

THE USE OF STOMATAL RESISTANCE, PHOTOPIGMENTS, NITROGEN,
WATER POTENTIAL, AND RADIATION TO ESTIMATE NET
PHOTOSYNTHESIS IN LIRIODENDRON TULIPIFERA L.
--A PHYSIOLOGICAL INDEX

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ABSTRACT

A physiological index is proposed as an indirect technique of estimating net CO₂ exchange. Multiple linear regression analysis is employed to estimate coefficients in a linear model relating physiological plant variables, site parameters, and dependent responses for tulip poplar (Liriodendron tulipifera L.) seedlings.

Objectives were to (1) ascertain which plant and environmental parameters were the most useful in predicting net photosynthesis under varied conditions, (2) determine the amplitudes of physiological processes (photorespiration, diurnal water flux, seasonal pigment development, stomatal resistance, leaf water potential and net CO₂ exchange) which characterize tulip poplar, and might prove useful in a predictive index, (3) investigate relationships between photopigment ratios and nutrient status, and (4) define empirical relationships between CO₂ uptake and physiological plant parameters and environmental variables.

Seasonal ranges of foliar pigments, nutrient content, radiation and precipitation were also measured in the Liriodendron forest to evaluate relationships and techniques that may prove useful in the development of predictive physiological indices for annual forest net photosynthesis.

Net photosynthetic rates were predicted from knowledge of several plant and environmental parameters: leaf chlorophyll (a+b) content, pigment absorbance ratio 460/663, percent leaf nitrogen, leaf water potential, soil water potential, radiation levels, and stomatal resistance. Highest correlation was obtained for sun leaves from a single time period when 99 percent of the variation in net photosynthesis was accounted for by these variables. In all the relationships tested, some indication of plant-water status was necessary to obtain accurate predictions. The removal of leaf and soil water potential reduced the R^2 to 19 percent. Inclusion of either of these increased the R^2 to 46 percent. The variation attributable to radiation alone for sun and shade leaves under no water stress (leaf water potential < -20 bars) was 20 percent. By separating sun and shade leaves, radiation accounted for 36 percent of the variation. A single regression was generated which accounted for 61 percent of the variation in net CO_2 exchange when data were utilized from 430 points covering the widest range of fluctuating water levels, shade environments and radiation intensities throughout the growing season. The most important plant and environmental factors for estimating net CO_2 exchange were (1) stomatal resistance, (2) radiation, (3) pigment absorbance ratio, and (4) chlorophyll content.

Empirical relations utilizing stomatal resistance,

chlorophyll content, absorbance ratio, and radiation were developed which accurately estimate photosynthetic capacity under a variety of conditions.

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I. INTRODUCTION

General Statement of the Problem

The quality and quantity of life in the biosphere are limited initially by primary production and in reality through available net productivity. Westlake (1965) defined primary production as "the weight of new organic material created by photosynthesis or the energy which this represents, and net productivity as the rate of accumulation of new organic matter, or stored energy." However, rapid expansion in the human population, increased use of inorganic fertilizers in forest management and agricultural practices, and higher levels of atmospheric pollutants have made production rates of vital interest, and have also made it of utmost importance that our lands be properly classified and utilized to maximize productivity.

Numerous direct and indirect methods have been developed to estimate productivity and to predict future production in changing or different environments. The most straightforward and accurate "direct method" of measuring production is the harvest technique, which was pioneered by Pearsall and Gorham (1956), Ovington (1957), Bray, Lawrence and Pearson (1959) and Odum (1960).

While such harvest methods are useful and accurate

over months or years, they are insensitive to rapid environmental fluctuations in time and space. Most direct methods also are generally destructive of the vegetation, and introduce irreversible alterations to the edaphic and meteorological environment. Numerous problems are also encountered when applying harvest methods to diverse vegetation types (many-layered forest) (Lieth, 1965).

Indirect techniques have been developed which do not alter the environment and which provide information on the effects of short-term and rapid environmental changes. They include gaseous exchange (cuvette methods and aerodynamic CO_2 flux), growth analysis, photopigment measurements and soil respiration flux. The methodology of these techniques has been discussed by Lieth (1965), Eckardt (1968), Newbould (1967), Larcher (1969a, 1969b) and Séstak, Catsky and Jarvis (1971). One of the more promising indirect techniques for investigating physiological processes in terrestrial ecosystems is the measurement of CO_2 exchange and subsequent conversion values of CO_2 fixed to carbohydrate production (Botkin, et al., 1970; Dinger, 1971).

In the past most photosynthesis information has been expressed descriptively as net CO_2 flux due to changes in environmental variables or more simply as diurnal fluctuations. Unfortunately, this kind of information provides little insight into the structure and

function of ecosystems and more importantly, does not allow prediction of net photosynthetic rates. Current approaches have centered on predicting net photosynthetic carbon fluxes through models validated on limited field measurements (Duncan, et al., 1967; Idso and Baker, 1968; Waggoner, 1969; Goldstein and Mankin, 1971; Sollins, 1971), or by direct monitoring of CO₂ exchange rates and concomitant environmental factors (Botkin, 1969; Helms, 1972). In the latter approach some form of regression analysis has been used to account for most of the statistical variation.

Development of the Problem

When looking at any indirect technique, two questions should be considered. First, is the plant response, e.g. net photosynthesis, the result of acclimation or adaptation? Physiological and morphological acclimations and/or adaptations may occur in conjunction with varying abiotic factors. Acclimations are environmentally induced somatic alterations and, as such, are not hereditary, while adaptations are genetically determined variations and are transferable (Prosser, 1967). The plant response in the first case is indicative only of that specific organism and environment, while plant responses in the latter case may be used in comparison of vegetational stands and in ecosystem analysis. Ecologists often study large-scale acclimations resulting from man's perturbations

(e.g. forest stand alterations, broadcasting fertilizers and pesticides and pollution effects). This information, although useful in the resolution of immediate problems, only gains full value when a knowledgeable comparison with the full genetic potential of the organism is realized.

Secondly, does the technique involved adequately estimate the physiological responses to plant-environment relationships? This is a main problem of most indirect approaches. Environmental parameters are often difficult to measure, overlooked, misinterpreted and/or wrongly weighted. When measured singly, environmental parameters give an imprecise estimate of the plant community production potential, and contribute little or no information concerning physiological rates, genetic variations or plant-environmental interactions. This is evident in failures to attain consistently high correlations between net photosynthesis values predicted solely on environmental parameters (Botkin, 1969; Helms, 1972).

The fact that plants integrate bits of information over long time intervals (Margalef, 1958, 1968) from the environment in which they live has proved useful in both harvest and dendrochronological studies. Little of this information has been utilized on a short-term basis, although both physiologists and ecologists have demonstrated CO_2 fluxes to be the result of diurnal, weekly and seasonal physiological changes (e.g., leaf water potential, stomatal

resistance, mineral content and chlorophyll content). While it is possible that too much "noise" in the system may lessen predictability of net photosynthesis solely from physiological parameters, and that physiological information is species specific, a cognizance of the holocoenotic approach -- "that the plant environment system is a dynamic unit in itself and reacts as a whole" (Billings, 1952; Idso, 1968) -- should place these factors in their proper perspective. Subsequent to the analyses of plant and environmental data, the physiological dynamics of the ecosystem can be dealt with quantitatively. This integrated approach of measuring physiological plant responses to plant-environmental interactions, which is referred to as a "physiological index," could establish present and potential CO₂ fixation rates as well as give some information concerning site influences on net photosynthesis.

The screening of physiological responses to plant and environmental relationships for high predictive value under a wide variety of conditions is a major problem. To be of real use the empirical relationships should be general in nature, should incorporate easily measurable variables, should require only a few forms and should estimate CO₂ exchange rates with reasonable accuracy.

In order to convert inorganic constituents to organic matter, the milieu of a plant must contain higher than threshold levels of radiant energy, CO₂, O₂, water,

nutrients and temperature. These abiotic factors along with such biotic properties as surface area at which energy reception and gaseous exchange occur, pigment diversity and concentration (chlorophylls a and b and carotenoids) for energy conversion and a transport system for uptake and dispersion of minerals, water and photosynthate comprise the plant-environment complex.

A skeletal framework of the important physiological responses to plant and environmental interactions is presented in Figure 1. Five primary factors that directly affect net photosynthesis, respiration and in turn net production are indicated in the five smaller boxes. The effects of these factors upon net photosynthesis can be measured experimentally and presented in the form of correlations (Idso, 1968). Wind speed, air temperature, relative humidity and vapor pressure deficit, shown in circles, are considered secondary factors that influence net photosynthesis through one or more of the five primary factors. Other parameters (pH, soil moisture, pollutants, et cetera) which directly or indirectly influence the secondary factors can also be placed in this hierarchy.

The composition of a physiological index is simplistic in principle. Plant responses to environmental fluctuations can be utilized in multiple regression analysis as independent variables to estimate changes in

Figure 1. The plant-environmental interrelationships which comprise the physiological index. The 5 smaller boxes indicate primary factors (modified from Idso, 1968). Primary correlations and secondary relationships are shown by the solid heavy arrows and dashed lines, respectively. Air and leaf temperature are considered environmental parameters of equal predictive value since both are influenced by radiation and are assumed to be equal inside a photosynthesis cuvette. Physiological plant parameters which influence net photosynthesis are shown in the outer circle. Photorespiration and dark respiration are not included in the diagram, but estimates of both are essential in obtaining estimates of gross photosynthesis.

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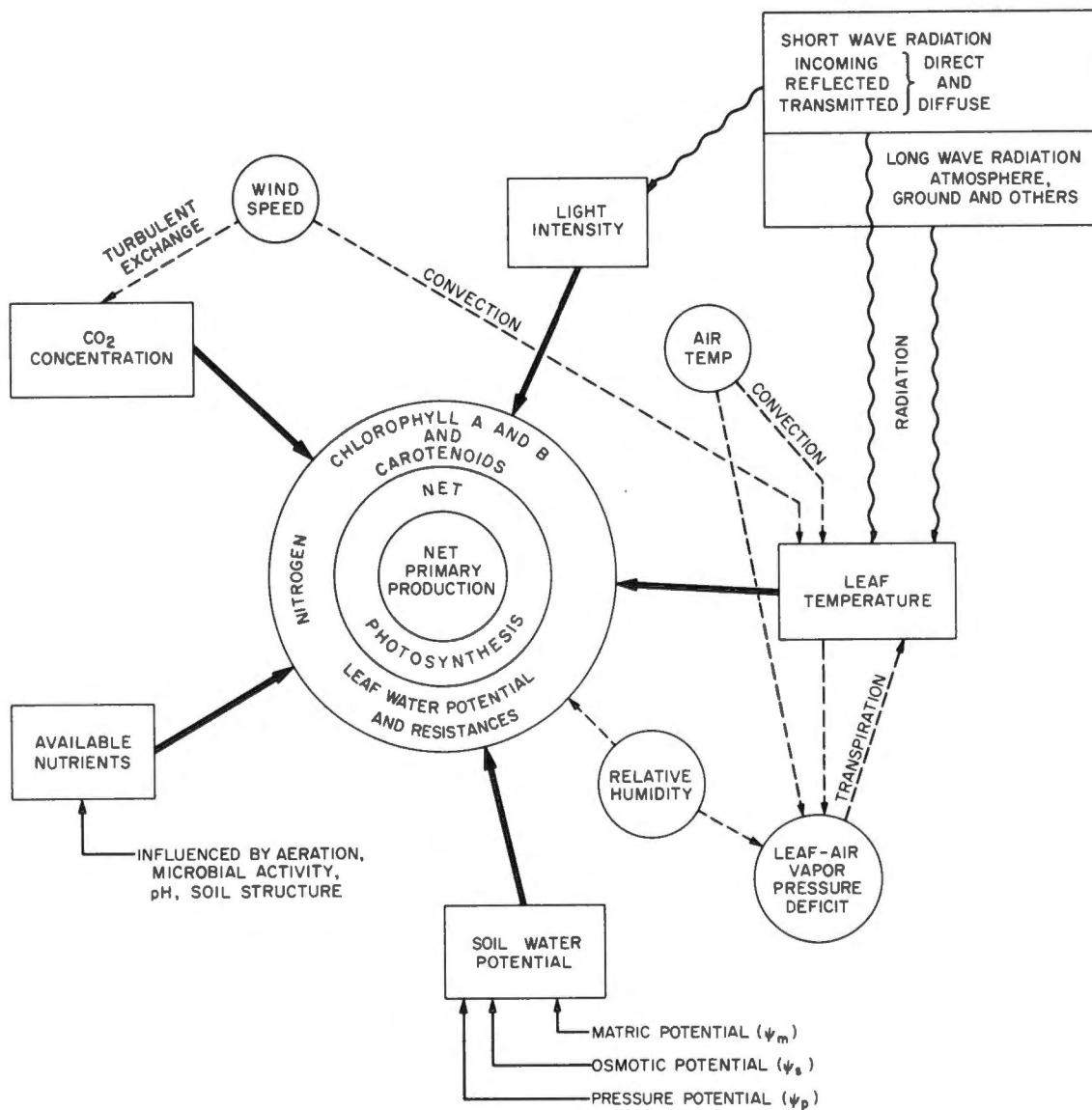


Figure 1

net photosynthetic or potential productivity. Thus, changes in such environmental site variables as soil water content, solar radiation, soil nutrient status, temperature, pollution effects, et cetera, which are reflected by changes in key physiological parameters such as pigment content and ratios, leaf water potential and leaf resistance are utilized to estimate concomitant CO₂ exchange and/or relative growth rates. The physiological rates, parameters and variations thereof are the direct result of site potential and/or modifications of it.

To be useful, a physiological plant parameter must reflect either rapid or substantial changes in one or more of the primary factors, and in turn must provide some reasonable estimate of resulting photosynthetic response. There are probably useful interactions and parameters which are omitted in this study and perhaps are yet to be described. In view of the relative crudeness of present techniques for environmental measurements in comparison to ecosystem complexity, empirically determined physiological responses to principal plant-environmental interactions seem to offer the most promising avenues for achieving predictability of net photosynthesis.

A Review and Theory of the Relationships between Plant Parameters, Environmental Factors, and Net Photosynthesis

An understanding of net production via CO_2 exchange analyses requires a knowledge of photosynthetic systems. Mechanisms of photosynthesis and photorespiration must be incorporated and utilized in any attempt at estimating production rates, since it has been shown that gross photosynthesis cannot be accurately estimated by merely assuming that dark and light respiration rates are equal (Decker, 1957; Rabinowitch and Govindjee, 1969; Hatch and Slack, 1970; Jackson and Volk, 1970). The accumulation and allocation of energy for growth, maintenance, reproduction and defense are inherent in each species and should be carefully considered when comparisons of primary production rates are made among species. Innate in these processes are a loss of biomass through respiration and a consequent differential accumulation of energy. For instance, one g of CO_2 may be fixed into 0.6 g of starch, 0.4 g of fat or 0.5 g of protein with a respective energy yield of $2.6 \text{ kcal g}^{-1} \text{ CO}_2$, $3.6 \text{ kcal g}^{-1} \text{ CO}_2$ and $3.0 \text{ kcal g}^{-1} \text{ CO}_2$ (Séstak, 1971).

Photosynthetic rates and in turn production are controlled by a number of external and internal factors. The internal factors (age, pigment content, enzymes, leaf water content, leaf morphology and leaf resistances) are often modified, either temporarily or on a long-term basis,

by a change in external factors (e.g., CO₂ concentrations, light quality and quantity, temperature, soil water content, nutrient supply and wind velocity). Since Blackman's (1905) early work on optima and limiting factors, there has been a myriad of photosynthesis studies (Rabinowitch, 1945, 1951, 1956; Heath, 1968; Rabinowitch and Govindjee, 1969). Most of these papers, however, have neither been concerned with CO₂ fixation under natural conditions nor with estimation or prediction of production rates.

Prediction of net photosynthesis in forest trees from measurement of light intensity and temperature has been made by Botkin (1969). By using these variables in regression analysis, prediction of daily CO₂ uptake to within a factor of two of observed values was possible. Helms (1972) studied net photosynthetic rates of Pinus ponderosa Laws under different conditions of air temperature, radiation, CO₂ concentration, humidity and saturation vapor pressure deficit (SVPD). He reported agreements of 80-90 percent between observed and predicted values under low stress conditions, but as stress increased, the accuracy of predicting daily CO₂ exchange from changes in radiation and temperature levels decreased to a R² of 45 percent. Improved correlations under stress conditions were attained by inclusion of saturation vapor pressure deficit in the regression analysis. Although important

steps, both of these studies met with limited success because of a lack of detailed physiological information concerning the plant species involved.

Including both physiological and environmental parameters should improve regression predictions of net CO₂ exchange rates on a daily or seasonal basis, and under stress or disturbed conditions. The selection of easily measured physiological and environmental parameters which explain most of the variation under heterogeneous conditions can be made in part from past physiological and ecological experiments. Some parameters will prove more useful than others, depending on the question being asked (i.e., seasonal trends, diurnal cycle and/or stress relationships).

One obvious parameter to consider is water, since every plant process is affected directly or indirectly by water content. The advent of pressure bomb (Scholander, et al., 1964, 1965; Waring and Cleary, 1967) and thermocouple psychrometry (Spanner, 1951; Boyer and Knipling, 1965; Barrs, 1968) techniques has made the determination of water potential¹ relatively easy. Representative studies by Wiebe, et al., (1970) and Waggoner and Turner

¹Water potential is defined as the difference in free energy or chemical potential between pure water and water in the system (i.e., leaf, soil). The potential of pure water is zero. Therefore, leaf and soil water potential is less than zero (negative) and is indicated by more negative values in bar units (Slayer, 1967; Kramer, 1969).

(1971) have defined the characteristic diurnal pattern of leaf water potentials. Values generally range from a high of -3 to -9 bars during the night to daytime values of -20 to -40 bars, depending primarily on soil water availability. Leaf or tissue water potential, presently the best single indicator of internal water balance, is highly correlated with diurnal changes in net photosynthesis (Hodge, 1967). He found statistically significant correlation coefficients of 0.96, 0.95, 0.90 and 0.95, respectively, in Douglas-fir (Pseudotsuga menziesii Mirb.), grand fir (Abies grandis Lindley), hemlock (Tsuga heterophylla Rafinesque) and sitka spruce (Picea sitchensis Bongard). Leaf water potentials also influence photosynthesis patterns by controlling stomatal and/or mesophyll resistances to CO₂ diffusion (Rawlins, 1963), and should prove to be a useful plant factor in explaining variations in net photosynthetic patterns.

Early use of pigment analysis (chlorophyll content) as an indicator of photosynthesis (Willstatter and Stoll, 1913, 1918; Emerson, 1929; Fleischer, 1935) was unproductive since data indicated that chlorophyll content was not the principal limiting factor controlling photosynthesis at high light and CO₂ concentrations. Gabrielsen (1948) found chlorophyll concentrations in 17 "leaf categories" to vary between 0.02 and 0.08 mg of chlorophyll (a+b) cm⁻² but the percentage of incident radiation fixed as chemical energy

reached an asymptote once the chlorophylls approached 0.04 to 0.05 mg (a+b) cm⁻². Most investigators have found chlorophyll contents to range from 0.02 to 0.10 mg chlorophyll (a+b) cm⁻². Bray (1960a, 1960b) compared chlorophyll content from crop and field communities in Minnesota. His estimates range from 0.01 mg cm⁻² in field weed crops and 0.02 mg cm⁻² in old field grasslands to 0.24 mg cm⁻² in an upland Quercus forest and 0.29 mg cm⁻² in a Typha marsh. Values in a Zea mays crop and Soja max were 0.16 and 0.09 mg cm⁻², respectively. These values can probably be considered typical of communities in the same latitude.

Generally any leaf under non-limiting conditions has more than enough chlorophyll for photon absorption. Consequently, pigment characteristics have not been utilized much for estimating net photosynthesis on a seasonal and/or structural and functional basis in the terrestrial ecosystem. The exception has been in the case of growth analyses, where the amount of chlorophyll represents a measure of the size of the assimilatory apparatus and can be correlated with the rate of dry matter production and with other growth characteristics (Kvet, et al., 1971). In most studies, finding a useful relationship between chlorophyll content and net photosynthesis activity has proved unsuccessful due in part to the techniques involved and/or the sole reliance on chlorophyll information

to explain photosynthetic variation, when in fact other plant or environmental parameters may have been limiting.

Low correlation between chlorophyll content and photosynthesis may be due to the irradiation levels utilized during growth and photosynthesis measurements (Séstak, 1966). He reported a decrease of unit chlorophyll activity as a result of changes in density and length of irradiance, and that high irradiance during the measurement of photosynthesis ensured finding a correlation of photosynthetic rate with chlorophyll content per unit leaf area. Séstak noted that the lower layers of chloroplasts were not light-saturated at lower irradiances or in thick leaves and that a higher correlation coefficient was found by relating photosynthetic rate to the percent of chlorophyll in dry weight rather than to the quantity of chlorophyll per unit leaf area.

Recent discoveries in photochemistry have brought new hope for relating production of photosynthate to chlorophyll content. A relationship between the number of photosynthetic units (quantasomes) and the number of chlorophyll molecules has been reported (Calvin and Bassham, 1962; Park and Biggins, 1964). With this information it is possible to relate potential photosynthetic rates to available photon flux, if the chlorophyll content is known. This information was largely ignored in ecology until Anderson (1967) related photon flux and chlorophyll

content in terms of available "photosynthetic machinery." Anderson calculated the total short wave energy (0.3 - 3.0 μm) sufficient to saturate the reactive centers and plotted this against the percentage of absorption (400 - 700 nm) for different chlorophyll contents (Figure 2). Sun leaves with typically more than 0.04 mg chlorophyll cm^2 will seldom receive enough photons to saturate all their reactive centers at once (Anderson, 1967). From Figure 2 and representative chlorophyll values, it appears that most plant communities contain sufficient quantities of chlorophyll per unit ground area to absorb naturally occurring photon flux levels. Tieszen and Johnson (1968) also suggest that chlorophyll per unit area increases with height and structural diversity, but is independent of species diversity.

It is now apparent that chlorophyll content takes on structural and functional meaning in the community only if considered in relation to photon flux. Chlorophyll content as an indicator of potential photosynthetic rates in a natural community or one time period is limited. Its real predictive value is manifest in disturbed communities (i.e., fertilization and pollution) which concomitantly alter their photosynthetic machinery.

Another form of pigment information that has largely been ignored in terrestrial ecosystems is the pigment ratio. Margalef (1962, 1968) successfully utilized a

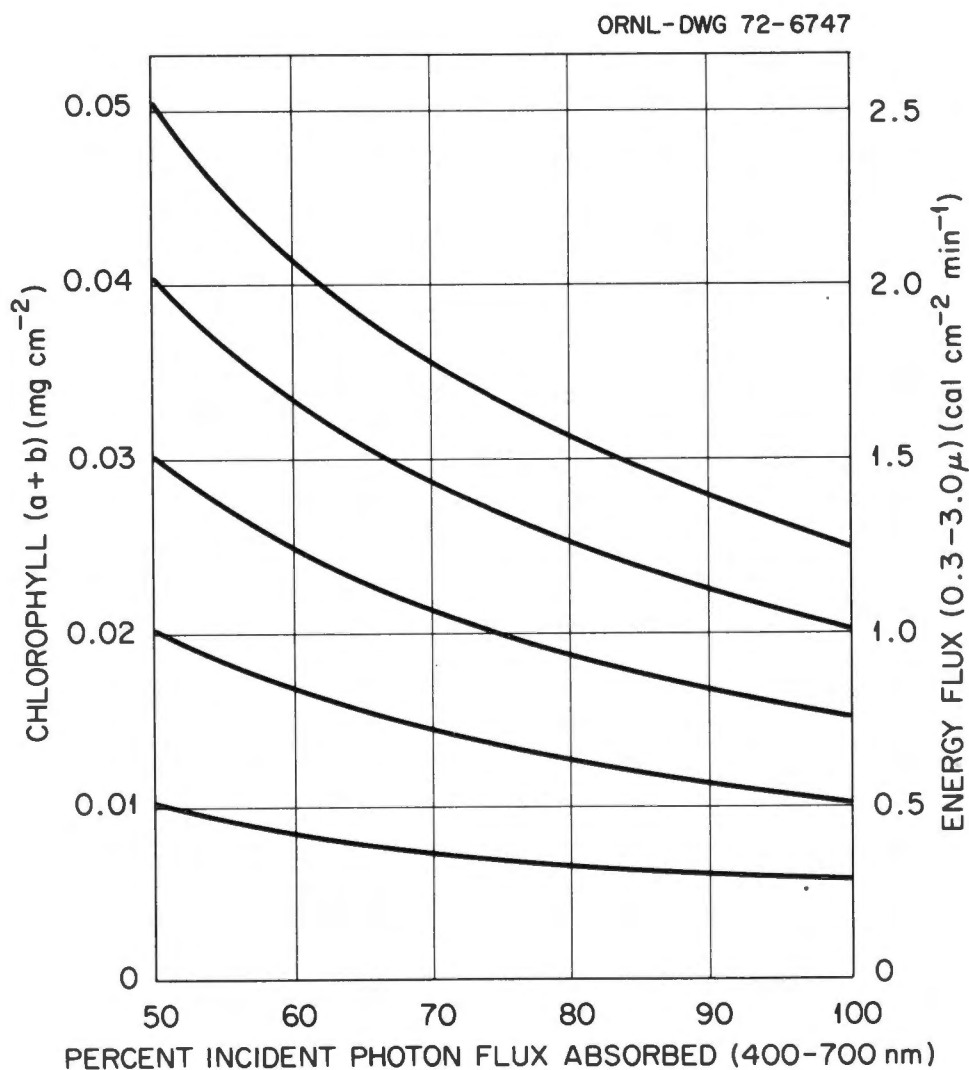


Figure 2. The total short-wave energy (0.3 - 3.0 μ m) sufficient to saturate a given amount of leaf chlorophyll (a + b) content as a function of the incident photon flux absorbed in the 400 - 700 nm waveband. (Adapted from Anderson, 1967.)

carotenoid-chlorophyll ratio (optical density readings of acetone pigment extractions, 430/665) in diversity, biomass and nutrient studies of marine ecosystems. Skeen (1969) has shown Margalef's ratio to be a poor indicator of community diversity in several terrestrial ecosystems in Georgia. Although seasonal trends in various pigment ratios were indicated, no attempt was made to relate them to nutrient status or net production. It is well known that both nutrient content and pigment ratios fluctuate throughout the season (Rodin and Bazilevich, 1967; Sanger, 1968; Rabinowitch and Govindjee, 1969). If consistent relationships could be established, then "chemical allometry" might be used to estimate nutrient status, relative leaf age and/or photosynthetic potential as a function of certain pigment ratios.

Foliar mineral content also has some predictive potential for estimating net photosynthesis. The influences of fertilizers on dry matter production are well known. Until recently, however, the dependence of gaseous exchange on mineral status in forest tree species had received minimal attention. It is evident that minimum nutrient levels in proper ratio must be maintained in order to attain adequate net photosynthetic rates. Keller (1967) showed that photosynthesis as well as transpiration rates in poplar were influenced by fertilization. He also stated that a proper balance of nutrient supply improved gaseous

exchange and water use, whereas excessive fertilization may cause physiological imbalances that result in decreased production or excessive water use or both. Even with balanced fertilization, however, total foliage, pigment content and water consumption increase.

The nutrient that seems the most feasible for predictive purposes is nitrogen. Nitrogen deficiency has the most pronounced depressing effect on photosynthesis and enzyme activity (Keller, 1967; Zelitch, 1971). Nitrogen also exerts a dominant role in chlorophyll biosynthesis and leaf size. Recent studies by Keller (1967), Murata (1969), Wada (1969) and Brix (1971) have shown a positive correlation between leaf nitrogen content on a dry weight basis and rates of CO_2 assimilation further suggesting the usefulness of nitrogen as an indicator of photosynthetic rates.

Of the environmental factors that have been used to predict CO_2 exchange rates in forest species, solar radiation, air and leaf temperature and saturation vapor pressure deficit (SVPD) are the most useful (Botkin, 1969; Helms, 1972). Radiation and CO_2 exchange rates have been successfully related over short intervals by employing either a logarithmic relation or the equation for a rectangular hyperbola (Blackman and Wilson, 1951; Rabino-witch, 1951; Hesketh and Musgrave, 1962; Hesketh and Moss, 1963; Duncan, et al., 1967; Botkin, 1969; Helms, 1972).

Both air and leaf temperature have been used linearly and with second-degree polynomial, logarithmic and sine transformations (Pharis and Woods, 1960; Botkin, 1969; Helms, 1972). In both Botkin's (1969) and Helms's (1972) studies, radiation accounted for most of the variation in net photosynthesis. The addition of temperature as an independent variable increased the R^2 of their regression equations only slightly. That is not surprising since both leaf and air temperature are somewhat dependent on radiation. Helms found SVPD to be the most useful under stress conditions, but predictive ability under stress remained generally poor.

Depending on which physiological responses are studied, additional plant and environmental variables may prove to be useful predictors. Among these are stomatal and mesophyll resistance, other nutrients (K and P), ribulose diphosphate, carboxylase, leaf carbohydrate accumulation, and ambient CO_2 levels. The usefulness of any empirical relationship established for predictive purposes is predicated on finding a few easily measured variables whose effect on net photosynthesis allow one to quickly approximate net CO_2 rates under a number of different conditions without destructive sampling.

Objectives

Although measurements of "plant biomass" may still be the best indicator of long-term plant-environmental

interactions, the physiological index, with inclusion of rate information, should expand the concept of the plant as a phytometer by increasing our ability to assess the effects of rapid environmental changes (pollution, ionizing radiation, fertilization, water stress, irradiance, et cetera).

In this study experiments were conducted with tulip poplar (Liriodendron tulipifera L.), a rapidly growing, shade intolerant, deciduous species of considerable importance in 16 U. S. forest cover types (Fowells, 1965). The objectives of this study were to (1) ascertain which plant and environmental parameters are the most accurate predictors of net CO₂ exchange under varied conditions, (2) determine the amplitudes of physiological processes (photorespiration, diurnal water flux, seasonal pigment development and stomatal response) which characterize tulip poplar, (3) establish background information and techniques that would enable utilization of the index in the forest, (4) investigate the relationships between photopigment ratios and nutrient status, and (5) define empirical relationships between CO₂ uptake and physiological responses to plant environmental relationships.

II. MATERIALS AND METHODS

Laboratory Studies

The general relationships between rate of net photosynthesis and plant-environmental parameters to be investigated are summarized in Figure 1, page 7. A series of laboratory experiments were conducted on tulip poplar seedlings (Liriodendron tulipifera L.) acclimated to known levels of light², soil water potential and soil nitrogen content at 30-day intervals during the 1971-72 growing season. Investigations centered on determining relationships between water potential (leaf and soil), chlorophyll content and ratios, leaf nitrogen content, stomatal resistance, irradiance and net photosynthesis. Dark and photorespiration values were measured also. To facilitate the logistics of sampling procedures, CO₂ concentrations and temperature during photosynthesis determinations were maintained at non-limiting levels of 320 ± 10 ppm and $26 \pm 2^\circ$ C, respectively. This removed two important environmental variables and may decrease the precision and usefulness of the index. However, ambient levels of CO₂ are generally non-limiting, and most naturally occurring

²In this study light level refers to the shading treatment the plants were acclimated to, while irradiance or radiation flux density is used when referring to the energy source for net photosynthesis measurements made in a cuvette inside a growth chamber.

tree species are adapted to their environment in such ways that temperatures seldom suppress CO₂ exchange rates severely.

Experimental design and procedure. In order to test the concept of the physiological index as applied to tulip poplar, various conditions of light, water, nitrogen and age and their interactions over the range of such conditions found in the environment were simulated in two factorial arrangements of treatments. Six hundred tulip poplar seedlings 60 days old were supplied by the state nursery at Pinson, Tennessee (July, 1971). Genetic variability was controlled to the extent that the seedlings were "half-sibs" (one known parent). Four hundred of these were transplanted into one-liter pots, one seedling per pot, in Emory silt loam and the remainder in a washed sand culture. Some physical and chemical properties of Emory silt loam are summarized in Table VI, Appendix A. The nutrient solution used in the sand culture (a modified Hoagland's solution) before the addition of nitrogen is given in Table VII, Appendix A.

Experiment one (I) consisted of analysis of water stress in a factorial arrangement composed of three light environments, three water levels, and three 30-day age periods in a randomized complete block design with three replications. The light environments consisted of full sunlight, 50 percent of full sun and 25 percent of full sun.

Full sun-grown plants were located in a non-shaded enclosure pen. Intermediate shading (50 percent of full sun) was achieved by growing the plants in a greenhouse of mylar-polyethylene construction. The addition of cheesecloth over greenhouse benches lowered the light intensity to approximately 25 percent full sun. Radiant flux density was measured continuously with a pyrliometer (Eppley) and dome solarimeters (Lintronic). A summary of the average daily irradiance for each environment is shown in Figure 22, Appendix A. Soil water potentials were monitored with thermocouple psychrometers (Wescor) (Figure 23, Appendix A) placed horizontally within the rooting zone (Spanner, 1951; Rawlins, 1966; Brown, 1970; Wescor, 1971). Soil water levels were adjusted weekly to maintain soil water potentials of <-1 bar, -2 to -9 bars and >-9 bars. Stress conditions under full sun were maintained by covering the soil surface with an inverted plastic funnel sealed at the stem with Duxseal. An aluminum foil covering prevented soil temperature from varying more than 2° C from uncovered soil.

Experiment two (II) was performed to alter the available chlorophyll content and photosynthetic rate by manipulating nitrogen levels. The design consisted of a factorial of three levels of nitrogen, three light regimes and two age classes of seedlings arranged in a randomized complete block design with two replications. A single nitrogen treatment was introduced 60 days after foliage

emergence to plants grown in a washed sand culture in the form of urea $[\text{CO}(\text{NH}_2)]$. Each pot received 400 ml of solution containing either 33, 100 or 300 ppm which correspond to 2.37, 7.14 and 21.42 millimolar solutions, respectively. The solution was adjusted to pH 6.0. Light regimes were identical to those outlined in Experiment I. Photorespiration, dark respiration and stomatal resistance experiments were conducted with a minimum of four replications on seedlings grown under full sun and no water stress.

Statistical analysis. Data analysis involved the following computer programs: BMD03V (Analysis of Covariance for Factorial Design) and BMD08V (Analysis of Variance for Factorial Design). These programs are part of the UCLA Health Sciences computing center statistics package modified for use at ORNL (Hull, 1967). Duncan's new multiple range test was used for separation of significance among means. Multiple regression analysis was used to develop linear predictive models of the physiological index (Draper and Smith, 1966). The final model and the fitting procedures in the index were produced with Regress, a multiple regression and correlation program (Van Dyne, 1965) and Mulgress, a step-wise deletion multiple regression analysis program (Martin, 1971). All summary statistics followed the procedures of Steel and Torrie (1960).

Harvest analysis. Plants from each treatment were randomly selected for harvest at 30-day intervals from the water stress experiment (I) in July, August, and September and from the nitrogen experiment (II) in July and August only. Leaf area was determined by measuring the maximum leaf blade length and width and then applying a regression equation (Harris, 1970) of the form $y = 0.72 + 0.72(x)$ where x is the product of maximum length and width in cm and y is the surface area of the leaf in cm^2 . This allometric relationship was verified with 50 planimetered leaves with a resultant R^2 of 98 percent. An allometric relationship between leaf area and leaf dry weight is presented in Figure 24, Appendix A.

Plant material from each treatment was separated (root, stem and leaf), dried (48 hours at 105°C), weighed, ground in a Wiley mill to pass a 20-mesh screen and stored for analysis.

Total leaf nitrogen was determined by Semimicro-Kjeldahl analysis (Black, 1965). To check the accuracy of results, U. S. Bureau of Standards Orchardgrass (Dactylis glomerata L.) leaves (2.76 percent N) were analyzed with each run.

Growth analysis (Watson, 1952; Radford, 1967; Květ, et al., 1971; Ondok, 1971) was measured along with determinations of net photosynthetic production (gross assimilation minus respiration) on duplicate plants and

utilized as a time integrator of net CO₂ fixation. Growth rate trends were compared to net photosynthetic responses for similarly acclimated plants. The basic concepts of growth analysis and their physiological implications are simple and are explained in detail by Květ, et al., (1971). Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) were calculated following the form:

$$\text{RGR} = 1/W \cdot dW/dt = d/dt (\log_e W) \quad (1)$$

$$\text{NAR} = 1/A \cdot dW/dt \quad (2)$$

where W and A represent biomass (W, total dry weight of the individual plant and A, total leaf area of plant), recorded at two sequential harvests made at times t_1 and t_2 .

Net photosynthesis and water potential measurements.

Measurements of CO₂ exchange were made utilizing an open-type (Sesták, et al., 1971) gaseous exchange system as follows. Four chambers (22-liter capacity each) were constructed of polyvinyl chloride (p.v.c.) following the design and recommendations of Harris (1970). Carbon dioxide flux was measured with a non-dispersive infrared analyzer (Beckman Model IR-215A) in conjunction with a ten-channel selector (Beckman Model 138 515w) and potentiometric recorder (Beckman Model 93500). Calibration of the analyzer and recording apparatus was carried out daily using standard gases supplied by Oak Ridge National Laboratory Analytical Chemistry Division (Figure 3).

Figure 3. Block diagram of equipment for the measurement of CO₂ assimilation. Dec, drying column (CaSO₄); EGC, environmental growth chamber; F, air flow meters; IRGA, infrared gas analyzer; P, air pumps; PVC, polyvinyl chloride plastic; Rec, recorder.

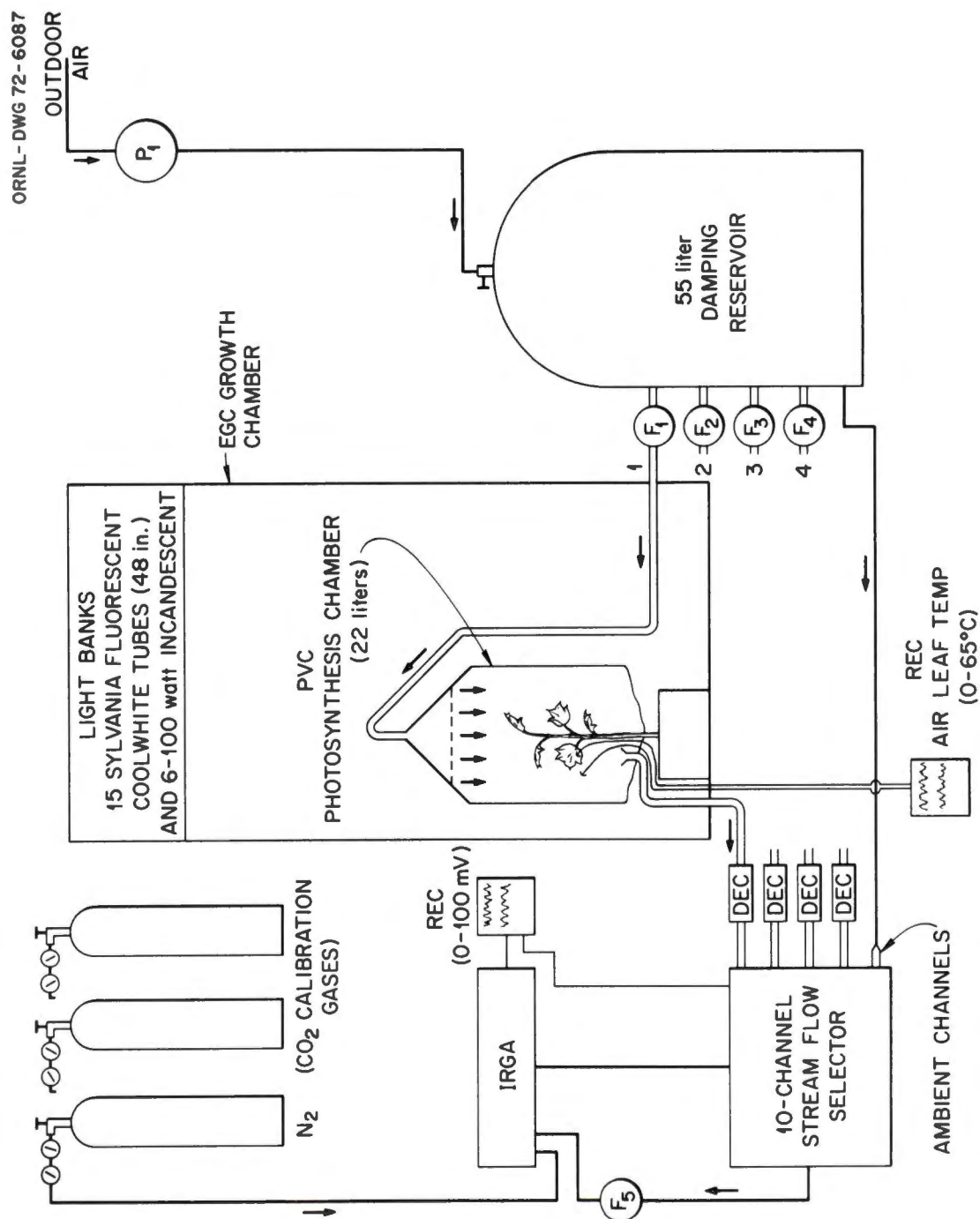


Figure 3

To dampen fluctuations in ambient CO₂ concentrations, outside air was pumped into a 55-liter mixing reservoir. A 1.5 liter/minute flow-rate into the top of each chamber was regulated by glass ball flowmeters (Fischer and Porter). An air baffle in the top of the chamber assured uniform air dispersion. Flow rates through the chambers were adjusted to 90 liters/hour to reduce boundary layer influences and minimize CO₂ depletion. Seedlings were placed in the chamber and two thermocouples and a CO₂ sampling funnel were attached to the stems below the foliage with Duxseal. The polyethylene base was drawn tightly around the stem and tied with nylon cord. The slight positive pressure maintained in the chamber minimized CO₂ influx from the soil. Chamber and ambient CO₂ differential were monitored at five-minute intervals until equilibrium rates were obtained (approximately one hour).

Soil and leaf water potentials were measured immediately after each photosynthesis run with soil and chamber psychrometers, respectively (Wescor, Figures 23 and 25, Appendix A). A copper-constantan (type T) thermocouple (0.003 inch) was attached to the underside of a representative leaf with Duxseal and a shielded thermocouple (0.01 inch) was used to monitor air temperature. Continuous values were recorded on a multichannel millivolt potentiometric recorder (Honeywell).

The chamber environment was controlled as follows.

Temperature control ($26 \pm 2^\circ \text{C}$) within the respective photosynthesis cuvettes was maintained by placing them in an environmental chamber (EGC Model 103). Relative humidity was maintained at 55 ± 5 percent in the growth chamber. The irradiance source consisted of varying numbers of fluorescent tubes (Sylvania cool white) and incandescent bulbs. Irradiance within each photo-chamber was measured between 400 - 700 nm at 25-nm intervals with an ISCO Model SR spectroradiometer (Figure 26, Appendix A). The use of polyvinyl chloride film did not result in detectable spectral modification. Apparent photosynthesis of all seedlings in the factorial experiments was measured at three successive radiation levels in watts (W m^{-2}) (18 W m^{-2} , 36 W m^{-2} and 51 W m^{-2} which corresponds approximately to 0.02 , 0.05 and $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$, 400 - 700 nm). Net photosynthesis was expressed on both a unit leaf area (dm^2 , one surface) and a dry weight (mg g^{-1}) basis.

Photopigment determinations. Following photosynthesis measurements, two sample discs (2.54 cm^2) were cut from random locations on each of five leaves. Fresh weight was determined for all discs. One five-disc subsample was dried for 48 hours at 105°C for percent dry weight. The other sample was used for pigment analysis. The discs selected for pigment determinations were homogenized in a homogenizer ("Vitriss 45") containing a 4:1 v/v (80 percent)

acetone-water solution with a few grains of CaCO_3 . The macerate was filtered on a Buchner funnel assembly to remove leaf tissue, diluted to a 100-ml volume and stored in the dark at 0°C . Before analysis, an aliquot of pigment extract was centrifuged for three minutes at approximately 3000 rpm in a clinical centrifuge. The supernatants (pigment solution) were decanted and optical densities (350 - 700 nm) of the respective samples were measured using a Beckman DBG scanning spectrophotometer (Figure 27, Appendix A). Centrifugation was repeated if the optical density at 750 nm revealed that the absorbance at this wavelength was greater than 1 percent of the absorbance at the red absorption peak of chlorophyll (Séstak, et al., 1971). Optical densities at 663 nm and 645 nm were utilized along with Arnon's equations (1949) for determination of chlorophyll a, b, and total chlorophyll. Absorbance at 652 nm (isosbestic wavelength) and Bruinsma's equations (1961) were used for comparative purposes. An estimate of carotenoids was determined at 460 nm using the equations of Myers and Kratz (1955). Optical densities at 430 nm, 450 nm, 460 nm and 663 nm were utilized in pigment ratios (430/663, 450/663 and 460/663) as indicators of carotenoid/chlorophyll (yellow/green) or nutrient fluxes.

Light saturation, stomatal resistance and photo-respiration. The physiological characteristics which influence CO_2 exchange rates in leaves of tulip poplar were

studied during the 1972 growing season. Background information on early season rates was collected from plants grown under water stress. Seedlings from the same tulip poplar population were grown under conditions similar to the 1971 series. Methodology was identical to that used for the 1971 series.

Additional light intensities during light saturation measurements were provided by six 250-watt flood lamps (Sylvania) filtered through a 10-cm water bath to remove a large part of the infrared component. A variac was utilized to provide irradiance levels from 0.005 to 0.6 cal cm⁻² min⁻¹, 400 - 700 nm.

Stomatal resistance (r_s) was measured abaxially since tulip poplar is hypostomatous. Stomatal resistance was measured with a diffusion porometer (Lambda Model LI-15S) in conjunction with a resistance meter (Lambda Model LI-60). Techniques and calibrations followed those of Kanemasu, Thurtell and Tanner (1969) and Lambda (1971).

A minimal estimate of photorespiration was obtained by the extrapolation technique (Decker, 1957; Brix, 1968; Ludlow and Jarvis, 1971; Zelitch, 1971). Carbon dioxide exchange was measured at CO₂ concentrations ranging from 500 ppm to the CO₂ compensation point, where there is no CO₂ uptake. This study was performed on four tulip poplar seedlings at three irradiance levels. The gas exchange system was similar to that described previously, except

that a semi-closed photochamber consisting of a double-walled polyfilm bag (1 liter), clamped and sealed to a plexiglass base, was used for measurements below 100 ppm external CO₂ (Dinger, personal communication). A reduced chamber size and positive internal pressure were employed in an attempt to keep CO₂ influx minimal. Enriched CO₂ with 21 percent oxygen and nitrogen balance was supplied by ORNL Analytical Chemistry Division. Lower CO₂ levels were produced by passing the air stream through a column of "Ascarite."

Field Studies

Site, soil and climate. Field studies in the Liriodendron forest were conducted to attain ambient canopy values for photopigments and nutrient content and to relate them to precipitation and radiant flux density through the 1971 growing season. The study site is an area of second-growth mesophytic forest located on the U. S. AEC Oak Ridge Reservation (35° 55' N. Lat., 84° 17' W. Long.) within the Ridge and Valley Physiographic Province in east Tennessee. Species composition is typical of a bottomland second-growth mesophytic hardwood forest of the Southern Appalachian region. Tulip poplar (Liriodendron tulipifera L.) is the dominant overstory species. Others obtaining canopy height included oaks (Quercus velutina Lam., Q. alba L., Q. coccinea Muench., Q. rubra L.

and Q. prinus L.), hickory (Carya tomentosa Nutt.), and short-leaf pine (Pinus echinata Mill.). Soils of the study area are of the Emory series of cherty silt loam and are alluvial in origin (Table VI, Appendix A). The study area is at an elevation of 290 meters and is located in a region of karst topography underlain by Knox dolomite. Monthly climatic data (1961-70) for the area are summarized in Table VIII, Appendix A.

Canopy characterization. Studies in the Liriodendron forest were undertaken in the spring of 1971 to establish background information about the forest canopy. Foliage collections were made at three canopy levels from a mobile lift truck. Seasonal development of plant photopigments was determined by collecting samples bi-weekly from April through September and analyzing them as described previously (page 31). To minimize errors caused by pigment decomposition, samples were sealed in polyethylene bags and stored in dry ice until extractions. Extractions were completed within 24 hours. Leaf material for mineral analysis was dried and milled as described previously.

Total nitrogen was measured by semimicro-Kjeldahl analysis (Black, 1965). A 500 mg subsample of leaf tissue was ashed and made to volume (100 ml) with 5 percent HCl for calcium and potassium analysis, both determined spectrophotometrically (Perkin-Elmer Model 403 atomic absorption spectrophotometer) following Kahn's method (1971).

Phosphorous was determined by the sulfo-molybdate method on an autoanalyzer (Technicon) as described by Lundgren (1960).

Daily variations in incoming solar radiation flux were monitored by dome solarimeters (Lintronic) sensitive over a 0.3 - 3.0 μm range. Precipitation data from an automatic precipitation collector (Wong) summarized on a bi-weekly basis were supplied by M. H. Shanks, ORNL Environmental Sciences Division.

III. RESULTS AND DISCUSSION

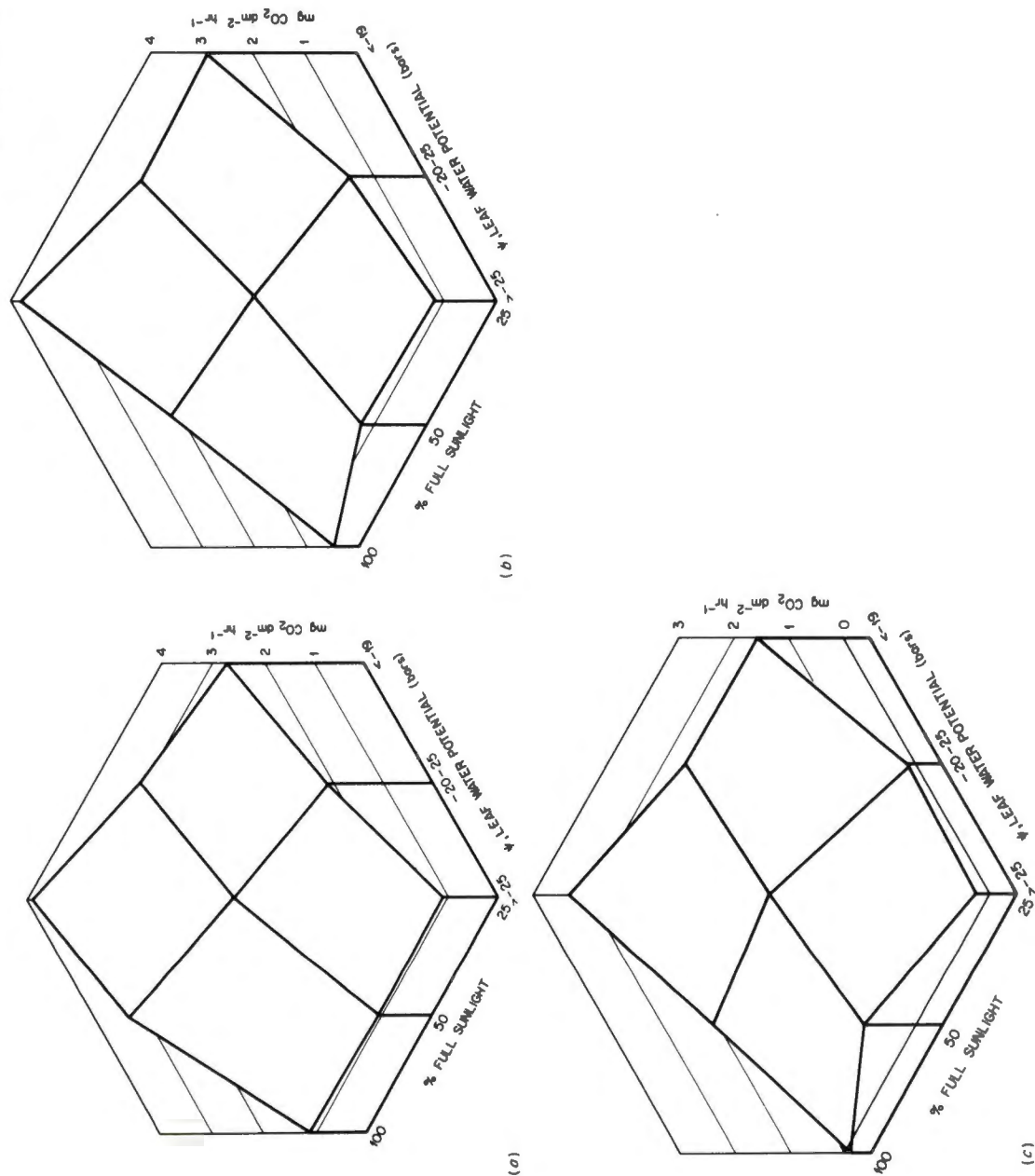
The Influence of Light, Age and Water on Physiological Dynamics of Tulip Poplar, Experiment I

The response of tulip poplar was analyzed in terms of physiological characteristics which control the fluxes of CO₂ and, in turn, net productivity. Treatment responses and physiological amplitudes in the laboratory experiments are presented as background information for tulip poplar. Further utilization of the data is realized in the main purpose of the study, predictions of net photosynthesis via physiological and environmental parameters. The combined effects of shading and water stress treatments on net photosynthesis measured at radiation levels of 0.07, 0.05 and 0.02 cal cm⁻² min (400 - 700 nm) in the laboratory are shown in isometric projections on Figures 4a, b, and c, respectively. At each radiation level the tulip poplar seedlings grown under full sunlight and no water stress maintained the highest CO₂ exchange rates. The decrease in rates of net photosynthesis with increasing water stress was statistically significant ($P < 0.05$) at all radiation levels. Analysis of variance of the data are summarized in Table XI, Appendix C. Increased soil moisture stress resulted in significant ($P < 0.05$) decreases in both net photosynthetic rates and growth. The light environment to which the seedlings were acclimated did not affect net CO₂

Figure 4. The effects of leaf water potential and light environment on net photosynthesis of tulip poplar seedlings under irradiance of (a) $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$, (b) $0.05 \text{ cal cm}^{-2} \text{ min}^{-1}$ and (c) $0.02 \text{ cal cm}^{-2} \text{ min}^{-1}$. Conditions: ambient CO_2 concentration $320 \pm 10 \text{ ppm}$, leaf temperature $26 \pm 2^\circ \text{C}$, and radiation (400 - 700 nm).

Figure 4

ORNL-DWG 72-8026



exchange significantly (Table XI, Appendix C). This absence of shade response in net photosynthesis was the result of the relatively low radiation levels utilized; thus the plants were not light saturated. However, significant interactions between light and age occurred at 0.07 and 0.05 cal cm⁻² min⁻¹ and between light and water at 0.02 cal cm⁻² min⁻¹ (Table XI, Appendix C).

Mean estimates of photosynthetic capacity through the 30- to 90-day period of the experiment are given in Table I. As the season progressed net photosynthetic capacity generally decreased for water stressed plants. A seasonal increase in photosynthetic rates of leaves grown under full sun and no water stress may be explained in part by the higher levels of leaf chlorophyll maintained (Figure 5a and b). Tulip poplar continually produced new leaves through the growing season in the absence of water stress. The sensitivity of tulip poplar to water stress is characterized by heavy foliage loss during periods of drought. A seasonal decrease in photosynthetic rate in 50 and 25 percent sun leaves corresponded to concomitant decreases in chlorophyll content per unit area (Table I and Table IX, Appendix B).

It is important to state whether chlorophyll content is being expressed on a dry weight or on a per unit area basis. For example, chlorophyll on a dry weight basis generally increases with heavy shading while it remains

TABLE I

RATES OF NET PHOTOSYNTHESIS OF TULIP POPLAR SEEDLINGS MEASURED AT THREE RADIATION LEVELS IN RESPONSE TO WATER AND SHADE TREATMENTS

Time Days Post Treatment	Light Treatment (Percent Full Sun)	Leaf Water Potential (Bars)	Radiation .07		Intensity .05		Cal cm ⁻² Min ⁻¹ (400-700 nm) .02	
			$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)
30	100	<-20 (1)	3.00	(0.43)	2.81	(0.42)	2.12	(0.47)
		-20 to -25 (2)	2.27	(1.21)	1.42	(1.20)	1.27	(0.84)
		>-25 (3)	1.46	(0.90)*	-0.28	(0.92)*	0.16	(0.53)*
	50	1	3.93	(0.90)	2.42	(0.48)	1.34	(0.42)
		2	3.24	(1.42)	1.81	(0.82)	1.33	(0.63)
		3	2.61	(0.45)	2.45	(0.50)	1.83	(0.46)
	25	1	3.24	(0.46)	2.97	(0.59)	2.05	(0.52)*
		2	2.12	(0.92)	1.71	(0.61)	0.77	(0.24)*
		3	1.18	(0.79)*	0.77	(0.42)*	1.14	(0.68)
60	100	1	3.74	(0.88)	3.06	(1.11)	2.14	(0.21)
		2	2.45	(0.19)	2.25	(0.68)	0.71	(0.12)*
		3	1.02	(0.06)*	0.82	(0.20)*	0.73	(0.26)*
	50	1	3.05	(0.77)	4.10	(0.43)	2.01	(0.40)
		2	1.62	(0.83)*	2.28	(1.13)	1.67	(0.72)
		3	1.09	(0.38)*	1.77	(0.48)	1.31	(0.65)
	25	1	2.93	(0.34)	3.04	(0.11)	2.04	(0.26)
		2	1.87	(0.26)*	1.55	(0.47)	1.17	(0.42)
		3	0.25	(0.89)*	1.37	(0.83)*	0.74	(0.44)*
100		1	5.03	(0.75)	5.26	(1.53)	2.72	(1.40)
		2	4.48	(1.31)	2.81	(0.54)	0.85	(1.14)*
		3	0.82	(0.53)*	0.84	(0.21)*	-1.03	(0.44)*

TABLE I (continued)

Time Days Post Treatment	Light Treatment (Percent Full Sun)	Leaf Water Potential (Bars)	Radiation .07		Intensity .05		Cal cm ⁻² Min ⁻¹ (400-700 nm) .02	
			$\bar{X}$	(SE)	$\bar{X}$	(SE)	$\bar{X}$	(SE)
50		1	2.09	(0.96)	2.31	(0.76)	1.12	(0.80)
		2	3.02	(2.02)	1.66	(0.53)	0.91	(0.55)*
		3	-0.54	(0.38)*	-0.17	(0.50)*	-0.10	(0.02)*
25		1	1.85	(0.22)*	2.74	(0.39)	0.83	(0.51)*
		2	2.05	(0.37)	1.06	(0.86)*	-0.90	(0.30)*
		3	1.18	(0.31)*	1.34	(1.20)*	-0.98	(0.39)*

*Denotes a mean value significantly different ($P < 0.05$) from the appropriate control value (100 percent full sun and <-20 bars).

-Negative values indicate an excess of respiration over photosynthesis.

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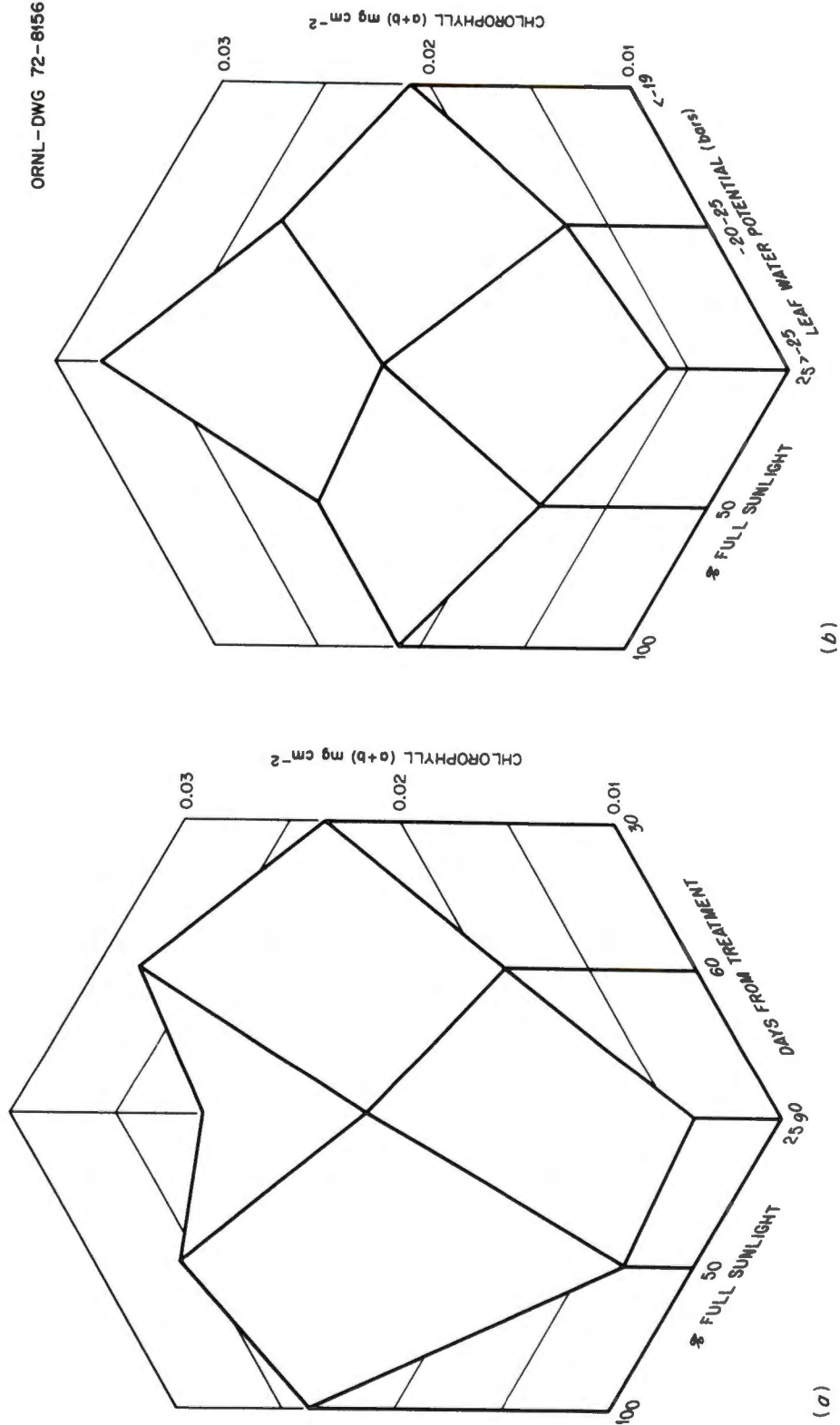


Figure 5. Response of total leaf chlorophyll content (a + b) of tulip poplar seedlings to (a) light environment and time post treatment and (b) light environment and leaf water potential.

fairly constant or decreases slightly on an area basis (Table IX, Appendix B). Increasing amounts of "photosynthetic machinery" on a unit area basis were found as water stress decreased and percent sunlight increased (Figure 5b, page 43). As expected, chlorophyll content of foliage on a dry weight basis increased with shading from 5.9 ± 0.42 mg/g dry weight under full sun and no water stress to 7.7 ± 0.47 mg/g dry weight under 25 percent sun and no water stress. A more detailed summary of the whole plant responses is included in Tables IX and X, Appendix B. The significance of main treatment and interactions on leaf characteristics is summarized in Table XI, Appendix C.

Kramer and Kozlowski (1960) reported reduced photosynthetic capacity at low light intensities with increased mutual shading. In this study leaf area of full sun seedlings under no water stress at 30 and 90 days post treatment was 310 ± 115 cm² and 78 ± 13 cm², respectively. Seedlings grown under 25 percent sun and no water stress maintained leaf areas of 280 ± 18 cm² and 178 ± 45 cm² at 30 and 90 days, respectively. Therefore, total leaf area was used as a covariate to analyze for possible bias in estimating photosynthetic rate due to foliage differences throughout the season. The F test of the adjusted mean was not significant at the 0.25 level and adjustments were not made.

Differential photosynthetic capacity of seedlings from full sun and 25 percent sun through the season was compared. Results of the t test comparisons are summarized in Table II and indicate that tulip poplar sun leaves generally had a higher photosynthetic capacity per unit leaf area than shade leaves through the growing season. The increased capacity of sun leaves was also reported for tulip poplar by Harris (1970).

The relation between net photosynthesis and various plant and environmental parameters when compared separately is somewhat perplexing. This is not surprising since mechanisms of CO₂ fixation are complex and yield a multidimensional response surface which, when expressed in two dimensions, present a scatter of points with little obvious pattern (Helms, 1972). Chlorophyll content by itself has little predictive value (Figure 6a). However, if water status and light environment are constrained (leaf water potential < -20 bars and leaves grown 100 percent full sun) a high linear correlation ($r = 0.86$) between net photosynthesis and chlorophyll content is obtained (Figure 6b). In the laboratory many of the controlling parameters are constrained. In the natural environment constraint is generally not possible or desirable, thus a more useful approach is to include some estimate of controlling factors in the analysis, which will allow development of a linear predictive model.

TABLE II

COMPARISON OF RATES OF NET PHOTOSYNTHESIS BETWEEN
SUN- AND SHADE-GROWN SEEDLINGS OF TULIP POPLAR
(CONTROL SEEDLINGS) AT 30, 60, 90, 120,
150 DAYS

Light Environment (Percent Full Sun)	Days	Rate of Net Photosynthesis ^a		t(df)	Probability of Greater t
		$\bar{X}$	(SE)		
100	30*	4.08	(0.07)	4.00(3)	0.02 < P < 0.05
25		2.32	(0.35)		
100	60*	4.66	(0.42)	3.38(3)	0.02 < P < 0.05
25		3.48	(0.13)		
100	90	3.00	(0.43)	0.04(3)	0.5 < P < 0.9
25		3.24	(0.46)		
100	120	3.74	(0.88)	0.86(3)	0.4 < P < 0.5
25		2.93	(0.34)		
100	150	5.03	(0.75)	4.06(3)	0.02 < P < 0.05
25		1.85	(0.22)		

^aNet photosynthetic rates are expressed as $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$
at $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400-700 nm).

*Data were collected in spring of 1972.

Figure 6. Relationship between chlorophyll content and net photosynthesis.

- a. Relationship between net photosynthesis and chlorophyll content of leaves grown under varying light and water levels.
- b. Relationship between net photosynthesis and chlorophyll of sun leaves only measured at leaf water potential less than -20 bars. Ambient conditions: radiation $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm), CO_2 concentration $320 \pm 10 \text{ ppm}$, and temperature $26^\circ \pm 2^\circ \text{ C}$.

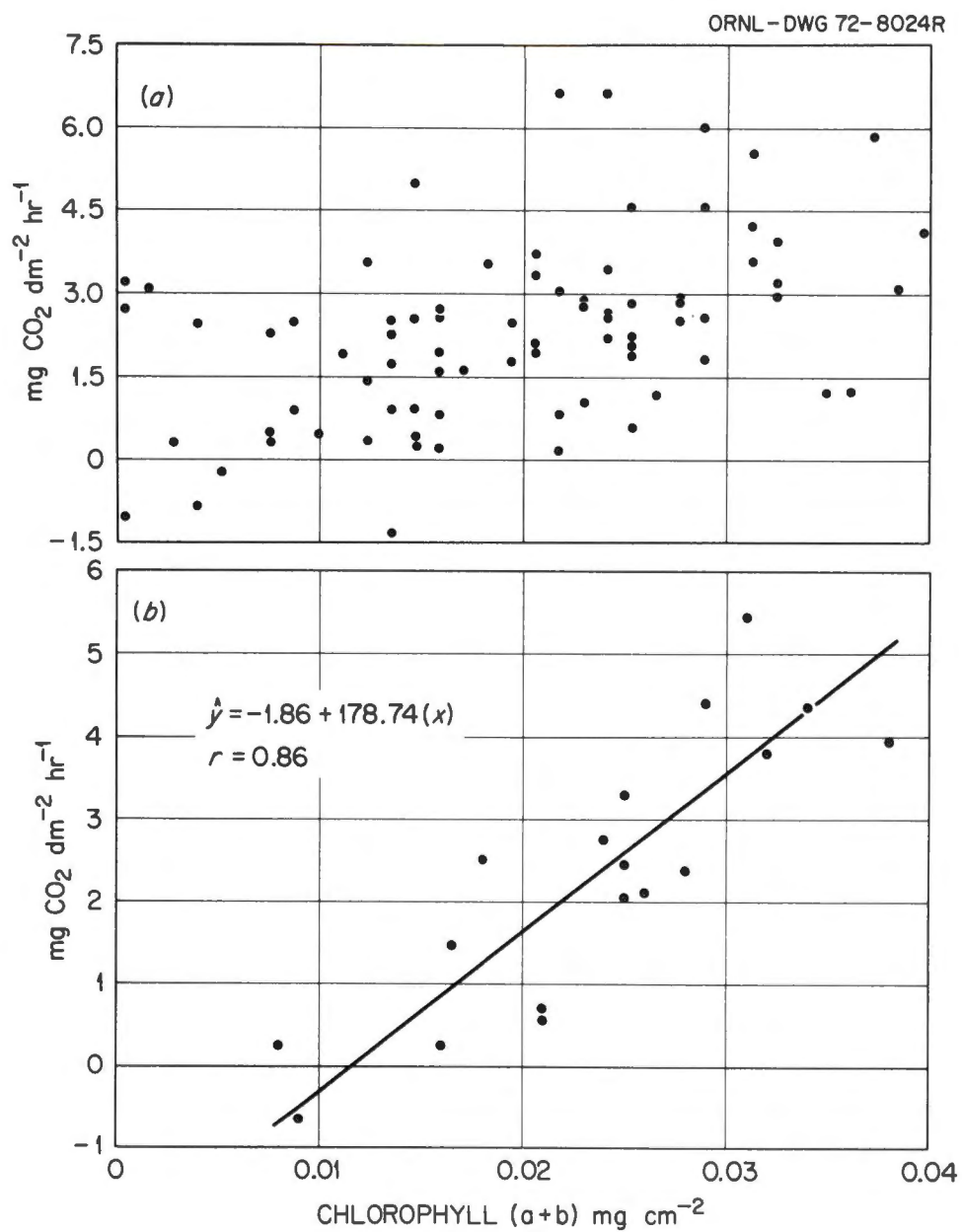


Figure 6

A means of explaining the combined effects of ranging levels of soil water potential, leaf water potential, chlorophyll content, and age, et cetera, and any perturbations which may affect photosynthetic rates through these physiological parameters is provided by multiple regression analysis.

Isometric projections of Relative Growth Rate (RGR) and Net Assimilation Rate (NAR) as functions of water stress and time post treatment, and of percent full sunlight and water stress are summarized in Figures 7a and b, respectively. The general shape of these surfaces was similar to net photosynthetic response, indicating that estimates of net photosynthesis in this study are good indicators of growth. The simple correlation between net photosynthesis and NAR was 0.42. Both NAR and RGR were significantly ($P < 0.05$) affected by the main effects of light, age and water (Table XI, Appendix C). As might be expected, growth was greater under full sun and no water stress early in the growing season.

The Influence of Light, Age and Nitrogen on Physiological Dynamics of Tulip Poplar, Experiment II

The responses in net photosynthesis at two irradiance levels by tulip poplar to nitrogen fertilization and shading are presented in Figures 8a and b. As shading increased, net CO_2 exchange reached a maximum at 100 ppm N

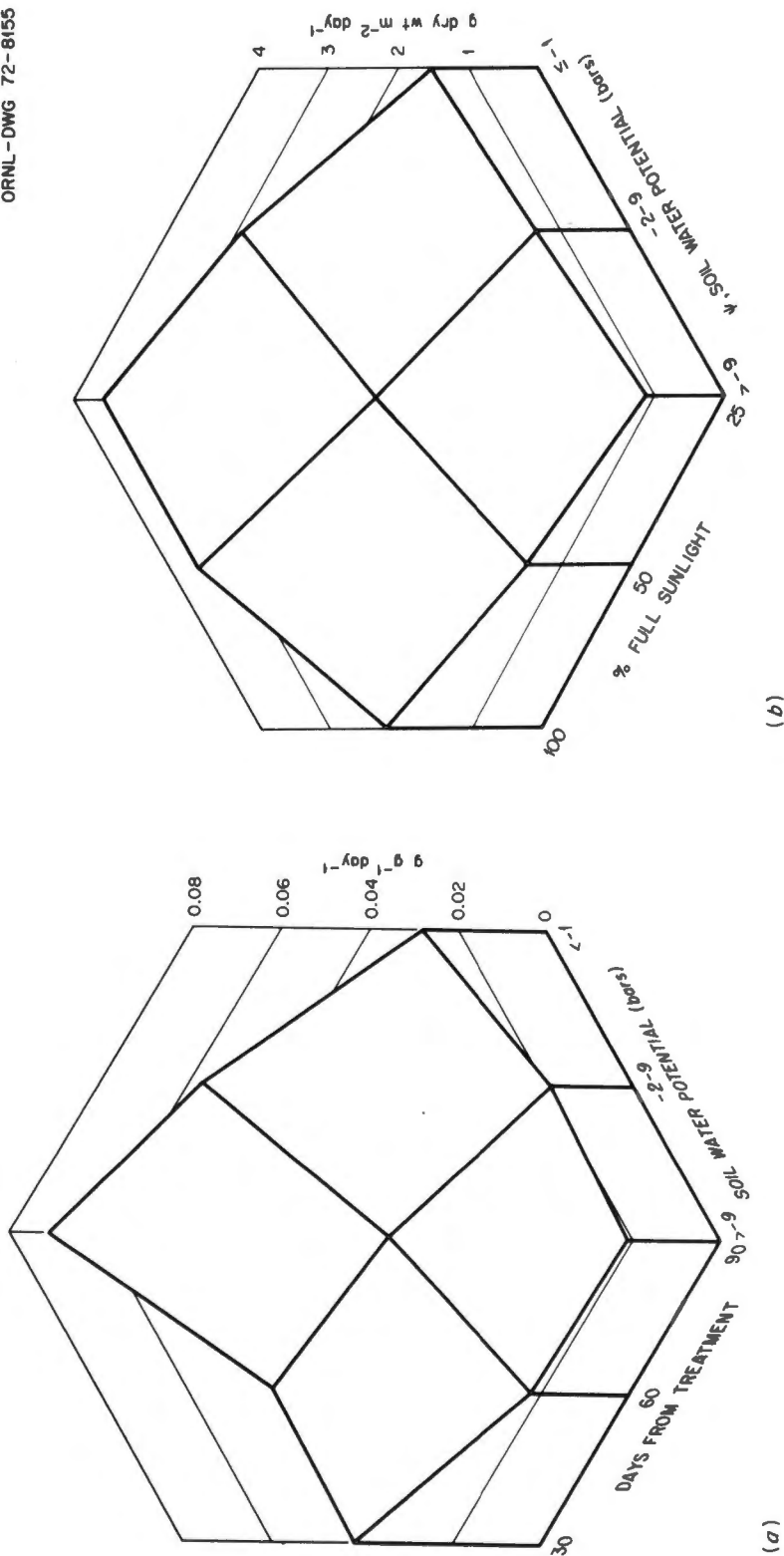


Figure 7. Growth responses of tulip poplar seedlings. (A) Relative growth rates ($\text{g g}^{-1} \text{ day}^{-1}$) of seedlings to soil water potentials and days from treatment. (B) Net assimilation rates ($\text{g m}^{-2} \text{ day}^{-1}$) of seedlings as a function of soil water potential and light environments.

Figure 8. The effects of nitrogen fertilization and light environment on net photosynthesis of tulip poplar seedlings (a) $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ (b) $0.02 \text{ cal cm}^{-2} \text{ min}^{-1}$. Conditions: ambient CO_2 concentrations $320 \pm 10 \text{ ppm}$, leaf temperature $26^\circ \pm 2^\circ \text{ C}$.

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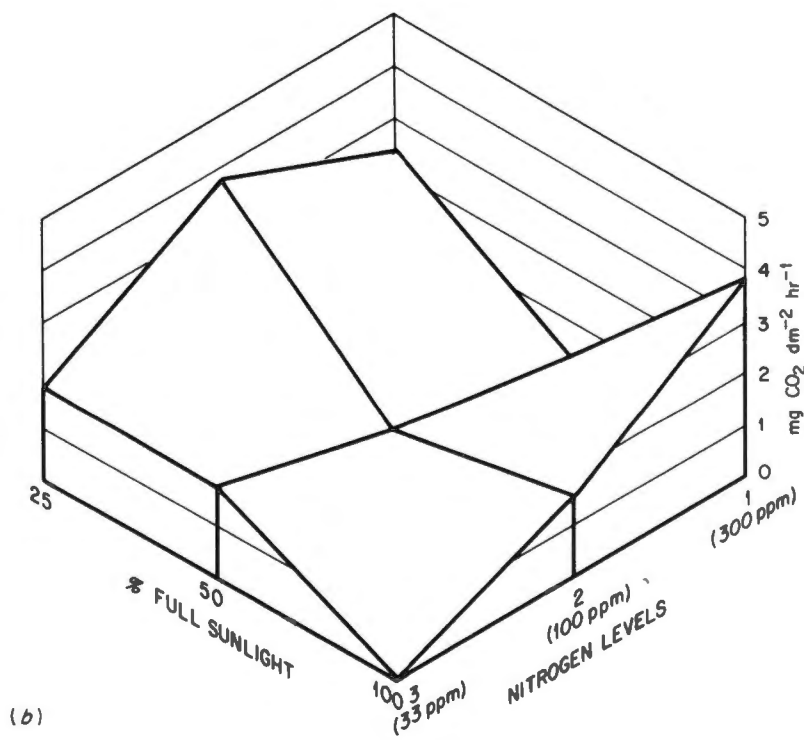
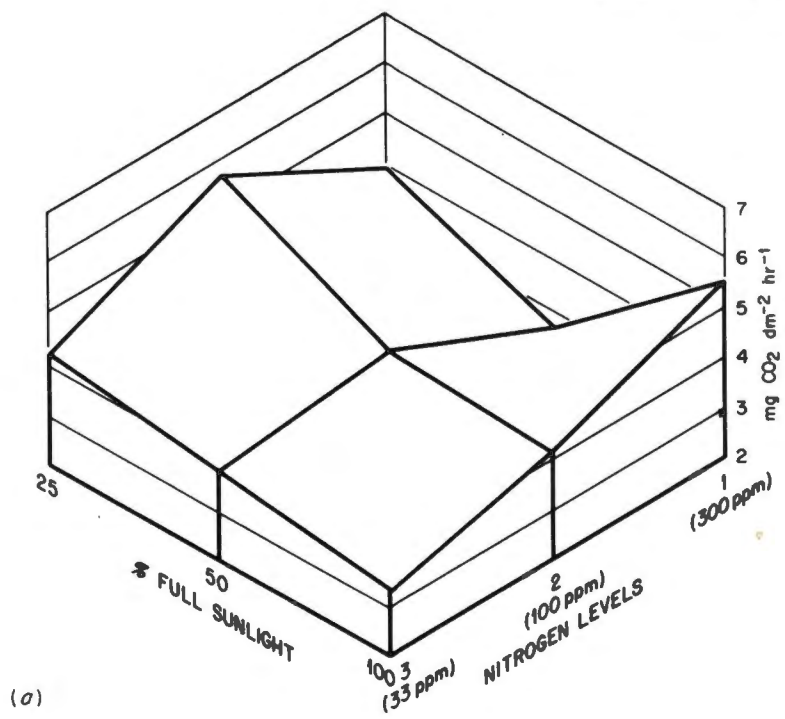


Figure 8

and decreased at 300 ppm N. A maximum CO_2 exchange rate of $5.5 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ was attained by full sun leaves at 300 ppm N under $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$. Photosynthesis increased significantly ($P < 0.10$) in sun leaves at higher nitrogen treatments at $0.05 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm), (Table XII, Appendix C). Navasero and Tanaka (1966) observed that crop plants supplied with abundant nitrogen and kept under reduced light lose their photosynthetic activity but retain their respiratory activity. This factor, which may account for the reduced photosynthesis levels found in this study under shading and high nitrogen levels, may also explain one of the causes of death of lower leaves noted in plants subjected to heavy shading. These responses suggest that high nitrogen fertilization in dense tulip poplar stands may result in little increase in net production.

The responses of chlorophyll content on a dry weight basis to fertilization and shading are shown in Figure 9. As expected, increased shading and nitrogen increased the "photosynthetic machinery."

Photosynthetic activity measured at light saturation and normal CO_2 concentrations had a high positive correlation ($r = 0.91$) with percent foliar nitrogen content over a wide range when the covariance of leaf age is considered (Figure 10). These results indicate that leaf nitrogen concentration is a useful indicator of potential

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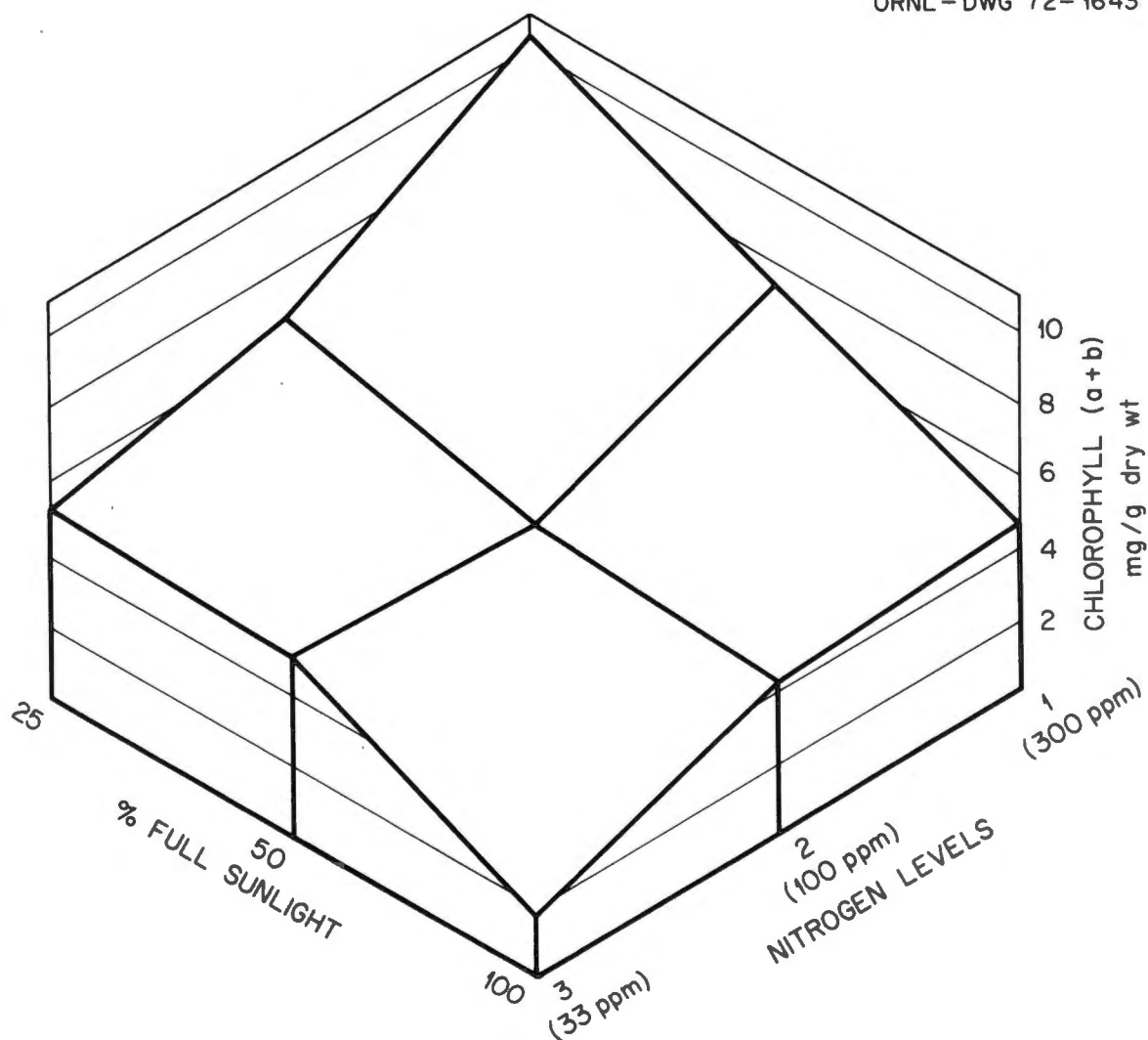


Figure 9. Relationship between nitrogen treatments and light environment on the total leaf chlorophyll concentration of leaves of tulip poplar seedlings.

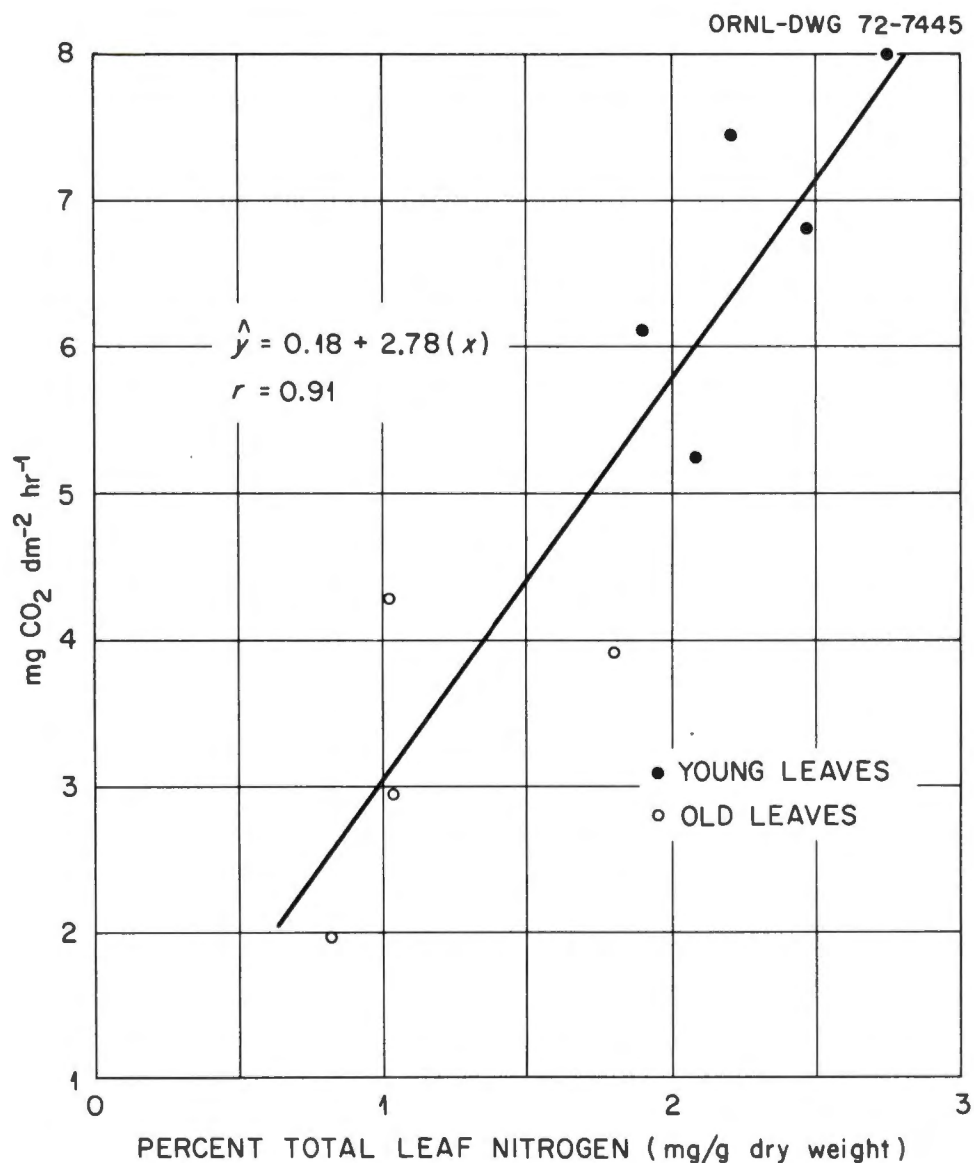


Figure 10. Relation between percent total leaf nitrogen and photosynthetic activity of tulip poplar leaves measured at $0.2 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm). Conditions: ambient CO₂ concentrations $320 \pm 10 \text{ ppm}$, leaf temperature $26 \pm 2^\circ \text{ C}$.

photosynthetic activity through the season. The photosynthetic capacity of tulip poplar at two levels of nitrogen fertilization is summarized in Figure 11. The photosynthetic rate of the "deficient" plants amounts to only 49 percent of the rate of the adequately supplied specimens. The role of nitrogen in chlorophyll formation also is apparent in that higher nitrogen concentrations resulted in a three-fold increase in chlorophyll content (Figure 11).

Dark respiration measurements were made on an irregular basis through the 1971 growing season, with rates averaging $0.63 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$. Harris (1970) reported dark respiration values of $0.76 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$.

In summary, net photosynthesis was reduced in seedlings acclimated to shade and water stress. Seasonal variations in values of NAR and RGR followed chlorophyll and nitrogen content as well as foliage development. Nitrogen fertilization altered CO_2 exchange rates mainly at higher light intensities and earlier in the growing season. Chlorophyll content increased with additional shade, water and nitrogen levels. Rates of photosynthesis were reduced earlier in the season in both experiments under stress conditions apparently as an expression of foliage senescence. Plant parameters shown to have prediction potential for estimating net photosynthesis are: chlorophyll concentration (a and b), percent foliar

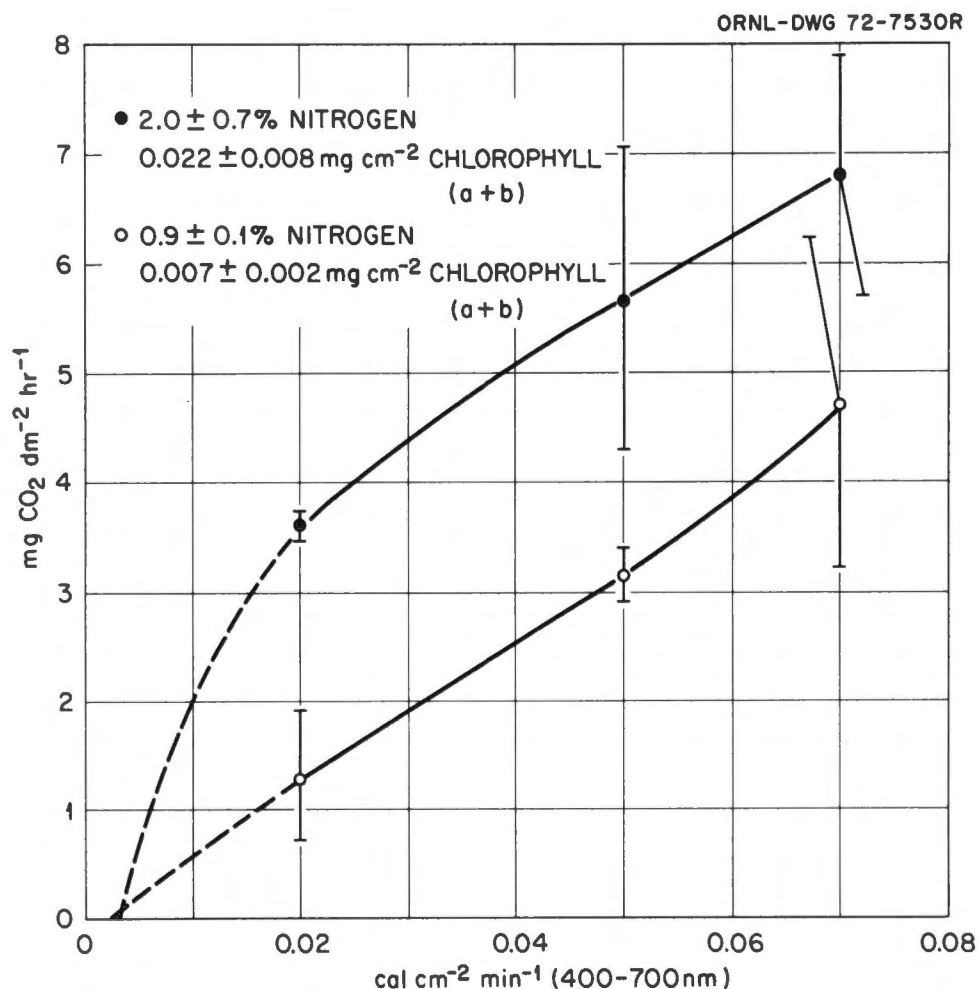


Figure 11. The influence of radiation flux density on photosynthesis in tulip poplar leaves at two nitrogen levels at 90 days post treatment. Conditions: ambient CO_2 concentrations 320 ± 10 ppm, leaf temperature $26 \pm 2^\circ \text{C}$, error bars ± 2 SE. (Curves hand-fitted to the light compensation point.)

nitrogen and leaf water potential. Environmental variables useful in predicting net CO₂ flux are radiation and soil water potential.

The predictive value of an individual parameter is somewhat limited since the CO₂ exchange rate of a plant is related to various dynamic plant and environmental interactions. To find which of the many plant and environmental parameters are significant for a given species, and to then determine the form of the empirical relationships necessary to predict the responses of the vegetation to them is the crux of this study. The data base for such an analysis would come from simultaneous field measurements of CO₂ exchange and various physiological and site variables. Greater resolution in laboratory studies on a few factors can prove invaluable in the selection of physiological plant parameters and in understanding cause and effect relationships. Exploration of the predictive value of stomatal resistance, and the previously studied parameters, singly and in various combinations, is the main content of the rest of this section.

Dependence of Stomatal Resistance on Radiation

The radiation dependent characteristics for photosynthetic light saturation and light compensation points, as well as stomatal response to photon flux, are important physiological parameters. They provide needed information

for supplemental experiments as well as for estimation of potential CO₂ fixation rates. The initial slope of the light curve provides an estimate of the efficiency of the photochemical portion of the photosynthesis curve, while the part of the curve beyond light saturation of the photosynthetic mechanism provides an estimate of the biochemical limitation of the leaf for fixing CO₂ (Harrison, 1970).

Photosynthetic light saturation for leaves of mature tulip poplar trees occurs at approximately 0.4 cal cm⁻² min⁻¹ (0.03 - 3.0 μm) under optimal temperature conditions of 26 ± 4° C (Dinger, 1971). It has been shown that 45 percent of the total short wave energy lies in the 400 - 700 nm waveband (Anderson, 1967); by applying this conversion, a light saturation value of 0.18 cal cm⁻² min⁻¹ (400 - 700 nm) may be calculated for tulip poplar. Conversion of total incident shortwave radiation to the photosynthetically active region (400 - 700 nm) allows rate comparisons to be made between natural and artificial radiation sources.

A light dependence curve for tulip poplar seedlings in the growth chamber is given in Figure 12. At 25° C, light saturation occurred at a radiation intensity of approximately 0.20 cal cm⁻² min⁻¹ (400 - 700 nm), corresponding to the saturation intensity of 0.40 cal cm⁻² min⁻¹ (0.3 - 3.0 μm) calculated for field conditions by Dinger

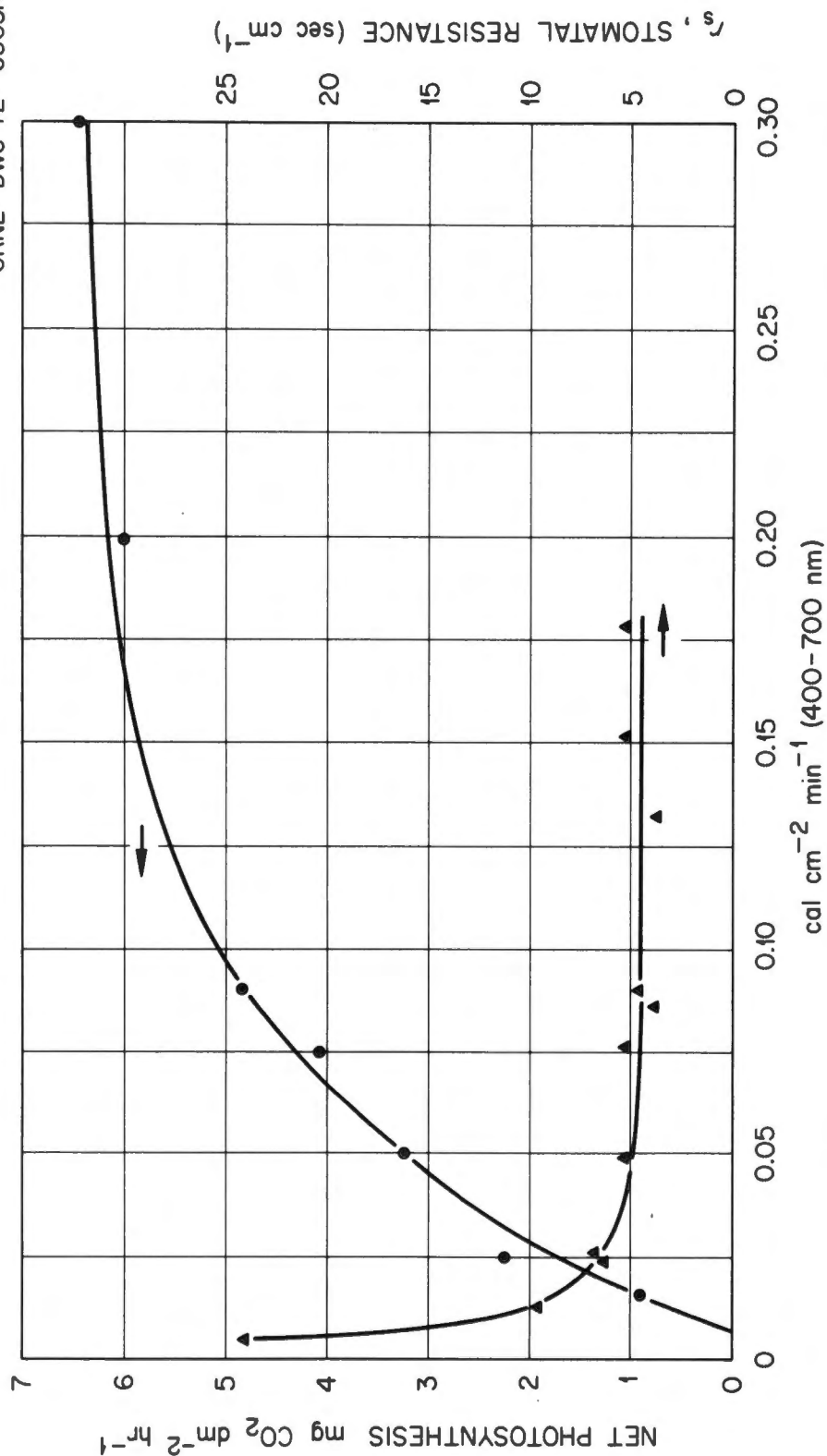


Figure 12. The influence of irradiance on net photosynthesis and on stomatal resistance. Conditions: ambient CO_2 concentrations 320 ± 10 ppm, leaf temperature $25 \pm 4^\circ \text{C}$. (Curve hand-fitted.)

(1971). Light compensation occurred at $0.006 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm) (38 foot candles). Stomatal resistance decreased from 24.5 sec cm^{-1} at $0.005 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm) to minimal resistances of approximately 4.5 sec cm^{-1} at $0.060 \text{ cal cm}^{-2} \text{ min}^{-1}$ (Figure 12, page 60). Further increases in radiation had little effect on resistance levels but net CO_2 exchange increased from $3.42 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ at $0.060 \text{ cal cm}^{-2} \text{ min}^{-1}$ to $6.0 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ at $0.21 \text{ cal cm}^{-2} \text{ min}^{-1}$.

The relation between stomatal resistance and radiation in tulip poplar seedlings grown under full sun and no water stress is summarized in Figure 13. At radiation levels greater than $0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$ (0.3 - 3.0 μm) there was little change in stomatal resistance, but the resistance increased sharply when incoming radiation was below $0.05 \text{ cal cm}^{-2} \text{ min}^{-1}$ (0.3 - 3.0 μm). Tulip poplar has stomates that open at extremely low radiation levels as well as a fairly low photosynthetic light compensation point, and yet the species is considered shade intolerant. This apparent anomaly is explained in part by its slow stomatal response to rapid changes in radiation levels such as sun flecks and clouds (Turner, 1969). The relationship between stomatal resistance and leaf water potential may give some insight into diurnal net CO_2 fluxes.

Diurnal Variation in Stomatal Resistance and Leaf Water Potential

Changes in radiation levels, saturation vapor pressure deficit, stomatal resistance and leaf water potential

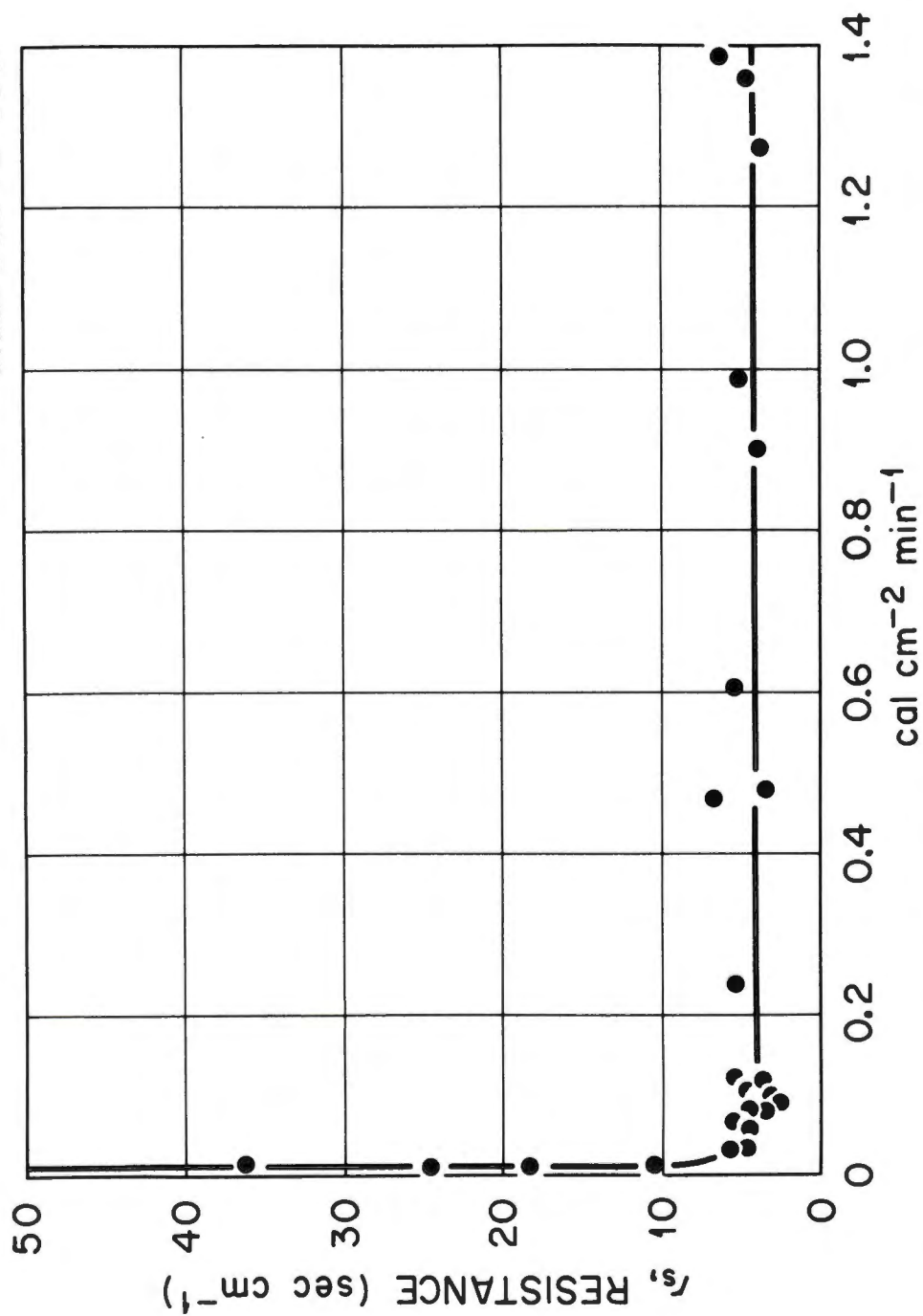


Figure 13. The relationship between stomatal resistance and radiation (0.3 - 3.0 μ) for tulip poplar. (Curve hand-fitted.)

for a 24-hour period in early May of 1972 for tulip poplar seedlings grown at three levels of soil moisture are presented in Figure 14. Changes in radiation near sunrise and sunset resulted in large decreases and increases in stomatal resistance, respectively. Little variation in stomatal resistance could be detected at leaf water potentials, between -6 and -16 bars, in seedlings grown under no stress (soil water potential < -1 bar) when radiation levels exceeded $0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$ ($0.3 - 3.0 \text{ } \mu\text{m}$). Stomatal resistance in the moderately stressed (soil water potential -7 ± 2 bars) plants increased to 24 sec cm^{-1} within one hour after sunrise, then rose to a high of 64 sec cm^{-1} at 12 p.m. Resistances in the extremely stressed (soil water potential -13 ± 2 bars) seedlings dropped rapidly at sunrise and then quickly rose to a daily high of 62 sec cm^{-1} within the hour. A slight decrease in resistance continued through the day (Figure 14). Little evidence for afternoon stomatal closure of non-stressed plants was observed despite leaf water potentials below -18 bars. Stomatal closure at sunset caused reductions in leaf water potentials to -4.4, -9.7 and -14.4 bars near dawn for non-stressed, moderately stressed and extremely stressed plants, respectively.

Stomatal activity varies among species and environments (Slatyer, 1967). Since leaf resistance changes little at leaf water potentials between -6 and -16 bars under no

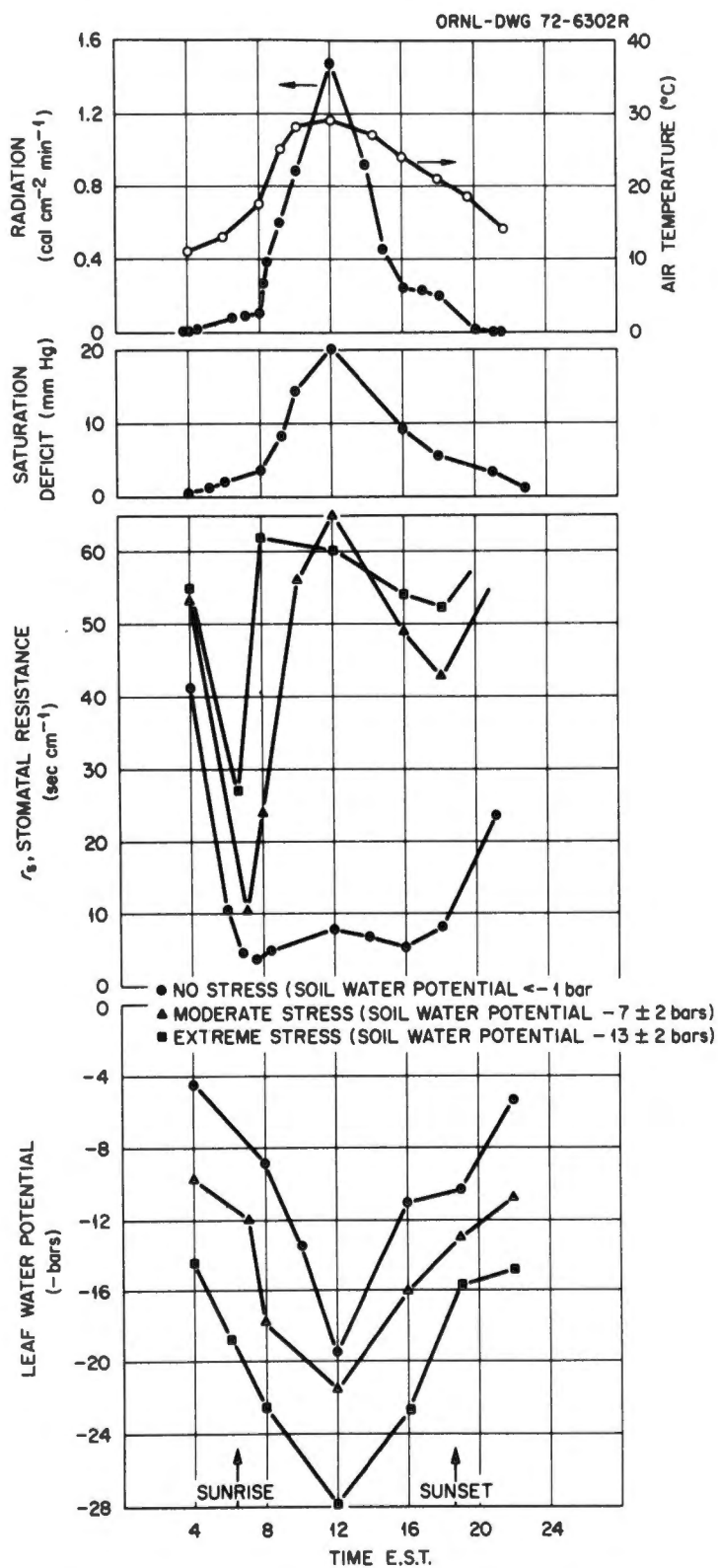


Figure 14. Diurnal variation in radiation, air temperature, saturation deficit, stomatal resistance and leaf water potential of stressed and non-stressed tulip poplar seedlings.

stress conditions, water deficit in itself does not affect stomates until a critical tension is reached. The relationship between stomatal resistance and leaf water potential under laboratory conditions at radiation levels above $0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm) is given in Figure 15. Stomatal resistance increased from approximately 5 to 20 sec cm^{-1} as the foliage potential decreased from -10 to -15 bars. Values <-20 bars consistently produced stomatal resistance values in excess of 45 sec cm^{-1} . The critical tension for stomatal closure in tulip poplar is reached at leaf water potentials <-20 bars. This critical tension value was similar under field conditions (Figure 14, page 64).

The relationship between net photosynthesis and stomatal conductance ($1/r_s$) for sun-grown tulip poplar seedlings is summarized in Figure 16. Photosynthetic rates are linearly related to stomatal conductance, which indicates that the influence of mesophyll resistance is a small part of total resistance, or that it is constant. A few shade leaves were also examined and photosynthesis increased little when stomatal conductance increased above 0.6 cm sec^{-1} . This may be the result of the limiting levels of radiation used or some aspect of mesophyll resistance. This study indicates that stomatal activity is highly correlated to changes in net photosynthetic rate ($r = 0.87$, Figure 16). The predictive value of stomatal information is further

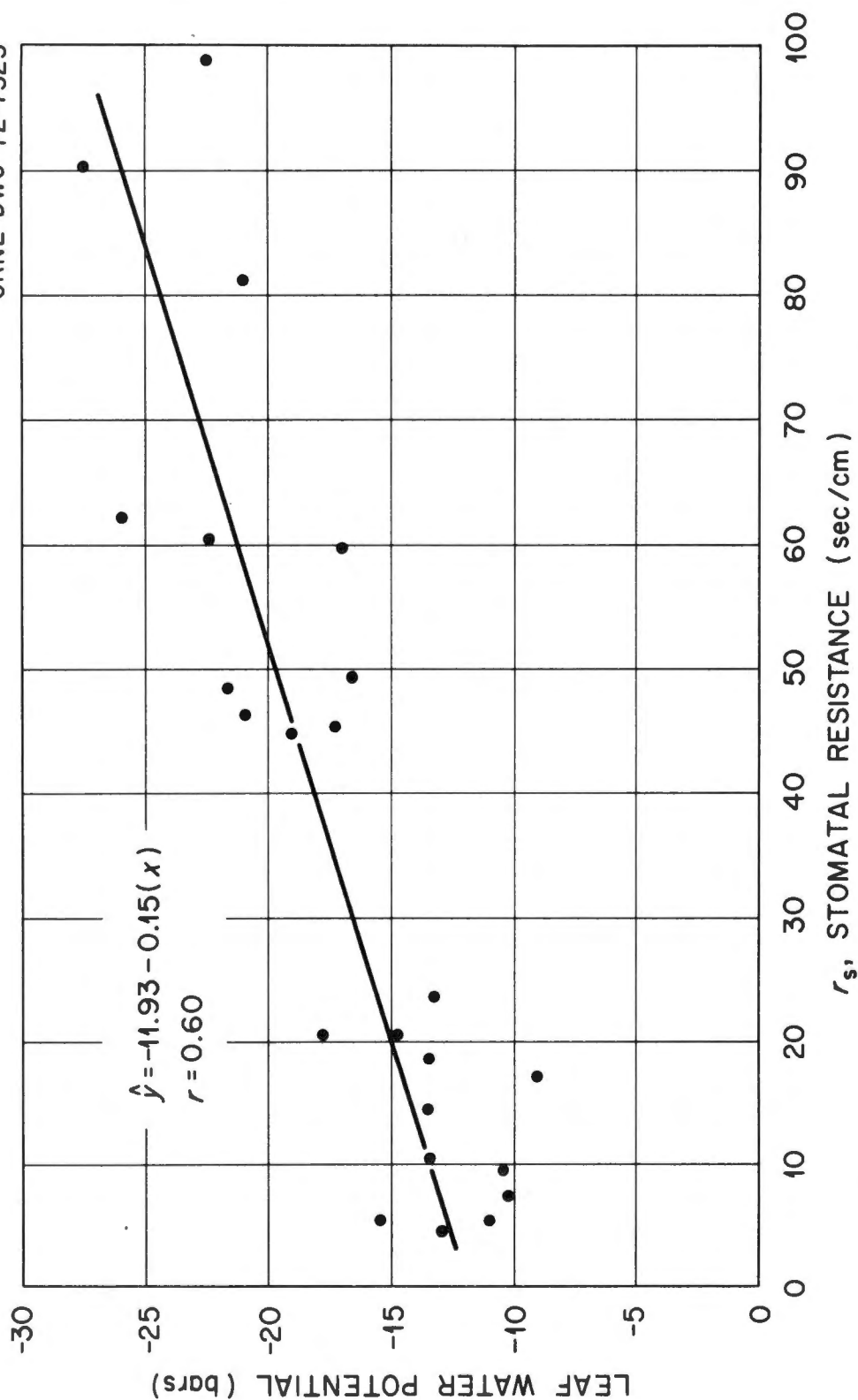


Figure 15. The relationship between leaf water potential and stomatal resistance of radiation levels exceeding $0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm). Conditions: ambient CO_2 concentrations $320 \pm 10 \text{ ppm}$, temperature $26 \pm 2^\circ \text{C}$.

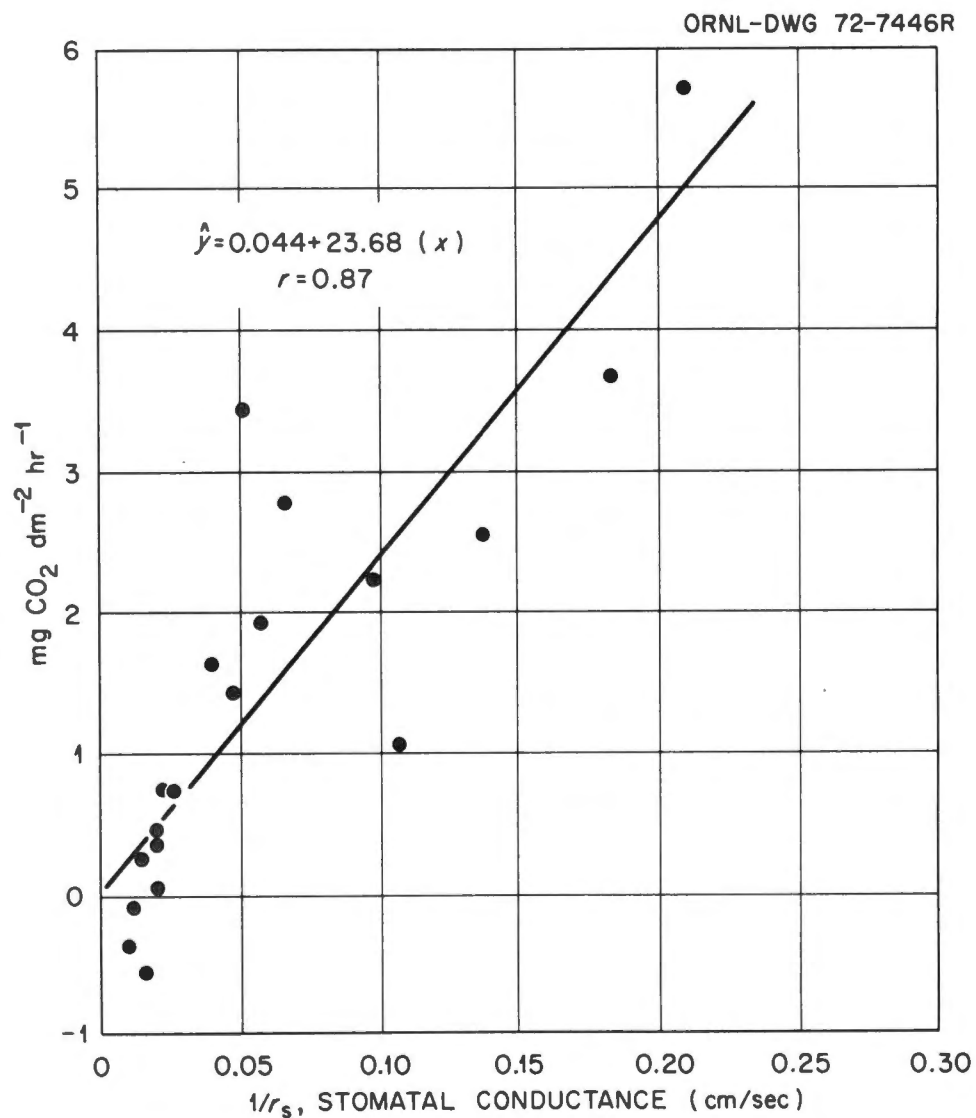


Figure 16. The relationship between net photosynthesis and stomatal conductance ($1/r_s$) for full sun grown tulip poplar seedlings. Conditions: ambient CO₂ concentrations 320 ± 10 ppm, leaf temperature $25 \pm 0.4^\circ$ C, irradiance $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm).

strengthened by recent studies indicating concurrent photosynthetic and stomatal oscillations which are slightly out of phase (Cowan, 1972, personal communication).

In summary, the stomates of tulip poplar open under extremely low radiation levels (Figure 13, page 62) but are slow to respond to rapid changes in intensity. Photosynthetic light saturation occurs well under normal radiation levels. The relationship between leaf water potential and stomatal resistance is highly dependent on previous water conditions (i.e., drying cycle vs. recharge cycle). Leaf water potential mainly restricts photosynthesis as leaf stress increases below -20 bars. The relationship between stomatal conductance and net photosynthesis may prove to be more useful than that of leaf water potential in determining variations in CO₂ uptake rate at the lower water stress levels. The relationship between stomatal resistance and ambient CO₂ concentrations plays an important role in the control of net CO₂ exchange. Determinations of this relationship can also be coupled with measurements of photorespiration, a physiological process which must be evaluated before estimates of gross photosynthesis are possible.

Photorespiration at Different Light Intensities

The rate of CO₂ exchange by tulip poplar seedlings at 25° C is plotted for different external CO₂ concentrations and light intensities in Figure 17. The changes in stomatal

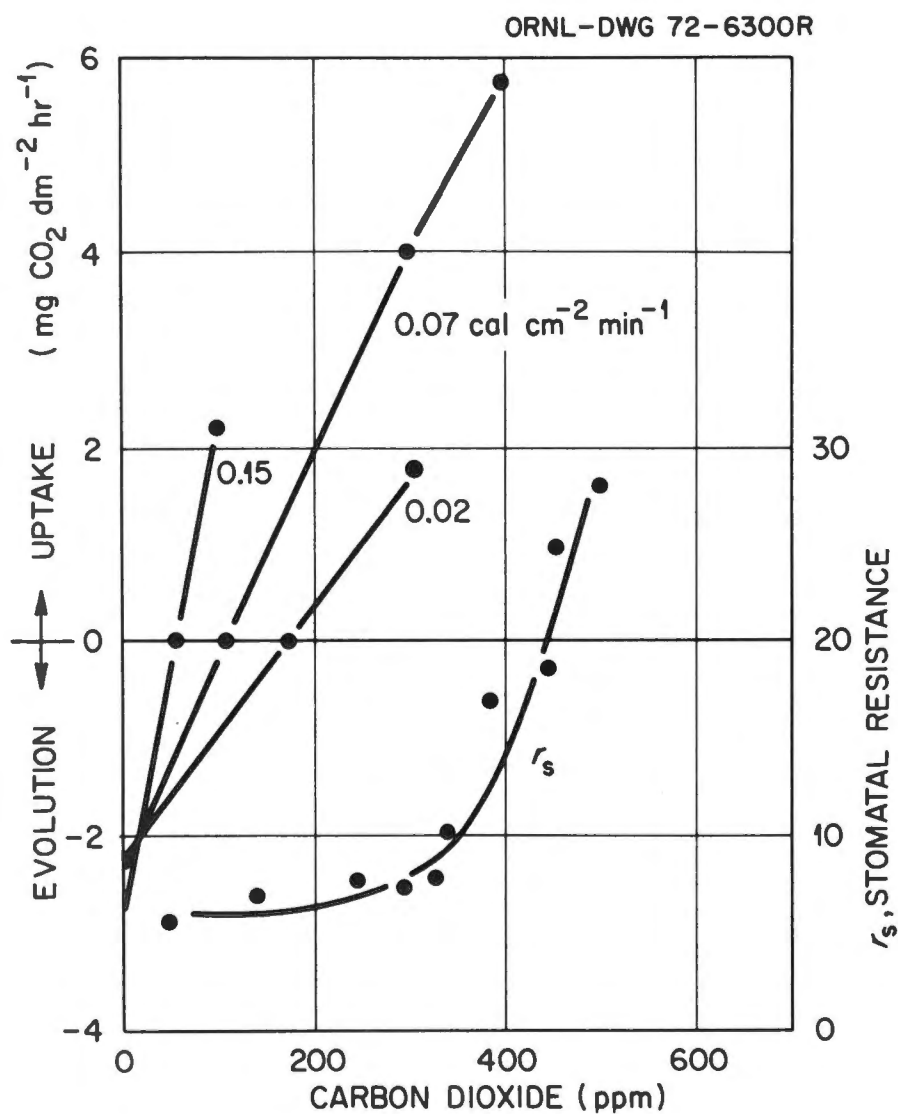


Figure 17. The influence of CO_2 concentrations and different radiation intensities on net photosynthesis and on stomatal resistance (r_s). Conditions: leaf temperature $26 \pm 2^\circ \text{C}$, irradiance measured 400 - 700 nm. (Curves hand-fitted.)

resistance with changing CO₂ concentrations are also shown. A minimal estimate of photorespiration was obtained by extrapolating the curves to zero CO₂ concentrations (Decker, 1957; Tregunna, et al., 1966; Brix, 1968). The technique yielded a value for mean photorespiration of 2.52 mg CO₂ dm⁻² hr⁻¹. This estimate was four times greater than that measured for dark respiration ($2.52/0.63 = 4.0$), which corresponds to the ratio of 4.1 ($2.95/0.72$) reported for tulip poplar by Decker (1957).

Several important inferences may be drawn from this type of analysis. First, the rate of CO₂ exchange was linear from zero to 350 ppm, indicating that CO₂ supply was limiting the photosynthetic rate. There was a general tendency toward increased stomatal opening with decreasing CO₂ concentration. The CO₂ compensation point, the concentration of CO₂ at which the rates of respiration and photosynthesis are equal, is changed if rates of either or both of these processes are altered. A CO₂ compensation point of approximately 59 ppm was observed at 0.15 cal cm⁻² min⁻¹ (400 - 700 nm). A decrease in light intensity from 0.07 to 0.02 cal cm⁻² min⁻¹ resulted in an increase of the CO₂ compensation point from 98 to 177 ppm, indicating that photosynthesis was more restricted by low radiation levels than was respiration.

In summary, a closer estimate of gross photosynthesis for tulip poplar can be obtained (under some conditions) by

multiplying dark respiration values by four. This study did not explore the relationships between photorespiration and respiration under water stress and temperature changes; thus, extrapolation to these conditions is unwarranted.

Seasonal Investigations of the Canopy in the Liriodendron Forest

Determinations of ambient levels of canopy photo-pigments and nutrient status are needed for estimating whether experimentally induced seedling fluctuations are realistic, and for background information for future photosynthesis evaluations in the canopy. Seasonal fluctuations in chlorophyll content have been reported by Schertz (1929), Goodwin (1958) and Sanger (1968). Laboratory experiments during this study have confirmed the sensitivity of pigment fluctuations to seasonal changes and environmental variations. Correlations of pigment acclimations to photosynthetic response were observed experimentally. By following the temporal changes in pigment concentrations and ratios, it may be possible to determine a more efficient means of assessing seasonal net photosynthetic rate changes. The more important portions of the growing season can also be established in order that site productivity comparisons via photopigments can be made while chlorophyll concentrations are at maximum levels.

Temporal changes in photosynthetic pigments from

spring through autumn of 1971 are presented in Figure 18. In early spring, at the time of rapid leaf development, there was an abrupt rise in concentrations of both chlorophylls. Total chlorophyll concentrations peaked in early June, declined during middle July and rose to near June levels by late August. Carotenoids remained fairly constant through the season, but decreased slightly in late September. Figure 19 presents a summary of pigment development in the Liriodendron canopy along with seasonal changes in radiation intensity, precipitation and foliar mineral analysis. For new leaves in early spring the ratio of chlorophyll a/b was relatively low. After leaf expansion, the ratio increased rapidly to 2.54 with little fluctuation thereafter. The summer levels of approximately 2.7 are comparable to values reported for higher plants by Smith and Benitez (1955). Total chlorophyll content (a and b) on an area basis increased rapidly in April from 0.009 mg cm² to a high of 0.045 mg cm² in early June. According to Anderson (1967) a concentration greater than 0.04 mg cm² will seldom receive enough photons to saturate all reactive centers at once (Figure 2, page 17). Thus, by early June the pigment development is sufficient to intercept a large portion of the incident radiation. Hutchison (1971) reported 94 percent interception of the direct beam component by the canopy in June.

To fully understand seasonal pigment development,

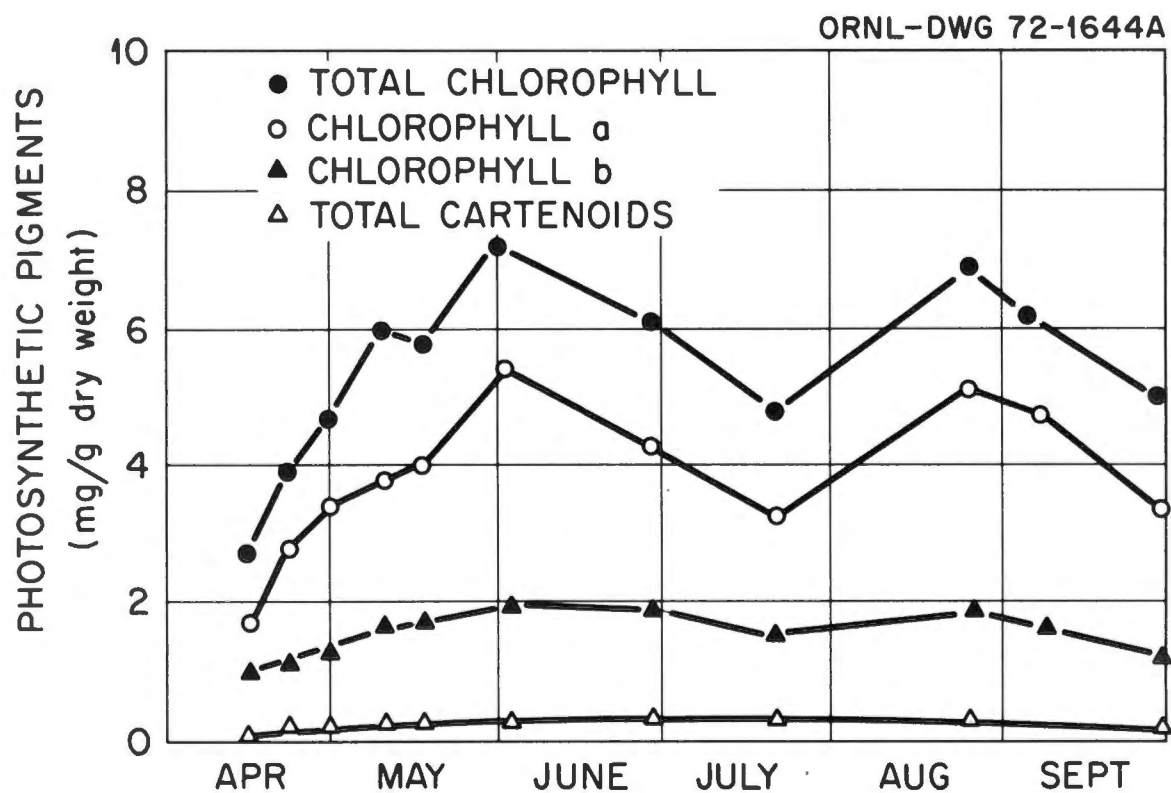


Figure 18. Temporal changes in photosynthetic pigments of tulip poplar during the 1971 growing season.

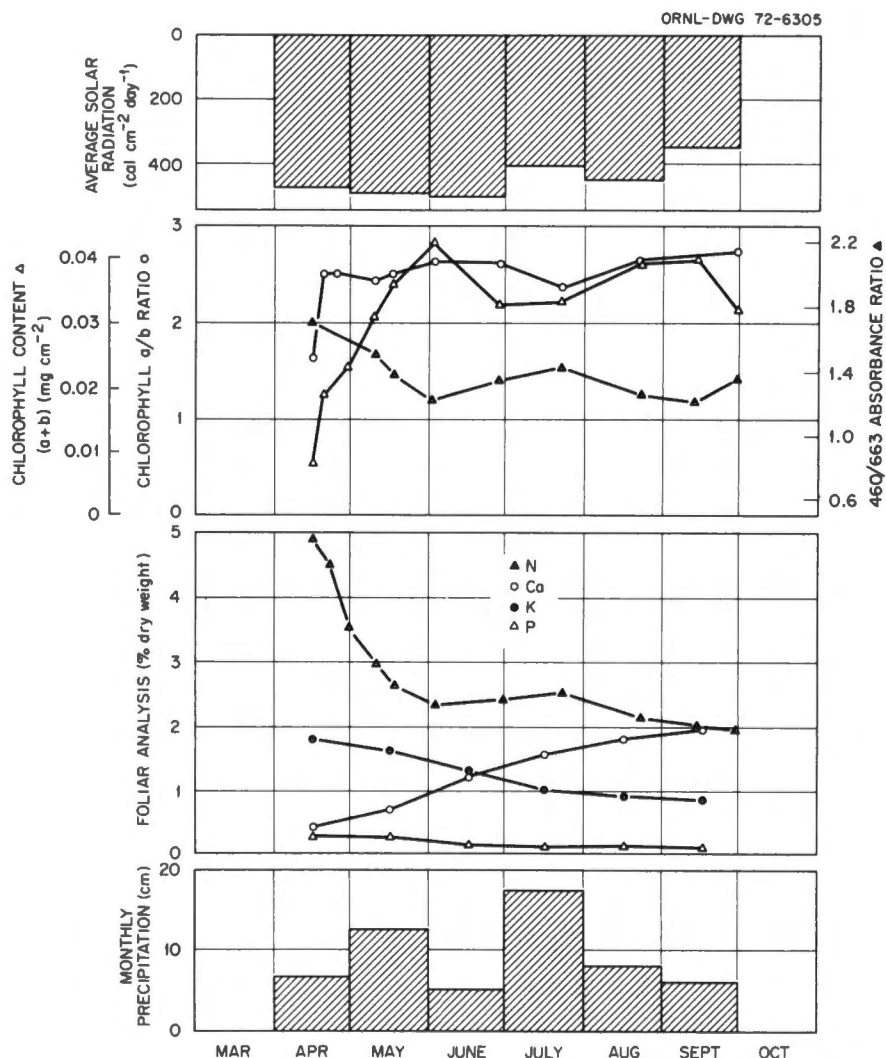


Figure 19. Seasonal foliar pigment and mineral concentrations in the tulip poplar canopy in relation to changes in radiation intensity and precipitation. Leaf chlorophyll content = Δ , chlorophyll a/b ratio = $\circ$, 460/663, absorbance ratio = $\blacktriangle$.

some knowledge of environmental interactions is essential. The drop in chlorophyll content from a high in June of 0.45 mg cm^2 to 0.34 mg cm^2 by mid-July was probably the result of low precipitation and high radiation in June (Figure 19, page 74). A three-fold increase in precipitation during July resulted in an increase in chlorophyll concentrations to near June levels by mid-August. The 460/663 absorbance ratio fluctuated from a seasonal high of 2 in mid-April to a low of 1.2 by mid-September. Both the chlorophyll content and pigment ratios fluctuated through the season and thus may prove useful when related to the seasonal photosynthetic dynamics and canopy development.

Nutrient concentrations associated with temporal leaf and pigment development behaved somewhat differently. As might be expected, calcium concentrations increased through the growing season. Phosphorus and K concentrations diminished through the growing season. Nitrogen decreased from a high of 4.9 percent mg g^{-1} dry weight in mid-April to 2.3 percent by early June. Dickson (1971) stated that N uptake does not appear to be hampered by drought conditions and that a decrease in available water usually decreases growth prior to affecting nutrient uptake. A decrease in growth would result in higher leaf concentration.

Although several pigment ratios were measured

(430/663, 450/663 and 460/663), the most useful ratio to simulate seasonal trends in nutrient status was the 460/663 ratio (Figure 19, page 74). The specific pigments that the ratios actually reflect can in part be determined from Table III. These peaks differ little from reported values (Rabinowitch and Govindjee, 1969), if allowances for variations between workers due to solvent concentrations and instruments are made. From this it would seem that Margalef's (1968) 430/665 ratio is not the carotenoid:chlorophyll ratio that he indicated. In reality it reflects the two peaks of chlorophyll a in the blue and red region. Skeen (1969) proposed the 450/660 ratio as a more meaningful comparison of the carotenoid:chlorophyll ratio of the community. Beta carotene, which is the most prevalent carotenoid in higher terrestrial plants, is reported to absorb mainly in the 450 nm region (Goodwin, 1958). However, unless the tedious carotenoid:chlorophyll separation is made, the carotene peak does not appear because it is masked by chlorophyll b absorbance. Skeen's 450/660 ratio may simply reflect a chlorophyll b:chlorophyll a ratio. The 460/663 ratio proposed by the author is a peak in 80 percent acetone which reflects the shoulder of chlorophyll b and both carotenes in the blue region (Figure 27, Appendix A).

The above arguments indicate that absorbance ratios in a pigment mixture do not directly reflect a carotenoid:

TABLE III

CHARACTERISTIC ABSORPTION PEAKS OF PIGMENTS FROM
TULIP POPLAR LEAVES IN 80 PERCENT ACETONE

Pigment	Blue Region Peak (nm)	Red Region Peak (nm)
Chlorophyll a	430	663
Chlorophyll b	454	645
Alpha carotene	446	475
Beta carotene	452	480

chlorophyll (yellow:green) ratio, a fact, however, which is of minor importance. What is of more importance is that pigment ratios may be useful predictive tools in analysis of terrestrial ecosystems. Seasonal fluctuations of 460/663 absorbance ratios measured in this study correspond closely to oscillations in leaf nitrogen content (Figure 20). The usefulness of this relationship in marine systems has been shown by Margalef (1958, 1968), but little is known about it in terrestrial systems. With the relationship between leaf nitrogen and net photosynthesis (Figure 10, page 55), it should be possible to indicate seasonal photosynthetic capacity indirectly from the absorbance ratio and nitrogen relationship. Jordan (1969) utilized the ratio concept in a structural context by assuming that: (1) leaves absorb more red than infrared and (2) the more leaves are present in the canopy, the greater is the absorption. He compared an 800 nm/675 nm ratio determined with a spectroradiometer on the forest floor in forest sites of varying leaf area indices. By regressing known leaf area index against ratios measured at the forest floor from several locations, he was able to predict the leaf area index throughout the entire forest. Thus, the ratio concept may prove useful both on a structural and a temporal basis in a terrestrial ecosystem. This is especially true for ecosystems with "photosynthetic machinery" altered as a result of fertilization, air

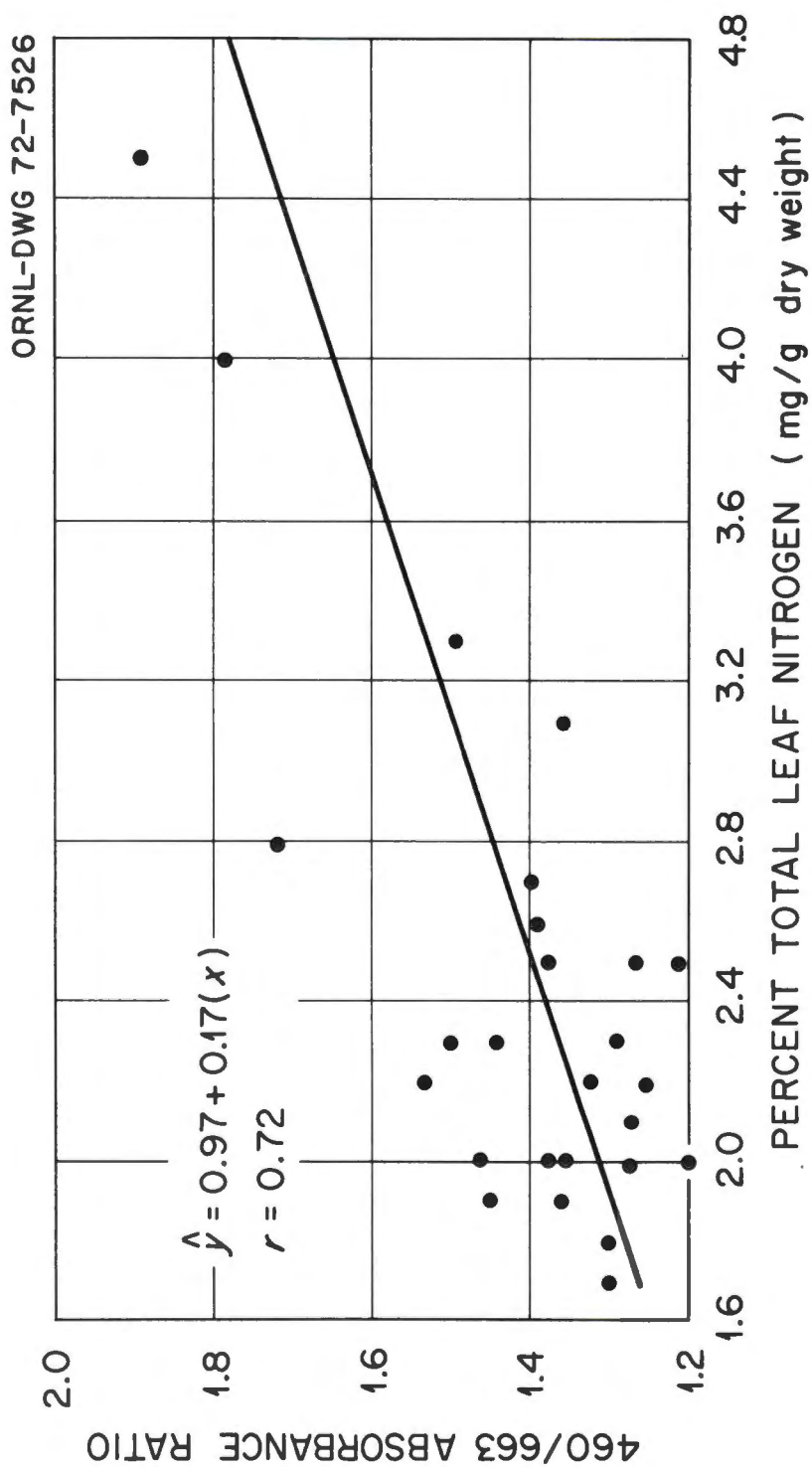


Figure 20. The correlation between seasonal changes in total leaf nitrogen of tulip poplar and the 460/663 absorbance ratio.

pollution, ionizing radiation, or other perturbations. The ratio concept also implies a functional meaning since it indicates potential for photon flux absorption in two regions of different quantum levels. This study definitely indicates that pigment ratios can be used to predict nutrient status. However, more work is needed in this area to explore potential species differences through the season, as well as responses to fertilization and pollution with resulting photosynthetic changes.

Predictions of Net Photosynthesis and Validation of the Index

With the physiological responses of tulip poplar as background, the physiological index was developed and evaluated statistically in terms of empirical relationships between CO₂ uptake, plant parameters, water stress and radiation through the growing season. The use of statistical methods requires a combination of sound ecological and statistical judgments. For example, poor correlations can often prove useful if such data are plotted, and comparisons of the distributions of the different variables disclose significant congruence of patterns (Margalef, 1965). All too often the proper dimensions, parameters, relationships, distributions and derivatives of the data are ignored. Photosynthetic capacity has the characteristic of a derivative of plant and environmental parameters relative to time. Seasonal trends in photosynthesis might

include a parameter which reflects nutrient status and senescence. An example of this is the absorbance ratio 460/663 (Figure 19, page 74). The ratio concept may prove especially useful when the same nutrient is not always limiting if different nutrient deficiencies are adequately reflected by predictable ratios. Daily variations in photosynthetic capacity might incorporate stomatal resistance as an estimator of CO₂ exchange capacity.

Prediction over any time interval will require some assessment of limiting or rate controlling parameters and will further require some prior knowledge of potential photosynthetic rates and their modification by water status, nutrient levels, chlorophyll content and ratios, radiation and temperature. The data often do not approach normal distributions and transformations are required. A logarithmic, reciprocal or quadratic transformation often appears appropriate, such that one can obtain a linear predictive model which, though it may be in some senses unrealistic, at least reproduces the main features of the behavior of the response under study (Draper and Smith, 1966).

Data from Experiment I and physiological information from other experiments provided the basis for preliminary parameter selection. The parameters and transformations used are shown in Table IV. The most accurate parameters for predicting net photosynthesis were selected through

TABLE IV

PLANT AND ENVIRONMENTAL PARAMETERS EXAMINED FOR
POSSIBLE INCLUSION IN THE PHYSIOLOGICAL INDEX

Variable Number	Variable Name	Units
Plant		
1	Chlorophyll (a + b)	mg cm ⁻²
2	Chlorophyll (a + b)	mg g ⁻¹ dry weight
3	Chlorophyll (a)	mg cm ⁻²
4	Chlorophyll (a)	mg g ⁻¹
5	Pigment ratio (optical density 430/663)	-
6	Pigment ratio (optical density 450/663)	-
7*	Pigment ratio (optical density 460/663)	-
8*	Leaf water potential	bars
9	Stomatal resistance**	sec cm ⁻¹
10*	Total leaf nitrogen	mg g ⁻¹ dry weight
11-20	Logarithmic (e) transformations	variables 1 - 10
21-30	Reciprocal transformation	variables 1 - 10
31-40	Quadratic transformation	variables 1 - 10
Environmental		
41*	Radiation	cal cm ⁻² min ⁻¹
42*	Soil water potential	bars
43-44	Log _e transformation	variables 41-42
45-46	Reciprocal transformation	variables 41-42

*These variables are included in general equation three (3),
page 83.

**This variable was measured only during the 1972 growing
season.

step-wise multiple regression analysis on the basis of variation accounted for (R^2) and significant (F).

Prediction of net photosynthesis (P) in the present study is presented as a function of several plant and environmental parameters: leaf chlorophyll (a + b) content (C), absorbance ratio 460/663 (A), percent total leaf nitrogen (N), leaf water potential ($L\psi$), soil water potential ($S\psi$) and radiation levels (R). The general equation tested is as follows:

$$P = b_0 + b_1(C) + b_2(A) + b_3(N) + b_4(L\psi) + b_5(S\psi) + b_6(R) \quad (3)$$

where (P) is measured in $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$, (C) in mg cm^2 , (A) the 460/663 ratio, (N) in mg/g dry weight , ($L\psi$) in bars, ($S\psi$) in bars and (R) in $\text{cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm).

The first analysis was conducted on data from 100 percent full sun leaves from the 1971 water stress experiment. Data from one harvest period (27 data points) and radiation level ($0.05 \text{ cal cm}^{-2} \text{ min}^{-1}$ 400 - 700 nm) during the height of the growing season were used. The correlation matrix between the physiological parameters for sun leaves and net photosynthesis is presented in Table V. As expected, there is a strong positive correlation (+0.854) between leaf and soil water potential, and both are negatively correlated (-0.718 and -0.768, respectively) with net photosynthesis. Due to the nature of the ratio there exists a strong negative correlation (-0.936) between

TABLE V

CORRELATION MATRIX BETWEEN SEVERAL PARAMETERS OF TULIP
 POPLAR (*LIRIODENDRON TULIPIFERA* L.) ASSIMILATING AT
 320 ± 10 PPM CO_2 , $26 \pm 2^\circ \text{C}$ AND 0.05 CAL CM^{-2}
 MIN^{-1} (400 - 700 NM); LOG TRANSFORMATION
 (LEAVES GROWN 100 PERCENT FULL SUN)

Parameters ^a	A	B	C	D	E	F
A	1	-0.936	-0.423	-0.507	0.376	0.358
B		1	0.339	0.434	-0.109	-0.441
C			1	0.854	-0.194	-0.718
D				1	-0.169	-0.768
E					1	-0.439
F						1

^aA = chlorophyll (a + b) mg cm^{-2} ; B = 460/663 (optical density of acetone pigment extractions); C = soil water potential (bars); D = leaf water potential (bars); E = percent nitrogen g/g dry weight; F = net photosynthesis $\text{mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$.

chlorophyll content and the absorbance ratio. Chlorophyll content is positively correlated (+0.358) with net photosynthesis. Both absorbance ratio and percent nitrogen, potential "seasonal indicators" (Figure 19, page 74) are negatively correlated (-0.441, -0.439) with net photosynthesis. The negative correlations of these "seasonal indicators" are due partially to a lack of data from other harvest periods, the nature of the ratio itself and the low light intensities utilized in this portion of the study. The highest order partial correlations for variables A-E (Table V, page 84) are 0.83, 0.70, -0.91, -0.93 and -0.99, respectively.

Multiple regression analysis resulted in a (R^2) of 99 percent. The equation for this relationship was:

$$\begin{aligned} \ln(P) = & 11.02 + 1.45 (C) + 1.01 \ln(A) - 0.18 \ln(S\psi) - 1.25 \\ & \ln(L\psi) - 2.5 \ln(N) \end{aligned} \quad (4)$$

where the terms are defined as above. A reciprocal transformation proved equally useful but the second order transformation did not contribute to the variation attributable to the regression. The use of this type of equation is limited in the environment since it does not take into account differences in canopy position or temporal rate changes. It does, however, show that photosynthetic variation can be described through the use of various plant parameters.

The combination of sun and shade leaf data from three harvest intervals (81 data points) during the growing season for one radiation level reduced the R^2 value to 69 percent, but provided a more useful equation for evaluating seasonal variation. The usefulness of chlorophyll content and leaf nitrogen to explain variation was reduced because of the low light intensities used in this part of the study. Significant correlation ($r = 0.829$ and $r = 0.613$) between chlorophyll a content and photosynthetic rate have been shown by Sésták (1966) under full irradiation and 50 percent irradiation, respectively. Results from the present study indicated a correlation ($r = 0.86$) between chlorophyll content (a + b) and net photosynthesis of sun leaves under conditions of no water stress and $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ (400 - 700 nm) (Figure 6b, page 47). A high correlation between leaf nitrogen and net photosynthesis under saturating light is shown in Figure 10, page 55. Therefore, both of these variables were useful in predicting potential CO_2 exchange rates variations under higher light intensities.

In all relationships tested, some indication of plant-water status was necessary to obtain realistic predicted values. The removal of leaf and soil water potential reduced the R^2 value to 19 percent. Inclusion of either of these increased the R^2 to 46 percent. The variation attributable to radiation alone for sun and shade

leaves under no water stress (< -20 bars) was only 20 percent. By separating sun and shade leaves, radiation accounted for 36 percent of the variation. Pooling of sun and shade leaf data from one growth period (three radiation levels and varied water stress levels) provided a single regression equation that accounted for 68 percent of the variation in net photosynthesis.

By using data from 430 data points covering the widest range of fluctuating water levels, shade environments and radiation intensities encountered during the latter part of the 1971 season, a single regression was generated which accounted for 61 percent of the variation in net CO_2 exchange. The best equation for this relationship was:

$$P = 6.82 + 1.07 \ln(C) + 0.41 \ln(L\psi) + 0.63 \ln(R) - 0.28 \ln(S\psi) \quad (5)$$

where variables are as previously defined. The non-significance and resulting deletion of the absorbance ratio from the equation may be due to a lack of sufficient variation of the ratio (Table IX, Appendix B) in the seedling data over the latter part of the season. The controlling variables were chlorophyll content, radiation intensity and soil water potential. From other phases of this study it was apparent that inclusion of stomatal information should increase the R^2 significantly.

Analysis under low water stress levels (drying vs.

wetting cycles), and readings from morning and afternoon measurements indicated problems with hysteresis effects between leaf and soil water potentials and net CO₂ exchange. It has been shown by many researchers that some estimate of leaf resistance is necessary when estimating rapid changes in photosynthetic potential (Zelitch, 1971). Waggoner and Turner (1971) have also shown increases in stomatal resistance from the top to the lower part of the canopy as well as the seasonal fluctuations. The high correlation ($r = 0.87$) between net photosynthesis and stomatal conductance ($1/r_s$) (Figure 16, page 67) indicated that the addition of this parameter could aid in daily estimates of CO₂ exchange.

Regression analysis on water stress and shade experiment data (46 observations) early in the 1972 growing season utilized stomatal resistance data along with previously mentioned variables. The best relationship on the basis of variation accounted for ($R^2 = 67$ percent) and significance (F) was:

$$\begin{aligned} \ln(P) = & -8.07 + 0.15 \ln(S\psi) - 3.20 \ln(C) - 0.05 \ln(A) - \\ & 0.33 \ln(L\psi) - 1.86 \ln(N) - 0.43 \ln(r_s) \end{aligned} \quad (6)$$

where r_s is the stomatal resistance in sec cm^{-1} and the other terms defined as in equation (3), page 83. An independent test of equation (6) used net photosynthetic data from 24 randomly selected tulip poplar seedlings selected

in May, which had been grown under varying levels of shade and water and analyzed at one radiation level. Figure 21 presents a comparison of observed and predicted photosynthetic values. In the above analysis the parameter accounting for most of the variation (36 percent) in net CO_2 exchange was stomatal resistance. The second most important parameter was chlorophyll content, which increased the R^2 significantly. The predictive value of stomatal resistance and the use of chlorophyll content early in the growing season are substantiated.

To further test the value of stomatal and other pigment ratios, stepwise deletion regression analysis was utilized to indicate the best relationship in terms of variation explained (R^2) and significant (F) from data of five harvest periods (varied shade, nutrient and water levels) in the 1972 growing season. The following relation was derived:

$$P = -12.20 + 36.00(A) + 3.32(M) - 27.90(\text{Ct}) - 0.04(r_s) \quad (7)$$

where (M) is the Margalef ratio (430/663), (Ct) is the carotenoid ratio (450/663) and the other terms are defined as before. The standard error of estimate was 0.74 mg $\text{CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$ and the R^2 71 percent. No transformations were needed because of the nature of the ratios and the elimination of radiation levels. This equation indicates that ratios may prove useful seasonally and in fertilization

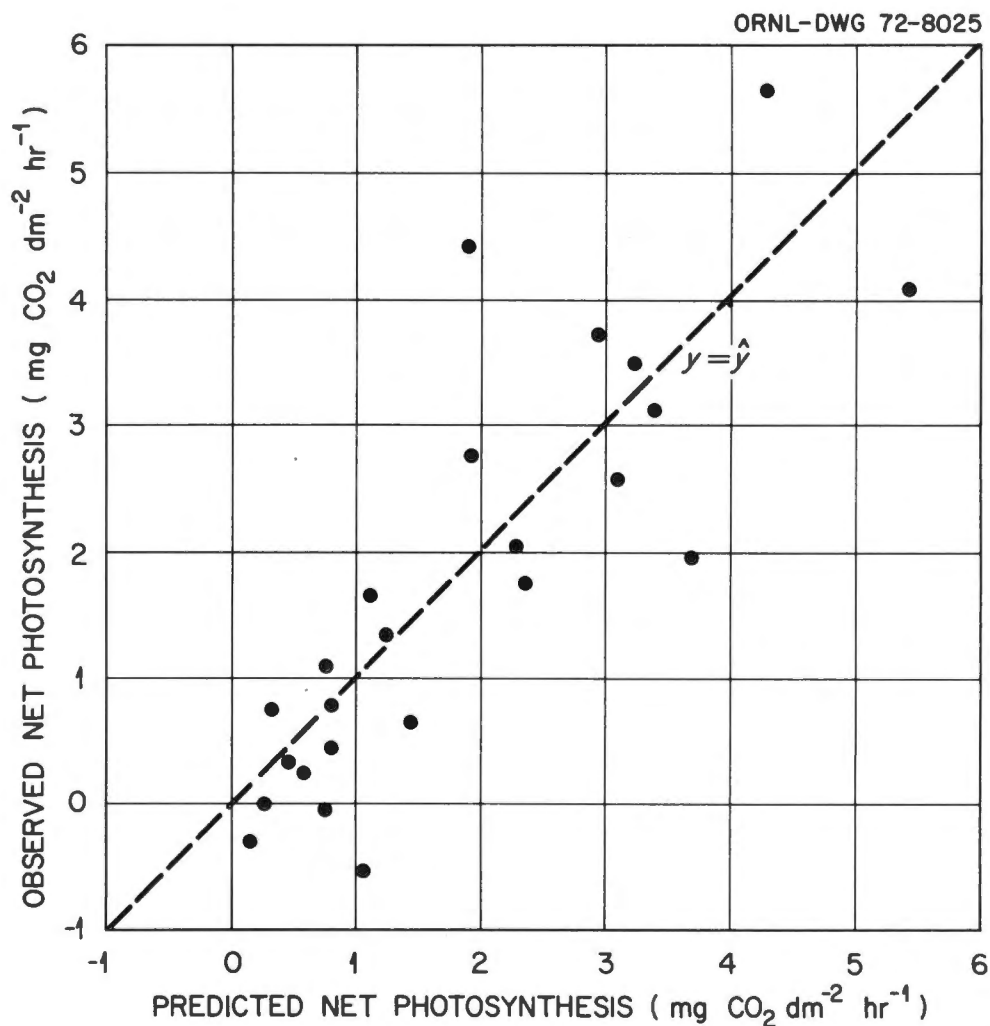


Figure 21. A comparison of observed and predicted photosynthesis values for tulip poplar seedlings. (Measured at $0.07 \text{ cal cm}^{-2} \text{ min}^{-1}$ 400 - 700 nm). Standard error of estimate $0.72 \text{ mg CO}_2 \text{ dm}^{-2} \text{ hr}^{-1}$.

studies if some estimate of leaf resistance is included.

While the equations are restricted to tulip poplar and the other constraints of this study, it is now evident that plant and environmental parameters can be combined for predictive purposes. It is apparent that simple empirical relations can be developed which accurately estimate net photosynthesis rates. One of the major strengths of this approach is the simplicity of the model. The most useful plant-environmental variables will vary as a result of species differences, site characteristics and perturbations. For example, this study indicates that forest fertilization studies should consider analysis of several ratios, chlorophyll content and possibly foliar nutrient concentrations (i.e., the solution can be weighted for these variables) along with some estimate of leaf resistance and radiation. Studies by Majernik and Mansfield (1972) and Wellburn, et al., (1972) have shown that stomatal resistance and pigment content are obviously affected by NO_x and SO_2 , thus both parameters should prove useful in NO_x and SO_2 studies. Environmental variables that have promise in this study are radiation and soil water potential. The inclusion of temperature (T^2) is questionable since its addition by both Botkin (1969) and Helms (1972) to an equation utilizing only radiation data did not significantly increase F or R^2 values.

For the sake of simplicity, a general equation of

use under a variety of conditions through the season is as follows:

$$\text{Ln}(P) = b_0 + b_1 \text{Ln}(C) + b_2 \text{Ln}(A) + b_3 \text{Ln}(r_s) + b_4 \text{Ln}(R) \quad (8)$$

where the variables are defined as before. This form should reflect photon flux absorption potential, nutrient status, water-temperature-resistance relationships and radiation influences.

The real value of this type of equation, besides predicting net CO_2 exchange at any one point in time, is that it provides an estimate of whether maximum net photosynthesis is or can be attained. For example, the amount, type and optimum time of fertilizer applications could be rapidly ascertained in terms of net photosynthetic response. Evaluation of photosynthetic potential in accordance with plantation planting densities (competition for nutrients, light, water, et cetera) could be used to develop more appropriate thinning procedures.

A potentially important use of this approach concerns plant responses to pollutants. The effect of heavy metals, gases, or particulate matter, et cetera, on physiological plant parameters and in turn net photosynthetic potential can be quantified rapidly without destructive sampling. Clones of plants sensitive to pollution could be placed at isopleths to "biologically integrate" critical fluctuations and/or accumulations of

the pollutant. Phytometers could be maintained over extended periods, and physiological information quantified once an index was established. Important estimates of biological effects of critical pollution levels, as well as evaluation of the functional aspects of the site, could then be determined rapidly and accurately.

The success of the index in determining photosynthetic rates and the method presented in this study, in spite of the constraints and incompleteness, should be encouraging to those interested in dealing quantitatively with the physiological dynamics of ecosystems.

Site Evaluation and Optimization of Net Photosynthesis by Integration of Multiple Linear Regression and Linear Programming

The optimization of production levels is the result of maintaining maximum net photosynthetic rates with minimum respiration (dark and photo) and maintenance costs. Assessment of the plant-environmental response and site evaluation is possible using linear programming. In this approach multiple linear regression functions can be used as objective functions along with appropriate physiological and site constraints on nutrients, "photosynthetic machinery," water balance, planting densities, et cetera. The methodology and ecological applications of linear programming were summarized by Hadley (1962) and Van Dyne (1966) and will not be covered here. A preliminary scheme

of quantifying the influence of site variables and maximizing physiological parameters is presented.

Most environmental site factors themselves cannot be manipulated. However, it is possible to regulate species composition, densities, fertilization levels and in some cases water status at the ecosystem level. A knowledge of the functional relation between the physiological and site conditions could be utilized to maximize or minimize vegetation parameters (i.e., biomass, protein yield, lignin composition, seed production), or for selection of the best species component in terms of production rates for that site.

The empirical model from multiple linear regression analysis in this study relates the independent physiological and site variables and the dependent plant response (equation 5, page 87). The relationship between the regression model and the linear programming model utilizing matrices may be formulated as follows:

$$Y_{1 \times 1} = X_{1 \times m} B_{m \times 1} \quad (9)$$

where the regression equation (9) becomes the object function (10),

$$f_{\max} = X_{1 \times m} B_{m \times 1} \quad (10)$$

which is maximized according to the constraints (11),

$$A_{1xm} X_{1xm} \leq C_{mx1} \quad (11)$$

where A and C are a matrix and column vector, respectively. The selection of values of the x variables in the regression equations which maximize or minimize the plant response can often be determined by a set of inequalities such as:

$$A_{11} X_1 + A_{12} X_2 + \dots + A_{1m} X_m \leq C_1 \quad (12)$$

$$A_{21} X_1 + A_{22} X_2 + \dots + A_{ij} X_j \dots + A_{im} X_m \leq C_i$$

$$A_{ni} X_1 + A_{n2} X_2 + \dots + A_{nm} X_m \leq C_n$$

where the A_{ij} and C_i are known constants (Spivey, 1963; Van Dyne, 1966). Linear programming also requires that the value of X_i variables be positive ($X_i \geq 0$ for all i).

In this study photosynthate could be expressed as a linear function of site and physiological variables. Constraints which are inherent relations (chlorophyll a + b = total chlorophyll, et cetera) imposed to make the solution realistic (i.e., relationship of percent leaf nitrogen 2 percent $\leq N \leq$ 4 percent mg/g dry weight, leaf chlorophyll (a + b) 0.01 $\leq C \leq$ 0.05 mg cm⁻², leaf water potential 8 $\leq L\psi \leq$ 20 bars, absorbance ratio 1.0 $\leq A \leq$ 4.0, soil water potential 1 $\leq S\psi \leq$ 15 bars, stomatal resistance 3 $\leq r_s \leq$ 40 sec cm⁻¹, radiation 0.4 $\leq R \leq$ 2.0 cal cm⁻² min⁻¹, and temperature 20 $\leq T \leq$ 32° C to net photosynthesis) and those used to evaluate biological factors could be

utilized with an object function (13) to maximize CO_2 exchange rates.

$$Y = b_0 + b_1 N_1 + b_2 C_2 + b_3 A_3 + b_4 r_{s4} \dots b_n X_n \quad (13)$$

Through this type of approach, the proper classification and utilization of land can be made so that production rates are maintained at maximum levels of efficiency. The complexities of ecosystems and the lack of current ecological techniques to adequately interpret these parameters may temporarily restrict the use of the physiological index.

IV. SUMMARY AND CONCLUSIONS

The objectives of the study were to develop and test the concept of a physiological index as a predictive tool in estimating potential production through analysis of net photosynthesis. Seedlings of tulip poplar were maintained under three light environments: 100, 50 and 25 percent full sunlight; three water environments: <-1 , -2 to -9 , and >-9 bars, and three nitrogen levels: 300, 100, and 33 ppm; throughout the growing season. The experiments had the following objectives: (1) to find which plant and environmental parameters were the most useful in estimating net CO_2 exchange under varied conditions, (2) to determine the amplitudes of physiological processes (photorespiration, diurnal water flux, seasonal pigment development, et cetera) which characterize tulip poplar, and might prove useful in an index, (3) to investigate possible relationships between photopigment ratios and nutrient status, and (4) to define empirical relationships between CO_2 uptake and plant environmental relationships (leaf chlorophyll content (a + b), percent total leaf nitrogen, stomatal resistance, radiation, and leaf and soil water potential).

Seasonal values of foliar pigments, nutrient content, radiation and precipitation were also collected in the Liriodendron forest to investigate relationships and

techniques that would enable future development of the index in the forest. Multiple linear regression analysis was employed to estimate coefficients in a linear model relating physiological and environmental variables and net CO₂ exchange.

A summary of the physiological dynamics of tulip poplar from the laboratory experiments are as follows:

(1) Net photosynthesis was reduced significantly in seedlings raised in the shade and under water stress.

(2) Seasonal values of Net Assimilation Rate and Relative Growth Rate followed chlorophyll and nitrogen content and foliage development.

(3) A high linear correlation ($r = 0.86$) between net photosynthesis and chlorophyll content ($a + b$) was found for leaves with leaf water potentials < -20 bars.

(4) Photosynthetic activity measured at light saturation had a high positive correlation ($r = 0.91$) with percent foliar nitrogen content over a wide range when the covariance of leaf age was considered.

(5) The photosynthetic rate of nitrogen "deficient" plants (< 1 percent N) amounted to only 49 percent of the rate of specimens supplied with adequate amounts (> 2.0 percent) of N.

(6) Light saturation and the light compensation point occurred at a radiation intensity of approximately 0.20 and 0.006 cal cm⁻² min⁻¹ (400 - 700 nm), respectively.

(7) At radiation levels greater than $0.1 \text{ cal cm}^{-2} \text{ min}^{-1}$ ($0.3 - 3.0 \text{ } \mu\text{m}$) there was little change in stomatal resistance, but the resistance increased sharply when incoming radiation decreased below $0.05 \text{ cal cm}^{-2} \text{ min}^{-1}$ ($0.3 - 3.0 \text{ } \mu\text{m}$).

(8) Leaf water potential significantly restricted net photosynthesis as leaf stress increased below -20 bars.

(9) Stomatal conductance ($1/r_s$) was highly correlated ($r = 0.87$) to changes in net photosynthesis.

(10) Photorespiration was calculated to be four times greater than dark respiration ($2.52/0.63 = 4.0$).

(11) The CO_2 compensation point of tulip poplar at light saturation was approximately 59 ppm.

Canopy analysis revealed that: (1) Both the chlorophyll content and the absorbance ratio fluctuated seasonally; (2) foliar nitrogen decreased from a high of 4.9 percent in April to approximately 2.3 percent in September; (3) absorbance ratio changes were closely correlated ($r = 0.70$) to seasonal fluctuations in nitrogen content; and (4) a decrease in mid-season chlorophyll content was related to mid-season drought conditions.

Prediction of net photosynthesis was presented as a function of several plant and environmental parameters: leaf chlorophyll (a + b) content, absorbance ratio 460/663, percent leaf nitrogen, leaf water potential, soil water

potential, stomatal resistance, and radiation levels. Highest correlations were obtained for sun leaves from one harvest period when 99 percent of the variation in net photosynthesis could be accounted for. The combination of sun and shade leaf data for three time periods reduced the R^2 value to 69 percent. In all the relationships tested, some indication of plant water status was necessary to obtain realistic predicted values. The removal of leaf and soil water potential reduced the R^2 value to 19 percent. Inclusion of either of these increased the R^2 to 46 percent. The variation attributable to radiation alone for sun and shade leaves under no water stress (<-20 bars) was only 20 percent. By separating sun and shade leaves, radiation accounted for 36 percent of the variation. A single regression was generated which accounted for 61 percent of the variation in net CO_2 exchange when data were utilized from 430 points covering the widest range of fluctuating water levels, shade environments and radiation intensities through the growing season. The most important plant and environmental factors for predictive purposes were: (1) stomatal resistance, (2) radiation, (3) absorbance ratio, and (4) leaf chlorophyll content.

In conclusion, simple empirical relations utilizing stomatal leaf resistance, absorbance ratios, chlorophyll

content, and radiation can be developed which accurately estimate photosynthetic capacity under a variety of conditions.

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APPENDIXES

APPENDIX A

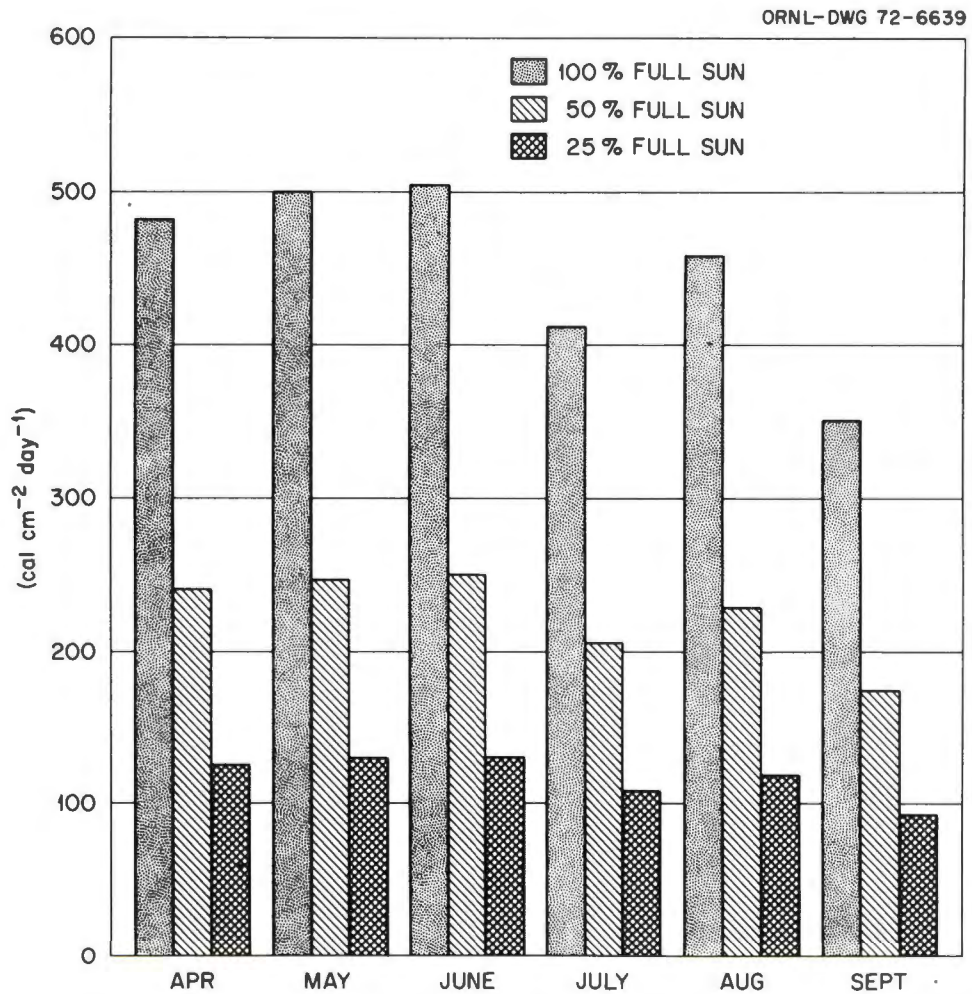
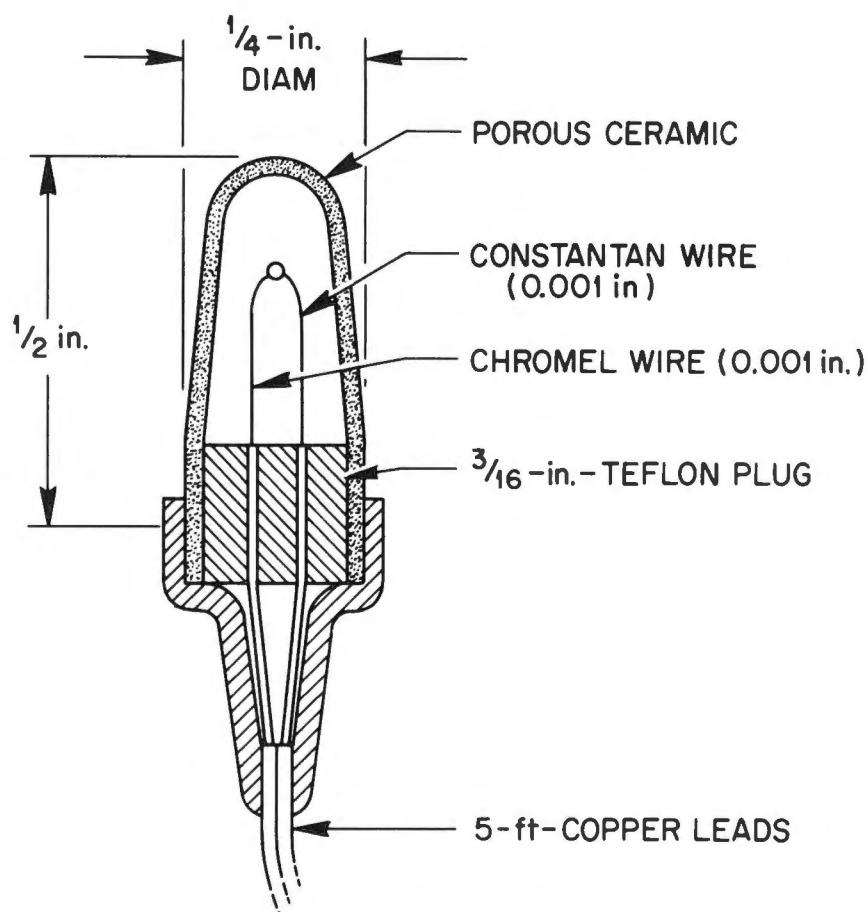


Figure 22. A summary of the average daily irradiance for each light treatment during the 1971 growing season.

ORNL - DWG 71-9706



OUTPUT: $0.47 \mu\text{v}/\text{bar} \pm 5\%$ AT 25°C

RANGE: -1 TO -50 bars WATER POTENTIAL

ACCURACY: ± 0.15 bar WITH INDIVIDUAL CALIBRATIONS

Soil Psychrometer (Wescor, Model PT51-05)

Figure 23. Thermocouple psychrometer used to measure soil water potential (bars).

TABLE VI
SUMMARY OF PHYSICAL AND CHEMICAL PROPERTIES OF EMORY SILT LOAM^a

Bulk Density (g/cm ³)	Soil Texture ^b		pH	Organic ^c Matter (Percent)	Availabled Phosphorous ppm	Exchangeable ^e Cations (meq/100 g)		Totals		
	Silt	Sand				K	Ca	(Per- cent) ^f N	ppm P	(Per- cent) K
0.94	58.62	14.77	13.56	5.9	4.65	12.1	0.32	8.2	0.21	440 0.53

^aModified from Francis (1971).

^bFrom Cox (1971).

^cBy Walkley-Black procedure as described by Jackson (1958).

^dAvailable phosphorous as determined by Bray No. 1, Soil-to-Volume ratio, 1 g:20 ml.

^eExtracted by 1 N NH₄OH, pH 7.

^fSemimicro-Kjeldahl analysis (Black, 1965).

TABLE VII

COMPOSITION OF NUTRIENT SOLUTIONS BEFORE N^a WAS ADDED

Salt	Mg/Liter	Major Ions	Molarity
Macronutrients			
MgSO ₄ · 7H ₂ O	246	Mg ++	.001
KCl	223	K +	.002
Ca(H ₂ PO ₄) ₂ · H ₂ O	252	Ca ++	.003
CaSO ₄ · 2H ₂ O	172	SO ₄ -	.002
		Cl -	.002
		H ₂ PO ₄ -	.003
Micronutrients			
H ₃ BO ₃	2.86		
MnCl ₂ · 4H ₂ O	1.81		
ZnSO ₄ · 4H ₂ O	0.22		
CuSO ₄ · 5H ₂ O	0.08		
MoO ₃	0.03		
Fe-EDTA (Sequestrene) ^b	1.00		

^aNitrogen source was Urea CO(NH₂)₂.^bFerric potassium ethylenediamine tetraacetate complex.

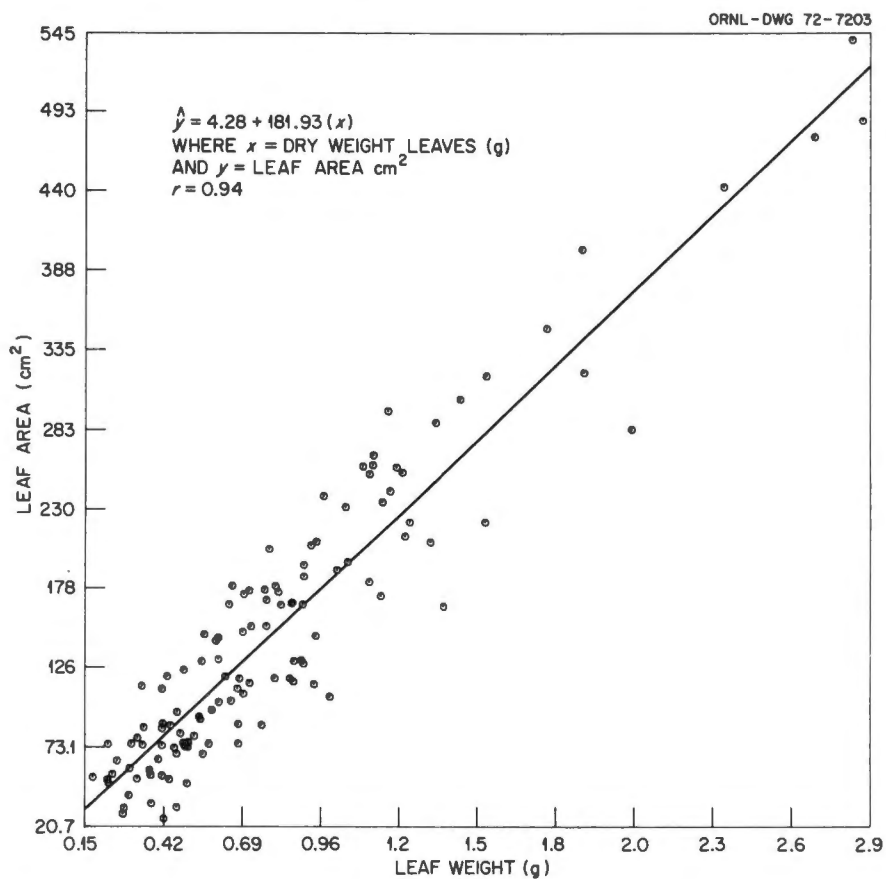
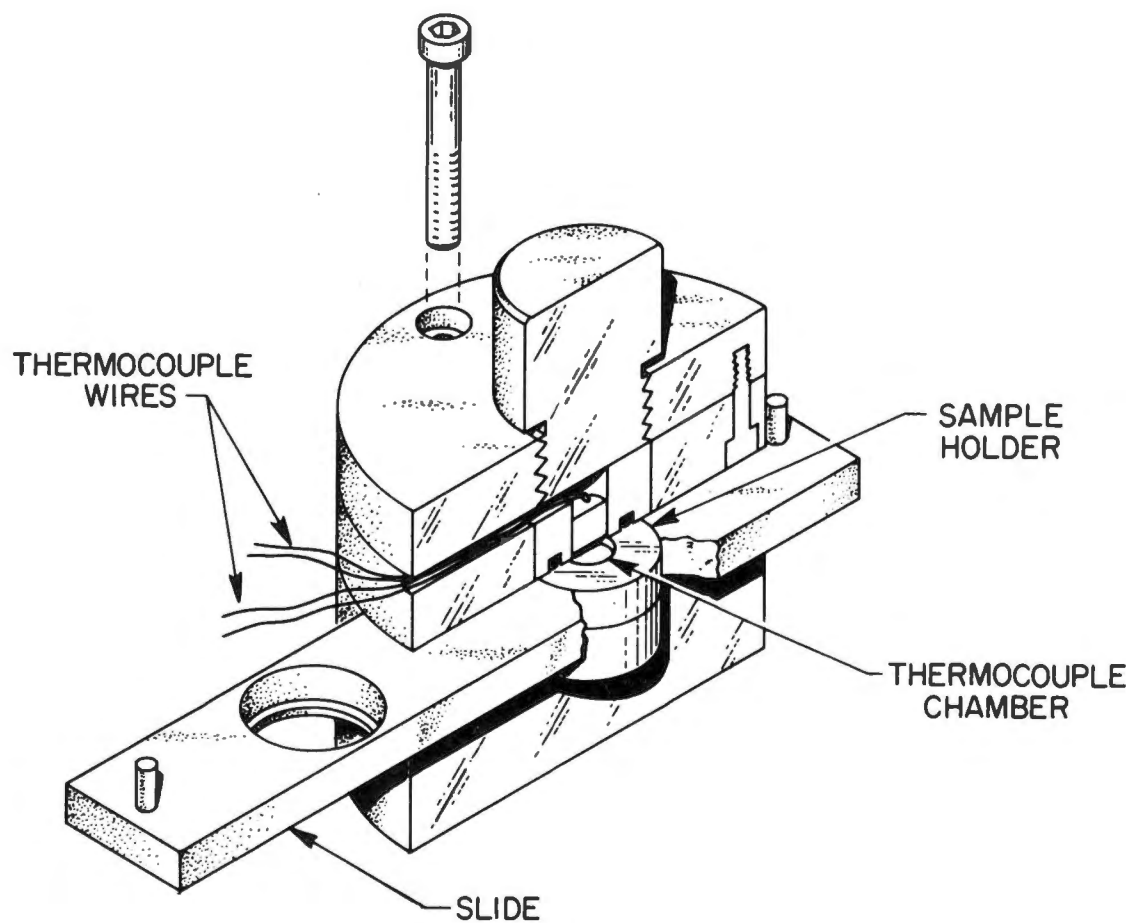


Figure 24. Allometric relationship of tulip poplar leaf dry weight to leaf area.

ORNL-DWG 71-9705



ACCURACY: ± 0.15 bar
RANGE: 0 TO -50 bars

Sample Chamber Psychrometer
(Wescor, Model C-51)

Figure 25. Leaf sample chamber psychrometer used to measure leaf water potential (bars).

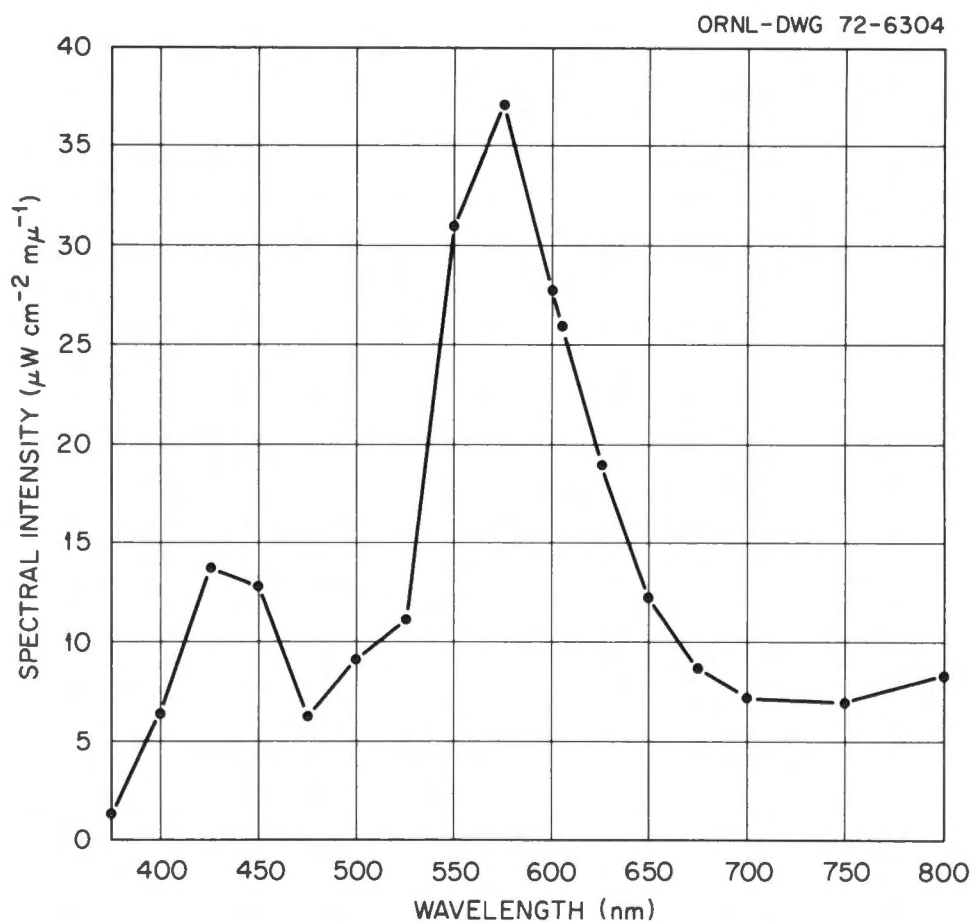


Figure 26. The spectral distribution of radiant energy inside one photosynthesis chamber utilized in the gaseous exchange measurements.

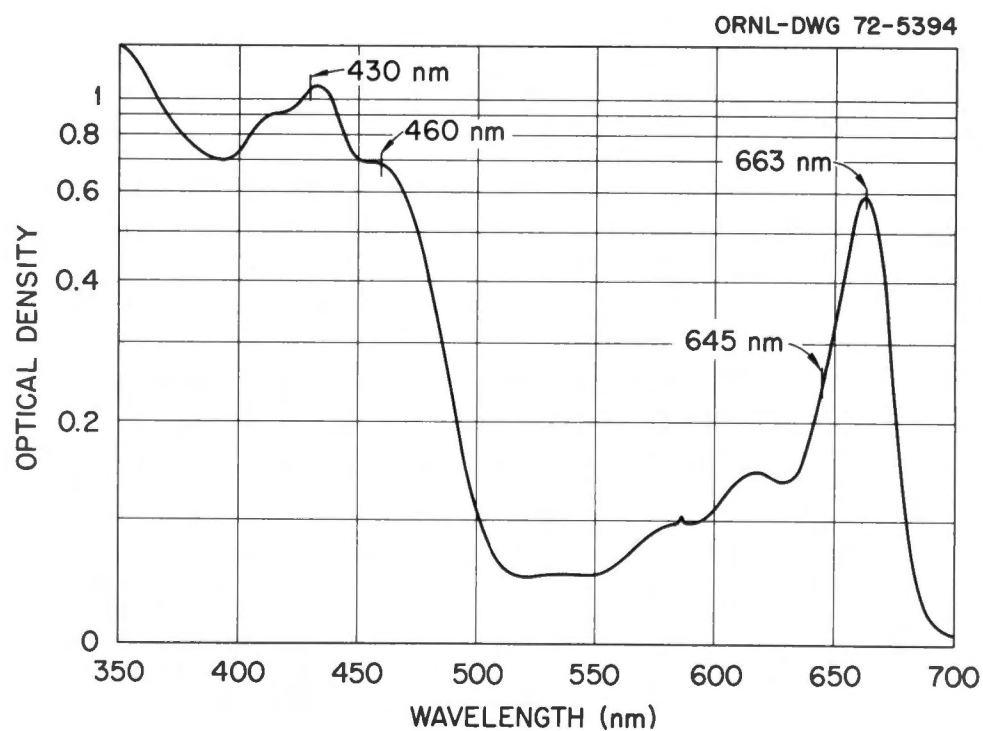


Figure 27. Typical absorbance curve for chlorophyll a, b, and carotenoid pigments from tulip poplar in 80 percent acetone.

TABLE VIII

MONTHLY CLIMATIC SUMMARY FOR THE OAK RIDGE AREA
 BASED ON 10-YEAR RECORD (1961-1970)*^a

Month	Temperature (°C)			Precipitation (cm)	Solar Radiation (kcal cm ⁻² Month ⁻¹)
	Mean**	Maximum	Minimum		
Jan.	1.8 ± 6.8	18.1 ± 2.0	-15.5 ± 4.2	9.3 ± 3.0	5.64 ± 0.72
Feb.	3.3 ± 6.6	18.8 ± 3.3	-13.2 ± 3.8	11.2 ± 5.6	6.73 ± 1.05
March	8.7 ± 7.1	25.5 ± 2.3	- 6.6 ± 2.3	12.4 ± 4.9	10.09 ± 1.59
April	14.7 ± 6.7	29.3 ± 1.6	- 1.1 ± 1.4	11.0 ± 3.9	12.27 ± 1.22
May	18.7 ± 6.1	31.6 ± 2.1	3.5 ± 1.6	8.4 ± 4.1	15.34 ± 1.23
June	22.3 ± 5.0	33.6 ± 1.5	10.3 ± 3.5	11.4 ± 4.9	15.14 ± 1.58
July	23.6 ± 4.6	34.0 ± 2.4	13.2 ± 2.5	16.2 ± 9.6	13.36 ± 3.71
Aug.	23.3 ± 4.6	34.0 ± 1.9	13.6 ± 1.9	10.1 ± 5.7	13.54 ± 1.64
Sept.	20.0 ± 5.6	32.2 ± 1.6	5.4 ± 2.4	7.1 ± 4.7	11.25 ± 1.10
Oct.	14.0 ± 6.5	28.5 ± 0.8	- 1.0 ± 2.3	6.3 ± 3.9	9.61 ± 0.90
Nov.	8.5 ± 6.4	23.9 ± 2.9	- 6.2 ± 2.6	9.2 ± 3.7	5.81 ± 0.82
Dec.	3.8 ± 6.3	18.7 ± 4.6	-10.9 ± 4.5	12.7 ± 8.2	4.65 ± 0.40
Mean annual total				126.5 ± 20.0	123.50 ± 10.90

*Data provided by W. M. Kulkowski, NOAA, Oak Ridge, Tennessee
 (in report by Sayrs and Sollins, 1972).

**N ≈ 6690 (6281 to 7407). Mean 13.30 ± 9.95.

***N = 8 × 10⁴.

^aLimits of variation are ± 1 standard deviation with N = 10 unless otherwise indicated. Maximum and minimum temperatures are averages for each month over the 10-year period. Precipitation and solar radiation are average totals for each month over the 10-year period.

APPENDIX B

TABLE IX

A SUMMARY OF LEAF CHARACTERISTICS OF TULIP POPLAR SEEDLINGS TO WATER AND SHADE TREATMENTS (EXPERIMENT I)

Time (Days Post Treatment)	Light Treatment (Percent Full Sun)	Weekly Soil Water Levels (Bars)	Chlorophyll (a+b)			Chlorophyll (a)			Absorbance Ratio (460/663)	Chlorophyll (a+b)		Percent Total Leaf Nitrogen	
			Mg/g		Dry Weight	Mg/g		Dry Weight		μg cm ²		Mg/g	
			$\bar{X}$	(SE)		$\bar{X}$	(SE)			$\bar{X}$	(SE)	$\bar{X}$	(SE)
30	100	< -1 (1)	6.55	(0.27)	4.64	(0.12)	1.36	(0.05)	28	(2.3)	1.83	(0.12)	
		-2 to 9 (2)	5.07	(0.46)	3.49	(0.33)	1.63	(0.14)	23	(3.5)	1.52	(0.35)	
		> -9 (3)	3.03	(0.70)	2.02	(0.53)	2.57	(0.37)	12	(4.2)	1.47	(0.20)	
	50	(1)	8.36	(1.15)	5.71	(0.86)	1.23	(0.05)	30	(4.2)	1.74	(0.21)	
		(2)	7.99	(0.53)	5.42	(0.36)	1.23	(0.05)	28	(0.6)	1.97	(0.22)	
		(3)	8.54	(0.24)	6.31	(0.19)	1.10	(0.02)	29	(1.5)	1.66	(0.33)	
25	(1)	8.61	(0.49)	6.10	(0.28)	1.23	(0.01)	29	(2.2)	1.71	(0.17)		
	(2)	6.15	(1.00)	4.43	(0.82)	1.48	(0.15)	18	(2.9)	1.67	(0.20)		
	(3)	6.46	(0.37)	4.56	(0.33)	1.26	(0.05)	22	(2.6)	1.94	(0.04)		
60	100	(1)	5.61	(0.41)	3.97	(0.34)	1.43	(0.09)	27	(2.0)	1.76	(0.12)	
		(2)	4.62	(0.87)	3.25	(0.59)	1.59	(0.12)	20	(4.0)	1.09	(0.06)	
		(3)	6.88	(0.71)	5.06	(0.45)	1.21	(0.04)	31	(3.5)	1.83	(0.20)	
	50	(1)	6.70	(0.34)	4.76	(0.17)	1.33	(0.03)	24	(1.2)	1.27	(0.03)	
		(2)	6.71	(1.40)	4.59	(0.96)	1.60	(0.34)	24	(6.3)	1.00	(0.14)	
		(3)	5.34	(1.10)	3.75	(0.82)	1.41	(0.15)	16	(2.4)	1.73	(0.33)	

TABLE IX (continued)

Time (Days Post Treatment)	Light Treatment (Percent Full Sun)	Weekly Soil Water Levels (Bars)	Chlorophyll1 (a+b)		Chlorophyll1 (a)		Absorbance Ratio (460/663)	Chlorophyll1 (a+b)		Percent Total Leaf Nitrogen		
			Mg/g		Mg/g			$\mu\text{g cm}^2$		Mg/g		
			Dry Weight	(SE)	Dry Weight	(SE)		$\bar{x}$	(SE)	Dry Weight	(SE)	
90	25	(1)	6.64	(0.65)	4.56	(0.51)	1.33	(0.08)	21	(1.8)	1.42	(0.21)
		(2)	6.07	(1.37)	4.16	(0.92)	1.39	(0.10)	18	(4.1)	1.24	(0.17)
		(3)	6.16	(0.59)	4.25	(0.46)	1.36	(0.11)	17	(1.0)	1.11	(0.01)
	100	(1)	5.71	(0.92)	3.97	(0.61)	1.34	(0.01)	29	(4.7)	1.33	(0.26)
		(2)	4.38	(0.42)	2.99	(0.29)	1.61	(0.06)	19	(1.9)	1.17	(0.21)
		(3)	4.42	(1.50)	3.11	(1.07)	2.02	(0.56)	19	(6.3)	1.88	(0.04)
	50	(1)	5.20	(0.86)	3.55	(0.59)	1.57	(0.08)	16	(2.4)	1.73	(0.33)
		(2)	5.01	(1.05)	3.54	(0.77)	1.47	(0.10)	15	(2.6)	1.31	(0.19)
		(3)	2.87	(0.71)	2.00	(0.52)	2.25	(0.52)	14	(3.2)	1.53	(0.24)
25	(1)	7.93	(0.30)	5.42	(0.27)	1.28	(0.02)	18	(1.9)	1.69	(0.13)	
	(2)	7.85	(4.31)	5.41	(3.00)	1.98	(0.88)	15	(7.2)	1.55	(0.38)	
	(3)	4.26	(2.27)	2.86	(1.48)	2.07	(0.58)	9	(3.8)	1.34	(0.14)	

TABLE X

A SUMMARY OF WHOLE PLANT RESPONSES OF TULIP POPLAR SEEDLINGS TO WATER AND SHADE TREATMENTS (EXPERIMENT I)

Time (Days Post Treatment)	Light Treatment (Percent Full Sun)	Weekly Soil Water Levels (Bars)	Soil Water		Leaf Water		Shoot/Root Ratio		Leaf Area cm ²		Margalef Ratio (430/663)	
			X	(SE)	X	(SE)	X	(SE)	X	(SE)	X	(SE)
30	100	<-1	1.96	(1.73)	14.88	(2.89)	0.91	(0.17)	310.21	(115.95)	2.60	(0.23)
		-2 to 9	5.09	(2.48)	19.87	(0.64)	0.91	(0.11)	259.17	(111.37)	2.86	(0.27)
		>-9	12.20	(3.75)	28.20	(1.07)	0.89	(0.18)	209.84	(30.08)	4.06	(0.38)
	50	(1)	1.32	(0.55)	20.48	(2.52)	0.53	(0.06)	248.62	(8.38)	2.01	(0.03)
		(2)	12.54	(5.63)	24.23	(3.37)	0.78	(0.06)	272.88	(12.70)	2.04	(0.16)
60	100	(3)	9.90	(1.55)	34.77	(6.65)	1.10	(0.03)	365.78	(66.91)	1.78	(0.02)
		(1)	0.34	(0.10)	17.12	(1.68)	0.82	(0.08)	280.94	(18.83)	2.06	(0.06)
		(2)	4.11	(0.40)	24.05	(2.85)	0.95	(0.16)	202.30	(48.41)	2.35	(0.23)
		(3)	13.40	(0.75)	30.98	(0.13)	1.49	(0.30)	336.19	(54.59)	2.07	(0.06)
		(1)	0.68	(0.48)	18.44	(0.83)	0.68	(0.15)	177.20	(37.14)	2.54	(0.31)
25	50	(2)	20.97	(7.43)	26.91	(0.55)	0.79	(0.21)	90.98	(7.97)	2.88	(0.24)
		(3)	27.02	(3.40)	28.83	(0.54)	0.98	(0.05)	188.64	(16.86)	2.15	(0.06)
		(1)	0.22	(0.01)	18.90	(1.46)	0.58	(0.12)	205.70	(22.08)	2.26	(0.12)
		(2)	11.94	(7.48)	24.34	(1.83)	0.80	(0.17)	125.38	(4.66)	2.45	(0.28)
		(3)	17.37	(5.91)	27.75	(2.45)	1.77	(0.67)	233.98	(126.81)	2.37	(0.29)
25	25	(1)	0.21	(0.00)	17.64	(1.47)	0.77	(0.10)	247.96	(76.78)	2.24	(0.21)
		(2)	17.09	(12.26)	25.32	(0.78)	1.52	(0.33)	208.30	(60.14)	2.37	(0.20)
		(3)	19.25	(3.47)	27.32	(3.36)	1.27	(0.16)	196.03	(6.79)	2.20	(0.15)

TABLE X (continued)

Time (Days) Post Treatment)	Light Treatment (Percent Full Sun	Weekly Soil Water Levels (Bars)	Soil Water		Leaf Water		Shoot/Root		Leaf Area		Margalef	
			Potential (Bars)	X (SE)	Potential (Bars)	X (SE)	Ratio	X (SE)	cm ²	X (SE)	Ratio (430/663)	X (SE)
100		(1)	2.03	(1.81)	17.41	(1.21)	0.54	(0.03)	78.71	(13.98)	2.21	(0.05)
		(2)	8.93	(4.99)	21.19	(1.59)	0.54	(0.06)	65.96	(14.44)	2.71	(0.10)
		(3)	18.86	(6.73)	24.12	(2.56)	0.80	(0.16)	99.84	(13.61)	2.97	(0.61)
90	50	(1)	0.21	(0.00)	17.95	(1.03)	0.62	(0.10)	154.05	(17.30)	2.48	(0.09)
		(2)	15.37	(6.87)	24.90	(0.71)	0.98	(0.24)	108.62	(36.33)	2.23	(0.11)
		(3)	27.86	(3.35)	30.90	(1.02)	0.54	(0.06)	163.15	(10.22)	3.31	(0.66)
25		(1)	0.21	(0.00)	16.06	(1.84)	0.96	(0.31)	173.89	(45.75)	2.01	(0.01)
		(2)	23.59	(6.56)	22.83	(3.23)	0.78	(0.18)	94.72	(29.14)	2.89	(1.11)
		(3)	24.60	(2.75)	31.46	(3.54)	0.77	(0.03)	100.70	(10.75)	3.02	(0.68)

APPENDIX C

TABLE XI

SUMMARY OF STATISTICAL ANALYSES FOR EXPERIMENT I
(ANALYSES OF VARIANCE)

Variable and Mode of Analysis	Source of Variation ^a	df	Mean Square	F Ratio	Probability Level
Net photosynthesis (mg CO ₂ dm ⁻² hr ⁻¹ 0.07 cal cm ⁻² min ⁻¹ 400 - 700 nm) (BMDO8V)	L	2	3.72	NS	
	A	2	2.13	NS	
	W	2	30.96	15.07	P < 0.005
	LxA	4	4.87	2.37	P < 0.10
	LxW	4	1.30	NS	
	AxW	4	2.10	NS	
	LxAxW	8	2.17	NS	
	Residual	54	2.05		
Net photosynthesis (mg CO ₂ dm ⁻² hr ⁻¹ 0.05 cal cm ⁻² min ⁻¹ 400 - 700 nm) (BMDO8V)	L	2	0.56	NS	
	A	2	1.45	NS	
	W	2	33.27	20.12	P < 0.005
	LxA	4	4.92	2.98	P < 0.05
	LxW	4	2.22	NS	
	AxW	4	0.61	NS	
	LxAxW	8	1.32	NS	
	Residual	54	0.65		
Net photosynthesis (mg CO ₂ dm ⁻² hr ⁻¹ 0.02 cal cm ⁻² min ⁻¹ 400 - 700 nm) (BMDO8V)	L	2	1.74	NS	
	A	2	8.77	8.43	P < 0.005
	W	2	13.85	13.26	P < 0.005
	LxA	4	1.34	NS	
	LxW	4	2.38	2.28	P < 0.10
	AxW	4	1.37	NS	
	LxAxW	8	0.46	NS	
	Residual	54	1.04		
Relative growth rate (RGR) (g g ⁻¹ d ⁻¹) (BMDO8V)	L	2	0.26 x 10 ⁻²	19.95	P < 0.005
	A	2	0.57 x 10 ⁻²	42.60	P < 0.005
	W	2	0.46 x 10 ⁻²	34.45	P < 0.005
	LxA	4	0.30 x 10 ⁻³	2.25	P < 0.10
	LxW	4	0.11 x 10 ⁻³	NS	
	AxW	4	0.68 x 10 ⁻³	5.14	P < 0.005
	LxAxW	8	0.30 x 10 ⁻³	2.25	P < 0.05
	Residual	54	0.13 x 10 ⁻³		

TABLE XI (continued)

Variable and Mode of Analysis	Source of Variation ^a	df	Mean Square	F Ratio	Probability Level
Net assimilation rate (NAR) (g m ² d ⁻¹) (BMDO8V)	L	2	0.22 x 10 ⁻⁶	40.26	P < 0.005
	A	2	0.16 x 10 ⁻⁷	2.89	P < 0.10
	W	2	0.88 x 10 ⁻⁷	15.61	P < 0.005
	LxA	4	0.36 x 10 ⁻⁷	6.45	P < 0.005
	LxW	4	0.95 x 10 ⁻⁷	NS	
	AxW	4	0.16 x 10 ⁻⁷	2.85	P < 0.05
	LxAxW	8	0.10 x 10 ⁻⁷	NS	
	Residual	54	0.56 x 10 ⁻⁸		
Total leaf area cm ² (BMDO8V)	L	2	16,077	NS	
	A	2	175,183	22.38	P < 0.005
	W	2	23,271	2.98	P < 0.10
	LxA	4	2,550	NS	
	LxW	4	4,510	NS	
	AxW	4	1,507	NS	
	LxAxW	8	6,713	NS	
	Residual	54	7,821		
Percent total leaf nitrogen (mg g dry weight ⁻¹) (BMDO8V)	L	2	0.06	NS	
	A	2	0.97	7.64	P < 0.005
	W	2	0.16	NS	
	LxA	4	0.23	NS	
	LxW	4	0.23	NS	
	AxW	4	0.19	NS	
	LxAxW	8	0.28	2.18	P < 0.05
	Residual	54	0.12		
Absorbance ratio (460/663) (BMDO8V)	L	2	0.24	NS	
	A	2	0.84	3.53	P < 0.05
	W	2	0.84	3.52	P < 0.05
	LxA	4	0.46	NS	
	LxW	4	0.13	NS	
	AxW	4	0.37	NS	
	LxAxW	8	0.26	NS	
	Residual	54	0.23		
Chlorophyll (a+b) (mg cm ²) (BMDO8V)	L	2	0.13 x 10 ⁻³	3.62	P < 0.05
	A	2	0.44 x 10 ⁻³	11.90	P < 0.005
	W	2	0.27 x 10 ⁻³	7.28	P < 0.005
	LxA	4	0.17 x 10 ⁻³	4.70	P < 0.005
	LxW	4	0.25 x 10 ⁻⁴	NS	
	AxW	4	0.23 x 10 ⁻⁴	NS	
	LxAxW	8	0.63 x 10 ⁻⁴	NS	
	Residual	54	0.37 x 10 ⁻⁴		

TABLE XI (continued)

Variable and Mode of Analysis	Variation ^a	df	Mean Square	F Ratio	Probability Level
Chlorophyll (mg gram dry weight ⁻¹) (BMDO8V)	L	2	17.40	3.78	P < 0.05
	A	2	14.43	3.14	P < 0.10
	W	2	13.79	3.00	P < 0.10
	LxA	4	11.98	2.60	P < 0.05
	LxW	4	1.30	NS	
	AxW	4	4.02	NS	
	LxAxW	8	3.76	NS	
	Residual	54	4.59		
Shoot/root ratio (BMDO8V)	L	2	0.47	3.65	P < 0.05
	A	2	0.60	4.67	P < 0.05
	W	2	0.85	6.62	P < 0.005
	LxA	4	0.06	NS	
	LxW	4	0.09	NS	
	AxW	4	0.26	2.08	P < 0.10
	LxAxW	8	0.25	NS	
	Residual	54	0.12		

^aThe following symbols are used to denote the source of variation: L = light, A = age, W = water.

TABLE XII

SUMMARY OF STATISTICAL ANALYSES FOR EXPERIMENT II
(ANALYSES OF VARIANCE)

Variable and Mode of Analysis	Source of Variation ^a	df	Mean Square	F Ratio	Probability Level
Net photosynthesis	L	2	3.32	NS	
(mg CO ₂ dm ⁻² hr ⁻¹)	A	1	71.83	13.19	P < 0.005
0.07 cal cm ⁻²	N	2	2.63	NS	
min ⁻¹ 400 - 700 nm)	LxA	2	16.30	2.99	P < 0.10
(BMD08V)	LxN	4	4.03	NS	
	AxN	2	2.34	NS	
	LxAxN	4	3.31	NS	
	Residual	18	5.44		
Net photosynthesis	L	2	4.02	NS	
(mg CO ₂ dm ⁻² hr ⁻¹)	A	1	78.01	15.23	P < 0.005
0.05 cal cm ⁻² min ⁻¹	N	2	14.65	2.85	P < 0.10
(BMD08V)	LxA	2	20.07	3.96	P < 0.05
	LxN	4	9.26	NS	
	AxN	2	5.90	NS	
	LxAxN	4	5.81	NS	
	Residual	18	5.12		
Net photosynthesis	L	2	9.66	6.39	P < 0.01
(mg CO ₂ dm ⁻² hr ⁻¹)	A	1	9.25	6.12	P < 0.02
0.02 cal cm ⁻² min ⁻¹	N	2	2.42	NS	
(BMD08V)	LxA	2	0.84	NS	
	LxN	4	6.97	4.61	P < 0.01
	AxN	2	4.45	2.94	P < 0.10
	LxAxN	4	1.23	NS	
	Residual	18	1.51		
Chlorophyll (a+b)	L	2	43.23	21.14	P < 0.005
(mg/g dry weight)	A	1	1.55	NS	
(BMD08V)	N	2	38.05	18.60	P < 0.005
	LxA	2	3.06	NS	
	LxN	4	5.42	2.66	P < 0.10
	AxN	2	0.10	NS	
	LxAxN	4	1.10	NS	
	Residual	18	2.04		

TABLE XII (continued)

Variable and Mode of Analysis	Source of Variation ^a	df	Mean Square	F Ratio	Probability Level
Percent total leaf nitrogen (mg g ⁻¹ dry weight) (BMD08V)	L	2	0.25	NS	P < 0.02
	A	1	0.04	NS	
	N	2	1.40	5.83	
	LxA	2	0.16	NS	
	LxN	4	0.21	NS	
	AxN	2	0.34	NS	
	LxAxN	4	0.20	NS	
	Residual	18	0.24		

^aThe following symbols are used to denote the source of variation: L = light, A = age, N = nitrogen.

VITA

Curtis John Richardson was born in Gouverneur, New York, on July 27, 1944. He attended schools in St. Lawrence County and was graduated from Ogdensburg Free Academy in 1962. The following September he entered State University College at Cortland and in June, 1966, received a Bachelor of Science degree in Biology. Upon graduation he accepted a teaching position in biology at West Genessee High School, Syracuse, New York.

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