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Photosynthetic electron-transfer reactions in the gametophyte of *Pteris multifida* reveal the presence of allelopathic interference from the invasive plant species *Bidens pilosa*

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Abstract

To date, the response of the fern gametophyte to its environment has received considerable attention. However, studies on the influence of plant invasion on the fern gametophyte are fewer. Allelopathy has been hypothesized to play an important role in biological invasion. Hence, it is necessary to study the allelopathy of invasive plant species to the fern gametophyte and elucidate the mechanisms by which invasive plants cause phytotoxicity. As one of the main invasive plants in China, *Bidens pilosa* exhibits allelopathic effects on the gametophytic growth of *Pteris multifida*. The root exudate plays an important role among various allelochemical delivery mechanisms in *B. pilosa*. The effect invasive plant species has on photosynthesis in native species is poorly understood. To elucidate this effect, the changes in photosynthesis in the gametophytes of *P. multifida* are analyzed to examine the mechanisms of the root exudates of *B. pilosa*. Meanwhile, a non-invasive plant, *Coreopsis basalis*, was also applied to investigate the effects on fluorescence and pigments in *P. multifida* gametophytes. We found that gametophytes exposed to both *B. pilosa* and *C. basalis* had decreased fluorescence parameters in comparison with the control, except for non-photochemical quenching. Furthermore, it was found that these parameters were markedly affected from day 2 to day 10 in the presence of both exudates at a concentration of 25% or above. *B. pilosa* exudate had a negative dose-dependent effect on chlorophyll *a*, chlorophyll *b*, carotenoid, and the total chlorophyll in the gametophyte. The inhibitory effects increased with increasing exudate concentrations of both species, exhibiting the greatest inhibition at day 10. In conclusion, *B. pilosa* irreversibly affected the photosynthesis of *P. multifida* on both PS I and PS II. Root exudates caused the primary damage with respect to the decrease of the acceptors and donors of photon and electron in photosynthetic units and the production and the relative yield of photochemical quantum in PS II. With the effects of exudates, part of the energy is released as heat in chloroplasts. The comparison of invasive and non-invasive plants in allelopathic experiments demonstrated that invasive plants were responsible for the critical damage to the photosynthetic process in local species.

Keywords: *Pteris multifida*; gametophyte; *Bidens pilosa*; *Coreopsis basalis*; photosynthesis; allelopathy

Abbreviations: PSII, quantum yield of photosystem II; Fv/Fm, lower quantum efficiency of open PSII reaction centers; qP, photochemical fluorescence quenching; ETR, electron transport rate; NPQ, non-photochemical quenching; Chl *a*, Chlorophyll *a*; Chlorophyll *b*, Chl *b*; Car, carotenoid.

Introduction

In recent years, biological invasion has become a phenomenon of environmental change with the increasing influence of human activity on the environment, and as such has become recognized as a major threat to the economy and environment worldwide because of its ecological impacts on ecosystems (Vitousek et al. 1996; Parker et al. 1999; Pimentel et al. 2005). In 2012, according to the preliminary statistics supplied by Ministry of Agriculture of PRC, the number of invasive species was over 448 in China, with a direct economic loss of about 20 billion dollars every year.

Invasive plant species can adversely affect the surrounding vegetation through allelopathic effects (Bais et al., 2003; Zhang et al., 2007, 2008a, 2008b). *Bidens pilosa* is one of the main invasive plants found in China. It can reduce agricultural production and alter plant community structures (Hao et al., 2009). Most studies on *B. pilosa* have focused on its deleterious effects on spermatophytes. It excretes allelochemicals and delivers them into the rhizosphere through leaching from the leaves and other aerial plant parts via different pathways that include volatile emissions, root exudate, and leaf litter. These allelochemicals hinder the germination and seedling growth of potential competitors (Khanh et al., 2009).

Ferns are the oldest extant vascular plants and the second-most diverse lineage of tracheophytes next to angiosperms (Lu et al. 2015). An important aspect separating ferns from seed plants is that ferns possess an independent haploid gametophyte stage. Otherwise, in comparison with the gametophytes of angiosperms and gymnosperms the fern gametophyte is a photosynthetic free-living entity that is often described as a small, simple, delicate, ephemeral stage of the fern life cycle. Fern gametophytes have unique advantages as a model in investigating response to environment and revealing the mechanism compared to sporophytes (de Groot et al. 2011).

So far, the effects of invasive plants on ferns have received less attention. As such, invasive plants have threatened the growth and development of fern gametophytes.

Hence, it is important to determine the allelopathic effects of invasive plants on fern gametophytes. In our previous work, we found that the cell membranes, antioxidant enzyme activities, and photosynthesis pigment contents of the gametophytes of the local fern species *P. multifida* were affected by leaf leachates of *B. pilosa* (Zhang et al. 2015). The results also showed that root exudates of *B. pilosa* adversely affected gametophytic survival, length of the rhizoid and biomass of gametophytes, and the conversion rate from gametophytic generation to sporophytic generation of *P. multifida* (Liu et al. 2015). Although (based on the evidence) *B. pilosa* is allelopathic to gametophytes of *P. multifida,* attention has been focused on determining secondary physiological processes such as germination, seedling growth, and the biomass of test species for most bioassays to assess the level of phytotoxic activity.

Environmental stress affects plant physiology at the whole plant and cellular levels (Ashraf and Foolad, 2007). However, the determination of other physiological and primary processes such as photosynthesis in native species affected by invasive species is still poorly understood (Aurélie et al., 2011). Photosynthesis inhibition is one of the best-characterized phytotoxic mechanisms induced by allelochemicals (Lorenzo et al., 2011; Hussain and Reigosa, 2011a). Root exudation plays an important role among the various mechanisms by which allelochemicals are delivered in *B. pilosa* (Khanh et al., 2009). To determine the potential involvement of allelopathic mechanisms of root exudates in *B. pilosa*, photosynthesis in the *P. multifida* gametophyte was investigated. Meanwhile, a non-invasive plant, *Coreopsis basalis* (Compositae), was selected as a control to determine the possible damage caused by the invasive plant.

Methods

Plant material

Root exudate collection of B. pilosa and C. basalis

The seeds of *B. pilosa* and *C. basalis* were rinsed carefully and thoroughly with

distilled water. Subsequently, the seeds were allowed to germinate in Baltisches Substrat at 25°C. After 15 days, 40 uniform seedlings (15 cm tall) from each plant species were selected and washed thoroughly with distilled water. The seedlings were cultivated in a root exudate collection machine with 1/100 strength Hoagland nutrient solution, with a day/night photoperiod of 16/8 h, temperature of 25/16°C, and humidity of 80/90%. The silica gel column chromatography was connected with the root exudate collection machine, and the solution was allowed to flow at 5 $ml \cdot min^{-1}$. After continuous collection for 2 days, chromatography was eluted with methanol. The eluent was evaporated, transferred to tubes, and frozen at -70°C for 72 h. The tubes were then put into the Alpha 1–4 LD plus freeze dryer (Marin Christ, Osterode, Germany) to yield powder root exudates. After that, the root exudates from each plant species were stored at -20 ° C for further analysis.

Fern gametophyte

Spores of *P. multifida* were collected from 15-20 fertile fronds with mature but closed sporangia on September 2012 at Nanjing Forestry University. The fronds were unfolded, placed in clean paper bags, and air-dried at room temperature. One week later, the spores were collected and screened using an 0.088-mm diameter mesh (Zhejiang Shangyu Yarn and Sieve Factory, Shangyu, China). Spores were spread evenly in plastic trays (measuring 25 cm × 20 cm × 5 cm) with a sieved mixture of dark soil and sand (Zhang et al., 2011) at an average density of 100–150 spores per cm^2. The trays were covered with transparent plastic film to avoid contamination and desiccation, with fluorescent light (photon flux density 1000 mol $m^{-2} \cdot s^{-1}$) at 25°C for a 12-h light photoperiod. After spore sowing for twenty days, healthy and uniform gametophytes (10 × 5 mm) were selected and rinsed carefully and thoroughly with distilled water.

Treatments application

Root exudates from each plant species were diluted with 1/1000 strength Hoagland

nutrition solution to produce five concentrations (0, 12.5, 25, 50 and 100%). The 1/1000 strength Hoagland nutrition solution was used as a control. Afterwards, all gametophytes were treated with 5 mL of exudates in a Petri dish (60-mm diameter). One hundred gametophytes were grown in each Petri dish with fluorescent light (37.5 mol $m^{-2} \cdot s^{-1}$) at 25°C for 12 h light photoperiod.

Fluorescence and pigment measurements

Fluorescence and pigments of fern gametophytes were analyzed every other day until growth for 10 days. The gametophytes were collected immediately before the fluorescence measurement. Samples were dark-adapted for 2 min. Fluorescence measurements were carried out using a Junior PAM (Heinz Walz GmbH, D91090, Effeltrich, Germany). The fluorometer was connected to a PAM Data Acquisition System PDA 100 (Walz) controlled by the software WINCONTROL v3 (Heinz Walz GmbH). Actinic light intensity (PAR) was 66 mol $m^{-2} \cdot s^{-1}$. $_{PSII}$, Fv/Fm, qP, ETR and NPQ were recorded and calculated by the formulas of Hoegh-Guldberg et al. (1999). The efficiency of dark-adapted PSII was calculated by Fv/Fm = (Fm-Fo)/Fm, where Fm and Fo are the maximum and the minimum fluorescence of dark-adapted samples, respectively.

For the pigment measurements, plant samples (0.025 g of fresh weight) from each plant species were used. Chlorophyll was extracted with a 5-mL mixture of ethanol and acetone (1:1, v/v) in darkness. A colorimetric procedure was performed until the samples were visibly colorless. The optical densities (OD) of the extracts were determined to be 470, 647 and 663 nm using a SP-2100P spectrophotometer (Spectroscopic Instrument Inc., Shanghai, China). Subsequently, the optical densities were converted to chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), carotenoid (Car), and total chlorophyll content following the formulas of Moran (1982).

Statistical analysis

One-way analysis of variance (ANOVA) was conducted on photosynthesis with exudate treatments for each plant species. Descriptive statistics were used to analyze central tendency and dispersion trends of the data. Mean differences were separated using least-squares difference (LSD) and Duncan's test with P = 0.05 indicating statistical significance. The comparative effect between invasive and non-invasive plant species on the fluorescence of the gametophyte was calculated by t-test for paired samples at P = 0.05. Statistical analyses were performed by using SPSS v.20.0 for Windows (IBM; Armonk, New York, USA); all graphs were drawn using Origin 8.0 (Origin Lab, Northampton, Massachusetts, USA). The detail data on the comparative effects between *B. pilosa* and *C. basalis* on PSII (A), Fv/Fm (B), qP (C), ETR (D) and NPQ (E) in the gametophyte described by t-test for paired samples is presented in Table 1, which is available online as Supplementary material in electronic copy of the journal as well as on our server because of the repetition with figures.

Results

Five fluorescence indexes of photosystem II (PS II) of the gametophytes were recorded with exposure of the plant samples to *B. pilosa* and *C. basalis* exudates. The chlorophyll pigments of the gametophytes were measured under the treatments of root exudates, and all the data were collected on days 2, 4, 6, 8, and 10 of the experiment.

PSII

$_{PSII}$ is an important index about the photosynthetic abilities of a given plant; the index reflects the ratio between the yield of PS II photochemical quantum and total photochemical quantum. The $_{PSII}$ of *P. multifida* decreased with increasing root exudate concentrations and exposure time after gametophytes were exposed to both *B. pilosa* and *C. basalis* exudates (Fig. 1A, B). For exposure of the 100% root exudate of *B. pilosa* at day 10, the $_{PSII}$ of the gametophyte reached the minimum at 0.10 (Fig. 1A).

In comparison with the control, $_{PSII}$ of *P. multifida* did not markedly decrease at day 2 with exposure to *C. basalis*, while $_{PSII}$ predominantly decreased during days 4-10. $_{PSII}$ was reduced to 27.5% at day 10 after gametophytes were exposed to 100% root exudate of *C. basalis* (Fig. 1B). In comparison with *C. basalis* exudate, after the application of *B. pilosa*, no significant difference in $_{PSII}$ of *P. multifida* was noted at day 2 at a 12.5% concentration (Table 1A). Afterwards, $_{PSII}$ differed significantly from day 4 to day 10 between the treatments of *B. pilosa* and *C. basalis*. Meanwhile, Table 1 shows that the changes in $_{PSII}$ of the gametophytes were significantly different after exposure to *B. pilosa* and *C. basalis* exudates at a concentration of 25% or above from day 2 to day 10.

Fv/Fm

Fv/Fm is an index to estimate the maximum portion of absorbed quanta used in PSII reaction centers. Fv/Fm of *P. multifida* was also affected by the root exudate of *B. pilosa* (Fig. 2A), gradually decreasing with the addition of root exudates. Fv/Fm leveled off in the control, while remarkable decreases were found at days 2-10 at all levels of root exudate exposure. In the control, Fv/Fm values were 0.70-0.69. However, Fv/Fm decreased to 0.52 at day 2 and 0.22 at day 10 under 12.5% treatments (Fig. 2A). Fv/Fm of *P. multifida* decreased with the increase of root exudate concentrations of *C. basalis* (Fig. 2B). Fv/Fm remarkably decreased during days 4-10 except for 12.5% treatment at day 4, compared with the control. Fv/Fm values were 0.77 and 0.68 in the control at day 2 and 100% treatment at day 10, respectively (Fig. 2B). The data points from the samples treated with *B. pilosa* and *C. basalis* exudates demonstrated differences with statistical significance (Table 1B).

qP

qP allows the quantification of both the effective photochemical state of the PSII regarding the fraction of PSII centers that remain open or oxidized at any time, and

the non-photochemical photosynthetic mechanisms involved in photo-protection, state 1 and state 2 transition quenching, photo-inhibition, and photo-damage. The inhibitory effect of *B. pilosa* exudates on the qP of gametophytes increased with increasing concentrations, indicating a negative dose-dependent effect between the exudate concentrations and qP (Fig. 3A). The differences were significant in inhibitory effects on gametophyte of *P. multifida* between the exudate treatments and the control. qP decreased by 81.3% after the gametophyte was exposed to 100% *B. pilosa* exudate for 10 days (Fig. 3A). The presence of high concentrations (especially a 100% concentration) of *C. basalis* exudates resulted in significant inhibition for the qP of the gametophytes (Fig. 3B). The average inhibitory rate was 67.9% for day 8 with the application of 100% root exudate. qP decreased by 35.7% upon exposure to 100% *C. basalis* exudate for 10 days. Comparative inhibition between *B. pilosa* and *C. basalis* of the qP of *P. multifida* followed a similar pattern as with $_{PSII}$ (Table 1C).

Electron transport rate (ETR)

Electron transport rate (or ETR) is valuable for many types of plant stress investigations. In this study, the variation in ETR was similar to that of qP, decreasing with the increase of root exudate concentrations of both species (Fig. 4A, B). Plants had their lowest ETR level (2.10 μ mol·m^{-2}·s^{-1}) when treated with 100% *B. pilosa* exudate at day 10. ETR decreased by 81.1% compared to the control with increases in *B. pilosa* exudate concentration to 100%. The highest ETR was 10.10 μ mol·m^{-2}·s^{-1} at day 4 with 12.5% *B. pilosa* exudates in comparison with the higher treatments (Fig. 4A). However, ETR remarkably decreased at days 6 and 10 in various concentrations of *C. basalis* exudates (Fig. 4B). Generally, *B. pilosa* exudates significantly decreased ETR compared with *C. basalis* exudates, except in the case of 12.5% concentration, day 2 (Table 1D).

Non-photochemical quenching (NPQ)

Non-photochemical quenching (NPQ) is more heavily affected in instances where NPQ reflects heat-dissipation of excitation energy in the antenna system. The NPQ of *P. multifida* was enhanced with increasing root exudate levels of *B. pilosa* and *C. basalis* (Fig. 5). Compared to the control, application of 12.5% root exudate of *B. pilosa* resulted in 1.14- to 1.55-fold increases in NPQ at days 2 and 10, respectively (Fig. 5A). The treatment of 100% root exudate of *B. pilosa* induced a 2-fold increase in NPQ at day 2 as well as a 2.45-fold increase at day 10 compared to the control. With regard to the treatments of *C. basalis*, the NPQ of *P. multifida* increased from 0 to 33.3% during the experimental period (Fig. 5B). In particular, NPQ was remarkably affected by increased root exudates of *C. basalis* at day 6. Fig. 5B showed that NPQ increased from 0.24 to 0.28 at 100% concentrations for *C. basalis* from days 2 to 10. A comparative influence between *B. pilosa* and *C. basalis* on NPQ of *P. multifida* followed a similar pattern as ETR (Table 1E).

Pigments of gametophytes

Chl *a*, Chl *b*, Car, and total chlorophyll content decreased as root exudate concentrations increased (Fig. 6A-D). This change was also reflected by the change in color of the gametophyte. When the applied root exudate increased from 12.5% to 25%, Chl *a* and Chl *b* content at day 10 were reduced by 18.85% and 12.50%, respectively ($P < 0.05$). Surprisingly, the changes of Chl *a* and Chl *b* were almost similar in samples treated with 25% and 50% root exudates (Fig. 6A-B).

Car content with 100% root exudate treatment exhibited the lowest levels compared to the control. The values of Car at day 2 and 10 with the addition of 100% root exudate were 0.19 and 0.16 mg/g, for a 33.34% and 44.83% reduction in comparison with the control (Fig. 6C). Treatment of 12.5% root exudate resulted in a greater decline in the total chlorophyll than other treatments (Fig. 6D). At day 2 and 10, 12.4% and 20% reductions in total chlorophyll were observed with the application of 12.5% root exudate compared with the control. In contrast, the total chlorophyll

decreased by 6.01% and 4.32% when root exudate concentration was increased from 25% to 50% (Fig. 6D).

Discussion

According to the results, the five fluorescence indexes and the pigments demonstrate a response with increases in the root exudates concentrations. In addition, the inhibitory effects on the chlorophyll fluorescence in gametophytes caused by *B. pilosa* root exudates are more significant and stronger than those caused by *C. basalis.* The environmental stress would enhance the extent of photo-inhibition, and the process is determined by the balance between the rate of photo-damage to PSII and the rate of its repair (Murata and Takahashi, 2008). It is found that Fv/Fm, $_{PSII}$, qP, and ETR are markedly decreased in the presence of *B. pilosa* exudates at a concentration of 12.5% or above. The value of NPQ increases at various concentrations.

In this study, with the decrease of Fv/Fm and $_{PSII}$, the absorbed *B. pilosa* root exudates in the gametophyte significantly damage the production of quantum yield and PS II photochemical quantum yield, as well as the relative quantum yield in the PS II process, with the ETR in PS II also declining. We assume that the decrease of photochemical quantum production and its yield retard the energy conversion from photon to electron, while the protective system also weakens with increasing concentrations of root exudates. This trend seems to endure and is not reversible, according to the results of the qP. In photosynthesis reaction centers, the production of quantum yield results in the fading of PS II, with subsequent decreases in ETR and the relative photochemical quantum yield. However, *C. basalis* root exudates cause little damage on the photosynthetic ETR of gametophyte, the trends of decline for Fv/Fm, $_{PSII}$, qP, and ETR seem smooth and steady in the treatments. These decreases are not significant at the same treatment, with the NPQ not having a significant increase as a result. The results clarify that the damage caused by invasive root exudates is more harmful than that from native plant species, especially with respect

to the photosynthetic electron-transfer reaction.

In addition, the pigments (Chl a, Chl b and Car) also decline with the increasing root-exudate concentrations, especially Chl a and Chl b, which are important parts in photosystems I and II (PS I and PS II). It can be inferred that the components of root exudates also have the function of promoting degradation or hindering of the synthesis of pigments. Photosystems I and II are the two major photosynthetic units, and they are mainly distributed at the non-stack battlements stroma and grana lamellae and stack battlements interstitial in the thylakoid membrane in chloroplasts. The pigments in chloroplasts have the functions of receiving, transferring, and converting energy in photosynthesis. In PS I, Chl a is the primary electron donor P700 and the original acceptor of the P700 excited state (Plato et al., 2003). In PS II, the chloroplast pigments Chl a, Chl b, and Car play an important role in light harvesting and energy transport (Leeuwen et al., 1991). In this study, the decrease of Chl a, Chl b, and Car in the presence of *B. pilosa* root exudates inhibits light harvesting and slows ETR in PS I and II. The concentration of pigments decreases due to allelochemicals, which leads to the degradation or depression of pigment synthesis under treatment.

Figure 7 provides the improved schema under root exudate treatment according to the Z-schema (zigzag schema) of the photosynthetic electron-transfer reaction chain. With the increasing effects of root exudates, some changes occur in PS I and II due to this stress. The electronic and energy chain transferring from PS II to PS I is caused by an oxidation-reduction reaction; the plant can use the light to produce chemical energy, and the chain is more reliant on the electron-transfer reactions in chloroplasts. H_2O is oxidized in O_2, and the electron is transferred to the PS II reaction center. With the energy of photon P680, the ground state of the accepter is excited enough to convert electric energy. P680 is mainly made up by Chl a, Chl b and Car, and these three pigments form a chain from accepter to donor, and this chain gives the electron to PS I through a series of compounds—PQ-PQH_2 (plastoquinones), Cybt6f (cytochrome complex), and PC (plastocyanin). Under the root-exudate treatments, the effective quantum yields in PS II reaction centers are limited, with the negative effects

resulting in reductions of the oxidation reaction. Meanwhile, this study reveals that the electron transport from PS II to PS I is interrupted as the effective excited electron decreases with the effects caused by root exudates on pigments. The result indicates that too much photonic energy is dissipated in a non-regulated manner (such as heat or uncoupling), as the method of regulation is interrupted. Weakened electron-transfer reaction is the main inhibition effect caused by *B. pilosa*, and reduction in the pigments of the gametophyte, Chl a, and Chl b are a decisive reason for ETR, the photon receive and electron transport. Decreases in the pigments of native species caused by *B. pilosa* root exudates are the primary cause of damage to the photosynthetic electron-transfer reaction.

It is well reported that extracts from *Ephedra equisetina* root are capable of inducing destruction of the thylakoid membrane, interruption of electron transport, and reduction of effective quantum yield, thus causing cessation of photosynthesis and inducing cyanobacterial death (Yan et al., 2012). In PS I, the system could use the electron from PS II and the photon to reduce $NADP^+$ (triphosphopyridine nucleotide), and store the energy in NADPH. The conversion is primarily based on the excitatory process of P700 so that the reaction center is concerned with Chl a, and the fluorescence indexes are applied to reflect this reaction. The chlorophyll fluorescence of *P. multifida* was adversely affected by *B. pilosa* root exudates, which is similar to the description of the chlorophyll fluorescence analysis of photosynthetic efficiency, quantum yield, and photon energy dissipation in photosynthesis antennae of *Lactuca sativa* leaves exposed to cinnamic acid given by Hussain and Reigosa (Hussain and Reigosa, 2011b). The reduced sensitivity of Fv/Fm to *B. pilosa* root exudates was due to the conserved F_0 in relation to lower chlorophyll content, indicating a low-level functioning of PS II with a low absorption rate of light in the gametophytes. The results for the chlorophyll contents are in line with those from preliminary studies on *Chlorella vulgaris* by Qian et al. (2009) and *Microcystis aeruginosa* NIES-843 (cyanobacteria) by Shao et al. (2011). Our results show that the contents of chlorophyll and Car decreased upon the exposure of *B. pilosa* root exudates. In this

study, we find that the effects of *B. pilosa* root exudates could cause damage to photosynthesis, both on PS I to PS II, and the components of the root exudates have the enduring and irreversible destruction on the energy transformation from photon to electron, and from electron to chemical energy in plants.

It is reported that the photosystem is more sensitive to herbicides, and the organics are harmful to the cyclic electron flow from PS I center to PS II center and the energy conversion from electron to chemicals, such as DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) and Gramoxone (Hosler and Yocum 1987; Allen and Nilsson 1997). However, the natural organics, such as the root exudates in this study, play a different role in the destruction of the photosystem. The organics affect photosynthesis by degrading the pigments; loss of pigments leads to photon acceptance, electron transport, and energy conversion. Chlorophyll plays an important role in the absorption, transfer, and transformation of light energy. Meanwhile, Car is the indispensable component of chromoprotein in photosynthesis, which is important for the protection of photosynthetic apparatus from injury caused by the presence of singlet oxygen. Decreases in Car block photon transfer in PS II, with the additional weakness of the overall antioxidant effect. The fluorescence indexes given in this study demonstrate that the root exudates have considerable damaging effects on PS I and PS II. The loss of pigments is the main reason for the destructive effects on the photosystem (caused by the allelochemicals) and the root exudates.

The donor invasive plant species could have fatal, weak or non-allelopathic effects on several target species, which belong to different taxonomic families. As a result, small but important differences are expected in their physiology associated with the allelochemical mode of action (Lorenzo et al., 2011). The observed loss of fern species in *B. pilosa* patches in the field, together with the results of our laboratory test, implies that the effects of *B. pilosa* on native species are deleterious and allelopathy may be partly responsible for the adverse effects. Meanwhile, in comparison with *B. pilosa* exudates, $_{PSII}$, Fv/Fm, qP, and ETR in *P. multifida* decreased slightly in *C. basalis* exudate treatments. The present comparative research on *B. pilosa* and *C.*

basalis reveals that *B. pilosa* is the invasive plant species responsible for the gametophyte damage.

The Novel Weapons Hypothesis set to explain the remarkable success of many exotic invasive species argues that invaders may possess novel chemicals that are more phytotoxic to plants in the invaded range than to adapted species in the invader's native range (Zhu et al., 2011). With regard to *B. pilosa*, many secondary metabolites (phenolics, polyacetylenes, and triterpenes) involved in allelopathic action were found; of these, phenylheptatriyne (PHT) and its derivatives amounts were highest in *B. pilosa* oil (Khanh et al., 2009). A total of 23 phenolic compounds (including salicylic acid, vanillin, *p*-hydroxybenzoic acid, *p*-coumaric acid, and ferulic acid) were identified and isolated in its shoots and roots. Contents of caffeic acid were highest (117.4, 298.7, and 350.3 g g^{-1} in leaves, stems and roots), followed by pyrocatechin (Khanh et al., 2009).

The detection and study of allelochemicals in *B. pilosa* root exudates, which are responsible for inhibition of *P. multifida* gametophyte, continue at our laboratory. About 30 allelochemicals were detected in *B. pilosa* root exudates (data not shown). Isolation, identification, and characterization of phytotoxic substances in *B. pilosa* root exudates should be further investigated. Determination of the mechanism and modes of action of these allelochemicals should be one area of particular focus. Moreover, allelopathic research of the impact of *B. pilosa* root exudates on other physiological and primary processes (except for photosynthesis) should be carried out, since root exudate of donor plant species can affect the receptor through several different mechanisms of action (Dayan et al., 2009). Besides, the effects of the stem, leaf, and flower of *B. pilosa* on the *P. multifida* gametophyte should be addressed due to the possible variety and concentrations of allelochemicals in different parts of plants. Although we are still in the early stages of understanding the gametophyte responses of *P. multifida* to the root exudates of *B. pilosa*, this study opens the door to understanding of the photosynthetic response of the fern to invasive plant species.

Conclusions

It is concluded that *B. pilosa* enduringly and irreversibly affects the photosynthesis of *P. multifida* via allelopathic interference, both on PS I and PS II. Decreases in the acceptors of light and electron transport in PS reaction centers and the production of photochemical quantum yield in PS II are the main factors of this that are precipitated by the stress caused by root exudates. This comparative research study of invasive and non-invasive plants in allelopathic experiments demonstrated that invasive plants are responsible for the critical damage to this local species.

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Additional files:

Additional file 1: Figure 1. Impacts of time and root exudations of *Bidens pilosa* (A) and *Coreopsis basalis* (B) on quantum yield of photosystem II (PSII) of *Pteris multifida* gametophytes. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 2: Figure 2. Comparison of results of the lower quantum efficiency of open PSII reaction centers (Fv/Fm) between the gametophyte treated with *Bidens pilosa* (A) and *Coreopsis basalis* (B) exudations. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 3: Figure 3. The reduction of photochemical fluorescence quenching (qP) using *Bidens pilosa* (A) and *Coreopsis basalis* (B) exudations. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 4: Figure 4. Electron transport rate (ETR) of *Pteris multifida* gametophytes in the treatment groups of *Bidens pilosa* (A) and *Coreopsis basalis* (B) exudations. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 5: Figure 5. Change of non-photochemical quenching (NPQ) of *Pteris multifida* gametophytes when exposed to *Bidens pilosa* (A) and *Coreopsis basalis* (B) exudations. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 6: Figure 6. The values of A) Chlorophyll *a*, B) Chlorophyll *b*, C) carotenoid and D) the total chlorophyll in the control and treated with *B. pilosa* root exudations. Statistically significant differences among treatments are indicated by different letters ($P < 0.05$).

Additional file 7: Figure 7. The improved Z-schema (Zigzag schema) of photosynthetic electron transport chain in fern under *Bidens pilosa* root exudations. (P680 and P700: chlorophyll in reaction centers)

Figures

Figure 1

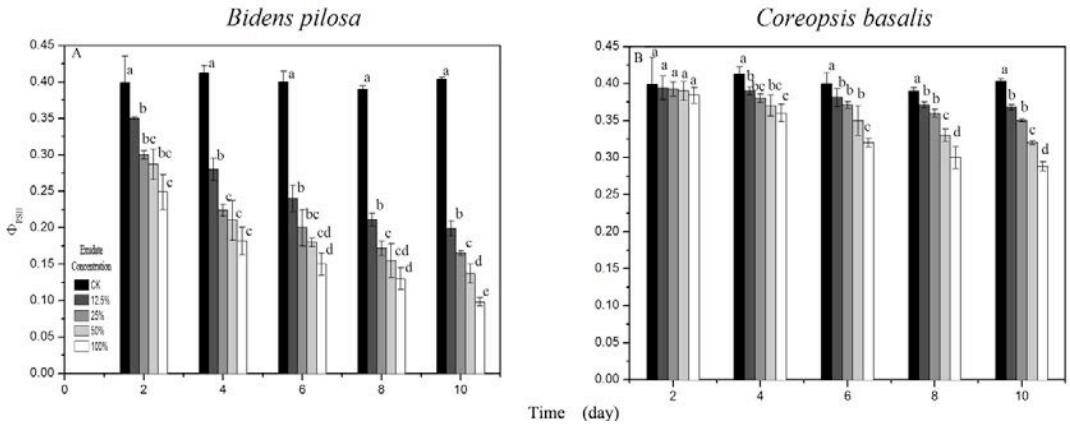


Figure 2

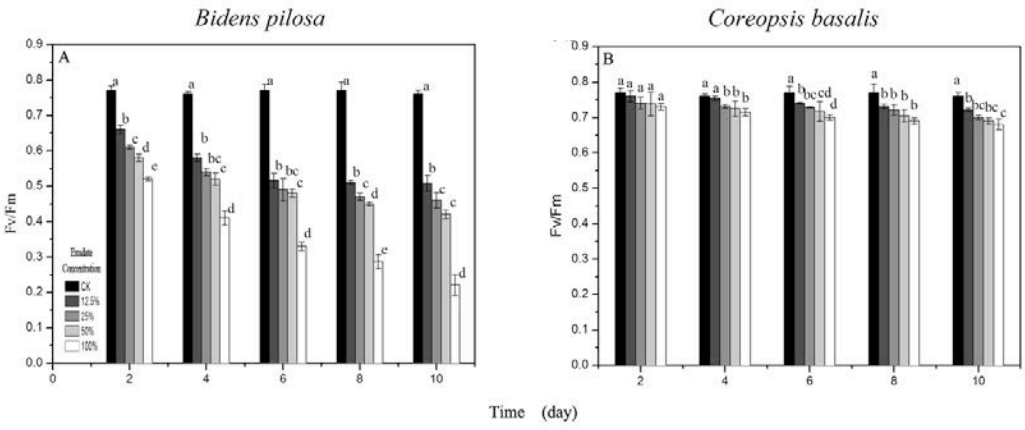


Figure 3

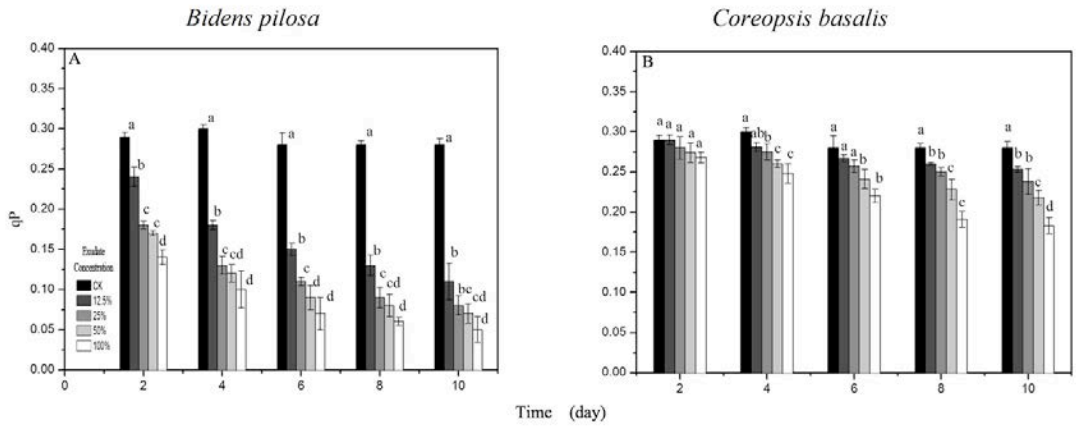


Figure 4

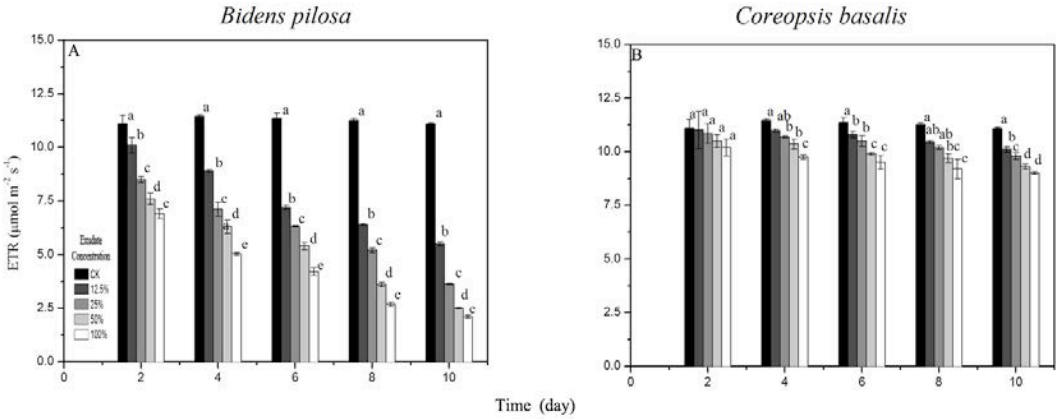


Figure 5

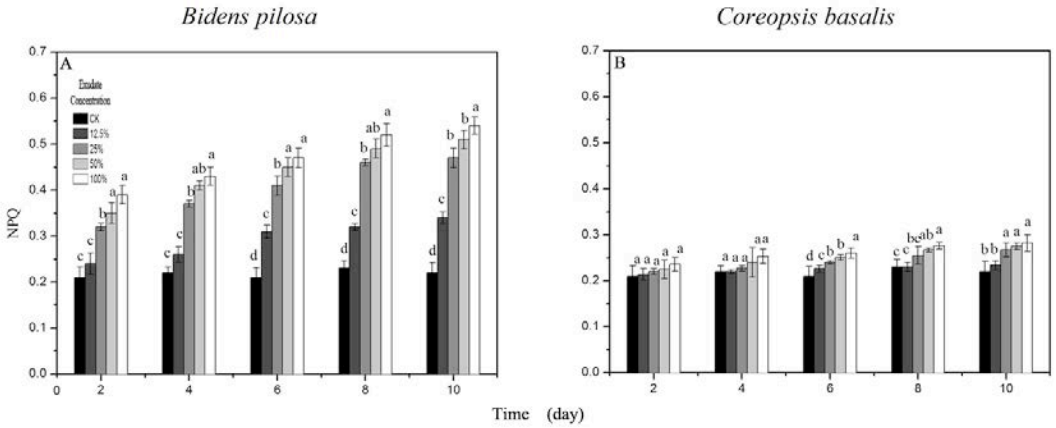


Figure 6

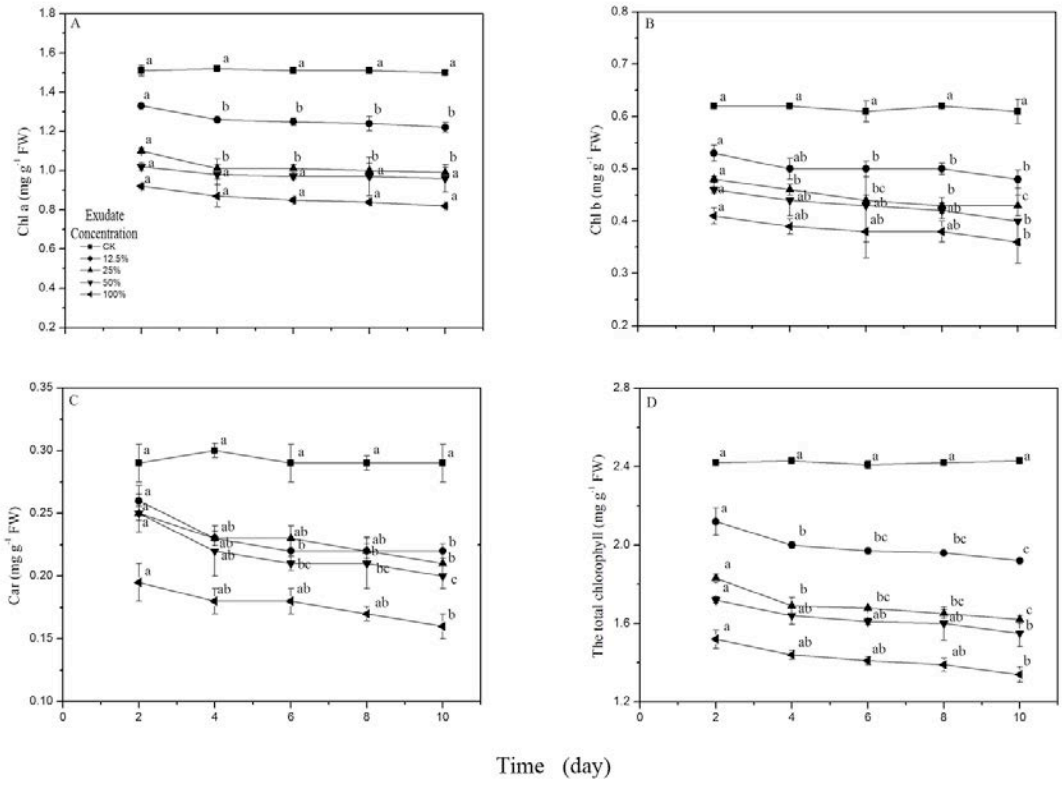
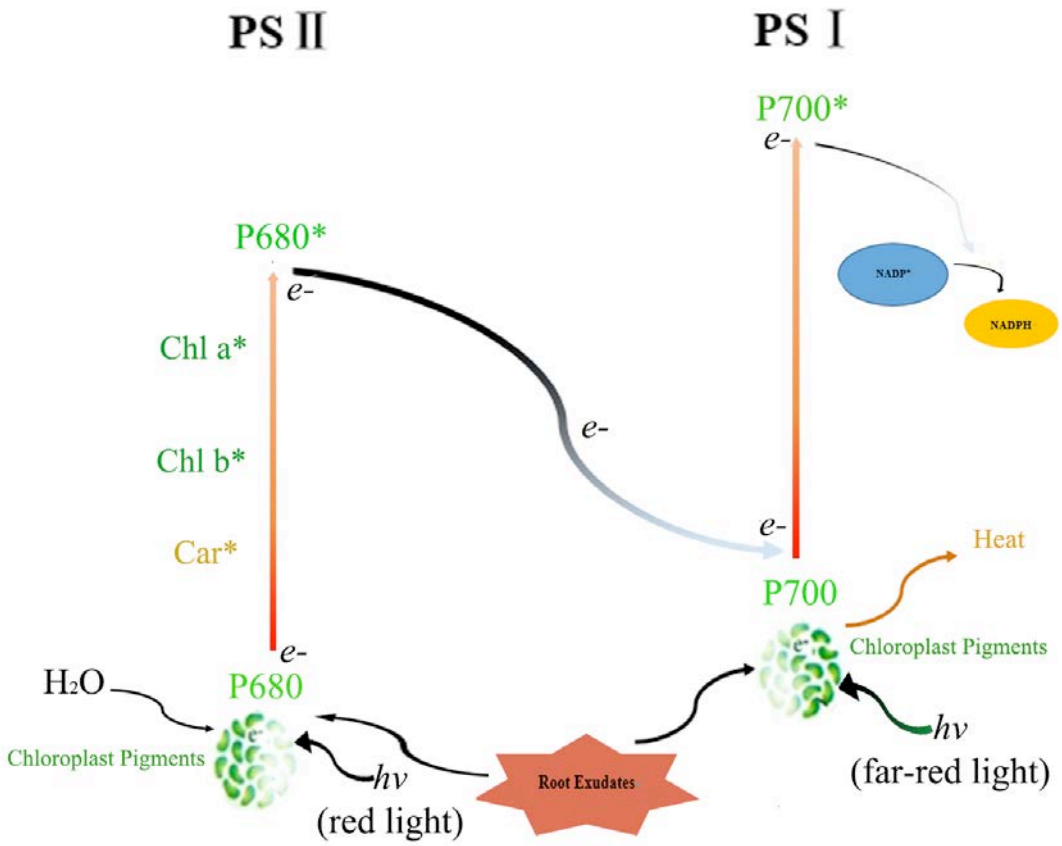


Figure 7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H2O
P680
Chloroplast Pigments
hv
(red light)
Root Exudates
hv
(far-red light)