Journal of Environmental Sciences Vol. 18, No. 1, pp. 101–108, 2006

ISSN 1001—0742
CN 11—2629/X

Article ID: 1001-0742(2006)01-0101-08 **CLC number:** X131 **Document code:**A

Effect of dissolved organic matter on the toxicity of chlorotoluron to *Triticum aestivum*

SONG Ning-hui[1], YANG Zhi-min[2], ZHOU Li-xian[3], WU Xin[1], YANG Hong[1,*]

(1. College of Science, Nanjing Agricultural University, Nanjing 210095, China. E-mail: hongyang@njau.edu.cn; 2. College of Life Science, Nanjing Agricultural University, Nanjing 210095, China; 3. College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China)

Abstract: Response of two wheat cultivars (*Triticum aestivum* cv. YM 158 and NM 9) to the herbicide chlorotoluron and the effect of two forms of dissolved organic matter on the chlorotoluron toxicity to the plants were characterized. Treatment with chlorotoluron at 10—50 μg/ml inhibited the seed germination and a dose-response was observed. The inhibition of seed germination was correlated to the depression of α-amylase activities. To identify whether chlorotoluron induced oxidative damage to wheat plants, the malondlaldehyde (MDA) content and electrolyte leakage were measured. Results showed that both MDA content and electrolyte leakage in the chlorotoluron-treated roots significantly increased. Activities of several key enzymes were measured that operate in citric acid cycle and carbohydrate metabolic pathway. Inhibited activities of citrate synthase and NADP-isocitrate dehydrogenase were observed in the chlorotoluron-treated roots as compared to control plants. We also examined malate dehydrogenase and phosphoenolpyruvate carboxylase in wheat roots exposed to 30 μg/ml chlorotoluron. However, none of the enzymes showed significant changes in activities. Application of 160 μg/ml dissolved organic matter (DOM) extracted from non-treated sludge(NTS) and heat-expanded sludge (HES) in the medium with 30 μg/ml chlorotoluron induced an additive inhibition of seed germination and plant growth. The inhibition of growth due to the DOM treatment was associated with the depression of activities of α-amylase, citrate synthase and NADP-isocitrate dehydrogenase, as well as the increase in malondlaldehyde content and electrolyte leakage. These results suggested that the presence of DOM might enhance the uptake and accumulation of chlorotoluron, and thus resulted in greater toxicity in wheat plants. The two forms of DOM exhibited differences in regulation of chlorotoluron toxicity to the wheat plants. Treatments with DOM-NTS induced greater toxicity to plants as compared to those with DOM-HES. In addition to DOM affecting chlorotoluron-induced toxicity to wheat plants, the cultivars could have also contributed to differences. Generally, NM-9 showed a higher sensitivity to chlorotoluron than YM 158 either in the absence or in the presence of DOM.

Keywords: dissolved organic matter; chlorotoluron; *Triticum aestivum*; toxicity

Introduction

Chlorotoluron, a phenylurea herbicide with high activity and low toxicity is worldwidely used for either pre- or post-emergence selective controlling of grass weeds in cereal, cotton and fruit productions (Lechón *et al.*, 1997). In china alone, more than thousands of tons of chlorotoluron each year have been applied for the spring and winter wheat production (Cai, 1999). Chlorotoluron is normally soil-applied and relatively soluble in water. It is therefore readily taken up by plant roots and transported to the leaves to perform the weeding effect (Sharples *et al.*, 1997). While chlorotoluron as one of indispensable elements of modern agriculture is applied to agricultural land to protect crops and plantation from weeds, it becomes a significant source of diffuse contaminants causing health implications on living organisms (Yazgan and Tanik, 2005). In recent years, there is an increasing concern about its fate (Walcr *et al.*, 2005). Research indicates that due to its moderate adsorption onto soil constituents, chlorotoluron is fairly mobile and leaching in soil and is detected in agricultural soils, rivers, lakes and streams (Deneer, 2000; El Sebai, 2004). Ecotoxicological data have demonstrated that chlorotoluron also readily accumulates in crops and vegetables and excess accumulation may cause toxicity to plant growth. Likewise, its existence in environments also has a toxic effect on aquatic invertebrates, freshwater algae and microbial activity (Pérés *et al.*, 1996; Ma *et al.*, 2002).

The performance of herbicides as soil contaminants is influenced by a varity of environmental conditions such as soil types, moisture, light, temperature and soil dissolved organic matter(DOM) (Sharples *et al.*, 1997). DOM is similar both structurally and functionally with surfactants and may influence herbicide mobilization, metabolism and binding properties in soils(Celis *et al.*, 1998; Zhou and Wong, 2000a). Therefore, DOM may affect bioavailability and toxicity of herbicides to plants (Bejarano *et al.*, 2005; Li *et al.*, 2005; Yang *et al.*, 2005). Several lines of studies show that DOM reduced the bioavailability to aquatic organisms of

Foundation item: The National Natural Science Foundation of China(30170537); *Corresponding author

hydrophobic contaminants (Businelli, 1997; Celis *et al.*, 1998). The original concentration of DOM in soil is 20—80 mgC/ml. However, some materials rich in organic carbon such as crop stalks, organic fertilizers and residuary roots of plants may produce high DOM concentrations in a short term (the concentration may increase from original 20—80 mgC/ml to thousands of mgC/ml). High DOM concentrations in soils may exert impact on the migration, biological accumulation and toxicity of herbicides to plants (Celis *et al.*, 1998).

Although it is well documented that the effect of DOM on herbicides is associated with its sorption, desorption and leaching in soils (Celis *et al.*, 1998; Seol and Lee, 2000; Li *et al.*, 2005), little information is available regarding the DOM-induced changes in toxicity of herbicide to plants. In this study, we characterized the susceptibility of two wheat cultivars to chlorotoluron toxicity. We also identified the role of two forms of DOM in regulating chlorotoluron-induced toxicity to wheat plants. The purpose of the investigation is to help us to understand how DOM biologically affects the response of plants to chlorotoluron.

1 Materials and methods

1.1 Materials

Seeds of wheat (*Triticum aestivum* L. cultivars YM 158 and NM-9) and the herbicide chlorotoluron [3-(3-chloro-*p*-tolyl)-1, 1-dimethylurea] examined in this study were obtained from the Academy of Agricultural Sciences in Jiangsu, China. The seeds were surface-sterilized with 1% NaClO for 10 min, rinsed several times with distilled water and placed on moistened filter papers(Whatman No.1) in a petri dish. The seeds were kept in the dark for germination. When seeds germinated, they were removed to another petri dish containing a freshly moistened filter paper with treatment solutions. Petri dishes with germinating seeds or seedlings were placed in a chamber with temperature at 20 ±1℃, a light intensity of 300 μmol/(m^2·s) and 14 h photoperiod. Usually, during the first 7 d, the plants were not fed because they contain enough nutrients for their growth.

Two forms of dissolved organic matter were used. One form of DOM was extracted from non-treated sludge(NTS) or intact sludge and the other from heat-expanded sludge (HES). The sludge was collected from Suzhou, Jiangsu, China. DOM was prepared according to a previous method described by Zhou and Wong (2000a). The sludges were extracted with Milli Q water using a solid:water ratio of 1:10 (w/v, dry weight basis) in a reciprocal shaker at 200 r/min and 4℃ for 16 h. The suspensions were centrifuged at 10000 g and 4℃ for 15 min, and filtered through a 0.45 μmol/L sterilized membrane (GN-6 Metrice, Gelman Sciences, Ann Arbor, MI). The filtrates were analyzed for pH and total organic carbon (TOC-5000A, Shimadzu, Japan). All DOM extracts were used immediately after preparation. The major properties are shown in Table 1.

Table 1 Selected properties of dissolved organic matter

DOM sample	TOC, mgC/L	pH
DOM from non-treated sludge(NTS)	337	7.13
DOM from heat-expanded sludge(HES)	1938	7.24

1.2 Chlorotoluron treatment and growth analysis

For the experiment of chlorotoluron impact on germination, seeds were sterilized, rinsed and soaked in distilled water for 1 h. This allowed seeds physically to take up enough water. The water then was removed and replaced by treatment solutions with chlorotoluron at 0, 10, 20, 30, 40 and 50 μg/ml (prepared in 10 mmol/L $CaCl_2$) and/or two forms of DOM at 160 μg/ml. Each petri dish contained 20 seeds for a treatment. Typically, a result (or an independent experiment) was the mean of at least three treatments.

To establish a dose-response curve, the roots of two-day old plants after germination were exposed to the various concentrations of chlorotoluron (0, 10, 20, 30, 40 and 50 μg/ml) and/or DOM at 160 mg C/L) for 7 d. The five concentrations of chlorotoluron were used because within the concentration 5% to 35 % inhibition of seed germination could be detected as compared with the control. The plants were sampled for analysis. Each experiment was repeated three times with at least 60 plants.

1.3 Measurement of electrolyte leakage of roots

Twenty 30-mm long segments of roots(from tips) were collected and rinsed three times, dried with filter papers and put into a test tube. 20—30 ml of deionized water was added to the tube to soak the root segments. The tubes were shaken at 80 r/min at(25 ±1)℃ for 2 h. Measurement of water conductance and calculation of electrolyte leakage percentage were based on the method described by Gong *et al.*(2001).

1.4 Determination of lipid peroxidation

The malondlaldehyde (MDA) content was determined by a procedure based on the method of Heath and Packer(1968).

1.5 Assay of enzyme activity

For α-amylase activity (EC 3.2.1.1) assay, 2 day-old germinating seeds were homogenized in an ice-old 100 mmol/L Tris-HCl buffer (pH 6.4). The homogenate was centrifuged at 12000 × g at 4℃ for 5 min. The supernatant was used to measure the activity of α-amylase. The enzyme activity was determined by the procedure of Agüloğlu *et al.*(2000).

Roots of 7 d old of plants were homogenized in an iced-cold 50 mmol/L HEPES-NaOH buffer(pH 7.5) containing 5 mmol/L $MgCl_2$, 5mmol/L EDTA, 10% (v/v) glycerol and 0.1%(v/v) Triton X-100(Yang *et al.*, 2004). The homogenate was centrifuged at 15000 ×g at 4℃ for 5 min. The supernatant was used to measure the activities of following enzymes. Citrate synthase (CS, EC 4.1.3.7) activity was spectrophotometrically assayed by monitoring a decrease in acetyl CoA at 412 nm for 4 min. The reaction mixture contained 100 mmol/L Tris-HCl buffer (pH 8.0), 5 mmol/L $MgCl_2$, 0.5 mmol/L 5,5-dithio-bis-2-nitrobenzoic acid, 0.2 mmol/L acetyl CoA, and 1 mmol/L oxalacetic acid (Yang *et al.*, 2003). Activity of NADP-isocitrate dehydrogenase (NADP-ICDH, EC 1.1.1.42) was assayed according to the method of Udvardi *et al.* (1993) by monitoring a reduction of NADH at 340 nm for 2—3 min with the reaction mixture containing 100 mmol/L Tris-HCl (pH 8.0), 5 mmol/L $MgCl_2$, 0.5 mmol/L $NADP^+$, 2.5 mmol/L DL-Na_3 isocitrate. For malate dehydrogenase (MDH, EC 1.1.1.37) activity measurement, the reaction mixture contained 50 mmol/L HEPES-KOH (pH 7.5), 0.5 mmol/L EDTA, 0.2 mmol/L NADH, and 1 mmol/L oxaloacetate (Macnicol and Jacobsen, 1992). The reaction was started by addition of oxaloacetate and the disappearance of NADH at A_{340} in the first minute was recorded. The phosphoenolpyruvate carboxylase (PEPCase, EC 4.1.1.31) activity was spectrophotometrically assayed by monitoring the disappearance of NADH at 340 nm for 3 min(Macnicol and Jacobsen, 1992). The reaction mixture contained 100 mmol/L Tris-HCl (pH 8.4), 5 mmol/L $MgCl_2$, 100 mmol/L $NaHCO_3$, 25 mmol/L PEP, 0.2 mmol/L NADH, 2 units of MDH. The protein in the enzyme extract was quantified by the method of Bradford (1976).

1.6 Statistical analysis

All the experiments were performed using at least three repetitive independent treatments. The values are expressed as means ± *SD*. The significance of the differences between the means was calculated by using the Student's *t* test. Statistical significance was set at $p<0.05$.

2 Results

2.1 Response of seed germination to chlorotoluron in the absence and presence of DOM

Fig.1 shows the seed germination of two wheat cultivars YM-158 and NM-9 under the treatment of chlorotoluron at 0, 10, 20, 30 and 50 μg/ml. In the absence of DOM, the inhibition of germination rates in both cultivars increased with the chlorotoluron concentrations applied. There was a linear correlation between the chlorotoluron concentration and inhibition rate, with correlation coefficients (r^2) of 0.9278 and 0.9752 for YM-158 and NM-9 respectively. However, the two cultivars showed a different sensitivity to chlorotoluron under the same condition. For example, if a threshold-value of 20% inhibition rates was introduced to calculate the difference, the chlorotoluron concentration for YM- 158 was 47.73 μg/ml, and the chlorotoluron concentration for NM-9 was only 36.25 μg/ml.

Application of either DOM-HTS or DOM-NTS

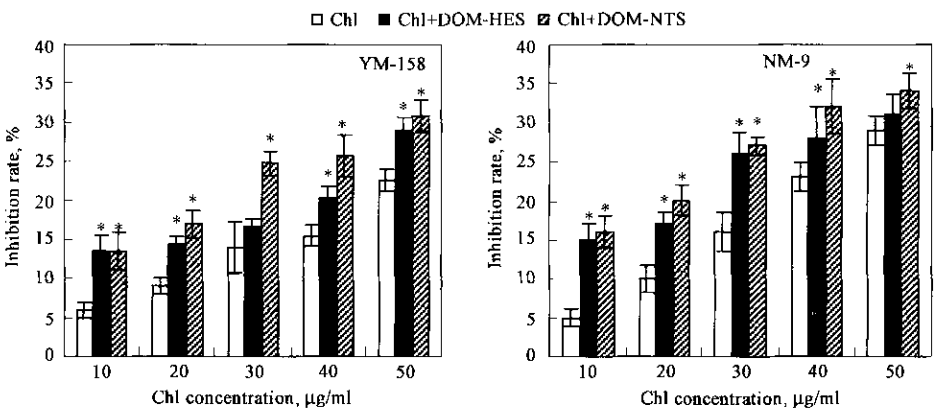

Fig.1 Effects of chlorotoluron and two types of DOM on the seed germination of wheat YM-158 and NM-9. Dry seeds were sterilized, rinsed and incubated in the solutions containing 0, 10, 20, 30, 40 and 50 μg/ml chlorotoluron and 160 μg/ml DOM (HES and NTS). The germinating seeds were counted and analyzed. Values are the means ± *SD* (n=60). Asterisks indicate that mean values are significantly different between the treatments of Chl+DOMs (DOM-HES or DOM-NTS) and Chl alone

(160 μg/ml) alone to the culture medium caused no significant changes in germination rate of YM 158 and NM 9 seeds as compared to controls(data not shown), however, in the presence of chlorotoluron, both forms of DOM induced general increases in inhibition of seed germination (Fig.1). DOM-NTS seemed to be stronger in inducing chlorotoluron-responsive inhibition of seed germination than that of DOM-HTS.

DOM-induced inhibition of wheat seed germination was cultivar-specific. The threshold-value (applied chlorotoluron concentrations) under 20% of inhibition rate of germination for YM 158 were 35.09 μg/ml (DOM-HES) and 21.97 μg/ml (DOM-NTS), while those for NM 9 were 22.09 μg/ml(DOM-HES) and 17.91 μg/ml(DOM-NTS).

Since the activity of α-amylase represents the capacity of seed germination, it was assayed in germinating seeds under the chlorotoluron treatment (Fig.2). Treatment with 30 μg/ml chlorotoluron significantly caused a decrease in α-amylase activities in both wheat cultivars. Simultaneous treatments with two forms of DOM induced slightly additional suppression of the enzyme activity. Compared to the α-amylase activity in cultivar YM 158, the activity in NM 9 was lower under the same treatment condition.

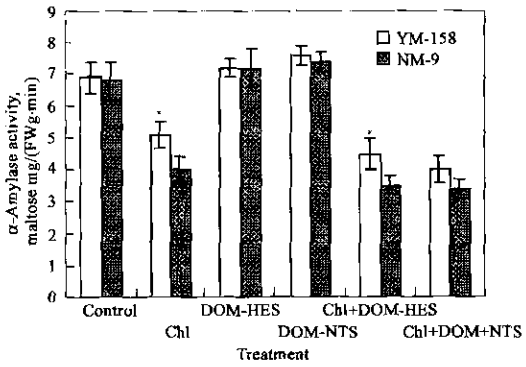

Fig.2 Response of α-amylase activities of germinating seeds of two wheat cultivars YM-158 and NM-9 to chlorotoluron and/or DOM. Dry seeds were sterilized, rinsed and incubated in the treatment solutions containing 0 (control) and 30 μg/ml chlorotoluron and/or 160 μg/ml DOM (HES and NTS). The treated plants were sampled for enzyme activity assays as described in "Materials and Methods". Values are the means ± *SD* (n=60). Asterisks indicate that mean values are significantly different between the activities of two cultivars

2.2 Growth response to chlorotoluron in the absence and presence of DOM

Effect of chlorotoluron on the growth of two wheat cultivars is presented in Fig.3. In general, both cultivar showed reduced elongation of leaves or roots after exposure to chlorotoluron at 10—50 μg/ml for 7 d. A concentration-dependent inhibition was observed.

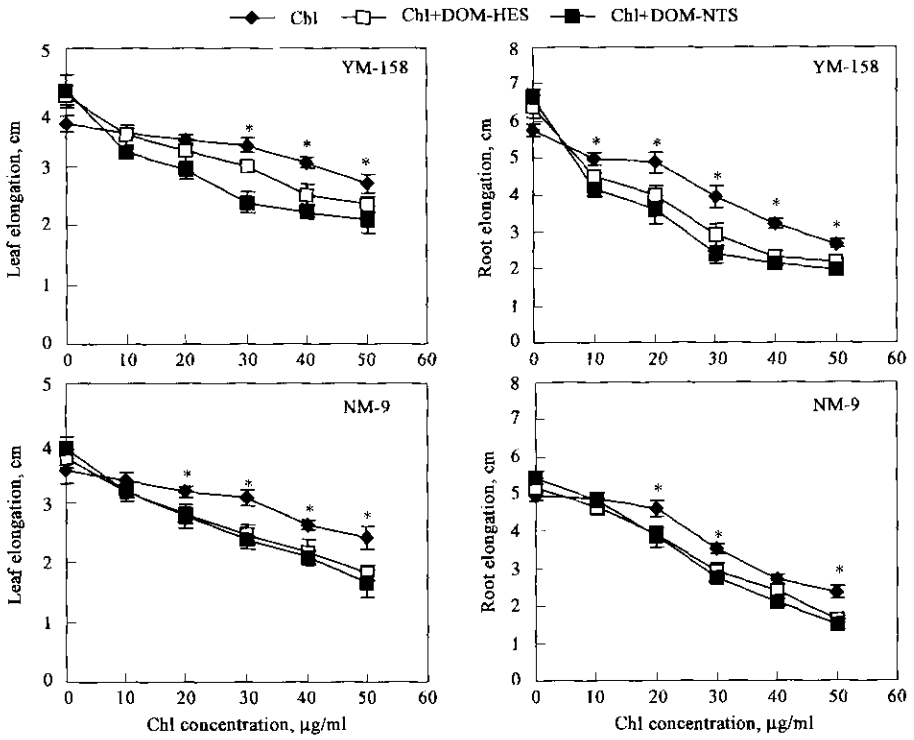

Fig.3 Effects of chlorotoluron and two forms of DOM on the elongation of wheat leaves and roots. Plants after germination were exposed to the treatment solutions containing chlorotoluron at 0, 10, 20, 30, 40, 50 μg/ml and DOM (HES and NTS) at 160 μg/ml for 7 d and then, the leaf and root elongation of the treated plants were measured. Values are the means ± *SD* (n=60). Asterisks indicate that mean values are significantly different between the treatments of Chl+DOMs(DOM-HES or DOM-NTS) and Chl alone

Treatment with DOM-HES or DOM-NTS(160 μg/ml) alone induced a slight increase in leaf and root elongation in YM 158 and NM 9 cultivars. However, in the presence of chlorotoluron, both forms of DOM caused additional inhibition of leaf or root elongation.

To confirm the roles of DOM in regulating the toxicity of chlorotoluron to wheat plants, we measured the fresh mass of plants under the combined treatments with chlorotoluron and DOM. As shown in Table 2, treatment of plants with 30 μg/ml chlorotoluron reduced the leaf fresh mass by 10.5% (YM 158) and 14.8%(NM 9) and root fresh mass by 4.8%(YM 158) and 6.1%(NM 9), respectively. The reduction in leaf fresh mass of both cultivars was greater than that of root, suggesting that leaves were more sensitive to the herbicide than roots. Treatment with DOM-HES or DOM-NTS alone slightly increased the fresh mass of both cultivars. However, the combined treatments with chlorotoluron and DOM exerted an additively inhibitory effect on the growth.

Table 2 Growth response of two wheat cultivars YM-158 and NM-9 to chlorotoluron and DOM

Treatment	YM-158		NM-9	
	Leaf	Root	Leaf	Root
	Fresh mass, mg/plant			
Control	35.2±2.3[a]	39.2±1.2[a]	35.8±2.1[a]	36.2±0.9[a]
Chl	31.5±1.4[b]	37.3±1.5[b]	30.5±1.2[b]	34.0±3.2[a]
DOM-HES	36.2±1.1[a]	41.5±1.5[a]	36.9±2.7[a]	37.8±1.7[a]
DOM-NTS	37.6±2.8[a]	40.2±1.2[a]	37.8±3.1[a]	37.2±1.5[a]
Chl+DOM-HES	29.1±1.1[b]	36.8±1.0[b]	28.2±2.6[b]	30.3±2.3[b]
Chl+DOM-NTS	27.6±2.8[b]	33.8±1.8[c]	25.2±1.7[c]	29.6±1.7[b]

Notes: Plants after germination were exposed to the treatment solutions containing chlorotoluron at 0 (Control) and 30 μg/ml and/or 160 μg/ml DOM for 7 d. Then, the treated plants were sampled and fresh leaf and root were weighted. Values are the means ±*SD* (n=30). Means with different superscript letters in columns are significantly different at p<0.05

2.3 Response of oxidative stress to chloroto-luron in the absence and presence of DOM

Concentration of lipid peroxides in the growing plants was measured in terms of MDA content(Table 3). Under the chlorotoluron treatment an elevated level of lipid peroxides was observed in roots compared to controls, with an increase from 12% to 19% in the two cultivars. Since lipid peroxides induced by the herbicide might be associated with plasma membrane injury, the root electrolyte leakage was detected. There was a concomitant increase in the chlorotoluron-treated roots of wheat plants. Addition of DOM caused a higher accumulation of MDA and greater leakage root tissues.

Table 3 Lipid peroxidation and root electrolyte leakage of plasma membrane in roots of the two wheat cultivars to chlorotoluron and DOM

Treatment	MDA content, nmol/g FW		Electrolyte leakage, %	
	YM15	NM9	YM15	NM9
Control	2.76±0.11[c]	2.71±0.09[c]	5.01±0.31[b]	4.30±0.24[b]
Chl	3.15±0.21[b]	3.34±0.07[b]	5.79±0.23[a]	5.22±0.24[a]
DOM-HES	2.73±0.13[c]	2.70±0.12[c]	5.11±0.12[b]	4.24±0.13[b]
DOM-NTS	2.66±0.24[c]	2.81±0.35[c]	5.02±0.23[b]	4.25±0.18[b]
Chl+DOM-HES	3.21±0.12[b]	3.55±0.12[a]	5.47±0.14[a]	5.18±0.31[a]
Chl+DOM-NTS	3.50±0.18[a]	3.59±0.15[a]	5.51 ± 0.26[a]	5.26±0.34[a]

Notes: Plants after germination were exposed to the treatment solutions containing chlorotoluron at 0 (CK) and 30 μg/ml and/or 160 μg/ml DOM for 7 d. Then, the treated plants were sampled for the physiological determination as described in "Materials and Methods". Values are the means ± *SD* (n=3). Means with different superscript letters in columns are significantly different at p<0.05

2.4 Enzymatic response to chlorotoluron in the absence and presence of DOM

The inhibited activities of citrate synthase (CS) and NADP-isocitrate dehydrogenase (NADP-ICDH) were observed in the chlorotoluron-treated roots as compared to control plants. Treatment of wheat plants with combination of DOM and chlorotoluron resulted in an additional inhibition of CS and NADP-ICDH activities(Table 4).

Table 4 Responses of activities of CS, NADP$^+$-ICDH, MDH and PEPCase in roots of two wheat cultivars YM-158 and NM-9 to chlorotoluron and DOM

Treatment	CS	NADP$^+$-ICDH	MDH	PEPCase
	μmol protein/(mg·min)			
	YM-158			
Control	0.81±0.07[a]	0.76±0.04[a]	5.2±0.2[a]	0.21±0.02[a]
Chl	0.65±0.05[b]	0.56±0.02[b]	4.8±0.4[a]	0.20±0.02[a]
DOM-HES	0.78±0.03[a]	0.70±0.06[a]	5.6±0.4[a]	0.24±0.03[a]
DOM-NTS	0.86±0.02[a]	0.80±0.01[a]	5.8±0.6[a]	0.22±0.02[a]
Chl+DOM-HES	0.61±0.05[b]	0.45±0.04[c]	5.0±0.5[a]	0.19±0.01[a]
Chl+DOM-NTS	0.50±0.02[c]	0.41±0.03[c]	4.9±0.6[a]	0.18±0.03[a]
	NM-9			
Control	0.88±0.04[a]	0.79±0.02[a]	5.8±0.4[a]	0.22±0.01[a]
Chl	0.60±0.03[b]	0.50±0.02[b]	5.2±0.5[a]	0.20±0.03[a]
DOM-HES	0.82±0.04[a]	0.78±0.03[a]	5.8±0.2[a]	0.23±0.02[a]
DOM-NTS	0.81±0.11[a]	0.79±0.04[a]	5.7±0.4[a]	0.23±0.02[a]
Chl+DOM-HES	0.46±0.03[c]	0.38±0.02[c]	5.0±0.2[a]	0.20±0.03[a]
Chl+DOM-NTS	0.41±0.02[c]	0.33±0.02[c]	4.8±0.5[a]	0.20±0.02[a]

Notes: Plants after germination were exposed to the treatment solutions containing chlorotoluron at 0 (CK) and 30 μg/ml and/or 160 μg/ml DOM for 7 d. Then, the treated plants were sampled for the enzyme activity assays as described in "Materials and Methods". Values are the means ± *SD* (n=3). Means with different superscript letters in columns are significantly different at p<0.05

In contrast, malate dehydrogenase (MDH) and phosphoenolpyruvate carboxylase (PEPCase) activities were hardly affected by the treatment with chlorotoluron. There was also no marked effect of DOM on the MDH and PEPCase activities in the chlorotoluron-treated and untreated roots.

3 Discussion

3.1 Response of growth and peroxidation to chlorotoluron

It is well known that herbicides like chlorotoluron are toxic to annual grass weed (Van Oorscho and Van Leeuwen, 1992; Menendez *et al.*, 1994; Sharples *et al.*, 1997). However, little information is available on the toxicity of herbicides to crops. The first aim of the investigation was to identify the toxicity of chlorotoluron to wheat plants. Our results showed that chlorotoluron at 10—50 μg/ml in the medium affected the seed germination of the two wheat cultivars YM 158 and NM 9 (Fig.1). This inhibition was dependent on the dosage of chlorotoluron. The inhibition of seed germination was supported by the evidence that α-amylase activity was depressed with the presence of 30 μg/ml chlorotoluron (Fig.2). These results indicated that the effect of the herbicide toxicity to wheat plants occurred at the very early stage of growth.

The growth response of the two wheat cultivars to chlorotoluron was also found. Elongation of roots and leaves was inhibited by chlorotoluron at the indicated concentrations (Fig.3). There was a concentration-dependent change. The inhibitory effect on growth was further confirmed by measuring fresh weight of plants that were exposed to 30 μg/ml chlorotoluron for 7 d (Table 2). The leaves seemed to be more sensitive to chlorotoluron than the roots.

Activation of lipid peroxidation in plants under the biotic and abiotic stresses like pathogen attack or other elicitors has been intensively investigated (Mittler, 2002). However, to our knowledge there has been no report on chlorotoluron-induced oxidative stress in plants. To understand the possible toxic mechanism by which chlorotoluron affected the growth of wheat plants, we measured the root MDA content and electrolyte leakage, two physiological parameters representative of cell plasma membrane damage(Wang *et al.*, 2004). The increase in MDA and electrolyte leakage of root plasma membrane suggested that chlorotoluron was able to induce oxidative stress in wheat plants. However, the reason why the herbicide induced the oxidation remains to be elusive.

The interference of herbicides on plant processes like photosynthesis has been reported (Sharples *et al.*, 1997). However, information of chlorotoluron on the processes linked to energy and carbohydrate metabolism in cells is not available. The present study examined the activities of some key enzymes in Krebs cycle(or citric acid cycle) and carbohydrate metabolic pathway. The first enzyme examined was the citrate synthase that catalyzes the condensation of acetyl CoA with oxaloacetate to form citrate. Our result showed that the CS activity in the two wheat plants appeared to be sensitive to chlorotoluron(Table 4). There was a difference between the cultivars, with more sensitivity in NM 9 than in YM 158. A similar result was found in activity of NADP-isocitrate dehydrogenase (Table 4). This enzyme catalyzes the oxidative decarboxylation of isocitrate to 2-oxglutarate in cytosol (Kruse *et al.*, 1998). The depression of CS and NADP-ICDH activities suggested that the interference of chlorotoluron with cell energy production possibly occurred.

Malate dehydrogenase converses of malic acid to oxalacetate. The latter is further converted to citric acid with CS in the presence of acetyl-CoA. Phosphoenolpyruvate carboxylase catalyzes the β-carboxylation of phosphenolpyruvate to yield oxaloacetate, the precursor of CS(Moing *et al.*, 1999). In contrast to CS and NADP-ICDH, activities of MDH and PEPCase in wheat plants were not significantly affected by the chlorotoluron exposure (Table 4). During the germination of seeds and early stage of seedling growth, the energy requirements must be partially or entirely met by respiration. The inhibition of key enzymes in citric acid cycle by chlorotoluron implied that the energy supply and production of metabolic intermediates (precursors for synthesis of other molecules) might be blocked. The inhibition of citric acid cycle would limit generation and supply of reducing equivalents for operation of the respiratory chain and this in turn is likely to diminish the capacity of seeds or seedlings to generate ATP through mitochondrial respiration, that is essential for the rapid growth of healthy seedlings(Bansal *et al.*, 2002). The effect of chlorotoluron might partly contribute to its toxicity to seed germination and early seedling growth.

3.2 Regulation of DOM in chlorotoluron-induced toxicity to wheat plants

Application of DOM extracted from the two forms of sludges led to a general reduction in the seed germination rate(Fig.1) and α-amylase activity(Fig.2). The inhibition of seed germination was associated

with decrease in the plant growth (Fig.3, Table 2) and some key enzymes related to energy metabolism (Table 4). These results suggested that the presence of DOM possibly induced an increase in permeability of the herbicide into germinating seeds and consequently resulted in greater phytotoxicity in the seedlings. It is reasonable to assume that the DOM with various chemical structures, composition and even steric conformations might interact with chlorotoluron and stimulate more chlorotoluron molecule translocation into the cells. In fact, DOM contains a variety of ligands, and these will facilitate the binding of organic contaminants in environments, thus affecting their bioavailability(Stangroom *et al.*, 2000; Bejarano *et al.*, 2005).

The two forms of DOM in the study exhibited differences in regulation of chlorotoluron toxicity to the wheat plants. In the most cases, treatment with DOM-NTS induced greater toxicity to plants as compared to those with the DOM-HES. This suggested that non-treated sludge might have a relative greater effect on the uptake of the herbicide chlorotoluron into plants. Although the different molecular properties or components of DOM might make the major differences (Zhou and Wong, 2000a), the mechanism by which the two forms of DOM affected the chlorotoluron toxicity remained to be elucidated.

In addition to DOM affecting chlorotoluron-induced toxicity to wheat plants, the cultivars could have also contributed to the differences. For example, the fresh mass and CS and NADP-ICDH activities in YM 15 under chlorotoluron and DOM treatments were higher than those in NM 9 (Table 2, 4). These suggested that with the same DOM, NM 9 was more sensitive to chlorotoluron than YM 15. The greater inhibition of growth and metabolism in NM 9 plants in the presence of the herbicide could possibly be explained by its increased uptake or less efficient reduction of toxicity(Table 1). Further research will be required for characterize the role of DOMs in regulating the herbicide uptake and bioconcentration in plants.

4 Conclusions

The herbicide chlorotoluron adversely affected the seed germination and seedling growth of two wheat cultivars YM 158 and NM 9. This was associated with the oxidative damage of plant root plasma membrane and hampered energy generation and decreased production of metabolic intermediates. Application of two forms of DOM aggravated the toxicity of chlorotoluron to wheat plants. DOM from non-treated sludge had a greater effect on chlorotoluron toxicity to wheat than DOM from heat-expanded sludge. Cultivar NM 9 showed a higher sensitivity to chlorotoluron than YM 158. These results indicated that the biological response of wheat plants to the herbicide depended on the DOM and cultivars.

References:

Agüloğlu S, Ensari NY, Uyar F *et al.*, 2000. The effects of amino acids on production and transport of α-amylase through bacterial membranes[J]. Starch/Stärke, 52: 290—295.

Bansal P, Sharma P, Goyal V, 2002. Impact of lead and cadmium on enzyme of citric acid cycle in germinating pea seeds [J]. Biologia Plant, 45: 125—127.

Bejarano A C, Decho A W, Chandler G T, 2005. The role of various dissolved organic matter forms on chlorpyrifos bioavailability to the estuarine bivalve *Mercenaria mercenaria* [J]. Marine Environ Research, 60: 111—130.

Bradford M M, 1976. A rapid and sensitive method for the quantitation of microgram of proteins utilizing the principle of protein-dye-binding[J]. Anal Biochem, 72: 248—254.

Businelli D, 1997. Pig slurry amendment and herbicide coapplication effects on s-triazine mobility in soil: An adsorption-desorption study[J]. J Environ Qual, 26: 102 108.

Cai D, 1999. Environmental toxicology of pesticides [M]. Beijing: Environmental Science Press. 34—38.

Celis R, Barriuso E, Houot S, 1998. Sorption and desorption of atrazine by sludge-amended soil: dissolved organic matter effects [J]. J Environ Qual, 27: 1348 1356.

Deneer J W, 2000. Toxicity of mixtures of pesticides in aquatic systems [J]. Pestic Manag Sci, 56: 516 520.

El Sebai T, Lagacherie B, Soulas G *et al.*, 2004. Isolation and characterisation of an isoproturon-mineralising *Methylopila* sp. TES from French agriculture soil [J]. FEMS Microbiol Letters, 239: 103—110.

Gong M, Chen B, Li Z G *et al.*, 2001. Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2 [J]. J Plant Physiol, 158: 1125—1130.

Heath R L, Packer K, 1968. Leaf senescense: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase [J]. J Expl Bot, 32: 93—101.

Kruse A, Fieuw S, Heineke D *et al.*, 1998. Antisense inhibition of cytosolic NADP-dependent isocitrate dehydrogenase in transgenic potato plants[J]. Planta, 205: 82 91.

Lechón Y, Sánchez-Brunete C, Tadeo J L, 1997. Influence of the laboratory incubation method on chlorotoluron and terbutryn degradation in soil[J]. J Agri Food Chem, 45: 951—954.

Li K, Xing B, Torello W A, 2005. Effect of organic fertilizers derived dissolved organic matter on pesticide sorption and leaching [J]. Environ Pollution, 134: 187—194.

Ma J Y, Zheng R Q, Xu L G *et al.*, 2002. Differential sensitivity of two green algae, *Scenedesmus obliquus* and *Chlorell-a pyrenoidosa*, to 12 pesticides[J]. Ecotoxicol Environ Safety, 52: 57—61.

Macnicol P K, Jacobsen J V, 1992. Endosperm acidification and related metabolic changes in the developing barley grain [J]. Plant Physiol, 98: 1098— 1104.

Menendez J, Jorrin J, Romera E *et al.*, 1994. Resistance to

chlorotoluron of a slender foxtail (*Alopecurus myosuroides*) biotype[J]. Weed Science, 42: 340—344.

Mittler R, 2000. Oxidative stress, antioxidants and stress tolerance [J]. Trends in Plant Science, 7: 405—410.

Moing A, Svanella L, Gaudillere M *et al.*, 1999. Organic acid concentration is little controlled by phosphoenolpyruvate carboxylase activity in peach fruit [J]. Aust J Plant Physiol, 26: 579—585.

Pérés F, Florin D, Grollier T *et al.*, 1996. Effect of the phenylurea herbicide IPU on periphytic diatom communities in freshwater indoor microcosms[J]. Environ Pollut, 94: 141—152.

Seol Y, Lee L S, 2000. Effect of dissolved organic matter in treated effluents on sorption of Atrazine and Prometryn by soils [J]. Soil Sci Soc Am J, 64: 1976—1983.

Sharples C R, Null M R, Cobb A H, 1997. Growth and phytosynthetic characteristics of two biotypes of the weed black-grass (*Alopecurus myosuroides* Huds) resistant and susceptible to the herbicide chlorotoluron[J]. Annals Botany, 79: 455—461.

Stangroom S J, Lester J N, Collins C D, 2000. Abiotic behavior of organic micropollutants in soils and the aquatic environments. A review: I. Partitioning[J]. Environmental Technology, 21: 845—863.

Udvardi M K, McDermott T R, Kahm M L, 1993. Isolation and characterization of a cDNA encoding $NADP^+$-specific isocitrate dehydrognase from soybean (*Glycine max*) [J]. Plant Molecular Biology, 21: 739- 752.

Van Oorschot J L P, Van Leeuwen P H, 1992. Use of fluorescence induction to diagnose resistance of *Alopecurus myosuroides* Huds. (Black-grass) to chlorotoluron [J]. Weed Research, 32: 473—482.

Waler A, Rodriguez-Cruz M S, Mitchell M J, 2005. Influence of residues on the availability of herbicides for leaching[J]. Envrion Pollution, 133: 43--51.

Wang S H, Yang Z M, Lu B *et al.*, 2004. Copper-induced stress and antioxidative responses in roots of *Brassica juncea* L. [J]. Botanical Bulletin of Academia Sinica, 45: 203—212.

Xu L B, Jin Z L, Xu H X, 1990. Environmental behavior and ecotoxicology of toxic organic substance [M]. Beijing: Science and Technology Press of China.

Yang Z M, Wang J, Wang S H *et al.*, 2003. Salicylic acid-induced aluminum tolerance by modulation of citrate efflux from roots of *Cassia tora* L.[J]. Planta, 217: 168—174.

Yang Z M, Yang H, Wang J *et al.*, 2004. Aluminum regulation of citrate metabolism for Al-induced citrate efflux in the roots of *Cassia tora* L.[J]. Plant Science, 166: 1589—1594.

Yang H, Wu X, Zhou L X *et al.*, 2005. Effect of dissolved organic matter on chlorotoluron sorption and desorption in soils [J]. Pedosphere, 15(4): 432 - 439.

Yazgan M S, Tanik A, 2005. A new approach for calculating the relative risk level of pesticides[J]. Environ Inter, 31: 687—692.

Zhang D D, Zhang X H, 2001. Pesticide contamination and protection [M]. Beijing: Chemical and Industrial Press.123—125.

Zhou L X, Wong J W C, 2000a. Microbial decomposition of dissolved organic matter and its control during a sorption experiment [J]. J Environ Qual, 29: 1852—1856.

Zhou L X, Yang H, Shen Q L *et al.*, 2000b. Fractionation and characterization of dissolved organic matter derived from sewage sludge and composted sludge[J]. Environ Tech, 21: 765—771.

(Received for review May 7, 2005. Accepted June 23, 2005)