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Effect of Elevated CO_2 on the Growth and Macronutrient (N, P and K) Uptake of Annual Wormwood (*Artemisia annua* L.)

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ABSTRACT

Annual wormwood (*Artemisia annua* L.) is the only viable source of artemisinin, an antimalarial drug. There is a pressing need to optimize production per cultivated area of this important medicinal plant; however, the effect of increasing atmospheric carbon dioxide (CO_2) concentration on its growth is still unclear. Therefore, a pot experiment was conducted in a free-air CO_2 enrichment (FACE) facility in Yangzhou City, China. Two *A. annua* varieties, one wild and one cultivated, were grown under ambient (374 μmol mol^{-1}) and elevated (577 μmol mol^{-1}) CO_2 levels to determine the dry matter accumulation and macronutrient uptake of aerial parts. The results showed that stem and leaf yields of both *A. annua* varieties increased significantly under elevated CO_2 due to the enhanced photosynthesis rate. Although nitrogen (N), phosphorus (P), and potassium (K) concentrations in leaves and stems of both varieties decreased under elevated CO_2, total shoot N, P, and K uptake of the two varieties were enhanced and the ratios among the concentrations of these nutrients (N:P, N:K, and P:K) were not affected by elevated CO_2. Overall, our results provided the evidence that elevated CO_2 increased biomass and shoot macronutrient uptake of two *A. annua* varieties.

Key Words: artemisinin, biomass, free-air CO_2 enrichment (FACE), medicinal plant, photosynthesis

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INTRODUCTION

The increasing atmospheric carbon dioxide (CO_2) concentration (IPCC, 2014) has led to many hypotheses and experiments on the possible effects of elevated CO_2 on the growth and development of plants (Ainsworth and Long, 2005). The current ambient level of atmospheric CO_2 is one of the limiting factors for photosynthesis; therefore, any rise in CO_2 above this level would have the potential to increase the rate of photosynthesis, decrease photorespiration (especially in C_3 plants), and generally enhance the levels of carbon (C) fixation and dry matter (DM) accumulation (Poorter, 1993; Ainsworth and Long, 2005; Booker *et al.*, 2007; Högy *et al.*, 2010; Kumari *et al.* 2013). Because there is no concomitant increase in nutrient uptake with DM accumulation, elevated CO_2 normally has an opposite effect on nutrient concentrations in plants, and reductions in nutrient levels would be observed (Loladze, 2002; Li *et al.*, 2013). These effects of elevated CO_2 have been studied in many crops, such as rice, wheat, soybean, and potato (Rogers *et al.*, 2004; Wu *et al.*, 2004; Long *et al.*, 2006). However, there are also other opinions that CO_2 fertilization effect on plant growth is not universal, especially for trees (Long *et al.*, 2006; Ellsworth *et al.*, 2012; Bader *et al.*, 2013); *e.g.*, CO_2-induced growth stimulation was not found in deciduous forest trees (Bader *et al.*, 2013), which may be offset by other environmental factors such as nutrient limitation in soils (Norby *et al.*, 2011).

Annual wormwood (*Artemisia annua* L.), a traditional Chinese herb, has been widely cultivated worldwide to meet the increasing demands for artemisinin since the World Health Organization (WHO) recommended artemisinin-based combination therapy (ACT) to treat multi-drug-resistant malaria in 2001 (Wyk and Wink, 2004). Synthetic production is not economically viable, and the only commercially feasible source of

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artemisinin is leaves of *A. annua*. The worldwide cultivated area of *A. annua* has expanded several times (White, 2008) and reached to 12 000 ha (Jessing *et al.*, 2013). Various approaches to conventional breeding and agronomic practices have been tried to increase artemisinin concentration or leaf biomass of *A. annua* (Weathers *et al.*, 2005; Aquil *et al.*, 2009; Zhang *et al.*, 2009).

Given the public health importance of this medicinal plant and the pressing need to optimize its production (Ferreira, 2007), the impact of projected CO_2 increases on the growth of *A. annua* must be known. Meanwhile, in view of down-regulation of plant growth responses induced by nutrient limitation found in some experiments (Oren *et al.*, 2001; Reich and Hobbie, 2013), it is also important to understand the macronutrient demands of *A. annua* under elevated CO_2. However, few studies have answered these questions. Thus, we conducted an experiment to determine whether elevated CO_2 could increase the leaf biomass and nutrient accumulation of a wild variety and a cultivated variety of *A. annua*. This study would be helpful to predict future yields and fertilizer needs of *A. annua*.

MATERIALS AND METHODS

Experiment description

This study was conducted at the free-air CO_2 enrichment (FACE) facility located at Zhongcun Village (119°42′0″ E, 32°35′5″ N), Yangzhou City, Jiangsu Province, China. Details of the design, operation, and performance of the FACE facility used in this study can be found in Liu *et al.* (2002). The experiment was laid out in a split plot design with elevated and ambient CO_2 levels as the main treatments, which were split into subplots of two cultivars. Three rings (replications) were for elevated CO_2 and three rings (replications) for ambient CO_2. Three pots per variety were placed within each of the three elevated CO_2 and ambient CO_2 rings. The target CO_2 concentration of the elevated CO_2 rings was controlled to approximately 200 μmol mol^{-1} above ambient during day and night by a computer system that factored in ambient CO_2 concentration, wind direction, wind speed, and canopy height during day changes. Average daytime CO_2 concentrations at canopy height during the experiment were 374 and 577 μmol mol^{-1} for the ambient and elevated CO_2 rings, respectively. Elevated CO_2 concentrations were within 80% of the set point, > 90% of those in each year.

Seeds of cultivated *A. annua* cv. Youyang were obtained from the Institute of Chinese Medicine in Chongqing City, China. Seeds of a wild *A. annua* variety were collected from populations in Wulong (107°45′42″ E, 29°21′17″ N), Chongqing City, China. The seeds were soaked in 2% (volume/volume) sodium hypochlorite for 15 min and then washed with distilled water. After germination in vermiculite-peat medium, two or three uniform seedlings (about 5-cm height) of each variety were transplanted into pots (25-cm diameter and 25-cm height) containing 4 kg soil on June 15, 2013 and then thinned to one plant per pot on June 30, 2013. The soil was locally obtained and classified as Shajiang Aquic Cambisol according to Chinese Soil Taxonomy, with 1.16 g cm^{-3} bulk density, 54% total porosity, 18.4 g kg^{-1} organic C, 1.45 g kg^{-1} total nitrogen (N), and 0.63 g kg^{-1} total phosphorus (P) (as P_2O_5). Before the transplanting, the soils in the pots were mixed with 0.43 g urea, 0.39 g KH_2PO_4, and 0.13 g K_2SO_4 per kg soil as basal fertilizers. The plants were watered with tap water to maintain soil moisture at about 70%–80%. The wild and cultivated varieties were harvested at initial anthesis on September 14 and September 19, 2013, respectively.

Measurement of plant growth

Plant heights were measured from plant base to top of stem prior to harvest and branch numbers were counted. At harvest, shoots were separated into stems and leaves, washed with distilled water, and dried in an oven at 75 °C for 72 h to determine the dry weight.

Measurement of photosynthesis parameters

Photosynthesis rate, stomatal conductance, and transpiration rate of leaves were measured with an LI-6400 portable photosynthesis system (LI-COR, Lincoln, USA) at 1 800 μmol m^{-2} s^{-1} light intensity at initial anthesis of plants. Leaf temperature in the leaf chamber was set to 30 °C, with humidity in the leaf chamber set to that in the field. Leaf chamber CO_2 concentration was set to 580 μmol mol^{-1} for the elevated CO_2 level and 380 μmol mol^{-1} for the ambient CO_2 level, with the flow rate set to 500 μmol s^{-1}. All measurements were taken during the periods of 10:00–11:30 a.m.

Analysis of plant macronutrients

After being ground and passed through a 5-mm mesh, plant samples were digested in concentrated