Photosynthesis in Three Provenances of Pinus merkusii

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Introduction

Intraspecific genetic variation has been observed in the morphological as well as in the physiological characteristics of forest trees (Ferrell, 1970). Short-term measurements have shown that within a given species of the northern temperate region trees from higher latitudes may photosynthetize at a faster rate per unit of foliage than trees of a more southern origin (Gordon and Gatherum, 1968; Pelkonen and Luukkanen, 1974). Growth rates are, however, often negatively or poorly correlated with short-term measurements of photosynthetic rates per unit of foliage, as discussed by Ferrell (1970).

Intraspecific variation in gas exchange has been studied in detail in Douglas-fir. In this species geographic variation is reflected in photosynthetic as well as in transpirational rates (Sorensen and Ferrell, 1973; Unterscheutz *et al.*, 1974). Little is known about the physiological variation of tropical species, such as Merkus pine *(Pinus merkusii)*. However, the grass stage which occurs in this as well as in several other pine species (Mirov, 1967) has received attention. The grass stage has been intensively studied in longleaf pine *(Pinus palustris)*. For instance, Brown (1964) observed that the formation of grass stage in this species is under strong genetic control but environmental factors determine the duration of this stage in a given seedling. The same author (Brown, 1958) also found that apices of P. *palustris* produce no auxin and that height growth was not promoted by spraying the shoots with gibberellic acid.

Within a species, variation in photorespiration and the CO, compensation point have been suggested as criteria for the selection of trees with potentially high photosynthetic performance (Decker, 1970). At least in some cases both photorespiration rate and the CO, compensation point do correlate (inversely) with net photosynthetic rates per unit of foliage. However, the significance of this relationship for evaluation and prediction of growth rates still needs further study (Luukkanen and Kozlowski, 1972; Luukkanen, 1976).

The purpose of this study was to obtain some preliminary information about the genetic variation in the CO, metabolism of *Pinus merkusii*. The relationship between CO, exchange and the capability to form the grass stage was also investigated in different genotypes.

Materials and Methods

Pinus merkusii De Vries seeds from two locations in Thailand, i.e. Omkoi (Lat. 17^0 52' N., Long. 98^0 20' E.) and Surin (Lat. 14^0 43' N., Long. 103^0 50' E.), and one location in Java, W. Pegalongan (Lat. 7^0 10' S., Long. 109^0 10' E.), were supplied by Mr. H. Keiding, Danish/FAO Forest Tree Seed Center at Humlebaek, Denmark. Sowing and subsequent studies were carried out in the laboratory of the Department of Silviculture, University of Helsinki, Finland. The seedlings were initially grown in large containers, filled with fertilized horticultural *Sphagnum* peat under the following conditions: day temperature 30^0 C, night temperature 200 C, day length 12 h, fluorescent light irradiance 10 Wm^{-2}. The seedlings were watered daily and given, after they had passed the germination stage, a nutrient solution once a week. After six months, the seedlings were transplanted into individual plastic containers, with a volume of approximately 200 ml. Subsequently, eight seedlings from each provenance were selected for the CO, exchange studies.

One month after transplanting, the CO_2 measurements were started using a URAS 1 infrared gas analyzer. At this stage, the development of new needle fascicles had generally ceased in all provenances, but the seedlings differed morphologically due to an inhibited apical bud development (grass stage) in Omkoi and Surin seedlings.

During the measurements the upper part of the seedling was enclosed in a water-jacketed plexiglass assimilation chamber with a volume of 300 ml. The total volume of the closed gas circuit system, including the gas analyzer, assimilation chamber and auxiliary equipment used to maintain the desired air flow, humidity and CO, concentration, was approximately 1000 ml. Temperature control was achieved using a pump which circulated water through the water jacket and a thermostat. During the measurements, the lower parts of the intact seedlings were subjected to the constant environmental conditions of the growth chamber in which the cuvette had been built.

The light source during the CO, measurements consisted of two 500 W incandescent/mercury vapor mixed light bulbs and ten 40 W fluorescent tubes. The irradiance within the assimilation chamber reached a level of 40 Wm^{-2}.

In the measurement procedure the CO, compensation point was first determined at 21^0 C by recording the equilibrium concentration of CO_2, after decreasing the CO, concentration to near the expected value using a KOH solution bypass in the air stream. The CO, concentration was then again raised to the normal atmospheric level, and after a half hour interval, the photosynthetic rate was measured at 16, 21, 26 and 31^0 C, by recording the time required to lower the CO, concentration from 315 to 265 ppm. The highest or lowest temperatures were used alternately as the initial temperatures. When the photosynthetic rates had been measured, dark respiration rates were determined in a similar manner at the same temperatures as already mentioned. However, only 25 ppm (from 282 to 307 ppm) was used as the CO, concentration range in the dark respiration determinations. Photosynthetic and dark respiration rates per seedling were converted to rates per unit of needle dry weight (mg CO, . $\mathrm{g}^{-1} \cdot \mathrm{h}^{-1}$), using weights obtained by drying the seedlings overnight at 105^0 C. Photorespiration rates were determined on the basis of CO, compensation points and photosynthetic rates, using the extrapolation method (Forrester *et al.*, 1966; Luukkanen and Kozlowski, 1972).

Results

Seedlings from Java (which in contrast to other provenances did not show a grass stage) had photosynthetic rates per unit of foliage nearly twice as high as those from Omkoi or Surin at all temperatures *(Fig. 1)*. This variation was pronounced ($P < 0.01$), whereas the two northern provenances did not show any significant difference in photosynthetic rates per unit of foliage. The optimum temperature for photosynthetic rate was also highest in the Javanese seedlings, as can be seen from the figure (31° C vs. 26° C in both Omkoi and Surin seedlings).

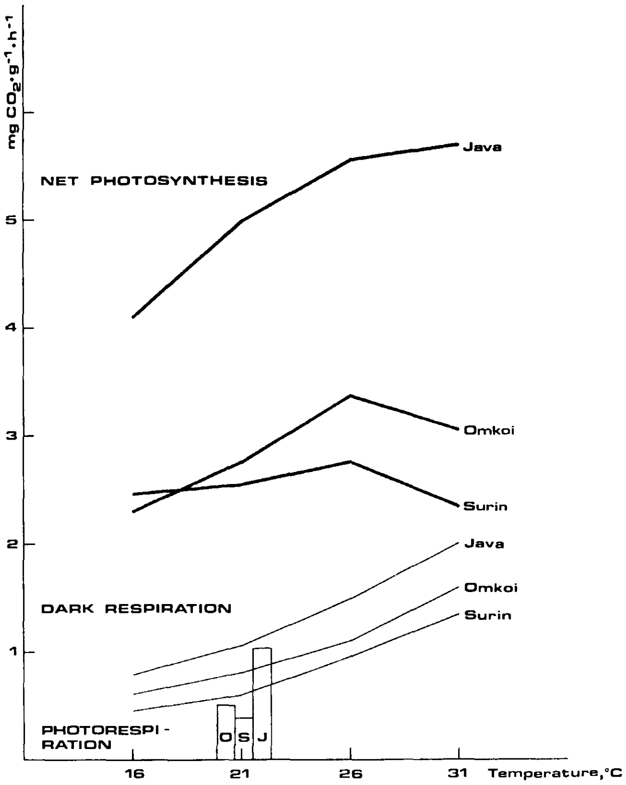

Fig. 1. — Net photosynthetic (thick lines), dark respiration (thin lines) and photorespiration rates (columns) per unit of foliage (dry wt.) in three provenances of *Pinus merkusii*. The variation among provenances was statistically significant ($P < 0.05$) for respiration rates at 16 and 26° C and highly significant ($P < 0.001$) for photorespiration rates; highly significant differences ($P < 0.01$) were also observed at the remaining temperatures in respiration measurements and in all photosynthesis measurements.

Dark respiration rates per unit of foliage were also highest in the southern seedlings. The variation among the three provenances was most clear ($P < 0.01$) at 21 and 31° C, and statistically significant ($P < 0.05$) at 16 and 26° C as well. These results are shown in *Fig. 1*, which also illustrates the variation in photorespiration rates. Photorespiration rates per unit of foliage (determined at 21° C only) were generally slightly lower than the dark respiration rates in all three provenances. Seedlings from Java had the highest photorespiration rates (about twice as high as were found in the two northern provenances), and the variation among provenances was statistically highly significant ($P < 0.01$).

Results for photosynthesis and dark respiration rates per whole seedling were different from those calculated per unit of foliage, as can be seen from *Fig. 2*. Seedlings from one northern source, Surin, were the largest having an average needle dry weight of 6.5 g vs. 3.2 g in both Omkoi and Javanese seedlings. The largest seedlings had photosynthetic and dark respiration rates per individual generally exceeding the rates of the Javanese seedlings, which, as shown above, had the most intensive photosynthetic and respiration rates per unit of foliage. The variation among provenances was confirmed statistically ($P < 0.05$) at 16 and 21° C as regards the photosynthetic rates and at all temperatures except 16° C for the respiration rates.

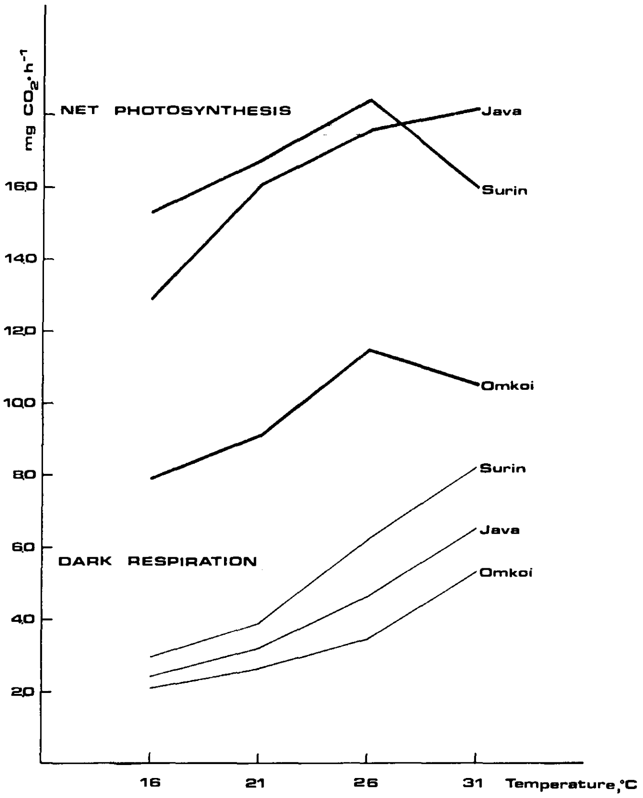

Fig. 2. — Net photosynthetic and respiration rates per seedling in three provenances of *Pinus merkusii*. At all temperatures (except at 16 and 21° C in photosynthesis measurements) the differences among provenances were statistically significant ($P < 0.05$).

The values of the CO$_2$ compensation points did not reveal any statistically significant differences among provenances when the results were subjected to analysis of variance. However, the Javanese seedlings appeared to have the highest CO$_2$ compensation points (49.5 ppm vs. 43.0 ppm in Surin and 46.4 ppm in Omkoi pines).

Relationships between various gas exchange parameters were also analyzed in further detail. It was found that the ratio of dark respiration to photosynthesis was lower in seedlings from Java as compared to the corresponding ratios in the two northern provenances. However, this difference was not statistically confirmed. The ratio between photorespiration and photosynthesis, on the other hand, was lowest in one of the northern sources, Surin, and highest in seedlings from Java. Again, this difference was not confirmed statistically. The photorespiration/dark respiration ratios at 21° C, however, were shown to vary significantly ($P < 0.05$) among provenances, the southern or Javanese seedlings having the highest and the northernmost ones from Omkoi having the lowest ratio.

Discussion

In the present study distinct differences were observed in the CO$_2$ exchange pattern between the southernmost Java provenance on the one hand and the two Thai provenances on the other. These physiological differences corresponded to morphological variation. Thai provenances were characterized by their ability to form a grass stage, which was lacking from the Javanese pines. The ability to form a grass stage was associated with lower than average photosynthetic rates per unit of foliage. Seedling size, after seven months of growth in a controlled environment was, however, largest in one of the provenances which exhibited grass stage formation (Surin). Therefore, photosynthetic rates per whole seedling did not differ between the two morphological types. Apart from photosynthetic rates, dark respiration and photorespiration rates per unit of foliage as well as the CO_2 compensation points were also higher than average in the Java provenance where no grass stages occurred. These results suggest that CO_2 metabolism is less active in *Pinus merkusii* seedlings which are in the grass stage condition as compared with normal seedlings.

Obviously, the CO_2 exchange should be followed over longer periods of time to exclude the effects of different seasonal rhythms in different provenances on the variation in CO_2 exchange. In *Pinus sylvestris,* for instance, needle development varies in different geographical populations and this in turn renders the variation in CO_2 exchange characteristics different at different times of the year (Zelawski and Kinelska, 1967).

In the present study the conditions during CO_2 measurements were close to optimal as far as water relations were concerned, whereas the light intensity was not saturating. Effects of various water and light regimes should conceivably also be analyzed before any conclusions about the ecological adaptation of *Pinus merkusii* seedlings with different morphology are made.

Photorespiration equalled 13.8% of total photosynthesis at 21⁰ C in the Surin seedlings or the provenance with the lowest photosynthetic rate per unit of foliage at this temperature. The highest photorespiration/total photosynthesis ratio (16.0%), on the other hand, was observed in the Javanese pines, which generally had the highest photosynthetic (and photorespiration) rates per unit of foliage. The variation in photorespiration/photosynthesis ratios was not statistically significant, however. This was different from the situation observed earlier in clones from different poplar species (Luukkanen and Kozlowski, 1972), where "relative" photorespiration rates (rates adjusted to average photosynthetic rates by means of covariance analysis) seemed to be highest in the genotype with the lowest photosynthetic efficiency (and with the lowest photorespiration rate prior to the adjustment). However, in these poplar clones higher photorespiration/photosynthesis ratios were associated with higher optimum temperatures for net photosynthesis, as also found in the present study. Obviously, photorespiration in *Pinus merkusii* should be investigated throughout the whole temperature range before any further conclusions about the diagnostic value of photorespiration or CO_2 compensation point measurements are to be made.

In the present study average photosynthetic rates in the three provenances varied between 2.3 and 5.8 mg $CO_2 \cdot g^{-1} \cdot h^{-1}$ within the applied temperature range of 16 to 31⁰ C. This corresponds well to values earlier reported for conifers but remains much lower than maximum rates (up to 35 mg $CO_2 \cdot g^{-1} \cdot h^{-1}$) obtained with Scots pine using a specially designed assimilation chamber which eliminates mutual shading of needles (Zelawski *et al.*, 1973).

Optimum temperatures for net photosynthesis clearly varied among the *Pinus merkusii* provenances. On an average, the highest net photosynthetic rates were measured at 26⁰ C in the two Thai pine sources, whereas seedlings from Java photosynthesized most rapidly at 31⁰ C. Differences in optimum temperature for net CO_2 uptake have earlier been reported by Ferrell (1970) and his coworkers between coastal and inland sources of Douglas-fir. In this species growing temperature and seedling age seem to affect the photosynthetic rate and optimum temperature even more than does seed source (Sorensen and Ferrell, 1973). In Norway spruce, no variation in optimum temperature seems to exist between populations from Southern Finland and the northern timberline (Pelkonen and Luukkanen, 1974).

In the grass *Festuca arundinacea,* growth temperature is known to affect enzymatic carboxylation and respiratory activity as well as stomatal conductivity when plants from different environments are investigated under identical conditions (Treharne and Nelson, 1975). Such differences in enzymatic or stomatal characteristics also affect the observed genetic variation in photosynthetic rates among climatic races of another grass, *Dactylis glomerata* (Treharne and Eagles, 1970).

As discussed by Dickmann *et al.* (1975), the ontogenic variation in photosynthetic rates of poplar leaves is also related, among other factors, to changes in enzymatic activity and stomatal resistance. In different poplar clones such changes vary distinctly and show different impacts on CO_2 exchange (Gordon and Promnitz, 1976). The next step in studying physiological intraspecific differences in *Pinus merkusii* would be to analyze such mechanisms.

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Summary

The CO_2 exchange of seven-month old *Pinus merkusii* seedlings grown under controlled environmental conditions was investigated using a URAS 1 infrared gas analyzer in a closed measurement system. Photosynthetic and dark respiration rates were measured at 16, 21, 26 and 31⁰ C and photorespiration rates were determined (from CO_2 compensation points by the extrapolation method) at 21⁰ C only. Among three provenances, the highest photosynthetic, dark respiration and photorespiration rates per unit of foliage were observed in seedlings from Java, whereas only small differences occurred between the rates of the two Thai provenances. The largest seedlings were those from one of the Thai sources and they exhibited the highest photosynthetic and respiration rates per seedling at all temperatures except for photosynthesis at the highest temperature.

The lower gas exchange rates per unit of foliage of the Thai seedlings were associated with the ability to form a grass stage, which was not observed in Javanese pines. Photosynthetic rates per entire seedling did not differ between the two morphological types, since the largest seedlings were found in one of the Thai sources. In conclusion, however, the results suggested that intraspecific differences in CO_2 metabolism occur in *Pinus merkusii* seedlings which are in the grass stage as compared with normal seedlings.

Key words: Photosynthesis, photorespiration, dark respiration, CO_2 compensation point, *Pinus merkusii*, provenances, grass stage.

Zusammenfassung

Bei Gaswechseluntersuchungen an 7 Monate alten Sämlingen von *Pinus merkusii* aus 2 Vorkommen Thailands (etwa 18° und etwa 15° nördlicher Breite) und aus einem Vorkommen der Insel Java (etwa 7° südlicher Breite) reagierten die thailändischen Herkünfte anders als die Herkunft aus Java. Gleichzeitig ergaben sich Wechselbeziehungen zu dem bei dieser Baumart auftretenden sog. Gras-Stadium.

Literature Cited

Brown, C. L.: Studies in the auxin physiology of longleaf pine seedlings. *In* The Physiology of Forest Trees (ed. Thimann, K. V.). Ronald Press, New York, pp. 511—525 (1958). — Brown, C. L.: The seedling habit of longleaf pine. Ga. Forest Res. Council Rep. 10 (1964). — Decker, J. P.: Photosynthetic efficiency, photorespiration and heterosis. Ariz. State Univ. Eng. Res. Cent. Bioeng. Bull. 12 (1970). — Dickmann, D. I., Gjerstad, D. H., and Gordon, J. C.: Developmental patterns of CO_2 exchange, diffusion resistance and protein synthesis in leaves of *Populus* × *euramericana*. *In* Biological Control of Photosynthesis (ed. Marcelle, R.). Junk, The Hague, pp. 171—181 (1975). — Ferrell, W. K.: Variation in photosynthetic efficiency within forest tree species. Proc. First North Amer. Forest Biol. Workshop, East Lansing, Mich. August 5—7 (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). — Gordon, J. C., and Gatherum, G. E.: Photosynthesis and growth of selected Scotch pine populations. Silva Fenn. 2: 183—194 (1968). — Gordon, J. C., and Promnitz, L. C.: Photosynthetic and enzymatic criteria for the early selection of fast-growing *Populus* clones. *In* Tree Physiology and Yield Improvement (ed. Cannell, M. G. R., and Last, F. T.). Academic Press, London (1976, in print). — Luukkanen, O.: Relationship between the CO_2 compensation point and carbon fixation efficiency in tree species. *In* Tree Physiology and Yield Improvement (ed. Cannell, M. G. R., and Last, F. T.). Academic Press, London (1976, in print). — Luukkanen, O., and Kozlowski, T. T.: Gas exchange in six *Populus* clones. Silvae Genet. 21: 220—229 (1972). — Mirov, N. T.: The Genus Pinus. Ronald Press, New York (1967). — Pelkonen, P., and Luukkanen, O.: Gas exchange in three populations of Norway spruce. Silvae Genet. 23: 160—164 (1974). — Sorensen, F. C., and Ferrell, W. K.: Photosynthesis and growth of Douglas-fir seedlings when grown in different environments. Can. J. Bot. 51: 1689—1698 (1973). — Treharne, K. J., and Eagles, C. F.: Effect of temperature on photosynthetic activity of climatic races of *Dactylis glomerata* L. Photosynthetica 4: 107—117 (1970). — Treharne, K. J., and Nelson, C. J.: Effect of growth temperature on photosynthetic and photo-respiratory activity in tall fescue. *In* Environmental and Biological Control of Photosynthesis (ed. Marcelle, R.). Junk, The Hague, pp. 61—69 (1975). — Unterscheutz, P., Ruetz, W. F., Geppert, R. R., and Ferrell, W. K.: The effect of age, pre-conditioning, and water stress on the transpiration rates of Douglas-fir *(Pseudotsuga menziesii)* seedlings of several ecotypes. Physiol. Plant. 32: 214—221 (1974). — Zelawski, W., Szaniawski, R., Dybczynski, W., and Piechurowski, A.: Photosynthetic capacity of conifers in diffuse light of high illuminance. Photosynthetica 7: 351—357 (1973). — Zelawski, W., and Kinelska, J.: Photosynthesis and respiration of Scots pine *(Pinus silvestris* L.) seedlings grown in water culture from seed of various provenance. Acta Soc. Bot. Pol. 35: 495—503 (1967).

Contributions to the genetics of empty grains in the seed of pine (Pinus silvestris)

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Introduction

Already when artificial extraction of seed from conifers was started the observations were made that a certain amount of empty grains was contained in the seed. Schotte (1905) speaks about dull seeds. Hagem (1917) stated that pine seed lots from the Vestland in Norway contained 21.4 ± 3.1% empty grains on an average with a variation from stand to stand between 7 and 33%. Heikinheimo (1937) found that the average percentage of empty grains in seed from Finnish stands was 18.2%. In the seeds from other Finnish stands Sarvas (1962) counted 13.7% empty grains with a stand to stand variation between 10 and 18%. Dengler (1932) observed that selfing was followed by a very noticeable increase in the frequency of empty grains. This experience has been confirmed later on by a number of authors, i. e. Plym-Forshell (1953) and Koski (1971).

The penomenon in question is also known in other gymnosperms. Thus Allen (1942) stated that selfing resulted in but few germinable seeds in *Pseudotsuga menziesii*, which was supported by Duffield (1950). Orr-Ewing (1957) examined the anatomical development of the ovules, resulting from selfing in the said species, and stated that the embryos collapsed at an earlier or later phase. He interpreted these events as a consequence of homozygotisation of recessive deleterious genes following self-fertilization. This theory has also been generally adopted for *Pinus silvestris* (Johnsson 1961, Sarvas 1962, Koski 1971) as for other *Pinus* species, i.e. *P. banksiana* (Fowler 1964), and can be postulated as being valid for the gymnosperms in general.

Koski (1971) with the frequencies of empty grains after selfing as basis, has carried out calculations of the number of recessive embryonic lethals. According to these calculations there is an individual range from 2—20 with 9.4 as an average in the investigated material and 70—80 in the populations. In *Pinus virginiana* Bramlett and Pepper (1974) estimated that 150—180 such genes would provide the most satisfactory explanation of the frequency of empty grains after individual crosses.

In this report, data, obtained after open pollination and individual crosses, are presented and treated from a quantitative genetic point of view. The grafted clones, on which the studies are based, are derived from plus trees, selected in natural stands in South Sweden between the latitudes 57° and 58°. Only in some few cases has more than one tree been selected in the same stand.