Response of eight Canadian spring wheat (*Triticum aestivum* L.) cultivars to copper: Copper content in the leaves and grain

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Owuoche, J. O., Briggs, K. G., Taylor, G. J. and Penney, D. C. 1995. **Response of eight Canadian spring wheat (*Triticum aestivum* L.) cultivars to copper: copper content in the leaves and grain.** Can. J. Plant Sci. **75**: 405–411. Concentrations of copper (Cu) in the youngest fully emerged leaves (YFEL) and grain of eight widely grown Canadian spring wheat (*Triticum aestivum* L.) cultivars, Biggar, Columbus, Conway, Katepwa, Laura, Oslo, Park and Roblin, were determined. Leaves were sampled at five growth stages from field plots grown in 1990 and 1991 on Cu-deficient soil or soil treated with 12.2 kg Cu ha^{-1} as Cu sulphate. Symptoms of Cu deficiency, mainly rolling and wilting of young leaves and twisting and terminal dieback, were noted on Katepwa, Park and Roblin at Zadok growth stage 24. Significant ($P \leq 0.01$) effects on Cu concentration in the YFEL were found due to cultivar, copper treatment, year and growth stage. The Cu concentrations in Katepwa, Park and Roblin not treated with Cu ranged between 4.6 and 5.7 $\mu g\ g^{-1}$ in 1990 and between 2.8 and 3.5 $\mu g\ g^{-1}$ in 1991 at Zadok growth stage 22. Cultivars Biggar, Columbus, Conway, Laura and Oslo did not show symptoms of Cu deficiency and had Cu concentrations in the range of 4.6–5.4 $\mu g\ g^{-1}$ in 1990 and 2.3–3.1 $\mu g\ g^{-1}$ in 1991. Deficiency symptoms were observed on Katepwa and Park supplied with Cu, although concentrations of Cu in the YFEL were relatively high. Grains sampled from the tillers generally had lower Cu concentrations than those from main stems, but the magnitude of this difference varied with the year. Significant correlations were found between Cu concentrations in the YFEL and grain yield ($r = 0.90$* in 1990 and 0.89* in 1991) and with floret fertility ($r = 0.74$* in 1990 and 0.94** in 1991). These large and significant correlations confirm the important role of Cu nutritional status in influencing floret fertility and grain yield. Critical levels of Cu in the leaves needed for unlimited growth could not be defined because of year-to-year variability. In this study, Cu concentration in the YFEL was not a useful indicator of potential Cu use efficiency in different wheat cultivars. However, for individual plants under Cu-deficiency stress, Cu concentration in the YFEL was a good indicator of the grain yield potential of different cultivars.

Key words: *Triticum aestivum*, copper, youngest fully emerged leaves, tissue analysis

Owuoche, J. O., Briggs, K. G., Taylor, G. J. et Penney, D. C. 1995. **Réaction de 8 cultivars canadiens de blé de printemps (*Triticum aestivum* L.) à l'égard du cuivre: teneur en cuivre des feuilles et du grain.** Can. J. Plant Sci. **75**: 405–411. Nous avons mesuré les concentrations de cuivre (Cu) dans la dernière feuille complètement sortie (DFCS) ainsi que dans le grain de 8 cultivars canadiens largement utilisés de blé de printemps (*Triticum aestivum* L.): Biggar, Columbus, Conway, Katepwa, Laura, Oslo, Park et Roblin. Les feuilles étaient prélevées à 5 stades de croissance dans des parcelles installées en 1990 et en 1991 dans un sol carencé en Cu ou dans un sol traité avec 12,2 kg ha^{-1} de cuivre apporté sous forme de sulfate. Les symptômes de carence cuprique, principalement l'enroulement et le flétrissement des jeunes feuilles et un dépérissement apical, étaient observés sur Katepwa, Park et Roblin au stade de croissance 24 de l'échelle de Zadok. Des effets significatifs ($P \leq 0,01$) des teneurs en Cu des DFCS étaient attribués au cultivar, au traitement cuprique, à l'année et au stade de croissance. Les concentrations de Cu chez les cultivars Katepwa, Park et Roblin non traités au Cu se situaient entre 4,6 et 5,7 $\mu g\ g^{-1}$ en 1990 et entre 2,8 et 3,5 $\mu g\ g^{-1}$ en 1991 au stade de croissance 22 de Zadok. Les cultivars Biggar, Columbus, Conway, Laura et Oslo ne manifestaient pas de symptômes de carence cuprique et leurs concentrations de Cu fluctuaient entre 4,6 et 5,4 $\mu g\ g^{-1}$ en 1990 et entre 2,3 et 3,1 $\mu g\ g^{-1}$ en 1991. Des symptômes de carence étaient notés sur Katepwa et sur Park fertilisés au Cu, bien que les concentrations de Cu dans la dernière feuille soient relativement élevées. Les grains prélevés des talles secondaires contenaient généralement moins de Cu que ceux provenant du chaume principal, mais l'amplitude de la différence variait selon l'année. On relevait des corrélations significatives entre les concentrations cupriques des jeunes feuilles et le rendement grainier ($r = 0,90$* en 1990 et 0,89* en 1991) ainsi que la fertilité des florules ($r = 0,74$* en 1990 et 0,94** en 1991). Ces importantes et significatives corrélations confirment le rôle important que joue le bilan cuprique sur la fertilité des florules et sur le rendement grainier. En raison de la variabilité observée d'une année à l'autre, nous n'avons cependant pas pu établir les niveaux critiques de cuivre dans les feuilles requis pour assurer une croissance non limitée. Les concentrations de Cu dans la dernière feuille complètement sortie ne se sont pas révélées des indicateurs utiles de l'efficacité éventuelle de l'utilisation du cuivre parmi les divers cultivars de blé étudiés. Toutefois, pour les plantes individuelles exposées à une carence cuprique, les concentrations dans les DFSC procuraient une bonne idée de la productivité en grain des divers cultivars.

Mots clés: *Triticum aestivum* L., cuivre, dernière feuille complètement sortie, analyse des tissus

Abbreviations: DAS, days after sowing; **YFEL**, youngest fully emerged leaves

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Plant tissue analysis can be a useful diagnostic tool for evaluating the nutritional status of plants. Copper (Cu) concentration in plants depends on the species, genotype, prevailing environmental conditions, plant organ sampled and age of plant at sampling time (Graham and Nambiar 1981). The critical Cu concentration was reported as being 3 $\mu g\ g^{-1}$ in *Trifolium subterranean* (Reuter et al. 1981) and 2.5 $\mu g\ g^{-1}$ for *Triticum aestivum* L. under field conditions (Nambiar 1976b).

Because of immobility of Cu at early stages of plant growth (Hill et al. 1979; Loneragan et al. 1980) and a wide range of Cu concentrations in different plant organs, assays conducted on the YFEL have been the most sensitive and accurate indicator of Cu status of wheat plants (Loneragan 1975; Nambiar 1976b; Gartrell et al. 1979). The ability of specific cultivars to concentrate different levels of Cu in different organs depends largely on the genotype (Nambiar 1976a; Graham 1991). Differences among cultivars in the uptake and translocation of Cu to the developing organs play a significant role in Cu use efficiency (Graham 1978; Graham et al. 1981a). Differences in Cu use efficiency in wheat have been demonstrated (Owuoche et al. 1994) for Canadian wheat cultivars grown in Cu-deficient field conditions. The objectives of this study were 1) to attempt to detect the critical Cu range in the YFEL and in grains from the main stems and tillers of eight Canadian spring wheat cultivars in response to Cu application and 2) to determine the Cu requirements of individual cultivars grown under field conditions at a Cu-deficient site.

MATERIALS AND METHODS

Details of the experimental site and cultural methods were described previously by Owuoche et al. (1994). At this site, yield responses to Cu in the range of 30–300% have been reported by Evans et al. (1994) and Solberg et al. (1994). The field site was at Stony Plain, Alberta, in 1990 and 1991 on an Orthic Dark Gray Chernozemic soil known as being Cu deficient for wheat and barley (Cu concentration in soil, 0.48 $\mu g\ g^{-1}$). From the same experiment and treatments, YFEL of the eight widely grown test cultivars (Biggar, Columbus, Conway, Katepwa, Laura, Oslo, Park and Roblin) were sampled (excluding leaf sheath) from one half of each subplot. This provided tissues throughout the growth cycle of the eight test cultivars grown in Cu-deficient soil (−Cu) or in soil supplied with 12.2 kg ha^{-1} Cu (+Cu) applied as Cu sulphate with the seed.

The YFEL were sampled 21, 28, 35, 42, and 51 d after sowing (DAS), when cultivars were mostly at or near Zadok growth stages 12, 22, 23, 24, 25 and 39 (Zadok et al. 1974). Sampled leaves from 10 plants $plot^{-1}$ were decontaminated of soil and dust by rinsing three times in distilled water and were then dried in a forced-air oven at 65°C for 7 d. The dried samples were chopped into small pieces, mixed thoroughly and stored in paper envelopes to await ashing. Samples of whole grains from the main stems and tillers, from which yield was determined, were subjected to the same procedure as the leaf samples. For the analysis of Cu concentration in the tissue, a modification of the method described by Taylor (1989) was used. About 0.5 g of dried sample was ashed at 500°C for 48 h, dissolved in 0.4 mL concentrated nitric acid, oxidized with 0.4 mL 50% hydrogen peroxide, and diluted to 10 mL volume. In the second year, when Cu levels were much lower than in 1990, 5 mL of diluted sample was concentrated to dryness in a Speed Vac high-speed concentrator and was then dissolved in 2 mL of deionized water to improve the precision of the assay. Copper concentration was determined by atomic absorption spectrophotometry under air–acetylene flame conditions.

Statistical Analyses

Analyses of variance for Cu concentration in the YFEL was used to assess the effects of cultivar, Cu level, year, growth stage and any interactions. The Cu concentration data from grains of main stems and tillers were similarly analyzed. To determine the relationship between Cu in the leaf blades at different growth stages and yield and yield components, Cu concentration values in the YFEL at five growth stages were correlated to grain yield, number of the grains $spike^{-1}$, and percentage fertile florets $spike^{-1}$ for main stems and tillers. Responses to Cu treatment for the correlated variables were presented in Owuoche et al. (1994).

RESULTS

Symptoms of Cu Deficiency

The symptoms of Cu deficiency noted in the field trials differed with the cultivar, stage of growth, and year. In 1990, symptoms of deficiency at 28 DAS included rolling and wilting of the young leaves before full emergence and twisting and terminal dieback of leaves of Katepwa and Park grown on the −Cu treatment. At 35 DAS, deficiency symptoms were prominent. These consisted mainly of rolling and dieback of emerging leaves, forming rat tails, and longitudinal splitting of the midrib on the young emerging and young fully opened leaves of Katepwa, Park and Roblin. Cultivar sensitivity to Cu deficiency was expressed as necrosis along the edge of the laminae of fully extended leaves, and this was observed on Conway, Katepwa, Laura, Park and Roblin. In 1990, no visual deficiency symptoms were noted at any stage on Biggar and Columbus, the two late-maturing cultivars. The intensity of Cu-deficiency symptoms for Katepwa, Park and Roblin decreased as the plants developed, but deficiency symptoms were observed on Katepwa, Park and Roblin in both +Cu and −Cu treatments. Spikes of Oslo, Park and Roblin particularly showed poor grain development and sterility. The effects of Cu deficiency were also evident on spikes from the tillers of Oslo and Park.

In 1991, Cu-deficiency symptoms appeared at 35 DAS. Symptoms were similar to those observed in 1990 and appeared first on Oslo, Park and Roblin from the −Cu treatments. However, the symptoms were more conspicuous at 51 DAS, with necrosis of the leaves also appearing on Biggar. In contrast to the 1990 results, the expression of symptoms increased as the plants matured. Stem and head melanosis (Evans et al. 1994) appeared on Katepwa, Oslo, Park and Roblin at maturity in both years. On Katepwa,

Table 1. Analysis of variance over time for Cu concentration in the YFEL of wheat (*Triticum aestivum*) grown in soil with two Cu levels

Source	df	Mean squares
Rep	3	5.92
Cultivar	7	8.37**
Cultivar × Rep ($error_a$)	21	1.52
Cu	1	97.50**
Cu × cultivar	7	1.53
Cu × Rep	3	1.01
Cu × cultivar × Rep ($error_b$)	21	0.78
Year	1	522.37**
Year × cultivar	7	1.33
Year × Cu	1	0.56
Year × Cu × cultivar	7	0.90
Year × Cu × cultivar × Rep ($error_c$)	48	1.73
GS[z]	4	331.37**
GS × cultivar	28	0.91**
GS × Cu	4	1.76**
GS × year	4	44.89**
GS × year × Cu	4	0.23
GS × year × cultivar	28	0.69
GS × year × Cu × cultivar	56	0.70*
Error (CV 17.0%)	384	0.48

[z]GS, growth stage at sampling (in days after seeding).
*,**Significant at the $P \leq 0.05$ and $P \leq 0.01$ probability levels, respectively.

Park and Roblin, senescence and maturity were also delayed in the −Cu treatment.

Copper Concentration in the YFEL

In a combined-years analysis of variance for Cu concentration in YFEL, significant ($P \leq 0.01$) main effects were found for cultivar, Cu, year and growth stage (Table 1). Cultivars generally responded similarly to Cu treatment, as indicated by non-significant ($P \leq 0.05$) interaction for Cu × cultivar and Cu × cultivar × year. Effects were significant (P Ŀ ≤ 0.05) for several interactions involving growth stage (i.e., growth stage × cultivar, growth stage × Cu, growth stage × year, and growth stage × cultivar × Cu × year).

In all test cultivars, Cu concentrations in the leaves were higher at earlier plant growth stages and decreased with the increasing maturity of the plants (Fig. 1). However, the cultivars translocated different amounts of Cu to the leaves in different seasons, with much higher values in the wetter season (1990). In nearly all cases, for all cultivars, higher levels of Cu were detected in the leaves of plants from the +Cu treatment than in those from the −Cu treatment.

Significant interactions of cultivar with sampling date (growth stage), growth stage × year, and growth stage × year × Cu level indicated that generalization about the Cu uptake in YFEL of specific cultivars could not be made. Description of this variability is presented in Fig. 1, in which variability in date of symptom appearance in different cultivars in different years is also evident.

Cultivars showed low Cu levels in the flag leaves, but the differences in Cu content in the flag leaves from +Cu and −Cu treatments were not significant for most cultivars. Copper levels in the flag-leaf blades were generally higher in 1990 than in 1991.

Copper Concentration in the Grain on Mainstems and Tillers

The assays of Cu concentration in the grain on mainstems and tillers were characterised by relatively high standard errors for individual treatments and very different ranges of values in the 2 yr (Table 2). Interpretation of these very variable data is difficult, but several trends were evident. Within treatments, Cu concentrations in the grain were generally lower in 1991 than in 1990, consistent with Cu concentrations found in the YFEL. Within treatments, Cu concentration in grain from tillers was generally lower (or not significantly different from) Cu concentration in grain from mainstems. This effect appeared independent of year or Cu treatment level. Significant cultivar differences were found in each year for each Cu treatment level. No significant difference was found between the Cu concentration in the grain of Oslo and Biggar in either the −Cu or the +Cu treatment, despite the previous finding (Owuoche et al. 1994) that these cultivars demonstrated the lowest and highest Cu efficiency of these eight cultivars in the field trial.

Relationship of Cu Concentration in the YFEL (51 DAS) with Cultivar Grain Yield Plant^{-1}, Number of Grains Spike^{-1}, and Percentage Fertile Florets Spike^{-1}

Significant and high correlation was found in both years for cultivar YFEL at 51 DAS with cultivar grain yield plant^{-1}, number of grains spike^{-1}, and percentage fertile florets spike^{-1} for the −Cu treatment (Figs. 2 and 3). The much smaller range and much lower values of Cu concentration in the YFEL (51 DAS) for the cultivars in 1990 than in 1991 were very evident from these data.

DISCUSSION

For the eight cultivars studied, the symptoms of Cu deficiency varied with the cultivar and season but were consistent with those reported in the literature. Foliar Cu-deficiency symptoms were observed earlier in 1990 than in 1991 but were more pronounced in the second season, perhaps owing to the influence of different moisture regimes in the two seasons and a resultant lower Cu level in the tissue. The intensity of foliar deficiency symptoms on Katepwa, Park and Roblin diminished as the plants matured in 1990, but in 1991 the intensity of symptoms increased. However, the appearance of deficiency symptoms even on the +Cu treatments on Katepwa, Park and Roblin in 1990 indicated that wheat cultivars have different requirements for Cu and that not all Cu requirements for all cultivars were met by the +Cu treatment applied.

Cultivars differed significantly in their accumulation of Cu in the YFEL, and addition of Cu to the soil resulted in an increase in Cu in the YFEL. Although this study was conducted on the same site in both years, Cu concentration in the YFEL of the cultivars varied significantly with the year. Thus, year-to-year variation influenced Cu nutrition of the tested cultivars and also contributed to the differences in Cu concentration in the YFEL. The significant effects of growth stage on the Cu concentration in the leaves provided clear evidence that the status of Cu in the wheat plants changes

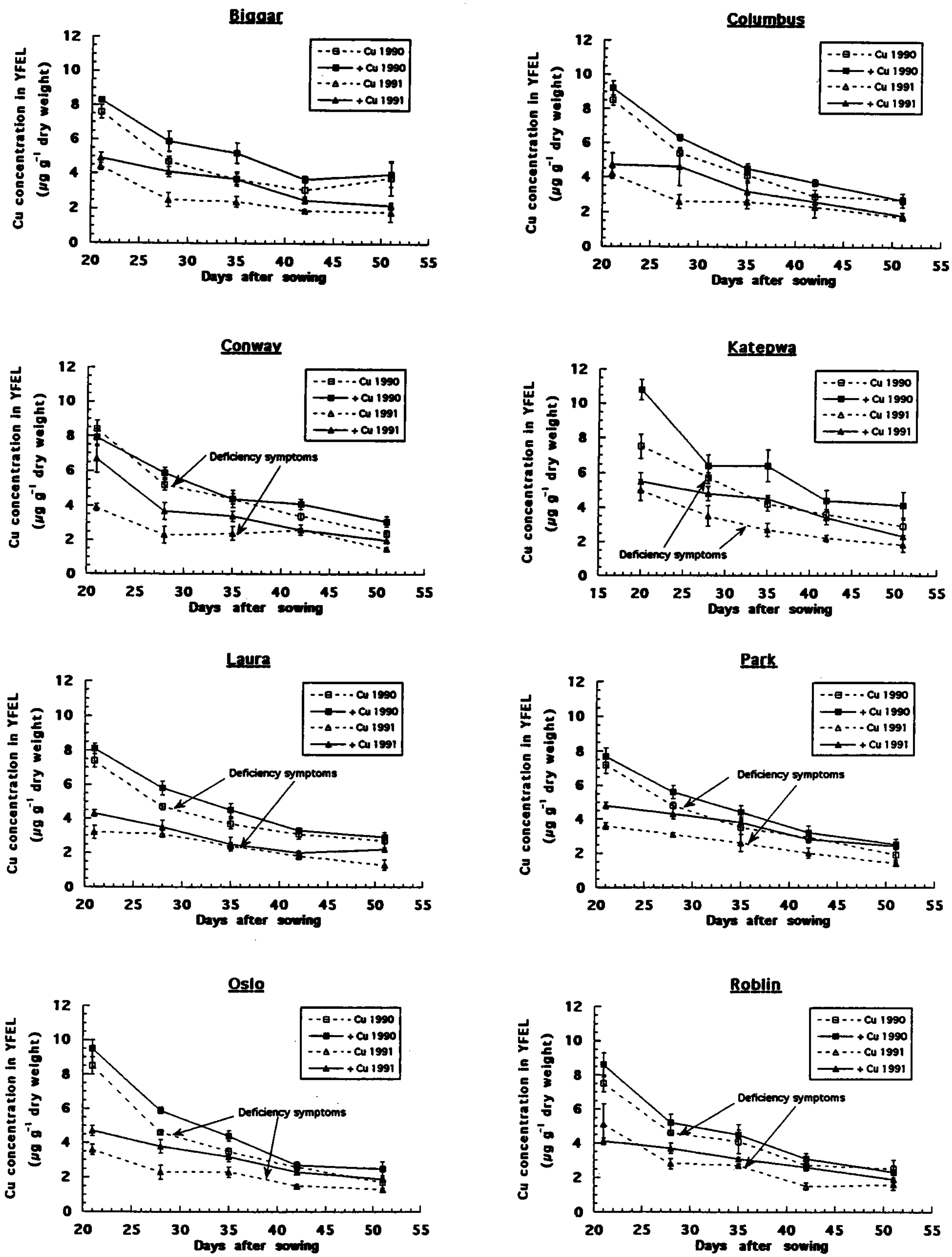

Fig. 1. The relationship between Cu concentration in the YFEL and DAS of eight Canadian spring wheats grown at Stony Plain, Alberta.

Table 2. Concentrations of Cu (μg g^{-1} dry weight) in grains from spikes of mainstems and tillers of eight Canadian spring wheat cultivars[z]

Treatment[y]		Katepwa	Roblin	Park	Laura	Conway	Oslo	Columbus	Biggar
						Spikes			
−Cu	1990	2.2±0.6	2.2±0.6	1.6±0.3	2.3±0.8	2.8±0.4	2.2±0.3	1.8±0.3	1.8±0.5
	1991	1.7±0.2	2.1±0.4	1.6±0.3	1.7±0.6	1.4±0.3	2.7±0.8	1.7±0.1	2.0±0.2
+Cu	1990	5.9±1.3	2.9±1.2	2.9±0.8	3.3±0.8	2.6±0.7	2.4±0.8	2.0±0.8	2.9±0.7
	1991	2.8±0.3	2.6±0.4	1.9±0.2	2.3±0.8	2.6±0.3	2.1±0.3	2.5±0.3	2.9±0.4
						Tillers			
−Cu	1990	2.2±0.5	2.2±0.6	1.3±0.2	2.1±0.7	2.2±0.5	1.5±0.4	1.6±0.4	1.8±0.7
	1991	1.0±0.2	2.1±0.3	1.5±0.2	1.7±0.5	1.1±0.3	1.6±0.3	2.0±0.4	2.2±0.3
+Cu	1990	1.7±0.8	1.4±0.8	2.4±0.4	2.7±2.2	2.2±0.6	2.8±0.7	1.7±0.7	2.6±0.5
	1991	2.0±0.2	2.1±0.5	1.4±0.3	2.4±0.5	2.0±0.3	1.8±0.3	1.8±0.4	2.6±0.5

[z]Values are means of four replicates ± standard errors.
[y]−Cu, Cu-deficient soil; +Cu, soil supplied with 12.2 kg ha^{-1} Cu.

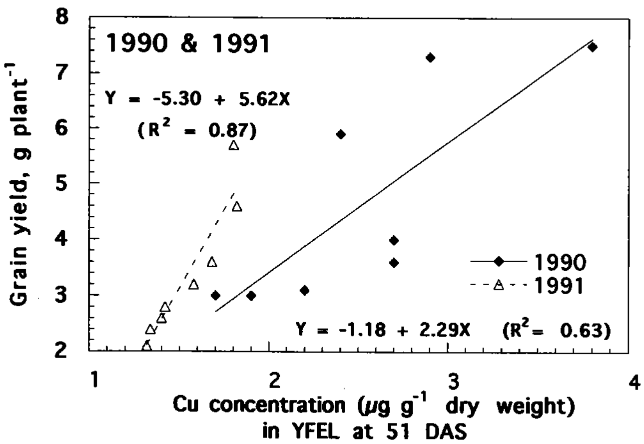

Fig. 2. Relationship between the grain yield and Cu concentration in the YFEL at 51 DAS of eight Canadian spring wheats, sampled from the −Cu treatment in 2 yr.

with growth stage. In this study, Cu concentration in the YFEL depended on cultivar, growth stage at sampling time, and year, in agreement with the findings of Bates (1971). It is, therefore, necessary to describe the stage of sampling, specify the cultivar, and conduct experiments for several seasons when attempting to establish Cu requirements for wheat production in these central Alberta soil and climatic conditions.

The use of tissue analysis as a diagnostic tool for detection of Cu deficiency only indicates the status of Cu in the plant at the time of sampling. In this study, the YFEL assays over time were used to determine the effect of the age of the plant and the part of the plant sampled. The gradual decrease of Cu concentrations in the YFEL with the increasing maturity of the plants (Fig. 1) was consistent with similar patterns of Cu accumulation detected in the leaf blades of oats as the plant matured (Williams and Moore 1952) and for subterranean clover (Reuter et al. 1981). In this study, cultivars showed genetic variation in their capability for Cu uptake and translocation to the leaves, agreeing with the findings of Nambiar (1976a) and Graham (1984).

The study showed that supplementation with Cu increased the level of Cu in the leaf blades of all cultivars. However, based on visual deficiency symptoms, the critical Cu concentration differed among cultivars and between seasons, with higher values in 1990 than in 1991. Although the differences in Cu uptake in the 2 yr showed that seasons have an influence on Cu nutrition, Cu use efficiency still varied among cultivars but was not correlated with Cu concentration in the YFEL.

Several physiological mechanisms may be responsible for cultivar differences in Cu efficiency. Nutrient efficiency may be expressed in terms of absorption, uptake, translocation, distribution within the plant systems, utilization, mobilization and reutilization efficiency (Clark 1990). Katepwa showed the highest Cu uptake and translocation to the YFEL and also showed a high Cu requirement. Oslo and Park were poor in Cu uptake. Because of small differences in Cu concentration in YFEL at 51 DAS, it seems that Cu efficiency for the test cultivars was mainly determined by the efficiency of redistribution and reutilization of Cu from Zadok growth stages 45–75, the booting to senescence stage. Gereloff and Gabelman (1983) argued that weak redistribution of elements would result in elements being retained in old inactive leaves instead of being reutilized in young growing tissues. Only those cultivars that are efficient in redistribution and reutilization of Cu would be Cu efficient. Since Cu is used in mobilization and remobilization of carbohydrates at the senescence stage (Brown and Clark 1977), it might be hypothesized that (except for Biggar) cultivars were poor in reutilizing Cu at later stages of growth. Because of this, Cu concentration in the flag leaves should not be used to assess and indicate critical Cu concentrations, since, as Nambiar (1976b) argued, Cu content in the flag leaves does not properly represent Cu status of the whole plant.

Supply of Cu to the plants also increased Cu content in the grain. In both years, the accumulation of Cu in the grains from the spikes of main stems was generally higher than in grain from the tillers, with exceptions for Cu-efficient Columbus and Cu-inefficient Oslo and Park.

Positive linear relationships of Cu concentration in the YFEL of cultivars with grain yield, grain number and floret fertility affirmed the importance of Cu supply as a limiting factor for plant growth at this site. Yield was improved because of improved floret fertility and, consequently, the number of grains spike^{-1}. It may be that when sufficient

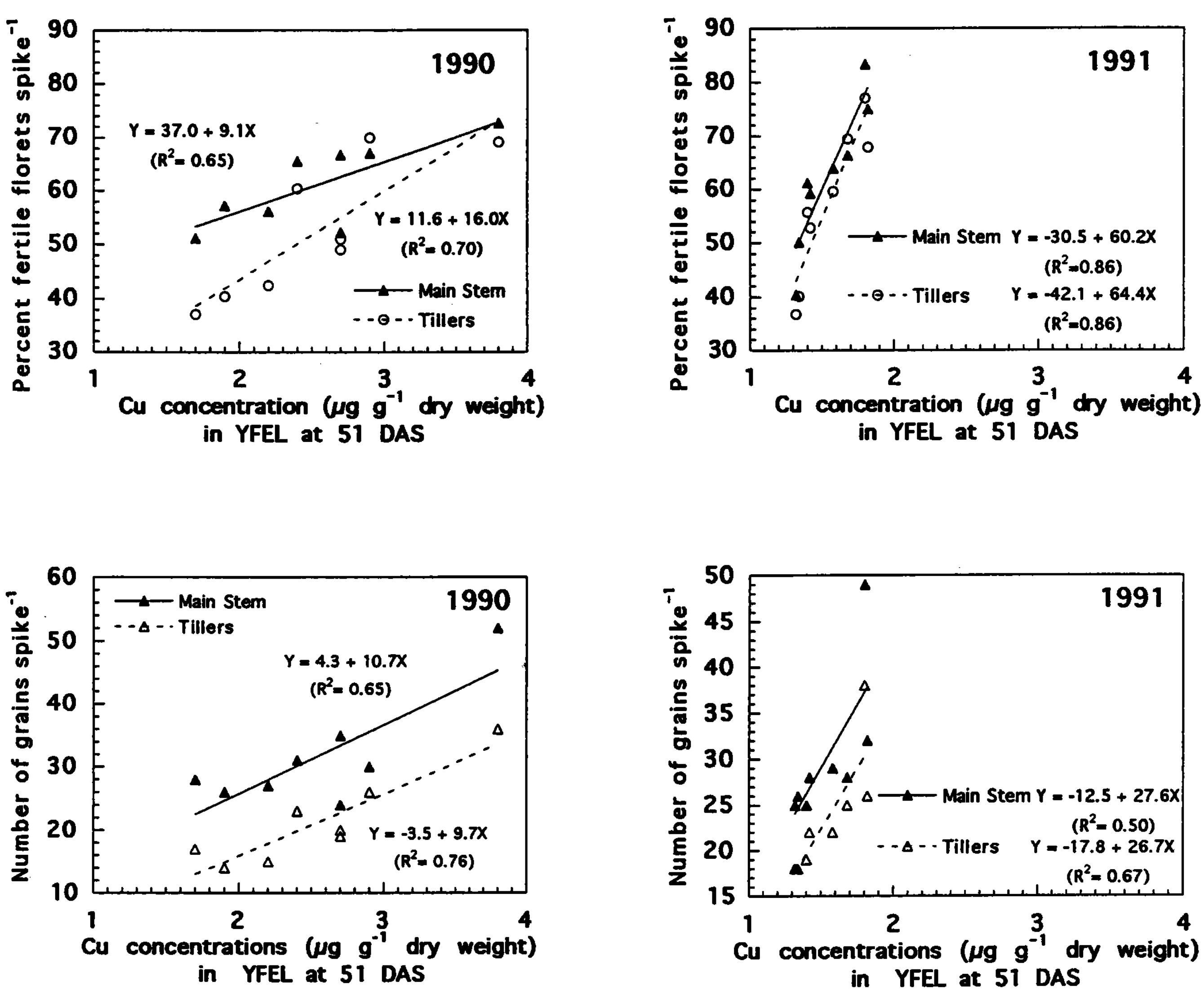

Fig. 3. Relationship of mean number of grains spike^{-1} and fertility of florets of the mainstem and tillers with Cu concentration in the YFEL at 51 DAS of eight Canadian spring wheats, sampled from the −Cu treatment in 2 yr.

Cu is supplemented, other factors become limiting, accounting for the non-significant correlations between tissue Cu level and grain yield in the +Cu treatment.

The level of Cu in the tissue of different wheat cultivars in this field study depended on the year, organ of the plant sampled, and stage of sampling. Therefore, the use of tissue analysis techniques to diagnose Cu deficiency cannot be recommended. This study demonstrated that Cu concentration in the YFEL decreased as the wheat plants matured. Secondly, Cu concentration in the plant leaves depended on cultivar, year, growth stage of sampling, and Cu status in the soil. Because of these effects and their interactions, no single critical Cu concentration in YFEL could be determined. Thirdly, Cu concentration in YFEL was not a useful indicator of potential Cu use efficiency of these wheat cultivars. However, Cu level in the leaves of cultivars 51 DAS was significantly related to cultivar grain yield and floret fertility and to grain set in both years, both under conditions of Cu-deficiency stress and when the cultivars were treated with Cu.

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