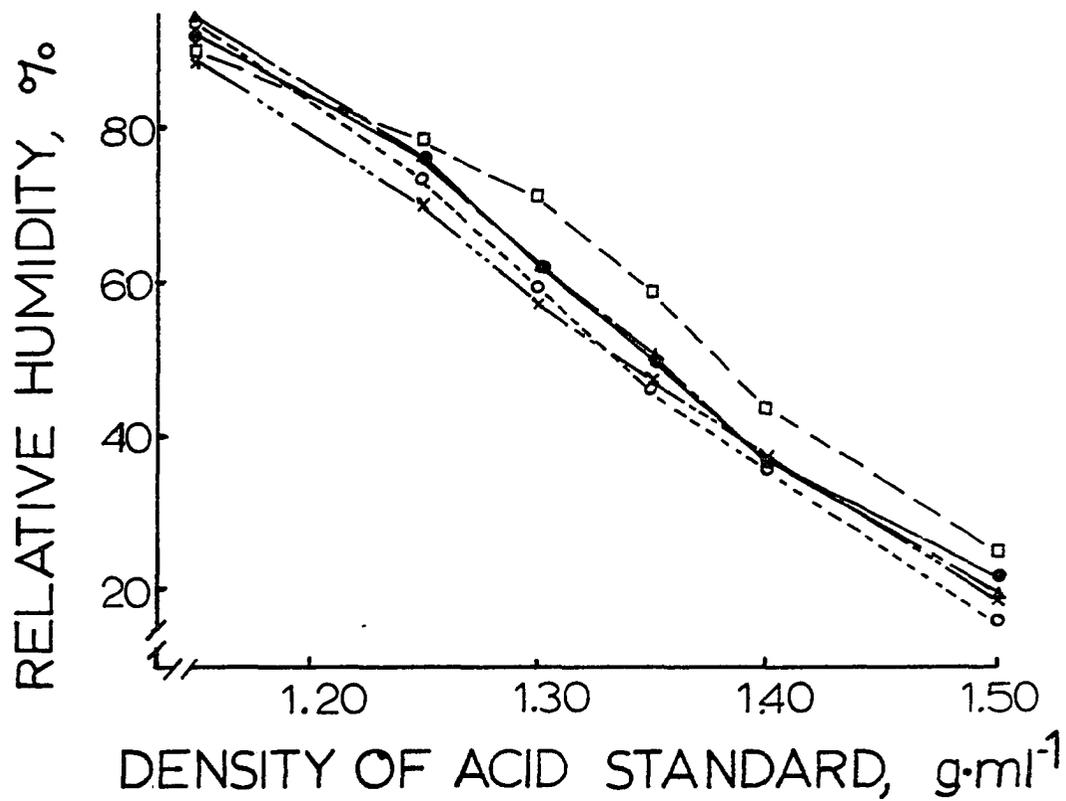
Air passed through submerged copper coils (CC<sub>1</sub>, CC<sub>2</sub>, CC<sub>3</sub>, and CC<sub>4</sub>, Figure 3) in the constant temperature bath in an attempt to standardize the temperature of the air before it passed across wet and dry bulb thermocouples in the psychrometer. A total length of 11.9 m for inlet air and 3.7 m for outlet air of 6.4 mm diameter copper tubing was used in 1968. In 1969 copper coils, constructed from 4.8 mm tubing, were used. The coils were 7.6 m long.

The water bath (CTB<sub>3</sub>) was maintained at a constant 20°C temperature in 1968. In 1969 condensation was found to occur in the outlet coils when temperature of the water bath was maintained at 20°C. This was because leaf chambers used in 1969 contained more transpiring leaf material, and the temperature of the outlet coils was below the dew point temperature of the outlet air. Consequently, temperature of the water bath was raised to 26°C in 1969. No further condensation problems were experienced.

Response of the psychrometer is rapid; however, the wet bulb depression is a function of the flow rate of air past the wick of the wet bulb thermocouple (Slatyer and Bierhuizen, 1964). Figure 5 shows a calibration curve for the four wet bulb thermocouples used in 1969. Air of known relative humidities, as obtained by various H<sub>2</sub>SO<sub>4</sub> solutions, was passed through the psychrometer. Tests showed the No. 1 wet bulb thermocouple was not functioning properly, consequently, it was rewrapped. As a consequence of the test the other three thermocouples also had to be rewrapped, because of degradation of the wicks by H<sub>2</sub>SO<sub>4</sub> fumes. At high humidities, all thermocouples gave slightly higher relative humidity readings than the computed acid standard values. However, tests were conducted at variable room temperatures rather than constant 20°C conditions upon which tabular values were based.

Wet bulb depressions were found initially to increase as the flow rate of air past the wet bulb thermocouple increased (Figure 5). Studies conducted in 1968 showed that excess thread or muslin on the thermocouple tip radically increased the flow rate of air necessary to

Figure 5. Relative humidities measured with the differential psychrometer in relation to various known relative humidities obtained with different density  $H_2SO_4$  standards (top). Shown below are wet bulb depressions obtained with various flow rates and aspiration speeds of air through the psychrometer. x = known humidity of acid standards; □, ○, ▲, and ● refer to thermocouples Nos. 1, 2, 3, and 4, respectively.



achieve maximum wet bulb depressions. In both 1968 and 1969 a flow rate of  $63.3 \text{ l}\cdot\text{hr}^{-1}$  was used through the psychrometer. At this flow rate maximum wet bulb depressions were obtained.

### Lighting

The light source for each chamber used in 1968 consisted of six 300 Watt reflector-flood incandescent lamps (L) mounted above and parallel to the length of the chamber. Three lamps, arranged triangularly above each chamber, were used in 1969. Light flux densities and quality are discussed in the section on measurements.

### Temperature control

The lamps emitted a large amount of infrared energy. Much of the infrared energy, however, was removed from the air-conditioned laboratory by an exhaust fan (EF) mounted in a cowling surrounding three sides of the lamps. The infrared radiation was partially absorbed by distilled water baths ( $\text{WB}_1$  and  $\text{WB}_2$ ) mounted 8 cm below the lamps. The depth of the water in the baths was maintained at 2.6 and 5.0 cm in 1968 and 1969, respectively. Equal depths of water in the two baths were maintained by a crossover tube (CR). Distilled water for the infrared radiation absorbing water baths was cooled by pumping the water through a radiator ( $\text{R}_2$ ) which was submerged in a  $16^\circ\text{C}$  water bath ( $\text{CTB}_4$ ).

Leaf chambers were cooled by water jackets through which distilled water was pumped. This was accomplished by pumping water out of a constant temperature distilled water bath ( $\text{CTB}_3$ ) and forcing it through a partially submerged radiator ( $\text{R}_1$ ) before passing the chilled water

through the jackets on the chambers. Once the water had passed through the chambers, it was returned to the water bath.

### Measurements

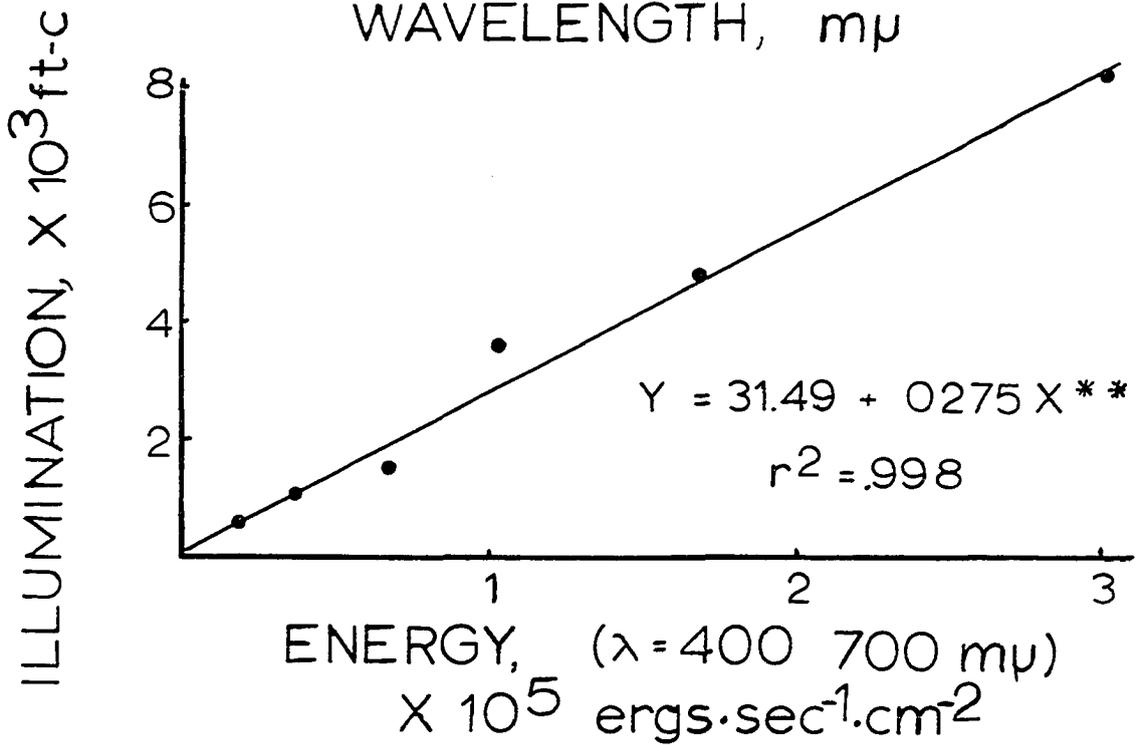
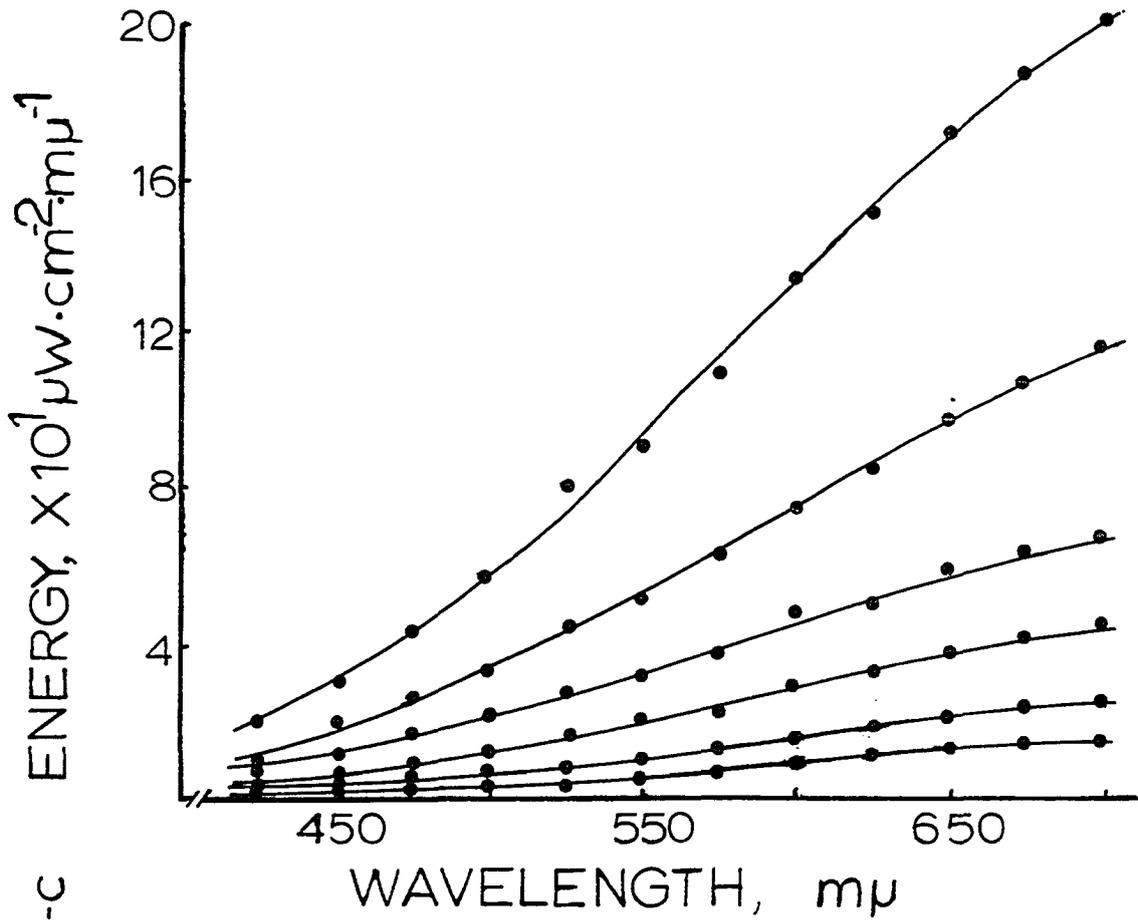
Many different variables were measured in 1968 and 1969. Some of the variables were measured directly. However, a large number were found indirectly through the use of various formulae. Light, temperature, CO<sub>2</sub> exchange, transpiration, and diffusion resistance measurements are discussed in this section. The procedure for determining the leaf area and leaf dry weight per unit area is also described.

#### Light measurements

In 1968 the light flux density was measured with an ISCO model SRR spectroradiometer at a position approximating the center of the leaf chamber. Illumination measurements were also taken with a Weston model 756 foot-candle meter. Light levels were varied by inserting various combinations of screens and perforated metal sheeting between the light source and the chambers.

Figure 6 shows typical spectra (400-700 mμ) of the incandescent light sources obtained with different screen and perforated metal sheet combinations. Each point represents the mean of two measurements taken at different positions on each chamber. Light quality was little affected by varying the amount of light. More energy was emitted at longer wavelengths than shorter wavelengths, but radiation in the region above 610 mμ is absorbed strongly by the chlorophyll and efficiently

Figure 6. Diagram of spectrums (400 to 700 mμ) obtained with the various screen combinations shown in Table 2. Spectrums were measured only in 1968 (top). Relationship between light flux density in energy units and illumination units (bottom).



converted to chemical energy by the photosynthetic process.

Light readings obtained in energy units were highly correlated with illumination measurements. Using illumination units as a dependent variable and light energy units as an independent variable the following equation was obtained by regression analysis:

$$Y = 31.49 + .0275X \quad (20)$$

where Y = illumination units, foot-candles, and

X = energy units,  $\text{ergs}\cdot\text{sec}^{-1}\cdot\text{cm}^{-2}$ .

This equation could explain essentially all the variation between the two types of light measurement with the two instruments ( $r^2 = .998$ ). Because the regression analysis gave a reasonably good fit and because illumination measurements were more easily obtained, only illumination measurements were taken in 1969. The regression equation was used to obtain energy estimates in 1969.

The 1968 light measurements, in terms of energy and illumination units, are shown in Table 2. Light flux density differed less than 6 and 2 percent between the two chambers in 1968 and 1969, respectively. A light flux density of  $3.02\cdot 10^5$  and  $1.73\cdot 10^5$   $\text{ergs}\cdot\text{sec}^{-1}\cdot\text{cm}^{-2}$  was used in 1968 and 1969, respectively. These values are equivalent to approximately 8,340 and 4,760 foot-candles.

#### Temperature measurements

Temperatures were measured by use of thermocouples constructed from No. 30 copper-constantan wire. The thermocouples were connected

Table 2. Light flux densities (400 to 700 m $\mu$ ) measured in 1968  
(values are averages of both chambers)

Screen combination	$10^4$ ergs. sec <sup>-1</sup> .cm <sup>-2</sup> <sup>a</sup>	Ly.min <sup>-1</sup> <sup>b</sup>	foot-candles <sup>a</sup>
No screen	30.2	.433	8,233
1 screen	16.8	.241	4,840
2 screens	10.6	.152	3,078
3 screens	6.7	.096	1,767
2 screens+sheet <sup>c</sup>	3.8	.054	1,019
3 screens+sheet <sup>c</sup>	2.4	.034	657

<sup>a</sup>Values obtained by direct measurement.

<sup>b</sup>Calculated from conversion factors.

<sup>c</sup>Perforated metal sheet.

to a multiple switching device which transmitted the output of any desired thermocouple to a Leeds and Northrup 8690 potentiometer. The potentiometer was sensitive to 0.3°C.

The potentiometer gave a millivolt output which had to be converted to temperature units. In 1968 this was laboriously done by the use of tables and charts supplied with the instrument; however, in 1969 a regression equation was developed which would accurately predict temperatures from millivolt outputs by the instrument. The response of the instrument was nearly linear, but the inclusion of a quadratic millivolt factor gave a slightly better fit when temperature (dependent variable)

was regressed against corresponding millivolt outputs (independent variable) supplied with the instrument. The following equation was developed:

$$Y = .0163 + 25.8995X - .6586X^2 \quad (21)$$

where Y = temperature, °C, and

X = potentiometer output, millivolts.

Temperature measurements included: wet and dry bulb readings of the air as it passed through the psychrometer, leaf temperature measurements, leaf chamber air temperature measurements, and air temperature measurements taken immediately before the air passed into the flowmeters regulating the air flow through the chambers.

In 1968 the leaf temperature was measured in only one location on each leaf. This was done by placing a thermocouple beneath the leaf. The resilience of the thermocouple wire provided the tension needed to hold the thermocouple against the leaf. In 1969 leaf temperatures were measured on two leaves, and the mean leaf temperature was used in calculations involving this factor. Radiation corrections for leaf thermocouples were not made in 1968 or 1969, because it was believed the large mass of the leaf--in relation to the thermocouple mass--determined the thermocouple temperature.

An estimate of direct radiation heating of the air temperature thermocouple in the leaf chamber was made in 1968 by shielding the thermocouple with a small piece of aluminum foil. Four measurements, taken at the light flux densities shown in Table 2, were made with and without shielding in 1968. Radiation correction factors, found by

subtraction of the shielded thermocouple temperature from the exposed thermocouple temperature, were found to be 1.4, .9, .7, .4, .3, and .1°C at each of the light flux densities. In 1968 a 1.0°C air temperature correction was made; however, the correction was not made in 1969 because the magnitude of the correction was small and consistently in one direction.

#### Net CO<sub>2</sub> exchange rates

CO<sub>2</sub> exchange rates were measured both in 1968 and 1969 with a Beckman 15A infrared gas analyzer. The CO<sub>2</sub> analyzer was of the absolute type, the sample tube being continuously compared to a sealed nitrogen tube. However, in 1969 it was converted to the differential type. This conversion slightly modified the calibration procedures, but calculations used to compute CO<sub>2</sub> exchange rates were the same.

Conversion of the analyzer To convert the absolute type analyzer to a differential type analyzer involved only slight modifications. The absolute reference cell was removed from the analyzer and the "window" on the end of the cell near the "chopper" disc was removed to prevent damage to it during the conversion process. A hole, the same size as the inside diameter of the stainless steel tubing through which air was to flow to and from the cell, was drilled in the "sensor" end of the analyzer reference cell. This hole was then slightly countersunk by enlarging the hole to the same diameter as the outside diameter of the stainless steel tubing. A stainless steel tube was then "cold soldered"

into the countersunk hole with a soldering compound having a slightly lower melting point than the gold plating on the cell. The end of the cell, normally near the "chopper" disc, already had a tapped hole. A plastic desiccant plug normally screwed into this hole. A second stainless steel tube, connected to a special male fitting, was inserted in place of the plastic plug. The housing of the analyzer was drilled in two places to accommodate inlet and outlet tubes added to the reference cell. The converted analyzer was checked against standard gases and was found to work satisfactorily (see section on calibration of the analyzer).

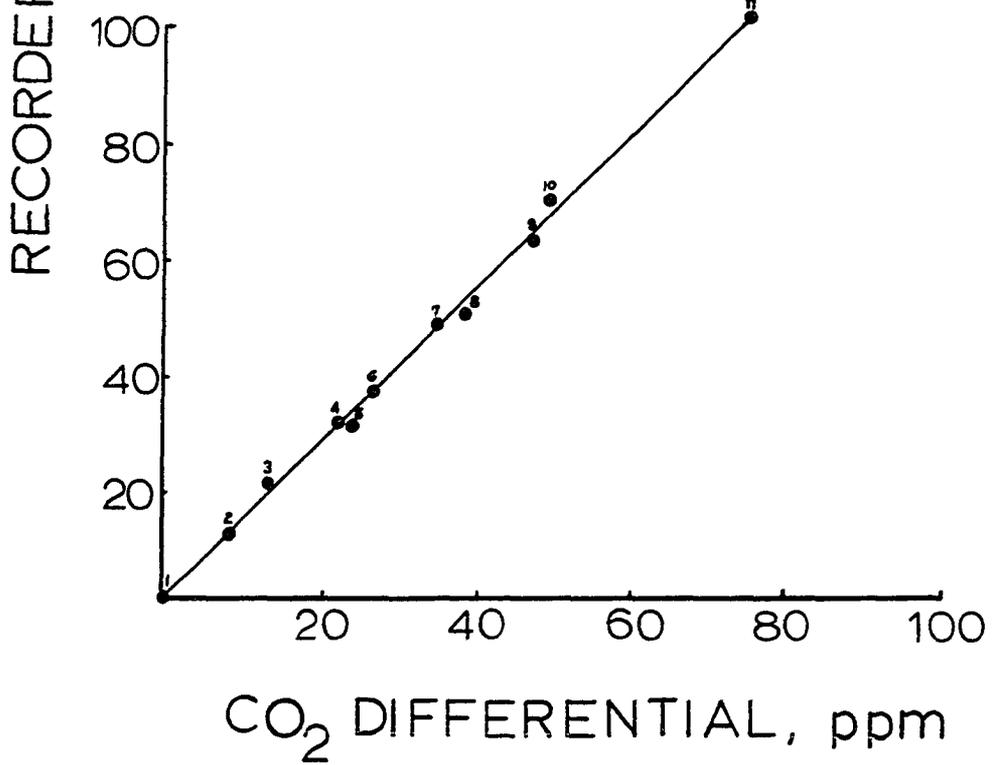
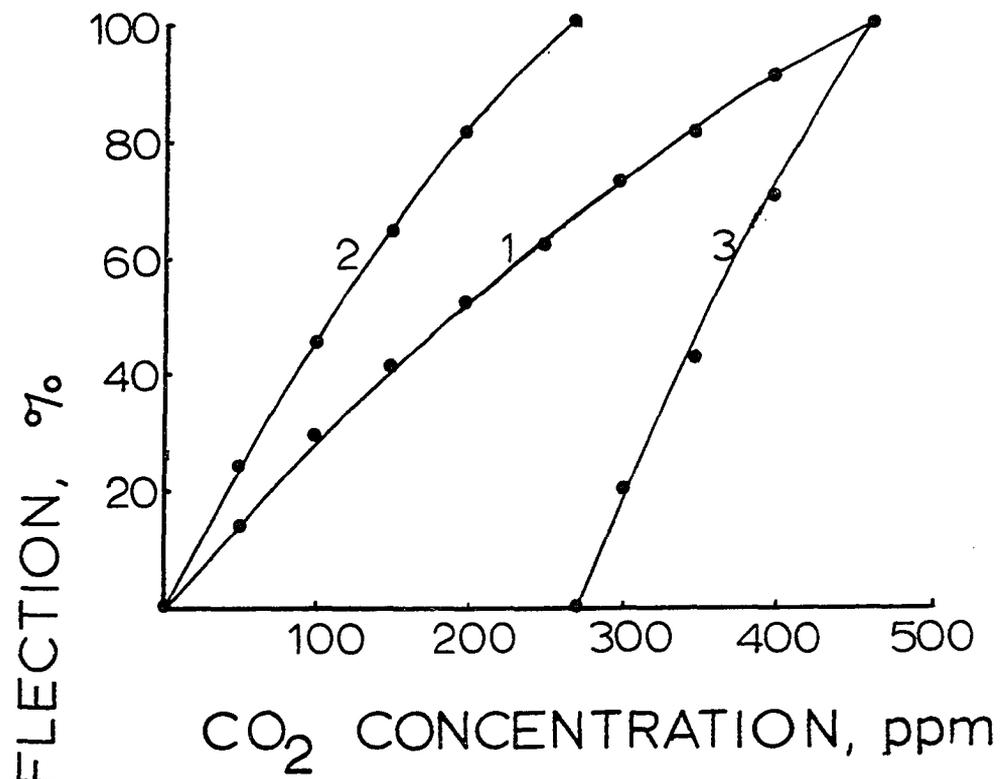
Another feature added to the analyzer was an opening in the side of the analyzer housing near the "chopper" disc. The opening was connected to a line through which dry, CO<sub>2</sub>-free air passed. This continually "purged" the analyzer housing as suggested by Brown and Rosenberg (1968). Since part of the path of the infrared radiation is through air in the analyzer housing, and because infrared radiation is absorbed by CO<sub>2</sub>, they believe the amount of radiation reaching the analyzer chamber is influenced by the concentration of CO<sub>2</sub> in the housing. Although specific tests were not conducted, there appeared to be little difference in analyzer stability before and after this modification was made. Placement of exposed Ascarite (a CO<sub>2</sub> absorbing compound) in the housing was not as satisfactory as the purging system, because the Ascarite absorbed moisture and had to be changed daily.

Calibration of the analyzer

The procedure for calibrating the CO<sub>2</sub> analyzer in 1968 differed from that used in 1969 because of the analyzer modifications. Prior to measuring each leaf in 1968, the analyzer (absolute type with 0 ppm CO<sub>2</sub> in the reference cell) was calibrated against 0 and 460 ppm CO<sub>2</sub> in N<sub>2</sub> air mixtures. For net CO<sub>2</sub> measurements taken at CO<sub>2</sub> concentrations below 270 ppm CO<sub>2</sub>, the span on a Leeds and Northrup Speed-O-Max variable-span, strip-chart recorder (connected to the CO<sub>2</sub> analyzer), was adjusted to give 100 percent deflection with 0 and 270 ppm CO<sub>2</sub> in N<sub>2</sub> standard gases. The full scale deflection of the recorder was reset for measurements of CO<sub>2</sub> exchange rates taken at CO<sub>2</sub> concentrations greater than 270 ppm. Standard gases containing 270 and 460 ppm CO<sub>2</sub> were used. Calibration curves were made (Figure 7) to account for the curvilinear response of the CO<sub>2</sub> analyzer. These could be read to 1.5 ppm CO<sub>2</sub> or better. The CO<sub>2</sub> analyzer has an accuracy of approximately 1.0 ppm.

In 1969 the CO<sub>2</sub> analyzer was calibrated with 0 and 376 ppm CO<sub>2</sub> in N<sub>2</sub> standards. For measurements taken at low CO<sub>2</sub> concentrations (CO<sub>2</sub>-free air), the recorder was set to give 100 percent span deflection with 0 and 85 ppm CO<sub>2</sub> in N<sub>2</sub> standards. Net CO<sub>2</sub> exchange measurements at higher CO<sub>2</sub> concentrations (approximately 320 ppm CO<sub>2</sub>) were made by adjusting the recorder span to give 100 percent full scale deflection with 277 and 376 ppm CO<sub>2</sub> in N<sub>2</sub> standards. Since only a small portion of the CO<sub>2</sub> response calibration curve was used for CO<sub>2</sub> exchange measurements at high and low CO<sub>2</sub> concentrations in 1969, the response of the CO<sub>2</sub> analyzer was assumed to be essentially linear (Figure 7).

Figure 7. Calibration curves used in 1968 (top). Curve 1 shows the curvilinear response obtained when the recorder was set to give 100 percent full scale deflection with 0 and 460 ppm CO<sub>2</sub> standards. Curve 2 shows the response curve obtained when the span of the recorder was adjusted to give 100 percent full scale deflection with 0 and 270 ppm CO<sub>2</sub> standards. Curve 3 shows the response curve obtained when the recorder span was set to give 100 percent full span deflection with 270 and 460 ppm CO<sub>2</sub> standards. Curves 2 and 3 were used in 1968 experiments because CO<sub>2</sub> differentials could be read more easily from the recorder chart paper. Points in the above figure were obtained by measuring differentials obtained by generating various CO<sub>2</sub> concentrations with the experimental apparatus. Shown below are CO<sub>2</sub> differentials obtained with the differential CO<sub>2</sub> analyzer in 1969. Little curvilinear response was evident over the range of CO<sub>2</sub> concentrations used in 1969. Points 1 through 11 refer to CO<sub>2</sub> differentials measured from the following standard gas comparisons: 293 vs. 293; 321 vs. 330; 320 vs. 344; 293 vs. 330; 344 vs. 370; 293 vs. 321; 293 vs. 330; 330 vs. 370; 327 vs. 370; 293 vs. 344; and 293 vs. 370 ppm CO<sub>2</sub>.



In all cases standard gas mixtures were obtained from the Matheson company. The CO<sub>2</sub>-free N<sub>2</sub> standards used in 1968 and 1969 were obtained either from the Matheson company or Chemistry Stores, Iowa State University. The standard gases were analyzed upon arriving at the laboratory because the CO<sub>2</sub> concentration usually differed from that ordered. Tests were conducted to determine if the CO<sub>2</sub>-free, N<sub>2</sub> gas standard contained any CO<sub>2</sub>. When one sample stream of the N<sub>2</sub> gas passed through a soda lime (CaO + NaOH) column and then through the reference cell of the CO<sub>2</sub> analyzer, and a second sample stream passed directly through the reference cell, the N<sub>2</sub> gas standard was found to contain a measurable amount of CO<sub>2</sub>. All CO<sub>2</sub> analyzer calibrations involving CO<sub>2</sub>-free air were made by first passing the bottled N<sub>2</sub> through a soda lime column to remove traces of CO<sub>2</sub> in 1969.

Procedure for measuring net CO<sub>2</sub> exchange      All experimental measurements were taken on flag leaves; however, preliminary experimental work, which was conducted to insure that analytical equipment was functioning properly, was conducted on flag leaves or fifth and sixth leaves. During the 1969 season, most of the preliminary tests were made on leaves of the varieties Burnett and Record. These two genotypes were used as border material. In 1968 the small pots could be easily rotated to eliminate border effects, and an excess of experimental plant material provided adequate test material.

Nine replications, of one flag leaf each, were taken per genotype in 1968. Flag leaves on three of the plants in each of the three pots were measured once. All 1968 measurements were taken within six days

prior to or after panicle emergence. Net CO<sub>2</sub> exchange rates were measured at inlet CO<sub>2</sub> concentrations of 0, 100, 200, 300, and 400 ppm. Measurements were taken in ascending order, because stomates have been reported to close under high CO<sub>2</sub> concentrations (Heath and Russell, 1954). Approximately 45 minutes were required to test one leaf (9 minutes per CO<sub>2</sub> concentration). It required about 3 minutes to adjust the inlet CO<sub>2</sub> concentration to the desired level and to obtain stable photosynthetic rates. Once steady state conditions were obtained, the inlet and outlet CO<sub>2</sub> concentrations were measured separately for 3 minutes each. The CO<sub>2</sub> differential was generally less than 20 ppm. Small CO<sub>2</sub> gradients over the leaf surface reduced the influence of systematic errors. (The reader is referred to papers by Gaastra (1969) and Avery (1966) if interested in systematic errors arising from low flow rates and improperly positioned leaves in assimilation chambers.)

The leaf chambers used in 1969 could accommodate the midportions of four flag leaves of a specific genotype. Eight replications of measurements, consisting of four flag leaves each, were taken in 1969. Measurements were taken during the morning over a four day period. Two replications of measurements being taken on each of the genotypes being tested each day until a total of eight replications were obtained. A total of 32 flag leaves (four leaves per replication) were tested per genotype in 1969. Measurements on a given genotype were initiated either on the day of panicle emergence or within one day after panicle emergence.

Plant material remained out-of-doors until immediately before flag leaves were tested in 1969, and when tests were completed, the plants were returned to the same location in the experimental site. In 1968

plant material to be tested was brought into the laboratory in the morning, and all tests on a given genotype were completed on one day. Although there was no evidence of stomatal closure on leaves of plants placed in the laboratory in 1968, it was believed the possibility of stomatal closure would be precluded in 1969 if plants to be tested were brought into the laboratory immediately before testing. It was hoped that, extending the 1969 testing period for each genotype over a four day period, would compensate for day-to-day variability of photosynthesis caused by low night temperatures (Izhar and Wallace, 1967b) or other uncontrollable external environmental factors.

Net CO<sub>2</sub> exchange measurements were taken only in CO<sub>2</sub>-free air and 320 ppm CO<sub>2</sub> air in 1969. CO<sub>2</sub> gradients were generally less than 20 ppm. The CO<sub>2</sub> gradient over the leaf was minimized by a mixing fan in the chamber. Gaastra (1959) has stated that when provisions are made which allow rapid air recirculation in the chamber, the mean CO<sub>2</sub> concentration over the leaf surface can be approximated by the following equation:

$$[\text{CO}_2]_m = [\text{CO}_2]_a + \frac{([\text{CO}_2]_b - [\text{CO}_2]_a)}{2(n - 1)} \quad (22)$$

where  $[\text{CO}_2]_m$  = mean CO<sub>2</sub> concentration over the leaf surface, ppm

$[\text{CO}_2]_a$  = outlet CO<sub>2</sub> concentration, ppm

$[\text{CO}_2]_b$  = inlet CO<sub>2</sub> concentration, ppm

$n$  = turnover rate of air in the chamber.

Unfortunately the chamber design did not allow turbulence measurements to be made within the chamber. However, the smoke tests seemed to indicate rapid air recirculation. Hence,  $n$  was assumed to be large,

and the outlet CO<sub>2</sub> concentration was considered to represent the mean CO<sub>2</sub> concentration in the chamber. In 1968 the mean CO<sub>2</sub> concentration over the leaf surface was assumed to be represented by the mean of the [CO<sub>2</sub>]<sub>b</sub> and [CO<sub>2</sub>]<sub>a</sub> concentrations.

It required about 30 minutes to test the midportions of four flag leaves in 1969. CO<sub>2</sub> differentials were first measured in CO<sub>2</sub>-free air for four minutes, and then the CO<sub>2</sub> concentration of the outlet air was raised to 320 ppm. After the photosynthetic rate was stabilized, the CO<sub>2</sub> differential was measured again for four minutes. The sample streams flowing into the CO<sub>2</sub> analyzer had to be reversed since CO<sub>2</sub> efflux occurs in CO<sub>2</sub>-free air, and CO<sub>2</sub> uptake takes place at normal atmospheric CO<sub>2</sub> concentrations (320 ppm CO<sub>2</sub>).

Net CO<sub>2</sub> exchange calculations      Net photosynthesis (P) and CO<sub>2</sub> evolution (R) rates in CO<sub>2</sub>-free air were calculated in the same manner in 1968 and 1969. The following equation was used:

$$\text{CO}_2 \text{ exchange} = \frac{\Delta\text{CO}_2 \cdot 10^6 \cdot F \cdot 44,010 \text{ mg CO}_2 \cdot \text{mole}^{-1}}{A \cdot R \cdot t} \quad (23)$$

where  $\Delta\text{CO}_2 = [\text{CO}_2]_b - [\text{CO}_2]_a$ , ppm

F = flow rate, l·hr<sup>-1</sup>

A = leaf area on one side, dm<sup>2</sup>

t = air temperature through the flowmeter, °K, and

R = gas constant, .08205 l·mole<sup>-1</sup>·°K at 1 atm.

Regression analyses conducted on individual leaf measurements taken in 1968 indicated the relationship between net CO<sub>2</sub> exchange rates and the CO<sub>2</sub> concentrations of the ambient air over the leaf surface was

linear up to CO<sub>2</sub> concentrations of at least 400 ppm. Only 5 of the 180 linear regression analyses carried out in 1968 were not significant at the .01 percent level (Table 26, Appendix). The r<sup>2</sup> values generally exceeded .95. It was for this reason that the number of CO<sub>2</sub> concentrations used in 1969 was reduced. Figure 8a shows the mean net CO<sub>2</sub> exchange rates for the 20 oat genotypes used in 1969 as a function of mean CO<sub>2</sub> concentration over the leaves. The CO<sub>2</sub> compensation point ( $\Gamma$ ) was found by regression analysis in 1968. The CO<sub>2</sub> concentration on the abscissa, where the regression line crossed, was assumed to represent  $\Gamma$ . Since CO<sub>2</sub> exchange rates were measured at only two CO<sub>2</sub> concentrations in 1969, the CO<sub>2</sub> concentration on the abscissa, crossed by a line connecting the two CO<sub>2</sub> exchange rates, was regarded as  $\Gamma$ . Because mean CO<sub>2</sub> concentrations over the leaf deviated slightly (5 to 10 ppm sometimes) from the desired CO<sub>2</sub> concentrations, the net CO<sub>2</sub> exchange rates were adjusted to comparable CO<sub>2</sub> concentrations by use of the slope (S). Statistical analyses were conducted on the corrected CO<sub>2</sub> exchange rates.

#### Transpiration measurements

Transpiration rates were measured by monitoring the change in vapor pressure of water in the air before and after it had passed through the leaf chamber. Dry bulb measurements of the air streams immediately before the air entered the psychrometer showed the temperatures of the inlet air streams to differ. Differences in dry bulb temperatures,

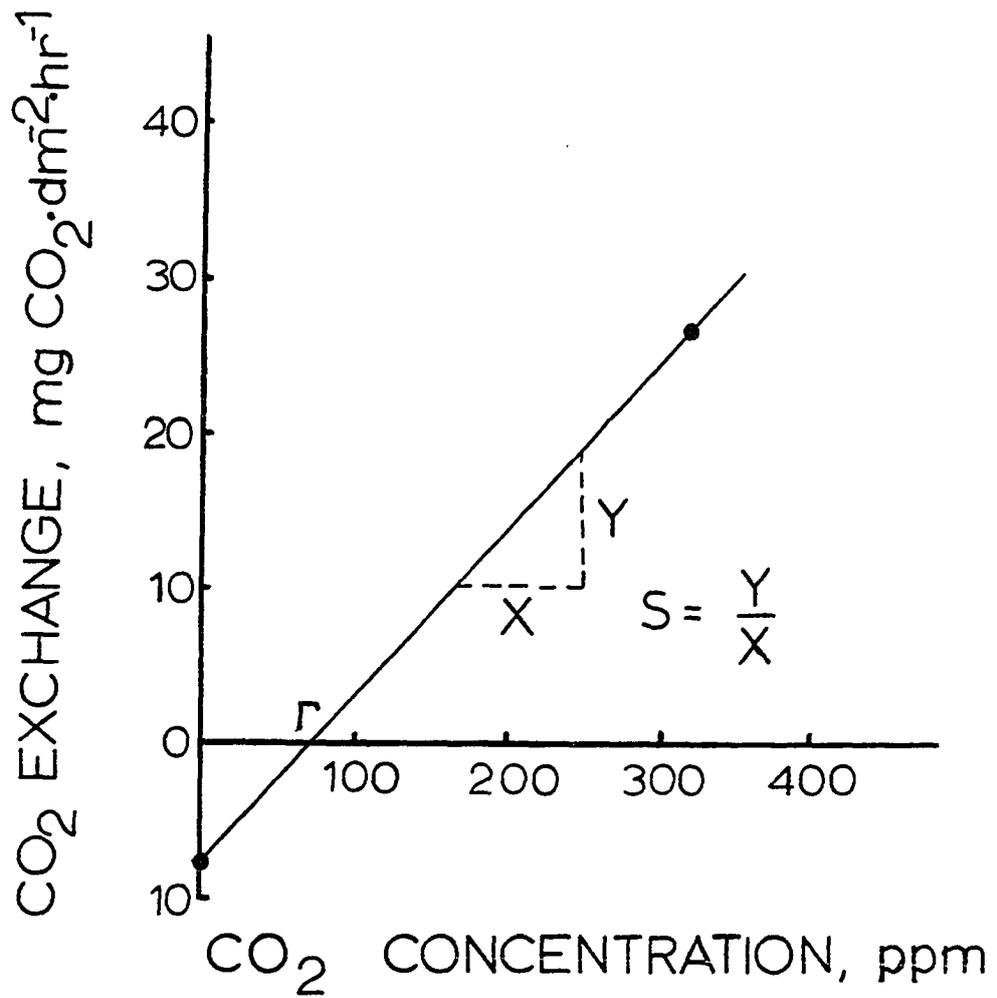


Figure 8a. Net CO<sub>2</sub> exchange as a function of mean CO<sub>2</sub> concentration over the leaves. The two points represent the mean CO<sub>2</sub> exchange rates for the 20 genotypes used in 1969.  $\Gamma$  = CO<sub>2</sub> compensation concentration;  $S$  = slope of the CO<sub>2</sub> response curve. Refer to text for evidence that the response is linear in the range of CO<sub>2</sub> concentrations shown in the graph.

therefore, had to be taken into consideration in the calculations.

Parameters measured for calculating transpiration were as follows:

flow rate, leaf area, and wet and dry bulb temperatures of the air entering the psychrometer before and after it had passed through the leaf chamber. Derivation of the transpiration formula began with the standard psychrometric equations as presented by Slatyer and Bierhuizen (1964).

$$e_{wa} - e_a = K(t - t_{wa}) \quad (24)$$

$$e_{wb} - e_b = K(t - t_{wb}) \quad (25)$$

where  $t$  = common temperature of the psychrometer and air stream, °C

$e_b$  = water vapor pressure of the air before entering the leaf chamber, mm Hg

$e_a$  = water vapor pressure of the air after leaving the leaf chamber, mm Hg

$t_{wb}$  = wet bulb temperature of the air before entering the leaf chamber, °C

$t_{wa}$  = wet bulb temperature of the air after leaving the leaf chamber, °C

$e_{wb}$  = saturation vapor pressure of the air before entering the leaf chamber, mm Hg

$e_{wa}$  = saturation vapor pressure of the air after leaving the leaf chamber, mm Hg

$K$  = psychrometric constant, .5 mm Hg.°C<sup>-1</sup>

The following equation was derived from the standard psychrometric equations to compute transpiration rates when dry bulb air stream temperatures entering the psychrometer were not the same. The equation is based on the assumption that the error introduced by not having

the psychrometer the same temperature as the air flowing through it is negligible. Errors in determining the common temperature of the air streams and psychrometer have been reported by Wylie (cited in Slatyer and Bierhuizen, 1964) to have little effect on determination of water vapor pressure.

$$T = \frac{F}{A} ([H_2O]_a - [H_2O]_b) \quad (26)$$

where  $T$  = transpiration rate,  $\text{mg H}_2\text{O} \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$

$F$  = flow rate,  $\text{l} \cdot \text{hr}^{-1}$

$A$  = leaf area on one side,  $\text{dm}^{-2}$

$[H_2O]_a$  = water concentration of the air leaving the chamber,  
 $\text{mg H}_2\text{O} \cdot \text{l}^{-1}$

$[H_2O]_b$  = water concentration of the air entering the chamber,  
 $\text{mg H}_2\text{O} \cdot \text{l}^{-1}$

The water vapor concentrations of the air leaving the leaf chamber and entering the leaf chamber are given by the following equations:

$$[H_2O]_a = \frac{\rho_a}{e_{sa}} (K(t_{wa} - t_a) + e_{swa}) \quad (27)$$

$$[H_2O]_b = \frac{\rho_b}{e_{sb}} (K(t_{wb} - t_b) + e_{swb}) \quad (28)$$

where  $t_a$  = dry bulb temperature of air leaving the leaf chamber,  $^{\circ}\text{C}$

$t_b$  = dry bulb temperature of air entering the leaf chamber,  $^{\circ}\text{C}$

$t_{wa}$  = wet bulb temperature of air leaving the leaf chamber,  $^{\circ}\text{C}$

$t_{wb}$  = wet bulb temperature of air entering the leaf chamber,  $^{\circ}\text{C}$

$\rho_a$  = density of water vapor in saturated air at  $t_a$ ,  $\text{mg H}_2\text{O} \cdot \text{l}^{-1}$

$\rho_b$  = density of water vapor in saturated air at  $t_b$ ,  $\text{mg H}_2\text{O} \cdot \text{l}^{-1}$

$e_{sa}$  = saturation vapor pressure of air at  $t_a$ ,  $\text{mm Hg}$

$e_{sb}$  = saturation vapor pressure of air at  $t_b$ , mm Hg

$e_{swa}$  = saturation vapor pressure of air at  $t_{wa}$ , mm Hg

$e_{swb}$  = saturation vapor pressure of air at  $t_{wb}$ , mm Hg.

The density of water vapor in saturated air and the saturation vapor pressure values were obtained from standard tables in 1968; however, equations which could compute these were incorporated into a Fortran program in 1969. Slatyer (1967) has stated that, if the temperature range is not too wide,  $\ln e_s = a + b/t_k$ , (where  $e_s$  is the saturation vapor pressure (mm Hg),  $t_k$  is the Kelvin temperature, and  $a$  and  $b$  are constants). Over the temperature range of from 0 to 50°C the following equation was developed to compute  $e_s$ .

$$e_s = e^{(20.9166 - (5,293.06/(273.16 + t)))} \quad (29)$$

where  $e_s$  = saturation vapor pressure of the air at  $T$ , mm Hg

$e$  = natural logarithmic constant, 2.7183

$t$  = temperature, °C

273.16 = constant to convert temperature from °C to °K.

This equation yielded an  $r^2$  value of .999 between temperatures taken at 5°C intervals (0 to 50°C temperature range) and tabular  $e_s$  values. The density of water vapor in saturated air was found from the following equation:

$$\rho = (2.89 \cdot 10^{-4} \cdot e_s) / (273.16 + t) \quad (30)$$

where  $e_s$  = saturation vapor pressure at  $t$ , mm Hg

$t$  = temperature, °C

$2.89 \cdot 10^{-4}$  = constant to convert water vapor pressure in mm Hg  
to absolute humidity in  $\text{cm}^3 \text{H}_2\text{O} \cdot \text{cm}^{-3}$  air

273.16 = constant to convert temperature from  $^{\circ}\text{C}$  to  $^{\circ}\text{K}$ .

### Resistance measurements

Resistance measurements were calculated on the basis of Gaastra's (1959) model. However, the  $\text{CO}_2$  concentration at the chloroplast site was assumed equal to the  $\text{CO}_2$  compensation concentration ( $\Gamma$ ) instead of zero. The reader is referred to the Literature Review section on the  $\text{CO}_2$  diffusion process in Part I if interested in the validity of this assumption.

The sum of the resistances were found from the following equation:

$$(\Sigma r)_{\text{CO}_2} = (r_a + r_s + r_m)_{\text{CO}_2} = \frac{[\text{CO}_2]_m - [\text{CO}_2]_{\text{chl}}}{P \cdot 1.4138} \quad (31)$$

where  $\Sigma r$  = sum of resistance to diffusion of  $\text{CO}_2$  from the ambient air to the chloroplast,  $\text{sec} \cdot \text{cm}^{-1}$

$r_a$  = laminar or boundary layer resistance,  $\text{sec} \cdot \text{cm}^{-1}$

$r_s$  = stomatal resistance,  $\text{sec} \cdot \text{cm}^{-1}$

$r_m$  = mesophyll resistance,  $\text{sec} \cdot \text{cm}^{-1}$

$P$  = net photosynthesis at  $[\text{CO}_2]_m$ ,  $\text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$

$[\text{CO}_2]_m$  =  $\text{CO}_2$  concentration of the air over the leaf surface, ppm

$[\text{CO}_2]_{\text{chl}}$  =  $\text{CO}_2$  concentration at the chloroplast (assumed to equal  $\Gamma$ ), ppm

1.4138 = constant for converting  $P$  to  $\text{cm}^3 \text{CO}_2 \cdot \text{cm}^{-2} \cdot \text{sec}^{-1}$ .

The quantity  $(r_a + r_s)_{\text{CO}_2}$  was calculated from the measurement of transpiration, mean water concentration over the leaf, and water concentration in the intercellular spaces. This calculation is based on

the assumption that the transpiration rate is not a function of  $r_m$  (i.e., the walls of the intercellular spaces are assumed to be at saturation vapor pressure).

$$(r_a + r_s)_{CO_2} = \frac{D_{H_2O}}{D_{CO_2}} \cdot \frac{[H_2O]_{int} - [H_2O]_a}{T} \quad (32)$$

where  $T$  = transpiration,  $cm^3 H_2O \cdot cm^{-2} \cdot sec^{-1}$

$D_{H_2O}$  = diffusion coefficient of water vapor in air,  $.24 cm^2 \cdot sec^{-1}$

$D_{CO_2}$  = diffusion coefficient of  $CO_2$  in air,  $.4 cm^2 \cdot sec^{-1}$

$(r_a + r_s)_{CO_2}$  = resistance to  $CO_2$  diffusion through the boundary layer and stomata and intercellular spaces,  $sec \cdot cm^{-1}$

$[H_2O]_{int}$  = water concentration in the intercellular spaces,  $cm^3 H_2O \cdot cm^{-3} air$

$[H_2O]_a$  = water concentration in air near the leaf surface,  $cm^3 H_2O \cdot cm^{-3} air$ .

For the calculation of  $(r_a + r_s)_{CO_2}$  it was necessary to measure  $T$ ,  $t_b$ ,  $t_a$ ,  $t_{wb}$ ,  $t_{wa}$ , and  $t_1$ . The mean leaf temperature ( $t_1$ ) was used in 1969 since leaf temperature measurements were taken on two flag leaves.

The working equation was as follows:

$$(r_a + r_s)_{CO_2} = \frac{.618934 \cdot 10^9}{T} ([H_2O]_{int} - [H_2O]_a) \quad (33)$$

where  $T$  = transpiration rate,  $mg H_2O \cdot dm^{-2} \cdot hr^{-1}$

$.618934 \cdot 10^9$  = constant to convert diffusion resistances in terms of  $H_2O$  vapor to  $CO_2$  diffusion resistances and to convert  $T$  measured in  $mg H_2O \cdot dm^{-2} \cdot hr^{-1}$  to  $cm^3 H_2O \cdot cm^{-2} \cdot sec^{-1}$ .

The quantities  $[H_2O]_{int}$  and  $[H_2O]_a$  were found from the following equations:

$$[\text{H}_2\text{O}]_{\text{int}} = \frac{2.89 \cdot 10^{-4} \cdot e_{s1}}{273.16 + t_1} \quad (34)$$

where  $2.89 \cdot 10^{-4}$  = constant to convert  $\text{H}_2\text{O}$  vapor pressure in mm Hg to absolute humidity in  $\text{cm}^3 \text{H}_2\text{O} \cdot \text{cm}^{-3}$

$273.16$  = constant to convert temperature from  $^{\circ}\text{C}$  to  $^{\circ}\text{K}$

$t_1$  = leaf temperature,  $^{\circ}\text{C}$

$e_{s1}$  = saturation vapor pressure at  $t_1$ , mm Hg .

In 1968 the  $[\text{H}_2\text{O}]_a$  was assumed equal to the mean water vapor concentration of the inlet and outlet air. However, because of the mixing fan in the chambers in 1969, the  $[\text{H}_2\text{O}]_a$  term was assumed equal to the water vapor concentration of the air leaving the chamber. The equation used to compute  $[\text{H}_2\text{O}]_a$  in 1969 was as follows:

$$[\text{H}_2\text{O}]_a = \frac{2.89 \cdot 10^{-4}}{273.16 + T_a} (e_{\text{swa}} + K(t_{\text{wa}} - t_a)) \quad (35)$$

where  $t_a$ ,  $t_{\text{wa}}$ ,  $K$ , and  $e_{\text{swa}}$ , are the same as in Equations 27 and 28.

In 1968 the following equation was used:

$$(\text{H}_2\text{O})_a = \frac{2.89 \cdot 10^{-4}}{273.16 + \left(\frac{t_b + t_a}{2}\right)} \cdot \left[ \frac{e_{\text{swa}} + e_{\text{swb}}}{2} - K \left( \frac{t_b - t_a}{2} - \frac{t_{\text{wa}} - t_{\text{wb}}}{2} \right) \right] \quad (36)$$

where symbols are the same as in Equations 27 and 28.

Boundary layer resistance ( $r_a$ ) was determined by a method adopted by Gaastra (1959) where  $r_a$  was assumed to be a function of evaporation ( $E$ ) from a saturated blotter. In this situation resistance is assumed a function of the boundary layer only, since there is no blotter resistance to diffusion of water. In 1968 it was observed that evaporation

was related to blotter size (Table 3). The measurements of  $r_a$  obtained in 1968, however, were believed in error. Condensation occurred in the leaf chamber when E from larger size blotters was measured, and uniform turbulence probably did not exist in the chambers because mixing fans were not used. Evaporation rates from blotters were not measured after every leaf, consequently, a constant value of evaporation was used in the calculations. The value of E used was  $8.3 \text{ g H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{hr}^{-1}$  in 1968, because a few of the genotypes had small flag leaves. The equation used to compute  $(r_a)_{\text{CO}_2}$  in 1968 was the same as the right side of Equation 32 used to compute  $(r_a + r_s)_{\text{CO}_2}$ , except that evaporation (E) was substituted in place of transpiration (T). This method of calculating  $r_a$  (with a constant E value) proved unsatisfactory. Differences in  $(r_a)_{\text{CO}_2}$ , because of variations in leaf size, were not accounted for, and fluctuations of chamber air temperature, which would be expected to affect the evaporation rate, were not considered in this method.

In 1969 a much better method of computing  $r_a$  was developed. Because the size of the experiment prevented measurements of evaporation after every leaf, some procedure had to be developed whereby estimates of evaporation could be obtained for leaves of various sizes at various air temperatures. Evaporation rates from saturated blotters of various sizes, ranging from .1 to .5  $\text{dm}^2$  were measured at various air temperatures in 1969. The data was then analyzed by regression analysis and the following equation was developed to predict E of various sized blotters

Table 3. Evaporation from the wet surface of different size blotters measured in 1968 at a mean blotter temperature of 34.2°C

Blotter size, dm <sup>2</sup>	Evaporation rate, g H <sub>2</sub> O·dm <sup>-2</sup> ·hr <sup>-1</sup> <sup>a</sup>
.20	2.3
.15	3.2
.10	4.3
.05	8.3

<sup>a</sup>Values represent means of measurements taken with both leaf chambers.

at various air temperatures in the chamber:

$$Y = 17,774.7 - 42,827.2X_1 + 45,139.9X_1^2 - 691.6X_2 + 23.2X_2^2 \quad (37)$$

where  $Y$  = evaporation, mg H<sub>2</sub>O·dm<sup>-2</sup>·hr<sup>-1</sup>

$X_1$  = area, dm<sup>2</sup>

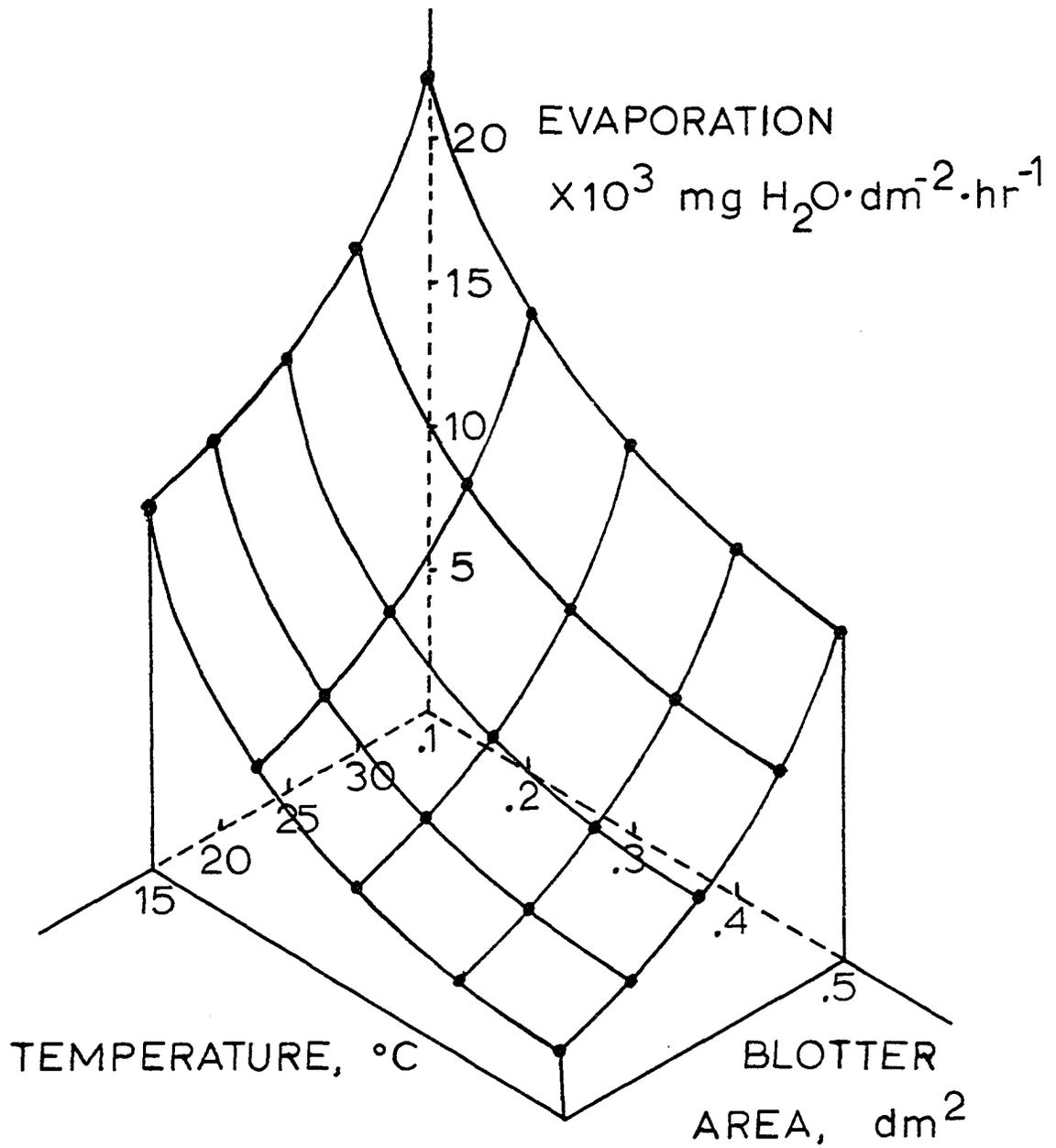
$X_2$  = chamber air temperature, °C.

The multiple  $r^2$  value of the equation was .898 (Figure 8b).

To estimate  $E$  for blotters the same size as the leaves tested and under similar chamber air temperature conditions, the leaf area was substituted in place of  $X_1$  and the air temperature in place of  $X_2$ . The predicted  $E$  rates were then substituted into the equation used to calculate  $(r_a + r_s)_{CO_2}$ , except  $E$  replaced  $T$ . Blotter temperature was assumed equal to leaf temperature.

$$(r_a)_{CO_2} = \frac{D_{H_2O}}{D_{CO_2}} \cdot \left( \frac{[H_2O]_{int} - [H_2O]_a}{E} \right) \quad (38)$$

Figure 8b. Response surface for evaporation from various sizes of saturated blotters at different chamber air temperatures. The regression equation and  $r^2$  value are also shown.



$$\text{EVAP.} = 17774.7 - 42827.2(\text{AREA}) + 45139.9(\text{AREA})^2 - 691.6(\text{AIR TEMP}) + 23.2(\text{AIR TEMP})^2$$

$$r^2 = .898$$

Mesophyll resistance  $(r_m)_{CO_2}$  and stomatal resistance  $(r_s)_{CO_2}$  were determined by subtraction by the following equations:

$$(r_s)_{CO_2} = (r_a + r_s)_{CO_2} - (r_a)_{CO_2} \quad (39)$$

$$(r_m)_{CO_2} = (r_a + r_s + r_m)_{CO_2} - (r_a + r_s)_{CO_2} \quad (40)$$

Because of the mathematical way  $r_m$  is computed, it can be regarded as a "residual" resistance.

Examination of the 1968 results indicated many of the  $r_m$  values were in error. Low values and sometimes negative values of  $r_m$  were obtained. The mean transpiration rate of the 20 genotypes in 1968 was  $3.1 \text{ g H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{hr}^{-1}$ , whereas in 1969 it was  $5.3 \text{ g H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{hr}^{-1}$ . Because the light flux density was greater in 1968 than in 1969 ( $3.02\cdot 10^5$  versus  $1.73\cdot 10^5 \text{ erg}\cdot\text{sec}^{-1}\cdot\text{cm}^{-2}$ ) and the leaf temperature was slightly greater (32.6 versus 31.0 °C), a greater transpiration rate would perhaps be expected in 1968 because of an increased radiation load on the leaf. The relative humidity conditions in the leaf chambers were not computed for every leaf tested in 1968. However, calculations made on leaves selected at random indicated relative humidity conditions of about 50 percent in the 1968 leaf chambers. Humidity conditions in the leaf chambers in 1969 averaged 40.3 percent across all 20 genotypes. Tests conducted in 1969 (see preliminary results on humidity) showed that transpiration decreased as the relative humidity increased. However, these differences in conditions could not explain the large discrepancy observed between 1968 and 1969 transpiration rates and, hence, the

observed differences in resistance data. Thus, it is concluded that the psychrometer was probably not functioning properly in 1968, and the negative  $r_m$  values were believed a result of artificially high  $(r_a + r_s)$  values resulting from inaccurate transpiration measurements. Therefore, the 1968 transpiration and diffusion resistances were considered invalid. The 1969 results appeared to be measured accurately. The mean  $r_a$ ,  $r_s$ , and  $r_m$  values, in terms of  $CO_2$  diffusion resistances, for the 20 genotypes were .94, 1.36, and 4.48  $sec \cdot cm^{-1}$ , respectively. These values agree favorably with those reported by El-Sharkawy and Hesketh (1965) for the oat variety Markton. They reported  $r_a$ ,  $r_s$ , and  $r_m$  values of 1.1, 1.7, and 4.1  $sec \cdot cm^{-1}$ , respectively.

#### Relative humidity measurements

In 1969 the relative humidities of the air streams passing through the psychrometer and leaf chamber were calculated for each set of four leaves tested. Since the air temperature in the leaf chamber exceeded that of air flowing through the psychrometer, measurements of relative humidity taken on air samples as they passed through the psychrometer did not represent relative humidity conditions in the leaf chamber. This is because relative humidity is temperature dependent. Hot air holds more water than cold air, but if the volume of water in the air (absolute humidity) is held constant, the cold air will have a higher relative humidity. Relative humidities of the inlet and outlet air streams flowing through the psychrometer were found by the following equations:

$$RH = \frac{e}{e_s} \cdot 100 = \frac{e_w - K(t - t_w)}{e_s} \cdot 100 \quad (41)$$

where RH = relative humidity, percent

$e$  = vapor pressure of the air at  $T$ , mm Hg

$e_s$  = saturation vapor pressure of the air at  $t$ , mm Hg

$e_w$  = saturation vapor pressure of the air at  $t_w$ , mm Hg

$K$  = psychrometric constant, .5 mm Hg·°C<sup>-1</sup>

$t$  = dry bulb temperature, °C

$t_w$  = wet bulb temperature, °C.

To calculate relative humidity conditions at leaf chamber air temperatures, it was assumed that density of water vapor in air (absolute humidity) passing through the inlet sample stream into the psychrometer was the same as that passing into the leaf chamber. Similarly, the absolute humidity of the outlet sample air stream was assumed to equal that of the air passing out of the leaf chamber. These assumptions are valid, providing condensation does not occur in the system. No condensation was believed to occur, because temperatures of tubes and coils, through which the air streams passed, were above the dew point temperature at the highest relative humidities used in the experiments in 1969.

Absolute humidity was calculated from the following equation:

$$\rho = \frac{2.89 \cdot 10^{-4} \cdot e}{273.16 + t} = \frac{2.89 \cdot 10^{-4} \cdot e}{273.16 + t_c} \quad (42)$$

where  $\rho$  = absolute humidity, cm<sup>3</sup> H<sub>2</sub>O·cm<sup>-3</sup> air

$e$  = vapor pressure of air at  $t$ , mm Hg

$e_c$  = vapor pressure of air at  $t_c$ , mm Hg

$t$  = dry bulb air temperature, °C

$t_c$  = chamber air temperature, °C

273.16 = constant to convert °C to °K

$2.89 \cdot 10^{-4}$  = constant to convert  $H_2O$  vapor pressure in mm Hg to absolute humidity in  $cm^3 H_2O \cdot cm^{-3}$  air.

Rearrangement of Equation 42 gave the following equation:

$$e_c = ((273.16 + t_c) \cdot e) / (273.16 + t) \quad (43)$$

from which the relative humidity at chamber temperatures could be calculated.

$$RH_c = \frac{e_c}{e_{sc}} \cdot 100 \quad (44)$$

where  $RH_c$  = relative humidity in the chamber at  $t_c$ , percent

$e_c$  = vapor pressure of air at  $t_c$ , mm Hg

$e_{sc}$  = saturation vapor pressure at  $t_c$ , mm Hg.

Relative humidity conditions in the chamber were assumed to be represented by relative humidity conditions of air leaving the chamber at chamber air temperatures. This was based on the assumption that  $n$  in Equation 22 was large.

The vapor pressure deficit between the leaf and chamber air was calculated from the following equation:

$$VPD = e_{s1} - e_c \quad (45)$$

where VPD = vapor pressure deficit between the leaf and chamber air, mm Hg

$e_{s1}$  = saturation vapor pressure at  $t_1$ , mm Hg

$e_c$  = vapor pressure of air at  $t_c$ , mm Hg.

The vapor pressure deficit of air in the chamber was computed from the following equation:

$$VPD = e_{sc} - e_c \quad (46)$$

where VPD = vapor pressure deficit of air in the chamber, mm Hg

$e_{sc}$  = saturation vapor pressure of air at  $t_c$ , mm Hg

$e_c$  = vapor pressure of air at  $t_c$ , mm Hg.

In 1969 the mean VPD between the leaf and the chamber air for the 20 genotypes was 19.6 mm Hg, and the mean VPD of the air was 21.1 mm Hg.

#### Leaf measurements

The procedure for determining laminar area and leaf weight per unit area was similar in 1968 and 1969.

Leaf area Leaf laminar area measurements in 1968 were taken by the procedure used by Stoy (1965). The width of each leaf at the base and at 50 mm intervals was measured, and calculations of the total laminar area were based on the assumption that each leaf lamina was composed of a number of trapezoids, with parallel sides 50 mm apart, and with a triangle at the tip of the leaf.

In 1969, widths of each of the four flag leaf midportions, enclosed in the chamber, were measured at 40 mm intervals. Laminar area was found by summing the areas of eight trapezoids. Occasionally differences in flag leaf insertion heights or very small flag leaves necessitated calculations involving triangulation formulae because the leaf did not occupy the full 80 mm width of the leaf chamber. Areas were expressed in  $dm^2$  units.

Leaf weight per unit area      Leaf fresh weight and leaf dry weight per unit area were measured in 1968 and 1969. Fresh weight measurements were taken at the end of the day with an analytical balance to the nearest ten thousandth of a gram. Leaves were sealed in moisture proof vials to prevent loss of moisture during the day as the samples were collected. After fresh weight measurements were made, the caps on the vials were removed, and the plant material was dried to constant weight at 77°C for 23 hours. The dry weight of the plant material was then determined. The leaf fresh weight and dry weight per unit area will hereafter be referred to as the specific leaf fresh weight (SLFW) and specific leaf dry weight (SLDW). Both are expressed as  $\text{g}\cdot\text{dm}^{-2}$  leaf area.

#### Preliminary Experiments

Before experimental results were obtained, a series of preliminary tests were conducted to insure that plant material was tested under near optimal conditions. Most of the tests were conducted in 1969, because time limitations prevented most of the tests in 1968. Preliminary tests were conducted during the day and analyzed at night. Consequently, if any part of the system was functioning improperly, remedial measures could be made the following day. A Fortran program facilitated rapid analysis of the data. Periodic analyses of data were also made during the 1969 experimental period to insure that the psychrometer and the remainder of the system were functioning properly.

Tests were usually designed to investigate only one aspect which might affect experimental results. Effects of different windspeeds, temperatures, relative humidities, and light conditions on net photosynthesis, transpiration, and CO<sub>2</sub> diffusion rates were investigated. Length of induction period required for maximum CO<sub>2</sub> exchange rates, stability of photosynthesis over long time periods, and CO<sub>2</sub> exchange rates in attached versus detached leaves were also examined.

### Windspeed

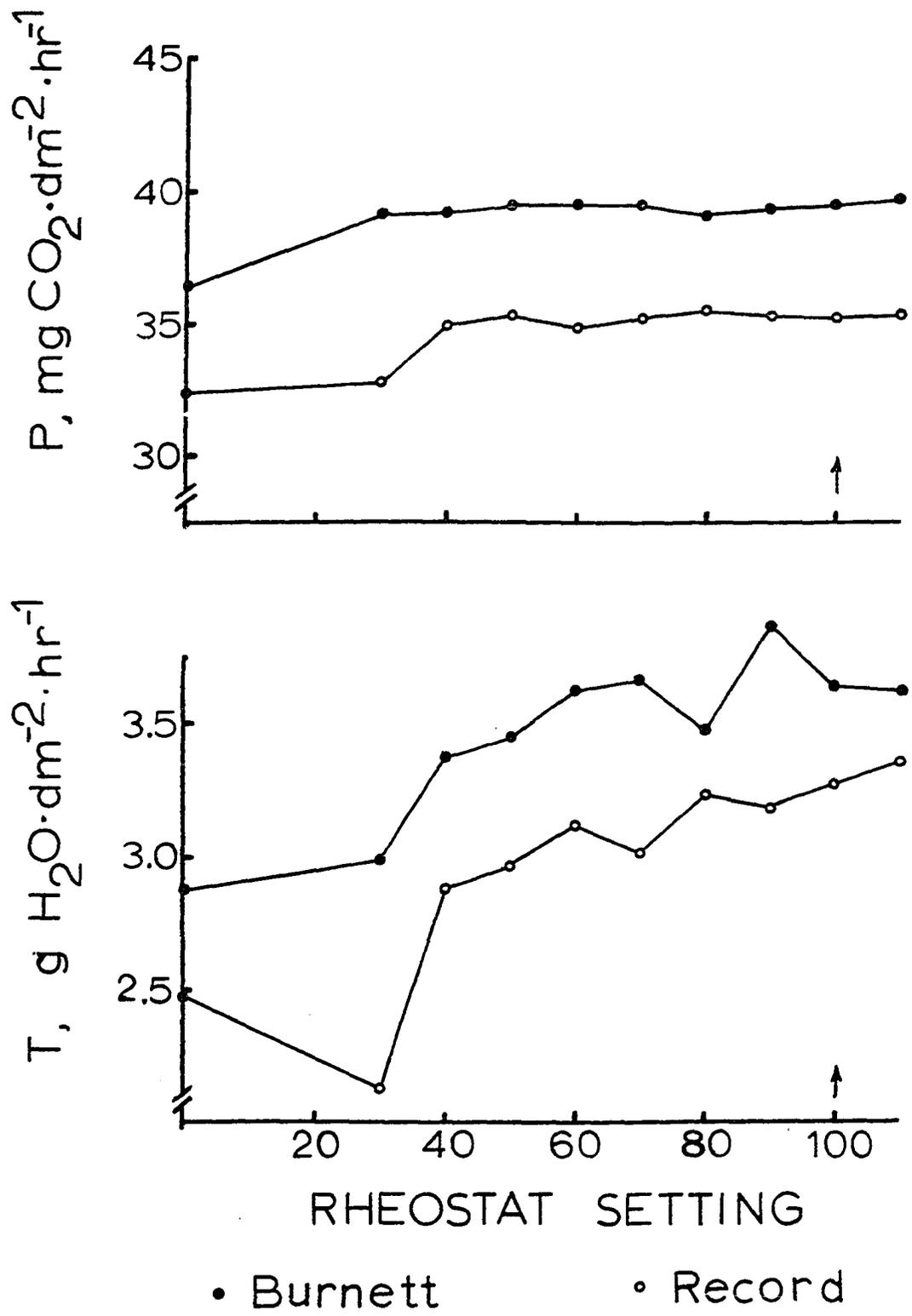
It is important to have enough turbulence so that laminar or boundary layer resistance to CO<sub>2</sub> diffusion is small. For this reason, windspeed tests were conducted on the fifth leaves of varieties Burnett and Record in 1969. Windspeed measurements could not be made inside the chambers; consequently, photosynthetic and transpiration measurements were expressed as a function of the rheostat setting used to control the internal fan speed (Figure 9). Transpiration was depressed more at lower rheostat settings than net photosynthesis. This is because in the equations:

$$T = \Delta[\text{H}_2\text{O}]/(r_a + r_s)_{\text{H}_2\text{O}} \quad (47)$$

$$P = \Delta[\text{CO}_2]/(r_a + r_s + r_m)_{\text{CO}_2} \quad (48)$$

the laminar resistance ( $r_a$ ) makes up a larger proportion of the denominator in Equation 47 than in the equation for photosynthesis. A rheostat setting of 100 was used in 1969 experiments. Evaporation rates from blotters, and all subsequent CO<sub>2</sub> exchange rate measurements were

Figure 9. Net photosynthesis (top) and transpiration rates (bottom) as a function of the rheostat setting used to control turbulence in the 1969 leaf chambers. Each point represents the mean of two tests per genotype. Conditions: CO<sub>2</sub> concentration = 320 ppm; light flux density =  $1.73 \cdot 10^5$  ergs·sec<sup>-1</sup>·cm<sup>-2</sup>; leaf temperature = 25.9°C; leaves = fifth; varieties = Record and Burnett; date = May 26, 1969.



made under similar turbulence conditions.

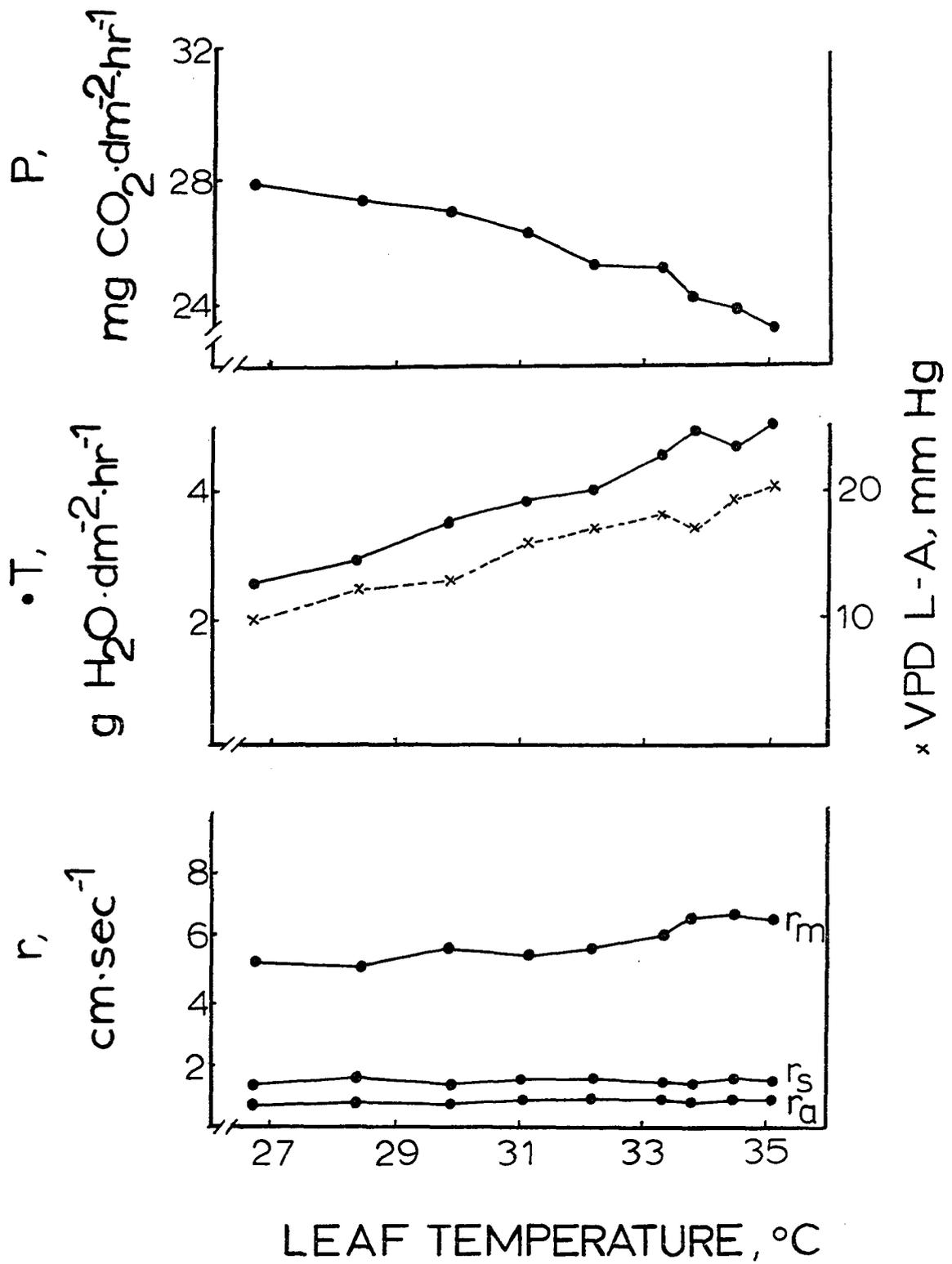
The aspiration speed of air over the leaf surface in 1968 was approximately  $40.7 \text{ cm}\cdot\text{min}^{-1}$ . Internal turbulence was dependent largely upon the flow rate of air through the tubular chamber.

### Temperature

Optimum temperature conditions for maximum  $\text{CO}_2$  exchange rates are important, because if enzymatic photosynthetic reactions are depressed by unfavorable temperature conditions, a high mesophyll resistance will result. This is because mesophyll resistance is a "residual" resistance by nature of the calculations.

It has been found that oat flag leaves show an optimum temperature for net photosynthesis of about  $25^\circ\text{C}$  (Criswell, 1968) which is similar to that reported in other temperate grass species (Murata and Iyama, 1963b). The temperature response curve for the photosynthetic process in oats is quite broad (Criswell, 1968). Limitations of the apparatus prevented varying the temperature over a wide range. However, temperature response for net photosynthesis could be obtained over a limited range by circulating warm water through the water jackets on the chambers. The temperature was continuously increased. Figure 10 indicates that at temperatures of  $31.1^\circ\text{C}$  the photosynthetic rate was only slightly reduced from that at  $26.7^\circ\text{C}$  ( $28.3$  versus  $29.8 \text{ mg CO}_2\cdot\text{dm}^{-2}\cdot\text{hr}^{-1}$ ). Four temperature response curves, conducted on fifth leaves of oats gave similar results. Leaf temperature of the 20 genotypes averaged  $32.6$  and  $31.0^\circ\text{C}$  in 1968 and 1969, respectively.

Figure 10. Net photosynthesis (top), transpiration and vapor pressure deficit (middle), and CO<sub>2</sub> diffusion resistances (bottom) as a function of leaf temperature. Only the results of one test are shown in the figure. Conditions: CO<sub>2</sub> concentration = 320 ppm; light flux density =  $1.73 \cdot 10^5$  ergs $\cdot$ sec<sup>-1</sup> $\cdot$ cm<sup>-2</sup>; leaves = fifth; variety = Record; date = May 31, 1969.



Transpiration increased linearly as the vapor pressure deficit between the leaf and air increased. The CO<sub>2</sub> diffusion resistances remained relatively stable until high temperatures were reached. Mesophyll resistance increased slightly at high temperatures.

#### Relative humidity

Four relative humidity tests were conducted on fifth leaves of oat varieties Burnett and Record. Relative humidity was increased progressively by increasing the temperature of the ethylene glycol bath CTB<sub>1</sub> (Figure 3). All four tests gave similar results.

Net photosynthesis was not affected by changes in concentration of water vapor in the atmosphere (Figure 11). The transpiration rate was observed to decline as the relative humidity of the air increased. Similar results have been noted in sugarbeet leaves by Nevins and Loomis (1970). The decline in transpiration was accompanied by a decrease in the vapor pressure deficit between the leaf and the air. Resistances to CO<sub>2</sub> diffusion remained relatively constant over the range of humidities used in the tests. Mean relative humidity of the air in leaf chambers for the twenty oat genotypes used in the screening experiment was approximately 50 percent in 1968 and 40.3 percent in 1969.

#### Induction and stability

Tests were conducted in 1969 to insure that leaves were fully induced before experimental observations were made. It was found that upon illumination, oat leaves (fifth leaves) required approximately 15 minutes to obtain stable net photosynthetic rates (Figure 12). Maximum

Figure 11. Net photosynthesis and leaf temperature as a function of relative humidity (top). Transpiration and the vapor pressure deficit as a function of relative humidity (middle) and the CO<sub>2</sub> diffusion resistances at various relative humidities (bottom). Only the results of one test are shown in the figure. Conditions: CO<sub>2</sub> concentration = 320 ppm; light flux density =  $1.73 \cdot 10^5$  ergs·sec<sup>-1</sup>·cm<sup>-2</sup>; leaves = fifth; variety = Record; date = May 30, 1969.

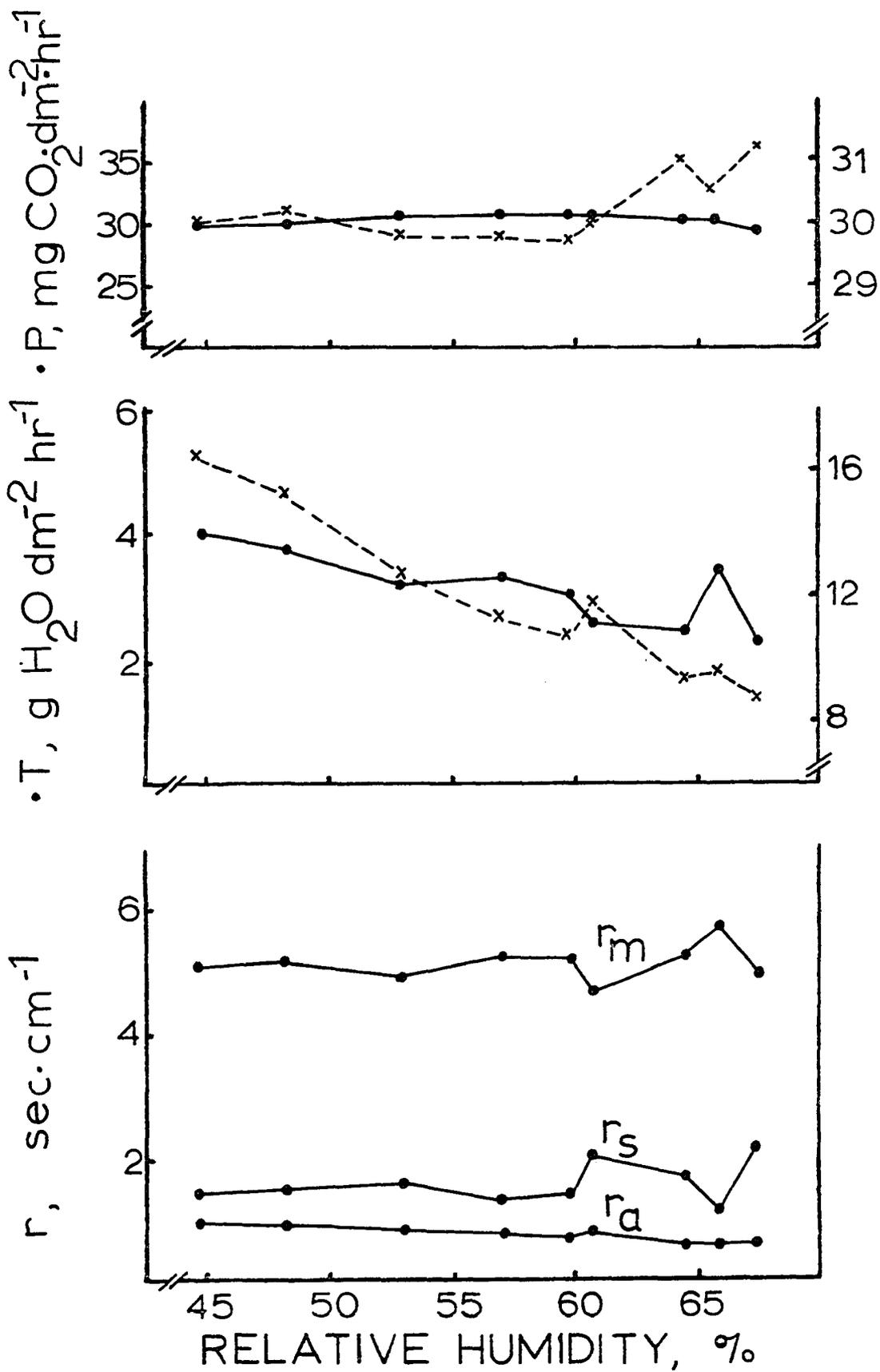
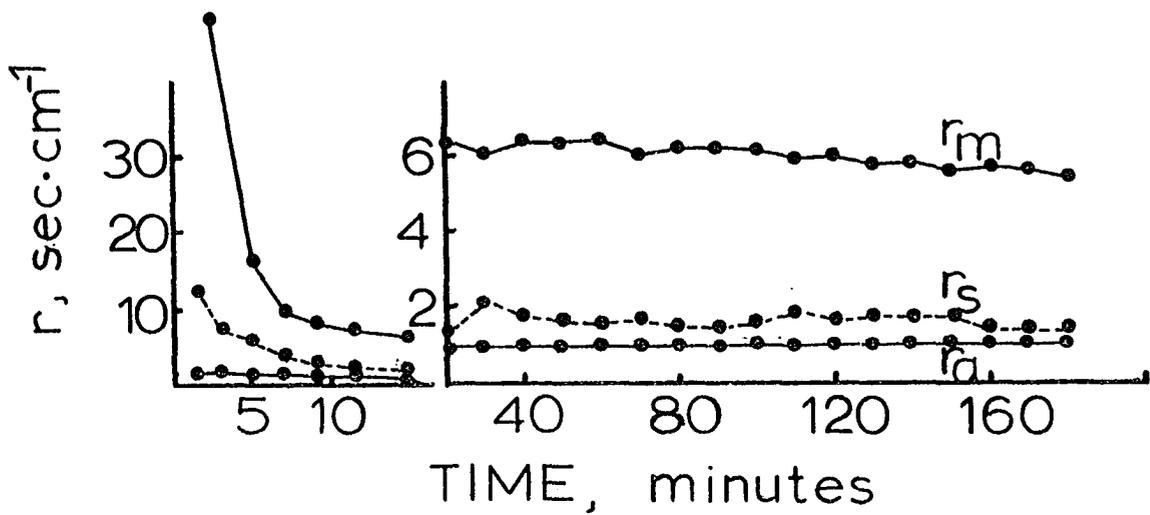
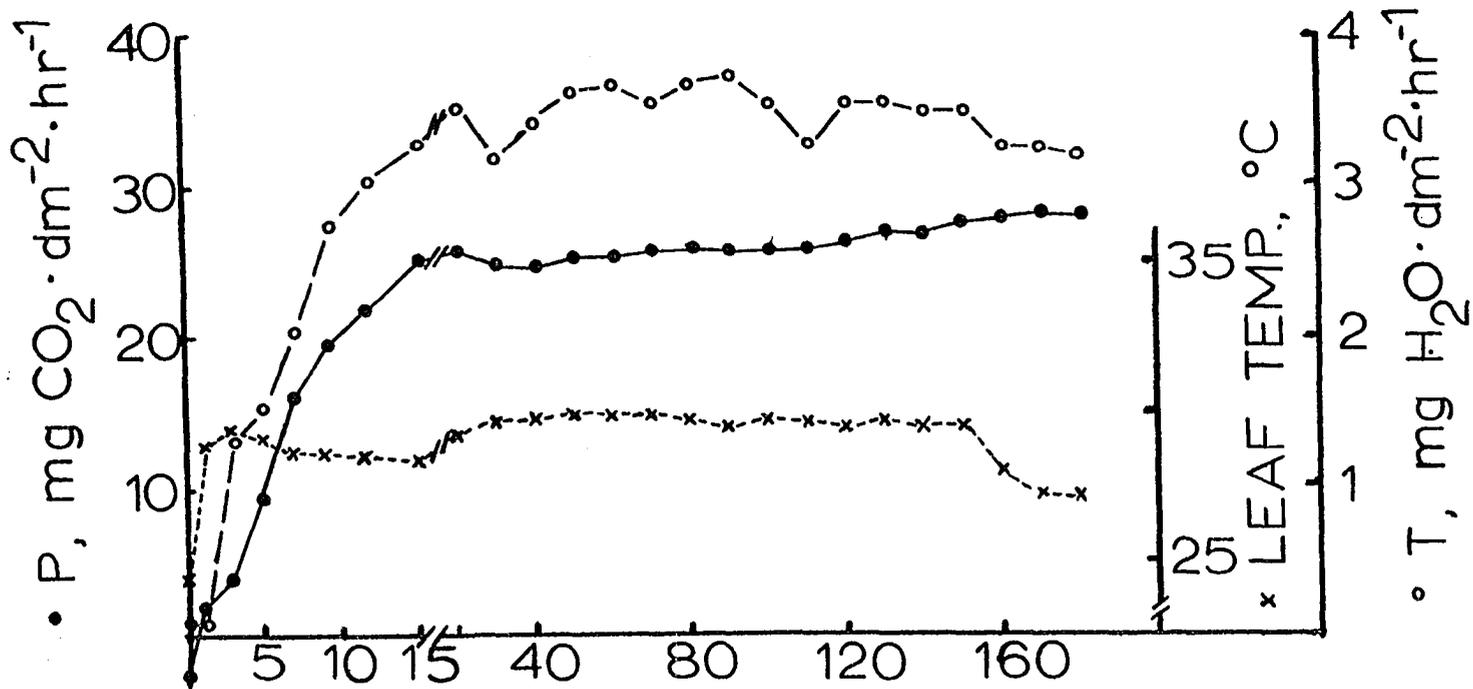


Figure 12. ● = net photosynthesis, ○ = transpiration, and x = leaf temperature as a function of time (top). Lights were turned on at time 0. CO<sub>2</sub> diffusion resistances are shown as a function of time (bottom). Only the results of one test are shown in the figure. Conditions: CO<sub>2</sub> concentration = 320 ppm; light flux density =  $1.73 \cdot 10^5$  ergs·sec<sup>-1</sup>·cm<sup>-2</sup>; leaves = fifth; variety = Burnett; date = June 2, 1969.



transpiration rates were also obtained after about 15 minutes. Leaf temperature initially increased, reaching a maximum after about 3 minutes. It is believed the initial rise of leaf temperature was because of the increased radiation load on the leaf which was transpiring slowly upon illumination. After the stomata opened, transpiration increased and eventually stabilized. When this occurred, leaf temperature declined slightly. Nevins and Loomis (1970) have interpreted this phenomenon in sugarbeets similarly. Evidence that stomates open upon illumination is supported by the fact that stomatal resistance decreased 356 percent from 3 to 15 minutes after illumination. All leaves tested in 1968 and 1969 were induced for periods of at least 15 minutes.

Stable net photosynthetic rates usually could be maintained for periods of 3 hours or longer. Test periods generally did not exceed 45 minutes in 1968 or 30 minutes in 1969. The photosynthetic rate of the leaf shown in Figure 12 actually increased 12.7 percent over the period of from 15 to 180 minutes. Another test showed a 7.5 percent increase in photosynthetic rate over this time period. Only two tests were conducted because each test required considerable time. The CO<sub>2</sub> diffusion resistances remained relatively stable throughout the duration of the test period. The reason for the increase in leaf temperature after about 15 minutes and the depression of leaf temperature after about 140 minutes is unknown.

#### Light flux density

One of the assumptions upon which the CO<sub>2</sub> diffusion equations of Gaastra (1959) is based is that photosynthesis is light saturated.

Tests conducted in 1969 on the sixth leaves of Burnett and Record oat varieties showed the photosynthetic process to be nearly light saturated at a light flux density of  $1.73 \cdot 10^5$  ergs $\cdot$ sec $^{-1}$  $\cdot$ cm $^{-2}$ , the light level used in 1969 (Figure 13). In 1968 a light flux density of  $3.02 \cdot 10^5$  ergs $\cdot$ sec $^{-1}$  $\cdot$ cm $^{-2}$  was used.

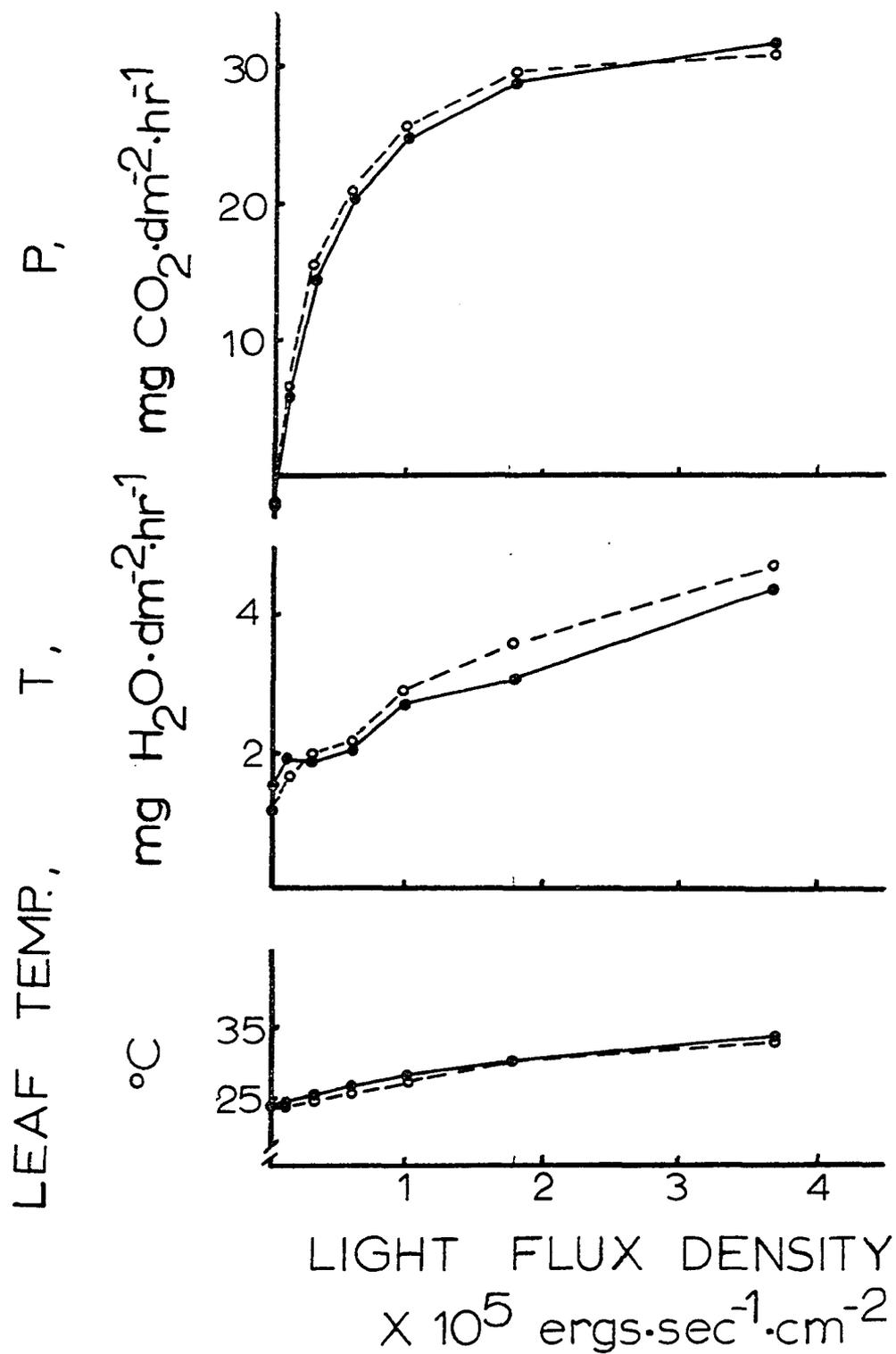
Transpiration increased linearly as the radiation load on the leaf increased. The increase was probably partially a result of greater leaf temperatures. Limitations of the apparatus prevented maintenance of constant leaf temperature conditions when the light flux density increased.

Shown in Figure 14 are the CO<sub>2</sub> diffusion resistances, plotted as a function of light. Laminar resistance remained relatively constant, whereas, at lower light levels, stomatal resistance decreased slightly as the light flux density increased. Mesophyll resistance decreased rapidly as the light flux density increased, indicating photochemical processes were limiting photosynthesis at low light levels. This is why the photosynthetic process should be light saturated if repeatable and meaningful CO<sub>2</sub> diffusion resistance data are to be obtained.

#### Leaf detachment

The experiments performed in 1968 and 1969 were conducted on attached leaves. It would appear from preliminary results, however, that detached leaves could have been used, providing high relative humidity conditions were maintained and measurements were not extended over long periods of time.

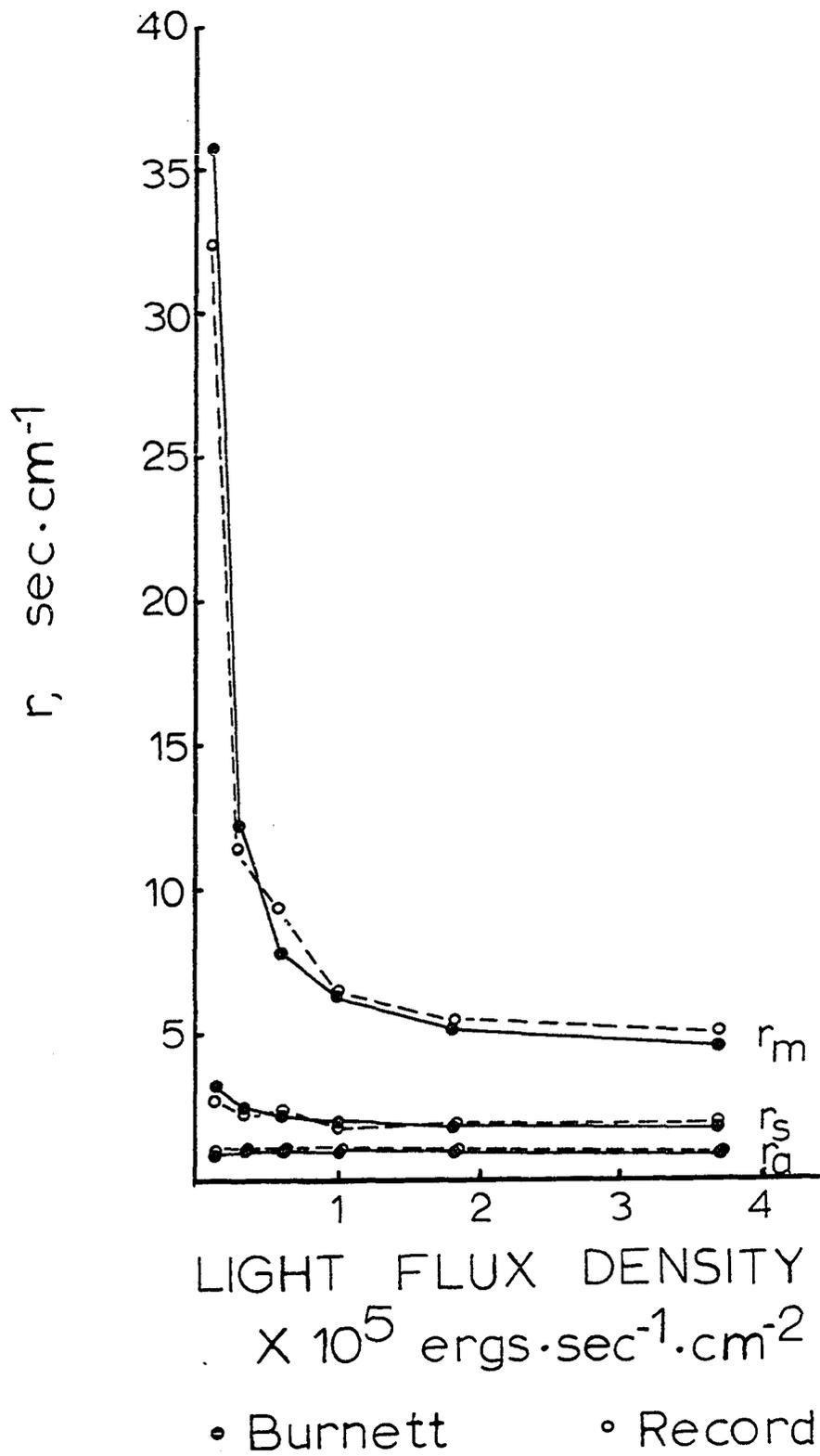
Figure 13. Net photosynthesis (top), transpiration (middle), and leaf temperature (bottom) as a function of light flux density. Each point represents the mean of three observations per genotype. The three observations were conducted on different leaves. Conditions: CO<sub>2</sub> concentration = 320 ppm; leaves = sixth; varieties = Burnett and Record; date = June 6, 1969.



• Burnett

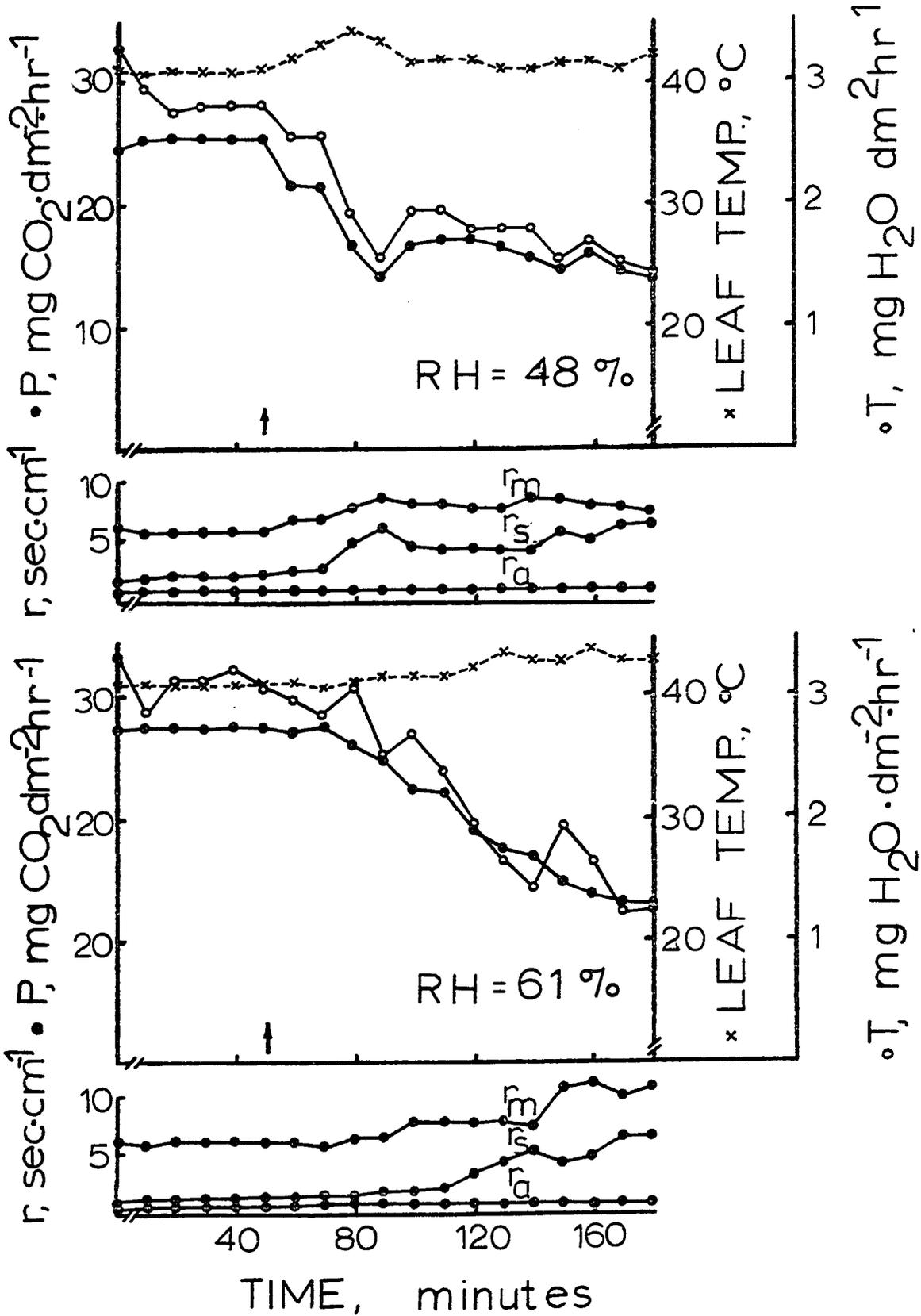
◦ Record

Figure 14. CO<sub>2</sub> diffusion resistances as a function of light flux density. Each point represents the mean of three observations per genotype. The three observations were conducted on different leaves. Conditions: CO<sub>2</sub> concentration = 320 ppm; leaves = sixth; varieties = Burnett and Record; date = June 6, 1969.



Experiments conducted on fifth leaves of varieties Burnett and Record showed reductions of photosynthesis and transpiration upon leaf detachment (Figure 15). Leaf blades were cut and recut under distilled water after they had been allowed to photosynthesize under steady state conditions for one hour. When leaves were excised under low relative humidity conditions, the net photosynthetic rate and transpiration rate rapidly declined. After approximately 30 to 50 minutes partial recovery of photosynthesis and transpiration occurred, followed by a slow gradual subsequent decline. When leaves were excised under higher relative humidity conditions, the photosynthetic rate and transpiration rates remained stable for 20 to 40 minutes before declining. It would appear from the stomatal resistance values obtained, that high humidities are needed to prevent stomatal closure in excised leaves. Evidence for stomatal closure at low relative humidities is also provided by the increase in leaf temperature, and depression of transpiration and photosynthesis, following leaf detachment. That the stomates reopened, or at least partially reopened following leaf excision under low relative humidities, is evident from the fact that  $r_s$  and leaf temperature declined, whereas transpiration and photosynthetic rates increased. The results also seem to indicate fluctuations in mesophyll resistance; however, it is not known why these occurred. At all times the leaves were well supplied with water.

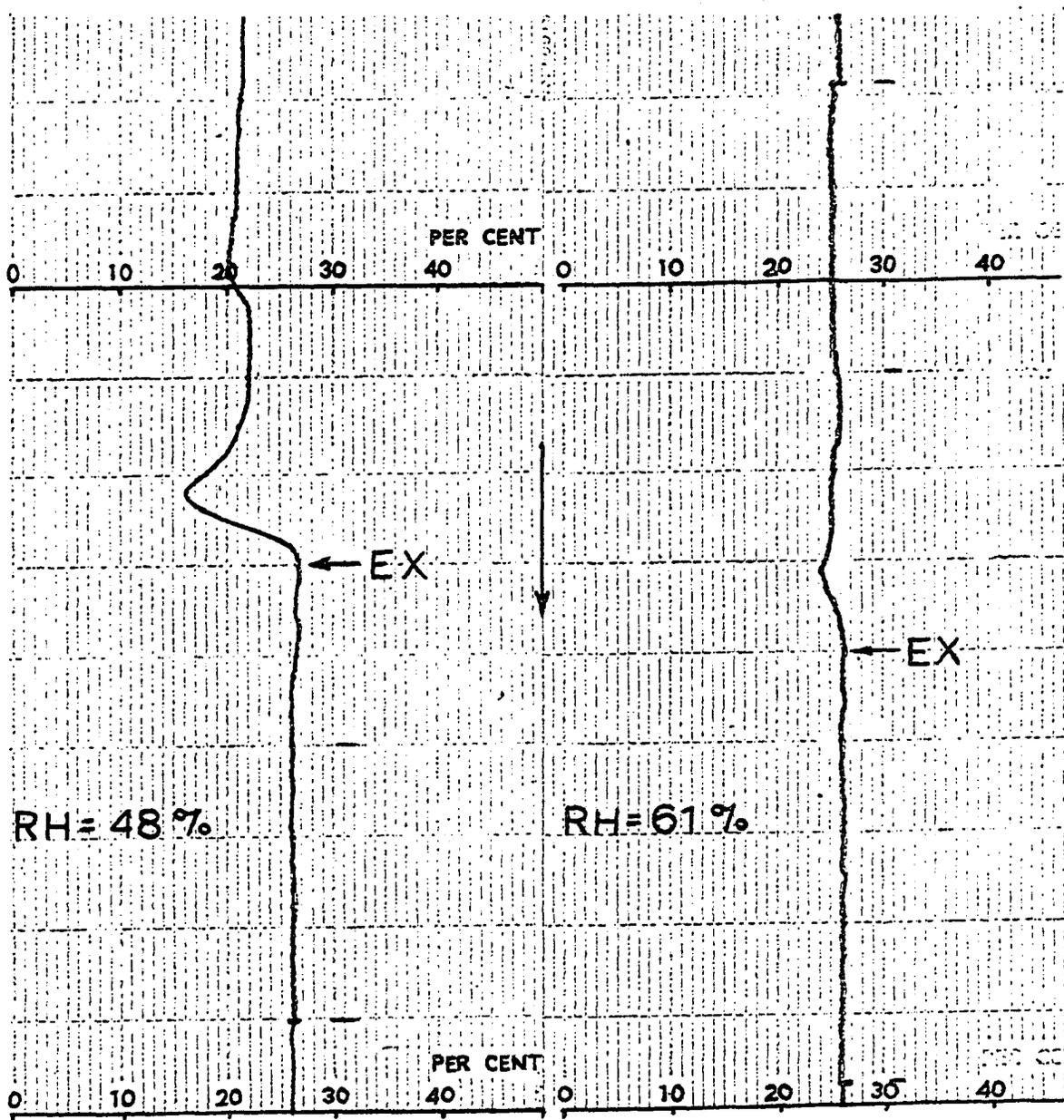
Figure 15. ● = net photosynthesis, ○ = transpiration, x = leaf temperature, and CO<sub>2</sub> diffusion resistances as a function of time after leaf detachment under low relative humidity (RH) conditions are shown above. The same variables are shown as a function to time below, except that the leaves were excised and tested under higher relative humidity conditions. The vertical arrows indicate the time of leaf excision. The leaves were excised while in the chamber. Conditions: CO<sub>2</sub> concentration = 320 ppm; light flux density =  $1.73 \cdot 10^5$  ergs·sec<sup>-1</sup>·cm<sup>-2</sup>; leaves = sixth; variety = Record; date = June 3-4, 1969.



An interesting feature, shown in Figure 16, was the variation in the response following leaf detachment under high and low relative humidity conditions. The  $\text{CO}_2$  differential was reduced almost immediately (within 10 to 20 seconds) when leaves were detached under low relative humidities, whereas under high relative humidities,  $\text{CO}_2$  differential depressions hardly could be observed. It would appear that perhaps some sort of a "shock" response occurred following leaf excision.

It is concluded from the preliminary results that most of the conditions for optimal photosynthetic rates were met in 1968 and 1969. Cooler leaf temperatures were desired. However, limitations of the apparatus prevented cooler leaf temperatures at the light flux densities used in these experiments. This was not believed to have influenced the experimental results, because temperature response curves for photosynthesis showed only slight photosynthetic rate depressions (1.4 to 6.6 percent) at temperatures of approximately  $31^\circ\text{C}$ , as opposed to lower temperatures--near  $25^\circ\text{C}$ . Also stable photosynthetic rates could be measured for periods up to at least 3 hours in length, indicating no detrimental effects from higher leaf temperatures.

Figure 16. Recorder traces showing the effect of leaf detachment on  $\text{CO}_2$  differentials under low (left) and high (right) relative humidity conditions. Each vertical division represents .99 ppm  $\text{CO}_2$ . The chart paper moved in the direction of the vertical arrow at a rate of one-half inch per minute. The point at which the leaves were excised is indicated by the symbol EX. Conditions are the same as those shown in Figure 15.



## RESULTS

The objectives of the oat genotype screening experiment were threefold: (1) to determine whether or not differences in net photosynthesis occurred, (2) to study the relationship between net photosynthesis and specific leaf weight, and (3) to study physiological factors affecting the net photosynthetic rate of oat flag leaves. To fulfill these objectives, the data were analyzed for genotypic differences in: net photosynthesis (P), CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light (R), CO<sub>2</sub> compensation concentration ( $\Gamma$ ), slope of the CO<sub>2</sub> response curves (S), specific leaf fresh weight (SLFW), specific leaf dry weight (SLDW), transpiration (T), photosynthesis/transpiration ratio (P/T), sum of CO<sub>2</sub> diffusion resistance ( $\Sigma r$ ), laminar resistance to CO<sub>2</sub> diffusion ( $r_a$ ), stomatal resistance to CO<sub>2</sub> diffusion ( $r_s$ ), and mesophyll resistance to CO<sub>2</sub> diffusion ( $r_m$ ).

## Statistical Analyses

Data was analyzed by a completely random design model. This design was chosen since all flag leaves were tested at or near the time of panicle emergence, and all tests were conducted under similar conditions. Single degree of freedom orthogonal comparisons were also conducted in 1968 and 1969. Simple correlation coefficients were obtained both years for all variables measured.

Analyses of variance

According to Steel and Torrie (1960), a completely random design is useful in many types of laboratory experiments where treatments are

randomly assigned and where environmental effects are much alike. The advantage of flexibility is achieved because the number of treatments and replicates is limited only by the number of experimental units available. The statistical analysis is simple, and the number of error degrees of freedom is maximum--an important factor in small laboratory experiments where the number of replicates may be limited. The main disadvantage of the completely random design is that, since randomization is unrestricted, experimental error includes the entire variation among experimental units except that due to treatments. However, in the oat screening experiment no source of variation, other than treatments (genotypes) and error, was known or anticipated. The oat genotypes were randomly assigned a location in the experimental site and grown under the same environmental conditions until tests were initiated. Because the genotypes were tested as each reached a specific stage of development, rather than randomly, the environmental conditions to which the genotypes were exposed immediately before testing were not necessarily the same. The flag leaves were tested under similar conditions, however. Twenty genotypes were used, and 9 and 8 test replications were measured in 1968 and 1969, respectively.

The F ratios obtained from the analyses of variance are shown in Table 4. Complete analyses of variance tables are presented in Table 23 of the Appendix. Because the differential psychrometer appeared to be functioning improperly in 1968, transpiration and CO<sub>2</sub> diffusion resistance measurements, which depended upon transpiration or evaporation

measurements, were considered invalid. The specific leaf fresh weight (SLFW) and specific leaf dry weight (SLDW) had to be analyzed with lesser degrees of freedom in 1968, because leaf samples of the variety Richland were lost. This occurred as the result of a thermostat failure on the oven used to dry the plant material which had been tested.

#### Single degree of freedom orthogonal comparisons

Steel and Torrie (1960) state that an F test with more than one degree of freedom for the numerator is an average test of as many independent comparisons as there are degrees of freedom (19 in the oat screening experiment). If only one of the comparisons is significantly different, and if this comparison is averaged with a number of non-real differences, then a test of this average might fail to detect the real difference. For this reason, single degrees of freedom orthogonal comparisons were conducted. It was believed this would be useful in detecting genotypic differences, if variation in the experimental variables tested was associated with differences in ploidy level, species (within hexaploids), origin, or productivity within Midwestern varieties. The restriction for orthogonality was met in that the sum of products of the coefficients of any two comparisons equalled zero. Meaningful comparisons, which were independent, in so far as it was possible, were made on mean values of each variable. The sums of squares value obtained for each comparison was then multiplied by the number of replications and tested against the error mean square value obtained with the completely random design for the variable of interest. This could be done since the following relationship exists:

$$\frac{(\sum c_i Y_i)^2}{r \sum c_i^2} \cong \frac{r(\sum c_i \bar{Y}_i)^2}{\sum c_i^2} = \begin{array}{l} \text{Sums of squares of} \\ \text{the comparison} \end{array} \quad (49)$$

where  $Y_i$  = treatment totals based on the same number of observations

$\bar{Y}_i$  = treatment means

$c$  = coefficients for orthogonal comparisons

$r$  = number of replications.

The results of the single degree of freedom orthogonal comparisons are shown in Tables 5 and 6. Statistically significant differences occurred both years; however, differences were not generally associated with differences in ploidy level, species (within hexaploids), origin, or productivity level with Midwestern varieties.

### Correlation

A correlation matrix of all variables was obtained for each genotype. A correlation matrix was also obtained for mean values of variables for each of the genotypes. This latter correlation matrix provided the relationship between variables over all genotypes, whereas the individual correlation matrices for each genotype gave the relationship of variables within a particular genotype. The correlation coefficients are shown in Tables 24 and 25 of the Appendix. Simple correlation coefficients of various factors between years are shown in Table 7.

### Genotypic Variation

#### Net photosynthesis

Within the range of CO<sub>2</sub> concentrations used (0 to 400 ppm) the rate of net CO<sub>2</sub> exchange was linearly related to the mean CO<sub>2</sub> concentration

over the leaf surface (Table 26 of the Appendix). Only 5 out of the 180 regression analyses of net CO<sub>2</sub> exchange versus the mean CO<sub>2</sub> concentration over the leaf surface were not significant at the 1 percent level. The proportions of the total sums of squares attributable to linear regression ( $r^2$ ) were high.

Significant differences in net photosynthetic rate measured at 300 and 320 ppm CO<sub>2</sub> in 1968 and 1969, respectively, were detected (Table 4). In 1968 the mean net photosynthetic rate of the genotypes ranged from 24.2 to 47.9 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup> (Table 8). Net photosynthetic rates measured in 1969 were generally lower, ranging from 21.8 to 31.9 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>. The genotypes ranked similarly both years, and the correlation of 1968 with 1969 net photosynthetic rates was significant at the 5 percent level ( $r = .51$ , Table 7).

Net photosynthetic rates were not associated with differences in ploidy level. Significant photosynthetic differences between ploidy levels were indicated both years in the orthogonal comparisons (Tables 5 and 6); however, the differences were not consistent between years. Mean net photosynthetic rates of genotypes of differing ploidy level are shown in Table 9. Stomata counts were not taken, but reduced numbers of stomata in hexaploids and tetraploids did not appear to limit photosynthesis, because stomatal resistance values between ploidy levels were not significantly different. Bjurman (1959) explained lower photosynthetic rates in tetraploid Ribes satigrum plants by the fact that tetraploids had fewer stomata per unit area than diploids. Only two

genotypes of Ribes sativum were used in his study, however.

Net photosynthetic rates differed between species. The two A. byzantina genotypes (A-465 and Curt) had a slightly higher mean net photosynthetic rate than the mean of A. sterilis genotypes (P.I. 296234 and P.I. 292546) used in this experiment (Table 9). This comparison was significantly different in 1969, but these species did not differ significantly in 1968 (Tables 5 and 6). The inference can be made that A. byzantina genotypes (A-465 and Curt) had a slightly higher mean net photosynthetic rate than the mean of A. fatua genotypes (C.I. 1779 and C.I. 2528) (Table 9). It should be noted that the variety Appler is an A. byzantina species; however, it could not be included in the single degree of freedom orthogonal comparisons with the other A. byzantina species (A-465 and Curt) if orthogonality was to be maintained. Mean net photosynthetic rate of the A. byzantina genotypes (A-465 and Curt) was approximately equal to the mean of the Midwestern adapted, A. sativa genotypes (Clintland-64 and Marion). Examination of Table 9 also shows that mean photosynthetic rate of A. fatua genotypes (C.I. 1779 and C.I. 2528) was similar to the mean of A. sterilis genotypes (P.I. 296234 and P.I. 292546), and did not appear to differ significantly. Thus, it was concluded that, of the four species tested in this study, the A. fatua and A. sterilis genotypes used had lower net photosynthetic rates than the A. byzantina and A. sativa genotypes tested. Almost identical results were reported earlier by Criswell (1968), when net photosynthetic rates of most of the same genotypes were measured at 31°C. However, it should be emphasized that only two genotypes of each species were tested in most cases.

Winter oat genotypes (Victorgrain and Appler) did not show a significantly different mean net photosynthetic rate from that of spring oat varieties (Bingham and Record) which are adapted to cool climates (Tables 5 and 6). The net photosynthetic rate of the variety Bingham significantly exceeded that of the variety Record by 11.7 and 10.1 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup> in 1968 and 1969, respectively.

It would seem that the net carbon balance of the plant would be a logical factor to consider when attempting to relate yield to physiological processes. Consequently, net photosynthetic rates of high and low yielding oat genotypes were examined. The high yielding Midwestern varieties (Garland and Burnett) showed a significantly greater mean net photosynthetic rate than the mean of low yielding Midwestern varieties (Richland and Goodfield) in 1968 (Table 5); however, significant differences did not occur in 1969 (Table 6). Of the two low yielding oat varieties, Goodfield showed the higher net photosynthetic rate both years (Table 5). The fact that the variety Goodfield showed a net photosynthetic rate approximately of the same magnitude to that of the high yielding genotypes, yet is a low yielding genotype, might be explained on the basis that yield is controlled by many factors. Photosynthesis is only one of the many interacting factors which ultimately determines yield.

#### CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light and CO<sub>2</sub> compensation points

The rate of CO<sub>2</sub> evolution into CO<sub>2</sub>-free air in light (El-Sharkawy and Hesketh, 1965), and the CO<sub>2</sub> compensation concentration (Tregunna *et al.*, 1966) have been accepted as estimates of photorespiration or

CO<sub>2</sub> not reassimilated. Therefore, it was of interest to measure these variables, because if differences in these processes occurred, such differences might be associated with genetic differences in net photosynthetic rates.

In both 1968 and 1969 highly significant differences in rates of CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light were observed (Table 4). Mean genotypic CO<sub>2</sub> efflux rates ranged from 3.1 to 9.1 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup> in 1968 and from 5.6 to 10.0 mg CO<sub>2</sub>·dm·hr<sup>-1</sup> in 1969 (Table 10). The 1968 and 1969 CO<sub>2</sub> efflux rates were positively correlated ( $r = .41$ ), but the correlation was not significant at the 5 percent level (Table 7).

Although the relationship between net photosynthesis (P) and CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light (R) was not tested within genotypes in 1968, because P and R data were analyzed in separate lots, the relationship was tested on genotype means. The 1968 correlation of P and R, using genotypic means, was positive, but it was not significant ( $r = .31$ ). In 1969 the correlation between P and R was tested both within and on genotype means. Within genotypes, the correlation coefficients were generally not significant, and they were variable (Tables 24 and 25 of the Appendix). The positive correlation of P and R, conducted on 1969 genotype means, was highly significant ( $r = .68$ ).

The mean CO<sub>2</sub> compensation points for the different oat genotypes ranged from 25.1 to 68.1 ppm CO<sub>2</sub> and 57.4 to 82.0 ppm CO<sub>2</sub> in 1968 and 1969, respectively (Table 11). CO<sub>2</sub> compensation points measured in 1968 were not significantly correlated with those measured in 1969 ( $r = -.11$ ).

The analysis of variance (Table 4) shows that CO<sub>2</sub> compensation points differed at the 5 percent level between genotypes in 1968; no significant differences were observed in 1969. Using the single degree of freedom orthogonal comparisons, however, a highly significant difference in CO<sub>2</sub> compensation points was found between the two A. sterilis genotypes P.I. 296234 and P.I. 292546, in 1969 (Table 6). The orthogonal comparisons gave a more sensitive test than the completely random design analysis. P.I. 292546 had a lower CO<sub>2</sub> compensation point than P.I. 296234 in 1969; examination of Table 11 showed the reverse occurred in 1968, however. In 1968 the differences were not significant (Table 5). Tetraploid genotypes (Glabrota and P.I. 193958) showed significantly lower mean CO<sub>2</sub> compensation points than the mean of the diploid genotypes (Saia and A. brevis) in 1968, but the difference between the two ploidy levels was not significant in 1969. Both of the average yielding Midwestern adapted genotypes (Clintland-64 and Marion) showed a significantly higher mean CO<sub>2</sub> compensation point than the high and low productivity genotypes (Richland, Goodfield, Garland, and Burnett). These were the only significant differences in CO<sub>2</sub> compensation points detected.

CO<sub>2</sub> efflux rates into CO<sub>2</sub>-free air in light (R) were positively correlated with the CO<sub>2</sub> compensation points ( $\Gamma$ ), within a genotype and on genotypic means. Correlation coefficients on genotype means were .80 and .75 in 1968 and 1969, respectively.

#### Transpiration rates and photosynthesis/transpiration ratios

Significant genotypic differences in transpiration rate were observed in 1969 (Table 4). Mean transpiration rates of various genotypes,

which ranged from 3.93 to 7.78 g H<sub>2</sub>O·dm<sup>-2</sup>·hr<sup>-1</sup>, are ranked from low to high in Table 12. Transpiration measurements were based on water vapor differentials at 320 ppm CO<sub>2</sub> and leaf temperatures of 31.0±1.5°C.

Plants utilizing water economically would be expected to show high photosynthesis transpiration (P/T) ratios. The P/T ratios of the 20 oat genotypes were found to differ significantly (Table 4). Table 12 shows mean P/T ratios ranked from high to low for the different genotypes. Mean P/T ratios ranged from 3.9 to 7.2 mg CO<sub>2</sub>·mg H<sub>2</sub>O<sup>-1</sup>·10<sup>-3</sup>. It is interesting that most Midwestern oat varieties and genotypes which originated in arid climates (A-465 and Curt) showed higher P/T ratios than many unadapted or unimproved genotypes. This, however, may be the result of an experimental artifact, which is explained in the Discussion section.

Net photosynthesis (P) generally was positively correlated with transpiration (T); however, the 1969 correlation between P and T on genotype means was not significant (Table 25 of the Appendix). Similarly, in no cases were correlations between R and T significant in 1969.

#### CO<sub>2</sub> diffusion resistances

Diffusion resistances were measured in both 1968 and 1969. However, because of psychrometer malfunctions and inability to accurately obtain laminar resistance estimates in 1968, confidence was placed in only the 1969 measurements. The sum of the CO<sub>2</sub> diffusion resistances ( $\Sigma r$ ) was calculated by the slope method of Holmgren *et al.* (1965);

consequently, the slope of the CO<sub>2</sub> response curve (S) was highly negatively correlated with  $\Sigma r$ . Genotypic differences of  $\Sigma r$  and S were found significant at the 1 percent level in both 1968 and 1969 (Table 4). The mean slopes of the various genotypes, ranked from highest to lowest, are shown in Table 13. The mean  $\Sigma r$  values, ranked from low to high, are also shown in the same table. Mean slopes, averaged across the 20 genotypes, were .131 and .106 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>·ppm CO<sub>2</sub><sup>-1</sup> in 1968 and 1969, respectively. Mean  $\Sigma r$  values for the different genotypes ranged from 4.48 to 8.53 sec·cm<sup>-1</sup> and 5.64 to 8.40 sec·cm<sup>-1</sup> in 1968 and 1969, respectively. The 1968 and 1969 slopes of the CO<sub>2</sub> response curves were significantly correlated at the 1 percent level ( $r = .64$ ), and the sum of the resistances to CO<sub>2</sub> diffusion were correlated over years at the 5 percent level ( $r = .46$ ).

Net photosynthesis (P) and the sum of the CO<sub>2</sub> diffusion resistances ( $\Sigma r$ ) were negatively correlated, both within genotypes and on genotype means. The correlation coefficients ( $r$ ) on genotype means were  $-.91$  and  $-.96$  in 1968 and 1969, respectively. Similarly, the rate of CO<sub>2</sub> efflux was negatively correlated with  $\Sigma r$ .

Highly significant genotypic differences were found in 1969 for laminar resistance ( $r_a$ ), stomatal resistance ( $r_s$ ), and mesophyll resistance ( $r_m$ ) (Table 4). Table 14 shows the genotypic means, ranked from low to high, of the three CO<sub>2</sub> diffusion resistances. Genotypic mean  $r_a$  values ranged from .80 to 1.02 sec·cm<sup>-1</sup>, and the mean  $r_s$  values ranged from .96 to 1.91 sec·cm<sup>-1</sup> in 1969. The  $r_m$  values were considerably

larger, and genotype means ranged from 3.43 to 5.48  $\text{sec}\cdot\text{cm}^{-1}$ . Mean values of  $r_a$ ,  $r_s$ , and  $r_m$  for all 20 genotypes were .94, 1.36, and 4.48  $\text{sec}\cdot\text{cm}^{-1}$ , respectively. The coefficient of variability was largest for the  $r_s$  determinations, which was apparently associated with greater error or variability in transpiration estimates. Examination of the orthogonal comparisons (Table 6) showed differences in  $\text{CO}_2$  diffusion resistances occurred at random; however, the comparisons in which significant photosynthetic and transpiration differences were found also often showed significant  $\text{CO}_2$  diffusion resistance differences.

Weak, none significant, negative correlations were found in 1969 between laminar resistance ( $r_a$ ) and P. However, highly significant negative correlations were found between P and stomatal resistance ( $r_s$ ) in 1969. Similarly, the correlation of P with  $r_m$  was found to be significantly negatively correlated. These correlations are shown in Table 25 of the Appendix.  $\text{CO}_2$  diffusion resistances were generally negatively correlated with  $\text{CO}_2$  efflux rates; however, of the correlations conducted on genotype means, only the correlation of R and  $r_m$  was significant ( $r = .76$ ).

Transpiration rates (T) were generally negatively correlated with  $\Sigma r$ ,  $r_a$ , and  $r_s$  within genotypes. However, only the correlations of T versus  $r_a$  and T versus  $r_s$  were found to be significant when correlations were conducted on genotype means ( $r = -.94$  and  $r = -.51$ , respectively). The correlation between T and  $r_m$  was not significant. This was because transpiration is a function of  $r_a$  and  $r_s$ , but not  $r_m$ . Also the calculation of  $r_m$  uses the residual of  $\Sigma r$  not attributable to  $(r_a + r_s)$ .

Specific leaf fresh weight and specific leaf dry weight

The specific leaf fresh weight (SLFW) and specific leaf dry weight (SLDW) were examined in both 1968 and 1969. There is evidence that fresh weight per unit area (Heichel and Musgrave, 1969) or dry weight per unit area (Pearce et al., 1969) may be positively correlated with net photosynthetic rates in some agronomic crops. SLFW and SLDW were found to differ significantly between genotypes in both 1968 and 1969 (Table 4). The mean SLDW and SLFW of each of the oat genotypes are shown ranked from high to low, in Table 15. The mean SLDW's and SLFW's were similar both years when averaged across all genotypes.

From Table 7 it can be noted that genotypic mean SLDW values were significantly correlated between years. SLFW values obtained in 1968 and 1969 were also significantly correlated.

The SLFW and SLDW were not consistently correlated in any manner with net photosynthesis (P) within genotypes (Tables 24 and 25 of the Appendix). When correlation coefficients on genotype means were examined, however, it was observed that correlations between P and SLDW were significant at the 1 percent level ( $r = .75$  and  $r = .58$  in 1968 and 1969, respectively). The SLFW, however, was not significantly correlated with P when correlation coefficients were obtained on genotype means ( $r = .12$  and  $r = .40$  in 1968 and 1969, respectively).  $\text{CO}_2$  efflux rates into  $\text{CO}_2$ -free air in light (R) were not significantly correlated with either the SLDW or SLFW. Within genotypes the SLDW's were often significantly positively correlated with the SLFW's, but the relationship was not significant when correlation coefficients were obtained on

genotype means.

It is interesting that mesophyll resistance ( $r_m$ ) was often negatively correlated with the SLDW within genotypes, and the correlation on genotype means was significant at the 5 percent level ( $r = -.55$ ). Stomatal resistance ( $r_s$ ) was not significantly correlated with the SLFW, and correlations within genotypes between  $r_s$  and SLDW were variable and generally not significant. The correlation of  $r_s$  versus SLDW on genotype means was not significant ( $r = -.17$ ). Laminar resistance ( $r_a$ ) was not associated with either SLFW or SLDW ( $r$  on genotype means were .23 and  $-.40$ , respectively). No correlation was expected, however, since  $r_a$  is an external resistance.

Flag leaves with large leaf areas did not have a significantly higher SLFW and SLDW. The correlations of mean leaf area with SLFW and SLDW were .14 and .15, respectively, in 1968. In 1969 the correlations of mean leaf area with SLFW and SLDW were .31 and  $-.39$ , respectively.

#### Leaf temperature and leaf area

In 1969 significant genotypic differences in leaf temperatures were found; however, when the genotypes were ranked, differences in mean leaf temperatures appeared to be small (Table 16). Over the range of leaf temperatures occurring in the experiment, photosynthesis was found to be significantly negatively correlated at the 5 percent level with leaf temperature ( $r = -.52$  and  $r = -.50$  in 1968 and 1969, respectively). This negative correlation was not unexpected, because photosynthetic measurements were made at temperatures slightly above

the optimum for net photosynthesis. The depression of net photosynthesis over this temperature range is small (approximately 5 percent), however, and it is believed that small differences in mean leaf temperatures did not influence the interpretation of the results appreciably (refer to Preliminary Experiments in the Methods and Materials section).

Dornhoff (1969) noted higher leaf temperatures in soybeans appeared to be associated with larger leaf areas. Mean laminar areas, which were measured, are ranked for the various genotypes in Table 16. The mean laminar area per leaf measured in 1969 was smaller than that measured in 1968, because only the midportions of the flag leaf laminae were measured. However, more total leaf area was generally measured because four lamina midportions were measured simultaneously in 1969. Mean genotypic leaf temperatures were not significantly positively correlated with mean genotypic leaf areas in 1968 ( $r = .17$ ), but in 1969 genotypic mean leaf temperatures were significantly negatively correlated with mean genotypic leaf areas ( $r = -.65$ ). Thus, it would appear that in oats, no consistent relationship between mean genotypic leaf temperature and leaf size exists.

#### Experimental Variability

The coefficients of variability were from 40 to 60 percent lower in 1969 than in 1968 (Tables 8, 10, 11, 12, 13, 14, and 15). The standard deviations between treatment (genotype) means were also reduced in 1969. The reduction in experimental variability in 1969 is believed largely attributable to the fact that a larger amount of plant

material from four different plants was used in the leaf chamber in 1969. In 1968, measurements were taken on only one oat flag leaf during each test. Conversion of the absolute type CO<sub>2</sub> analyzer to a differential type CO<sub>2</sub> analyzer probably also reduced errors involved in CO<sub>2</sub> exchange measurements.

Table 4. Analyses of variance results obtained in 1968 and 1969

Source	F ratio	
	1968	1969
Net photosynthesis, P	5.14**	4.75**
CO <sub>2</sub> efflux, R	2.40**	2.42**
CO <sub>2</sub> compensation concentration, $\Gamma$	1.69*	.98
Slope, S	5.54**	6.68**
Specific leaf fresh weight, SLFW	4.80**	22.53**
Specific leaf dry weight, SLDW	6.49**	13.48**
Sum of resistances, $\Sigma r$	2.71**	5.66**
Transpiration, T		7.73**
Photosynthesis/transpiration ratio, P/T		11.81**
Laminar resistance, $r_a$		8.72**
Stomatal resistance, $r_s$		2.15**
Mesophyll resistance, $r_m$		6.26**

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

Table 5. Single degree of freedom orthogonal comparisons in 1968<sup>a</sup>

Comparison	P	R	Γ	S	SLFW	SLDW	Σr
1	**			**	**	* *	**
2		**	**				
3	**	**		**		**	
4	*		*			**	*
5	**			**	*		
6					**		
7					*	**	
8							
9						*	
10							
11	**			**	**	*	
12							
13	*				*		
14							
15						*	
16	**	*		**			*
17						**	
18	**			**	--	--	**
19							

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

<sup>a</sup>Comparisons: 1 = diploid and tetraploid genotypes versus hexaploid genotypes; 2 = diploids versus tetraploids; 3 = unadapted hexaploids versus Midwest hexaploids; 4 = average versus low and high yielding Midwestern *A. sativa* spp.; 5 = low versus high yielding Midwestern *A. sativa* spp.; 6 = unadapted *A. fatua*, *A. sterilis*, and *A. byzantina* spp. versus cool climate and winter oat genotypes; 7 = *A. fatua* spp. versus *A. sterilis* and *A. byzantina* spp.; 8 = *A. sterilis* spp. versus *A. byzantina* spp.; 9 = cool climate versus winter oat genotypes; 10 = Saia versus *A. brevis*; 11 = Glabrota versus P.I. 193958; 12 = C.I. 1779 versus C.I. 2528; 13 = P.I. 296234 versus P.I. 292546; 14 = A-465 versus Curt; 15 = Clintland-64 versus Marion; 16 = Record versus Bingham; 17 = Victorgrain versus Appler; 18 = Richland versus Goodfield; 19 = Garland versus Burnett.

Table 6. Single degree of freedom orthogonal comparisons in 1969<sup>a</sup>

Comparison	P	R	r	T	S	SLFW	SLDW	$\Sigma r$	r <sub>a</sub>	r <sub>s</sub>	r <sub>m</sub>
1				**		**	**		**		
2	*			**	*	*	**	*	**		**
3	**	**	*	*	**	*		**	**		**
4											
5							*		*		
6						**	**				
7	*				*	**	**	*	**		**
8	*			**	*			**	**		**
9						**	*				
10		*		*	**	*		*			
11	**			**	**	**	**	**	**		*
12						**		*		**	
13	*		**								
14						**	**				*
15						**					
16	**	**		**	**	**		**	**	*	**
17						**	**				
18						**					
19					*						

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

<sup>a</sup>Comparisons: 1 = diploid and tetraploid genotypes versus hexaploid genotypes; 2 = diploids versus tetraploids; 3 = unadapted hexaploids versus Midwest hexaploids; 4 = average versus low and high yielding Midwestern A. sativa spp.; 5 = low versus high yielding Midwestern A. sativa spp.; 6 = unadapted A. fatua, A. sterilis, and A. byzantina spp. versus cool climate and winter oat genotypes; 7 = A. fatua spp. versus A. sterilis and A. byzantina spp.; 8 = A. sterilis spp. versus A. byzantina spp.; 9 = cool climate versus winter oat genotypes; 10 = Saia versus A. brevis; 11 = Glabrota versus P.I. 193958; 12 = C.I. 1779 versus C.I. 2528; 13 = P.I. 296234 versus P.I. 292546; 14 = A-465 versus Curt; 15 = Clintland-64 versus Marion; 16 = Record versus Bingham; 17 = Victor-grain versus Appler; 18 = Richland versus Goodfield; 19 = Garland versus Burnett.

Table 7. Simple correlation coefficients (r) of various factors between years (Degrees of freedom = 18)

Variables correlated 1968 versus 1969	r
Net photosynthesis	.51*
CO <sub>2</sub> efflux into CO <sub>2</sub> free air in light	.41
CO <sub>2</sub> compensation concentration	-.11
Slope of the CO <sub>2</sub> response curve	.64**
Specific leaf fresh weight	.74** <sup>a</sup>
Specific leaf dry weight	.55* <sup>a</sup>
Sum of CO <sub>2</sub> diffusion resistances	.46*

\* r value exceeds 5% level of significance.

\*\* r value exceeds 1% level of significance.

<sup>a</sup>Degrees of freedom = 17 since leaves of one genotype were lost.

Table 8. Mean net photosynthetic rates (P) in  $\text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ 

1968		1969	
Genotype	$P^a$	Genotype	$P^b$
Glabrota	47.9	Bingham	31.9
Goodfield	40.7	Burnett	29.8
Burnett	40.4	<u>A. brevis</u>	29.3
Garland	39.4	Goodfield	28.9
Saia	38.4	Glabrota	28.1
Bingham	36.2	A-465	28.0
P.I. 193958	35.3	Marion	27.3
Victorgrain	34.4	Clintland-64	26.8
<u>A. brevis</u>	34.1	Garland	26.5
A-465	34.1	Victorgrain	26.4
P.I. 296234	33.6	P.I. 292546	26.3
Appler	31.7	Curt	26.3
Clintland-64	31.2	Saia	26.3
C.I. 2528	31.0	Richland	25.7
Marion	29.8	C.I. 2528	24.8
Curt	28.8	Appler	24.5
C.I. 1779	26.7	P.I. 296234	22.9
P.I. 292546	24.8	P.I. 193958	22.7
Record	24.5	C.I. 1779	22.5
Richland	24.2	Record	21.8
$\bar{X}$	33.3		26.3
$S_{\bar{d}}$	3.8		1.7
CV	24.3		13.0

<sup>a</sup>Net photosynthesis at 300 ppm.

<sup>b</sup>Net photosynthesis at 320 ppm.

Table 9. Net photosynthetic rates of oat genotypes differing in ploidy level, species (within hexaploids), origin, and productivity level (Net photosynthetic rates are expressed in units of  $\text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ )

Comparison	Photosynthetic rate	
	1968	1969
Differences in ploidy level		
Diploids		
Saia	38.4	26.3
<u>A. brevis</u>	34.1	29.3
$\bar{X}$	36.3	27.9
Tetraploids		
Grabrota	47.9	28.1
P.I. 193958	35.3	22.4
$\bar{X}$	41.6	25.4
Hexaploids		
C.I. 1779	26.7	22.5
C.I. 2528	31.0	24.8
P.I. 296234	33.6	22.9
P.I. 292546	24.8	26.3
A-465	34.1	28.0
Curt	28.8	26.3
Clintland-64	31.2	26.8
Marion	29.8	27.3
Record	24.5	21.8
Bingham	36.2	31.9
Victorgrain	34.4	26.4
Appler	31.7	24.5
Richland	24.2	25.7
Goodfield	40.7	28.9
Garland	39.4	26.5
Burnett	40.4	29.8
$\bar{X}$	32.0	26.3

Table 9. (Continued)

Comparison	Photosynthetic rate	
	1968	1969
Differences in species (within hexaploids)		
<u>A. fatua</u>		
C.I. 1779	26.7	22.5
C.I. 2528	31.0	24.8
$\bar{X}$	28.9	23.7
<u>A. sterilis</u>		
P.I. 296234	33.6	22.9
P.I. 292546	24.8	26.3
$\bar{X}$	29.2	24.6
<u>A. bysantina</u>		
A-465	34.1	28.0
Curt	28.8	26.3
$\bar{X}$	31.5	27.2
<u>A. sativa</u>		
Clintland-64	31.2	26.8
Marion	29.8	27.3
$\bar{X}$	30.5	27.1
Differences in origin		
Cool climate		
Record	24.5	21.8
Bingham	36.2	31.9
$\bar{X}$	30.4	26.9
Red oat region		
Victorgrain	34.4	26.4
Appler	31.7	24.5
$\bar{X}$	33.1	25.5

Table 9. (Continued)

Comparison	Photosynthetic rate	
	1968	1969
Differences in productivity		
Low productivity		
Richland	24.2	25.7
Goodfield	40.7	28.9
$\bar{X}$	32.5	27.3
High productivity		
Garland	39.4	26.5
Burnett	40.4	29.8
$\bar{X}$	39.9	28.2

Table 10. Mean CO<sub>2</sub> efflux rates (R) into CO<sub>2</sub>-free air in light in mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>

1968		1969	
Genotype	R	Genotype	R
C.I. 2528	3.1	Record	5.6
P.I. 296234	3.7	P.I. 292546	5.9
Record	3.7	C.I. 1779	6.4
Glabrota	4.1	P.I. 193958	6.8
P.I. 193958	4.4	Appler	6.8
C.I. 1779	4.4	C.I. 2528	6.9
Richland	4.6	Curt	7.3
P.I. 292546	4.8	Victorgrain	7.4
A-465	5.5	Saia	7.5
Appler	6.0	Garland	7.7
Curt	6.0	P.I. 296234	7.8
A. brevis	6.1	Clintland-64	7.8
Victorgrain	6.2	A-465	7.9
Goodfield	6.6	Bingham	8.3
Burnett	6.8	Goodfield	8.4
Marion	7.3	Burnett	8.5
Bingham	7.4	Glabrota	8.7
Garland	7.9	Richland	8.9
Clintland-64	8.1	Marion	9.3
Saia	9.1	<u>A. brevis</u>	10.0
$\bar{X}$	5.8		7.8
$S_d$	1.5		1.0
CV	56.1		26.3

Table 11. Mean CO<sub>2</sub> compensation concentration ( $\Gamma$ ) in ppm CO<sub>2</sub>

1968		1969	
Genotype	$\Gamma$	Genotype	$\Gamma$
Glabrota	25.1	P.I. 292546	57.4
C.I. 2528	27.2	Record	64.3
P.I. 296234	31.1	Bingham	66.0
P.I. 193958	32.2	C.I. 2528	69.5
Record	40.6	Victorgrain	69.7
Goodfield	42.7	Appler	69.9
C.I. 1779	43.0	Curt	70.0
Victorgrain	47.1	Saia	70.1
Burnett	47.6	A-465	70.4
A-465	47.8	C.I. 1779	70.7
Richland	48.2	Burnett	71.0
Appler	48.5	Garland	72.5
<u>A. brevis</u>	49.8	Clintland-64	72.7
<u>P.I. 292546</u>	50.1	Goodfield	73.6
Garland	50.7	P.I. 193958	74.8
Bingham	53.6	Glabrota	76.6
Curt	54.4	P.I. 296234	80.8
Saia	55.5	Marion	81.2
Marion	58.1	<u>A. brevis</u>	81.5
Clintland-64	68.1	<u>Richland</u>	82.0
$\bar{X}$	46.1		72.2
$S_{\bar{d}}$	11.7		8.8
CV	53.6		24.3

Table 12. Mean 1969 transpiration rates (T) in  $\text{g H}_2\text{O}\cdot\text{dm}^{-2}\cdot\text{hr}^{-1}$  and photosynthesis/transpiration ratios (P/T) in  $\text{mg CO}_2\cdot\text{mg H}_2\text{O}^{-1}\cdot 10^{-3}$

Genotype	T	Genotype	P/T
A-465	3.93	A-465	7.2
Record	4.38	Burnett	6.4
Garland	4.46	Goodfield	6.3
Clintland-64	4.47	Garland	6.0
Goodfield	4.60	Clintland-64	6.0
Curt	4.62	Curt	5.7
Appler	4.67	Bingham	5.7
Burnett	4.69	Richland	5.4
C.I. 1779	4.73	Marion	5.4
Richland	4.80	Appler	5.2
P.I. 193958	4.91	C.I. 2528	5.0
C.I. 2528	4.95	Record	5.0
Marion	5.04	C.I. 1779	4.9
Victorgrain	5.42	Victorgrain	4.9
Bingham	5.66	P.I. 193958	4.6
P.I. 296234	5.95	P.I. 296234	4.5
Saia	6.50	Saia	4.2
P.I. 292546	6.65	P.I. 292546	4.1
Glabrota	6.99	Glabrota	4.1
<u>A. brevis</u>	7.78	<u>A. brevis</u>	3.9
$\bar{X}$	5.26		5.2
$S_{\bar{d}}$	.51		.4
CV	19.6		14.2

Table 13. Mean slopes (S) of the CO<sub>2</sub> response curves, mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>·ppm CO<sub>2</sub><sup>-1</sup>, and sums of CO<sub>2</sub> diffusion resistances (Σr), sec·cm<sup>-1</sup>, for each of the genotypes

1968				1969			
Genotype	S	Genotype	Σr	Genotype	S	Genotype	Σr
Glabrota	.174	Glabrota	4.48	Bingham	.126	Bingham	5.64
Garland	.158	Garland	4.56	<u>A. brevis</u>	.123	<u>A. brevis</u>	5.89
Goodfield	.158	Goodfield	4.63	Burnett	.120	Burnett	5.91
Burnett	.158	Saia	4.73	Goodfield	.117	Goodfield	6.12
Saia	.157	Burnett	4.81	Glabrota	.115	Marion	6.21
Bingham	.156	Victorgrain	5.43	Marion	.114	Glabrota	6.27
Victorgrain	.136	<u>A. brevis</u>	5.71	A-465	.111	A-465	6.32
<u>A. brevis</u>	.136	Bingham	5.73	Richland	.108	Clintland-64	6.56
Clintland-64	.132	Appler	5.75	Clintland-64	.108	Richland	6.57
P.I. 193958	.132	Clintland-64	5.77	Garland	.107	Garland	6.65
A-465	.132	P.I. 193958	5.87	Victorgrain	.106	Victorgrain	6.74
Appler	.126	P.I. 296234	6.09	Curt	.105	Curt	6.77
P.I. 296234	.125	A-465	6.10	Saia	.105	Saia	6.83
Marion	.124	Curt	6.60	P.I. 292546	.101	P.I. 292546	7.21
Curt	.116	C.I. 1779	7.08	C.I. 2528	.099	C.I. 2528	7.21
C.I. 2528	.114	Marion	7.28	Appler	.098	Appler	7.34
C.I. 1779	.104	C.I. 2528	7.68	P.I. 296234	.096	P.I. 296234	7.58
P.I. 292546	.099	P.I. 292546	8.01	P.I. 193958	.092	P.I. 193958	7.77
Richland	.096	Record	8.50	C.I. 1779	.090	C.I. 1779	8.13
Record	.094	Richland	8.53	Record	.085	Record	8.40
$\bar{X}$	.131		6.17		.106		6.81
$S_{\bar{d}}$	.013		1.11		.006		.45
CV	21.6		38.4		11.3		13.2

Table 14. Means of CO<sub>2</sub> diffusion resistances for genotypes, in sec·cm<sup>-1</sup>, measured in 1969 (Laminar resistance = r<sub>a</sub>, stomatal resistance = r<sub>s</sub>, mesophyll resistance = r<sub>m</sub>)

Genotype	r <sub>a</sub>	Genotype	r <sub>s</sub>	Genotype	r <sub>m</sub>
<u>A. brevis</u>	.80	Bingham	.96	A-465	3.43
Glabrota	.80	Glabrota	.99	Burnett	3.68
Saia	.85	<u>A. brevis</u>	1.07	Bingham	3.76
P.I. 292546	.87	<u>C.I. 2528</u>	1.16	Goodfield	3.86
P.I. 296234	.87	Victorgrain	1.17	Marion	3.98
Bingham	.91	Burnett	1.23	<u>A. brevis</u>	4.02
Victorgrain	.92	Goodfield	1.28	<u>Clintland-64</u>	4.23
Richland	.95	Marion	1.28	Garland	4.30
Marion	.95	Garland	1.33	Richland	4.30
Clintland-64	.96	Richland	1.33	Curt	4.43
Curt	.96	Clintland-64	1.37	Saia	4.43
C.I. 1779	.97	Curt	1.38	Glabrota	4.47
A-465	.97	P.I. 193958	1.42	Victorgrain	4.64
Appler	.98	Saia	1.54	Appler	4.74
C.I. 2528	.98	Record	1.60	P.I. 292546	5.04
Goodfield	.98	Appler	1.62	P.I. 296234	5.04
P.I. 193958	.99	P.I. 292546	1.67	C.I. 2528	5.06
Burnett	1.00	P.I. 296234	1.67	C.I. 1779	5.32
Record	1.01	C.I. 1779	1.84	P.I. 193958	5.36
Garland	1.02	A-465	1.91	Record	5.48
$\bar{X}$	.94		1.36		4.48
$\bar{S}_d$	.03		.26		.36
CV	6.8		37.5		16.0



Table 16. Mean leaf temperatures in °C and mean laminar areas in dm<sup>2</sup> measured in 1968 and 1969  
(Laminar areas measured in 1969 refer to only the mean area of one of the four leaf mid-  
portions upon which photosynthesis was measured simultaneously)

1968				1969			
Genotype	Leaf temp.	Genotype	Leaf area	Genotype	Leaf temp.	Genotype	Leaf area
Appler	30.6	P.I. 296234	.041	Clintland-64	30.2	Saia	.019
P.I. 193958	31.1	Saia	.042	Goodfield	30.4	<u>A. brevis</u>	.020
Curt	31.5	<u>A. brevis</u>	.043	Burnett	30.4	Glabrota	.027
Goodfield	31.6	Glabrota	.045	Garland	30.5	P.I. 296234	.028
Glabrota	31.7	P.I. 292546	.046	Richland	30.5	P.I. 292546	.034
Saia	31.7	Appler	.056	Curt	30.6	Victorgrain	.051
<u>A. brevis</u>	31.8	Victorgrain	.072	Marion	30.7	Marion	.056
<u>C.I. 1779</u>	32.4	Clintland-64	.079	Victorgrain	30.7	Bingham	.057
P.I. 296234	32.6	C.I. 1779	.083	Bingham	30.8	C.I. 1779	.057
Garland	32.6	P.I. 193958	.084	A-465	30.8	A-465	.058
Burnett	32.6	Marion	.085	C.I. 2528	30.9	Appler	.058
A-465	32.6	Curt	.094	Glabrota	30.9	Richland	.058
Marion	32.7	A-465	.095	Record	31.3	P.I. 193958	.062
Bingham	32.9	Richland	.098	Appler	31.4	Curt	.062
C.I. 2528	32.9	Bingham	.099	<u>A. brevis</u>	31.4	Clintland-64	.065
Victorgrain	33.1	Record	.104	<u>C.I. 1779</u>	31.4	Burnett	.069
Record	33.5	Burnett	.123	P.I. 292546	31.5	C.I. 2528	.070
Clintland-64	33.6	Goodfield	.125	P.I. 193958	31.6	Goodfield	.070
P.I. 292546	34.2	Garland	.134	Saia	32.2	Record	.070
Richland	35.5	C.I. 2528	.155	P.I. 296234	32.3	Garland	.072
$\bar{X}$	32.6		.085		31.0		.053

## DISCUSSION

Before a critical discussion of the results from the genotype screening experiment is presented, experimental techniques used to obtain net CO<sub>2</sub> exchange and transpiration measurements will be discussed. Environmental factors were of most importance with regard to interpretation of the results, because some factors affected measurements of photosynthesis or transpiration.

## Evaluation of Experimental Techniques

Plant material

Both years (1968 and 1969) CO<sub>2</sub> exchange measurements were taken on attached flag leaves of oat plants grown under natural light conditions. There is evidence that greenhouse-grown plants often differ in characteristics of photosynthesis, compared to plants grown under natural field conditions (Elmore et al., 1965). It is concluded that attached leaves were most desirable to use, because preliminary experiments showed depressed net photosynthetic rates in detached oat leaves (Figure 15).

Since attached flag leaves were tested in a laboratory situation, plant material was grown in pots. Best growth was obtained in the plastic "waste-paper-baskets" used in 1969. These held considerably more soil than did the six-inch clay pots used in 1968, and it is believed the larger volume of soil for root expansion was partially responsible for more vigorous plants in 1969.

Early summer 1969 temperatures were slightly lower than normal, and it appeared growth and development were better.

### Environmental factors

In general the system worked quite well with respect to CO<sub>2</sub> exchange and transpiration measurements. Preliminary experiments showed stable net photosynthetic and transpiration rates could be maintained for periods as long as at least 3 hours after a 15 minute induction period (Figure 12). This indicated near optimum environmental conditions in the leaf chambers. Tests conducted in 1969, as well as earlier (Criswell, 1968), demonstrated that leaves tested in this experiment were approaching light saturation of the photosynthetic process. Over the range of CO<sub>2</sub> concentrations used, photosynthesis was found linearly related to CO<sub>2</sub> concentration. Thus, two of the requirements required for accurate CO<sub>2</sub> diffusion estimates, as suggested by Gaastra (1959), were met.

The cooling capacity of the system was limited; consequently, leaves were tested at temperatures slightly above the optimum for net photosynthesis. Mean genotypic flag-leaf temperatures were relatively constant ( $32.6 \pm 3.0^{\circ}\text{C}$  in 1968 and  $31.0 \pm 1.5^{\circ}\text{C}$  in 1969). Lower leaf temperatures would have been desirable. However, temperature response curves for net photosynthesis (Figure 10) indicated only small depressions of net photosynthesis at these temperatures. The  $r_m$  values obtained in the four temperature response curves were found to increase by only about 4 to 8 percent when leaf temperatures were increased from approximately 27.0 to 32.0°C. This small increase was believed largely the result of a depression of temperature-dependent biosynthetic reactions. Stomatal closure was not believed to have occurred at the test temperatures used,

because  $r_s$  values remained stable as leaf temperatures were increased. Values of  $r_a$  were found relatively independent of leaf temperature. Because  $r_a$  is an external physical resistance, this response was not unexpected.

Relative humidity conditions in the chambers could only be controlled within a limited range. Whereas air which entered the leaf chambers was approaching constant relative humidity conditions, the relative humidity of air in the leaf chambers also was dependent upon amount and transpiration rate of plant material in the chambers. Differences in relative humidity conditions did not alter the ranking of genotypes with respect to  $CO_2$  exchange rates, because tests showed that over the range of atmospheric water vapor concentrations used in the experiments, photosynthesis was little affected (Figure 11).

It is believed, however, that rankings of mean genotype transpiration rates and P/T ratios were affected by different relative humidity conditions existing within the leaf chambers for the various genotypes. Transpiration rates were dependent upon the vapor pressure deficit maintained between the leaf and the atmosphere (Figure 11). Correlations within genotypes, between transpiration and the vapor pressure deficit between the leaf and the air, were often negative. Negative correlations were found in 13 out of the 20 genotypes tested in 1969. Only 4 of the 13 negative correlations were significant. None of the positive correlations were significant, however. Calculations made in 1969 showed genotypic mean vapor pressure deficits between the leaf and the air ranged between 17.3 and 24.5 mm Hg. Because higher vapor pressure

deficits between the leaf and the air occurred when a small amount of plant material was enclosed in the assimilation chambers, higher transpiration rates were expected from genotypes having small leaves. Examination of Tables 12 and 16 indicate such a relationship in 1969. The negative correlation between mean transpiration and mean leaf area of the flag leaf midportions tested was significant at the 1 percent level ( $r = .89$ ). Thus, few inferences can be made regarding the relative rankings of mean genotype transpiration rates. The high P/T ratios of many Midwestern varieties and genotypes adapted to warm climates may also be somewhat of an artifact, because many of the genotypes showing high P/T ratios had large flag leaf midportion areas. Transpiration from the larger amount of leaf area resulted in reduced vapor pressure deficits between the leaf and the air. This reduction ultimately reduced transpiration rates, and as a result, high P/T ratios resulted.

The stomatal resistance ( $r_s$ ) remained relatively constant at various relative humidities. Thus, it is concluded that if stomatal changes, which would affect transpiration rates, did occur the changes were not large, because  $r_s$  and net photosynthesis were not affected significantly. Correlations of  $r_s$  and net photosynthesis with mean flag leaf midportion areas were not significant ( $r = .13$  and  $r = -.10$ , respectively). It is concluded that  $CO_2$  exchange and diffusion resistance processes were not appreciably affected by differences in relative humidity conditions within the leaf chambers. Hence, the ranking of the genotypes, with respect to these factors, is considered relatively unaffected.

The 1969 leaf chambers are considered superior to those used in 1968 in that turbulent air transport was provided by an internally-mounted fan. Transport of  $\text{CO}_2$  to the leaf lamina enclosed in the 1968 leaf chambers was dependent primarily upon laminar flow of air through the tubular chambers. Turbulent air transport in the 1969 leaf chambers probably reduced  $r_a$  values some; however, reliable estimates of  $r_a$  were not obtained in 1968. Hence, comparisons could not be made between years.

### Apparatus

The conversion of the absolute type  $\text{CO}_2$  analyzer to a differential type  $\text{CO}_2$  analyzer was advantageous, because  $\text{CO}_2$  differentials could be measured directly. Inlet and outlet  $\text{CO}_2$  concentrations did not have to be measured alternately in 1969. According to Brown and Rosenberg (1968) as the error in leaf area and flow rate measurement is decreased, the advantage in accuracy of an otherwise comparable differential analyzer over an absolute analyzer is increased. Standard gases used were cross checked against other standard gases, and errors introduced by inaccurate standard gases were believed less than 5 ppm  $\text{CO}_2$ . When  $\text{CO}_2$  differentials were measured at 0 and 100 ppm  $\text{CO}_2$  concentrations in 1968, small  $\text{CO}_2$  gradients were recorded. Because mean ratio of flow rate to mean leaf area was reduced from  $1,234 \text{ l}\cdot\text{hr}^{-1}\cdot\text{dm}^{-2}$  in 1968 to  $943 \text{ l}\cdot\text{hr}^{-1}\cdot\text{dm}^{-2}$  in 1969, larger, more accurately-estimated  $\text{CO}_2$  gradients were generally obtained in 1969. One of the advantages of a supplemental fan within the leaf chamber is that low flow rates through the chamber can be used, and large  $\text{CO}_2$  differentials can be measured. This can be done because chamber turbulence is not dependent upon flow rate. With

this technique the exit gas can be sampled as the measurement level of  $\text{CO}_2$ , rather than utilizing the mean of inlet and exit  $\text{CO}_2$  concentrations as the measurement level (Nevins and Loomis, 1970).

The psychrometer failure in 1968 was believed to result from unsuitable wick material. The cotton thread, which was used for wick material, often dried out, and it was believed that maximum wet bulb depressions were not obtained in many cases. Cotton muslin was found to be a superior wick material; hence, it was used in 1969. Because of the larger flow rate to leaf area ratio maintained in 1968, water vapor differentials between inlet and outlet air streams were not large. The smaller flow rate to leaf area ratio, maintained in 1969, consequently, allowed for larger water vapor differentials. Since wet bulb temperatures could only be determined to the nearest  $0.3^\circ\text{C}$ , it seemed of paramount importance to reduce the sensitivity required in 1969 by measuring larger water vapor differentials. Absolute accuracy of temperature measurements was not affected. One of the problems encountered with large water vapor differentials, however, was that inlet air water vapor concentrations had to be quite low to avoid condensation on the inner walls of the leaf chamber or, if low water bath temperatures were used, to avoid condensation in outlet copper coils submersed in the constant temperature water bath. Condensation of moisture in the system at any point between the psychrometer and the leaf chambers would affect the measured transpiration rates. If high water vapor concentrations were used, leaf chamber and coil temperatures had to be maintained at temperatures above the dew point of the outlet air. To avoid exposing the leaf

to severe vapor pressure deficits or high leaf temperatures, a balance of bath temperatures was used which would provide moderate relative humidities and reasonable leaf temperatures, yet avoid condensation problems. Unfortunately, this compromise resulted in slightly higher than optimal leaf temperatures for net photosynthesis.

#### Genotypic Variation in Net Photosynthesis

The first objective of the oat screening experiment was to determine whether or not differences in net photosynthesis occurred between the 20 oat genotypes. Significant genotypic differences for the net photosynthetic process were found in both 1968 and 1969. The correlation of genotypic mean net photosynthetic rates across years was significant ( $r = .51$ ). This indicated a similar ranking of genotypes, with respect to net photosynthesis, both years.

Tests were conducted under somewhat artificial laboratory conditions; however, environmental conditions were similar to those which might occur under field conditions. It was believed that reasonably valid inferences can be made between laboratory experimental results and those which might be obtained under field conditions. This conclusion was arrived at because the plant material was grown under conditions approaching field conditions, and all tests were conducted under repeatable experimental conditions.

Net photosynthetic rates measured in 1969 were approximately 21 percent lower than those measured in 1968. Mean  $\text{CO}_2$  efflux rates and  $\text{CO}_2$  compensation concentrations measured in 1968 were lower than those

measured in 1969. Although light flux densities used in 1969 were 43 percent lower than those used in 1968, tests showed the photosynthetic process was essentially light saturated (Figure 13). Preliminary experiments showed net photosynthesis was not affected by differences in relative humidities over the range of relative humidities used in the experiment (Figure 11). Temperature conditions within the leaf chambers were similar both years (Table 16). Consequently, it is concluded that variation of net photosynthetic rates across years may have resulted from different environmental conditions under which the plants developed in the field.

The relationship between net  $\text{CO}_2$  exchange and the mean  $\text{CO}_2$  concentration of air over the leaf was extremely linear. Even at  $\text{CO}_2$  concentrations as high as 400 ppm, the relationship did not deviate from linearity. This may be interpreted to mean that genotypes will photosynthesize in the same relative manner under field conditions, even though atmospheric  $\text{CO}_2$  levels fluctuate or  $\text{CO}_2$  gradients within the crop canopy develop.

Photosynthetic capacity of oats did not appear consistently associated with ploidy level, area of origin and adaptation, or yielding capacity. However, photosynthetic rates differed significantly between species. Although the mean net photosynthetic rate of two genotypes of one species may have been higher than the mean of two other genotypes of another species, this higher mean sometimes consisted of both a high and reasonably low photosynthesizing genotype. The genotype having

the low photosynthetic rate often showed a rate which appeared to be similar to that of the genotypes of the low photosynthesizing species. Hence, the fact that A. byzantina and A. sativa species showed greater net photosynthetic rates than A. fatua and A. sterilis species may have been coincidental since only a very limited number of genotypes were used in each comparison.

#### Relationship of Specific Leaf Weight and Photosynthesis

The second objective of the oat screening experiment was to study the relationship between net photosynthesis and leaf anatomical characteristics, as revealed by the specific leaf dry weight (SLDW) or specific leaf fresh weight (SLFW).

#### Net photosynthesis as related to SLFW

Net photosynthetic rates measured in 1968 and 1969 were not significantly correlated with the SLFW, regardless of whether or not the correlations were conducted within genotypes or on genotypic mean values. The correlation of genotypic mean net photosynthetic rates with mean SLFW values was .12 and .40 in 1968 and 1969, respectively. Heichel and Musgrave (1969a) suggested the correlation they obtained between lamina fresh weight and net photosynthesis in different corn genotypes was due to a greater quantity of photosynthetic enzymes in thicker leaves. Perhaps variations in the leaf water status obscured such a correlation in this experiment; however, it is not believed that this was the case, because at no time during the experimental period were the plants under

obvious moisture stress. The fact that 1968 and 1969 SLFW genotypic mean values were highly significantly correlated ( $r = .74$ ) might be interpreted to mean that there are genotypic differences in plant water status which are not associated with genotypic differences in net photosynthetic rates.

#### Net photosynthesis as related to SLDW

The correlation of mean genotypic net photosynthetic rates with mean SLDW values was highly significant in 1968 and 1969. The  $r$  values were .75 and .58 in 1968 and 1969, respectively (Figure 17). Correlations of net photosynthesis (P) with SLDW within genotypes were generally variable and inconsistent (Tables 24 and 25). This was not surprising, because little variation of SLDW and P would be expected to exist within genetically purified genotypes. More variation between genotypic mean P and SLDW values would, perhaps, be expected to exist.

The correlation of P with SLDW was greater in 1968 than in 1969, and it is believed this was the consequence of more variation in genotypic net photosynthetic rates. The SLDW of the various genotypes tested remained relatively stable across years. This was indicated by the fact that the correlation of 1968 with 1969 mean genotypic SLDW values was significant ( $r = .55$ , Figure 18).

Friend (1966) has reported that high light levels (8 to 16  $\text{cal}\cdot\text{cm}^{-2}\cdot\text{hr}^{-1}$ ) and cool temperatures (10 to 20°C) tend to promote the

Figure 17. Relationship of mean genotypic net photosynthetic rates with mean specific leaf dry weight (SLDW) measurements.

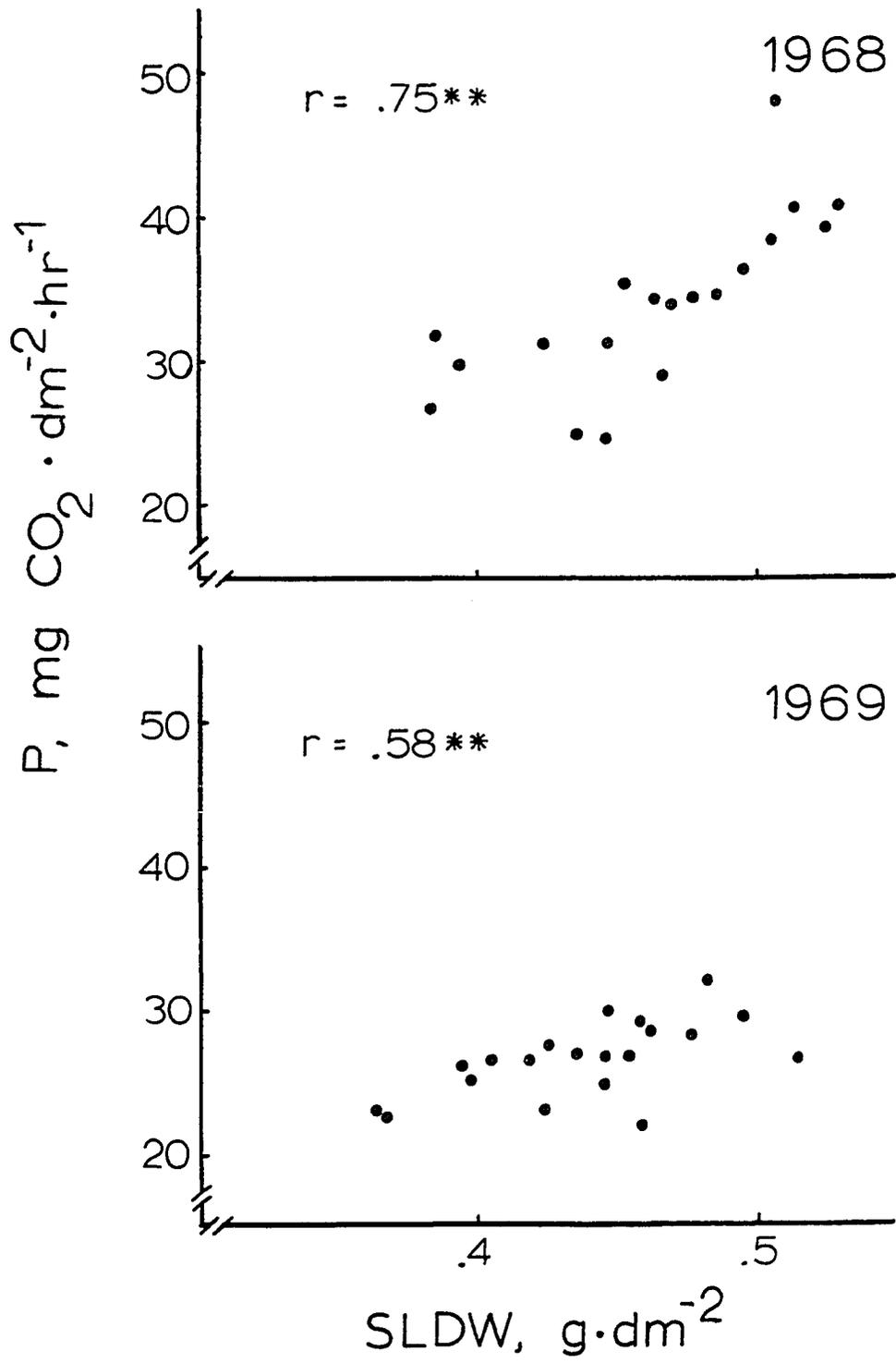
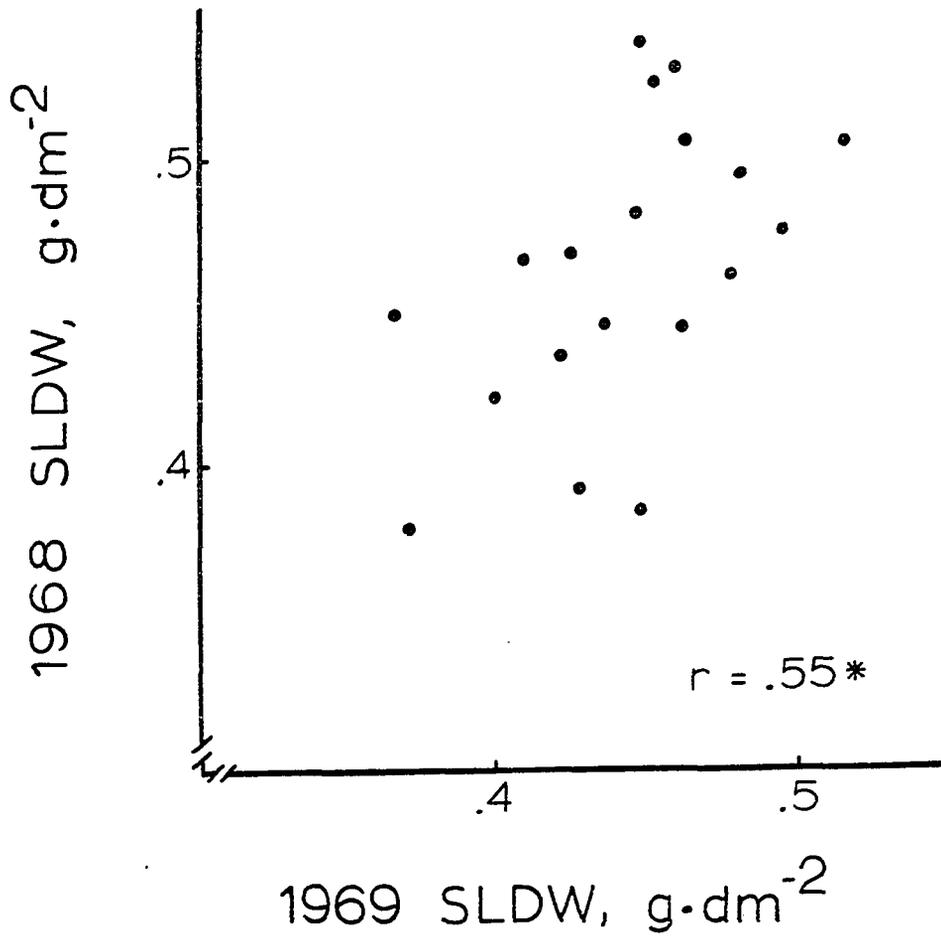


Figure 18. Relationship of mean genotypic specific leaf dry weight (SLDW) measurements obtained in 1968 with those obtained in 1969.



development of thick leaves in wheat, whereas low light levels (0 to  $8 \text{ cal}\cdot\text{cm}^{-2}\cdot\text{hr}^{-1}$ ) and high temperatures (20 to  $30^{\circ}\text{C}$ ) resulted in thinner wheat leaves. Monthly solar radiation data and mean temperatures under which the leaves developed in this experiment are shown in Table 17. Most of the flag leaves of genotypes used in this experiment were probably developing during the latter part of June, the period during which environmental conditions were the most different. Yet, the specific leaf dry weights across years were similar. The effect of a lower mean light flux density during June in 1969 may have been partially offset by the low temperatures, if environmental factors were effective in affecting leaf morphology.

The fact that the SLDW remained relatively constant across two different years would seem advantageous if the SLDW was to be used as a selection index for isolating photosynthetically efficient genotypes. The SLDW and SLFW were often significantly correlated within genotypes, but the correlations of SLDW with SLFW conducted on genotypic means was not significant in either 1968 or 1969. A large leaf size was not necessarily associated with a high SLFW or SLDW. Thus, it is postulated the SLDW is relatively independent of leaf size, the SLFW, or environmental factors.

In this experiment all flag leaves were measured at the time of panicle emergence; consequently, no time trends or changes in the SLDW values could be observed over the experimental test period. Dornhoff and Shibles (1970) observed a linear increase in the dry weight per unit

Table 17. Mean light flux density conditions and temperatures at Ames, Iowa in 1968 and 1969

Month	Light flux density, $\text{cal}\cdot\text{cm}^{-2}\cdot\text{hr}^{-1}$		Temperature, $^{\circ}\text{C}$	
	1968	1969	1968	1969
April	15.3	16.2	10.5	10.4
May	18.6	18.7	14.2	16.5
June	22.0	19.1	21.8	18.5
July	21.6	18.6	22.5	23.4

area in soybeans when different fully expanded terminal leaves were tested over the period of from July 11 to August 21.

It was reported by Barnes et al. (1969) that leaf dry weight per unit area could be used routinely in breeding programs to select for increased photosynthetic efficiency. This suggestion was based on reports of positive correlations of net photosynthesis with leaf dry weight per unit area in some agronomic crops (Hayashi, 1968; Pearce et al., 1969). There have been additional reports of positive correlations of net photosynthesis with leaf fresh weight per unit area (McClendon, 1962; Heichel and Musgrave, 1969a). Both the specific leaf dry weight (SLDW) and the specific leaf fresh weight (SLFW) were measured in this experiment. Lamina thickness has been reported to be positively correlated with net photosynthesis (Pieters, 1960; Irvine, 1967); however, leaf thickness was not measured in this experiment. It is believed the SLDW or SLFW may have served as an indicator of leaf thickness, since high correlations between leaf thickness, as measured by a micrometer, and leaf dry weight per unit area have been observed in leaves of spring wheat

(Friend et al., 1965), a crop which might be expected to show leaf morphological characteristics similar to those which are believed to occur in oats.

It was reasoned that if net photosynthetic rates of the oat genotypes were associated with the SLFW or the SLDW, perhaps these factors could prove beneficial to the plant breeder as a selection index for isolating photosynthetically efficient lines. Because the measurement of net photosynthetic rates by infrared gas analysis techniques is quite time consuming and laborious, and thus, only a limited number of lines can be tested, the use of the SLFW or SLDW as a selection index for photosynthetic efficiency would be attractive. Also a minimum amount of equipment would be needed to test large numbers of lines. Essentially all the equipment which would be needed to measure the SLFW and SLDW would be an accurate analytical balance, a leaf punch, and a drying oven. All these items are more modestly priced than the financial outlay needed to design a photosynthesis system such as was used in these experiments. Moreover, most laboratories are already equipped with these basic equipment items.

#### The Physiological Basis of Variation in Net Photosynthesis

The third objective of the oat genotype screening experiment was to explain the physiological basis for differences in net photosynthetic rates between oat genotypes. Since the photosynthetic rate is dependent upon three processes (i.e., a photochemical process, biochemical processes,

and a CO<sub>2</sub> diffusion process), it was believed that, by investigating factors associated with some of these processes, the physiological basis for genotypic differences in net photosynthesis could be more clearly understood.

#### Photochemical process

The photochemical process was not studied in this experiment. Thus, there is little evidence for or against the photochemical process as a photosynthetic limitation in oats. Gabrielsen (1948) found that chlorophyll (a + b) contents of most leaves exceeded values required for maximum photosynthesis, and work by Hesketh (1963) demonstrated that differences in chlorophyll efficiency in converting light energy into chemical energy did not differ significantly between species. Therefore, it was assumed that photochemical processes were not limiting net photosynthetic rates in oats.

#### Biochemical processes

Oats can be classified as a "non-efficient" type of plant, photosynthetically. This conclusion is based on the fact that the CO<sub>2</sub> compensation point did not approach zero, and CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light occurred. Thus, it seems likely that oats utilize the Calvin cycle for the reduction of CO<sub>2</sub> in the photosynthetic process, as opposed to the "four-carbon" pathway proposed by Hatch and Slack (1966). That this is a valid conclusion is supported by the fact that oats have been shown to lack the chlorophyllous, parenchymatous bundle sheath, and photosynthetic rates are enhanced by the use of oxygen-free

air (Akita and Miyasaka, 1969). Oats also show a broad optimum temperature response curve for net photosynthesis (Criswell, 1968) similar to those observed in other temperate grasses, such as barley and wheat (Murata and Iyama, 1963b). "Non-efficient" plants, which are believed to operate via the Calvin cycle, show such characteristics. The aforementioned properties have also generally been associated with the presence of "photorespiration" or incomplete  $\text{CO}_2$  reassimilation.

In this experiment the activities of enzymes involved in the photosynthetic process, such as carbonic anhydrase or ribulose diphosphate carboxylase, were not measured. It is believed, however, that if differences in activities of either of these two enzymes, or any other enzyme limiting the photosynthetic process, were present, such differences would be apparent in the  $r_m$  values of the various genotypes. The fact that mean genotypic  $r_m$  values were highly correlated with mean net photosynthetic rates would seem to indicate the possible occurrence of differing activities of enzymes involved in the photosynthetic process; however, the possibility of differing physical resistances to  $\text{CO}_2$  transport is not discounted.

Although possible differences in activities of enzymes associated with the biochemical pathway used to reduce  $\text{CO}_2$  may occur, differences in activities of enzymes associated with respiratory processes, which occur in darkness and light, might also be related to differences in net photosynthesis between oat genotypes. Dark respiration and factors associated with photorespiration and/or efficiency of  $\text{CO}_2$  reassimilation,

are discussed in the following paragraphs.

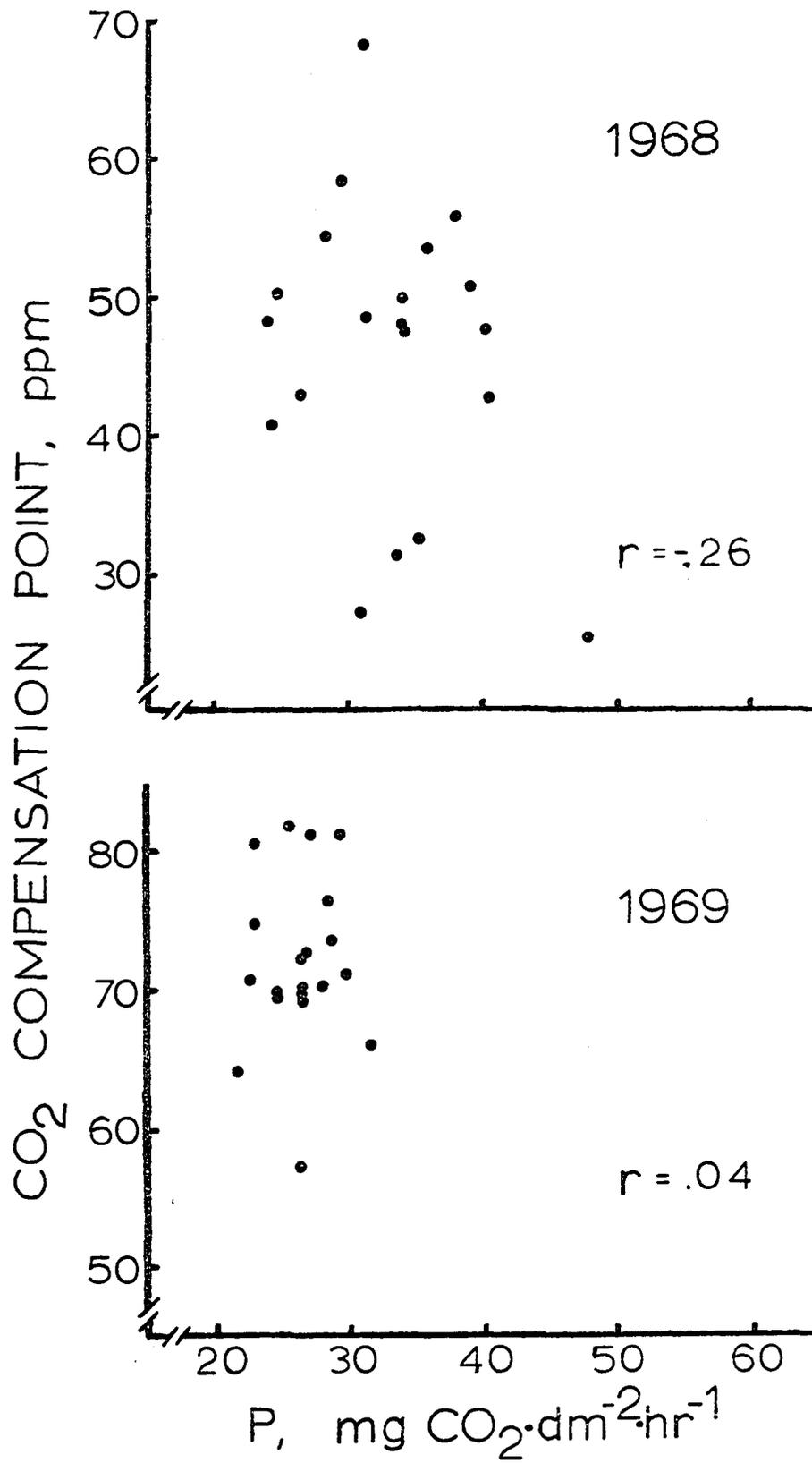
It is believed that genotypic variation of the dark respiration process was not responsible for genotypic differences in net photosynthesis. Although dark respiration rates were not measured in each of the 20 oat genotypes used in this experiment, Stoy (1965) has shown that at 30°C dark respiration rates of leaves of three varieties of wheat (Dala, Diamant, and Sv 01200) ranged from 1.19 to 1.81 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>, whereas net photosynthetic rates ranged from 32.7 to 38.9 mg CO<sub>2</sub>·dm<sup>-2</sup>·hr<sup>-1</sup>. Dark respiration rates were only 3 to 6 percent the magnitude of net photosynthetic rates in wheat leaves. In this experiment net photosynthetic rates of the different oat genotypes varied by approximately 98 and 46 percent in 1968 and 1969, respectively. It would seem reasonable that any process, such as dark respiration, which accounts for such a small percentage of the total carbon exchanged in the net photosynthetic process would not be associated with large genotypic differences in net photosynthesis. Thus, even if considerable genotypic variability for the dark respiration process was apparent, variability of the photosynthetic process between genotypes probably would not be accounted for. The above conclusion is based on the assumption that dark respiration rates in oats and wheat are of the same order of magnitude. That this is a valid assumption is supported by dark respiration measurements on two genotypes. Dark respiration rates of leaves of the two oat genotypes tested (Burnett and Record) were only 6 to 8 percent those of net photosynthetic rates measured at near saturating light flux densities (Figure 13).

It was anticipated that genotypic differences in the light respiratory process might be related to genotypic variations of net photosynthesis. Estimates of photorespiration or CO<sub>2</sub> not reassimilated ("CO<sub>2</sub>-leakage" from the leaf) have been obtained by measuring the CO<sub>2</sub> compensation concentration (Tregunna et al., 1966) and the rate of CO<sub>2</sub> evolution into CO<sub>2</sub>-free air in light (El-Sharkawy and Hesketh, 1965). Consequently, the association of these two variables with net photosynthesis was examined.

The CO<sub>2</sub> compensation point ( $\Gamma$ ) was the least variable of all the experimental variables measured. In 1968  $\Gamma$  values were significantly different at the 5 percent level, but in 1969 significant differences were not detected. Since significant differences in  $\Gamma$  values were obtained in 1968, and because there was some variation in  $\Gamma$  values measured in 1969, it was of interest to study the relationship of  $\Gamma$  with net photosynthesis (P). There appeared to be a negative trend between P and  $\Gamma$  within genotypes; however, the correlation of mean P rates with mean  $\Gamma$  values was not significant either year (Figure 19). Thus, it was concluded that the net photosynthetic differences noted between the 20 oat genotypes used in this experiment were not associated with  $\Gamma$ .

Although  $\Gamma$  was the least variable factor measured in this experiment, the CO<sub>2</sub> compensation concentrations were more variable than those measured in different soybean genotypes by Cannell et al. (1969). They measured  $\Gamma$  with a closed system which allowed photosynthesis to remove CO<sub>2</sub> from the system until the CO<sub>2</sub> compensation point was reached. The mean  $\Gamma$  value of the 44 soybean genotypes they studied was  $73 \pm 0.9$  ppm

Figure 19. Relationship of mean genotypic CO<sub>2</sub> compensation concentrations with mean net photosynthetic rates.



CO<sub>2</sub>. Similar studies with 100 genetic lines of wheat and 20 lines of barley showed  $\Gamma$  values of  $52 \pm 2.0$  and  $55 \pm 2.0$  ppm CO<sub>2</sub>, respectively (Moss et al., 1969). The temperature conditions under which the soybeans were tested was 30°C, whereas the wheat and barley were tested under 23°C temperature conditions.

I believe, however, that  $\Gamma$  values obtained by closed system measurements may not necessarily be equivalent to those obtained from the point of intersection of the CO<sub>2</sub> response curve with the abscissa (CO<sub>2</sub> concentration axis). The  $\Gamma$  values obtained in this experiment were found by the "intercept" method. That the above conclusion may be correct is supported by the work of Kriedemann (1968). He obtained abscissa intersection values of 24 and 22 ppm CO<sub>2</sub> when net CO<sub>2</sub> exchange rates of orange and lemon leaves, respectively, were plotted as a function of the external CO<sub>2</sub> concentration over the leaf. Immediately after the CO<sub>2</sub> response curves had been measured, he measured the CO<sub>2</sub> compensation concentration ( $\Gamma$ ) with a closed system. The leaves were undisturbed, and only minor alterations were required to close the gas circuit. Under the same light and temperature conditions, he noted  $\Gamma$  values measured with the closed system were 60 and 65 ppm CO<sub>2</sub> for orange and lemon leaves, respectively.

The interpretation of  $\Gamma$  in relation to the "intercept" method of obtaining it has been discussed by Koller (1969). He states that  $\Gamma$ , as measured by the "intercept" technique, is not an independent plant characteristic, but the product of two such characteristics, mainly photorespiration and  $r_m$ , and that any attempt to use  $\Gamma$  as a unique

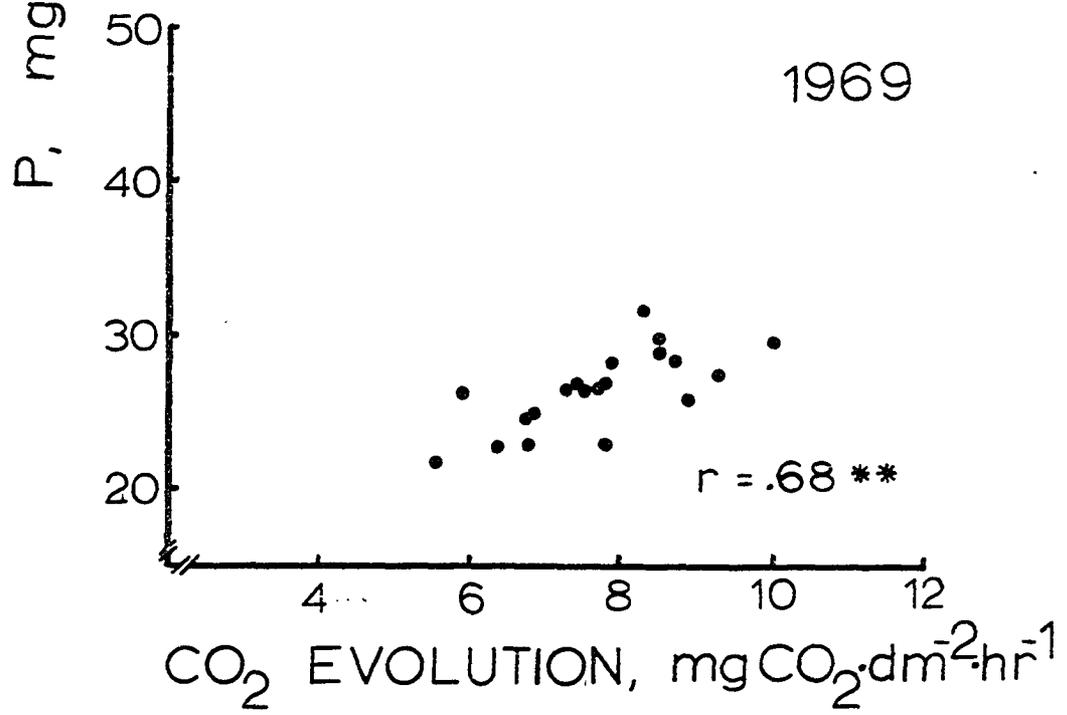
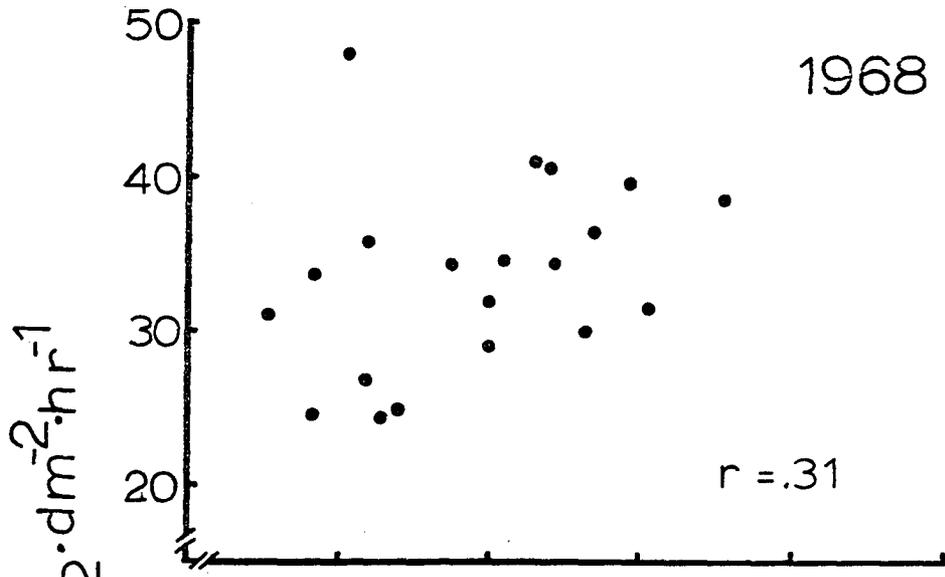
indicator of photorespiration may be misleading. Lake (1967a) has also postulated that  $\Gamma$  may increase as photosynthesis increases because of greater light respiration. From the above discussion it can be concluded that  $\Gamma$ , as measured by the "intercept" method, is a dynamic quantity which is dependent on many factors. Perhaps closed system measurements provide a better estimate of  $\Gamma$ .

The interrelationships between photorespiration or  $\text{CO}_2$  reassimilation efficiency, and the  $\text{CO}_2$  diffusion resistances  $r_s$  and  $r_m$  appeared to be quite complicated and difficult to interpret. Stomatal resistance ( $r_s$ ) values tended to be positively correlated with  $\Gamma$  values when correlations were conducted within genotypes. Thus, it is believed that high  $\Gamma$  values were effective in increasing the intercellular  $\text{CO}_2$  concentration. This probably resulted in partial stomatal closure and higher  $r_s$  values. However, the relationship of  $r_s$  with  $\Gamma$ , conducted on genotypic means, showed no correlation ( $r = -.01$ ). This infers that genotypic differences in  $r_s$  were not caused by genotypic differences in  $\Gamma$ . Mesophyll resistances within genotypes tended to be negatively correlated with  $\Gamma$  values. The negative correlations of  $\Gamma$  with  $r_m$ , conducted both within genotypes and on genotypic means, were not significant. The relationship of  $\Sigma r$  with  $\Gamma$  within genotypes was highly variable. Since laminar resistance ( $r_a$ ) is a small external resistance that shows little genotypic variation, it was believed that the interaction of  $r_m$  with  $\Gamma$  and  $\Gamma$  with  $r_s$  largely determined the relationship of  $\Sigma r$  with  $\Gamma$ . The  $r_s$  and  $r_m$  values were generally associated with  $\Gamma$  in a diametrically opposed manner (Table 25).

It is concluded that  $\Gamma$ , as measured in this experiment, was not associated with genotypic differences in net photosynthetic rates. There was a trend for  $\Gamma$  to be negatively correlated with P rates within genotypes, however. Furthermore, it is believed the  $\Gamma$  is not an indicator of photorespiration or  $\text{CO}_2$  reassimilation per se, because  $\Gamma$  is confounded by  $r_m$ , and photorespiratory processes, which may vary as a function of photosynthesis when the "intercept" method of computing is used.

As was mentioned previously, the  $\text{CO}_2$  efflux rate into  $\text{CO}_2$ -free air in light has sometimes been used as an estimate of photorespiration or  $\text{CO}_2$  reassimilation efficiency. Consequently,  $\text{CO}_2$  efflux (R) rates might be expected to be negatively correlated with net photosynthetic rates (P). This relationship was not found, however. Instead, there appeared to be a positive trend for high  $\text{CO}_2$  efflux rates to be associated with high net photosynthetic rates. This relationship was not apparent within genotypes, but the relationship was evident when genotypic mean P and R values were correlated (Figure 20). The correlation of genotypic mean P with R values was not significant in 1968; however, examination of Figure 20 seems to indicate this is largely the result of one genotype (Glabrota) which had an exceptionally high P rate in relation to R. If this value is not considered in the correlation analysis, the r value increases from .31 to .55. Even without the exclusion of this value, there appears to be an obvious trend in 1968 for R to increase as P increases. The correlation of mean 1969 P rates with mean R rates was highly significant.

Figure 20. Relationship of mean genotypic net photosynthetic rates with mean rates of CO<sub>2</sub> evolution into CO<sub>2</sub>-free air in light.



It is believed that the positive correlation of P with R is related to the  $\text{CO}_2$  diffusion resistances. The fact that genotypes with high net photosynthetic rates and  $\text{CO}_2$  efflux rates into  $\text{CO}_2$ -free air in light had low  $\Sigma r$  values would indicate that the association of high  $\text{CO}_2$  efflux rates into  $\text{CO}_2$ -free air in light in genotypes having high net photosynthetic rates is a consequence of a lower  $\text{CO}_2$  diffusive resistance between respiratory sites and atmosphere (Figure 21). A similar interpretation of this phenomenon has been postulated by Dornhoff and Shibles (1970) who worked with 20 soybean varieties.

Examination of the simple correlation coefficients presented in Table 25 seem to indicate that mesophyll resistance is most strongly associated with the rate of  $\text{CO}_2$  efflux into  $\text{CO}_2$ -free air in light. The  $\text{CO}_2$  efflux rates into  $\text{CO}_2$ -free air in light were associated with  $\Gamma$  (Figure 22). Both of the estimates of light respiration (R and  $\Gamma$ ) appear to be confounded by  $\text{CO}_2$  diffusion processes, however. It appears that light respiratory processes are less important than  $\text{CO}_2$  diffusion resistance processes in relation to the photosynthetic differences which were observed between genotypes.

#### $\text{CO}_2$ diffusion process

The resistances encountered in the transport of  $\text{CO}_2$  from the external air to the chloroplast site were measured in this experiment. The sum of all the  $\text{CO}_2$  resistances encountered can be partitioned into three different resistance terms. These three terms are: laminar or boundary layer resistance ( $r_a$ ), stomatal resistance ( $r_s$ ), and mesophyll

Figure 21. Relationship of mean genotypic sums of CO<sub>2</sub> diffusion resistances ( $\Sigma r$ ) with mean CO<sub>2</sub> evolution rates into CO<sub>2</sub>-free air in light.

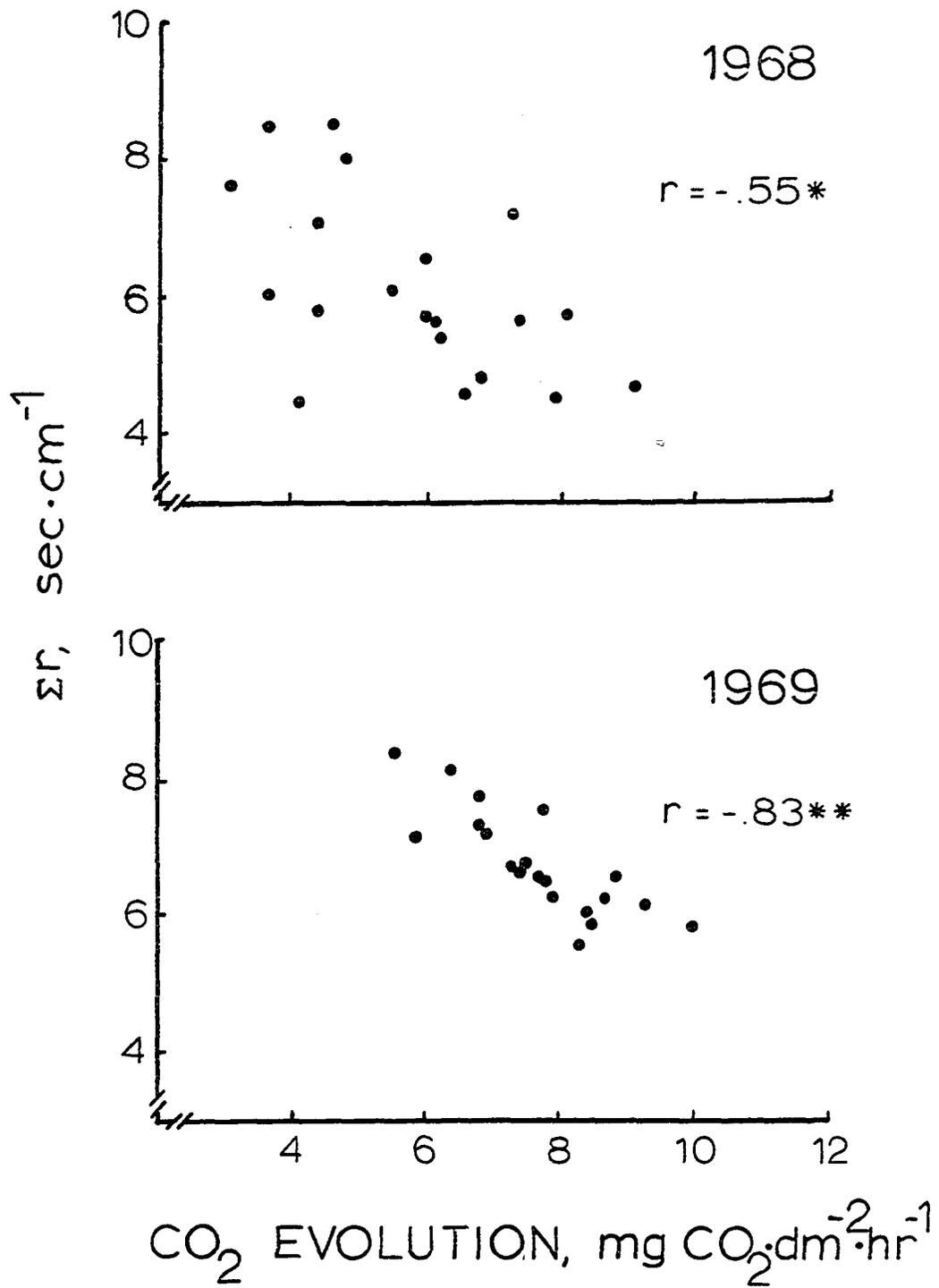
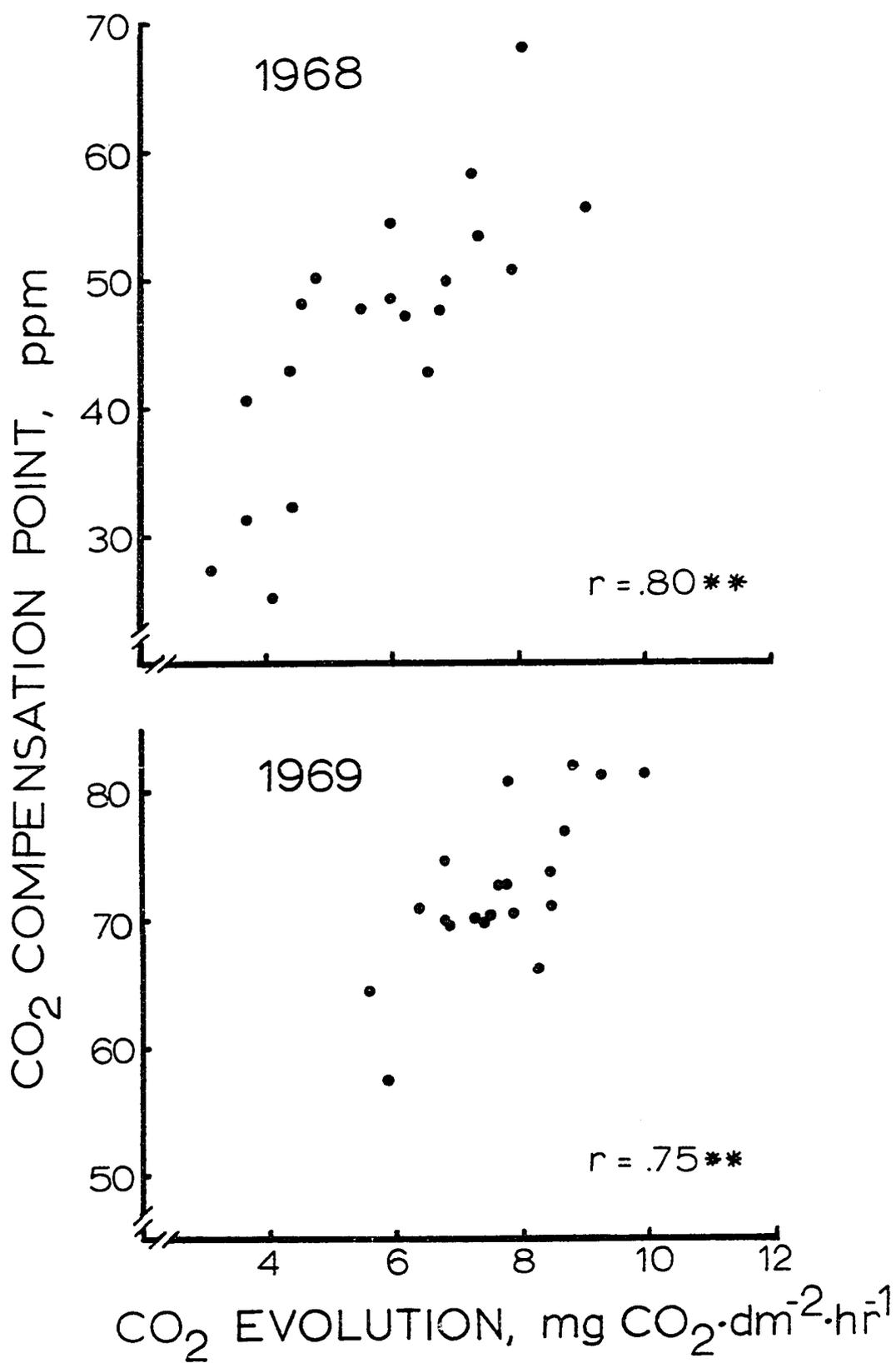


Figure 22. Relationship of mean genotypic  $\text{CO}_2$  compensation points with mean rates of  $\text{CO}_2$  evolution into  $\text{CO}_2$ -free air in light.



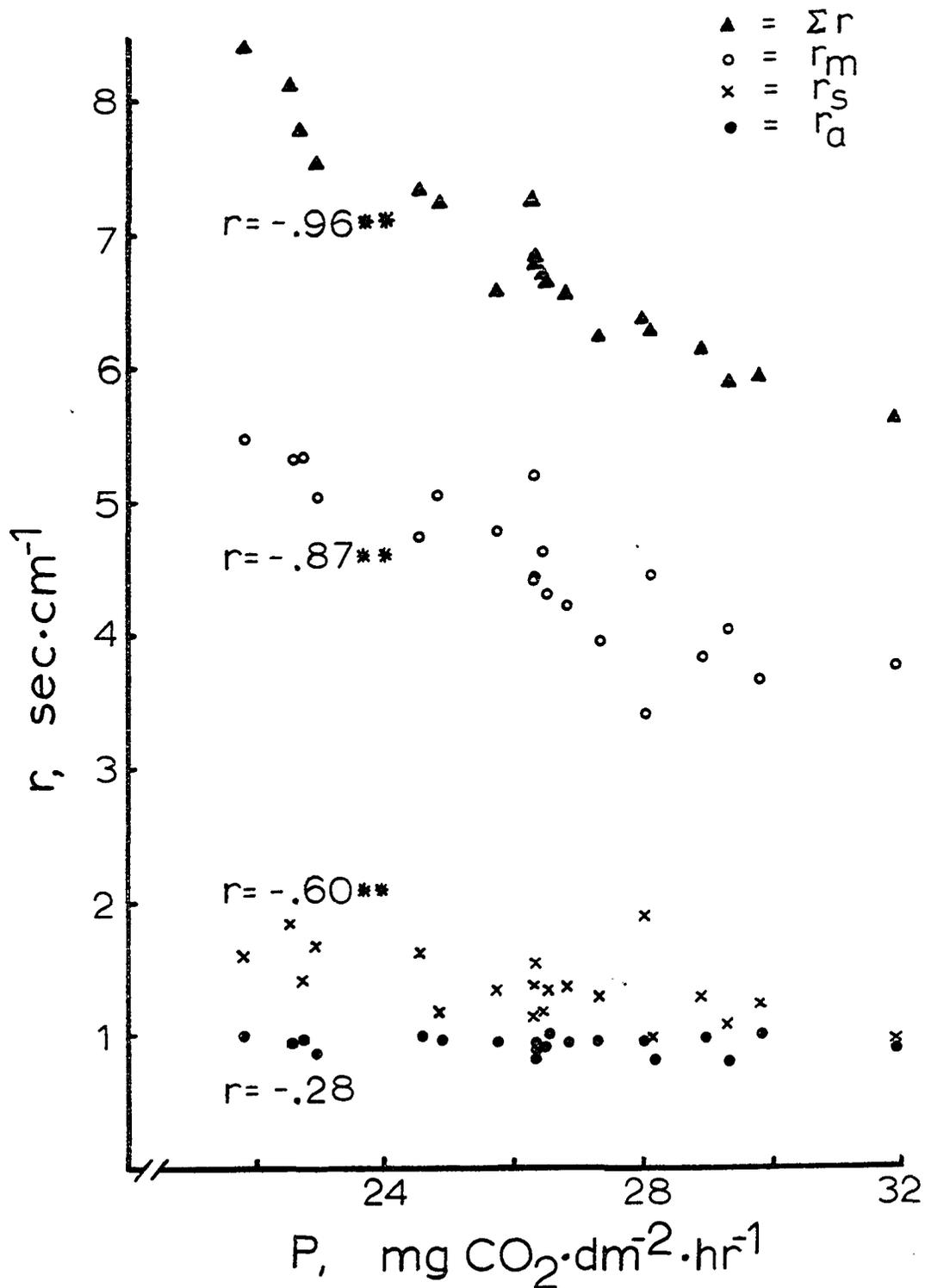
resistance ( $r_m$ ). Each diffusion resistance term is discussed separately in relation to photosynthetic limitations.

Sum of resistances The sums of the  $CO_2$  diffusion resistances were significantly different between genotypes in 1968 and 1969. The ranking of the genotypes with respect to  $\Sigma r$  was similar across years. This was indicated by the fact that the correlation of genotypic mean  $\Sigma r$  values across years was significant at the 5 percent level ( $r = .46$ ). Genotypic mean net photosynthetic rates were significantly correlated in a negative manner with mean  $\Sigma r$  values (Figure 23), indicating the  $CO_2$  diffusion process is probably limiting for net photosynthesis ( $r = -.91$  and  $-.96$  in 1968 and 1969, respectively).

The genotypic mean  $\Sigma r$  values ranged from 4.48 to 8.53 and from 5.64 to 8.40  $sec \cdot cm^{-1}$  in 1968 and 1969, respectively. The mean  $\Sigma r$  values, averaged across all 20 oat genotypes, were 6.17 and 6.81  $sec \cdot cm^{-1}$  in 1968 and 1969, respectively. El-Sharkawy and Hesketh (1965) reported a  $\Sigma r$  value of 6.9  $sec \cdot cm^{-1}$  for the oat variety Markton. The  $\Sigma r$  values reported in this experiment were slightly lower; however, the above figures represent mean  $\Sigma r$  values for the 20 oat genotypes, whereas the figure reported by El-Sharkawy and Hesketh (1965) was for only one genotype. Because in calculating  $\Sigma r$ , the  $CO_2$  concentration at the chloroplast site was assumed to equal the  $CO_2$  compensation concentration, rather than zero, lower than reported  $\Sigma r$  values resulted.

Since the  $\Sigma r$  values were mathematically equal to the reciprocal of the slope of the  $CO_2$  response curve ( $S$ ), high negative correlations of

Figure 23. Relationship of mean genotypic CO<sub>2</sub> diffusion resistance estimates with mean net photosynthetic rates. The  $r_a$ ,  $r_s$ , and  $r_m$  values were measured in 1969 only.



$\Sigma r$  with  $S$  resulted. Net photosynthetic rates were also highly correlated with  $S$  ( $r = .96$  and  $.97$  in 1968 and 1969, respectively). It is evident that genotypic differences in net photosynthesis are associated with  $\Sigma r$  or  $S$ .

Laminar resistance It was mentioned in the Literature Review section that laminar or boundary layer resistance ( $r_a$ ) is influenced primarily by leaf shape and surface characteristics. In this experiment  $r_a$  was found to increase as a function of leaf size. Although the increase in  $r_a$ , as leaf size increased was not large, differences in  $r_a$  values between genotypes were significant. The correlation of genotypic mean  $r_a$  values with mean leaf area was significant at the 1 percent level ( $r = .94$ ). Because of the manner in which  $r_a$  was calculated in this experiment, the correlation of  $r_a$  with leaf area was not unexpected. Small saturated blotters had larger evaporation rates than large saturated blotters because of a lower boundary layer resistance.

Laminar resistance is not believed a factor responsible for large genotypic differences in net photosynthetic rates in oats. Laminar resistance is the smallest of the three  $CO_2$  diffusion resistances, and it is the least variable term. The  $r_a$  values measured in this experiment ranged from only  $.80$  to  $1.02 \text{ sec}\cdot\text{cm}^{-1}$ . El-Sharkawy and Hesketh (1965) reported an  $r_a$  value of  $1.1 \text{ sec}\cdot\text{cm}^{-1}$  for the oat variety Markton.

In general diploid, tetraploid (with the exception of P.I. 193958), and A. sterilis genotypes had small leaves and low  $r_a$  values, whereas many of the Midwestern adapted genotypes with large leaves showed higher

$r_a$  values. Mean genotypic net photosynthetic rates (P) were not significantly correlated with mean  $r_a$  values ( $r = -.28$ ); however, the correlation was negative (Figure 23). There appeared to be a trend for negative correlations of  $r_a$  with P, even within specific genotypes (Table 25). Most of the correlations within genotypes were not significant.

The fact that  $r_a$  was the smallest and least variable of the three  $\text{CO}_2$  diffusion resistances, coupled with the fact that  $r_a$  was not significantly correlated with P, would seem to preclude the use of genetic modifications directed toward lowering  $r_a$  by reducing leaf widths in oats. In most small grains the flag leaf is considered the most important leaf in supplying carbohydrates to the panicle or ear. Consequently, it would seem that narrow flag leaves might in fact be undesirable from the aspect of reducing the active photosynthetic flag leaf area. Baker and Myhre (1969) similarly concluded that genetic manipulations designed to alter  $r_a$  in cotton would most likely not increase rates of photosynthesis. They also emphasize the fact that  $r_a$  is small in relation to the other  $\text{CO}_2$  diffusion resistances.

Stomatal resistance Stomatal resistance ( $r_s$ ) is a physical resistance to  $\text{CO}_2$  diffusion. Miller (1938) reported that there were approximately 54,000 stomata per square inch on an oat leaf, and the ratio of stomata on the top of the leaf surface versus the bottom was approximately one. Bonnett (1961), however, has reported more stomata on the upper than on the lower surface of the oat leaf lamina. Because the

mesophyll of monocot leaves shows no distinct differentiation into spongy and palisade parenchyma (Esau, 1966), and since monocotyledonous leaves have approximately equal numbers of stomata on the upper and lower surfaces of the leaf lamina, the error incurred when the component resistances were calculated from measurements of photosynthesis and transpiration on the entire leaf, as opposed to measurements of photosynthesis and transpiration on the two leaf surfaces separately, was believed insignificant. Gale and Poljakoff-Mayber (1968) believe considerable error may be incurred when the component leaf resistances to gases are calculated from the rates of photosynthesis and transpiration measured for the entire leaf. This, they believe, is due to the fact that there are two parallel pathways for diffusion--through the upper and lower leaf surfaces--which in most leaves are asymmetrical.

Significant differences in  $r_s$  values were observed, and the negative correlation of mean genotypic net photosynthetic rates with mean  $r_s$  values was highly significant ( $r = -.60$ , Figure 23). Correlations of  $r_s$  with P within genotypes were generally negative (Table 25). The  $r_s$  values ranged from .96 to 1.91  $\text{sec}\cdot\text{cm}^{-1}$ . The mean  $r_s$  value for the 20 genotypes was 1.36  $\text{sec}\cdot\text{cm}^{-1}$ . This mean  $r_s$  value compares favorably with the value of 1.7  $\text{sec}\cdot\text{cm}^{-1}$  reported by El-Sharkawy and Hesketh (1965) for the oat variety Markton. The fact that mean  $r_s$  values were significantly correlated in a negative manner with mean net photosynthetic rates, and that  $r_s$  values were more variable than  $r_a$  values can be interpreted to mean that a significant amount of genotypic variation in net photosynthetic rates in oats can be explained by genotypic differences in  $r_s$ . Dornhoff

(1969) has noted a similar correlation of net photosynthesis with  $r_s$  in soybeans.

Since  $r_m$  is the largest of the  $\text{CO}_2$  diffusion resistances in oats, it is believed that changes in  $r_s$  would have a more profound effect on transpiration than on net photosynthesis. If  $r_m$  were small compared with  $r_s$ , then photosynthetic rates would be affected relatively more by changes in stomatal aperture.

Mesophyll resistance Mesophyll resistance ( $r_m$ ) is calculated by subtracting ( $r_a + r_s$ ) from  $\Sigma r$ . It has been stated by Moss (1968a) that  $r_m$ , calculated by this technique, is a function of light intensity or any other factor which controls net photosynthesis. This is because most techniques for calculating  $\Sigma r$  assume the  $\text{CO}_2$  concentration at the chloroplast equals zero (see Equation 1, Literature Review section), whereas Moss (1968a) states, in fact, the partial pressure of  $\text{CO}_2$  can only be zero above sugar. Thus, it is concluded that  $r_m$ , as calculated using zero  $\text{CO}_2$  at the chloroplast, is a "residual" resistance, which is a function of the biochemical processes, as well as physical resistances to  $\text{CO}_2$  transport in the leaf mesophyll.

The  $\text{CO}_2$  concentration at the chloroplast site is difficult to measure; however, it is believed not to equal zero, even though the partial pressure of  $\text{CO}_2$  above the photosynthetic product is probably equal to zero. It is believed that the  $\text{CO}_2$  compensation concentration ( $\Gamma$ ) more accurately represents the actual  $\text{CO}_2$  concentration at the chloroplast site. Such a conclusion has been supported by the work of Bierhuizen

and Slatyer (1964) and Whiteman and Koller (1968). The slope procedure, introduced by Holmgren et al. (1965) and employed in this study, for calculating  $r_m$  (Equation 7, Literature Review section), makes the implicit assumption that the  $CO_2$  concentration at the chloroplast site is equal to  $\Gamma$ . Based upon mean net photosynthetic rate and  $\Gamma$  values for all 20 oat genotypes, it was calculated that  $r_m$  values were reduced by approximately 30 percent when the  $CO_2$  concentration at the chloroplast site was assumed to equal  $\Gamma$ . Thus, the estimate of  $r_m$  was minimized and it is hence, believed more likely to represent the physical resistance of the leaf mesophyll.

Differences between genotypic  $r_m$  values were significant at the 1 percent level. The mean  $r_m$  value, obtained as an average across all 20 genotypes, was  $4.48 \text{ sec}\cdot\text{cm}^{-1}$  and genotypic means ranged from 3.43 to  $5.48 \text{ sec}\cdot\text{cm}^{-1}$ . El-Sharkawy and Hesketh (1965) reported an  $r_m$  value of  $4.1 \text{ sec}\cdot\text{cm}^{-1}$  for the oat variety Markton. The fact that, (1) variation of  $r_m$  was large, (2)  $r_m$  values were significantly inversely correlated with net photosynthetic rates ( $r = -.87$ , Figure 23), and (3)  $r_m$  was considerably greater in magnitude than either  $r_s$  or  $r_a$ , would seem to justify the conclusion that a major part of the variation of net photosynthetic rates between genotypes can be explained by differences in  $r_m$ .

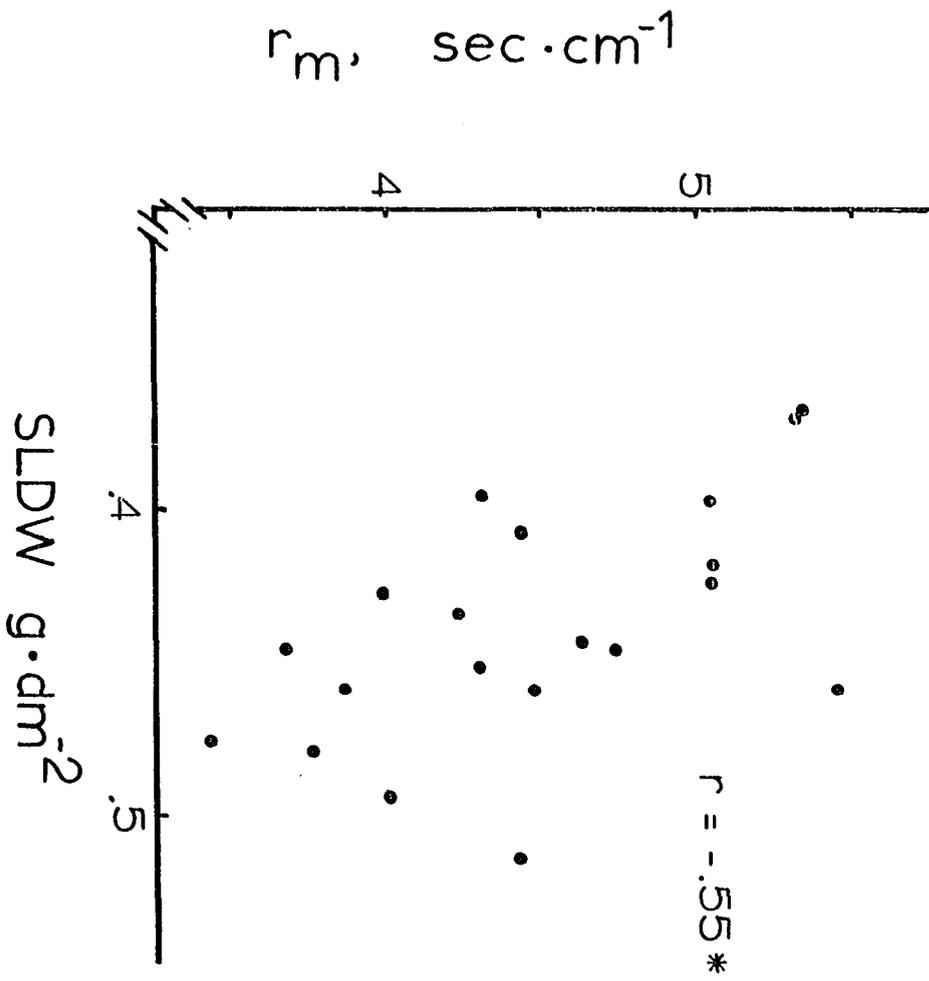
Leaf anatomy as related to the diffusion process      The mechanism of increased net photosynthetic rates in the genotypes with a high specific leaf dry weight was not studied in this experiment. Therefore, only possible mechanisms will be suggested. One definite relationship

seemed to exist. The mesophyll resistance and SLDW were significantly correlated in a negative manner ( $r = -.55$ , Figure 24). Holmgren (1968) has also reported a highly significant correlation between mesophyll conductance ( $1/r_m$ ) and leaf dry weight per unit area in leaves of different populations of Solidago virgaurea L. Consequently, it would appear that mesophyll resistance is associated with the internal morphology of the leaf as revealed by the SLDW--at least in some plant species. Since the  $r_m$  diffusion resistance component includes both physical and biochemical factors, mechanisms related to each of these factors will be discussed.

Leaves having a high SLDW and low  $r_m$  may show greater net photosynthetic rates as a result of structural differences which are associated with physical transport of  $CO_2$  from the external air to the chloroplast site. The following reasons might account for the relationship. First, leaves with a high SLDW are probably thicker. This might result in more parallel pathways for  $CO_2$  transport. Second, if leaf-thickness does increase, the assumed increase in the volume of intercellular spaces with thickness might facilitate the transport of  $CO_2$ . The third hypothesis is that if leaf thickness was not increased, leaves with a high SLDW might be expected to have smaller mesophyll cells, which might presumably promote transfer of  $CO_2$  because of a larger ratio of cell surface area to external leaf surface area.

The first explanation was proposed by Holmgren (1968). He believed that such a relationship was indicated by an increase in the number of palisade cell layers and palisade cell length in leaves of Solidago

Figure 24. Relationship of mean genotypic mesophyll resistance ( $r_m$ ) with mean specific leaf dry weight (SLDW) measurements obtained in 1969.



virgaurea L. This presumably resulted in an increased ratio of internal to external surface area. In oats there is no well differentiated palisade layer (Bonnett, 1961); consequently, increases in palisade cell length, such as reported by Holmgren (1968) or Pieters (1960) would not be present. Larger spongy mesophyll cells might conceivably be present, however.

Wilson and Cooper (1967) state that the ratio of internal surface area to external surface area could be an important factor governing CO<sub>2</sub> transport; however, they suggest a second factor favoring CO<sub>2</sub> transport may also be associated with thicker leaves. They believe thicker leaves might be expected to show greater photosynthetic rates because the volume of intercellular spaces, which would facilitate the transport of CO<sub>2</sub>, would be increased. However, the fact that Irvine (1967) found no correlation in sugar cane leaves between leaf porosity and leaf thickness would not lend support to this hypothesis.

Contrary to the two hypotheses mentioned above, Hiechel and Musgrave (1969a) state that thicker leaves should exhibit a greater mesophyll diffusion resistance. Presumably CO<sub>2</sub> diffusion resistances would increase with increasing leaf thickness because of longer CO<sub>2</sub> diffusion path lengths (El-Sharkawy and Hesketh, 1965). There was no evidence to support such an occurrence in oats in this experiment.

The third hypothesis was proposed by El-Sharkawy and Hesketh (1965). They observed that species having small diameter palisade mesophyll cells showed greater maximum net photosynthetic rates than those with

large diameter mesophyll cells. This suggested the ratio of internal cell surface to cell volume ratio was important in determining the maximum net photosynthetic rate. Loach (1969), however, has experimental evidence that the cell surface to cell volume ratio is poorly correlated with net photosynthesis.

Leaf thickness was not measured in this experiment, but it might be assumed, reasonably, that genotypes having a high SLDW would have thicker leaves. Friend (1966) noted that thicker leaf laminae in wheat were characterized by larger mesophyll cells with a higher degree of lobing. In oats the mesophyll cells are bilobed or multilobed (Bonnett, 1961). Such lobing would be expected to increase the external surface area of the cell without increasing its diameter. Should more lobing occur in oat leaves with a high SLDW, an increased cell surface to cell volume ratio might result, and the volume of intercellular spaces might be increased, too. It would seem reasonable that the number of parallel pathways for CO<sub>2</sub> transport might also be increased in a leaf with such anatomical characteristics.

The observation that the SLDW is correlated with  $r_m$  might suggest biochemical differences as well as physical differences in CO<sub>2</sub> diffusion. Loach (1969) reported a significant correlation between maximum photosynthesis and volume of mesophyll cells, and he postulated that biochemical factors, rather than physical diffusion factors, limited the net photosynthetic rate. It is interesting to speculate that large mesophyll cells might contain a larger total volume of active enzymes than small mesophyll cells. Because enzymes are proteins, and because extremely high correlations of leaf nitrogen content and dry weight

per unit area have been reported ( $r = .97$ ) by Holmgren (1968) in sun and shade populations of Solidago virgaurea L., such a hypothesis might seem attractive.

Though the reason for the inverse relationship between SLDW and  $r_m$  was not clear, it is suggested internal morphological differences may interact in such a manner so as to depress the physical resistance to  $CO_2$  transport. Biochemical factors may also be associated with leaf anatomical characteristics.

## SUMMARY

Experiments were conducted during the summers of 1968 and 1969 to study, (1) whether or not genotypic variation of net photosynthesis existed in oats, (2) the relationship between net photosynthesis and specific leaf weight, and (3) the physiological basis for photosynthetic variation. To fulfill these objectives the net CO<sub>2</sub> exchange rates of 20 oat genotypes differing in, (1) ploidy level, (2) species (within hexaploids), (3) origin, and (4) productivity level within Midwestern adapted genotypes were measured by the conventional infrared analysis technique.

Significant differences in net photosynthetic rates of flag leaves were found in both 1968 and 1969. In general, photosynthetic differences were not associated with differences in ploidy level, origin, or productivity. There appeared to be a trend for the A. byzantina and A. sativa species to show greater net photosynthetic rates than A. fatua and A. sterilis species. However, because only two genotypes of each species were tested, it is believed such differences may have merely been coincidental.

The results of this experiment indicate that a major portion of the photosynthetic variation between genotypes can be explained by differences in mesophyll resistance ( $r_m$ ) to CO<sub>2</sub> transfer. Stomatal resistance ( $r_s$ ) was also significantly, negatively, correlated with the net photosynthetic rate, but  $r_s$  was much smaller than  $r_m$  in all genotypes. Results show no indication that photorespiration and/or CO<sub>2</sub> re-assimilation

efficiency were related to genotypic variation of net photosynthesis.

Net photosynthetic rates, as measured at normal atmospheric CO<sub>2</sub> concentrations, were significantly correlated with specific leaf dry weight (SLDW). No correlation between the specific leaf fresh weight (SLFW) and net photosynthesis was found. The fact that the SLDW and mesophyll resistance ( $r_m$ ) were negatively correlated suggests leaf anatomical characteristics may be associated with genotypic photosynthetic differences. It is suggested that the SLDW should receive further attention as a possible index to select for photosynthetically efficient oat genotypes.

PART II. THE SINK-SOURCE EXPERIMENT

## LITERATURE REVIEW

The control of leaf net photosynthetic rate by assimilate concentration in the leaf is an old hypothesis. However, there is now limited experimental evidence which indicates, because of limited size of storage organs, leaves may operate at levels below the maximum of which they are capable. Neales and Incoll (1968) state that to establish a satisfactory proof of this hypothesis, it must be shown that there is a negative correlation between photosynthesis and assimilate level in the leaf and biochemical mechanisms involved should be demonstrated. There is, currently, little information on biochemical mechanisms involved. However, there is evidence that growth substances produced by growing organs might exert a controlling influence on the photosynthetic rate (Sweet and Wareing, 1966).

It is necessary to define the terms "source" and "sink" before further discussion of the subject. The assimilatory surfaces have been referred to as the "source" of assimilates, and Beevers (1969) has defined the "sink" as any location in the plant where products of photosynthesis are utilized. The sink may take the form of underground plant parts (roots or tubers), non-green aerial plant parts (buds, flowers, or fruits), or any other plant part which constitutes a drain on photosynthetic products. Sucrose is the major transport form of assimilate that moves from source to sink.

Experiments dealing with sink-source relationships have been partitioned into two categories; those designed to test the hypothesis that

sink demand controls photosynthetic rate and those which have invoked this hypothesis to explain results of leaf photosynthetic measurements which were acquired for other purposes (Neales and Incoll, 1968).

#### Indirect Evidence of a Sink Effect

Early evidence, based upon the hypothesis that assimilate accumulation in the leaf reduced photosynthesis, first resulted from measurements taken on attached and detached leaves (Neales and Incoll, 1968). It was reasoned that accumulation of assimilates in the leaf would be more marked in detached than attached leaves and the assimilate accumulation could eventually depress the photosynthetic rate.

Neales and Incoll (1968) also state that "midday depression" of photosynthesis has been interpreted as carbohydrate accumulation effects on photosynthesis. However, in experiments where net photosynthesis has been measured under constant environmental conditions "midday depressions" have not been found associated with assimilate accumulations in the leaf. Bohning (1949) observed a daily cycle in the photosynthetic rate of apple tree leaves exposed to atmospheric CO<sub>2</sub> concentrations and continuous light; however, when tanks of compressed air were used as CO<sub>2</sub> source, the fluctuations did not occur. He concluded the fluctuations of photosynthesis were due to changes in the atmospheric CO<sub>2</sub> content rather than assimilate accumulation in the leaf. Stable photosynthetic rates were measured for periods of up to 18 days in "sun" adapted leaves exposed to a light flux density of 3,200 foot-candles; but, at a light flux density of 5,800 foot-candles, the photosynthetic rate decreased 40 percent by

the fourteenth day. End product inhibition was considered, but as translocation was not restricted, bleaching of chlorophyll was considered by Bohning (1949) to be the major reason for the photosynthetic depression. Murata and Iyama (1963a) and Iyama et al. (1964) have also observed diurnal variations of photosynthesis in various crop species, but they found the variations could be explained by atmospheric CO<sub>2</sub> fluctuations.

Heath and Orchard (1957) have associated the "midday depression" with stomatal apertures, and Hopkinson (1964) has associated the depressions of photosynthesis he observed in cucumber leaves (Cucumis sativus) after lengthy light exposures partly to stomatal closure. Hopkinson (1964) observed that capacity of cucumber leaves to export assimilates decreased with age. This was indicated by the fact that the photosynthetic rate of older leaves declined more rapidly than younger leaves when exposed to continuous light. He suggested this response was related to a decreased ability of the leaf to phosphorylate sugars because the photosynthetic decline became more pronounced as the phosphorus content of aging leaves decreased.

Neales and Incoll (1968) state that before "midday depression" in assimilation rate can be ascribed to accumulation of assimilate in the leaf, the accumulation should be demonstrated. This was not done in the above experiments. They also mention that the two factors should be shown to be causally associated. To date there seems to be only correlative evidence.

### Direct Evidence of a Sink Effect

If photosynthesis is influenced by the demand for assimilates, experiments designed to alter the sink-source ratio would seem to be useful in testing the hypothesis. Four types of plant manipulations have been used to study relationships between sinks and sources. Manipulation of the sources of assimilates, manipulation of the translocating system to impede or prevent translocation, manipulation of sinks for assimilates, and treatments of the intact plant in a manner to increase assimilate accumulation in leaves have been used to study sink-source relationships.

#### Manipulation of the source

Two types of experiments have been conducted by manipulating the source of assimilates. The first type has involved complete removal of the source from normal sinks. When this is done the leaf then becomes the sink. Beevers (1969) has stated that even within leaves there are many cells lacking chloroplasts, and these autotrophic cells consume photosynthate in their own growth and respiration.

Using barley (Hordeum) leaf segments, Nātr (1967) was able to measure constant assimilation rates for periods as long as 78 hours. The leaf segments showed an approximate 200 percent increase of initial dry weight and the net assimilation rate was not observed to decline until leaf segments began to yellow. Working with detached sugar cane (Saccharum) leaves, Hartt (1963) noted that leaves of several varieties

maintained maximum photosynthetic rates for only about two hours before the rate fell. The photosynthetic depression was not associated with stomatal closure. Subsequent experiments showed that photosynthesizing sugar cane leaves, with the stem base immersed in water, showed a 34 percent reduction of photosynthesis and 84 percent increase in leaf sucrose at the end of three hours, whereas leaves exposed to light for the same period of time, but with the leaf base immersed in a 5 percent sucrose solution, showed a 90 percent reduction in photosynthetic rate and 230 percent increase in leaf sucrose content. It was concluded that absorption of  $\text{CO}_2$  by leaves of sugar cane was negatively associated with high leaf sugar contents, and coordination of translocation and photosynthesis was essential for maximum yields.

The second type of experiment involving source manipulation is generally achieved by leaf removal or shading treatments designed to alter the sink-source ratio. Loomis (1935) noted that, if maize (Zea mays L.) was defoliated at the time of ear silking, grain yield was found to increase as a linear function of the percent remaining leaf area, except at high percentages of remaining leaf areas. He postulated that at large leaf areas translocation to the same or slightly larger number of developing ovules probably became a limiting factor, and with the delay in removal of photosynthate from the leaves there may have been a tendency toward the reversal of some photosynthetic reactions, with a consequent loss of leaf efficiency. He emphasized that evidence for this occurrence was not available.

There is evidence that leaves may normally photosynthesize below their potential. Kiesselbach (1948) observed that a midseason reduction of 50 percent of the leaf area in corn reduced dry-matter production by 22 percent but increased the net assimilation rate of remaining leaves by 56 percent. That the photosynthetic efficiency of leaves of partially defoliated corn plants may increase has also been observed by Allison and Watson (1966).

Womack and Thurman (1962) found that in wheat (Triticum aestivum L.) leaf removal one week before the boot stage depressed yields the most, but removal of at least 10 percent of the leaf area was necessary to significantly reduce grain yield. In oats (Avena sativa L.) removal of 30 to 40 percent of the leaf area was necessary to significantly reduce yields below that of the check. Stoy (1965), however, observed that in wheat elimination of even one leaf (flag leaf) decreased total grain weight. This indicated to him that there was no surplus productive capacity and that during the grain filling period all reserves were drained to the utmost.

Evidence of a sink effect has been observed in trees. Sweet and Wareing (1966) suggested that instead of there being a one-way relationship of photosynthesis controlling growth, the reverse phenomenon of growth controlling photosynthesis might also occur. They observed that, when all fully expanded leaves were removed from 11 week old pine seedlings (Pinus radiata D. Don) six days prior to photosynthetic measurements, photosynthetic rates of defoliated plants at saturating light flux densities were 27.4 percent greater than those of undefoliated

controls. When fully expanded leaves were removed immediately before measurements of photosynthesis were made, the plants showed rates of photosynthesis similar to those of the controls. They state that since the sink for photosynthates in defoliated plants was comparable to that of the controls, photosynthetic activity of remaining leaves might be expected to increase with time. Higher net assimilation rates have also been observed in partially defoliated apple trees (Maggs, 1964).

Wardlaw et al. (1965) have shown, by the use of labelled carbon, that removing or shading photosynthetic organs in wheat, results in compensatory movement of carbohydrates from other plant parts, and the photosynthetic contribution of the shaded or defoliated part is likely to be underestimated. Also, Frey-Wyssling and Buttrose (1959) state that one of the major difficulties of shading experiments designed to evaluate photosynthetic contributions of various plant parts is compensation for the absence of photosynthesis by other plant parts.

#### Manipulation of translocation from source to sink

Blockage of translocation of assimilates moving out of the leaf has been used as a means of regulating the carbohydrate content of the leaf to study the role of assimilate accumulation on photosynthesis. In an attempt to simulate wind injury effects in sugar cane (Saccharum), Hartt (1963) observed that six hours after breaking only the leaf midrib, translocation of assimilates was reduced 34 to 38 percent and photosynthesis was inhibited 30 percent. When both the midrib and

lamina were broken, translocation of assimilates was almost completely inhibited and photosynthesis was reduced 84 percent. Moisture determinations indicated leaf water content was not altered by the treatments.

Neales and Incoll (1968) state that a more elegant way of inactivating the phloem is to chill a portion of the conducting stem of petiole. This method offers a valuable means of regulating the carbohydrate content of a leaf in a reversible fashion. Research by Thrower (1965) has shown resumption of translocation in petioles of chilled soybean (Glycine max (L.) Merr.) to occur with 5 to 20 minutes after warming. Using this method, Webb and Gorham (1965) found that varying the primary leaf node temperature of straight-necked squash (Cucurbita milopepo torticollis Bailey) from 0 to 55°C did not alter the rate of  $^{14}\text{CO}_2$  assimilation or transpiration rate. However, the export of labelled carbon from the leaf was reduced in a reversible fashion at low temperatures and irreversibly at high temperatures, showing an optimum export rate at about 25°C. Burt (1966) found, however, that when developing potato tubers (Solanum tuberosum L.) were exposed to a temperature regime of 17 to 32°C the net assimilation rate was 1.40  $\text{mg}\cdot\text{cm}^{-2}\cdot\text{day}^{-1}$ ; when the temperature of the ambient air surrounding the tubers was reduced to 7 to 13°C, the net assimilation rate was reduced to .91  $\text{mg}\cdot\text{cm}^{-2}\cdot\text{day}^{-1}$ . Measurement of leaf dry weight area ratios failed to indicate any detectable carbohydrate accumulation in leaves, and it was believed that growth and net assimilation rate were controlled by growth of the major carbohydrate sinks.

### Manipulation of sinks

Removal of sinks to which leaf assimilates move might be expected to depress leaf assimilation rates. Development of more or larger sinks would be expected to perhaps increase assimilation rates of leaves, if they are operating below capacity.

Both Burt (1964) and Nösberger and Humphries (1965) found that removal of potato tubers reduced net assimilation rates. Burt (1964) observed that after 13 days the leaf dry weight-leaf area ratio and stem weight increased, but growth of the entire plant was reduced 44 percent. Nösberger and Humphries (1965) stated that as the amount of nitrogen supplied was increased, the effect of tuber removal on net assimilation rate was diminished, presumably because other sinks for carbohydrate accumulation developed.

Tsuno and Fujise (1965) found that if sweet potato tuber growth was prevented by exposing them to light, the photosynthetic rate was depressed and the starch content of the leaves increased. They also found, however, that the photosynthetic rates of sweet potato leaves showed no marked diurnal fluctuations even though the carbohydrate contents of the leaves were greater in the afternoon than in the morning. On the other hand, in cases where plants were cultured under differential nutrient conditions, or where the plants were at different growth stages, leaves having high photosynthetic rates were shown to have lower carbohydrate contents than leaves with low photosynthetic rates. From these results they concluded that it is not the accumulation of photosynthates in the leaf, but the rate of movement of photosynthates from

the leaf (source) that is essential in controlling the photosynthetic rate.

Kiesselbach (1948) observed that in maize, removal of the ear shoot at silking increased stover yield 59 percent but reduced total dry-matter production 27 percent. This suggested greater storage of photosynthates in the stalk, but less photosynthetic output. Moss (1962a) found that if pollination of the double-cross corn hybrid Conn. 870 was prevented, the CO<sub>2</sub> assimilation rate was only 55 percent of that of nonbarren plants and the barren plants had a higher sugar content. Barren plants showed a slightly greater photosynthetic rate at the end of the season because of delayed maturity but the advantage was small, in absolute terms, compared to the disadvantage measured earlier in the season. Sugar accumulation and depression of photosynthesis were also successfully shown in the determinant tomato variety Tiny Tim. Indeterminant plants have storage mechanisms or organs other than fruit or seeds, and Moss (1962a) reasoned that barrenness would have little effect on such plants. Contrary to Moss's and Kiesselbach's research, Allison and Watson (1966) found that when pollination of corn was prevented, net assimilation during the first month after flowering was unaffected and dry-matter which would have passed into the grain accumulated in the stem and husks.

Nösberger and Thorne (1965) observed that removal of half the florets from barley (Hordeum spp.) depressed photosynthesis of flag

leaves slightly ( $2.0 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ ) between 10 and 17 days after ear emergence, but slightly increased photosynthesis ( $1.3 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ ), and delayed leaf senescence 31 to 42 days after ear emergence. Leaf weight per unit area was unaffected, which they believed indicated floret removal did not decrease movement of assimilate from the flag leaf. They concluded that removal of half the florets had only a slight effect on photosynthesis because the total dry weight of shoot, roots, and late tillers increased and net assimilation rate was little affected. In this case alternative sinks appeared as a result of treatments designed to remove them (Neales and Incoll, 1968). King et al. (1967), who prevented the development of alternative sinks in wheat (Triticum aestivum L.) by clipping the plants so that only the intact main culm and two tillers, from which the ears were removed, remained, showed 50 percent photosynthetic reductions within 3 to 15 hours after the ear from the main culm was removed. They also state that stomatal resistance was significantly higher in plants without ears than initial and control values. Subsequent darkening of all other leaves on plants without ears led to recovery of flag leaf photosynthesis and increased translocation of assimilates to roots and young shoots. Inhibition of ear photosynthesis by spraying them with DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea), a specific inhibitor of photosynthesis, was found to restore depressed photosynthetic rates of flag leaves which had been exposed to a week of continuous high irradiance.

Considerable work on sink effects has been done on apple trees.

Maggs (1963) found redistribution of carbohydrates in deblossomed apple trees and less total dry-matter production per unit leaf area. This was attributed to a greater "demanding power" of the fruiting trees in diverting photosynthates from lower portions of the tree and increased rates of removal of photosynthates from leaves. Working with apple trees Hansen (1967) observed that amount of  $^{14}\text{CO}_2$  taken up per spur shoot was unaffected by the presence or absence of fruit. However, more recent work (Hansen, 1969) has shown significant negative correlations ( $r = -.81$  to  $-.92$ ) between percent labelled carbon fixation in the fruit and the leaf-fruit ratio of branches. Kazaryan et al. (1965) have been able to detect higher net photosynthetic rates in apple tree leaves located near fruits, and assimilatory activity was observed to almost double during years of fruit bearing. Leaves located near fruits had a lower soluble carbohydrate content but protein content was unaffected. Greater metabolic activity in leaves of fruit bearing branches was believed to be supported by the fact that higher phosphorus and ash contents were found in such leaves. They state that phosphorus plays an active part in the translocation of sugars synthesized in the leaves.

Sweet and Wareing (1966) removed stem apices from shoots of pine trees and found that after eight days leaves of treated plants showed significantly lower photosynthetic rates. They suggested this was either a "metabolic sink effect" or an effect of auxin level on photosynthetic rate. Turner and Bidwell (1965) noted increased photosynthetic rate in bean leaves (Phaseolus vulgaris L.) was associated with breaking

of dormancy of an axial bud. Similar increases of photosynthetic rate were observed when the leaf was sprayed with an indoleacetic acid solution. However, Neales and Incoll (1968) were unable to reproduce this response. Alvin (1960) has associated greater net assimilation rates in bean plants receiving gibberellic acid spray treatments with more rapid translocation of photosynthates from leaves to stem tissue. King et al. (1967) found that application of TIBA (2,3,5-triiodobenzoic acid) to the culm of wheat plants directly below the ear inhibited auxin movement, but had no effect on flag leaf photosynthesis, suggesting to them that control of photosynthesis was regulated directly by demand for assimilates from the flag leaf rather than through the action of leaf auxins produced in sink regions.

The sink effect has also been studied by treatments designed to alter the sink capacity of the roots. Using single, rooted leaves of dwarf bean, (Phaseolus vulgaris L.), Humphries (1963) postulated that if assimilatory area remained constant and because carbohydrates could be transported only to a single sink (roots), inhibition of assimilation by accumulation of carbohydrates could be easily studied. Using this approach, Humphries (1963) and Humphries and Thorne (1964) observed that net assimilation rates of bean plants (single leaf plus root) increased as the size of the sink (root) increased. The net assimilation rate decreased when root growth was restricted by high temperature or kinetin treatments, but net assimilation rate increased when root growth resumed following partial removal of roots or when root growth was promoted by indoleacetic acid treatments. Ryle and Hesketh (1969) have observed photosynthetic increases in maize plants amply supplied

with nitrogen following repotting from 15 cm to 25 cm pots and partial leaf removal.

Attempts have been made to increase sink size. Thorne and Evans (1964) attempted to determine whether the greater root size of sugarbeet was the result of or cause of greater net assimilation rates in sugarbeet, as opposed to spinach beet. They grafted tops and roots of the two types of plants in all four possible combinations. Grafted plants with sugarbeet roots were observed to have greater net assimilation rates, less leaf area, and lower lamina sugar contents than those with spinach beet roots, irrespective of the top. They believe sugarbeet roots probably increased photosynthesis by providing a better sink for assimilates than spinach beet roots. Recent work has shown that sugarbeet plants germinated in a growth cabinet at 20°C, and transplanted into the field after three weeks, developed larger roots than plants grown from drilled seed. At the end of the season root size was 39 percent greater in plants started in the growth cabinets, which Humphries and French (1969) attributed to a "sustained increase" in photosynthesis as a result of a larger initial sink for photosynthates.

It is interesting to note that with the evolution of cultivated plants there has been an enormous increase in size of plant parts of special interest to man. Stoy (1969) states that the increase in size of these parts has sometimes been accompanied by enlargement of photosynthetic organs, but in most cases increase in photosynthetic efficiency has been achieved. The increased storage organ growth is believed

to be accompanied by a stronger flow of assimilates from photosynthetic organs.

It was suggested by Bingham (1966) that yield of winter wheat might be limited by either the assimilative capacity of the plant after anthesis or the size of the sink for photosynthates. When reciprocal crosses of two varieties with differing seed size were made, it was found that the genotype of the grain was the determinant of grain size and yield. He concluded, however, that the genetic limitation of sink size prevented the assimilative capacity of the plant from being fully exploited.

Carr and Wardlaw (1965) noted increases in photosynthesis by the ear of wheat up to 15 days after anthesis, which might indicate increased assimilatory capacity with increased sink size. However, Stoy (1965) mentioned that he could observe no signs of increased photosynthesis in wheat during the grain filling stage.

Dornhoff and Shibles (1970) noted an increase in mean net photosynthetic rates of recently expanded leaves of 20 soybean varieties (Glycine max (L.) Merr.) at about the time of pod filling, and they speculate that perhaps both changes in sink demand, as well as decreased CO<sub>2</sub> diffusion resistances, explain the phenomenon.

#### Manipulation of the entire plant

In some instances the entire plant has been subjected to various types of treatments designed to show a sink effect. Warren-Wilson (1966) observed that net assimilation rates, relative growth rates, and leaf

area ratios of Oxyria digyna Hill were depressed when grown in an arctic environment compared with similar plants grown in a temperate climate. The arctic grown plants were found to have greater sugar and starch concentrations, which led him to propose that depression of net assimilation rates in arctic grown plants was due to low temperatures, which, especially when associated with soil nitrogen deficiencies, reduced the rate at which assimilates could be used in respiration and new growth. Reduced utilization of assimilates was believed to result in sugar accumulations to levels which could depress photosynthesis.

Waldron et al. (1967) found that if sugar cane (Saccharum) was exposed to a 17/10°C day-night temperature regime for a prolonged period of time, stalk elongation ceased and an initial rise in the level of stored sugar resulted, followed by a decline in photosynthesis. The photosynthetic response decline was found to be slowly reversible when the temperature was raised.

\* \* \* \* \*

It appears that there is good evidence for partial regulation of photosynthesis by assimilate level in the leaf and/or sink demand. A negative correlation between photosynthesis and assimilate level has been demonstrated, but as Neales and Incoll (1968) state, it is unknown if the two factors are causally associated. The biochemical basis for such a relationship remains obscure.

## METHODS AND MATERIALS

The experimental procedures used in the sink-source experiment were quite similar, in many respects, to those used in the oat genotype screening experiment. There were fundamental differences, however, because the purposes of the two experiments were somewhat unrelated. Experimental treatments designed to alter the sink-source ratio were used, and tests were conducted over a time period of approximately one month.

## Plant Material

The varieties Richland and Burnett were chosen for study in the sink-source experiment. Burnett is a variety having a high net photosynthetic rate and specific leaf dry weight (see Part I). In the 1968 genotype survey experiment the variety Richland showed a lower net photosynthetic rate. Both genotypes are adapted to the Midwest; however, Burnett is a variety which has consistently yielded high in Iowa oat variety performance trials, while the variety Richland has generally yielded less well. The variety Burnett is characterized by large upright leaves and coarse main culms, whereas the variety Richland has smaller somewhat drooping leaves and weaker main culms.

Plant material was grown in plastic "waste-paper-baskets", and soil fertility conditions were the same as those described in the Methods and Materials section in Part I. The material was planted April 22, 1969 adjacent to the oat genotype screening experiment. Twenty-four seeds of each genotype were planted in a row down the center of

each "waste-paper-basket", and on May 7, 1969, at the two leaf stage, the population was reduced to 18 plants per pot.

#### Treatments

The purpose of the experiment was to determine how the net photosynthetic rate and carbohydrate partitioning patterns in oats were influenced by various sink-source ratios. Treatments applied to both genotypes were therefore designed to alter the sink-source ratios. The five treatments consisted of the following:

1. Control--normal plant development.
2. Removal of half the spikelets at the time of full panicle emergence.
3. Removal of three-quarters of the spikelets at the time of full panicle emergence.
4. Removal of the outer glumes from the spikelets and all leaves below the flag leaf at the time of full panicle emergence.
5. Growth in a CO<sub>2</sub> enriched atmosphere during the period of panicle differentiation.

Treatments 2 and 3 were designed to decrease the sink-source ratio. It was believed that if the net photosynthetic rate was influenced by the sink-source ratio, perhaps net photosynthetic depressions would result from these treatments which would reduce the sink capacity. Sources of assimilates would be reduced relatively less. Spikelets were removed randomly from the panicles by clipping off the appropriate number of spikelets. Spikelet counts were made on each panicle to determine how

many spikelets should be removed.

Treatments 4 and 5 were designed to increase the sink-source ratio. If increases could be obtained, perhaps greater than normal net photosynthetic rates of flag leaves would result. Treatment 4 was accomplished by carefully removing the outer glumes from each of the spikelets on the panicle. All leaves below the flag leaf on the main culms which received the treatment were also removed. Treatment 5 involved the application of CO<sub>2</sub> as a gas, so as to enrich the CO<sub>2</sub> concentration of the atmosphere in which the plants were growing during the period of panicle differentiation. It was anticipated that larger panicles with more spikelets might be obtained, thus increasing the sink-source ratio. Because maximum tillering is determined in oats within two weeks after planting (Frey and Wiggans, 1957), it was not until 18 days after planting that CO<sub>2</sub> treatments were initiated. It was thought earlier applications of CO<sub>2</sub> might have resulted in greater vegetative growth in the form of tillers, instead of increased reproductive growth. According to Bonnett (1961), the reproductive stage begins with the initiation of the panicle. He states that approximately 18 days are required for spikelet differentiation. CO<sub>2</sub> applications were made during daylight hours (9:00 a.m. to 5:00 p.m. C.D.T.) from May 16 to June 5, 1969. Application of CO<sub>2</sub> was accomplished by supplying the plants with an air source containing approximately 1,200 ppm CO<sub>2</sub>. Plants receiving the CO<sub>2</sub> fertilizer treatment were placed under a transparent polyvinyl-chloride plastic chamber, through which air was passed at a rate of approximately 1,750 l·min<sup>-1</sup>. The chamber used was similar to the plant chambers

described by Jeffers and Shibles (1969), and the chamber could accommodate all the potted oat plants which received the fifth treatment. To achieve CO<sub>2</sub> concentrations of approximately 1,200 ppm CO<sub>2</sub> inside the chambers, 100 percent CO<sub>2</sub> was metered into the inlet atmospheric air stream at a rate of about 92.5 l·hr<sup>-1</sup>. Although temperature measurements inside the chamber were not taken, it was believed that the flow rate of the CO<sub>2</sub> enriched atmospheric air through the chamber was sufficient to prevent excessive air temperatures inside the chamber. The CO<sub>2</sub> was applied all 21 days during the treatment period; however, 8 out of the 21 days were heavily overcast or partially cloudy. The atmospheric CO<sub>2</sub> fertilization was believed to have been less effective on these days, because the photochemical process was probably more limiting for photosynthesis than was the CO<sub>2</sub> diffusion process. When CO<sub>2</sub> fertilization treatments were concluded, plants which had been placed under the chamber were returned to their original position in the experimental site.

The control plants were allowed to develop normally.

Four pots of each genotype received each treatment; consequently, a total of 40 pots were used. Treatments were assigned at random within the experimental site. Of the 18 plants (main culms) in each pot, 16 received a particular treatment. Plants near the edges of the "waste-paper-baskets" were often the plants which were left untreated. Main culms of "edge" plants were usually shorter and differences in flag leaf insertion heights did not allow the testing of such leaves in combination with leaves from plants located more centrally in the pots. Tillers

were not treated, except in the case of Treatment 5 where treatment was unavoidable. Tillers which developed following treatment applications were allowed to develop normally. All treatments involving defoliation of plant parts were applied between June 20 and June 23, 1969.

#### Measurements

All photosynthetic measurements were taken during midafternoon hours, because it was believed that if high concentrations of sugar in the leaf affected net photosynthetic rates of flag leaves, such concentrations would be most pronounced at that time. Measurements of net photosynthesis (P), transpiration (T), and specific leaf dry weight (SLDW) were obtained only on flag leaves. Laminar ( $r_a$ ), stomatal ( $r_s$ ), and mesophyll ( $r_m$ )  $CO_2$  diffusion resistances were calculated. Carbohydrate determinations were conducted on flag leaves, peduncles, culms, and stem bases of plants which had been tested.

A total of 12 replications of measurements were taken on each of the two genotypes on all five treatments. Four of the 12 replications were taken prior to the application of defoliation Treatments 2, 3, and 4. Plants receiving Treatment 5 had previously received the  $CO_2$  fertilization treatment. Measurements were initiated on June 11, 1969, and they were concluded on July 12, 1969. Only five measurements could be taken per afternoon; consequently, genotypes receiving the various treatments were tested on alternate days, with the exception of Sunday.

### Net photosynthetic measurements

The apparatus used to measure net photosynthetic rates was the same as that used to measure CO<sub>2</sub> exchange rates of the oat genotypes in the 1969 oat screening experiment (Part I, Methods and Materials section). Measurements of net photosynthesis were taken only at 320 ppm atmospheric CO<sub>2</sub> concentrations.

### Transpiration and diffusion resistance measurements

Transpiration measurements were taken by use of the differential psychrometer described in Part I, Methods and Materials section. Calculations of CO<sub>2</sub> diffusion resistances were made using the equations presented in Part I, Methods and Materials section. Since net photosynthesis was measured at only one CO<sub>2</sub> concentration (320 ppm CO<sub>2</sub>), the CO<sub>2</sub> compensation concentration, CO<sub>2</sub> efflux into CO<sub>2</sub>-free air in light, and slope of the CO<sub>2</sub> response curve could not be estimated. Consequently, the value of the  $[CO_2]_{chl}$  in Equation 31 was assumed equal to zero in lieu of a better estimate. It was recognized that this probably overestimated  $r_m$ .

### Carbohydrate determinations

It was believed that if end product inhibition effects were operative in depressing net photosynthetic rates, perhaps carbohydrate accumulations might be evident in leaf tissue. Alternatively, if carbohydrates were transported out of the leaf, carbohydrate accumulations might be evident in some other plant part. Consequently, after the photosynthetic

measurements were concluded, carbohydrate levels were determined in flag leaves and 10 cm peduncle, culm, and stem base sections which had been rapidly frozen and stored in a deep freeze throughout the experimental test period.

Preparation of samples Carbohydrate determinations were conducted only on plants which had been tested. Immediately after photosynthetic measurements were taken, the plant material was sectioned, placed in coin envelopes, and transferred to a "styrofoam" container, where the samples were frozen almost instantly with "dry ice". This sectioning and freezing process required only 2 to 3 minutes. Samples were stored in a deep freeze at approximately  $-25^{\circ}\text{C}$  until they could be "freeze-dried". Burns et al. (1964) reported that oven drying of plant material could produce undesirable chemical changes which affect carbohydrate determinations. Since "freeze-drying" has been reported by Burns et al. (1964) to be superior to oven drying, the plant material was "freeze-dried". After the plant material was "freeze-dried", it was placed in a  $70^{\circ}\text{C}$  drying oven for approximately 4 hours to remove any surface moisture which may have accumulated on the samples while they were being removed from the "freeze-drier". The samples were stored in desiccators until they could be ground.

Because the four flag leaves of each test did not provide adequate plant material for carbohydrate determinations, samples tested on adjacent dates were bulked together for each genotype before grinding (i.e., samples on either side of a mid-date were bulked together). The plant material was ground with a Thomas micro mill using a 40 mesh screen.

Ground plant material was stored in desiccators until carbohydrate determinations could be conducted.

The carbohydrate determinations were divided into three parts, an extraction process, a hydrolysis process, and a reducing-power test. Approximately 30 samples could be analyzed per day with this procedure.

Extraction procedure Grasses of the Aveneae tribe accumulate predominately long chain fructosans which are readily extracted with water (Smith, 1969). Free sugars, such as glucose, fructose, and other simple sugars are soluble in water; however, amylose is largely soluble only in hot water. Thus, it was believed that a hot water extraction would remove most of the nonstructural carbohydrates. An extraction procedure similar to that used by Brown and Blaser (1965) was used. Since the amount of plant material available for the analyses was limited, only 50 mg plant samples were used. The hot water extraction period was extended from 30 to 60 minutes. The extraction procedure was carried out as follows:

1. A 50 mg dry plant sample was weighed into a 125 ml Erlenmeyer flask.
2. Next, 30 ml of distilled H<sub>2</sub>O was added to the flask.
3. A Petri dish lid 4 cm in diameter was then placed over the top of each flask to reduce evaporation losses while carbohydrates were being extracted. Extraction was accomplished by placing the flask in a boiling water bath for 60 minutes.
4. The contents of the Erlenmeyer flask were next filtered "hot" through number 30 Whatman filter paper. Sides of the flask were

washed down with exactly 20 ml of distilled water which was also filtered. Filtrations were made into 100 ml volumetric flasks. All of the filtered extracts were relatively clear.

5. A 1.0 ml aliquot of the extract was removed for a reducing-power test before acid hydrolysis.

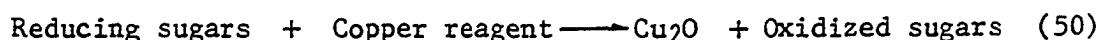
Acid hydrolysis      The reducing-power test was sensitive only to reducing sugars. Fructosans, amylose, sucrose, and other nonreducing sugars, therefore, had to be hydrolyzed if they were to be measured. This is easily accomplished by weak acid concentrations (Smith, 1969). The procedure used consisted of the following steps:

1. To the remaining 49 ml of extract solution, .5 ml of 1.0 N HCl and .5 ml of distilled H<sub>2</sub>O were added. The resulting extract was .01 N.
2. The volumetric flask containing the sugar extract was then placed in a boiling water bath for 60 minutes to hydrolyze long chain fructosans and nonreducing sugars.
3. After 60 minutes, the hydrolyzed extracts were removed from the water bath, cooled, and neutralized with .5 ml of 1.0 N NaOH.
4. The extract solution was then diluted to volume (100 ml) and a 1.0 ml aliquot was removed for the reducing power test of nonstructural carbohydrates.

Preliminary tests showed HCl concentrations of at least .005 N were required to obtain complete hydrolysis of the nonreducing sugars.

The percentage of reducing sugars measured after acid hydrolysis in 0.1 N HCl was not increased. Thus, it was assumed that complete hydrolysis of nonstructural carbohydrate polymers was obtained with the .01 N HCl concentration used in the experiment.

Reducing-power tests      The reducing-power test was carried out by the Somogyi-Nelson procedure as described by Broida (1965). The test is clinically accurate and specific for reducing sugars only. The colorimetric test is based on the following reactions:



The procedure for conducting the test was as follows:

1. A 1.0 ml aliquot of the sugar extract was pipetted into a 50 ml test tube.
2. To the sugar extract aliquot in the test tube, 1.0 ml of copper reagent was added.
3. The test tube was then partially immersed in a boiling water bath for 20 minutes to reduce the copper. To minimize evaporation losses, a marble was placed on the top of each test tube before the test tube was placed in the boiling water bath.
4. After the copper was reduced, the test tube was removed and cooled in cold tap water for about 3 minutes.
5. After cooling, 1.0 ml of arsenomolybdate reagent was added to the test tube. The contents in the tube were mixed to dissolve all the precipitate that was formed.

6. The colored contents in the tube were diluted by adding 22 ml of distilled H<sub>2</sub>O.
7. After about 2 hours, the percent absorbance was read on a Bausch and Lomb "Spectronic 20" spectrometer at a wavelength of 580 nm.

In all cases a working standard was prepared at the same time the reducing tests were being performed. This was prepared from a .2 mg-glucose-per-ml stock solution which was normally kept refrigerated. The working standard ranged from 0.00 to .20 mg glucose. About .60 and .01 mg of glucose are maximum and minimum values which can be analyzed accurately with this method (Somogyi, 1952). There is satisfactory proportionality between color density and reducing sugar content, and differences in results obtained by colorimetric and copperiodometric titration methods are small (Nelson, 1944). The test has the virtue of developing color density almost instantaneously, and the color complex remains stable for many hours (Somogyi, 1952). A wavelength was selected such that the .2 mg glucose standard gave absorbance values between .7 and .9 (Broida, 1965). This resulted in a standard curve with optimal accuracy over the major part of the curve.

Calculations of the percent reducing sugars were made from the following formula:

$$\text{Percent reducing sugars} = \frac{\text{RS}}{\text{SW}} \cdot \text{DF} \cdot 100$$

where RS = mg reducing sugar present in the sample (obtained from the standard curve)

SW = sample weight, mg

DF = dilution factor, 50 for reducing sugar determinations prior to acid hydrolysis, 100 for reducing sugar determinations after acid hydrolysis.

The arsenomolybdate reagent was prepared by the procedure described by Nelson (1944). The arsenomolybdate color reagent was prepared by dissolving 25 g of ammonium molybdate in 450 ml of distilled H<sub>2</sub>O. To this solution, 21 ml of concentrated H<sub>2</sub>SO<sub>4</sub> was added and mixed. Next, 3 g of Na<sub>2</sub>HAsO<sub>4</sub>·7H<sub>2</sub>O, which had been previously dissolved in 25 ml of distilled H<sub>2</sub>O, was added. The solution was then placed in a 37°C incubator for 48 hours and stored in an amber bottle. The color reagent is stable for one year, or longer, when stored at room temperature.

Nelson (1944) also described a preparation of the copper-carbonate-tartrate reagent, but Somogyi (1952) observed self reduction of the copper in the copper-carbonate-tartrate reagent because of the large amounts of tartrate needed to keep the copper in solution. Therefore, Somogyi (1952) modified the colorimetric copper reagent by decreasing the copper content of the solution. The copper reagent was prepared as two solutions. Solution I was prepared by dissolving 12 g of Rochelle salt (potassium sodium tartrate), 24 g of Na<sub>2</sub>CO<sub>3</sub>, 16 g of NaHCO<sub>3</sub>, and 144 g of Na<sub>2</sub>SO<sub>4</sub> in H<sub>2</sub>O; the solution was diluted to 800 ml. The Na<sub>2</sub>SO<sub>4</sub> had to be first dissolved in about 500 ml of H<sub>2</sub>O and boiled to expel air. Solution II was prepared by dissolving 4 g of CuSO<sub>4</sub>·5H<sub>2</sub>O and 36 g of Na<sub>2</sub>SO<sub>4</sub> in H<sub>2</sub>O; the solution was diluted to 200 ml. Again the Na<sub>2</sub>SO<sub>4</sub> was previously dissolved in boiling H<sub>2</sub>O and allowed to cool before the copper sulfate was added. Prior to use, 4 volumes of Solution I were mixed with 1 volume of Solution II.

#### Specific leaf dry weight measurements

Because the leaf laminae tested were frozen immediately after photosynthetic measurements had been completed, specific leaf fresh weight determinations were not made. Specific leaf dry weight measurements were taken after the plant material had been "freeze-dried".

#### Dry weight measurements of other plant parts

Panicle dry weights of tested plants were measured throughout the experimental test period. Spikelet counts were also made, and the dry weight per spikelet was calculated. When all tests were completed (July 14, 1969), four treated plants from each pot were harvested and spikelet and tiller counts were recorded. Panicle, culm, and tiller dry weights were also recorded.

#### Statistical Analyses

The genotypes were tested on alternate days. Therefore, the data collected on each genotype was analyzed separately. A modified randomized complete block design was used. Blocking was done across the 12 dates of testing.

Preliminary examination of the data indicated there might be a significant block (dates) X treatment interaction during the latter test dates. Consequently, it was anticipated that if the analyses were conducted across all 12 test dates an inflated error term might result. An attempt was made to test for such an interaction by conducting separate analyses across the first 4 and last 8 dates of testing.

When this was accomplished, it was found that error mean square terms in analyses blocked across the last 8 test dates were generally much larger than error mean square terms in analyses blocked over only the first 4 test dates. This indicated the error terms in analyses blocked over the last 8 test dates were not random, and it was believed such error terms were generally large because of a block (date) X treatment interaction. Because most treatments were applied after date 4, it was reasoned the error term in analyses conducted over the first 4 test dates would be a more representative random error term. With the advice of Dr. D. Jowett, consulting statistician, this error term was used on analyses conducted across all 12 test dates. As a result, there were two additional interaction terms. These interaction terms consisted of a date X treatments-in-late-dates and an (early vs. late dates) X treatments terms. The error term consisted of the date X treatments-in-early-dates interaction. Early and late dates refer to tests conducted over the first 4 and last 8 dates, respectively.

The carbohydrate data was analyzed by a similar modified randomized complete block design. The number of degrees of freedom of most of the source terms was reduced, however, because plant material tested on adjacent dates was bulked together to provide ample plant material for the carbohydrate determinations.

Spikelet number, tiller number, and dry weight of various plant portions harvested at the termination of the experiment (July 14, 1969) were analyzed by a 2 X 5 factorial design. Because both genotypes were harvested on the same date, separate analyses were not conducted on

each genotype. Use of the factorial design permitted the study of genotype effects (factor A), treatment effects designed to alter the sink-source ratio (factor B), and the interaction of these factors.

## RESULTS

The measurements of various factors in the sink-source experiment were divided into three groups, mainly: (1) photosynthesis and related measurements taken over time, (2) carbohydrate measurements, and (3) harvest data.

Photosynthesis and Related Measurements  
Taken over Time

The analyses of variance results of various factors measured in the sink-source experiment are shown in complete form in Table 27 of the Appendix. In every case the effect of blocking over the 12 test dates removed a significant amount of variability. The source of variation which was of primary interest, however, was that of the treatment effects. The F ratios of the treatment effects for the various factors measured over the 12 test dates (June 11 till July 12) are shown in Table 18.

Net photosynthesis

The measurement of net photosynthesis was of primary interest, because it was hypothesized that if sink effects were present, such effects might be reflected in the magnitude of net photosynthetic rates.

Highly significant treatment effects were observed in both varieties. Photosynthetic rates of treatments designed to decrease the sink-source ratio are plotted as a function of time in Figure 25, whereas photosynthetic rates of treatments designed to increase the sink-source ratio are plotted as a function of time in Figure 26. Photosynthetic

Table 18. F ratios for treatment effects obtained in the analyses of variance of several variables measured in the sink-source experiment

Source	F ratio	
	Burnett	Richland
Net photosynthesis	11.65**	6.75**
Transpiration	1.07	3.16
Specific leaf dry weight	5.26*	8.64**
Sum of resistances	142.77**	79.90**
Mesophyll resistance	110.09**	61.89**
Stomatal resistance	3.33*	8.23**
Laminar resistance	.86	8.28**

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

rates of the control treatment (normal plant development) are shown in both figures. The period during which defoliation treatments were applied is indicated by the black bar in the figures. Individual photosynthetic rates are shown in Table 28 of the Appendix. Mean net photosynthetic rates, averaged across treatments and test dates, are also shown in the same table.

It is immediately obvious that net photosynthetic rates of all flag leaves tested, regardless of treatment, began to decline about 20 days after flag leaf emergence. Differences in net photosynthetic

Figure 25. Net photosynthetic rates of flag leaves of the oat varieties Burnett (top) and Richland (bottom) plotted as a function of time. Each point represents an individual measurement on four flag leaves. Day 1 and day 32 correspond to June 11 and July 12, 1969; **■** indicates date of treatment (June 20 and June 22, 1969, days 10, 11, and 12). **—▲—** = Treatment 1 (normal plant development); **--x--** = Treatment 2 (one-half spikelet removal); **—⊖—** = Treatment 3 (three-quarters spikelet removal).

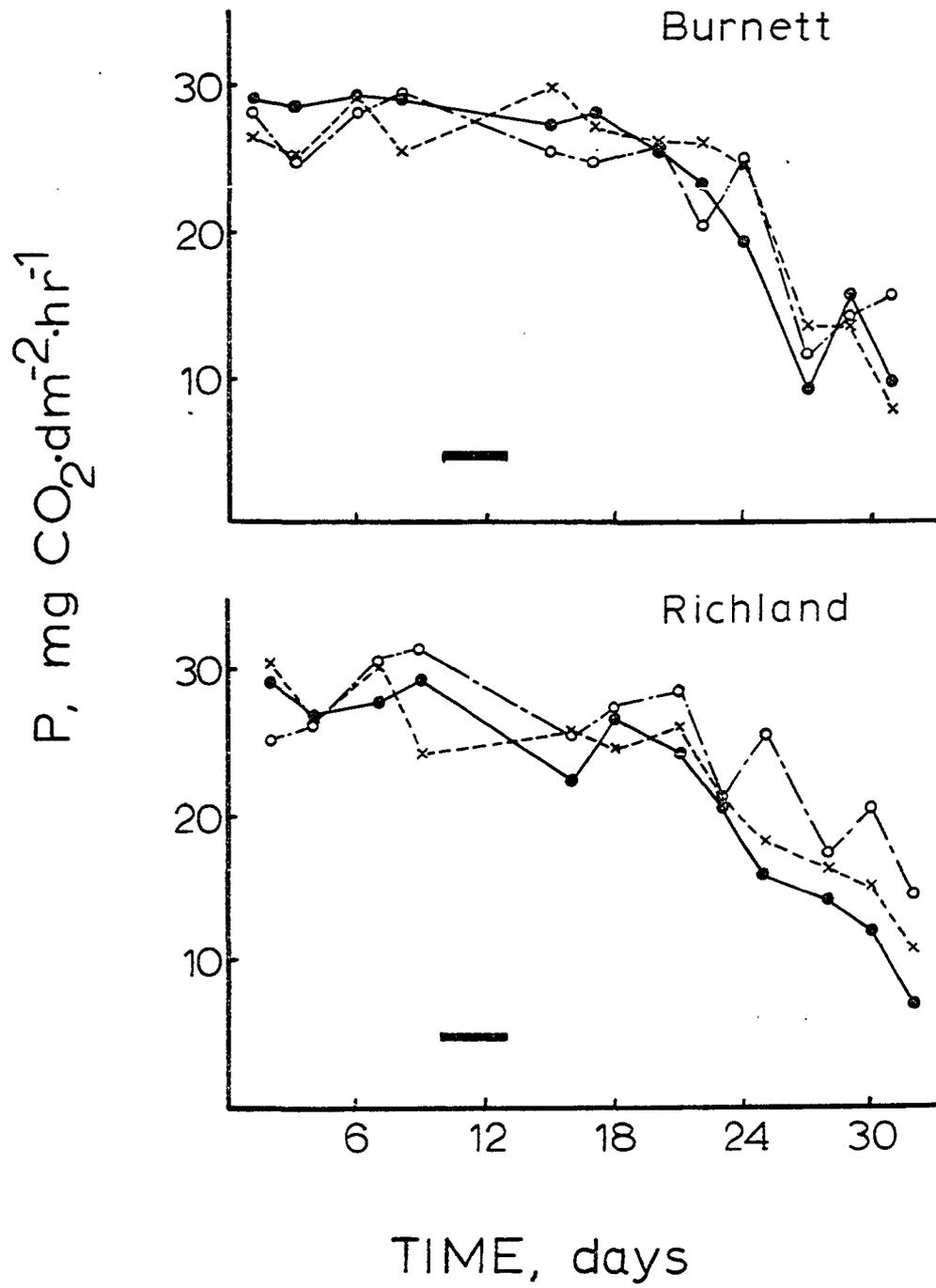
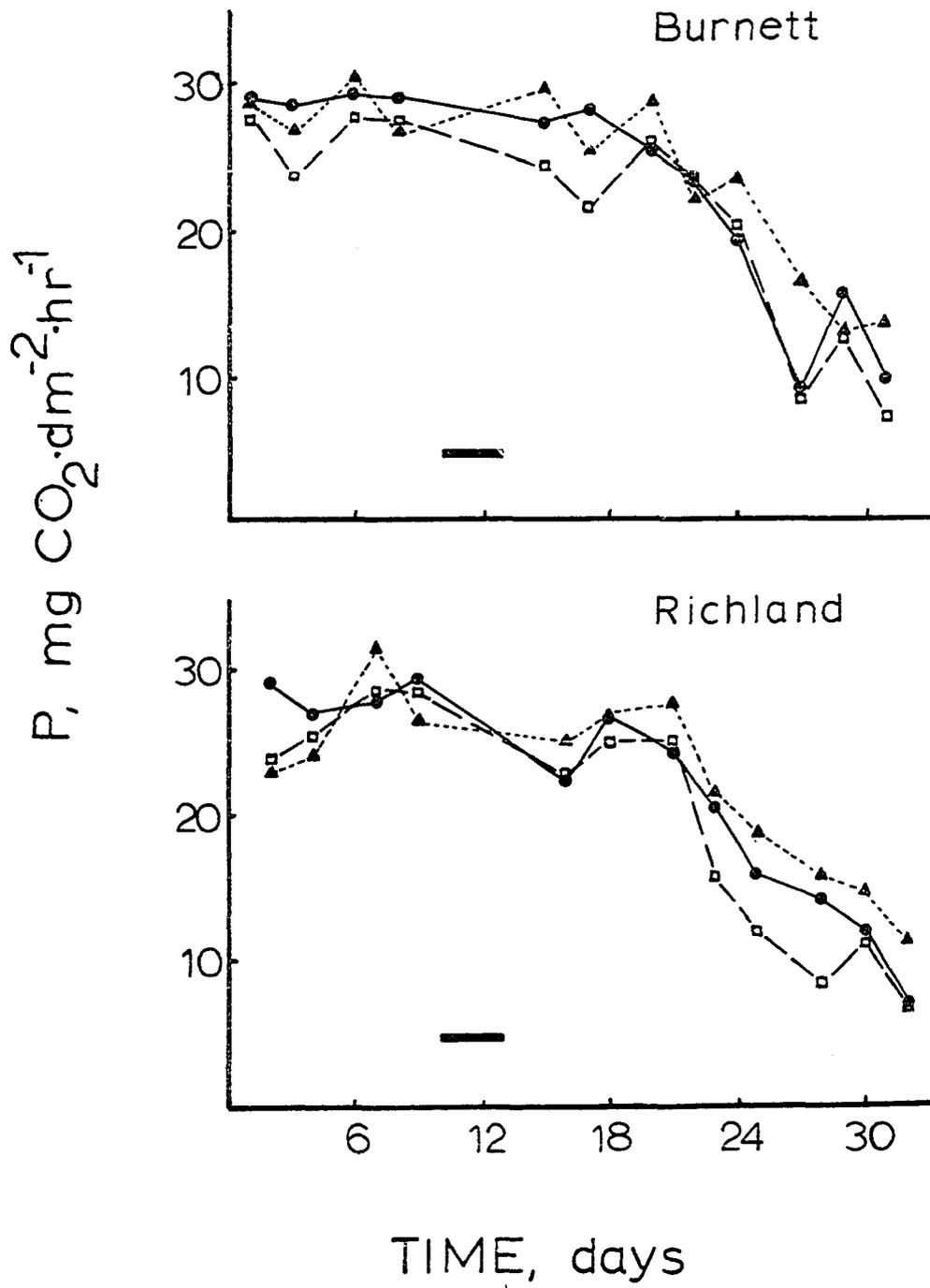


Figure 26. Net photosynthetic rates of flag leaves of oat varieties Burnett (top) and Richland (bottom) plotted as a function of time. Each point represents an individual measurement on four flag leaves. Day 1 and day 32 correspond to June 11 and July 12, 1969, respectively. — indicates date of treatment (June 20 and June 22, 1969; days 10, 11, and 12). Treatment 5 was applied from May 16, 1969 to June 5, 1969. —○— = Treatment 1 (normal plant development); --▲-- = Treatment 4 (all outer glumes and leaves below the flag leaves removed); -□- = Treatment 5 (CO<sub>2</sub> fertilization during the period of panicle differentiation).



rates within a variety, because of treatment, were not as obvious, even though highly significant treatment effects occurred. Examination of Figure 25 indicates, particularly in the variety Richland, that there was a tendency for flag leaves of plants from which one-half and three-quarters of the spikelets had been removed to show higher net photosynthetic rates than the control plants towards the end of the experimental test period. Examination of Figure 26 indicates that net photosynthetic rates of flag leaves of plants from which outer glumes and all leaves below the flag leaf had been removed were generally greater than photosynthetic rates of control plants. However, flag leaves of plants which received CO<sub>2</sub> fertilization during the period of panicle differentiation often showed lower net photosynthetic rates than control plants towards the end of the season. This latter response was most obvious in Richland. There was a significant dates X treatments-in-late-dates interaction in Burnett, and this is probably explained by the increase in photosynthesis in plants of Treatments 1, 3, and 5 on test date 11.

Mean flag leaf net photosynthetic rates of the control Burnett plants were found to be only slightly higher than the controls of Richland (22.9 versus 21.3 mg CO<sub>2</sub> dm<sup>-2</sup>·hr<sup>-1</sup>, respectively) when rates were averaged across the 12 test dates. Larger genotypic differences were reported in the oat genotype screening experiment (Table 8, Results section in Part I). The reason for this discrepancy is not known.

### Transpiration

The experimental treatments had no significant effect on transpiration rates in either variety (Table 18). Examination of the transpiration rates (Table 28, Appendix) showed there was a trend for slightly lower transpiration rates to occur early during the experimental test period, when leaf expansion presumably still may have been occurring. Transpiration rates decreased towards the end of the season as the leaves began to senesce. It was believed these time trends resulted in the significant date effect (Table 27, Appendix).

### Specific leaf dry weight

The specific leaf dry weight (SLDW) has sometimes been used to indicate the accumulation of assimilates in leaf laminae (Burt, 1964; Nösberger and Thorne, 1965). Consequently, it was of interest to measure the SLDW over the 12 test dates. In most cases the SLDW increased linearly over the first part of the experimental test period (Figures 27 and 28). However, after test date 6 the SLDW of leaves of plants receiving various treatments remained relatively constant, and ~~often~~ a slight decrease in the SLDW was observed toward the end of the season.

Significant treatment effects on the SLDW were found in both varieties (Table 18). Specific treatment comparisons were not made; however, it is obvious from Figure 27 that flag leaves from plants from which three-quarters of the spikelets had been removed had the highest SLDW, particularly towards the end of the season. The SLDW of flag leaves

Figure 27. Specific leaf dry weights of flag leaves of the oat varieties Burnett (top) and Richland (bottom) plotted as a function of time. Each point represents an individual measurement on four flag leaves. Day 1 and day 32 correspond to June 11 and July 12, 1969, respectively. — indicates date of treatment (June 20 and June 22, 1969; days 10, 11, and 12). —●— = Treatment 1 (normal plant development); --x-- = Treatment 2 (one-half spikelet removal); —○— = Treatment 3 (three-quarters spikelet removal).

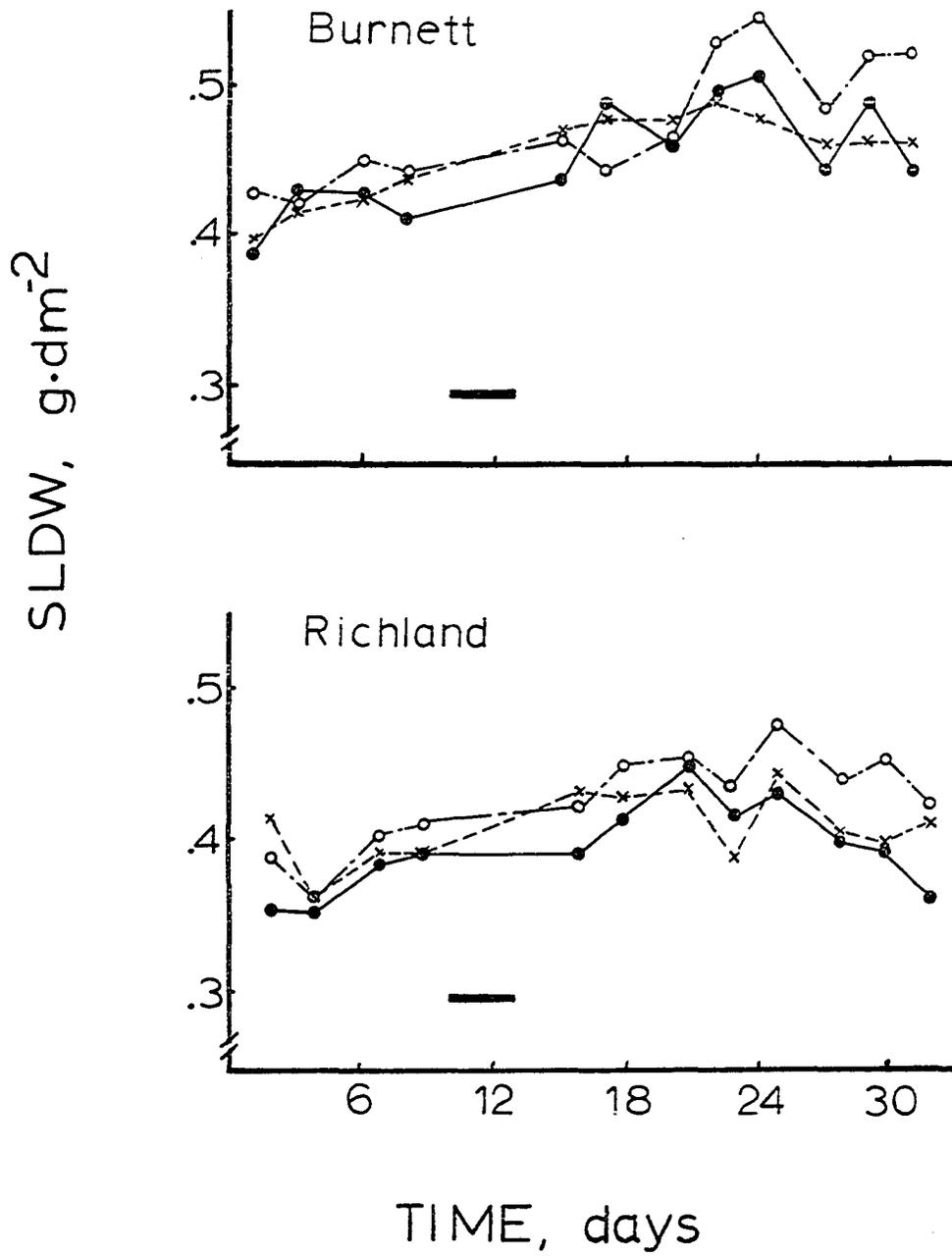
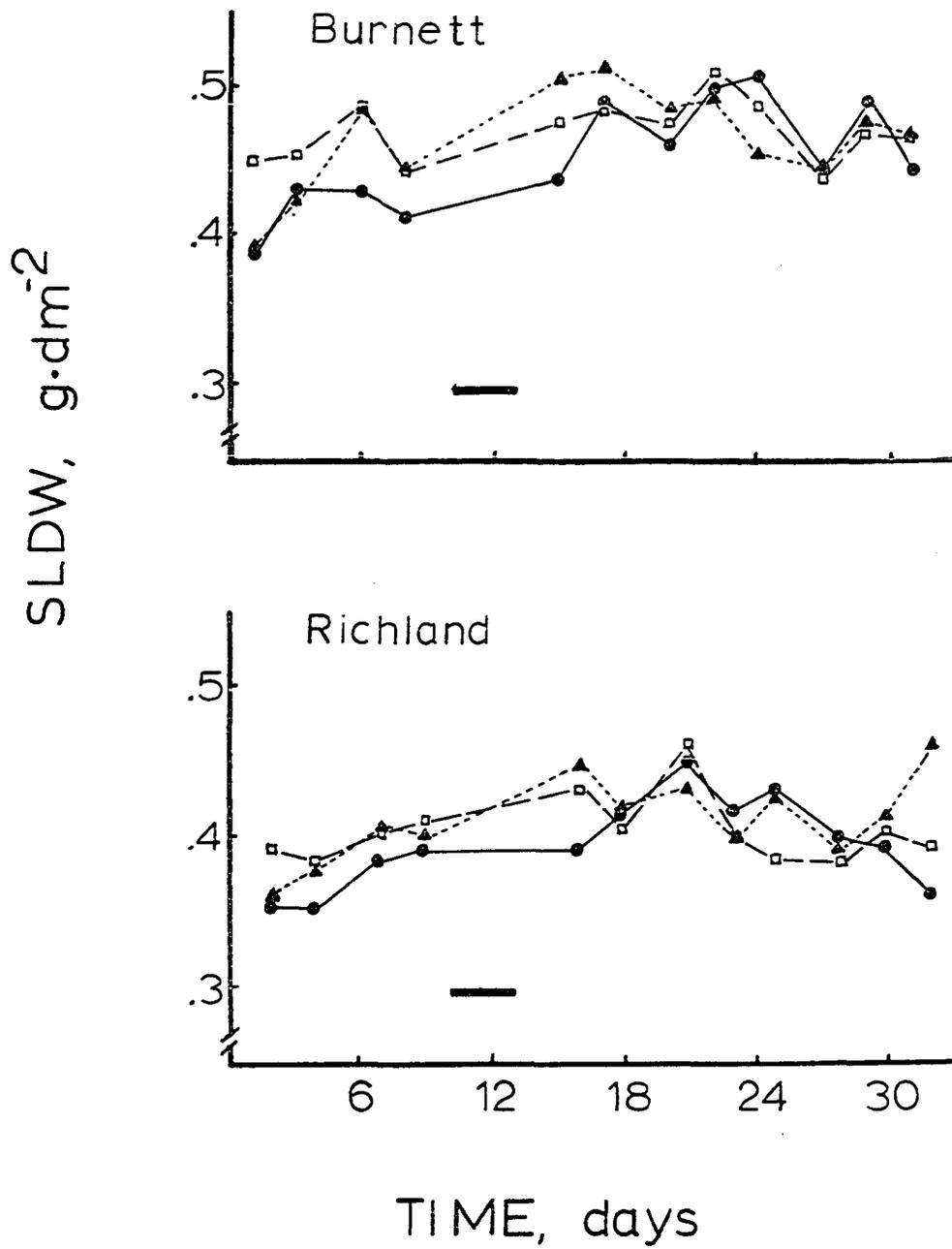


Figure 28. Specific leaf dry weights of flag leaves of oat varieties Burnett (top) and Richland (bottom) plotted as a function of time. Each point represents an individual measurement on four flag leaves. Day 1 and day 32 correspond to June 20 and July 12, 1969, respectively. — indicates date of treatment (June 20 and June 22, 1969; days 10, 11, and 12). Treatment 5 was applied from May 16, 1969 to June 5, 1969. —●— = Treatment 1 (normal plant development); ---▲--- = Treatment 4 (all outer glumes and leaves below the flag leaves removed); —□— = Treatment 5 (CO<sub>2</sub> fertilization during the period of panicle differentiation).



from plants which received treatments designed to increase sink-source ratios did not appear to vary greatly from the SLDW of control plant flag leaves (Figure 28).

Mean SLDW values for flag leaves of the control Burnett and Richland plants were .452 and .396  $\text{g}\cdot\text{dm}^{-2}$ , respectively. The SLDW values reported for varieties Burnett and Richland in the 1969 oat screening experiment were .446 and .395  $\text{g}\cdot\text{dm}^{-2}$ , respectively.

#### CO<sub>2</sub> diffusion resistances

It was believed that if photosynthetic depressions could be detected as the result of a sink effect, the reason for such depressions might be better understood by comparing CO<sub>2</sub> diffusion resistance terms of plants subjected to various treatments. Consequently, CO<sub>2</sub> diffusion resistances were measured and analyzed.

The effect of blocking over dates removed a significant amount of variation in all cases when the resistance data for  $\Sigma r$ ,  $r_m$ ,  $r_s$ , and  $r_a$  were analyzed. The increase in  $\Sigma r$  and  $r_m$  over the experimental period was associated with photosynthetic depressions during the latter part of the season. Stomatal resistance ( $r_s$ ) was generally slightly higher during the initial test dates, and there was also a slight increase in  $r_s$  as the flag leaves senesced. Laminar resistance ( $r_a$ ) showed a seasonal trend similar to that observed for  $r_s$ . The reader is referred to Tables 29, 30, and 31 in the Appendix if interested in the data on trends in the CO<sub>2</sub> diffusion resistances.

There were significant treatment differences in  $\Sigma r$ ,  $r_m$ , and  $r_s$  in both varieties, and also in  $r_a$  in Richland (Table 18). In general, there was an inverse relationship between net photosynthetic rates and  $r_m$ . For example, flag leaves of plants which received the CO<sub>2</sub> fertilization treatment generally showed higher  $r_m$  values. Other treatment effects where photosynthetic differences were observed appeared to be associated with differences in  $r_m$ . Although  $r_s$  values differed significantly between treatments, the  $r_s$  values ranged from only 1.6 to 1.8 sec·cm<sup>-1</sup> for Burnett and from 1.4 to 1.9 sec·cm<sup>-1</sup> for Richland. This range of  $r_s$  values is probably inadequate to account for photosynthetic differences due to treatments. Laminar resistances differed significantly between treatments in Richland. Individual mean values of the CO<sub>2</sub> diffusion resistances are presented in Tables 29, 30, and 31 of the Appendix.

#### Carbohydrate Measurements

It was believed that, if large photosynthetic depressions could be produced following treatments designed to reduce the sink-source ratio, perhaps a carbohydrate accumulation might be evident in flag leaves or some other portion of the plant. Moss (1962a) was able to associate lower photosynthetic rates with sugar accumulations in barren corn plants, and other researchers have experimental evidence that such an inverse relationship occurs (Thorne and Evans, 1964; Kazaryan et al., 1965).

The experimental results, however, showed no significant differences in free, hot-water extractable sugars in any plant parts as a result of experimental treatments (Table 19). Upon acid hydrolysis of hot-water extractable carbohydrates, which were believed to consist primarily of long chain fructosans, the only significant treatment effects were associated with carbohydrate contents in the main culms and peduncles of the variety Burnett (Table 19). Examination of individual carbohydrate determinations (Tables 32 and 33, Appendix) indicates there was a tendency for higher carbohydrate levels to occur in culms and peduncles of the Burnett plants which received CO<sub>2</sub> fertilization.

Table 19. F ratios obtained in the analyses of variance conducted on carbohydrate data (Error degrees of freedom = 4)

Source	F ratio	
	Burnett	Richland
Free sugars		
Main culms	1.194	.345
Flag leaves	1.568	1.650
Stem bases	3.537	.259
Peduncles	3.577	.046
Free sugars plus hydrolyzed carbohydrates		
Main culms	8.130*	.499
Flag leaves	.818	2.386
Stem bases	2.657	1.570
Peduncles	15.704*	.353

\*F value exceeds 5% level of significance.

Because treatment differences were generally not significant, mean carbohydrate values of all the treatments were plotted as a function of time for the various plant parts (Figure 29). It can be noted from the figure that the percentages of carbohydrate in the main culms, stem bases, and peduncles rapidly decreased as the season progressed. The decrease, however, was not as evident in the flag leaves. The carbohydrate contents in flag leaves of Burnett decreased slightly, but the decrease in percentage of carbohydrates (free plus hydrolyzed carbohydrates) in Richland was not significant across dates (Table 34, Appendix).

#### Harvest Maturity Data

Dry weights of various plant parts harvested at the termination of the experiment are shown in Table 20. Spikelet numbers and the number of tiller buds which elongated are also shown in the table. Analyses of variances of the various characters measured during the final harvest are shown in Table 21.

Richland tillered significantly more than Burnett. There appeared to be a significant inverse relationship between the number of spikelets removed and the number of tiller buds which elongated and, in some cases, developed panicles. The effect appeared to be most pronounced in the Richland. Leaf removal and CO<sub>2</sub> fertilization, which were designed to increase the sink-source ratio, had little effect on tillering. Because Richland tillered more profusely, tiller dry weights were

Figure 29. Percentage of carbohydrates (based on dry weight), plotted as a function of time, in various parts of the oat plant. Day 2 and day 31 correspond to June 12 and July 11, 1969, respectively. The broken lines refer to free sugars extracted with hot water. Each point represents the mean value of the 5 treatments. The solid lines refer to free sugars plus hydrolyzed carbohydrates extracted with hot water. ● = Burnett; ○ = Richland.

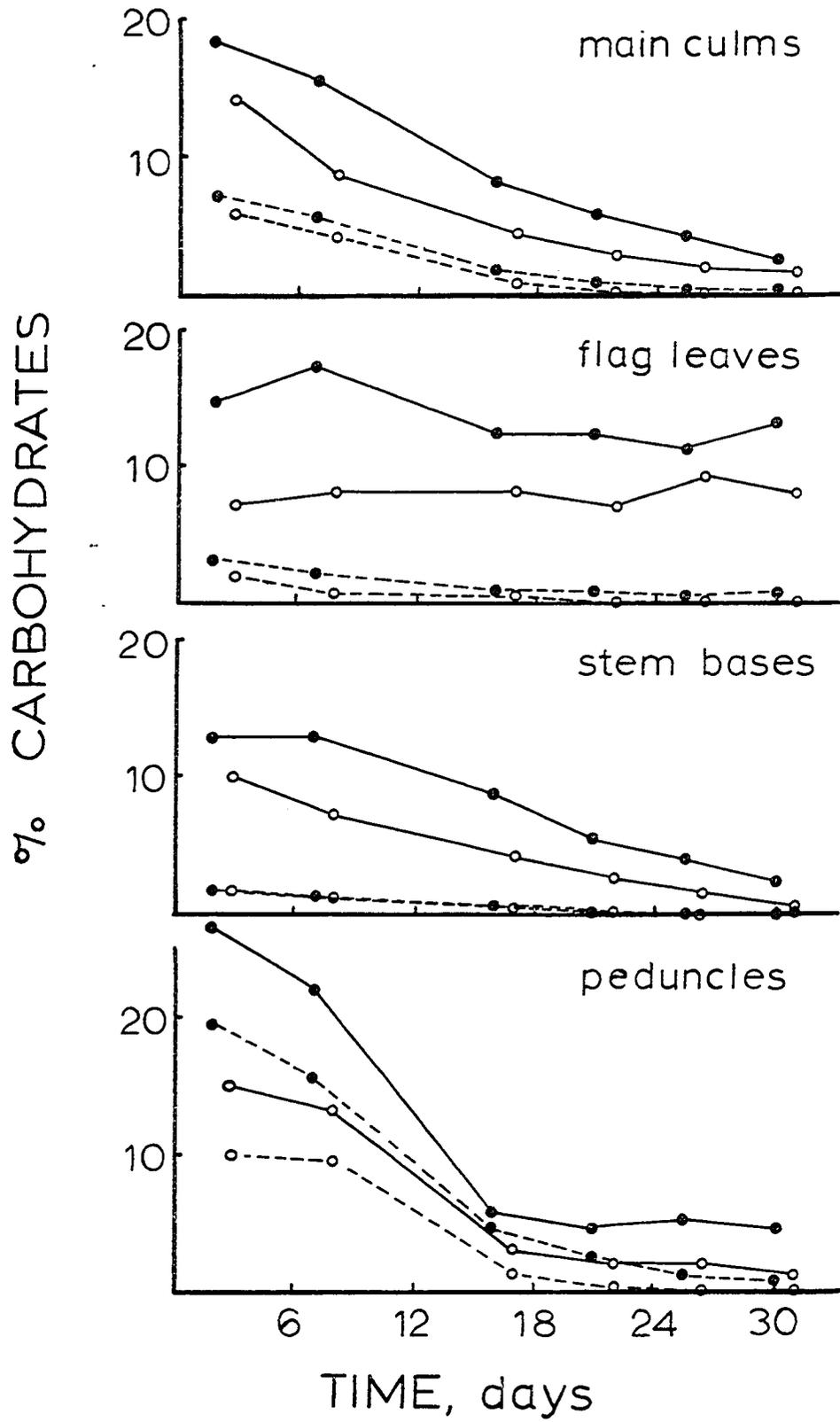


Table 20. Spikelet number, tiller number, and dry weights of various plant parts at termination of the experiment (July 14, 1969)

Treatment	Spikelets per 4 plants	Tillers per 4 plants	Dry weight per spikelet, mg	Dry weight per 4 plants, g			
				Panicle weight	Culm weight	Tiller weight	Total weight
Burnett							
1	121	4	57.0	6.98	8.20	3.48	18.65
2	56	7	62.0	3.53	7.52	4.56	15.61
3	30	7	72.0	2.17	7.96	5.49	15.61
4	116	4	37.0	4.22	5.24	3.44	12.90
5	116	3	71.0	8.19	10.54	2.47	21.20
Richland							
1	142	5	45.0	6.31	5.27	5.43	17.46
2	72	8	52.0	3.76	5.67	8.42	17.85
3	34	12	58.0	1.93	5.32	8.83	16.08
4	139	5	30.0	4.21	4.51	5.56	14.28
5	121	6	47.0	5.63	6.22	6.47	18.32

Table 21. Analyses of variance of spikelet number, tiller number, and dry weights of various plant parts at termination date of the experiment (July 14, 1969)

Source	df	Mean squares	F ratio
Spikelet number			
Genotype	1	1,876.8987	14.962**
Treatment	4	15,764.8203	125.675**
G X T	4	162.9624	1.299
Error	30	125.4414	
Tiller number			
Genotype	1	46.2250	13.366**
Treatment	4	45.2250	13.077**
G X T	4	5.9750	1.728
Error	30	3.4583	
Weight per spikelet			
Genotype	1	1,822.5000	93.229**
Treatment	4	1,128.2236	54.714**
G X T	4	85.0000	4.348**
Error	30	19.5486	
Panicle weight			
Genotype	1	4.2250	6.975*
Treatment	4	34.1325	56.352**
G X T	4	2.4927	4.116*
Error	30	.6057	
Culm weight			
Genotype	1	57.6479	56.515**
Treatment	4	12.4320	12.188**
G X T	4	3.4287	3.361*
Error	30	1.0201	
Tiller weight			
Genotype	1	93.4217	15.614**
Treatment	4	13.7014	2.290
G X T	4	1.8498	.309
Error	30	5.9832	
Total weight			
Genotype	1	.0003	.000
Treatment	4	43.1557	3.613*
G X T	4	8.4497	.708
Error	30	11.9431	

\*F value exceeds 5% level of significance.

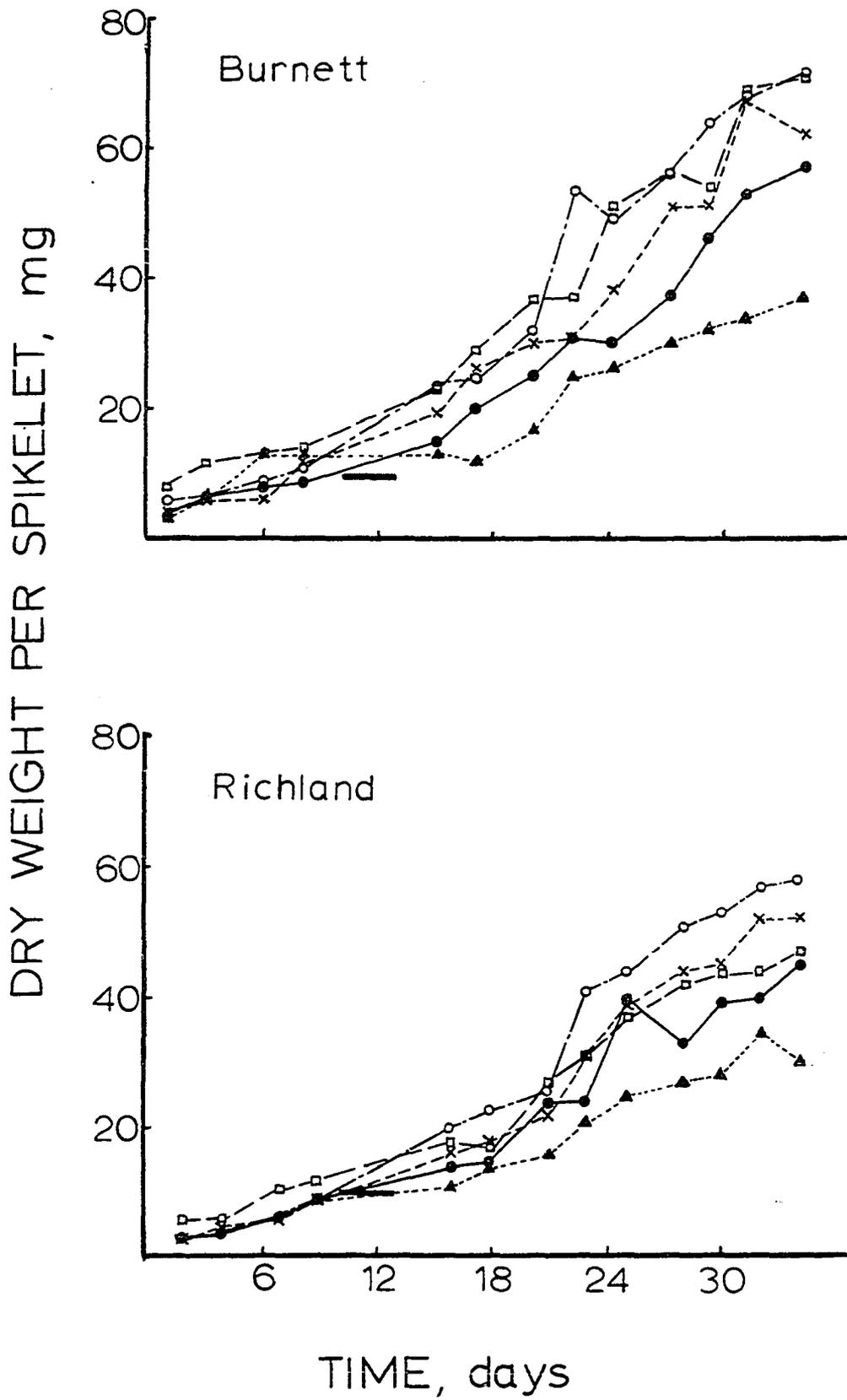
\*\*F value exceeds 1% level of significance.

generally higher. Although treatment effects on tiller dry weight were not significant, there was a trend for tiller dry weight to increase as the number of spikelets decreased--i.e., in spikelet removal treatments.

Significantly different spikelet numbers were found between the two varieties. Richland, the low yielding variety, had more spikelets per panicle than Burnett. Because spikelet removal was involved in two of the experimental treatments, significant treatment effects upon spikelet number were expected.

Spikelets of Burnett were significantly heavier than those of Richland. The experimental treatments had a significant effect on the weight per spikelet. Within both varieties it was found that spikelet removal (Treatments 2 and 3) and CO<sub>2</sub> fertilization (Treatment 5) increased spikelet weight, whereas leaf removal (Treatment 4) decreased weight per spikelet. Treatment 2, 3, and 5 increased the spikelet weight by approximately 8.8, 26.3, and 24.6 percent, respectively, in Burnett; spikelet weights were increased 15.6, 28.9 and 4.4 percent in Richland. Frey (1962) reported a 20 percent increase in oat seed weights when 50 percent of the spikelets were removed. Treatment 4, however, depressed weight per spikelet in Burnett and Richland by approximately 35.1 and 33.3 percent, respectively. Dry weights of spikelets were also measured over the experimental test period, and these data are shown as a function of time in Figure 30. There was a significant genotype X treatment interaction, and examination of treatment means

Figure 30. Dry weight per spikelet plotted as a function of time in oat varieties Burnett (top) and Richland (bottom). Day 1 and day 34 correspond to June 11 and July 14, 1969, respectively. Each point represents the mean dry weight per spikelet of spikelets from four panicles. — indicates date of treatment (June 20 and June 22, 1969; days 10, 11, and 12). —◆— = Treatment 1 (normal plant development); —x— = Treatment 2 (one-half spikelet removal); —○— = Treatment 3 (three-quarters spikelet removal); —▲— = Treatment 4 (all outer glumes and leaves below the flag leaf removed); —□— = Treatment 5 (CO<sub>2</sub> fertilization during the period of panicle differentiation).



seems to indicate a greater response of Burnett to the CO<sub>2</sub> fertilization treatment. Greater spikelet weights and panicle weights resulted from treatment in Burnett. CO<sub>2</sub> fertilization resulted in only slight increases in spikelet weight in Richland, and panicle weights in Richland actually were depressed.

The main culms of Burnett were significantly larger and heavier than those of Richland. Treatment effects were significant. Spikelet removal treatments had little effect on the dry weight of the main culm; however, leaf removal depressed the weight of the main culm because some plant material (leaf laminae below the flag leaf) had been removed. The dry weight of the main culms was substantially increased as a result of the CO<sub>2</sub> fertilization treatment. The increase in culm dry weight was more pronounced in Burnett than Richland, and this appeared to explain the significant genotype X treatment interaction.

The total dry matter produced in the above-ground plant portions did not differ significantly between genotypes. For the most part, only partial defoliation and CO<sub>2</sub> fertilization appeared to significantly affect dry matter production. Partial defoliation, obviously, would be expected to result in a lower total dry weight. On the other hand, CO<sub>2</sub> fertilization increased the total dry matter produced in above-ground plant portions. This was believed to be the result of enhanced photosynthetic rates during the CO<sub>2</sub> fertilization period. The Burnett and Richland plants receiving CO<sub>2</sub> fertilization produced 13.6 and 4.9 percent more total dry matter, respectively, in the above-ground portions.

## DISCUSSION

Evans (1968) states that in most small grains, yield is dependent largely upon photosynthates formed after earing. Experiments with oats (Jennings and Shibles, 1968) and wheat (Archbold, 1942) have demonstrated that non-lamina tissues may contribute even more assimilates to the grain than leaves. Nevertheless, a substantial amount of carbon assimilated by the flag leaf blade does move to the grain. For example, Jennings and Shibles (1968) found flag leaf photosynthesis accounted for approximately 18 and 10 percent of the grain weight in oat varieties A-465 and Goodfield, respectively. In wheat it has been reported that about half the carbon assimilated in the flag leaf blade moves directly to the grain (Carr and Wardlaw, 1965; Evans, 1968). Therefore, it was reasoned that if the sink-source ratio could be altered in this experiment, corresponding changes in net photosynthetic rate might result.

Measurements which were of primary interest in this experiment were those of net photosynthesis, specific leaf dry weight, and percent carbohydrates as influenced by various treatments designed to alter the sink-source ratio.

## Net Photosynthesis

The most obvious feature of the net photosynthetic rates was their decline from anthesis onwards, regardless of treatments designed to alter the sink-source ratio. A similar observation has been reported in flag

leaves of wheat by Carr and Wardlaw (1965). The declination of the flag leaf photosynthetic rates with increasing maturity is attributed to leaf senescence effects. The decrease of net photosynthetic rates over the season was associated with an increase in the mesophyll resistance.

Examination of the figures indicates a departure of various treatments from control photosynthetic rate. There were significant treatment effects with respect to all three CO<sub>2</sub> diffusion resistances (laminar, stomatal, and mesophyll resistance), but variation of laminar and stomatal resistance between treatments was small. Mesophyll resistance varied inversely as a function of the net photosynthetic rates of the various treatments.

#### Treatments designed to decrease the sink-source ratio

It was postulated the plants receiving the one-half and three-quarter spikelet removal treatments (Treatments 2 and 3, respectively) might show depressed net photosynthetic rates. However, these plants did not show lower net photosynthetic rates than controls. In fact, there was a trend for the plants, from which the spikelets had been removed, to show slightly greater net photosynthetic rates toward the end of the season. This effect was most evident in Richland (Figure 25). The increases in rates were not large, however.

Contrary to the results obtained in this experiment, Nösberger and Thorne (1965) found that removal of half the florets (12 versus 24 florets) slightly reduced the rate of photosynthesis of barley flag leaves between 10 and 17 days after ear emergence. Because the net

assimilation rate was unchanged, they concluded the effects of floret removal on net photosynthesis were small. The photosynthetic rates they report appeared to be extremely low, however, ( $5.2-5.7 \text{ mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$ ).

Possible reasons for the greater net photosynthetic rates toward the end of the season are: First, and probably the most logical explanation, is that flag leaf senescence was slowed by the spikelet removal treatments. Leaves of treated plants, which remained green longer, would be expected to show greater net photosynthetic rates and possibly higher cytokinin levels than flag leaves of control plants. It is believed the growth regulator balance of the plants may have been disturbed, because there have been reports (Leopold, 1964) that destruction of strong mobilizing centers for nutrients (i.e., the spikelets in this experiment) delays senescence. Slightly higher net assimilation rates have been observed in barren corn plants approaching maturity (Moss 1962a). He attributed this response to an obvious difference in leaf maturity. Similar maturity effects were believed present in this experiment. The second hypothesis is based on the observation that tiller dry weight increased as a result of spikelet removal (Table 20). Spikelet removal decreased panicle weight, and the tiller dry weight and panicle weight were inversely related. Total dry weight of above-ground plant parts was little affected by spikelet removal treatments. Perhaps the net photosynthetic rates of the plants from which the spikelets had been removed were higher because active meristematic regions near the crown, together with the remaining spikelets,

became a stronger sink for assimilates than the normal panicle and tillers in the untreated control plants. Aspinall (1963) has stated that nutrients, both minerals and carbohydrates, may be preferentially transported to regions of high auxin content, and developing grains might be expected to contain a high concentration of growth substances. However, since auxin occurs most abundantly in actively growing meristems (Leopold, 1964), it might be expected that tiller meristems in the crown region became a major sink for assimilates.

King et al. (1967) found in wheat that removal of the ear from the main culm two weeks after anthesis reduced the photosynthetic rate of the flag leaf about 50 percent within 3 to 15 hours. Subsequent darkening of all other leaves on plants without ears led to recovery of flag leaf photosynthesis. This is one of the few examples where a large sink effect has been shown in a small grain crop, and I believe that the results they obtained may have been dependent, largely, upon the fact that development of alternative sinks was prevented. That this may be true is supported by Moss's (1962a) statement that barrenness would be expected to have little effect in plants which never have many fruit or seed, are non-determinant in growth habit, or have storage mechanisms other than fruit seeds. It would seem that oats could be classified as a plant with an alternative "storage" mechanism (i.e., the tillers). Wheat, also, could probably be classified as such a plant, because alternative sinks (tillers) may develop if normal sinks (grains) for assimilates are removed. However, the wheat plants used by King et al. (1967) did not have alternative sinks because the plants had been cut back, leaving only the main culm and two tillers, from

which the ears had been removed. In this experiment tillers of the oat plants were allowed to develop normally, and it was believed the effect of spikelet removal in reducing sink-source ratios was largely destroyed, because of the elongation and development of late tillers.

The fact that tiller growth increased more when spikelets were removed and that the weight per spikelet was only slightly increased would seem to indicate that translocation patterns in the plants were affected more by spikelet removal treatments than net photosynthesis. There was probably increased movement of assimilates to the crown regions and less to the panicle. Wardlaw (1965) found that removing grains from the head of wheat reduced the velocity of  $C^{14}$  moving up the stem and increased the downward velocity of labelled assimilates in the stem. He found the rate of movement of labelled assimilates out of the leaf was unaffected by grain removal.

The level of free sugars and free sugars plus acid-hydrolyzable carbohydrates in flag leaves of plants, from which spikelets had been removed, did not differ significantly from those of control plants. Carbohydrate levels in other plant parts (peduncles, main culms, and stem bases) also appeared similar. Hence, there was little evidence that carbohydrate level was related to photosynthetic rate.

#### Treatments designed to increase the sink-source ratio

Partial defoliation and  $CO_2$  fertilization (Treatments 4 and 5) were both designed to increase the sink-source ratio. However, the photosynthetic responses of the two treatments were quite different. Removal

of the outer glumes from the spikelets and leaf laminae below the flag leaf increased net photosynthetic rates of flag leaves in both varieties (Figure 26). It is believed this occurred, largely, because of an increased sink-source ratio; i.e., perhaps flag leaf photosynthetic efficiency was increased because the flag leaf compensated for loss of other photosynthetic tissue. Other researchers have noted that in partially defoliated plants remaining plant parts appear to compensate for loss of photosynthetic tissue (Kiesselbach, 1948; Allison and Watson, 1966).

The possibility that dynamic growth regulator interactions may have been responsible for greater net photosynthetic rates in flag leaves of partially defoliated plants is not discounted, however. For example, Wareing et al. (1968) observed that leaves of partially defoliated dwarf bean, maize, and willow showed stimulated net photosynthetic rates, increased levels of leaf protein, and greater carboxylating enzymatic activity. To them this suggested that partial defoliation not only increased the relative demand for photosynthates on the remaining leaves, but also increased the photosynthetic efficiency of the latter by reducing competition between leaves for mineral nutrients and, possibly also, for specific growth regulation factors, such as cytokinins supplied by the roots. In this experiment, defoliation of various photosynthetic tissues would have increased the root-shoot ratio and, presumably, the relative level of cytokinin in the remaining vegetative tissue may have been increased. There is no evidence for or against this hypothesis; however, if higher levels of cytokinins in the flag leaves of defoliated

oat plants did occur, this may have led to increased protein synthesis in these leaves, and a higher level of carboxylating enzymes may have resulted.

The CO<sub>2</sub> fertilization treatment was not effective in increasing the number of spikelets. Because sink strength has been reported to be primarily dependent upon size and number of grains (Evans, 1968), it is believed CO<sub>2</sub> fertilization was not effective in increasing the sink-source ratio. In fact, in Richland the sink-source ratio appeared to be decreased. Vegetative tissues (main culms and tillers) comprised 69.3 percent of the total dry weight of the above-ground portions in plants which received CO<sub>2</sub>, whereas they accounted for only 61.3 percent in the control Richland plants at the end of the season. Main culms and tillers of the Burnett control plants accounted for 62.6 percent of the total dry weight of the above-ground portions, whereas vegetative tillers and culms accounted for 61.4 percent in plants receiving CO<sub>2</sub> fertilization. Thus, it was concluded the sink-source ratios were not significantly increased in either variety as a result of CO<sub>2</sub> fertilization.

The CO<sub>2</sub> fertilization treatment hastened maturity of the oat plants. It is believed the more rapid senescence of flag leaves of plants receiving CO<sub>2</sub> fertilization, coupled with the fact that sink-source ratios were not increased, can explain the depressed net photosynthetic rates in CO<sub>2</sub> treated plants.

The level of free sugars and free sugars plus acid-hydrolyzable carbohydrates in flag leaves of partially defoliated or CO<sub>2</sub> treated

plants did not differ significantly from those of control plants. Thus, no evidence for or against a carbohydrate accumulation effect on photosynthesis was obtained. Burnett plants receiving the CO<sub>2</sub> fertilization treatment did appear to show slightly higher carbohydrate levels (free sugars plus hydrolyzed carbohydrates) in peduncles and main culms, but these accumulations evidently had little effect on the photosynthetic rate. More rapid leaf senescence in CO<sub>2</sub> fertilized plants would seem a more likely explanation for the lower rates of photosynthesis.

#### Specific Leaf Dry Weight

Flag leaves of plants from which three-quarters of the spikelets had been removed generally had higher SLDW's and net photosynthetic rates than control plants, (Figure 27), toward the end of the experimental test period. This response was unexpected. Perhaps there is export of nutrients from leaves of plants as they senesce. The fact that leaves of plants from which the spikelets had been partially removed showed delayed senescence might explain their higher SLDW toward the end of the experimental period.

Net photosynthetic rates as altered by treatment effects, appeared to be associated with a high SLDW, and correlations between the two factors were generally positive (Table 22). It is believed a positive relationship, similar to the association of net photosynthesis with the SLDW as discussed in the Discussion section in Part I, probably existed. There was no evidence of a carbohydrate accumulation effect on SLDW--

Table 22. Correlation coefficients ( $r$ ) of net photosynthesis with specific leaf dry weights of flag leaves after treatments had been applied (Degrees of freedom = 3)

Genotype	Test date							
	5	6	7	8	9	10	11	12
Burnett	.30	.11	.81	-.76	.11	.18	.52	.74
Richland	.66	.39	-.33	.36	.94*	.61	.87	.68

\* $r$  value exceeds 5% level of significance.

because, even though the SLDW's differed significantly between treatments, the carbohydrate contents in flag leaves of plants subjected to various treatments did not differ significantly. Consequently, it is concluded the various treatments imposed on the plants did not decrease the movement of assimilates from the leaf. Nösberger and Thorne (1965) found in barley, that floret removal did not affect the leaf dry weight per unit area, and they, similarly, concluded the movement of assimilates from the flag leaf was unaffected.

#### Carbohydrate Levels

There was a general tendency for partially defoliated plants (Treatment 4) to show lower than normal carbohydrate levels (free plus acid-hydrolyzable) in the culms and peduncles, whereas plants which received CO<sub>2</sub> fertilization generally showed greater than normal carbohydrate contents in the culms and peduncles. This effect was most pronounced in the Burnett (Table 33, Appendix). Apparently, removal of

photosynthetic tissue resulted in a carbohydrate "drain", but subjecting plants to CO<sub>2</sub> fertilization caused a slight accumulation of assimilates in the main culms and peduncles.

It is readily apparent from Figure 29 that the carbohydrate contents in leaf tissues of both varieties did not change with time. However, carbohydrate contents decreased with time in the main culms, stem bases, and peduncles. It is believed the declination of carbohydrates in these tissues is the result of a redistribution of carbohydrates to other plant organs, the conversion of sugars to polysaccharides within the stem, and, in part, respiration. Wardlaw and Portor (1967), using labelled C<sup>14</sup>, found that wheat-stem sugars (including fructosans) were lost by remobilization and conversion to polysaccharides, and only a small part was lost by respiration. However, Archbold (1942) believed that sugar not available for conversion to starch in the ear was lost from the stem by respiration and other metabolic processes not involving translocation. The reasons for the decrease of hot water extractable carbohydrates in the main culms, stem bases, and peduncles of oats used in this experiment was not investigated. Consequently, the fate of the stem carbohydrates can only be speculated upon.

Even though the carbohydrate contents in the stem decrease during the grain filling period (Figure 29), it appears only a small percentage of the final grain weight is derived from material stored in the stems prior to anthesis. Wardlaw and Porter (1967) state that only 5 to 10 percent of the grain weight in wheat can be attributed to remobilization of stored stem sugar to the grain. Stoy (1963) found that only 12 to 15

percent of assimilates stored in the stem were remobilized and transported to the grain in wheat; however, he noted there was preferential transport of labelled assimilates to the reproductive organs. Jennings and Shibles (1968) obtained little evidence in oats for accumulations of large amounts of assimilates in vegetative tissues which might later be transported to the grain. Rawson and Hofstra (1969) concluded that remobilization of assimilates was not an important factor in determining grain yields in wheat, and Archbold (1942) concluded the carbohydrates supplied to the barley ear were supplied from directly assimilated material and were transported immediately from the various organs. Thus, the majority of the experimental evidence supports the contention that the immediate fate of the assimilates formed during the period of grain development is of major importance in determining the potential economic yield in oats. Assimilates formed prior to anthesis, which are later remobilized and moved from the stem to the grain, appear to be of only secondary importance in determining the maximum economic yield obtainable in small grains.

Carr and Wardlaw (1965) state that in wheat the expanding top internode of the stems act as a sink for assimilates from both the flag leaf and the ear. The results of this experiment also suggest the top internode (peduncle section) served as a sink for assimilates. The highest concentration of free sugars and acid-hydrolyzable carbohydrates were found in the peduncle sections just prior to full panicle emergence. However, carbohydrate levels in the peduncle sections decreased to

levels below those found in the main culms and stem bases within about 5 days after full panicle emergence. The percentage of free sugars in the peduncles generally exceeded those found in the main culm and stem bases.

In all cases the percentages of free sugars and acid-hydrolyzable carbohydrates in Burnett exceeded those found in Richland. Apparently, Burnett either accumulated more carbohydrates, or the carbohydrates which were accumulated were of some form more readily extractable with hot water.

## SUMMARY

Photosynthetic rate and carbohydrate partitioning patterns in oats, as influenced by various treatments designed to alter the sink-source ratio were studied in 1969. Treatments were as follows:

(1) control-normal plant development, (2) one-half spikelet removal, (3) three-quarters spikelet removal, (4) removal of outer glumes from spikelets and all leaf laminae below the flag leaf, and (5) CO<sub>2</sub> fertilization during the period of panicle differentiation. Treatments 2, 3, and 4 were applied at the time of full panicle emergence. The treatments involving partial spikelet removal were designed to decrease the sink-source ratio, whereas Treatments 4 and 5 were designed to increase the sink-source ratio.

Oat varieties Burnett and Richland were used. CO<sub>2</sub> exchange rates of flag leaves were measured at normal atmospheric CO<sub>2</sub> concentrations (320 ppm) by the infrared gas analysis technique, and the amount of hot water extractable carbohydrates was found by the Somogyi-Nelson procedure.

Significant treatment effects on the net photosynthetic rate were observed. Plants receiving treatments designed to decrease the sink-source ratio showed a slightly greater net photosynthetic rate and SLDW as the oats approached maturity. This is believed, largely, the result of delayed leaf senescence and possibly higher cytokinin levels in leaves of treated plants. It is postulated that the development of alternative

sinks for assimilates, namely tillers, prevented the occurrence of photosynthetic depressions in plants from which spikelets had been removed. The fact that flag leaves of plants subjected to partial defoliation showed slightly greater net photosynthetic rates than the controls may be attributable to an increased sink-source ratio, but that complex growth regulator interactions also might be involved is not discounted. The CO<sub>2</sub> fertilization treatment was not effective in increasing the size of the reproductive sink, and it is believed lower photosynthetic rates observed in plants receiving CO<sub>2</sub> fertilization were due largely to increased senescence rates. There was no evidence that a carbohydrate accumulation occurred in flag leaf tissue and depressed photosynthesis in any of the treated flag leaves tested. The decrease in net photosynthesis as the season progressed in all the plant material was associated with an increase in mesophyll resistance.

The peduncles accumulated a higher percentage of carbohydrates than any of the other plant parts, and much of this was in the form of free sugars. Carbohydrate contents, both in terms of free sugars and acid-hydrolyzable carbohydrates decreased in the peduncles, stem bases, and main culms during grain filling. Burnett consistently showed a higher percentage of hot water extractable carbohydrates than Richland.

## BIBLIOGRAPHY

- Akita, Shigemi and Miyasaka, Akira  
1969 Studies on the differences of photosynthesis among species. II. Effect of oxygen-free air on photosynthesis. Crop Science Society of Japan Proceedings 38:525-533.
- Akita, Shigemi, Miyasaka, Akira, and Murata, Yoshio  
1969 Studies on the differences of photosynthesis among species. I. Differences in the response of photosynthesis among species in normal oxygen concentration as influenced by some environmental factors. Crop Science Society of Japan Proceedings 38:507-523.
- Allison, J. C. S. and Watson, D. J.  
1966 The production and distribution of dry matter in maize after flowering. Annals of Botany, New Series 30:365-382.
- Alvin, P. deT.  
1960 Net assimilation rate and growth behavior of beans as affected by gibberellic acid, urea and sugar sprays. Plant Physiology 35:285-288.
- Apel, P. and Lehmann, C. O.  
1967 The intensity of photosynthesis in winter wheat hybrids (F<sub>1</sub>) and their parents (translated title). Züchter 37:377-378.  
1967. Original not available; abstracted in Plant Breeding Abstracts 38:761.
- Archbold, H. K.  
1942 Physiological studies in plant nutrition. XIII. Experiments with barley on defoliation and shading of the ear in relation to sugar metabolism. Annals of Botany, New Series 23:487-531.
- Aspinall, D.  
1963 The control of tillering in the barley plant. II. The control of tiller-bud growth during ear development. Australian Journal of Biological Sciences 16:285-304.
- Avery, D. J.  
1966 The supply of air to leaves in assimilation chambers. Journal of Experimental Botany 17:655-677.
- Baker, D. N. and Myhre, D. L.  
1969 Effects of leaf shape and boundary layer thickness on photosynthesis in cotton (Gossypium hirsutum L.). Physiologia Plantarum 22:1034-1049.

- Barnes, D. K., Pearce, R. B., Carlson, G. E., Hart, R. H. and  
 1969 Hanson, C. H. Specific leaf weight differences in alfalfa  
 associated with variety and plant age. *Crop Science* 9:421-423.
- Barua, D. N.  
 1964 Effect of light intensity on assimilation characteristics of  
 detached tea leaves. *Journal of Agricultural Science* 63:  
 265-271.
- Beevers, Harry  
 1969 Metabolic sinks. P. 169-180. In Jerry D. Eastin, F. A.  
 Haskins, C. Y. Sullivan, and C. H. M. van Bavel (editors)  
 Physiological aspects of crop yield. American Society of  
 Agronomy, Crop Science Society of America, Madison, Wisconsin.
- Bertsch, Andreas and Domes, Wolfhardt  
 1969 CO<sub>2</sub>-gaswechsel amphistomatischer blätter I. Der einfluss  
 unterschiedlicher stomaverteilung der beiden blattepidermen  
 auf den CO<sub>2</sub> transport. (English summary). *Planta* 85:183-193.
- Bierhuizen, J. F. and Slatyer, R. O.  
 1964 Photosynthesis of cotton leaves under a range of environ-  
 mental conditions in relation to internal and external dif-  
 fusive resistances. *Australian Journal of Biological  
 Sciences* 17:348-359.
- Bingham, J.  
 1966 Paternal effect of grain size in wheat. *Nature* 209:940-941.
- Bisalputra, T., Downton, W. J. S., and Tregunna, E. B.  
 1969 The distribution and ultrastructure of chloroplast in leaves  
 differing in photosynthetic carbon metabolism I. Wheat,  
Sorghum, and Aristida (Gramineae). *Canadian Journal of Botany*  
 47:15-21.
- Björkman, Olle  
 1966 The effect of oxygen concentration on photosynthesis in higher  
 plants. *Physiologia Plantarum* 19:618-633.
- Björkman, Olle  
 1968a Carboxydismutase activity in shade-adapted species of higher  
 plants. *Physiologia Plantarum* 21:1-10.
- Björkman, Olle  
 1968b Further studies on differentiation of photosynthetic properties  
 in sun and shade ecotypes of Solidago virgaurea. *Physiologia  
 Plantarum* 21:84-99.

- Björkman, Olle and Holmgren, Paul  
 1963 Adaptability of the photosynthetic apparatus to light intensity in ecotypes from exposed and shaded habitats. *Physiologia Plantarum* 16:889-914.
- Bjurman, Barbro  
 1959 The photosynthesis in diploid and tetraploid *Kibes satigrum*. *Physiologia Plantarum* 12:183-187.
- Black, C. C., Chen, T. M., and Brown, R. H.  
 1969 Biochemical basis for plant competition. *Weed Science* 17:338-343.
- Bohning, R. H.  
 1949 Time course of apple leaves exposed to continuous illumination. *Plant Physiology* 24:222-240.
- Bonnett, O. T.  
 1961 The oat plant: Its histology and development. Illinois Agricultural Experiment Station Bulletin 672.
- Broida, Dan  
 1965 The colorimetric determination in blood of urea nitrogen (BUN) at 400-480 m $\mu$  and glucose at 480-600 m $\mu$  based on the Somogyi-Nelson procedure. Sigma Chemical Company (St. Louis, Missouri) Technical Bulletin 14.
- Brown, K. W.  
 1969 A model of the photosynthesizing leaf. *Physiologia Plantarum* 22:620-637.
- Brown, K. W. and Rosenberg, N. J.  
 1968 Errors in sampling and infrared analysis of CO<sub>2</sub> in air and their influence in determination of net photosynthetic rate. *Agronomy Journal* 60:309-311.
- Brown, R. H. and Blaser, R. E.  
 1965 Relationships between reserve carbohydrate accumulation and growth rate in orchardgrass and tall fescue. *Crop Science* 5:577-582.
- Brun, W. A. and Cooper, R. L.  
 1967 Effects of light intensity and carbon dioxide concentration on photosynthetic rate of soybean. *Crop Science* 7:451-454.
- Bull, T. A.  
 1969 Photosynthetic efficiencies and photorespiration in Calvin cycle and C<sub>4</sub>-dicarboxylic acid plants. *Crop Science* 9:726-729.

- Burns, J. C., Noller, C. H., and Rhykerd, C. L.  
 1964 Influence of method of drying on the soluble carbohydrate content of alfalfa. *Agronomy Journal* 56:364-365.
- Burnside, Christel A. and Bohning, R. H.  
 1957 The effect of prolonged shading on the light saturation curves of apparent photosynthesis in sun plants. *Plant Physiology* 32:61-63.
- Burt, R. L.  
 1964 Carbohydrate utilization as a factor in plant growth. *Australian Journal of Biological Sciences* 17:867-877.
- Burt, R. L.  
 1966 Some effects of temperature on carbohydrate utilization and plant growth. *Australian Journal of Biological Sciences* 19:711-714.
- Cannell, R. Q., Brun, W. A., and Moss, D. N.  
 1969 A search for high net photosynthetic rate among soybean genotypes. *Crop Science* 9:840-841.
- Carr, D. J. and Wardlaw, I. F.  
 1965 The supply of photosynthetic assimilates to the grain from the flag leaf and ear of wheat. *Australian Journal of Biological Sciences* 18:711-719.
- Criswell, Jerome Glenn  
 1968 Net photosynthesis in Avena. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Curtis, P. E., Ogren, W. L., and Hageman, R. H.  
 1969 Varietal effects in soybean photosynthesis and photorespiration. *Crop Science* 9:323-327.
- Decker, John P.  
 1955 A rapid, postillumination deceleration of respiration in green leaves. *Plant Physiology* 30:82-84.
- Dornhoff, Gary Marvin  
 1969 Genotypic variation in net photosynthesis of Glycine max (L.) Merr. leaves. Unpublished M.S. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Dornhoff, Gary M. and Shibles, R. M.  
 1970 Varietal differences in net photosynthesis of soybean leaves. *Crop Science* 10:42-45.

- Downton, W. J. S., Bisalputra, T., and Tregunna, E. B.  
 1969 The distribution and ultrastructure of chloroplast in leaves differing in photosynthetic carbon metabolism II. Atriplex rosea and Atriplex hastata (Chenopodeaceae). *Canadian Journal of Botany* 47:915-919.
- Downton, W. J. S. and Tregunna, E. B.  
 1968 Carbon dioxide compensation--its relation to photosynthetic carboxylation reactions, systematics of the Gramineae, and leaf anatomy. *Canadian Journal of Botany* 46:207-215.
- Dreger, R. H., Brun, W. A., and Cooper, R. L.  
 1969 Effect of genotype on the photosynthetic rate of soybean (Glycine max (L.) Merr.) *Crop Science* 9:429-431.
- Duncan, W. G. and Hesketh, J. D.  
 1968 Net photosynthetic rates, relative leaf growth rates, and leaf numbers of 22 races of maize grown at eight temperatures. *Crop Science* 8:670-674.
- Elmore, D., Hesketh, J., and Muramoto, H.  
 1965 Rates of leaf area development among species of different leaf photosynthetic rates. *American Society of Agronomy Abstracts* 1965:26.
- El-Sharkawy, M. and Hesketh, J. D.  
 1964 Effects of temperature and water deficit on leaf photosynthetic rates of different species. *Crop Science* 4:514-518.
- El-Sharkawy, M. and Hesketh, J. D.  
 1965 Photosynthesis among species in relation to characteristics of leaf anatomy and CO<sub>2</sub> diffusion resistances. *Crop Science* 5:517-521.
- El-Sharkawy, M., Hesketh, J. D., and Muramoto, H.  
 1965 Leaf photosynthetic rates and other growth characteristics among 26 species of Gossypium. *Crop Science* 5:171-186.
- El-Sharkawy, M. A., Loomis, R. S., and Williams, W. A.  
 1967 Apparent reassimilation of respiratory carbon dioxide by different plant species. *Physiologia Plantarum* 20:171-186.
- Esau, Katherine  
 1966 *Anatomy of seed plants*. 5th edition. New York, N.Y., John Wiley and Sons, Inc.
- Evans, L. T.  
 1968 In search of the limitations to yield. Unpublished paper presented at the symposium of the Australian Institute of Agricultural Science. Bl-B10. C.S.I.R.O. Canberra, A.C.T., Australia.

- Everson, R. G. and Slack, C. R.  
1968 Distribution of carbonic anhydrase in relation to the C<sub>4</sub> pathway of photosynthesis. *Phytochemistry* 7:581-584.
- Fock, H. and Krotkov, G.  
1969 Relation between photorespiration and glycolate oxidase activity in sunflower and red kidney bean leaves. *Canadian Journal of Botany* 47:237-240.
- Forrester, Marlene L., Krotkov, G., and Nelson, C. D.  
1966a Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. I. Soybeans. *Plant Physiology* 41:422-427.
- Forrester, Marlene L., Krotkov, G., and Nelson, C. D.  
1966b Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. II. Corn and other monocotyledons. *Plant Physiology* 41:428-431.
- Fousová, S. and Avratovščuková, N.  
1967 Hybrid vigour and photosynthetic rate of leaf disks in Zea mays L. *Photosynthetica* 1:3-12.
- Freeland, R. O.  
1948 Photosynthesis in relation to stomatal frequency. *Plant Physiology* 23:595-600.
- Frey, K. J.  
1962 Influence of leaf blade removal on seed weight of oats. *Iowa State Journal of Science* 37:17-22.
- Frey, K. J. and Wiggans, S. C.  
1957 Tillering studies on oats: IV. Effect of rate and date of nitrogen fertilizer application. *Iowa Academy of Science* 64:160-167.
- Frey-Wyssling, A. and Buttrose, M. S.  
1959 Photosynthesis in the ear of barley. *Nature* 184:2031-2032.
- Friend, D. J. C.  
1960 The control of chlorophyll accumulation in leaves of Marquis wheat by temperature and light intensity I. The rate of chlorophyll accumulation and maximum absolute chlorophyll contents. *Physiologia Plantarum* 13:776-785.
- Friend, D. J. C.  
1961 Control of chlorophyll content by daylength in leaves of Marquis wheat. *Canadian Journal of Botany* 39:51-63.

- Friend, D. J. C.  
1966 The effect of light and temperature on the growth of cereals. Pp. 181-199. In F. L. Milthorpe and J. D. Ivins (editors). The growth of cereals and grasses. Butterworths, London.
- Friend, D. J. C., Helson, V. A., and Fisher, J. E.  
1962 Leaf growth in Marquis wheat, as regulated by temperature, light intensity, and daylength. Canadian Journal of Botany 40:1299-1311.
- Friend, D. J. C., Helson, V. A., and Fisher, J. E.  
1965 Changes in the leaf area ratio during growth of Marquis wheat as affected by temperature and light intensity. Canadian Journal of Botany 43:15-28.
- Gaastra, P.  
1959 Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. Mededelingen van de Landbouwhogeschool te Wageningen, Nederland 59, No. 13:1-68.
- Gaastra, P.  
1962 Photosynthesis of leaves and field crops. Netherlands Journal of Agricultural Science 10:311-324.
- Gabrielsen, E. K.  
1948 Effects of different chlorophyll concentrations on photosynthesis. Physiologia Plantarum 1:5-37.
- Gale, J. and Poljakoff-Mayber, Alexandra  
1968 Resistances to the diffusion of gas and vapor in leaves. Physiologia Plantarum 21:1170-1176.
- Hansen, Poul  
1967 <sup>14</sup>C-studies on apple trees. I. The effect of the fruit on the translocation and distribution of photosynthates. Physiologia Plantarum 20:382-391.
- Hansen, Poul  
1969 <sup>14</sup>C-studies on apple trees. IV. Photosynthate consumption in fruits in relation to the leaf-fruit ratio and to the leaf fruit position. Physiologia Plantarum 22:186-198.
- Hartt, Constance E.  
1963 Translocation as a factor in photosynthesis. Naturwissenschaften 21:666-667.
- Hatch, M. D. and Slack, C. R.  
1966 Photosynthesis by sugar-cane leaves. A new carboxylation reaction and the pathway of sugar formation. Biochemical Journal 101:103-111.

- Hatch, M. D. and Slack, C. R.  
1968 A new enzyme for the interconversion of pyruvate and phosphopyruvate and its role in the C<sub>4</sub> dicarboxylic acid pathway of photosynthesis. *Biochemical Journal* 106:141-146.
- Hatch, M. D. and Slack, C. R.  
1969 Studies on the mechanism of activation of pyruvate, phosphate dikinase. A possible regulatory role for the enzyme in the C<sub>4</sub> dicarboxylic acid pathway of photosynthesis. *Biochemical Journal* 112:549-558.
- Hayashi, Ken-ichi  
1968 Response of net assimilation rate to differing intensity of sunlight in rice varieties. *Crop Science Society of Japan Proceedings* 37:528-533.
- Heath, O. V. S., Meidner, Hans, and Spanner, D. C.  
1967 Compensation points and carbon dioxide enrichment for lettuce grown under glass in winter. *Journal of Experimental Botany* 18:746-751.
- Heath, O. V. S. and Orchard, B.  
1957 Midday closure of stomata. Temperature effect on the minimum intercellular space carbon dioxide concentration "Γ". *Nature* 180:180-181.
- Heath, O. V. S. and Russell, J.  
1954 Studies in stomatal behaviour VI. An investigation of the light responses of wheat stomata with the attempted elimination of control by the mesophyll. Part 2. Interactions with external carbon dioxide, and general discussion. *Journal of Experimental Botany* 5:269-292.
- Heichel, Gary H. and Musgrave, Robert B.  
1969a Varietal differences in net photosynthesis of Zea mays L. *Crop Science* 9:483-486.
- Heichel, Gary H. and Musgrave, Robert B.  
1969b Relation of CO<sub>2</sub> compensation concentration to apparent photosynthesis in maize. *Plant Physiology* 44:1724-1728.
- Hesketh, J. D.  
1963 Limitations to photosynthesis responsible for differences among species. *Crop Science* 3:493-496.
- Hesketh, J. D.  
1968 Effects of light and temperature during plant growth on subsequent leaf CO<sub>2</sub> assimilation rate under standard conditions. *Australian Journal of Biological Sciences* 21:235-241.

- Hesketh, John D. and Moss, Dale N.  
1963 Variation in the response of photosynthesis to light. *Crop Science* 3:107-110.
- Hesketh, John D. and Musgrave, Robert G.  
1962 Photosynthesis under field conditions IV. Light studies with individual corn leaves. *Crop Science* 2:311-315.
- Hew, C. S. and Krotkov, G.  
1967 Effect of temperature on apparent photosynthesis, CO<sub>2</sub> evolution in light and in darkness by attached leaves of sunflower, soybean and egg plants. *Plant Physiology Abstracts* 42:S-47.
- Hew, Choy-Sin, Krotkov, G. and Calvin, David T.  
1969 Effects of temperature on photosynthesis and CO<sub>2</sub> evolution in light and darkness by green leaves. *Plant Physiology* 44: 671-677.
- Hofstra, G. and Hesketh, J. D.  
1969 Effects of temperature on the gas exchange of leaves in light and dark. *Planta* 85:228-237.
- Holmgren, Paul  
1968 Leaf factors affecting light-saturated photosynthesis in ecotypes of *Solidago virgaurea* from exposed and shaded habitats. *Physiologia Plantarum* 21:676-698.
- Holmgren, Paul and Jarvis, Paul G.  
1967 Carbon dioxide efflux from leaves in light and darkness. *Physiologia Plantarum* 20:1045-1051.
- Holmgren, Paul, Jarvis, Paul G., and Jarvis, Margaret S.  
1965 Resistances to carbon dioxide and water vapour transfer in leaves of different plant species. *Physiologia Plantarum* 18: 557-573.
- Hopkinson, J. M.  
1964 Studies on the expansion of the leaf surface IV. The carbon and phosphorus economy of a leaf. *Journal of Experimental Botany* 15:125-137.
- Humphries, E. C.  
1963 Dependence of net assimilation rate on root growth of isolated leaves. *Annals of Botany, New Series* 27:175-183.
- Humphries, E. C. and French, S. A. W.  
1969 Photosynthesis in sugar beet depends on root growth. *Planta* 88: 87-90.
- Humphries, E. C. and Thorne, Gillian  
1964 The effect of root formation on photosynthesis of detached leaves. *Annals of Botany, New Series* 28:391-400.

- Irvine, J. E.  
1967 Photosynthesis in sugarcane varieties under field conditions. *Crop Science* 7:297-300.
- Iyama, Junichiro, Murata, Yoshio, and Homa, Tsutomu  
1964 Studies on the photosynthesis of forage crops III. Influence of the different temperature levels on diurnal changes in the photosynthesis of forage crops under constant conditions (English tables, figures, and summary). *Crop Science Society of Japan Proceedings* 33:25-28.
- Izhar, S. and Wallace, D. H.  
1967a Studies of the physiological basis for yield differences III. Genetic variation in photosynthetic efficiency of Phaseolus vulgaris L. *Crop Science* 7:457-460.
- Izhar, S. and Wallace, D. H.  
1967b Effect of night temperature on photosynthesis of Phaseolus vulgaris L. *Crop Science* 7:546-547.
- Jackson, W. A. and Volk, R. J.  
1968 Oxygen uptake by illuminated corn leaves. *American Society of Agronomy Abstracts* 1968:35.
- Jeffers, D. L. and Shibles, R. M.  
1969 Some effects of leaf area, solar radiation, air temperature, and variety on net photosynthesis in field-grown soybeans. *Crop Science* 9:762-764.
- Jennings, V. M. and Shibles, R. M.  
1968 Genotypic differences in photosynthetic contributions of plant parts to grain yield in oats. *Crop Science* 8:173-175.
- Jolliffe, P. A., Bulley, N. R., and Tregunna, E. B.  
1969 Photorespiration and the post illumination CO<sub>2</sub> burst in wheat, soybean, and Amaranthus edulis. *International Botanical Abstracts* 11:130.
- Kazaryan, V. O., Balagezyan, N. V., and Karapetyan, K. A.  
1965 Influence of the fruits of apple trees on the physiological activity of the leaves. *Soviet Plant Physiology* 12:265-269.
- Kiesselbach, T. A.  
1948 Endosperm type as a physiological factor in corn yields. *Journal of the American Society of Agronomy* 40:216-236.
- King, R. W., Wardlaw, I. F., and Evans, L. T.  
1967 Effect of assimilate utilization on photosynthetic rate in wheat. *Planta* 77:261-276.

- Kisaki, T. and Tolbert, N. E.  
1969 Glycolate and glyoxylate metabolism by isolated peroxisomes or chloroplasts. *Plant Physiology* 44:242-250.
- Kleese, R. A.  
1966 Photophosphorylation in barley. *Crop Science* 6:524-527.
- Koller, Dov  
1969 Discussion: Mechanisms of carbon fixation and associated physiological responses. Pp. 226-231. In Jerry D. Eastin, F. A. Haskins, C. Y. Sullivan, and C. H. M. van Bavel (editors) *Physiological aspects of crop yield*. American Society of Agronomy, Crop Science Society of America, Madison, Wisconsin.
- Kortschak, H., Hartt, C., and Burr, G.  
1965 Carbon dioxide fixation in sugar cane leaves. *Plant Physiology* 40:209-219.
- Krenzer, E. G. and Moss, Dale N.  
1969 Carbon dioxide compensation in grasses. *Crop Science* 9:619-621.
- Kriedemann, P. E.  
1968 Some photosynthetic characteristics of citrus leaves. *Australian Journal of Biological Sciences* 21:895-905.
- Krotkov, G., Rumeckles, V. C., and Thimann, K. V.  
1958 Effect of light on the CO<sub>2</sub> absorption and evolution by *Kalanchoe*, wheat, and pea leaves. *Plant Physiology* 33:289-292.
- Kumura, Atsuhiko  
1968 Studies on dry matter production of soybean plant. IV. Photosynthetic properties of leaf as subsequently affected by light conditions (English tables, figures, and summary). *Crop Science Society of Japan Proceedings* 37:583-588.
- Laetsch, W. M. and Price, Ian  
1969 Development of dimorphic chloroplasts of sugar cane. *American Journal of Botany* 56:77-87.
- Lake, J. V.  
1967a Respiration of leaves during photosynthesis. I. Estimates from an electrical analogue. *Australian Journal of Biological Sciences* 20:487-493.
- Lake, J. V.  
1967b Respiration of leaves during photosynthesis. II. Effects on the estimation of mesophyll resistance. *Australian Journal of Biological Sciences* 20:495-499.

- Leonard, D. L. and Bidwell, R. G. S.  
1969 The effect of oxygen on carbon dioxide fixation. *Plant Physiology Abstracts* 44:12.
- Leopold, Carl A.  
1964 *Plant growth and development*. New York, N.Y., McGraw-Hill Book Company, Inc.
- Loach, K.  
1969 Light-saturated photosynthesis in sun and shade raised seedlings of five tree species in relation to leaf structure. *International Botanical Congress Abstracts* 11:130.
- Logan, K. T. and Krotkov, G.  
1969 Adaptation of the photosynthetic mechanism of sugar maple (*Acer saccharum*) seedlings grown in various light intensities. *Physiologia Plantarum* 22:104-116.
- Loomis, W. E.  
1935 The translocation of carbohydrate in maize. *Iowa State College Journal of Science* 9:509-520.
- Lupton, F. G. H.  
1964 Synthesis and translocation of carbohydrate in wheat (abstract). *Plant Breeding Abstracts* 35:414.
- Madsen, Erik  
1968 The effect of CO<sub>2</sub>-concentration on the accumulation of starch and sugar in tomato leaves. *Physiologia Plantarum* 21:169-175.
- Maggs, D. H.  
1963 The reduction in growth of apple trees brought about by fruiting. *Journal of Horticultural Science* 38:119-128.
- Maggs, D. H.  
1964 Growth-rates in relation to assimilate supply and demand I. Leaves and roots as limiting regions. *Journal of Experimental Botany* 15:574-583.
- McClendon, J. H.  
1962 The relationship between the maximum thickness of deciduous leaves and their maximum photosynthetic rate. *American Journal of Botany* 49:320-322.

- McNaughton, S. J.  
1967 Photosynthetic system II. Racial differentiation in Typha latifolia. *Science* 156:1363.
- Meidner, Hans  
1964 The minimum intercellular-space CO<sub>2</sub>-concentration ( $\Gamma$ ) of maize leaves and its influence on stomatal movements. *Journal of experimental Botany* 13:284-293.
- Meidner, Hans  
1967 Further observation on the intercellular space carbon-dioxide concentration ( $\Gamma$ ) of maize leaves and postulated roles of "photorespiration" and glycolate metabolism. *Journal of Experimental Botany* 18:177-185.
- Menz, Kenneth M., Moss, Dale N., Cannell, Robert Q., and Brun, William A.  
1969 Screening for photosynthetic efficiency. *Crop Science* 9:692-694.
- Mifflin, B. J. and Hageman, R. H.  
1966 Activity of chloroplast isolated from maize inbreds and their F<sub>1</sub> hybrids. *Crop Science* 6:185-187.
- Miller, Edwin C.  
1938 *Plant physiology*. 2nd edition. New York, N.Y., McGraw-Hill Book Company, Inc.
- Milner, H. W. and Hiesey, W. M.  
1964 Photosynthesis in climatic races of Mimulus l. Effect of light intensity and temperature on rate. *Plant Physiology* 39:208-213.
- Miyazaki, Tokuzo and Tatemichi, Yoshiro  
1968 The change of photosynthetic activity and the varietal difference in the ripening of tobacco (English tables, figures, and summary). *Crop Science Society of Japan Proceedings* 37:135-139.
- Mooney, H. A. and Billings, W. D.  
1961 Comparative physiological ecology of arctic and alpine populations of Oxyria digyna. *Ecological Monographs* 31:1-29.
- Moss, Dale N.  
1962a Photosynthesis and barrenness. *Crop Science* 2:366-367.
- Moss, Dale N.  
1962b The limiting carbon dioxide concentration for photosynthesis. *Nature* 193:587.
- Moss, Dale N.  
1964 Optimum lighting of leaves. *Crop Science* 4:131-136.

- Moss, Dale N.  
1966 Respiration of leaves in light and darkness. *Crop Science* 6:351-354.
- Moss, Dale N.  
1967 High activity of the glycolic acid oxidase system in tobacco leaves. *Plant Physiology* 42:1463-1464.
- Moss, Dale N.  
1968a Photorespiration and glycolate metabolism in tobacco leaves. *Crop Science* 8:71-76.
- Moss, Dale N.  
1968b Relation in grasses of high photosynthetic capacity and tolerance to atrazine. *Crop Science* 8:774.
- Moss, Dale N., Krenzer, Eugene G., Jr., and Brun, William A.  
1969 Carbon dioxide compensation points in related plant species. *Science* 164:187-188.
- Moss, Dale N., Musgrave, Robert B., and Lemon, Edgar R.  
1961 Photosynthesis under field conditions. III. Some effects of light, carbon dioxide, temperature, and soil moisture on photosynthesis, respiration, and transpiration of corn. *Crop Science* 1:83-87.
- Moss, Dale N. and Rasmussen, H.  
1969 Cellular localization of CO<sub>2</sub> fixation and translocation of metabolites. *Plant Physiology* 44:1063-1068.
- Muramoto, H., Hesketh, J., and El-Sharkawy, M.  
1965 Relationships among rate of leaf area development, photosynthetic rate, and rate of dry matter production among American cultivated cottons and other species. *Crop Science* 5:163-166.
- Murata, Yoshio and Iyama, Junichiro  
1963a Studies on the photosynthesis of forage crops. I. Diurnal changes in the photosynthesis of several grasses and barley seedlings under constant temperature and light intensity. *Crop Science Society of Japan Proceedings* 31:311-314.
- Murata, Yoshio and Iyama, Junichiro  
1963b Studies on the photosynthesis of forage crops. II. Influence of air temperature upon the photosynthesis of some forage and grain crops. *Crop Science Society of Japan Proceedings* 31:315-322.

- Murata, Yoshio, Iyama, Junichiro, and Honma, Tsutomu  
 1965 Studies on the photosynthesis of forage crops. IV. Influence of air-temperature upon the photosynthesis and respiration of alfalfa and several southern type forage crops. Crop Science Society of Japan Proceedings 34:154-158.
- Nátr, L.  
 1964 Vynosu zrna u obilnin. I. Odrudove rozdilyue fotosyntese ozime pšenice. Rostlinna Vyroba 10:5-10.
- Nátr, L.  
 1967 Time-course of maximum photosynthesis and maximum figures for the accumulation of assimilates in barley leaf segments. Photosynthetica 1:29-36.
- Neales, T. F. and Incoli, L. D.  
 1968 The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: A review of the hypothesis. Botanical Review 34:107-125.
- Nelson, Edward B., Cenedella, Arlene, and Toibert, N. E.  
 1969 Carbonic anhydrase levels in Chlamydomonas. Phytochemistry 8:2305-2306.
- Nelson, Norton  
 1944 A photometric adaptation of the Somogyi method for the determination of glucose. Journal of Biological Chemistry 153:375-380.
- Nevins, D. J. and Loomis, R. S.  
 1970 A method for determining net photosynthesis and transpiration of plant leaves. Crop Science 10:3-6.
- Nielsen, Steeman E.  
 1960 Uptake of CO<sub>2</sub> by the plant. Handbuch der Pflanzenphysiologie (Encyclopedia of Plant Physiology) 1:70-84.
- Nobel, Park S., Chang, Diane T., Wang, Cheng-teh, Smith, Steven S.,  
 1969 and Barcus, Donald E. Initial ATP formation, NADP reduction, CO<sub>2</sub> fixation, and chloroplast flattening upon illuminating pea leaves. Plant Physiology 44:655-661.
- Nösberger, J. and Humphries, E. C.  
 1965 The influence of removing tubers on dry-matter production and net assimilation rate of potato plants. Annals of Botany, New Series 29:579-588.
- Nösberger, J. and Thorne, Gillian N.  
 1965 The effect of removing florets or shading the ear of barley on production and distribution of dry matter. Annals of Botany, New Series 29:635-644.

- Ojima, Mutsu and Kawashima, Ryoichi  
 1968 Studies on the seed production of soybean 5. Varietal differences in photosynthetic rate of soybean (English tables, figures, and summary). Crop Science Society of Japan Proceedings 37:667-675.
- Ojima, Mutsu, Kawashima, Ryoichi, and Sakamoto, Shin-ichi  
 1968 Studies on the seed production of soybean 6. Relationship between the activity of photosynthesis of improved varieties and that of parent lines (English tables, figures, and summary). Crop Science Society of Japan Proceedings 37:676-679.
- Osada, A.  
 1964 Studies on the photosynthesis of Indica rice. Crop science Society of Japan Proceedings 33:69-76.
- Osada, A. and Murata, Y.  
 1965 Varietal differences in the rate of photosynthesis of rice plant and its relation to dry-matter production (English tables, figures, and summary). Crop Science Society of Japan Proceedings 33:454-459.
- Pearce, R. B., Carlson, G. E., Barnes, D. K., Hart, R. H. and Hanson, C. H.  
 1969 Specific leaf weight and photosynthesis in alfalfa. Crop Science 9:423-426.
- Pearce, R. B. and Lee, D. R.  
 1969 Photosynthetic and morphological adaptation of alfalfa leaves to light intensity at different stages of maturity. Crop Science 9:791-794.
- Pieters, G. A.  
 1960 On the relationship between the maximum rate of photosynthesis and the thickness of the mesophyll in sun and shade leaves of Acer pseudoplatanus L. Mededelingen van de Landbouwhogeschool to Wageningen, Nederland 60, No. 17:1-6.
- Rabinowitch, Eugene I.  
 1948 Photosynthesis. Scientific American 179, No. 2:24-35.
- Rahmankulov, S.  
 1967 Intensity of photosynthesis and respiration in maize hybrids and inbreds (translated title). Uzbek. biol. Zh. 5:19-24. 1963. Original not available; abstracted in Plant Breeding Abstracts 37:749.
- Rawson, H. M. and Hofstra, G.  
 1969 Translocation and remobilization of C<sup>14</sup> assimilated at different stages by each leaf of the wheat plant. Australian Journal of Biological Sciences 22:321-331.

- Reed, M. L. and Graham, D.  
1968 Control of photosynthetic carbon dioxide fixation during the induction phase in Chlorella. Plant Physiology Abstracts 43:S-29.
- Rodionov, V. S.  
1963 Comparative rates of photosynthesis and respiration in different tomato species and varieties. Soviet Plant Physiology 10:544-549.
- Ryle, G. J. A. and Hesketh, J. D.  
1969 Carbon dioxide uptake in nitrogen-deficient plants. Crop Science 9:451-454.
- Salisbury, Frank B. and Ross, Cleon  
1969 Plant physiology. Belmont, California, Wadsworth Publishing Company, Inc.
- Sarkissian, I. V.  
1962 Enhanced photosynthetic carboxylation as a mechanism of heterosis (abstract). West Virginia Academy of Science Proceedings 34:50.
- Schultz, G.  
1964 The significance of assimilation capacity as a breeding character. Investigations on sugar beets (translated title). Züchter 33:116-122. 1963. Original not available; abstracted in Plant Breeding Abstracts 34:96.
- Shibles, R. M. and MacDonald, H. A.  
1962 Photosynthetic area and rate in relation to seedling vigor of birdsfoot trefoil (Lotus corniculatus L.). Crop Science 2:299-302.
- Slack, C. R.  
1969 Localization of certain photosynthetic enzymes in mesophyll and parenchyma sheath chloroplasts of maize and Amaranthus palmeri. Phytochemistry 8:1387-1391.
- Slack, C. R. and Hatch, M. D.  
1967 Comparative studies on the activity of carboxylases and other enzymes in relation to the new pathway of photosynthetic carbon dioxide fixation in tropical grasses. Biochemical Journal 103:660-665.
- Slatyer, R. O.  
1967 Plant-water relationships. New York, N.Y., Academic Press.

- Slatyer, R. O. and Bierhuizen, J. F.  
1964 A differential psychrometer for continuous measurements of transpiration. *Plant Physiology* 39:1051-1056.
- Smith, Dale  
1969 Removing and analyzing total nonstructural carbohydrates from plant tissue. University of Wisconsin Research Report 41.
- Somogyi, Michael  
1952 Notes on sugar determination. *Journal of Biological Chemistry* 195:19-23.
- Sparling, J. H.  
1967 Assimilation rates of some woodland herbs in Ontario. *Botanical Gazette* 128:160-168.
- Srugoenyte, A. V. and Shpokene, A. P.  
1969 Carboanhydrase activity in plants. *Soviet Plant Physiology* 16:120-121.
- Steel, Robert G. D. and Torrie, James H.  
1960 Principles and procedures of statistics with special reference to the biological sciences. New York, N.Y., McGraw-Hill Book Company, Inc.
- Stevenson, Kenneth Ross  
1969 The effects of environmental variables and plant morphology on leaf resistances, leaf temperatures and relative water content in soybeans. Unpublished Ph.D. thesis. Ames, Iowa, Library, Iowa State University of Science and Technology.
- Stoy, Volkmar  
1963 The translocation of C<sup>14</sup>-labelled photosynthetic products from the leaf to the ear in wheat. *Physiologia Plantarum* 16:851-866.
- Stoy, Volkmar  
1965 Photosynthesis, respiration and carbohydrate accumulation in spring wheat in relation to yield. *Physiologia Plantarum Supplementum* 4:1-125.
- Stoy, Volkmar  
1969 Interrelationships among photosynthesis, respiration, and movement of carbon in developing crops. Pp. 185-202. In Jerry D. Eastin, F. A. Haskins, C. Y. Sullivan, and C. H. M. van Bavel (editors). *Physiological aspects of crop yield*. American Society of Agronomy, Crop Science Society of America, Madison, Wisconsin.
- Sweet, G. B. and Wareing, P. F.  
1966 Role of plant growth in regulating photosynthesis. *Nature* 210:77-79.

- Sybanbekov, K. Z.  
 1967 Comparative data on the rate of photosynthesis and transpiration of different organs in awned and awnless forms of wheat (translated title). *Bot. Zh.*, Moskva 51:1628-1632. 1966. Original available but not translated; abstracted in *Plant Breeding Abstracts* 37:493.
- Thorne, Gillian N.  
 1963 Varietal differences in photosynthesis of ears and leaves of barley. *Annals of Botany, New Series* 27:155-174.
- Thorne, Gillian N. and Evans, Audrey F.  
 1964 Influence of tops and roots on net assimilation rate of sugar-beet and spinach beet grafts between them. *Annals of Botany, New Series* 28:499-508.
- Thrower, Stella L.  
 1965 Translocation of labelled assimilates in the soybean IV. Some effects of low temperature on translocation. *Australian Journal of Biological Sciences* 18:449-461.
- Tieszen, E. L.  
 1969 The effect of light intensity on photosynthesis diffusion resistances and carboxylation activity. *International Botanical Congress Abstracts* 11:219.
- Tolbert, N. E.  
 1969 Leaf peroxisomes, peroxisomal respiration and net photosynthesis. *International Botanical Congress Abstracts* 11:220.
- Tregunna, E. B.  
 1966 Flavin mononucleotide control of glycolic acid oxidase and photorespiration in corn leaves. *Science* 151:1239-1241.
- Tregunna, E. B. and Downton, W. J. S.  
 1969 Carbon dioxide compensation in members of the Amaranthaceae and some related families. *Canadian Journal of Botany* 45: 2385-2387.
- Tregunna, E. B., Downton, W. J. S., and Berry, J. S.  
 1969 Variation of photorespiration among species. *International Botanical Congress Abstracts* 11:221.
- Tregunna, E. B., Krotkov, G., and Nelson, C. D.  
 1961 Evolution of CO<sub>2</sub> by tobacco leaves during the dark period following illumination with light of different intensities. *Canadian Journal of Botany* 39:1045-1056.

- Tregunna, E. B., Krotkov, G., and Nelson, C. D.  
 1966 Effect of oxygen on the rate of photorespiration in detached tobacco leaves. *Physiologia Plantarum* 19:723-733.
- Troughton, J. H.  
 1969 Plant water status and carbon dioxide exchange of cotton leaves. *Australian Journal of Biological Sciences* 22: 289-302.
- Troughton, J. H. and Slatyer, R. O.  
 1969 Plant water status, leaf temperature, and the calculated mesophyll resistance to carbon dioxide of cotton leaves. *Australian Journal of Biological Sciences* 22:815-827.
- Tsuno, Yukindo and Fujise, Kazuma  
 1965 Studies on the dry matter production of the sweet potato VIII. The internal factors influence on photosynthetic activity of sweet potato leaf (English summary). *Crop Science Society of Japan Proceedings* 33:230-235.
- Turner, Wendy B. and Bidwell, R. G. S.  
 1965 Rates of photosynthesis in attached and detached bean leaves, and the effect of spraying with indoleacetic acid solution. *Plant Physiology* 40:446-451.
- Voříšek, V. and Hudeová, M.  
 1970 Pigment contents in leaves and photosynthetic rates in lines and simple hybrids of *Zea mays* L. "Čejčský raný" variety (translated title). *Acta Univ, Agri. Fac. agron., Brno* 16: 559-556. 1968. Original not available; abstracted in *Plant Breeding Abstracts* 40:96.
- Waggoner, Paul E. and Zelitch, Israel  
 1965 Transpiration and the stomata of leaves. Water loss through leaf pores is controlled by pore size which varies with environment and chemical sprays. *Science* 150:1413-1420.
- Waldron, J. C., Glasziou, K. T., and Bull, T. A.  
 1967 The physiology of sugar-cane IX. Factors affecting photosynthesis and sugar storage. *Australian Journal of Biological Sciences* 18:269-281.
- Wardlaw, I. F.  
 1965 The velocity and pattern of assimilate translocation in wheat plants during grain development. *Australian Journal of Biological Sciences* 18:269-281.

- Wardlaw, I. F., Carr, D. J., and Anderson, M. Jean  
 1965 The relative supply of carbohydrates and nitrogen to wheat grains, and an assessment of the shading and defoliation techniques used for these determinations. *Australian Journal of Biological Sciences* 16:893-901.
- Wardlaw, I. F. and Portor, H. K.  
 1967 The redistribution of stem sugars in wheat during grain development. *Australian Journal of Biological Sciences* 20:309-318.
- Wareing, P. F., Khalifa, M. M., and Trehearne, K. J.  
 1968 Rate limiting processes in photosynthesis at saturating light intensities. *Nature* 220:453-457.
- Warren-Wilson, J.  
 1966. Effects of seasonal variation in radiation and temperature on net assimilation and growth rates in an arid climate. *Annals of Botany, New Series* 31:41-57.
- Warren-Wilson, J. and Wadsworth, R. M.  
 1958 The effect of wind speed on assimilation rate--a reassessment. *Annals of Botany, New Series* 22:285-290.
- Webb, J. A. and Gorham, P. R.  
 1965 The effect of node temperature on assimilation and translocation of C<sup>14</sup> in the squash. *Canadian Journal of Botany* 43:1009-1020.
- Whiteman, P. C. and Koller, D.  
 1968 Estimation of mesophyll resistance to diffusion of carbon dioxide and water vapor. In U.N.E.S.C.O. *Functioning of terrestrial ecosystems at the primary production level*. Edited by F. E. Eckardt, *Proceedings of the Copenhagen Symposium*. Iowa State University Library, Ames, Iowa.
- Wilson, D. and Cooper, J. P.  
 1967 Assimilation of *Lolium* in relation to leaf mesophyll. *Nature* 214:989-991.
- Womack, David and Thurman, R. L.  
 1962 Effect of leaf removal on the grain yield of wheat and oats. *Crop Science* 2:423-426.
- Zelitch, Israel  
 1966a Increased rate of net photosynthetic carbon dioxide uptake by the inhibition of glycolate oxidase. *Plant Physiology* 41:1623-1631.
- Zelitch, Israel  
 1966b Increased rate of photosynthesis in tobacco at 35°C by inhibition of glycolate oxidase. *Plant Physiology* 41:xxxviii.

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APPENDIX

Table 23. Analyses of variance results in 1968 and 1969

Source	df	Mean squares	F ratio
1968 net photosynthesis			
Genotypes	19	336.515	5.136**
Error	160	65.518	
1969 net photosynthesis			
Genotypes	19	55.361	4.751**
Error	140	11.654	
1968 CO <sub>2</sub> efflux into CO <sub>2</sub> -free air			
Genotypes	19	25.372	2.396**
Error	160	10.591	
1969 CO <sub>2</sub> efflux into CO <sub>2</sub> -free air			
Genotypes	19	10.143	2.416**
Error	140	4.198	
1968 CO <sub>2</sub> compensation concentration			
Genotypes	19	1030.143	1.685*
Error	160	611.289	
1969 CO <sub>2</sub> compensation concentration			
Genotypes	19	302.401	.979
Error	140	308.778	
1968 Slope of the CO <sub>2</sub> response curve			
Genotypes	19	.00467	5.543**
Error	160	.00084	
1969 Slope of the CO <sub>2</sub> response curve			
Genotypes	19	.00096	6.682**
Error	140	.00014	
1968 Specific leaf fresh weight			
Genotypes	18	.1146	4.801**
Error	152	.0239	
1969 Specific leaf fresh weight			
Genotypes	19	.1088	22.529**
Error	140	.0048	

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

Table 23. (Continued)

Source	df	Mean squares	F ratio
1968 Specific leaf dry weight			
Genotypes	18	.0184	6.489**
Error	152	.0028	
1969 Specific leaf dry weight			
Genotypes	19	.0125	13.484**
Error	140	.0009	
1968 Sum of CO <sub>2</sub> diffusion resistances			
Genotypes	19	15.194	2.709**
Error	160	5.608	
1969 Sum of CO <sub>2</sub> diffusion resistances			
Genotypes	19	4.599	5.660**
Error	140	.813	
1969 Transpiration			
Genotypes	19	8.221	7.726**
Error	140	1.064	
1969 Photosynthesis/transpiration ratio			
Genotypes	19	6.529	11.810**
Error	140	.553	
1969 Laminar CO <sub>2</sub> diffusion resistance			
Genotypes	19	.0355	8.719**
Error	140	.0041	
1969 Stomatal CO <sub>2</sub> diffusion resistance			
Genotypes	19	.5586	2.145**
Error	140	.2604	
1969 Mesophyll CO <sub>2</sub> diffusion resistance			
Genotypes	19	3.2319	6.261**
Error	140	.5162	

Table 24. Simple correlation coefficients (r) obtained in 1968 (Degrees of freedom within a genotype = 7, degrees of freedom for r on genotypic means = 18)

Genotype	P					R			S	SLDW
	r	Σr	S	SLFW	SLDW	r	Σr	S	Σr	SLDW
Saia	.23	-.81**	.82**	-.52	-.22	.98**	-.81**	.83**	-.99**	.38
<u>A. brevis</u>	-.63	-.76*	.68*	-.43	-.36	.91**	-.35	.43	-.95**	.23
Glabrota	-.77*	-.82**	.84**	.17	.66	.97**	-.38	-.20	-.99**	-.39
P.I. 193958	.63	-.98**	.99**	-.61	-.63	.98**	-.81**	.85**	-.98**	.59
C.I. 1779	-.17	-.93**	.94**	-.54	-.66	.91**	-.58	.55	-.99**	.81**
C.I. 2528	-.09	-.94**	.98**	-.56	-.33	.92**	-.54	.62	-.98**	.86**
P.I. 296234	-.41	-.96**	.95**	-.76*	-.13	.91**	-.27	.29	-.98**	.06
P.I. 292546	-.38	-.97**	.97**	-.03	.35	.69*	-.49	.56	-.98**	.82**
A-465	-.90**	-.96**	.98**	-.07	.03	.69*	.07	-.12	-.99**	.93**
Curt	-.65	-.92**	.93**	-.23	-.19	.84**	-.26	.24	-.99**	.92**
Clintland-64	-.85**	-.97**	.96**	-.77*	-.41	.88**	.38	-.31	-.98**	.43*
Marion	.30	-.91**	.99**	-.09	.40	.75*	-.79*	.91**	-.91**	.68*
Record	-.37	-.96**	.98**	.60	.55	.67*	-.53	.58	-.97**	.85**
Bingham	-.64	-.91**	.95**	.90**	-.88**	.67*	-.35	.43	-.94**	.96**
Victorgrain	-.41	-.94**	.98**	-.15	-.57	.79*	-.52	.43	-.99**	.65
Appler	-.66	-.74*	.77*	.23	.08	.98**	-.28	.17	-.99**	.20
Richland	-.23	-.95**	.97**	--	--	.63	-.49	.78*	-.90**	--
Goodfield	-.52	-.94**	.96**	-.68*	.18	.89**	-.27	.22	-.99**	.30
Garland	-.52	-.83**	.81**	-.25	-.53	.95**	-.45	.39	-.99**	.41
Burnett	-.94**	-.96**	.98**	-.71*	-.72*	.77*	.31	-.34	-.98**	.88**
r on genotype means	-.26	-.91**	.96**	.12 <sup>a</sup>	.75** <sup>a</sup>	.80**	-.55*	.53*	-.94**	.23 <sup>a</sup>

\*r value exceeds 5% level of significance.

\*\*r value exceeds 1% level of significance.

<sup>a</sup>Degrees of freedom = 17 since leaves of one genotype were lost.

Table 25. Simple correlation coefficients (r) obtained in 1969 (Degrees of freedom within a genotype = 6, degrees of freedom for r on genotypic means = 18)

Genotype	P									
	R	r	S	T	SLFW	SLDW	$\Sigma r$	$r_a$	$r_s$	$r_m$
Saia	.06	-.34	.81*	.77*	-.34	.24	-.83*	-.93**	-.76*	-.32
<u>A. brevis</u>	.03	-.50	.87**	.76*	.62	.80*	-.83*	-.86**	-.78*	-.41
Glabrota	-.29	-.68	.89**	.82*	.12	.63	-.84**	-.73*	-.79*	-.77*
P.I. 193958	.35	-.77*	.99**	.28	.57	.39	-.99**	-.53	-.49	-.92**
C.I. 1779	.30	-.34	.93**	.89**	-.39	-.26	-.94**	-.73*	-.87**	-.48
C.I. 2528	-.07	-.50	.80*	.02	-.07	.44	-.84**	-.02	-.50	-.63
P.I. 296234	-.03	-.47	.85**	.46	-.38	-.01	-.82*	-.79*	-.57	-.41
P.I. 292546	-.09	-.48	.75*	.29	-.62	-.48	-.85*	-.77*	-.70	-.69
A-465	-.75*	-.86**	.61	.48	.23	-.10	-.60	.00	-.67	-.29
Curt	-.65	-.84**	.90**	.50	.27	.70	-.88**	-.72*	-.76*	-.82*
Clintland-64	-.12	-.52	.91**	.21	-.31	-.36	-.91**	-.42	-.32	-.38
Marion	.24	-.66	.82*	.29	.19	-.08	-.83*	-.01	-.65	-.55
Record	.17	-.22	.81*	.01	.16	.51	-.87**	-.16	-.66	-.81*
Bingham	.25	-.07	.84**	.18	.01	.68	-.82*	-.50	-.45	-.46
Victorgrain	.51	.28	.89**	.61	.26	.17	-.89**	-.26	-.60	-.82*
Appler	.01	-.58	.92**	.80*	-.52	-.90**	-.92**	-.56	-.93**	-.66
Richland	-.28	-.58	.70	.37	.24	.41	-.68	-.40	-.48	-.56
Goodfield	.58	-.83*	.98**	.72*	-.46	-.70	-.99**	-.48	-.87**	-.79*
Garland	-.45	-.71*	.77*	-.23	.48	.29	-.77*	.53	-.66	-.72*
Burnett	-.83*	-.91**	.53	.32	-.17	.37	-.56	.05	-.69	.19
r on genotype means	.68**	.04	.97**	.25	.40	.58**	-.96**	-.28	-.60**	-.87**

\*r value exceeds 5% level of significance.

\*\*r value exceeds 1% level of significance.

Table 25. (Continued)

Genotype	R									SLEW SLDW
	r	S	T	SLEW	SLDW	$\Sigma r$	$r_a$	$r_s$	$r_m$	
Saia	.91**	.64	.21	-.28	.26	-.58	.16	.08	-.74*	.80*
<u>A. brevis</u>	.88**	.47	.07	.57	.57	-.50	-.15	-.01	-.72*	.89**
Glabrota	.90**	.18	.03	.15	-.26	-.27	-.18	-.15	-.32	.73*
P.I. 193958	.32	.50	-.30	.17	.47	-.41	.05	.18	-.54	.40
C.I. 1779	.78*	.63	.22	-.03	-.01	-.58	-.26	-.24	-.79*	.41
C.I. 2528	.90**	.55	-.13	-.16	.52	-.48	.12	.31	-.62	.46
P.I. 296234	.88**	.50	-.40	.06	.54	-.53	.16	.47	-.75*	.74*
P.I. 292546	.91**	.59	.26	.34	.20	-.44	-.02	-.05	-.57	.42
A-465	.98**	.07	-.69	-.01	.33	-.08	.37	-.83*	-.75*	.44
Curt	.96**	-.24	-.18	-.47	-.54	.20	.41	.20	.15	.55
Clintland-64	.86**	.40	.03	-.26	.25	.40	.22	.23	-.49	.73*
Marion	.89**	.35	-.27	-.41	-.29	-.35	.24	.31	.53	.84**
Record	.92**	.71*	.28	.42	.60	-.62	.06	.00	-.68	.80*
Bingham	.95**	.73*	.46	-.31	.50	-.75*	-.34	.02	-.68	.13
Victorgrain	.96**	.85**	.54	-.25	-.12	-.85**	-.12	-.66	-.75*	.79*
Appler	.81*	.39	-.14	-.25	.05	-.39	-.08	.00	-.68	.57
Richland	.95**	.49	.47	-.48	.13	-.51	-.57	-.14	.51	.68
Goodfield	.51	.27	.24	-.09	.19	-.22	-.01	.03	-.39	.46
Garland	.95**	.22	.56	-.41	-.08	-.22	-.70	.06	-.19	.11
Burnett	.99**	.29	-.03	.22	-.36	.00	-.06	.49	-.49	.52
r on genotype means	.75**	.83**	.31	-.01	.36	-.83**	-.36	-.41	-.76**	.43

Table 25. (Continued)

Genotype	T						S			
	$\Sigma r$	$r_a$	$r_s$	$r_m$	SLFW	SLDW	$\Sigma r$	$r_a$	$r_s$	$r_m$
Saia	-.65	-.70	-.95**	.01	-.23	.20	-.98**	-.62	-.54	-.68
<u>A. brevis</u>	-.68	-.90**	-.95**	-.03	.66	.71	-.98**	-.83*	-.70	-.71*
Glabrota	-.84**	-.90**	-.95**	-.67	.57	.83*	-.99**	-.84**	-.88**	-.94**
P.I. 193958	-.28	-.85**	-.95**	.08	-.20	-.14	-.99**	-.50	-.42	-.95**
C.I. 1779	-.86**	-.92**	-.97**	-.16	-.56	-.62	-.99**	-.70	-.80*	-.69
C.I. 2528	.06	-.81*	-.82*	.46	-.33	-.06	-.99**	.10	-.23	-.91**
P.I. 296234	-.10	-.77*	-.96**	.48	-.13	-.09	-.99**	-.60	-.24	-.76*
P.I. 292546	-.46	-.68	-.87**	-.08	-.47	-.57	-.98**	-.64	-.60	-.94**
A-465	.10	-.62	-.95**	.89**	-.61	-.30	-.99**	.44	-.02	-.46
Curt	-.58	-.52	-.85**	-.32	.43	.12	-.99**	-.67	-.85**	-.96**
Clintland-64	-.19	-.78*	-.94**	.65	-.20	-.42	-.99**	-.32	.23	-.52
Marion	-.12	-.87**	-.89**	.38	.05	.26	-.99**	.17	.43	-.85**
Record	-.15	-.83*	-.28	-.04	-.68	-.31	-.99**	-.08	.46	-.98**
Bingham	-.39	-.84**	-.77*	.24	-.31	.39	-.99**	-.55	.32	-.69
Victorgrain	-.64	-.86**	-.84**	-.33	-.05	-.33	-.99**	-.20	.70	-.92**
Appler	-.71	-.69	-.95**	-.27	-.53	-.94**	-.99**	-.56	-.86**	-.87**
Richland	-.68	-.71*	-.83*	-.37	-.55	-.07	-.99**	-.79*	.54	-.88**
Goodfield	-.73*	-.86**	-.75*	.42	-.74*	-.74*	-.99**	-.45	.83*	-.85**
Garland	-.15	-.78*	-.41	.13	-.50	-.52	-.99**	-.06	-.69	-.92**
Burnett	-.54	-.32	-.81*	.45	.27	.62	-.99**	-.03	-.50	-.37
r on genotype means	-.22	-.94**	-.51*	.07	.32	.36	-.99**	-.33	-.60**	-.89**

Table 25. (Continued)

Genotype	$r_m$				$r_s$				$r_a$	
	SLFW	SLDW	$r_a$	$r_s$	SLFW	SLDW	$r_a$	$r_m$	leaf temp.	area/leaf
Saia	.30	.12	.73*	-.23	.16	-.27	.75*	-.23	.73*	.42
A. brevis	.56	-.72*	.65	-.03	-.73*	-.70	.95**	.03	.91**	.58
Glabrota	-.06	-.32	.70	-.72*	-.43	-.63	.85**	.72*	.52	-.27
P.I. 193958	-.67	-.54	.49	.14	.15	.09	.84**	.14	.34	.47
C.I. 1779	-.22	-.29	.52	.14	.55	.53	.87**	.14	.89**	.68
C.I. 2528	.05	-.63	-.19	-.18	.14	-.13	.55	-.18	-.06	.14
P.I. 292234	.08	-.34	.58	-.45	.29	.19	.75*	-.45	.34	-.41
P.I. 292546	.62	-.06	.68	.33	.59	.68	.87**	.33	.20	-.39
A-465	-.47	-.37	-.67	-.88**	.36	.26	.61	-.88**	-.27	.16
Curt	.15	-.54	.72*	.68	-.42	-.49	.59	.68	.40	.17
Clintland-64	.16	-.21	-.20	-.71*	.13	.43	.81*	-.71*	.11	-.43
Marion	.20	.40	-.29	-.10	-.21	-.15	.62	-.10	.38	.11
Record	-.44	-.76*	.10	.35	.07	-.15	.52	.35	.20	-.06
Bingham	.00	-.45	-.17	-.45	.20	-.25	.82*	-.45	.37	-.24
Victorgrain	-.18	-.32	-.10	.39	.34	.55	.62	.39	.31	-.23
Appler	.48	.49	.46	.50	.57	.97**	.70	.50	.71*	-.31
Richland	.10	-.56	.25	.11	.26	.15	.52	.11	-.14	-.46
Goodfield	.35	.17	.29	.42	.31	.91**	.54	.43	.04	-.64
Garland	.25	.37	-.64	.38	.31	.68	.06	.38	-.34	-.56
Burnett	.20	.63	-.16	-.57	-.25	-.73*	-.08	-.57	-.09	-.30
r on genotype means	-.43	-.55*	.06	.22	-.03	-.17	.38	.22	-.44	.94**

Table 25. (Continued)

Genotype	$r$		
	$r_r$	$r_m$	$r_s$
Saia	-.21	-.55	.35
A. brevis	-.04	-.42	.36
Glabrota	.17	.11	.25
P.I. 193958	.73*	.57	.64
C.I. 1779	.04	-.51	.36
C.I. 2528	-.06	-.28	.50
P.I. 296234	-.10	-.51	.71*
P.I. 292546	-.04	-.24	.27
A-465	.11	-.66	.82*
Curt	.48	-.41	.45
Clintland-64	.13	-.22	.35
Marion	.12	-.15	.55
Record	-.28	-.37	.25
Bingham	-.51	-.55	.16
Victorgrain	-.68	-.61	-.56
Appler	-.22	-.18	.56
Richland	-.20	-.25	.06
Goodfield	.73*	.47	.77*
Garland	.10	.10	.29
Burnett	.16	-.43	.57
r on genotype means	-.27	-.28	-.01

Table 26. Proportion of the total sums of squares attributable to linear regression ( $r^2$ ) in 1968 (Dependent variable =  $\text{CO}_2$  exchange; independent variable = mean  $\text{CO}_2$  concentration over the leaf, error degrees of freedom = 3)

Genotype	Replication								
	1	2	3	4	5	6	7	8	9
Saia	.985	.988	.990	.975	.982	.998	.985	.899 <sup>†</sup>	.985
A. brevis	.930	.951	.980	.920	.964	.963	.795 <sup>†</sup>	.980	.998
Glabrota	.954	.961	.975	.988	.986	.960	.989	.975	.987
P.I. 193958	.975	.994	.988	.997	.959	.959	.982	.983	.994
C.I. 1779	.990	.997	.985	.993	.994	.988	.988	.984	.997
C.I. 2528	.978	.999	.995	.999	.991	.999	.999	.997	.994
P.I. 296234	.967	.991	.947	.992	.993	.985	.941	.973	.981
P.I. 292546	.980	.983	.929	.951	.959	.973	.942	.986	.958
A-465	.990 <sup>†</sup>	.989	.991	.994	.990	.984	.956	.995	.994
Curt	.899 <sup>†</sup>	.997	.991	.999	.962	.975	.981	.931	.989
Clintland-64	.971	.999	.989	.997	.956	.993	.933	.995	.998
Marion	.994	.994	.983	.990	.988	.970	.995	.986	.912 <sup>†</sup>
Record	.995	.990	.991	.960	.990	.998	.999	.945	.974
Bingham	.983	.988	.998	.995	.935	.987	.988	.988	.996
Victorgrain	.989	.993	.989	.992	.966	.983	.992	.968	.985
Appler	.955	.990	.999	.937	.989	.996	.977	.949	.991 <sup>†</sup>
Richland	.991	.980	.983	.953	.980	.992	.973	.990	.834 <sup>†</sup>
Goodfield	.994	.993	.999	.989	.993	.989	.999	.999	.974
Garland	.987	.993	.978	.996	.995	.989	.995	.986	.994
Burnett	.992	.997	.990	.990	.999	.993	.999	.999	.979

<sup>†</sup>Regressions where F ratios did not exceed the 1% level of significance.

Table 27. Analyses of variance results obtained for various variables in the sink-source experiment

Source	df	Mean squares	F ratio
Net photosynthesis			
Burnett			
Dates	11	216.6300	168.803**
Treatments	4	15.1687	11.658**
Dates X Treatments-in-late-dates	28	5.1377	3.949**
(Early vs. late dates) X Treatments	4	6.0498	4.650*
Error	12		
Richland			
Dates	11	200.1500	33.455**
Treatments	4	40.3850	6.750**
Dates X Treatments-in-late-dates	28	3.3730	.564
(Early vs. late dates) X Treatments	4	13.7049	2.291
Error	12	5.9827	
Transpiration			
Burnett			
Dates	11	2.4060	33.638**
Treatments	4	.0766	1.072
Dates X Treatments-in-late-dates	28	.1802	2.520*
(Early vs. late dates) X Treatments	4	.1285	1.796
Error	12	.0715	
Richland			
Dates	11	2.7503	16.110**
Treatments	4	.5387	3.155
Dates X Treatments-in-late-dates	28	.2059	1.206
(Early vs. late dates) X Treatments	4	.1600	.937
Error	12	.1707	
Specific leaf dry weight			
Burnett			
Dates	11	.0038	13.003**
Treatments	4	.0015	5.256*
Dates X Treatments-in-late-dates	28	.0005	1.843
(Early vs. late dates) X Treatments	4	.0010	3.515*
Error	12	.0003	
Richland			
Dates	11	.0023	12.609**
Treatments	4	.0016	8.641**
Dates X Treatments-in-late-dates	28	.0004	2.283
(Early vs. late dates) X Treatments	4	.0007	3.696*
Error	12	.0002	

\*F value exceeds 5% level of significance.

\*\*F value exceeds 1% level of significance.

Table 27. (Continued)

Source	df	Mean squares	F ratio
Sum of resistances			
Burnett			
Dates	11	142.0854	1181.092**
Treatments	4	17.1753	142.771**
Dates X Treatments-in-late-dates	28	10.6098	88.195**
(Early vs. late dates) X Treatments	4	6.7694	52.271**
Error	12	.1203	
Richland			
Dates	11	132.8915	235.707**
Treatments	4	45.0495	79.903**
Dates X Treatments-in-late-dates	28	9.8193	17.416**
(Early vs. late dates) X Treatments	4	20.3774	36.143**
Error	12	.5638	
Mesophyll resistance			
Burnett			
Dates	11	112.9075	798.497**
Treatments	4	15.6824	110.091**
Dates X Treatments-in-late-dates	28	9.3578	66.180**
(Early vs. late dates) X Treatments	4	5.4334	38.699**
Error	12	.1414	
Richland			
Dates	11	116.1057	198.573**
Treatments	4	36.1848	61.886**
Dates X Treatments-in-late-dates	28	7.4835	12.799**
(Early vs. late dates) X Treatments	4	16.0688	27.482**
Error	12	.5847	
Stomatal resistance			
Burnett			
Dates	11	1.9656	68.014**
Treatments	4	.0964	3.336*
Dates X Treatments-in-late-dates	28	.1522	5.266**
(Early vs. late dates) X Treatments	4	.0487	1.685
Error	12	.0289	
Richland			
Dates	11	2.2867	34.232**
Treatments	4	.5500	8.234**
Dates X Treatments-in-late-dates	28	.2255	3.376*
(Early vs. late dates) X Treatments	4	.1934	2.895
Error	12	.0668	

Table 27. (Continued)

Source	df	Mean squares	F ratio
Laminar resistance			
Burnett			
Dates	11	.0286	12.381**
Treatments	4	.0020	.866
Dates X Treatments-in-late-dates	28	.0033	1.429
(Early vs. late dates) X Treatments	4	.0037	1.602
Error	12	.0023	
Richland			
Dates	11	.0219	9.125**
Treatments	4	.0199	8.292**
Dates X Treatments-in-late-dates	28	.0201	8.375**
(Early vs. late dates) X Treatments	4	.0079	3.292*
Error	12	.0024	

Table 28. Net photosynthetic rates and transpiration rates measured in the sink-source experiment

Treatment	Test dates												$\bar{X}$	
	1	2	3	4	5	6	7	8	9	10	11	12		
Net photosynthesis, $\text{mg CO}_2 \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$														
Burnett	1	29.2	28.8	29.6	29.2	27.4	28.2	25.4	23.2	19.2	9.1	15.9	9.9	22.9
	2	26.6	25.4	29.4	25.6	29.9	27.2	26.2	26.1	24.6	13.7	13.7	7.9	23.0
	3	28.4	25.1	28.3	29.5	25.5	24.8	25.9	20.4	25.0	11.7	14.4	15.9	22.9
	4	28.9	26.8	30.2	26.7	29.2	25.2	28.8	22.1	23.5	16.4	13.0	13.4	23.7
	5	27.3	23.6	27.7	27.1	24.4	21.6	26.0	23.2	20.1	8.2	12.4	7.1	20.7
	$\bar{X}$	28.1	25.9	29.0	27.6	27.3	25.4	26.5	23.0	22.5	11.8	13.9	10.8	
Richland	1	29.2	26.7	27.9	29.3	22.4	26.8	24.4	20.4	15.7	14.2	12.0	6.8	21.3
	2	30.5	26.6	30.1	24.2	25.4	24.5	26.0	20.3	18.2	16.3	15.1	10.8	22.3
	3	25.2	26.4	30.2	31.6	25.1	27.1	28.5	20.9	25.4	17.3	20.6	14.6	24.4
	4	22.9	24.0	31.3	26.5	25.0	26.8	27.4	21.3	18.7	15.9	14.8	11.0	22.1
	5	23.9	25.3	28.3	28.1	22.8	24.7	25.0	15.8	12.0	8.3	11.0	6.8	19.3
	$\bar{X}$	26.3	25.8	29.6	27.9	24.1	26.0	26.3	19.7	18.0	14.4	14.7	10.0	
Transpiration, $\text{g H}_2\text{O} \cdot \text{dm}^{-2} \cdot \text{hr}^{-1}$														
Burnett	1	3.16	3.56	4.12	3.78	4.54	5.11	4.63	5.20	4.75	2.93	5.55	2.93	4.19
	2	3.90	3.95	3.54	3.50	5.13	3.92	4.99	5.27	4.10	3.39	5.35	2.77	4.15
	3	3.56	3.50	3.63	3.51	5.20	3.98	4.60	4.90	5.38	3.42	4.57	3.67	4.16
	4	3.62	3.90	3.63	3.78	4.94	4.31	4.80	4.99	4.83	3.92	5.57	3.24	4.30
	5	4.07	3.53	3.80	3.92	5.26	3.78	4.76	4.77	4.42	3.62	3.98	2.98	4.08
	$\bar{X}$	3.66	3.69	3.74	3.70	5.02	4.22	4.76	5.03	4.70	3.45	5.01	3.12	
Richland	1	3.00	3.72	4.45	4.28	5.29	4.92	5.18	3.55	4.81	4.90	4.20	3.05	4.28
	2	3.32	3.83	4.34	4.36	5.22	5.15	4.81	4.14	4.51	5.30	5.01	3.66	4.47
	3	3.33	4.25	4.12	4.66	5.20	5.09	5.69	3.86	6.46	5.31	5.45	4.34	4.81
	4	2.47	3.53	3.73	5.37	5.16	5.22	5.13	4.36	4.99	4.52	4.22	3.40	4.34
	5	3.12	3.61	4.12	5.46	5.21	5.17	5.92	4.22	4.27	3.80	4.56	2.92	4.37
	$\bar{X}$	3.05	3.79	4.15	4.83	5.22	5.11	5.34	4.03	5.01	4.77	4.69	3.48	

Table 29. Specific leaf dry weights and sums of resistances ( $\Sigma r$ ) measured in the sink-source experiment

Treatment	Test dates												$\bar{X}$	
	1	2	3	4	5	6	7	8	9	10	11	12		
Specific leaf dry weight, $g \cdot dm^{-2}$														
Burnett	1	.390	.431	.429	.412	.439	.489	.458	.495	.506	.441	.489	.444	.452
	2	.398	.416	.425	.439	.472	.478	.478	.490	.477	.460	.464	.462	.455
	3	.429	.420	.451	.449	.466	.446	.468	.556	.546	.486	.522	.521	.480
	4	.394	.424	.489	.448	.505	.513	.486	.495	.453	.442	.476	.466	.466
	5	.451	.459	.489	.443	.473	.482	.476	.507	.483	.437	.466	.463	.469
	$\bar{X}$	.412	.430	.457	.438	.471	.482	.473	.509	.493	.453	.483	.471	
Richland	1	.356	.353	.387	.393	.392	.416	.452	.417	.433	.400	.395	.362	.396
	2	.414	.362	.391	.393	.434	.429	.436	.390	.446	.404	.397	.413	.409
	3	.390	.362	.405	.413	.424	.450	.453	.436	.478	.441	.455	.426	.428
	4	.361	.379	.408	.401	.447	.417	.434	.400	.428	.392	.415	.462	.412
	5	.393	.383	.405	.413	.432	.405	.462	.397	.384	.382	.405	.394	.405
	$\bar{X}$	.383	.368	.399	.403	.426	.423	.447	.408	.438	.404	.413	.411	
Sum of resistances, $sec \cdot cm^{-1}$														
Burnett	1	7.8	7.9	7.7	7.8	8.3	8.1	9.0	9.8	11.8	25.1	14.4	23.0	11.7
	2	8.6	8.9	7.8	8.8	7.6	8.3	8.7	8.7	9.3	16.6	16.5	28.9	11.6
	3	8.0	9.1	8.0	7.7	8.9	9.2	8.8	11.4	9.1	19.5	15.9	14.4	10.8
	4	7.9	8.5	7.6	8.6	7.8	9.0	7.9	10.3	9.7	13.9	17.5	17.0	10.5
	5	8.3	9.6	8.2	8.4	9.3	10.6	8.7	9.8	11.3	27.8	18.3	32.2	13.5
	$\bar{X}$	8.1	8.8	7.8	8.3	8.4	9.0	8.6	10.0	10.2	20.6	16.5	20.1	
Richland	1	7.8	8.5	8.2	7.8	10.2	8.5	9.4	11.1	14.5	16.0	19.0	33.7	12.9
	2	7.5	8.6	7.6	9.4	9.0	9.3	8.7	11.2	12.5	14.0	15.0	21.0	11.1
	3	9.0	8.6	7.5	7.2	9.1	8.4	7.9	10.9	9.0	13.2	11.0	15.6	9.8
	4	9.9	9.5	7.2	8.6	9.1	8.5	8.3	10.7	12.2	14.2	15.4	20.7	11.2
	5	9.5	9.0	8.1	8.1	10.0	9.2	9.1	14.4	19.0	27.5	20.5	33.5	14.8
	$\bar{X}$	8.7	8.8	7.7	8.2	9.5	8.8	8.7	11.7	13.4	17.0	16.2	24.9	

Table 30. Mesophyll ( $r_m$ ) and stomatal ( $r_s$ )  $CO_2$  diffusion resistances measured in the sink-source experiment.

Treatment	Test dates												$\bar{X}$	
	1	2	3	4	5	6	7	8	9	10	11	12		
Mesophyll resistance, $sec \cdot cm^{-1}$														
Burnett	1	4.7	5.0	5.2	5.4	6.3	6.2	6.9	7.8	9.1	20.1	12.3	18.1	8.9
	2	5.8	6.5	5.1	6.2	5.7	6.1	6.9	6.7	6.5	12.7	14.2	24.5	8.9
	3	5.4	6.2	5.5	5.1	6.9	6.7	6.9	8.9	7.0	15.7	13.1	11.2	8.2
	4	5.0	5.9	4.9	6.1	5.8	6.8	5.8	8.1	7.1	10.6	15.3	13.0	7.9
	5	5.8	6.8	5.7	6.1	7.5	7.9	6.7	7.5	9.0	24.3	14.9	27.6	10.8
	$\bar{X}$	5.3	6.1	5.3	5.8	6.4	6.7	6.6	7.8	7.7	16.7	14.0	18.9	
Richland	1	4.1	5.7	5.7	5.5	8.1	6.4	7.3	7.9	12.1	13.9	15.8	28.5	10.1
	2	3.6	5.8	5.3	7.2	6.8	7.3	6.7	8.4	9.7	11.7	12.6	17.5	8.6
	3	5.6	6.3	5.0	5.1	7.0	6.3	6.1	7.7	7.3	11.1	10.8	12.8	7.6
	4	5.3	6.5	4.6	6.7	7.0	6.6	6.4	8.2	10.1	11.8	12.6	16.5	8.5
	5	5.8	6.0	5.7	6.3	7.8	7.2	7.4	11.9	16.2	24.2	17.7	28.1	12.0
	$\bar{X}$	4.9	6.0	5.3	6.1	7.3	6.7	6.8	8.8	11.1	14.5	13.9	20.7	
Stomatal resistance, $sec \cdot cm^{-1}$														
Burnett	1	2.1	1.8	1.5	1.4	1.1	1.1	1.0	1.1	1.8	3.8	1.1	3.7	1.8
	2	1.7	1.5	1.7	1.6	1.0	1.3	.9	1.0	1.8	2.7	1.2	3.4	1.7
	3	1.6	1.8	1.6	1.6	1.1	1.6	1.0	1.3	1.3	2.7	1.7	2.1	1.6
	4	1.8	1.5	1.6	1.5	1.1	1.3	1.1	1.3	1.6	2.1	1.2	2.8	1.6
	5	1.5	1.7	1.5	1.4	.9	1.7	1.0	1.3	1.4	2.4	2.3	3.4	1.7
	$\bar{X}$	1.7	1.7	1.6	1.5	1.1	1.4	1.0	1.2	1.6	2.8	1.5	3.1	
Richland	1	2.8	1.9	1.5	1.4	1.2	1.2	1.1	2.3	1.5	1.3	2.2	4.1	1.9
	2	2.9	1.8	1.4	1.3	1.3	1.2	1.1	1.8	1.8	1.3	1.6	2.5	1.7
	3	2.4	1.5	1.6	1.2	1.2	1.2	.9	2.2	.8	1.2	1.2	1.9	1.4
	4	3.6	2.0	1.8	1.1	1.2	1.0	1.1	1.6	1.2	1.6	1.9	3.1	1.8
	5	2.7	1.9	1.5	1.0	1.3	1.2	.9	1.7	1.8	2.2	1.8	4.3	1.9
	$\bar{X}$	2.9	1.8	1.6	1.2	1.2	1.2	1.0	1.9	1.4	1.5	1.5	3.2	

Table 31. Laminar ( $r_a$ )  $CO_2$  diffusion resistances measured in the sink-source experiment

Treatment	Test dates												$\bar{X}$	
	1	2	3	4	5	6	7	8	9	10	11	12		
						Laminar resistance, $sec \cdot cm^{-1}$								
Burnett	1	.94	1.00	.98	.95	.93	.81	1.06	.93	.88	1.17	.91	1.18	.98
	2	1.13	.97	1.03	1.00	.90	.91	.93	.93	.95	1.12	1.05	1.10	1.00
	3	.98	1.09	.97	1.01	.95	.95	.88	.92	.81	1.14	1.00	1.03	.98
	4	1.08	1.03	1.02	.96	.94	.86	.98	.91	.96	1.07	1.00	1.08	.99
	5	1.03	1.04	.97	.94	.86	.97	.97	.92	.95	1.13	1.11	1.16	1.00
	$\bar{X}$	1.03	1.03	.99	.97	.91	.90	.96	.92	.91	1.13	1.01	1.11	
Richland	1	.95	.96	.93	.92	.91	.91	.94	.95	.90	.89	.95	1.05	.94
	2	.98	1.05	.88	.90	.88	.82	.88	.93	.95	.88	.90	.95	.92
	3	1.04	.90	.93	.84	.86	.89	.93	1.00	.81	.89	.91	.93	.84
	4	1.02	1.02	.91	.96	.90	.89	.85	.95	.90	.92	.92	1.00	.94
	5	1.01	1.02	.85	.83	.87	.85	.80	.88	.96	1.06	.99	1.09	.94
	$\bar{X}$	1.00	.99	.90	.89	.88	.87	.88	.94	.90	.92	.97	1.00	

Table 32. Percent free sugars measured in various plant parts in the sink-source experiment

Treatment		Test dates, Burnett							Test dates, Richland						
		1-2	3-4	5-6	7-8	9-10	11-12	X	1-2	3-4	5-6	7-8	9-10	11-12	X
Main culms	1	6.9	7.7	2.5	.9	.4	.2	3.1	6.5	3.7	1.4	.3	.0	.1	2.0
	2	8.4	6.5	2.5	1.3	.2	.2	3.2	5.9	2.2	.7	.1	.1	.0	1.5
	3	8.6	7.4	1.6	1.2	.4	.2	3.2	7.1	4.3	1.0	.1	.0	.1	2.1
	4	8.6	4.6	1.9	.3	.5	.2	2.7	6.0	5.4	.6	.0	.4	.0	2.1
	5	3.9	3.3	1.9	1.4	.6	.3	1.9	5.5	5.4	.8	.5	.4	.3	2.2
	X	7.3	5.9	2.1	1.0	.4	.2		6.2	4.2	.9	.2	.2	.1	
Flag leaves	1	4.1	2.1	1.1	1.0	.8	1.2	1.7	1.8	1.2	.6	.0	.3	.0	.6
	2	3.5	2.2	1.1	1.1	.8	2.0	1.8	2.1	1.0	.3	.0	.2	.0	.6
	3	3.7	2.7	1.2	2.3	.7	1.5	2.0	2.5	1.1	.6	.0	.1	.0	.7
	4	3.0	2.2	2.0	.7	.9	1.5	1.7	3.3	1.3	.4	.0	.1	.1	.9
	5	2.6	2.4	.7	.6	.9	.9	1.4	1.8	.2	.0	.0	.0	.0	.2
	X	3.4	2.3	1.2	1.1	.8	1.0		2.3	1.0	.4	.0	.1	.0	
Stem base	1	2.6	1.9	1.6	.0	.1	.0	1.0	1.8	1.3	.6	.1	.0	.0	.6
	2	2.8	1.3	.7	.0	.0	.0	.8	1.6	1.4	.4	.2	.0	.0	.6
	3	2.2	1.3	.5	.2	.0	.0	.7	2.8	1.1	.1	.3	.0	.0	.7
	4	1.4	1.3	.0	.0	.0	.0	.4	1.7	1.4	.2	.4	.0	.0	.6
	5	1.1	.9	.7	.0	.0	.0	.5	2.3	1.5	.7	.2	.0	.0	.7
	X	2.0	1.3	.7	.0	.0	.0		2.0	1.3	.4	.2	.0	.0	
Peduncle	1	20.8	17.4	7.2	2.3	1.5	.6	8.3	10.5	8.8	1.8	.2	.2	.0	3.6
	2	18.3	15.7	4.8	3.9	1.1	1.9	7.6	10.2	8.3	2.2	.7	.2	.0	3.6
	3	21.7	15.8	4.3	4.4	1.7	2.3	8.4	9.9	9.4	1.7	.5	.1	.0	3.6
	4	20.3	16.1	2.3	.7	.8	.7	6.8	11.9	10.9	.4	.0	.0	.1	3.9
	5	18.1	14.4	5.9	2.7	1.7	.6	7.2	8.1	11.8	2.8	.1	.0	.1	3.8
	X	19.8	15.9	4.9	2.8	1.4	1.2		10.1	9.8	1.8	.3	.1	.0	

Table 33. Percent free sugars plus hydrolysed carbohydrates measured in various plant parts in the sink-source experiment

Treatment		Test dates, Burnett							Test dates, Richland						
		1-2	3-4	5-6	7-8	9-10	11-12	$\bar{X}$	1-2	3-4	5-6	7-8	9-10	11-12	$\bar{X}$
Main culms	1	15.9	16.7	6.7	5.0	3.7	3.3	8.6	15.2	8.2	3.8	4.0	2.2	1.3	5.8
	2	19.3	15.2	5.8	5.5	1.4	3.5	8.5	15.3	7.7	3.7	4.2	1.8	2.6	5.9
	3	19.7	14.8	5.8	7.1	2.6	3.8	9.0	15.8	6.7	5.9	3.3	3.5	2.7	6.3
	4	19.5	18.2	6.4	.6	3.4	.8	8.2	12.4	9.9	1.8	.8	1.4	1.2	4.6
	5	18.6	14.8	17.8	12.0	11.1	2.6	12.8	12.6	11.0	7.2	2.6	1.8	1.4	6.1
	$\bar{X}$	18.6	15.9	8.5	6.0	4.4	2.8		14.3	8.7	4.5	3.0	2.1	1.8	
Flag leaves	1	15.6	16.0	11.6	14.5	12.1	13.1	13.8	4.8	8.6	7.9	10.4	8.6	7.6	8.0
	2	16.5	17.0	10.3	14.4	10.3	15.5	14.0	7.3	7.7	8.6	7.2	10.5	9.5	8.5
	3	19.1	17.5	10.3	16.3	11.5	15.1	15.0	7.2	8.2	8.0	6.4	11.3	9.0	8.4
	4	15.7	19.2	17.3	8.3	12.4	11.2	14.0	10.3	9.4	9.8	6.8	9.8	8.9	9.2
	5	19.1	18.0	13.3	9.0	10.6	11.7	13.6	7.2	7.3	7.8	5.4	7.6	6.2	6.9
	$\bar{X}$	17.2	17.5	12.6	12.5	11.4	13.3		7.3	8.2	8.4	7.2	9.6	8.2	
Stem base	1	11.3	14.0	9.9	5.1	4.4	2.2	7.8	10.2	7.2	3.9	3.7	1.4	.9	4.6
	2	14.7	13.0	8.5	4.1	1.4	3.8	7.6	10.3	6.5	4.3	4.7	1.6	1.3	4.8
	3	14.3	11.2	7.2	9.3	3.9	2.9	8.1	10.3	5.3	6.2	3.3	2.6	1.0	4.8
	4	11.5	14.0	5.4	3.6	2.6	.0	6.2	9.4	7.8	1.0	.8	1.0	.2	3.4
	5	13.2	12.7	14.0	7.0	8.0	2.4	9.6	10.1	9.5	5.9	2.0	1.2	.6	4.9
	$\bar{X}$	13.0	13.0	9.0	5.8	4.1	2.3		10.1	7.3	4.3	2.9	1.6	.8	
Peduncle	1	27.8	22.6	7.6	4.4	4.4	3.3	11.7	16.4	14.4	2.2	2.5	2.0	1.2	6.4
	2	27.0	21.1	5.7	5.8	4.6	5.0	11.5	15.8	12.6	2.8	2.9	2.0	1.6	6.3
	3	27.2	20.7	4.3	7.0	7.0	6.6	12.1	14.7	11.1	3.9	3.3	3.8	1.6	6.4
	4	26.7	22.8	2.4	1.4	3.7	3.1	10.0	15.6	13.7	1.0	.8	1.4	1.6	5.7
	5	28.6	23.8	10.0	5.7	7.5	3.5	13.2	13.8	14.8	5.6	1.4	1.2	1.2	6.3
	$\bar{X}$	27.4	22.2	6.0	4.9	5.4	4.3		15.3	13.3	3.1	2.2	2.1	1.4	

Table 34. Analyses of variance results obtained for free sugars and free sugars + hydrolyzed sugars in various plant parts in the sink-source experiment

Source	df	Mean squares	F ratio
Free sugars, main culms			
Burnett			
Dates	5	45.6855	29.175**
Treatments	4	1.8695	1.194
Dates X Treatments-in-late-dates	12	.1003	.064
(Early vs. late dates) X Treatments	4	4.2894	2.745
Error	4	1.5659	
Richland			
Dates	5	33.8443	27.798**
Treatments	4	.4203	.345
Dates X Treatments-in-late-dates	12	.0455	.037
(Early vs. late dates) X Treatments	4	.5876	.483
Error	4	1.2175	
Free sugars, flag leaves			
Burnett			
Dates	5	4.6019	21.013**
Treatments	4	.3433	1.568
Dates X Treatments-in-late-dates	12	.2022	.923
(Early vs. late dates) X Treatments	4	.0992	.453
Error	4	.2190	
Richland			
Dates	5	3.9693	28.577**
Treatments	4	.2292	1.650
Dates X Treatments-in-late-dates	12	.0163	.117
(Early vs. late dates) X Treatments	4	.2481	1.786
Error	4	.1389	
Free sugars, stem bases			
Burnett			
Dates	5	3.4397	28.929**
Treatments	4	.4205	3.537
Dates X Treatments-in-late-dates	12	.0843	.709
(Early vs. late dates) X Treatments	4	.2725	2.292
Error	4	.1189	
Richland			
Dates	5	3.5360	19.375**
Treatments	4	.0472	.259
Dates X Treatments-in-late-dates	12	.0193	.106
(Early vs. late dates) X Treatments	4	.0606	.332
Error	4	.1825	

\*\*F value exceeds 1% level of significance.

Table 34. (Continued)

Source	df	Mean squares	F ratio
Free sugars, peduncles			
Burnett			
Dates	5	328.3211	434.090**
Treatments	4	2.7058	3.577
Dates X Treatments-in-late-dates	12	1.1923	.709
(Early vs. late dates) X Treatments	4	2.8938	2.292
Error	4	.7565	
Richland			
Dates	5	120.5177	45.809**
Treatments	4	.1212	.046
Dates X Treatments-in-late-dates	12	.2028	.077
(Early vs. late dates) X Treatments	4	1.5347	.583
Error	4	2.6309	
Free sugars plus hydrolyzed carbohydrates, main culms			
Burnett			
Dates	5	207.6715	74.796
Treatments	4	22.5720	8.130*
Dates X Treatments-in-late-dates	12	6.9255	2.494
(Early vs. late dates) X Treatments	4	18.7126	6.740*
Error	4	2.7765	
Richland			
Dates	5	118.7268	21.679**
Treatments	4	2.7333	.499
Dates X Treatments-in-late-dates	12	1.2989	.237
(Early vs. late dates) X Treatments	4	1.0365	.208
Error	4	5.4765	
Free sugars plus hydrolyzed carbohydrates, flag leaves			
Burnett			
Dates	5	34.3803	17.351**
Treatments	4	1.6208	.818
Dates X Treatments-in-late-dates	12	7.8421	3.958
(Early vs. late dates) X Treatments	4	3.5414	1.787
Error	4	1.9815	
Richland			
Dates	5	3.5918	2.090
Treatments	4	4.0995	2.386
Dates X Treatments-in-late-dates	12	1.6367	.952
(Early vs. late dates) X Treatments	4	2.2478	1.308
Error	4	1.7185	

Table 34. (Continued)

Source	df	Mean squares	F ratio
Free sugars plus hydrolyzed carbohydrates, stem bases			
Burnett			
Dates	5	103.8948	36.618**
Treatments	4	8.7303	2.657
Dates X Treatments-in-late-dates	12	3.6562	1.113
(Early vs. late dates) X Treatments	4	5.0252	1.529
Error	4	3.2859	
Richland			
Dates	5	63.4780	41.762**
Treatments	4	2.3863	1.570
Dates X Treatments-in-late-dates	12	1.1193	.736
(Early vs. late dates) X Treatments	4	2.4927	1.640
Error	4	1.5200	
Free sugars plus hydrolyzed carbohydrates, peduncles			
Burnett			
Dates	5	531.8495	1,060.729**
Treatments	4	7.8738	15.704*
Dates X Treatments-in-late-dates	12	2.8345	5.653
(Early vs. late dates) X Treatments	4	3.6237	7.227*
Error	4	.5014	
Richland			
Dates	5	198.3371	122.136**
Treatments	4	.5738	.353
Dates X Treatments-in-late-dates	12	2.0029	1.233
(Early vs. late dates) X Treatments	4	3.0783	1.901
Error	4	1.6239	