T1988PRO15

PRE-ANTHESIS ASSIMILATES AND GRAIN FILLING IN IRRIGATED AND WATER-STRESSED SUNFLOWER CROPS : QUANTIFICATION USING LABELLED CARBON.

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SUMMARY
Estimates of pre-anthesis assimilate contributions to seed yield were made in sunflower crops (density 44.400 plants.ha^{-1}) irrigated throughout the season or droughted during grain filling. In order to do this, the partitioning of labelled carbon, applied at intervals during growth, between the seed and the rest of the crop (including roots) was determined at maturity. Mean partitioning for the pre- and post-anthesis periods was then weighted using crop dry weight changes over the same periods. Three plots per treatment were labelled on each of ten occasions at weekly intervals during the growth period commencing 23 days before anthesis. Weekly harvests of above-ground biomass were made over the same period. Root biomass was estimated from core samples taken at three developmental stages and the dynamics of the fine root: tap root ratios determined in another experiment. The dynamics of carbon and nitrogen contents of all organs were followed. Oil content of the grain during growth, and the water soluble carbohydrate content of stem and tap roots were also monitored. Biomass values were corrected for oil synthesis costs. The resulting estimates showed that out of total seed carbon (corrected for oil synthesis costs), about 70 gC.m^{-2} originated in pre-anthesis assimilation, treatment having little effect on this contribution. This amount was equivalent to about 23 or 35 % of the carbon content of seeds in irrigated and droughted crops, respectively. During grain filling the losses in dry weight of the non-seed portion of the crops were equivalent to 73 gC.m^{-2}. Over the same period the loss in water-soluble carbohydrates stored in stem, tap root and receptacle was equivalent to 60 gC.m^{-2}, and that of nitrogen-associated carbon to 14 gC.m^{-2}. The amount of water-soluble carbohydrate stored at anthesis was equivalent to 86 gC.m^{-2}. Thus labile carbon stored in the crop at anthesis considerably exceeded that required to sustain the estimated transfer to seeds, and observed losses from non-seed organs were similar in magnitude to this estimate. A comparison with the absolute amounts of pre-anthesis C translocated to grain in cereal crops (measured by others using the same technique) suggests that this contribution is usually considerably greater in sunflower.

INTRODUCTION
Some of the carbon in mature grain may derive from pre-anthesis assimilates (PAA), and there have been some attempts to measure the size of this contribution in cereals (*e.g.* Bidinger *et al*., 1977: Simmons and Jones, 1985). Direct measurements of this source of carbon for grain growth in field-grown crops are lacking in sunflower, although Blanchet and Merrien (1982) have used plant and organ dry weight changes between anthesis and maturity to estimate PAA utilization during grain filling.

We have used the technique of Bidinger *et al*. (1977) to estimate the contribution made by PAA to grain yield in crops of sunflower irrigated throughout the season or subjected to drought during grain filling. Changes in dry weight, in water-soluble carbohydrates and in nitrogen of the non-grain organs over the grain filling period were also determined. These data served to fix an upper limit to the amount of labile carbon available at anthesis and allowed us to estimate the net change in labile carbon of these organs during grain filling.

MATERIALS AND METHODS
A sunflower crop (cv. Pioneer Flora, density 44.400 plants.ha^{-1}, row spacing 0.75 m) was grown on a Shepparton fine sandy loam at the Institute for Irrigation and Salinity Research at Tatura during the 1985-86 summer season. There were two treatments (irrigated throughout and droughted during grain filling) and three replicate 6-row x 16 m plots per treatment. At least two further rows on either side of each plot, and at least one additional metre of row at each end of the rows, were left as buffer strips.

Cumulative Class A pan evaporation during the season was 731 mm and the irrigated treatment received 596 mm of water between rain and irrigation (average 42 mm. wk^{-1} during the last 13 weeks of the season) over this period. The crop reached 50% eight-row anthesis at 72 days after sowing (DAS) and maximum embryo weight was achieved in both treatments by 112 DAS. Grain filling was taken as occuring between these two dates.

Commencing 48 DAS, harvests of above-ground and tap-root biomass were taken at approximately weekly intervals throughout the season. Fine-root biomass was estimated from tap-root weights using seasonal dynamics of the fine root: tap root ratio measured for plants growing in 33-1 pots (Hall, unpublished). These estimates agreed well with direct measurements of fine root biomass made at 48 DAS, anthesis and maturity using a root-core technique described by van Noordwijk *et al.* (1985).

A previously unlabelled sub-plot of each of the main plots was exposed to 270 μCi of $^{14}CO_2$ during 30 minutes at weekly intervals commencing 49 DAS. Each of these sub-plots, as was the case for the biomass harvest sub-plots, contained four contiguous plants within a row and was buffered on all sides. At maturity, labelled sub-plots were harvested and the partitioning of label between the grain and the remainder of the plant was determined.

Label in milled material was determined combusting 200-mg samples in a Packard TriCarb B306 Sample oxidizer and counting recovered label in a Packard TriCarb 300 scintillation counter. Organ N-contents were determined by Kjehldahl analysis, and C-contents using a Leco CHN-600 CHN analyser. Water soluble carbohydrates in stem, tap root and receptacle were determined after hot water extraction by the anthrone method (Yemm and Willis, 1954) using fructose standards. Oil content of seeds was determined using nuclear magnetic resonance.

Allowance was made for the higher synthesis costs (relative to non-grain organs) of the grain in the calculation of partitioning of label to the grain and of the change in crop biomass during the grain filling period. This was done to avoid the effects of underestimation of total and labelled carbon in the grain and in biomass changes during grain filling associated with the changes in composition of material laid down before and after anthesis. For this purpose, non-oil grain dry weight was assumed to have 2.5% lipid associated with it, and the remaining oil was replaced by a carbohydrate : lipid mixture (97.5: 2.5 w/w) using the assumptions given by Penning de Vries *et al.* (1974) for carbohydrate composition and oil and carbohydrate synthesis costs. The resulting "grain" contained 26 % and 22 % more carbon than the harvested grain in the irrigated and stressed crops, respectively.

The contribution of PAA to grain filling was estimated using the expression (Bidinger *et al.*, 1977)

$$\Delta W_1 . P_{ic1} \Big/ [(\Delta W_1 . P_{ic1}) + (\Delta W_2 . P_{ic2})] \tag{1}$$

where ΔW is the change in oil-synthesis corrected biomass, P_{ic} is the mean partitioning index (corrected for oil-synthesis costs), and the subscripts 1 and 2 refer to pre- and post-anthesis periods, respectively. This expression serves to weight the partitioning indices for pre-and post-anthesis by the changes in crop biomass over the same periods. Crop biomass changes are used as an approximation to the relative rates of crop gross photosynthesis in the two periods.

RESULTS

Exposure to stress caused a slight decrease in crop total biomass and grain number at maturity, and significant (P = 0.05) decreases in grain yield, oil content and oil yield (Table 1).

The partitioning index corrected for oil-synthesis costs increased with time from the start of labelling, showed a broad maximum between 85 and 105 DAS, and then fell again (Fig. 1). With the possible exception of the last labelling in stressed crops, exposure to stress had no effect on this index.

Total crop biomass corrected for oil-synthesis costs increased from 48 DAS to maturity in the irrigated crops, but dry weight accumulation tended to become negative in droughted crops after the start of stress (Fig. 2). Dry weights of the non-seed portions of the crop did not differ significantly between treatments at anthesis or at maturity, but there was a significant (P=0.05) loss in dry weight in the non-seed (roots, stem, leaves, receptacle) compartment for pooled values of both treatments (Table 2, line 1) over that period. At an individual organ level, significant (P=0.05) losses between anthesis and maturity were found in both treatments only in stem, which contributed almost 57 % of the total non-seed dry weight loss . During the stress period, dry weights of organs of droughted crops were usually lower than those of irrigated ones, but these differences were significant (P=0.05) for receptacles only, with droughted receptacles at maturity weighing about 75% of irrigated ones (data not shown).

Table 1 . Treatment effects on crop variables as measured at maturity. Values are means and standard errors (n=3).

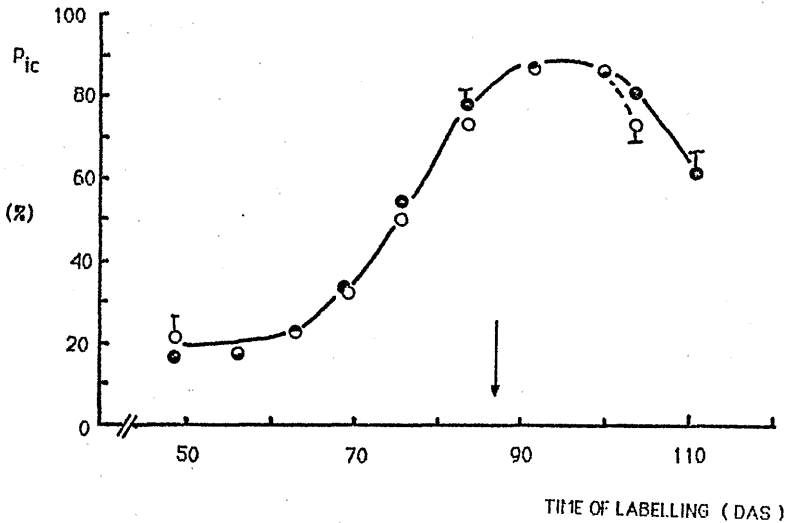| Variable	Treatment	
	Irrigated	Stressed
Total dry weight (g.m^{-2})	1020±35.6	903±52.1
Grain yield (g.m^{-2})	381±18.2	287±14.0
Grain number (cypselae.m^{-2})	8064±452	7272±300
Grain oil content (%)	50.2±0.09	41.0±0.42
Oil yield (g.m^{-2})	192±9.3	118±6.2
Grain carbon content (%)	61.3±1.06	59.5±0.15
Grain carbon (g.m^{-2})	233±11.1	171±8.4
Total carbon (g.m^{-2})	498±15.8	430±24.2

Fig. 1 . Partitioning indices (corrected for oil synthesis costs) for sunflower crops irrigated throughout (●) and stressed during grain filling (O). Error bars are standard errors (n=3), and are not shown where smaller than symbols. Arrow shows start of stress period . Curves fitted by eye.

The data collected in this experiment allowed the construction of plots equivalent to Figs. 1 and 2 for each of the three replicates per treatment. Applying expression (1) to these plots yielded mean values of 0.23 ± 0.010 and 0.35 ± 0.041 for irrigated and stressed crops, respectively. The corresponding estimates for carbon derived from PAA were 63 ± 1.03 and 76.7 ± 10.64 gC. m^{-2}, respectively. These do not differ between treatments, although the values for expression (1) shown above do differ significantly (P=0.05). The mean values of the denominator in expression (1) represented 92 and 94% of the oil-synthesis corrected seed biomass in stressed and irrigated treatments, respectively (*cf.* Bidinger *et al.,* 1977).

During the grain-filling period non-seed organs lost water-soluble carbohydrates and nitrogen. Values for the interval 76 to 114 DAS are shown in Table 2.
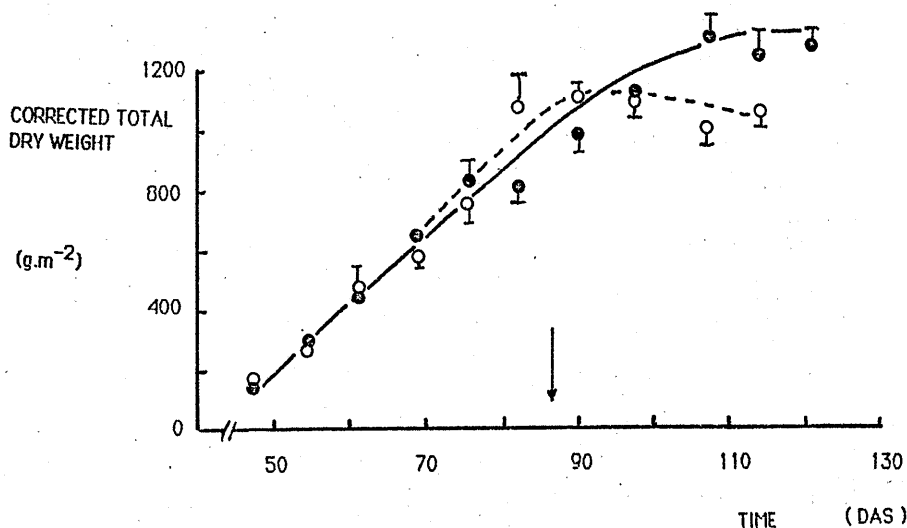

Fig. 2. Dynamics of total dry weight corrected for seed oil synthesis costs in sunflower crops irrigated throughout or stressed during grain filling. Symbols as in Fig. 1. Curves fitted by eye.

Table 2. Non-seed dry weight (Dwt) and nitrogen (N), and water-soluble carbohydrates in (Stem +Tap root + Receptacle) (WSC) immediately after anthesis and at maturity. Changes in weight for each variable(Δ) and carbon equivalent changes (ΔC) over this period also shown. Values followed by different letters differ significantly (P=0.05).

| | Harvest date | | | |
	76 DAS	114 DAS	Δ	ΔC (gC. m^{-2})
Dwt (g. m^{-2})	740.4a	586.8b	153.6	72.7
N (g. m^{-2})	11.4a	4.5b	6.9	13.8[1]
WSC (g. m^{-2})	214.1a	64.6b	149.5	59.8[2]

[1]Nitrogen assumed to be exported as glutamine and asparagine.
[2]Carbon equivalent assuming WSC as fructose.

Discussion

Total biomass yield of the irrigated treatment (Table 1) was within the range of values found by Connor *et al.* (1985) for crops of sunflower at the same location, although seed yield in our crops was somewhat lower. The effects of stress on oil yield and its components (Table 1) indicate that we were

able to achieve our aim of significant responses to stress while minimizing effects on grain number (and hence potential assimilate demand). This is important in sunflower, as in this species adjustment of grain number in response to stress occurs over a longer period after the start of anthesis than in cereals (*e.g.* Hall *et al.*, 1985).

In our sunflower crops PAA made a significant contribution to grain filling, whether taken on a relative or on an absolute basis, in both irrigated and droughted treatments. These contributions clearly warrant attention in relation to management, modelling and selection . Use of dry weight changes as an estimator of crop gross photosynthesis can lead to overestimates of the PAA contribution under conditions in which the crop is losing dry weight over 24-hour periods but still fixes carbon during the daytime. We have measured crop carbon dioxide exchanges over the season in the crops used for the experiment described herein. Estimates of PAA contributions to yield obtained by weighting P_{ic} by the best estimate of gross photosynthesis suggest that at least 50 $gC.m^{-2}$ was derived from PAA in these crops (Hall, Connor and Whitfield, unpublished). This continues to be a very substantial fraction of grain carbon (corrected for oil-synthesis costs). The relative insensitivity of the absolute PAA contribution to water stress was unexpected (*cf.* Hall *et al.* , 1985). This may reflect a very high demand for assimilate in the irrigated crop, although other plausible causes exist.

The carbon equivalent of non-seed dry weight changes (estimated from organ carbon contents) between 76 DAS and 114 DAS proved to be of similar magnitude to the labelled-carbon estimates of pre-anthesis contributions to grain yield (Table 2). Over the same period, the sum of the carbon equivalents of the nitrogen loss from the non-seed portion (assuming nitrogen was exported as glutamine and asparagine in a 2:1 ratio (Pate, 1980)) and of the WSC loss from stem, tap root and receptacle yielded a similar value (Table 2). If total "labile" carbon at anthesis is assumed to be equal to the sum of stored WSC (in stem, tap root and receptacle) and the nitrogen-associated carbon later translocated from the non-seed portion (i.e. $(214.1 \text{ g m}^{-2} \times 0.4) + (13.8 \text{ g m}^{-2}) = 99.4 \text{ g C m}^{-2}$), this amount considerably exceeded all estimates of pre-anthesis contributions to grain filling (Table 2).

There are several reports for cereals in which PAA contributions to yield have been estimated using the Bidinger *et al* (1977) technique or some close approximation to it. It is instructive to compare our estimates for sunflower with these (Table 3). No corrections for oil-synthesis costs have been made for the cereals in this comparison. Under non-stressed conditions the absolute contribution in sunflower was about twice that of the cereals: this difference does not appear to be associated with

Table 3 . Labelled carbon estimates of PAA contributions to grain yield. Carbon content of grain of wheat, barley and maize derived from data given by Sinclair and de Wit (1975). assumptions given in text of this paper, and a value of .523 for proportion of C in protein.

Species	Treatment	Contribution $(g C.m^{-2})$	Anthesis aboveground biomass $(g. m^{-2})$	Source
Wheat	Irrigated	35.1	760	(1)
	Stressed	35.6	610	(1)
Barley	Irrigated	32.6	750	(1)
	Stressed	27.2	655	(1)
Barley	Wet year	33.2	634	(2)
	Dry year	59.3	521	(2)
Maize	1982	27.9	580[a]	(3)
	1983	31.9	490[a]	(3)
Sunflower	Irrigated	63.0	724	
	Stressed	76.7	649	

Sources: (1) Bidinger *et al*. 1977, (2) Austin *et al*. 1980, (3) Simmons and Jones 1985.
[a] Our estimate using expression (1) and Simmons and Jones' data on total biomass at harvest and P_{ic}.

proportional differences in above-ground biomass at anthesis. Under stress conditions, one of the three available reports (Austin *et al* 1980) gives an absolute contribution similar to that of sunflower, the other two are much lower. There are some difficulties of interpretation here. Austin *et al* calculated their values on the basis of pre-anthesis labelling only, commencing 12 days before anthesis, and their stress situation was poorly defined. Bidinger *et al*'s data refer to crops stressed from before anthesis through to maturity (and therefore may not be representative in terms of the potential pre-anthesis store nor maximum demand during grain filling). It would seem that the question of the effects of stress on the pre-anthesis contribution to grain yield in cereals has yet to be resolved. What can be stated, given these uncertainties, is that our irrigated sunflower crops depended to a greater extent than cereals on pre-anthesis carbon for grain filling. This difference between species may also hold for the comparison between stressed sunflower and cereal crops.

ACKNOWLEDGEMENTS

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