BIOLOGIA PLANTARUM (PRAHA)

21 (4): 302—306, 1979

Study of CO_2 Exchange Processes, Resistances to Carbon Dioxide and Chlorophyll Content during Leaf Ontogenesis in Poplar

R. CEULEMANS and I. IMPENS

Department of Biology, University of Antwerp, UIA, Wilrijk*

Abstract. Net photosynthetic (P_N) and dark respiration (R_D) rate, stomatal (r_s') and internal (r_i') resistances to carbon dioxide were measured by gas exchange methods on leaves of different ages, expressed in leaf plastochron index units (LPI) for a fast growing poplar cultivar Unal 2. Although the optimal leaf age differs slightly for the different gas exchange parameters, leaf ontogeny is reflected in the same way in these different parameters. Maximal P_N and minimal r_s' and r_i' values were found at LPI between 6 and 10. Chlorophyll concentrations were lowest at LPI lower than 10 although an increase in two steps was found, when leaf age increases up to maturity.

Additional index words: Populus deltoides S. 9-2 × *P. nigra* cv. Ghoy; photosynthesis; dark respiration; stomatal resistance; internal resistance.

The developmental stage of a leaf has an important impact on its gas exchange processes and characteristics (FREELAND 1952). Many investigators have found that the gas exchange rates of young leaves tend to increase with increasing leaf age, attain an optimum and then decline when senescing (ČATSKÝ *et al.* 1976, DICKMANN 1971a, MALKINA 1976, VÁCLAVÍK 1975).

In the present communication, the influence of leaf age on net photosynthesis, P_N, dark respiration, R_D, stomatal, r_s' and internal, r_i' resistance to carbon dioxide and chlorophyll content of poplar leaves was studied in order to describe their changes during leaf ontogeny and to determine the functionally optimal leaf age for the cultivar studied.

MATERIAL AND METHODS

Cuttings of poplar plants (*Populus* cv. Unal 2 = *P. deltoides* S. 9-2 × *P. nigra* cv. Ghoy) were grown under glasshouse conditions at 20 °C and 50 % relative air humidity before they were transferred to a controlled environment chamber with a 12 h photoperiod, day/night air temperatures of 22.5/19.0 °C and day/night relative air humidity of 80/70 %. Photon flux density was around 260 μeinstein m^{-2} s^{-1} (400 to 700 nm). The plants were watered well and they were given a modified Hoagland nutrient solution twice a week.

Received February 13, 1979; accepted February 26, 1979

* *Address:* UIA, Universiteitsplein 1, B-2610 Wilrijk, Belgium.

The leaf plastochron index, LPI was determined by measuring the lamina length of the leaves at regular intervals (ERICKSON and MICHELINI 1957, LARSON and ISEBRANDS 1971). A lamina length of 20 mm was chosen as reference length.

The gas exchange measurements were performed on single leaves of different leaf ages expressed as LPI-units, from very young (LPI 1) up to old leaves (LPI $\pm$ 24). During the gas exchange measurements the leaf was exposed in a ventilated, Peltier cooled assimilation chamber (Siemens Sirigor cuvette, described by KOCH *et al.* 1968) to a saturating photon flux density of 800 to 1000 μeinstein m^{-2} s^{-1} supplied by three sodium lamps. Net photosynthetic CO_2 uptake and transpiration rates were measured as carbon dioxide and water vapour fluxes in an open system using a UNOR infrared gas analyser (Maihak) and LiCl sensors. Air temperature in the cuvette during the experiments was 25.0 °C, relative air humidity 77 %, wind velocity 1.6 m s^{-1} and carbon dioxide concentration of the incoming air 386 $\pm$ 23 μl l^{-1}. Leaf temperatures were recorded with three chromel — alumel thermocouple junctions pressed to the abaxial side of the leaf. Leaf area was measured with a Lambda-area meter (LiCor).

Stomatal and internal resistances to CO_2 were calculated according to GAASTRA (1959) and GALE and POLJAKOFF-MAYBER (1968).

Chlorophyll content was determined by spectrophotometry while chlorophyll *a* and *b* concentrations were calculated according to MCKINNEY (1941).

RESULTS AND DISCUSSION

Net carbon dioxide uptake rate shows a typical pattern influenced by the developmental stage of the leaves, having an initial increase with leaf expansion up to an optimal leaf age at which P_N is maximal (DICKMANN 1971a, VÁCLAVÍK 1975). When further senescing, carbon dioxide uptake rate decreases and this decline can be fast (DICKMANN *et al.* 1975) or slow (ČATSKÝ *et al.* 1976, VÁCLAVÍK 1975). The optimal leaf age for the poplar cultivar studied here (Fig. 1) was between LPI 6 and 10. For other poplar cultivars optimal leaf ages were found between 5 to 12 (CEULEMANS unpublished data). DICKMANN and GORDON (1975), working with $^{14}CO_2$ found maximal P_N at LPI 4 to 5 for *Populus* × *euramericana*. In our experiments maximal P_N coincides with the moment at which the leaf reaches maximal leaf length, as measured during the PI and LPI determinations. In contrast with P_N, dark respiration continually decreases with increasing leaf age (Fig. 2). For young leaves this decline is very steep, but levels off from a certain leaf age (from LPI 6 on for poplar cv. Unal 2) (ČATSKÝ *et al.* 1976, DICKMANN *et al.* 1975, MALKINA 1976). DICKMANN and GORDON (1975) and GJERSTAD (1975) found with very old poplar leaves (LPI over 40) again a marked increase in R_D.

Since foliar senescence influences P_N which in turn is determined by stomatal and internal resistances, r_s' and/or r_i' must be also dependent upon leaf age (ČATSKÝ *et al.* 1976, MALKINA 1976, TICHÁ and ČATSKÝ 1977). The LPI at which r_s' and r_i' attain minimal values (Fig. 3) coincides with the optimal LPI for P_N (Fig. 1) namely between LPI 6 and 10. Internal resistance to CO_2 is always higher than stomatal resistance (15 compared to 3 s cm^{-1}), as found already by many investigators (EL-SHARKAWY and HESKETH 1965, MALKINA 1976). An equation of the form $Y = A + BX + CX^2$ was fitted

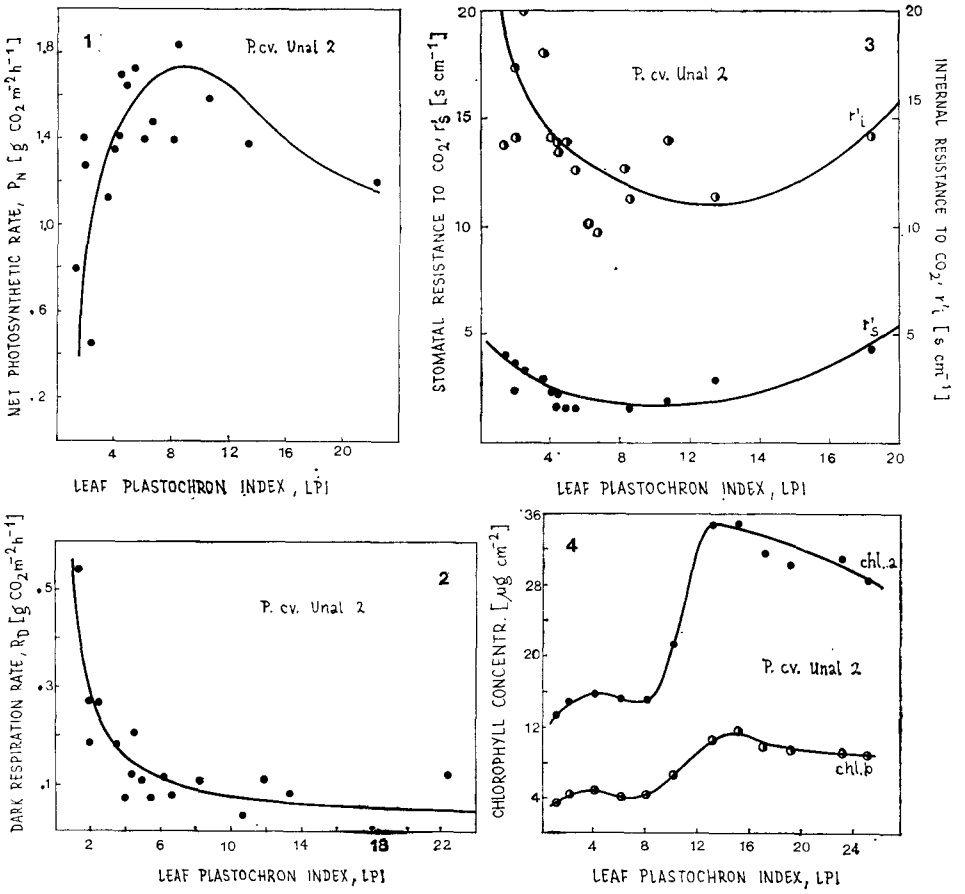

Fig. 1. Net photosynthetic rate (P_N) as amount of CO_2 absorbed by one leaf surface, at 25 °C during leaf ontogenesis of poplar cultivar Unal 2.
Fig. 2. Dark respiration rate (R_D) during leaf ontogenesis of poplar cultivar Unal 2. Fitted curve significant at 0.001.
Fig. 3. Stomatal (r_s') and internal (r_i') resistances to carbon dioxide during leaf ontogenesis for poplar cultivar Unal 2. Curve fittings are significant at 0.01.
Fig. 4. Chlorophyll *a* and chlorophyll *b* concentrations during leaf ontogenesis of leaves of poplar cultivar Unal 2. Chlorophyll contents are expressed per unit leaf area.

through the two sets of points in Fig. 3. Both fittings are significant at the 0.01 level.

Since there is no photosynthesis, no captation of solar energy and transformation into chemical energy without chlorophyll *a* or related pigments (ŠESTÁK 1977), the determination of chlorophyll content with leaf age was an essential part of this study. Generally during leaf ontogenesis, chlorophyll accumulates up to some maximum level and afterwards the rate of degradation processes overtakes the rate of synthetic processes. These changes are also reflected in the rate of chlorophyll *a* and chlorophyll *b* synthesis (LEWANDOWSKA and JARVIS 1977, ŠESTÁK 1977). The age of the leaf tissues deter-

mines the rate of chlorophyll accumulation and according to ŠESTÁK (1977) is also the main factor responsible for its distribution. The rate of increase in chlorophyll *a* and *b* is usually more rapid than the rate of decrease, especially in the leaves formed in the later phases of plant ontogenesis whose life spans are longer (DICKMANN 1971b, LEWANDOWSKA and JARVIS 1977, MALKINA 1976, ŠESTÁK 1977). For our poplar cultivar an increase in two steps was found (Fig. 4). A first plateau was reached at the optimal LPI (here at LPI around 8), while a second was found around LPI 12. When the leaf is young, the complete photosynthetic apparatus has to be built up: fully developed chloroplast lamellae, reaction centres and antennae chlorophyll for light capturing. This stage is reached at the first plateau and is responsible for maximal P_N values. Afterwards, further chlorophyll synthesis only serves to expand the amount of antennae chlorophyll, which only enhances light capturing but does not influence light conversion processes (DE GREEF personal communication). This is shown by the second, higher maximum in Fig. 4. This second maximum lies after the optimal photosynthesis leaf age. The values of the chlorophyll concentrations reported here are slightly higher than those obtained by DICKMANN (1971b) on poplar.

It may be concluded that the study of gas exchange processes and characteristics and of chlorophyll concentrations can be used in studying and understanding leaf ontogenesis and in determining the leaf age at which the leaves perform optimally, although small variation exists in the leaf age data, suggesting that the different parameters studied do not reach their optimum at exactly the same leaf age. Inversely, comparative studies of gas exchange processes and characteristics of leaves from different plant species and cultivars should be made with leaves of comparable relative morphological, physiological and biochemical maturity.

Acknowledgements

The authors are indebted to ir. V. Steenackers, Director of the Rijksstation voor Populierenteelt (Geraardsbergen) for providing the plant materials and to Miss D. Verschooren for her technical help. IWONL-Brussels allowed a grant (No 76031) to R. Ceulemans.

REFERENCES

ČATSKÝ, J., TICHÁ, I., SOLÁROVÁ, J.: Ontogenetic changes in the internal limitations to bean-leaf photosynthesis. 1. Carbon dioxide exchange and conductances for carbon dioxide transfer. — Photosynthetica **10** : 394—402, 1976.

DICKMANN, D. I.: Photosynthesis and respiration by developing leaves of cottonwood (*Populus deltoides* BARTR.). — Bot. Gaz. **132** : 253—259, 1971a.

DICKMANN, D. I.: Chlorophyll, ribulose-1,5-diphosphate carboxylase and Hill reaction activity in developing leaves of *Populus deltoides*. — Plant Physiol. **48** : 143—145, 1971b.

DICKMANN, D. I., GJERSTAD, D. H., GORDON, J. C.: Developmental patterns of CO_2 exchange, diffusion resistance and protein synthesis in leaves of *Populus* × *euramericana*. — In: MARCELLE, R. (ed.): Environmental and Biological Control of Photosynthesis. Pp. 171—181. Dr. W. Junk, Publ., The Hague 1975.

DICKMANN, D. I., GORDON, J. C.: Incorporation of ^{14}C-photosynthate during leaf development in young *Populus* plants. — Plant Physiol. **56** : 23—27, 1975.

EL-SHARKAWY, M., HESKETH, J.: Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2-diffusion resistances. — Crop Sci. **5** : 517—521, 1965.

ERICKSON, R. O., MICHELINI, F. J.: The plastochron index. — Amer. J. Bot. **44** : 297—305, 1957.

FREELAND, R. O.: Effect of age of leaves upon the rate of photosynthesis in some conifers. — Plant Physiol. **27** : 685—690, 1952.

GAASTRA, P.: Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature

and stomatal diffusion resistance. — Mededel. Landbouwhogesch. Wageningen **59** : 1—68, 1959.

GALE, J., POLJAKOFF-MAYBER, A.: Resistances to the diffusion of gas and vapour in leaves. — Physiol. Plant. **21** : 1170—1176, 1968.

GJERSTAD, D.: Photosynthesis, photorespiration and dark respiration in *Populus* × *euramericana*: effects of genotype and leaf age. — Ph. D. dissertation, Iowa State University, 1975.

KOCH, W., KLEIN, E., WALZ, H.: Elektronische Gaswechsel-Messanlage für Pflanzen in Laboratorium und Freiland. — Siemens Z. **42** : 392—404, 1968.

LARSON, P. R., ISEBRANDS, J. G.: The plastochron index as applied to developmental studies of cottonwood. — Can. J. For. Res. **1** : 1—11, 1971.

LEWANDOWSKA, M., JARVIS, P. G.: Changes of chlorophyll and carothenoid content, specific leaf area and dry weight fraction in Sitka spruce in response to shading and season. — New Phytol. **79** : 247—256, 1977.

MALKINA, I. S.: Changes in the light curves of photosynthesis with aging in the leaf of Norway maple. — Soviet Plant Physiol. **23** : 208—212, 1976.

MCKINNEY, G.: Absorption of light by chlorophyll solutions. — J. biol. Chem. **140** : 315—322, 1941.

ŠESTÁK, Z.: Photosynthetic characteristics during ontogenesis of leaves. 1. Chlorophylls. — Photosynthetica **11** : 367—448, 1977.

TICHÁ, I., ČATSKÝ, J.: Ontogenetic changes in the internal limitations to bean-leaf photosynthesis. 3. Leaf mesophyll structure and intracellular conductance for carbon dioxide transfer. — Photosynthetica **11** : 361—366, 1977.

VÁCLAVÍK, J.: Comparison of the changes in net photosynthetic CO_2 uptake and water vapour efflux during leaf ontogenesis with the differences between the leaves according to their descending insertion level. — Biol. Plant. **17** : 411—415, 1975.