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Effects of CO_2 on growth and photosynthesis of *Pyrrosia piloselloides* (L.) Price gametophytes

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Abstract

The effects of CO_2 concentration on spore germination, growth, and net photosynthetic rate (P_N) of gametophytes of a tropical epiphytic fern, *Pyrrosia piloselloides*, were investigated over a 100-d period. Increasing CO_2 concentration stimulated spore germination and enhanced gametophytic growth. The appearance of sexual organs and formation of sporophytes were accelerated with higher CO_2 during growth. Radiant energy saturated P_N and dark respiration rate also increased with increasing CO_2 concentrations during growth.

Additional key words: chlorophyll; dry mass; fern; respiration; spore germination; sporophyte.

Introduction

The global atmospheric CO_2 concentration is predicted to double by the end of the next century. Under favourable irradiance, water and nutrient conditions, plants grown under elevated CO_2 for short periods generally exhibit an increase in net carbon accumulation (Cure and Acock 1986, Bazzaz 1990, Rogers and Dahlman 1993, Veste and Herppich 1995). Over longer durations, this enhancement in P_N often decreases (DeLucia *et al.* 1985, Cure and Acock 1986, Sage *et al.* 1989, Tissue *et al.* 1993, Marek *et al.* 1995). Acclimation of the photosynthethic system to high CO_2 frequently results in the re-allocation of protein nitrogen from ribulose-1,5-bisphosphate carboxylase/oxygenase to the enzymes of light-harvesting, ribulose-1,5-bisphosphate regeneration, and saccharide synthesis (Sage *et al.* 1989). Moreover, in CO_2-enriched plants nitrogen content often decreases; carbon partitioning also changes to result in increased root mass in some plants (Norby *et al.* 1987). Elevation of CO_2 concentration also affects germination of seeds and initial size and growth of certain plants, suggesting that the final size and survival of the species might be affected by future increases in CO_2 (Woodward *et al.* 1991, Ziska and Teramura 1992, Morse and Bazzaz 1994).

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While numerous studies report on the responses of crop plants, tree seedlings, grasses, and algae to elevated CO_2, very little attention has been focused on ferns. This study investigated the effects of elevated CO_2 on the growth and photosynthesis of gametophytes of *P. piloselloides* (L.) Price, a common epiphytic fern in Singapore.

Materials and methods

Spores of *P. piloselloides* were collected from a naturally-growing population, surface-sterilised with 5 % *Clorox*™ for 4 min, and then washed with sterile distilled water. Two thousand spores were introduced into a 6-cm diameter Petri-dish containing 5 cm^3 sterile culture solution (Hoagland solution at 0.1 strength) (Tuite 1969). Petri-dishes of spores were then put into *GA 7* vessels with different CO_2 concentrations. To encourage synchrony in spore germination, spores were subjected to 24 h darkness. The initial protruberance of a rhizoid was taken as the first visible sign of spore germination.

Changes in spore germination were followed by determining the number of spores germinated per 1000 spores counted. Gametophytes obtained from the above spore cultures were used for further studies. Changes in gametophytic length and breadth, and time of appearance of sex organs were noted.

P_N of gametophytes was determined with an oxygen electrode (*Rank Brothers*, Cambridge, U.K.). Five gametophytes were suspended in 4 cm^3 Tris-HCl buffer (pH 7); the rates of photosynthetic oxygen exchange were determined at 0-600 μmol m^{-2} s^{-1} PPFD (photosynthetic photon flux density). Irradiation was from a 100 W *Tungram* incandescent bulb, and temperature was kept constant at 25 °C. Carbon dioxide was supplied in the form of sodium bicarbonate (7.1 mM) dissolved in the buffer (Umbreit *et al.* 1964). P_N was measured during the first two hours of the light period. From the irradiance-response curves, compensation and saturation irradiances, and radiant energy saturated P_N and dark respiration rate (R_D) were determined (Walker 1989).

Following the determination of P_N, gametophytes were recovered from the electrode chamber and their chlorophyll (Chl) was extracted with pure acetone and determined spectrophotometrically at 645 and 663 nm (Harborne 1973). Simultaneously, another five gametophytes were oven-dried at 70 °C and their dry mass was determined after constant mass was achieved. These determinations were done only with gametophytes older than 40 d.

To study the effects of CO_2 on spore germination, growth, and P_N of gametophytes, chambers were set up using *Magenta GA 7*-polycarbonate containers (340 cm^3; *Sigma Chemical*). Two vials in each *GA 7* vessel, each containing 10 cm^3 of 0.1 M sodium carbonate/0.1 M sodium bicarbonate buffer, were used to produce atmospheres containing 219, 350, 515, and 3360 $cm^3(CO_2)$ m^{-3} according to Umbreit *et al.* (1964). An additional condition was set up with 4 M potassium hydroxide in the vials, creating an atmosphere of 0 $cm^3(CO_2)$ m^{-3}. Sterilised spores or gametophytes were placed in an uncovered bottom half of a 6-cm Petri-dish (containing 5 cm^3 culture medium), put into the *GA 7* containers, and supported in the air space by

placing them over the vials. With the aid of an ADC infrared CO_2 analyzer (*The Analytical Development Co.*, Hoddesdon, U.K.), it was established that the desired CO_2 concentration in the *GA 7* vessels remained constant for three days. Thus, the buffer solutions in the vials were changed every three days. To prevent overcrowding, gametophytes were regularly transferred to new Petri-dishes whenever they covered about 75 % of the total area of each Petri-dish.

All cultures were kept at 25 °C and 30 μmol m^{-2} s^{-1} PPFD (provided by fluorescent tubes) with a 12/12 h light/dark regime. All experiments were replicated three times. Multifactor analysis of variance was used to test the effects of gametophytic and CO_2 concentration on the different parameters tested. A least significant difference test ($p < 0.05$) was used to compare the treaments.

Results and discussion

Spores of *P. piloselloides* kept under 219-3360 $cm^3(CO_2)$ m^{-3} germinated four days after sowing; per cent germination increased with time after sowing and ambient CO_2 concentration (Table 1). Spores kept under zero CO_2 germinated only on the sixth day after sowing (Table 1). Seventeen days after sowing, spore germination was maximum under all CO_2 concentrations and did not increase further even after 30 d after sowing (Table 1). In *P. piloselloides*, CO_2 seemed to have a slight stimulatory effect on spore germination. The effect of CO_2 on spore germination seems to vary among fern species. For instance, spore germination of the fern, *Onoclea sensibilis*, was reported to be unaffected in an atmosphere containing 0-20 000 $cm^3(CO_2)$ m^{-3} (Edwards 1977).

Table 1. CO_2 concentration [cm^3 m^{-3}] and spore germination [%] in *Pyrrosia piloselloides*. All values are means ± S.E. Means followed by the same letter do not differ significantly at the 0.05 level of probability.

Time after sowing [d]	CO_2 concentration 0	219	350	515	3360
2	0.00a	0.00a	0.00a	0.00a	0.00a
4	0.00a	3.27±0.27a	3.30±0.18a	3.63±0.20a	3.93±0.38a
6	13.60±0.40b	22.30±1.00cd	23.10±1.00d	21.17±1.45cd	22.40±1.07cd
8	17.00±4.40bc	33.65±1.85e	34.45±3.15e	36.97±1.27ef	40.60±2.05f
10	46.33±3.88g	56.13±4.88ij	57.73±2.71ij	55.43±2.81i	60.00±3.13jk
12	51.10±3.77h	67.00±1.50lm	68.00±1.00mn	70.00±1.50mn	71.80±4.15op
14	63.00±1.67kl	75.67±3.71p	75.25±3.75p	75.50±4.31p	75.60±4.67p
17	63.60±2.20kl	93.03±3.59q	95.45±3.05q	93.46±2.99q	93.05±4.53q

Following spore germination, the gametophytes of *P. piloselloides* became cordate-shape 33, 31, 30 and 27 d after sowing when grown in 219, 350, 515, and

3360 $cm^3(CO_2)$ m^{-3}, respectively. Gametophytes grown under zero CO_2 did not show much growth and did not survive longer than 20 d after sowing. The size (in terms of length and breadth) of the gametophytes increased with time and CO_2 concentration inside the *GA 7* containers. The maximum length of cordate-shape gametophytes grown under 219, 350, and 515 $cm^3(CO_2)$ m^{-3} were 2.55±0.11, 2.87±0.13, and 3.13± 0.18 mm, respectively; gametophytes grown in 3360 $cm^3(CO_2)$ m^{-3} were smaller (1.61±0.09 mm). Maximum breadth of the cordate-shape gametophytes grown under 219, 350, 515, and 3360 $cm^3(CO_2)$ m^{-3} were, respectively, 3.87±0.20, 4.23±0.13, 4.93±0.27, and 3.67±0.13 mm. The gametophytes reached their maximum size 100 and 60 d after sowing when grown under 219-515 and 3360 $cm^3(CO_2)$ m^{-3}, respectively. The amount of dry matter accumulated (μg dry mass) per gametophyte also increased with the age of gametophytes and CO_2 concentration (Table 2). Compared to those grown under 350 $cm^3(CO_2)$ m^{-3}, dry mass increased by 431 and 142 % 80 d after sowing under 3360 $cm^3(CO_2)$ m^{-3} and 100 d under 515 $cm^3(CO_2)$ m^{-3}, respectively, and decreased by 45 % in those grown under 219 $cm^3(CO_2)$ m^{-3}.

The increases in growth and dry mass accumulation of gametophytes at elevated CO_2 were associated with faster development and earlier transition into the sporophytic phase. Antheridia and archegonia first appeared in 50 % of the gametophytes grown under 219, 350, and 515 $cm^3(CO_2)$ m^{-3} 40 d after sowing; and in gametophytes grown under 3360 $cm^3(CO_2)$ m^{-3} 32 d after sowing. Sixty days after sowing, sporophytes developed in gametophytes grown under 219 and 350 $cm^3(CO_2)$ m^{-3}; gametophytes grown under 515 and 3360 $cm^3(CO_2)$ m^{-3} showed sporophytic formation 50 d after sowing. No observations were taken after 100 d for gametophytes grown under 219, 350,and 515 $cm^3(CO_2)$ as 95 % of them developed into sporophytes. Ninety-five percent of gametophytes grown under 3360 $cm^3(CO_2)$ m^{-3} developed into sporophytes within a shorter period of 60 d.

Reproductive characters of the gametophytes were associated with earlier senescence of the gametophytes grown under 515 and 3360 $cm^3(CO_2)$ m^{-3}. The onset of earlier senescence in these gametophytes was also physiologically indicated by a decrease in total Chl concentration [g kg^{-1}(dry mass)] (Table 2). Gametophytes grown under lower CO_2 showed delayed maturation and no decrease in the Chl concentration was observed (Table 2), indicating that senescence in these gametophytes was not induced during this study. Induction of earlier flowering and senescence were accelerated in *Layia platyglossa* and *Clarkia rubicunda* as their life spans were significantly reduced when they were grown under elevated CO_2 (St Omer and Horvath 1983). However, the effect of elevated CO_2 on Chl concentration varied according to plant species (Sage *et al.* 1989, Holbrook *et al.* 1993, Sicher *et al.* 1994).

Radiant energy saturated P_N of gametophytes of *P. piloselloides* generally decreased with increasing age (Table 2). Such age-dependent changes in photosynthesis are well-documented in leaves of higher plants: P_N usually increases and then decreases as leaves mature (Suzuki *et al.* 1987, Mebrahtu and Hanover 1991, Šiffel *et al.* 1993). However, rates of light-saturated photosynthesis of gametophytes increased with CO_2 concentrations (Table 2). The rates of photosynthesis were higher in gametophytes grown under 515 and 3360 $cm^3(CO_2)$

m^{-3} throughout the experiment; however, this enhancement of photosynthesis decreased with increasing age of gametophytes. Such decreases in photosynthesis as a result of increasing plant age and time of exposure to CO_2 were also observed in water hyacinth (Spencer and Bowes 1986), two species of tomato (Yelle *et al.* 1989), and geranium (Kelly *et al.* 1991).

Table 2. Characteristics of gametophytes of *Pyrrosia piloselloides* grown under different CO_2 conditions. *Number of days after sowing of spores germination. All data are means ± S.E. Means in the same column followed by the same letter do not differ significantly at the 0.05 level of probability.

Ambient CO_2 concentrations during growth [cm^3 m^{-3}]	Dry mass per gametophyte [μg]	Chlorophyll concentration [g kg^{-1}(d.m.)]	Rate of dark respiration [mmol(O_2) kg^{-1}(Chl) s^{-1}]	Irradiance-saturated photosynthesis [mmol(O_2) kg^{-1}(Chl) s^{-1}]
40 d*				
219	0.39±0.19a	7.64±0.55ab	2.17±0.13ab	10.77±1.30bc
350	0.58±0.19a	8.55±0.56bcd	2.69±0.89bc	12.28±1.75cd
515	0.76±0.19a	9.73±0.73def	2.05±0.01ab	15.03±4.89d
3360	1.92±0.39b	8.64±1.46bcde	3.34±0.66c	26.20±3.63e
60 d*				
219	2.31±0.19b	9.73±0.73def	2.10±0.61ab	6.77±0.98a
350	4.23±0.19c	8.90±0.56bcde	2.08±0.29ab	7.91±0.99ab
515	5.00±0.58cd	9.09±0.82cde	2.40±0.38abc	11.34±4.49bcd
3360	9.62±0.00f	10.00±1.18ef	2.97±0.31c	12.45±0.70cd
80 d*				
219	5.58±0.58d	11.09±0.46fg	2.59±0.65abc	8.65±1.10abc
350	6.92±0.39e	9.09±0.55cde	2.11±0.38ab	11.98±1.46cd
515	9.23±0.39f	13.82±0.55h	2.17±0.19ab	10.04±0.93abc
3360	29.61±1.54h	6.55±0.00a	6.59±0.61d	24.32±0.64e
100 d*				
219	5.39±0.58d	11.55±0.73g	1.81±0.44ab	6.81±0.23a
350	9.42±0.58f	9.27±0.70cde	1.55±0.25a	10.45±1.18abc
515	13.46±0.77g	8.18±1.46bc	3.45±0.45c	11.52±1.54bcd

The observed changes in dry matter accumulation, P_N, and Chl concentration in gametophytes might reflect a reduced need for more Chl under elevated CO_2 (Cui *et al.* 1993) as the efficiency of radiant energy utilization increased (Idso *et al.* 1993). With an increase in CO_2 concentration resulting in lower photorespiration coupled with higher photosynthesis rates, it is expected that the compensation irradiance of plants will decrease. Compensation and saturation irradiances of *P. piloselloides* gametophytes grown under various CO_2 concentrations generally did not differ significantly and ranged from 7-20 and 65-100 μmol m^{-2} s^{-1}; such observations were also recorded in water hyacinth (Spencer and Bowes 1986).

Information on the effects of elevated CO_2 on respiration is conflicting. Ramets of water hyacinth (Spencer and Bowes 1986) and seedlings of deciduous trees (Bunce 1992) showed decreased R_D under elevated CO_2. In contrast, canopy R_D of rice increased with increasing daytime CO_2 concentration (Baker *et al.* 1992). R_D increased in gametophytes of *P. piloselloides* grown under 515 and 3360 $cm^3(CO_2)$ m^{-3}; with increasing age, lower R_D was observed (Table 2). The increase in R_D under elevated CO_2 might be a reflection of an increased demand for the supply of carbon skeletons and resources for biosynthesis in growing gametophytes (Thomas and Griffin 1994), and energy for structural growth and maintenance of the phytomass (Amthor 1991).

Thus, increases in growth and photosynthesis of gametophytes of *P. piloselloides* suggest that they could establish themselves faster under elevated CO_2 conditions. The accelerated appearance of sexual organs and the faster transition of gametophytes into sporophytes, and thus the acceleration of the entire fern life cycle, would ensure the survival and growth of the fern in the new environment. The gametophytes are normally not exposed to saturating irradiances. Enhancement of P_N under elevated CO_2 could, therefore, offset the negative effects of lower irradiances during growth (Idso *et al.* 1993). Hence the sum responses of *P. piloselloides* gametophytes to elevated CO_2 concentration suggest greater success against competitors in the future environment, enabling this fern to continue to establish itself in a future world with high atmospheric CO_2.

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