

# The residual effects of bensulfuron-methyl on growth and photosynthesis of soybean and peanut

W.C. SU<sup>\*,#</sup>, L.L. SUN<sup>\*,#</sup>, Y.H. GE<sup>\*</sup>, R.H. WU<sup>\*,+</sup>, H.L. XU<sup>\*,+</sup>, and C.T. LU<sup>\*</sup>

*Institute of Plant Protection, Henan Academy of Agricultural Sciences; Henan Key Laboratory of Crop Pest Control, IPM Key Laboratory in Southern Part of North China for Ministry of Agriculture. Zhengzhou 450 002, China\**

## Abstract

The effects of various concentrations of bensulfuron-methyl residues (BSM, 0–500  $\mu\text{g kg}^{-1}$ ) on the growth and photosynthesis of soybean and peanut were studied. Shoot length, root length, root-to-shoot ratio, and biomass of soybean and peanut seedlings declined with the increase of BSM residue concentrations. As the concentration of BSM increased, SPAD value, net photosynthetic rate, stomatal limitation, stomatal conductance, and transpiration rate also declined with varying extent, but dark respiration rate and intercellular  $\text{CO}_2$  concentration increased gradually. PSII maximum quantum yield, actual quantum yield, and electron transport rate were significantly reduced by the BSM residues in soil, and the reduction was mostly attributed to the decrease in photochemical quenching coefficient. The results showed that photosynthesis in both crops was limited by nonstomatal factors. The residues of BSM caused reversible damage in PSII reaction centers and decrease the proportion of available excitation energy used for photochemistry.

*Additional key words:* *Arachis hypogaea*; chlorophyll fluorescence; gas exchange; *Glycine max* (Linn.) Merr.; phytotoxicity; relative chlorophyll content.

## Introduction

Bensulfuron-methyl [BSM, 2-(4,6-dimethoxypyrimidin-2-carbamoylsulfamoyl)-o-toluic acid methyl ester], discovered by the *DuPont Co.* in the late 1970s, is a type of sulfonylurea herbicide, characterized by broad-spectrum weed control at very low use rates (30–100  $\text{g ha}^{-1}$ ), high herbicidal activity, good crop selectivity, and low mammalian toxicity (Brown 1990, Brusa *et al.* 2001, Sabater *et al.* 2002). All these characteristics make sulfonylurea herbicides such as BSM an ideal substitute for other older herbicides (Pimentel *et al.* 1991). The use of BSM in southeast China, California, Japan, and many other areas in the world as a herbicide in rice paddies has steadily increased over the past several years (Sarmah *et al.* 1998, Sabater *et al.* 2002), making great contributions to crop protection and production. However, BSM is neither

particularly volatile nor photodegradable (Boschin *et al.* 2003, Si *et al.* 2004) and therefore may persist for a long time (over 100 d) when applied under specific climatic and/or soil conditions (Blair and Martin 1998) and can be leached into the environment or its residues may damage other crops in the rotation (Si *et al.* 2004).

Crop rotation is common in China. For example, in most wheat-growing areas of the country, wheat is rotated with peanut, soybean, *etc.* Over large cultivated tracts in southern China, farming is even more intensive and consists of three crops a year: wheat, rice, and soybean (in autumn, after rice). In some areas in the south, owing to recent droughts, a rotation of rice with peanut has been promoted vigorously to boost the production of food grains as well as of edible oil.

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\*Corresponding authors; phone/fax: +86-371-65731962, e-mails: [renhai.wu@163.com](mailto:renhai.wu@163.com), [xuhongle86@126.com](mailto:xuhongle86@126.com)

*Abbreviations:* ALS – acetolactate synthase; a.i. – active ingredient; BSM – bensulfuron-methyl; Chl – chlorophyll;  $C_i$  – intercellular  $\text{CO}_2$  concentration;  $E$  – transpiration rate; ETR – electron transport rate;  $F_m$  – maximum fluorescence yield of the dark-adapted state;  $F_0$  – minimal fluorescence yield of the dark-adapted state;  $F_v$  – variable fluorescence;  $F_v/F_m$  – maximum quantum yield of PSII photochemistry;  $g_s$  – stomatal conductance;  $L_s$  – stomatal limitation; LSD – least significant difference;  $P_N$  – net photosynthetic rate;  $q_p$  – photochemical quenching coefficient; R:S – root-to-shoot ratio; SDM – shoot dry mass;  $R_D$  – dark respiration rate; RDM – root dry mass; SD – standard deviation; TDM – total dry mass;  $\Phi_{\text{PSII}}$  – the actual quantum yield of PSII.

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<sup>#</sup>These authors contributed equally to this work.

A great deal of research has been conducted on the adsorption, degradation, and leaching of BSM in soils and its photolysis on soil surface (Saeki and Toyota 2004, Si *et al.* 2004, Zhu *et al.* 2005, Luo *et al.* 2008, Lin *et al.* 2010, Lin *et al.* 2012), including the residual phytotoxicity of sulfonylureas to rotation crops, such as maize, sunflower, sugar beet, and dry beans (Anderson and Humburg 1987, Curran *et al.* 1991). Zhang *et al.* (2016) reported that residual BSM mainly affected plant height, leaf width, and the activity of amylase and peroxidase, but had little effect on the physiological activity in tobacco. Ye *et al.* (2005) found that residual BSM inhibited root length in rape. Su *et al.* (2016) reported that plant height, root length, and net photosynthetic rate of maize seedlings decreased with increased concentrations of BSM residue.

BSM acts by inhibiting the enzyme acetolactate synthase (ALS), which is involved in the synthesis of branched-chain amino acids, leading to the cessation of cell division and of subsequent growth processes in plants. Although photosynthesis is not a major target of herbicides, which inhibit ALS, changes in photosynthesis

parameters were detected in plants treated with herbicides (Sousa *et al.* 2014). Xia *et al.* (2006) found that these parameters were inhibited to various degrees upon imazaquin application. Sousa *et al.* (2014) reported that the organs of photosynthesis in rice may be damaged before visible injury by imidazolinone, lowering the photosynthesis capabilities of the crop. Tomar *et al.* (2015) reported that anthracene lowered the performance index of PSII in soybean significantly. Imazapic residues in soil inhibited the growth of wheat seedlings and significantly reduced the rate of photosynthesis in wheat (Su *et al.* 2013).

Although some information is available on the toxicity of sulfonylurea herbicides to weeds and crops (French and Buckley 2008, Kleter *et al.* 2011, Muszynski *et al.* 2014), the toxic effects of BSM residues on soybean and peanut have been hardly investigated. In order to fill this research gap, the objective of this study was to understand the response of soybean and peanut to BSM residue by assessing its potential phytotoxicity through examining photosynthesis characteristics so as to provide a theoretical basis for practical use of BSM in rice or wheat rotations.

## Materials and methods

**Plant material and herbicide:** The soil used in this study was the top soil (0–10 cm) collected from Yuanyang, in Henan province, China. The soil (pH 8.4, soil:water ratio of 1:2.5, w/v), which contained 5.5 g(organic matter) kg<sup>-1</sup>, 29.8 mg(available N) kg<sup>-1</sup>, 6.5 mg(available P) kg<sup>-1</sup>, and 78.3 mg(available K) kg<sup>-1</sup> – measured according to the method described by Bao (2000) – was air-dried and passed through a 2-mm sieve to remove debris and stones.

Soybean (*Glycine max* (Linn.) Merr., cv. Xudou 18) and peanut (*Arachis hypogaea*, cv. Yuhua 15) were selected as the test species. Seeds of soybean and peanut were pregerminated for 24 h at 25°C prior to sowing by placing the seeds in Petri dishes on wet paper towels and keeping the dishes in darkness. BSM (98.5% pure) was purchased from Hangzhou Tianyi Pesticide Manufactory, Zhejiang province, China.

**Soil treatment and culture conditions:** Stock solutions of BSM were prepared by placing a known quantity of BSM in approximately 50 mL of acetone and then diluting with water to the 1-L mark in a volumetric flask (Eliason *et al.* 2004). Standard solutions were created from the stock solutions to produce solutions with concentrations of 0.5, 1.5, and 2.5 mg of active ingredient (a.i.) L<sup>-1</sup>(BSM). Soil samples (each 200 g) were placed into pots (70 mm tall and 83 mm in diameter) and 40 mL of the standard solution was added to the untreated soil to produce concentrations of BSM up to two or four times those achieved by following the recommended field application rate [74 µg(a.i.) kg<sup>-1</sup>]. This resulted in concentrations of 100, 300, and 500 µg(a.i. of BSM) kg<sup>-1</sup>(soil). Throughout the paper, the concentrations of BSM was referred to the concentrations of its active ingredient. For the control,

50 mL of distilled water was added to the untreated soil. The soil samples were then manually mixed to ensure uniform distribution of the added BSM throughout the soil and allowed to equilibrate for 24 h. Three pregerminated seeds of soybean or two of peanut, of similar size and comparable length of the radicle, were selected and placed onto the soil surface and covered with a small amount of soil (a layer approximately 0.5 cm thick), which was lightly packed. The pots were watered daily to 20% of soil moisture content by adding distilled water to reach a pre-determined mass. The seedlings were grown in a growth chamber under controlled conditions [12 h of light at 27°C alternating with 12 h of darkness at 25°C; light intensity of 150 µmol(photon) m<sup>-2</sup> s<sup>-1</sup>; relative humidity of 70–75%].

**Growth parameters:** After 14 d of growth, plant height and root length of soybean and peanut seedlings were measured and recorded. Each plant was separated into its roots and shoots. These samples were placed in an oven at 80°C for 48 h before the dry mass of each seedling component was recorded. The root-to-shoot ratio (R:S) was calculated from the shoot dry mass (SDM) and root dry mass (RDM), total dry mass (TDM) being the sum of RDM and SDM.

**Relative chlorophyll (Chl) content and gas exchange:** After 14 d, five to ten successive readings (depending on the area) in SPAD units were taken by a Chl meter SPAD-502 (Konica Minolta Camera Co. Ltd., Japan) across the whole surface of leaves, taking one leaf at a time. Net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ), and transpiration rate ( $E$ ) of single leaves were measured from the central part of

the first young, fully mature, healthy leaf using a portable photosynthesis system (*Li-6400*; *Li-Cor Inc.*, Lincoln, NE, USA). The CO<sub>2</sub> concentration, temperature, and light intensity in the leaf chamber were kept at 400 µmol mol<sup>-1</sup>, 28 ± 0.5 °C, and 150 µmol(photon) m<sup>-2</sup> s<sup>-1</sup>, respectively. Stomatal limitation was calculated as  $L_s = 1 - C_i/C_a$ , where  $C_a$  is the ambient CO<sub>2</sub> concentration. Each treatment was replicated three times. Dark respiration rate ( $R_D$ ) was measured with the photosynthetic system after placing the leaves in darkness for 2 min. Individual leaves were irradiated at a PPFD of 0 µmol m<sup>-2</sup> s<sup>-1</sup> until a steady state  $P_N$  was reached.

**Chl fluorescence** was measured simultaneously with an auxiliary attachment to *Li-6400* (*Li-6400-40* leaf chamber fluorometer). After dark adaptation of samples for 1 h, minimal fluorescence ( $F_0$ ) was measured with weak modulated irradiation [ $<0.1$  µmol(photon) m<sup>-2</sup> s<sup>-1</sup>]. A 600-ms saturating flash [ $>7,000$  µmol(photon) m<sup>-2</sup> s<sup>-1</sup>] was applied to determine the maximum Chl fluorescence yield ( $F_m$ ). Variable fluorescence ( $F_v$ ) was calculated as  $F_v = F_m - F_0$ . The maximum quantum yield of PSII photochemistry

( $F_v/F_m$ ) was calculated as  $F_v/F_m = (F_m - F_0)/F_m$ ; the actual quantum yield of PSII ( $\Phi_{PSII}$ ) was calculated as  $\Phi_{PSII} = (F_m' - F_s)/F_m'$ ; and photochemical quenching coefficient ( $q_P$ ) was calculated as  $q_P = (F_m' - F_s)/(F_m' - F_0)$ . The electron transport rate (ETR) was calculated as  $ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$ , where PAR is the intensity (µmol m<sup>-2</sup> s<sup>-1</sup>) of photosynthetically active radiation incident on the sample, 0.5 is the proportion of light energy assigned to PSII, and 0.84 denotes that 84% of the incident light is absorbed by leaves (Genty *et al.* 1989, Lazár 2015). All these calculations are performed automatically by the instrument.

**Statistical analysis:** All the data presented here are the mean values of two independent experiments with three replications and are given as mean ± standard deviation (SD). All data were subjected to one-way analysis of variance (*ANOVA*) with *SPSS (Version 17.0, SPSS Inc.)*. The differences between treatments were separated by the method of least significant difference (*LSD*) at 0.05 probability level ( $P < 0.05$ ).

## Results

**Growth:** The effect of BSM residues at its different doses on the growth of soybean and peanut is shown in Fig. 1. The results clearly demonstrated that roots were quite sensitive to the residue of BSM. The greater effect of BSM residues was found in the soybean seedlings. Shoot length, root length, and dry mass of both soybean and peanut seedlings decreased with increase in BSM residue concentrations.

Dry mass decreased with the increase in BSM residue concentrations. At BSM concentrations of 100–500 µg kg<sup>-1</sup> (soil), shoot length (Fig. 1A) and R:S (Fig. 1F) of soybean seedling were not significantly different from their corresponding values in the control. At 300 and 500 µg kg<sup>-1</sup>, SDM, RDM, and TDM of soybean seedling decreased significantly (Fig. 1C–E). The effect of BSM residue was greater on root length of both crops: root length was significantly reduced in both at all concentrations of BSM when compared to that in the control (Fig. 1B). In peanut, shoot length at 500 µg kg<sup>-1</sup> decreased significantly, by as much as 21% (Fig. 1A). Compared with the untreated controls, RDM at 100, 300, and 500 µg kg<sup>-1</sup> decreased significantly, by 18, 26, and 29%, respectively (Fig. 1D). However, no significant difference was found in the values of SDM, TDM, and R:S at any of the BSM residue concentrations (Fig. 1C,E,F).

**Relative Chl content and gas exchange:** SPAD readings have been shown to be strongly correlated to leaf Chl content (Marquard and Tipton 1987, Markwell *et al.* 1995, Wang *et al.* 2004). At a low residue concentration of BSM (100 µg kg<sup>-1</sup>), SPAD values of soybean and peanut seedling leaves were significantly lower than those in the

control, the extent of decrease being 13 and 18%, respectively. As the BSM residue concentration increased, SPAD values decreased even further: the decrease at 300 and 500 µg kg<sup>-1</sup> in soybean was 19 and 24%, respectively; in peanut, the corresponding values were 29 and 30% (Fig. 3E).

As the residue concentration of BSM increased,  $P_N$  decreased significantly in both soybean and peanut in all the treatments (Fig. 2A), compared with the control: the extent of decrease was 38–62% in soybean and 22–52% in peanut.

$R_D$  in soybean and peanut leaves rose gradually with the residue concentration of BSM. Compared with the untreated controls,  $R_D$  of soybean leaves at 300 and 500 µg kg<sup>-1</sup> increased significantly, by 87 and 144%, respectively, and that of peanut leaves at 100, 300, and 500 µg kg<sup>-1</sup> increased significantly, by 64, 158, and 197%, respectively (Fig. 2B).

The application of BSM increased  $C_i$  to varying degrees in soybean and peanut (Fig. 2C). Compared with the control,  $C_i$  in soybean was not significantly different at 100–300 µg kg<sup>-1</sup> but increased significantly at 500 µg kg<sup>-1</sup>;  $C_i$  of peanut leaves at 100, 300, and 500 µg kg<sup>-1</sup> increased significantly, by 28, 31, and 38%, respectively.

Concerning  $L_s$ , its value decreased in both crops as the residue concentration of BSM increased (Fig. 2D). Although the difference between soybean and the control was not significant at 100 µg kg<sup>-1</sup>, the differences were significant at 300–500 µg kg<sup>-1</sup>. In peanut, the decrease was not significant at 100–300 µg kg<sup>-1</sup> but it was significant at 500 µg kg<sup>-1</sup> (the extent of decrease was 33%). Thus, the pattern of changes in  $L_s$  in the two species was just the opposite of that seen in  $C_i$ .

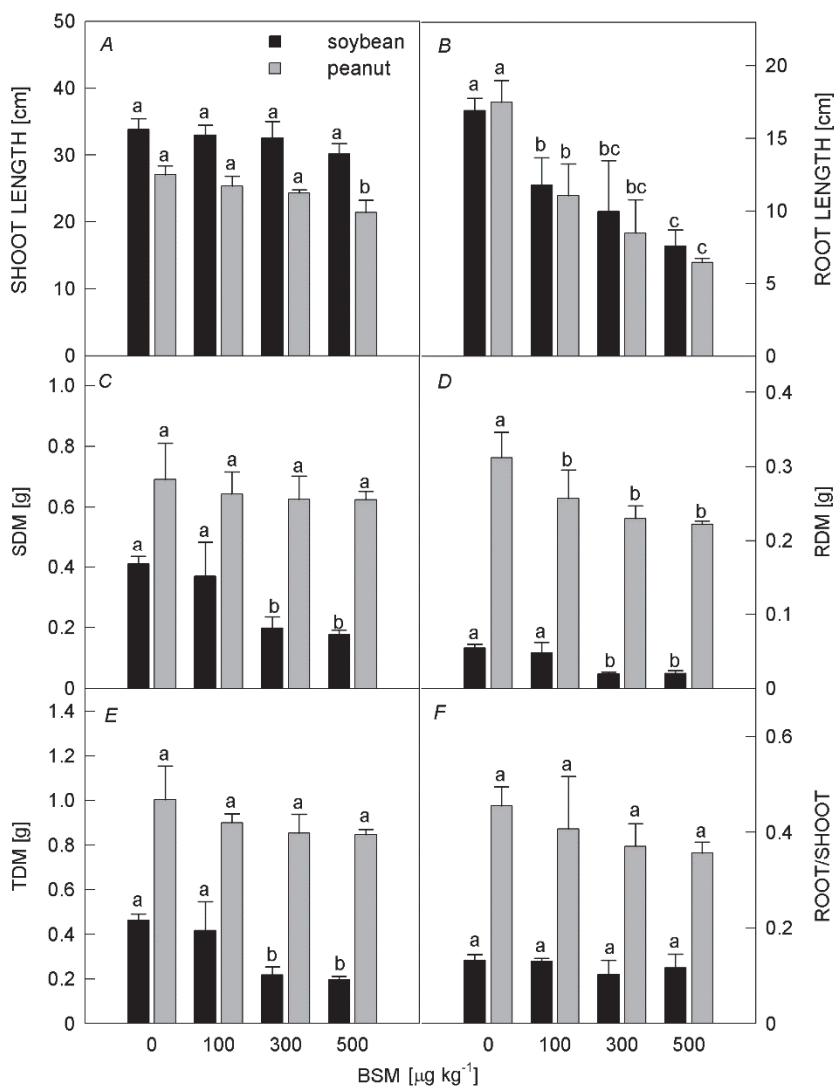


Fig. 1. (A) Shoot length, (B) root length, (C) shoot dry mass (SDM), (D) root dry mass (RDM), (E) total dry mass (TDM), and (F) root/shoot ratio of soybean and peanut seedlings under different soil concentrations (0, 100, 300, and 500  $\mu\text{g kg}^{-1}$ ) for the herbicide bensulfuron-methyl. Values are means of four replicates  $\pm$  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  $P < 0.05$ .

As the residue concentration of BSM increased,  $g_s$  of soybean leaves decreased. The difference in  $g_s$  between the treated plants and the control was not significant at 100  $\mu\text{g kg}^{-1}$  but it was significant at 300 and 500  $\mu\text{g kg}^{-1}$ . Compared with the control,  $g_s$  of peanut leaves in 100, 300, and 500  $\mu\text{g kg}^{-1}$  decreased by 40, 52, and 74%, respectively (Fig. 2E).

As shown (in Fig. 2F),  $E$  decreased significantly in both crops as the residue concentration of BSM increased. The decrease at 100, 300, and 500  $\mu\text{g kg}^{-1}$  in soybean was 40, 56, and 58%, respectively; in peanut, the corresponding values were 30, 53, and 67%. The pattern of changes in  $E$  in each crop thus essentially matched to that in  $g_s$ , which also suggests that BSM affected photosynthesis in soybean and peanut significantly.

## Discussion

Herbicide persistence is an important fact in crop production since residues can potentially injure sensitive

**Chl fluorescence** parameters, such as  $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $q_p$ , and ETR in both crops decreased as the residue concentrations of BSM increased (Fig. 3A–D). Compared with the untreated controls,  $F_v/F_m$  and  $\Phi_{PSII}$  of soybean leaves at 100–500  $\mu\text{g kg}^{-1}$  decreased by 2–6% and 16–28%, respectively,  $q_p$  and ETR at 300 and 500  $\mu\text{g kg}^{-1}$  were also significantly lower, by 13–18% and 24–25%, respectively. In peanut,  $F_v/F_m$  was significantly lower at 300–500  $\mu\text{g kg}^{-1}$  by 1–2%,  $\Phi_{PSII}$  decreased significantly at 100–500  $\mu\text{g kg}^{-1}$  by 4–7%, and  $q_p$  and ETR were significantly lower only at 500  $\mu\text{g kg}^{-1}$  by 8%. These results showed that fluorescence parameters of soybean were more sensitive than those of peanut to BSM residue.

crops grown in rotation (Riddle *et al.* 2013). A laboratory test helps to predict potential herbicide residue problems

so that proper decisions can be taken about crop rotation, herbicide selection, planting date, and other cultural practices. In the present study, the residue of BSM lowered plant height and root length in both soybean and peanut seedlings significantly, the extent of decrease being directly proportional to the concentration of BSM added to the soil. Roots proved to be more sensitive to the residue of BSM than shoots, probably because the seedlings absorbed it through their roots and then transported it to the stems and leaves. As the concentration of the residual BSM increased, SDM, RDM, and TDM declined gradually. The root-to-shoot ratio in both soybean and peanut decreased as BSM concentration increased, showing that BSM had a

greater effect on below-ground biomass than on above-ground biomass. These results agree with analysis of Lou *et al.* (2005) that BSM inhibition of root length is greater than that of plant height. Our results showed that BSM residue strongly inhibited the growth of soybean and peanut seedlings, and that soybean was more sensitive to BSM residue.

Stress factors, such as temperatures too high or too low, insufficient light, and heavy metals are known to affect photosynthesis (Berry and Bjorkman 1980, Burzyski and Klobus 2004, Kosobrukhov *et al.* 2004, González-Naranjo *et al.* 2015, Tomar *et al.* 2015). Some herbicides can also affect the growth and development of crops by impairing

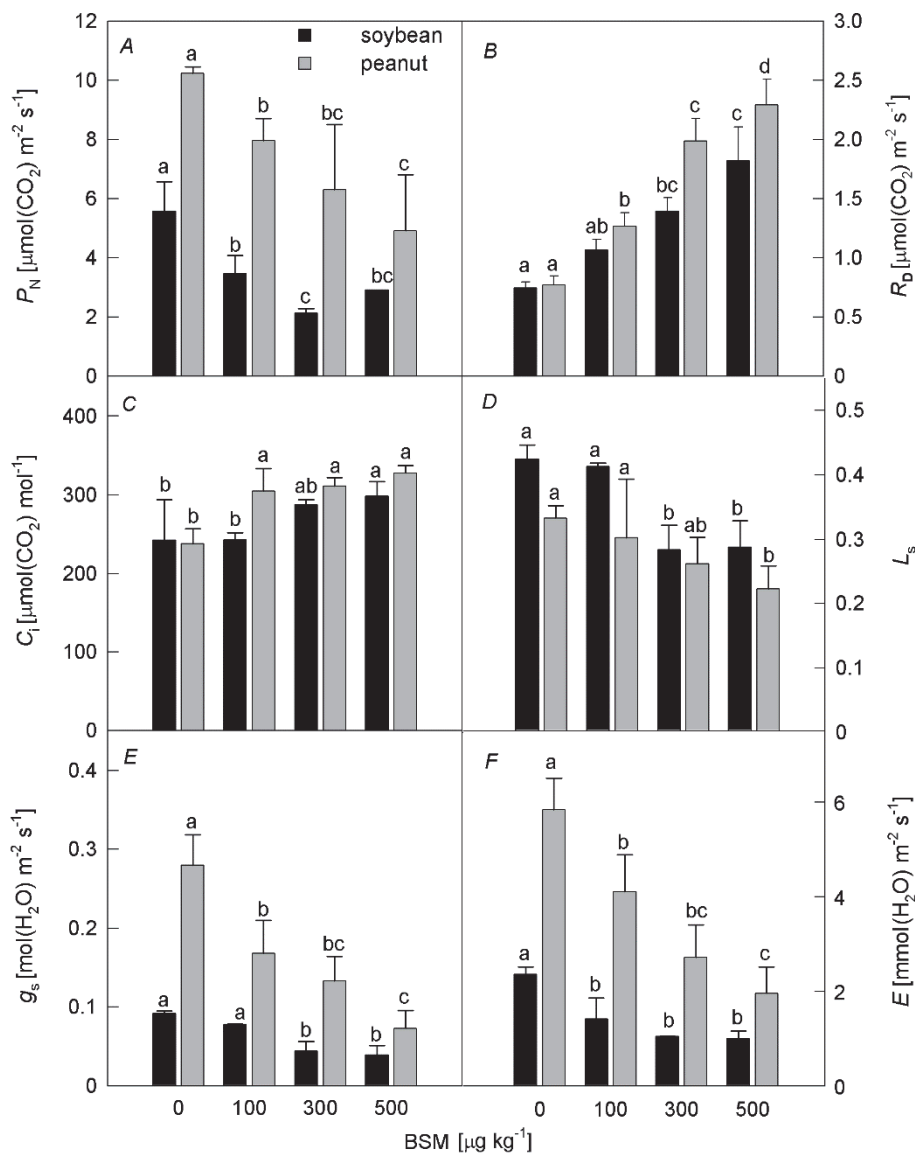


Fig. 2. (A) Net photosynthetic ( $P_N$ ), (B) dark respiration rate ( $R_D$ ), (C) intercellular  $\text{CO}_2$  concentration ( $C_i$ ), (D) stomatal limitation ( $L_s$ ), (E) stomatal conductance ( $g_s$ ), and (F) transpiration rate ( $E$ ) of soybean and peanut seedlings under different soil concentrations (0, 100, 300, and 500  $\mu\text{g kg}^{-1}$ ) for the herbicide bensulfuron-methyl. Error bars show standard deviation ( $n = 4$ ). Different letters on the bars indicate significant differences at  $P < 0.05$ , according to Duncan's multiple range test.

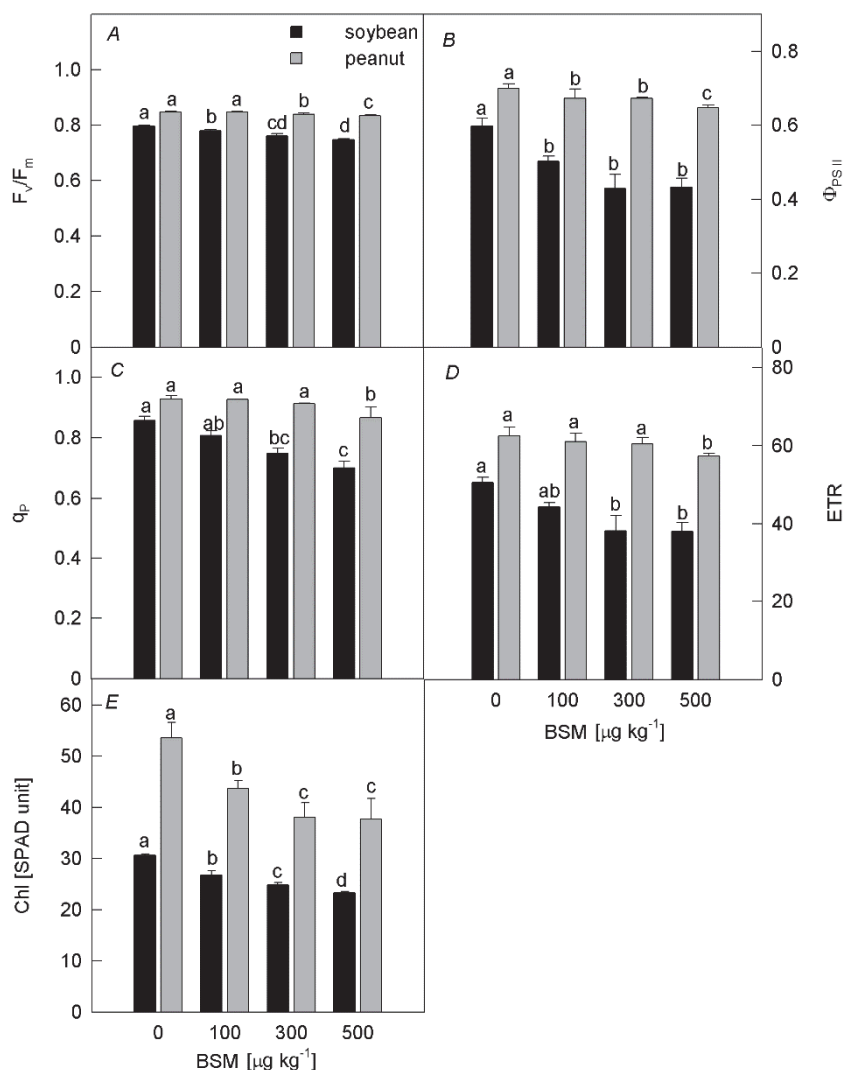


Fig. 3. (A) Maximum quantum yield of PSII photochemistry ( $F_v/F_m$ ), (B) actual quantum yield of PSII under actinic light of  $150 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$  ( $\Phi_{PSII}$ ), (C) photochemical quenching coefficient ( $q_p$ ), (D) electron transport rate (ETR), (E) SPAD value of soybean and peanut seedlings under different soil concentrations (0, 100, 300, and  $500 \mu\text{g kg}^{-1}$ ) for the herbicide bensulfuron-methyl. Error bars show standard deviation ( $n = 4$ ). Different letters on the bars indicate significant differences at  $P < 0.05$ , according to Duncan's multiple range test.

normal physiological parameters or functions. For example, flumioxazin can cause a sharp drop in  $P_N$  and  $g_s$  in grapes (Bigot *et al.* 2007). Leaf SPAD values are a direct reflection of the relative leaf Chl and are positively correlated to Chl content. Chl content is closely related to  $P_N$ , and herbicides have been shown to reduce the content of photosynthetic pigments and  $P_N$  in leaves (Su *et al.* 2013). Both  $P_N$  and Chl content showed a similar trend, and the decrease in Chl might also be one of the major reasons for the decrease in  $P_N$  in the present experiment. The decrease in  $P_N$  and the increase in  $R_D$  in both the crops suggest that increased respiration could be a mechanism to alleviate the damage to photosynthesis caused by BSM (Catriona *et al.* 2002, Ohe *et al.* 2005, Sun *et al.* 2010).

Farquhar and Sharkey (1982) examined whether stomatal or nonstomatal factors were the main cause of reduced  $P_N$  as can be judged by the changing pattern of both  $C_i$  and  $L_s$ . If both  $C_i$  and  $P_N$  decreased, accompanied by an increase in  $L_s$ , the decrease of  $P_N$  was mainly caused by stomatal limitation. On the contrary, when  $P_N$  decreased,  $C_i$  may increase or be constant despite lower  $g_s$ ,

and is accompanied by a decrease in  $L_s$ . Thus it is the photosynthetic activity of mesophyll cells rather than  $g_s$  that was regarded as the critical factor in reducing  $P_N$ .

According to the above theory, the residual effects of BSM resulted in decreased  $P_N$  and  $L_s$  and increased  $C_i$ , indicating that photosynthesis in both crops was limited by nonstomatal factors. These results were consistent with earlier findings showing that the suppression of  $P_N$  induced by cyazofamid was mostly due to nonstomatal factors (Xia *et al.* 2006).

In order to further investigate the nonstomatal factors involved, Chl fluorescence parameters were analyzed. Chl fluorescence is a major technique for detecting and analyzing photosynthesis, which can provide a wealth of information on the photosystem and the electron transfer activities (Krause and Weis 1991). Earlier studies have shown that herbicides damage the PSII complex, block photosynthetic electron transfer, reduce  $F_v/F_m$ ,  $\Phi_{PSII}$ , and  $q_p$  significantly, and increase the nonphotochemical quenching coefficient in wheat leaves (Wang *et al.* 2011). The residual BSM caused significant decreases in  $F_v/F_m$ ,

$\Phi_{PSII}$ ,  $q_P$ , and ETR in both crops. The effect on these parameters indicates that the residues of BSM caused reversible destruction in PSII reaction centers. A decrease in  $q_P$  indicated a higher proportion of closed PSII reaction centers, *i.e.*, an increase in the proportion of the reduced state of  $Q_A$ , which probably leads to a decrease in the proportion of available excitation energy used for photochemistry (Genty *et al.* 1989). Dayan and Zaccaro (2012) observed a decreased ETR in cucumber plants exposed to light and bentazon. An increase in the non-

photochemical quenching coefficient was observed concomitantly with a drop in  $\Phi_{PSII}$ ,  $q_P$ , and ETR, confirming that the energy absorbed by the antenna system was not used for photochemical effect in photosynthesis (Maxwell and Johnson 2000, Müller *et al.* 2001, Roháček 2002).

The accumulation of biomass represents the net effect of carbon assimilation and maintenance. In soybean and peanut, the SDM, RDM, and TDM were significantly reduced by BSM residue concentration, suggesting that growth of was negatively influenced by BSM residue.

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