

## Effects of glyphosate on photosynthesis, chlorophyll fluorescence and physicochemical properties of cogongrass (*Imperata cylindrical* L.)

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### Abstract

Cogongrass is one of the most destructive weed in China. It has been reported as one of the ten most troublesome weed species in the world. Therefore, developing an effective method to control this weed has become a significant and worthwhile practice. The effect of glyphosate on this weed growth was investigated during the 2009-2011 years. The photosynthesis, chlorophyll fluorescence, chlorophyll a and b content, proline content and shikimic acid content were assessed at the second, fifth, and ninth day after treatment with different concentration levels of glyphosate (0%, 0.3%, 0.5%, 1.0%, and 2.0%) in this study. Our results showed that the chlorophyll a and b content, the net photosynthetic rate ( $P_N$ ), effective quantum yield of photochemical energy conversion (Yield), and the relative rate of electron transport through PSII (ETR) decreased significantly after treated with different concentration levels of glyphosate. However, the photochemical quenching (qP) and the transpiration rate (E) increased significantly with damage of glyphosate. Therefore, glyphosate could constrain the growth of Cogongrass effectively by causing adverse effects on photosynthetic pigments, photosynthesis, photochemical activity and the shikimic acid pathway.

**Keywords:** *Imperata cylindrical* L., glyphosate, photosynthesis, chlorophyll fluorescence, proline, shikimic acid.

**Abbreviations:** Chl-chlorophyll;  $P_N$ -net photosynthetic rate; E-transpiration rate; PPFD-photosynthetic photon flux density; Yield-the effective quantum yield of photochemical energy conversion; ETR-the relative rate of electron transport through PSII; qP-photochemical quenching; NPQ-non-photochemical quenching.

### Introduction

Cogongrass [*Imperata cylindrical* (L.)], also called Japgrass, blady grass, speargrass, *alang-alang*, and *lalang-alang*, is a C4, rhizomatous and perennial weed species with culms that grow erect to ascending and typically reaches heights of 1.2 m but can grow to heights of 3 m (Holm et al., 1977). It has been reported as one of the ten most troublesome weed species in the world (Tran et al., 2009). This weed has received great interest for cause harm to the production of 35 crops in 73 countries (Udensi et al., 1999; Holm et al., 1977). It was found throughout tropical and subtropical regions, generally in areas disturbed by human activities (Macdonal et al., 2004). Because of its long and strong root, it is very hard to root out and has spread over large areas of China. Cogongrass spreads mainly by way of seeds and rhizomes (Dozier et al., 1998). Once established, it is extremely competitive with crops and neighboring plant communities. In corn (*Zea mays* L.), grain yield reductions of 80 to 100% have been observed (Koch et al., 1990; Udensi et al., 1999; Koger et al., 2004). In China, the fast invasiveness of this weed is hindering agricultural practices and increasing the cost of its control because a strictly limited use of herbicides, as the glyphosate is necessary. Glyphosate (N-[phosphonmethyl] glycine) is being widely used as a nonselective, broad-spectrum, postemergence herbicide that is

readily translocated in plants and is biodegradable by soil microorganisms (Gresshoff et al., 1979). This herbicide inhibits the biosynthesis of the aromatic amino acids in plant, such as tryptophan, tyrosine, and phenylalanine. This study aims to determine how the glyphosate control this weed by evaluating the effect of glyphosate damage on photosynthesis, chlorophyll fluorescence and physicochemical properties of cogongrass. In addition, provide some theory evidences for the agriculture.

### Results

#### Survival rates

The mortality rate of cogongrass treated with 0 %, 0.3 %, 0.5 %, 1.0 % and 2% glyphosate after 18 days and the survival rates next spring are shown in Table 1. In this study we observed that all the treatments with glyphosate resulted in mortality rate of 100 % in the aboveground parts of these plants. In addition, the treatments with glyphosate concentrations of 0.3 %, 0.5 %, 1.0 % and 2.0% in March of the next year showed survival rates were 41 %, 13 %, 2 % and 1%, respectively.

### ***Chlorophyll contents and Chlorophyll fluorescence parameters***

The Chl a and Chl b contents were remarkably decreased by increase in concentration and treatment time of glyphosate (Figs. 1 and 2). In addition, the increase in the glyphosate concentrations also promoted significant changes on chlorophyll fluorescence parameters (Fig. 3). For example, with the increase of PAR, the Yield and ETR of glyphosate-treated plants were reduced when compared to control plants. On the other hand, the influence of glyphosate treatment on qP had a considerable difference with the control plants. On the second day, we observed an increase of qP in the 2.0% glyphosate treatment between 400 to 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR values, however it decreased between 200 to 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR values on the fifth and ninth day when compared to control plants. On the fifth and ninth days, the qP decreased in the 0.5% and 2.0% glyphosate treatments and enhanced in the afterward. In parallel, all the glyphosate-treated plants showed increase in the NPQ on the second day, however on the fifth day, it showed low values of NPQ when compared to control. On the ninth day only the 0.5% glyphosate treatment had lower value of NPQ and the 0.3% glyphosate treatment had higher value of NPQ between 400 to 1600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of PAR values while the others treatments did not show significant changes in comparison to untreated plants.

### ***Photosynthesis***

Considerable changes in net photosynthetic rate ( $P_N$ ) and transpiration rate (E) were noticed in cogongrass leaves. Specifically, diurnal changes in the  $P_N$  of cogongrass leaves significantly decreased when compared to untreated leaves (Fig. 4). Transpiration rate (E) of the leaves witch treated with low concentration glyphosate had no significant difference with the control leaves on the second day, however, the E value of leaves witch treated with 2.0% glyphosate was higher than the control between about 10:00 h to 12:00h on the fifth and ninth day, and this difference increased with the treatment time.

### ***Shikimic acid content***

The effects of glyphosate on changes in the shikimic acid content of cogongrass plants are shown in Fig. 5. According to Fig.5, there is a remarkable increase in shikimic content caused by increase in concentration and treatment time of glyphosate application.

### ***Proline Content***

The effects of glyphosate on changes of proline content are shown in Fig. 6. On the second day, the proline content of cogongrass increased with the glyphosate concentration increasing. On the fifth day, all the glyphosate treatments showed remarkable difference when compared to control plants, mainly in the most elevate concentration (2.0%). On the ninth day, the proline content progressively increased with the glyphosate concentration increasing.

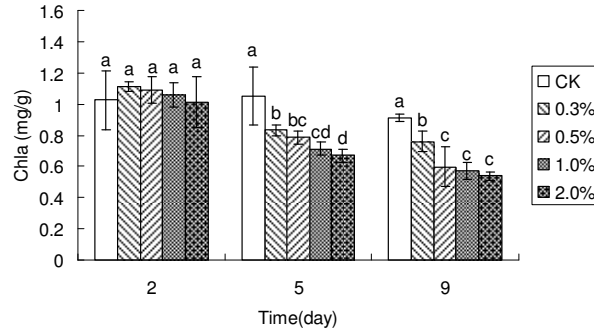
### ***Discussion***

The control of Cogongrass is very difficult because it produces strong and large quantities of rhizomes. Glyphosate is transferred to the underground parts after daubed in the leaf of this weed. Therefore, if the glyphosate concentration is high, it will quickly kill the aboveground parts of this weed and the glyphosate can not be transferred in amounts sufficient to the

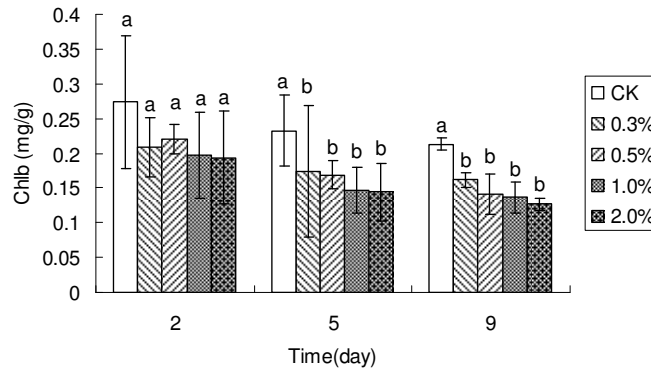
rhizome and can not kill it. So an appropriate herbicide concentration is very important to apply on the rhizomatous weeds. Although the mortality rate of the aboveground parts of cogongrass was 100 % for all glyphosate treatments, the shoot ratios of the 0.3 % and 0.5 % glyphosate treatments were significantly higher than the 1.0% and 2.0% glyphosate treatment in middle March of the next year. Our data showed that 1.0 % glyphosate will be the optimum concentration for the control of this weed, however the 0.3 % and 0.5 % glyphosate concentrations does not seem effective. Pigment contents and particularly chlorophyll fluorescence parameters were used to elucidate the mode of action of herbicides on plant physiology (Conrad et al., 1993. The contents of Chla and Chlb in leaves markedly decreased, according the concentration and time of application of glyphosate (Fig. 1 and Fig. 2). Such changes in Chla and Chlb contents after herbicide application have been demonstrated in several experiments (Pline et al., 1999; Wong, 2000; Mateos-Naranjo et al., 2009; Cakmak et al., 2009). The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis (Santos 2004). It was previously reported that herbicide stress induced a reduction in the number of chloroplasts (Marchner et al., 1975). The glyphosate effect on chlorophyll fluorescence parameters of cogongrass was also severe and dependent of concentration and time of application. In our results, the change more prominent occurred in the NPQ. It is worthy of notice that NPQ of cogongrass which treated with different concentration of glyphosate is increased on the second day, but decreased after treated 5 days, compared with the control. It is reported in some studies that the NPQ can be increased by application of norflurazon (Jung et al., 2000; Tschiersch et al., 2002; Frankart et al., 2003). An increase in NPQ indicates the presence of a non-radiative energy dissipation mechanism using thermal processes (Ribeiro et al., 2009). This photoprotective mechanism helps to maintain the high oxidative state of the primary electron acceptors of PSII, lowering the probability of photo-damage and photo-oxidative stress in chloroplast components (Silva et al., 2010). The results show that the 2.0% glyphosate treatment had decreased the qP on the fifth and ninth day. The qP is an indication of the proportion of PSII reaction centers that are open (Maxwell et al., 2000) and is the balance between excitation of PSII centers, and removal of electrons from PSII by the electron transport chain (Campbell et al., 1998). So in this study the glyphosate treatment damaged this mechanism. The apparent electron transport rate (ETR) was also adversely affected by glyphosate in our experiment. This response may be associated with blocking of the electron transport from the primary to secondary plastoquinone (Qa to Qb), resulting in an increase in fluorescence emission (Conrad et al., 1993). Since this herbicide may block the reoxidation of Qa, absorbed energy cannot be used in photochemistry, inhibiting the electron transport shortly after PSII. Our experiment demonstrated that quantum yield (Yield) declined after damage by glyphosate. The result may be associated to an efficient permeability displayed by thylakoid membrane (Dai et al., 2009b). The results showed that with increased the concentration and time of application of glyphosate, PS II reaction centers gradually closed resulting in a decline of electron transport rate and quantum yield. Thus, the capacity of protecting the PS II will weakened, as well as damage of thylakoid membrane integrity (Dai et al, 2009b). Glyphosate produced deleterious effects on photosynthesis of cogongrass. In our experiment, the results showed that glyphosate induced a significant decline on  $P_N$  and E with application time. As demonstrated by other researchers (Bigot et al., 2007;

**Table 1.** The mortality rate of the aboveground parts of cogongrass and recurrence ratio of rhizome after 0 %, 0.3 %, 0.5 %, 1.0% and 2.0% glyphosate treatment

Glyphosate concentration	Mortality rate (%)	Survival rate (%)
Control	0	100
0.3%	90	41
0.5%	100	13
1.0%	100	2
2.0%	100	1



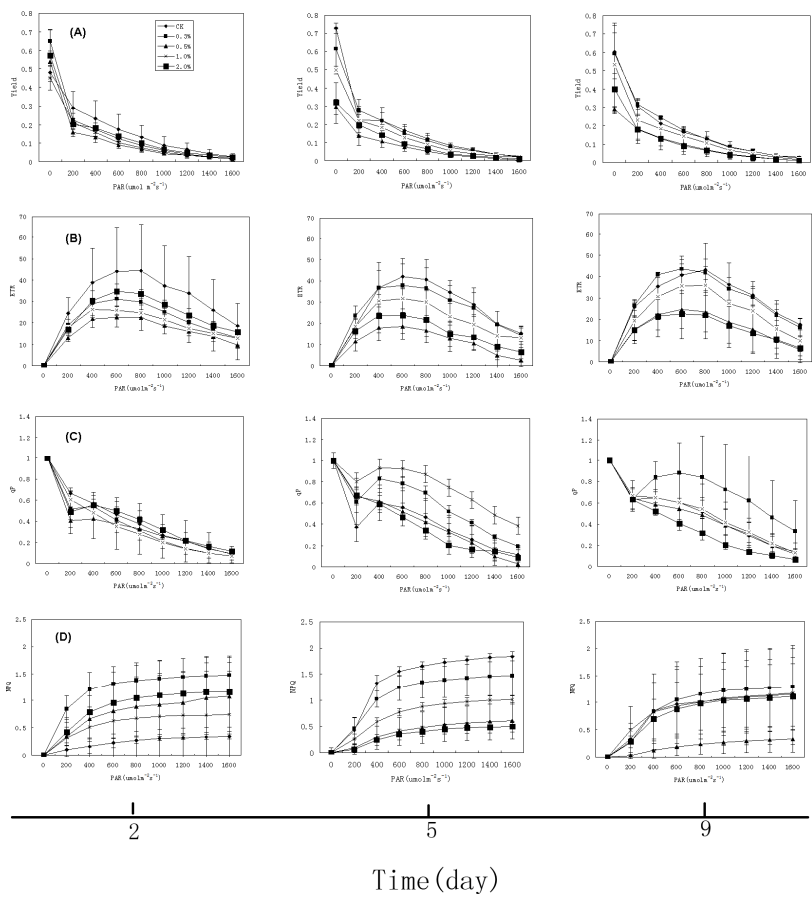
**Fig 1.** Effect of glyphosate on photosynthetic pigments of *Imperata cylindrical* L. Changes in Chla at 2, 5 and 9 days after 0 % (□), 0.3 % (▨), 0.5 % (▩), 1.0% (■) and 2.0 % (▤) glyphosate treatments. Different letters represent significant differences as determined by Student's t-test ( $P < 0.05$ ).



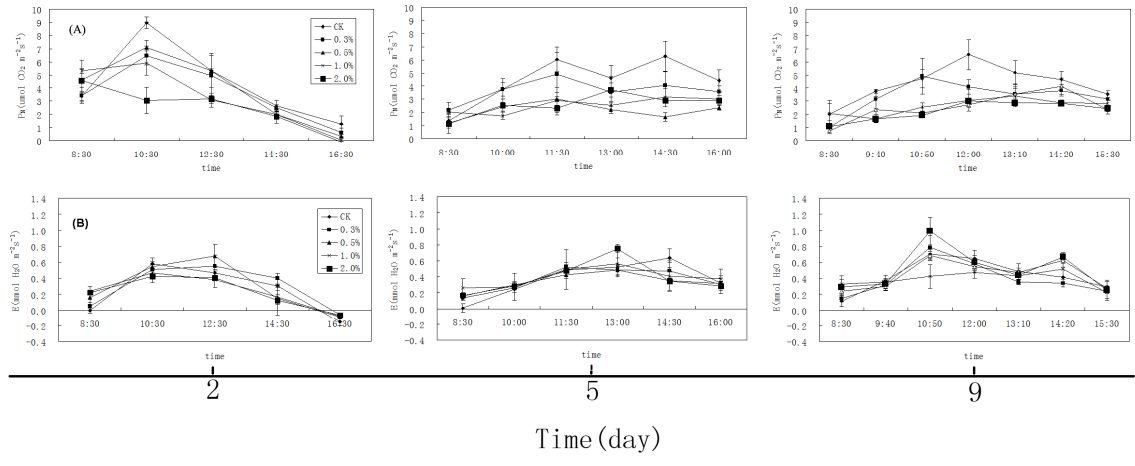
**Fig 2.** Effect of glyphosate on photosynthetic pigments of *Imperata cylindrical* L. Changes in Chlb at 2, 5 and 9 days after 0 % (□), 0.3 % (▨), 0.5 % (▩), 1.0% (■) and 2.0 % (▤) glyphosate treatments. Different letters represent significant differences as determined by Student's t-test ( $P < 0.05$ ).

Mateos-Naranjo et al., 2009),  $P_N$  was adversely affected by herbicides. In addition, the changes observed in transpiration rate may be associated to epidermal and stomatal damage. Moreover, Ghosh (2004) also showed that the glyphosate effect on the epidermis cell and stomatal of leaves was severe and progressive. Our result suggest that the effect of glyphosate on photosynthesis is due to the reduction in the content of photosynthetic pigments (Chla and Chlb) and damages in the PSII and in leaf structure. The changes in the shikimic acid content of cogongrass treated with different concentration of glyphosate observed in this study are similar to the results obtained by Wendy et al. (2002) in cotton (*Gossypium hirsutum* L.) plants. They pointed that accumulation of high levels of shikimic acid may be the result of a loss of feedback control of the shikimic acid pathway by a downstream product that regulates the activity of 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase. In our experiment, the glyphosate caused a minor accumulation of

proline. This accumulation of proline in response to different concentration of glyphosate is well documented. Proline contributes the osmotic adjustment (Rudulier et al., 1984; Zhang et al., 2001), help in protection of macromolecules during dehydration (Yancey et al. 1982), and act as a hydroxyl radical scavenger (Smirnoff et al., 1989; Zhang et al., 2001). The accumulation of proline in plants under abiotic or biotic stresses can be considered a protective mechanism. Proline is one of the most common compatible osmolytes in stressed plants (Mafakheri et al., 2010). In our study, the plants resist the glyphosate with enhanced levels of proline biosynthesis; however, the proline content in cogongrass in the ninth day was lower than in the fifth day. One possible explanation for these results is that the weed began to wither with the concentration and time increased, and the physiological indexes began to decrease. Such changes in proline content after glyphosate treatment have been demonstrated in several experiments.



**Fig 3.** Effect of glyphosate on Chl fluorescence of *Imperata cylindrical* L. (A) The change of the effective quantum yield of photochemical energy conversion (Yield) at 2, 5 and 9 days after treatment with 0 % (◆), 0.3 % (■), 0.5 % (▲), 1.0% (\*) and 2.0 % (■) glyphosate. (B) The change of the relative rate of electron transport through PSII (ETR) at 2, 5 and 9 days after glyphosate treatments. (C) The change of the photochemical quenching (qp) at 2, 5 and 9 days after glyphosate treatments. (D) The change of the nonphotochemical quenching (NPQ) at 2, 5 and 9 days after glyphosate treatments. Values are means ± SE (n = 5).



**Fig 4.** Effect of glyphosate on photosynthesis of *Imperata cylindrical* L. (A) The change of the net photosynthetic rate (PN) at 2, 5 and 9 days after treatment with 0 % (◆), 0.3 % (■), 0.5 % (▲), 1.0% (\*) and 2.0 % (■) glyphosate. (B) The change of the transpiration rate (E) at 2, 5 and 9 days after glyphosate treatments. Values are means ± SE (n = 5).

Evidence supporting the role of proline during herbicide stress was obtained in maize plants (Sergiev. et al., 2006).

## Materials and methods

### Plant material and culture conditions

Cogongrass was collected from the Zhejiang Normal University campus. The experiment was arranged on the lawn of the campus, chose five group witch has the same density of the cogongrass. Each group has about 1×1m<sup>2</sup> with four replications (0.5m×0.5m). In each group the leaves were treated with concentrations of 0.3 %, 0.5 %, 1.0 % and 2.0% (v/v) glyphosate, respectively. In the control was used distilled water. The physiological indexes were measured 2 d, 5 d and 9 d after treatment with glyphosate, respectively.

### Chlorophyll a and b content

Chlorophyll contents were extracted by grinding leaves in 80% acetone. Absorbance of extracts was recorded at 663 nm, 645 nm and 470nm with a UV-VIS spectrophotometer (Lambda 5, Perkin-Elmer, USA). Chlorophylls levels were expressed as mg/g from the equations of Dai (2009b) and Porra (2002).

### Shikimic acid content determination

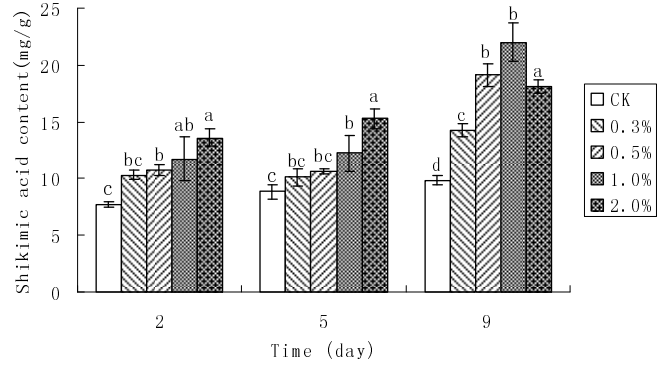
The shikimic acid content of cogongrass was extracted with 0.25mol/L HCl according to Bijay (1998). After washing and airing of cogongrass leaves, samples were homogenized in liquid nitrogen and extracted with 0.25mol/L HCl (w/v). for 30min on a shaker. Following it was centrifuged at 25000g for 15min at 4 °C. The supernatant was centrifuged and stored at 4 °C until analysis. After 100µl supernatant was used to analyze the shikimic acid accumulation, add 1 ml 1% of the hepta-iodic acid, 1ml 1mol/L of the NaOH and 0.3ml 0.1mol/L of the glycine. The absorbance of admixture was recorded at 380 nm with a UV-VIS spectrophotometer (Lambda 5, Perkin-Elmer, USA).

### Photosynthetic parameters

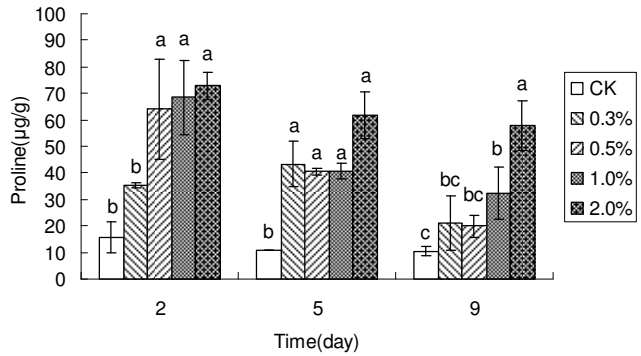
Photosynthetic photon flux density (PPFD) response curves were developed using a GFS-3000 portable photosynthesis system (WALZ, Effeltrich, Germany) as described by Dai et al. (2009a, b). The photosynthetic parameters were measured on fully expanded leaves on a clear, cloudless day from 08:00 to 17:00. The air cuvette temperature and the air CO<sub>2</sub> concentration were maintained at 25 °C and 750 µl l<sup>-1</sup>, respectively. Five replications were used for each weed.

### Chlorophyll fluorescence

Chlorophyll fluorescence parameters was measured with a MINIPAM (pulse-amplitude modulation) fluorometer (WALZ, Effeltrich, Germany) as described by Dai et al. (2009a, b). Fluorescence measurements were taken simultaneously with gas exchange measurements, because the fiber optic bundle of the fluorometer was fitted with a gas-tight seal within the gas exchange cuvette. Leaves were dark-adapted for approximately 30 min prior to measurements of the effective quantum yield of photochemical energy conversion (Yield), photochemical (qP) and non-photochemical (NPQ) quenching. Measurements were obtained over a range of PAR values between 0 and 1600 µmol



**Fig 5.** Effect of glyphosate (0 % (□), 0.3 % (▨), 0.5 % (▧), 1.0% (■) and 2.0 % (■)) on Shikimic acid content (mg/g) of *Imperata cylindrical* L. Different letters represent significant differences as determined by Student's t-test ( $P < 0.05$ ).



**Fig 6.** Effect of glyphosate (0 % (□), 0.3 % (▨), 0.5 % (▧), 1.0% (■) and 2.0 % (■)) on proline content (µg/g) of *Imperata cylindrical* L. Different letters represent significant differences as determined by Student's t-test ( $P < 0.05$ ).

m<sup>2</sup> s<sup>-1</sup>. PAR values were increased from 0 to 1600 µmol m<sup>-2</sup> s<sup>-1</sup>. The relative effective quantum yield of photochemical energy conversion at steady-state photosynthesis was calculated as:

$$Yield = \frac{(F_m' - F_s)}{F_m'} \quad (1)$$

where  $F_s$  and  $F_m'$  are fluorescence at steady-state photosynthesis and maximum fluorescence in the light, respectively.

qP was calculated as:

$$qP = \frac{F_m - F_m'}{F_m' - F_0} \quad (2)$$

where  $F_0$ , minimum fluorescence in the dark-adapted state;  $F_m$ , maximum fluorescence in the dark-adapted state.

NPQ quenching was calculated as (Genty et al., 1989):

$$NPQ = \frac{F_m - F_m'}{F_m'} \quad (3)$$

The relative rate of electron transport through PSII (ETR) was calculated as (Krall & Edwards, 1992):

$$ETR = Yield \times PPF \times 0.5 \quad (4)$$

where, PPF is absorbed light ( $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ) by leaf.

#### Proline content determination

Determination of free proline content was done according to Bates et al. (1973). Leaf samples (0.5 g) from each group were homogenized in 3% (w/v) sulphosalicylic acid and homogenate filtered through filter paper. After addition of acid ninhydrin and glacial acetic acid, resulting mixture was heated at 100 °C for 1 h in water bath. Reaction was then stopped by using ice bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from liquid phase was read at 520 nm with a UV-VIS spectrophotometer (Lambda 5, Perkin-Elmer, USA).

#### Statistical analysis

Statistical analysis was carried out using SAS Version 9.0. Quantitative values are presented as mean  $\pm$  standard error of the mean (SE). Significance values were assessed by Student's t-test. Statistical significance was at the 0.05 level.

#### Conclusion

In conclusion, the results show that the glyphosate has a strong effect on cogongrass. With the increase of glyphosate concentration, physiological indexes of this weed have significant difference, especially in the 1.0% and 2% concentration. In order to save wealth and root up this weed, we recommend using the 1.0% of the glyphosate to deal with the cogongrass.

#### Sources of Materials

<sup>1</sup>SPSS software, Version 17.0, SPSS Inc., Shanghai, China.

#### Acknowledgements

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