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Effects of CO_2 Atmospheric Fertilization on Greenhouse Production of Olive Trees (*Olea europaea* L. 'Arbequina')

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Summary

The efficacy of CO_2 enrichment for greenhouse production of woody plants has received scant attention. We present the results of studies to test the hypothesis that CO_2 enrichment would increase the growth of vegetatively-propagated olive trees during greenhouse production during winter in a Mediterranean climate. One-month-old rooted cuttings of *Olea europaea* 'Arbequina' were grown for three months in greenhouses that were maintained at ambient CO_2 concentrations or enriched to CO_2 concentrations of 500 or 750 μmol mol^{-1}. CO_2 enrichment at either level enhanced growth, with no differences in effects of the two elevated CO_2 concentrations. Enrichment of greenhouse air to 500 μmol mol^{-1} did not increase the growth rate of one-year-old trees in comparison to that of plants grown at the ambient CO_2 concentration. Use of CO_2 enrichment can facilitate rapid nursery production of olive trees during the winter season under Mediterranean conditions.

Key words. biomass accumulation – greenhouse culture

Introduction

Brief exposure of plants to high concentrations of atmospheric carbon dioxide (CO_2) generally stimulates increased rates of photosynthetic fixation of carbon and accelerates plant growth (Bassirirad et al. 1997). One practical application of this has been the enrichment of greenhouse air with carbon dioxide (ECO_2). Woody plant species respond to ECO_2, although the increase in growth rate is smaller than that of herbaceous plants, and woody species tend to redistribute the acquired carbon into storage structures (Ceulemans and Mousseau 1994; Cornelissen et al. 1999). When trees with different resource availability were exposed to elevated CO_2 levels for brief periods (less than 6 months), mean total biomass increased 38 % in conifers and 63 % in deciduous trees (Ceulemans and Mousseau 1994; Tissue et al. 1997). Longer ECO_2 treatment can increase tree biomass and allometric relations (Bazzaz et al. 1993; Bassirirad et al. 1997; Will and Teskey 1997).

In mild, Mediterranean climates, such as that found in much of Spain, ECO_2 use is not widespread because greenhouse crop producers rely extensively on open ventilation for cooling, thereby making it difficult to maintain high [CO_2]. Research on effects of ECO_2 on woody species in this climate has focused mainly on aspects of biomass distribution (Llusià et al. 1996; Savé et al. 2002; Biel et al. 2004; Cortés et al. 2004). Olive (*Olea europaea* L.) has been the subject of very few studies of CO_2 effects (Tognetti et al. 2001; Sebastiani et al. 2002). These studies addressed the response of 1- or 5-year old olive trees to elevated CO_2 under field conditions, rather than in a greenhouse. In both cases, there was a positive response of photosynthetic rate to ECO_2, but no effect of ECO_2 on rate of biomass accumulation.

Here we present research on the application of ECO_2 to the intensive nursery production of olive trees. The scientific interest in this research was to test the hypothesis that CO_2 enrichment would increase the growth of vegetatively-propagated olive trees during greenhouse production during winter in a Mediterranean climate, when greenhouse vents are closed for a longer fraction of the day than in other seasons. The work is also pertinent to nurseries that seek to hasten the growth of young trees during unfavourable seasons and thereby shorten the production cycle.

Material and Methods

Two experiments were conducted between Winter 1997 and Spring 2000 in a greenhouse at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Cabrils (Barcelona), Spain (latitude 41 º N and longitude 2 º23 ', altitude 55 m). The greenhouse was oriented along a north-south axis, and had the same levels of radiation in each of its three 77 m^2 modules (Cortés et al. 2004). Temperatures in the modules were between 10 and 35 °C, and differences in mean temperature among the modules was 1 °C or less throughout the experiments. There was a heating system to maintain temperature up to 10 °C. The following procedures were used in each experiment, except as noted below. CO_2 treatments in each experiment were randomly assigned to the modules. One

module was maintained at ambient CO2 concentrations, approximately 350 μmol mol^{-1}. Pure CO_2 gas was injected into the other two greenhouse modules to elevate atmospheric CO_2 concentrations to 500 or 750 μmol mol^{-1}. A computer program (Carburos Metálicos, Barcelona, Spain) controlled the monitoring and injection of CO_2. CO_2 concentrations were tested and recorded every 3 minutes by automatic sampling and analysis using an infrared gas analyzer (Model 3600; MSA, Barcelona, Spain). When needed to maintain the desired CO_2 concentrations, liquified CO_2 was injected through a solenoid valve and distributed through emitters in a network of polyethylene irrigation lines located close to the plants.

For each experiment, rooted cuttings of *Olea europaea* 'Arbequina' were obtained from Agromillora Catalana S.A. (Subirats, Barcelona, Spain) one month after they had been planted in 252-cm^3 (6x6x7 cm) plastic containers filled with peat as a substrate. Prior to each experiment, 20 replicate plants were randomly selected for determination of their initial properties. After their height and basal stem diameter had been recorded, these plants were separated into leaf, stem, and root fractions, then dried in an oven at 65 °C for 1 week before the biomass of each fraction was determined.

The plants used in each experiment were placed on benches in the greenhouse modules. The pots were placed to give a plant density of 100 m^{-2}. Plants were irrigated as needed through microsprinklers that delivered a nutrient solution containing 8.2 mM NO_3^-, 1.3 mM NH_4^+, 0.45 mM HPO_4^{2-}, 1.65 mM SO_4^{2-}, 4.2 mM K^+, 3.9 mM Ca^{+2}, and 1.5 mM Mg^{+2}, at pH 6.5.

Experiment 1, a study of the effect of CO_2 fertilization on olive tree growth, was carried out during consecutive years: from 2 Dec. 1997 to 4 Mar. 1998, and from 11 Nov. 1998 to 18 Feb. 1999. Each year we put 100 plants into each greenhouse module and applied CO_2, fertilizer, and irrigation as described above. Experiment 2 was a study of the response of one-year-old olive trees to ECO_2. Twenty one-year-old olive trees were transplanted into a peat:coconut coir (1:1, v:v) substrate in 20-L containers on 11 Nov. 1998; half were then grown in a greenhouse module at ambient CO_2 levels, and the other half were grown in a module enriched with CO_2 to 500 μmol mol^{-1}. Growth was assessed monthly during a three-month period by measuring the increase in length and diameter of three branches on each plant.

At the conclusion of each experiment, fifteen plants were randomly selected from each treatment. Plant height and basal diameter were measured, after that the plants were harvested and separated into root, stem, and leaf fractions. Leaf area was determined by analysis of digital images (Digital Image Analysis System, Delta T Devices, Cambridge, UK). Biomass was determined as described above. Relative growth rates were calculated using the equation,

$$\text{Relative growth rate} = \frac{1n(\text{final weight}) - 1n(\text{initial weight})}{\text{time interval in days}}$$

All data in Experiment 1 were subjected to analysis of variance, with CO_2 as a fixed factor. Means comparisons in Experiment 1 were made using the Tukey-Kramer Honestly Significant Difference test ($P<0.05$). Data from Experiment 2 were analyzed by a t-test. All statistical analyses were performed with JMP 7.0 (SAS Institute, Cary, N.C.).

Results

Experiment 1

CO_2 enrichment of the greenhouse air resulted in significantly greater relative growth rates, biomass production, plant height, and leaf area compared with plants in ambient CO_2 (Table 1). The response to ECO_2 at 500 μmol mol^{-1} was not significantly different from the response at 750 μmol mol^{-1}. The average total plant biomass gain in the ECO_2 treatments was 67 % greater than in the control plants. Plant height, the characteristic typically used as a grading measure in commercial production of olive trees, doubled during the experiment in the ECO_2 treatments, but increased by less than 50 % in the controls. Partitioning of dry matter among leaves, stems, and roots was not significantly affected by ECO_2.

Experiment 2

ECO_2 at 500 μmol mol^{-1} had no significant effect ($t_{0.05}$) on the relative growth rate of one-year-old trees. The relative rate of shoot elongation was of 0.0075 d^{-1} at the ambient CO_2 concentration (350 μmol mol^{-1}) and 0.0069 d^{-1} in ECO_2. The relative rates of increase in basal diameter were 0.0055 d^{-1} and 0.0050 d^{-1} in ambient and ECO_2, respectively.

Discussion

The results support the hypothesis that month-old rooted cuttings of olive trees produced in CO_2-enriched air would grow more rapidly than those in ambient conditions. The growth enhancement we measured in olive trees was similar to that reported for deciduous tree crops, such as cherry (Centritto et al. 1999b) and peach (Centritto et al. 2002), and also consistent with the findings for several non-crop tree species (Ainsworth and Long 2005). In the only previous study of young olive trees, CO_2 enrichment to 560 μmol mol^{-1} caused a decrease in the above-ground biomass of one-year-old plants of one cultivar and had no effect on the biomass of the other (Sebastiani et al. 2002). Although procedural differences between that study and ours could confound direct comparisons (in particular, theirs was a free-air study conducted in summer and early fall on plants growing in small containers), our results also showed no effect of ECO_2 on year-old plants.

There have been reports of enhanced growth of year-old deciduous trees in response to ECO_2, particularly when plants were grown in relatively large containers. Year-old birch trees (*Betula pendula*) in 5-L containers were less responsive to elevated CO_2 than plants in 25-L containers (Centritto 2000). Others have reported that biomass production of year-old deciduous trees in CO_2-enrichment experiments was positively correlated with container volume, but found no interaction between [CO_2] and container volume (Kerstiens and Hawes 1994; McConnaughay et al. 1996). In the latter studies, all tree species had greater biomass gains when grown in CO_2-enriched environments. In general, annual plants are less responsive to CO_2 in free-air enrichment experiments than in chambers (Ainsworth and Long 2005), but that relationship has not been shown clearly for very young trees.

Table 1. Effect of CO_2 fertilization during three winter months in a greenhouse on growth of rooted cuttings of 'Arbequina' olive trees. The experiment was first conducted in 1997–1998, and then repeated the following winter. Treatments included greenhouse CO_2 concentrations of 350, 500, or 750 μmol mol^{-1}. Initial growth measurements of rooted cuttings are also presented.

	1997–1998				1998–1999						
	Initial	350	500	750	Initial	350	500	750	CO_2	Year	CO_2*Year
Biomass per plant											
Leaves (g)	0.15	0.89 a+	1.63 b	1.39 b	0.51	1.42 a	2.18 b	2.37 b	***	***	NS
Stems (g)	0.07	0.18 a	0.46 b	0.35 b	0.17	0.34 a	0.53 b	0.58 b	***	***	NS
Roots (g)	0.08	0.44 a	0.78 b	0.71 b	0.40	0.53 a	0.68 b	0.85 b	***	NS	NS
Total (g)	0.30	1.50 a	2.86 b	2.45 b	1.08	2.29 a	3.40 b	3.81 b	***	***	NS
Height (cm)	6.7	9.3 a	15.2 b	15.5 b	11.2	14.8 a	22.7 b	17.9 ab	***	***	NS
Stem diameter (mm)	2.2	2.6 a	3.7 b	3.0 ab	2.7	3.6 a	3.8 a	3.9 a	*	***	NS
Leaf area (cm^2)	14.3	55.3 a	97.5 c	73.3 b	42.5	85.3 a	120.8 b	111.3 b	***	***	NS
Leaf mass fraction	0.50	0.58 a	0.56 a	0.57 a	0.47	0.62 a	0.64 a	0.62 a	NS	***	NS
Stem mass fraction	0.25	0.12 a	0.16 a	0.14 a	0.16	0.14 a	0.16 a	0.15 a	NS	NS	NS
Root mass fraction	0.25	0.30 a	0.28 a	0.29 a	0.37	0.23 a	0.20 a	0.23 a	NS	***	NS
Relative growth rate (d^{-1})		0.016 a	0.024 b	0.022 b		0.009 a	0.014 b	0.014 b	***	***	NS

***, *, NS: Significant at P<0.0001, 0.05, or not significant, respectively.
+Values followed by a common letter in a row for each year are not significantly different at P=0.05.

The positive response of month-old olive trees, in contrast to the lack of response of year-old trees, may be due to differences in leaf age. Young leaves generally are more responsive than old ones to ECO_2 (Medlyn et al. 1999). Therefore, in an evergreen species like olive, young plants from cuttings that have new leaves are likely to have higher maximum carbon assimilation rates than older plants. It is worth noting that the significant year effects in our experiments may reflect less sensitivity to elevated CO_2 among the second-year plants, which were made from larger cuttings of older plant material than that used in the first year.

The lack of a greater response of the month-old olive trees to CO_2 at 750 μmol mol^{-1} than at 500 μmol mol^{-1} could indicate that the plants acclimate quickly to ECO_2. Acclimation to high CO_2 has been well documented (Medlyn et al. 1999; Ainsworth and Long 2005), and others have also found that the effect of ECO_2 on trees may occur at the earliest stages of development (Centritto et al. 1999a). For tree crops, ECO_2 may be most useful during short-term nursery production.

In conclusion, CO_2 enrichment of greenhouse air during winter in Mediterranean conditions can be useful for rapid nursery production of olive trees. This evergreen species, whose young leaves are responsive to ECO_2, is particularly well suited to this method of production because there is a growing market for small plants that can be transplanted to the field in early spring.

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