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Diagnosis of Copper Deficiency in Wheat by Plant Analysis

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Abstract

A glasshouse experiment was conducted to define critical concentrations of copper in young leaves of wheat and to investigate the effect of water stress after anthesis on the relationship between yield and copper concentrations in young leaves. The concentration of copper in the youngest fully emerged leaf was a sensitive and accurate indicator of the copper status of wheat. The critical concentration for copper in the youngest fully emerged leaf did not change with the age of the plant. Copper deficiency occurred whenever the concentration of copper in the youngest fully emerged leaf fell below 1·3 $\mu g\ g^{-1}$ (dry weight). Water stress after anthesis did not change the relationship between copper concentrations in young leaves and grain yield, although this stress markedly decreased grain yield.

In the field there was considerable variability among plants given the same copper treatment in copper concentrations in young leaves. Nevertheless, whenever copper deficiency decreased growth, the average concentration of copper in the youngest fully emerged leaf was less than 1·3 $\mu g\ g^{-1}$.

Introduction

A diagnostic procedure for copper deficiency in cereals would be valuable. Firstly, such a procedure would assist where cereals are grown on copper-deficient soils. Secondly, while the residual value of applied copper appears to be very long-lasting (Gartrell 1980), it is difficult to decide when to reapply copper. Thirdly, where the nitrogen supply is increased, either by fertilizer application or by nitrogen fixation by legumes, copper deficiency may develop in situations where the copper supply had previously been adequate. Increasing the nitrogen supply increases the external requirement of cereal plants for copper (Chaudhry and Loneragan 1970; Hill *et al.* 1978; Gartrell 1981). Finally such a procedure would be useful in evaluating the efficacy of soil application of copper where lack of moisture in the zone of copper application limits uptake by plants (Nambiar 1977; Grundon 1980).

Copper deficiency in wheat is difficult to diagnose by existing techniques. The symptoms of copper deficiency in wheat can be similar to those of advanced manganese deficiency, frost, moisture stress at anthesis and take-all (Gartrell *et al.* 1979). Soil analysis does not appear to discriminate between responsive and non-responsive sites in many situations (see review by Robson and Reuter 1981). Current techniques for plant analysis involving analysis of whole tops or whole grain may sometimes be misleading.

From a study of the distribution of copper in wheat in relation to supply throughout its life cycle, it was suggested that copper concentrations in the

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youngest leaves may be a more sensitive and accurate predictor of copper status than copper concentrations in whole tops or older leaves (Loneragan *et al.* 1980).

We report here a glasshouse experiment designed to (*a*) define critical concentrations of copper in the young leaves of wheat, and (*b*) investigate the effect of water stress after anthesis on the relationships between yield and copper supply and between yield and copper concentrations in young leaves. We also report results obtained in a field experiment in which we tested the value of copper concentrations in the youngest leaves as a diagnostic index of copper status.

Methods

Glasshouse Experiment

The experimental design was a completely randomized factorial combination of (*a*) seven rates of copper application (Cu_0, 0; Cu_{50}, 50; Cu_{100}, 100; Cu_{200}, 200; Cu_{400}, 400; Cu_{800}, 800; and Cu_{1600}, 1600 μg Cu per pot applied as $CuSO_4.5H_2O$) and (*b*) two watering treatments (soil maintained at field capacity throughout; soil maintained at field capacity until anthesis before applying a water stress). There were four replicates of each treatment combination. In the water stress treatment, plants were not watered for 3–4 days (flag leaves of copper-adequate plants wilted and curled) before rewatering to field capacity. This water stress was repeated on two occasions with pots being watered to field capacity between each occasion.

Because copper deficiency delayed maturity, the water stress treatments were commenced when copper-adequate plants had reached anthesis. Four replicate pots were harvested on four occasions when plants were at the following respective stages on the Feekes scale (Large 1954): 2 (beginning of tillering), 6 (first node of stem visible), 10.5.1 (heading), and 11.4 (maturity). However, the third harvest (at heading) was taken when plants in each treatment reached heading so that copper-deficient plants were harvested up to 12 days after copper-adequate plants. Cu_0 plants that did not form heads were harvested at the same time as Cu_{50} plants.

A bulk sample from the surface 0–20 cm of a virgin copper-deficient sand (from Lancelin; see Brennan *et al.* (1980) for soil properties) was air-dried, sieved through a 3·9-mm stainless steel sieve, and thoroughly mixed. Aliquots of 3 kg were weighed into polythene bags in undrained plastic pots of 16·5 cm surface diameter. Basal fertilizer dressings of 522 mg K_2SO_4, 280 mg NH_4NO_3, 514 mg $CaCl_2.2H_2O$, 64 mg $MgSO_4.7H_2O$, 272 mg KH_2PO_4, 253 mg KNO_3, 34 mg $ZnSO_4.7H_2O$, 34 mg $MnSO_4.H_2O$, 2·4 mg H_3BO_3, 1·2 mg $CoSO_4.7H_2O$, and 0·6 mg $Na_2MoO_4.2H_2O$ were applied in solution to the surface of each pot (on a surface area basis 214 mg $pot^{-1} = 100$ kg ha^{-1}). All macronutrient salts were purified by complexing with dithizone in carbon tetrachloride (Hewitt 1952). Copper solutions were also applied to the surface. After the solutions had dried, all nutrients were mixed throughout the soil by shaking in a plastic jar. A further dressing of NH_4NO_3 (285 mg per pot) was applied in solution to the soil surface immediately after harvest 2 (32 days from emergence).

At sowing (16 August) 13 wheat seeds (*Triticum aestivum* cv. Gamenya) were sown in each pot. Each pot was thinned to six plants 3 days after germination. The soil was watered to field capacity (14·5%) with deionized water throughout the experiment (except for the water stress treatment).

At the first two harvests the oldest leaf (blade and sheath), the youngest fully emerged leaf (blade and sheath) and the new growth (leaves enclosed within the youngest fully emerged leaf) were separated from the rest of the shoots. At the third harvest the flag leaf and various stem segments (Table 4) were separated from the rest of the shoots. At the final harvest main shoots (that is, the first shoot which developed from the seed) were separated from the tillers. All plant material was oven-dried at 70°C.

Two replicates were digested in a nitric-perchloric acid mixture (Johnson and Ulrich 1959) and analysed for copper using atomic absorption spectroscopy (Gladstones *et al.* 1975) for samples over 0·1 g dry weight and using the heated graphite atomizer (Simmons and Loneragan 1975) for samples of 0·01–0·1 g dry weight.

Field Experiment

Selected treatments were sampled on five occasions (Table 1) in 1974 from a field experiment (block 4, experiment 5, Gartrell 1980). At most harvests the treatments sampled were those which had not been fertilized with copper before 1974 and which received one of four rates of copper application (0, 2·75, 5·5, 8·25 kg $CuSO_4$ ha^{-1}) at seeding in 1974. Each plot was 60 m long and contained 12 rows 17 cm

apart. Samples were taken from the middle eight rows after leaving unsampled the terminal 4 m at the end of each plot. Plants were sampled in a regular pattern, taking 32 plants per plot. Plants harvested at ground level were approximately 1 m apart and in adjacent rows. That is, each plot was traversed four times in a zig-zag pattern to give four replicates each of eight plants collected sequentially.

The youngest fully emerged leaf (blade and sheath) was separated from the rest of the plant. At harvests 2 and 3 the plant parts were bulked into four replicates each of eight plants, whereas at the other harvests the parts of some individual plants were kept separate.

Table 1. Details of harvests for field experiment

Harvest	Date	Days from sowing	Feekes scale
1	23.vii	33	1
2	14.viii	55	6
3	9.ix	81	9
4	8.x	110	10·5
5	25.xi	158	Maturity

At harvest 4 we sampled two sets of plants for analysis. One set was selected so that plants were at a common growth stage (when the basal spikelets of the main head had just reached the flag leaf auricle). For these plants the main stem was cut immediately below the flag leaf node and 2-cm segments from below the head and above the node were taken. These samples, together with the flag leaf and the head, were analysed for copper. Additionally, at harvest 4, we sampled plants using the procedures previously described.

Results

Glasshouse Experiment

Symptoms and growth

Symptoms of copper deficiency first appeared on new leaves of plants grown without copper at 22 days after germination. By 29 days, marked symptoms (withering of tips) were evident on plants grown at the lowest four copper levels (0, 50, 100, 200 μg Cu pot^{-1}). Microscopic examination of the growth apices at 32 days after germination indicated that development was slower in copper-deficient plants than in copper-adequate plants. There did not appear to be any abnormality in the development of the apices of copper-deficient plants. Copper deficiency delayed ear emergence by 2, 6 and 11 days in treatments receiving 200, 100 and 50 μg Cu pot^{-1} respectively.

Copper application increased the growth of shoots at all harvests (Figs 1 and 2). The magnitude of the response increased with time so that plants not given copper produced 80, 46, 20 and 10% of the maximum fresh weight of shoots at harvests 1, 2, 3 and 4 respectively. At all harvests maximum fresh weight of shoots occurred at a copper application of 400 μg pot^{-1}; however, there was only a slight depression when the level of copper application was decreased to 200 μg^{-1}. At the first three harvests, plants given 200 μg Cu pot^{-1} produced more than 95% of maximum growth. At maturity, plants given 200 μg pot^{-1} produced approximately 90% of maximum growth.

Effects of copper supply on grain yield were more marked than those on vegetative growth (Fig. 2). Little or no grain was produced at the lowest three levels of applied copper (0, 50, 100 μg Cu pot^{-1}). Plants given 200 μg Cu pot^{-1} produced 83 and 79% of the maximum grain yield achieved at each level of water supply,

respectively, for plants with and without water stress after anthesis. Irrespective of water supply after anthesis, plants supplied with 400 or more μg Cu pot^{-1} produced more than 90% of maximum grain weight. Water stress after anthesis decreased grain yield at the higher rates of copper application, but did not affect the external requirement of copper for maximum grain yield.

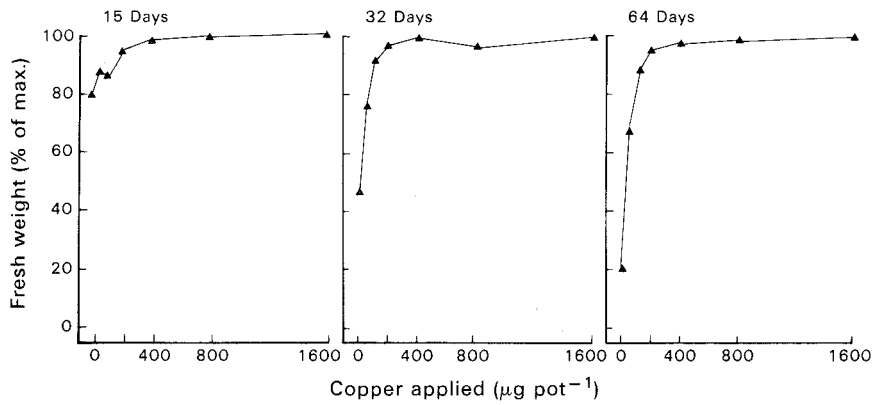

Fig. 1. Glasshouse experiment: the effect of copper supply on the fresh weight of wheat shoots 15, 32 and 64 days after germination.

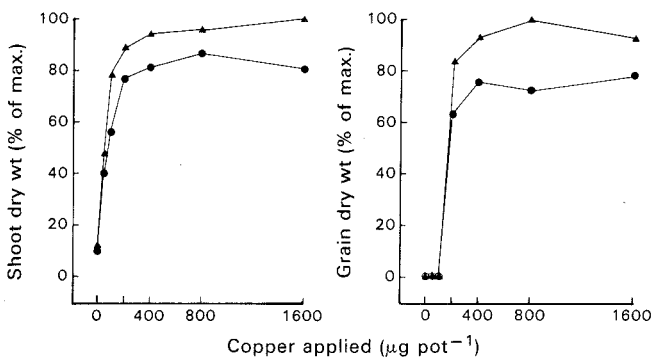

Fig. 2. Glasshouse experiment: the effect of copper supply on the total dry weight of shoots at maturity and grain dry weight for wheat plants with (●) and without (▲) water stress after anthesis.

Where copper was not applied, plants did not produce ears (Table 2). No grain was set in the ears of plants given 50 μg Cu pot^{-1}. Very few grains were set in the ears of plants given 100 μg Cu pot^{-1}. Increasing the level of copper application from 100 to 200 μg Cu pot^{-1} increased grain yield per plant by increasing the number of grains per spikelet in the main ear for both levels of water supply after anthesis and the weight per grain for those plants adequately supplied with water. Further increasing the level of copper application to 400 μg pot^{-1} increased grain yield per plant mainly by increasing the weight per grain of grains produced on tillers. Water stress after anthesis decreased grain yield mainly by decreasing the number of grains per spikelet, particularly in heads on tillers.

Copper concentrations in the plant

Copper supply influenced the distribution of copper within wheat plants. Copper-deficient plants had much higher concentrations in old leaves than in young leaves (Table 3), whereas, in copper-adequate plants, concentrations of copper in

Table 2. Glasshouse experiment: the effect of copper supply and moisture stress after anthesis on the yield components of wheat

Within columns, values with same letter are not significant ($P > 0{\cdot}05$)

Main effect of water stress on No. of tiller heads per plant, No. of spikelets per head (main stems and tillers), and grain weight (main stem) were not significant ($P > 0{\cdot}05$); but was significant ($P < 0{\cdot}05$) for No. of grains per spikelet

Interaction between water supply and copper supply was significant ($P < 0{\cdot}05$) only for grain weight (main stem) and No. of grains per spikelet (tiller heads)

	No water stress							Water stress after anthesis						
Copper applied (μg pot^{-1})	No. of tiller heads per plant	No. of spikelets per head		No. of grains per spikelet		Grain weight (mg/grain)		No. of tiller heads per plant	No. of spikelets per head		No. of grains per spikelet		Grain weight (mg/grain)	
		MS[A]	T[B]	MS	T	MS	T		MS	T	MS	T	MS	T
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
50	1·4b	14a	13a	0	0	0	0	1·1a	16a	12a	0	0	0	0
100	1·6c	18b	16b	0·1a	0·1a	16a	22a	1·9c	17b	14b	0·1a	0·1a	24a	18a
200	1·3ab	19c	18c	1·9b	1·2b	29b	21a	1·2a	20e	17c	1·6b	0·9cd	28ab	19a
400	1·1a	19c	18c	2·0b	1·4b	32b	29b	1·2a	19d	18cd	1·7b	0·8bc	29ab	25b
800	1·4b	19c	17bc	1·9b	1·3b	32b	29b	1·2a	19d	18cd	1·7b	0·6b	31b	29b
1600	1·2a	19c	16b	2·2b	1·3b	32b	28b	1·3b	18c	16c	1·9c	1·1d	28ab	26b

[A] MS, main stem. [B] T, tillers.

old leaves were either similar to or less than those in young leaves. Increasing copper supply did not increase copper concentrations in old leaves to the same extent as concentrations in young leaves. Indeed, at 32 days after germination, there was no effect of copper supply on the concentration of copper in the oldest leaf. Copper concentrations in the old leaves of copper-adequate plants had declined to the levels of those of copper-deficient plants.

Copper concentrations in whole shoots were intermediate in their sensitivity to copper supply to concentrations in old and young leaves. Concentration in whole shoots declined markedly with increasing plant age at all levels of copper supply.

Table 3. Glasshouse experiment: effect of copper supply on the concentration of copper in the shoots (μg per g dry weight) of wheat harvested 15 and 32 days from germination

Copper applied (μg pot^{-1})	Leaf 1	Youngest fully emerged leaf[A]	New growth[A]	Whole shoots	Leaf 1	Youngest fully emerged leaf[A]	New growth[A]	Whole shoots
	15 Days from Germination				*32 Days from Germination*			
0	4·1	0·8	1·4	1·7	3·4	0·6	0·9	1·0
50	4·9	1·2	1·6	2·1	2·8	0·7	1·3	1·1
100	5·3	1·6	2·1	2·6	3·3	0·7	2·4	1·2
200	7·4	3·0	3·7	4·1	2·7	1·8	4·2	1·6
400	7·5	4·3	5·5	5·5	4·1	3·6	7·2	2·8
800	9·6	7·6	8·8	8·4	4·0	5·4	10·8	4·0
1600	11·2	9·8	9·8	10·0	3·7	6·3	9·3	5·6

[A] At 15 days from sowing, leaf 3 was youngest fully emerged leaf and leaf 4 was new growth. At 32 days from sowing, leaf 6 was youngest fully emerged leaf and leaf 7 was new growth, except for the Cu_0 treatment where leaf 5 and leaf 6 were the youngest fully emerged leaf and new growth respectively.

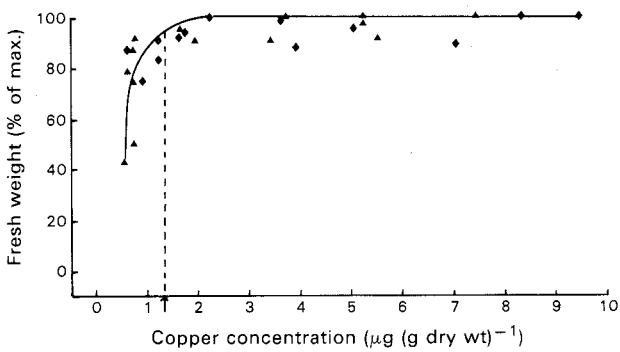

Fig. 3. Glasshouse experiment: the relationship between copper concentrations in the youngest fully emerged leaf (YFEL) and fresh weight of shoots of wheat at 15 (♦) and 32 (▲) days after germination. Arrow indicates the critical copper concentration (1·3 μg g^{-1}).

At the first two harvests the concentration of copper in the youngest fully emerged leaf was a sensitive and accurate indication of the adequacy of copper supply for the growth of wheat (Fig. 3). For both harvests, plants with copper concentrations in this leaf of less than 1·3 μg g^{-1} dry weight generally produced less than 90% of maximum fresh weight of shoots, whereas plants with concentrations greater than this generally produced more than 90% of maximum fresh weight of shoots.

At heading, copper supply also influenced the distribution of copper within the plant (Table 4). At this harvest the youngest fully emerged leaf was the flag leaf

and at the time of harvest this leaf was no longer the youngest tissue within the plant. The relationship between copper concentration in the flag leaf and shoot weight exhibited Piper-Steenbjerg curvature in that concentrations in leaves of severely deficient plants exceeded those in leaves of moderately deficient plants. Nevertheless, as at the first two harvests, deficient plants (i.e. those producing less than 90% of maximum fresh weight of shoots) had copper concentrations in their youngest fully emerged leaf of $<1{\cdot}3$ $\mu g\ g^{-1}$ dry weight.

Table 4. Glasshouse experiment: copper concentrations (μg per g dry weight) in plant parts at heading
Data are for main stem only, and were taken when the head of the main stem had just emerged from leaf

	No. of replicates	Copper applied ($\mu g\ pot^{-1}$): 50	100	200	400	800	1600
Flag leaf	2	1·3	0·8	1·3	1·7	3·9	5·0
Flag leaf node	2	1·6	1·2	1·8	2·1	4·0	9·8
Stem below flag leaf node (0–2 cm)	3	1·8	0·8	1·5	1·5	3·0	5·1
Stem above flag leaf node (0–2 cm)	3	4·8	2·2	2·6	4·0	7·0	16·8
Stem below basal spikelet of ear (0–2 cm)	3	1·4	1·5	1·3	2·0	2·6	5·6

Copper concentrations in stem sections varied markedly with their position on the stem. Young, actively growing sections (stem above flag leaf node) had much higher concentrations of copper than did older sections of stem at all levels of copper supply, in contrast to the situation with young leaves. The concentrations in the young stem section also far exceeded concentrations in the flag leaf, which were similar to those in old stem sections. The relationship between concentration in all stem sections and shoot growth exhibited Piper-Steenbjerg curvatures.

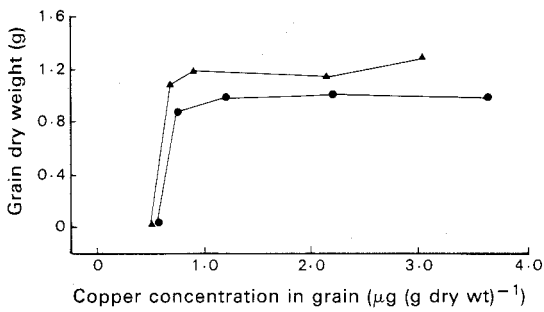

Fig. 4. Glasshouse experiment: relationship between copper concentration in grain of main stem and dry weight of grain of main stem for plants with (●) or without (▲) water stress after ear emergence.

For both levels of water stress after anthesis, copper concentrations in grain in plants producing maximum grain yield were *c.* 1 $\mu g\ g^{-1}$ dry weight (Fig. 4). Copper concentrations in the grain of plants producing 80% of maximum grain yield were $0{\cdot}7$ $\mu g\ g^{-1}$ dry weight.

Field Experiment

Symptoms and growth

Symptoms of copper deficiency (Gartrell *et al.* 1979) were observed only where copper had never been applied. Symptoms were first observed 55 days from sowing.

At subsequent harvests copper application increased dry weight of shoots (Table 5). Copper application also markedly increased grain yield.

Whenever copper deficiency decreased shoot weight, the concentration of copper in the youngest fully emerged leaf of deficient plants was $<1{\cdot}3$ $\mu g\ g^{-1}$ (Table 5). Copper application in the year of sowing increased the average copper

Table 5. Field experiment: effect of copper application on dry weight of shoots, grain yield, and the concentration of copper in the youngest fully emerged leaf (YFEL) and grain of wheat
Data are from plots to which copper had not been previously applied

Copper applied (kg $CuSO_4$ ha^{-1})	Days from sowing: 33 Dry wt shoots (g $plant^{-1}$)	33 Cu concn YFEL ($\mu g\ g^{-1}$)	55 Dry wt shoots (g $plant^{-1}$)	55 Cu concn YFEL ($\mu g\ g^{-1}$)	81 Dry wt shoots (g $plant^{-1}$)	81 Cu concn YFEL ($\mu g\ g^{-1}$)	Maturity Grain yield (kg ha^{-1})	Maturity Cu concn grain ($\mu g\ g^{-1}$)
0	0·039	2·4	0·21	1·1	2·3	1·2	994	0·8
2·75	0·042	4·4	0·24	2·7	3·6	2·2	1634	0·7
5·5	0·037	4·8	0·26	2·7	3·5	1·9	1765	0·7
8·25	0·037	7·3	0·26	3·1	3·6	1·9	1590	1·2
l.s.d. ($P=0{\cdot}05$)	n.s.		n.s.		0·5		340	

concentration in the youngest fully emerged leaf (in this case, the flag leaf) above the suggested critical level of $1{\cdot}3$ $\mu g\ g^{-1}$. However, values for individual leaves varied and a proportion of plants at all levels of currently applied copper had levels $<1{\cdot}3$ $\mu g\ g^{-1}$. Copper application decreased the proportion of plants with copper concentrations in flag leaves $<1{\cdot}3$ $\mu g\ g^{-1}$ from 79 to 6%.

Table 6. Field experiment, harvest 4: the effect of previous and current application of copper on copper concentrations in flag leaves of wheat at heading
Data are for 32 individual plants

Copper applied 4 years previously (kg $CuSO_4$ ha^{-1})	Copper applied at sowing (kg $CuSO_4$ ha^{-1})	Copper concn ($\mu g\ g^{-1}$) Mean	Copper concn ($\mu g\ g^{-1}$) Range	Coeff. of variation	Percentage of plants with concn $<1{\cdot}3$ $\mu g\ g^{-1}$
0	0	1·1	0·6–2·1	29	79
	2·75	2·2	1·0–4·6	31	6
	5·5	1·8	1·1–3·4	22	6
	8·25	1·8	0·9–4·1	35	16
2·75	0	3·0	1·9–4·7	20	0
	2·75	2·7	1·9–4·0	17	0
8·25	0	2·9	1·6–3·9	20	0
	8·25	2·7	1·5–4·5	20	0

Where copper had been applied 4 years previously, copper concentrations in flag leaves were $>1{\cdot}3$ $\mu g\ g^{-1}$ in all plants. Mean concentrations of copper in flag leaves in these plants were greater than those in plants receiving equivalent amounts of copper in the year of sowing. Coefficients of variation for copper concentrations in flag leaves were generally greater where copper had not been previously applied than where copper had been applied 4 years previously (Table 6), irrespective of the amount of copper applied in the year of sowing.

Copper application did not markedly increase copper concentrations in stem segments at heading (Table 7). Unlike the situation for plants in the glasshouse experiment, the copper concentration in stem segments immediately above the flag leaf node were similar to those immediately below the basal spikelet.

Copper concentrations were similar in grain from the plants which had not received copper (copper-deficient) and plants receiving 2·75 and 5·5 kg $CuSO_4$ ha^{-1} (copper-adequate) (Table 5).

Table 7. Field experiment, harvest 4: effect of copper application on copper concentrations (μg per g dry weight) of stem sections, flag leaf and head when basal spikelet has just passed through the flag leaf auricle

Data are from plots to which copper had not been applied previously

Copper applied (kg $CuSO_4$ ha^{-1})	Basal stem segment[A]	Terminal stem segment[B]	Flag leaf	Head
0	1·1	1·2	1·2	0·8
2·75	1·0	1·2	2·2	1·0
5·5	1·3	1·4	1·9	1·0
8·25	1·4	1·7	1·9	1·2

[A] Segment 2 cm above flag leaf node.
[B] Segment 2 cm below basal spikelet.

Discussion

The copper concentration in the youngest fully emerged leaf appears to be a sensitive and accurate indicator of the copper status of wheat. Copper concentrations in young leaves also appear to give a good indication of copper status in other experiments with wheat (Nambiar 1976) and other species (peanuts, Nualsri *et al.* 1977; chrysanthemum, Graves 1978; subterranean clover, Reuter *et al.* 1981*a*, 1981*b*).

The critical concentration for copper in the youngest fully emerged leaf of wheat did not change with the age of the plant. Copper deficiency in wheat occurred whenever the concentration of copper in the youngest fully emerged leaf fell below 1·3 μg g^{-1}. This critical value has been confirmed in subsequent glasshouse (Brennan *et al.* 1980) and field (J. W. Gartrell, unpublished data) experiments. Critical copper concentrations in whole shoots of wheat (Loneragan *et al.* 1980), peanuts (Nualsri 1977) and subterranean clover (Reuter *et al.* 1981*a*) declined considerably as plants aged. The critical concentration in young leaves did not decrease for wheat in this study, nor for subterranean clover in the study by Reuter *et al.* (1981*a*).

In some situations the concentration of copper in the plant may increase as the degree of deficiency increases from marginal to severe (see, for example, Piper 1942; Steenbjerg 1951), giving rise to Piper-Steenbjerg effects (Rosell and Ulrich 1964; Bates 1971). In the present experiment this Piper-Steenbjerg effect occurred for the relationship between copper concentrations in young leaves and shoot weight only when the youngest fully emerged leaf was the flag leaf and the ear had emerged. Notwithstanding the Piper-Steenbjerg curvature in the relationship, copper-deficient plants at this harvest could be identified by using a critical concentration in young leaves of 1·3 μg g^{-1}. Furthermore, severely deficient plants displayed characteristic symptoms of copper deficiency and failed to produce ears.

Piper-Steenbjerg curvature in the relationship between copper concentrations in the plant and shoot or grain dry weights results from effects of copper supply on the distribution of copper within the plant (see Loneragan 1978). Certainly, at ear emergence, copper concentrations varied widely within stem sections and between stem sections and the flag leaf.

Water stress after ear emergence did not change the nature of the relationship between copper concentrations in young leaves and grain yield, although this stress markedly decreased grain yield. In the field, effects of water stress on the response of grain yield to copper application may be more complex than in our glasshouse experiment. Coppper deficiency delayed ear emergence by up to 12 days in our experiment. Hence copper-deficient plants may pass through critical stages of grain development (e.g. anthesis) at different times and with a different (usually a less favourable) water supply. Moreover drying of the surface soil may restrict root activity and the uptake of applied copper that is retained in the surface soil (Nambiar 1977). In our experiment the critical concentration of copper in young leaves for maximum grain yield was unaffected by severe water stress after anthesis.

Copper concentrations in grain from bulk samples appeared to be of little value in the diagnosis of copper deficiency. Moreover concentrations of copper in grain of copper-adequate plants in our experiments were only one-half of values associated with copper deficiency in wheat elsewhere (Caldwell 1971; Davies *et al.* 1971; King and Alston 1975). The reason for this discrepancy is not obvious.

In field-grown plants there was considerable variability among plants in copper concentrations in the flag leaf. This variability has implications for sampling of crops for assessment of nutrient status. Samples taken may contain plants with deficient and adequate supplies of copper, and the mean value for copper concentrations may reflect the proportion of plants in each category. For this reason it is probably desirable to apply the values for critical concentrations in a conservative way; it may be desirable to apply copper if the mean copper concentration in young leaves is less than $1{\cdot}5\ \mu g\ g^{-1}$.

In the field copper applied at relatively high rates did not completely eliminate copper deficiency in the year of application, whereas the same rate of copper applied 4 years previously did eliminate copper deficiency. The greater availability of previously applied copper compared with that drilled with the seed, as occurred in our experiments and elsewhere (Gartrell 1980), appears to be due to effects of cultivation in mixing the copper throughout the soil and thus increasing the opportunity for plant roots to contact fertilized soil (see Gartrell 1981; Gilkes 1981).

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