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Growth and Photosynthesis of Two Field-Grown Populus Clones During The Establishment Year

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GROWTH AND PHOTOSYNTHESIS OF TWO FIELD-GROWN <u>POPULUS</u> CLONES DURING THE ESTABLISHMENT YEAR

By

Donald A. Michael

## A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Forestry

#### ABSTRACT

### GROWTH AND PHOTOSYNTHESIS OF TWO FIELD-GROWN <u>POPULUS</u> CLONES DURING THE ESTABLISHMENT YEAR

By

#### Donald A. Michael

Photosynthesis and growth under field conditions were monitored for Populus x euramericana cv. "Eugenei" (Eugenei) and P. tristis x P. balsamifera cv. "Tristis #1" (Tristis) during their first growing season. Photosynthetic rates 14 were measured using a portable CO apparatus which allowed intensive sampling within individual trees. Diurnal photosynthesis patterns were determined throughout the growing season for four positions within the crown: (1) an expanding leaf (prior to budset), (2) a recently mature leaf, (3) a leaf in the center of the mature leaf zone, and (4) a lower In addition, photosynthetic rates were detercrown leaf. mined for the entire leaf complement of trees selected periodically throughout the growing season. The microenvironment of measured leaves was quantified by measuring photosynthtically-active photon flux density, leaf temperature, and relative humidity. In addition, stomatal conductance and CO compensation points were determined. Weekly measurements of a permanent growth plot morphological and periodic destructive sampling were used to monitor the field development of the two clones.

Leaf orientation was quantified using a weighted protractor and compass. Vectors normal to the leaf lamina were mathmatically constructed and used to determine the area of each leaf projected toward the sun.

The clones exhibited widely different growth patterns. Tristis grew rapidly for 48 days before setting bud in mid-July. In contrast, Eugenei grew at a slower rate than Tristis but maintained this rate for 75 days before setting bud in September. Eugenei exceeded Tristis' total leaf area and dry weight by 56 and 37 %, respectively. Eugenei had a higher harvest index than Tristis throughout most of the growing season. The product of stem height and squared stem diameter (measured 2.5 cm above the point where the stem originated on the cutting) was highly correlated with total leaf area in both clones.

Photosynthetic rates were low in immature leaves; increased basipetally and peaked in recently-mature leaves; and thereafter declined basipetally in both clones. Diurnal and within-tree photosythesis patterns were highly variable due to differential light interception between leaves. In general, Tristis produced smaller leaves that had higher unit-area photosynthesis rates than Eugenei. Total photosynthesis integrated over the growing season closely matched dry matter production in both clones.

Leaves in Tristis were displayed nearly horizontally, whereas leaves were displayed more vertically in Eugenei. Within-tree mutual shading was more pronounced in Tristis; however, light interception in the crown of Eugenei was also reduced since some leaves were situated at oblique angles to the sun. Reductions in light and photosynthesis occured in the lower-crown in Tristis due to mutual shading whereas light and photosynthesis reductions occurred largely in upper and middle-crown leaves in Eugenei due to the oblique angles formed between the sun and certian leaves within those regions. This work is dedicated to my wife, Jan, in gratitude for her unqualified love and support.

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I wish to express my sincere thanks to Dr. Donald I. Dickmann for his support, guidance and patience during the course of my graduate program. I would also like to give special thanks to Drs. Jud Isebrands and Neil Nelson for their continued encouragement and support.

Appreciation is extended to Drs. Flore, Hanover, and Heins for their suggestions, critical review of this manuscript, and for serving on my graduate committee.

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#### INTRODUCTION

A predicted shortage of wood fiber due to increased demand (1) and a decreasing land base (10) has prompted interest in improving tree yields. Yields have been vastly increased in agricultural crops by optimizing plant structure and environmental factors which increase a crop's usable components (3). In a similar manner, experimental intensive cultural systems have been used in an attempt to provide optimum conditions for tree growth (2,3,9). Intensive culture systems use some combination of geneticallyimproved planting stock, irrigation, fertilization, weed and pest control, short rotations, and dense spacings to promote rapid stand growth and high yields.

Intensive culture short-rotation (SRIC) systems have several advantages over conventional forest management. For example, SRIC provides : 1) rapid and complete utilization of the site, 2) a secure, sustained, and controlled fiber source, 3) a rapid return on investment, 4) high yields, and 5) an opportunity to produce fiber tailored to a specific end-use. Of course, there are also problems associated with SRIC: 1) SRIC plantations can be genetic monocultures which are prone to disease and insect problems, 2) SRIC requires high inputs of energy and capital, and 3) SRIC has a relatively low return on investment (4). However, these problems are not insurmountable. Disease and insect problems

can be minimized by careful species and clonal selection, by planting mixtures of species and clones, and by matching specific species and clones to individual sites; energy and capital inputs can be minimized by developing more efficient cultural techniques; and the return on investment can be improved by increasing per hectare yield.

Yield is a critical factor affecting the economic feasibility of SRIC plantations (11). Maximum wood yield can be obtained under SRIC by selecting species which have rapid juvenile stem growth and by optimizing growing conditions. Since, on certain sites, <u>Populus</u> species are the fastest growing trees in the Lake States (U.S.A) and produce fiber which is readily usable in the forest products industry (5), poplars have become one of the most promising species for SRIC systems (12). Poplar yields can be systematically increased only if the physiological components of yield are identified and if knowledge of how these components are influenced by cultural and environmental variables is obtained.

Biomass yield in trees is based on the integrated production and utilization of photosynthate by individual leaves. Factors which influence yield do so by directly or indirectly influencing photosynthate production or partitioning. Therefore, an examination of the photosynthetic process is required if yield is to be understood. Earlier work (6,7,8) established base-line data which described the processes of leaf initiation and development, and photosyn-

thate production and partitioning in young <u>Populus</u> trees grown under controlled environmental conditions. As a logical extension of this work, experiments were conducted to examine the growth, CO fixation, and autecology of two 2contrasting <u>Populus</u> clones growing under field conditions.

The objectives of this research were to determine how much CO was fixed, where it was fixed, and what were the major factors influencing CO fixation. More specifically, the intent was to quantify diurnal and seasonal changes in single leaf and whole-tree photosynthesis in relation to tree development in the first growing season. Data were also gathered in two- and three-year-old plantations, but these data are not reported in this dissertation. It was hoped that some of the data and techniques developed in the field examination of these two poplar clones would lead to the development of principles that would apply to other clones and species. In addition, it was hoped that field experimentation would provide insight into which factors or groups of factors merit further controlled environment investigation.

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# CHAPTER I

DETERMINING PHOTOSYNTHESIS OF TREE LEAVES

IN THE FIELD 14 USING A PORTABLE CO APPARATUS: 2 PROCEDURES AND PROBLEMS

# Submitted to PHOTOSYNTHETICA

### Abstract

A field approach for studying photosynthesis in Populus Photosynthetic rates were measured leaves is described. 14 CO apparatus. A paired comparison inusing a portable 14 dicated that photosynthetic rates measured with the CO device exceeded those measured with an infrared gas analyzer by 5 percent. One hundred to 150 single-leaf photosynthesis and companion environmental measurements could be completed within one day, allowing intensive sampling within trees. Measurements from permanent growth plots located within the experimental plantation were used to identify sample trees. In the first growing season, four leaf positions were sampled on a diurnal and seasonal basis. Lateral branches and current terminals were sampled in a similar manner in older trees. Boundary line analysis was used to establish photosynthetic response curves. Field response curves established with the boundary line technique compared favorably with those established under laboratory conditions for similar leaves.

### Introduction

Biomass yield in trees is ultimately determined by the integrated production and utilization of photosynthate by individual leaves. To study the physiological basis of yield, patterns of CO assimilation and photosynthate dis-2 tribution must be identified through field experiments which determine the effects of leaf development, leaf position and orientation, leaf aging, and tree development on total photosynthate production. In addition, the sensitivity of leaves to numerous environmental conditions must be assessed on a diurnal and seasonal basis.

Tree crowns are very complex, with leaves of several different age classes located on different orders of branches (Isebrands and Nelson 1982). An equally complex sampling scheme is required to obtain data from these different leaf populations, necessitating a measurement system that produces rapid and accurate determinations of photosynthe-Several researchers have experimented with simple. sis. 14 methods of measuring photosynthesis rapid using CO (Austin and Longden 1967, McWilliam <u>et al</u>. 1973, Bell and Incoll 1981). These methods use miniature chambers to ex-14 sections of leaf lamina to a short pulse of a Dose 12 CO gas mixture. The exposed discs are excised and as-14 sayed CO activity. In this manner, several leaf for positions throughout a tree crown can be quickly measured with a minimum of leaf disturbance. Moreover, the photosynthetic contribution of individual leaves in different

portions of the crown can then be assessed on a diurnal and seasonal basis.

Several CO devices do not permit light interception 2 on the abaxial leaf surface during measurement (e.g., Bell and Incoll 1981). However, abaxial light can be important in driving photosynthesis (Moss 1964), especially in plants 14 with upright leaf displays. Therefore, a CO apparatus is needed which permits both adaxial and abaxial light interception.

14

This paper describes a CO technique modified from 2 those described by Incoll and Wright (1969) and McWilliam <u>et</u> <u>al</u>. (1973). The equipment is inexpensive, relatively easy to construct, simple to operate, allows adaxial and abaxial light interception during measurement, and provides accurate field measurements of photosynthesis. In addition, field sampling procedures are discussed and a technique is described which can be used to analyze field photosynthesis data.

## <u>Methods</u>

## 14 The <u>CO</u> <u>Apparatus</u> 2 14

The CO appartatus consists of two components: (1) 2 the gas system and (2) the handpiece (Fig. 1).

<u>The Gas System</u>-- The CO - CO gas mixture used in 2 2this system can be generated as described by Shimshi (1969), McWilliam <u>et al</u>. (1973), and Neilson (1977) or premixed and analyzed gas can be obtained from commercial producers. The gas used in our field experiments was

A - complete CO gas apparatus. 1 - 0.3 1 2 Figure 1. stainless steel field tank with quick connect coupling; 2 brass two-stage regulator; 3 - flow control valve; 4 - flow meter; 5 - handpiece; 6 - leather holster. B - Closeup of handpiece. 1 - upper and lower plastic jaws; 2 - recessed gas chambers with silicone rubber gaskets; 3 - clamping mechanism; 4 - gas inlet to tire valve and transfer chamber; 5 - outlet line; 6 - CO absorption column; 7 - vent;  $2^{2}$ 8 -alumninum handle.



purchased from Matheson Gas Products. Our mixture contained 3 - 3 CO, with a specific activity of 185 KBq l 322 cm m at The gas was stored in a 7 21.1 C 1 atmosphere. and 1 aluminum tank purchased from Matheson. A smaller 0.3 1 stainless steel cylinder (Matheson Model 8x) was filled from the storage tank for use in the field. The field tank was sealed by a hand-operated valve and connected to a Swagelok quick-connect coupling by brass and stainless steel fittings (Fig. 1A). Pressure was reduced by a brass 2-stage regulator (Matheson Model 3322) which was supplied with an outlet needle valve. Gas flow was further controlled by a high resolution flow control valve (Airco Series 32 HRV). Tygon tubing was used to connect the flow valve to a flow meter (Matheson Series 7360. Model 602) and from the flow meter to the handpiece. The entire gas system and handpiece was supported by a leather holster which can be clipped to a strap or belt. Total weight of the gas system was 3 kg.

<u>The Handpiece</u> -- The pistol-shaped handpiece was modified from that described by McWilliam <u>et al</u>. (1973) and consisted of two transparent plastic jaws and an aluminum clamping lever and handle (Fig. 1B). Each jaw was recessed to accept a silicone rubber gasket. When pressed together, the gaskets formed a miniture leaf chamber with a diameter of 11 mm and a volume of 0.19 cm. The lower jaw contained a tire valve and gas transfer chamber. The clamping lever closed the plastic jaws, sealed the leaf chamber, triggered the tire valve and released gas from the field tank through

the transfer chamber and into the upper and lower leaf chambers. The gas then exited through outlet tubes in the leaf chamber, passed through a sodium hydroxide CO -absorp-2 tion column in the pistol grip, and vented to the atmosphere. The sodium hydroxide was changed frequently to en-14 sure adequate absorption of the outgoing CO, especially when the apparatus was used indoors or in poorly ventilated areas. The total weight of the handpiece was 455 g.

### Field Operation

The field tank was filled to approximately 7 X 10 kg  $^2$  m pressure from the large storage tank. This was enough gas to make 100 to 120 measurements. After setting the delivery pressure to 0.5 X 10 kg m, the aluminum handle on the field gas regulator was removed to prevent accidental adjustment. The flow control valve was also protected.

In the field, the outlet needle values were opened fully and the flow control value adjusted to provide a flow -3 -1rate of 1.3 X 10 1 s . Flow rates between 1.0 and 2.0 X -3 -110 1 s are required to ensure that photosynthesis is not limited by the supply of CO (Strebeyko 1967, McWilliam <u>et</u> <u>2</u> <u>al</u>. 1973, Naylor and Teare 1975). An optimum flow rate should be determined for each species studied from preliminary experimentation.

The upper and lower rubber chamber gaskets were coated with a thin layer of silicone grease to provide a seal between the leaf and gaskets and to mark the location of the exposed disc. A section of leaf lamina free from large

veins was selected midway between the leaf tip and base, and the exposure chamber clamped firmly onto the leaf (Fig. 2). A 20 s pulse of gas, timed with a stopwatch, was then administered simultaneously to both sides of the leaf. During the treatment period, the leaf was held in its natural orientation. At the end of the 20 s pulse, the chamber was quickly removed and the center of the exposed disc excised using a #4 cork borer (diameter = 7 mm). The leaf disc was then forced from the cork borer with a glass rod into a scintillation vial containing 1.5 ml NCS tissue solubilizer (Amersham/Searle). The vial was tightly capped and taken to 14the laboratory for C-analysis.

Several companion measurements were taken to quantify the leaf's environment and condition during the measurement period. These measurements included:

1. Leaf temperature - Measured on the abaxial leaf surface with a YSI Model 427 stainless steel thermistor.

2. Air temperature - Measured in the shade of the leaf as above.

3. Diffusive resistance - Measured on the abaxial and adaxial leaf surfaces with a LiCor Model LI-65 autoporometer.

4. Photosynthetically-active photon flux density (PPFD, 400 to 700 nm) - Measured at leaf level in the adaxial and abaxial leaf planes, horizontally at leaf level, horizontally above the tree, and toward the sun with a LiCor Model LI-185 quantum sensor and meter.

14 Figure 2. Closeup of handpiece during field use. Note CO 2 gas treatment chamber enclosed by silicone rubber gasket.

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5. Relative humidity - Measured within the tree crown for each sample tree using an American Instrument Company hygrometer and narrow range hygrosensors.

6. Leaf orientation - Vertical angles of leaf axes parallel and perpendicular to the leaf midvein were measured using a protractor and weighted nylon cord (Max 1975). Leaf direction was determined using a Silva compass.

Finally, sampled leaves in our studies were excised at the base of the petiole, the cut surface placed in a vial of distilled water, and taken to the laboratory for determination of CO compensation points (Dickmann and Gjerstad 1973) 2 and measurement of leaf area (LiCor Model LI 3000 leaf area meter) and dry weight (oven-dried at 100 C).

## Assay for Radioactivity

After returning to the laboratory, 1.5 ml of 0.5% benzyl peroxide in toluene were added to each vial to bleach out color and the leaf discs were digested for 24 h in an oven at 50 °C. After the digestion period, three drops of glacial acetic acid and 13 ml of scintillation cocktail containing 63 ml Spectrafluor (Amersham/Searle) in 1 l toluene were added to the vials. The vials were placed in a darkened chamber for 3 h to reduce the effects of chemilluminescence and were then counted with a liquid scintillation spectrometer (Beckman Model LS 150).

## Calculating Photosynthetic Rate

Photosynthesis was calcualted from the CO assay 2 from:

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Pg = (CPM/CE X CCO x 1.18)/(SA x LA x T)(1)where: Pg = gross photosynthesis rate (mg CO m ) S CPM = sample counts per minute (corrected for background radiation) CE = counting efficiency of the liquid scintillation spectrometer (expressed as a decimal) CCO = concentration of CO at a standard temperature and 2 pressure (determined in our case from Matheson's calibration) (mg CO 1 2 1.18 = a discrimination factor to account for diffusive and biochemical discrimination against CO (Van Norman and Brown 1952, Austin and Longden 1967) 14 12 SA = the specific activity of the CO - CO gas mixture at the same standard temperature and pressure as in CCO (dpm -1 l gas mixture) LA = the area of the excised leaf disc (m ) 14 T = time length of theCO pulse (s) 2 Photosynthetic rate on a dry weight basis (mg CO g ) was calculated by substituting oven-dry weight of the excised leaf disc in g for LA in (1), using specific leaf weight to estimate the weight of the disc. In addition. -1 photosynthesis can be expressed per leaf (mg CO s leaf ), or per tree (mg CO s tree 2 14 Comparison of Photosynthetic Rates Determined with the <u>C0</u>

#### Apparatus and Infrared Gas Analysis

Photosynthesis was measured on identical areas of the

same leaf using an infrared gas analyzer (IRGA) and the 14 14 device to test the reliability of the CO CO method. The air entry connection of the CO handpiece was linked to an electric switching solenoid (Skinner Electric Valves). The solenoid was used to alternate the entry of outside air (322 cm m) or radioactive gas (311 cm m CO . 194 KBa at 21.1 C and 1 atm.) into the photosynthetic 1 chamber 14 The exit connection of the of the handpiece. CO handpiece was linked to a differential IRGA and then to a CO absorbing column. The gas was then vented to the atmosphere.

In 1981, photosynthetic measurements were made on ten mature leaves from three different trees of <u>Populus x eur-</u> <u>americana</u> cv. 'Eugenei' (NC 5326), grown in pots in the field for four months under natural light conditions. The leaf plastochron index (LPI; Larson and Isebrands 1971) of sampled leaves ranged from 5 to 9 and the plants had 25 to 30 leaves. Multiple samples were taken on some leaves.

Measurements of photosynthesis were made in sequence. 14 first by the IRGA method, followed by the CO method. The handpiece was clamped onto a portion of leaf lamina located midway between the leaf tip and base. Air from outside the laboratory was then passed through the chamber and into the IRGA at a flow rate of 2.4 X 10 The rate of photosynthesis was determined from the IRGA after equilibrium had been attained. The solenoid switch was then triggered, shutting off the outside air and releasing a 20s

pulse of CO -labelled air which passed over the same 2 -3 -1 portion of the leaf at a flow rate of 1.3 X 10 l s . The exposed leaf disc was then quickly excised and processed as -2 described earlier. PPFD varied from 135 to 636 u mole m -1 s during the experiment, but was constant for each sample.

A similar comparison was conducted in 1979 in which thirteen leaves (LPI's ranging from 3 to 12) on two greenhouse-grown P. <u>balsamifera x P. tristis</u> cv. 'Tristis #1' (NC 5260) trees (with 18 and 23 leaves) were measured. In the 1979 comparison, a 150 mm plexiglass cuvette was used for the IRGA measurement (Nelson and Ehlers 1983) instead of 14 using the handpiece's chamber for both the IRGA and CO 2 measurements.

## Field Sampling Scheme

Several leaves were sampled within systematically selected "average" trees. An "average" tree was defined as a tree whose height and number of leaves (or crown size) approximated the plantation mean. Mean values were obtained from weekly measurements of trees in permanent growth plots in our plantation.

In one-year-old trees, diurnal photosynthesis measurements were conducted on two-hour increments at four physiologically important leaf positions (Fig. 3), comprising three oblique and one horizontal age series (Dickmann 1971). The first oblique age series, A, was located in the upper portion of the crown and consisted of an expanding or immature leaf (i.e., LPI 3 or 4). The second oblique series,

Figure 3. Within tree sampling scheme for <u>Populus</u> trees during the first growing season. A - immature leaf, B recently-mature leaf, C - leaf in center of mature leaf zone, D - lower crown leaf.

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B, was located in the mid-upper crown region and consisted of a recently-mature leaf. The third oblique series, C, was located in the mid-lower crown region and consisted of a leaf which had reached maturity several days prior to mea-The horizontal age series, D, was located in the surement. lower-most crown region and consisted of a leaf which reached maturity early in the growing season. Daily leaf length measurements were taken on all leaves on sample trees beginning three days prior to the Pg measurement to ensure that this sampling scheme was maintained throughout the growing season. Within-tree photosynthetic patterns were determined by measuring Pg of each leaf on selected trees at 10 to 12 am (solar time) on days spaced equally throughout the growing season.

In two-year-old trees, diurnal measurements were taken at four leaf positions (A,B,C,D; Fig. 3) on the current terminal and on first-order lateral branches in the upper, middle and lower crown regions. Odd-numbered leaves (i.e. LPI 1,3,5, etc.) were measured on these shoots from 10 to 13 am (solar time) throughout the growing season to examine developmental Pg patterns. Three-year-old trees were sampled in a similar manner, although the inclusion of secondorder lateral branches increased the complexity by an additional order of magnitude.

#### Boundary Line Analysis of Field Photosynthesis Data

Boundary line analysis (Webb 1972, Hinckley <u>et al</u>. 1978) was used to interpret the field photosynthesis data.

The validity of the boundary-line technique was tested on seven Popululs x euramericana cv. 'Eugenei' trees with 23 to 28 leaves grown in pots in the field. The boundary line for Pg and PPFD in the field was established by measuring 23 mature leaves (LPI 15 or 16) with the device. CO A Pg/PPFD saturation curve was also established in the laboratory using an open IRGA system (Nelson and Ehlers 1983) and one mature leaf (LPI 15) from two 'Eugenei' trees selected from the same population of potted field plants. Leastsquare curves were then fit to the data by computer using the Gaussian method of successive approximations to an asymptotic model:

> PPFD Pg = a - bD

where: Pg = gross photosynthetic rate (mg CO m s ) a,b,D = regression parameters -2 -1 PPFD = photon flux density (umoles m s )

### Results and Discusion

In general, photosynthetic rates measured by the  $\begin{array}{c} 2\\ 2\\ \end{array}$ technique exceeded those measured by the IRGA by 5 percent (Fig.4). A paired t-test indicated, however, that there was not a significant statistical difference between the two methods (P = 0.05). A slight difference between the two methods was expected since it is generally assumed that a 14 short exposure of CO results in an approximation of gross 2 photosynthesis rather than net photosynthesis, as measured Figure 4. Photosynthetic rates (Pg) of mature <u>Populus</u> x <u>euramericana</u> cv. 'Eugenei' (circles - 1981 experiment) and <u>P. balsamifera x P. tristis</u> cv. 'Tristis #1' (stars - 1979 14 experiment) leaves measured with the CO and IRGA tech-2 niques. The linear equation of best fit for the combined 14 data was: CO = 0.16 + 0.68 (IRGA) (r =0.7).



by the IRGA (Turner and Incoll 1971. McWilliam et al. 1973. Naylor and Teare 1975, Zelawski and Walker 1976, Incoll 1977). However. an underestimate of gross photosynthesis is more likely since there are possible sources of error associated with the use of isotopic methods due to physical and 14 biochemical discrimination against CO at the diffusion sites and perhaps at the carboxylation sites within the leaf mesophyll (Van Norman and Brown 1952, Yemm and Bidwell 1969, 14 12 Incoll 1977):dilution of CO within the leaf by CO evolved from the respiratory pathways (Vozensenskii et al. 14 1971. Incoll 1977); and evolution of CO from photorespiration (Roberts and Keys 1978, D'Aoust and Canvin 1972). The 14 extent of these errors is not known; however, CO the device described here provides estimates of photosynthesis for Populus leaves which closely compare to those determined using the IRGA method. Photosynthesis rates measured with 14 CO device probably lie somewhere between gross the and net photosynthesis.

A major task in field photosynthesis research is designing a sampling strategy. In most cases, environmental conditions cannot be controlled in the field, but rather, only monitored. When leaves are studied in their natural orientation, dramatic differences in microenvironment occur, even between adjacent leaves of the same tree. In addition, continuous changes are induced by cloud movements and the sun's diurnal and seasonal movements. As a result, true replications of any measurement are difficult, if not impos-

sible, to obtain in the field. Lack of replication may trouble workers who are accustomed to controlled-environment laboratory research; however, as Helms (1976) suggested, problems in the field may be resolved with a lesser degree of precision, but a high degree of ecological relevance is usually attained.

The first step in designing a sampling strategy is to develop criteria for selecting sample trees. A limited number of leaves can be sampled in the field during a day. This sample can consist of a few leaves from several randomly selected trees or several leaves from a few trees selected systematically. Given the difficulties in obtaining true replication in the field, we found that it was more informative to sample several leaves on a few systematically selected "average" trees.

Sampled leaves must represent physiologically important populations within the crown. The leaf sampling scheme used here comprised three oblique and one horizontal age series (Dickmann 1971). The oblique age series yielded information on the effects of time of season, stage of plant development, and leaf position on diurnal photosynthetic capacity. The horizontal series yielded information on the effect of leaf aging at the same leaf position on diurnal photosynthetic capacity. By using this sampling scheme, the photosynthetic activity of leaves of various ages and positions was monitored on a diurnal and seasonal basis and under a wide range of climatic conditions. This information

Figure 5. Boundary line relationship between gross photosynthesis (Pg) and photosynthetically-active photon flux density (PPFD) for recently-mature leaves of fieldgrown <u>Populus</u> x <u>euramericana</u> cv. 'Eugenei' trees. The starred data points were used to establish the nonlinear PPFD regression line: Pg =  $0.85157 - (0.88654 \times (0.99689))$ .



provided a dymanic view of the photosynthetic development of average <u>Populus</u> trees.

When data obtained from field measurements of Pg are plotted against a single environmental parameter such as PPFD, a scatter of points always results (Fig. 5). The scatter occurs because uncontrolled variables influence the relationship between the two plotted variables. Boundary line analysis is a technique, proposed by Webb (1972) and used extensively by others (e.g., Hinckley et al. 1978) where all values for two variables are plotted and a line enclosing these points is established. This line represents the limiting effect of the independent variable on the dependent variable; it is assumed that values below this line result from the influence of another independent variable or a combination of limiting variables (Webb 1972. Hinckley et al. 1978).

Boundary line analysis is a useful tool for analyzing data from studies where interacting variables cannot be controlled or, in many cases, even identified (Hinckley <u>et al</u>. 1978). However, definition of the exact shape and limits of the boundary line is difficult and often very subjective. When possible, the shape of the boundary line should be established from controlled environmental studies that are either done by the researcher or obtained from the literature.

If the boundary line is drawn to enclose all points, no allowance is made for sampling error, i.e. the deviation

above and below the "true" boundary line. Instead, a body of exterior points must be identified through which a regression line can be derived using standard statistical techniques. An exterior body of points can usually be identifed for relationships which are either linear or asymptotic. For example, if a relationship is asymptotic with a positive slope, an increase in the independent variable (x) corresponds to an increase or stabilization of the dependent variable (y). Thus, after an increase in x, any x-y pair which shows a decrease in y below any previous y value can be excluded from the exterior zone of points. Exterior points are thereby selected along the increasing x-axis whose y coordinate is greater than or equal to the y coordinate of any previous point. The star-shaped symbols in Figure 5 were chosen in this way and were used to establish the non-linear regression line representing optimal leaf Pg response to adaxial PPFD. Deriving a body of exterior points by this analytical process will minimize the contribution of error to the effect being studied but will not provide a true estimate of error in the statistical sense (Webb 1972). Despite this limitation, the method will alleviate the need to hand-fit boundary line curves and will assist in mathematically describing observed relationships.

There was good agreement between the shape of the IRGA 14 light saturation curve and the CO boundary line curve 2 (Fig. 6). However, the boundary line technique must be used with caution in situations where two or more independent Figure 6. Relationship between gross photosynthesis (Pg) and photosynthetically-active photon flux density (PPFD) for mature Populus x euramericana cv. 'Eugenei' leaves using the 14 CO technique and boundary-line analysis (dashed line and 2 points; Pg = 0.88514 - (0.99372)starred data X PPFD ))) and the IRGA technique (solid line and open (0.99810 PPFD circles;  $Pg = 0.7904 - (0.83279 \times (0.99796))$ ))).



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variables are highly correlated. For example, a stomatal conductance/temperature boundary line plot may indicate that temperature directly influences conductance, when in fact vapor pressure deficit, which is strongly influenced by temperature, may actually exert the direct effect.

The general shape of the Pg curve determined for a single field-grown <u>Populus</u> tree agrees with those published for laboratory-grown <u>Populus</u> trees by Larson and Gordon (1969) and Dickmann (1971) (Fig. 7). Variations in Pg between adjacent leaves and among other leaves within the crown reflect the effect of leaf aging and differential 14 light interception. The sensitivity of the CO technique 2 is demonstrated by its ability to detect within-tree variations in leaf Pg. Information of this type is important for physiological studies of tree crowns.

In addition to entire-tree Pg profiles, diurnal Pg 14 patterns have also been identified using the CO technique. Since the method samples a portion of the leaf destructively, preliminary experiments are necessary to determine whether a single leaf can be measured several. times without introducing confounding factors, or whether different leaves must be sampled to establish a diurnal We have found that a single mature leaf can be pattern. used for at least two Pg determinations, one or more on each side of the midrib. If successive sampling of the same leaf would impair the leaf's structural integrity, then adjacent leaves should be sampled.

Figure 7. Gross Photosynthesis (Pg) and photosyntheticallyactive photon flux density (PPFD) profile for a 46-leaf <u>Populus x euramericana</u> cv. 'Eugenei' tree measured in the field from 10 to 12 am (solar time), August 30, 1979. LPI = leaf plastochron index (LPI 0= the first 30 mm leaf below the apex).



Light saturation curves were developed for Eugenei leaves at three of the four positions described earlier using the boundary line technique (Fig. 8). Leaves at all crown positions reached light saturation at approximately -2 -1 umole m s . or about 50% full sunlight. 1100 However. Mature upper-crown Pmax varied markedly within the tree. leaves (B) attained Pmax rates above 0.83 mg CO m The 9 older lower- crown and the immature upper-crown leaves **(A**) -2 -1and D) had Pmax rates below 0.69 mg CO m S 2

# <u>Conclusions</u>

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The CO technique described in this paper gives 2 rapid, accurate estimates of photosynthesis for <u>Populus</u> leaves. The device is inexpensive, portable and facilitates extensive sampling of tree crowns. Field data obtained from this device can be analyzed using the boundary line technique. Sampling schemes must be designed to detect important physiological changes which occur during leaf and crown development. Field conditions impose severe sampling constraints since rapidly changing conditions make replications difficult, or nearly impossible, to obtain. Figure 8. Boundary-line relationship between gross photosynthesis (Pg) and photosynthetically-active photon flux density (PPFD) for field grown P. x <u>euramericana</u> cv. 'Eugenei' leaves. (A - expanding leaf, Pg =  $0.62766 - (0.73957 \times PPFD)$ (0.99736 )); B - recently-mature leaf, Pg = 0.85157 - PPFD(0.88654 x (0.99698 )); D - lower crown leaf, Pg = PPFD0.70224 - (0.73277 x (0.99653 ))).







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CHAPTER II

GROWTH AND DEVELOPMENT OF TWO FIELD-GROWN <u>POPULUS</u> CLONES DURING THE ESTABLISHMENT YEAR

Prepared for Submission

to

PHOTOSYNTHESIS RESEARCH

Abstract. Weekly morphological measurements of trees within permanent growth plots and periodic destructive sampling were used to monitor the field development of two Populus clones during their first growing season. Tristis (P. tristis x P. balsamifera) grew rapidly for 48 days before setting bud in July. In contrast. Eugenei (P. X euramericana) grew at a slower rate than Tristis but maintained this rate for 75 days before setting bud in September. The total leaf area and dry weight of Eugenei exceeded that of Tristis by 56 and 37 percent, respectively. In addition, Eugenei had a larger harvest index than Tristis throughout most of the growing season because a greater proportion of photosynthate produce was directed to shoot growth in Eugenei than Tristis; however, a high shoot-root ratio in Eugenei predisposed it to water stress. Differences in above-ground biomass between clones were largely attributable to clonal differences in seasonal leaf area development.

Introduction

Tree biomass yield is ultimately dependent upon photosynthate production, redistribution, and utilization. Among other factors, a tree's photosythetic output is related to the size, arrangement, duration, and photosynthetic capacity of its leaf component (21). Photosynthate is then redistributed within the plant in response to the demands of various sinks, which in turn respond to several external and internal factors (3). Knowledge of such a complex system is best obtained from experiments which quantify patterns of growth, photosynthesis, and photosynthate distribution in the same or similar plants (4,13). We have used this integrated physiological approach in our field studies of hybrid poplar. This paper summarizes growth data collected as part of a large-scale field examination of the photosynthetic properties of two poplar clones during their first growing season. It is important to identify how a tree develops and functions during the establishment year, since growth during this period can greatly influence performance in later In addition, knowledge of seasonal changes in leaf years. and tree characteristics aids in assessing measured patterns of photosynthesis and photosynthate distribution, which will be discussed in later papers.

Methods and materials

# Plant Material and Cultural Methods

Two hybrid poplar clones, <u>Populus</u> x <u>euramericana</u> cv."Eugenei" (NC 5326) and <u>P. tristis x P. balsamifera</u> cv.

"Tristis #1" (NC 5260) were grown under a short-rotation, intensive culture (SRIC) system (8). The clones (hereafter. Eugenei and Tristis) were established on May 22, 1979 at the U.S. Forest Services' Harshaw Experimental Farm near Rhinelander. Wisconsin, U.S.A. (45 N 89 W) using 1.040 25 cm unrooted hardwood cuttings per clone planted in blocks at a 0.6 m x 0.6 m spacing. The cuttings were inserted into the soil so that one to two buds were exposed above the soil level. Some trees were later pruned to provide a single - 1 main stem. Nitrogen fertilizer (total = 187 kg N ha 88 NH NO ) was applied through a gun irrigation system. Soil moisture status was monitored at a depth of 15 cm using soil tensiometers (Soil Moisture Equipment Corp. Model 2725) and the plantation was irrigated with the gun system when the soil tension dropped below -0.5 bar. Herbicides (trifluralin and glyphosate) plus additional hand weeding were used to control weed growth. Survival after 20 weeks was 96 and 94 percent for Tristis and Eugenei, respectively.

## Growth Measurements

Permanent plots located within each clonal block in the main plantation were used to monitor growth. Six 4-tree plots per clone spaced evenly over the plantation were measured weekly to obtain estimates of tree height, total number of leaves, and stem diameter. Height was measured with a meter stick from the soil surface to the main stem's apex; total number of leaves was determined by counting the leaves present on the main stem in addition to the scars of

abscised leaves; and stem diameter was measured with calipers at a permanently marked location 2.5 cm above the main stem's point of insertion on the cutting. These measurements were used to periodically select representative trees from the interior of the plantation on which leaf length, leaf area, and leaf dry weight were measured for all leaves. Periodically, representative trees (one tree per clone per date) were harvested and the dry weight of their components (i.e., leaves, stem, cutting, roots) determined. A representative tree was defined as a tree whose height and total number of leaves approximated the permanant plot mean. Also, the heights, diameters, and leaf numbers of all trees sampled throughout the growing season in the photosynthesis portion of the study were measured in addition to the length, area, and weight of each leaf sampled.

# Environmental Measurements

Hourly measurements of soil temperature, incident irradiance, precipitation, and air temperature were obtained from a weather station located 280 m from the plantation.

#### Results

## Growth Environment

Daily totals of irradiance, irrigation, precipitation, soil tension, and maximum and minimum air temperatures for the 1979 growing season are shown in Figure 1. Three periods of moderately high soil tension (30 June, 13 July,

Figure 1. Environmental conditions during the 1979 growing season at the Harshaw Experimental Farm near Rhinelander, Wisconsin. Precip. = precipitation (dased lines represent irrigation); ST = soil tension at a depth of 15 cm; Air Temp. = air temperature (squares = maximum, stars = minimum); IFD = irradiant flux density; RH = mean relative humidity.



and 14 August) were alleviated by rain or irrigation. Mean o daytime air temperatures ranged between 11 and 21 C; however, night temperatures below 4 C were recorded on three occasions (5 June, 15 August, and 15 September). Irradiance peaked in mid-June and thereafter declined.

# Height Growth, Leaf Initiation, and Diameter Growth

Both clones first produced leaves around 29 May. Tristis grew rapidly for 48 days after initial leaf flush and reached its maximum growth rate by 17 July (Fig. 2). Height growth during this period peaked at 2.5 cm d (Fig. 2a) and leaf production reached a maximum rate of 0.8 leaves -1 d (Fig. 2C).

Tristis diameter growth reached a maximum rate of 0.24 mm d sixteen days after height growth and leaf production rates peaked (Fig. 2B). The development of Tristis declined rapidly after 16 July as budset approached; however, it continued to surpass Eugenei in height, total number of leaves, and stem diameter until mid-August. Visible terminal bud formation began in Tristis trees on 23 July. 55 days after intial leaf flush. Ninety-four percent of the Tristis trees in the permanant plots set bud and ceased height and diameter growth by 13 August. The remaining 6% continued to grow until 21 August. Temporal variation in terminal bud formation resulted in dramatic height differences between trees in the planatation and strongly influenced the growth rates presented in Figure 2.

Eugenei grew at a slower rate than Tristis during the

Figure 2. Changes in height (A), diameter (B) and number of leaves (C) for Tristis (stars) and Eugenei (squares) trees during their first growing season. Twenty-four trees per clone were measured on each date shown. Budset dates are shown with arrows. Dashed lines indicate rates of change. TNL = total number of leaves; D = stem diameter 2.5 cm above the main stem's point of insertion on the cutting; Ht = total tree height.


first 59 days after leaf flush (Fig. 2); however, Eugenei produced new leaves and height and diameter increment at this slower rate for 27 days after growth declined in Tristis. During this period, height growth reached a maximum rate of 1.8 cm d (Fig. 2A) and leaf production reached a maximum rate of 0.6 leaves d (Fig. 2C). Eugenei produced leaves for 23 days after budset occured in Tristis. Height growth and leaf production gradually declined with the onset of terminal bud formation; however, the rate of diameter growth continued to increase and reached a maximum  $(0.17 \text{ mm d}^{-1}; \text{ Fig. 2B})$  prior to budset. Measurable diameter growth ceased after budset in both clones.

The timing of budset in Eugenei was more regular than in Tristis. Approximately 40% of the Eugenei trees in the permanant plots began to form terminal buds on 13 September and all trees attained budset by 24 September.

# Within-Tree Leaf Composition

Varying proportions of mature (i.e., not expanding) and immature (i.e., expanding) leaves occurred within the crowns of both clones throughout the growing season. The number of leaves and proportions of leaf types differed between clones as a result of the different growth patterns described above; however, the general developmental pattern was similar between clones.

Early in the growing season over 50% of the leaves present in both clones were immature (Fig. 3A,B). The immature leaf zone increased in absolute size (but decreased Figure 3. Within-tree changes in the number of expanding, mature, and abscised leaves for Tristis (A) and Eugenei (B) trees during their first growing season. Budset dates are indicated by arrows. TNL = total number of leaves.



as a proportion of total leaf number) as the number of leaves increased. The immature leaf zone in Tristis decreased rapidly in size after 17 July (Fig. 3A). probably due to a slower rate of leaf production at the apex in conjunction with a constant rate of acropetal leaf maturation. This decrease directly preceded terminal bud form-The last 2 to 3 leaves produced by the apex contination. ued to expand slightly for 1 to 2 days after the terminal bud began to form; however, they failed to attain normal mature leaf size and in most trees, these latter-formed leaves quickly abscised. After budset, leaf production and leaf expansion ceased; as a consequence, Tristis had mature leaves ranging in age from 1 to 70 days. Its mean foliage age increased thereafter.

The developmental pattern in Eugenei was similar to that observed in Tristis, however, the entire sequence was extended over a longer period of time and involved more leaves (Fig. 3B). The immature leaf zone in Eugenei increased in size to approximately 10 leaves; the size of the immature leaf zone slowly decreased after 10 August as the rate of leaf intiation declined prior to budset; and, after budset, all leaves were mature.

Leaf abscission began in mature lower-crown leaves and proceeded acropetally in both clones. Abscission began during mid-July and mid-August for Eugenei and Tristis, respectively. Eugenei lost a greater number of leaves in the lower crown than Tristis; however, as of 10 September,

both clones had lost approximately 10% of TNL. <u>Marssonina</u> leaf spot and <u>Melampsora</u> rust were associated with deteriorating leaves of both clones.

# Specific Leaf Weight Development

Specific leaf weight (SLW, leaf dry weight/leaf area) varied between clones and within individual trees. In addition, within-crown SLW patterns changed over the growing season. Average SLW per tree (SLW) ranged between 68 and 78in late July in both clones (Fig. 4). SLW increased gm \_2 abruptly in Tristis during August, exceeding 90 g m . This increase occured after budset.  $\overline{SLW}$  ranged between 72 and 75 -2 in Eugenei throughout August and September and ing creased to above 90 g m in October after budset (Fig. 4).

These shifts in SLW resulted from changes in withincrown SLW patterns. On 23 July, both clones had similar within-crown SLW patterns (Fig. 5B): SLWs of leaves near -2 the apex (LPI 0) were above 58 g m in both clones (since the edges of these leaves were curled, their leaf areas were underestimated using the leaf area meter and their SLWs were overestimated; significant leaf curling did not occur beyond LPI 2); SLW then declined basipetally in rapidly expanding leaves (LPI 1 to 6) of both clones; and SLW thereafter increased basipetally, reaching a maximum in the lower-crown leaves. SLW increased in Tristis during late July and August, largely due to SLW increases in its upper- and middle-crown leaves (Fig. 5B). In addition, SLWs of Figure 4. Changes in mean specific leaf weight (SLW) for Tristis (stars) and Eugenei (squares) trees during their first growing season. Budset dates are indicated by arrows. Each point represents the average SLW of all leaves of one tree.

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Figure 5. Changes in specific leaf weight (SLW) with leaf plastochron number (LPI) for Tristis (stars) and Eugenei (squares) trees for three dates during their first growing season. The location of the first mature leaf below the apex is shown by arrows. LPI 0 is the first leaf below the apex with a length > 29 mm.



lower-crown leaves increased slightly during this period. In a similar fashion, SLW increased in Eugenei during late September and early October as a result of SLW increases in its upper-crown leaves (Fig. 5C). SLWs in the lower-crown of Eugenei were lower on 5 October than 20 August due to abscission of several high-SLW lower-crown leaves (Fig. 5B,C).

## Leaf Area Development

New leaves matured quickly in July in both clones (Fig. 2 6). Mature leaves attained 20 to 25 cm and 30 to 40 cm for Tristis and Eugenei, respectively, during this period. The area of the first fully mature leaf increased with increasing tree size in both clones (Fig. 6). The area of the first mature leaf reached a maximum when growth rates declined prior to budset. Although variation existed among 2 -1 trees, this maximum leaf area was 60 to 70 cm leaf and 80 2 -1 to 90 cm leaf for Tristis and Eugenei, respectively.

Total tree leaf area production in the two clones was similar up to early August (Fig. 7). Eugenei produced fewer, yet larger leaves than Tristis during this period. Leaf area production peaked in Tristis after budset at 1600 cm -1 tree ; leaf area peaked in Eugenei at 2850 cm tree prior to setting bud in early September. The rate of leaf area production varied greatly throughout the growing season in both clones (Fig. 7B).

## Dry Weight Yield

The hardwood cutting was the principal dry weight

Figure 6. Within-crown leaf area patterns for Tristis (A) and Eugenei (B) trees at four dates during their first growing season. The location of the first mature leaf below the apex is indicated by an arrow. LNFB = leaf number from the tree base; LA = leaf area.



Figure 7. Total leaf area (A) and the rate of leaf area development ( $\Delta$ LA) (B) for Tristis (stars) and Eugenei (squares) trees during their first growing season. Budset dates are indicated by arrows.

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component in both clones during July (Table 1). The remaining dry matter was equally divided among the roots. leaves. and stem. As the season progressed, Eugenei increasingly partitioned its dry matter into above-ground (i.e., leaves and stem) parts whereas Tristis partitioned its dry matter more equally into above-ground and below-ground (i.e., cutting and roots) parts. Tristis produced more total dry matter than Eugenei through early August; however, Eugenei surpassed Tristis thereafter (Table 1). The leaf area ratio (leaf area/total dry weight), a measure of leafiness (19), was higher in Eugenei than Tristis throughout the growing season (Table 2) indicating that Eugenei reinvested a greater proportion of its dry matter into photosynthetic tissue. In addition, Eugenei had a higher shoot-root ratio (leaf + stem dry weight / cutting + root dry weight) and harvest index (leaf + stem dry weight / total dry weight) than Tristis (Table 2).

#### Discussion

The field performance of Tristis and Eugenei was greatly affected by their respective budset dates. Eugenei more fully utilized the growing season in northern Wisconsin by extending apical growth six weeks beyond the budset date of Tristis. Pauley and Perry (23) found that the timing of budset in several <u>P. trichocarpa</u> and <u>P. deltoides</u> clones planted in Massachusetts was directly correlated to the length of the frost-free season in the clone's native habitat. Differences in budset date in the present clones were

		Dry	<u>Dry Weight (g) by Date</u>					
		<u>23 Jun</u>	<u>20 Aug</u>	<u>10 SEP</u>	<u>5 OCT</u>			
Clone	<u>Component</u>							
Tristis	Leaves Stem Cutting	5 4 10	14 16 17	14 20 27	14 16 22			
	Roots Total	3  22	4  51	12  73	17 69			
Eugenei	Leaves Stem Cutting Roots	4 2 8 4	12 11 8 3	24 30 18 10	23 40 17 12			
	Total	 18	 34	82	92			

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Table 1. Dry weight (g) by date for harvested 1-year-old Tristis and Eugenei trees. One representative tree was harvested per clone on each date shown.

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Table 2. Comparison of developmental parameters of harvested Tristis and Eugenei trees during the first growing season.

		Date					
<u>Clone</u>	<u>Trait</u>	<u>23 JUL</u>	<u>20 AUG</u>	<u>10 SEP</u>	<u>5 OCT</u>		
Tristis	Leaf Area Ratio 2 (cm g-1)	33	30	20	22		
	Shoot-Root Ratio# _1 (g g )	0.7	1.4	0.9	0.8		
	Harvest Index## -1 (g g )	0.4	0.6	0.4	0.4		
Eugenei	Leaf Area Ratio 2 (cm g-1)	44	50	38	28		
	Shoot-Root Ratio# _1 (g g )	0.5	2.1	2.0	2.2		
	Harvest Index <b>**</b> -1 (g g )	0.4	0.7	0.7	0.7		

Shoot/Root = (leaf + stem dry wt.)/(cutting + root dry wt.)
##
Harvest Index = (leaf + stem dry wt.)/total dry wt.

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likewise attributable to differences in clonal parentage. Although the exact parentage of Tristis is controversial, it did originate in the northern plains of Saskatchewan where the frost-free growing season is short, whereas Eugenei originated in the more temperate climate of France. As a result, Tristis set bud much earlier than Eugenei in northern Wisconsin. Therefore, Tristis and Eugenei provided a comparison between clones adapted to relatively short and long growing seasons.

The pattern of leaf area development within single trees of both clones was consistant with that described by others for <u>P. deltoides</u> seedlings (2,17). Leaves produced early in the growing season mature quickly and are important sources of photosynthate for initial shoot survival and development (2,10,15). As a poplar tree grows it can support a greater number of expanding leaves (2,16,17) and, as a result, each successive leaf attains greater leaf area at maturation by increasing the rate and duration of leaf expansion (17). Since Eugenei set bud much later than Tristis, Eugenei produced twenty sucessively larger leaves after Tristis ceased leaf production and attained its maximum leaf size. Consequently, Eugenei had a larger, younger, and presumably more productive zone of large leaves than Tristis after July. Since leaf area and wood weight are highly correlated in poplar trees (10,11,18), it is reasonable to assume that the rapid total dry weight increase observed in Eugenei between August and September was attrib-

utable to its large total leaf area and rapid rate of leaf area expansion. In some clones, therefore, the late summer and early fall periods are important for wood production and should not be culturally ignored.

The temporary reduction in leaf area expansion and height growth rates observed in Eugenei during early August was correlated with moderately low soil water tension and low night air temperature. Although Tristis had a similar reduction in leaf area production during this period, it was associated with budset processes rather than low soil water tension. Water stress has been shown to adversely affect leaf production in several crops (1,9). It is possible that the high shoot-root ratio developed by Eugenei predisposed it to water deficits.

SLW has been associated with productivity in Populus (5,6,22) and other species (15,24). SLW patterns within trees changed dramatically in both clones throughout the Increases in SLW occurred as leaves growing season. attained maturity and as mature leaves aged. A portion of this increase in SLW may have resulted from continued development of leaf thickness after leaf expansion ceased (25). Increases in SLW as leaves aged may also be due to retention of current photosynthate in mature leaves (20), adsorption and retention of calcium and other mineral elements (26), or shrinkage (7). Changes in SLW with leaf ontogeny and tree phenology must be considered when SLW is used as an indicator predictor of photosynthetic potential or yield. or

Failure to recognize that several leaf populations exist within poplar trees could result in erroneous clonal comparisons and yield predictions.

The harvest index of a plant is an indication of how effectively dry matter is partitioned into usable compo-In contrast to Tristis, Eugenei invested a larger nents. proportion of its dry matter into additional photosynthetic tissue and stem growth throughout the growing season, resulting in a higher harvest index. Isebrands and Nelson (12) recently analysed these growth patterns and found that Tristis exports appreciable quantities of photosynthate to the roots and cutting after budset. Therefore, the roots and cutting comprise the most active sinks for photosynthate in Tristis for nearly half of the first growing season. In contrast, Eugenei exports photosynthate for stem and leaf development throughout most of the growing season (11, 12, 13).

The growth patterns described above represent genetic adaption to growing seasons of different lengths. Tristis, which appears adapted to a relatively short growing season, concentrated its height growth into the first few weeks of the growing season in northern Wisconsin. Thereafter, dry matter was allocated for root and cutting development. In contrast, Eugenei, which is adapted to a longer growing season, more fully utilized the growing season for leaf area and shoot development. Both growth patterns have advantages and disadvantages. The extensive root system produced by Tristis may enable it to grow under droughty conditions and may facilitate rapid growth in subsequent growing seasons. However, it is questionable whether such extensive root development is an economically desirable trait for SRIC trees grown under irrigated conditions. Clones such as Eugenei that have rapid and prolonged shoot growth rather than extensive root development are more suitable for the SRIC system. Full utilization of the growing season for shoot growth is an important trait for SRIC trees if maximum yields are to be obtained. However, a balance between shoot and root development is important, especially under drought conditions. References

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CHAPTER III

# CHARACTERIZATION OF PHOTOSYNTHESIS WITHIN TWO FIELD-GROWN <u>POPULUS</u> CLONES DURING THE ESTABLISHMENT YEAR

Prepared for Submission

to

PHOTOSYNTHESIS RESEARCH

Abstract. Diurnal and seasonal photosynthesis patterns were established for two Populus clones during their first field CO device. Photosynthetic rates were low season using a 2 increased basipetally and peaked in immature leaves: in recently-mature leaves; and thereafter declined in lowercrown leaves in both clones. Photosynthesis was strongly associated with leaf age and stomatal conductance in immature leaves; adaxial irradiance and leaf temperature in recently-mature leaves; and leaf age and adaxial irradiance lower-crown leaves. Diurnal photosynthesis patterns in within trees were highly variable due to differential light interception between leaves. Results of clonal comparisons of photosynthetic rates were dependent upon which leaves were pooled for comparison and how photosynthesis was expressed. Tristis (P. tristis x P. balsamifera) produced smaller leaves which had higher unit-area photosynthesis rates than Eugenei (P. x euramericana) which produced leaves which had lower unit-area photosynthesis larger rates. Eugenei outgrew Tristis principally by fully utilizing the growing season for leaf area production. Photosynthetic production integrated over the growing season closely matched dry matter production in both clones.

Introduction

A major goal in forestry research is to develop procedures to predict yield. To achieve this goal, plant and environmental factors that influence and control yield must be fully understood. All factors which influence yield must do so by directly or indirectly influencing photosynthesis or photosynthate distribution (39). Therefore, a thorough understanding of the photosynthetic process under field conditions is required if yields are to be reliably predicted.

A logical approach for investigating photosynthesis is to develop baseline data under controlled environmental conditions, test this data through field experimentation, and then refine concepts developed in the field with further controlled- environment work (27). This approach has been used to examine the photosynthetic physiology of Populus species. Early controlled-environment work established baseline physiological data for young poplar trees (4,5,7,19,28,31). As a logical extension of this work, field experiments were conducted to examine growth, CO fixation, and dry matter distribution in two hybrid poplar clones during their first growing season. Growth and dry matter distribution have been described in earlier papers (20, Chapter II).

The objective of the present study was to characterize photosynthesis within two field-grown hybrid poplar clones and determine how much CO was fixed, where it was fixed,

and what were the major factors influencing CO fixation. 2 More specifically, our intent was to quantify diurnal and seasonal changes in single leaf and whole-tree photosynthesis throughout the first growing season.

#### Methods and materials

<u>Plant Material</u>. Two hybrid poplar clones, <u>Populus</u> x <u>euramericana</u> cv. "Eugenei" (NC 5326) and <u>P. tristis x P.</u> <u>balsamifera</u> cv. "Tristis #1" (NC 5260) (hereafter, Eugenei and Tristis, respectively) were grown under a short-rotation intensive culture system (14). The trees were established on May 22, 1979 at the U.S. Forest Service's Harshaw Experimental Farm near Rhinelander, Wisconsin (U.S.A.) using 1,040 25 cm unrooted hardwood cuttings per clone. Cultural treatments have been described in an earlier paper (Chapter II).

14 14 Technique and Companion Measurements. The <u>C0</u> CO technique described earlier (Chapter I) was used to measure photosynthesis in the field. Briefly, the technique involv-2 ed exposing a 0.95 cm section of leaf lamina located midway -3 14 between the leaf tip and base to 322 cm of CO --1 2 labelled air with a specific activity of 185 kBq 1 (at 21 C and 1 atm.)(Matheson Gas Products) for 20 s at a constant rate (1.3 ml s ) and then subsampling the exposed flow section with a sharp #4 cork borer (diameter = 7 mm). After excision, the leaf disk was placed into a 20 ml scintillation containing 1.5 NCS solubilizer vial ml tissue

(Amersham/Searle). In the laboratory, 1.5 ml of 0.5% benzyl peroxide was added to each vial and the disks were digested for 24 h in an oven at 50 C. After the digestion period, three drops of glacial acetic acid and 13 ml of scintillatcocktail ion containing 63 ml of Spectrafluor (Amersham/Searle) in 1 l toluene were added to the vials. The vials were placed in a darkened chamber for 3 h to reduce chemilluminescence and were then counted with а liquid scintillation spectrometer (Beckman model LS 150).

Photosynthetic rate on a unit area basis (PgA, mg CO m 2 -1 s ) was then calculated from:

# $PgA = (CPM/CE \times CCO \times 1.18)/(SA \times LA \times T)$ 2

where: CPM = sample counts per minute (corrected for background radiation); CE = counting efficiency of the liquid scintillation spectrometer (expressed as a decimal); CCO = concentration of CO (mg CO 1 ) at a standard temperature and pressure (determined in our case from Matheson's calibration); 1.18 = a discrimination factor to account for 14 diffusive and biochemical discrimination against CO 12 14 (1,43); SA = the specific activity of the CO gas mixture at the same standard temperature and pressure as in CCO (dpm 1 gas mixture); LA = the area of the excised 14 T = length of the CO pulse (s). Experileaf disc (m ); ments conducted in 1979 and 1980 indicated that photosynthe-14 CO technique exceeded those sis rates measured by the 2 an infrared gas analyzer by ca. measured by 5 percent;

however, there was not a significant difference between the two methods (P = 0.05) (Chapter I).

Several companion measurements quantified the condition of the leaf and environment during the photosynthesis measurement. These were:

1. Photosynthetically active photon flux density (PPFD, -2 -1 u mole m s , 400 to 700 nm) - measured normal (i.e., perpendicular) to the adaxial (PPFD ) and abaxial (PPFD ) AD leaf planes and also above and below the tree crown using a LiCor model LI-185 meter and quantum sensor (hereafter, the terms "PPFD" and "light" will be used interchangeably). All leaves were measured in their natural orientation.

2. Diffusive resistance to H O (s mm) - measured 2 with a LiCor model LI-65 autoporometer and horizontal sensor (24) on the abaxial leaf surface. Both clones were amphistomatous, but with fewer stomata on the upper than lower leaf epidermis.

3. Leaf temperature (LT, C) - measured by apressing a YSI model 427 stainless steel thermistor against the abaxial leaf surface. Air temperature was measured as above approximately 5 cm below the abaxial leaf surface in the shade of the leaf.

4. Relative humidity (%) - measured within the crown of each tree using an American Instrument Co. hygrometer and appropriate narrow range humidity sensor.

5. Leaf orientation - measured for selected leaves using a weighted protractor (34).

After completion of all daily field measurements, three leaves at each of four crown positions (see below) were excised and their petioles placed immediately into scintillation vials containing 15 ml distilled water. CO compen-3 -3 sation points ( $\Gamma$ , cm m CO ) were measured in the laboratory by placing each leaf into a mylar bag inflated with air containing 350 cm m CO (6) (LT= 27 C, PPFD =660 u -1 After one hour the contents of the bag were mole m s ). expelled through an infrared gas analyzer (Beckman model 215A) and  $\Gamma$  was determined. Unpublished experiments conducted in 1978 indicated that compensation points of leaves treated in this manner were not significantly different from those determined on leaves in situ.

Each sampled leaf was excised and its area measured with a leaf area meter (Lambda Instruments model LI-3000). Leaf dry weight was then determined by oven-drying the leaves at 100 C for 24 h and weighing the samples on a Sartorious balance.

<u>Calculated</u> <u>Parameters</u>. Several parameters were calculated from the measured variables. These were: (1) specific leaf -2 weight (2) photosynthesis on a unit (SLW, g m ), dry weight basis (PgW, g s ), (3) photosynthesis on a mg CO whole-leaf basis (PgL, ug CO s leaf ), (4) whole-leaf interception (photosynthetically active photon flux light (PPF, u moles s leaf )), and (5) stomatal conductance to (CON, mm s ) calculated from:  $CON = (1/r) \times 0.623$ , CO where 0.623 is the ratio of diffusion coefficients for 2

and H O in air (21). In addition, effective PPFD (PPFDe, u 2 -2 -1 moles m s ) was calculated from: PPFDe = PPFD + (PPFD AD AB x e), where e is a conversion factor to adjust for the different effeciencies of abaxial and adaxial light in driving photosynthesis. e has not been experimentally determined for poplars, therefore, 0.5 was selected after reviewing our data and data presented by others (36). Also, light-use efficiency (LUE, mg CO per u mole incident PPFDe) was calculated from: LUE = PgL/PPF.

Sample Tree Selection. Measurements from a permanent growth plot located within the main plantation were used to guide selection of sample trees. Twenty-four trees per clone spaced evenly over the plantation were measured weekly to obtain estimates of mean tree height, total number of leaves, and stem diameter. At each photosynthesis sampling date, trees which represented the average of these measurements were selected from the main plantation. This assured that "average" trees in the plantation were sampled at each measurement date. An "average" tree was defined as a tree whose height and total number of leaves approximated the plantation mean.

<u>Photosynthesis</u> <u>Measurements</u>. Diurnal photosynthesis patterns were established for four crown regions, comprising two oblique age series and one horizontal age series (5, Chapter I). The first oblique series, A, was located in the upper crown (i.e., leaf plastochron index (LPI) 3 (29) and

consisted of an immature leaf (i.e., expanding) prior to budset. After budset, leaves in the A region were morphologically mature (i.e., not expanding). The second oblique series, B, was located in the upper-middle crown and consisted of a recently-mature leaf. Prior to budset, B was the first fully mature leaf below the apex: after budset . B was standardized at LPI 9. The third oblique series, C, was located in the middle-lower crown and was a leaf which had reached maturity several days prior to measurement. The C leaf was the central leaf between the B and D leaf positions. The horizontal series, D, was a lower-crown leaf which attained maturity early in the growing season. The D leaf was the sixth leaf up from the lowermost leaf. Successive daily leaf length measurements ensured that this sampling pattern was maintained throughout the growing season.

Since the CO device measured a portion of the leaf lamina destructively, photosynthesis was not measured on the same leaf at each crown position throughout the day. Instead, measurements were taken at the four crown positions on leaves from similar trees. Two leaves per position per tree were measured during the diurnal period. For example, if the diurnal measurement consisted of eight sample periods (i.e., one measurement every two hours from 6 am to 20 pm solar time (ST)), then four trees per clone were selected as sample trees. Within each sample tree, two adjacent leaves were selected at each crown position. One tree and one leaf per position were selected randomly from this pool for each

two-hour measurement.

One tree per clone was selected on six different dates and the photosynthetic rate of each leaf determined. These entire-leaf series measurements began at 10 am (ST) to avoid possible midday photosynthesis depressions.

Boundary Line Analysis. Boundary line analysis (18,46,Chapter I) was used to establish response curves for the measured variables. All measured values for several twovariable combinations were plotted and least-squares lines were established through the exterior points using linear or non-linear regression (Chapter I). These lines represented the limiting effect of the independent variable on the dependent variable; it was assumed that points below the boundary lines resulted from the influence of another unplotted variable or combination of variables (18). Field laboratory tests have shown that the boundary line and technique provides reliable response curves for the PgA/PPFDe relationship (Chapter I).

Integration of Photosynthesis. An estimate of the daily total carbon uptake of each leaf within a tree was obtained from the diurnal photosynthesis measurements using the measured leaves within each crown section to estimate PgA for unmeasured leaves. PgA rates were averaged within each measurement period for each crown region, e.g., if three leaves were sampled in the A crown region between 7 and 8 am (ST), then the average of their PgA rates was used as the
7:30 am PgA rate. For each measurement period within a day. average PgA rates were plotted against LPI and smooth curves were established through the points. These curves were used to estimate PgA rates of unmeasured leaves. Whole-leaf photosynthetic rates were then calculated. The photosynthetic output of each leaf during the measurement period was calculated by multiplying each leaf's whole-leaf photosynthesis rate by the number of hours between measurement periods (in most cases, two hours). Photosynthesis was integrated in this manner for a 14 hour diurnal period, beginning at 6 am and ending at 20 pm (ST). Integrations were performed for four sunny-day diurnal measurements to obtain estimates of total daily carbon fixation (TDCF, mg -1 CO tree day ). The growing season was then divided into four periods and these TDCF values were used to estimate TDCF for all sunny days (radiant flux density 97 Wm 2 ) within each period. One-half of the sunny day TDCF was used as an estimate of cloudy day performance. The total photosynthetic output of both clones between 28 May 1979 and 10 September 1979 was then estimated. These data were then used to estimate cummulative TDCF for days which whole trees were harvested and their component dry weights determined (Chapter II). The dry-weight equivalent of cummulative TDCF was estimated using 1.63:1 as the ratio of g CO fixed to g carbohydrate produced (2).

Growth Analysis Harvests. "Average" trees (one tree per

clone per date) were periodically harvested and the dry weight of their leaves, stem, cutting, and root determined (Chapter II). "Average" trees were selected using data from the growth plot as guidelines.

## Results

Expression and Comparison of Photosynthetic Rates. Comparisons within and between clones were influenced by how photosynthesis was expressed and by which sets of leaves were compared. To illustrate, photosynthesis was expressed on a unit area, unit dry weight and whole-leaf basis and compared between clones using different sets of "similar" leaves for the 30 August 1979 entire leaf series measurement (Fig. 1). "Similar" leaves were selected using similar LPIs, similar crown regions or strata, and similar leaf numbers from the base of the crown (LNFB).

When LPI was used to identify "similar" leaves, PgA rates in Eugenei surpassed those in Tristis in the LPI 11 to 20 and 21 to 30 leaves, however, PgA rates were greater in Tristis than Eugenei in the LPI 0 to 10 leaves (Fig. 1C). When expressed as PgW, photosynthesis was similar between clones in the LPI 0 to 10 leaves and differences were accentuated in the LPI 11 to 20 and 21 to 30 leaves. PgL rates in Eugenei were greater than those in Tristis in all leaves (Fig. 1C). When "similar" leaves were selected Figure 1. Expression of photosynthetic rates on a unit area (PgA), unit weight (PgW) and whole-leaf (PgL) basis for Tristis (squares) and Eugenei (stars) leaves measured 30 August 1979. Leaves were pooled for comparison by leaf number from the tree base (LNFB) (A), crown section (B), and leaf plastochron index (LPI) (C).

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using crown regions or vertical strata (Fig. 1B) (each crown region comprised 25% of the total number of leaves) photosynthesis was higher in upper leaves of Tristis than Eugenei when expressed as PgA but not when expressed as PgW or PgL: photosynthesis rates were greater in Eugenei than Tristis in the second crown region, regardless of how it was expressed: photosynthesis rates were similar in region #3; and photosynthesis rates in the lowest leaf region of Tristis (#4) surpassed those in Eugenei, regardless of how expressed. When similar-aged leaves were compared by grouping leaves acropetally (Fig. 1A), Tristis lower leaves (LNFB 0 to 20) had higher photosynthetic rates than Eugenei lower leaves, however, LNFB 21 to 40 Eugenei leaves had equivalent or higher photosynthetic rates than Tristis leaves in the same Tristis did not have leaves in the LNFB 41 to 50 region. region for comparison.

Photosynthesis Within Individual Trees. Both clones were in a similar stage of growth during the 10 July 1979 entire leaf series measurement, i.e., a new 30 cm leaf was produced by the apical meristem on ca. two day intervals; height -1 growth was proceeding at a rate of 1.5 cm d ; and both clones had ca. 8 expanding and 12 mature leaves (Chapter II). Within-tree PgA patterns were also similar between clones during this period (Fig. 2): rapidly expanding leaves near the apex had low PgA rates regardless of microenvironment; PgA increased basipetally in the rest of the expanding leaves and peaked in the last expanding or

Figure 2. Photosynthesis (PgA) by leaf number from the tree base (LNFB) for three measurement dates on which each leaf within each Tristis (squares) and Eugenei (stars) tree was sampled.







Table 1. photon fl (LUE) for	Within- ux (PPF) Eugenei	crown com , whole-l and Tris	parison of t eaf photosyn tis measured	cotal leaf area thesis (PgL), 1 10 July 1979.	I, photosynthet and light use	ically-active efficiency
Clone	LNFB +	Leaves	Leaf Area	PPF 2	PgL	LUE
			-2 cm	-1 umole s	-1 ug CO s 2	ug CO mole 2
Tristis	11-20 1-10	10	215 (67) 105 (33)	33 (75) 11 (25)	12.1 (61) 7.8 (39)	0.4 0.7
TOT	AL	20	320	<u>t</u> t	19.9	
Eugenei	11-19 1-10	9 10	274 (58) 201 (42)	40 (62) 25 (38)	18.9 (56) 14.9 (44)	0.5 0.6
TOT	AL	21	475	65	33.8	
1 LNFB =	leaf num	ber from	tree base			

Values in parentheses are percent of total

first mature leaf; and PgA then declined basipetally in the lower-mature leaves.

Although both clones had similar within-crown PgA patterns on 10 July 1979. differences existed in whole-tree fixation (Pg carbon ug CO S tree ) and whole-tree tot 2 light interception (PPF u moles s tree ) (Table 1). tot Both clones intercepted over 60% of PPF and fixed ca. 60% tot of Pg in the upper crown region; however, Eugenei Pg tot surpassed Tristis Pg by 41% on 10 July 1979, largely due to 33% greater leaf area in Eugenei in the upper region.

Prior to setting bud on 30 July 1979, Tristis had a more rapid rate of height and diameter growth than Eugenei. After budset, leaf initiation and significant leaf expansion ceased. As a result, Tristis leaves were morphologically mature and its leaf area and total number of leaves were fixed until leaf abscission began in mid-August. In contrast, Eugenei maintained leaf production until setting bud on 10 September 1979. Nine of 33 Eugenei leaves were immature on 8 August 1979 (Chapter II). The 8 August 1979 entire leaf series measurement (Fig. 2), therefore, compared trees with similar physical but different phenological characteristics. The general within-crown photosynthesis pattern described for the 10 July 1979 entire leaf series measurement was observed on 8 August 1979 (Fig. 2). As the trees grew, the size of the immature and recently-mature leaf zones and the average area of individual leaves in these zones increased (Chapter II). Therefore, each clone

Clone	1 LNFB	Leaves	Area		PPF	01	2 PgL	LUE
							-1	
		-	E C	I	umole		ug CO s 2	ug CO mole 2
Tristis	31-34	4 C	148	(13)	15	(14) (54)	8.0 (9) 117 0 (56)	0.5 8
	11-20	200	362	(14)	500 000	(8)	22.0 (26) 9.0 (10)	
TOT	AL	34	1,234		110		86.0	
Eugenei	31-32 21-30 11-20 1-10	٥ <u></u> ٥ ٥	53 489 310	(4) (42) (33) (21)	34 26 26	(1) (38) (26)	0.3 ( 1) 36.0 (43) 31.0 (37) 17.0 (20)	0.9 0.1 0.7
TOT	AL	31	1,477		98		84.3	

Values in parentheses are percent of total LNFB = leaf number from tree base 2

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had a broader plateau of leaves with high PgA rates on 8 August 1979 than on 10 July 1979 (Fig. 2).

PgA rates were higher in Tristis than Eugenei in the upper crown on 8 August 1979 (Fig. 2), but Pg was simtot ilar for the two clones (Table 2). Eugenei compensated for its lower PgA rates by producing larger leaves than Tristis. was 5% greater in Tristis than Eugenei on 8 Overall. Pg August 1979. Uppermost leaves in Tristis were morhpologically mature, however, they did not attain PgA rates equivalent to leaves which matured before budset. However, those upper leaves had higher PgA rates than leaves at similar positions measured before budset (Fig. 2) and their PgA rates were higher than Eugenei upper leaves. Tristis surpassed Eugenei in Pg on 8 August 1979 largely due to greater productivity of its upper leaves. The two clones had roughly equal PPF on 8 August 1979, but light interception in Tristis tot was concentrated in its upper crown while in Eugenei, light was distributed more evenly (Table 2). Light was utilized with different effeciencies within trees and between clones. LUE's were highest in the lower-crown region of Tristis, indicating that its older leaves responded well to low light levels. In contrast, LUE's peaked in the upper-middle crown region of Eugenei and declined basipetally.

Terminal buds had been set for 30 days in Tristis by the time of the 30 August 1979 entire leaf series measurement (Fig. 2) and its leaf area did not increase between 8 August 1979 and 30 August 1979. Rather, abscission of four

lower-crown leaves had reduced its leaf area by about 10%. Eugenei was still producing new leaves on 30 August 1979, but leaf initiation rates were declining and budset occurred 10 days later (Chapter II). The Eugenei leaf complement consisted of 8 expanding and 29 mature leaves on 30 August 1979. Seven lower-crown leaves had abscised (14% of to-The more indeterminate growth pattern of Eugenei tal). produced a 44% leaf area advantage over Tristis on 30 August 1979 (Table 3). This leaf area difference produced compar-(41**%)** and Pg (44%) able differences in PPF between ot. Eugenei and Tristis. The lower-crown regions in Eugenei were less productive on 30 August 1979 than on 8 August 1979 due to leaf aging and abscission. However, this loss in productivity was compensated for by the addition of younger, larger leaves in its upper-crown. The lower-crown region in Tristis received more light on 30 August 1979 than on 8 August 1979 (Tables 2,3) because the sampled tree was inadvertently selected near an opening in the plantation created when a neighboring tree was harvested. Thus, leaves directed towards the opening received full sunlight. Tristis lower-crown leaves responded to this increased light than measured earlier. However, reduced with a higher Pg LUE's indicated that much of the additional light was used inefficiently.

<u>CO</u> <u>Compensation</u> <u>Point</u>.  $\Gamma$  ranged from 56 to 95 and 65 to <u>2</u> 3 -3 92 cm m in Tristis and Eugenei mature leaves,

Table 3. photon flu for Eugene	Within- ux (PPF) ei and T	crown cou , whole- ristis me	mparison of leaf photosy easured 30 A	total leaf are nthesis (PgL), ugust 1979.	a, photosynthet and light use	ically-active efficiency (LUE)
Clone	1 LNFB	Leaves	Leaf 2 Area	P P F	PgL_	LUE
			C - 5	-1 umole s	ug co s 2	ug CO mole
Tristis	21-30 11-20 1-10	000	450 (35) 495 (38) 346 (27)	53 (34) 54 (34) 50 (32)	38.0 (44) 31.0 (36) 17.0 (20)	0.7 0.6 0.3
TOT	AL	<u> 0</u>	1,291	157	86.0	
Eugenei	41-46 31-40 21-30 11-20 1-10	60 11 20 20 20	342 (15) 827 (36) 747 (32) 324 (14) 80 (3)	35 (13) 87 (33) 96 (37) 24 (9) 20 (8)	14.0 (9) 60.0 (40) 53.0 (36) 17.0 (11) 6.0 (4)	4.0 0.6 0.7 0.3 0.3
TOT	AL	11	2,320	262	<u>150.0</u>	

LNFB = leaf number from tree base 2

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Values in parentheses are percent of total

					-3	-2		
Table	4.	CO	compensat	ion points	(cm	m )	for	varoius
		2	-	-				
leaf p	ositi	ons f	or Tristis	and Eugen	ei tre	es by	date.	Each
value	repre	sents	the avera	ige of thre	e dete	rminat	ions.	•

Clone	Leaf Position	7 Jul	20 Jul	Date 15 Aug	29 Aug	8 Sep
				-3 -2 cm m		
Tristis	A B C D	 56 53	70 70 73	61 58 57 58	57 57 56 85	55 55 60 95
Eugenei	A B C D	72 92 65	76 85 70	227 70 70 72	191 80 70 73	85 72 75 70

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respectively (Table 4). Mean  $\Gamma$ 's were significantly lower in Tristis than Eugenei in the A and pooled B and C regions, however, no difference existed in the D region means.

Γ was highest in the immature Eugenei leaves, although it declined in those leaves as the season progressed. A slight

Γ increase was observed in lower Tristis leaves but not in Eugenei lower leaves as the season progressed (Table 4). Environmental Effects on Photosynthesis. Light saturation -2 -1was reached at ca. 1100 and 1700 u moles m s for Eugenei and Tristis leaves, respectively (Fig. 3C). Although LUE's were similar at all leaf positions, the PgA rate at saturating PPFDe (Pmax) varied markedly in both clones (Fig. 3C).

Both clones had the same photosynthetic response to leaf temperature (Fig. 3B). PgA increased linearly with increasing LT at all leaf positions reaching a maximum near 0 25 C and 30 C for Eugenei and Tristis, respectively. Temperature optima are uncertian since leaf temperatures 0 rarely exceeded 32 C.

<u>Stomatal</u> <u>Conductance</u>. Photosynthesis increased linearly with increasing conductance at low conductance levels (below -10.8 mm s ) in both clones (Fig. 4C). Photosynthesis reached a plateau (Pmax) at conductance levels above 0.8 mm s . Pmax and the rate at which Pmax was approached ( $\alpha$ ) varied within and between clones. The upper leaf position (A) in Tristis had the highest Pmax and steepest  $\alpha$ ; Pmax and  $\alpha$  declined basipetally within Tristis. The same trend

Figure 3. Boundary line plots of photosynthesis (PgA) versus photosynthetically-active photon flux density (PPFD) (B) and leaf temperature (LT) (A) for Tristis and Eugenei leaves at various positions within the crown. Only data near the boundary lines are shown. Stars (A) = immature leaf; circles (B) = recently-mature leaf; asterisks (C) = leaf in center of mature leaf zone; triangles (D) = lower-crown mature leaf.



Figure 4. Boundary line plots of photosynthesis (PgA) versus stomatal conductance (CON) (C), stomatal conductance versus photosynthetically-active photon flux density (PPFDe) (B), and stomatal conductance versus leaf temperature (LT) (A) for Tristis and Eugenei leaves at various locations within the crown. Only data near the boundary lines are shown. Stars (A) = immature leaf; circles (B) = recentlymature leaf; asterisks (C) = leaf in center of mature leaf zone; triangles (D) = lower-crown mature leaf.



was observed within Eugenei; however, its A leaf had a lower Pmax and flatter  $\alpha$  than Tristis. Tristis  $\alpha$ 's were steeper than similar leaf positions in Eugenei.

Conductance increased rapidly with increasing light in leaves of both clones (Fig. 4B). The slope of the CON/PPFD at low light levels was flattest in Eugenei upper and curve lower leaves (A and D) indicating that stomata in those leaves were less responsive to light fluctutions than other Tristis and Eugenei leaves. Tristis leaves attained higher CON rates at light saturation than Eugenei leaves, but the rate at which this maximum was approached was similar for the B and C leaf positions in both clones. CON measurements at low light levels are absent from our data since stomatal resistance could not be measured in early morning with the porometer due to water condensation on the leaves. As a result, threshold levels for stomatal opening could not be determined.

CON increased rapidly in response to increasing leaf temperature in all leaves (Fig. 4A). The response of CON to LT was the same in all Tristis leaves. Some differentiation occured in Eugenei: CON rates responded rapidly to LT in mature mid-crown leaves (B) and slowly in immature uppercrown leaves (A).

<u>Interrelationships</u> <u>Between Variables</u>. PgA increased linearly in the uppermost leaves of both clones (Fig. 5) and was strongly correlated with CON and LPI (Tables 5,6). CON and LPI were strongly intercorrelated in those leaves. PPFDe

Figure 5. The relationship between leaf number from the tree base (LNFB) and photosynthesis (PgA), photosynthetically-active photon flux density (PPFDe), stomatal conductance (CON), and leaf temperature (LT) for each leaf on single Tristis and Eugenei trees measured 30 August 1979.



and LT were weakly associated with PgA in the upper leaves. PgA fluctuated widely in the recently-mature leaves in response to changes in leaf microenvironment (Fig. 5). PPFDe, CON and LT were strongly associated with PgA in the recently-mature leaves, whereas LPI had a weak association with PgA. PPFDe, CON, and LT were highly intercorrelated in both clones in those leaves. PgA in the middle-mature region was also strongly associated with PPFDe, CON, and LT in both clones, while the correlation between PgA and LPI became stronger in Eugenei middle-mature leaves. The correlation between PPFDe and PgA was exceptionally high in Tristis middle-mature leaves (Table 5), indicating that light was the major factor limiting photosynthesis. LPI had a stronger negative relationship with PgA in the lower-crown leaves of both clones, especially Eugenei (Table 6). Correlation coefficients indicate that PgA was more closely associated with microenvironment in lower leaves of Tristis than Eugenei (Tables 5,6).

Diurnal Photosynthesis Patterns. On a sunny day, PgA, LT, and CON increased linearly in early morning (6 to 8 am ST) in response to rapidly increasing light. PgA rates were similar at all leaf positions in early morning (Figs. 6,7,8). As light levels increased in late morning, (8 to 10 am ST) PgA differences developed between leaves in accordance with their varying photosynthetic capacities, i.e., leaves at the B and C positions typically had higher PgA

Table 5. Simple correlation coefficients between photosyn-
thetic rate (PgA), photosynthetically-active photon flux
density (PPFD), leaf temperature (LT), stomatal conductance
(CON), and leaf number from the tree base (LNFB) for leaves
pooled acropetally in a 1 year-old Tristis tree measured at
10 am (solar time) on 30 August 1979.

LNFB		Parameter 1				
Range	PgA -	PPFD	LT	CON		
20-27 14-19 8-13 1-7	53 .85 .96 .69					
20-27 14-19 8-13 1-7	25 .82 .52 .51	.60 .96 .59 .95				
20-27 14-19 8-13 1-7	.40 .94 .75 .57	21 .83 .85 .80	26 .89 .75 .89			
20-27 14-19 8-13 1-7	.84 .11 .01 48	56 .59 09 07	16 .54 63 12	.62 .12 16 42		
	Range 20-27 14-19 8-13 1-7 20-27 14-19 8-13 1-7 20-27 14-19 8-13 1-7 20-27 14-19 8-13 1-7 20-27 14-19 8-13 1-7	RangePgA $20-27$ $53$ $14-19$ .85 $8-13$ .96 $1-7$ .69 $20-27$ $25$ $14-19$ .82 $8-13$ .52 $1-7$ .51 $20-27$ .40 $14-19$ .94 $8-13$ .75 $1-7$ .57 $20-27$ .84 $14-19$ .11 $8-13$ .01 $1-7$ .48	RangePgAPPFD $20-27$ $53$ $14-19$ .85 $8-13$ .96 $1-7$ .69 $20-27$ $25$ $1-7$ .69 $20-27$ $25$ $1-7$ .52 $20-27$ $25$ $1-7$ .51 $95$ $20-27$ .40 $1-7$ .51 $20-27$ .40 $1-7$ .51 $20-27$ .40 $1-7$ .57 $8-13$ .75 $1-7$ .57 $80$ $20-27$ .84 $1-7$ .57 $8-13$ .01 $09$ $1-7$ $48$ $07$	RangePgAPPFDLT $20-27$ 53		

Natural log transformation of PPFD

Table 6. Simple correlation coefficients between photo	syn-
thetic rate (PgA), photosynthetically-active photon flu	X
density (PPFD), leaf temperature (LT), stomatal conduct	ance
(CON), and leaf number from the tree base (LNFB) for le	aves
pooled acropetally in a 1 year-old Eugenei tree measure	d at
10.50 am (solar time) on 30 August 1979.	

	LNFB		Parame	ter	
Parameter	Range	PgA	PPFD	LT	CON
PPFD	41-47 29-40 12-28 1-11	.03 .76 .80 .18			
LT	41-47 29-40 12-28 1-11	17 .60 .71 .63	.74 .54 .66 .82		
CON	41-47 29-40 12-28 1-11	.86 .63 .31 .26	.29 .43 .35 .41	.19 .64 .65 .60	
LNFB	41-47 29-40 12-28 1-11	.96 .22 36 89	.13 .35 31 .06	01 13 25 50	.96 26 04 37

Natural log transformation of PPFD

Figure 6. The relationship between solar time and photosynthesis (PgA), photosynthetically-active photon flux density (PPFDe), stomatal conductance (CON) and leaf temperature (LT) for Tristis and Eugenei leaves at various crown positions measured during 17 July 1979. Stars (A) = immature leaf; circles (B) = recently-mature leaf; asterisks (C) = leaf in center of mature leaf zone; triangles (D) = lowercrown mature leaf.



Figure 7. The relationship between solar time and photosynthesis (PgA), photosynthetically-active photon flux density (PPFDe), stomatal conductance (CON) and leaf temperature (LT) for Tristis and Eugenei leaves at various crown positions measured during 15 August 1979. Stars (A) = immature leaf; circles (B) = recently-mature leaf; asterisks (C) = leaf in center of mature leaf zone; triangles (D) = lowercrown mature leaf.



Figure 8. The relationship between solar time and photosynthesis (PgA), photosynthetically-active photon flux density (PPFDe), stomatal conductance (CON) and leaf temperature (LT) for Tristis and Eugenei leaves at various crown positions measured during 29 August 1979. Stars (A) = immature leaf; circles (B) = recently-mature leaf; asterisks (C) = leaf in center of mature leaf zone; triangles (D) = lowercrown mature leaf.



rates in full sunlight than leaves at the A and D positions. However, this ranking varied considerably throughout the day, principally due to differential light interception between leaves.

Photosynthesis rates peaked between 10 am and 14 pm (ST); however, several leaves of both clones exhibited noon photosynthesis reductions. On some days, a noon PgA depression and corresponding CON reduction occurred in Eugenei (Fig. 6E,G). In most cases, leaves recovered quickly from these PgA depressions. PgA depressions associated with reductions in CON were not observed in Tristis; however, Tristis lower leaves frequently had reduced PgA rates during mid-afternoon associated with light reductions (Fig. 6,7A,B). Maximum photosynthetic rates attained by mature leaves during noon varied considerably between days (Figs. 6,7,8).

Leaf temperature usually reached a peak in mid-afternoon in both clones (Figs. 6,7,8D,H). Leaf temperature was closely correlated with air temperature. On windy days, all leaves had similar leaf temperatures, but leaf temperatures varied as much as 8  $^{\circ}$  C between leaves within the same tree on windless days (lower leaves generally had higher LT's than upper leaves). Stomatal conductance usually reached a plateau before 10 am (ST) and thereafter fluctuated with changes in light, leaf temperature, and presumably, endogenous factors (Fig. 6,7,8,C,G).

The volume beneath the surfaces shown in Figure 9

Figure 9. Total daily carbon uptake for single Tristis and Eugenei trees on 15 August 1979. (PgL - whole leaf photosynthesis; LNFB - leaf number from the tree base).



represents total carbon fixation for each clone during 15 August 1979. This integrated total was 2060 and 1995 mg CO -1 -1 tree d for Tristis and Eugenei, respectively.

## Total Photosynthesis and Yield

Diurnal photosynthesis integrations showed that Tristis out-performed Eugenei until late August (Table 7). Dry weight estimated from cumulative TDCF was strongly correlated with measured dry weight (overall r = 0.98; Table 8), although early season estimates were less accurate than those later in the season.

## Discussion

A growing tree is a complex system, so it is difficult to isolate the simple effects of individual factors on physiological processes. Problems arise in analyzing and interpreting changes in photosynthesis observed in the field since extreme interdependency among variables precludes using many standard statistical techniques (e.g., multiple regression) due to the presence of multicollinearity (23). As a result, it is difficult to statistically identify the proportion of change in photosynthesis attributable to a single factor. However, variation in crop yield can only be described by investigating crops growing in the field. A1though a low degree of statistical precision may be derived from field experiments, a high degree of ecological relevance is usually attained (15).

Differences in leaf area and specific leaf weight

Table 7. Total diurnal photosynthetic production (mg CO ) for single Tristis and 2 Eugenei trees on four days. Whole-leaf photosynthesis rates of each leaf within a tree were integrated over a 14 h diurnal period and summed to obtain total daily photosynthetic production per tree.

Date	Clone Tristis	Eugenei	
	mg C	:0	
3 Jul	149	202	
17 Jul	1,071	668	
15 Aug	2,056	1,948	
29 Aug	3,258	4,855	
Clone	Date	Estimated Cumulative Dry Weight	Measured Cumulative Dry Weight
---------	------------------------------------	---------------------------------------	--------------------------------------
Tristis	3 Jul 23 Jul 20 Aug 9 Sep	2 15 40 72	22 51 73
Eugenei	3 Jul 23 Jul 20 Aug 9 Sep	2 11 35 83	18 34 82

Table 8. Comparison of estimated and measured cumulative dry weight yield for Tristis and Eugenei trees by date.

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within individual trees and between clones made it difficult to compare photosynthesis on an equal basis. Results of photosynthetic comparisons in the present study were strongly dependent on how photosynthesis was expressed and which sets of leaves were pooled for comparison. Great care must be taken to provide a complete description of the history, condition, microenvironment, and position of leaves selected for clonal comparisons. It is also important to develop criteria for selecting those leaves. The LPI system was designed to select leaves at similar developmental stages and has been used successfully in several controlledenvironment studies. By definition, the LPI system requires that successive leaves are formed at equal intervals and have exponential growth (32). These criteria are not met (or rather, measurement is difficult) after budset. Therefore, it is difficult to use the LPI system to select "similar" leaves after budset. Instead, the crown region system, which compares leaves in similar light strata, or the LNFB system, which compares leaves initiated at similar times, should be used when comparing clones.

In addition, a relevant basis for expressing photosynthesis must be selected. Photosynthesis is commonly expressed on a unit area basis because light is intercepted and expressed on an area basis (42). However, Nelson and Ehlers (38) have shown that photosynthesis should be expressed on a unit dry weight basis when comparing leaves grown in different environments. Expressing photosynthesis on a whole-leaf

basis has the advantage of accounting for differences in intrinsic photosynthetic rates and leaf area.

The general within-tree ontogenetic pattern of photosynthesis observed in this study was consistent with patterns defined under controlled-environment conditions for Populus species (3,5,7,8,28). However, in the present study, changing environmental conditions were imposed upon this general developmental pattern resulting in greater variability in photosynthesis within single trees. In general, during active leaf production the photosynthetic capacity of young immature leaves increased in direct correlation with leaf Other studies have shown that the development of the age. photosynthetic system in expanding Populus leaves closely parallels RuBP carboxylase and Hill reaction activity (4). In addition, the level of stomatal maturity attained by each leaf may have influenced photosynthetic activity. as indicated by the close relationship between photosynthesis and conductance and also between conductance and leaf position. After budset, the upper most leaves ceased expansion, their specific leaf weights increased, and they attained photosynthetic rates between those of expanding leaves and leaves which reached full expansion prior to budset.

Recently-mature leaves combined high photosynthetic rates with large assimilatory surfaces and comprised the major production center within trees of both clones. The photosynthetic output of these leaves was extremely variable since they were responsive to small changes in leaf microenviroment. Light was the principal factor governing PgA, but CON and LT were also important, especially at their extremes, e.g., during cool mornings or hot, dry afternoons. In general, recently-mature Tristis leaves had higher PgA rates than recently-mature Eugenei leaves. Lower  $\Gamma$ 's in recently-mature and lower-crown Tristis leaves may indicate that they had lower photorespiration rates (38) and higher photosynthetic efficiencies than comparable Eugenei leaves.

Lower-crown leaves responded to fluctuations in PPFD and their stomata remained functional early in the growing season, but as the season progressed PgA rates and stomatal responsiveness declined. Other workers have found that partial stomatal closure accompanied PgA reductions in senescing <u>P</u>. <u>deltoides</u> leaves (9). Slightly reduced conductance rates were also observed in this study in aging lower-crown leaves, although, conductance rates in several of these leaves were equivalent to those found in recentlymature leaves. Stomata in lower leaves with high conductance rates appeared to be unresponsive to light fluctuations.

In general, reductions in conductance did not appear to fully account for observed declines in photosynthesis in aging leaves. It has been observed that internal (or mesophyll) resistances were higher than stomatal resistance in aging <u>Populus</u> leaves (3,37) suggesting that internal resistance is a major limitation to photosynthesis. Decreases in RuBP carboxylase synthesis (8) and in the performance of the ATP-synthesizing system (16) may increase internal resistances, thereby limiting photosynthesis in aging leaves (37).

Response curves between PgA, PPFD, LT, and CON reflected the developmental patterns described above. PgA/PPFD response curves indicated that all leaves within a clone responded to light at approximately the same rate; however, Pmax varied widely with leaf age. Pmax values were higher in Tristis than Eugenei at all leaf positions, suggesting that Tristis leaves better utilized high light; leaves in the upper mature leaf zone attained the highest Pmax rates. supporting earlier controlled-environment findings (5,28): and lower-crown leaves had reduced Pmax rates due to leaf The ranking of the upper-crown leaves depended on aging. the phenology of the tree: prior to budset, upper leaves were immature and had low Pmax rates: after budset. upper leaves had Pmax rates somewhere between those of immature and mature leaves. The PgA/PPFD curves reported here compare favorably with other data reported for Tristis and Eugenei (38) and for P. deltoides (40).

Extremes in leaf temperature can impose limitations on photosynthesis in the field. Extremely high air and leaf temperatures are rare in northern Wisconsin, but the effects of low leaf temperature on photosynthesis were evident in these data. Leaves of both clones had the same linear photosynthetic response to increasing leaf temperature, consistent with data reported elsewhere for <u>Populus</u> species (9,12,33).

The general shape of the PgA/CON curves conformed to those reported for other species (11,22,44). Both clones had similar photosynthetic responses to conductance: PgA quickly increased in response to increasing CON up to a -1maximum near 0.8 mm s<sup>-1</sup>; thereafter, further increases in CON did not influence PgA directly; however, further CON increases probably had an adverse effect on water-use efficiency (i.e., the amount of water transpired per unit of CO fixed (48)).

Within-crown diurnal photosynthesis patterns must be measured throughout the growing season to estimate the contribution of each leaf and crown region to dry matter production. The diurnal photosynthesis patterns reported here were more irregular than commonly-reported bell-shaped diurnal patterns because changing cloud conditions, leaf displays, and mutual shading patterns produced extremely variable diurnal light interception. As a consequence, each diurnal pattern was unique. In general, however, the importance of environmental variables shifted throughout the day according to the pattern described by Kramer and Kozlowski (26). In early morning, PgA rates of mature leaves quickly increased with increasing PPFD and LT; in mid-afternoon, PPFD and, occasionally, CON were principal factors controlling PgA; PPFD and LT were again important in late afternoon as PPFD and LT declined. LT became an increasingly important variable late in the growing season as the mornings and late afternoons became cooler. Since CON

rates of 0.8 mm were typically attained by 8 am (ST), the rate-limiting effect of CON was restricted to early morning, unless noon CON depressions occurred. Although it is difficult to separate the individual roles of these highly intercorrelated variables, light appeared to be the principal environmental factor controlling photosynthesis in mature leaves since it directly influenced all other environmental parameters. In general, photosynthesis, conductance, and leaf temperature patterns within trees mirrored light interception patterns. The overwhelming influence of light on photosynthesis has been demonstrated for several other field crops (25,35,45).

The two clones appeared to have different leaf area development and photosynthetic "strategies". Eugenei produced many large leaves while Tristis produced fewer smaller, but thicker leaves (using SLW as an indication of leaf thickness) which had higher PgA rates. Due to its horizontal leaf display, light interception and photosynthetic activity were concentrated in the youngest, most productive upper-crown leaves within Tristis at the expense of its lower-crown leaves. In contrast, the vertical leaf display in Eugenei permitted a more even distribution of light and photosynthesis throughout its crown. It is difficult to determine which "strategy" was "best" since other factors confound such a comparison; e.g., clonal differences in intrinsic PgA rates, total leaf area, leaf area duration. and tree growth patterns. Several investigators have pro-

posed that photosynthetic output is maximized when light is distributed uniformly over many leaves (13,39,47). However, much of the dispersed light in Eugenei was intercepted by relatively unproductive leaves which became senescent and abscised soon after midseason. Moreover, light interception was reduced in the photosynthetically efficient upper mature Eugenei leaves due to their vertical display. When aging effects produce a rapid basipetal decline in photosynthetic capacity, it may be more efficient to maximize light interception within the younger, upper-crown mature leaves rather than disperse light evenly throughout the crown.

Lower leaves in Eugenei intercepted such a large proportion of the total intercepted light that it would appear advantageous to culturally or chemically delay their senescence and abscission. Increases in root development and stem growth may be realized from better leaf retention in the lower-crown (20). However, lower-crown leaf abscission may improve water-use efficiency and growth in Eugenei because it was susceptible to periodic water stress, possibly resulting from its high shoot/root ratio.

To estimate the total photosynthetic output of a tree, the leaf area over which photosynthesis occurs must be known. Only then can an estimate of diurnal and seasonal carbon production be obtained. Intuitively, dry matter accumulation should be directly related to the integrated product of PgA and leaf area. However, several studies have failed to show a relationship between photosynthesis and

yield (10). In many cases, an adequate sample was not obtained in the field to quantify diurnal and seasonal shifts in photosynthesis and leaf area. Sampling is one of the greatest challenges in field research. Laboratory studies often fail to relate photosynthesis to field productivity because the duration of leaf area and photosynthetic activity are not considered. The close association between cummulative total daily carbon fixation and cummulative dry weight reported here resulted from knowledge of diurnal and seasonal changes in photosynthetic rate and leaf area. Leaf area was highly correlated with estimated total daily carbon fixation and dry weight in both clones. Eugenei outgrew Tristis principally by better-utilizing the growing season for leaf area development. A strong positive correlation between wood weight and leaf area has been reported for 1year-old poplar sprouts by Larson and Isebrands (30). In the present study, knowledge of leaf area changes were at least as important as knowledge of PgA changes. Without knowing the pattern of leaf area development in both clones, it would be difficult to estimate total daily carbon fixation. Models which predict long-term dry weight accumulation cannot be developed until factors which regulate photosynthate partitioning into roots, stem and new leaves in the field are better understood.

Due to complex interactions between the factors which govern yield, it is doubtful whether any single gas exchange or morphological variable will reliably indicate yield potential. Instead, leaf area development, the rate and duration of photosynthesis, and patterns of photosynthate distribution and partitioning be considered in together. Knowledge of how these processes operate under field conditions should improve the effectiveness of future <u>Populus</u> tree breeding programs. References

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# CHAPTER IV

# LEAF ORIENTATION, LIGHT INTERCEPTION AND PHOTOSYNTHESIS IN TWO <u>POPULUS</u> CLONES

## DURING THE ESTABLISHMENT YEAR

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ABSTRACT

influence of leaf orientation and leaf area on The light interception and photosynthesis was examined for two Populus clones during their first growing season. Field leaf angle measurements were used to construct leaf normals describing each leaf's orientation and angle with respect to the sun's rays. The proportion of leaf area projected toward the sun was estimated on a diurnal basis. The erectophile clone (Eugenei, P. x euramerica) projected its leaf area evenly throughout the day while the planophile clone (Tristis, <u>P. tristis x P. balsamifera</u>) had a peak leaf area projection at solar noon. Total diurnal leaf area projections for the two clones were similar, even though the erectophile clone had more actual leaf area than the planophile clone. The abaxial leaf surface comprised a greater proportion of the diurnal leaf area projection in the erectophile clone than the planophile clone.

Photosynthesis rates were calculated from measured and estimated light interception rates to assess the importance of leaf orientation on total-tree photosynthesis. Withintree mutual shading was the most significant cause of light reductions in the planophile clone, whereas the direct effect of leaf orientation was most responsible for light reductions in the erectophile clone. Declines in photosynthesis were proportionately less per unit light reduction in the planophile clone, since reductions occured in its less productive lower-crown leaves, whereas reductions in light

and photosynthesis occurred in the productive upper and middle-crown leaves in the erectophile clone. Leaf orientation was as important as leaf area in accounting for clonal differences in total-tree photosynthesis.

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INTRODUCTION

The yield of a tree growing in the field is largely determined by its ability to capture radiant energy and convert this energy into useable biomass through photosynthesis. It is especially important to understand how leaf display and canopy structure influence light interception and photosynthate production in the first growing season since the success or failure of a tree in subsequent years is greatly influenced by first-year performance.

The manner in which leaves are displayed profoundly influence single-leaf light interception and photosynthesis and also determine the distribution of light and photosynthesis within individual <u>Populus</u> trees (Chapter III). Since a detailed investigation of the geometrical structure of a crop is prerequisite to an examination of photosynthesis (Ross and Vlasova, 1967), leaf display and light interception were quantified under a variety of field conditions. Canopy geometry, light interception, and photosynthesis have frequently been evaluated in unison for agronomic crops (e.g. deWit, 1965; Hesketh and Baker, 1967); however, the interrelationships between these factors are poorly understood for individual trees growing in the field, principally because of the difficulty of making field phytometric measurements.

A technique for quantifying leaf orientation and evaluating its influence on single-leaf and whole-tree photosynthesis is presented here for single-stemmed, one year-old

Populus trees.

## MATERIAL AND METHODS

# <u>Plant Material</u>

Two hybrid poplar clones, <u>Populus x euramericana</u> cv. "Eugenei" (NC 5326) and <u>P. tristis x P. balsamifera</u> cv. "Tristis #1" (NC 5260) were grown under a short-rotation intensive culture system (Hansen et al., 1979). The trees were established at the U.S. Forest Service's Harshaw Experimental Farm near Rhinelander Wisconsin (U.S.A.) on a 0.6 m x 0.6 m spacing using unrooted hardwood cuttings. Separate plantations were established during May in 1979 and 1980. The trees in each plantation were sampled throughout their first growing season. Cultural treatments have been described in an earlier paper (Chapter II).

#### Field Measurements

Figure 1. Leaf axes and vectors used to quantify leaf orientation in <u>Populus</u> leaves. V = vector along the leaf 1 midrib (leaf axis #1); V = vector perpendicular to V in 2 the lamellar plane (leaf axis #2); N = vector normal (per-L pendicular) to V and V. 1 2



leaf when facing the leaf tip. The north azimuth of leaf axis #1 was measured with a Silva compass. Each leaf's vertical distance from the base of the stem, its length, and area (LA) were also determined. Tree height was then recorded for each tree sampled.

Photosynthetically-active photon flux density (PPFD, u -2 -1 mole m s , 400 to 700 nm) was measured normal to the (PPFD ) and abaxial (PPFD ) surfaces of each samadaxial pled leaf using a LiCor light meter and quantum sensor (hereafter, the terms "PPFD" and "light" will be used interchangeably). Leaves with over 50 \$ of their area in shade were recorded as "shaded" leaves. In addition, above-crown PPFD was measured: (1) in a horizontal plane (PPFDh) and. (2) perpendicular to the sun's rays (PPFD ). Total PPFD DS for individual leaves was obtained from : PPFD = PPFD tot A D PPFD . Photosynthetically-active photon flux on a whole-AB leaf ) was calculated from leaf basis (PPF, u mole s PPF = PPFDx LA. The PPFD effective in driving photosyntat + (PPFD x CF). thesis was calculated as: PPFD = PPFDAD AB where CF was a conversion factor to adjust for the different efficiencies of abaxial and adaxial light in driving photosynthesis. CF has not been experimentally determined for poplars; therefore, 0.5 was selected after reviewing our field photosynthesis data and data presented by Moss (1964). Effective photon flux on a whole-leaf basis was calculated PPF = PPFD x LA. Total photosynthetically-active as: -1 -1 flux (PPF , u mole s tree ) and total effective photon

photosynthetically-active photon flux (PPF, u mole s -1 et tree ) were obtained by summing the PPF and PPF, e respectively, of all leaves within a tree.

# Calculation of Leaf and Sun Vectors

Unit vectors describing leaf axis #1 and #2 (V and V, 1 2 respectively) and a vector normal (i.e., perpendicular) to V and V (N, Fig. 1) were calculated using equations given 1 2 L in Appendix A. The north azimuth and zenith angle of N L were then calculated (Appendix A). A vector describing the sun's rays (V) as a function of date and solar time (ST) s was obtained using methods described in Appendicies A and B.

# Projected Leaf Area

The area of each leaf projected onto a plane perpendicular to V was calculated according to Max (1975): PROJLA s = LA x  $\phi$  where: PROJLA is the leaf area projected onto a plane perpendicular to V , LA is the actual leaf area, and  $\phi$  is the angle between N and V (Appendix A). Leaf area L s projections were performed for both clones over a diurnal period by varying V. The ratio of total PROJLA to LA S (PROJLA/LA) was computed for both clones for each hour.

## Light Interception Model

A model which predicts PPFD and PPFD as a function AD AB of leaf orientation, leaf position, and V was developed and run on an IBM-PC microcomputer. Components of this model were: (1) direct light, (2) diffuse light received from the sky, (3) diffuse light received from the surrounding vegetation, and (4) diffuse light reflected from the soil surface.

The direct and diffuse components of total light available to unobstructed leaves were estimated by establishing transmission coefficients for direct and diffuse light for specific solar times and atmospheric conditions. To obtain the atmospheric transmission coefficient for direct light, the total instantaneous direct solar radiation on a horizontal surface above the Earth's atmosphere was calculated (Gates, 1980)

hSo =  $\overline{So} (d/\overline{d})^2 (\sin \lambda \sin \delta + \cos \lambda \cos \delta \cos \eta)$  (1) where: hSo= instantaneous total solar radiation (Wm<sup>-2</sup>);  $\overline{So} = -\frac{2}{3}$ solar constant (1353 Wm<sup>-2</sup>; Birth, 1975);  $\overline{d}$  = mean Earth-sun distance (1.495 X 10 km; Duffett-Smith, 1981); d = Earth to sun distance (km);  $\lambda$  = latitude;  $\delta$  = solar declination; and  $\eta$  = hour angle. hSo found from (1) represents total irradiance (i.e., irradiance integrated across all wave-

units in the 400 to 700 nm wavelength range using

$$hPPFDo = 4.57 x (hSo x 0.567)$$
 (2)

lengths) expressed in Wm ; hSo was converted into quantum

where: hPPFDo = photosynthetically-active photon flux density on a horizontal surface above the Earth's atmosphere -2 -1 -2 (umole m s , 400-700 nm); 4.57 = a factor to convert Wm (400-700 nm) to PPFD (400-700 nm) (Biggs and Hansen, 1979); and 0.567 = a factor to convert total hSo to hSo in the 400 to 700 nm range for skylight (Thimijan and Heins, 1983).

$$\mathbf{T} = PPFDh/hPPFDo$$
(3)

where: T = direct light transmittance and PPFDh = PPFD measured on a horizontal surface beneath the Earth's atmosphere. The transmittance of the atmosphere to diffuse light d (T) was estimated from the relationship established by Liu and Jordan (1960)

$$\begin{array}{c} d & m \\ T &= 0.271 - 0.294 \ T \end{array}$$
 (4)

Average diffuse skylight (PPFD ) was then estimated from dif

To find d in (1), the sun's true anomaly (V) was calculated using (Duffett-Smith, 1981)

$$V = M + \{(360/\pi) \times e\} \sin M$$
 (6)

where: M = mean anomaly and e = eccentricity of orbit = 0.016718. M was found from

$$M = N + Eg - Wg$$
(7)

where: N = {(360/365.2422) x D} (note: multiples of 360 must be added or subtracted until N lies in the range of 0 to 360); D = the number of days since the 1980 epoch; Eg = mean longitude of the sun at epoch = 278.83354; and Wg = mean longitude of sun at perigee = 282.596403. Then, to find d

$$d = \{r (1 - e)\}/(1 + e \cos V)$$
(8)

where: r = semi-major axis = 1.495985 X 10 km.

Two types of diffuse light were considered: (1) diffuse light from the open sky (PPFD ) and, (2) diffuse light dif.s received from the surrounding vegetation (PPFD ). If a dif,v leaf was directed above the vegetation, it received diffuse light from the sky; if it was directed into the surrounding vegetation, it received diffuse light which had filtered through other leaves. A leaf received diffuse light from the open sky when: C + LDFB > TH where: C = tan Nalt x D; D = the distance between trees; Nalt = the altitude of the leaf normal (Nalt = 90 - Nza; Appendix A); LDFB = the leaf's vertical distance from the base of the tree; and TH =tree height. Otherwise, the leaf received diffuse light from the surrounding vegetation.

If the leaf was directed towards the open sky, it received the full diffuse light from the sky calculated in (6) (i.e., in this case, PPFD = PPFD ). This assumes dif,s dif an isotropic sky for diffuse light. If the leaf was directed toward the surrounding vegetation, then PPFD was dif attenuated according to Beer's Law

where: PPFD = diffuse light received from the surrounddif,v ing vegetation; k = extinction coefficient for diffuse light; and LAI = cummulative leaf area index above the leaf -2 -2 (m m ). k was obtained from (Monteith, 1969)

$$\mathbf{k} = \cos \bar{\boldsymbol{\phi}} \, \cos \boldsymbol{\epsilon} \, \boldsymbol{\beta} \tag{10}$$

where:  $\bar{\phi}$  = the mean angle between the leaf normals and the sun's rays (Appendix A) and  $\beta$  = the sun's altitude (Appendix B).

Direct PPFD incident on a leaf (PPFD ) was found dir,l from Lambert's cosine law (Robinson, 1966)

Soil reflection was calculated by attenuating PPFD as ps it passed through the vegetation to the soil surface, and then by attenuating the reflected light as it passed up through the canopy to the vicinity of the leaf. The downward attenuation of light was calculated from

$$PPFD = \sin\beta \times PPFD \times e \times r$$
(12)  
sd ps s

where: PPFD = downward attenuation of PPFD ; LAI = cummusd ps lative LAI to the soil level; and r = the reflectivity of s the soil surface (r was 0.07 for the soil in this study). Light available to the leaf (PPFD ) was then calculated soil from

where: LAI = cummulative LAI from the soil to the leaf. When the adaxial leaf surface was directed toward the

PPFD = PPFD + PPFD AD dir,1 dif where: PPFD = adaxial PPFD and PPFD = PPFD dif dif,s · AD . Abaxial light was then obtained from dif,v PPFD = (PPFD x cos  $\rho$ ) + (cos Nza x PPFD ) (15) AB dif,v soil 0 where: PPFD = abaxial PPFD and  $\rho = 90$  - Nza. AB

(14)

or

The

assumption was made in (15) that abaxial diffuse light was received horizontally from the surrounding vegetation when the adaxial surface was directed toward the sun.

When the abaxial leaf surface was directed toward the sun

```
PPFD = PPFD
                                           (16)
   AD
        dif
were: PPFD = PPFD or PPFD , and
      dif dif,s dif,v
PPFD = (PPFD \times \cos v) + PPFD
                                           (17)
          dir,1
                           dif
   AB
          + (cos Nza x PPFD )
                        soil
```

where:  $\upsilon =$  the angle between the normal to the abaxial leaf surface and the sun's rays.

<u>Clonal Comparisons</u>

Trees with similar numbers of leaves were measured on

155

sun

PPFD

8-20-79 and 7-22-80 (one tree per clone per date); these trees were the basis for clonal comparisons. Although the sampled trees had similar leaf numbers, they were at different phenological stages in both years, i.e., Eugenei was actively producing new leaves at its apex whereas budset had occurred in Tristis.

The influence of leaf orientation and leaf size on within-tree light interception was assessed by comparing measured light interception values against estimates of PPFD for unobstructed leaves. The light interception model was used to estimate PPFD for leaves within the crown which were shaded. In this manner. an estimate of light interception for an unshaded leaf complement was obtained. The light interception model was also used to estimate PPFD leaves mathmatically rotated so that their laminae were for perpendicular to the sun (i.e., N was parallel to V ).

The influence of leaf orientation and leaf size on -1 single-leaf and whole-tree photosynthesis (PgL, ug CO s -1 and PgT, ug CO s tree , respectively) was assessleaf ed by substituting measured and estimated PPF values into light response curves (i.e., PPFD versus PgA (mg CO m )) developed earlier for these clones (Chapter III; Fig. 2). To discern how differences in leaf area, leaf orientation, and photosynthetic response to light influenced clonal PgT differences, PgT was recalculated for Tristis using: (1) Eugenei's leaf area and PPFD/PgA curves (to

Figure 2. The relationship between photosynthesis (PgA) and PPFD for field-grown Tristis and Eugenei leaves during their first growing season. A = upper-crown leaf (LPI 3); B = recently-mature leaf (LPI 9); C = mature leaf midway between B and D; and D = sixth mature leaf from the base of the stem. These curves were generated from data presented in Chapter III.





examine the separate effect of leaf orientation), (2) Eugenei's leaf orientation and leaf area (to examine the separate effect of the PPFD/PgA curves) and, (3) Eugenei's leaf orientation and PPFD/PgA curves (to examine the separate effect of leaf area). For example, to isolate the seperate effect of leaf orientation, PPFD rates measured in Tristis for each leaf were substituted into PPFD/PgA curves developed for Eugenei and the resultant PgA rates were extrapolated over Eugenei's leaf area.

#### RESULTS

The two poplar clones had widely contrasting leaf displays: leaves were oriented vertically (i.e., erectophile) in Eugenei and horizontally (i.e., planophile) in Tristis (Fig. 3). Leaf direction was largely controlled by phyllotaxy in both clones; however, slight deviation from a strictly phyllotactic series occurred due to twisting and bending along the petioles of a few leaves. Midrib angles gradually increased basipetally in both clones causing leaves to be nearly upright near the apex, more horizontal in the middle-crown region, and sloped slightly downward in the lower crown. Leaves exhibited greater variation from the horizontal (i.e., leaf axis #1 was horizontal when the midrib angle equaled 90 ) in Eugenei than in Tristis. Lamina angles differed dramatically between clones. Lamina angles varied only slightly from the horizontal (i.e., leaf axis #2 was horizontal when the lamina angle equaled 90 ) in

Figure 3. Leaf azimuth, midrib angle, and lamina angle by LPI for the entire leaf complement of single Tristis and Eugenei trees measured on July 22, 1980.


Tristis; in contrast, lamina angles varied up to 90 from the horizontal in Eugenei. Eugenei's more vertical leaf display was derived largely through rotation around leaf axis #1, i.e., by adjustment of the lamina angle.

The azimuth and zenith angles of N are plotted in L Figure 4 for all leaves of one tree per clone measured on July 22, 1980. Figure 4 is a two-dimensional representation of three-dimensional N projections onto the celestial sphere and illustrates to which region of the celestial sphere each leaf was directed. Tristis leaves were directed near the zenith and had N zenith angles less than 45°. The two exceptions to this occured at leaf plastochron index (LPI, Larson and Isebrands, 1971) 0 and 3 which were vertical leaves near the apex with N zenith angles of 50 and 65°. In contrast, N zenith angles in Eugenei ranged from 0° to 80°. Leaves did not appear to have an azimuthal preference in either clone.

Diurnal leaf area projected onto a plane perpendicular to V differed greatly between clones (Fig. 5). Tristis had a bell-shaped pattern with a peak occurring at solar noon. The adaxial surface comprised most of the projected leaf area in Tristis, although a small proportion represented the abaxial leaf surface directed toward the sun during early morning and late afternoon. The PROJLA/LA ratio varied from 0.26 to 0.84 for Tristis leaves (Fig.6). Total projected leaf area also peaked near solar noon in Eugenei, but

Figure 4. Equal-area projection of the azimuth and zenith angles of N for Tristis and Eugenei leaves measured on L August 20, 1979. Latitude lines denote zenith angle and longitude lines denote north azimuth angle.





Figure 5. Diurnal leaf area projections onto a plane perpendicular to the sun's rays for Tristis and Eugenei leaves measured on August 20, 1979.

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Figure 6. Ratio of total leaf area/total projected leaf area (PROJLA/LA) for Tristis and Eugenei leaves for the diurnal period of August 20, 1979.



Eugenei projected less leaf area toward the sun than Tristis during the noon period (Fig. 5). Eugenei projected substiantially more leaf area toward the sun than Tristis during early morning and late afternoon, and abaxial projections comprised a greater proportion of its total projected leaf area than observed for Tristis. The PROJLA/LA ratio in Eugenei was lower and less variable than in Tristis, ranging between 0.54 to 0.66 (Fig.6). Although Eugenei had 10% more actual leaf area than Tristis on August 20, 1979 (LA = 1691 and 1527 cm for Eugenei and Tristis, respectively), Eugenei had only 3% more leaf area projected toward the sun over the diurnal period.

The separate effects of leaf orientation and mutual shading were assessed by comparing the light interception of leaves measured under natural conditions against estimates of light interception for a totally unshaded leaf complement, using the light interception model to estimate PPFD AD in full sun for all leaves that were shaded. and PPFD A AB good correlation between known and estimated PPFD and AD PPFD (r = 0.7) was obtained for unobstructed leaves using the light interception model.

The difference between estimated PPF on PgT for an t unshaded versus a shaded leaf complement represented the effect of within-tree mutual shading. Mutual shading resulted in a 14 % reduction in PPFt and a corresponding 6 % reduction in PgT in Tristis on July 22, 1980 (solar time (ST) = 10.00 to 14.00 h) (Table 1). PPFt was reduced in

Table 1. Tot: Tristis and F on July 22, leaves orient	al-tree light interc Eugenei leaves measu 1980 compared to e ced perpendicular to	eption (PPFt) and red between 10.00 stimates for total the sun.	photosynthesis (PgT) for and 14.00 h (solar time) ly unshaded leaves and
	8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8		
CLONE/CONDIT	n) (n	mole s tree )	(ug CO2 s tree )
<u> Tristis</u>			
Measured		336	204
Unshaded		392	216
Unshaded,	Perpendicular to su	n 483	221
Eugenei			
Measured		304	161
Unshaded		313	167
Unshaded,	Perpendicular to su	n 537	196

Eugenei by 3 %, which corresponded to a 4 % PgT reduction.

The separate influence of leaf orientation was assessed by comparing the unshaded leaf complement in its natural orientation against estimates of PPF for a complement of t unshaded leaves oriented perpendicular to the sun. PPF for t unshaded Tristis leaves in their natural orientation was 19% less than PPF for unshaded leaves facing the sun (Table 1). t This reduction corresponded to a 2 % reduction in PgT. PPF for naturally oriented unshaded Eugenei leaves was 42 % less than for leaves facing the sun, resulting in a 15 % PgT reduction (Table 1).

PgT was 21 % greater in Tristis than Eugenei during midday on July 22, 1980 (Table 1). When both clones were given a common leaf area and PPFD/PgA curves, PgT was greatest in Tristis by 15 % (Table 2); when both clones had the same leaf orientation (and therefore, the same within-tree PPFt) and leaf area, PgT was greatest in Tristis by 23 %; and when both clones were given the same leaf orientation and PPFD/PgA curves, Eugenei exceeded Tristis' PgT by 17 %.

## DISCUSSION

This examination of photosynthesis within one-year-old poplar trees revealed that leaf orientation and leaf size were important determinates of light interception and photosynthesis at the level of the single leaf as well as the whole tree. Even in the first growing season, significant within-tree mutual shading occured and light was attenuated due to the direct effect of leaf orientation.

Table 2. Tristis and Eugenei's measured total-tre estimates of PgT when leaf orientation (LO), leaf ar to light (PPFD/PgA) were held constant between clone time), July 22, 1980. Numbers in parenthesis Eugenei's measured rate.	e photosynthesis (PgT) compa ea (LA), or photosynthetic a s. Measured 10.00 to 14.00 h indicate percent difference	ared to response h (solar ice from
CLONE/CONDITION	BET-1EACTOL-1-1EACTOL(ug COstree	DR TESTED
EUGENEI - measured	161	
Tristis w/ Eugenei's LA & PFD/PgA curves	190 (15) Tr	0
Tristis w/ Eugenei's LO & LA	210 (23) PFD,	/PgA
Tristis w/ Eugenei's LO & PFD/PgA curves	134 (-17) LJ	A.
TRISTIS - measured	204	

In the first growing season, single-stemmed trees were examined whose crowns were essentially isolated from their neighbors. This may, at first, appear to be a simplistic approach; however, as Thornley (1976) emphasized, the isolated plant is generally a more difficult theoretical problem than the crop growing as a stand. In addition, it is essential to identify the geometrical and biological parameters which control light interception and photosynthesis within a realtively simple crown before advancing to older, more complex trees and whole stands.

A leaf area projection pattern which promotes photosynthesis during early morning and late afternoon may be more conducive to Eugenei's growth than one which maximizes leaf exposure during noon, since Eugenei had a high shoot/root ratio which predisposed it to water stress during the hot noon period. The low shoot/root ratio in Tristis may have allowed its horizontal leaves to take advantage of the favorable light environment occuring during solar noon without suffering from stresses associated with high leaf temperatures.

The influence of leaf orientation on light interception can be examined on at least two levels: (1) direct effects the influence of leaf orientation on individual-leaf light interception, and (2) indirect effects - the influence of leaf orientation on mutual shading within the tree. Tristis' leaf display produced greater light losses from mutual shading but less reductions due to leaf orientation

than Eugenei's leaf display. In effect, the Tristis leaf display is a compromise, with irradiation of its lower crown sacrificed so that upper-crown leaves are fully irradiated. In contrast, full irradiation of the upper-crown leaves in Eugenei was compromised so that leaves could be irradiated throughout its crown. Since upper-crown Eugenei leaves fail to intercept much of the available light, very little mutual shading occurred.

Reductions in PPF resulting from the combined effects of mutual shading and leaf orientation were remarkably similar in the two clones. However, the crown regions in which these reductions occurred differed markedly: lower Tristis leaves and upper- and middle-crown Eugenei leaves experienced reduced PPFD rates. Leaves in different crown regions respond differently and in a non-linear fashion to intercepted light. Therefore, the impact of these light interception patterns can only be assessed by considering the photosynthetic response of leaves in specific crown regions to intercepted light. The PgT reduction per unit PPFt reduction was proportionately less in Tristis than Eugenei because Tristis PPFD reductions occurred in its less productive, lower-crown leaves, whereas the majority of Eugenei's PPF reductions occurred in its productive upperand middle-crown leaves. Several investigators have suggested that photosynthetic production would be maximized in a crown which disperses light so that a large number of leaves throughout the crown are irradiated below light sat-

uration (e.g., deWit, 1965). However, leaves in the lowercrown region must be photosynthetically responsive to light received for this type of dispersal pattern to be effective. Although an even distribution of light occurred in Eugenei, its lower-crown leaves did not photosynthetically respond to this favorable light environment to the extent that losses incurred in upper-crown leaves were offset. In fact, Eugenei lost 10 % of its leaf complement during midseason as a result of senescence in the lower-crown (Chapter II), negating any beneficial effect of its light dispersal pattern.

It would be difficult to experimentally isolate the separate effects of leaf orientation, leaf area, and intercepted light without mechanically or genetically manipulating the two clones to vary one factor while holding the others constant. Although these factors can probably be manipulated genetically over a wide range, genetic manipulation could be hampered by pleiotropy. Mechanical manipulation of leaf orientation or leaf area may induce unwanted plant responses which would confound the comparison. As an alternative, an estimate of the separate effects of these variables was obtained by mathmatically varying one factor while holding the others constant.

To predict adaxial and abaxial PPFD, the direct and diffuse light received by each leaf must be estimated. Estimating the diffuse light component has traditionally been the most diffucult task confronting modelers, since

light emitted from each region of the sky varies with atmospheric conditions, solar azimuth, and solar altitude. In addition, diffuse light impinging upon a leaf is dependent upon the orientation and position of the leaf within the tree and degree of shade. Prediction of the diffuse light contribution of each sky region under a wide range of plant and atmospheric conditions would be difficult, if not impossible. Therefore, a more generalized approach to estimating diffuse light was employed in the model presented here. The model supplied adequate predictions of adaxial and abaxial direct and diffuse light for unobstructed <u>Populus</u> leaves within one-year-old trees; however, patterns of intra- and inter-tree shading were not considered. A much more sophisticated model than the one presented here would be required to predict shading patterns within individual trees; such a model would facilitate the development of ideotypes for Populus trees.

The PPFD/PgA response of leaves was found to be the most important factor contributing to the observed PgT difference, followed closely by leaf area and leaf orientation. Clonal differences in the PPFD/PgA curves may be due to several factors: (1) different leaf anatomy (e.g., different mesophyll thickness per unit leaf area, different chlorophyll concentrations), (2) different residual resistance to CO movement into the leaf (Nelson and Ehlers, 2) different leaf aging patterns. The initial slopes of the PPFD/PgA curves may profoundly affect PgT since many leaves were oriented so they received less than saturating PPFD in the linear region of the curve.

There is an indication that leaf orientation was as important as leaf area in accounting for the observed PgT difference. Tristis compensated for its smaller leaf area by arranging its leaves to maximize light interception within the productive region of its crown. Although total leaf area per se is known to be an important determinate of growth in poplar trees (Larson and Isebrands, 1972), the orientation of leaves within a tree's canopy is an important factor which cannot be ignored.

It would appear from our results that the horizontal leaf display of Tristis was best adapted to the relatively open growing conditions present during the first growing This conclusion supports results obtained from season. computer simulations for several other crops (deWit, 1965; Duncan et al., 1967; Ross, 1970; Oker-Blom and Kellomaki, 1982). However, extreme caution must be used in attempting to determine which strategy was "best" between the two This discussion has centered on the solar noon clones. period, which may have produced a bias toward Tristis. The entire diurnal period must be considered before a "best" strategy could be identified. Even then, ideal crown structure depends upon several dynamic, intercorrelated factors. The influence of leaf display, crown structure, leaf area development, and environment on photosynthesis must be examined together on a diurnal and seasonal basis before an

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optimum crown structure can be identified.

APPENDIX A. CALCULATION OF LEAF AND SUN VECTORS.

Consider a X-Y-Z coordinate system where the positive X axis is directed south, the positive Y axis is directed east, and the positive Z axis is perpendicular to X and Y and directed upward. To quantify a leaf's three-dimensional orientation, the angles between these major axes and two principal vectors which describe a leaf (Fig.2) must be determined. The leaf vectors are: (1) V, which extends along the midrib from the leaf's base to tip (leaf axis #1), and (2) V, which is perpendicular to V in the lamellar 2 plane and occurs at the point of greatest leaf width (leaf axis #2) (Max, 1975).

Typically, V and V can be described using direction 1 2 cosines (Flanders and Price, 1973)

$$V = \cos \alpha \quad (i) + \cos \beta \quad (j)$$

$$1 + \cos \gamma \quad (k) \qquad (1)$$

 $V = \cos \alpha \quad (i) + \cos \beta \quad (j)$ +  $\cos \gamma \quad (k)$  (2)

where:  $\alpha$  = angle from the X axis;  $\beta$  = angle from the Y axis; and  $\gamma$  = angle from the Z axis. V and V can also be 1 2 described using angles which are more readily measured in the field

$$V = \sin \Theta \sin \Psi (i) + \cos \Theta \sin \psi (j) + \cos \psi (k)$$
(3)

 $V = -\cos \Theta \sin \Omega$  (i) +  $\sin \Theta \sin \Omega$  (j) +  $\cos \Omega$  (k) (4) where:  $\Psi =$  midrib angle, the vertical angle of leaf axis #1;  $\Omega =$  lamina angle, the vertical angle of leaf axis #2; and  $\Theta =$  the east azimuth of leaf axis #1 (Max, 1975). These formulae differ from those derived by Max (1975) since a different X-Y-Z coordinate system was used.

The vector normal (i.e., perpendicular) to V and V 1 2 (N) can be found from: N = V X V, the vector product of L 1 2 V and V (Thomas, 1969; Max, 1975). More specifically 1 2 N = { $\cos \theta \sin \psi \cos \Omega - \sin \theta \cos \psi \sin \Omega$  } (i) (5) L + { $-\cos \theta \cos \psi \sin \Omega - \sin \theta \sin \psi \cos \Omega$  } (j) + { $(\sin \theta)^{2} \sin \psi \sin \Omega + (\cos \theta)^{2} \sin \psi \sin \Omega$ }(k) N must be normalized to unit length by L N = (i/|N|) + (j/|N|) + (k/|N|) (6) where: i, j, and k denote the i, j, k components of N L defined in (5) and

$$2 2 2 0.5$$
  
|N| = (i + j + k) (7)

To find the north azimuth of N (Nazm), the angle L between the projection of N onto the X-Y plane and any L major X or Y coordinate axis must be determined

$$\sigma = \tan |i/j|$$
(8)

where: || denotes the absolute value. Then, if i>0 and j>0,

Nazm = 90 +  $\sigma$ ; if i>0 and j<0, Nazm = 270 -  $\sigma$ ; if i>0 and j<0, Nazm = 90 -  $\sigma$ ; and, if i<0 and j<0, Nazm = 270 +  $\sigma$ . To find the zenith angle of N (Nza) L Nza = 90 -  $\tau$  (9)

where

$$\tau = \tan |k/c|$$
(10)

and

$$2 2 0.5$$
  
c = (i + j) (11)

Calculation of a vector describing the sun's rays (V) s proceeds as described for V

$$V = \sin \omega \sin \zeta (i) + \cos \omega \sin \zeta (j) + \cos \zeta (k)$$
(12)

where:  $\omega$  = the sun's east azimuth and  $\zeta$  = the sun's zenith angle.  $\omega$  and  $\zeta$  can be found using the method described in Appendix B. V must then be normalized to unit length using the the approach described in (6) and (7).

The angle between N and V (  $\phi$  ) can be obtained from L s the inverse cosine of the dot product of N and V (Flanders L s and Price, 1973; Max, 1975)

$$\phi = \cos \left\{ (N + V) / (|N| + |V|) \right\}$$
(13)  
L s L s

where

$$N * V = (N (i) \times V (i))$$

$$L s L s$$
(14)

and |N| and |V| are found as in (7). L s APPENDIX B. CALCULATION OF SOLAR ALTITUDE, AZIMUTH, ZENITH ANGLE, AND HOUR ANGLE

Solar declination can be obtained using (deWit, 1978)

$$\delta = (\pi/180) \times -23.4 \times \cos \{2 \times \pi \times (DAY + 10)/365\}$$
(1)

where:  $\delta$  = solar declination (radians) and DAY = Julian date (i.e., the number of days since January 0). Solar altitude can then be obtained from

$$\beta = \sin \{\sin \delta \sin \lambda + (2) \\ \cos \delta \cos \lambda \cos \{2\pi (t + 12)/24\}\}$$

where:  $\beta$  = solar altitude (radians);  $\lambda$  = latitude (radians); and t = solar time (h).

Solar azimuth can then be calculated from (Smart, 1962) -1 $\omega = \cos \{\sin \delta - \sin \beta \sin \lambda\} / \cos \beta \cos \lambda \}$  (3)

Equation (3) gives the eastwardly azimuth from north when t

12.00 h and the westwardly azimuth from north when t 12.00 h.

The sun's zenith angle (5, radians) can be calculated from

$$\delta = 1.5708 - \beta \tag{4}$$

The hour angle  $(\eta)$  of the sun can be calculated from (Duffett-Smith, 1981)

-1 $\eta = \cos \{ (\sin \beta - \sin \lambda \sin \delta) / (\cos \lambda \cos \delta) \}$ (5) REFERENCES

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