

Effects of irradiance on growth, photosynthetic characteristics, and artemisinin content of *Artemisia annua* L.

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Abstract

With an increase in growth irradiance (from 15 to 100 % of full sunlight, I_{15} to I_{100}), the maximum net photosynthetic rate (P_{max}), compensation (CI) and saturation irradiances of *A. annua* increased. At full sunlight, *A. annua* had a high capacity of photosynthesis, while at low irradiance it maintained a relatively high P_{max} with a low CI. The height and diameter growth, total and leaf biomass, and artemisinin content of *A. annua* decreased with the decrease in irradiance, which might be connected with lower photosynthesis at lower than at higher irradiance. Irradiances changed biomass allocations of *A. annua*. The leaf/total mass ratio of *A. annua* increased with decreasing irradiance, but the root/total mass ratio and root/above-ground mass generally increased with increasing irradiance. Thus *A. annua* can grow in both weak and full sunlight. However, high yield of biomass and artemisinin require cultivation in an open habitat with adequate sunshine.

Additional key words: biomass allocation; diameter; growth; root; seasonal course.

Introduction

Irradiation is one of the most important environmental factors affecting plant survival, growth, reproduction, distribution, and yields. When irradiance becomes a limiting factor to growth, a plant allocates more biomass to leaves, increases leaf/mass ratio (LMR), reduces dark respiratory rate and compensation irradiance (CI) (Boardman 1977). When irradiance is adequate but nutrients and water are limiting, plants allocate more biomass to roots, and their potential photosynthetic ability (P_{max}) and saturation irradiance (SI) increase (Poorter 2001).

Artemisia annua L. is an annual herb in the family Asteraceae. It has been used in Chinese medicine for hundred of years (Perazzo *et al.* 2003). It produces endo-

peroxide sesquiterpene lactone antimalarial, artemisinin (qinghaosu) (Picaud *et al.* 2005), the first drug for anti-malarials recommended by World Health Organisation (Liu *et al.* 1999). Ecology (Wang and Liang 2003), biology (Wei *et al.* 1997, 1998), tissue culture (Wang 2004), and pharmacology (Liu *et al.* 1999) of the species have been studied, but not the effects of irradiance on its growth, photosynthetic characteristics, and artemisinin content. This is why we measured growth, biomass allocation, photosynthetic response to irradiance, and artemisinin content of plants grown under different irradiances to provide basic information for better cultivation of *A. annua* for high yield (Wei *et al.* 2005) and good quality.

Materials and methods

The study was conducted in the experimental farm of Guangxi Institute of Botany in Yanshan, Guilin city, Guangxi (Wei *et al.* 1997). The seeds of *A. annua* were sown on 15 February 2006. Seedlings of uniform sizes were selected and transplanted at 30-cm spacing on March 31 when the average plant height was 15–17 cm. Two weeks later, 4 irradiances were applied with black nylon net: 15, 30, 50, and 100 % of full sunlight (I_{15} , I_{30} , I_{50} , and

I_{100}). The plants were fertilized (*Osmocote 15-11-13*) and watered to ensure optimum growth. Starting on April 20 and to August 27, 20 plants were randomly selected, their heights were measured with a ruler to 1 mm, and their diameters at the soil surface were measured with a caliper to 0.1 mm every 15 d.

On August 10, when plants under I_{100} started to grow flower buds and those under other irradiances generally

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stopped vegetative growth, 20 plants from each treatment were randomly selected and harvested, and their leaf dry mass (DM), supporting structure (stem and twigs) DM, and root DM were measured to 0.1 mg after the parts were dried at 80 °C for 48 h. Then the following parameters were calculated: root mass ratio (RMR, root/total plant mass); leaf mass ratio (LMR, leaf/total plant mass); supporting structure biomass ratio (SBR), and R/C (root/above-ground biomass). At the same time, 50 g of fresh leaves were sampled from each treatment to determine artemisinin content by reverse phase high performance liquid chromatography (RP-HPLC; Liu *et al.* 2004).

On July 15, one mature leaf per plant and 4 plants per irradiation treatment were selected to measure photosynthesis of *A. annua* leaves with *Li-6400* portable photosynthesis system (*LI-COR*, Lincoln, Nebraska, USA). The selected leaves were oriented westward and horizontal. The leaves were put under 1 500 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ with the red and blue radiation source in the photosynthesis system for 10 min, and then photosynthesis with an open air source was measured at flow rate of 500 $\text{cm}^3 \text{min}^{-1}$, leaf temperature of 25 °C, CO_2 concen-

Results

Photosynthesis in response to irradiance: When irradiance was below 200 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, P_N of plants increased linearly with irradiance and P_N was not significantly different among different irradiance treatments (Fig. 1). When the irradiance was above this value, P_N increased with increase in irradiance with decreased margins until reaching P_{max} . Within this range P_N of *A. annua* showed a relatively large change under different irradiances: P_N of plants grown under higher irradiances was higher than under low irradiances (Fig. 1). As the growth irradiance increased, the P_{max} , SI, and CI increased, but the increase of SI was not always significant from one to other irradiance (Table 1). AQY was significantly lower at I_{15} than at I_{30} , but it did not change significantly among I_{30} , I_{50} , and I_{100} .

Height and diameter growth: From April 27 to August 12, the growth in height and stem diameter of *A. annua* increased with growth irradiance, but the growth showed little increase after August 12 (Fig. 2). As the growing period became longer, the differences of the height and stem diameter of *A. annua* became generally larger among the irradiance treatments: the higher the growth irradiance, the greater was the growth. For instance, at the first measurement the plant height among irradiances was similar, but on August 27, the heights at I_{50} and I_{15} were 88 and 57 % of that at I_{100} , respectively.

Biomass and artemisinin content: These values increased significantly with the increase in growth irradiance (Fig. 3). The total biomass and leaf biomass at I_{100} were about 2.56 and 2.12 times those at I_{50} , and 20.83

times those at I_{15} , respectively (Fig. 3). The artemisinin contents at I_{50} and I_{15} were about 80 and 69 % of that at I_{100} , respectively (Fig. 3).

tration of 400 $\mu\text{mol} \text{mol}^{-1}$, and irradiances of 1 800, 1 500, 1 200, 1 000, 800, 600, 400, 200, 100, 50, 20, 10, and 0 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. For I_{100} plants, photosynthesis was first measured at 2 000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$. Since the leaves was lobed, leaf area was determined with a *Li-3000A* Portable Area Meter (*LI-Cor*, Lincoln, NE, USA) and used to calculate the photosynthetic data.

The apparent quantum yield (AQY) was the slope of the linear regression of the irradiance response curve below 200 $\mu\text{mol} \text{m}^{-2} \text{s}^{-1}$ (Li *et al.* 1997). Using *SigmaPlot 9.0* (*SPSS*, USA), the P_N -PPFD (net photosynthetic rate–photon flux density) curve fitting was made according to $P_N = P_{\text{max}} (1 - C_0 e^{-\Phi \text{PPFD}/P_{\text{max}}})$ (Bassman and Zwier 1991), where P_{max} is the maximum net photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$], Φ is AQY, and C_0 is an index when P_N approaches 0 under weak irradiance. Then, CI was calculated as $\text{CI} = P_{\text{max}} \ln(C_0)/\Phi$, and SI was calculated as $\text{SI} = P_{\text{max}} \ln(100 C_0)/\Phi$ assuming SI was the PPFD when P_N reached 99 % of P_{max} . The photosynthetic data and growth variables were analyzed by one-way ANOVA with *SPSS13.0* (*SPSS*, USA).

and 27.03 times those at I_{15} , respectively (Fig. 3). The artemisinin contents at I_{50} and I_{15} were about 80 and 69 % of that at I_{100} , respectively (Fig. 3).

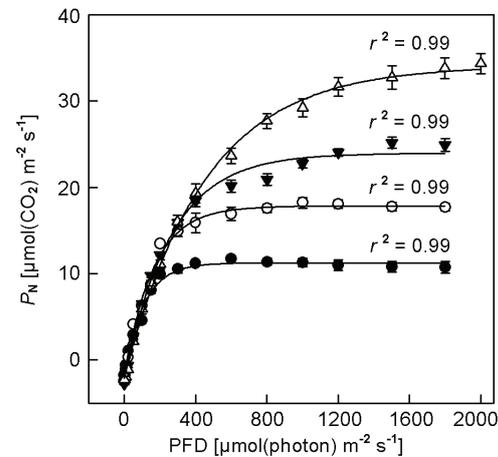


Fig. 1. Responses of net photosynthetic rate (P_N) to photon flux densities (PPFD) in *A. annua* grown under 15 (●), 30 (○), 50 (▲), and 100 (△) % of sunlight.

Biomass allocation: The RMR and R/C of *A. annua* were significantly lower at I_{15} and I_{30} than at I_{50} , but they were not significantly different between I_{100} and other irradiances (Fig. 4). LMR was significantly lower at I_{100} than at other irradiances and at I_{50} than at I_{15} , but it was not so between I_{30} and I_{50} (Fig. 4). SBR was significantly higher at I_{100} than at other growth irradiances. There was no significant difference in SBR among other growth irradiances (Fig. 4).

Table 1. Photosynthetic parameters derived from P_N -PFD curves of *A. annua* grown under different irradiances (I_{15} to I_{100}). AQY – apparent quantum yield [mol mol^{-1}]; CI – compensation irradiance [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]; P_{\max} – irradiance saturated photosynthetic rate [$\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$]; SI – saturation irradiance [$\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. Within each column, values followed by the same letter are not significantly different at $p < 0.05$ according to LSD multiple test.

Irradiance	P_{\max}	AQY	CI	SI
I_{15}	11.3±1.0 d	0.056±0.005 b	5.6±2.7 d	532±44 c
I_{30}	17.8±1.2 c	0.074±0.001 a	19.2±1.1 c	753±39 bc
I_{50}	24.0±0.8 b	0.075±0.002 a	22.2±3.5 b	1241±135 ab
I_{100}	34.2±2.3 a	0.069±0.009 a	25.8±3.7 a	2113±177 a

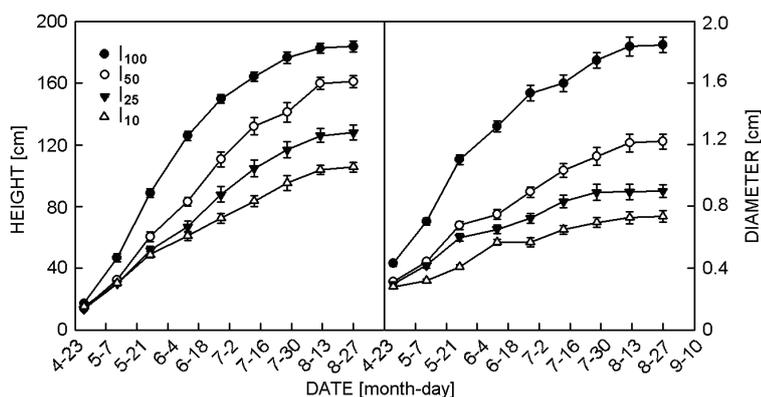


Fig. 2. Seasonal course of mean height and diameter (\pm SD, $n = 20$) of *A. annua* grown under different irradiances.

Discussion

Generally, for typical shade intolerant herbaceous plants, the SI, CI, and P_{\max} are 1 000–1 500 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, 10–40 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, and 15–30 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, respectively (Zhai *et al.* 1997). For well-grown plants, AQY is 0.04–0.07 mol mol^{-1} (Long *et al.* 1994). We observed that under I_{100} , the SI, CI, P_{\max} , and AQY of *A. annua* were 2 113 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, 25.8 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, 34.2 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$, and 0.069 mol mol^{-1} . Thus, under full irradiance, *A. annua* could use photon energy more efficiently than other typical shade intolerant herbaceous plants. Our results show that CI of *A. annua* decreased as growth irradiance decreased, which indicated that *A. annua* plants did adapt to weak irradiance by decreasing CI to use weak irradiance efficiently. Even under I_{15} , *A. annua* had a P_{\max} of 11.3 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ in this study. Hence *A. annua* can grow in a wide range of irradiances. It has a high photosynthesis potential under full sunlight and at the same time, it also maintains reasonable photosynthesis under weak irradiance due to its low CI.

As irradiance decreases, growth and biomass accumulation of sun plants decrease (Hoeft *et al.* 1996, Stuefer and Huber 1998). Our results support this conclusion as the biomass and leaf mass, height and diameter growth, and artemisinin content of *A. annua* decreased significantly as growth irradiance decreased.

On August 27, the biomass, leaf mass, and artemisinin

content at I_{50} were 39, 47, and 80 % of those at I_{100} , and at I_{15} , they were 4.8, 3.7, and 69 %, respectively. Such decreases in growth with decreasing irradiance may be at least partially attributable to the higher P_N of the *A. annua* growing at higher than at lower irradiances as mentioned above. These might be part of the reasons that in nature *A. annua* is often found in open habitats, since at low irradiance *A. annua* does not grow so good as at high irradiance, and therefore, it might be out-competed by other shade tolerant species in shade conditions.

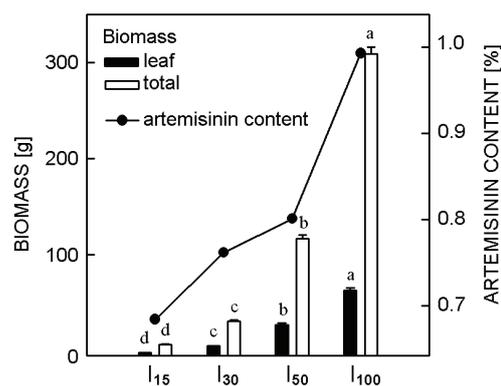


Fig. 3. Mean (\pm SD, $n = 20$) biomass and artemisinin content [%] of *A. annua* grown under different irradiances. Different letters indicate significant differences among irradiances.

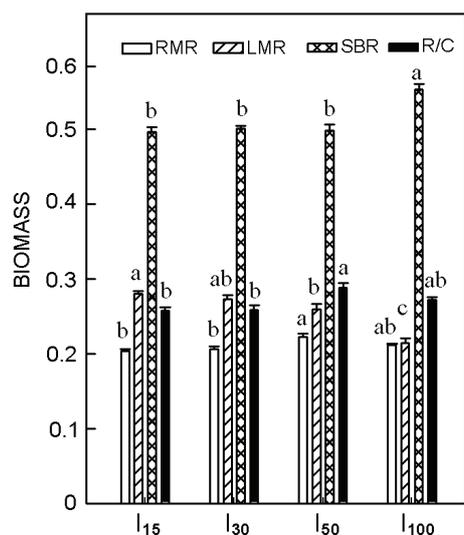


Fig. 4. Biomass allocation of *Artemisia annua* grown in different irradiances. RMR – root/mass ratio; SBR – biomass ratio of supporting organs; LMR – leaf/mass ratio; R/C – root/crown ratio.

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