as many testors as logistically possible when testing first generation seed orchards. A design using four or five testors will aid in maintaining a broad biological base for future improvement work but in many cases will not meet financial criteria.

Abstract

The financial impact of inbreeding depression on the number of testors that maximizes the profitability of second generation forest-tree seed orchard production has been determined under various assumptions. Sensitivity analysis of these assumptions indicates that optimal testor numbers vary mainly with changes in clone number in the first generation orchard, the interest rate used, the seed yield from the second generation orchard, and the site index for the resulting plantations. The range of optimal testor numbers was from one to eight.

Key words: Economics, Testor Numbers, Second Generation Seed Orchards.

Literature Cited

BERGMAN, A.: Variation in flowering and its effect on seed cost. N. C. State Univ. Technical Report No. 38, 63 pp., 1968. — COMSTOCK, R. E., and H. F. ROBERTSON: "Estimation of Average Dominance of Genes". Heterosis, Ames: Iowa State College Press, pp. 494–516, 1952. — DAVIS, LAWRENCE S.: Investments in Loblolly Pine Clonal Seed Orchards. Journal of Forestry 65: 882–887 (1967). — FRANKLIN, E. C.: Survey of mutant forms and inbreeding depression in species of the family *Pinaceae*. USDA, Forest Service Research Paper SE-61, 21 pp., 1970. — GANSEL, C. R.: Effect of several levels of inbreeding on growth and oleoresin yield in slash pine. Proc. 11th South. Conf. Forest Tree Impr., p. 173–177, 1971.

Gas Exchange in Six Populus Clones[1])

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Introduction

Short-time measurements of photosynthetic performance have often been used as an index of growth potential of trees (BOURDEAU, 1958; FERRELL, 1970; KOZLOWSKI, 1971a, 1971b; KOZLOWSKI and KELLER, 1966). Such measurements sometimes have limitations because both high and low (and even negative) correlations have been reported for Populus (HUBER and POLSTER, 1955; GATHERUM et al., 1967), Pseudotsuga menziesii (CAMPBELL and REDISKE, 1966), Pinus elliottii (WYATT and BEERS, 1964), and Pinus taeda (MCGREGOR et al., 1961). By comparison, poor correlation between photosynthesis and growth has been reported for *Larix* (NEUWIRTH, 1967), Pseudotsuga menziesii (SORENSEN 1964; BRIX, 1967), Pinus contorta (SWEET and WAREING, 1968), and Pinus banksiana (LOGAN, 1971). Because dry weight increment varies greatly with experimental conditions, there are difficulties in comparing photosynthetic efficiency of different species based on production data of various investigators (HELLMERS, 1964).

In addition to photosynthetic rates alone, at least three important physiological considerations determine growth potential (dry weight increment). These include the relation of photosynthesis to respiration, the distribution of photosynthate within the tree, and duration of growth or the seasonal pattern of assimilation (LEDIG, 1969). The relationship between photosynthesis and respiration is of particular interest in tree improvement becausc heterotic plants have more effective photosynthetic systems than do phenotypically normal plants. DECKER (1970) suggested that there may be phenotypically normal individuals within a species that have unusually high photosynthetic capacity but this is combined with high photorespiration with the net result of normal growth rate. There may also be phenotypically normal individuals with low photosynthetic rates and low photorespiration. If such individuals could be identified and if the two processes are genetically separable, genetic recombination of high-low might be possible.

As emphasized by DECKER (1957, 1970), the CO, compensation point, at which a plant is gaining and losing CO, at the same rates, is determined in strong light largely by photo-

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Table 1. — The identification numbers and origins of the experimental clones.

Identification No.	Species	Section	Origin
D 40	P. deltoides	*Aigeiros*	Sandy shore of L. Ontario, Canada.
M 12	P. maximowiczii	Tacamahaca	Hokkaido, Japan; collected from natural stands by Kórnik Arboretum, Poland, in cooperation with the Oji Institute, Kuriyame, Japan.
M 13	P. maximowiczii	Tacamahaca	
MN 1	P. maximowiczii X P. nigra	Tacamahaca X Aigeiros	Arnold Arboretum (hybrid by E. Chalmere Smith).
N 2	P. nigra	*Aigeiros*	Kunovice, Moravia, Czechoslovakia.
T 6	P. trichocarpa	Tacamahaca	Poplar Institute, Geraardsberger, Gramont, Belgium.

respiration. Hence, the possibility exists for using the compensation point as evidence of genotypic differences, with low-low genotypes having low compensation points and high-high genotypes having high compensation points. Because photorespiration is more temperature-dependent than photosynthesis (DECKER, 1959), a shifting of the compensation point with temperature also provides useful information. For example, a normal phenotype of high-high genotype will show greater change in compensation point with a given temperature change than will a normal phenotype of low-low genotype.

With the foregoing considerations in mind, total and net CO_2 uptake (as photosynthetic efficiency), photorespiration, dark respiration, and CO_2 compensation points were measured and compared for six *Populus* clones under controlled environmental conditions. An attempt was also made to relate variations in gas exchange to leaf arrangement and structure.

Materials and Methods

The six clones used in this study belonged to different species of *Aigeiros* and *Tacamahaca* sections of the genus *Populus*, except one which was an intersectional hybrid between two species (but not the particular clones) included in this material. The scions were supplied by and ortets were grown at Maple, Ontario, Canada. The identification numbers and origins of the experimental clones are given in *Table 1*.

Healthy cuttings (about 20 cm long) which had been shipped to Madison, Wisconsin, on January 5, 1971, were planted the following day in a greenhouse, each in a plastic container 14.5 cm high and 11 cm in diameter. The method used in rooting was described by SIWECKI (1969). The rooting medium consisted of three parts of horticultural soil to one part sand. During the first week after planting, the cuttings were watered daily and maintained at a temperature of about 12° C. On January 13, the temperature was increased to 15° C and on February 3 to 18° C. On March 22, well-rooted cuttings were transferred to a growth chamber, where they were arranged in seven replications, six randomized clones in each, under the following conditions: Temperature 18° C, light intensity 1100 ft.-c. (4.0×10^4 erg · cm^{-2} · sec^{-1}) on a 12 hr. day, relative humidity 70—90%. Beginning on April 21, the cuttings were each given 100 ml of Hoagland nutrient solution, with added micronutrients (iron as FeEDTA), once each week.

Gas exchange was studied in a closed system with a Beckman 15A infrared CO_2 analyzer and a Speedomax W recorder. The analyzer was connected to a plant chamber by copper tubing. A galvanized iron cylinder (volume of 26.0 l) served as the plant chamber (cuvette). The light source (with an irradiance of $5 \cdot 10^4$ erg · cm^{-2} · sec^{-1} at the bottom of the chamber) was a Lucalox 400/BU lamp described by WUENSCHER and KOZLOWSKI (1970). The apparatus was basically that described by SASAKI and KOZLOWSKI (1967). However, several modifications were introduced to improve temperature control and facilitate movement of plants into and out of the chamber. These are described by LUUKKANEN (1971).

Air flow was maintained at 3 l/min with a pump, valves, Gelman flowmeter, a water manometer connected to the air outlet from the plant chamber, and a mercury manometer. Since the air entering the analyzer was dried by passing it through Drierite (anhydrous $CaSO_4$), water was added to the air returning to the plant chamber, to maintain a favorable water balance in the experimental plants. Humidity in the plant chamber was periodically monitored with a Vap-Air dewpoint analyzer.

For measurement of the CO_2 compensation point, net photosynthesis, and dark respiration, well watered plants were transferred from the growth chamber to the plant chamber and first allowed to reach the compensation point at 15° C. This usually required 2 to 4 hours, depending on plant size. At a steady state of photosynthesis and respiration (when no changes could be detected at least during 10 minutes), the CO_2 compensation point at 15° C was recorded. Then the temperature was raised to 20° C and CO_2 compensation point was again recorded. The procedure was repeated at 25 and 30° C. The instrument was calibrated during these measurements, usually after recording compensation point at 20° C, and previous recordings were corrected if necessary. After the compensation point at highest temperature was recorded, CO_2 concentration was increased to near 600 ppm by injecting a few ml of CO_2-enriched air into the system. Then the modified methods of SASAKI and KOZLOWSKI (1967) for measurement of net photosynthesis was followed. In this procedure the time for decrease of CO_2 concentration from 90% (512 ppm; converted by using the calibration curve) to 60% (288 ppm) of full scale on the recorder was determined. After measuring net photosynthesis of a plant at 30° C, dark respiration was determined at the same temperature after turning off the light and covering the chamber with a black cloth. The time for increasing CO_2 concentration from 60% (288 ppm) to 65% (321 ppm) was recorded and the procedure for measuring photosynthesis and respiration was repeated at 25, 20, and 15° C. Rates were converted to mg CO_2/hr by using coefficients obtained from determination of total air volume in the system. All measurements for one plant usually were made during the natural light period (12 hours). Plants were watered frequently during the procedure.

Following measurement of gas exchange, leaf areas and dry weights were determined. Leaf areas (one side) were obtained by exposing the leaves on photographic paper and determining leaf area by proportionality of weight and area of the cutout leaf outline to weight and area of the whole sheet. Gas exchange data were expressed as mg CO_2/dm^2/hr for one side of the leaf and mg CO_2/g dry weight of leaves/hr.

The study consisted of three experiments. Experiment A included determination of total and net photosynthesis, photorespiration, dark respiration, and CO_2 compensation point at 25° C. In Experiment B similar determinations were made at 15, 20, 25, and 30° C. Experiment A was made using seven and Experiment B four replications (plants). Rates obtained at 25° C in Experiment B were included as the last four replications in Experiment A. In Experiment C the correlations between various parameters of gas exchange were further analyzed.

By using values from direct measurements of net photosynthesis and the compensation point, actual photorespiration was calculated by the modified extrapolation method (DECKER, 1957; FORRESTER *et al.*, 1966 a-b; TREGUNNA *et al.*, 1966; BRIX, 1967; ZELAWSKI, 1967). In this method photorespiration (PR) is determined by "carboxylation efficiency" (CE) and CO_2 compensation point ($CO_{2\,comp}$) (FORRESTER *et al.*, 1966 a):

$$PR = CE \cdot [CO_{2\,comp}]. \qquad (1)$$

Carboxylation efficiency is assumed to be the linear slope of increase in net photosynthesis with increasing CO_2 concentration and is expressed as a function of both ap-

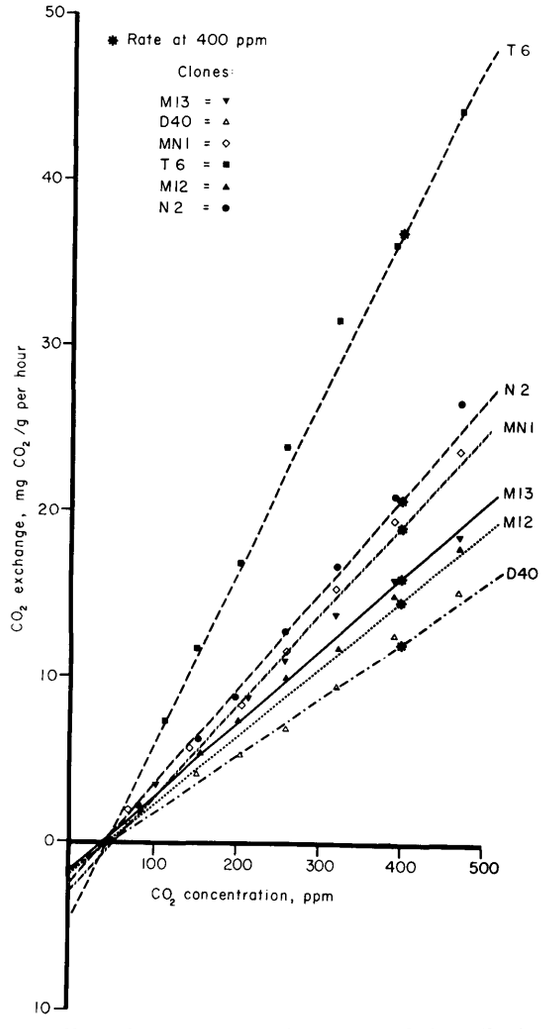

Fig. 1. — Effect of CO_2 concentration on net photosynthesis of six poplar clones at 25° C. Clone symbols indicate rates measured at gradually decreasing CO_2 concentration; lines showing "carboxylation efficiency" are drawn through rates at 400 ppm and CO_2 compensation points and extrapolated to zero CO_2 concentration where the rate equals the negative value of photorespiration.

parent (or net) photosynthesis (APS) and the difference between actual and compensation concentrations of CO_2:

$$CE = \frac{APS}{[CO_2 - CO_{2\,comp}]} \qquad (2)$$

Net photosynthesis was generally measured at only one concentration of CO_2 (400 ppm). Therefore, a separate experiment was conducted to determine how well one measured rate of photosynthesis would represent the whole range of photosynthesis with changing ambient concentration of CO_2, *i. e.* to determine the linearity of carboxylation efficiency response. When (in one plant per clone) plotted rates of photosynthesis at gradually decreasing concentrations of CO_2 were compared with the straight line drawn through the compensation point and the rate of photosynthesis at 400 ppm, good conformity was found in each clone *(Fig. 1)*. This was true throughout the range, including the highest concentration of CO_2 (about 500 ppm) at which deviations from linearity might have been expected. Thus, comparable values for carboxylation efficiency, and thereby also for extrapolated photorespiration rates, were obtained by using the single value for net photosynthesis in Equations *(1)* and *(2)*.

After determining photorespiration, total photosynthesis was obtained by adding the rates of net photosynthesis and photorespiration. Differences between clonal means of gas exchange rates were determined by analysis of variance and TUKEY's test for "honestly significant difference" (hsd) (STEEL and TORRIE, 1960). In Experiments B and C regression analyses were also used. Equations for relationships presented below are given by LUUKKANEN (1971).

Results

Experiment A. — Photosynthesis, Photorespiration, and Dark Respiration at 25° C. Both total and net photosynthesis at 25° C, whether CO_2 uptake was expressed per unit of leaf dry weight or leaf surface area, were highest in T 6 (18.6 mg CO_2/dm²/hr) and lowest in D 40 (6.7 mg CO_2/dm²/hr). These clones differed significantly ($P < 0.05$) from all remaining ones when rates per leaf area unit were used. In general, the rate of photosynthesis was lowest in the two *Aigeiros* clones (D 40 and N 2), and highest in the *Tacamahaca* clones (T 6, M 12, and M 13). The two *P. maximowiczii* clones (M 12, M 13) had very similar photosynthetic rates. Photosynthesis of the intersectional hybrid MN 1 approximated that of either the *Aigeiros* or *Tacamahaca* poplars, depending on whether the rate was expressed per unit of leaf dry weight or surface area.

Photorespiration varied in much the same way as total or net photosynthesis. For example, D 40 had the lowest rate of photorespiration (1.01 mg CO_2/dm²/hr) and T 6 the highest rate (2.28 mg CO_2/dm²/hr); this difference was also significant. Expressing photorespiration (as well as photosynthesis) on a leaf area basis resulted in more distinct differences among clones than when rates were expressed on a leaf dry weight basis. Also the rate of photorespiration in the hybrid MN 1 seemed to depend more on the unit chosen for expressing the rate than it did in any other clone. Possibly this was the result of heavier leaves in this clone.

Compensation points of the six clones showed some variation but differed less than rates of photosynthesis or photorespiration. Clonal means of compensation points seemed to follow the same pattern as the other parameters, but in a reverse order. Thus T 6, with the highest rate of photosynthesis and photorespiration, also had the lowest compensation point (43.7 ppm CO_2), whereas D 40, with very low photosynthesis and photorespiration, had the highest compensation point (52.9 ppm). These clones differed significantly ($P < 0.05$) from each other. Also the compensation points of the *Aigeiros* clones were higher than those of the *Tacamahaca* clones. The hybrid MN 1 had a compensation point close to that of the black poplars *(Aigeiros)* as found in photosynthesis and photorespiration with rates expressed per unit of leaf dry weight rather than leaf area.

There was less interclonal variation in dark respiration than in photosynthesis or photorespiration. Furthermore, when respiration rates of the six clones were compared, rates per unit of dry weight and per unit of leaf area had quite different orders. For example, T 6 had a relatively low dark respiration rate per unit of leaf dry weight and a much higher one per unit of leaf area. On the contrary, D 40 had a relatively low respiration rate on a leaf area basis. As was true for photosynthetic and photorespiration rates of MN 1, these inconsistencies might also be explained by differences in leaf weights. Both T 6 and MN 1 had heavy leaves, whereas the lightest leaves were found in

D 40. The rate of dark respiration of MN 1, however, was relatively high regardless of the units used.

Rates of apparent dark respiration (means over six clones, 1.34 mg CO_2/dm²/hr and 1.83 mg CO_2/g dry weight/hr, respectively) generally were lower than the calculated values of photorespiration (respective means of 1.64 mg CO_2/dm²/hr and 2.17 mg CO_2/g dry weight/hr). The one exception was D 40 for which CO_2 evolution in the dark was much greater than photorespiration in the light. In all other clones, photorespiration exceeded dark respiration, although in MN 1 the difference was smaller than in the remaining four clones.

Experiment B. — Photosynthesis, Photorespiration, and Dark Respiration at 15, 20, 25, and 30° C. Rates of total and net photosynthesis, *(Figs. 2 and 3)*, gave nearly identical information on the variation between clones at a given temperature, or on the photosynthetic response of a particular clone to temperature. In both cases some significant ($P < 0.05$) variation was always found in each individual comparison, except between clones at 30° C. As in Experiment A, expressing gas exchange on a leaf area basis resulted in greater differences among rates of photosynthesis of the six clones, or among mean rates at each temperature than when rates were expressed on a dry weight basis. Also, since error variance increased with increasing temperature, larger differences among clonal means of photosynthetic rates were found at lower temperatures.

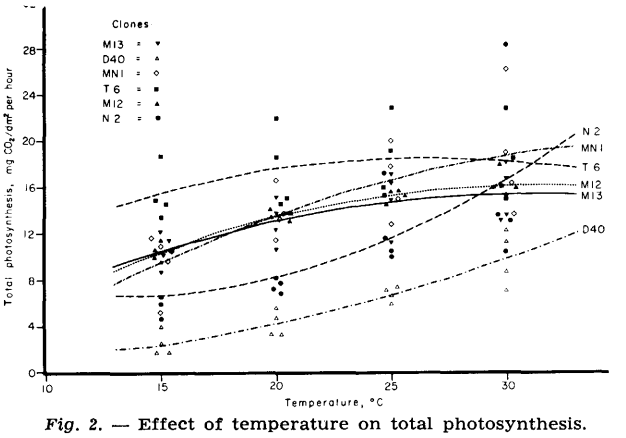

Fig. 2. — Effect of temperature on total photosynthesis.

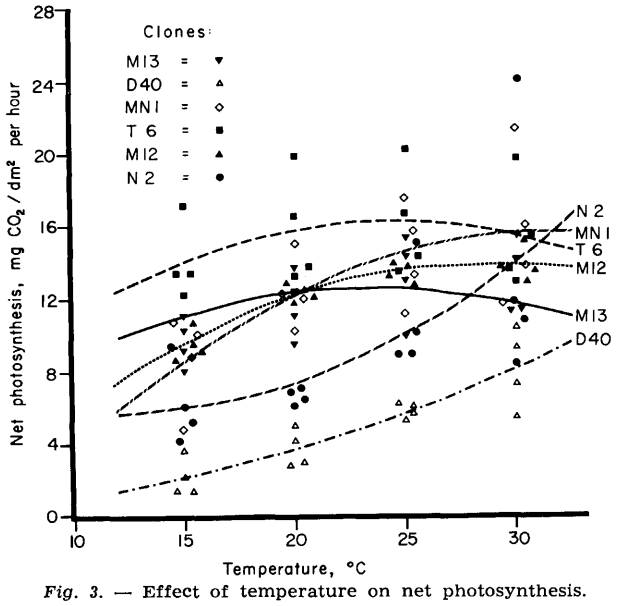

Fig. 3. — Effect of temperature on net photosynthesis.

Clones from the *Tacamahaca* section reached maximum photosynthesis rates at lower temperatures than the *Aigeiros* clones. Maximum net photosynthesis rate was reached at the lowest temperature (24° C) by T 6. This clone also showed highest rates throughout the lower temperature range, especially when rates were expressed per unit of leaf area (only this unit is shown in *Figs. 2 and 3*). The hybrid clone MN 1 had a low rate of net photosynthesis at 15° C and a high rate at 30° C and thus resembled N 2. However, MN 1 reached maximum rate at about 30° C, whereas in N 2 no maximum rate was found in the temperature range used. At 20 and 25° C the two sections showed the largest differences in photosynthesis and the hybrid clone was either intermediate (rates per unit of leaf dry weight) or similar to the *Tacamahaca* clones (rates per unit of leaf area). Of the two *Aigeiros* clones, N 2 showed greater variation among individual values of photosynthetic rates than was found in any other clone of either section. This was especially apparent at 30° C for net photosynthesis per unit of leaf dry weight. At this temperature N 2 had the highest mean rate of net photosynthesis on a leaf dry weight basis. Although considerable variation existed, the effect of temperature on photosynthesis was established in all clones over 15 to 30° C ($P < 0.05$). When both net and total photosynthesis were considered, the multiple correlation coefficients for the quadratic response curves in *Figs. 2 and 3* ranged from 0.31 to 0.95.

Effects of temperature on photorespiration are shown in *Fig. 4*. The finding in Experiment A that photorespiration in general followed the rate of photosynthesis when the six clones were compared also appeared to hold at temperatures other than 25° C. Thus, at 15 and 20° C photorespiration and photosynthesis were highest in T 6, but both decreased in relation to rates in other clones at higher temperatures. Similarly, D 40 had the lowest rates of photosynthesis at all temperatures. Also photorespiration of this clone remained lowest throughout the temperature range used. The general pattern of the effect of temperature on photorespiration was unlike the variation in photosynthesis. A nearly linear temperature response was found in photorespiration of all six clones.

When rates of photorespiration per unit of leaf dry weight and leaf area were compared, differences similar

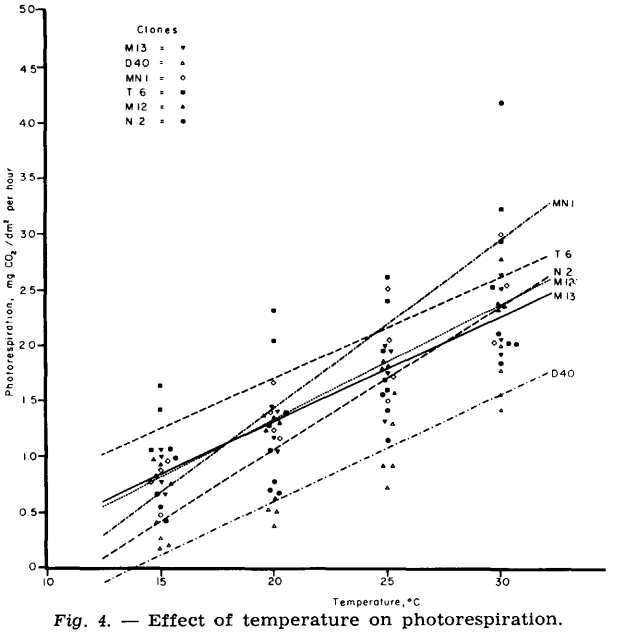

Fig. 4. — Effect of temperature on photorespiration.

to those in photosynthesis were found. The differences among rates of the six clones were greater at each temperature as well as in overall means, when leaf area instead of dry weight was used. In contrast to photosynthesis, however, means of photorespiration rates at each temperature (with clones pooled) differed more when leaf area rather than when dry weight was used. As was true on the basis of photosynthesis rates, there was a change in the ranking of clones by photorespiration rates when different units for gas exchange were used. Again this may have reflected differences in leaf weight or structure. For example, at 25 and 30° C T 6, in comparison to other clones, had relatively much lower photorespiration rates per unit of leaf dry weight than per unit of leaf area.

Differences in photorespiration between *Aigeiros* and *Tacamahaca* poplars were not as clear as those in photosynthesis. At lower temperatures, however, the two *Aigeiros* clones D 40 and N 2 — together, in part, with the hybrid MN 1 — had the lowest rates of photorespiration (and photosynthesis). At 30° C the rapid increase of photorespiration in N 2 to near maximum mean value altered this relation. Also MN 1, with a photosynthetic rate that was intermediate to that of the two sections, exhibited rapidly accelerating photorespiration with temperature increase and at 30° C reached the highest recorded means. The total range of photorespiration, when compared with minimum rate, was considerably greater than that of photosynthesis.

As was the case with photosynthesis, variations among photorespiration rates at high temperatures (especially 30° C) were marked. This made it difficult to compare variations between clones. Nevertheless, some significant variation ($P < 0.05$) was found among clones at each temperature except 30° C. The linear temperature effect on the variation of gas exchange was more significant for photorespiration than was the total curvilinear regression component of photosynthesis. The lowest correlation coefficient for the calculated relationships between photorespiration and temperature shown in *Fig. 4* was 0.78 (in N 2) and the highest was 0.97 (in M 12).

CO_2 compensation points at four temperatures are given in *Fig. 5*. Since all clones except D 40 had a significant quadratic component in the regression, curvilinear models are shown for each clone. As in Experiment A, ranking of clones by average compensation points resulted in a nearly reverse order when compared to ranking by photosynthesis or photorespiration. D 40 which also had the lowest rates of photosynthesis and photorespiration, consistently exhibited the highest compensation point. At low temperature, the compensation point for N 2 also was higher than for other clones. The latter had almost identical compensation points at 15° C, and again, although somewhat higher, at 20° C. At the two highest temperatures MN 1 also reached a high compensation point (at 30° C, even slightly surpassing that of N 2). This resembled the observation in Experiment A that MN 1, together with N 2 and D 40, had a high compensation point. In experiment B, however, the compensation point of MN 1 at 25° C was closer to those of *Tacamahaca* poplars.

The high compensation point of MN 1 was the main exception to a general relationship of high compensation point associated with low photosynthetic rate. The rate of photosynthesis (as well as photorespiration) of MN 1 was high also at 30° C. Another distinctive clone was T 6 which at high temperatures had low rates of photosynthesis and photorespiration, at least per unit of leaf dry weight.

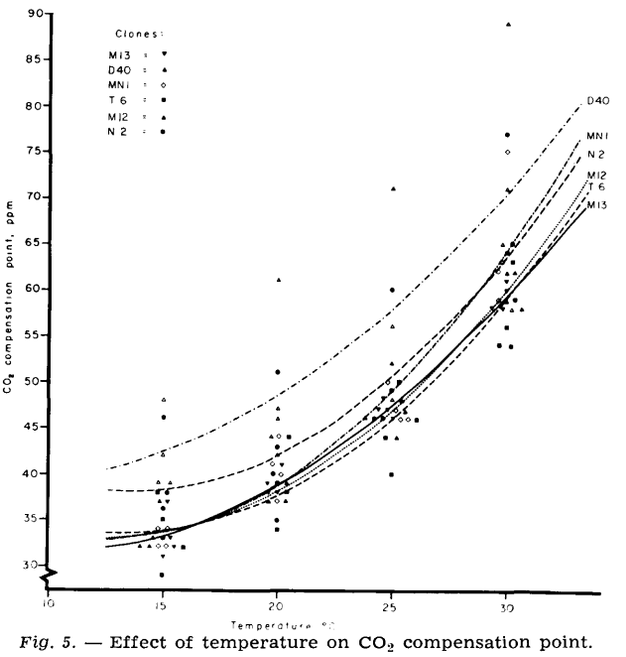

Fig. 5. — Effect of temperature on CO_2 compensation point.

Nevertheless, it also had a low compensation point at all temperatures. In general, the regression curves for the compensation point were strikingly uniform in shape *(Fig. 5)*.

At different temperatures (with clones pooled) a significant difference was found between average compensation points at any two temperatures. In contrast, the variation of compensation points between clones at a given temperature generally was less apparent, although significant ($P < 0.05$) at all temperatures except 30° C. A common trend was that clonal differences in compensation points (as in photosynthesis and photorespiration) were more significant at each lower temperature. At 15° C, the highest compensation point (that of D 40) differed significantly ($P < 0.05$) from each of the four *Tacamahaca* or hybrid means but not from the mean of N 2.

The average increase in compensation point indicated a marked increase (from 0.12 to 0.16) in the photorespiration total photosynthesis ratio when temperature was raised from 25 to 30° C. A highly significant ($P < 0.001$) relationship was found between CO_2 compensation point and temperature in all clones. The multiple correlation coefficients for quadratic models shown in *Fig. 5* ranged from 0.79 to 0.99. These values, as well as the narrow scatter of observations, show that for compensation points as for photorespiration (and dark respiration, as discussed next), error variance was much less than for total or net photosynthesis.

Measurements of dark respiration are shown in *Fig. 6*. Slightly larger differences were found among clones for dark respiration rates calculated on a leaf dry weight than on a leaf area basis, which was contrary to the findings in Experiment A and to photosynthesis and photorespiration rates. By both methods of calculation some highly significant ($P < 0.001$) differences were found between clonal means of dark respiration. Particularly, the two lowest respiration rates (in *P. maximowiczii* clones M 12 and M 13) were different from the remaining values. This was true not only for overall rates but at each individual temperature as well, although the significance of these differences in the latter cases was not as well established. The remaining four clones with higher respiration rates did not differ from each other when overall means or means at individual

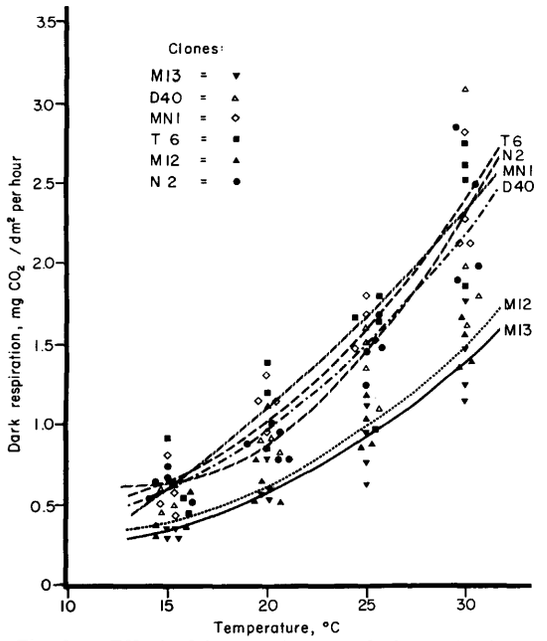

Fig. 6. — Effect of temperature on dark respiration.

temperatures were compared. The order of these four clones (M 12 and M 13 always having the lowest rates) differed somewhat, depending on temperature and method of calculation. For example, N 2 had the highest respiration rate at 15° C. At 30° C this clone also had the highest mean respiration rate per unit of leaf dry weight. When, instead, the rate was expressed per unit of leaf area, the highest mean at 30° C was that of T 6. Yet, next to M 12 and M 13, T 6 had the lowest mean at all temperatures on a leaf dry weight basis.

The regression curves in *Fig. 6*, as those for photorespiration or compensation point but not photosynthesis, were quite uniform for all clones and were concave with slope increasing with temperature. Only M 12 and M 13 differed slightly from the general trend. In addition to showing lower rates of dark respiration throughout the temperature range used, they exhibited a less pronounced increase in slope as temperature was raised. The influence of temperature on differences among clonal means of dark respiration was unlike its effect on clonal differences in photosynthesis or compensation point. At 15° C, only few differences between mean respiration rates of clones were significant; at 20° C they were very distinct, and less significant at 25 and 30° C. The increase of overall respiration mean (with clones pooled) was successively larger for each 5° C temperature increase. The temperature regression component of the total variance of dark respiration was highly significant ($P < 0.001$) in all clones (multiple correlation coefficients for curves in *Fig. 6* varied from 0.88 to 0.96, respectively). Thus, temperature regression in dark respiration comprised, as in photorespiration or compensation point, a clearly larger proportion of the total variance of gas exchange than that of photosynthesis. One obvious cause was the smaller error variance in dark respiration than in photosynthesis.

Rates of dark respiration generally were lower than calculated photorespiration rates (the ratio photorespiration/dark respiration having a value above one). As in Experiment A. D 40 was an exception and always exhibited lower photorespiration than dark respiration rates (the mean ratio over the temperature range being 0.61 in this clone). In contrast to the first experiment, MN 1 did not have the next lowest ratio, but was intermediate to that of the *Aigeiros* and *Tacamahaca* sections, with the former having low ratios and the latter high ones. The second lowest ratio was found in N 2, except at 30° C where T 6, a *Tacamahaca* clone, came next to the lowest ratio of D 40. The average trend as in clones T 6, M 12, and M 13 was toward a decreasing ratio of photorespiration to dark respiration with increasing temperature. An opposite, increasing trend was observed in D 40, whereas the ratios in N 2 and MN 1 were unaltered by temperature or slightly decreased in the middle range. The highest ratio of photorespiration to dark respiration was found in both *P. maximowiczii* clones (M 12 and M 13, with means 2.0 and 2.1, respectively). They nevertheless differed in that M 13 had a wider range of the ratio than M 12.

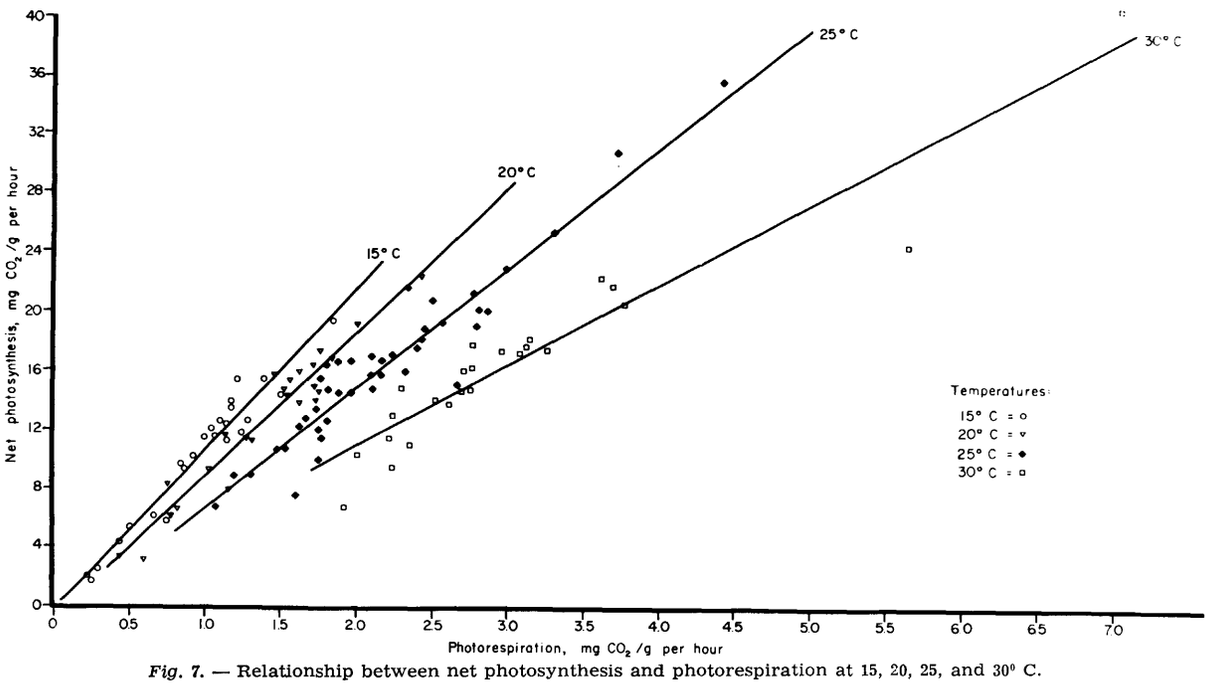

Fig. 7. — Relationship between net photosynthesis and photorespiration at 15, 20, 25, and 30° C.

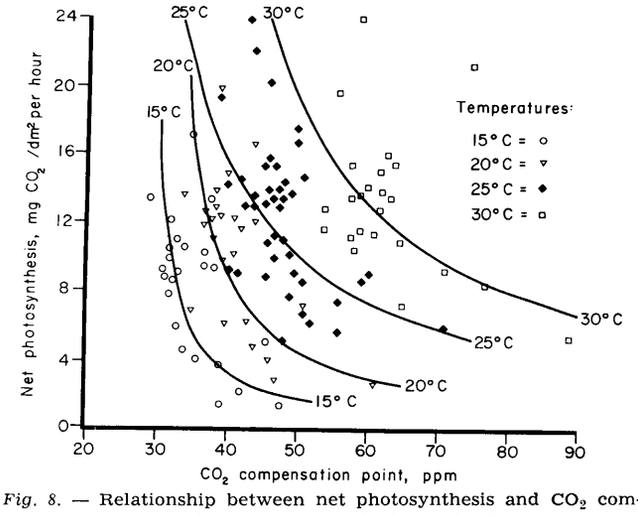

Fig. 8. — Relationship between net photosynthesis and CO_2 compensation point at 15, 20, 25, and 30° C.

Experiment C. — Relationships Between Gas Exchange Parameters. Relationships were analyzed between (1) net photosynthesis and photorespiration, (2) net photosynthesis and dark respiration, (3) net photosynthesis and compensation points, and (4) compensation points and photorespiration or total photosynthesis.

Net photosynthesis as a function of photorespiration is shown in *Fig. 7*, which demonstrates the relationship at four temperatures with data pooled for all clones. Clones did not clearly differ in the slope of the regression line but rather could be assumed to form a common population of variables. Photosynthesis increased linearly with photorespiration ($r = 0.95+$). Hence estimated photorespiration predicted net photosynthesis in an unexpected pattern (greater net gain of carbon was associated with increased loss of respiratory CO_2).

The regression of net photosynthesis on dark respiration was nonsignificant at all temperatures (with clones pooled as above). Multiple correlation coefficients of the best curvilinear model ranged from 0.09 at 25° C to 0.44 at 15° C. However, this regression (in contrast to that of photorespiration) appeared to be negative, showing decreasing net photosynthesis with increasing dark respiration.

Net photosynthesis of clones was also compared with corresponding compensation points. *Fig. 8* shows individual observations (with clones pooled) and regression curves at four temperatures as y-reciprocal hyperbolic functions, which gave the highest coefficients of determination of all regression models tested. Photosynthetic efficiency was negatively correlated with compensation point (r ranging from —0.56 to —0.73; $P < 0.001$).

To study further the nature of the CO_2 compensation point as an indicator of the rate of carbon fixation (fixed CO_2 per unit of leaf tissue and time) compensation points were plotted as a function of total photosynthesis or photorespiration at 25° C *(Figs. 9 and 10)*. Total photosynthesis was determined as the sum of measured net photosynthesis and calculated photorespiration. It was found that total photosynthesis and photorespiration, with either one increasing, caused a decrease in the CO_2 compensation point. The regression between compensation point and total photosynthesis *(Fig. 9)* was highly significant (for the best model, the double reciprocal transformation, $P < 0.001$ and $r = -0.61$). This relationship can be explained by assuming that the compensation point is the equilibrium between total photosynthesis and photorespiration. Increase in photosynthesis lowers the compensation point by increasing CO_2 uptake.

Less obvious is the cause for decrease in compensation point with increasing photorespiration. The negative curvilinear regression of *Fig. 10* was significant ($P < 0.05$), even though the correlation coefficient remained low ($r = -0.31$). At lower temperatures somewhat higher correlation coefficients were found. This relationship cannot be explained by the assumption that the compensation point is determined directly by rates of total photosynthesis and photorespiration. Instead, the explanation discussed by Jackson and Volk (1970), that photosynthesis and photorespiration are not independent processes, must be considered. If it is assumed that photorespiration utilizes mainly the substrate produced in the light by photosynthesis, this becomes understandable as does the relationship between net photosynthesis and photorespiration shown in *Fig. 7* by assuming that total photosynthesis is considerably greater than photorespiration and thus is the main contributor to net photosynthesis. The dependence of photorespiration on total photosynthesis dominates and counteracts even the relationship between net photosynthesis and photorespiration.

Since some significant variation in photorespiration was found between clones, an attempt was made to study further some causes of this variation. Since the rates of both net and total photosynthesis also varied, and since a very strong positive correlation was established between photosynthesis and photorespiration, it seemed possible that all or part of the variation in photorespiration would be due primarily to differences in rates of total photosynthesis. Therefore, observed clonal means of photorespiration were adjusted by means of covariance analysis (Steel and Torrie, 1960) where the effect of either net or total photosynthesis was eliminated. The results were similar whether rates of net or total photosynthesis were applied.

The range of photorespiration at each temperature was greatly reduced by the recalculation, and no significant differences between adjusted clonal means were established. Thus it appeared that the variation of photorespiration among clones was caused primarily by differences in photosynthesis. The fact that both net and total photosynthesis explained equally well the variation in photorespi-

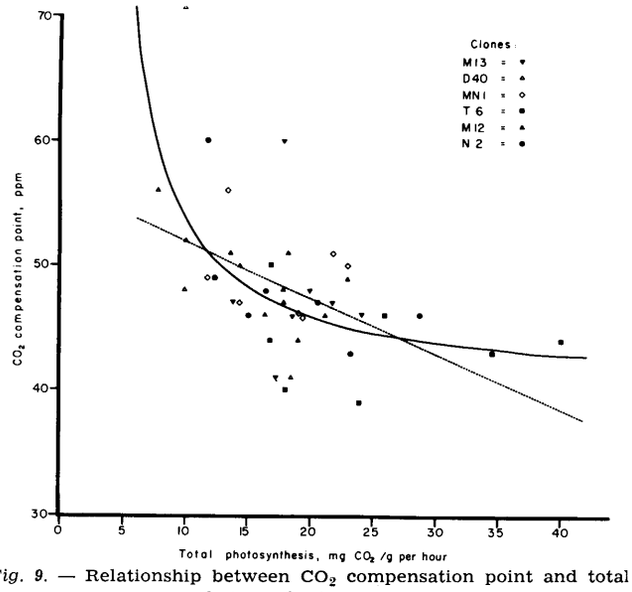

Fig. 9. — Relationship between CO_2 compensation point and total photosynthesis at 25° C.

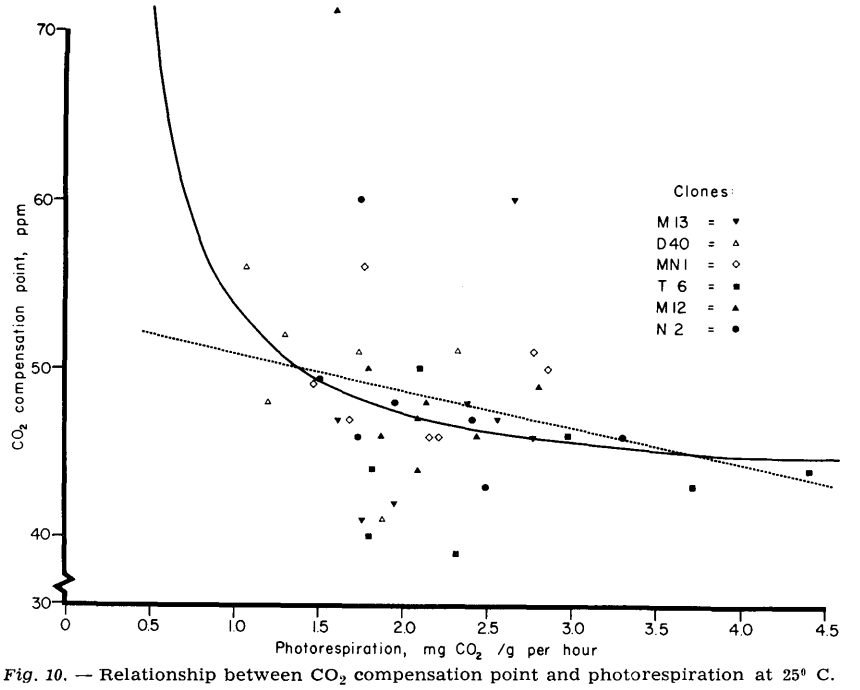

Fig. 10. — Relationship between CO_2 compensation point and photorespiration at 25° C.

ration is conceivable because of the close relationship between these two rates of photosynthesis in the temperature range used. The most striking result from the analysis of adjusted photorespiration rates was that the recalculation almost exactly reversed the previously determined ranking of clones according to average photorespiration rates. Thus, in a relative sense, T 6 had the lowest and D 40 the highest photorespiration rate (as an average over temperatures). Throughout the temperature range T 6 had the lowest adjusted rate, whereas the rate of D 40 at 25 and 30° C was surpassed by that of the hybrid clone MN 1.

For comparison, an analogous covariance analysis was used to study rates of net photosynthesis adjusted for variation in photorespiration. It was found, however, that assuming photorespiration to be similar (the average over clones, at each temperature separately), net photosynthesis did not change from rates found earlier, at least with respect to order of photosynthetic efficiency of clones.

Discussion

Marked variations were shown among *Populus* clones in rates of total and net photosynthesis, photorespiration, dark respiration, and the CO_2 compensation point. When each clone was placed in a high or low total photosynthesis or relative photorespiration category, a distinctive grouping was obtained:

Relative photorespiration	Total photosynthesis	Clones
High	Low	D 40, N 2
High	High	MN 1
Low	High	M 12, M 13, T 6
Low	Low	None

The overall mean of photorespiration, photosynthesis, or the compensation point in every case separated the two *Aigeiros* clones (D 40, N 2) from the three *Tacamahaca* clones (M 12, M 13, T 6). The intersectional hybrid, *P. maximowiczii* × *P. nigra* (MN 1), which was intermediate in these gas exchange parameters, seemed to combine high photorespiration and high photosynthesis found in the two parental species (which were not, however, represented by the actual parental clones). If this variation of the gas exchange parameters in question should be more than coincidence, it would reinforce Decker's (1955, 1970) suggestion that photosynthesis and photorespiration are at least partly genetically separate and separable characteristics.

The grouping of clones was further compared with calculated average ratios of photorespiration to total photosynthesis (based on clonal means rather than individual observations, both expressed as mg CO_2/g dry weight/hr). Actual instead of relative photorespiration rates were used in calculating the ratios. These ratios also justified the assignment of clones, according to photosynthetic efficiency, separately for photorespiration and photosynthesis. Lowest ratios were found in the *Tacamahaca* clones, both *Aigeiros* clones had high ratios, and the hybrid MN 1 was intermediate but close to N 2.

While the variation in the compensation point was difficult to explain by rates of photorespiration, the relative rates of photorespiration were in accord with observed differences of compensation points in different clones and with the general concept of the compensation point as an equilibrium of total uptake and output of CO_2. Despite the relative nature of the equilibrium concentration of CO_2 which need not imply any direct relation to net uptake of CO_2, this study showed a significant (inverse) relationship between the compensation point and net photosynthesis. This conclusion was compatible with the common emphasis on the compensation point as an indicator of relative photorespiration, or relative loss of CO_2 (Jackson and Volk, 1970; Decker, 1955, 1970). The same reasoning is seen in the accepted distinction of "low compensation point plants" (sugar cane, maize, sorghum, *etc.*, with the C_4-dicarboxylic acid pathway for CO_2 fixation), and "high compensation point plants" (the majority of plants, with the Calvin cycle as the predominant CO_2-fixing pathway). Hence the present study supports the possibility of using CO_2 compensation points in selecting poplar clones for

high photosynthetic efficiency. Nevertheless, as emphasized by FERRELL (1970), high photosynthetic efficiency does not necessarily imply rapid growth or rapid dry weight increment.

Gas Exchange and Leaf Anatomy. The experimental clones differed markedly not only in gas exchange characteristics but also in leaf arrangement, shape, and anatomy, with leaves within a section *(Aigeiros* or *Tacamahaca)* resembling each other. *Aigeiros* clones, including *P. deltoides* (D 40) and *P. nigra* (N 2) had broadly triangular, truncate or subcordate leaves with long flattened petioles and a more or less irregular, drooping arrangement of leaves. In general, D 40 also formed a distinctly shorter stem during one season of growth. *Tacamahaca* clones, including *P. maximowiczii* (M 12 and M 13) and *P. trichocarpa* (T 6), had ovate or elliptical to lanceolate leaves with rounded to cuneate bases and short, unflattened petioles. The leaf blades of *Tacamahaca* clones were also borne nearly horizontally in more regularly alternating positions. Leaf shape and arrangement of *P. maximowiczii* × *P. nigra* (MN 1) were intermediate between clones of the two sections.

Leaf thickness, as expressed by unit of dry weight per unit of surface area, differed significantly in the six clones. The heaviest leaves were found in T 6 and the lightest ones in D 40. The difference between *Aigeiros* and *Tacamahaca* poplars was not, however, quite clear, and a further discrepancy from the general morphological distinction was the hybrid MN 1, which had the second heaviest leaves. Thus, instead of being intermediate, this clone exceeded both parental species in leaf weight.

There was some evidence that the observed variation in leaf weight did not correspond to actual leaf thickness. Using cuttings from the same population as the present study, SIWECKI and KOZLOWSKI (1973) reported that D 40 had the thickest leaves of the six clones, followed by T 6 and M 13. The remaining clones had somewhat thinner leaves. One feature that renders a study on leaf weight (but not necessarily actual leaf thicknesses) difficult is the change of weight with aging; both increases and decreases of leaf dry weight may occur during ontogeny, as discussed by KOZLOWSKI (1971 a). In the present experiment, average leaf dry weight per unit of surface area increased by 46% during the experimental period of two months. New leaf formation was prevented by light and temperature treatments — thus predominantly mature leaves from the same (first) shoot elongation cycle were used.

SIWECKI and KOZLOWSKI (1973) emphasized variations in both palisade and spongy parenchyma layers of the six clones discussed here. They found that D 40 had the thickest palisade layer both as a percentage of total leaf thickness and in absolute units. On the contrary, T 6, which also had thick (and according to the present study, also heavy) leaves, had much less palisade tissue: as an average 61 μ, or 32% of the total thickness, compared with 114 μ, or 56% in D 40.

Perhaps the most important structural features affecting gas exchange, reported by SIWECKI and KOZLOWSKI (1973) were stomatal size, distribution, and function. They found that all clones had amphistomatous leaves, with fewer stomata on the upper than on the lower epidermis. The proportion of stomata on the upper side varied between clones. Over a third of the stomata were found on the upper epidermis in D 40, whereas in MN 1 this proportion was about 10%. Remaining clones had about 20% of their stomata on the upper epidermis.

The large variation in stomatal frequency among clones did not seem to explain much of the observed variation in photosynthesis. When rates of net photosynthesis per unit of leaf area were converted to rates per unit of lower leaf surface only (using data of SIWECKI and KOZLOWSKI, 1973), the ranking of clones by photosynthesis rates was unaltered. The clone with a large percentage of its stomata on the upper epidermis (D 40) had a considerably lower net photosynthetic efficiency (compared to other clones) after this recalculation. As for the remaining clonal means of net photosynthesis after the adjustment, T 6 seemed to show the highest photosynthetic rates less distinctly than before recalculation, which at least in part was caused by the increase of net photosynthesis rate of MN 1 after the correction.

SIWECKI and KOZLOWSKI (1973) found that T 6, with the fewest stomata per unit of leaf area, had the largest stomata. Hence rapid photosynthesis of this clone might be explained by the large stomata allowing rapid gaseous exchange. Using transpiration decline curves of excised leaves the same investigators demonstrated differences between clones in duration of the stomatal phase of transpiration. In D 40 a rapid decrease in transpiration of excised leaves indicated prompt closure of stomata under water stress. Also N 2 tended to close its stomata rapidly, whereas T 6 maintained open stomata longer than any other clone. Since these differences were correlated with rates of gas exchange (rapid closure of stomata was associated with slowly photosynthetizing clones), it seems likely that the observed differences in CO_2 fixation among clones were at least partly due to variation in stomatal aperture rather than in stomatal distribution, stomatal number, or arrangement of mesophyll tissues.

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Summary

Rates of photosynthesis, dark respiration, and photorespiration, and CO_2 compensation points were studied in six clones of *Populus*. The experimental material included one clone from each of the species *P. deltoides, P. nigra,* and *P. trichocarpa,* and two clones of *P. maximowiczii.* In addition, one hybrid *P. maximowiczii* × *P. nigra,* which also represented an intersectional cross between black *(Aigeiros)* and balsam *(Tacamahaca)* poplars, was included. Differences among clones were found in rates of net and total photosynthetic efficiency, photorespiration, dark respiration (all expressed as exchange of CO_2 per unit of leaf surface area or dry weight), and the CO_2 compensation point. Representatives of *Aigeiros* and *Tacamahaca* sections differed in that the former had lower net and total rates of photosynthesis and higher compensation points. Rates of photorespiration were positively correlated with net and total photosynthesis, which explained most of the differences in photorespiration between clones. When rates of photorespiration were adjusted for average (over all clones) photosynthesis, these relative photorespiration rates were correlated positively with CO_2 compensation points and negatively with rates of photosynthesis. Dark respiration differed among clones but less obviously than photosynthesis. No apparent differences were found in dark respiration between clones of *Aigeiros* and *Tacamahaca* sections.

Photosynthetic efficiency of the hybrid clone, *P. maximowiczii* × *P. nigra,* was intermediate between the parental species. *P. nigra* had lower than average rates of photo-

synthesis and high relative rates of photorespiration, whereas *P. maximowiczii* had low relative rates of photorespiration but high photosynthetic rates. The intermediate rate of net photosynthesis in the hybrid appeared to result from combining the high photosynthetic rate of *P. maximowiczii* with high relative photorespiration rate of *P. nigra*. It is suggested that the CO_2 compensation point be used in further studies as an index of photosynthetic performance.

Photosynthetic efficiency was correlated better with stomatal size than stomatal frequency, and (inversely) with rate of stomatal closure.

Key words: Photosynthesis, photorespiration, dark respiration, CO_2 compensation point, poplar clones, *Populus deltoides, P. nigra, P. trichocarpa, P. maximowiczii, P. maximowiczii* × *P. nigra,* section hybrid.

Zusammenfassung

Bei 6 Pappelklonen wurden die Photosynthese, die Respiration im Dunkeln und im Licht und die CO_2-Kompensationspunkte untersucht. Es waren dies bei je 1 Klon von *Populus deltoides, P. nigra* und *P. trichocarpa,* ferner bei 2 Klonen von *P. maximowiczii* und bei 1 Bastardklon *P. maximowiczii* × *P. nigra.* Die Untersuchungsergebnisse zeigten in allen Fällen Unterschiede zwischen den Klonen. Die *Aigeiros*-Klone hatten geringere Photosynthese-Raten und höhere Kompensationspunkte als die *Tacamahaca*-Klone. Es gab aber keine Unterschiede zwischen ihnen bei der Respiration im Dunkeln. — Die photosynthetische Leistung des Hybridklones lag intermediär zwischen denen der Elternarten. Sie wird als die Kombination der hohen Photosynthese-Rate von *P. maximowiczii* und der hohen relativen Respirationsrate im Licht von *P. nigra* aufgefaßt. Es wird vorgeschlagen, den CO_2-Kompensationspunkt bei späteren Untersuchungen als Index für die Photosynthese-Leistungen zu verwenden. — Zusammenhänge mit der Größe und der Anzahl der Stomata werden u. a. diskutiert.

Literature Cited

Bourdeau, P.: Photosynthesis and respiration rates in leaves of male and female quaking aspen. Forest Sci. **4**: 331—334 (1958). — Brix, H.: An analysis of dry matter production of Douglas-fir seedlings in relation to temperature and light intensity. Can. J. Bot. **45**: 2063—2072 (1967). — Campbell, R. K., and Rediske, J. H.: Genetic variability of photosynthetic efficiency and dry matter accumulation in seedling Douglas-fir. Silvae Genetica **15**: 65—72 (1966). — Decker, J. P.: The uncommon denominator in photosynthesis as related to tolerance. Forest Sci. **1**: 88—89 (1955). — Decker, J. P.: Further evidence of increased carbon dioxide production accompanying photosynthesis. J. Solar Energy Sci. and Eng. **1**: 30—33 (1957). — Decker, J. P.: Some effects of temperature and carbon dioxide concentration on photosynthesis of mimulus. Plant Physiol. **34**: 103—106 (1959). — Decker, J. P.: Photosynthetic efficiency, photorespiration and heterosis. Ariz. State University Eng. Res. Center Bioengineering Bull. **12** (1970). — Ferrell, W. K.: Variation in photosynthetic efficiency within forest tree species. Proc. First North American Forest Biology Workshop, East Lansing, Michigan (1970). — Forrester, M. L., Krotkov, G., and Nelson, C. D.: Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. I. Soybean. Plant Physiol. **41**: 422—427 (1966 a). — Forrester, M. L., Krotkov, G., and Nelson, C. D.: Effect of oxygen on photosynthesis, photorespiration, and respiration in detached leaves. II. Corn and other monocotyledons. Plant Physiol. **41**: 428—431 (1966 b). — Gatherum, G. E., Gordon, J. C., and Broerman, B. F. S.: Effects of clone and light intensity on photosynthesis, respiration and growth of aspen-poplar hybrids. Silvae Genetica **16**: 128—132 (1967). — Hellmers, H.: An evaluation of the photosynthetic efficiency of forests. Quart. Rev. Biol. **39**: 249—259 (1964). — Huber, B., and Polster, H.: Zur Frage der physiologischen Ursachen der unterschiedlichen Stofferzeugung von Pappelklonen. Biol. Zentralbl. **74**: 370—420 (1955). — Jackson, W. A., and Volk, R. L.: Photorespiration. Ann. Rev. Plant Physiol. **21**: 385—432 (1970) — Kozlowski, T. T.: Growth and development of trees. Vol. I. Seed germination, ontogeny, and shoot growth. Academic Press, New York (1971 a). — Kozlowski, T. T.: Growth and development of trees. Vol. II. Cambial growth, root growth, and reproductive growth. Academic Press, New York (1971 b). — Kozlowski, T. T., and Keller, T.: Food relations in woody plants. Bot. Rev. **32**: 293—382 (1966). — Ledig, F. T.: A growth model for tree seedlings based on the rate of photosynthesis and the distribution of photosynthate. Photosynthetica **3**: 263—275 (1969). — Logan, K. T.: Monthly variations in photosynthetic rate of jack pine provenances in relation to their height. Can. J. Forest Res. **1**: 256—261 (1971). — Luukkanen, O.: Studies of photosynthesis, photorespiration, and dark respiration in six *Populus* clones. M. S. Thesis, University of Wisconsin, Madison, Wisconsin (1971). — McGregor, W., Allen, J., and Kramer, P. J.: The effect of photoperiod on growth, photosynthesis, and respiration of loblolly pine seedlings from two geographic sources. Forest Sci. **7**: 342—348 (1961). — Neuwirth, G.: Gasstoffwechselökologische Untersuchungen an Lärchenprovenienzen (*Larix decidua* Mill.). I. Vergleichende Stoffwechselanalysen an Pfropflingen. Photosynthetica **1**: 219—231 (1967). — Sasaki, S., and Kozlowski, T. T.: Effects of herbicides on carbon dioxide uptake by pine seedlings. Can. J. Bot. **45**: 961—971 (1967). — Siwecki, R.: Badania dziedzczenia odporności kórnickich mieszańców topoli na porazenie grzybem *Chondroplea (Dothichiza) populea* (Sacc.) Kleb. Arboretum Kórnickie XIV: 219—274 (1969). — Siwecki, R., and Kozlowski, T. T.: Leaf anatomy and water relations in six poplar clones. Arboretum Kórnickie (1973, in press). — Sorensen, F. C.: Photosynthesis, respiration, and dry matter accumulation of Douglas-fir seedlings from different geographic sources and grown at different temperatures. Ph. D. Thesis, Oregon State University, Corvallis, Oregon (1964). — Steel, R. G. D., and Torrie, J. H.: Principles and procedures of statistics. MacGraw-Hill, New York (1960). — Sweet, G. B., and Wareing, P. F.: A comparison of the rates of growth and photosynthesis in first-year seedlings of four provenances of *Pinus contorta* Dougl. Ann. Bot. **32**: 735—751 (1968). — Tregunna, E. B., Krotkov, G., and Nelson, C. D.: Effect of oxygen on the rate of photorespiration in detached tobacco leaves. Physiol. Plantarum **19**: 723—733 (1966). — Wuenscher, J. E., and Kozlowski, T. T.: Carbon dioxide transfer resistance as a factor in shade tolerance of tree seedlings. Can. J. Bot. **48**: 453—456 (1970). — Wyatt, W. R., and Beers, W. L.: A growth chamber study of plus-tree progeny. Tappi **47**: 305—309 (1964). — Zelawski, W.: A contribution to the question of the CO_2 evolution during photosynthesis in dependence on light intensity (*Pinus sylvestris* seedlings). Bull. Acad. Pol. Sci. Ser. Sci. Biol. **15**: 565—570 (1967).