DOI: 10.1007/s11099-015-0071-z PHOTOSYNTHETICA 53 (1): 23-28, 2015

Chlorophyll fluorescence characteristics and the growth response of *Elaeocarpus glabripetalus* to simulated acid rain

M.H. LIU[*, **], L.T. YI[*, **, +], S.Q. YU[*, **], F. YU[*, **], and X.M. YIN[*]

School of Forestry and Biotechnology, Zhejiang Agriculture and Forestry University, Lin'an, 311300, China[*]
The Naturing Station for the State Key Laboratory of Subtropical Silviculture, Lin'an, 311300, China[**]

Abstract

Acid rain causes damages to forest ecosystems. Here, we reported that acid rain could promote plant growth. From 2006 to 2009, one-year-old *Elaeocarpus glabripetalus* seedlings were sprayed with simulated acid rain (AR) (pH 2.5, 4.0, and 5.6). The maximum quantum yield efficiency of PSII and the actual photochemical quantum efficiency of PSII increased with rising AR acidity, which facilitated chlorophyll fluorescence and plant growth, as shown by a declining minimal fluorescence yield of dark-adapted state with little damage to the PSII reaction center. After the second experimental year, the plant height and ground diameter were greater at pH 2.5 than those found at pH 4.0 and 5.6. This showed the positive effects of AR on the seedling growth and photosynthesis of *E. glabripetalus*, revealing that this species exhibited a stronger resistance to acid deposition than some other tree species. This implies that *E. glabripetalus* is an acid-tolerant species.

Additional key words: acid stress; growth adaptability; photosynthetic efficiency.

Introduction

Acid rain has been a serious and worldwide environmental problem for several decades (Abbasi *et al*. 2013, Singh and Agrawal 2008). Along with Europe and North America, China has become a region severely polluted (Larssen *et al*. 2006) and about 40% of its territory has been affected by acid rain (Menz and Seip 2004). It has been reported that *Pinus massoniana* and *Cunninghamia lanceolata* forests were largely destroyed by acid rain in Sichuan Province, China (Feng 2000). Other reports showed great damage to broad-leaved forests caused by acid rain in the tropical and subtropical regions of China (Feng 2000, Larssen and Carmichael 2000, Liu *et al*. 2011). The impact of acid rain on plants and natural ecosystems is one of increasing environmental concerns.

Acid rain can cause leaf damage, reduction in photosynthesis, and defoliation (Larssen and Carmichael 2000). It leads to further decreases in vertical growth, stem incremental growth, and in total tree biomass (Fan and Wang 2000, Feng 2000). Under environmental stress, plant growth becomes restricted, mainly due to stress toxicity, leading to a decrease in plant tolerance capacity, an imbalance of nutrient uptake, and the toxicity of ions (van Hoorn *et al*. 2001, Neves *et al*. 2009, Dias *et al*. 2010). The significant differences in plant responses to acid rain stress are dependent on the physiological characteristics of the plant species. Generally, exposure to different stresses affects cell activity and may damage photosynthetic membranes leading to a reduction in the net photosynthetic rate, especially in acid-sensitive areas (Sheng *et al*. 2008). Previously, it was reported that different tree species show some level of tolerance to acid rain, which indicates various mechanisms behind a tolerance to acid rain (Ceron *et al*. 2009, Dias *et al*. 2010, Liu *et al*. 2011).

Previous studies showed that some species had been seriously damaged by acid rain in various forest ecosystems in China and that acid rain is a reason for decreasing population sizes of some species (Feng 2000,

---

Received 2 May 2013, *accepted* 2 May 2014.

[+] Corresponding author; phone: 86-571-6374-2067, fax: 86-571-6374-0809, e-mail: yilita@126.com

Abbreviations: Chl – chlorophyll; D – ground diameter; F_m – maximal fluorescence yield in the dark-adapted state; F_m' – maximal fluorescence yield in the light-adapted state; F_s – steady-state fluorescence yield; F_v/F_m – maximal photochemical quantum efficiency of PSII ; F_0 – minimal fluorescence yield in the dark-adapted state; F_0' – minimal fluorescence yield in the light-adapted state; H – plant height; q_N – nonphotochemical quenching coefficient; AR – simulated acid rain ; Φ_{PSII} – actual photochemical quantum efficiency of PSII.

Acknowledgements: The authors thank the editor and two anonymous reviewers for their helpful comments and suggestions. This study is supported by Zhejiang Provincial Natural Science Foundation (Y5110226, Y3110200) and National Natural Science Foundation of China (Grant No. 31100325). The authors are grateful to Prof. P. Lovell for her helpful comments on manuscript writing.

Liu *et al.* 2007). However, it is enough evidence to prove that *E. glabripetalus* continues to grow well under acid rain conditions.

E. glabripetalus is a dominant, heliophilous species in the forest ecosystems of China. This species is widely distributed in warm and humid regions and is planted in neutral or slightly acidic soil. It is fast-growing and has a well-developed, deep root system. None of studies have addressed until now the question how *E. glabripetalus* adapts to acid rain and grows better than other species. However, methods for analyzing the photosynthetic apparatus state were used in the early detection of plant damage and the prediction of tolerance to acid rain during plant development.

Although much effort has been devoted to investigate the impact of acid rain on plant species (Liu *et al.* 2007, Ceron *et al.* 2009, Dias *et al.* 2010, Liu *et al.* 2011), a comprehensive literature search revealed that few studies have focused on the impact of AR on plant photosynthetic characteristics. Our specific objectives were: (*1*) to investigate the variations in chlorophyll (Chl) fluorescence and to discover how AR affects photosynthesis at different pH; and (*2*) to compare the growth characteristics after long-term acid stress at different pH and determine whether acidity stress plays a role in the growth of *E. glabripetalus*. The results could provide an insight into the possible protective mechanisms of this species against acid rain. In addition, the results could be used to establish a risk management strategy for acid rain stress on forest ecosystems in China

Materials and methods

Plant material and stress treatments: In June 2006, healthy, one-year-old seedlings of *E. glabripetalus* were collected and transplanted into pots with an inner diameter of 30 cm, a depth of 30 cm, and a cover area for each seedling of 0.07 m^2. Each seedling was planted in a separate pot. The pots were filled with a mixture of reddish brown forest soil, which is the typical soil type for the area in southern China that receives acid rains. The soil was collected from the top layer (0–20 cm) of a forest near the study site. The composition of the soil included 9.3 g(N) kg^{-1}, 0.3 g(P) kg^{-1}, 1.62 g(K) kg^{-1}, 1.83 g(Fe) kg^{-1}, 0.17 g(Zn) kg^{-1}, and 1.17 g(Mg) kg^{-1} (Pan *et al.* 2011). To avoid a nutrient insufficiency during the acid leaching process, we repotted seedlings and displaced the soil every six months. The plants grew under a canopy at the experimental fields of the Zhejiang Agriculture and Forestry University (119°44′E, 30°16′N), eastern China. The temperature under the canopy was close to the local, natural conditions. In this area, the mean annual temperature is 16.4°C, and the mean daily temperature ranges from 3.8°C in the winter to 28.6°C in the summer. The mean annual rainfall (46 years recorded) is 1,628.6 mm, and the mean monthly rainfall is 130 mm. The rain falls mainly in the spring and summer (April to August), which accounts for 70% of the total precipitation; there is less rain in autumn and winter.

Seedlings were sprayed with three different rain acidities (pH 2.5, 4.0, and 5.6, with pH 5.6 being the control) during their vegetative stages for four years. Each treatment included 50 seedlings. The AR solution was prepared as described by Fan and Wang (2000) using a solution of 1 mmol(H_2SO_4) L^{-1} and 1 mmol(HNO_3) L^{-1} at a ratio of 5:1 by chemical equivalents, which is similar to the general anion composition of rainfall in southern China. Based on the mean annual rainfall and the mean monthly rainfall, daily watering per pot was adjusted to 0.3 L AR. The experiment was conducted from June 2006 to December 2009.

Chl fluorescence was measured in leaves from the top of the seedling during each October from 2006 to 2009. Chl fluorescence in dark- and light-adapted leaves was measured using a *PAM 2000* Chl fluorometer (*WALZ GmbH*, Effeltrich, Germany). The fluorometer was connected to a computer by the data acquisition system (*PAMWIN*, *Walz*, Germany). Prior to each measurement, the leaf was dark-adapted for 30 min (Guo *et al.* 2006). The minimal fluorescence yield of the dark-adapted state (F_0) was measured by the modulated light of 6 nmol(photon) m^{-2} s^{-1}, which was too low to induce significant physiological changes in the plant, and was recorded after dark adaptation. Subsequently, a saturating pulse [6,000 μmol (photon) m^{-2} s^{-1}, 2 s] was given to measure the maximal fluorescence yield of the dark-adapted state (F_m) (Qin *et al.* 2011). The steady-state fluorescence yield (F_s), the maximal fluorescence yield of the light-adapted state (F_m'), and the minimal fluorescence yield of the light-adapted state (F_0') after far-red illumination were determined as described by Guo (2005). The maximal photochemical quantum efficiency of PSII (F_v/F_m) was determined after a 20-min dark acclimation period in selected leaves using a dark leaf clip. The actual photochemical quantum efficiency of PSII (Φ_{PSII}) was recorded using fully expanded leaves that were illuminated with actinic light [600 μmol(photon) m^{-2} s^{-1}] after a 20-min dark adaptation period, with six measurements conducted per replication. The nonphotochemical quenching coefficient (q_N) was calculated according to Genty *et al.* (1989) as $q_N = (F_m - F_m')/(F_m' - F_0')$.

Chl content and growth measurements: The relative Chl content was assessed on the same leaf where Chl fluorescence was determined using a portable Chl content instrument (*SPAD-502*, *Konica Minolta Inc.*, Japan) and taking ten measurements per leaf. Since the SPAD readings significantly correlated with the Chl content, the

SPAD meter could be used to estimate the photosynthetic capacity of the leaves (Kumagai *et al.* 2009). Plant height (H) was measured with a measuring tape and ground diameter (D) was measured using a vernier caliper.

Measurement of the element composition of plants: At the end of the acid-spraying experiment, leaf samples were oven-dried at 80°C to a constant mass, weighed, and then ground to pass through a 30-mesh (0.5 mm) screen. To analyze N, P, K, Fe, Zn, and Mg contents, dry leaf samples were wet-ashed with H_2SO_4. Water extracts from plant digestion were used for N determination by the micro-Kjeldahl method (Stuart 1936). P, K, Fe, Cu, Zn, and Mg were measured using the *Perkin Elmer Optima 7000 DV* Inductively Coupled Plasma Optical Emission Spectrometer (*PerkinElmer Inc.*, Shelton, CT, USA).

Data analysis: The data were subjected to a two-way analysis of variance (*ANOVA*), and a general linear model of univariate analysis was used. Differences between treatments were detected and mean values were compared by *Tukey*'s test (p=0.05). A statistical analysis was conducted using the statistical software package *SPSS 13.0* for *Windows* (*SPSS Inc.*, Chicago, IL, USA). *Sigma Plot 10.0* was used for graphing (*SPSS Inc.*, Chicago, IL, USA).

Results

Chl fluorescence: *Elaeocarpus glabripetalus* showed a higher F_v/F_m value and lower F_0 and q_N values under serious acid stress (Fig. 1). F_v/F_m values at pH 2.5 were higher than those of pH 5.6, except during 2007. However, the difference between F_v/F_m values at pH 2.5 and pH 4.0 was not significant, except in 2009. The response pattern of Φ_{PSII} was the same as that of F_v/F_m, which showed a decrease with time and an increase as the acidity increased, with higher values at pH 2.5 than at pH 5.6 (Fig. 1).

The q_N showed a different response to acid stress compared with F_v/F_m and Φ_{PSII}. The value of q_N decreased with increasing pH, and the value at pH 2.5 was lower than that at pH 5.6 in the same year. There was a significant difference among the values of q_N for the three acidity treatments in 2006 and 2009. However, there were no significant difference between values of q_N at pH 4.0 and pH 5.6 during 2007 and 2008.

Chl content: The total Chl content showed significant changes at different pH (Fig. 2). Under acid stress, the Chl content decreased during the first three years and then increased in the last year, with a larger significant change at pH 2.5 than at the other pH levels. Changes in Chl content showed the same trend as F_v/F_m and Φ_{PSII} with a decrease over time and an increase as the acidity increased, and the highest value occurring at pH 2.5.

Plant growth: Plant H and D showed different changes with different pH values over four years of acid rain stress; with decreasing magnitude in following order: pH 2.5 > pH 4.0 > pH 5.6 (Fig. 3). However, there were differences among the years. The difference in plant H among the three pH levels was not significant, while D showed a significant difference in 2006. In 2007, the difference in H and D among pH 2.5 and the other treatments (pH 4.0 and pH 5.6) was significant. Increased acidity could improve the growth of *E. glabripetalus* (Fig. 3). At pH 2.5, plant H and D were significantly stimulated.

Elemental composition of plants showed no differences in the P, Zn, or Mg contents in leaves at different pH values (Table 1). However, N and K contents showed a significant difference between the control plants (pH 5.6) and the other treatments (pH 2.5 and pH 4.0). The N, K, and Fe contents in the pH 5.6 treated plants were lower than those receiving the pH 2.5 and pH 4.0 treatments. The Fe content was the highest at pH 2.5 and the difference was statistically significant.

Discussion

Many stressful conditions, such as aridity, salinity, and high or low temperatures, can disrupt the components of photosynthetic apparatus, *e.g.*, its membrane integrity, and decrease its photosynthetic capacity (Sheng *et al.* 2008). Chl fluorescence analysis is the most powerful and widely used technique for studying photosynthetic performance in plants (Maxwell and Johnson 2000). The F_v/F_m, which is particularly sensitive to a variety of environmental stress-inducing factors, is a measure of the capacity of the primary PSII photochemistry (Figueroa *et al.* 1997), and it has been shown to be a reliable indicator of stress (Liu *et al.* 2011, Qin *et al.* 2011). The results of this study indicated that acid rain positively affected the photosynthetic performance of *E. glabripetalus* and that the F_v/F_m was significantly higher at pH 2.5 than at pH 5.6. Together with the lower F_0 and Φ_{PSII} values in the leaves of *E. glabripetalus*, these results imply that this species has highly efficient PSII photochemistry under extremely acidic AR conditions.

Any change in q_N corresponds to a change in the efficiency of heat dissipation. Broadly, an increase in q_N can occur as a result of processes that protect the leaf from light-induced damage (Maxwell and Johnson 2000, Liu *et al.* 2011). At lower pH, *E. glabripetalus* maintained a

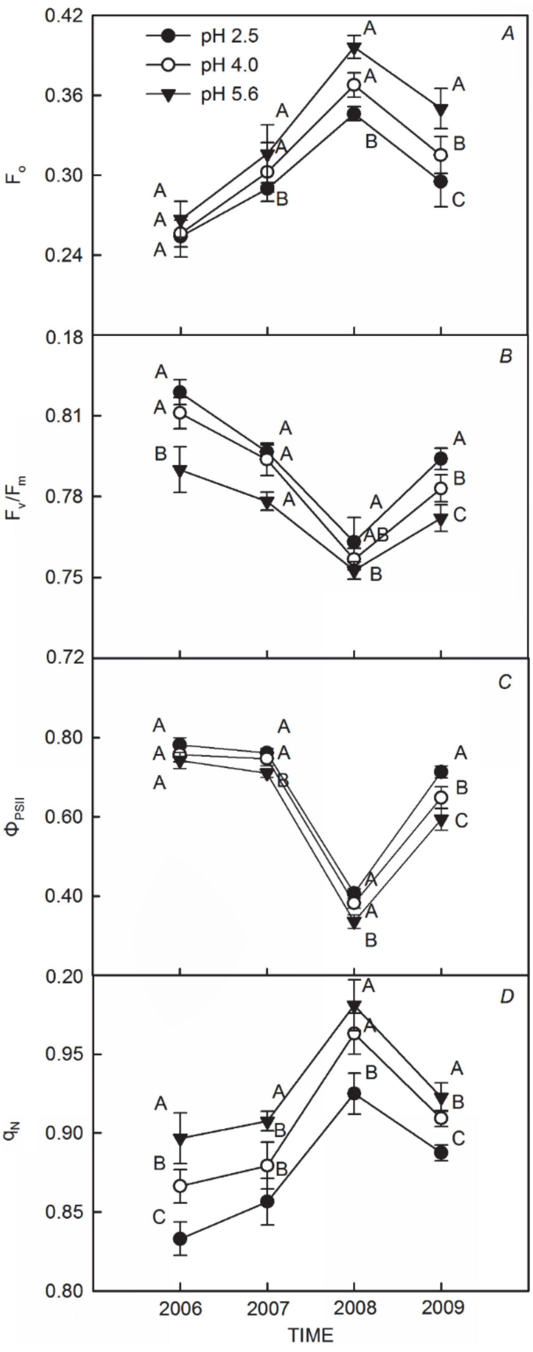

Fig. 1. Effect of acid rain on chlorophyll fluorescence parameters of dark- and light adaptation for *Elaeocarpus glabripetalus* in different years. F_v/F_m – maximal quantum yield of PSII photochemistry; F_0 – minimal fluorescence yield of the dark-adapted state; q_N – nonphotochemical quenching coefficient; Φ_{PSII} – actual photochemical quantum efficiency of PSII.

lower q_N value, suggesting that a protective or stimulatory mechanism was acting in this species. This allowed the leaves to dissipate harmlessly excessive excitation energy as heat. Hence, it suggests that *E. glabripetalus* was able to protect its leaves from light-induced damage. In addition, our data pointed out a rising trend in F_0 with increasing acid spray time. F_0 measures the minimal fluorescence in the dark-adapted state. An increase in F_0 due to stressful conditions might indicate the destruction of the PSII reaction center (Bolhar-Nordenkampf *et al*. 1989). If true, the significantly increased F_0 after the pH 4.0 and pH 5.6 treatments would indicate that PSII functions of *E. glabripetalus* were partially inactivated.

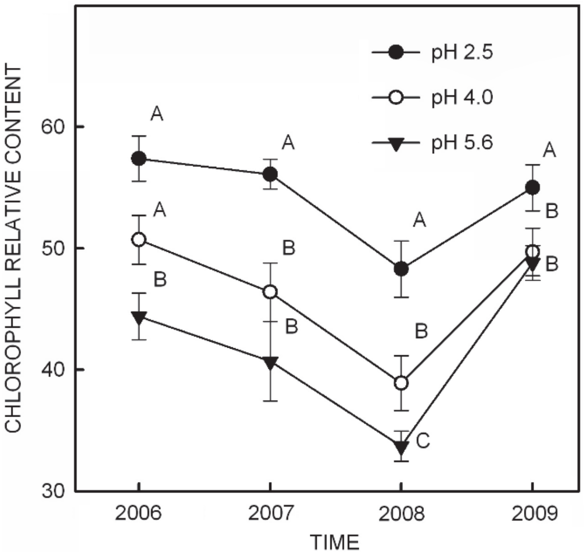

Fig. 2 Effect of different pH levels of acid rain on the relative chlorophyll content of *Elaeocarpus glabripetalus*.

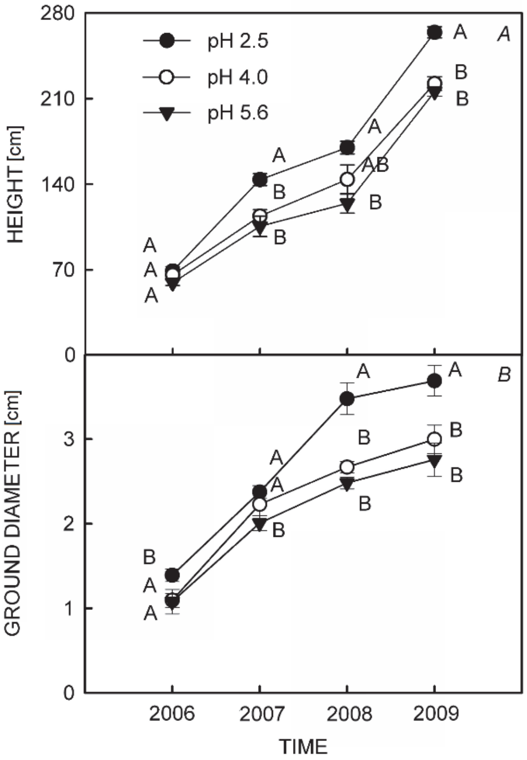

Fig. 3 Effect of different pH levels of acid rain on the height and ground diameter of *Elaeocarpus glabripetalus*.

Table 1. Leaf element contents of *Elaeocarpus glabripetalus* seedlings after the acid-spraying experiment. Values are mean values ± SE, $n = 4$. *The same letters* assign no difference at $p = 0.05$.

pH	N [g kg^{-1}]	P [g kg^{-1}]	K [g kg^{-1}]	Fe [mg kg^{-1}]	Zn [mg kg^{-1}]	Mg [mg kg^{-1}]
2.5	14.22 ± 1.83^{ab}	0.63 ± 0.02^{a}	12.57 ± 0.24^{b}	468.43 ± 9.87^{b}	33.20 ± 1.19^{a}	604.29 ± 46.49^{a}
4.0	14.83 ± 0.94^{b}	0.63 ± 0.10^{a}	13.42 ± 0.57^{b}	379.70 ± 30.64^{a}	39.20 ± 19.32^{a}	782.04 ± 45.74^{a}
5.6	10.57 ± 1.09^{a}	0.66 ± 0.07^{a}	10.16 ± 0.55^{a}	360.66 ± 13.69^{a}	37.36 ± 5.24^{a}	750.60 ± 26.83^{a}

The finding that the F_0 at pH 2.5 was substantially lower than that at pH 4.0 and pH 5.6 shows that the plants subjected to high acidity suffered less damage; it could be a physiological mechanisms of acid tolerance.

Generally, photosynthetic rates are positively correlated with the Chl content in leaves (Fleischer 1935). In this study, there was a strong positive relationship between the leaf Chl content and Chl fluorescence, as indicated by the high Chl content of *E. glabripetalus* when subjected to high acidity. This phenomenon was detected in other tree species under AR, such as *Cryptomeria fortunei* and *Cunninghamia lanceolata* (Liu *et al*. 2011). Another piece of evidence that *E. glabripetalus* was less damaged by high acidity rain was the growth of these trees. In previous studies, many plants showed damage under different stresses. *Cryptomeria fortunei* at Tianmu mountain became weak and even died because of gall disease caused by acid rain (Ma 2007). When exposed to acid rain, the photosynthetic rates of *Schima superba* and *P. massoniana* decreased as both species were susceptible to acid rain (Liu *et al*. 2007). However, the growth of *E. glabripetalus*, including H and D, showed an increase with time and rising acidity. A significant decrease in Chl content and changes in fluorescence induction parameters were found in 2008. The anomalous fluorescence induction parameters in 2008 were likely related to the significantly decreased Chl content. In addition, the drop in plant height in 2008 suggested that there might be a long-term, as yet unidentified, stress imposed on the plants that resulted in a much reduced yearly growth. From the elemental composition of the leaves, such as the Fe content, we could see that the changes were indeed a result of tolerance to acidity. Providing that there are enough nutrients, a lower pH level facilitates Fe uptake (Morrissey and Guerinot 2009). After the pH 2.5 treatment, the Fe content was the highest, F_0 decreased and Chl content increased; it could be a result of increased Fe availability. Therefore, the growth of *E. glabripetalus* under AR stress was improved, indicating a resistance and/or adjustment to the AR conditions.

Recent studies have shown that adaptation to environmental stress can be accurately tested by means of Chl fluorescence. The high Chl fluorescence found in *E. glabripetalus* under AR indicated that the species was able to adjust its metabolism to cope with acidic conditions. Hence, the ability to tolerate enhanced acidity is advantageous for maintaining productivity. The enhanced Chl fluorescence activity of *E. glabripetalus* might be a part of the physiological strategy to cope with highly acidic rain. Undoubtedly, further studies on light interactions, as well as assessment of chemical, morphological, and functional components of photoprotection are required to fully understand their ecological role in this species and in the productivity of ecosystems affected by increased acid rain.

Conclusion: The Chl fluorescence and growth of *E. glabripetalus* under AR were investigated for four years. At pH 2.5, the higher Chl content, F_v/F_m, and Φ_{PSII} were found; the declining F_0 indicated little damage to the PSII reaction center. According to our data, when compared with other species, *E. glabripetalus* can grow well in seriously acid rain–affected regions and can be considered an acid-tolerant species.

References

Abbasi T., Poornima P., Kannadasan T. *et al.*: Acid rain: past, present, and future. – Int. J. Environ. Eng. **5**: 229-272, 2013.

Bolhar-Nordenkampf H.R., Long S.P., Baker N.R. *et al.*: Chlorophyll fluorescence as a probe of the photosynthetic competence of leaves in the field: a review of current instrumentation. – Funct. Ecol. **3**: 497-514, 1989.

Ceron R.M., Ceron J.G., Guerra J.J. *et al.*: Effects of simulated acid rain on tropical trees of the coastal zone of Campeche, Mexico. – WIT Trans. Ecol. Envir. **126**: 259-270, 2009.

Dias B.B., Leite M.L., Farago P.V. *et al.*: Sulfur effect by simulated acid rain on morphophysiological parameters of the bean plant. – Acta Sci.-Agron. **32**: 433-439, 2010.

Fan H.B, Wang Y.H.: Effects of simulated acid rain on germination, foliar damage, chlorophyll contents and seedling growth of five hardwood species growing in China. – Forest Ecol. Manag. **126**: 321-329, 2000.

Feng Z.W.: [Impacts and control strategies of acid deposition on terrestrial ecosystems in China.] – Eng. Sci. **9**: 5-11, 2000. [In Chinese]

Figueroa M.E., Fernández-Baco L., Luque T. *et al.*: Chlorophyll fluorescence, stress and survival in populations of Mediterranean grassland species. – J. Veg. Sci. **8**: 881-888, 1997.

Fleischer W.E.: The relation between chlorophyll content and rate of photosynthesis. – J. Gen. Physiol. **18**: 573-597, 1935.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochem. Biophys. Acta **990**: 87-92, 1989.

Guo D.P., Guo Y.P., Zhao J.P. *et al.*: Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsatsai*) after turnip mosaic virus infection. – Plant Sci. **168**: 57-63, 2005.

Guo Y.P., Zhou H.F., Zhang L.C.: Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. – Sci. Hortic.-Amsterdam **108**: 260-267, 2006.

Kumagai E., Araki T., Kubota F.: Correlation of chlorophyll meter readings with gas exchange and chlorophyll fluorescence in flag leaves of rice (*Oryza sativa* L.) plants. – Plant Prod. Sci. **12**: 50-53, 2009.

Larssen T., Carmichael G.R.: Acid rain and acidification in China: the importance of base cation deposition. – Environ. Pollut. **110**: 89-102, 2000.

Larssen T., Lydersen E., Tang D.G. *et al.*: Acid rain in China. – Environ. Sci. Technol. **40**: 418-425, 2006.

Liu J.X., Zhou G.Y., Yang C.W. *et al.*: Responses of chlorophyll fluorescence and xanthophyll cycle in leaves of *Schima superba* Gardn. & Champ. and *Pinus massoniana* Lamb. to simulated acid rain at Dinghushan Biosphere Reserve, China. – Acta Physiol. Plant. **29**: 33-38, 2007.

Liu T.W., Wu F.H., Wang W H. *et al.*: Effects of calcium on seed germination, seedling growth and photosynthesis of six forest tree species under simulated acid rain. – Tree Physiol. **31**: 402-413, 2011.

Ma Y.: Effects of simulated acid rain on *Cryptomeria fortunei* of Tianmu Mountain. – Master´s Thesis, East China Normal University, Shanghai 2007.

Maxwell K., Johnson, G.: Chlorophyll fluorescence-a practical guide. – J. Exp. Bot. **51**: 659-668, 2000.

Menz F.C., Seip H.M.: Acid rain in Europe and the United States: an update. – Environ. Sci. Policy **7**: 253-265, 2004.

Morrissey J., Guerinot M.L.: Iron uptake and transport in plants: the good, the bad, and the ionome. – Chem. Rev. **109**: 4553-4567, 2009.

Neves N.R., Oliva M.A., Centeno D.C. *et al.*: Photosynthesis and oxidative stress in the restinga plant species *Eugenia uniflora* L. exposed to simulated acid rain and iron ore dust deposition: potential use in environmental risk assessment. – Sci. Total Environ. **407**: 3740-3745, 2009.

Pan W.H., Zhao Y.P., Wu J.S. *et al.*: [Analysis on the nutrient elements in litters of different forest vegetations and soil fertility.] – J. Anhui Agri. Sci. **39**: 5828-5829, 2011. [In Chinese]

Qin L.Q., Li L., Bi C. *et al.*: Damaging mechanisms of chilling and salt stress to *Arachis hypogaea* L. leaves. – Photosynthetica **49**: 37-42, 2011.

Sheng M., Tang M., Chen H. *et al.*: Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. – Mycorrhiza **18**: 287-296, 2008.

Singh A., Agrawal M.: Acid rain and its ecological consequences. – J. Environ. Biol. **29**: 15-24, 2008.

Stuart N.W.: Adaption of the micro-Kjeldahl method for the determination of nitrogen in plant tissues. – Plant Physiol. **11**: 173-179, 1936.

van Hoorn J.W., Katerji N., Hamdy A. *et al.*: Effect of salinity on yield and nitrogen uptake of four grain legumes and on biological nitrogen contribution from the soil. – Agr. Water Manage. **51**: 87-98, 2001.