

THE EFFECT OF WATER STRESS ON THE ASSIMILATION AND ALLOCATION OF CARBON
DURING SEED FILLING IN SUNFLOWER

By

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Abstract

Two cultivars of sunflower, Pacific Sunfolia 68/2 (SF68/2) and Pacific Hysun 10 (PH10), were subjected to cycles of moisture stress during early and late flowering. Observations were made on the response to stress of the photosynthetic capacity of leaves, of the ability of the cultivars to maintain leaf area and on the accumulation of recently fixed assimilate in the seed.

Although PH10 had a similar potential photosynthesis rate to SF68/2 at early flowering, its photosynthetic capacity was more rapidly lost as the leaves aged and it was more seriously affected by water stress than was SF68/2. Unlike SF68/2 its stomatal mechanism was not able to recover fully following the release from stress. At the early flower stage, both cultivars directed a similar proportion of recently fixed assimilate to the seed and in both cultivars this process was equally sensitive to water stress. However, whereas SF68/2 retained its ability to export assimilate to the seed, this ability was drastically reduced in PH10 at the late flower stage. Both varieties had comparable leaf survival to the early flower stage and at this stage both were equally sensitive to water stress. However whereas SF68/2 maintained its leaf area into late flowering, even under stress conditions, PH10 had a rapid leaf senescence under well watered as well as under stress conditions. The loss of leaf area due to stress was not, however, as extreme in the capacity of the leaves to export assimilate to the seed.

Introduction

In grain crops, yield is determined by three inter-related processes. These are the net production of assimilate in the leaves, its translocation away from the leaves, and finally its allocation to a variety of functions in the other organs of the plant. One such function is the accumulation of biomass in the structural and subsequently the storage tissues of the reproductive system, part of which comprises the economic yield. The rates of these processes are largely dependent upon the same environmental factors but their sensitivities differ and furthermore they change during the growth and development of the crop.

The studies reported here were carried against a fairly limited experience (CAWOOD 1978, JESSOP 1977) with sunflower as a potential summer, dryland crop

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in the winter rainfall, traditionally wheat growing Wimmera country of the State of Victoria, Australia. Here, latitude $35^{\circ} 30'$ to $37^{\circ}S$, mean annual rainfall 446 mm (136 mm November to March), annual pan evaporation 1515 mm, the soils are typically deep grey cracking clay soils with a more or less friable surface soil (BADAWY 1977, STACE et al 1968). Wheat production is successful, providing yields from autumn sown crops in the range 1.5 to 4.0 tn/ha, but the capacity of the soil to store large amounts of water (up to 86 cm to a depth of 180 cm) following winter fallow make sunflower a potential summer crop provided that its demand for water can be met largely from soil reserves. Experience to date has indicated that yields in the range 0.6 to 1.5 tn/ha (40-46% oil) can be achieved, the higher yields occurring when crops receive the benefit of low and unpredictable summer rains. Under these cultural conditions, water stress during the later stage of crop development becomes the dominant factor determining yield. For these reasons we were concerned to study the effect of water shortage on the assimilation of carbon and upon its allocation to seed during the seed filling phase. We chose two promising cultivars which had previously demonstrated morphological and phenological differences in plot and field trials (CAWOOD 1978). The work itself was, because of its nature, carried out on plants grown in pots in the open but great care was taken to ensure that the plants under investigation were equivalent to the field phenotype.

Methods

The open-pollinated Pacific Sunfola 68/2 (SF68/2) and the hybrid Pacific Hysun 10 (PH10) were planted in 457 pots on 30 August 1976. Seedlings were thinned to two per pot, both of the same variety, and except for the period 28 to 38 days after sowing were grown in the open. The pots were arranged to maintain a plant density equivalent to $60,000 \text{ ha}^{-1}$ and were watered freely until treatments were imposed. Since treatment involved the withholding of water, both control plants and those undergoing treatment were transferred during that time into an evaporatively cooled glasshouse.

For either cultivar, water stress was imposed on two occasions, during early and late flowering respectively. For SF68/2 this commenced on days 92 and 107 after sowing and for PH10 on days 87 and 107 respectively. The plants were allowed to pass through three (early flowering) or two (late flowering) drying cycles each of approximately 4 days duration. All except the last cycle of each series was terminated by rewatering when the dawn leaf water potential, determined by the pressure cylinder technique, was in the range -1700 to -2000 kPa. At this point groups of plants were treated in different ways as follows.

One group was transferred to a laboratory where the gas exchange (CO_2 and H_2O) of an upper, expanded leaf was measured at a leaf temperature of 25°C and a photon flux density of $2250 \text{ uEm}^{-2}\text{s}^{-1}$. A second group was rewatered and their gas exchange behavior measured five days later. In a third group, an upper, expanded leaf of similar position was exposed to 50 μCi of $^{14}\text{CO}_2$ for 15 min. in an airtight chamber when the air temperature did not exceed 30°C and the photon flux density was greater than $700 \text{ uEm}^{-2}\text{s}^{-1}$. Twenty-four hours later these plants were harvested, divided into separate parts, deep frozen, later dried

and the distribution of label throughout the plant determined by scintillation counting. At the early flower stage only, a fourth group of plants was re-watered after they had passed through the stress cycles and then 5 days later the distribution of recently fixed assimilate was measured in the same way as described above.

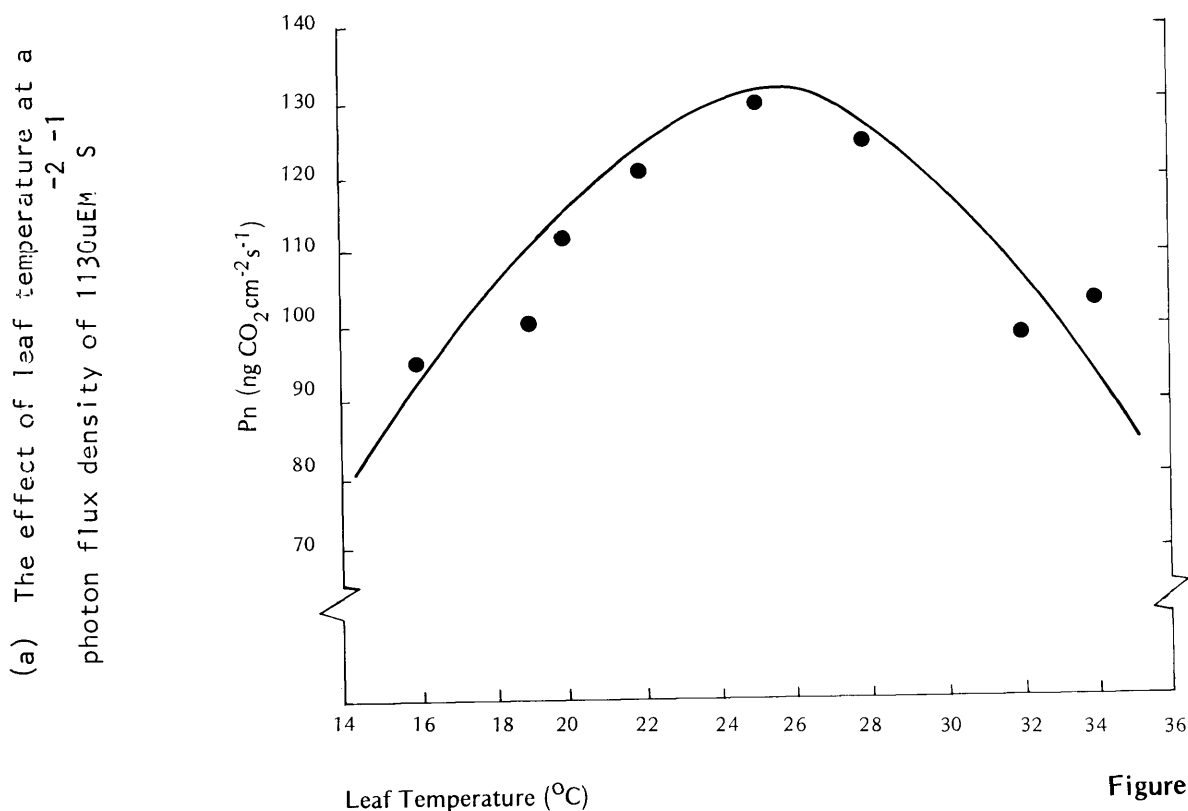
Observations were also made on the expansion of leaf area and its response to the stress treatments.

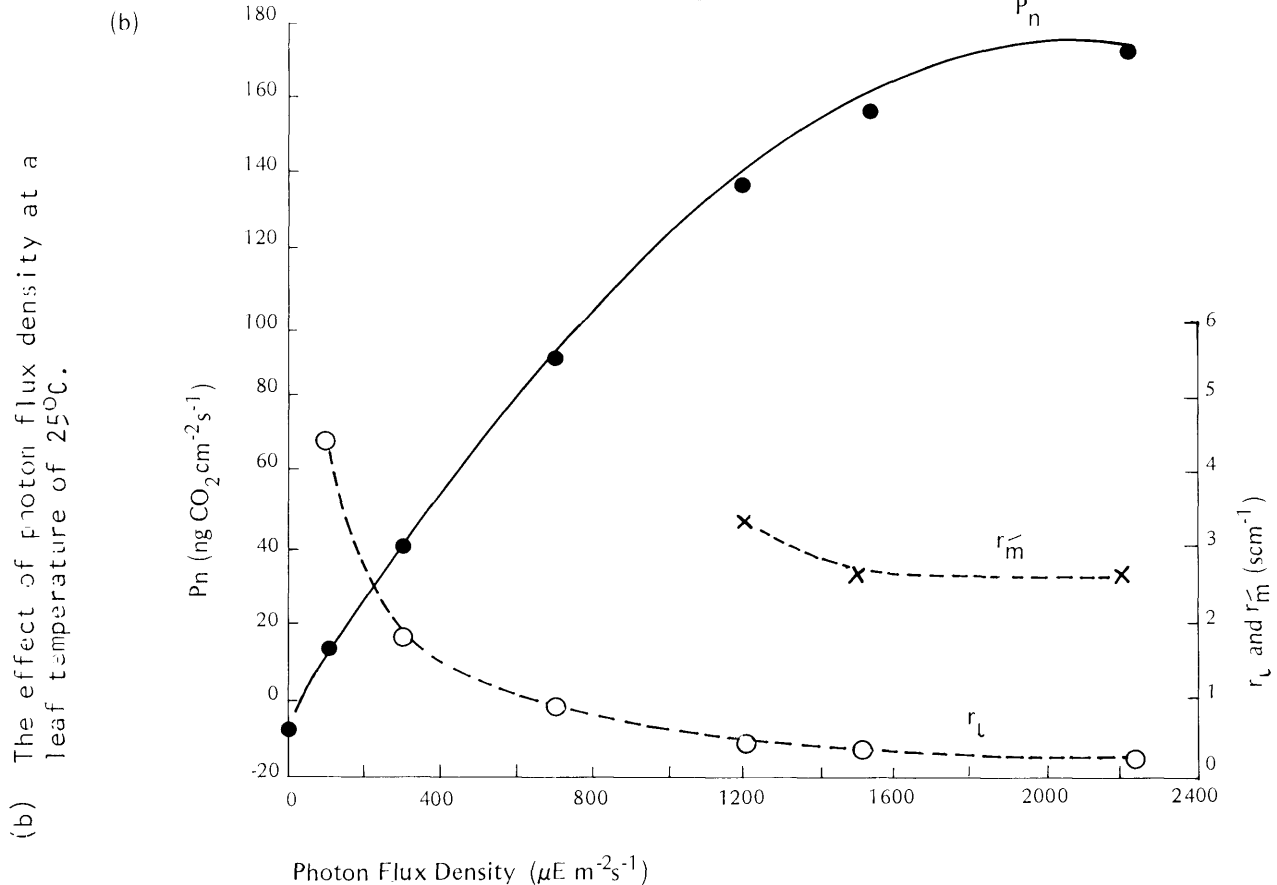
Results

A. Response of Leaf Photosynthesis to Photon Flux Density and Temperature.

The photosynthetic response of leaves of well watered plants was determined in order to establish appropriate conditions under which the response of leaf photosynthesis to water stress could be assessed. The two cultivars behaved similarly and the results of these observations are presented as the mean response in Figure 1. Maximum photosynthesis rates were obtained at leaf temperatures around 25°C (Figure 1a) and responded to increasing photon flux density up to 2250 $\mu\text{Em}^{-2}\text{s}^{-1}$ (Figure 1b). The combined photosynthesis/transpiration analysis showed that the response of photosynthesis to high photon flux density was a result of continued stomatal opening (reduction in leaf diffusive resistance, r_l) rather than an improvement in the efficiency of the internal fixation machinery (reduction in mesophyll resistance, r'_m).

On the basis of these results the subsequent gas exchange measurements designed to assess the effect of water stress were made at a leaf temperature of 25°C and a photon flux density of 2250 $\mu\text{Em}^{-2}\text{s}^{-1}$.





B. The Effect of Water Stress on the Photosynthesis Rate.

The results of these observations are summarized in Table 1. Here the effect of equivalent levels of plant water stress (dawn values of leaf in the range, -1700 to -2000 kPa falling to -1900 to -2100 during the day), is compared with the controls at both early and late flowering. Also included is the response of leaf photosynthesis 5 days after rewatering when dawn leaf was in the range -460 to -570 kPa.

The analysis of the response of both photosynthesis and transpiration to water stress and to recovery enables a separation of the separate effects on gas exchange of the physical diffusion pathway associated with stomatal activity and the biochemical fixation machinery itself. Changes in stomatal activity can be seen in the leaf diffusive resistance (r_L) whereas the internal behavior is depicted in changes in mesophyll resistance (r'_m).

Table 1 shows that whilst the photosynthesis rates of the two cultivars under non-stress conditions were similar at the early flowering stage, stress reduced photosynthesis dramatically by increases in both stomatal and mesophyll resistances. Whereas SF68/2 could recover completely from stress imposed at early flowering, PH10 did not and the effect lies in a deterioration of stomatal activity rather than in the internal chemical processes of photosynthesis. By late flowering both cultivars had lost some photosynthetic ability, but PH10 was affected much more than was SF68/2. Both cultivars were able to recover completely from stress at the late flowering stage.

TABLE 1. The Effect of Water Stress on Leaf Gas Exchange of Sunflower (Means of Four Replicates)

Parameter	Stage of Flowering	SF68/2			PH10			Pooled Standard Error of Mean
		Control	Stress	Recovery	Control	Stress	Recovery	
Photosynthesis ($\text{ng cm}^{-2}\text{s}^{-1}$)	E	112.1	45.8	123.2	121.2	23.4	99.8	9.6
	L	93.9	30.4	91.8	52.0	19.4	59.6	
Transpiration ($\mu\text{g cm}^{-2}\text{s}^{-1}$)	E	13.9	5.2	12.1	14.1	2.3	9.5	1.2
	L	11.8	2.7	10.9	8.2	2.0	7.6	
Leaf Diffusion Resistance (scm^{-1})	E	0.8	3.4	1.0	0.6	9.1	1.3	0.9
	L	0.8	3.9	0.9	1.4	10.5	1.6	
Mesophyll Resistance (scm^{-1})	E	4.1	7.5	2.8	3.8	13.8	3.9	2.6
	L	5.7	18.0	4.9	9.6	21.3	7.2	
% Survival of Leaf Area	E	93	91	-	96	81	-	7.1
	L	95	64	-	55	48	-	

An important part of the photosynthetic capacity of a plant lies in the extent of the leaf area available for photosynthesis. For completeness the survival of leaf area in the post-flowering period is included in Table 1. In both cultivars, maximum leaf area was attained at the commencement of flowering and in the control treatments the leaf areas were similar in the two cultivars ($0.55\text{m}^2/\text{plant}$). It is noticeable in the results presented in Table 1 that PH10 had a more rapid leaf senescence even under well-watered conditions than did SF68/2 and this was even further exaggerated in PH10 by water stress during late flowering. Since the leaf area declined more rapidly in PH10 than in SF68/2 it is likely that the treatments which were based on drying down following rewatering to a fixed level, would have slightly favored PH10 in the later flowering stage since at this time its total transpirational load would have been similarly reduced.

C. Effect of Water Stress on the Allocation of Recently Fixed Assimilate.

The labelled leaves were exposed to $50\text{ uCi}^{14}\text{CO}_2$ for a sufficient period (15 min) such that, regardless of the vast variation in individual leaf photosynthesis rate in response to treatment (Table 1) all leaves were essentially able to fix all the applied label. This is shown in Table 2. The average uptake across all treatments was 48.7 uCi and the deviation amongst treatment, although statistically significant, was small. The advantage of this is that in all plants, regardless of assimilation rate, a significant and equitable amount of label was available with which to follow the allocation of assimilate.

TABLE 2. Fate of Administered Label. Percentage Uptake of the Administered Dose (50uCi) and Percentage Recovery of Fixed Label 24 Hours Later. (Means of Three Replicates)

Parameter	Flower Stage	SF68/2		PH10		Pooled Standard Error of Mean
		Control	Stress	Control	Stress	
% Uptake	E	98.8	98.8	98.3	96.7	1.5
	L	99.1	97.2	97.7	91.3	
% Recovery	E	63.9	35.2	67.6	58.0	4.9
	L	73.0	40.7	37.8	41.7	

The fate of the fixed label is detailed in Table 2. In many experiments of this type only the relative distribution of recovered label is presented. Such results can be misleading, because as is seen in Table 2, if the amount lost during the grow-on period is responsive to treatment, then a false pattern may emerge. Here it is clear that a greater proportion of label is lost under stress conditions and this is taken to represent a relatively higher respiratory loss at lower assimilation rates. For this reason the label recovered from each organ is presented in Table 3 as the proportion of the label fixed by the plant rather than of the total label that was subsequently recovered. Because of uncertainty in the attempts to completely separate root material from soil it was not possible to measure with confidence the movement of label into the root system. However estimates based upon specific activity of root

samples and highest root/shoot ratios suggest that the recently fixed assimilate with the general patterns of assimilate movement in annual plants during the final growth stages.

TABLE 3. The Effect of Water Stress on the Carbon Allocation Pattern of Recently Fixed Assimilate. Recovery as % of Fixed Label. (Means of Three Replicates)

Component	Stage of Flowering	SF68/2		PH10		Pooled Standard Error of Mean
		Control	Stress	Control	Stress	
Target Leaf	E	16.4	14.8	15.8	21.1	4.0
	L	15.8	19.8	15.5	8.3	
Target Zone Petiole and Stem	E	2.2	3.3	3.0	11.0	1.0
	L	1.0	1.5	2.8	2.5	
Lower Leaves, Petioles and Stems	E	1.3	2.0	2.1	7.2	0.03
	L	0.7	1.4	1.0	0.5	
Upper Stem*	E	2.6	5.2	2.0	6.2	1.1
	L	0.4	1.1	1.2	0.9	
** Capitulum (incl. seed)	E	41.3	9.8	44.5	12.2	4.4
	L	55.0	16.8	17.2	29.4	
Seed	E	25.9	2.0	25.8	2.8	6.4
	L	48.5	10.1	8.6	8.5	

* Negligible activity (<0.10% in upper leaves and petioles).

Of particular interest in the movement of assimilate out of the leaf is an analysis of the proportion that moves upward through the stem and capitulum into the seed. Water stress is seen to have a dramatic effect on this movement. Using the total label fixed as a marker of assimilate use and allocation, it can be seen that the proportion reaching the seed is the greatest and also the most sensitive to stress of all the upward flows. Under stress, the proportion in the transport route itself (upper stem and capitulum) may be increased. This increase may simply represent a slower transport of assimilate under stress and hence not a complete loss to seed yield. The amount involved, however, is relatively small and, like the changes occurring in the small amount of assimilate directed at these late growth stages to the lower plant parts, does not dominate allocation patterns.

Observations were also made of the allocation pattern of label administered to plants five days after the release from stress at the early flower stage only. These experiments showed that, as with leaf photosynthetic capacity, the pre-stress pattern was quickly re-established.

D. Effect of Water Stress on Seed Filling.

The separate estimates of leaf photosynthetic capacity, relative allocation of recently fixed assimilate to seed and the survival of leaf area can

lead to a ranking of the two cultivars with respect to their capacity to fill seed under both control and water stressed conditions. Such a ranking is presented in a stepwise manner in Figure 2. In order to develop the relationship in Figure 2(d) it is necessary to assume that the photosynthetic capacity of the total leaf area on a plant and its relative allocation of assimilate to seed is proportional to the behavior of those individual leaves that were measured.

The striking feature of the relationship (Figure 2) is that although both cultivars were able to maintain substantial photosynthesis rates under stress, especially at early flowering, and although a large proportion of leaf area survived the stress treatment, the drastic reduction in the proportion of assimilate allocated to seed dominates seed filling under stress. Although SF68/2 had substantial advantages over PH10 in terms of photosynthetic capacity and maintenance of leaf area, particularly at early flowering, the advantage was largely lost in the comparison of the supply of assimilate from the total plant to the seed.

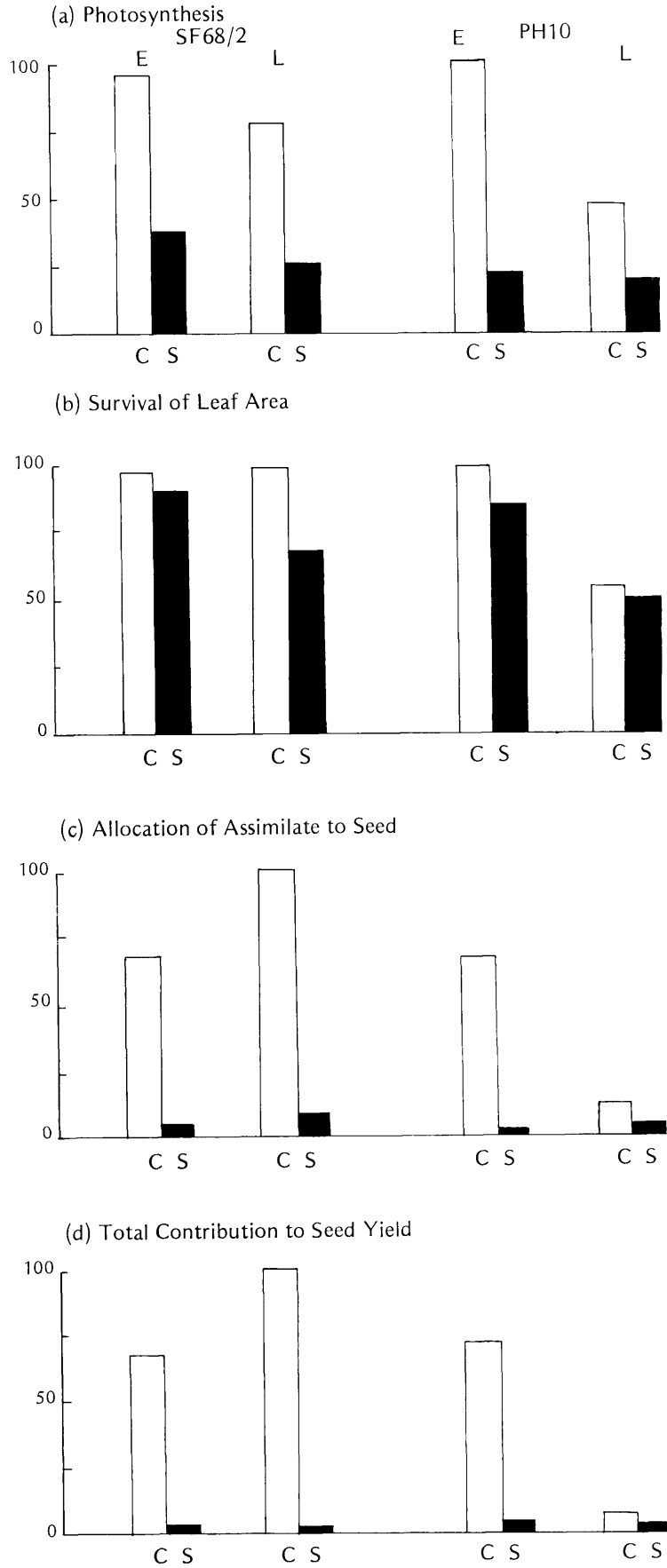
Discussion

The high photosynthesis rates that were observed in these experiments attest to the success of the efforts that were made to grow plants consistent with the field phenotype. It was felt at the outset that unless such plants could be grown successfully in containers then the significance of the stress treatments would be in doubt. The rates reported here compare with the highest rates reported in the literature (HIROI and MONSI 1966, WILSON 1966) and are consistent with the observation that sunflower ranks highly in the maximum productivity estimates for C_3 plants at least (WILSON 1966).

The observations on the effect of water stress on photosynthesis demonstrate that sunflower can maintain a positive carbon balance even under considerable levels of leaf water stress (-2000kPa). They also demonstrate that recovery from stress can be complete in both the stomatal system and at the chemical sites of photosynthesis within the leaf. Not all previous observations on the photosynthesis of sunflower support this view. BOYER (1971) for example reported that sunflower was unable to recover photosynthetic capacity once leaf water potential had fallen below -1800kPa . However observations on the behavior of sunflower crops in our local environment (CAWOOD 1978) show that not only do stomata remain open at leaf water potentials at which visible wilting occurs (-1800kPa) but also that their productive capacity is not halted by this experience.

Between the two cultivars studied here there was a significant difference in the response of leaf photosynthesis to water stress. SF68/2 maintained a higher photosynthesis rate under stress than did PH10 at both early and late flowering stage. Unlike SF68/2, PH10 was unable to recover completely from stress and the analysis implicates a failure in the stomatal mechanism as well as in the chemical capacity of the photosynthetic system. Under well watered conditions, SF68/2 maintained a high photosynthesis rate longer than did PH10 relative to their individual stage of flowering.

Figure 2



The relative effect of water stress (S) compared with the control (C) on (a) the leaf photosynthesis rate, (b) the survival of leaf area, (c) the allocation of recently fixed assimilate to seed, and (d) the total contribution of assimilate to seed production, for two cultivars of sunflower SF68/2 and PH10 at both early (E) and late (L) flowering stages.

In many studies of the effect of water stress on photosynthesis little attention is paid to the effect of stress on the survival of leaf area and hence on the extent of the photosynthetic system. In annual grain crops such as sunflower the leaf system has ceased to expand at or before flowering so that seed filling proceeds at a time when the natural pattern is one of leaf senescence. Water stress at this time has the potential to accelerate this natural process and thereby have a marked effect on the total photosynthetic capacity of the crop. Whereas the unit leaf photosynthesis rate was able to completely recover in SF68/2 at least, in both species the additional leaf senescence induced by stress was an irrecoverable loss. The more rapid leaf senescence that was characteristic of PH10 under well watered conditions in these experiments parallels its more rapid decline in leaf photosynthesis rate. Under stress conditions and particularly at late flowering, when the natural rate of senescence is increasing the effect of stress is amplified and in the cultivar comparison made here, points to a further advantage of SF68/2 as an accumulator of assimilate for seed filling under stress conditions.

Compared with the effect of stress on the leaf photosynthetic capacity and on the survival of leaf area, the effect on the allocation patterns of recently fixed assimilate to the seed was extreme. In these experiments the allocation patterns of recently fixed assimilate were based on the fate, 24 hours after labelling, of the label actually taken up by the plant. Since the early losses of label were markedly increased under stress this form of analysis, which is not a universal feature of experiments concerning movement of assimilate within plants, is an essential basis of the conclusion that under stress, the proportion of recently fixed assimilate allocated to the seed was drastically reduced. There is evidence that a part of this reduction was caused by a slower translocation of assimilate out of the leaf and through the upper stem to the capitulum and seed, since the proportion of assimilate remaining in these organs 24 hours after labelling increased under stress. However, the major effect was seemingly one of a proportionately greater relative respiratory loss from leaves of lower assimilate status leaving less assimilate available for translocation to other organs.

At early flowering under well watered conditions, both cultivars directed 25% of assimilate to the seed whereas under the lower photosynthesis rate associated with water stress, only 2% of the smaller assimilate supply reached the seed. At late flowering the effect was less marked but the results of these experiments become unclear at this point. The lower allocation of assimilate in PH10 under control conditions is consistent with its overall reduced assimilate supply resulting from a markedly lower leaf photosynthesis rate. At this time, whereas SF68/2 allocated a massive 49% of its high photosynthetic yield to seed, PH10 directed only 8%. Under stress the contribution from SF68/2 was reduced to 25% of the control rate whereas in PH10 it was unaffected. It might be that at this stage PH10, at a relatively late stage of leaf senescence, was remobilizing assimilate in the final stages of seed filling. The technique of labelling adopted here does not permit any conclusions to be drawn concerning the significance of stored assimilates in the development of yield in sunflower and particularly of its importance to the maintenance of yield under stress.

There is clearly a great deal to be learned about the assimilate relationships in sunflower and of the variation that might be exploited in the develop-

ment of improved cultivars. In the experiments reported here it is clear that differences between cultivars exist in the response of leaf photosynthesis rate, of leaf survival rate and to a lesser extent in the allocation of assimilate to seed under water stress. The effect of water stress on allocation is so extreme as to effectively remove any assimilate gains destined for seed filling under stress conditions. However the assimilate supply under stress is also closely linked with the survival of the leaf area itself, and in this, the effect of short term stresses have cumulative effects on the total assimilatory potential of the plants particularly in late flowering. In the limited experience to date in dryland sunflower production in the Wimmera, SF68/2 has maintained a yield advantage over PH10 and certainly a characteristic of the cultivar has been its ability to maintain an active, photosynthetic system for a longer period post-flowering when soil water reserves are approaching exhaustion and intermittent periods of high evaporative demand place great stresses on the plant. It is hoped that the observations reported here on the assimilate relationships of sunflower during seed filling will contribute to our understanding of the response of the crop to water stress and to the more effective selection of superior cultivars.

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