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Effect of imazapic residues on photosynthetic traits and chlorophyll fluorescence of maize seedlings

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Abstract

The influence of various concentrations of imazapic residues (0–800 μg kg^{-1}) on the growth, chlorophyll content, and photosynthetic characteristics of maize seedlings was studied in a greenhouse pot experiment. Plant height, root length, shoot dry mass, root dry mass, and total dry mass of maize declined with the increase of imazapic residue concentrations. The root/shoot ratio initially decreased and then increased in presence of imazapic, which indicated that the effects of imazapic residues on plant height and root length might differ in maize seedlings. Lowered chlorophyll content and net photosynthetic rate were observed in leaves of maize seedlings in all treatments and indicated a dose-response relationship to imazapic concentrations. Intercellular carbon dioxide concentration, transpiration rate, and stomatal conductance also declined to varying extents, but the chlorophyll *a*/*b* ratio increased gradually together with the increase of imazapic residue concentrations. Generally, the maize seedlings were negatively affected by the imazapic residues in soil. Response of root length and biomass to imazapic residues could be the important index for maize variety selection.

Additional key words: chlorophyll fluorescence; gas exchange; growth characteristics; photosystem II.

Introduction

Imazapic is one of the imidazolinone class of herbicides and is registered for use on peanut and sugar cane with a broad spectrum of herbicidal activity. It can control the majority of broadleaf and grass weeds and has become the main herbicide for peanut fields in China. The extent to which imazapic is degraded by sunlight is believed to be minimal when applied to terrestrial plants or soil. Moreover, imazapic is not volatile when applied in the field. Thus, in combination with their long residual period, this class of herbicides may often cause injury, significant reductions in yield or quality, or even mortality to succeeding crops (Su and Song 1996). However, in the many areas of northern China, imidazolinone residues in soil may limit agricultural rotations due to the traditional rotation of peanut, wheat, maize, and soybean. The labels of imazapic products clearly indicate that wheat can be cultivated following a four-months interval after application at the recommended rate; corn, cotton, and barley after 18 months; cucumber, rape, and spinach after 24 months; banana and sweet potato after 36 months. Maize or cotton cultivated in the following year can be injured if imazapic application in peanut fields is delayed or the recommended rate exceeded.

Crop injury from imazapic residues in soil has been identified by a number of methods. Su *et al.* (2014) reported that cucumber and rape were the most sensitive to imazapic, followed by wheat and maize, while soybean showed the strongest tolerance. Imazaquin caused more injury in cotton than that in maize following application during the previous year for soybean in the same field (Monks and Banks 1991). Maize was seriously injured when planted in a field treated with imazaquin for one or two years (Renner *et al.* 1988). Rape, cabbage, sugar beet, flax, red pepper, tomato, and other crops are very sensitive to imidazolinone herbicides (Huang *et al.* 2001, Aichele and Penner 2005, Sousa *et al.* 2012). Su *et al.* (2013) reported that the height, plant fresh mass, and whole plant dry mass of wheat seedlings decreased with the increase of imazapic residue concentrations, and the net photo-

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Abbreviations: ALS – acetolactate synthase; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; E – transpiration rate; FM – fresh mass; F_v/F_m – maximum photochemical efficiency of PSII; g_s – stomatal conductance; PHT – plant height; P_N – net photosynthetic rate; RDM – root dry mass; RL – root length; SDM – shoot dry mass; TDM – total dry mass.
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synthetic rate was significantly inhibited by imazapic treatments. Wixson and Shaw (1992) found in Mississippi, USA that immediate planting of maize, sorghum, cotton, rice, wheat or ryegrass in a field treated with imazapic at 35 g of active ingredient (a.i.) ha^{-1} had no effect on aboveground mass of these crops. However, under greenhouse conditions, maize, sorghum, cotton, rice, and wheat were sensitive to imazapic at 11.6 g(a.i.) ha^{-1}. The shoot mass of maize and sorghum was reduced as well. In addition, the tolerance concentration of cotton, rice, and wheat were within the range of 19–38 g(a.i.) ha^{-1} (Wixson and Shaw 1992).

Imazapic acts through inhibition of the enzyme acetolactate synthase (ALS) by foliar or root uptake following post-emergence application. Imazapic kills plants by inhibiting the production of branched chain amino acids, which are necessary for protein synthesis and cell growth. Although photosynthesis is not a major target of ALS-inhibitor herbicides, the parameter changes for photosynthesis can be detected in plants treated with this class of herbicides (Sousa *et al.* 2014). Xia *et al.* (2006) found that parameters of photosynthesis were inhibited to various degrees upon imazaquin application. Sousa *et al.* (2014) reported that the photosynthetic organs of rice may be damaged before visible injury by imidazolinone, leading to a decrease of its photosynthesis capabilities.

Recently, the planted area of maize has increased yearly in China and the optimal crops following peanut are of the grass family (Gramineae), such as wheat and maize. Thus, it is of practical significance to investigate the effect of imidazolinone residues on maize. By adding imazapic to soil in a pot experiment in a greenhouse, this study aimed to understand the response of maize to imidazolinone residues through assessing its potential phytotoxicity risks by means of photosynthetic characteristics in order to provide a theoretical basis for the practical use of imidazolinone in peanut rotations.

Materials and methods

Plant material, soil treatment and culture conditions: The soil used in this study was taken from top soil (0–10 cm) in Yuanyang, Henan Province, China. The soil (pH 8.4, soil/water ratio of 1:2.5, w/v), containing 5.5 g(organic matter) kg^{-1}, 29.8 mg(available nitrogen) kg^{-1}, 6.5 mg(available phosphorus) kg^{-1}, and 78.3 mg kg^{-1} of available potassium-measured according to the method described by Bao (Bao 2000) was air-dried and passed through a 2-mm sieve to remove debris and stones. Maize (*Zea mays* L. cv. 'Zhengdan 958') was selected as the test species. Maize seeds were pregerminated for 24 h prior to seeding by placing the seeds in a Petri dish on wetted paper towel and placed in the darkness at 25°C.

Stock solutions of the imazapic (*BASF Corp*., Research Triangle Park, NC, USA) were prepared by placing a known quantity of imazapic in approximately 50 ml of acetone then diluting with water to the 1-L mark in a volumetric flask (Eliason *et al*. 2004). Standard solutions were prepared from the stock solution to produce solutions with concentrations of 6.25, 12.5, 25, 50, 100, and 200 μg(a.i.) L^{-1} of imazapic. Two hundred and fifty grams of each soil, replicated four times, were placed into pots (70 mm height × 85 mm diameter). For each pot, 50 ml of the standard solution was added to the untreated soil to produce the concentrations of imazapic to reach soil concentrations up to two or four times of the recommended field application rate [166 μg(a.i.) L^{-1}]. This resulted in imazapic concentrations of 25, 50, 100, 200, 400, and 800 μg(a.i.) L^{-1}(soil). The control was prepared by adding 50 ml of distilled water to the untreated soil. The soils were then manually mixed to ensure uniform distribution of the added herbicides throughout the soils, and allowed to equilibrate for 24 h. Five pregerminated seeds of similar size and radicle protrusion were selected and placed onto the soil surface, covered with a small amount of soil (approximately 0.5 cm) and were lightly packed. The plants were watered daily to 20% soil moisture content by adding distilled water to a predetermined mass. Seedlings were grown in a growth room under controlled conditions [12-h light at 27°C and 12-h darkness at 25°C; light intensity: 150 μmol(photon) m^{-2} s^{-1}; relative humidity: 70–75%].

Growth: After 10 d of growth, plant height and root length of maize seedlings were measured and recorded. IC_{50} (*i.e.*, the herbicide concentration required to cause a 50% reduction in plant height or root length) values were calculated using a log-logistic model in *SigmaPlot 12.0*:

$$Y = C + \{(D - C)/[(1 + X/IC_{50})^B]\}$$

where Y represents plant height or root length expressed as a percentage of the untreated control, X represents herbicide concentration, C is the lower limit for Y, D is the upper limit for Y, IC_{50} is the herbicide concentration required to cause a 50% reduction in plant height or root length, and B is the slope of the line at IC_{50}.

Root and shoot dry mass: Shoots and roots were separated, then dried at 80°C for 48 h and weighed to determine the root dry mass (RDM) and shoot dry mass (SDM).

Photosynthetic pigments: A fresh leaf sample of 0.1 g was ground and extracted with 5 ml of 80% (v/v) acetone in the dark. The slurry was filtered, centrifuged at 5,000 × g for 10 min and absorbancies were determined at 663, 646, and 470 nm, for Chl *a*, Chl *b*, and carotenoid (Car) concentrations, respectively, using a spectrophotometer (*UV550, ThermoSpectronic*, Cambridge, UK). Concentrations of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car were determined according to Lichtenthaler (1987).

Gas exchange: A portable photosynthesis system (*Li-6400, Li-Cor Inc*., Lincoln, NE, USA) equipped with a light source (*6400-02B LED, Li-Cor*) was used to measure photosynthesis parameters of maize seedlings. The system was operated under the following conditions: ambient CO_2 concentration of 400 μmol mol^{-1}, leaf temperature of 28°C, and light intensity of 150 μmol(photon) m^{-2} s^{-1}. The net photosynthetic rate (P_N), intercellular CO_2 concentration (C_i), transpiration rate (E), and stomatal conductance (g_s) of maize leaves were determined. Four replicates were made for each treatment.

Chlorophyll (Chl) *a* fluorescence was measured simultaneously on the same leaves (n = 4) used for the gas-exchange measurements, with a leaf chamber fluorometer (*LI-6400-40*), a LED-based fluorescence accessory for the portable photosynthesis system *LI-6400* (*LI-COR Bioscience Inc*., Lincoln, NE, USA). After dark adaptation of samples for 1 h, the minimal fluorescence (F_o) was measured with weak modulated irradiation [< 0.1 μmol (photon) m^{-2} s^{-1}]. A 600-ms saturating flash [> 7,000 μmol (photon) m^{-2} s^{-1}] was applied to determine the maximum Chl fluorescence yield (F_m). The variable fluorescence (F_v) was calculated as $F_v = F_m - F_o$. The maximum efficiency of PSII photochemistry in the dark-adapted state (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_o)/F_m$ (Genty *et al*. 1989, Lazár 2015).

Statistical analysis: All data presented here are the mean values of two independent experiments with four replicates. Data are presented as mean ± standard deviation (SD). Statistical analyses were performed by the analysis of variance (*ANOVA*) using the *SPPS* software. Differences between treatments were separated by least significant difference (LSD) at a 0.05 probability level.

Results

Plant growth: Imazapic treatments inhibited the growth of maize seedlings (Fig. 1). With increasing concentration of imazapic residues, both the plant height and root length of maize seedlings significantly declined. At imazapic treatment by 25 and 50 μg(a.i.) kg^{-1}, the seedling height was not significantly different from control; however, there were significant decreases in height as the residue concentration reached 100–800 μg(a.i.) kg^{-1}. There was a greater effect of imazapic residues on root length of maize seedlings. For example, at 25 μg(a.i.) kg^{-1}, the inhibition rate for root length was 17.0% and root growth was greatly reduced. Moreover, the inhibitory effect of imazapic residue was more pronounced as its concentration rose, with inhibition rates of 28.2–82.4%. The IC_{50} values of plant height and root length were 235.86 and 105.46 μg(a.i.) kg^{-1}. This suggested the root of maize seedlings was more sensitive to imazapic residues than plant height.

Dry mass decreased with the increase of imazapic residue concentrations. For the imazapic soil concentrations within the range of 25–800 μg(a.i.) kg^{-1}, SDM, RDM, and TDM of maize seedling decreased significantly (Fig. 1*C*,*D*,*E*). SDM was reduced to 74.2% of the control with a low imazapic dose, while the high dose decreased SDM to 19.5% of the control. Moreover, RDM was reduced to 41.5% of the control with the high imazapic dose. For imazapic of < 100 μg(a.i.) kg^{-1}, the root/shoot ratio decreased as the concentration increased; in contrast, this ratio increased with residual imazapic concentrations of > 200 μg(a.i.) kg^{-1}. Thus, imazapic had a more pronounced effect on RDM than that on SDM at lower concentrations.

Photosynthetic pigments: Different treatments caused significant reductions in all pigments. The contents of Chl *a*, Chl *b*, Chl (*a*+*b*), and Car gradually decreased as the residual imazapic concentration rose (Fig. 2). At concentrations within the range of 25–200 μg(a.i.) kg^{-1}, there was no significant difference between the treatment and control in contents of Chl *a*, Chl *b*, and Chl (*a*+*b*) (Fig. 2*A*–*C*). However, these parameters were greatly reduced by imazapic at higher concentrations of 400–800 μg(a.i.) kg^{-1}. The pattern of imazapic effect on Car varied; the Car content was not significantly affected by imazapic at 25–100 μg(a.i.) kg^{-1}, but greatly decreased at 200–800 μg(a.i.) kg^{-1} (Fig. 2*D*). Our results suggested that the Car content in maize seedlings may be more sensitive to imazapic. Chl *a/b* demonstrated a trend of increase with the increment of residual imazapic concentration. However, for all treatments, there was no significant difference between the treatment and control. The increase of Chl *a/b* ratios were related to the decrease in Chl *b* rather than that of Chl *a* content in imazapic-treated leaves (Fig. 2*E*).

Gas exchange and Chl *a* fluorescence: The P_N for maize seedlings initially increased and then declined as the imazapic residues in the soil rose (Fig. 3*A*). For imazapic residue concentrations within the range of 25–200 μg(a.i.) kg^{-1}, there was no difference in P_N between the treatments and control. In contrast, relative to control, there were significant decreases of 26.5 and 47.8% for treatments at 400 and 800 μg(a.i.) kg^{-1}, respectively. Both P_N and Chl contents showed a similar trend, indicating that the decline in the Chl content may be one underlying reason for the observed decrease in photosynthesis.

With increased imazapic residue concentration, there was a trend of initial increase followed by decrease for g_s of seedling leaves (Fig. 3*B*). In addition, there was no significant difference between treatments and control until the residual imazapic treatment reached 100 μg(a.i.) kg^{-1}, at which g_s was significantly elevated. In contrast, g_s decreased at imazapic residue concentrations of 200–800 μg(a.i.) kg^{-1} and, specifically, an inhibition rate was very

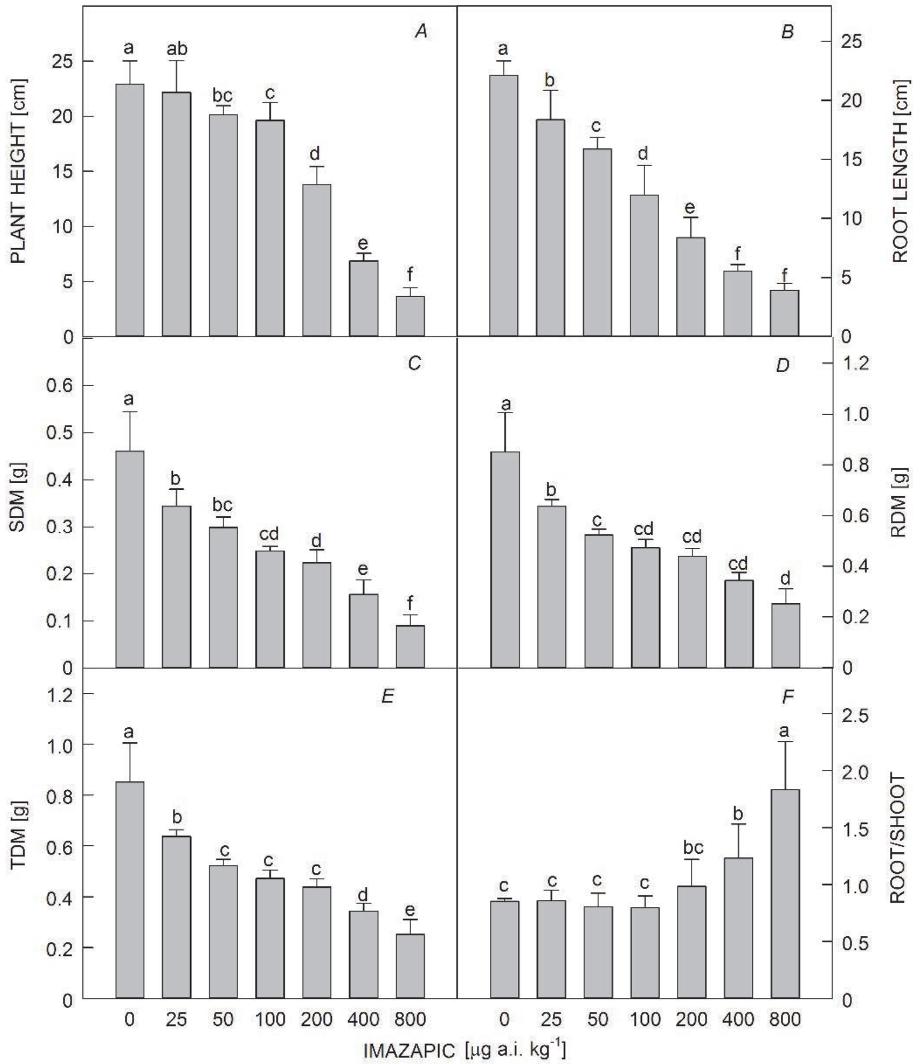

Fig. 1. (*A*) Plant height, (*B*) root length, (*C*) shoot dry mass (SDM), (*D*) root dry mass (RDM), (*E*) total dry mass (TDM), and (*F*) root/shoot ratio of maize seedlings under different soil concentrations [0, 25, 50, 100, 200, 400, and 800 μg(a.i.) kg^{-1}] for the herbicide imazapic. Values are means of four replicates ± SD. Means of each parameter were analyzed using *Duncan*'s multiple range test to check the significance of differences between treatments. Columns marked with different *lowercase letters* indicate a significant difference between treatments at $P<0.05$.

marked at 55.4% for 800 μg(a.i) kg^{-1}. Although C_i differed between treatments, there was no significant difference between treatments and control (Fig. 3*C*). The *E* of maize seedling leaves increased to various levels upon treatments at 25–400 μg(a.i.) kg^{-1} (Fig. 3*D*). Notably, imazapic at 800 μg(a.i.) kg^{-1} significantly lowered *E* to 32.7%. There was the insignificant negative effect of imazapic on F_v/F_m (Fig. 3*E*).

Discussion

Carry-over of herbicides to rotational crops can be a significant problem. Because of a high potency of the ALS-herbicides, the minuscule residual concentrations may remain active and may exert an effect on sensitive crops. Moyer and Esau (1996) reported that imazethapyr application increased the risk of yield loss in flax, corn, meadow bromegrass, mustard, sunflower, wheat, conola, sugar beet, and potato seeded between one to three years later. Our results demonstrated that residual activity of imazapic in soil could inhibit the plant growth and photosynthetic capacity in maize seedlings. With the increase in residue imazapic concentrations, plant height, root length, SDM, RDM, TDM, Chl *a*, Chl *b*, Chl (*a*+*b*), Car, P_N, and g_s decreased, while root/shoot ratio and Chl *a/b* exhibited an increasing trend.

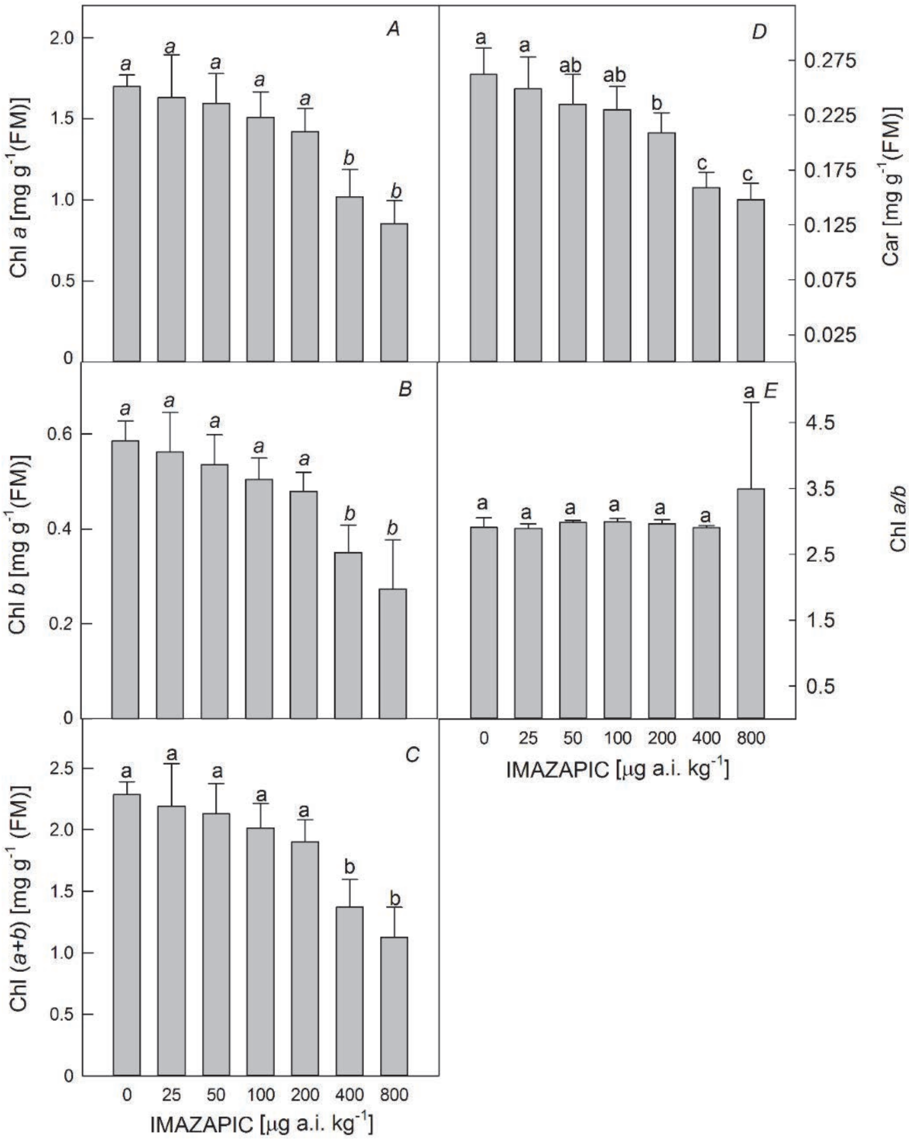

Fig. 2. (*A*) Chlorophyll (Chl) *a*, (*B*) Chl *b*, (*C*) Chl (*a*+*b*), (*D*) carotenoid content (Car), and (*E*) Chl *a*/*b* of maize seedlings under different soil concentrations [0, 25, 50, 100, 200, 400, and 800 μg(a.i.) kg^{-1}] of the herbicide imazapic. Values are means of four replicates ± SD. Means of each parameter were analyzed using *Duncan*'s multiple range test to check the significance of difference between treatments. Columns marked with different *lowercase letters* indicate a significant difference between treatments at $P<0.05$.

There is a direct relationship between Chl content and light transformation in photosynthesis. Herbicides affect cell metabolism of plants, specifically their photosynthetic machinery; changes in leaf color or bleaching due to stress are mostly related to alterations in photosynthetic pigment contents (Radwan and Soltan 2012). In this experiment, the contents of pigments were lowered due to imazapic residues in soil. In support, Yuan *et al*. (2013) reported that herbicide *Sigma Broad* could significantly reduce Chl content in leaves of *Radix isatidis* (*Isatis indigotica* Fort.). The reduction of Chl content might be a good indicator for monitoring damage to the plant growth and development (Yin *et al*. 2008). Wiatrek *et al*. (2009) reported that cotton height was reduced by the high rates of imazapic in one year but not in another. Grey *et al*. (2005) reported a negative exponential trend where cotton height decreased as imazapic dose increased. Matocha *et al*. (2003) reported a reduction in cotton height with imazapic applied at 140 and 210 g(a.i.) ha^{-1} during the previous year. In agreement with above, our results also showed that plant height and root length of maize seedlings decreased as the imazapic residue rate increased.

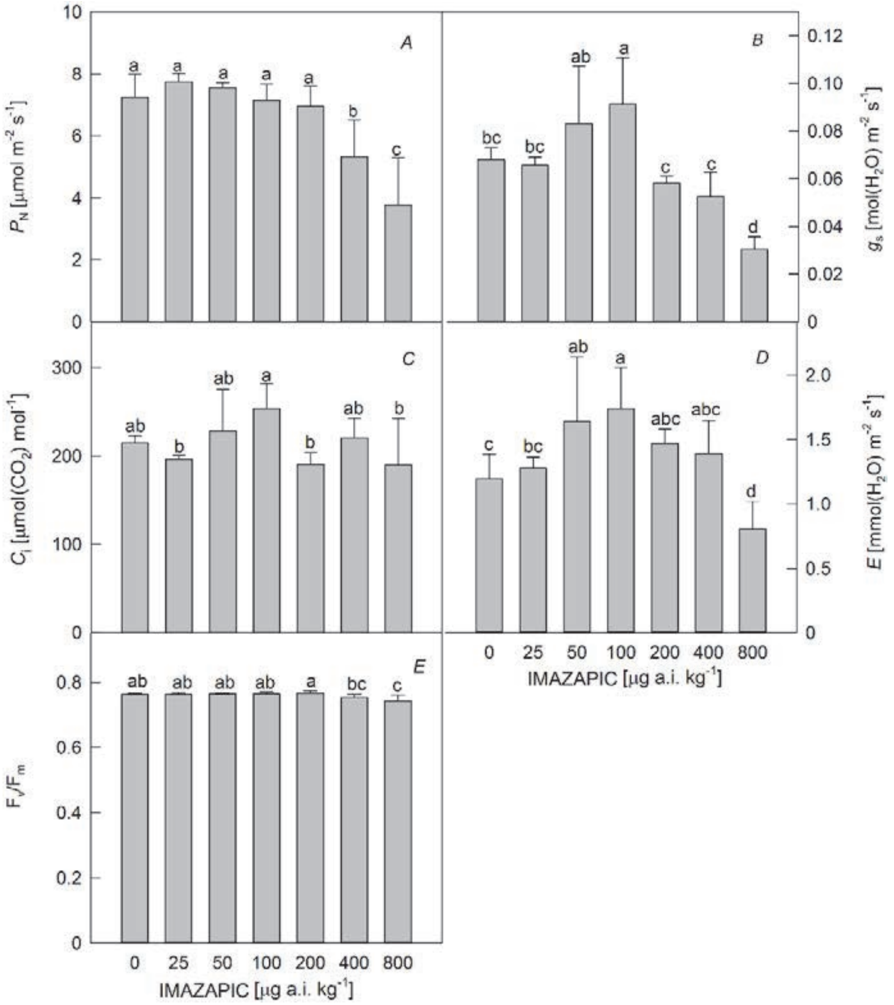

Fig. 3. (*A*) Net photosynthetic rate (P_N), (*B*) stomatal conductance (g_s), (*C*) intercellular CO_2 concentration (C_i), (*D*) transpiration rate (*E*), and (*E*) F_v/F_m of maize seedlings under different soil concentrations [0, 25, 50, 100, 200, 400, and 800 μg(a.i.) kg^{-1}] for the herbicide imazapic. Values are means of four replicates ± SD. Means of each parameter were analyzed using *Duncan*'s multiple range test to check the significance of difference between treatments. Columns marked with different *lowercase letters* indicate a significant difference between treatments at $P<0.05$.

Table 1. Correlation tests of plant height (PHT), root length (RL), dry mass (DM), chlorophyll content (Chl), and net photosynthetic rate (P_N) on the leaves of maize seedling. *Correlation is significant at the 0.05 level, ** correlation is significant at the 0.01 level.

Parameter	PHT	RL	DM	Chl	P_N
PHT	1	0.93**	0.87**	0.99**	0.94**
RL	0.93**	1	0.96**	0.91**	0.80*
DM	0.87**	0.96**	1	0.87**	0.74*
Chl	0.99**	0.91**	0.87**	1	0.97**
P_N	0.94**	0.80*	0.74*	0.97**	1

The majorities of herbicides not only inhibit the growth of crops, but also affect their photosynthesis. Herbicides targeting photosynthesis tend to affect crop photosynthesis more than other families of herbicides. For example, flumioxazin belongs to the protoporphyrinogen oxidase-inhibiting herbicides and causes a sharp decline in P_N and g_s of grape leaves (Bigot *et al.* 2007). The content of Chl is closely related to photosynthetic performance. Previous studies concluded that herbicides could decrease the content of photosynthetic pigments and P_N (Ralph 2000, Wang *et al.* 2011, Su *et al.* 2013, Hu *et al.* 2014). In order to confirm the change in photosynthetic machinery caused by imazapic, the P_N and other gas-exchange parameters were analyzed for both control and treated plants in this experiment. A significant reduction in P_N was observed and this reduction was concomitant with the imazapic dose in soil, suggesting that the photosynthesis was inhibited. The decreasing trend of P_N coincided with that of the Chl content, indicating that the decrease in photosynthetic pigment was one of the main factors that resulted in the P_N reduction (Yordanova *et al.* 2001). However, F_v/F_m was not affected by imazapic (Fig. 1*E*), implying that the reduction in P_N was not caused by the reduction of PSII photochemical activity (Baker 2008), but it was probably caused by the energy deficiency for carbon assimilation in dark reaction of photosynthesis.

The accumulation of biomass represents the net effect of carbon assimilation and maintenance. The SDM, RDM, and TDM of maize seedlings were significantly reduced by imazapic residues (Fig. 1), suggesting that the growth of the maize seedlings would be negatively influenced by the imazapic. Results of statistical analysis showed that the biomass loss positively correlated with the relative loss in the Chl content and P_N (Table 1), which could be easily understood since the P_N is an important resource of the biomass. The significantly changed root/shoot ratio (Fig. 1*F*) indicated that imazapic residue might result in the change of biomass partition.

To conclude, maize seedlings were negatively affected by imazapic residues in soil. Imazapic treatment with 25 μg kg^{-1} could decrease significantly root length, SDM, RDM, and TDM. Response of root length and biomass to imazapic residue might be the important index for maize variety selection.

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