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THE INTERACTION OF COPPER AND SULPHUR DIOXIDE IN PLANT INJURY

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Barley cultivars were assessed for relative sensitivity to SO_2. The most SO_2-sensitive cultivar, Laurier, was used to study the effects of Cu in the rooting medium on plant growth. Copper stunted growth, especially of the roots, and induced leaf injury starting at the leaf tips. The cultivar Laurier, grown in a medium watered with 0, 10, 50 and 100 ppm Cu as $CuSO_4 \cdot 5H_2O$, was exposed to 1.0 ± 0.1 ppm SO_2 for 6 and 7 h on 2 consecutive days. Less injury due to SO_2 was observed at the higher Cu concentration. The higher levels of Cu greatly increased stomatal resistance, thereby reducing SO_2 uptake.

Nous avons évalué la sensibilité relative de plusieurs cultivars d'orge envers SO_2. Le cultivar le plus sensible, Laurier, a été utilisé pour étudier les effets de la présence de cuivre dans le substrat de culture. Le cuivre a atrophié la croissance, particulièrement celle des racines, et a provoqué des lésions foliaires commençant aux extrémités. Le cultivar Laurier cultivé dans un substrat arrosé de solution de 0, 10, 50 et 100 ppm de Cu, apporté sous forme de $CuSO_4 \cdot 5H_2O$, a été exposé à 1.0 ± 0.1 ppm SO_2 deux jours de suite pendant 6 et 7 h. Les dommages causés par SO_2 ont été moins prononcés aux fortes concentrations de Cu dans le substrat. En effet, à ces concentrations, le Cu a accru la résistance stomatique, réduisant du fait même l'absorption de SO_2.

Smelting processes produce a pollutant complex which often includes sulphur dioxide gas and heavy metal particulates as major components (Hutchinson and Whitby 1974). The most obvious impact of smelter emissions on the ecosystem is that of damage to vegetation (Katz 1939; Whitby and Hutchinson 1974). While sulphur dioxide and heavy metals occur together in the field, there has not been much investigation into their combined effect. Krause and Kaiser (1977) reported the enhancement of heavy metal toxicity by sulphur dioxide, when the metals were applied to foliage as oxide dusts. In the mining and smelting area of Sudbury, Ontario, the metals also accumulate to high concentrations in the soil (Warren et al. 1971; Hutchinson and Whitby 1974). Vegetation in the Sudbury area is also subject to occasional sulphur dioxide fumigations at damaging levels (Dreisinger 1970; Ontario Ministry of the Environment 1975), but no one has studied possible interactions between the polluting metals and sulphur dioxide. The objective of this paper is to investigate the effect of copper in the rooting medium on the subsequent response of plants to acute sulphur dioxide fumigation.

Copper was chosen as the heavy metal contaminant in the study since it is a prevalent contaminant in the Sudbury area (Hutchinson and Whitby 1974), and is also quite phytotoxic (Day et al. 1977). The study was approached in three steps: (1) the characterization and quantification of acute SO_2 injury on the foliage of potential plants to be used in this study, (2) the characterization and quantification of the effects of soil copper on these plants, and (3) the study of the interaction of soil copper and acute sulphur dioxide injury on the foliage.

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MATERIALS AND METHODS

Sulphur Dioxide Study

Twelve cultivars of barley (*Hordeum vulgare* L.), listed in Table 1, were sown in 10-cm pots containing a mixture of Guelph loam:peat:perlite (2:2:1). Seedlings were thinned to four per pot after 7 days. Plants were grown in Controlled Environment Model E15 growth cabinets with a day/night temperature regime of 22/15°C, respectively. A relative humidity of 70 ± 10% and light intensity of 18 klx (at pot height) were maintained. The photoperiod was 16 h with an abrupt light/dark change. The pots were fertilized with 100 ml of a 1 g/*l* solution of 20–20–20 soluble fertilizer once every 2 wk; otherwise they were watered with deionized water as necessary. Twenty-five days after sowing, the six pots of each cultivar were fumigated in plexiglas chambers as described by Ormrod et al. (1971) at a concentration of 0.35 ppm SO_2 for 9 h. Foliar injury was assessed the day following fumigation by visually estimating the percentage of the total leaf area that was chlorotic or necrotic.

Table 1. The relative sensitivity of 12 barley cultivars to sulphur dioxide treated 9 h at 0.35 ppm SO_2

Cultivar	% injury rating
Herta	1.7 *abc*
OB 123-28	2.7 *abcd*
Vanier	3.5 *bcd*
AB 15-1	4.3 *def*
Keystone	4.4 *def*
Conquest	5.1 *def*
Trent	6.9 *efg*
Perth	7.8 *efg*
Bonanza	8.5 *fg*
Peguis	9.7 *fg*
OB 148-1	10.0 *g*
Laurier	12.3 *g* (most sensitive)

a-g Numbers followed by the same letter are not significantly different at the 5% probability level (Duncan's new multiple range test).

Copper Study

To determine the response of barley to copper, Laurier barley was sown in 10-cm pots containing Turface (IMC Chemical Group (Canada)) and grown under similar conditions to the previous study. After germination, the pots were separated into four treatments: (1) 0 ppm Cu, (2) 10 ppm Cu, (3) 50 ppm Cu, and (4) 100 ppm Cu in the watering solution. The balance of each watering solution was of the composition defined by Winzeler et al. (1976). Plants were watered eve 2 days with the watering solution. Harvests we taken at 18, 23 and 33 days of growth of four po each time and dry weight, leaf area, and tiss copper content determined. Copper content w determined by dry-ashing 1.25 g of tissue 450°C for 10 h, dissolving the ash in 2 N HCl an measuring the copper by atomic absorptio (Varian Techtron AA175).

Copper and Sulphur Dioxide Interaction

To investigate the interaction between copper an SO_2, Laurier barley was sown in a mixture o Turface and perlite (1:1). The plants were grow under the environmental conditions and copp treatments already described. Twenty-five day after sowing, the plants were fumigated at 1.00 ± 0.1 ppm SO_2 for 13 h (6 h on day 1 + 7 h on day 2) to produce at least some injury on all copp treatments. Stomatal resistance readings were taken of 20 plants in each treatment with a Li-Co Model L 160 diffusive resistance meter equipped with a Li-Cor sensor. The instrument was calibrated and used with a reduced aperture (5 × 20 mm) to fit the leaves. Readings were taken before the first fumigation and before and after the second fumigation.

RESULTS AND DISCUSSION

Effect of Sulphur Dioxide

The injury symptoms due to sulphur dioxide involved necrotic streaking which developed near the leaf tip at first. As the injury extended down the leaf blade, it followed along the midrib region. If bending of some leaves occurred, the necrotic streaking developed from that point of bending. The necrotic tissue was gray-green in color at first; after a few hours it changed to an ivory color and after 24 h it bleached white. Young, expanded leaves showed the most damage, older leaves less, and unexpanded leaves the least. The oldest leaves tended to show injury much further down the leaf blade than did the younger leaves.

The results of the assessment (Table 1) indicate a range of sensitivities of the 12 barley cultivars. Laurier and OB 148-1 barley were the two most sensitive cultivars. Laurier barley was chosen for continued study because of its sensitivity to SO_2.

Effect of Copper

Copper toxicity symptoms developed in the two higher concentrations of copper treatment. There was a general stunting of the plant and decreased root development with increasing copper concentration of the watering solution. The oldest leaves developed an ivory-colored tip dieback. At 100 ppm copper after 33 days, this dieback extended along most of the oldest leaf and symptoms began to develop on younger leaves.

There was little effect of Cu on growth in terms of dry weight accumulation during the first 3 wk. After that, growth was significantly reduced, with the higher Cu concentrations having the greatest effect (Table 2). The Cu inhibited root growth: (1) it stunted growth of the primary roots, (2) it reduced the number and growth of secondary roots, and (3) it inhibited root hair formation. Root inhibition has been reported as a major effect of Cu toxicity (Brenchley 1938; Hunter and Welkie 1976; Whitby and Hutchinson 1974). The growth of roots was affected more than the growth of shoots as indicated by the reduction of root:shoot ratio with increasing copper levels. The ratio decreased from 0.70 at 0 ppm copper, to 0.45 at 100 ppm copper in the medium. The size of individual leaves was also reduced by the Cu treatments (Table 3). Initially, when the effect on growth was small the effect on final leaf size was small, but the effect increased with each additional leaf and with Cu concentration. However, the ratio between leaf length and width did not change with Cu treatment.

Absorbed Cu apparently accumulated in the roots and was not translocated to the shoot until levels in the medium were high (Table 4). The root accumulation of Cu may explain why root growth was more affected than shoot growth in copper treatments. The reduction in shoot growth may be related to impaired root function.

Combined Effects of Copper and Sulphur Dioxide

The two higher levels of Cu in the rooting medium significantly reduced the amount of visible, foliar injury from SO_2 treatment (Table 5). Some foliar injury was apparent on the 0 and 10 ppm Cu treatments at the end

Table 2. The effect of copper in the rooting medium on the growth of barley (Laurier) in terms of dry weight per plant

Time (days after sowing)	Cu treatment (ppm)			
	0	*10*	*50*	*100*
	Dry wt/plant (g)			
18	0.06 *a*	0.08 *ab*	0.09 *ab*	0.09 *b*
23	0.15 *c*	0.17 *cd*	0.14 *c*	0.15 *c*
33	0.36 *e*	0.25 *d*	0.20 *cd*	0.15 *c*

a–e Weights followed by the same letter are not significantly different at the 5% probability level (Duncan's new multiple range test).

Table 3. The effect of Cu in the rooting medium on leaf area in Laurier barley

	Cu treatment (ppm)			
	0	10	50	100
	Leaf area (cm^2)			
Leaf 1	37.9 *a*	38.9 *a*	74.8 *a*	28.4 *b*
2	80.7 *a*	73.9 *b*	58.7 *b*	43.5 *c*
3	139.7 *a*	128.5 *a*	111.0 *b*	64.7 *c*

a–c Values followed by the same letter for any one leaf are not significantly different at the 5% probability level (Duncan's new multiple range test).

Table 4. Copper content of Laurier barley (ppm on dry weight basis)

Cu treatment (ppm)	Tops (ppm)	Roots (ppm)
0	60 *a*	163†
10	61 *a*	249
50	59 *a*	1040
100	171 *b*	2272

a,b Numbers followed by the same letter are not significantly different at the 5% probability level (Duncan's new multiple range test).
† Root sample bulked; therefore no statistical tests were performed.

of the first fumigation period but none on the 50 and 100 ppm Cu. All treatments exhibited SO_2 injury at the end of the second SO_2 fumigation, but injury was more severe in control and 10 ppm Cu treatment.

Table 5. The effect of Cu in the rooting medium on foliar injury of barley exposed to sulphur dioxide (1.00 ± 0.10 ppm) for 13 h

	Percent injury	
Cu treatment (ppm)	After 1st fumigation	After 2nd fumigation
0	2.7 *a*	9.0 *a*
10	1.7 *a*	9.3 *a*
50	0 *b*	3.1 *b*
100	0 *b*	1.5 *c*

a-c Numbers followed by the same letter in a column are not significantly different at the 5% probability level (Duncan's new multiple range test).

The stomatal resistance of the plants treated with 50 and 100 ppm Cu was consistently higher both at the beginning and during the SO_2 treatments (Table 6). With Cu treatments, the stomatal resistance di not change significantly during the fumig: tion for the 0 and 10 ppm Cu whereas th stomatal resistance for the 50 and 100 ppı increased significantly as a result of the fir: day fumigation. The resistances for the 5 and 100 ppm had decreased again to value similar to those at the beginning of the fir: exposure to SO_2. The higher resistances i: the 50 and 100 ppm Cu treatments appeare: to reflect the severe reduction in root size an impaired water uptake of these plant through increased stomatal resistance.

The significance of this study lies in it: simulation of the field situation. Coppe: levels comparable to that found in the leave: of plants treated with 100 ppm Cu have been found in the field in *Deschampsia flexuosa* growing in the Sudbury area (Hutchinson and Whitby 1974) and so interactions found in this study could quite conceivably occur in the field. Exposure to SO_2 somewhat increased the chlorosis induced by the 50 and 100 ppm Cu treatments, this being similar to the results of Krause and Kaiser (1977) where SO_2 increased Cu toxicity when the Cu was applied to the foliage as dust.

In summary, high levels of copper in the rooting medium suppressed growth, especially of the rooting system. With high copper, stomatal resistance was increased and plants were injured less by sulphur dioxide. Plants growing in mining and smelting areas may be showing less sensitivity to sulphur dioxide because of elevated levels of copper in the soil.

Table 6. The effect of Cu (in the rooting medium) and SO_2 treatment on stomatal resistance in barley leaves

	Cu treatment (ppm)			
Time	0	10	50	100
		($s.cm^{-1}$)		
Before 1st exposure to SO_2	3.1 *a*	3.4 *a*	13.8 *bc*	24.3 *d*
Before 2nd exposure to SO_2	4.5 *a*	5.5 *a*	22.8 *d*	47.6 *e*
At end of 2nd exposure to SO_2	3.6 *a*	9.2 *ab*	13.8 *bc*	19.1 *cd*

a-e Values followed by the same letter are not significantly different at the 5% probability level (Duncan's new multiple range test).