Distribution and Remobilization of Iron and Copper in Wheat

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• *Background and Aims* The amount of iron (Fe) and copper (Cu) that is loaded into grains of wheat (*Triticum aestivum*) depends on both the amount of nutrient taken up by the plant post-anthesis and the amount that is remobilized from vegetative organs as they senesce. Previous reports have shown that these two micronutrients behave quite differently in wheat in that Cu is readily remobilized to the grain whilst Fe shows poor remobilization. The object was to quantify the distribution of Fe and Cu in wheat and to show how this distribution changes from anthesis to grain maturity.
• *Methods* The uptake and distribution of both Fe and Cu were investigated in wheat grown at two levels, adequate and low, of both micronutrients. Plants were grown in sand culture and the main culms were harvested at anthesis, 18 days post-anthesis and at maturity. Plants were separated into various organs and analysed for Fe and Cu using ICP-OES.
• *Key Results* There was good remobilization of Fe from the rest of the shoot to the grain with 77 % of the total shoot Fe in the grain at maturity. In the adequate-Cu treatment there was 62 % of the total plant Cu in the grain at maturity, whereas in the low-Cu treatment this was only 40 %. There was no net Fe taken up into the above-ground plant parts post-anthesis whilst for Cu there was. The remobilization evident for Fe and Cu was greater than that found for zinc and much greater than evident for manganese in the same material.
• *Conclusions* The results reported here represent good evidence for the high reproductive mobility of both Fe and Cu in wheat.

Key words: Iron, copper, wheat, distribution, remobilization, *Triticum aestivum*, zinc, manganese, grain.

INTRODUCTION

Deficiencies of essential micronutrients such as iron (Fe) and zinc (Zn) are widespread in humans. In many regions of the world, a large proportion of dietary intake of these nutrients is derived from grains. As grain levels of these micronutrients are generally low, increasing the micronutrient content of grains has been identified as a way of addressing human micronutrient deficiencies (Graham and Welch, 1996).

The amount of micronutrients [e.g. Fe, Zn, manganese (Mn) and copper (Cu)] in the grain will depend on the amount taken up by the roots during grain development and the amount redistributed to the grain from vegetative tissue via the phloem. The amount remobilized via the phloem is greatly dependent on the phloem mobility of each element. Pearson and Rengel (1994) investigated Zn and Mn transport into wheat grains and found that Zn showed good remobilization from leaves whilst Mn remobilization was poor. The poor remobilization of Mn was thought to be due to the poor phloem mobility of Mn. It should be noted that all nutrient transport into the grain must at some stage pass through the phloem due to xylem discontinuity in the grain stalk (O'Brien *et al*., 1985).

Iron has been described as having intermediate phloem mobility (Kochian, 1991). Averaged over all growth stages Fe generally has higher phloem mobility than Cu. However,

in field-grown wheat crops it has previously been shown that there is very limited Fe remobilization to the grain. Only 5 % of the shoot Fe in irrigated wheat at maturity was found in the grain compared with 32 % for Cu (Hocking, 1994). Miller *et al*. (1994) found that 20 % of the shoot Fe was in the grain at maturity in hard red spring wheat, whilst this figure was 60 % for Cu. Rice also appears to be very inefficient at transporting Fe to grain as only 4 % (cf. Cu 45 %) of total shoot Fe was found in the grain (Marr *et al*., 1995). Whereas wheat and rice appear to have poor Fe remobilization, other crops have good Fe remobilization. Zhang *et al*. (1995) found good remobilization of Fe out of mature leaves of bean. In peas as much as 75 % of the total shoot Fe was found in the seeds (Grusak, 1995).

Copper has been described as variably mobile in the phloem (Loneragan, 1981). It has earned this description because it can show all the symptoms of being phloem immobile (no remobilization from old to young tissue under deficiency) or phloem mobile (upon senescence of old leaves). The distribution and remobilization of Cu in wheat has been thoroughly investigated by Hill and co-workers (Hill *et al*., 1978, 1979*a–c*). In this series of papers it was found that Cu was remobilized from senescing leaves in conjunction with nitrogen. In this way the majority of total plant Cu at maturity was found in the grain.

This study is an investigation into the distribution of Fe throughout wheat plants, how this distribution changed during grain filling, and how varying the availability of Fe influenced these processes. At the same time Cu was studied as a comparison.

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TABLE 1. *Growth characteristics of plants with different iron and copper treatments*

	Harvest	Non-grain weight (g)	Grain weight (g)	Grain number	Individual grain weight (mg)	Days to anthesis
Control	Anthesis	4·16 (0·14)				58·8 (0·6)
	Anthesis + 18 d	4·11 (0·12)	2·30 (0·19)	115·3 (1·7)	20	
	Maturity	3·51 (0·04)	3·36 (0·03)	115·5 (3·1)	29	
No added Fe	Anthesis	3·87 (0·10)				57·7 (0·6)
	Anthesis + 18 d	3·86 (0·12)	2·13 (0·10)	113·3 (2·1)	19	
	Maturity	3·55 (0·11)	3·34 (0·10)	114·3 (1·9)	29	
Low Cu	Anthesis	2·03 (0·08)				62 (0·7)
	Anthesis + 18 d	1·78 (0·16)	0·96 (0·16)	75·3 (8·3)	13	
	Maturity	2·00 (0·15)	1·66 (0·21)	81 (6·6)	20	

Numbers in parentheses are s.e.m. ($n = 4$).

MATERIALS AND METHODS

Wheat (*Triticum aestivum* 'Warigal') seeds were surface sterilized by soaking in 70 % ethanol for 1 min followed by 5 min in NaClO (1 % Cl). Seeds were then rinsed thoroughly and germinated in aerated Millipore H_2O for 48 h. Five seeds were planted into individual pots ($27 \times 9 \times 9$ cm) containing 3·2 kg of siliceous sand (Laffer sand) collected from undisturbed scrub soil near Tintinara, South Australia (Thongbai *et al.*, 1993). The sand was sieved (2 mm stainless-steel mesh) to remove organic matter, washed six times in reverse-osmosis H_2O, and then air-dried. After this treatment the typical micronutrient concentrations of the soil were (DTPA-extractable, mg kg^{-1}): Fe, <1; Cu, 0·02; Zn, 0·06; Mn, 0·20. Nutrients were supplied as solutions at watering. Macronutrients were supplied to the sand as two additions, one initial and one at 6 weeks, totalling (in mg pot^{-1}): NH_4NO_3 (750), K_2SO_4 (260), $MgSO_4.7H_2O$ (150), $CaSO_4$ (91) and KH_2PO_4 (231). Micronutrients were added to pots in one initial addition amounting to: (in mg pot^{-1}) $MnSO_4$ (17·5), $H_2MoO_4.H_2O$ (1·40), H_3BO_3 (7), $CoSO_4.6H_2O$ (1·32), NaCl (20·3) and $ZnSO_4$ (26·43). Cu and Fe treatments were applied as either (in mg pot^{-1}): control (added Fe and Cu) – $CuSO_4.5H_2O$ (15·72) and $FeSO_4.7H_2O$ (21); no added Cu – $FeSO_4.7H_2O$ (21); and no added Fe – $CuSO_4.5H_2O$ (15·72). The no-added-Cu plants were growing so poorly at 5 weeks that some Cu ($CuSO_4.5H_2O$, 1·57 mg pot^{-1}) was added with the second addition of macronutrients to avoid the possibility of no grain yield (Graham and Pearce, 1979). The no-added-Cu treatment was thus referred to as the low-Cu treatment. The sand in pots was maintained at close to 12 % water content (w/w) with Millipore H_2O. Plants were grown in a temperature-controlled growth room with a 21/15 °C, 14/10 h day/night cycle with light supplied at 600 μmol m^{-2} s^{-1} PAR by high pressure sodium lamps (Philips SON-T Agro 400W, Philips, Osnabrück, Germany). Five days after planting the plants were thinned down to two plants per pot.

The main culms of the plants were harvested at anthesis, 18 days post-anthesis and at maturity. Main culms were separated into different plant parts and dried at 80 °C for 48 h. Dried plant material was weighed and digested using a nitric acid/perchloric acid method (Zarcinas *et al.*, 1987). Iron and Cu concentrations were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES: ARL 3580 B, ARL, Lausanne, Switzerland).

RESULTS

Growth

There was no difference in the growth characteristics (grain yield, total biomass, days to anthesis) between the control and no-added-Fe plants (Table 1). Low-Cu plants had lower total plant matter and lower grain yields than the control and no-added-Fe plants. The grain weight at maturity of the low-Cu plants was one-third less than the control or no-added-Fe plants. The low-Cu plants also took longer to reach anthesis than the control and no-added-Fe plants. Senescence associated with maturation was delayed in low-Cu plants. In these plants there were no dead leaves at anthesis, whilst in the control and adequate-Cu plants at anthesis only the flag leaves showed no sign of senescence.

Fe and Cu Concentration

As with the growth characteristics, at no stage was there any difference in tissue Fe concentration between the two Fe treatments, control and no Fe added. Only the Fe added results are shown (Fig. 1). The highest Fe concentrations were in the lower leaves and flag leaves at anthesis. All plant parts, apart from the grain, showed large decreases in concentration between harvest 1 and harvest 3. This was most evident in the peduncle, flag leaf and rachis where there was up to a ten times reduction in the Fe concentration. The tissue with the lowest concentration was the stem but even this concentration was greatly reduced between harvests 1 and 3.

Unlike the Fe treatments, there were large differences in tissue Cu concentrations between the high and low-Cu treatments (Figs 2 and 3). The difference in tissue concentration was generally a factor of 10, reflecting the differences in Cu supply between the high and low treatments (15·72 cf. 1·57 mg pot^{-1}). In the low-Cu plants, there was almost no difference in tissue Cu concentration between harvests, whilst in the high-concentration plants there was a reduction between harvests. The greatest decrease in Cu concentration was seen in the peduncle. The Cu levels in the rachis of the
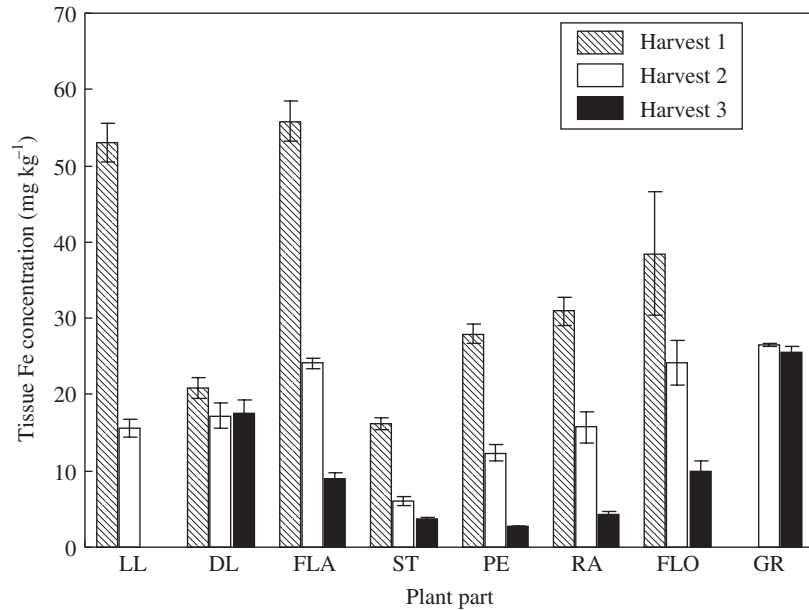
FIG. 1. Iron concentration in tissues of control plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are: LL, lower leaves, DL, dead lower leaves; FLA, flag leaves; ST, stems; PE, peduncle; RA, rachis; FLO, florets; GR, grain.
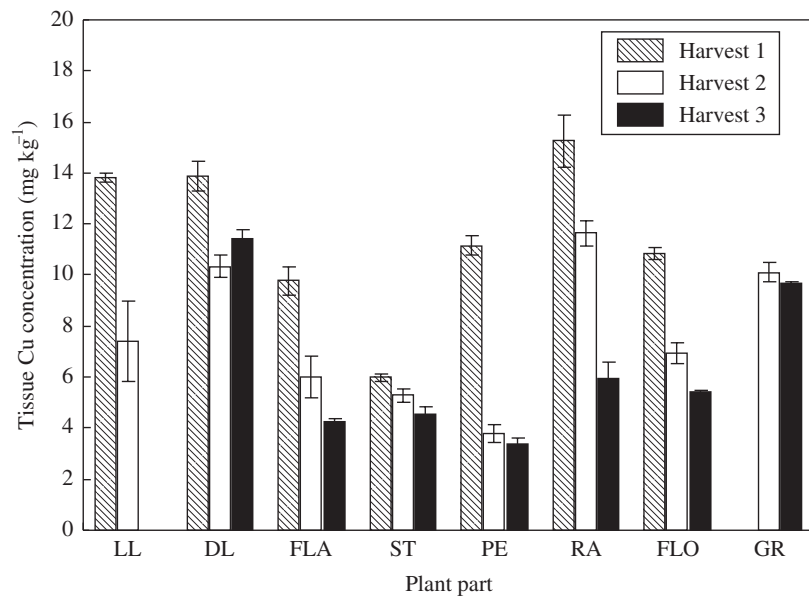

FIG. 2. Copper concentration in tissues of control plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.

low-Cu treatment were so low as to be below the detection limit of the ICP-OES. It would appear that the peduncle of the control plants showed the earliest remobilization of Cu as the peduncle concentration was at harvest 3 levels by harvest 2. The Cu concentration in the grain of the low-Cu treatment was the lowest of any tissue, as compared with the control treatment where the grain Cu concentration was almost as high as any other plant tissue.

Tissue Fe concentrations in the control treatment were generally two to five times higher than tissue Cu concentrations (Figs 1 and 2). The variation in Fe concentrations between plant parts that was evident, say between the flag leaf and the stem, was much greater than the variation in Cu concentrations. The reduction in concentration between harvest 1 and harvest 3 was also less for Cu in the control plants than for Fe in the same plants. The comparison between Fe and Cu concentration of the dead and live leaf tissue is made difficult because there is a gradient between when a leaf is either live or dead (i.e. a leaf which is half necrotic). Hence, the best indicator of leaf
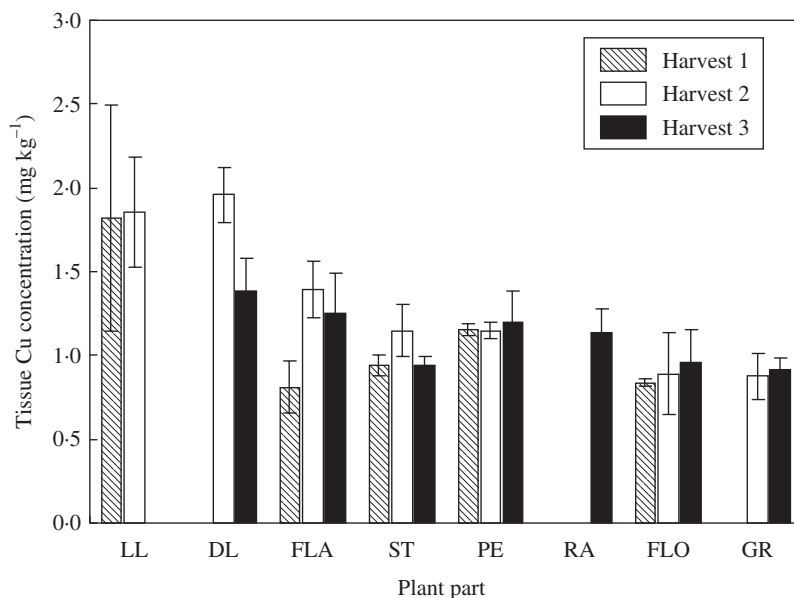
FIG. 3. Copper concentration in tissues of low copper plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.
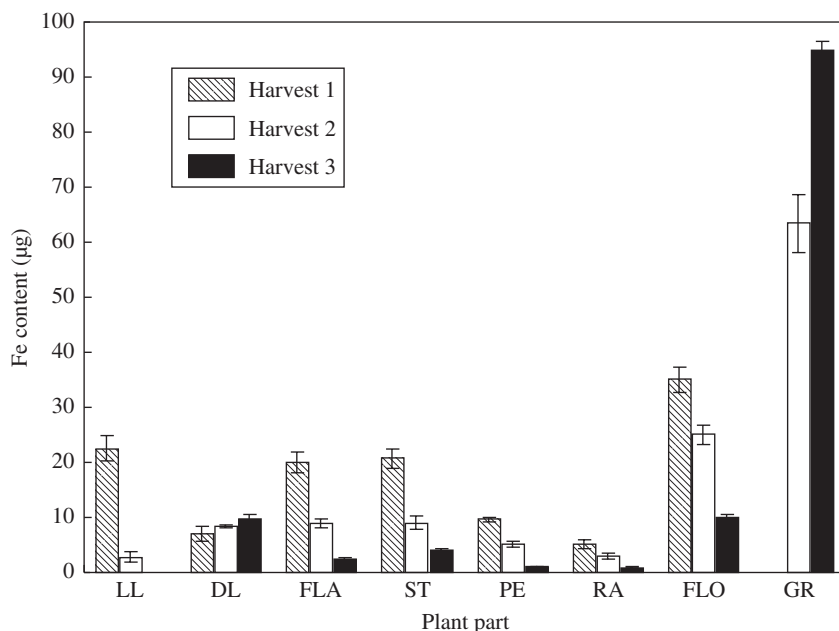

FIG. 4. Iron content of tissues of control plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.

tissue content/concentration is perhaps the flag leaf, which generally showed no sign of necrosis at harvest 2.

Fe and Cu Content

Calculation of Fe and Cu content of whole organs allows the assessment of the extent of remobilization to and from organs. In terms of content, the grain contains by far the most Fe and Cu in the mature plant (Figs 4 and 5). Although the grain concentration did not change between harvests the grain content did (Figs 4–6). Between harvests 2 and 3, in the control treatment the Fe and Cu content of the grain increased by 30 %, whilst in the low-Cu treatment the increase was almost 50 %. As the concentration of Fe and Cu did not change in this time (Figs 1–3), this implies that the Fe and Cu were loaded into the grain at the same rate as the development of the grain.

Timing of uptake of Fe and Cu differed markedly (Table 2). In the control treatment, the total amount of Fe in the tissue did not differ between harvests 1 and 3.
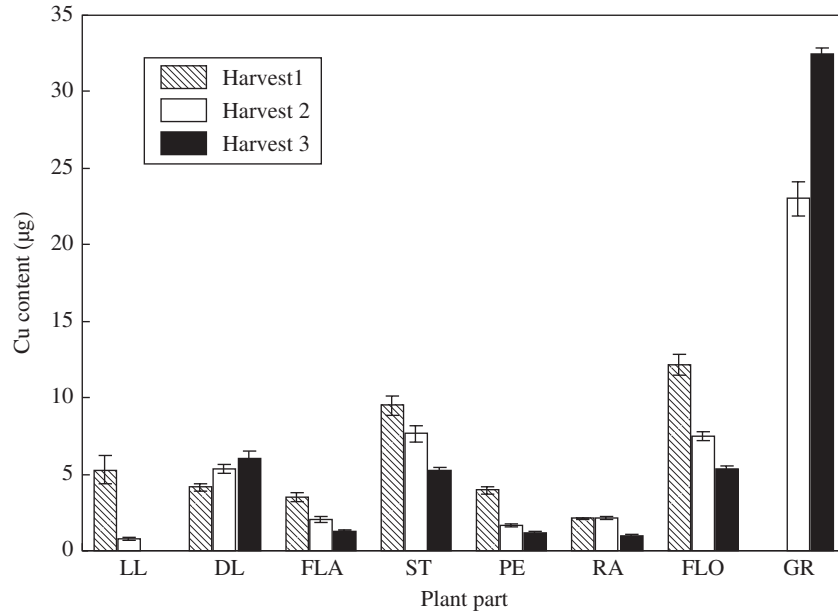
FIG. 5. Copper content of tissues of control plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.
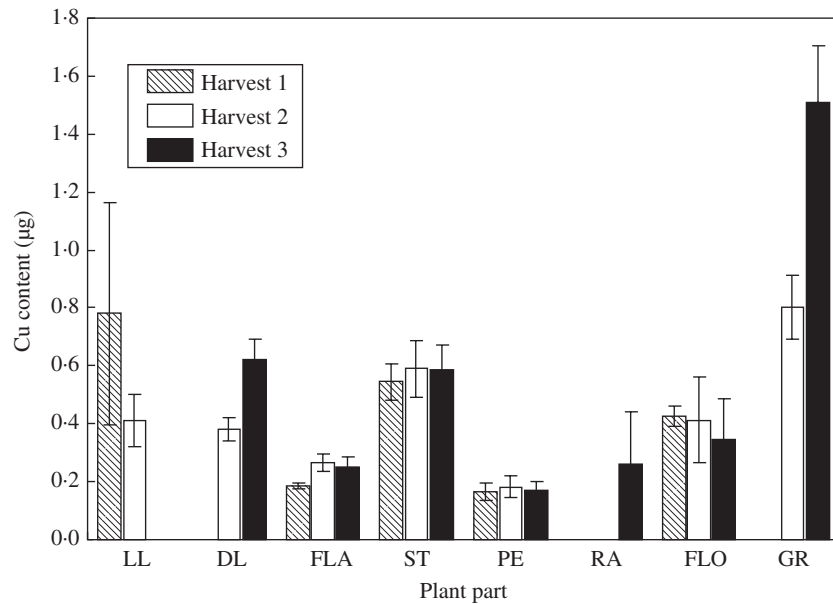

FIG. 6. Copper content of tissues of low copper plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.

The amount of Fe remobilized from the maternal tissue is enough to account for the Fe content of the grain, and the plant took up no new Fe. The Cu in the aerial parts of plants kept increasing post-anthesis. In the control treatment the total Cu increased between harvests 1 and 2 but not after that. In the low-Cu treatment the amount of Cu increased steadily between anthesis and maturity. This Cu was either being remobilized from the roots or taken up from the soil by the roots and new to the plant. The majority of the grain Fe was remobilized from the non-ear parts of the plant. Only 20 % of the grain content came from within the head itself. There was no net remobilization of Cu from vegetative parts of the shoot to the grain at all in the low-Cu treatment.

As a proportion of total metal in the plant within the grain, Fe was highest with 77 % of the plant Fe located within the grain 77 % (Table 2). For Cu this amount was less, with the high Cu plants having 62 % of the plant Cu in the grain. The low-Cu treatment had only 40 % of the total plant Cu located in the grain, and this came essentially from below ground during grain fill. For the control treatment,

TABLE 2. *Nutrient content of wheat shoots at three harvests expressed as: total content; content at each harvest as a percentage of total content at maturity; and content in plant parts as a percentage of the total at each harvest*

Treatment	Control Fe			Control Cu			Low Cu		
Harvest	1	2	3	1	2	3	1	2	3
Total content (µg)	120	125	123	41	50	52	2·5	3·0	3·7
% maturity	98	102	100	77	95	100	66	81	100
% plant part									
LL	19	2	0	13	2	0	32	13	0
DLL	6	7	8	10	11	12	14	13	17
(TLL)	(25)	(9)	(8)	(23)	(12)	(12)	(46)	(26)	(17)
FLA	17	7	2	9	4	2	8	9	7
ST	17	7	3	23	15	10	22	19	16
PE	8	4	1	10	3	2	7	6	5
RA	4	2	1	5	4	2	0	0	7
FLO	29	20	8	30	15	10	17	14	9
GR	0	50	77	0	46	62	0	26	40

Plant parts: LL, lower leaf; DLL, dead lower leaf; TLL, combined live and dead lower leaves; FLA, flag leaf; ST, stems; PE, peduncle; RA, rachis; FLO, florets; GR, grain.

TABLE 3. *Mn and Zn content of wheat shoots at three harvests expressed as: total content; content at each harvest as a percentage of total content at maturity; and content in plant parts as a percentage of the total at each harvest*

Treatment	Control Mn			Control Zn		
Harvest	1	2	3	1	2	3
Total content (µg)	565	708	796	232	342	397
% maturity	71	89	100	77	95	100
% plant part						
LL	18	3	0	8	1	0
DLL	30	31	28	14	11	8
(TLL)	(48)	(34)	(28)	(22)	(12)	(8)
FLA	5	7	7	6	2	2
ST	32	15	14	40	35	30
PE	7	4	3	8	3	8
RA	0	2	2	3	4	2
FLO	7	11	20	21	9	8
GR	0	27	27	0	35	42

Plant parts: LL, lower leaf; DLL, dead lower leaf; TLL, combined live and dead lower leaves; FLA, flag leaf; ST, stems; PE, peduncle; RA, rachis; FLO, florets; GR, grain.

a considerable part of the plant content of both Fe (29 %) and Cu (30 %) at anthesis (harvest 1) was in the floret. By maturity (harvest 3), this amount in the floret dropped to 8 % for Fe and 10 % for Cu.

Zn and Mn

Zinc and manganese content of the tissue was measured as part of the tissue analysis and provide useful comparisons with Fe and Cu. Both Mn and Zn had a lower proportion of the total plant content in the grain (27 % and 42 %, respectively; cf. Fe, 77 %; Cu, 62 %) (Table 3). For Zn this is mainly due to the high levels of Zn in the stems (Fig. 7). The amount of Zn in the grain was five to ten times greater than most plant parts but the stem levels were of almost the same

magnitude as the grain. For Mn (Fig. 8) the grain content was similar to the dead leaves, stems and florets. Compared with Fe and Cu, both Mn and Zn showed minimal remobilization. The Mn in the florets kept increasing throughout grain filling. There appeared to be some Zn remobilization from the lower leaves and the florets following the first harvest but this amount was small compared with the total amount in the plant. For Mn there was a drop in the stem content after the first harvest, but the general trend was for no remobilization and increasing content. This trend is most evident in the florets where the content at maturity was three times the level at anthesis. There was as much Mn in the dead leaves at maturity as there was in the grain.

DISCUSSION

Growth

The reduction in growth of the low-Cu plants is indicative of the very low Cu levels in the Laffer soil used. Based on the reduced yield it is postulated that the low-Cu plants were just above that level where Cu deficiency-induced sterility occurs (Graham and Pearce, 1979). That the low-Cu treatment was Cu deficient means that caution must be applied when comparing Fe levels in this treatment with the control treatment.

Tissue concentrations

The tissue Cu concentrations in the control treatment were comparable to those found in other pot-grown (Hill *et al*., 1978; Loneragan, 1981), as well as field-grown, wheat (Hocking, 1994). Copper concentrations in these plants were well above the critical levels suggested in the literature (Reuter and Robinson, 1997). In the low-Cu treatment the Cu concentrations were around the critical level given for youngest expanded blades (Graham and Pearce, 1979; Reuter and Robinson, 1997),
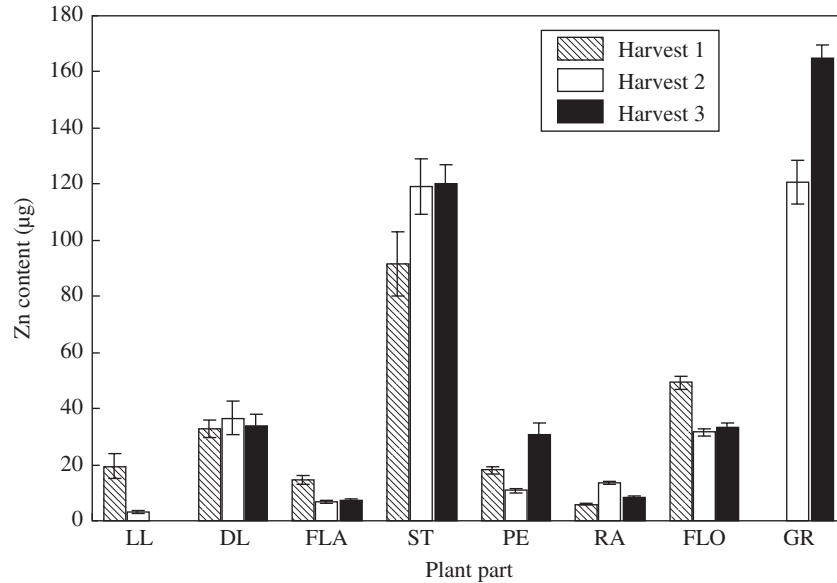
FIG. 7. Zinc content of tissues of low copper plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.
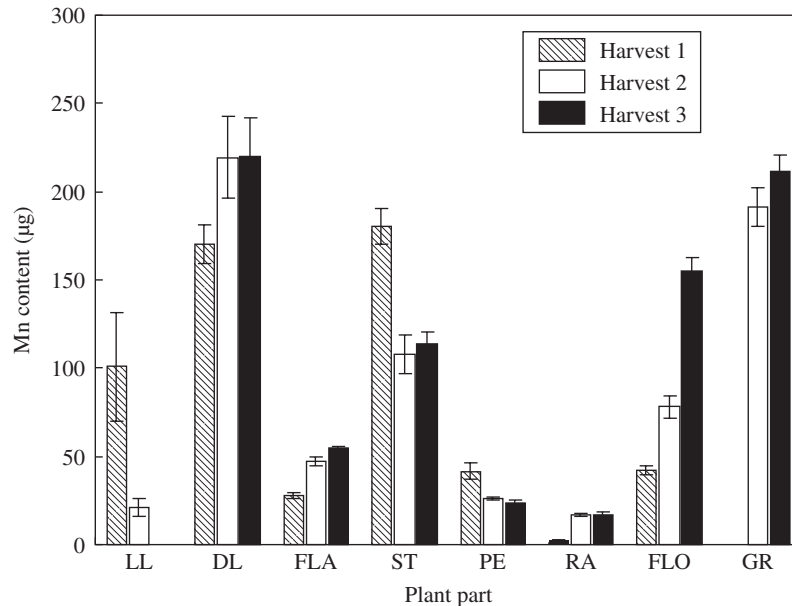

FIG. 8. Manganese content of tissues of low copper plants when harvested at anthesis (harvest 1), 18 days post-anthesis (harvest 2) and maturity (harvest 3). Values are the means of four replicates (±s.e.m.). Plant parts are as in Fig. 1.

although all leaves in this study were physiologically considerably older. Between the control and low-Cu treatments the seed concentrations showed a similar pattern to that found by Loneragan (1981) where seed content in adequate-Cu plants was 10-fold higher than in deficient plants.

That neither the tissue Fe concentrations nor plant growth differed between the control and no-added-Fe treatment highlights both the inefficiency of fertilizing with $FeSO_4$ and the Fe efficiency of wheat. It is assumed that all the Fe^{2+} added would have been oxidized to Fe^{3+} and precipitated within a few days leaving both Fe treatments basically the same in terms of Fe availability. The $FeSO_4$ was used to avoid chelates such as EDTA, which have been shown to enter plants (Vassil *et al*., 1998) and thus could affect Fe distribution and mobilization. However, adding $FeSO_4$ only showed that there was indeed no advantage to adding $FeSO_4$. The Fe content of the soil was low but high enough to allow non-Fe-limited growth. Iron concentrations in plant tissue fell within the adequate range (Reuter and Robinson, 1997) but were between five and ten times lower than field-grown wheat (Hocking, 1994;

Miller *et al.*, 1994), and possible reasons for this difference are discussed below.

Remobilization

Apart from the grain, the concentration of Fe in all plant organs dropped over time during grain filling, as expected, due to reproductive remobilization. This was evident in all tissues, but particularly in the peduncle, flag and rachis where there was up to a 10-fold reduction in the Fe concentration. This pattern was also evident with Cu in the control treatment but not to the same extent. Based on these results, both Fe and Cu would be described as phloem mobile. Both Fe and Cu have been described as intermediately mobile (Kochian, 1991; Marschner, 1995). Copper is generally considered phloem immobile unless it is linked to senescence whereupon it becomes mobile (Loneragan, 1981). Iron is thought to be more mobile than Cu and can be redistributed to seeds (Grusak, 1994), although, like Cu, Fe retranslocation can be assisted by senescence (Zhang *et al.*, 1995).

The Fe distribution and remobilization patterns found were very different from those found with field-grown wheat. In two field studies, Fe appeared to be phloem immobile and there was no apparent reproductive mobilization with Fe concentrations being highest in dead tissue (Hocking, 1994; Miller *et al.*, 1994). This pattern of little reproductive mobilization was also evident in field-grown rice (Marr *et al.*, 1995). In the present results the tissue concentration, in the flag leaves for example, showed a drop in concentration of approx. 5-fold between anthesis and maturity. Iron concentration was reduced by a factor of approx. 2·5 between dead and live leaves. In the field-grown plants of Hocking (1994), the Fe concentration in the dead leaf tissue at anthesis was 2·3 times higher than live leaf tissue and only 5 % of the total Fe in the above-ground tissue was found in the grain. It is important to note that, although the non-grain tissues in the study of Hocking (1994) were much higher than in the present study (dead leaves 948 mg kg^{-1} *vs.* 20 mg kg^{-1}), the grain concentrations were the same (26 mg kg^{-1}). The differences between field-grown wheat and those in the present experiments appear similar to the comparison between wild-type peas and a hyper-accumulating mutant pea (Grusak, 1994). In the hyper-accumulating mutant, leaf levels were raised 35-fold with no increase in seed content.

There are a number of plausible reasons to explain the low Fe mobilization to the grain in the field-grown plants with high Fe levels. Firstly, the Fe levels may have been high enough to be toxic. The Fe levels in the leaves in the present study ranged from 55 µg g^{-1} d. wt in live leaves to 20 µg g^{-1} d. wt in dead leaves. In dead leaf blades in the study of Hocking (1994) the Fe concentration was as high as 948 µg g^{-1} d. wt. The dead leaves in the study of Miller *et al.* (1994) also had high (415 µg g^{-1} d. wt) Fe levels. These levels in wheat tissue have been classed as high (Reuter and Robinson, 1997) and 500 µg g^{-1} d. wt has lead to toxicity symptoms in rice (Yamauchi, 1989). Plants can buffer internal Fe using mechanisms such as ferritin (Briat and Lobreaux, 1997). However, the amount of Fe buffering capacity must be limited and, at high concentrations, Fe could be detoxified by precipitation in the apoplasm (Becker

et al., 1995). It seems unlikely that internally precipitated Fe could be remobilized and this may lead to the apparent non-remobilization in the field-grown crops. The amount of Fe that is remobilized would be the Fe stored in normal physiological forms (active Fe), whilst that not remobilized would represent the precipitated Fe (inactive). Although this is feasible, is seems more likely to occur in flooded rice where Fe availability is higher than in wheat.

It could also be that the apparent lack of mobilization was related to saturation of either the grain loading or phloem loading process. Due to xylem discontinuity in the grain stalk (O'Brien *et al.*, 1985) Fe transported into the wheat grain must do so via a phloem step. However, if it were the grain loading process that was saturating then it would be expected that there would still be a drop in concentration of the leaves and increases in Fe content of the non-grain floral structures which was not evident. Remobilization of Fe from the leaves is dependent upon it being loaded into the phloem. Phloem loading in the non-grain tissue may have been restricted by availability of endogenous chelates (Grusak, 1994).

Another reason for the distribution patterns of Fe in the field studies could be that soil was contaminating the plant tissue. It has long been known that contamination can lead to erroneously high Fe in leaf tissue analysis and that rinsing in detergent can reduce this contamination (Nicholas *et al.*, 1957). Soil dust is high in Fe and only small amounts would lead to erroneously high Fe levels. It appears reasonable to assume that, especially nearing maturity, the field-grown plants under drying conditions at the beginning of summer would have been exposed to high amounts of dust. Both Hocking (1994) and Miller *et al.* (1994) washed leaves briefly in deionized water. It is unclear as to how much contaminant this process would have removed. If surface contaminant were left on the tissue then this would explain the high levels of Fe.

An observation that supports the contamination theory is the post-anthesis uptake of Fe by the aerial parts. In field-grown wheat the percentage of total plant Fe accumulated by anthesis was only 60 % (Hocking, 1994), whilst in the present study no further Fe was accumulated post-anthesis. With the continued uptake of Fe post-anthesis in the field-grown wheat there would be no need for remobilization from other tissue. However, once again it is possible that the continued increase in tissue Fe may be caused by dust contamination. Drying conditions at the end of the season would increase the likelihood of tissue Fe levels being increased by contamination.

The remobilization of Cu was minimal in the low-Cu treatment. The levels of Cu in the tissue were at or below the critical level for wheat (Robson *et al.*, 1984). Their value of 1·3 µg g^{-1} is for the youngest expanded blades, which should be similar to the flag leaf at anthesis in the present study. It seems likely that the concentration in the tissue was so low that the Cu could not be remobilized at senescence. Loneragan (1981) suggested that perhaps this Cu is bound in a structural form that cannot be remobilized. As the Cu levels were low in all the tissues, including the grain, it would seem that the tissue had the minimum amount of Cu that was physiologically possible.

Compared with Zn and Mn

The behaviour or Fe and Cu in this case was different to that of Zn and Mn. The behaviour of Zn was closer to Fe and Cu in that it did show some remobilization but certainly not of the same magnitude of Fe and Cu. For both Zn and Mn most of the grain content at maturity can be accounted for by Mn and Zn entering the shoots after anthesis, whether this be from uptake new to the plant or remobilization from the roots. The high Mn levels in the leaves and florets and the floret Mn increasing throughout the harvests are symptomatic of transport of Mn to the endpoints of the xylem stream with no concurrent retranslocation in the phloem. The amount of shoot Mn and Zn contained in the grain at maturity (nutrient harvest index) was similar to that found in field-grown wheat by Zubaidi *et al.* (1999).

It is useful to compare the results for Mn and Zn with those found by Pearson and Rengel (1994). Their study was different from the present study in two major ways in that, firstly, the levels of Mn and Zn were lower and, secondly, in their study the roots were removed from soil at anthesis and transferred to solution culture containing no Mn and Zn. Pearson and Rengel found that zinc was remobilized from vegetative parts. They also found the stems contained considerable Zn at anthesis and this Zn was readily remobilized to the grain. This result, together with the present results, suggests that the stem is a store of Zn that can be utilized when Zn uptake from the roots is low and also that the stem is the main location of Zn loading into the phloem. The results for Mn are directly comparable and highlight the phloem immobility of Mn.

Conclusions

These results show that, in wheat plants, there can be good reproductive remobilization of Fe and Cu to the grain. When comparing the present results with field studies it appears that increased Fe availability in the soil does not increase its content in the grain. This implies that simply increasing Fe levels by increased root uptake capacity or foliar fertilization would not be an effective way of increasing grain levels of Fe. As the phloem transport pathway works then it is clear that breeding for better expression of the phloem pathway would be the best approach to improve the nutritional value of wheat grains and, by implication, other cereal grains. The characterization of the phloem transport pathways Fe in and Cu in wheat is the subject of further work.

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