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SHADE ADAPTATION OF THE HAWAIIAN TREE-FERN (CIBOTIUM GLAUCUM (Sm.) H. & A.)

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ABSTRACT

Shade adaptation of both gametophytes and sporophytes of a Hawaiian tree-fern, (Cibotium glaucum (Sm.) H. & A.) was measured by growing plants under a range of light intensities and at 2 daylengths, at 20 or 25°C.

Three ecophysiological parameters of shade adaptation and adjustment, initial slope of the photosynthesis curve in response to increasing light intensity (α), rate of light saturated photosynthesis (P_N max), and rate of photosynthesis at the intensity given during growth (P_N growth), were determined by infra-red gas analysis. Both sporophytes and gametophytes showed shade adaptation by a decline in α with increasing irradiation during growth and shade adjustment by a light saturation value for shade-grown plants that was well above the level of light at which the plants were grown. Sporophytes exhibited one feature of sun plants; P_N max increased with increasing irradiation during growth.

Morphological adaptations to low light intensity included a narrowing of the gametophyte, higher chlorophyll contents on a fresh weight basis of both gametophytes and sporophytes, and reduced thickness of sporophyte fronds. A greater total frond area of shade-grown sporophytes was brought about by a greater area of individual fronds and a longer retention time of fronds. Rates of frond production and expansion were little affected by light intensity.

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INTRODUCTION

Cibotium sp. (principally C. glaucum (Sm.). H. & A.) form a conspicuous understory in the Koa-Metrosideros dominated rain forest of the Kilauea Forest Reserve on the island of Hawaii. This forest, which has been studied intensively by several participants of the Island Ecosystems IRP of the U. S. International Biological Program, has been fully described in several Technical Reports, vegetation in No. 31 (Maka), climate in No. 22 (Bridges and Carey), and phenology in No. 24 (Lamoureux).

The present study was initiated as part of a study in the autecology of Cibotium. Major emphasis has been placed on defining the effect of the light environment on the rate of photosynthesis and growth of both gametophytes and sporophytes.

The ability to adapt to extreme shade conditions is an obvious advantage in the establishment of gametophytes and in enabling sporophytes to emerge above the level of other ferns and shrubs and to grow in the less restricted environment of the understory of the dominant trees, Acacia koa and Metrosideros collina.

In an experimental study of the nature of shade adaptation in Cibotium one can distinguish several possible strategies. Morphological adaptations for shading such as the growth of a high leaf area ratio (leaf area x total plant dry weight^{-1}), diversion of a high proportion of photosynthate into leaf growth (high percentage weight of leaf, leaf wt x plant dry wt^{-1}) and formation of a thin lamina (high ratio leaf area x leaf dry wt^{-1}) are common adaptations to shading, as shown for example in a series of studies on the environmental physiology of wheat, (Friend 1966, 1969, Friend, Helson and Fisher, 1962, 1965). This increase in leaf area may partly compensate for the lower rate of net photosynthesis at low light intensities. When examining physiological components of shade adaptation, such as the response of the net photosynthesis of the leaf to light intensity, one can distinguish between short term effects, such as the ability of a leaf to increase its rate of photosynthesis

at a certain rate in response to a given increase in light intensity until the saturating light intensity is reached, and long term effects integrating the morphological adaptations to previous growing conditions. Three distinctions have been formally summarized in a recent paper by Prioul and Bourdu (1973). They use these parameters to characterize photosynthetic adaptations and adjustment of plants to radiation. The first of these, the initial slope (α), measures the relationship between light intensity and net leaf photosynthesis close to the light compensation point. The second parameter measures the rate of light-saturated photosynthesis (P_N max). Both parameters vary according to the light intensity at which the plants have been grown and provide a measure of adaptation to shade during growth. A third parameter, the difference between the rate of light saturated photosynthesis and the rate of photosynthesis at the intensity at which the plants were grown (P_N max - P_N growth) provides a measure of the ability of the plant to respond to short-term changes in the light environment, and is termed "adjustment" by Prioul and Bourdu (1973). One major difference between the three shade species and one shade ecotype investigated by them was that the shade ecotype had a high value of P_N max even when grown at low light intensities, giving it a high adjustment ability (P_N max - P_N growth) when grown at low light intensities.

In the investigations reported here, the nature of shade adaptation in Cibotium has been examined at the morphological and eco-physiological level for both gametophytes and sporophytes.

MATERIAL AND METHODS

A. Gametophytes - growth

Spores were collected from fertile fronds of Cibotium glaucum growing in the Kilauea Forest Reserve, and stored at 3°C. To obtain an even sowing, spores were shaken through lens paper onto the surface of agar medium in plastic Petri dishes

9 cm diameter and 1 cm deep. The composition of the medium was:

NH_4NO_3	0.5 g
KH_2PO_4	0.2 g
$MgSO_4.7H_2O$	0.2 g
$CaCl_2$	0.1 g
Fe citrate	5 mg
Bacto agar	10 g
H_2O	to 1000 ml

Agar plates were placed at the desired light intensity and temperature and spore germination and growth of the gametophytes was followed by sampling 2 dishes for each condition at 2-weekly intervals. Ten prothalli were removed at random from each dish, mounted in glycerin jelly and examined with a projection microscope. The outline of the prothallus was traced from the ground-glass screen of the micro-projector and the width (dimension at right angles to the apical notch) of the prothallus was measured at its widest dimension.

Although fern spores were not surface-sterilized before sowing, fungal or algal contamination was not generally a problem. It was difficult to obtain an even sowing of spores and the number of prothalli per dish varied from about 50 to several hundred. In selecting prothalli for measurement, especially in the later stages of growth, preference was given to prothalli not touching a neighbor.

Cultures were grown at constant temperatures of either 20 or 25°C in controlled temperature rooms or cabinets. Temperatures inside the dishes were about 1 or 2°C higher than the stated air temperature at the highest light intensities in each series. White fluorescent light was used as the source of illumination, the photo period being maintained at either 12 or 24 hours. A range of intensities was obtained by varying the distance of the agar plates from the light source, and by shading plants by placing dishes in packets of white paper with the desired number

of layers. Measurement of the quanta received in the photosynthetically active part of the spectrum (photosynthetically active radiation, referred to as PhAR) was carried out using a quantum sensor (Lambda instruments, sensor LI-1905). Values of PhAR are given as quanta expressed in μ E $m^{-2}s^{-1}$ for the region of the spectrum at wave lengths between 400 and 700 nm. A Weston illumination meter was used as a secondary light measuring instrument when making periodic adjustments to the distance between the plants and the light source during growth and photosynthetic determinations. For white fluorescent light, 10 lux was equivalent to 0.206 μ E $m^{-2}s^{-1}$, and for incandescent light (used in photosynthetic measurements), 1 ft-c was equivalent to 0.328 μ E $m^{-2}s^{-1}$. Both light measuring instruments were cosine corrected.

B. Gametophytes - photosynthetic rates

Rates of photosynthesis were measured for prothalli grown for 16 weeks at a range of light intensities at a daylength of 12 hours and a temperature of 20 or 25°C. Agar plates with lids removed were placed within a transparent plastic photosynthesis chamber linked to a Beckman infra-red gas analyzer. The rate of air flow was maintained at 4 liters per minute. Further increases in the rate of air flow did not result in any further increase in the rate of photosynthesis. Temperatures were maintained at 20 or 25°C at the surface of the plants by immersing the photosynthesis chamber 4 cm under the surface of a controlled temperature water bath. A range of light intensities was obtained by raising or lowering a 500 W internal reflector flood lamp (Sylvania Cool-Ray) above the chamber. The reflecting surface of these lamps allows the transmission of infra-red radiation through the back of the lamp, reducing heating effects in the light beam. Photosynthetic rates were determined for two replicate agar plates of gametophytes from each growing condition. Each determination took between 5 and 8 hours. The order in which dishes were sampled was randomized over a three week period. The general procedure for each photosynthetic measurement was as follows.

Plants were introduced into the photosynthetic chamber, the light intensity was held at PhAR 66 μ E $m^{-2}s^{-1}$ and the system was then checked for leaks. Outside air was then flushed through the system for about 30 minutes, the air entering the photosynthesis chamber being humidified by bubbling through water in a flask held in the water bath. Previous experiments had established that the photosynthetic rate of prothalli remained constant for periods up to 24 hours after being maintained at this intensity for about 30 minutes. The light intensity was then set at the desired level, not exceeding 200 μ E $m^{-2}s^{-1}$ to avoid photo-damage to the prothalli, and the air flow system was closed. Rates of photosynthesis were calculated by measuring the amount of CO_2 removed from the system over a 10 to 20 minute period. Readings were begun when the rate of photosynthesis was constant, which usually occurred within 1 to 3 minutes of changing the light conditions. The CO_2 concentration was not allowed to fall below 250 ppm, as at this concentration the rate of photosynthesis began to decline because of CO_2 depletion. For most runs, the CO_2 concentration was maintained above 300 ppm.

After measuring the rate of photosynthesis at one light intensity, the chamber was flushed with outside air to restore the CO_2 content to about 320 ppm. For ease of operation light intensities were not selected at random. Instead, the light intensities were first increased in steps from about 70 to 300 μ E $m^{-2}s^{-1}$ then reduced in steps to darkness, then increased again up to the highest intensities used. The effects of from 10 to 20 intensities were examined for each plate.

The infra-red gas analyzer was calibrated by substituting 0.5 ml of 0.01 M barium hydroxide solution into the humidifying vessel in the closed system. CO_2 uptake was hastened by rapid shaking of the flask, and completed within 5 minutes. As the volume of the closed system remained constant, within the small limits of barometric variation, the rate of photosynthesis expressed as reduction in CO_2 concentration in the system could be calibrated in absolute terms of mg CO_2 removed.

Recalibration was carried out at two-weekly intervals, three replicate calibrations were made on each occasion.

Because of the highly convoluted surface of prothalli it was difficult to measure the total area of the prothalli on an agar plate. Photosynthetic rates were expressed on the basis of prothallial fresh weight, dry weight, and chlorophyll content. Prothalli were carefully scraped from the agar surface and the fresh weight was measured. The chlorophyll content was determined by grinding the prothalli in absolute acetone, filtering, and making the solution up to volume. The chlorophyll content in an aliquot of the extract was measured by reading the absorption at 600 nm in a Spectronic 20 spectrophotometer. Ether extracts of chlorophyll were used to standardize the Spectronic 20 readings, using a Beckman spectrophotometer to measure the absorption at 660 and 530 nm (Horowitz, 1970). Chlorophyll extracts and plant debris were transferred to an aluminum dish and evaporated to dryness at air temperature. Dry weights of the prothalli were determined from these extracts after oven drying for 24 hours at 80°C.

C. Sporophytes - growth

Small sporophyte plants, with fronds less than 1 meter in length and stems about 10 to 15 cm in height were collected in the Kilauea Forest Reserve and transferred to a greenhouse. Plants were placed in a mixture of 50% shredded hapuu (weathered tree fern stem) and 50% peat, in 10 cm diameter plastic pots. Plants were placed under mist spray in a glass house at a temperature of about 30°C day and 20-25°C night. Plastic shading was used to reduce the light intensity to about 50% daylight. After one month under these conditions, when new fronds had started to emerge, 40 plants were randomized between 4 different light environments. The light intensity varied from unshaded (about 80% daylight) 50% of unshaded, 25%, to 10% of unshaded. Mist spray was maintained during the day at 5 minute intervals. The following

records were taken over a 1 year period:

(a) Rate of frond emergence
(b) Rate of frond death
(c) Number of fronds retained
(d) Rate of frond expansion

D. Sporophytes - photosynthetic rates

The effect of light intensity on the rate of photosynthesis of a newly expanded frond was determined for three replicate plants from each shading treatment. The procedure was the same as that described for the measurement of photosynthetic rates of gametophytes, except that detached pieces of frond were used instead of agar plates of gametophytes. The basal pinnule of a newly expanded frond was placed in water immediately after detachment and then placed in the photosynthesis chamber with the petiole in water and the lamina horizontal.

It was assumed that detaching the pinnule had no adverse effect on the rate of photosynthesis as the photosynthetic rate of detached pinnule remained constant in preliminary experiments, even after 24 hours. In subsequent photosynthetic determinations no pinnule was kept in the chamber for more than 8 hours. Pinnule area was measured with a grid of 4 mm^2 holes cut in a pattern of 1 hole for every 16 mm^2. Holes that were not fully filled when placed over the lamina were recorded as half full. The total area of the lamina was estimated as 16 mm^2 (number of full holes plus half the number of half-filled holes). Fresh weights were determined, and the lamina was ground and extracted in acetone for the determination of total chlorophyll content as before. Dry weights were obtained from oven drying the residues and evaporated extracts. Rates of photosynthesis were expressed on an area, fresh or dry weight, or unit chlorophyll basis.

RESULTS AND DISCUSSION

A. Gametophytes - growth

The upper light intensity limit for growth of prothalli was between 200 and 800 μE $m^{-2}s^{-1}$ at a daylength of 12 hours and a temperature of 20°C (FIG. 1). The rate of growth in width was close to linear at all light intensities and was similar at intensities of 103 and 227 μE $m^{-2}s^{-1}$. At 7 μE $m^{-2}s^{-1}$ the rate of growth was only about one half of that 21 μE $m^{-2}s^{-1}$. The shape of prothalli varied from broadly cordate at high intensities to narrowly cordate at low (FIG. 2A, B).

When gametophytes were grown at 20°C under a 24 hour daylength there was again little difference in growth between intensities 165 to 82 μE $m^{-2}s^{-1}$ but a reduction in growth at the later dates at 21 μE $m^{-2}s^{-1}$. The rate of growth under the 24 hour daylength was about three times that under the 12 hour daylength (TABLE 1). Prothalli grown under a 24 hour daylength were more lobed and folded than those grown under a 12 hour daylength (FIG. 2).

The effect of temperature as well as light intensity was investigated in a a second series of experiments at a daylength of 12 hours. The rates of growth of the prothallus are not strictly comparable to those given in FIG. 1, because the experiments were done at a different time, with spores from a different frond. At 20°C, as before, the rate of increase in breadth was greatest at intensities close to 100 μE $m^{-2}s^{-1}$ (FIG. 3). At 25°C the optimal light intensity was lower, about 50 μE $m^{-2}s^{-1}$ and an intensity of 165 μE $m^{-2}s^{-1}$ reduced the rate of growth to less than 50% of the maximal. An intensity as low as 2.1 μE $m^{-2}s^{-1}$ was still above the compensation point at both temperatures.

The shape of the prothalli was broadly cordate at high intensities, becoming progressively more narrowly cordate at lower intensities (FIG. 4A, B) as was also evident in the experiments recorded in FIG. 2A and 2B. This effect of light intensity

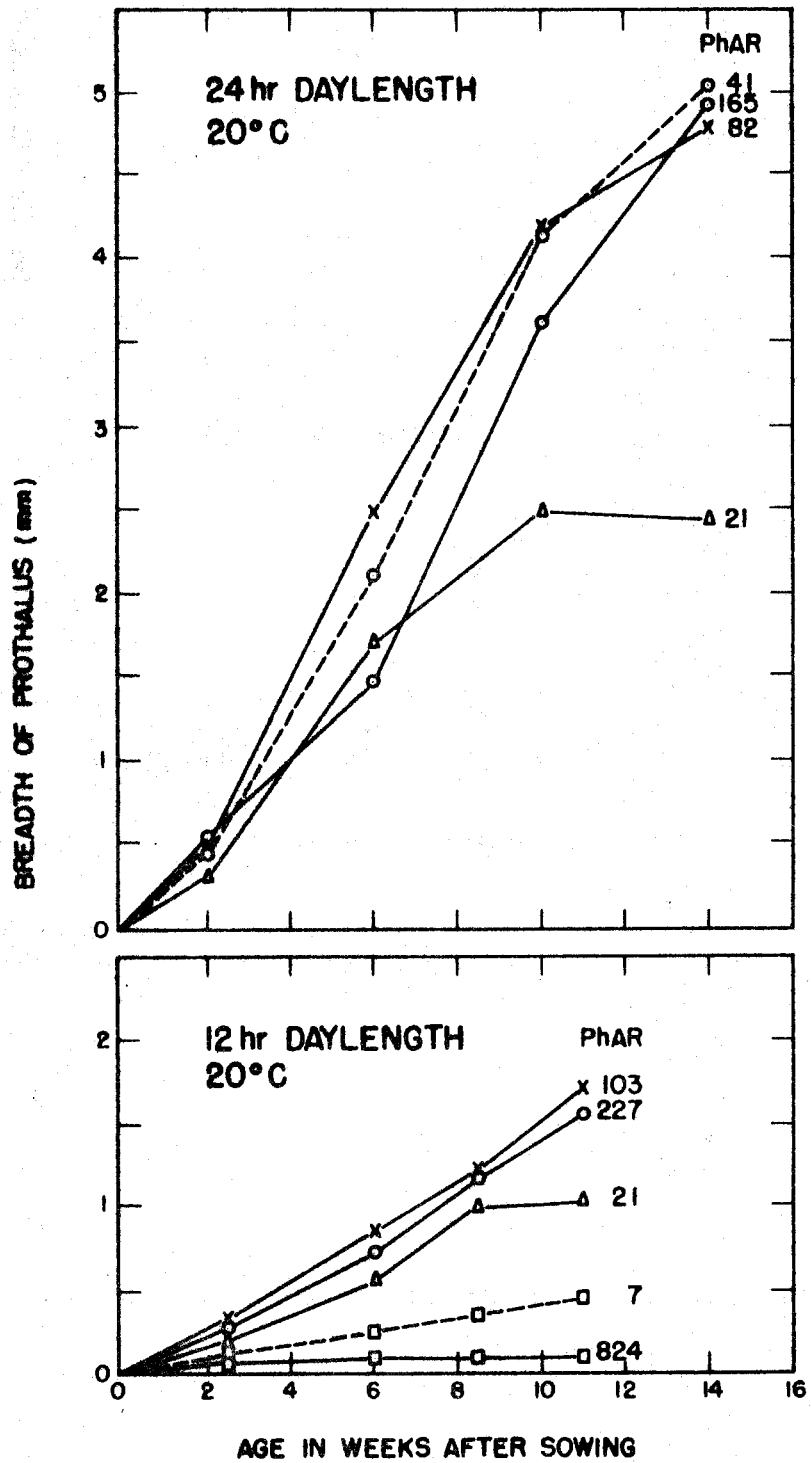
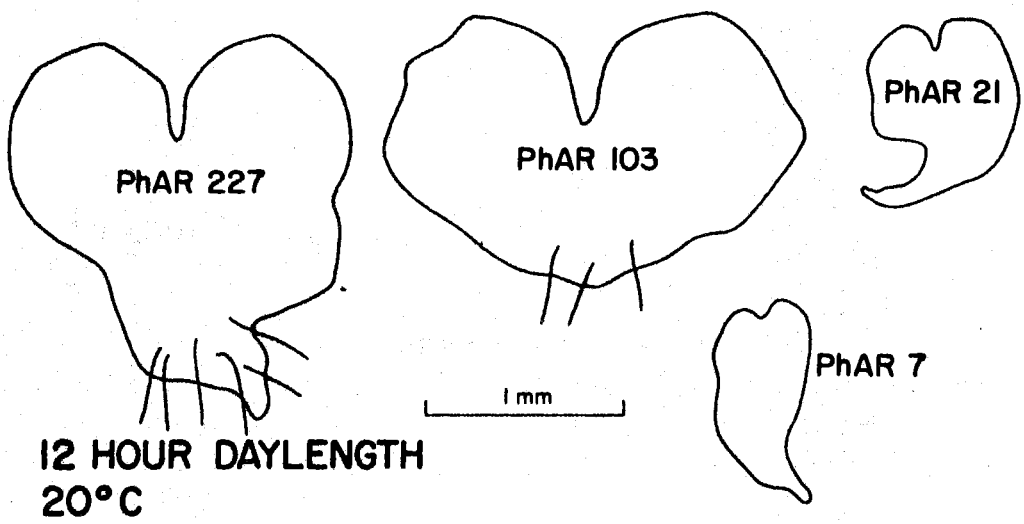
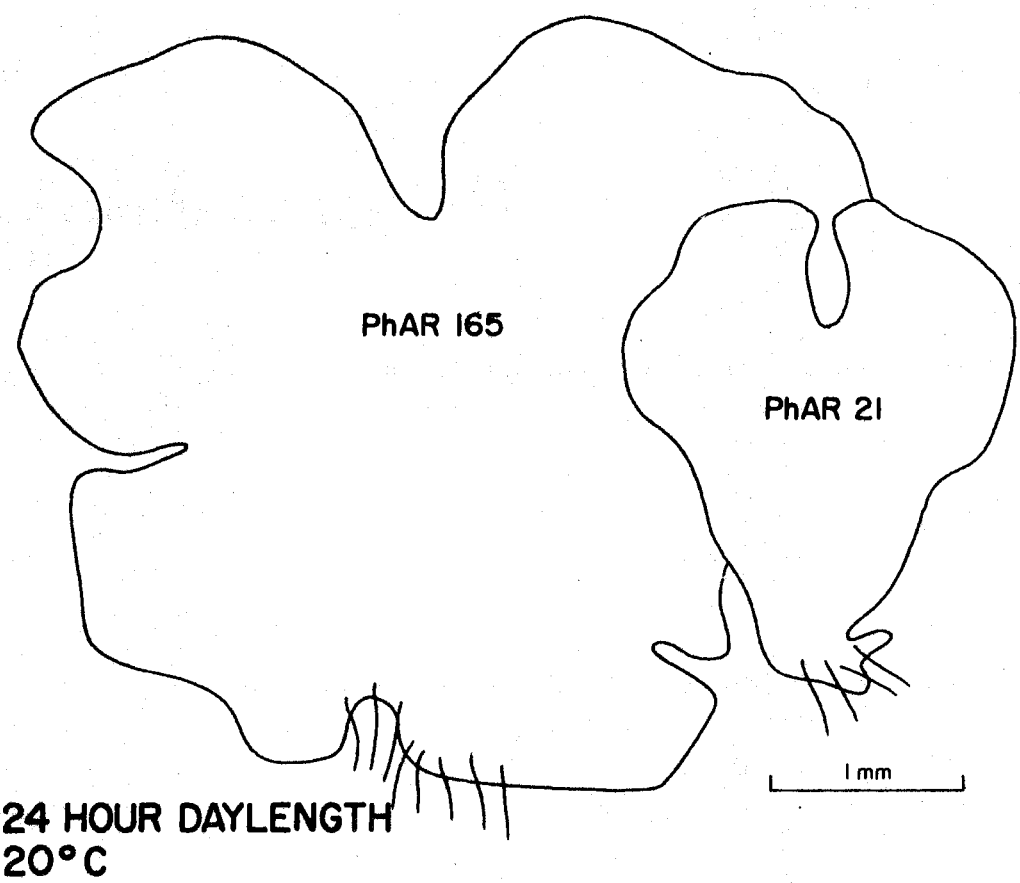


FIG. 1. Breadth increase of prothalli grown at different light intensities under 24 or 12 hour daylengths at 20°C. PhAR = photosynthetically active radiation, E $m^{-2}s^{-1}$.



2A 12 HOUR DAYLENGTH
20°C



2 B 24 HOUR DAYLENGTH
20°C

FIG. 2. Shape of prothalli grown at different light intensities under 12 and 24 hour daylengths at 20°C. PhAR in $\mu E\ m^{-2}s^{-1}$.

TABLE 1. Effect of daylength on mean* rates of growth (width in $mm \cdot wk^{-1}$) of prothalli grown at 20°C.

PhAR $\mu E\ m^{-2}\ s^{-1}$	24 hr daylength	PhAR $\mu E\ m^{-2}\ s^{-1}$	12 hr daylength
41	0.84 $mm \cdot wk^{-1}$	103	0.29 $mm \cdot wk^{-1}$
82	0.91 $mm \cdot wk^{-1}$	227	0.21 $mm \cdot wk^{-1}$
165	0.81 $mm \cdot wk^{-1}$		

* For 24 hr series, mean rates over wks 4-8, 8-12, 12-16 weeks.

For 12 hr series, mean rates over wks 5-7, 7-10, 10-12 weeks.

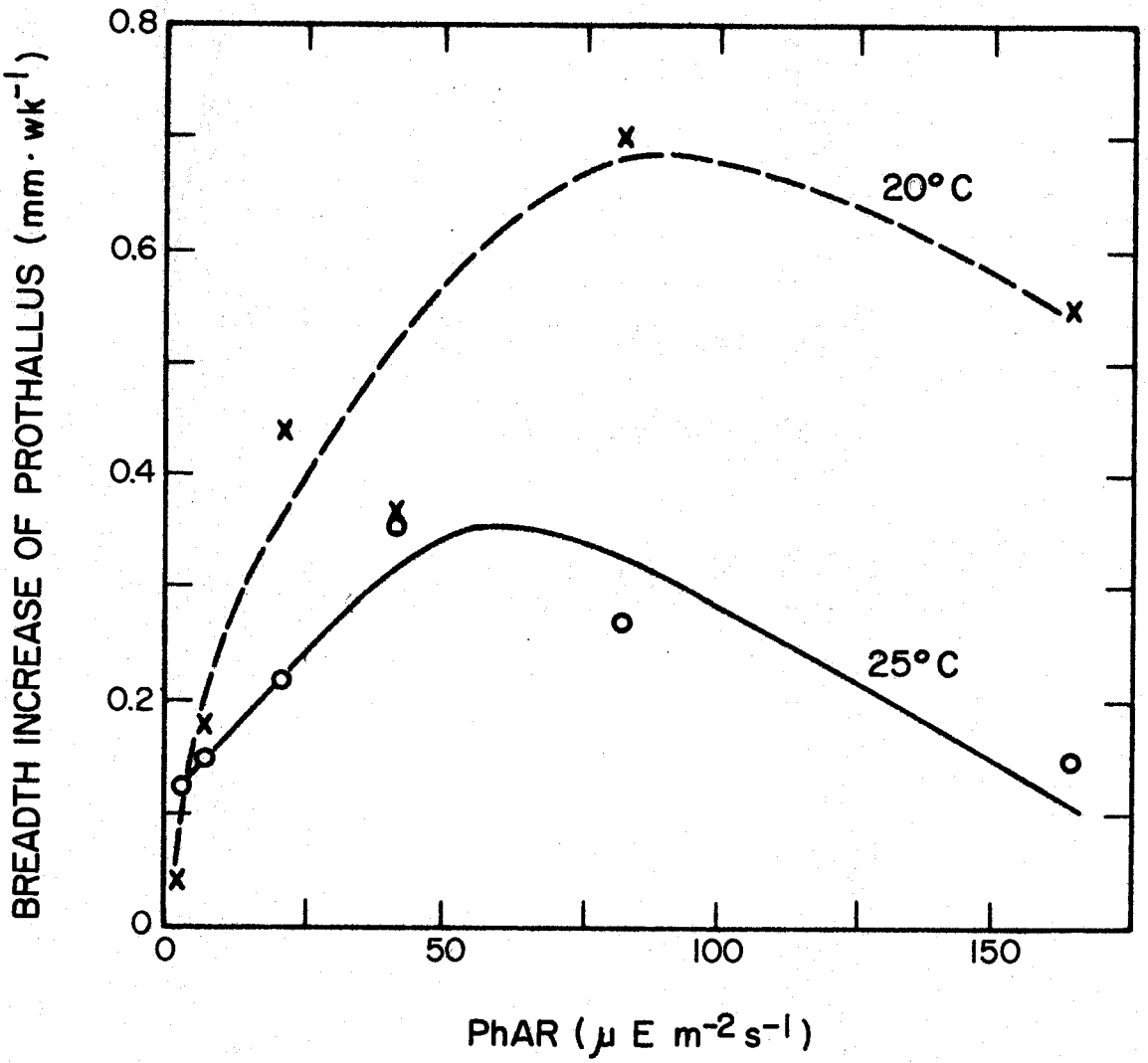
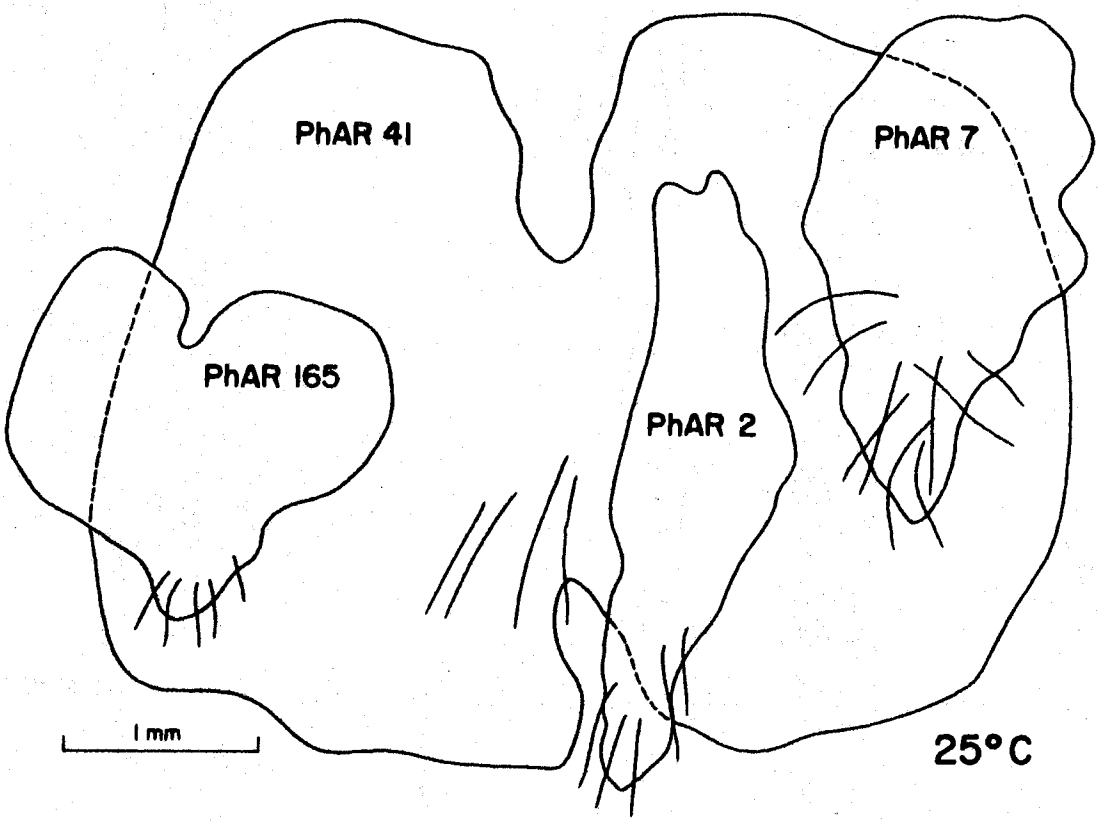
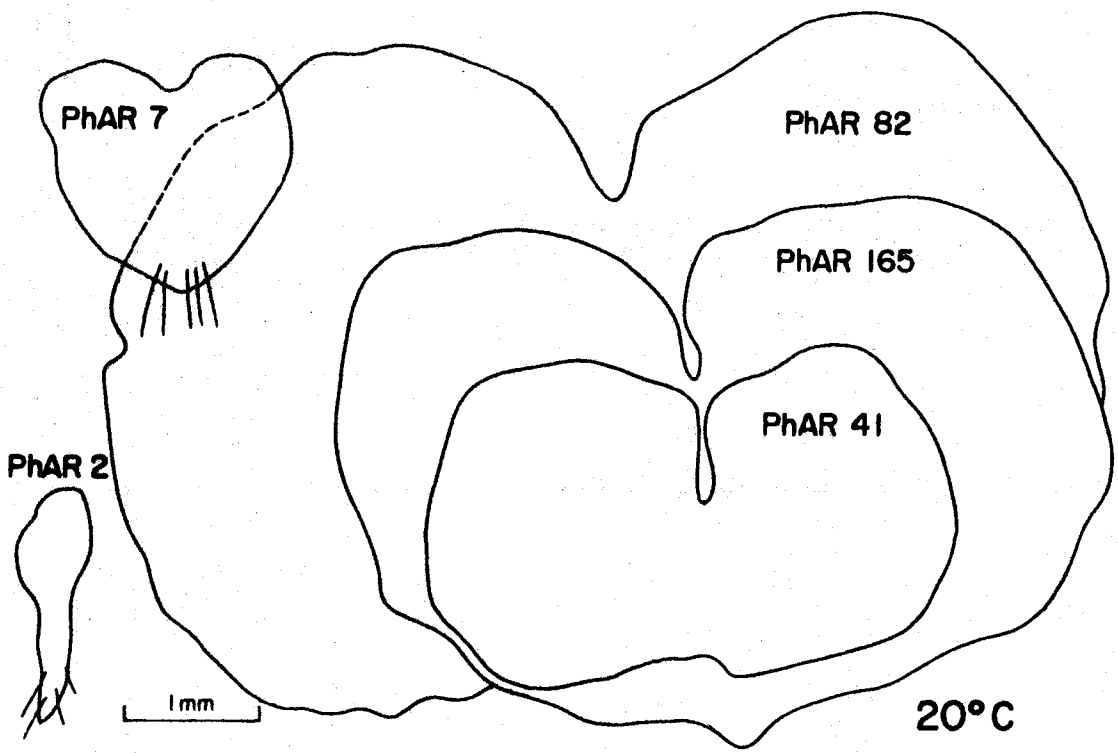


FIG. 3. Rate of increase in breadth of prothalli grown at different light intensities at 20 or 25°C under a 12 hour daylength.



4A



4B

FIG. 4. Shape of prothalli grown at different light intensities at 25 (A) or 20°C (B) under a 12 hour daylength. PhAR in $\mu E\ m^{-2}s^{-1}$.

on prothallial shape was not caused by ontogenetic changes in prothallial shape associated with slower cell divisions at low light intensities. The length of prothalli grown at low light intensities was greater than that of prothalli of younger age but the same width grown at high intensities (FIG. 5).

The lower light limit for growth of prothalli was determined in a further set of experiments by growing plants either in darkness or at intensities ranging from .4 to 2.1 μE $m^{-2}s^{-1}$ at temperatures of 20 and 25°C under a 12 hour daylength. Germination was first stimulated by exposure to fluorescent light for 24 hours, at an intensity of 21 μE $m^{-2}s^{-1}$. Plants were examined after 3 months growth. Spores kept in darkness had germinated but died. In all other treatments, even at 0.41 μE $m^{-2}s^{-1}$ spores had germinated and made some prothallial development. At the lowest intensity prothalli consisted simply of a file of three to six cells. At 1.0 and 1.6 μE $m^{-2}s^{-1}$ an apical cell was differentiated to make a prothallus three or four cells wide, and at higher intensities prothalli were narrowly cordate as previously illustrated (2 μE $m^{-2}s^{-1}$ prothallus in FIG. 4B). There was no significant difference in growth of prothalli at 20 or 25°C (FIG. 6). The reduced length of prothalli between 1 and 1.5 μE $m^{-2}s^{-1}$ was related to the development of the cordate shape (FIG. 7).

From these results, growth of the prothallus (measured as increase in breadth, FIGS. 1 to 4) was greatest at values of PhAR of about 100 μE $m^{-2}s^{-1}$ at 20°C. This corresponds to the PhAR value of about 3% sunlight. The mean annual average temperature under the canopy in the Kilauea rain forest is about 12°C (Bridges and Carey, Tech. Rept. No. 22, Suppl. 1). As there is evidence for an inverse relationship between the optimal PhAR for growth and temperature (FIG. 3) it is possible that under field conditions the optimal PhAR would be somewhat higher than that obtained at 20°C.

The damaging effects of high levels of PhAR (824 μE $m^{-2}s^{-1}$ in FIG. 1) were

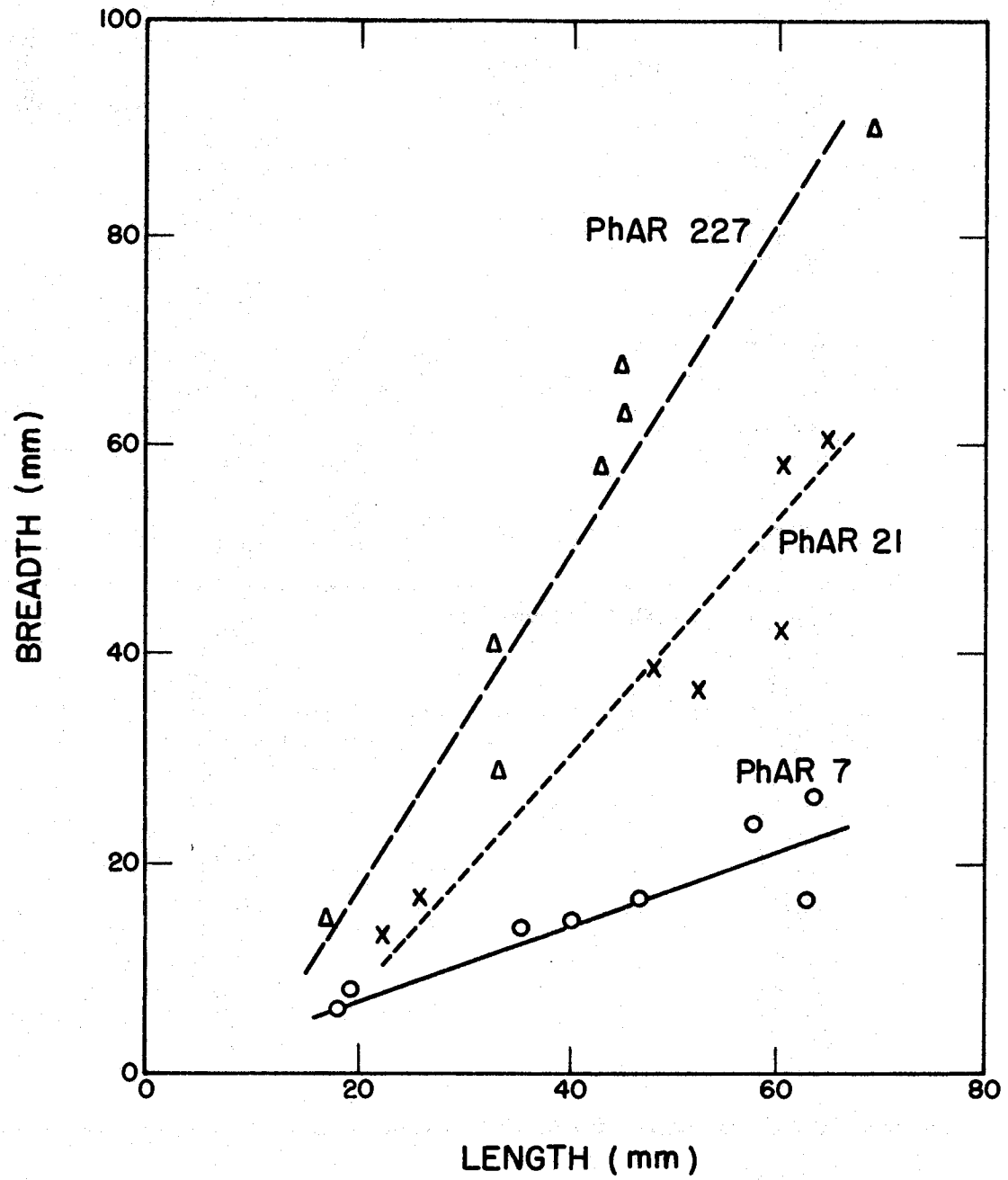


FIG. 5. Relationship between length and breadth of prothalli grown at different light intensities at 20°C under a 12 hour daylength. PhAR in $\mu E\ m^{-2}s^{-1}$.

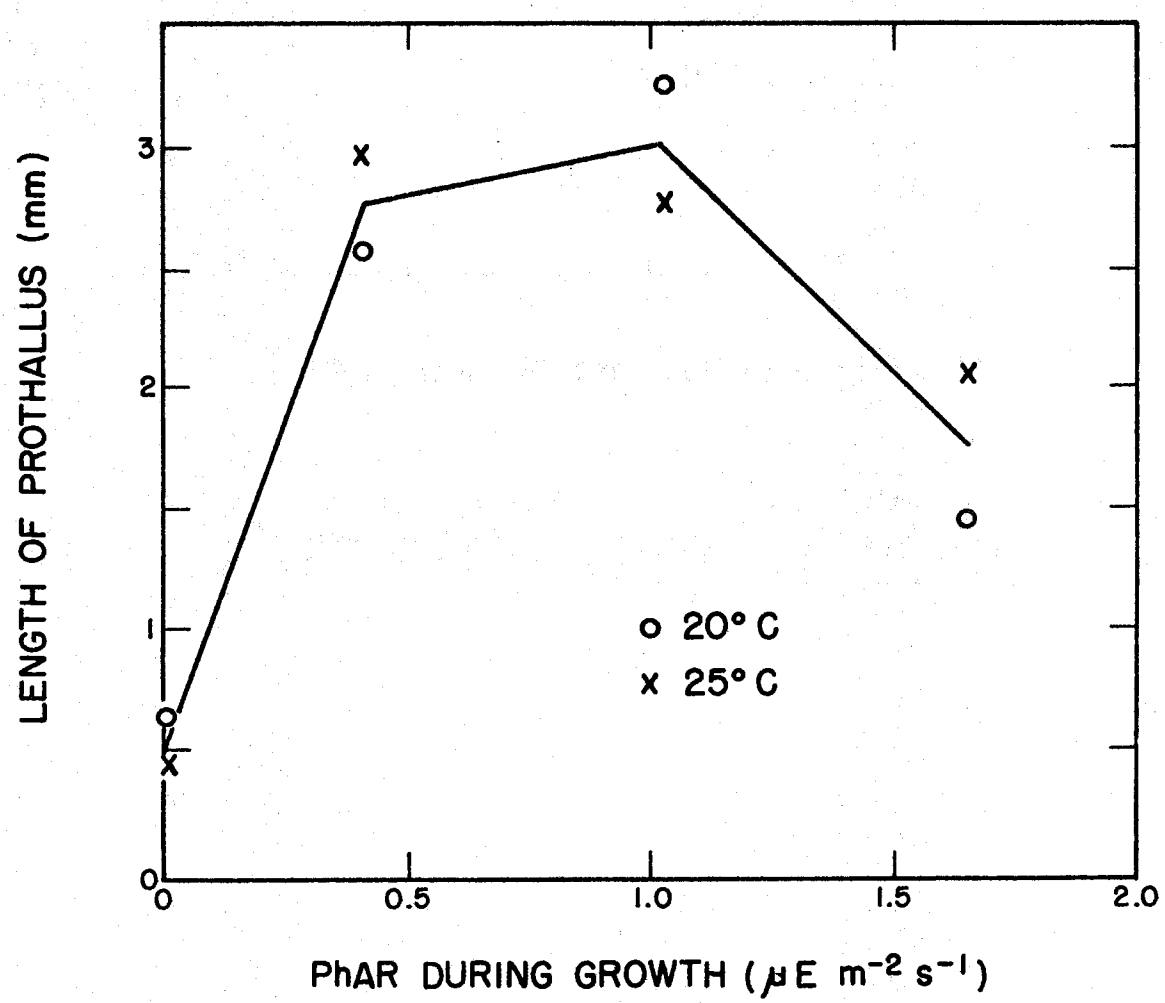


FIG. 6. Length of prothalli grown at intensities of PhAR near the light compensation point. Plants measured 3 months after spore germination. The daylength was 12 hours; each point is a mean from 32 prothalli.

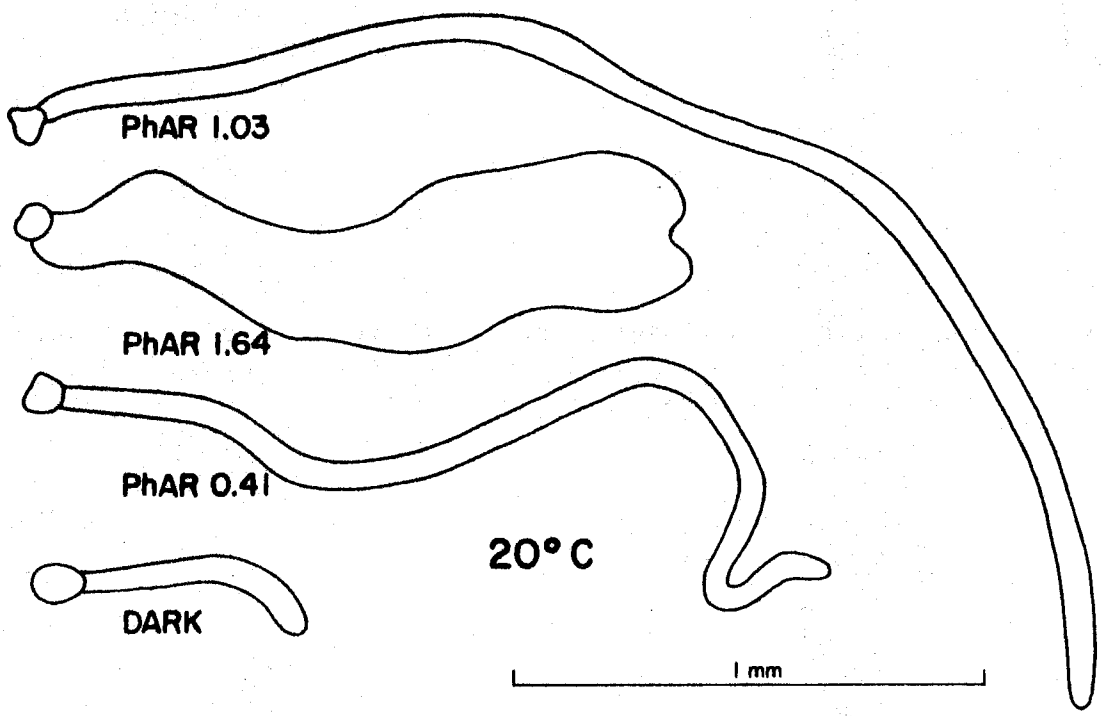


FIG. 7. Shape of prothalli grown at intensities of PhAR near the light compensation point. Plants 3 months old, grown under a 12 hour daylength at 20°C. Prothalli represented were close to the mean lengths.

probably caused by photobleaching of photosynthetic pigments. In the field, this level of PhAR (equivalent to about the PhAR of 25% sunlight) would also have a strong desiccating effect.

Survival and slow growth of prothalli was possible at extremely low values of PhAR of about 4 μ E $m^{-2}s^{-1}$; this lower light limit to growth would be exceeded in almost all places in the natural habitat, other than directly beneath completely opaque objects. Although survival of prothalli is possible at these low intensities, development of the cordate prothallus apparently necessary for gamete production needed a higher intensity, in the region of 1.6 μ E $m^{-2}s^{-1}$ (FIG. 7).

The reproductive biology of the prothalli was not followed in detail in these experiments but no gametangia were present in cultures maintained for 18 months at intensities of 1.6 μ E $m^{-2}s^{-1}$ or lower.

B. Gametophytes - photosynthetic rates

The sets of curves relating the rate of CO_2 uptake to light intensity were of the usual shape, with a close to linear increase in the rate of uptake with increasing light intensity from the light compensation point to about two thirds of the saturating light intensity. Two representative curves are presented in FIG. 8.

The curves relating the effect of the light intensity during growth to the rate of CO_2 uptake at 66 μ E $m^{-2}s^{-1}$ were similar whether expressed on a fresh or dry weight or unit chlorophyll basis (FIG. 9).

As the rate of CO_2 uptake was least variable between replicates when expressed on a dry weight basis, this was used in the further presentation of results.

The light compensation point and the saturating light intensity were estimated by interpolation from a set of curves similar to those in FIG. 8. The rates of CO_2 uptake given in FIG. 8, and the rates of CO_2 uptake at the saturating light intensity were corrected by the addition of the rate of CO_2 emission in darkness.

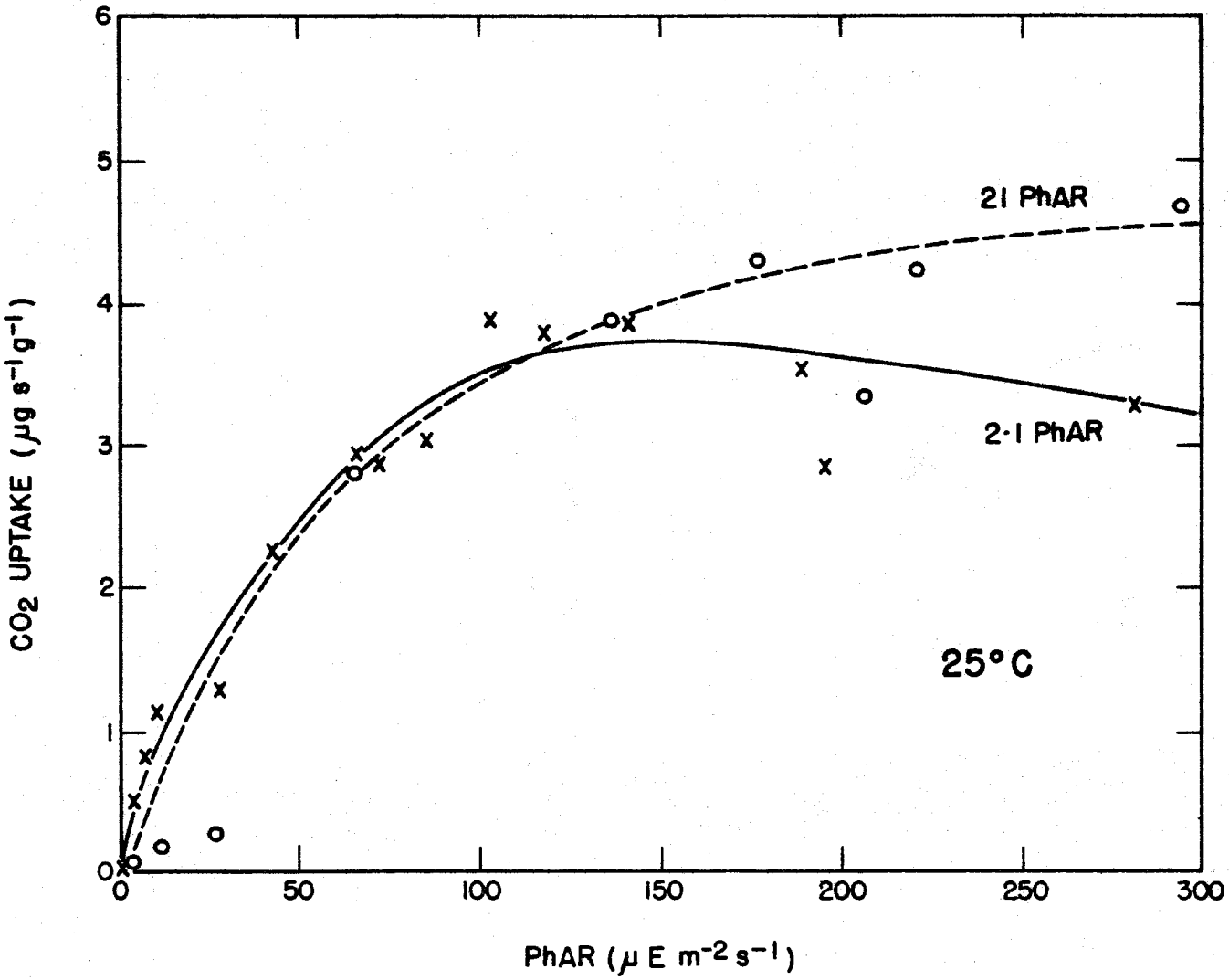


FIG. 8. Effect of light intensity on CO_2 uptake of prothalli grown at PhAR of 21 and 2.1 $\mu E\ m^{-2}s^{-1}$ under a 12 hour daylength. CO_2 uptake corrected for dark respiration, as in subsequent figures.

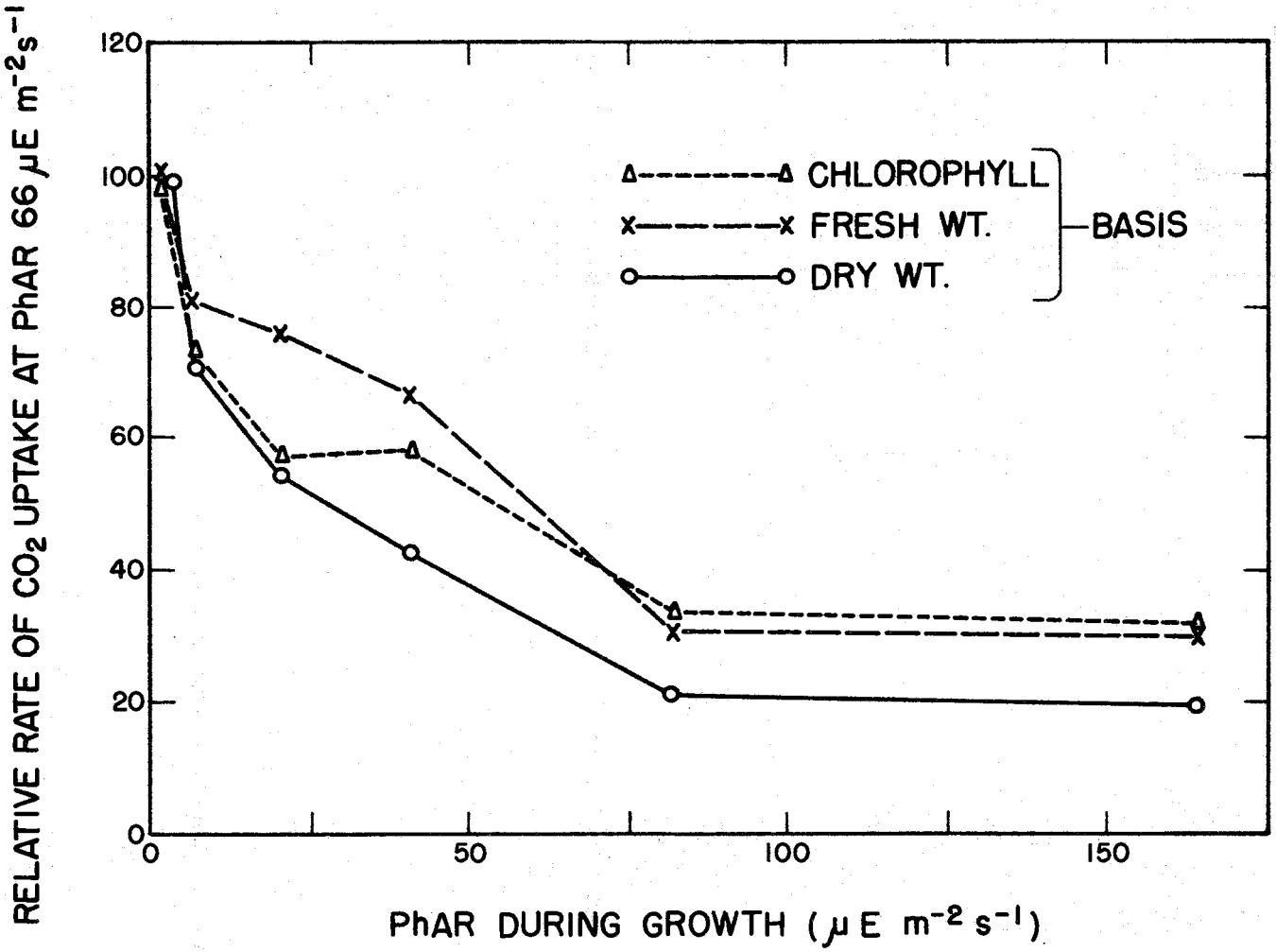


FIG. 9. Relative rate of CO_2 uptake at PhAR of 66 μE $m^{-2}s^{-1}$ for prothalli grown at different light intensities at 20°C under a 12 hour daylength. CO_2 uptake expressed on unit chlorophyll, fresh and dry weight basis.

Elevation of the measured rate of respiration of prothalli caused by the respiration of microbial contaminants was thereby avoided. The rate of dark respiration of prothalli was below the limits of measurement in several cases, where microbial respiration was presumably missing. No attempt was made to measure the rate of photorespiration.

The saturating light intensity increased the higher the intensity at which plants were grown over the range of 2 to 42 μ E $m^{-2}s^{-1}$ at both 20 and 25°C (FIG. 10). At 20°C the saturating light intensity continued to increase for plants grown at intensities up to 165 μ E $m^{-2}s^{-1}$.

With these short-term measurements, the rate of CO_2 uptake at 20°C was actually highest at over 900 μ E $m^{-2}s^{-1}$, an intensity resulting in photobleaching of prothalli exposed continuously to this radiation.

The light compensation point was highest for plants grown at 165 μ E $m^{-2}s^{-1}$ in the 20°C series, and progressively decreased with plants grown at lower intensities (FIG. 10). For plants grown at 25°C the results were more variable.

The optimal rate of CO_2 uptake at light saturation was for plants grown at about 21 μ E $m^{-2}s^{-1}$ at both 20 and 25°C. Further increases in light intensity resulted in a lower rate of CO_2 uptake (FIG. 11). Rates of CO_2 uptake were higher for plants grown at 25°C than for plants grown at 20°C.

Chlorophyll concentration in general was lowest at the higher light intensities at which plants were grown (FIG. 12). When measured on a dry weight basis, chlorophyll concentration at 20°C was consistently higher than at 25°C. When measured on a fresh weight basis, the concentration was higher at 25°C than at 20°C at all except one light intensity.

Prothalli are adapted to grow at low light intensities, as shown by the low light compensation points, below 12 μ E $m^{-2}s^{-1}$ even for plants grown at PhAR levels near the optimal (FIG. 10). The lower the intensity of PhAR at which plants were

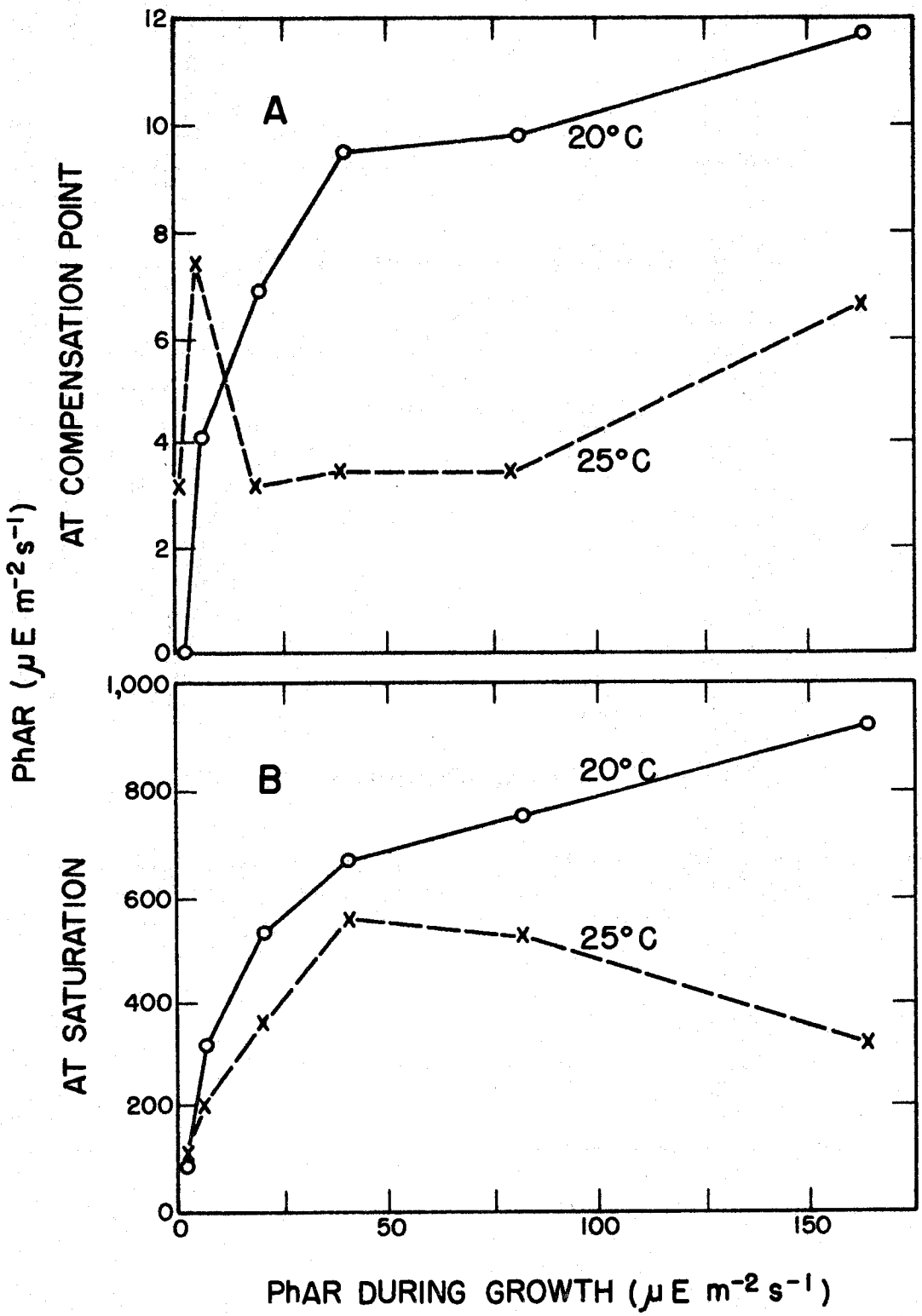


FIG. 10. Light compensation point (A) and saturating light intensity (B) for CO_2 uptake of prothalli grown at different light intensities at 20 or 25°C under a 12 hour daylength.

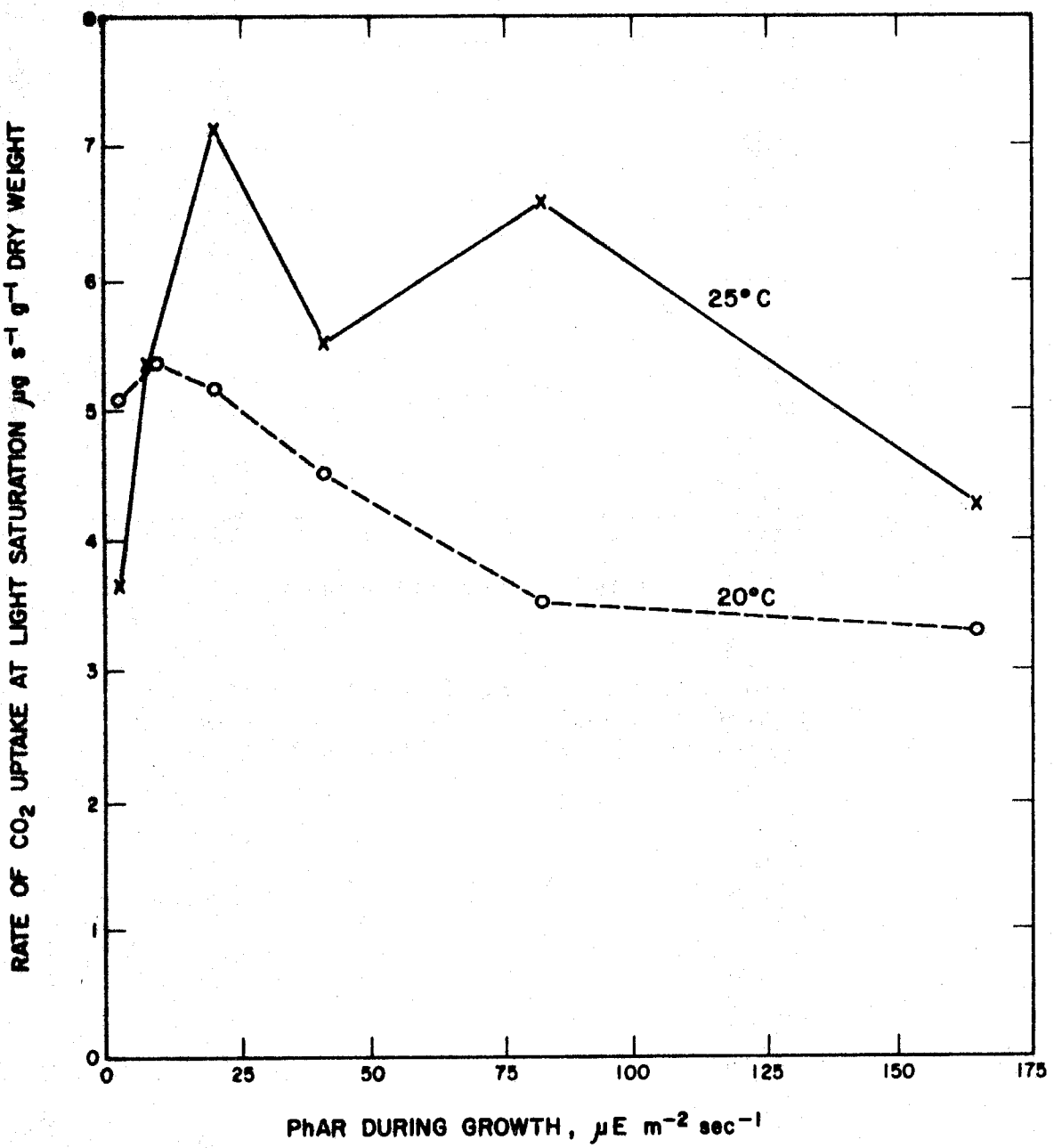


FIG. 11. Rate of CO_2 uptake at light saturation of photosynthesis of prothalli grown at different light intensities at 20 or 25°C under a 12 hour daylength. CO_2 uptake expressed on unit dry weight basis.

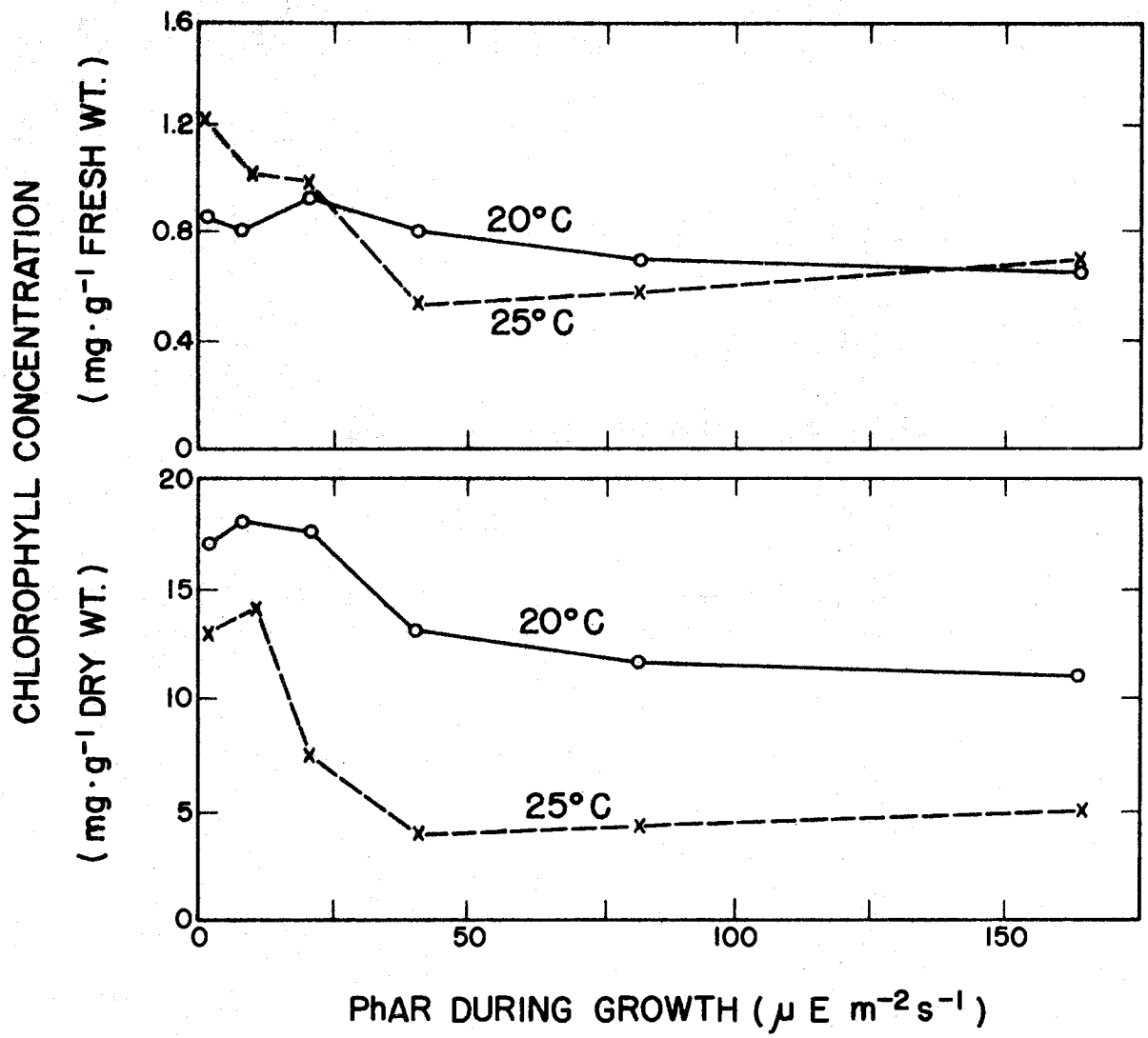


FIG. 12. Chlorophyll concentration on fresh and dry weight basis, of prothalli grown at different temperatures and light intensities under a 12 hour daylength.

grown, the lower was the light compensation point. This lowering of the light compensation point was correlated with an increase in the chlorophyll content per unit fresh or dry weight (FIG. 12).

A further measure of the physiological plasticity of the prothalli is provided by the relationship between the rate of CO_2 uptake at 66 μE $m^{-2}s^{-1}$ and the PhAR during growth (FIG. 9). Part of this apparent adaptability of the photosynthetic rate of the prothallus to low light conditions is probably caused by ontogenic declines in the rate of photosynthesis with increasing size of prothalli, as shown in other species.

The saturating light intensity for all prothalli was higher than the intensity at which they had been grown (FIG. 10) but these curves were obtained during short-term measurements and do not reflect the continued behavior of plants maintained at these high intensities. Although at 20°C the rate of CO_2 uptake at light saturation was greatest for plants grown at PhAR 8 (FIG. 11) the optimal intensity for long periods of growth was about PhAR 100 (FIG. 1).

C. Sporophytes - growth

The rate of frond emergence was little affected by shading, the average interval being about 38 days (FIG. 13). The rate of frond expansion was similarly little affected by shading. The number of fronds retained by the plant increased with increasing shading, and varied from 3.6 in full daylight to 5.0 in 12.5% daylight. This increase in the number of fronds retained at the lower light intensities was caused by the increased longevity of individual fronds. The average age of the first frond to emerge was 24, 27 and 27 weeks for plants grown under 100, 50, 25 and 12.5% daylight respectively.

Plants grown under 100% greenhouse daylight were yellow-green in color, with stiff, leathery fronds, in marked contrast to the deep green, pliable fronds grown

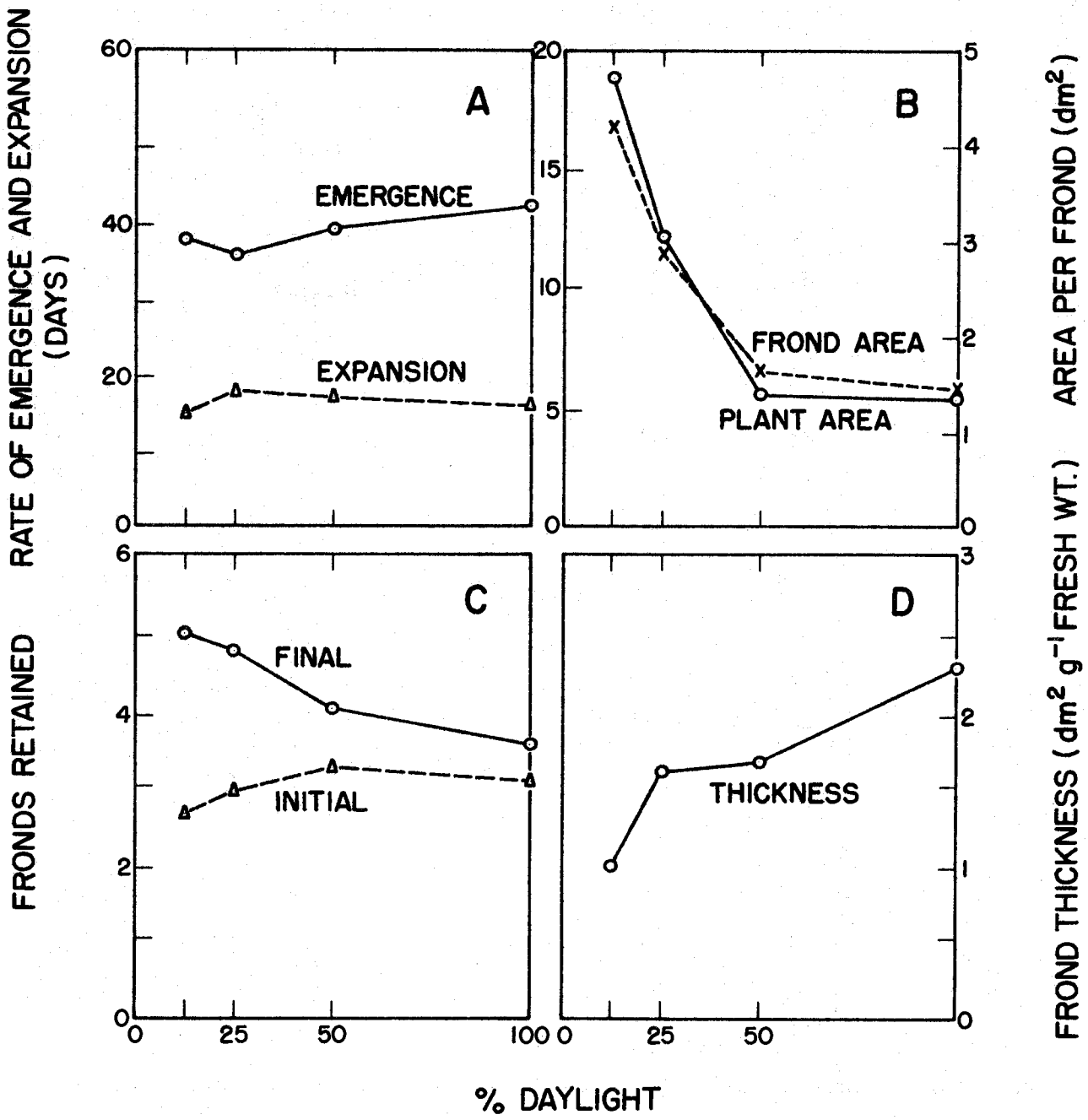


FIG. 13. Rate of frond emergence and expansion (A), frond area and plant area (B), number of fronds retained (C), and frond thickness (D) after six months growth of sporophyte at different light intensities under natural daylengths in a greenhouse.

at the lower intensities. The chlorophyll content decreased with increasing light intensity, when expressed on a fresh or dry weight basis (FIG. 14). The trend was similar, but more variable, when expressed on a unit area basis. Leaf thickness, expressed as fresh weight per unit area of lamina, decreased with decreasing light intensity (FIG. 16).

Adaptations of the sporophyte frond to shading were mainly an increase in the area of individual fronds (not measured in this experiment) and the formation of thinner lamina. Increased retention of fronds under shaded conditions would also increase the leaf area ratios. Severe photobleaching in full sunlight obviously restricted growth in the greenhouse, and from observation, in nature.

D. Sporophytes - photosynthetic rates

Sets of curves relating the rate of CO_2 uptake to light intensity were constructed in a similar manner as was done for the gametophytes. Two representative curves are presented in FIG. 15. The effect of light intensity during growth on the rate of photosynthesis was examined by comparing photosynthetic rates at a light intensity of 66 $\mu E\ m^{-2} s^{-1}$. At this intensity, the rate of photosynthesis was linearly related to light intensity for plants in 100% daylight and in deep shade. The rate of CO_2 uptake measured at 66 $\mu E\ m^{-2} s^{-1}$ increased with decreasing daylight, the relationship being close to linear when CO_2 uptake was measured on a fresh or dry weight or unit area basis (FIG. 16). When measured per unit chlorophyll, the rate of CO_2 uptake at 66 $\mu E\ m^{-2} s^{-1}$ was greatest under 100% daylight, and was lower, with similar values, at 50 to 12.5% daylight. Both the saturating light intensity and the light compensation point were positively and linearly related to the percentage of daylight at which plants were grown (FIG. 17).

The rates of CO_2 uptake at light saturation were obtained by interpolation in sets of curves similar to those presented in FIG. 17. When measured on a unit

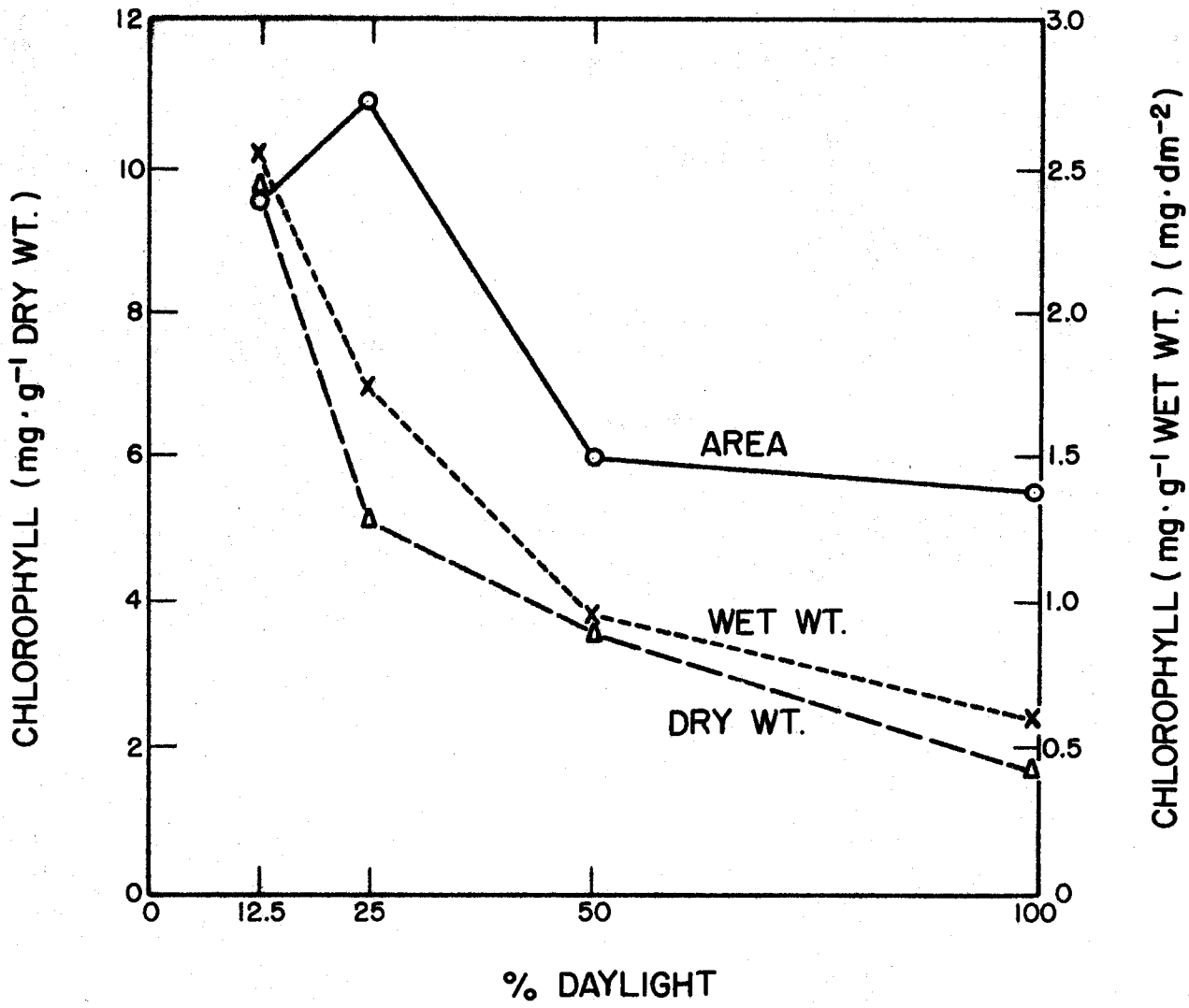


FIG. 14. Chlorophyll concentration of fully expanded sporophyte fronds grown at different light intensities under natural daylengths in a greenhouse. Chlorophyll concentration expressed on fresh and dry weight and unit area basis.

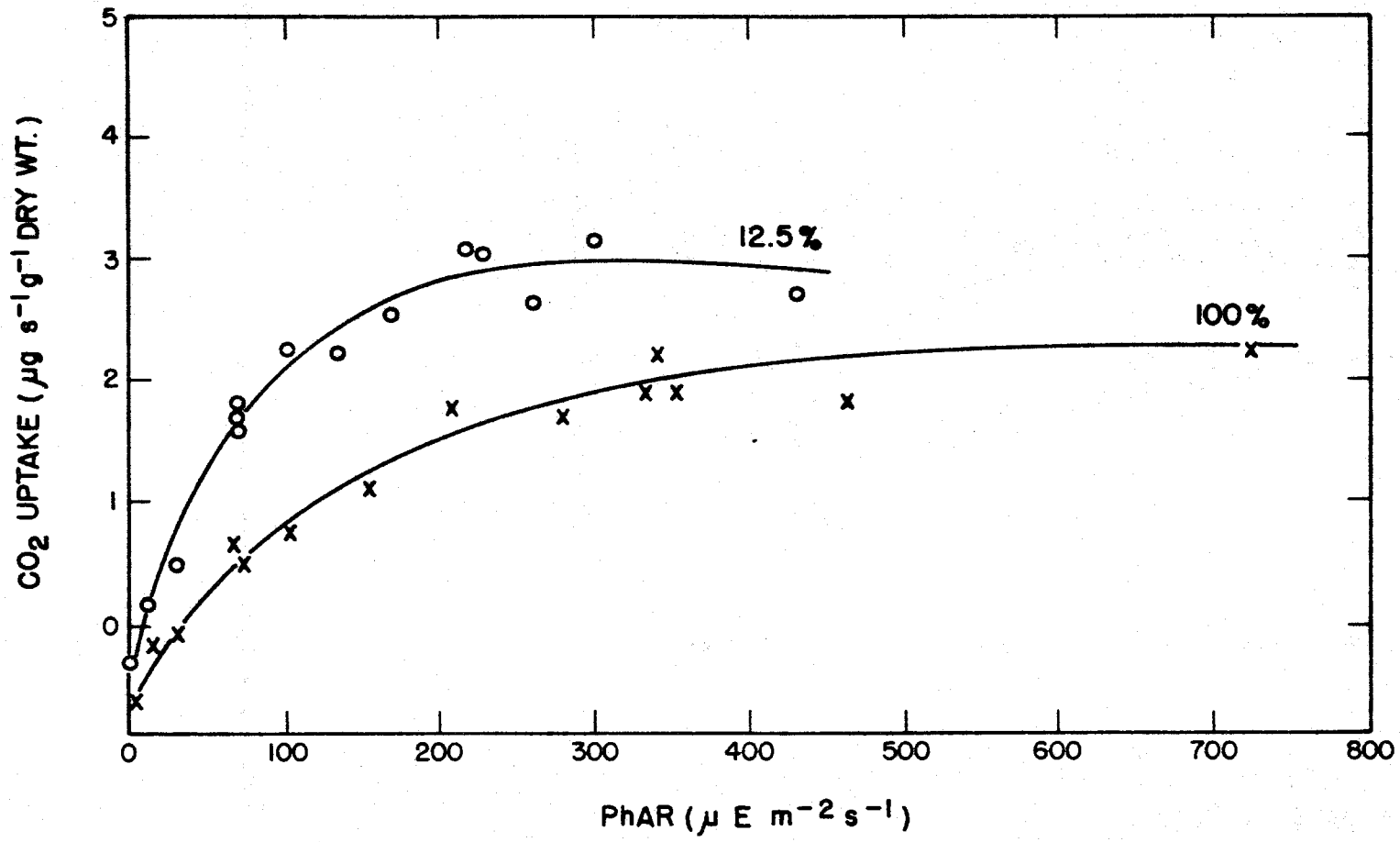


FIG. 15. Effect of light intensity on CO_2 uptake of sporophyte fronds grown at 100 or 12.5% daylight. CO_2 uptake corrected for dark respiration and in subsequent figures.

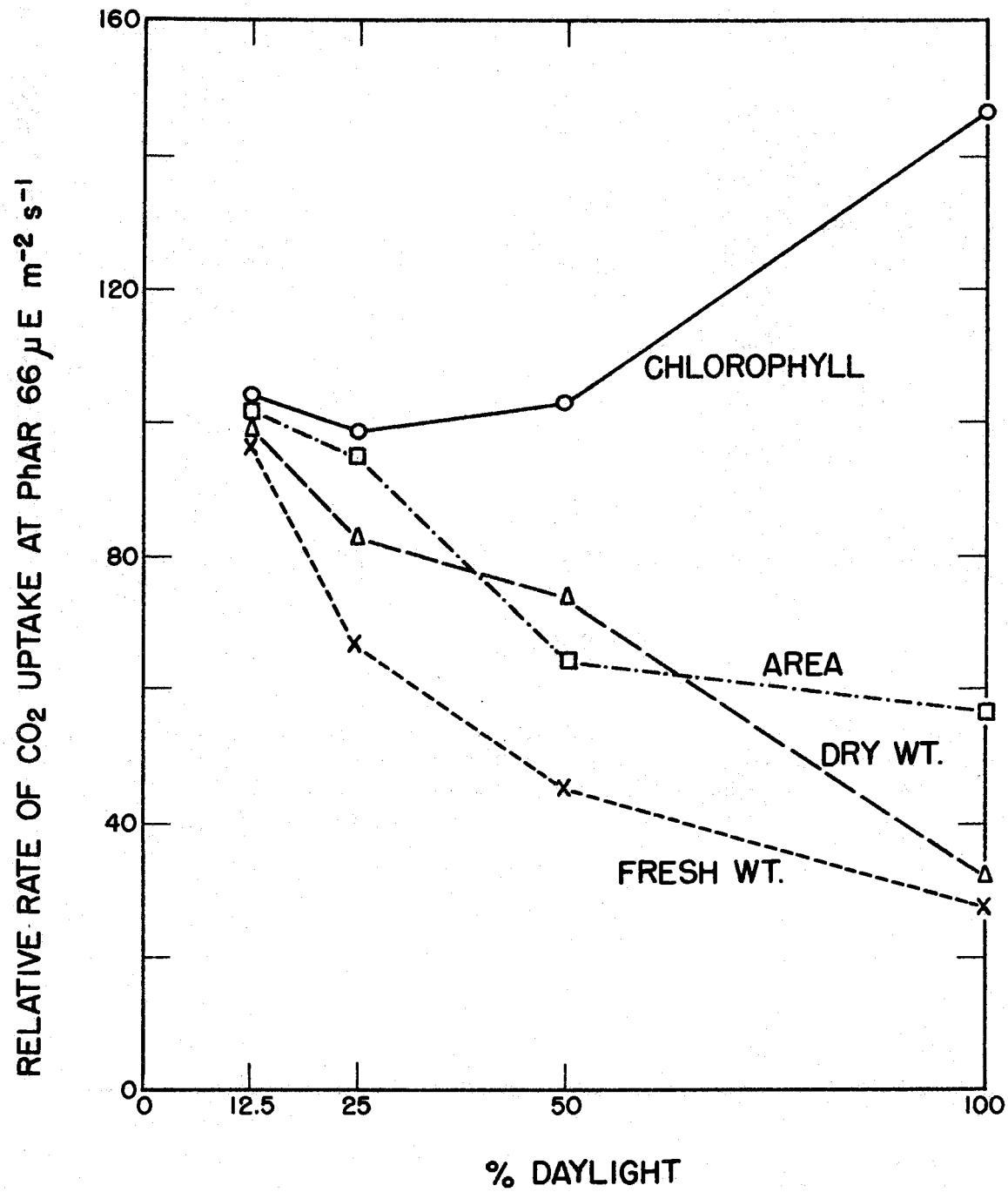


FIG. 16. Relative rate of CO_2 uptake at PhAR of 66 μE $m^{-2}s^{-1}$ for sporophyte fronds grown at different light intensities under natural daylengths in a greenhouse. CO_2 uptake expressed on unit chlorophyll, area, dry and fresh weight basis.

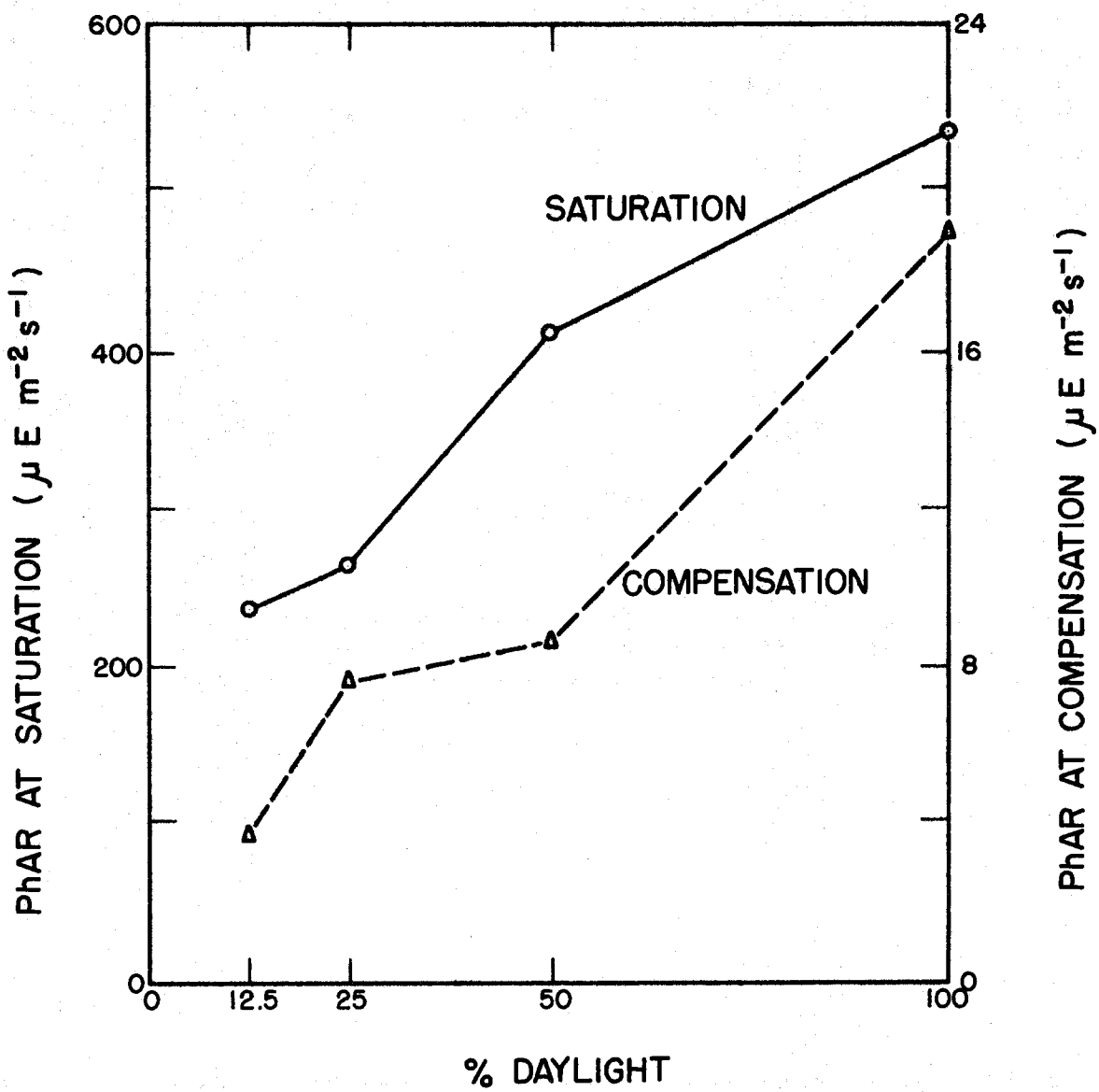


FIG. 17. Saturating light intensity for CO_2 uptake and light compensation point for sporophyte fronds grown at different light intensities under natural daylengths in a greenhouse.

chlorophyll basis, CO_2 uptake at saturation was highest for plants grown with the highest light intensity (FIG. 18). When measured on a unit fresh or dry weight basis, CO_2 uptake at light saturation showed a general decrease the higher the light intensity at which plants had been grown. When measured on a unit area basis, there was less photosynthesis at light saturation for plants grown in the deepest shade.

As with the gametophyte, adaptation to shade is shown by the low light compensation point of shade-grown plants (FIG. 17) accompanied by a high chlorophyll concentration (FIG. 14). Like the gametophyte, both the rate of CO_2 uptake (on a fresh or dry weight basis) at light saturation (FIG. 18) and the CO_2 uptake at PhAR 66 μ E $m^{-2}s^{-1}$ (FIG. 17) were greatest for shade-grown plants. In the case of the sporophyte results are uncomplicated by ontogenic changes in photosynthetic rates, as measurements of photosynthesis were restricted to recently expanded fronds.

GENERAL DISCUSSION

The distribution of tree ferns is strongly related to the light environment of the habitat. Gametophytes will not grow in full sunlight even if desiccation is prevented, because of photobleaching of the photosynthetic pigments. This bleaching takes place at an intensity of 824 μ E $m^{-2}s^{-1}$, about half the PhAR in full sunlight (FIG. 1). A similar bleaching of gametophytes of several ferns was reported by Hill (1971). The lower light limit for survival of the prothallus is so low (about 0.4 μ E $m^{-2}s^{-1}$ PhAR, about 0.03% full sunlight) that there can be few microhabitats within the rain forest in which prothalli cannot become established, as long as sufficient moisture is available. About four times this intensity is needed for development of the cordate prothallus, and probably an even higher intensity for the formation of gametes and the development of the sporophyte. These limits to reproduction have not yet been fully determined for Cibotium, but the lower limit over a

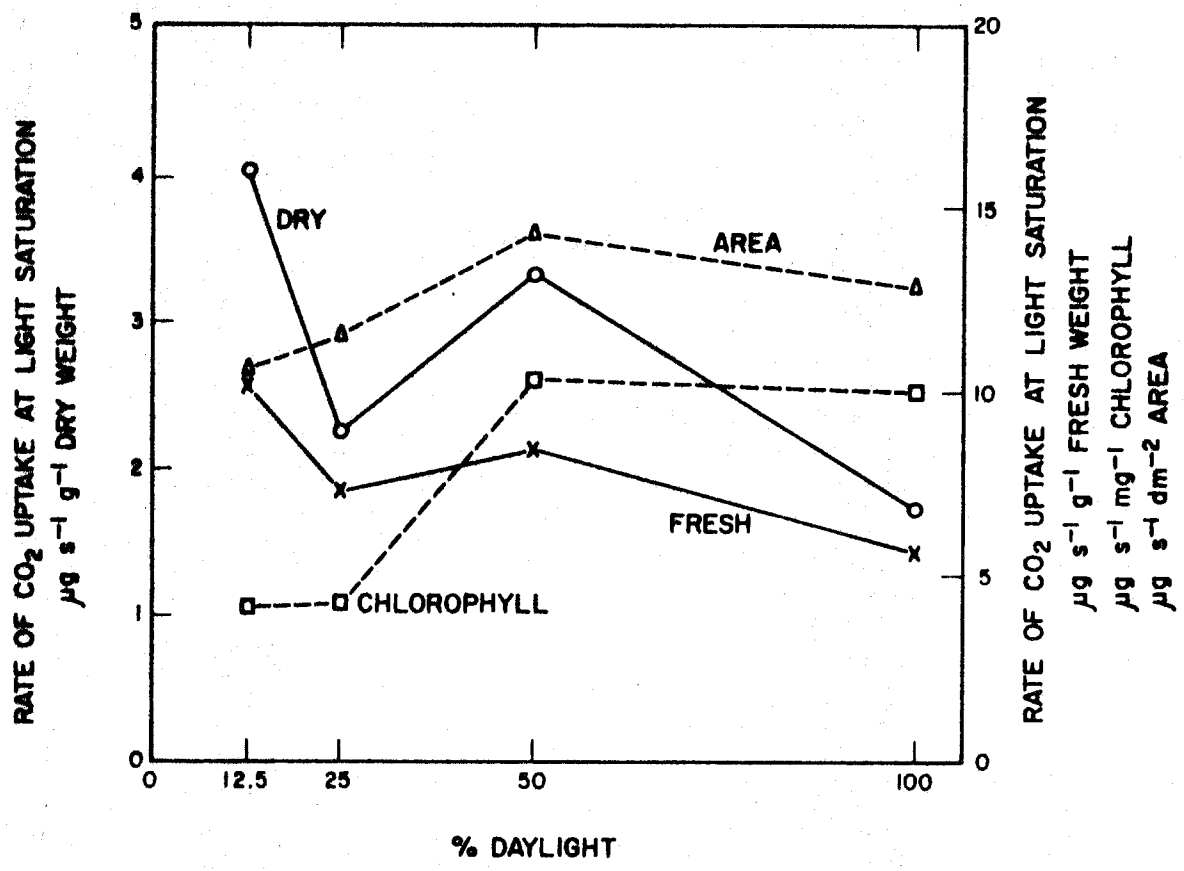


FIG. 18. Rate of CO_2 uptake at light saturation of photosynthesis of sporophyte fronds grown at different light intensities under natural daylengths in a greenhouse. CO_2 uptake expressed on unit chlorophyll, area, dry and fresh weight basis.

one year period exceeded PhAR 1.6 μE $m^{-2}s^{-1}$.

The young sporophyte (stem about 10 cm) can survive in full sunlight at natural daylengths although the reduced chlorophyll content of the lamina, presumably caused by photobleaching, slows down growth. Measurements on growth rate of sporophytes were limited to measurements of rates of frond emergence, expansion and retention. In full sunlight the faster death of fronds shortened their retention time. Visual observations suggest that the leaf area ratio was greatest in the deepest shade used in this experiment (12% daylight). The reduced leaf area ratio in full daylight was caused by a smaller area of individual fronds as well as the more rapid senescence and loss of fronds.

The lowest light intensity at which sporophytes can survive was not determined in these experiments, but from the information on light compensation points (FIG. 17) must be higher than 4 μ E $m^{-2}s^{-1}$, the light compensation point for a portion of frond. A whole plant would have a higher compensation point than a portion of frond because of the necessity to maintain non-photosynthetic tissue such as roots and stem, and because of respiration of the whole plant at night.

Both the gametophyte and sporophyte of Cibotium can be classed as shade requiring plants both on the basis of their low light compensation points and on the basis of the photobleaching and restricted growth of the sporophyte or even death of the gametophyte in full sunlight.

In terms of Prioul's analysis of adaptability to irradiance (Prioul and Bourdu, 1973) the response of the gametophyte supports their suggestion, derived from only one shade ecotype of Solidagor virgaurea, that shade species show high adjustment when grown at low intensities. For gametophytes grown under 12 hour daylengths at both 20 and 25°C, P_N max - P_N growth increased the higher the intensity at which the plants were grown.

This means that gametophytes grown at low intensities can make greatest use of

short periods of higher irradiance, an obvious advantage to plants grown in a low light environment. The sporophyte on the other hand, shows characteristics of a sun species in that there was a low degree of adjustment (no difference between P_N max and P_N growth in FIG. 20). Even at the lowest light intensity used in these experiments, 12.5% daylight, the rate of photosynthesis during growth was light saturated. It would be interesting to know whether adjustment would occur at lower intensities.

The adaptability to light conditions during growth is measured by P_N max and by α, the initial slope of photosynthesis. For the gametophytes, there was a general decline in P_N max (apart from some high values at the lowest two irradiances at 25°C) with increasing irradiance during growth (FIG. 19). This may be a characteristic of shade plants, as it also occurred in the Solidago shade ecotype described by Prioul and Bourdu (1973). The sporophyte in contrast showed "sun plant" characteristics, in that P_N increased by about 30-40% when the light intensity during growth was increased from 12.5 to 100% daylight (FIG. 20). Examining the relationship between α and increasing irradiance during growth, both gametophytes (FIG. 19) and sporophytes (FIG. 20) showed a decline, most marked with the sporophytes. This may be regarded as a "shade plant" characteristic, as the three "sun plant" species examined by Prioul and Bourdu (1973) showed slight or marked increases in α with increasing irradiation during growth.

To summarize, while the gametophytes show the wide adjustment and adaptation typical of shade plants, the sporophytes have the adjustment typical of shade plants but an adaptation that shows features of sun plants, presumably related to their emergence from the extreme shade habitat characteristic of the gametophyte.

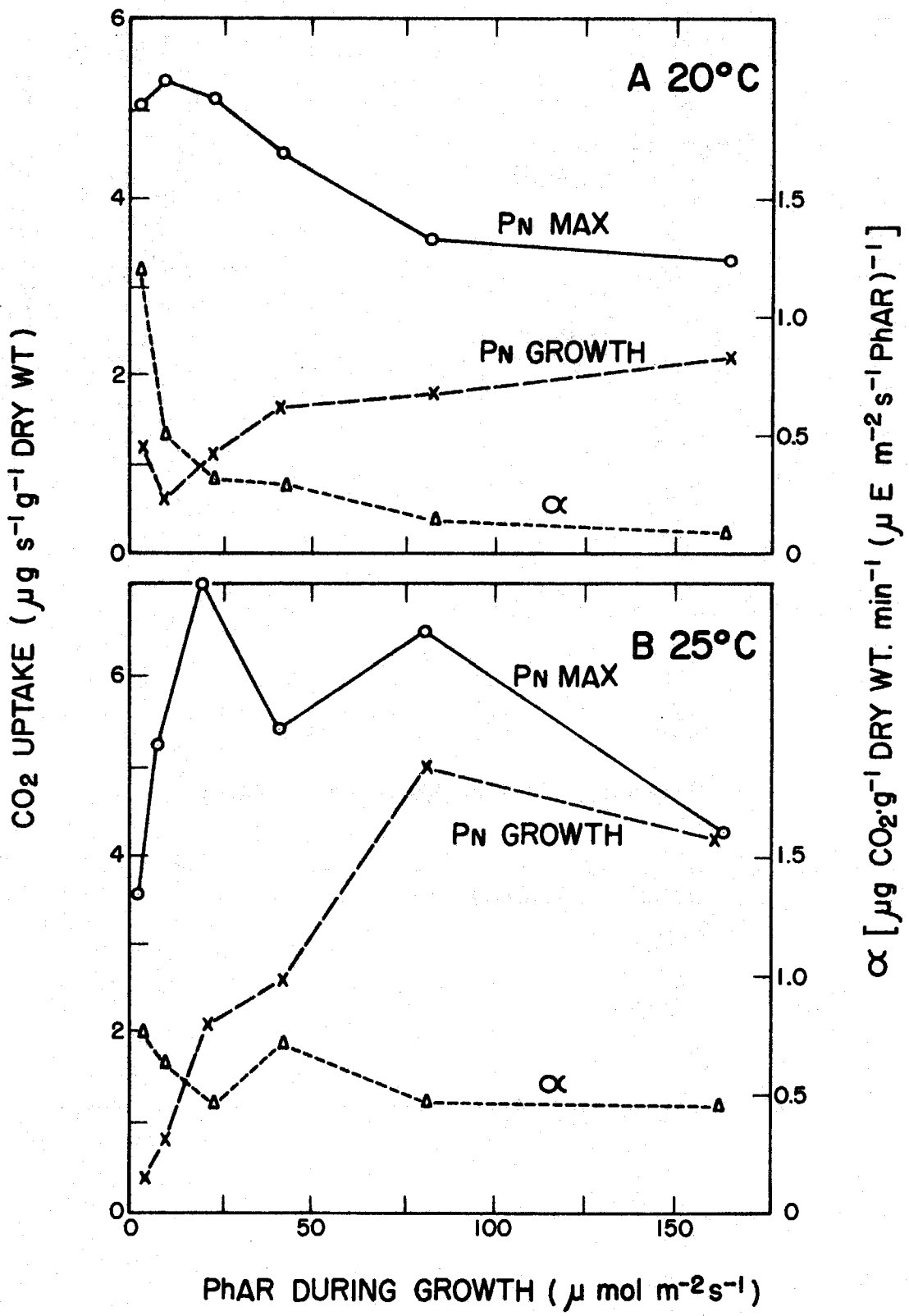


FIG. 19. Gametophytes, summary of effect of light intensity during growth at 20°C (A) and 25°C (B) on adaptation and adjustment.

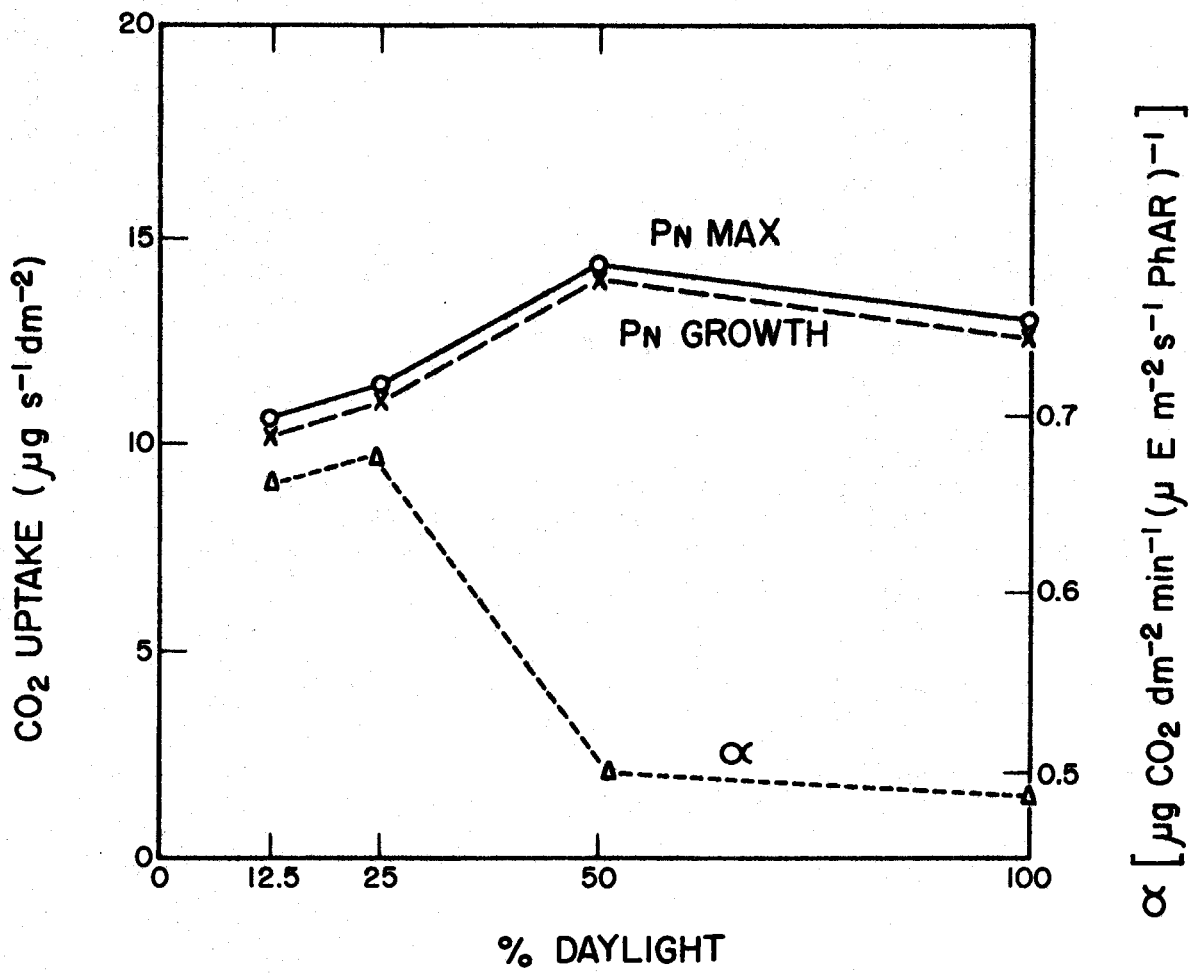


FIG. 20. Sporophytes, summary of effect of light intensity during growth on adaptation and adjustment.

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REFERENCES

Friend, D. J. C. 1966. Effects of light and temperature on growth of cereals. In F. L. Milthorpe and J. D. Ivins, (eds.). The growth of cereals and grasses. Butterworth Scientific Publications. pp. 181-199.

_____. 1969. Net assimilation rate of wheat as affected by light intensity and temperature. Can. J. Bot. 47:1781-1787.

Friend, D. J. C., V. A. Helson and J. E. Fisher. 1962. The rate of dry weight accumulation in Marquis wheat, as affected by temperature and light intensity. Can. J. Bot. 40:939-955.

_____. 1965. Changes in the leaf area ratio during growth of Marquis wheat, as affected by temperature and light intensity. Can. J. Bot. 43:15-28.

Hill, R. H. 1971. Spore germination and prothallial growth. Amer. Fem J. 61:171-182.

Horowitz, W. (ed.). 1970. Official methods of analysis of Association of Official Analytical Chemists, 11th Edition. Pub. AOAC, Wash., D. C. USA.

Prioul, J. L. and Bourdu, R. 1973. Graphical display of photosynthetic adaptability to irradiance. Photosynthetica 7:405-407.

TECHNICAL REPORTS OF THE US/IBP ISLAND ECOSYSTEMS IRP

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