EFFECTS OF LIGHT AND TEMPERATURE DURING PLANT GROWTH ON SUBSEQUENT LEAF CO_2 ASSIMILATION RATES UNDER STANDARD CONDITIONS

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Summary

The rate of photosynthetic CO_2 assimilation and transpiration as measured under standard conditions was determined for *Zea mays*, *Amaranthus palmeri*, *Helianthus annuus*, *Triticum aestivum*, *Gossypium hirsutum*, and *Phaseolus vulgare* after culture at a range of temperatures (15/10 to 36/31°C day/night temperatures) and natural (seasonal) or artificial light conditions. In general, the relative classification of the six species as to rate of CO_2 uptake was the same for plants grown under a wide range of conditions. CO_2 assimilation was depressed at the lower temperature limits for growth in *Z. mays* and *G. hirsutum* where plants were chlorotic. CO_2 assimilation shifted from one class of rates to another in *H. annuus* and *G. hirsutum* as seasonal radiation increased from winter to summer. Some of this change was caused by a factor inside the leaf, after seasonal variations in transpiration were accounted for. Much of the variation in CO_2 assimilation within and between *H. annuus*, *T. aestivum*, *G. hirsutum*, and *P. vulgare* was associated with differences in transpiration or the conductance for diffusion of CO_2 into the leaf.

I. INTRODUCTION

El-Sharkawy, Hesketh, and Muramoto (1965) and Elmore, Hesketh, and Muramoto (1967) have reported 50–80% increases in leaf photosynthetic rates of cotton and sunflower when comparing plants grown in the summer with plants grown in the winter in glasshouses. This suggests that at least part of the differences between species in leaf photosynthetic rates (El-Sharkawy and Hesketh 1965; El-Sharkawy, Hesketh, and Muramoto 1965; Elmore, Hesketh, and Muramoto 1967; El-Sharkawy, Loomis, and Williams 1967) could be complicated by such a seasonal effect.

The experiments presented here examine this possibility for a number of species grown at various controlled temperature regimes both in summer and winter in the CSIRO phytotron (CERES) at Canberra. A constant day length of 16 hr was used in the glasshouses; hence the only seasonal variable during growth was the amount and quality of light. Transpiration rates were also measured in an attempt to separate diffusion characteristics in air from factors inside the leaf which could also limit leaf photosynthetic rates.

Six species from earlier studies, each differing from the others in some respect, were evaluated. Two, *Amaranthus palmeri* and *Zea mays*, have very high CO_2 assimilation rates (El-Sharkawy and Hesketh 1965). The other four species have lower CO_2 assimilation rates in air because of apparent photorespiration (see

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Forrester, Nelson, and Krotkov 1963). This apparent respiration in the light ceases in O_2-free air where CO_2 assimilation rates of *Helianthus annuus* and *Triticum aestivum* approach those of *A. palmeri* and *Z. mays* (Hesketh 1967). *Gossypium hirsutum* and *Phaseolus vulgare* have even lower CO_2 assimilation rates, suggesting other limiting factors associated either with the conductance of CO_2 diffusion in the air phase, or with factors inside the leaf. All species evaluated had stomata in both surfaces of the leaf.

II. Methods

Plants were grown at several constant day/night temperature regimes in summer and winter in a 1 : 1 mixture of vermiculite and perlite provided with Hoaglands No. 2 solution. Day/night temperatures ranged from 15/10 to 36/31°C. Day temperatures were held for the middle 8 hr of light and day lengths were extended to 16 hr with incandescent lamps. Plants were also grown at a 16-hr day length at 27/22°C in a growth cabinet under fluorescent and incandescent lights. Available light energy inside the glasshouses was estimated by multiplying readings from a Kipp solarimeter outside by 0·8 (see Morse and Evans 1962). *G. hirsutum* did not grow at 15/10°C. In midsummer plants were grown at 18/13 and 24/19°C as well as at the other temperatures.

In an attempt to expose plants to greater light intensity than 3000 f.c. fluorescent light, some plants under a 16-hr day length were exposed to 8–10 hr of fluorescent light (3000 f.c.) and 6–8 hr of incandescent light (0·9 cal cm^{-2} min^{-1}) per day for a week before determination of photosynthesis and transpiration.

CO_2 assimilation rates were measured under 0·9 cal cm^{-2} min^{-1} light from a source consisting of seven 300-W incandescent lamps and a 7·5-cm water filter. CO_2 was measured with a Grubb–Parsons IMI infrared gas analyser. Water vapour was determined from relative humidity measurements with a Hygrodynamics electric hygrometer. Air flow rates of about 30 litres/min were measured with Scientific Glass rotameters. Leaf temperatures of about 30°C were determined with a thermocouple touching the underside of the leaf. Vapour pressure differences between the leaf (assumed to be at 100% R.H.) and the air were calculated, and transpiration rates were calculated per unit vapour pressure difference (see Gaastra 1959). Both CO_2 assimilation and transpiration were calculated from the concentration difference of CO_2 or water vapour between the leaf chamber intake and exhaust and from air-flow rates, leaf areas, and gas constants. Relative humidities inside the leaf chamber were usually 50–60%. CO_2 concentrations were about 0·65 mg/l.

Young but fully expanded leaves were selected from plants which had developed a leaf area of 3–10 dm^2 (one-tenth of this in the case of wheat). Single attached leaves, or three leaves of *T. aestivum*, were placed in a chamber under intense light immediately after plants were removed from the glasshouse or cabinet. Maximum photosynthetic rates were usually attained within 20 min. For each of the six species in midwinter, and for *G. hirsutum*, *H. annuus*, and *Z. mays* in midsummer, 15–25 leaves were measured for photosynthetic rate. Transpiration rates were obtained with about half of the photosynthetic determinations. In the other experiments, about five leaves for each species were tested for both photosynthesis and transpiration.

III. Results

Mean CO_2 assimilation rates and transpiration rates measured simultaneously for the six plant species grown at different light regimes and temperatures (excluding *Z. mays* at 15/10°C and *G. hirsutum* at 21/16°C) are given in Table 1. The CO_2 assimilation rates were relatively independent of temperature (Fig. 1).

Table 1

Leaf CO_2 assimilation rates P (mg $dm^{-2}hr^{-1}$) and transpiration rates T (cm sec^{-1}) at 30°C for plants grown in different light regimes and temperatures

Light regimes were either seasonal [midwinter, spring, midsummer (clear skies)] or artificial (growth cabinet) and average radiations received by the plants were 250, 400, and 650 cal $cm^{-2}min^{-1}$ for the seasonal conditions, respectively, and 256* and 522 cal $cm^{-2}sec^{-1}$ respectively for growth cabinet without and with supplement from an incandescent light source. Where both P and T were measured, mean values are given for plants grown at different temperatures. Values for *Z. mays* and *G. hirsutum* grown at temperatures below 21/16°C are excluded from these results. Letters denote different populations as determined by the least significant difference test at the 5% level

Plants	Midwinter		Spring		Midsummer		G.C.†		G.C.+L.†	
	P	T	P	T	P	T	P	T	P	T
Z. mays L. OH45 × Ky hybrid	49a	0·52y	50a	0·53	57e	0·6	48ai‡	0·44	54	0·57
A. palmeri Watts	49a	0·55y	49a	0·60	—	—	51ai	0·53	58e	0·60
H. annuus L. cv. Jupiter	32b	0·76x	32b	0·67	42hi	0·8	32b	0·60	30b	0·67
T. aestivum L. cv. Gabo	33b	0·65xy	32	0·74	27	0·8	37bh	0·98	—	—
G. hirsutum L. cv. Deltapine 15	23c§	0·54y	30bd	0·62	33bh	0·7	23cd‡	0·39	34	0·62
P. vulgare cv. Westralia	19c	0·44y	21c	0·62	—	—	21c	0·36		

* This light intensity is approximately equal to the photosynthetically active fraction of sunlight of intensity 480 cal $cm^{-2}min^{-1}$, fluorescent light being mostly in the visible spectrum.

† G.C., growth cabinet without light supplement; G.C.+L., growth cabinet with light supplement.

‡ In *Z. mays* for a T value of 0·6 this P value would be about 57, or the same as that at midsummer. In *G. hirsutum* for a T value of 0·7 this value would be about 33.

§ In *G. hirsutum* in midwinter, for a T value of 0·7 this value would be about 25.

In general, the classification of these six species on the basis of CO_2 assimilation rate confirm similar classification attempts reported by El-Sharkawy and Hesketh (1965), Elmore, Hesketh, and Muramoto (1967), El-Sharkawy, Loomis, and Williams (1967), Hesketh (1967), and an important conclusion from these data is that the classification holds for plants grown under a wide range of conditions. Two important exceptions to this conclusion were the behaviour of *H. annuus* where the CO_2 assimilation rate increased from the minimum values of 32 mg CO_2 $dm^{-2}hr^{-1}$ in midwinter to 42 mg CO_2 $dm^{-2}hr^{-1}$ under clear skies in midsummer and that of *G. hirsutum* where the CO_2 assimilation rate increased from the winter minimum of

23 to 30–33 mg dm^{-2} hr^{-1} in September and midsummer. These two changes in leaf photosynthesis will now be evaluated in relation to changes in transpiration.

According to Gaastra (1959), transpiration as calculated here (Table 1) is closely associated with the conductance for the diffusion of CO_2 from the air to the wet surfaces of cells inside the leaf via the stomata. The CO_2 assimilation rate of *H. annuus*, *T. aestivum*, *G. hirsutum*, and *P. vulgare* is greatly limited by an apparent photorespiration inside the leaf (see above). *Z. mays* and *A. palmeri* have greater CO_2 assimilation rates since this phenomenon is not evident in their leaves. As a consequence of this, the ratio between CO_2 assimilation and transpiration is almost

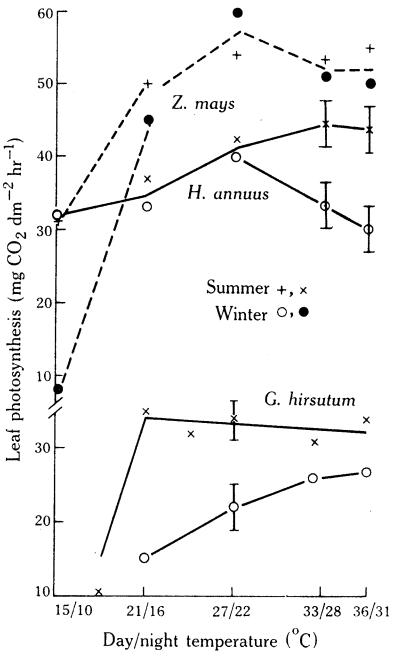

Fig. 1.—Leaf photosynthesis at 30°C for *Z. mays*, *H. annuus*, and *G. hirsutum* plants grown at day/night temperatures ranging from 15/10 to 36/31°C at light intensities of 250 (winter) and 650 (summer) cal $cm^{-2}min^{-1}$. Two standard deviations are given as errors for leaf photosynthetic rates in *H. annuus* at 33/28 and 36/31°C and in *G. hirsutum* at 27/22°C for summer and winter conditions.

twice as large in *Z. mays* and *A. palmeri* as in the other four species (Table 1; see also El-Sharkawy and Hesketh 1965) and, therefore, these two species were excluded when attempting to correlate CO_2 assimilation and transpiration among and within the other four species. The correlation coefficient between mean values of CO_2 assimilation and transpiration in *H. annuus*, *G. hirsutum*, *P. vulgare*, and *T. aestivum* plants grown under different conditions was 0·74; 55% of the inter- and intraspecific variation reported in Table 1 was thus associated with different conductances of CO_2 diffusion into the leaf. For the same plants under fluorescent lights, the correlations among individual determinations was 0·84, with 70% of the variation associated with transpiration. Presumably some of the difference between 70% for plants in one experiment and 55% in the overall experiment could be due to light-induced differences in photosynthetic potential inside the leaf. For example, transpiration in June–July for *H. annuus* was not much less than that in midsummer, nor was that for *G. hirsutum* much less than that in September or in plants exposed to intense

artificial light; yet in both species, the CO_2 assimilation was much lower in midwinter. The CO_2 assimilation data in Table 1 were plotted graphically against transpiration and the projected assimilation values which would have been associated with a higher transpiration rate were estimated for some species (see footnotes, Table 1). For example, if the leaves of *G. hirsutum* had a transpiration rate in winter of 0·7 cm sec^{-1}, the CO_2 assimilation rate would have been 25 mg $dm^{-2}hr^{-1}$ from such an interpolation. The seasonal change in CO_2 assimilation not associated with transpiration would then be 8/25 or 32%.

Details of the effects of temperature of growth on leaf photosynthesis at 30°C are given in Figure 1 and in Table 2. For *T. aestivum*, *P. vulgare* and *A. palmeri* in midwinter, photosynthetic rate was little affected by growth temperatures between 15/10 and 36/31°C (Table 2). The CO_2 assimilation rate was quite low in *Z. mays* for plants grown at 15/10°C in midwinter and was not as low in midsummer (Fig. 1). The winter-grown plants at 15/10°C were chlorotic. CO_2 assimilation values for *Z. mays* in midsummer (Table 1) are significantly greater than those determinations in midwinter, whereas inspection of the data in Figure 1, which includes many more determinations, does not suggest this.

TABLE 2

LEAF PHOTOSYNTHETIC RATES (P) FOR WINTER-GROWN PLANTS OF *A. PALMERI*, *T. AESTIVUM*, AND *P. VULGARE* GROWN AT DIFFERENT DAY TEMPERATURES

Day Temperature of Plant Growth (°C)	P (mg CO_2 $dm^{-2}hr^{-1}$) at 30°C		
	A. palmeri	*T. aestivum*	*P. vulgare*
15	51	32	19
21	55	32	19
27	60	37	19
33	48	32	19
36	58	30	20

CO_2 assimilation rates in *G. hirsutum* at the lower temperatures were greatly depressed in midwinter but little affected in midsummer between 21/16°C and 36/31°C; in fact, the highest CO_2 assimilation rates were determined in the plants grown at 21/16°C. Again, depressions in CO_2 assimilation rate were associated with changes in leaf colour and from dark green to yellow.

In *H. annuus* CO_2 assimilation rates were highest at 33/28 and 36/31°C in midsummer, but the optimum temperature was lower in winter.

IV. DISCUSSION

From an analysis of the classification of species on the basis of photosynthetic rate, differences in leaf photosynthetic rates have been associated with the presence or absence of apparent photorespiration and with variations of stomatal conductance

(El-Sharkawy and Hesketh 1965). The classification of species based on photosynthetic rates was derived from measurements in glasshouses and in the field in a semi-arid desert climate (Tucson, Arizona) and in a temperate climate (Ithaca, N.Y., and New Haven, Conn.). The photosynthetic rates were much higher in the summer in the desert climate, although relative differences among groups of species were about the same. Most of the CO_2 assimilation rates in Tables 1 and 2 fall within four classes: 50–60, 40–45, 30–35, and 20–25 mg CO_2 $dm^{-2}hr^{-1}$. Both *G. hirsutum* and *H. annuus* shifted from one class to another during the experiments. Many of the differences in individual measurements of photosynthesis in *H. annuus*, *T. aestivum*, *G. hirsutum*, and *P. vulgare* when grown under one set of conditions were associated with differences in transpiration. In earlier experiments (El-Sharkawy and Hesketh 1965) the correlation between rates of photosynthesis and transpiration among much the same species was 0·6. In the 1965 experiments, *H. annuus* and the cool-climate cereal *Avena sativa* (oats) had much the same transpiration rate but photosynthesis in *H. annuus* was 60% greater.

In order to explain seasonal changes in photosynthesis, it was desirable to separate changes in the conductance of CO_2 diffusion from changes inside the leaf. Transpiration rates per unit difference in vapour pressure between the wet surfaces inside the leaf and the air provide an estimate of the relative condutance of CO_2 diffusion in the opposite direction (see Gaastra 1959; Slatyer 1967, pp. 256–60). If photosynthesis and transpiration are not closely correlated, then some factor inside the leaf probably limits photosynthesis more in some species than in others. An example of such a factor is the photorespiration effect; *Z. mays* and *A. palmeri* leaves apparently do not have photorespiration (Forrester, Nelson, and Krotkov 1963; Hesketh 1967). In our experiments these two species consistently had much higher photosynthetic rates at a given transpiration rate than other species examined. Photosynthesis of *H. annuus*, a plant showing photorespiration, increased by 30%, but transpiration by only 10% or less from June–July and September to midsummer (Table 1); hence some of the increase in photosynthesis must have been caused by a change in some factor in the leaf.

With plants grown under fluorescent lights, photosynthesis and transpiration were closely correlated. Among such plants the conductance of CO_2 diffusion was apparently a dominant limiting factor. The relative importance of limitations in conductance of CO_2 diffusion in air compared with the limitation inside photosynthetic cells depended upon light conditions during growth. The limiting factor inside leaves was most evident when comparing *H. annuus* plants grown in intense light with other species which have the photorespiration limitation. However, some differences in this factor were found earlier when comparing other species (El-Sharkawy and Hesketh 1965); hence the apparent relative importance of the CO_2 diffusion conductance seen in the present study may also have been caused by the choice of species.

The seasonal change in photosynthesis is probably physiologically similar to the difference in photosynthesis found between shade and sun leaves as recently evaluated by Burnside and Bohning (1957) and Bjorkman and Holmgren (1963), although shade leaves in such studies were grown in less than the equivalent of 250 cal $cm^{-2}min^{-1}$ sunlight. For example, Bjorkman and Holmgren (1963) grew their

sun leaves in 200 cal $cm^{-2}min^{-1}$ light between wavelengths of 400–799 mμ, which is approximately equivalent to 500 cal $cm^{-2}min^{-1}$ sunlight, whereas their shade leaves were grown at one-fifth of this light intensity. If the seasonal change in photosynthesis is closely related to available sunlight in some species, then the photosynthetic potential of sun leaves may change between periods of cloudy and clear weather in midsummer. Some of the variation in photosynthetic rates in this study for plants in the glasshouses probably was associated with fluctuations in available light, as the day-to-day variation in CO_2 assimilation rates encountered in previous inter- and intraspecific surveys may well have been (El-Sharkawy, Hesketh, and Muramoto 1965).

Summer-grown *H. annuus* plants had leaf photosynthetic rates approaching those of winter-grown *A. palmeri* and *Z. mays*. This was seen earlier in the semi-arid environment where photosynthetic rates for field-grown *H. annuus* plants were similar to those of greenhouse-grown *Z. mays* in midwinter (Elmore, Hesketh, and Muramoto 1967). Summer-grown *Z. mays* and *A. palmeri* had higher leaf photosynthetic rates than summer-grown *H. annuus*, both in Tucson, Arizona, and in the present experiment. Thus it seems that plants such as *P. vulgare*, *T. aestivum*, *G. hirsutum*, and *H. annuus* cannot possess leaf photosynthetic rates similar to *A. palmeri* or *Z. mays* when comparisons are made for plants grown in any one environment between 21/16 to 36/31°C temperatures and 250–650 cal $cm^{-2}min^{-1}$ light intensity.

V. References

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