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Enantioselectivity in the Phytotoxicity of Herbicide Imazethapyr

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Chiral compounds usually behave enantioselectively in phyto-biochemical processes. With the increasing application of chiral herbicides, their enantioselective phytotoxicity to plants merits further study, and little information is available in this area. The purpose of this study was to examine the enantioselective phytotoxicity of the herbicide imazethapyr (IM) on the roots of maize (*Zea mays* L.) seedlings. Enantiomers of IM were separated by HPLC, and their absolute configurations were confirmed as *S*-(+)-IM and *R*-(−)-IM by the octant rule. Plant growth measurements and morphological, microscopic, and ultrastructural observations were conducted after treatment with individual IM enantiomers and the racemate. Observations of root morphology showed that the root diameter significantly increased, whereas the root volume, surface area, and number of root tips decreased significantly. IM enantiomers selectively damaged root hair growth and significantly reduced the sloughing of border cells from the tips. IM also had adverse effects on cell organelles, such as statocytes, mitochondria, dictyosomes, and endoplasmic reticulum in maize roots. Moreover, cell membranes and cell walls were thicker than usual after IM treatment. All of the results showed the same trend that the *R*-(−)-IM affected the root growth of maize seedlings more severely than the *S*-(+)-IM. The inhibition abilities of (±)-IM was between *S*-(+)- and *R*-(−)-IM. The behavior of the active enantiomer, instead of just the racemate, may have more relevance to the herbicidal effects and ecological safety of IM. Therefore, enantiomeric differences should be considered when evaluating the bioavailability of the herbicide IM.

KEYWORDS: Enantioselectivity; border cell; root hair; cell organelle; statocytes

INTRODUCTION

About 25% of currently used pesticides are chiral, and this ratio is increasing as compounds with more complex structures are introduced into use (*1*). It has been estimated that chiral pesticides account for more than 40% of currently used pesticides in China because of the popular use of chiral pesticides such as synthetic pyrethroids, organophosphorus insecticides, imidazolinones, and diphenyl ether herbicides (*2*). However, these pesticides are usually sold and widely entered into the environment as racemic compounds (i.e., a 1:1 ratio of enantiomers). Enantiomers of the same compound have identical physical and chemical properties in an achiral environment (e.g., air−water exchange, sorption, and abiotic transformation), but they interact with biological systems enantioselectively and may behave as drastically different compounds (*3*, *4*). Often, one enantiomer is solely active, or it is more active than the other enantiomer, which is inactive or less active and simply adds an extra chemical load to the environment (*1*, *3*). Studies on chiral insecticides gradually have become a concern since the early 1990s because these compounds are more directly related to human health. The enantioselectivities of toxicity, teratogenicity, mutagenicity, carcinogenicity, endocrine disrupting activity, immunotoxicity, and environmental fate of a number of chiral insecticides were studied (*5*, *6*). However, although of equal or even greater importance, the enantioselective eco-effects and toxicities of chiral herbicides in plants have not received as much attention as insecticides in animals. Notable exceptions include two herbicides, dichlorprop and mecoprop, which have limited portions of their total worldwide production as single enantiomers (*7*). Only the active *R* enantiomers of dichlorprop and mecoprop can be used in some European countries (*8*). The application of the pure active enantiomer reduces the dosage and possible unwanted effects of the racemates. Among all pesticides (fungicides, herbicides, and insecticides), herbicides rank highest in user expenditures as well as the volume of active ingredient (US EPA 2004, available online at http://www.epa.gov/oppbead1/pestsales/). As important ecological receptors in

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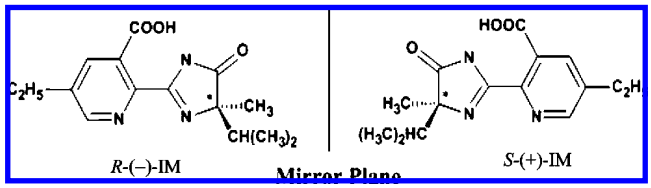

Figure 1. Chemical structures of imazethapyr (IM) with * indicating asymmetric position.

the ecosystem, plants are immobile and cannot avoid harmful effects. Therefore, enantioselective phytotoxicity of herbicides on plants merits more study.

Imidazolinones are widely used because of their low application rate, nontoxicity to animals, and broad spectrum of weed control activity (*9*). The use of imidazolinones has increased steadily since their introduction into the market in the early 1980s. They are used not only in farmlands but also in forests, railways, and freeways to control weeds. However, because of their long persistence and relatively high water solubility, they can enter the environment and cause phytotoxicity to nontarget plants.

All imidazolinone herbicides are chiral and typically consist of two enantiomers. However, they are often sold commercially as racemates. Little information is available on the enantioselectivity of imidazolinones and their toxicity on plants. It has been reported that the imidazolinone enantiomers have different herbicidal activities, with the *R* enantiomer being 8 to 10 times more inhibitory of the enzyme acetolactate synthase (ALS) than the *S* enantiomer (*10*, *11*). Imazethapyr (IM), one of the imidazolinones, which is chiral and is number one among all of the imidazolinones in sales, was used in this article as a model herbicide to study the enantioselective effects on maize seedling growth.

Imazethapyr is usually absorbed through the roots of plants. Most of the past studies on imidazolinones were on the mode of action of herbicides on ALS and the biosynthesis of valine, leucine, and isoleucine in plants (*12*−*16*). However, few records are available concerning IM toxicity and root growth such as morphological changes and ultrastructural destruction. As IM is a soil-applied herbicide, the radicle is the first organ to contact the herbicide in the soil. IM phytotoxicity may damage plant roots first. Because the enantiomers show different activity toward the target enzyme, root growth could be enantioselectively inhibited. In this study, maize (*Zea mays* L.) was used as a model plant for its common use in phytotoxicity studies of IM. The objectives of the present study were to determine the enantioselective phytotoxicity of the IM herbicide by measuring changes in the root morphology and subcellular structure of the root tips of tender maize seedlings.

MATERIALS AND METHODS

Chemicals. Analytical standards of racemate imazethapyr (98%) (IUPAC name, 2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid; trade names include Contour, Hammer, Overtop, Passport, Pivot, Pursuit, Pursuit Plus, and Resolve) were kindly donated by the Shenyang Research Institute of Chemical Industry (Shenyang, China). The stereo configuration of IM is given in **Figure 1**. Other solvents or chemicals used in this study were of analytical or HPLC grade.

Preparation and Absolute Configuration Determination of IM Enantiomers. Enantiomers were separated with the method developed in a previous study (*17*). Briefly, a Jasco LC-2000 series HPLC system (Jasco, Tokyo, Japan) with a chiral column OJ was used, and a hexane/ethanol/acetic acid solution (75/25/0.5 by volume) was used as the mobile phase with a flow rate of 1 mL·min^{-1}. A volume of 20 μL was injected for analysis. The circular dichroism (CD) detector was operated at 250 nm for detection. Chromatographic data were acquired and processed with the ChromPass software (Jasco, Tokyo, Japan). The first peak of the graph was the (+)-enantiomer, and the second peak was the (−)-enantiomer (**Figure 2**). The resolved enantiomers were manually collected into separate glass vials at the HPLC outlet. The fractions were dried under a stream of nitrogen and redissolved in ethanol. The purity and concentration of the recovered enantiomers were verified by HPLC with the same conditions used for separation. IM enantiomers were stable, and there were no signs of enantiomer conversion or degradation occurred during the experiment.

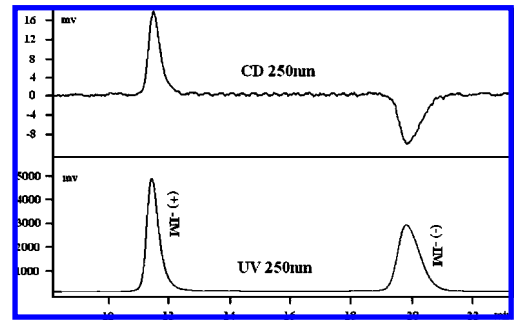

Figure 2. HPLC chromatogram for the enantiomeric separation of IM.

The CD detector was used to characterize the enantiomers after elution. Its detection is based on an absorption difference between right and left circularly polarized light. Recently, the coupling of HPLC and CD has been successfully applied for the determination of absolute configuration and elution order of a number of chiral compounds (*18*). The octant rule was used to establish the absolute configuration of IM enantiomers. The CD spectra showed that the stable configuration of *S*-IM was the geometry optimization based on the MMFF94 force-field calculation (*19*). The carbonyl group of IM could be placed into the origin of the octants. When the substituted phenyl group is located in the upper right back octant, it should give a positive Cotton effect. Thus, the absolute configuration of *S*-IM is (+)-IM, or *S*-(+)-IM. The absolute configuration of the other enantiomer *R*-IM is (−)-IM, or *R*-(−)-IM.

Plant Materials. Seeds of maize (*Zea mays* L.) were bought from the Hanzhou Seed Station, China. They were surface-sterilized by 10% sodium hypochlorite (NaOC1) for 10 min and rinsed thoroughly with distilled water. After soaking in distilled water for 12 h at room temperature, seeds were then placed on moist gauze for germination until the emergence of the radicle. Maize seedlings of all experiments were grown in a growth chamber under controlled environmental conditions with a 12 h light period (light intensity of 10,000 l×), a 25 °C/20 °C light/dark temperature regime and 60% relative humidity.

Root Growth Observations. When the primary roots of maize were approximately 3 cm long, the seedlings were transferred to an herbicide solution (*S*-(+)-, *R*-(−)-, or (±)-IM solved in distilled water) with various concentrations (0, 100, 200, 400, and 800 μg L^{-1}). The IM-treated seedlings were harvested five days after the treatment. The lengths of shoots were recorded. Samples of roots and shoots were oven-dried at 70 °C for approximately 72 h, and the dry weights were recorded. Three replicates were used in each treatment, and every replicate contained 10 seedlings. The relative inhibition rate of root elongation by IM enantiomers and racemate was measured for 72 h at 400 μg L^{-1} after the primary roots of maize were approximately 3 cm long. The relative inhibition rate (%) is $1 - (X_n - X_0)/((\overline{A_n - A_0})) \times 100$, where A_0 and X_0 represent the root length in the control (CK) and treatments, respectively, at 0 h. A_n and X_n are the root length in CK and treatments, respectively, at various times. Three replicates were used in each treatment, and every replicate consisted of 10 seedlings.

Measurement of Root Morphological Parameters. When the primary roots of maize were approximately 3 cm long, the seedlings were transferred to a herbicide solution (*S*-(+)-, *R*-(−)-, or (±)-IM) with various concentrations (0, 100, 200, 400, and 800 μg L^{-1}). The root morphological parameters were determined after five days. Three replicates were used in each treatment, and each replicate consisted of

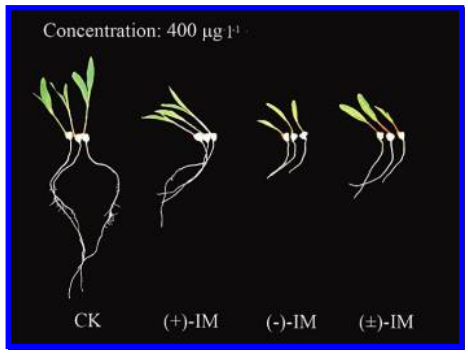

Figure 3. Enantioselective inhibition of growth in maize exposed to IM enantiomers and the racemate at 400 μg L^{-1} for five days.

10 seedlings. In each treatment, plant samples were selected randomly. The total root length, surface area, volume, diameter, and number of root tips of each plant were determined using an automatic root scan apparatus (MINMAC, STD1600+), equipped with WinRHIZO software offered by the Regent Instruments Corporation.

Observation of Border Cells and Root Hair. When the primary roots of maize were approximately 2 mm long, the seedlings were transferred to a herbicide solution (*S*-(+)-, *R*-(−)-, or (±)-IM) with a concentration of 0 or 400 μg L^{-1}. After 48 h of treatment, the root tips and the most dense root hair part of the roots of each treatment were observed under a microscope (Nikon eclipse E600) and photographed.

Observation of Subcellular Structure. Seedlings with a root length of 2 mm were selected and transferred into sterilized Petri dishes filled with distilled water (1 mm depth) containing 0 or 400 μg L^{-1} *S*-(+)-, *R*-(−)-, or (±)-IM. After 48 h, seedlings were selected for the transmission electron microscopy (TEM) studies. Small sections of root tips, 1−3 mm in length, were fixed in 4% glutaraldehyde (by volume) in 0.2 M PBS (sodium phosphate buffer, pH 7.2) for 6−8 h and postfixed in 1% OsO_4 (osmium(VIII) oxide) for 1 h and then in 0.2 M PBS (pH 7.2) for 1−2 h. Dehydration was performed in a graded ethanol series (50, 60, 70, 80, 90, 95, and 100%) followed by acetone, and the samples were then infiltrated and embedded in Spurr's resin. Ultrathin sections (80 nm) were prepared and mounted on copper grids for viewing in the transmission electron microscope (JEOL TEM-1200EX) at an accelerating voltage of 60.0 kV.

Data Analysis. All data processing and graphical work were carried out by Origin software (OriginLab Corporation, MA, USA, Version 6.0). The analysis of variance was conducted according to the experimental designs employed, and the treatment means were compared by Fisher's protected least significant difference (LSD) at the 5% level of probability.

RESULTS

Enantioselective Effects of IM on Plant Growth. In a hydroponics experiment, plant growth was enantioselectively inhibited by IM enantiomers (**Figure 3** and Table. **1**). After five days of treatment, visible crop injury symptoms were observed, including chlorosis of leaves, reduced plant growth, and putrescence on the root tips. The injury generally increased with increasing dose. In addition, *R*-(−)-IM was the most effective inhibitor in the damage of maize growth with the shortest shoot and root length, lowest dry weight, and most obvious chlorosis in comparison to those of the *S*-(+)-IM and racemate mixtures at equal concentrations.

Dry weight responses showed that the root was more sensitive than the shoot to IM herbicide during the treatment. At low concentrations of 100 and 200 μg L^{-1}, the root biomass was significantly decreased by *S*-(+)-, *R*-(−)-, and (±)-IM in comparison with that of the control. However, the shoot biomass was significantly reduced by *R*-(−)- and (±)-IM and not affected by *S*-(+)-IM. The shoot and root dry weights exposed to *R*-(−)-

Table 1. Effects of IM on Dry Weights, Shoot Height, and Root Length (Average of Three Replicates ± S.D.)[a]

concentration (μg L^{-1})	shoot dry weight (mg 10 plants^{-1})			root dry weight (mg 10 plants^{-1})			shoot height (cm)			total root length (cm plant^{-1})		
	(±)	(+)	(−)	(±)	(+)	(−)	(±)	(+)	(−)	(±)	(+)	(−)
0 (control)		101.2 ± 4.5			42.8 ± 0.7			6.89 ± 0.30			12.32 ± 1.46	
100	92.4 ± 5.5 ab*	100.0 ± 2.5 a	90.3 ± 0.7 b*	36.1 ± 2.2 ab*	36.6 ± 1.1 a*	33.8 ± 0.6 b*	6.3 ± 0.40 a*	6.5 ± 0.47 a*	6.2 ± 0.43 b*	8.1 ± 0.77 b*	9.4 ± 0.78 a*	7.7 ± 0.52 b*
200	107.8 ± 2.5 a*	101.3 ± 1.0 b	91.9 ± 0.7 c*	34.6 ± 0.6 a*	35.1 ± 1.2 a*	30.5 ± 1.8 b*	6.2 ± 0.34 a*	6.3 ± 0.32 a*	5.9 ± 0.33 b*	7.4 ± 0.78 b*	8.9 ± 0.82 a*	6.4 ± 0.53 b*
400	83.3 ± 2.0 a*	85.4 ± 2.7 a*	61.4 ± 3.5 b*	26.4 ± 0.8 b*	30.3 ± 0.5 a*	25.7 ± 1.0 b*	4.6 ± 0.40 b*	5.7 ± 0.38 a*	3.9 ± 0.39 c*	5.5 ± 0.38 b*	7.2 ± 0.46 a*	4.9 ± 0.10 c*
800	73.4 ± 1.5 a*	75.6 ± 3.3 a*	57.1 ± 2.6 b*	29.0 ± 1.4 a*	29.6 ± 3.5 a*	25.2 ± 1.2 b*	4.0 ± 0.30 b*	5.1 ± 0.31 a*	3.4 ± 0.22 c*	5.2 ± 0.39 b*	6.1 ± 0.21 a*	4.8 ± 0.16 b*

[a] Each replicate contains 10 seedlings. The total weight of 10 seedlings of each replicate was used for data analysis. Different letters in the same row indicate significant differences ($P < 0.05$) among the (−)-, (+)-, and (±)-IM. * indicates significant differences ($P < 0.05$) between the treatment and control.

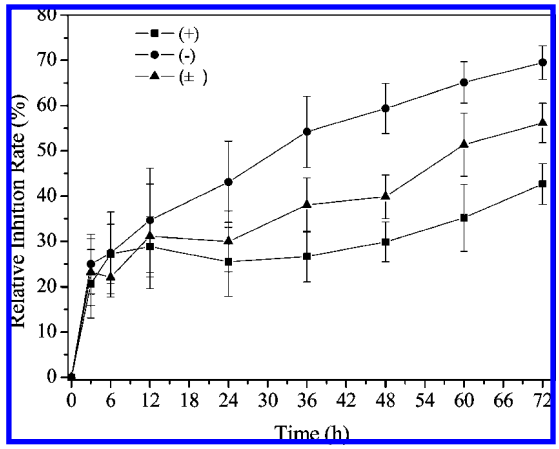

Figure 4. Relative inhibition rate of root elongation of maize to IM enantiomers and the racemate at 400 μg L^{-1} during a 72 h experiment. Data points and error bars represent the means ± SD of three replicates. Each replicate consists of 10 seedlings.

and *S*-(+)-IM showed similar trends that significant differences ($P < 0.05$) between *S*-(+)-and *R*-(−)-IM were observed at both doses, and *R*-(−)-IM was much more inhibitory than *S*-(+)-IM. However, there was no significant difference between *S*-(+)-IM and the racemate.

The shoot height and root length of maize were adversely affected by herbicide treatments and were decreased significantly from the control. The inhibition also showed enantioselectivity. The *R*-(−)-IM treatment was significantly different from the *S*-(+)-IM treatment. At low concentrations of 100 and 200 μg L^{-1}, there were no significant differences between *S*-(+)-and (±)-IM. With increased concentrations, the amount of the *R*-(−)-enantiomer in (±)-IM also increased, and the (±)-IM became more inhibitory. Therefore, significant differences were observed between *S*-(+)-and (±)-IM at 400 and 800 μg L^{-1} concentrations. The root was more sensitive to IM than the shoot. The root length was inhibited by nearly 50% in 100 μg L^{-1} *R*-(−)-IM, while little inhibition could be observed at the same concentration of *S*-(+)-IM. The significant difference between *S*-(+)-and (±)-IM could be observed at each concentration.

Root growth inhibition was observed after exposure to IM (**Figure 4**). After 24 h, significant differences of root growth inhibition could be observed between *S*-(+)- and *R*-(−)-IM. The lag of relative inhibition rate between the enantiomers became larger with time. *R*-(−)-IM appeared almost two times more toxic than *S*-(+)-IM to the root during the time course experiment. At 72 h after treatment, the relative inhibition rate of root exposed to *R*-(−)-IM was 70%, while the *S*-(+)- and (±)-IM rates were 42% and 55%, respectively.

Enantioselective Effects of IM on Root Morphology. The root diameter was significantly increased by the application of various IM treatments, with *R*-(−)- and (±)-IM being more toxic than *S*-(+)-IM (**Figure 5a**). Different IM concentrations had variable impacts on root volume (**Figure 5b**) and surface area (**Figure 5c**), which were insignificantly decreased ($P < 0.05$) from those of the control at lower concentrations and decreased significantly with higher concentrations. Data on root volume and surface area showed a similar trend with higher inhibition found for *R*-(−)-IM than for *S*-(+)-IM (**Figure 5b** and **c**). The number of root tips of all treatments was dramatically decreased from that of the control. A significant difference was observed between the two enantiomers with *R*-(−)-IM being five times more toxic than the *S*-(+)-enantiomer at a concentration of 100 μg L^{-1}(**Figure 5d**).

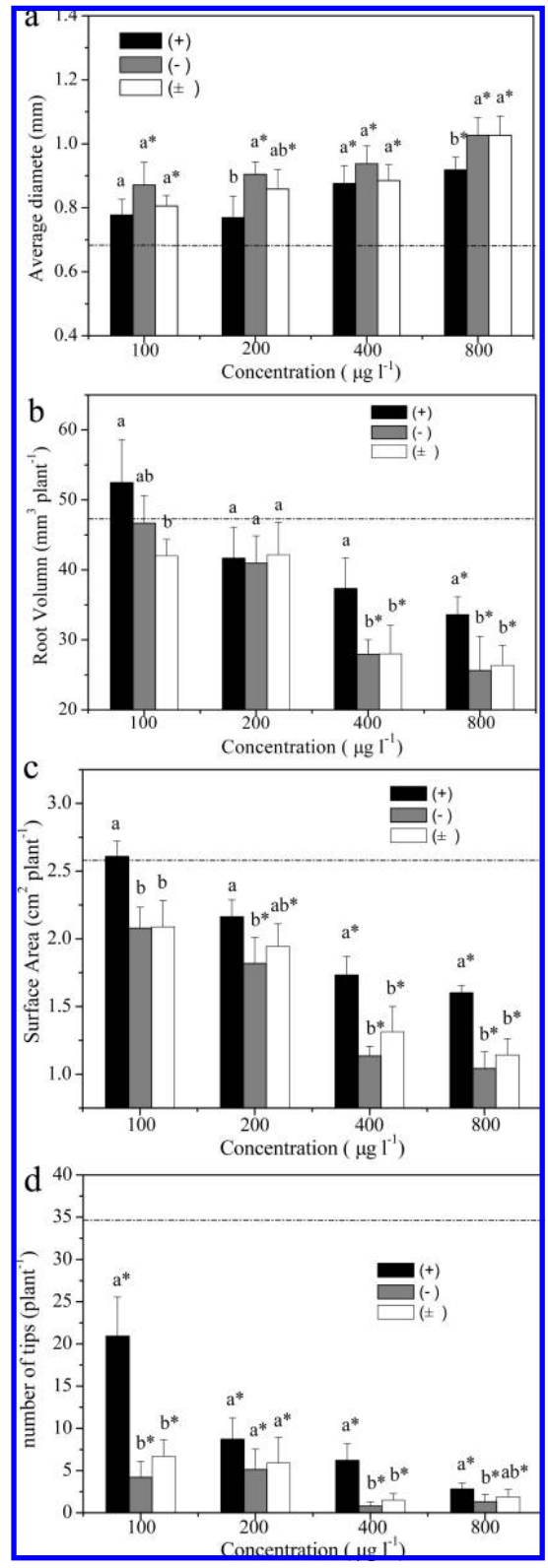

Figure 5. Average diameter (**a**), root volume (**b**), surface area (**c**), and number of root tips (**d**) of maize under different levels of IM enantiomers and the racemate for five days. Data points and error bars represent the means ± SD of three replicates. Each replicate consists of 10 seedlings. The dashed line indicates the mean of the control. Different letters indicate significant differences ($P < 0.05$) among the *R*-(−)-, *S*-(+)-, and (±)-IM. * indicates significant differences ($P < 0.05$) between the treatment and control.

Enantioselectively Inhibition on Border Cells and Root Hair. The microscopic observations of the roots revealed the

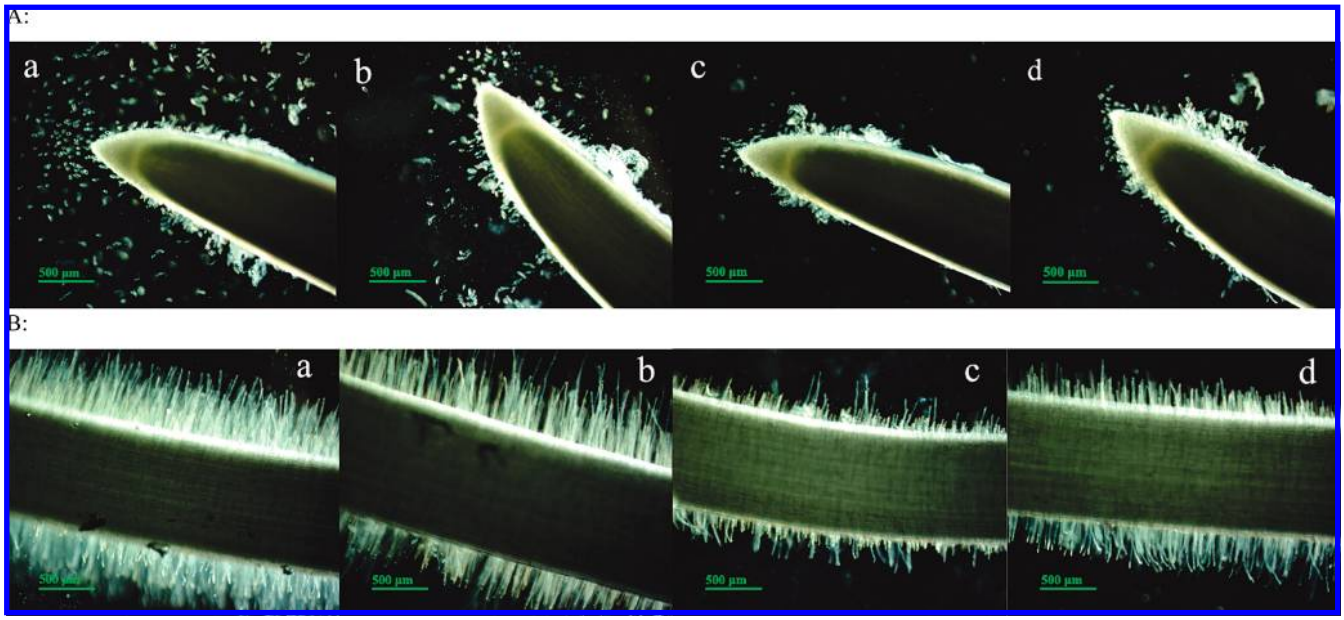

Figure 6. **Aa**−**d** are micrographs of root tips of maize (*Zea mays*) primary roots. **a**, control, shows characteristic sloughing of cells from the tips; **b**, 400 μg L^{-1} *S*-(+)-IM; **c**, 400 μg L^{-1} *R*-(−)-IM, the least border cells are produced; **d**, 400 μg L^{-1} (±)-IM. **Ba**−**d** are micrographs of root hairs of maize primary roots. **a**, control; **b**, 400 μg L^{-1} *S*-(+)-IM; **c**, 400 μg L^{-1} *R*-(−)-IM; **d**, 400 μg L^{-1} (±)-IM. Bar = 500 μm.

different effects of the IM enantiomers (**Figure 6**). The root tips of CK released abundant border cells, which were much higher than after the treatments. *R*-(−)-IM had the strongest effect on the root tips, leading to a reduction of the sloughing of border cells from the tips (**Figure 6**A**a**−**d**). **Figure 6Ba**−**d** shows the densest root hair part of roots of each treatment. Root hair growth was damaged by the herbicide, with *R*-(−)-IM being more active than the *S*-(+)-enantiomer. Root hair treated with *R*-(−)-IM was obviously sparser and shorter than that of *S*-(+)-IM and (±)-IM.

Enantioselective Effects of IM on the Subcellular Structure of the Root Tip Cell. Ultrastructural studies revealed that IM had adverse effects on cell organelles in maize root, and *R*-(−)-IM treatment caused the most pronounced damage. The inhibition ability of the racemate was between *S*-(+)-IM and *R*-(−)-IM. The following changes in treated roots were observed in comparison with those in the control. In the control, root cells had rich cytoplasm and organelles, including numerous statocytes, mitochondria, dictyosomes, well-developed endoplasmic reticulum, and smooth and continuous cell membrane and cell wall (**Figures 7Aa**, **Ba**, and **Ca**). *S*-(+)-IM did not change the ultrastructure of the statocytes at a concentration of 400 μg L^{-1} of the herbicide (**Figure 7Ab**). The starch grains were as large as the control and still surrounded by the amyloplast envelope membranes. However, *R*-(−)-IM damaged the ultrastructure of the statocyte seriously (**Figure 7Ac**). The starch grains, which were still visible, were much smaller and no longer enclosed by envelope membranes, releasing the starch grains into the cytoplasm. (±)-IM also damaged the statocyte with smaller starch grains compared with that in the *S*-(+)-IM treatment (**Figure 7Ad**). *S*-(+)-IM also had the weakest effect on other cell organelles, having rich and well-developed mitochondria and endoplasmic reticulum, and only some dictyosomes were swollen (**Figure 7Bb**). The meristematic cells treated by *R*-(−)-enantiomer and the racemate had much sparser and smaller organelles (**Figure 7C**). Mitochondria of *R*-(−)-IM and (±)-IM were much smaller than that of the control and *S*-(+)-IM treatment (**Figures 7Ca**−**d**). Additionally, the mitochondria envelope membranes were destroyed, and the dictyosomers were severely swollen (**Figures 7Bc**−**d**). The cell membranes and cell walls were thicker than usual after the IM treatment. The cell membrane of the *R*-(−)-enantiomer appeared to be the thickest, showed the phenomenon of fibrosis, and lacked flexibility (**Figure 7C**).

DISCUSSION

Because enantiomers of chemicals may interact with biological systems that are usually enantioselective and behave as drastically different compounds, it is imperative to assess the effects of chiral pesticides using pure individual enantiomers. Unfortunately, the enantioselective toxicity of the IM enantiomers to plants is still poorly understood. Furthermore, to our knowledge, the roots of maize have not yet been examined in relation to the action of the IM herbicide, although this tissue has very important functions, e.g., perception of gravity, mechanical protection of the meristematic root tip region, and protective slime secretion (*20*).

As a soil-applied herbicide, the pre-emergent application of IM was supposed to affect crop seedlings during very early stages of their development. The radicle is the first organ to contact the herbicide in the soil. Most of the previous studies reporting deleterious effects of IM on root growth were merely based on the racemate of IM. Various authors have reported inhibition of plant growth, including shoot and root length and dry weight caused by IM racemate (*21*−*23*). However, there is little information available on its enantioselectivity. In the present study, we found IM retarded maize growth, which is in agreement with the previous studies. Furthermore, the inhibition of the IM enantiomer is enantioselective. *R*-(−)-IM exhibited the strongest inhibition, and *S*-(+)-IM caused a slight inhibition on maize growth. It has been reported that the *R* enantiomer was almost 8−10 times more inhibitory against the enzyme ALS than the *S* enantiomer (*10, 11*). Our results showed a similar trend, i.e., *R*-(−)-IM was the more effective inhibitor. However, our results showed only a 2-fold difference, which may be caused by the fact that the plant and the target enzyme (in vitro) have different sensitivities to herbicides.

Most of the previous studies reporting deleterious effects of IM on the root growth were based merely on parameters such

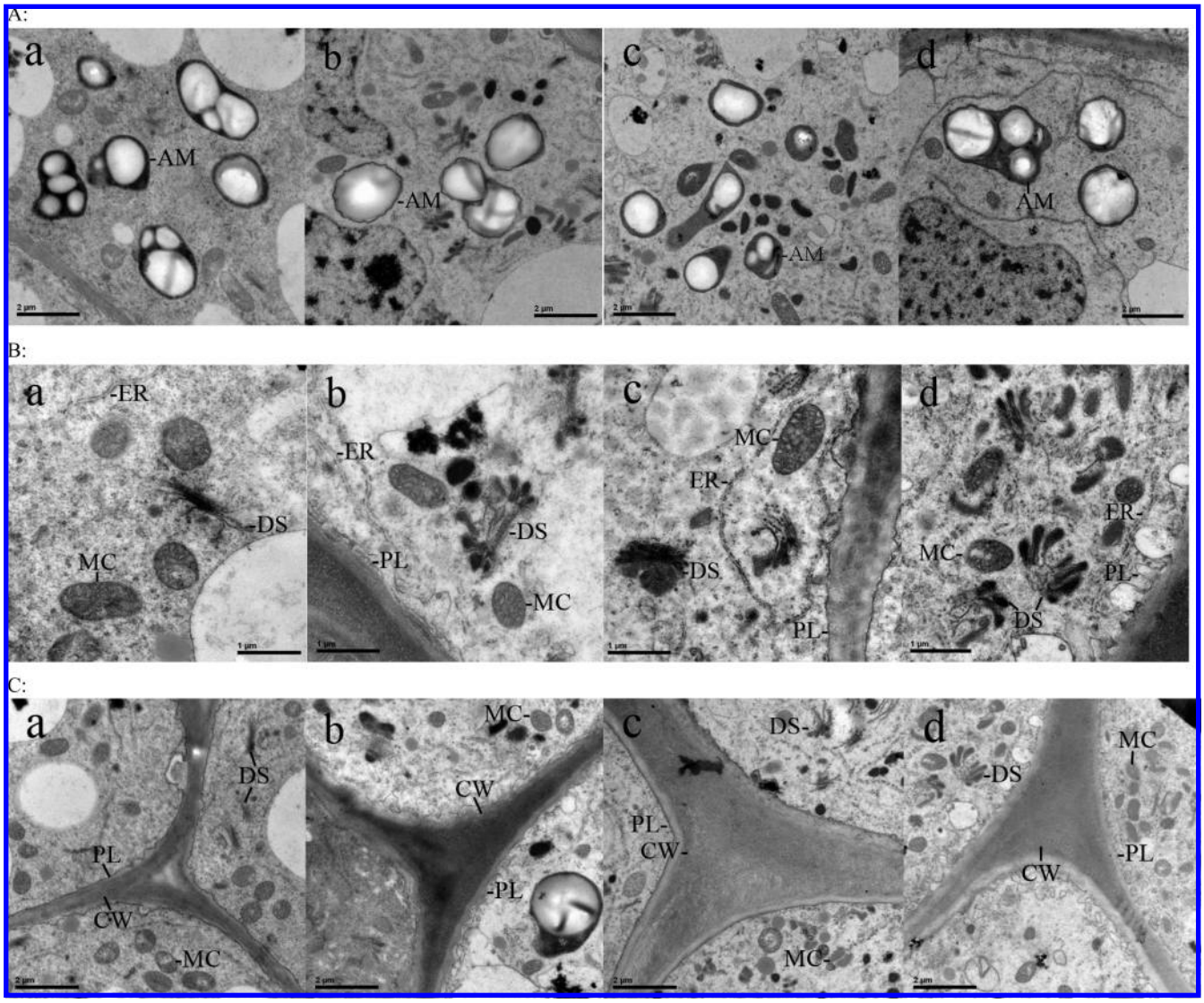

Figure 7. **Aa**−**d** show electron micrographs of root cap cells. **a**, control, the statocytes show the characteristic amyloplasts containing large starch grains; **b**, 400 μg L^{-1} *S*-(+)-IM; **c**, 400 μg L^{-1} *R*-(−)-IM, the least border cells are produced; **d**, 400 μg L^{-1} (±)-IM. Labels: AM, amyloplast. **Ba**−**d** show electron micrographs of organelles of root cap cells. **a**, control; **b**, 400 μg L^{-1} *S*-(+)-IM; **c**, 400 μg L^{-1} *R*-(−)-IM; **d**, 400 μg L^{-1} (±)-IM. Labels: MC, mitochondria; ER, endoplasmic reticulum; DS, dictyosomer; PL, plasmalemma. **Ca**−**d** show electron micrographs of organelles of root cap cells. **a**, control; **b**, 400 μg L^{-1} *S*-(+)-IM; **c**, 400 μg L^{-1} *R*-(−)-IM; **d**, 400 μg L^{-1} (±)-IM. Labels: CW, cell wall; PL, plasmalemma; MC, mitochondria; DS, dictyosomer.

as root elongation and fresh and dry weights, whereas in response to IM toxicity, roots can also respond via changes in surface area, volume and diameter, production or inhibition of lateral roots and tips, and variation in other morphological parameters potentially indicative of herbicide toxicity. Some studies were also conducted on root morphology. They showed that environmental stresses, such as temperature, nutrition, organic contaminants, and heavy metals, may cause root morphological changes (*24*−*28*). However, there is little information available on the relationships between root morphology and herbicide injury. Results from the present study showed that morphological parameters of roots changed enantioselectively by the IM enantiomers. The *S*-(+)-enantiomer was less toxic than the *R*-(−)-enantiomer. The root diameter was enantioselectively increased by the application of the IM enantiomers. This result is in agreement with Subramanyan and Rangaswamy (*29*), who also showed that the herbicide dichlobenil increased root diameter by causing an enlargement of cortical cells, extension of pith, and stelar aberrations in the primary root. The root morphological changes of surface area, volume and numbers of lateral roots to IM enantiomers were similar to that of plant growth. Growth cessation of maize may also be attributed to the destruction of root structure in response to the various toxicities of the IM enantiomer.

The root tip is primarily exposed to IM toxicity after its application. Plant roots release a large number of border cells into the rhizosphere, which are believed to play key roles in root development and health. Various evidence have suggested the involvement of border cells in protecting the root system against abiotic stress (*30*−*34*). They reported that the number of border cells released from roots treated with Al^{3+} is significantly less than that from roots without Al^{3+} treatment. Our results show similar trends with IM toxicity, reducing the sloughing of border cells from the tips. The reduction rate of sloughing was in proportion to the toxic degree of the IM enantiomers. The inhibition of IM on the sloughing of border cells caused roots to be more apt to be injured by IM.

Root hairs are subcellular extensions from the root epidermis that facilitate the uptake of nutrients by increasing the absorptive surface area of the root and allowing the root to explore a greater

soil volume (*35*). Root hairs are closely related to plant nutrition absorption. It has been reported that visible crop injury symptoms caused by IM including the chlorosis of leaves, reddening of the veins on the lower side of the leaf, leaf distortion, and reduced plant growth are often related to poor root growth and nutrient uptake in plants (*22*). Therefore, the herbicide IM could inhibit maize growth by damaging root hair enantioselectively development.

The horizontal growth of roots was observed in maize treated by *R*-(−)-IM, even at the lowest concentration. According to the starch-statolith theory, the displacement of and-or pressure from amyloplasts is the signal for gravity perception. Therefore, we hypothesized that IM may damage the amyloplasts in the statocytes of the root cap so that the root cap would no longer be able to respond correctly to gravity, resulting in a transverse growth of the root. In the present study, IM enantioselectively damaged the ultrastructure of the statocyte seriously and finally caused the horizontal growth of roots. IM also enantioselectively led to strong structural perturbations in the root caps. These phenomena had also been found in other studies. Fayez and Kristen (*20*) reported that there was a herbicide concentration-dependent progression of tissue destruction from the root cap periphery toward the root cap center for herbicides chlorsulfuron, norflurazon, and triallate. The ultrastructural observations of the cell membranes and cell walls being several times thicker than usual also corresponded to the root morphological observation of increasing root diameter. Thicker cell membranes and cell walls caused them to lack flexibility. Brittle roots would no longer be able to protect the initial cell complex against the mechanical, chemical, and pathogenic effects of the environment; therefore, the plant would be much more apt to be injured.

Conclusions. The results from the ultrastructural observations, plant growth measurements, and microscopic observations support the hypothesis that IM causes phytotoxicity in maize by destroying root growth. Herbicides damaged cell organelles and reduced root hair growth and the sloughing of border cells from the tips in a very short time (less than 48 h). All of these damages finally led to visible phytotoxicity symptoms, including chlorosis of leaves, reduced plant growth, and putrescence on the root tips. Another important finding is that the *R*-(−)- and *S*-(+)-enantiomers have different toxicities, with *R*-(−)-IM more severely affecting maize seedlings in the very early stages of their development. The inhibition ability of the racemate was between *S*-(+)-IM and *R*-(−)-IM. Like IM, most chiral compounds are still used as racemates. When applied, the chiral compounds are involved in biochemical interactions, and receptors generally distinguish between their enantiomers, which causes enantioselectivity in the chiral environment. The results obtained in this study fully support the need for investigating the enantioselective toxicity to plants and the environmental fate for each chiral herbicide. The development of new enantiopure products may thus benefit from this information.

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