

PHOTOSYNTHESIS, RESPIRATION AND TRANSPIRATION OF RADIATA PINE

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ABSTRACT

Gas exchange measurements of photosynthesis, respiration, and transpiration of radiata pine (*Pinus radiata* D. Don) seedlings have been used to study diurnal patterns and responses to temperature, subjection to drought, flooding, and decapitation of the shoot.

The diurnal patterns of net photosynthesis and transpiration in a controlled environment showed a rapid initial increase followed by a slower increase to a maximum after about three hr of illumination. This was followed by a gentle decrease, consistent with an increasing leaf water deficit, until the end of the photoperiod. This diurnal pattern exhibited by plants grown in a controlled environment should be taken into account in planning short term experiments.

The effects of temperature included an increase in maximum photosynthetic capacity between 11° and 23°C, possibly due to effects on the enzymatic reactions, and a depression at elevated temperatures attributed to disorganisation of the photochemical systems.

Repeated cycles of droughting produced expected stomatal responses and less obvious cumulative effects on gas exchange, some of which seemed non-stomatal. Plants which were flooded behaved in most respects as though suffering a water deficit.

Gas exchange of the shoots of seedlings severed from their roots suggested that whilst subsequent transpiration was much affected, photosynthesis was only slightly affected.

INTRODUCTION

The limitations to be borne in mind in interpreting measurements of gas exchange of higher plants by the methods used in this and a previous paper (Brittain and Cameron, 1973) are even more stringent in the case of *Pinus* species than they are when broad-leaved plants are used. The geometrical complexity of the space occupied by a pine plant is substantial as is the complexity of the gas flow patterns and the heat and light gradients which result when the plant is enclosed in an illuminated assimilation chamber and swept with a stream of air.

Whilst it might be preferable to work with less complex plant material than a whole

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pine plant, it is impractical to use a single needle as one might use one leaf of a broad-leaved plant. The need to obtain physiological data relative to whole plants on which forestry practices may be based necessitates adopting available methods despite their recognised limitations.

MATERIALS AND METHODS

The seedlings used for these experiments were raised from seed derived from a controlled cross-pollination and were grown in soil under glasshouse conditions. Their age varied from eight to 16 months in different experiments, with some two-year-old stock being used in examining the effects of drought and flooding. This stock was grown for eight months under standard nursery conditions and thereafter in a glasshouse.

The gas exchange of the seedling material was determined by enclosing a shoot or part of a shoot in a cylindrical perspex chamber, 25.4 cm in diameter and 45.7 cm high, through which an air stream was passed. This chamber and the potted seedling were placed in a controlled environment chamber (Morse and Evans, 1962) receiving light at approximately 18.8 mW cm^{-2} for a photoperiod of 12 hr at a temperature of 25°C . The night temperature was 16°C . Photosynthesis and respiration were measured with an infra-red gas analyser*. Wet- and dry-bulb thermocouple psychrometers were included in the gas line before and after the chamber to estimate transpiration. The psychrometers were similar to those described by Bierhuizen and Slatyer (1964) but were constructed of standard glass joints and tubing and the wet- and dry-bulb temperatures were measured separately rather than differentially.

Gas exchange has been calculated as $\text{mg CO}_2 \text{ cm}^{-3}\text{hr}^{-1}$ where the volume measurement is that of the foliage. Foliage volume was estimated by a displacement technique (Clark, 1961), the accuracy of the apparatus being checked with objects of known volume and found to be $\pm 1\%$.

The method of Wood (1971) for severing a shoot under water was used for the work on detached shoot material.

RESULTS

The Diurnal Course of Net Photosynthesis and Transpiration

An 11-month-old seedling was removed from the glasshouse and placed in the controlled environment cabinet a week prior to the shoot being sealed in the assimilation chamber. Net photosynthesis and transpiration were then determined from 0600 hr to 1800 hr, and dark respiration from 0530 to 0600 hr and from 1800 to 1830 hr over five successive days.† The results expressed as a percentage of the maximum rate are the means of these five determinations (Fig. 1a).

The initial response to light was rapid and both net photosynthesis and transpiration reached 80% of their maximum rates within half an hour. Thereafter rates continued to increase slowly to reach their maxima after three hr of illumination. They then decreased gently for the remainder of the photoperiod with the fall never exceeding 5% of the maximum rate per hour.

* Hilger-I.R.D. Ltd, Null Balance Infra-Red Gas Analyser, Type SB/K.

† Times quoted throughout this paper are Australian Eastern Standard Time.

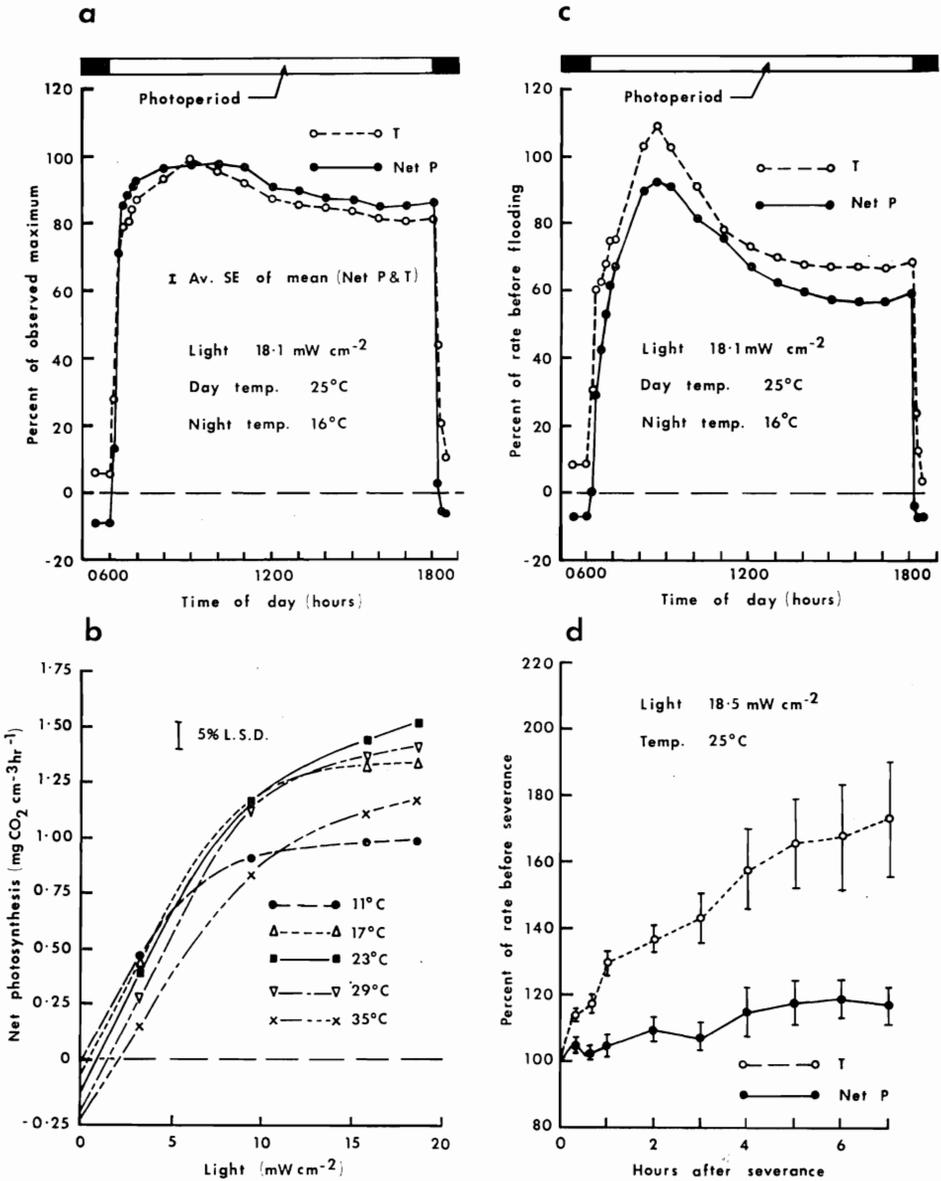


FIG. 1—Gas exchange of radiata pine seedlings.

- a. Time course of net photosynthesis and transpiration under a normal watering regime,
- b. Light response curves at various temperatures,
- c. Time course of net photosynthesis and transpiration during the fourth day of flooding,
- d. Transpiration and net photosynthesis of shoots severed from root systems.

Since it seemed the slow increase to the maximum might be associated with a lag in the rate of rise of the temperature of the root system compared with that of the shoot, this was investigated separately. Thermocouples were inserted in a pot of soil which had been watered to field capacity and in another which was flooded by being placed in a tub of tap water. The pots were then subjected to the same environmental regime as used previously. Substantial temperature lags occurred. At the end of the 16°C dark period of 12 hr, the bulk of the moist soil was at air temperature ($\pm 0.1^\circ\text{C}$) although the surface of the soil was some 1.5°C cooler. Fifteen minutes later, when the air temperature had risen to 24.8°C, the soil in middle of the pot had only increased in temperature by 0.2°C. After over 6 hr the bulk of the soil reached its final temperature of 24.1°C, i.e., 0.7°C less than air temperature. For the flooded pot the soil temperature at the end of the 16°C dark period was 14.9°C; 15 min later when the air temperature had stabilised at 24.8°C the flooded soil had not begun to respond but subsequently its temperature rose more slowly than the moist soil, requiring a little more than 9½ hr to reach its final temperature of 22.8°C, i.e., 2°C less than air temperature. The differences between soil and air temperature at equilibrium are assumed to be due to evaporative cooling.

Effects of Temperature on the Photosynthetic Light Response

Twenty seedlings eight months old were allotted at random to five temperature treatments to be investigated, viz., 11°, 17°, 23°, 29°, and 35°C. The four replicates of a treatment were placed in the apparatus at approximately 1000, 1200, 1400, and 1600 hr respectively on a given day and the five treatments applied on successive days. In this way compensation was made for the diurnal variation while keeping the replicates close together and the whole experiment within reasonable time limits.

For each determination, the seedling was first exposed to the highest light intensity until a steady photosynthetic rate was established. The radiation was then reduced in steps, time being allowed for the establishment of a new steady state at each level, and finally dark respiration was estimated. The results are summarised in Fig. 1b as light response curves. The slopes of the light-limited sections of these curves differ little except for that at 35°C being significantly different from the others. The compensation point increases with temperature largely because of a concomitant increase of dark respiration.

Saturating light intensities were approached only for the 11° and 17°C treatments; for the 23° and 29°C treatments they are obviously greater than at 17°C although they cannot be estimated or distinguished.

The optimum temperature for net photosynthesis at higher light intensities is about 23°C. This is supported by estimates of maximum photosynthetic potential obtained by plotting the double reciprocal transformations of the light response curves and extrapolating to zero.

Effects of Drought and Flooding on Photosynthesis

The soil of a two-year-old potted seedling was allowed to dry out over a period of 13 days while the photosynthetic rate at 17.8 mW cm⁻² was followed. The relative water content (RWC) of foliage, and the soil moisture content as determined with individually calibrated gypsum blocks, were estimated.

The rate of photosynthesis remained high for the first four days while the RWC of needles was stable at approximately 89%. However, on the fifth day when the RWC decreased to 85%, net photosynthesis decreased sharply to about 62% of its previous maximum and on the sixth day with a RWC of 83%, net photosynthesis was near the compensation level. For the remainder of the drought period RWC continued to decline steadily, reaching 39% on day 13 while net photosynthesis remained below compensation. After watering on day 13, RWC increased but net photosynthesis did not exceed compensation.

In another experiment, a similar seedling was subjected to three successive drying cycles of 4, 12, and 17 days respectively while again following net photosynthesis at 17.8 mW cm^{-2} , transpiration, needle RWC, and soil moisture.

During the severe second and third cycles of drought, soil moisture fell rapidly at first and then much more gradually suggesting an asymptotic approach to the permanent wilting point of the soil. RWC also declined during the droughts but lagged markedly behind soil moisture. On each occasion, transpiration decreased and net photosynthesis fell steeply to or below compensation level when RWC dropped below about 80%. With watering at the end of the second drought period, the RWC of foliage was quickly restored to about 88% and net photosynthesis and transpiration both reached 77% of the original rates. However, after the third drought, watering restored the RWC of leaves to about 85%, transpiration to 87% but net photosynthesis only to 61% of the original rate.

In an experiment designed to obtain information on the effect of flooding of the root system, a one-year-old potted seedling was placed in the growth cabinet. One week later, the terminal 20-cm section of the shoot was inserted in the assimilation chamber and the rates of net photosynthesis and transpiration were established. Twenty-four hours later, these measurements were repeated and the pot was then immersed in a container of tap water and was maintained thus for a period of six weeks. During the first 16 days of this treatment the flooding water was changed every fourth day; this was discontinued after 16 days and the water was allowed to stagnate. Anaerobic conditions were promoted by bubbling nitrogen gas through the water for one hour daily from the third to the sixth week. The flood bath was then removed and the pot allowed to drain. Normal watering was resumed for two weeks before terminating the experiment.

Rates of photosynthesis and transpiration were monitored continuously on the first day of flooding for seven hr after submergence and from 0900 to 1700 hr on the second and third days. On the fourth day, measurements were made continuously throughout the photoperiod from 0600 to 1800 hr and for the first half hour of the dark period. During the subsequent three weeks, brief measurements were made at 1200 hr at intervals of one to three days, and thereafter at intervals of one week. The experiment was then terminated, but the plant was kept under observation for three months by which time it was judged to have survived the treatment.

Immediately after flooding there was a brief increase in transpiration to about 106% of the pre-flood rate and a slight increase in net photosynthesis. Two hours after flooding, both processes were reduced to 65% of their initial rates; thereafter during the first day gradual recovery to about 75%-85% of the initial rates occurred. The

time course of net photosynthesis and transpiration observed on the fourth day of flooding is presented in Fig. 1c. The curves are rather similar to those for a normal plant (Fig. 1a) except that the decrease in both net photosynthesis and transpiration in the latter part of the day is more pronounced.

Regarded over a long term, the results were rather erratic, varying quite markedly from day to day, but on average both net photosynthesis and transpiration measured at noon were about 60%-70% of the pre-flood rate during the first five weeks of flooding. A general downward trend in both processes then set in and by the end of the sixth week both were only 10% of the pre-flood rate and they did not rise above this during the two weeks of post-flood observation.

Gas Exchange of a Decapitated Shoot

In one experiment the uppermost 12 cm of an eight-month-old seedling were removed, the cut stump covered with petroleum jelly and aluminium foil to prevent water loss, and the next lower 8 cm of the shoot were enclosed in the assimilation chamber. The foliage from a second and comparable seedling was removed from the uppermost 12 cm of the shoot except for the terminal rosette and apical bud. The bare stem was likewise coated with petroleum jelly and wrapped in foil to prevent water loss and the 8 cm section of the stem below this was enclosed in the assimilation chamber. For a control, the terminal 20 cm of a third untreated seedling were enclosed in the assimilation chamber.

Net photosynthesis was measured immediately after preparation of each seedling and again on the 4th, 8th, and 15th days. All treatment including the control showed some increase of photosynthetic rate over the 15 days of the experiment but the rates of the two treated shoots increased much more than the control.

In a subsequent experiment a 15-month-old seedling was decapitated retaining the youngest fully expanded foliage. A second comparable seedling was decapitated lower down, the foliage retained being more mature. The cut stems were coated with petroleum jelly and foil as before and the photosynthetic light response curves and transpiration of the first 10 fascicles below the cut were determined immediately and again after 17 days.

Immediately after severance, both shoots approached light saturation at 18.6 mW cm^{-2} , the one cut lower down the shoot having a rate of net photosynthesis 19% greater than the one cut higher up the stem. During the following 17 days, net photosynthesis for both shoots increased by about one third, but the relative difference between them remained. Transpiration showed a similar difference between the two shoots cut at different levels—24% greater for that cut lower down the shoot—but the rates did not rise with time after severance.

Gas Exchange of a Shoot Severed from its Root System

Approximately 20 cm of the apical part of the shoot of a 16-month-old potted seedling were enclosed in the assimilation chamber and after one hour the rates of net photosynthesis and transpiration were established at a light intensity of 18.5 mW cm^{-2} . The shoot was then cut under water below the assimilation chamber and the rates of net photosynthesis and transpiration were followed continuously for one hour and then at hourly intervals until the seventh hour. This entire procedure was repeated on four other similar seedlings on subsequent days.

The data have been calculated as a percentage of the rate before severance of the shoot, and the mean results of the five replicates are presented in Fig. 1d. After an initial rather abrupt surge transpiration increased considerably during the seven hours of observation: photosynthesis also increased but only to a moderate degree.

DISCUSSION

The diurnal photosynthetic and transpirational patterns revealed in Fig. 1a present no surprising features. In the initial dark period, carbon dioxide output amounted to *ca.* 9% of the maximum photosynthetic uptake, a proportion which is well within the normal range found in many plants. In the dark, transpiration amounted to about 6% of the maximum observed.

The initial rapid increase of transpiration and the onset of net photosynthetic gas exchange is interpreted as being due to stomatal opening in response to light. The slower drift upwards to the eventual maximum during the next $2\frac{1}{2}$ hr probably results from the change of environmental temperature imposed at the same time as the change from dark to light. The temperature settings of the controlled environment cabinet were arranged to provide a rise from 16° to 25°C at the time of turning on the lights, this temperature change being completed in the air space within 15 min. But the temperature of the root system in the pot increased much more slowly and it seems that a gradual increase in root and soil water temperature contributed to a gradually increasing availability of water to the plant (Kuiper, 1964; Cox and Boersma, 1967; Babalola *et al.*, 1968). Such an effect could result in a gradual widening of stomatal aperture and hence to gradually increased gas exchange.

There is no suggestion of a midday depression of gas exchange. Since the curves for net photosynthesis and transpiration remained parallel through the afternoon, it is unlikely that photosynthesis could have been affected by accumulation of its own end products. The steady and parallel decrease of net photosynthesis and transpiration may be due to gradual stomatal closure in response to increasing leaf water deficit. In the final phase of the measurements carbon dioxide exchange appears to have altered more rapidly than water vapour loss. Presumably this reflects the immediate cessation of the photochemical reactions of photosynthesis and the slower closure of the stomata during the early part of the dark period.

The diurnal pattern observed indicates some precautions should be taken in planning day-to-day experiments. No doubt it would be safest to restrict experimentation to the second half of the 12-hr photoperiod, but where this is not feasible, the choice of a standard starting time, at least one hour after placing the plant in the experimental environment, is probably satisfactory unless a substantial temperature change is involved. In such a case several hours should elapse before measurements begin.

The effects of temperature on the photosynthetic light response curves in Fig. 1b are complex. Light saturation at 11°C was achieved at about 12.6 mW cm^{-2} whilst at 17°C saturation was reached at about 18.5 mW cm^{-2} with the slope of the light-limited section unchanged. It seems that while the photochemical reactions were unaltered, either some other part of the photosynthetic complex of reactions was stimulated or the stomatal or mesophyll resistances decreased at 17°C.

Although the curves for 23°C and 29°C are incomplete, it is obvious that the slopes of the light-limited sections are still the same and that the saturating intensities would

be higher than that at 17°C; so the same interpretation holds. As the initial slope at 35°C is less, some damage to the photosynthetic apparatus affecting the photochemical reactions may have occurred at this temperature.

For the plants subjected to drought, the abrupt drop in net photosynthesis and transpiration when needle RWC fell below a critical value of about 80% can be attributed to stomatal closure. This accounts for the failure of RWC to decrease as soon and as steeply as soil moisture. Apart from these effects, there was a cumulative effect of drought in that after each drought cycle was ended with watering, net photosynthesis did not fully recover. This might also seem a stomatal effect since transpiration followed the same general trend but in the last recovery phase transpiration was large and photosynthesis small. Presumably repeated subjection to drought had produced an effect on photosynthesis such as that noted by Nir and Poljakoff-Mayber (1967) in depressed photochemical activity of chloroplasts.

Some of the observations made on the plant flooded for six weeks call for comment. The brief stimulation of about 6% of the rate of transpiration observed in the first half hour after flooding could reasonably be interpreted as a stomatal response. This might be a stomatal opening due to a temporary increase of water supply as suggested by Parker (1950) or a temporary hydropassive opening of the stomata caused by inequilibrium between the epidermal cells and the guard cells consequent on an abrupt reduction of the water supply. This would be analogous to the Iwanoff effect (Brittain and Nagarajah, 1971). The latter explanation seems more probable since the stimulation was soon followed by marked depression which would be expected on the re-establishment of equilibrium between guard and epidermal cells under conditions of reduced water supply.

The partial recovery from depression observed after five hr of flooding might occur for three reasons. Firstly, there could have been overshoot of the stomata in their closing response to leaf water deficit but, since the subsequent recovery from this is likely to have a time scale of the order of half an hour, this is an unlikely explanation. Secondly, there could have been a partial recovery from leaf-water deficit when stomatal closure reduced water loss, and a consequent partial stomatal reopening. This is different from recovery from overshoot and would tend to have a significantly longer time scale. Thirdly, a slowly rising root temperature consequent on raising the cabinet temperature could have been the cause. This temperature change seems responsible for the slow rise of photosynthesis observed between 0630 and 0900 hr (Fig. 1a). In the present case the temperature of the flooded soil would have continued to increase slowly until 1530 hr so that there is again a parallelism between rising soil temperature and rising photosynthetic rate. The recovery did not occur on days 2, 3, and 4.

The time course study performed on day 4 compares well with that done with a normal plant and discussed earlier, the decline from the maximum being somewhat steeper. This observation would be consistent with the suggestion that the plant in flooded conditions is in a state of reduced water supply.

The long-term effect of flooding, i.e., an irregular reduction in both net photosynthesis and transpiration, is presumably attributable to the cumulative effects of water stress and to anoxia causing a gradual deterioration of the root as a sink for photosynthate (Crawford, 1969). However, the flooded plant is not fully comparable with a plant

subjected to drought since on day 42, when its net photosynthesis and transpiration had fallen to less than 10% of the pre-flood rate, the RWC of the foliage was still 93%. Since both photosynthesis and transpiration were depressed to about the same extent one must infer this was caused by stomatal closure occurring despite an apparently favourable leaf water balance.

The experiment investigating the effects of severing apical parts of shoots was directed to practical ends related to accommodating material from specific sections of a shoot in the plant chamber. The result indicated little error is likely to be introduced by this treatment at least for several days after decapitation. Some release from self-shading and possibly partial relief of water deficit, both of which tend to increase the photosynthetic rate of the decapitated shoot relative to that of the intact one, might have been expected. Such effects were insignificant.

At the end of this experiment, some terminal growth was evident in both the defoliated and control material and would involve needle extension. Since foliage volume was measured at the end of the experiment, photosynthetic rates during its course would have been underestimated. This would tend to remove some of the upward trend with time seen in the control and in some of the experimental treatments. Nevertheless, a distinctly higher photosynthetic rate had developed in shoots 15 days after either decapitation or defoliation relative to the intact control which suggests a disturbance of a developmental process, cf. Sweet and Wareing (1966).

The second experiment, which dealt with decapitation effects, was unreplicated. Since evidence exists that photosynthetic activity may reach a maximum in two-year-old foliage (Wood, 1969), the difference in photosynthetic response between these two treatments immediately after decapitation could be due either to individual variation or to a difference in foliage age rather than to the treatment applied.

The main point at issue in investigating the effect of severing the shoot from the root system is to decide whether or not this is a technique which will permit one to obtain meaningful gas exchange estimates by detaching branches or parts of branches from trees and introducing them into the equipment. The result obtained, being the mean of five replicates, implies this might be possible in *radiata pine* for measurements of net photosynthesis, provided measurements are made at a standard time interval soon after severance, and a rather low level of accuracy is acceptable. The use of data on transpiration obtained from severed shoots is obviously unacceptable.

The rise in transpiration after severance of the shoot might have resulted from release from water deficit when the stem was cut under water, allowing the stomata to open more widely with concomitant increase in water loss. If this was so, stomatal resistance was presumably not limiting photosynthesis since photosynthesis did not rise in a parallel course to transpiration. Unfortunately, this hypothesis could not be tested since it was not possible to raise the light intensity when the steady trend of photosynthesis was established after the fifth hour of stem severance.

These experiments, in which potted seedlings were detached from their root systems while their net photosynthesis and transpiration were observed, suggest it might be possible to obtain useful data on the photosynthetic capacity of foliage of established trees by detaching branches under water and transporting them to the laboratory. While it would be dangerous to claim that data obtained in this way are representative of

in situ conditions, the trends observed tend to support the belief that photosynthetic rates so measured may at least be compared one with another and may be used to construct some general picture of the photosynthetic capacity within the crown. Such results are likely to be qualitatively correct even if quantitatively in error.

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REFERENCES

- BABALOLA, O., BOERSMA, L. and YOUNGBERG, C. T. 1968: Photosynthesis and transpiration of Monterey pine seedlings as a function of soil water suction and soil temperature. **Plant Physiology** 43: 515-21.
- BIERHUIZEN, J. F. and SLATYER, R. O. 1964: An apparatus for the continuous and simultaneous measurement of photosynthesis and transpiration under controlled environmental conditions. **Division of Land Research and Regional Survey, CSIRO, Australia, Technical Paper No. 24.**
- BRITTAI, E. G. and CAMERON, R. J. 1973. Photosynthesis of leaves of some **Eucalyptus** species. **New Zealand Journal of Botany** 11 (1).
- BRITTAI, E. G. and NAGARAJAH, S. 1971: Iwanoff effect in leaves of cotton (**Gossypium hirsutum**). **Physiologia Plantarum** 25: 441-7.
- CLARK, J. 1961: Photosynthesis and respiration in white spruce and balsam fir. **State University College of Forestry, Syracuse University, Technical Publication 85.**
- COX, L. M. and BOERSMA, L. 1967: Transpiration as a function of soil temperature and soil water stress. **Plant Physiology** 42: 550-6.
- CRAWFORD, R. M. M. 1969: The physiological basis of flooding tolerance. **Berichte der Deutschen Botanischen Gesellschaft** 82: 111-4.
- KUIPER, P. J. C. 1964: Water uptake of higher plants as affected by root temperature. **Mededelingen Landbouwhogeschool Wageningen** 64-4: 1-11.
- MORSE, R. N. and EVANS, L. T. 1962: Design and development of CERES—an Australian phytotron. **Journal of Agricultural Engineering Research** 7: 128-40.
- NIR, I. and POLJAKOFF-MAYBER, A. 1967: Effect of water stress on the photochemical activity of chloroplasts. **Nature** 213: 418-9.
- PARKER, J. 1950: The effects of flooding on the transpiration and survival of some southeastern forest tree species. **Plant Physiology** 25: 453-60.
- SWEET, G. B. and WAREING, P. F. 1966: Role of plant growth in regulating photosynthesis. **Nature** 210: 77-9.
- WOOD, G. B. 1969: Photosynthesis and growth in **Pinus radiata** D. Don as affected by environmental factors and inherent qualities. (Ph.D. Thesis lodged in the Australian National University library).
- 1971: A simple means of detaching tree shoots under water for physiological study. **Australian Forest Research** 5: 37-8.