

STUDIES OF GRAIN PRODUCTION IN *SORGHUM VULGARE*

II.* SITES RESPONSIBLE FOR GRAIN DRY MATTER PRODUCTION DURING THE POST-ANTHESIS PERIOD

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Abstract

The relative contributions of different photosynthetic sites to the filling of the grain in grain sorghum (*Sorghum vulgare* cv. Brolga) were estimated by measuring the ^{14}C in the grain after exposing various leaves and the head to radioactive carbon dioxide. Methods for preventing photosynthesis were also used.

Of the grain yield, 93% was due to assimilation by the head and upper four leaves. The head contribution of 18% was due equally to direct assimilation of atmospheric carbon dioxide and to re-assimilation of carbon dioxide released within the grain by respiration of material translocated from the leaves. The remaining 75% was equally assimilated by the upper four leaves, the flag leaf being the most efficient contributor per unit area and the third uppermost leaf the least efficient.

The percentage contributions to the grain by the flag leaf and fourth leaf, estimated from the decrease in grain yield when they were shaded, agreed closely with the estimates obtained by using $^{14}\text{CO}_2$.

I. INTRODUCTION

The final grain weight of cereals is formed from assimilate that moves to the grain from the leaves plus that directly assimilated by the head from atmospheric carbon dioxide. Part of this material, from both sources, is used as a respiratory substrate in the head, but not all of the carbon dioxide evolved is lost; some is re-assimilated by the chlorophyllous tissue of the head.

The photosynthetic sites involved and their relative contribution to post-flowering dry matter accumulation in the grain have been studied in such cereals as barley, wheat, rice, and maize (Thorne 1965). However, a careful analysis has not been made in grain sorghum. Li and Liu (1935) indicated the importance of the upper three leaves in an unspecified type of *Sorghum vulgare*, and Stickler and Pauli (1961) demonstrated that the leaves of the upper half of the plant are associated with maximum yield.

The present work was undertaken to examine yield in terms of the components set out above, including the separation of the role of individual leaves. Only the post-anthesis period was examined, the earlier period having been shown to make only a very small contribution (Fischer and Wilson 1971).

Measurement of the contribution by various sites has generally depended on yield reduction techniques (prevention of photosynthesis), but there are doubts about their validity (Thorne 1965). A method based on the use of $^{14}\text{CO}_2$, which avoids

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these difficulties, was employed here although the assessment of the head re-assimilation component required prevention of photosynthesis.

In the usual method, known as shading, light is excluded from the site in question. As this method is widely used because of its simplicity, and it was desired to use it in later field studies in this programme, an assessment of its reliability was made by comparing results obtained respectively from this and the ^{14}C method.

II. EXPERIMENTAL

(a) Sources of Grain Dry Matter

The work was based on the following scheme of grain dry matter accumulation.

Photosynthate from the various leaves is translocated to the head and this material is treated as either going to grain storage or as being used in respiratory activity. That part going to storage is indicated as L_F (from flag leaf), L_2 (from second uppermost leaf), etc. and the total from this source, $L_{F,2,\dots}$. Of the respiratory carbon dioxide, some is re-fixed and stored owing to photosynthetic activity by the head, and is identified by its original leaf source as rL_F etc.

Similarly, the head fixes carbon directly from the atmosphere, part (H) going to storage while some is respired, of which a fraction rH is re-assimilated and stored. Thus the total contribution to grain resulting from head photosynthesis is ${}^rL_{F,\dots} + H + {}^rH$. The head component (${}^rL_{F,\dots}$) is also considered within the concept of initial contribution by the leaves.

The material represented by L and rL has its origin in leaf photosynthesis, occurring not only concurrently (during grain filling) but prior to anthesis, and stored in vegetative structures. Previous work (Fischer and Wilson 1971) showed such storage to be relatively unimportant, and it is ignored here. This study examines grain filling only in terms of post-anthesis activities.

Total grain yield is therefore given by $L_{F,\dots} + {}^rL_{F,\dots} + H + {}^rH$.

Various combinations of treatments, involving the labelling of photosynthesis in separate leaves and in the head with $^{14}\text{CO}_2$, and the prevention of photosynthesis in the head by the exclusion of light or carbon dioxide, allowed the quantitative identification of the separate sources of supply of grain material, with the exception of the rH component.

(b) Radioactive Carbon Dioxide Technique

The application of this technique is based on the assumption that if a plant is subjected to a $^{14}\text{CO}_2$ environment during the period of assimilation of grain carbon, the activity in the grain at maturity is a relative measure of grain yield. The percentage of this ^{14}C which can be attributed to assimilation by the various sites will then be a measure of the contribution by each site to the grain dry matter yield. Thus the percentage of the grain yield contributed by any one site is given by

$$\frac{{}^{14}\text{C content of grain of plant with that site exposed to } {}^{14}\text{CO}_2}{{}^{14}\text{C content of grain of plant with all sites exposed to } {}^{14}\text{CO}_2} \times 100.$$

To maintain a $^{14}\text{CO}_2$ supply throughout the whole growth period would require attention for all of the daylight hours. This was not practicable, and it was considered that brief exposure at intervals would be representative of the whole

photosynthetic performance. All plant parts to be treated were exposed to the same environment of $^{14}\text{CO}_2$ for a period of 1 hr every second day throughout the grain-filling period. Differences in ^{14}C uptake by plants will therefore reflect differences in photosynthetic capacities of these parts, and differences in the ^{14}C content of grain at maturity will reflect both these photosynthetic capacities and the pattern of distribution of assimilates.

The plant parts to be exposed to $^{14}\text{CO}_2$ were enclosed in large Polythene bags which were connected in parallel and, with an air pump, formed a closed system. Treatments were usually based on the application of $^{14}\text{CO}_2$ to the head, or to the head together with increasing numbers of leaves down the stem. In each case the portion of the plant below the $^{14}\text{CO}_2$ enclosure was separately enclosed and aerated with unlabelled air. In this way the whole plant was always enclosed, which ensured uniformity of conditions, particularly light profile, between the plants in the different treatments.

$^{14}\text{CO}_2$ was generated by the reaction of ^{14}C -labelled barium carbonate with lactic acid. Weighed amounts of radioactive barium carbonate (specific activity 1.48 mCi/mmmole) were mixed with c. 0.5 g barium carbonate and placed in a glass chamber connected into the closed circulatory system. A 10% lactic acid solution was admitted into the chamber from a burette, which thus allowed control of the reaction. The circulating air was monitored with an infrared gas analyser placed in parallel so that only a small fraction of the air passed through it. Carbon dioxide concentration within the system was maintained at 300 ± 50 p.p.m. (v/v). During the initial period of application of isotope the photosynthesized carbon dioxide was replaced from the lactic acid/barium carbonate reaction, and thereafter by manually admitting carbon dioxide from a cylinder.

After each hourly treatment with $^{14}\text{CO}_2$ the closed circulatory system was opened and connected to an external air supply, which not only provided a continuous supply of air of ambient temperature and carbon dioxide concentration but also flushed away from the plants and the glasshouse any respiratory ^{14}C released into the Polythene enclosures.

At maturity, the ^{14}C content of the grain was determined by the procedure previously reported by Fischer and Wilson (1971).

(c) *Techniques for Prevention of Photosynthesis*

Heads were shaded by enclosure in a 6 in. diameter cylinder 15 in. long, with an aluminium foil exterior and a darkened paper interior. The top and bottom of the cylinder were enclosed in such a way as to allow air movement through the cylinder but to exclude light. Temperatures increased within the cylinder to a maximum of 4°C above ambient. Leaves and leaf sheaths were shaded by wrapping in aluminium foil. There was no air movement to these shaded parts.

Prevention of photosynthesis by excluding carbon dioxide was practised only for the head. Carbon dioxide-free air was passed through a Polythene bag in which the head was enclosed.

(d) *Experiment 1*

Experiment 1 was designed to measure the relative contributions to the yield of

(i) $L_F + {}^1L_F, L_2 + {}^1L_2$, etc. and (ii) $H + {}^1H$.

TABLE 1
 DATA OF EXPERIMENT 1
 For meaning of H , $'H$, L_F , etc. see Section II(a)

Treatment Sites Exposed to $^{14}\text{CO}_2$	Source of ^{14}C in Grain	Allocation of Radioactivity in Grain to Assimilation by Individual Sites		Contribution to Grain Yield (%)	Area of Leaf (cm ²)	Efficiency of Leaves per unit area (c.p.m. cm ⁻²)	Grain Dry Matter (g)
		Activity in or Differences between Treatments	Photosynthetic Source				
(1) Head	$H + 'H$	Treatment (1)	$H + 'H$	5.5			46.1
(2) Head and flag leaf	$H + 'H + L_F + 'L_F$	Treatment (2) - (1)	$L_F + 'L_F$	17.2	104	90.0	45.9
(3) Head, flag leaf, and leaf 2	$H + 'H + L_{F,2} + 'L_{F,2}$	Treatment (3) - (2)	$L_2 + 'L_2$	19.0	208	49.5	45.2
(4) Head, flag leaf, leaves 2 and 3	$H + 'H + L_{F-3} + 'L_{F-3}$	Treatment (4) - (3)	$L_3 + 'L_3$	20.0	251	45.5	43.2
(5) Head, flag leaf, leaves 2, 3, and 4	$H + 'H + L_{F-4} + 'L_{F-4}$	Treatment (5) - (4)	$L_4 + 'L_4$	31.2	251	71.5	43.7
(6) Whole plant	$H + 'H + L_{F-x} + 'L_{F-x}$	Treatment (6)	$H + 'H + L_{F-x} + 'L_{F-x}$	100.0	1317		
Least significant difference: $P < 0.05$				4.9		23.3	NS
$P < 0.01$				6.8		31.7	

Plants of *Sorghum vulgare* cv. Brolga were grown in a glasshouse as previously described (Fischer and Wilson 1971). Thirty plants were selected for uniformity, and five replicate plants subjected to each of six $^{14}\text{CO}_2$ treatments in a randomized block design. The plant parts exposed to $^{14}\text{CO}_2$, the source of ^{14}C in the grain, and the method of allocation of radioactivity in the grain to assimilation by the individual sites are shown in Table 1.

TABLE 2
DESIGN OF EXPERIMENTS 2a AND 2b
For meaning of H , ${}^{\prime}H$, L_F , etc. see Section II(a)

Treatment Sites Exposed to $^{14}\text{CO}_2$	Heads Supplied with		Source of ^{14}C (expt. 2a) and Dry Matter (expt. 2b) in Grain	Allocation of Radioactivity (expt. 2a) and Dry Matter (expt. 2b) in Grain to Assimilation by Individual Sites	
	Light	CO_2		Activity in or Differences between Treatments	Photosynthetic Source
<i>Experiment 2a</i>					
(1) Whole plant minus head	+		$L_{F-x} + {}^{\prime}L_{F-x}$	Treatment (7) - (1)	$H + {}^{\prime}H$
(2) Head only	+		$H + {}^{\prime}H$	Treatment (2)	$H + {}^{\prime}H$
(3) Flag leaf	-		L_F	Treatment (3)	L_F
(4) Flag leaf and leaf 2	-		$L_{F,2}$	Treatment (4) - (3)	L_2
(5) Flag leaf and leaves 2 and 3	-		L_{F-3}	Treatment (5) - (4)	L_3
(6) Flag leaf and leaves 2, 3, and 4	-		L_{F-4}	Treatment (6) - (5)	L_4
(7) Whole plant	+		$H + {}^{\prime}H + L_{F-x} + {}^{\prime}L_{F-x}$	Treatment (7)	$H + {}^{\prime}H + L_{F-x} + {}^{\prime}L_{F-x}$
<i>Experiment 2b</i>					
(7) Whole plant	+	+	$L_{F-x} + {}^{\prime}L_{F-x} + H + {}^{\prime}H$	Treatment (7)	$L_{F-x} + {}^{\prime}L_{F-x} + H + {}^{\prime}H$
(8) Whole plant minus head	+	-	$L_{F-x} + {}^{\prime}L_{F-x}$	Treatment (7) - (8)	$H + {}^{\prime}H$
(3) to (6)	-	+	L_{F-x}	Treatment (7) - (3 to 6)	${}^{\prime}L_{F-x} + H + {}^{\prime}H$

Results are also shown in Table 1. There was no effect on the grain yield of the different methods of enclosure. Of the grain material, 93% was assimilated by sites above leaf 5. The head component $H + {}^{\prime}H$ provided significantly less than any of the leaves, while leaf 4 contributed more than any other leaf.

The efficiency of grain production, expressed as radioactivity in the grain per unit leaf area, showed that the flag leaf was the most efficient followed by leaf 4, and then by leaves 2 and 3 which were similar.

(e) Experiment 2

Experiment 2a was designed to measure by the $^{14}\text{CO}_2$ method (i) L_F , L_2 , etc., and (ii) $H + {}^{\prime}H$; experiment 2b by yield reduction to measure (iii) ${}^{\prime}L_{F,2,\dots} + H + {}^{\prime}H$, (iv) $H + {}^{\prime}H$, and (v) ${}^{\prime}L_{F,\dots}$ (estimated by difference between (iii) and (iv)).

Forty plants were selected for uniformity, providing a randomized block design with five replicates of eight treatments. In five treatments the heads were shaded,

while in the other three the heads were enclosed in Polythene bags and supplied with either CO₂-free air, ¹⁴CO₂, or ¹²CO₂ air. For experiment 2*a*, the plant parts exposed to ¹⁴CO₂, the plants with heads shaded, the source of ¹⁴C present in the grain, and the allocation of radioactivity in the grain to assimilation by the individual sites are shown in Table 2*a*.

For experiment 2*b* the treatments, the source of grain dry matter, and the calculated contribution by sites are shown in Table 2*b*.

Grain dry matter attributed directly to photosynthesis by the individual leaves (L_F etc.) and total photosynthesis by the head ($'L_{F,\dots} + H + 'H$) is given in the upper part of Table 3. As in experiment 1, 93% of the grain yield was assimilated by the

TABLE 3

CONTRIBUTION TO GRAIN YIELD BY THE DIFFERENT SITES OF PLANTS IN EXPERIMENT 2 ESTIMATED BY BOTH ¹⁴CO₂ AND SHADING TECHNIQUES, AREAS OF LEAVES, AND EFFICIENCY OF CONTRIBUTION PER UNIT AREA OF LEAF

For meaning of H , $'H$, L_F , etc. see Section II(*a*)

Method	Source	Contribution to Grain Yield (%)	Areas of Leaves (cm ²)	Efficiency of Leaves per unit area ‡ (c.p.m. cm ⁻²)
¹⁴ CO ₂	Flag (L_F)	18.0	164	118.0
¹⁴ CO ₂	Leaf 2 (L_2)	17.4	267	70.0
¹⁴ CO ₂	Leaf 3 (L_3)	18.6	350	57.0
¹⁴ CO ₂	Leaf 4 (L_4)	20.8	329	68.0
Shading	Head ($'L_{F-x} + H + 'H$)	17.9		
	Subdivision of head contribution:			
Shading	$H + 'H$	10.6		
¹⁴ CO ₂ (Treatment (7) - (1))	$H + 'H$	9.5		
¹⁴ CO ₂ (Treatment (2))	$H + 'H$	10.9		
Shading	$'L_{F-x}$	7.3		

† NS, differences between contributions not significant.

‡ Least significant difference between efficiencies: $P < 0.05$, 9.1; $P < 0.01$, 12.5.

parts above leaf 5. The total contributions by the head and the leaves were all similar. Again, as in the previous experiment, the flag leaf was the most efficient per unit area (Table 3), but the relative order of the others was a little different in that on this occasion the efficiency of leaf 2 was similar to that of leaf 4.

A measure of the importance of the two components of the total head photosynthesis, assimilation of atmospheric carbon dioxide ($H + 'H$) and re-assimilation of translocated material ($'L_{F,\dots}$), is presented in the lower part of Table 3. The two independent estimates (by ¹⁴CO₂ and yield reduction) of the atmospheric carbon dioxide assimilation component ($H + 'H$) do not differ significantly. This component and the other head component, $'L_{F,\dots}$ (estimated by difference), have equal importance in the head's activity.

(f) Comparison of the [^{14}C]Carbon Dioxide and Shading Techniques (Experiment 3)

Plants from the same population as those of experiments 2a and 2b were used in a 3×15 randomized block to measure by shading the contributions to grain yield of the flag leaf ($L_F + {}^rL_F$) and leaf 4 ($L_4 + {}^rL_4$). The estimates were then compared with those available from experiment 2a. Leaves were shaded at three-quarter anthesis.

The effect of shading on grain yield, and the percentages of yield attributable to photosynthesis by the flag leaf and leaf 4, as calculated by yield reduction, are shown in Table 4. To compare these results with those of the concurrent experiment 2a, it is necessary to convert the measurements of contribution by the flag leaf (L_F) and leaf 4 (L_4), to values of $L_F + {}^rL_F$ and $L_4 + {}^rL_4$ respectively. It is assumed that the respiratory processes within the head are non-discriminatory as to the source of

TABLE 4
EXPERIMENT 3: GRAIN DRY MATTER YIELD OF PLANTS AND CONTRIBUTION BY LEAVES TO GRAIN YIELD AS ASSESSED BY $^{14}\text{CO}_2$ AND SHADING TECHNIQUES

Source	Grain Dry Matter Yield (g)†	Contribution to Grain Yield (%)		
		Shading Expt. 3	$^{14}\text{CO}_2$ Method Expt. 1	Expt. 2a
Flag leaf ($L_F + {}^rL_F$)	36.1	15.4 [‡]	17.2 ^a	19.8 ^a
Leaf 4 ($L_4 + {}^rL_4$)	35.7	16.7 ^{b‡}	31.2 ^c	22.8 ^b
Whole plant	42.9			

† Least significant difference between yields: $P < 0.05$, 3.78; $P < 0.01$, 5.18.

‡ ^a Flag yield contributions not significantly different. ^b Significantly less than value *c* ($P < 0.05$).

respiratory substrate, and therefore that the components of rL_F , rL_2 , etc. will be proportional to the ratio of substrate, L_F , L_2 , etc. which is available. The individual rL_F etc. values can be obtained by using the formulae:

$${}^rL_x = (L_x/L_{F,\dots}) \times {}^rL_{F,\dots}$$

These estimated values of the grain yield dependent on photosynthesis by the leaves are given in Table 4, and are compared with those obtained by the shading method. Corresponding estimates from the data of experiment 1 are also shown.

Estimates of the flag leaf contribution by the two methods do not differ significantly. The contribution by leaf 4, as measured in experiment 1, differs from that obtained by shading, but it also differs from that obtained in experiment 2a in which the same method was used. Those two experiments were conducted at different times, at which plants may have had different photosynthetic outputs down the profile. What is important, however, is the agreement between the estimates obtained by the two methods concurrently.

III. DISCUSSION

(a) *Contribution by Sites*

Assimilation by the head and upper four leaves was responsible for 93% of the grain yield. At anthesis, the lower leaves comprised 40% of the total leaf surface but contributed only 7% of the yield. During grain development some of the lower leaves senesced, but leaves 5, 6, and 7 remained green at maturity. Without measurements of photosynthetic activity, however, their potential for providing assimilate is not known. In wheat (Lupton 1966) and rice (Shen 1960), the lower leaves supply the lower stem tissues and roots, but it is not known to what extent the demand by these lower parts in sorghum competes with that of the grain.

Yield attributable to photosynthesis by the upper four leaves varied from 82% in experiment 2a to 87.6% in experiment 1, the remainder being due to direct assimilation by the head. Each of these leaves was of similar importance in grain dry matter production, although in experiment 1, leaf 4 produced a greater amount than any of the other three leaves. As previously mentioned, these two experiments were conducted at different times, and probably under different weather conditions, but no records were kept. While the environmental factors might alter somewhat the contribution from particular sites, as shown in wheat (Kriedemann 1966), the overall importance of the upper four leaves and the head, and the similar importance of these sites, is indicated in both experiments.

These upper four leaves differed in their efficiency of production per unit area. In both experiments the flag leaf was the most efficient and leaf 3 the least. The higher efficiency of the flag leaf may be due to both a higher rate of photosynthesis and a larger amount of its assimilate moving to the grain. Being uppermost, it has maximum illumination, and as sorghum leaves are not light-saturated at full sunlight intensities (El-Sharkawy, Loomis, and Williams 1967), it will have higher photosynthetic rates. Also, as a younger leaf, it may have a higher capacity.

It is not possible to assess the relative importance of factors such as light environment, leaf angle, leaf age, and relative role in supplying grain or other plant parts, in determining the efficiencies of contribution of various leaves down the plant to grain filling.

It is clear that the head of sorghum makes an important contribution. In experiment 2a it accounted for 18% of the yield, made up equally of two components, direct fixation of atmospheric carbon dioxide and re-fixation of respired carbon dioxide. These results accord with Kriedemann's (1966) findings for ear photosynthesis in wheat.

(b) *Techniques*

While radioactive carbon dioxide has been widely employed in general studies of photosynthetic movement, and has been used by a number of workers to indicate the source of grain dry matter in cereals, it has not been used to quantify the contribution from each site. It avoids the objection to yield reduction techniques that the balance of processes in the plant may be upset. While enclosure in Polythene bags reduces the light intensity, the light profile remains the same in plants subjected to all treatments. That $^{14}\text{CO}_2$ did not have a harmful effect on plant growth is indicated by grain dry matter yields, which were independent of the number of sites exposed

and thus of the amount of ^{14}C assimilated by the plant. This method does not attempt to measure rates of photosynthesis, but is only a relative measure of photosynthetic activity. The isotopic effect of preferential assimilation of $^{12}\text{CO}_2$ (van Norman and Brown 1952) should not bias the estimates, as each site would be similarly affected. ^{14}C gives a positive identification of the source of assimilate for the grain. The contribution by the ear from assimilation of atmospheric carbon dioxide and the contributions by the individual leaves can be accurately determined. However, as seen in experiment 2*b*, there are some situations in which the method cannot provide a complete analysis.

The method as used here is restricted in its use to single plants, the more convenient technique for field assessment being shading. Assuming that $^{14}\text{CO}_2$ gives an accurate assessment, results indicate that shading provides satisfactory estimates. While the method tends to underestimate the contribution by the leaves, the differences between these two techniques were not significant. There is, however, a much greater variance when shading is used, necessitating a large number of replicates.

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