THE PHLOEM OF THE WHEAT STEM IN RELATION TO REQUIREMENTS FOR ASSIMILATE BY THE EAR

By L. T. Evans,* R. L. Dunstone,* H. M. Rawson,* and R. F. Williams*

[*Manuscript received February* 13, 1970]

Summary

The cross-sectional area of the phloem and the number of vascular bundles at the top of the main stem were determined in 22 diploid, tetraploid, and hexaploid wheats and related wild species grown at 21/16°C in 16-hr days of high light intensity.

The number of vascular bundles was smallest in the wild diploids *Aegilops speltoides* and *Triticum boeoticum*, highest in the hexaploid *T. aestivum*. Differences between lines in bundle number bore no simple relation to spikelet number. Phloem area per stem was smallest in the two species of *Aegilops* (as low as $7 \cdot 6 \times 10^{-3}$ mm^2) and increased with evolutionary level to *T. aestivum*, with up to $79 \cdot 1 \times 10^{-3}$ mm^2.

For the range of lines examined the relation between phloem area and estimates of the maximum rates of import of assimilates by their ears was equivalent to a specific mass transfer rate of 3·3 g per square centimetre of phloem per hour, close to that found previously for dicotyledonous tuber stems and fruit peduncles.

With plants of Late Mexico 120 wheat grown in the standard conditions, increase in the duration of seed vernalization from 0 to 12 weeks halved the number of spikelets, grains, and grain weight per ear; the number of vascular bundles was reduced from 57·2 to 39·5, and the phloem area from 98·1 to $41 \cdot 2 \times 10^{-3}$ mm^2. Both the number and the size of phloem cells decreased. Thus, both bundle number and phloem area within a single line can vary over a considerable range in proportion to spikelet number and subsequent grain yield.

I. Introduction

Considerable attention is currently being given to the limitations on yield in wheat imposed by the rate of photosynthesis on the one hand, and on the other by the capacity of the ears to utilize assimilates. Ears of the wild diploid progenitors of wheat may be largely self-supporting, but with evolutionary advance there has been a marked increase in the amount of assimilate that must be translocated through the stem to meet the needs of grain growth (Evans and Dunstone 1970). There is the possibility, therefore, that the capacity of the stem to transport assimilates may limit grain development.

As a first step in assessing the likelihood of this limitation we have examined phloem development in the peduncle (the top internode) of the main stem of a number of species from various stages in the evolution of wheat, and that within one modern cultivar when spikelet and grain number per ear were varied over a twofold range by varying the duration of seed vernalization. We also examine the relation between phloem area and estimates of the maximum rate at which assimilates must be imported by the ear during grain growth.

* Division of Plant Industry, CSIRO, P.O. Box 109, Canberra City, A.C.T. 2601.

II. Materials and Methods

(*a*) *Plant Materials*

The lines examined were selected from among those used in other experiments in which ear development was related to ear and leaf photosynthesis (Evans and Dunstone 1970; Rawson 1970; Rawson and Evans, unpublished data). They are listed in Table 1. Eight lines were wild plants, 14 cultivated. Nine were diploid, four were tetraploid, and nine hexaploid. All plants were grown from seed which developed at 21/16°C.

Table 1*

PHLOEM CHARACTERISTICS AT THE TOP OF THE PEDUNCLE IN SOME WILD AND CULTIVATED WHEAT PLANTS GROWN IN LONG DAYS AT 21/16°C

Serial No.	Species and Line	No. of Spikelets	No. of Bundles	No. of Inner Bundles	$10^3 \times$ Phloem Area (mm^2)
		Diploid			
	T. boeoticum				
1	C64/145	21	23	10·5	30·0
2	6625	17·5	30	14	30·5
3	TBI	19·5	22	11	25·4
	T. monococcum				
4	W10	23	32	15	26·1
5	W292	22·5	33	16	29·7
	A. speltoides				
6	AS1	10·5	22	9	13·4
7	6001	6·5	20	8	7·6
	A. squarrosa				
8	G46	11	35	10·5	14·3
9	G90	8	28	10	12·4
		Tetraploid			
	T. dicoccoides				
10	W1043	14·5	33	13·5	30·0
	T. dicoccum				
11	W12	22·5	26	13	33·3
	T. durum				
12	W8	19	40	18·5	53·4
13	W9	20·5	39	19	52·6
		Hexaploid			
	T. aestivum				
14	Gabo	16·5	40	18·5	48·5
15	Late Mexico 120	19	46	21	54·4
16	Cappelle Desprez	20	46	21	76·8
17	Sunset	15·7	48	20·8	36·7
18	Mexico Triple Dwarf	14·7	48	22·5	45·4
19	Sonora	15·7	46	20·8	51·8
20	Nainari	17·0	55	23	68·0
21	Pitic	19·3	61	25	59·0
22	Mexico 120	27·2	54	25·5	79·1

* Statistical analysis of the data here and in Table 2 was carried out for most of the attributes listed, but is not presented because transformations were necessary in many cases, and because interest centred on the general trends. Transformations were not necessary for phloem area, and mean significant differences ($P = 0·05$) for the final six entries in Table 1, and for those of Table 2, were 7·9 and 10·6 respectively. The trends in other attributes are also well established.

(*b*) *Growing Conditions*

The plants were grown singly in 8-cm diameter pots containing a mixture of perlite and vermiculite, at 21/16°C in summer days of high light intensity extended to 16 hr with incandescent light of 50 f.c. intensity. They were given nutrient solution and water daily.

Peduncles and ears were taken from plants in three experiments, which differed in the duration of the vernalization treatment prior to planting out. The first experiment included all the diploid and tetraploid species, and three hexaploid cultivars—Gabo, Late Mexico 120, and Cappelle Desprez. In this the seedlings were vernalized by growing them at 7/4°C in 8-hr days for 10 weeks.

In the second experiment the imbibed seeds of six hexaploid wheats, Sunset and five Mexican cultivars, were held at 4°C for 3 weeks. In the third experiment only one cultivar, Late Mexico 120, was used and different lots of imbibed seed were kept at 4°C for between 0 and 12 weeks before planting out.

(*c*) *Histological Procedures*

At anthesis, the ear and the top 10 cm of the main stem were fixed in formalin–acetic acid–alcohol. Two replicates of all lines in the first experiment were fixed, and six of each cultivar or treatment group in the other two experiments. In the latter, the diameter at the junction of the peduncle and the ear was measured, and the four median peduncles were selected for sectioning.

A piece of stem 3 mm long was cut 1 cm below the top of each peduncle and kept for 24 hr in equal parts of 10% glycerol and 10% dimethylsulphoxide (DMSO) at room temperature, before embedding in 15% gelatin + 0·5% DMSO + 1% glycerol. Sections 10 μm thick were then cut in a freezing microtome at −26°C, and stained by the procedure of Sharman (1943) by which the phloem areas can be clearly delimited from the surrounding fibrous tissue. Using a projection microscope, outlines were drawn of the phloem tissue in all bundles within two large sectors of the stem section, and the area of these was subsequently determined over a grid.

(*d*) *Maximum Import of Assimilates by the Ears*

The rate of import of carbohydrate through the peduncle by the ears of all lines during the period of their most rapid grain growth was estimated as follows:

(1) The course of grain growth in main stem ears was measured by twice-weekly harvests of 4–6 replicates of the six hexaploid wheats in the second experiment (Rawson and Evans, unpublished data), and weekly harvests of 8–15 replicates in the experiment which included all the diploid and tetraploid species (cf. Evans and Dunstone 1970). In the latter case the maximum requirements for grain growth were estimated from the weekly interval in which growth was most rapid, while for the hexaploid experiment it was computed from regressions for changes in grain weight with time.

(2) Ear photosynthesis at 21°C was measured by gas analysis under light of 3200 f.c. intensity from fluorescent and incandescent lamps, at atmospheric CO_2 concentration, prior to each harvest. It was assumed that photosynthesis under the natural light prevailing during grain growth was approximately the same as that under light of 3200 f.c. for 11 hr each day. The justification for this assumption was that wheat plants growing under artificial light of 3200 f.c. intensity for 12 hr each day, at the same time and temperature as the plants in the glasshouse, increased in dry weight slightly faster than plants of the same cultivar in the glasshouse. The error introduced by this assumption is likely to be small, since the dominant term in the balance sheet is that for increase in grain weight.

The rate of dark respiration by the ears was also measured, and subtracted to give an estimate of net daily photosynthesis by the ears, which was converted from CO_2 to carbohydrate equivalents (by dividing by 1·6) and subtracted from the requirements for grain growth to give estimates of the net amount of carbohydrate imported each day by the ears. These estimates are used in the horizontal scale of Figure 2.

III. Results

Table 1 summarizes some of the measurements made on all lines, for the ears and peduncles which were preserved. There was some variation between individual plants of a line in the number of vascular bundles present, but this was small compared with the differences between lines, even within one species such as *Triticum boeoticum*. The number of bundles was lowest in *Aegilops speltoides* and highest in the hexaploid wheats. In all lines, half or less of the total number of bundles were the larger bundles of the inner ring, which project into the central pith (see Figs. 1 and 3). The remainder were the smaller bundles embedded in the peripheral cylinder of sclerenchyma between the bands of chlorenchyma. This chlorenchyma differed to some extent between lines, occupying a greater proportion of the stem surface in some (e.g. *A. squarrosa*, G90) than in others (e.g. *T. dicoccoides*, W1043), and having more tightly packed cells in some (e.g. *T. dicoccum*, W12) than in others (e.g. all forms of *Aegilops*).

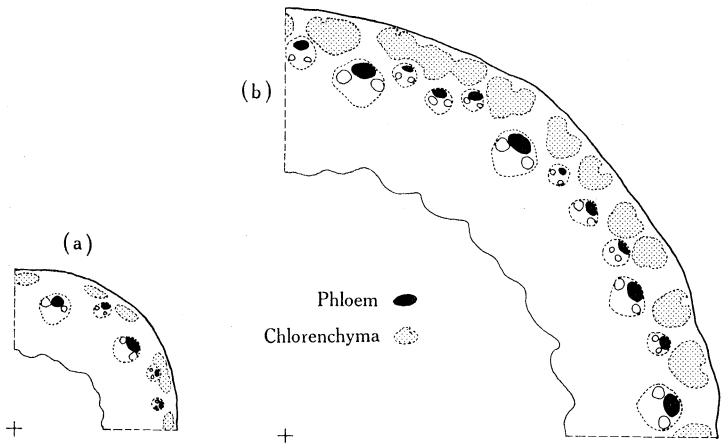


Fig. 1.—Line drawings based on cross sections at the top of the main stem of two extreme types—the diploid *Aegilops speltoides* AS1 (*a*) and the hexaploid Cappelle Desprez (*b*), both at the same magnification. The basic structure is similar in the two forms, but modern wheat has much larger stems, more bundles, and a relatively greater proportion of chlorenchyma. Within each vascular bundle only the phloem and the two metaxylem vessels are indicated.

The total number of bundles was almost always the same as the number of bands of chlorenchyma, but bore no consistent relation to the number of spikelets (cf. the three lines of *T. boeoticum*). Nor was there any simple relation between the number of inner or outer bundles and the number of spikelets (cf. *T. dicoccoides* and *T. dicoccum*).

Although there was only a threefold range between lines in bundle number, there was a tenfold range in phloem area per stem. This was smallest in the two species of *Aegilops*, largest in the hexaploid wheats and *T. durum*. As a proportion of the stem area, phloem area was least in *A. squarrosa* (less than 1%) and highest (3·1–3·5%) in *T. boeoticum*. Comparing only species of *Triticum*, the average phloem area per stem was highest in the hexaploids, least in the diploids.

The relation between total phloem area in the stem and the estimated maximum requirements for import of assimilates by the ears of the various lines is shown in Figure 2, and is discussed below.

Rawson (1970) has described the marked effects of prior vernalization on the ear of Late Mexico 120 wheat. As the results in Table 2 show, with increase in the duration of seed vernalization from 0 to 12 weeks, the number of spikelets, the number of grains, and grain weight per ear were approximately halved. The number of bundles

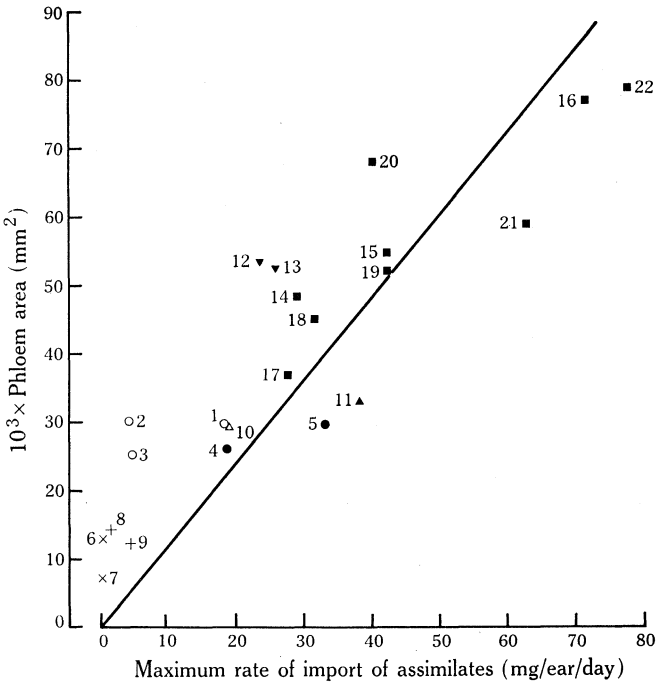


Fig. 2.—Relation between phloem area per culm and the estimated rates of import of carbohydrates by the ears of wild and cultivated wheats during most rapid grain growth. The method of estimating assimilate import by the ears is described in Section II(*d*). The line indicates the expected relation on the basis of calculations given in Section IV. ○ *Triticum boeoticum*; ● *T. monococcum*; × *Aegilops speltoides*; + *A. squarrosa*; △ *T. dicoccoides*; ▲ *T. dicoccum*; ▼ *T. durum*; ■ *T. aestivum*. Individual lines can be identified by reference to the serial numbers of Table 1.

was also reduced progressively with increasing vernalization, but the fall was only 31% for the total number and 34% for the number of large inner bundles. Total phloem area per stem, however, fell by 58%. The course of grain growth was not followed in these plants, but it is likely that imports of assimilate by the ears would be approximately proportional to grain number and weight. Phloem area in the peduncles of Mexico 120 wheat was also measured in the first and second experiments, after vernalization for 10 and 3 weeks respectively, and these agreed closely with those measured in comparably vernalized plants in the third experiment, as may be seen by

comparing Tables 1 and 2. For plants of Mexico 120 in the first two experiments the estimated import of assimilates by the ear during most rapid grain growth was approximately proportional to spikelet number (cf. Fig. 2), and is likely to have been so also in the third experiment. Thus, there was probably a close relation between ear requirements for assimilate and phloem area within this variety, in spite of the considerable differences in ear structure generated by the vernalization pretreatments in the third experiment.

TABLE 2

EFFECT OF DURATION OF SEED VERNALIZATION ON SOME CHARACTERISTICS OF THE EAR AND STEM OF LATE MEXICO 120 WHEAT

Vernalization (weeks)	Ear			Peduncle		
	Spikelet No.	Grain No.	Grain Wt./Ear (mg)	Total No. of Bundles	No. of Inner Bundles	$10^3 \times$Phloem Area (mm^2)
0	30·6	71·2	3346	57	26	98·1
2	28·1	62·2	2563	52	24	83·7
4	27·9	64·4	2906	52	25	83·2
6	25·2	60·2	2345	51	23	74·1
8	18·1	44·6	2012	45	21	60·3
10	16·8	38·0	1795	42	18	54·7
12	15·9	34·1	1667	40	17	41·2

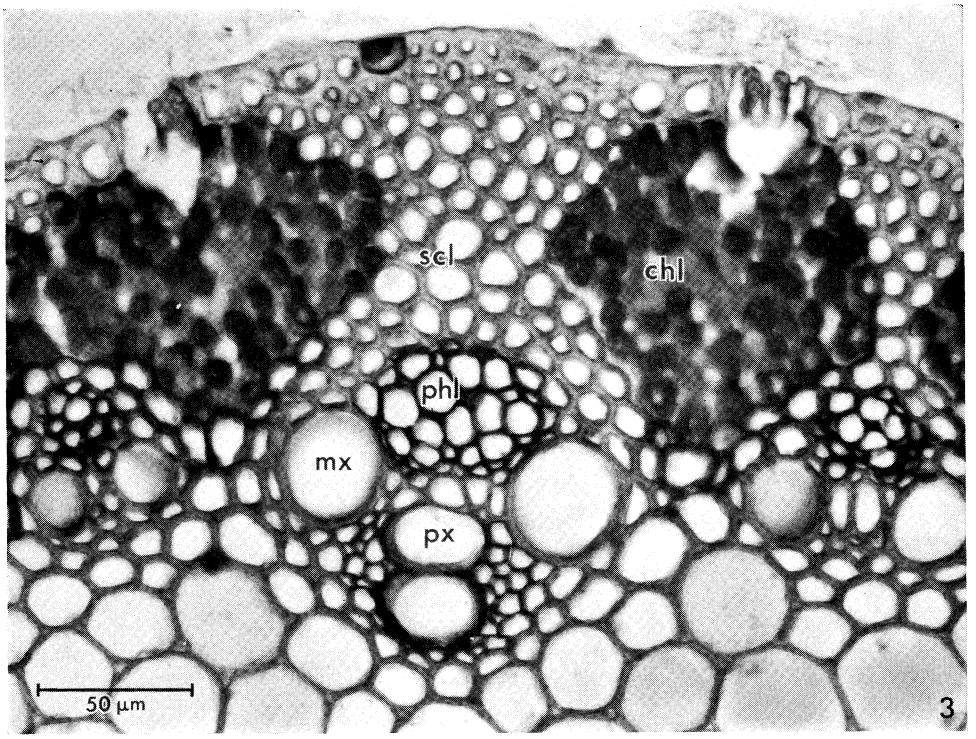
The modal area of both the large inner and the small outer bundles decreased progressively with increasing vernalization, and these changes involved both the number and the size of phloem cells. Mean cell numbers per inner bundle decreased from 23·4 in unvernalized plants to 18·4 in fully vernalized ones, and from 14·8 to 10·6 respectively in the outer bundles. Both decreases were significant at $P < 0·01$. In unvernalized plants the phloem areas of the inner bundles were composed of three or more irregular rows of large metaphloem vessels (Fig. 4). In fully vernalized plants, on the other hand, the phloem areas of the inner bundles were often elliptical in outline, with only two fairly regular rows of vessels (Fig. 5).

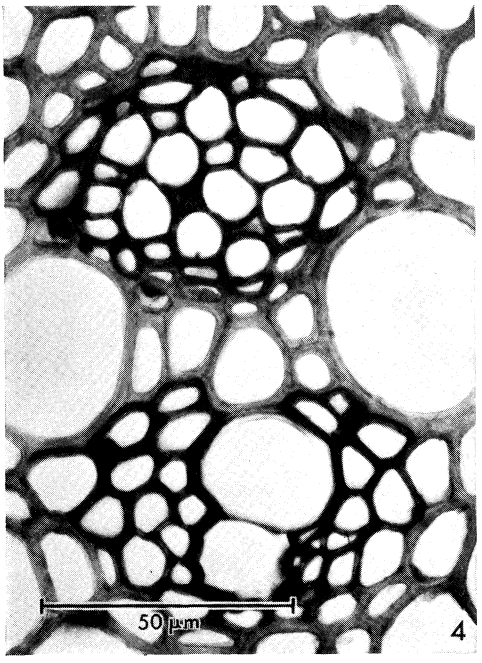
IV. DISCUSSION

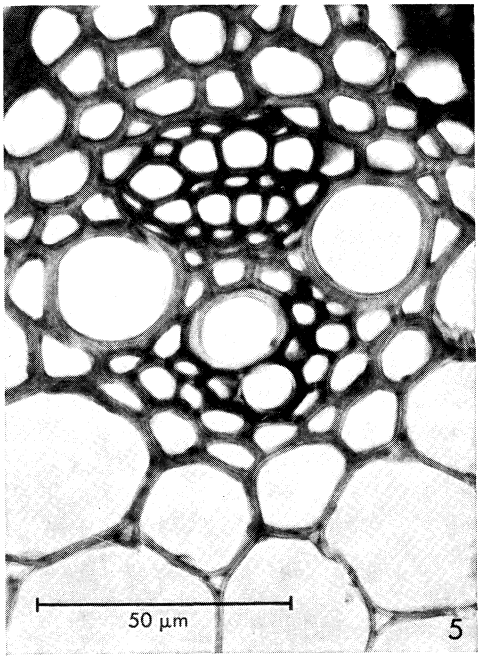
Blaringhem (1921) and Blaringhem and Miège (1913) considered the number of vascular bundles in the peduncle of various species of wheat to have diagnostic value in taxonomy. However, the differences we have found between lines of a species (eg. *T. boeoticum*) and even within one variety, depending on condition of growth, do not support their conclusion.

Fig. 3.—Segment of the stem of *Triticum boeoticum* (6625) with a large vascular bundle near the middle and smaller vascular bundles more closely associated with the areas of chlorenchyma.

Figs. 4 and 5.—Large vascular bundles of Late Mexico 120 wheat illustrating the differences in phloem structure associated with vernalization. The bundle in Figure 4 is from an unvernalized plant, that in Figure 5, with a smaller, more regularly arranged phloem area, is from a plant vernalized for 12 weeks. *mx*, metaxylem vessel; *px*, protoxylem; *phl*, phloem area; *chl*, chlorenchyma; *scl*, sclerenchyma.


scl
chl
phl
mx
px
50 µm
3


50 µm
4


50 µm
5

Examination of sections of young ears of wheat (*T. aestivum* cv. Nabawa) used by one of us for another purpose (Williams 1966) suggested that each spikelet usually added one major bundle to the complement of bundles in the rachis at that level. However, even in these ears, which were only 10 mm long, there were two major and from six to eight minor bundles which appeared to serve the rachis itself. The number of bundles at the top of the peduncle, though clearly dependent on the number of spikelets present, was always in excess of that number. This is also true for all of the lines of the present study, though the excess was slight in some lines (Nos. 1, 3, and 11 of Table 1), but very large in others (e.g. all the hexaploids).

From Table 2 it can be shown that there are about 25 bundles in excess of spikelet number and that this number is virtually independent of treatment within Late Mexico 120, even though there is a twofold range in spikelet number. This could mean that a single bundle tends to be related to each spikelet, and there is a constant number serving other purposes.

However, neither the total number of vascular bundles in the peduncle, nor the number of large, inner bundles, bore any simple relation to the number of spikelets. Nor was there evidence that spikelet number, determined before elongation of the top internode begins, is closely related to phloem area. That different bundles in the peduncle may have different roles in translocation remains an open question, and our distinction between "inner" and small peripheral bundles has not helped to elucidate this point. Further discussion of the relations between phloem area and the number of bundles, spikelets, and grains must await more information, especially that relating to the developmental anatomy of the ear.

Figure 2 indicates the relation for the various lines between the measured phloem area in the peduncle and the estimated maximum rates of daily assimilate import by the ears. The slope of the line is equivalent to a specific mass transfer rate of 3·3 g per square centimetre of phloem per hour, which is close to the average rate of 3·6 g per square centimetre of phloem per hour calculated by Canny (1960) from earlier experiments with tuber stems of *Solanum* and *Dioscorea* and fruit peduncles of *Cucurbita* and *Kigelia*. Thus, the relation between phloem area in the peduncles of a wide range of wild and cultivated wheats and the transfer of assimilates through them is similar to that measured in several very different dicotyledonous systems.

The relation between these two parameters was also estimated on the assumption that the translocation of assimilates involved mass flow of a sugar solution rather than surface movement of sugar molecules. The concentration of the sugar solution was assumed to be 10%. Aphid exudates from other plants (Mittler 1958; Weatherley, Peel, and Hill 1959) suggest this is a reasonable assumption, but much higher concentrations are sometimes found (Zimmermann 1969). Extrapolation of some results of Jenner (1968) on rates of starch synthesis in detached ears of Gabo in a range of sucrose solutions suggests that the rate in ears on intact plants would have been the same as that in ears on a solution of 11% concentration. However, movement to the detached ears may not have been only through the phloem. We further assumed that translocation continued for 24 hr each day, on the grounds that there is no evidence in wheat of a diurnal cycle of starch deposition, and because Jenner (1968) found detached ears on water in darkness for 24 hr to produce almost as much starch as ears provided with sucrose. The velocity of translocation was assumed to be 100 cm/hr,

since Wardlaw (1965) measured velocities of 87–109 cm/hr upwards through the culm of Gabo wheat at 21°C. The final assumption was that 33% of the measured phloem area was active in translocation, the remainder being occupied by cell walls, companion cells, crushed protophloem, and non-functional metaphloem. Earlier work with yams and cucurbits suggested that sieve tubes occupied about one-fifth of the phloem (Canny 1960), but grid counts over a number of phloem areas in peduncles of Late Mexico 120 wheat indicated that the lumina of metaphloem vessels occupied about one third of the phloem area.

The line drawn in Figure 2 indicates the relation between phloem area and ear imports based on these assumptions. The agreement between the estimated and the measured relations for the range of lines in no way establishes that translocation is by mass flow, but shows that, on reasonable assumptions, the phloem areas present could accommodate the required mass flow, in lines spanning all stages in the evolution of wheat. Although we cannot determine whether the extent of phloem development in the peduncle actually limits grain development, the data in Figure 2 suggest that phloem area is more likely to be limiting in some of the highly productive modern wheats than in the diploids where the amount of phloem in relation to ear needs is high.

The results presented in Table 2 indicate the considerable range in phloem area of the peduncle that can be induced within a cultivar by varying the growing conditions. As the data for the same cultivar in the first two experiments suggest, the extent of phloem development in Mexico 120 at anthesis appeared to be closely related to the likely rate of assimilate import required by the ear to support subsequent grain growth. The extent of phloem development could not have been determined directly by the demands of the ear, since it was measured before the demands were expressed. Presumably it was determined, at an early stage of development of the top internode, by an early differentiating determinant of yield. Spikelet number seems the most likely candidate. Although not closely related to phloem area in comparisons between genotypes, it was so within Late Mexico 120.

V. Acknowledgments

We are particularly grateful to Mrs. T. Wallace who prepared all the stem sections and measured the phloem areas. We also thank K. Finlay, G. Hollamby, A. T. Pugsley, J. R. Syme, I. A. Watson, and D. Zohary for supplies of seed, C. Totterdell for the photographs, I. F. Wardlaw for discussion of the results, and the Wheat Research Council for support.

VI. References

Blaringhem, L. (1921).—Sur les caracteres anatomiques des chaumes des genes *Triticum*, *Secale* et *Haynaldia*. *Bull. Soc. bot. Fr.* **68**, 563–9.

Blaringhem, L., and Miège, E. (1913).—Études sur les pailles de Blé. *C. r. hebd. Séanc. Acad. Sci., Paris* **157**, 1457–9.

Canny, M. J. (1960).—The rate of translocation. *Biol. Rev.* **35**, 507–32.

Evans, L. T., and Dunstone, R. L. (1970).—Some physiological aspects of evolution in wheat. *Aust. J. biol. Sci.* **23**, 725–41.

Jenner, C. F. (1968).—Synthesis of starch in detached ears of wheat. *Aust. J. biol. Sci.* **21**, 597–608.

MITTLER, T. E. (1958).—Studies on the feeding and nutrition of *Tuberolachnus salignus*. II. The nitrogen and sugar composition of ingested phloem sap and excreted honey dew. *J. exp. Biol.* **35**, 74–84.

RAWSON, H. M. (1970).—Spikelet number, its control and relation to yield per ear in wheat. *Aust. J. biol. Sci.* **23**, 1–15.

SHARMAN, B. C. (1943).—Tannic acid and iron alum with safranin and orange G in studies of the shoot apex. *Stain Technol.* **18**, 105–11.

WARDLAW, I. F. (1965) —The velocity and pattern of assimilate translocation in wheat plants during grain development. *Aust. J. biol. Sci.* **18**, 269–81.

WEATHERLEY, P. E., PEEL, A. J., and HILL, G. P. (1959).—The physiology of the sieve-tube. Preliminary experiments using aphid mouth parts. *J. exp. Bot.* **10**, 1–16.

WILLIAMS, R. F. (1966).—The physiology of growth in the wheat plant. III. Growth of the primary shoot and inflorescence. *Aust. J. biol. Sci.* **19**, 949–66.

ZIMMERMANN, M. H. (1969).—Translocation velocity and specific mass transfer in the sieve tubes of *Fraxinus americana* L. *Planta* **84**, 272–8.