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Symbiotic Vesicular-Arbuscular Mycorrhizae Influence Maximum Rates of Photosynthesis in Tropical Tree Seedlings Grown Under Elevated CO_2

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Abstract. To investigate the importance of phosphorus and carbohydrate concentrations in influencing photosynthetic capacity of tropical forest tree seedlings under elevated CO_2, we grew seedlings of *Beilschmiedia pendula* (Sw.) Hemsl. (Lauraceae) under elevated CO_2 concentrations either with or without vesicular-arbuscular (VA) mycorrhizae. VA-mycorrhizae increased phosphorus concentrations in all plant organs (leaves, stems and roots). Maximum rates of photosynthesis (A_{max}) measured under saturating levels of CO_2 and light were correlated with leaf phosphorus concentrations. VA-mycorrhizae also increased leaf carbohydrate concentrations, particularly under elevated CO_2, but levels were low and within the range observed in naturally occurring forest species. Root carbohydrate concentrations were reduced in VA-mycorrhizal plants relative to non-mycorrhizal plants. These results indicate an important role for VA-mycorrhizae in controlling photosynthetic rates and sink strength in tropical trees, and thus in determining their response to future increases in atmospheric CO_2 concentrations.

Introduction

The extent to which tropical forests will store carbon as atmospheric CO_2 rises is currently a matter of debate. Both low soil nutrient availability and feedback inhibition of photosynthesis due to high leaf carbohydrate concentrations have been proposed as factors that will limit the ability of tropical forest trees to utilise elevated levels of atmospheric CO_2 for growth (Bazzaz 1990; Ceulemans and Mousseau 1994; Körner *et al.* 1995). Phosphate concentrations are particularly low in many tropical soils (Vitousek and Sanford 1986; Yavitt *et al.* 1993) and may impose limitations on growth and photosynthesis under present and future elevated atmospheric CO_2 concentrations.

Vesicular-arbuscular (VA) mycorrhizae are present in the roots of most tropical forest trees (Redhead 1980). Little is known of their functional significance in tropical trees (Janos 1980; Arnone and Körner 1995) but, based on work with temperate species (Norby *et al.* 1986; Conroy *et al.* 1990; Morgan *et al.* 1994; O'Neill 1994; Ineichen *et al.* 1995), it is likely that VA-mycorrhizae will have major effects on tree growth responses to elevated CO_2 because of the influence they may have on phosphorus nutrition and carbon accumulation in tropical trees (reviewed by Smith 1980; Newsham *et al.* 1995). In addition to contributing to the phosphate nutrition of plants, VA-mycorrhizae may also alter the carbohydrate allocation patterns of plants by providing an additional respiratory sink for photosynthetic products (Baas *et al.* 1989 for VA-mycorrhizae in *Plantago major*; Rygiewicz and Andersen 1994 for ectomycorrhizae in conifers). An additional sink for carbohydrates under conditions of elevated CO_2 could prevent the build-up of carbohydrates in leaves in concentrations sufficient to lead to reductions in rates of photosynthesis (van Oosten and Besford 1995). A test of this in tropical trees is particularly important because they have been proposed to be limited in their capacity to utilise elevated levels of CO_2 owing to high leaf carbohydrate concentrations (Körner and Arnone 1992).

In this study we manipulated the VA-mycorrhizae of seedlings of the tropical forest tree species *Beilschmiedia pendula* (Sw.) Hemsl. (Lauraceae) growing under elevated and ambient levels of atmospheric CO_2 to test the following hypotheses: (1) VA-mycorrhizae would increase P concentrations within plants, (2) A_{max} would be sensitive to leaf P concentrations, (3) growth under elevated CO_2 would lead to increased levels of carbohydrates, and (4) VA-mycorrhizae would increase the sink strength for carbohydrates in roots. We assessed maximum rates of photosynthesis of leaves and investigated how these were related to the mineral element concentration and carbohydrate status of both leaves and whole seedlings. The results indicate an important role for VA-mycorrhizae in phosphorus nutrition and in the control of maximum rates of

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photosynthesis. Furthermore, VA-mycorrhizae altered carbohydrate allocation patterns within plants, particularly in those growing under elevated CO_2.

Materials and Methods

Plants and Growth Conditions

Seedlings of *Beilschmiedia pendula* (Sw.) Hemsl. (Lauraceae), a common species of humid forests of Panamá, were grown without VA-mycorrhizae (controls) or with VA-mycorrhizae for 20 weeks in large pots (15 L) of sterilised forest soil. Inoculum of VA-mycorrhizae was obtained from a mix of forest soil and feeder roots of the palm *Oenocarpus panamanus* (Janos 1980). All soil and half the inoculum were sterilised using methyl bromide gas. To ensure similar carbon addition to each pot, half the plants were inoculated with sterilised and the other half with unsterilised inoculum. In order to reintroduce soil bacteria to the controls a solution of the unsterilised inoculum filtered through a fine mesh (40 μm) to remove VA-mycorrhizal fungal spores was added. Plants were watered daily. Three times a week 50 mL of dilute nutrient solution (1/5 strength Hoaglands solution), that either included phosphate or was phosphate-free, was added to each pot after watering.

Twenty plants (five plants per treatment combination of ± VA-mycorrhizae and ± phosphate fertiliser) were randomly assigned to four, 1.5 m diameter open-top chambers in a forest clearing on Barro Colorado Island, Panamá (9°10′N, 79°51′W). The open-top chambers were arranged in pairs across the clearing and were ventilated with either ambient air (two chambers where CO_2 concentrations varied between 350 and 400 μL L^{-1}), or air in which the CO_2 concentration had been doubled (two chambers where CO_2 concentrations were 790±70 μL L^{-1}). CO_2 was injected at a constant flow rate (1.2 L min^{-1}) into the tubing connecting the ventilators and the open-top chambers. Photon flux densities within the chambers were approximately 30% of natural sunlight, varying between 4 and 10 mol m^{-2} d^{-1} depending on cloud cover. Air temperatures ranged between 25 and 33°C, and leaf temperatures between 24 and 36°C. Air temperatures within the chambers were approximately 1°C above ambient. Relative humidity was ambient, varying between 60 and 100%.

Harvest

At the end of the experiment, plants were harvested in the early morning and divided into roots, stems, and leaves. Subsamples of the roots (approximately 3 cm long) were checked for the extent of VA-mycorrhizae infection. Infection was scored using the following classes: 0 (0%), 1 (0–5%), 2 (6–25%), 3 (26–50%), 4 (51–75%), 5 (76–100%) as described elsewhere (Lovelock *et al.* 1996). No VA-mycorrhizal infection was detected in the non-mycorrhizal treatment. The average infection class for VA-mycorrhizal plants grown under ambient levels of CO_2 was 2.28, while for plants grown under elevated CO_2 it was 3.19 (Kruskal-Wallis statistic = 5.037, differences significant at $P = 0.0248$) (Lovelock *et al.* 1996). Leaf discs of 10 cm^2 area were sampled in the early morning from the most recently matured leaf for determination of maximum rates of photosynthetic O_2 evolution (A_{max}). Directly after harvesting leaf discs, all remaining plant material was placed in a microwave oven for 1 min on full power to denature enzymes. Plant material was then dried to a constant weight in an oven at 60°C. Subsamples of the dried plant material were used for mineral element and carbohydrate analysis. For measurements of A_{max}, carbohydrates, and mineral elements, plants from one pair of chambers were used.

Photosynthesis and Mineral Element Analysis

Maximum rates of photosynthetic O_2 evolution (A_{max}) were measured at 30°C in saturating CO_2 concentrations (5% CO_2 in air) and saturating light levels (1000 μmol m^{-2} s^{-1}) using a Hansatech LD2 Leaf Disc Electrode System (Hansatech Ltd, Kings Lynn, UK). For mineral element analysis, plant material was ground and analysed at the University of Würzburg (Germany) using a CHN Elemental Analyzer (Heraeus, Hanau, Germany) and an ICP spectrometer (JY 70 Plus, ISA, München, Germany).

Carbohydrates

Analysis of carbohydrates was done using samples finely powdered in a sample mill (MM2, Retsch, Idar-Oberstein, Germany) and extracted with hot water. Aliquots of this extract were analysed by high pressure liquid chromatography (HPLC) on an anion-exchange column (Carbopac PA100, 250 × 4 mm, Dionex, Sunnyvale, USA). Low molecular weight carbohydrates and polyols were eluted by 50 mM NaOH at 32°C and detected by PAD (pulsed amperometric detection; ED40, Dionex). For determination of starch content, 20 mg of the finely ground powder was extracted with 1 mL distilled water at room temperature, centrifuged and the pellet reextracted with 1mL of 80% ethanol and 1 mL 90% ethanol at 60°C for 5 min. The pellets were dried and incubated with 8 μkat heat stable α-amylase (from *Bacillus licheniformis*, Sigma, St Louis, USA) in 1 mL distilled water at 85°C for 30 min. The samples were centrifuged and 100 μL aliquots of the supernatant incubated with 160 nkat amyloglucosidase (from *Aspergillus niger*, Boehringer-Mannheim, Mannheim, Germany) in 0.5 mL of 20 mM sodium acetate (pH 4.6) at 55°C. The reaction was terminated after 30 min by addition of 0.5 mL chloroform. Glucose was quantified in aliquots of the supernatants by HPLC-PAD (Carbopac PA100, 250 × 4 mm, 150 mM NaOH at 32°C). Shikimate was determined in hot water extracts (3% w/v) by ion chromatography with suppressed conductivity detection (CD-20 and ASRS-1, Dionex). Separation of anions was achieved by a linear gradient of NaOH (0.5 mM to 37.5 mM within 14 min) on an anion-exchange column (Ionpac AS11, 250 × 4 mm, Dionex).

Data Analysis

Data were analysed by analysis of variance (ANOVA). VA-mycorrhizae, phosphate fertilisation and CO_2 concentrations were considered as fixed effects. There were five replicate plants for each treatment combination. An analysis of covariance of A_{max} was performed with leaf P concentrations as the covariate in order to test for effects of the treatments on A_{max} in addition to increasing leaf P concentrations.

Results

Mineral Nutrition

Fertilisation with P had no effect on mineral element concentrations within plants ($P > 0.50$). Therefore, mean mineral element concentrations of plants were calculated by

pooling plants grown with and without added P within CO_2 and VA-mycorrhizal treatments. VA-mycorrhizae increased P concentrations within all plant parts in both elevated and ambient CO_2 concentrations, although more so in stems ($F_{1,30}$ 7.65, $P = 0.009$) and roots ($F_{1,30}$ 8.11, $P = 0.008$) than in leaves ($F_{1,30}$ 5.38, $P = 0.027$) (Fig. 1). Growth under elevated CO_2 had no significant effect on P concentrations in leaves when expressed on either a dry weight (Fig. 1) or area basis (Table 1), or in roots and stems (Fig. 1) ($P > 0.05$). P concentrations were greatest in stems of VA-mycorrhizal plants grown under elevated CO_2 concentrations (Fig. 1). VA-mycorrhizae also increased the concentration of magnesium (Mg) and calcium (Ca) within roots while decreasing concentrations of B, Zn, Mn, Fe, Al and Na in leaves (Table 2).

VA-mycorrhizae had no significant effect on the nitrogen concentration in leaves ($P > 0.05$) (Table 1). Increases in leaf P concentrations were correlated with increases in N (Fig. 2; $r^2 = 0.37$, $P < 0.001$), with a doubling of N being associated with a 6-fold increase in P. Growth in elevated CO_2 increased the carbon to nitrogen ratio of the leaves ($F_{1,30}$ 10.15, $P = 0.003$) (Table 1).

Carbohydrates

Carbohydrate concentrations over the whole plant varied depending on the CO_2 concentration at which plants were grown and whether or not plants had VA-mycorrhizae. Fertilisation with P had no effect on carbohydrate concentrations within plants ($P > 0.05$); therefore, mean carbohydrate concentrations of plants were calculated by pooling plants grown with and without phosphate within CO_2 and VA-mycorrhizal treatments (Fig. 3). Leaf and stem sucrose concentrations were higher in VA-mycorrhizal plants compared to non-mycorrhizal plants ($F_{1,29}$ 6.78, $P = 0.014$; $F_{1,29}$ 12.13, $P = 0.001$ for leaves and stems respectively), while sucrose concentrations in the roots were similar in VA-mycorrhizal plants compared to non-mycorrhizal plants (Fig. 3). Elevated CO_2 had no influence on early morning sucrose concentrations. Thus the gradient in sucrose concentrations between the leaves and the roots was steeper in VA-mycorrhizal plants than non-mycorrhizal plants, and was similar at both ambient and elevated CO_2 concentrations. The concentrations of glucose, fructose, *myo*-inisitol and bornesitol were similar for all plants (Table 3). Shikimate is a product of carbohydrate metabolism that

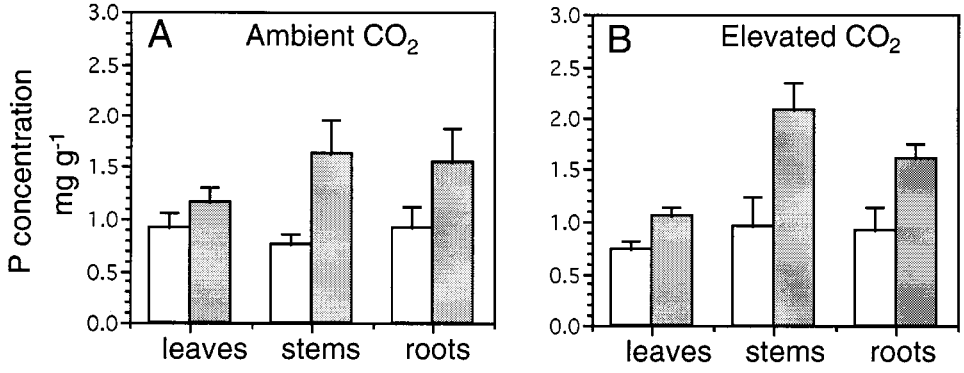

Fig. 1. Phosphorus concentration of organs (leaves, stems and roots) of seedlings of *B. pendula* growing under ambient (*A*) and elevated CO_2 concentrations (*B*). Plants were grown either with VA-mycorrhizae (shaded bars) or without VA-mycorrhizae (open bars). Values are the means of 10 plants with standard error bars.

Table 1. Leaf N and P concentrations
Mean concentrations (± standard error) of leaf P and N, and the ratio of C:N in leaves of *Beilschmiedia pendula* which were either VA-mycorrhizal (+VAM) or not (–VAM) and grown under elevated or ambient concentrations of CO_2. Means are values for 10 plants

Mineral element	Ambient CO_2		Elevated CO_2	
	–VAM	+VAM	–VAM	+VAM
P (g m^{-2})	0.0517 ± 0.0083	0.0591 ± 0.0083	0.0495 ± 0.0088	0.0683 ± 0.0045
N (g m^{-2})	1.07 ± 0.07	1.05 ± 0.09	1.05 ± 0.10	1.11 ± 0.05
N (mg g^{-1})	19.2 ± 1.0	21.1 ± 1.7	15.8 ± 0.8	17.3 ± 0.8
C:N	26.7 ± 1.3	24.5 ± 1.5	32.3 ± 2.0	28.9 ± 1.3

Table 2. Mineral nutrient concentrations in leaf, stem and root tissue of *Beilschmiedia pendula*
Plants were grown at elevated or ambient CO_2 concentrations, with or without VA-mycorrhizae (VAM) and with or without the addition of phosphate fertiliser (P). Concentrations are in μmol g^{-1}

	Ambient CO_2				Elevated CO_2			
	–VAM		+VAM		–VAM		+VAM	
	–P	+P	–P	+P	–P	+P	–P	+P
				Leaf				
S	58.9 ± 4.8	56.6 ± 4.1	52.9 ± 2.4	76.8 ± 9.7	52.6 ± 8.0	49.1 ± 2.8	56.5 ± 5.1	62.6 ± 5.8
Al	6.1 ± 0.6	40.3 ± 3.0	3.0 ± 1.8	10.6 ± 1.9	16.6 ± 6.2	12.4 ± 4.4	10.5 ± 4.0	6.7 ± 1.1
B	6.3 ± 0.6	6.1 ± 0.5	4.4 ± 0.4	5.0 ± 0.5	5.2 ± 0.8	7.2 ± 0.6	5.2 ± 0.6	4.4 ± 0.4
Fe	8.7 ± 3.3	24.4 ± 2.8	8.3 ± 1.7	10.2 ± 1.4	12.0 ± 3.9	11.9 ± 2.0	7.7 ± 2.2	5.9 ± 0.9
Mg	163 ± 13	190 ± 28	153 ± 7	204 ± 30	145 ± 43	155 ± 23	173 ± 18	172 ± 12
Mn	2.8 ± 0.5	4.0 ± 0.6	2.0 ± 0.3	3.0 ± 0.3	2.3 ± 0.6	3.5 ± 0.3	2.8 ± 0.4	2.2 ± 0.3
Zn	1.5 ± 0.3	2.0 ± 0.4	1.2 ± 0.2	1.4 ± 0.1	1.2 ± 0.3	1.4 ± 0.3	0.8 ± 0.1	0.6 ± 0.1
Ca	109 ± 13	129 ± 15	99 ± 8	139 ± 17	108 ± 31	108 ± 20	112 ± 12	111 ± 10
Cl	36.0 ± 14.6	20.7 ± 2.6	18.9 ± 3.9	15.7 ± 2.8	16.2 ± 3.0	20.0 ± 3.2	12.8 ± 1.9	9.6 ± 0.8
K	402 ± 78	389 ± 39	369 ± 26	436 ± 9	276 ± 9	255 ± 27	298 ± 30	291 ± 23
Na	34.7 ± 5.7	36.8 ± 3.8	27.0 ± 1.2	35.1 ± 3.9	35.6 ± 2.6	37.1 ± 3.8	24.7 ± 1.9	23.6 ± 4.0
				Stem				
S	55.3 ± 4.1	50.3 ± 4.1	54.0 ± 2.4	72.2 ± 12.2	51.6 ± 13.1	60.0 ± 5.3	60.9 ± 4.7	87.2 ± 6.6
Al	13.5 ± 3.4	23.9 ± 7.7	19.5 ± 8.6	14.8 ± 13.8	13.8 ± 3.4	15.1 ± 4.5	9.9 ± 1.8	7.8 ± 1.7
B	2.0 ± 0.3	2.8 ± 0.7	2.1 ±0.5	2.8 ±0.1	3.1 ± 1.1	3.5 ± 1.0	4.1 ± 0.6	4.1 ± 0.9
Fe	10.4 ± 2.2	16.8 ± 4.5	13.2 ± 4.9	12.1 ± 0.8	10.2 ± 2.9	11.4 ± 3.0	7.6 ± 1.4	5.8 ± 1.3
Mg	209 ± 16	206 ± 16	277 ± 34	259 ± 30	201 ± 72	209 ± 33	240 ± 24	295 ± 19
Mn	8.3 ± 0.9	8.0 ± 0.8	5.8 ± 0.7	7.1 ± 0.3	10.0 ± 7.9	11.2 ± 0.9	5.6 ± 0.5	4.0 ± 0.34
Zn	3.2 ± 0.2	3.6 ± 0.5	3.2 ± 0.4	3.5 ± 0.2	2.6 ± 0.4	2.7 ± 0.4	2.6 ± 0.5	2.0 ± 0.2
Ca	88 ± 8	113 ± 20	106 ± 7	124 ± 20	130 ± 15	110 ± 23	119 ± 16	139 ± 31
Cl	28.4 ± 4.5	39.3 ± 2.1	37.1 ± 7.4	30.7 ± 8.8	32.9 ± 4.4	31.2 ± 5.7	26.3 ± 5.0	27.9 ± 4.3
K	431 ± 72	483 ± 9	501 ± 53	615 ± 122	402 ± 119	478 ± 78	565 ± 73	648 ± 47
Na	41.5 ± 5.7	41.8 ± 8.1	35.3 ± 8.4	29.1 ± 5.2	34.8 ± 9.2	31.6 ± 6.9	21.7 ± 4.6	14.3 ± 1.6
				Root				
S	58.4 ± 4.1	67.1 ± 11.3.	56.9 ± 4.1	98.8 ± 23.1	70.6 ± 15	56.3 ± 5.9	96.6 ± 7.5	116 ± 18.7
Al	85.2 ± 18.9	39.6 ± 5.9	61.5 ± 11.1	70.7 ± 10.4	54.8 ± 3.0	61.1 ± 9.3	66.3 ± 24.4	75.2 ± 18.1
B	0.9 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Fe	40.7 ± 8.6	30.2 ± 14.3	25.6 ± 4.7	28.1 ± 3.8	23.1 ± 1.9	26.1 ± 3.8	27.8 ± 3.9	31.0 ± 8.4
Mg	120 ± 16	138 ± 23	148 ± 15	183 ± 28	126 ± 27	144 ± 30	202 ± 23	231 ± 29
Mn	11.1 ± 1.7	12.2 ± 3.2	7.4 ± 0.6	11.3 ± 1.0	14.3 ± 2.2	10.3 ± 2.2	12.7 ± 4.5	10.4 ± 1.4
Zn	8.9 ± 2.4	9.8 ± 3.6	7.4 ± 0.7	10.8 ± 2.4	11.0 ± 1.2	9.1 ± 1.4	10.6 ± 0.8	8.1 ± 1.7
Ca	51 ± 5	51 ± 7	54 ± 2	73 ± 7	50 ± 3	55 ± 2	63 ± 3	61 ± 5
Cl	58.0 ± 3.4	48.9 ± 45.9	62.5 ± 10.0	57.9 ± 5.3	55.0 ± 16.2	61.7 ± 7.3	61.5 ± 1.6	60.1 ± 3.5
K	561 ± 31	545 ± 32	504 ± 33	561 ± 31	532 ± 33	548 ± 18	426 ± 49	401 ± 47
Na	103 ± 13	96 ± 10	111 ± 14	123 ± 24	97 ± 11	97 ± 12	160 ± 20	117 ± 15

is a precursor for many secondary plant compounds. Under elevated CO_2 leaf shikimate concentrations were increased in VA-mycorrhizal plants ($F_{1,31}$ 4.48, $P = 0.042$) (Table 3).

In non-mycorrhizal plants grown at both elevated and ambient CO_2, tissue starch concentrations increased toward the roots (i.e. roots>stem>leaves, Fig. 3). In VA-mycorrhizal plants grown under ambient CO_2, the concentration of starch within the plant had a similar distribution to non-mycorrhizal plants, but was slightly lower in the roots ($F_{1,31}$ 5.89, $P = 0.021$). In contrast, in VA-mycorrhizal plants grown at elevated CO_2, the concentration of starch was reduced in the roots relative to the concentration in leaves (Fig. 3). Thus the gradient in starch concentrations between roots and shoots was reversed when VA-mycorrhizal plants were grown under elevated CO_2.

Maximum Rates of Photosynthesis

Maximum rates of photosynthesis (A_{max}) measured at 5% CO_2 were greater in VA-mycorrhizal plants relative to controls ($F_{1,30}$ 11.85, $P = 0.002$), particularly in plants grown under elevated CO_2 (Fig. 4). A_{max} was positively correlated with the concentration of P within leaves (Fig. 5*A*, $r^2 = 0.54$, $P < 0.001$). In addition to contributing to increased phosphate uptake in plants, an analysis of covariance where

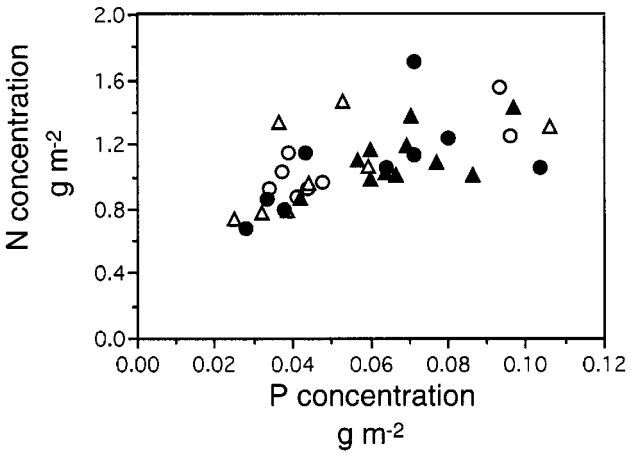

Fig. 2. Correlation between leaf nitrogen and leaf phosphorus concentrations of *B. pendula* ($r^2 = 0.37$, $P < 0.001$) growing under ambient (○, ●) or elevated (△, ▲) CO_2 concentrations, and either with VA-mycorrhizae (●, ▲) or without VA-mycorrhizae (○, △).

leaf P concentrations were used as the covariate showed that VA-mycorrhizae had an additional positive affect on A_{max} beyond the improvement of P concentrations within the leaves ($F_{1,40} = 6.97$, $P = 0.0136$). Over the range of nitrogen concentrations within leaves (0.6–1.8 g m^{-2}), there was a very low correlation between leaf nitrogen and A_{max} (Fig. 5*B*, $r^2 = 0.08$, $P = 0.048$).

Discussion

This study aimed to determine the physiological importance of VA-mycorrhizae to tree seedlings of *B. pendula*, and to determine whether VA-mycorrhizae modified plant responses to elevated CO_2. We were interested in whether VA-mycorrhizae would increase P concentrations within plants leading to increased A_{max}, whether elevated CO_2 would lead to altered carbohydrate concentrations, and whether VA-mycorrhizae provide a sink for carbohydrates, that could prevent the build-up of carbohydrates in concentrations sufficient to 'down-regulate' photosynthesis.

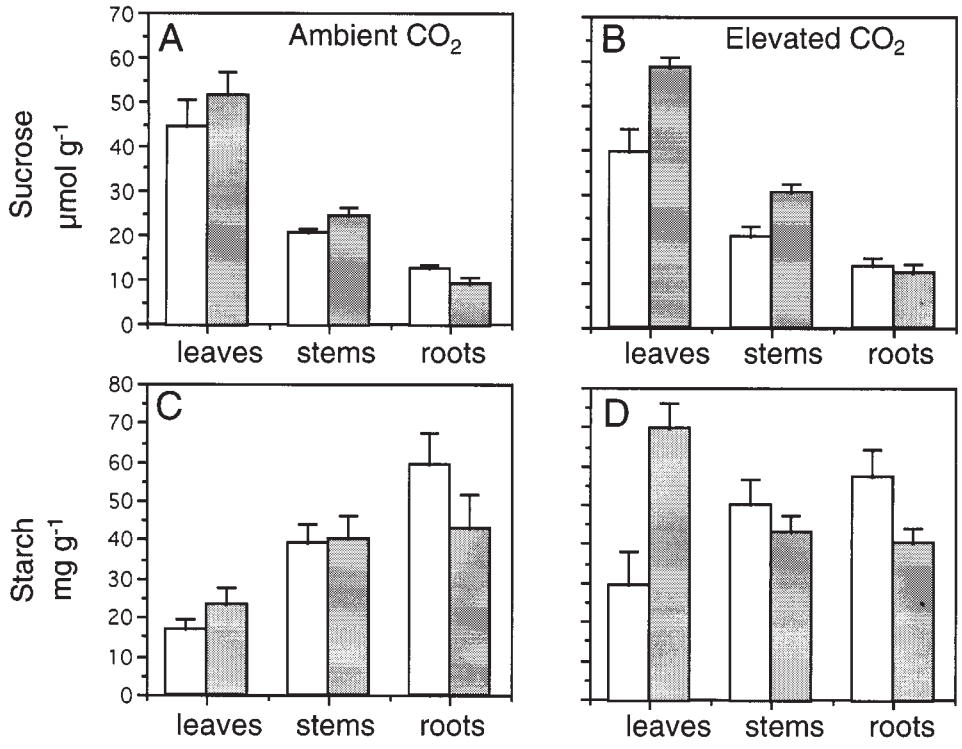

Fig. 3. Sucrose (*A* and *B*) and starch (*C* and *D*) concentrations of organs (leaves, stems and roots) of seedlings of *B. pendula* growing under ambient (*A* and *C*) and elevated CO_2 concentrations (*B* and *D*). Plants were grown either with VA-mycorrhizae (shaded bars) or without VA-mycorrhizae (open bars). Values are the means of 10 plants with standard error bars.

Table 3. Concentrations of carbohydrates and shikimate
Mean concentrations (± standard error) of carbohydrates in leaves, stems and roots of *Beilschmiedia pendula* which were either VA-mycorrhizal (+VAM) or not (–VAM) and grown under elevated or ambient concentrations of CO_2. Concentrations are in μmol g^{-1} and are for 10 plants

Compound	Ambient CO_2		Elevated CO_2	
	–VAM	+VAM	–VAM	+VAM
		Leaves		
Glucose	61.7 ± 15.2	41.2 ± 10.9	65.7 ± 9.9	54.9 ± 9.9
Fructose	28.3 ± 3.7	23.7 ± 3.0	22.9 ± 2.3	24.9 ± 2.2
Bornesitol	2.9 ± 0.4	2.6 ± 0.2	2.5 ± 0.3	2.0 ± 0.2
myo-Inisitol	14.3 ± 1.4	16.6 ± 1.4	11.0 ± 1.3	14.4 ± 0.6
Shikimate	66.6 ± 12.7	68.6 ± 9.0	53.5 ± 8.5	80.1 ± 9.9
		Stems		
Glucose	69.2 ± 8.3	45.3 ± 7.1	57.3 ± 6.0	69.3 ± 9.5
Fructose	58.3 ± 5.6	38.6 ± 4.7	41.4 ± 3.6	59.5 ± 7.6
Bornesitol	4.1 ± 0.7	4.1 ± 0.4	3.4 ± 0.4	4.0 ± 0.4
myo-Inisitol	14.9 ± 2.0	13.0 ± 1.4	14.9 ± 1.7	14.5 ± 1.2
Shikimate	11.2 ± 1.5	14.3 ± 2.6	13.0 ± 2.2	15.9 ± 3.1
		Roots		
Glucose	43.3 ± 3.4	40.9 ± 4.5	38.4 ± 5.7	53.0 ± 6.2
Fructose	37.0 ± 3.7	40.2 ± 4.4	33.3 ± 6.4	54.9 ± 6.4
Bornesitol	2.6 ± 0.3	2.7 ± 0.2	1.9 ± 0.2	2.5 ± 0.2
myo-Inisitol	12.0 ± 1.3	9.1 ± 0.7	9.7 ± 1.3	11.1 ± 0.6
Shikimate	5.8 ± 0.5	8.3 ± 0.9	8.4 ± 1.4	8.6 ± 0.6

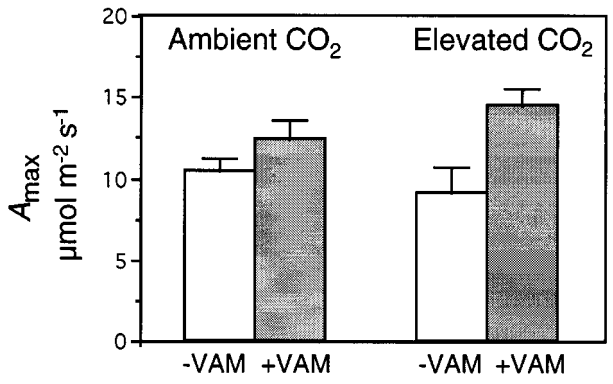

Fig. 4. Maximum rate of photosynthetic oxygen evolution (A_{max}) in *B. pendula* growing under ambient (*A*) or elevated (*B*) CO_2 concentrations, and either with VA-mycorrhizae (+VAM) or without VA-mycorrhizae (–VAM). Values are means and standard errors of 10 plants.

Effect of VA-Mycorrhizae on Mineral Nutrition and A_{max}

VA-mycorrhizae are important for P nutrition in *B. pendula*, resulting in increased leaf P concentrations and enhanced rates of A_{max}. In a variety of tropical species, light-saturated photosynthetic CO_2 uptake under ambient CO_2 is correlated with 24 h photosynthetic carbon gain, and thus with gross primary production (Zotz and Winter 1996). The correlation observed between A_{max} and leaf P concentrations over a range of leaf N concentrations suggests P availability is likely to be a major limitation to A_{max}, and thus to photosynthetic carbon gain in the soils used in this study. Other studies of tropical plants have found strong correlations between leaf N and A_{max} over a similar range of leaf N as observed here (0.6–1.8 g N m^{-2}, Fig. 3*B*) (Medina 1984; Field 1988; Thompson *et al.* 1992). Correlations between leaf P and A_{max} have also been observed in other shade tolerant tropical forest species growing on soil low in P (Raaimakers *et al.* 1995; Reich *et al.* 1995), *Eucalyptus grandis* (Kirschbaum and Tompkins 1990) and *Pinus radiata* (Conroy *et al.* 1988). The small response of A_{max} to leaf N concentrations in the current study could be because P concentrations were limiting A_{max} over all N concentrations, underscoring the importance of VA-mycorrhizae for P nutrition and high rates of carbon gain in tropical tree species growing in soils low in P.

In addition to increasing leaf P concentrations, VA-mycorrhizae also led to doubled concentrations of P in stems and roots. Storage of P in stem tissue has been observed in other species and is viewed as 'luxury consumption' of available P to be used at a later time when P may become limiting (Chapin 1980). Therefore, VA-mycorrhizae may contribute to plant fitness in *B. pendula* by providing a buffer against P deficiency that may occur seasonally, or due to tissue loss caused by herbivore damage.

Unlike the concentration of leaf N, which was reduced under elevated CO_2 (Table 1), concentrations of leaf P were not influenced by growth under elevated CO_2 in either VA-mycorrhizal or non-mycorrhizal plants (Fig. 1; Table 1). Because plants growing under elevated CO_2 were larger, this indicates elevated CO_2 grown plants increase their whole-plant P uptake. In *Quercus alba* (Norby *et al.* 1986) and *Pinus* (Conroy *et al.* 1990), both with ectomycorrhizae, and in VA-mycorrhizal tallgrass (Owensby *et al.* 1993), similar tissue P concentrations among elevated and ambient CO_2 grown plants have also been observed while, in a VA-mycorrhizal C_4 grass, P concentrations were reduced under elevated CO_2 (Morgan *et al.* 1994). Increased whole-plant P uptake under elevated CO_2 has been attributed to greater fine root development under elevated CO_2 rather than to enhanced ectomycorrhizae development (Norby *et al.* 1986), and also to changes in the composition of the mycorrhizal community facilitating P uptake (Conroy *et al.* 1990). In *B. pendula*, enhanced P uptake due to enhanced fine root development is unlikely because this species has no fine roots; however, changes in the degree of root branching were not assessed, and may have been sufficient under elevated CO_2 to maintain P concentrations similar to those of

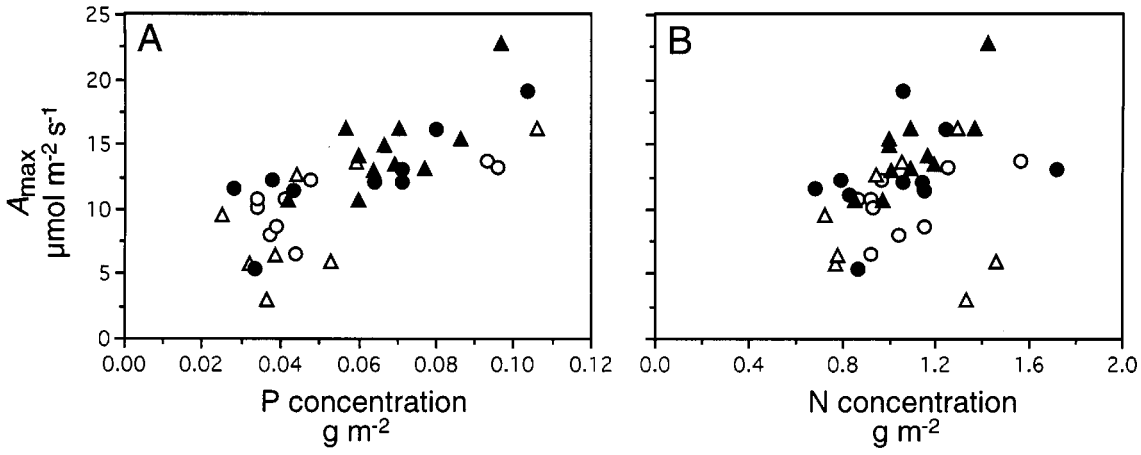

Fig. 5. Correlation between the maximum rate of photosynthetic oxygen evolution (A_{max}) and the leaf phosphorus (*A*, $r^2 = 0.54$, $P < 0.001$) and nitrogen (*B*, $r^2 = 0.08$, $P = 0.048$) concentrations of *B. pendula* growing under ambient (○, ●) or elevated (△, ▲) CO_2 concentrations, and either with VA-mycorrhizae (●, ▲) or without VA-mycorrhizae (○, △).

ambient CO_2 grown plants. Enhanced fungal infection of roots in *B. pendula* under elevated CO_2 (Lovelock *et al.* 1996) may partially explain enhanced whole plant P uptake, although this cannot be the case in non-mycorrhizal plants (Fig. 1).

Fertilisation with P in the absence of VA-mycorrhizae did not result in increased concentrations of P within *B. pendula*, probably due to the structure of the roots. This species, along with other tropical tree species within the Magnoliales and Laurales, have low frequencies of fine roots (roots less than 0.3 mm diameter) and have no root hairs (St John 1980). This morphology, which is believed to be common in other tropical forest tree species, reduces the plants' ability to explore the soil volume, so making added nutrients largely inaccessible (Koide 1991). In *B. pendula*, VA-mycorrhizae appear to be necessary for storage of P, and for leaf P concentrations sufficient to maintain high rates of photosynthesis. These results are consistent with the proposal that species with little fine root development are dependent on VA-mycorrhizae for adequate nutrient supply (Newsham *et al.* 1995).

In addition to increasing P concentrations within plants, VA-mycorrhizae also influenced the concentration of other mineral elements (Table 2). Concentration of S was increased in VA-mycorrhizal plants. This has been observed in other species (Cooper and Tinker 1978; Rhodes and Gerdemann 1978). Concentration of Al, Zn, B, Mn, Fe and Na were higher in non-mycorrhizal plants than in VA-mycorrhizal plants, indicating VA-mycorrhizae may limit the uptake of these ions, some of which are toxic. Evidence for a role for mycorrhizae in protecting against the uptake of toxic metal ions has also been found in plants with ericoid mycorrhizae (reviewed by Newsham *et al.* 1995). Interestingly, in a study of mineral element composition of tropical tree species, Reich *et al.* (1995) reported that early successional species had higher concentrations of Al in their leaves than later successional species, despite the higher concentrations of Al in late successional soils. VA-mycorrhizae occur more commonly in late successional than early successional species; thus differences in leaf Al concentrations in the study of Reich *et al.* (1995) may reflect the influence of VA-mycorrhizae on uptake of metal ions.

Effect of Elevated CO_2 on Carbohydrate Concentrations and Root Sink Strength

Our third hypothesis was that carbohydrate concentrations would be increased in plants grown under elevated CO_2 without VA-mycorrhizae. The concentrations of glucose, fructose, bornesitol and *myo*-inisitol were not influenced by elevated CO_2 or VA-mycorrhizae (Table 3). In contrast, leaf and stem sucrose concentrations and leaf starch concentrations where greater in VA-mycorrhizal plants than in non-mycorrhizal plants under elevated CO_2 (Fig. 3). Interestingly, even when starch concentrations were doubled under elevated CO_2, leaf starch concentrations were within the range measured for understory species within nearby forest (Tissue and Wright 1995). The relatively low carbohydrate concentrations observed in *B. pendula* are unlikely to cause any product-related feedback inhibition of photosynthesis. This contrasts with feedback inhibition of photosynthesis by high concentrations of carbohydrates observed in agricultural species growing under elevated CO_2 (van Oosten *et al.* 1994; Xu *et al.* 1994).

Shikimate is a compound derived from carbohydrate metabolism from which many secondary plant compounds are formed (e.g. phenolics), some of which are important in

deterring plant herbivores (Coley *et al*. 1985). It is estimated that 60% or more of plant carbon traverses the shikimate pathway (Jensen 1985). The concentration of shikimate in leaves was increased under elevated CO_2 in VA-mycorrhizal plants by approximately 15% (Table 3). This may lead to greater concentrations of shikimate-derived secondary compounds in plants grown under elevated CO_2, as has been shown in another species within the Lauraceae (Cipollini *et al*. 1993). Thus future elevated concentrations of CO_2 could influence populations of plant herbivores in tropical forests.

Our fourth hypothesis was that VA-mycorrhizae would increase the sink strength for carbohydrate in the roots due to the symbiosis. VA-mycorrhizal plants generally had higher leaf starch and sucrose concentrations and lower root starch and sucrose concentrations than non-mycorrhizal plants at both ambient and elevated CO_2 (Fig. 3). This indicates carbohydrate production and/or storage in the leaves, and carbohydrate usage, or leakage, from roots was increased in VA-mycorrhizal plants. High leaf starch concentrations in VA-mycorrhizal plants were particularly evident at high CO_2, while root starch concentration in VA-mycorrhizal plants were lower that in non-mycorrhizal plants under both ambient and elevated CO_2 (Fig. 3). These data can be interpreted in at least two ways.

Firstly, under elevated CO_2 concentrations, leaves of VA-mycorrhizal plants may accumulate more carbohydrates during the light period than can be transported to the roots in the phloem. Limitations to phloem transport of carbohydrates in plants growing under elevated CO_2 has been proposed as a possible mechanism explaining species differences in their response to elevated CO_2, with more basal taxa, to which *B. pendula* belongs, being more limited in their phloem carbohydrate transport capacity than more herbaceous, modern taxa (Körner *et al*. 1995). Higher leaf sucrose concentrations under elevated CO_2 concentrations (Fig. 3) provide support for this interpretation, as do the similar levels of starch in roots at both ambient and elevated CO_2 concentrations.

A second interpretation is based on the assumption that differences between leaf and root carbohydrate concentrations represent the balance between carbohydrate production and the sink strength of the roots. Differences in sucrose and starch concentrations between leaves and roots are generally greater in VA-mycorrhizal plants, particularly under elevated CO_2 (Fig. 3). The possible significance of this result could be that the sink strength of roots for carbohydrates is increased in VA-mycorrhizal plants, particularly under elevated CO_2. This could be due to higher levels of fungal infection under elevated CO_2 (Ineichen *et al*. 1995 and Tingey *et al*. 1995 for ectomycorrhizae; Morgan *et al*. 1994 and Lovelock *et al*. 1996 for VA-mycorrhizae), and the subsequently higher demand for sugars for growth and respiration of the fungus. The additional positive effect of VA-mycorrhizae on A_{max}, above that explained by increased leaf P concentrations, may be evidence for the importance of remote sinks for carbohydrates in influencing A_{max} in plants growing under elevated CO_2.

Accounting for increased starch concentrations under elevated CO_2 in leaves of VA-mycorrhizal plants will require further investigation of how VA-mycorrhizae influence phloem transport and daily fluctuations in carbohydrate concentrations, and how P concentrations directly effect carbohydrate accumulation. Because starch concentrations of naturally occurring forest understory shrubs were similar to those observed in *B. pendula* grown with VA-mycorrhizae (Tissue and Wright 1995), and because phloem transport is more likely to be limited by low temperatures in temperate regions rather than at the higher temperatures that occur in the tropics, the observed distribution of carbohydrates in *B. pendula* are probably the result of source–sink relationships influenced by VA-mycorrhizae rather than reflecting limitations to phloem transport.

Conclusions and Implications for Seedling Growth Under Elevated CO_2

It has been proposed that phosphorus and carbohydrate status of plants will modify plant responses to elevated CO_2, and may influence the effects of elevated atmospheric CO_2 on tropical forests. In *B. pendula,* VA-mycorrhizae increased leaf P concentrations resulting in a linear increase in A_{max} with increasing leaf P concentrations. As light saturated CO_2 uptake correlates with 24 h carbon gain over a wide range of tropical species, VA-mycorrhizae and P nutrition of plants are likely to be important factors influencing forest productivity.

VA-mycorrhizal plants grown under elevated CO_2 had higher carbohydrate concentrations within leaves but showed no decline in A_{max}. Starch concentrations in roots of VA-mycorrhizal plants were reduced. Therefore, VA-mycorrhizae increased photosythetic rates in leaves and also altered the sink strength for carbohydrates in roots. As shade tolerant seedlings in tropical forests are normally VA-mycorrhizal, the results presented here suggest that, in the future under elevated atmospheric CO_2, A_{max} will not be reduced. Future seedling growth rates could then be expected to increase in proportion to increases in atmospheric CO_2 concentrations. For *B. pendula* this was not the case, possibly because the cost of sustaining the VA-mycorrhizae symbiosis was increased (Lovelock *et al*. 1996). Because tree species with varying levels of shade tolerance also have varying levels of VA-mycorrhizae, the presence and/or change in costs of sustaining VA-mycorrhizae may be a mechanism that partially explains differences in species growth responses to elevated CO_2.

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