Effects of Atmospheric Carbon Dioxide Concentrations on *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis*

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**Abstract**

Seedlings of *Thymus vulgaris*, *T. zygis* and *T. hyemalis* were grown for 17 weeks in a greenhouse under three different CO₂ gas concentrations: high (750 ppm), intermediate (500 ppm) and ambient (350 ppm). Four variables were studied: total biomass, biomass allocation, and essential oil quantity and composition. Biomass production was different between species, but not between CO₂ treatments, only the roots had a response positive to CO₂ increase. *Thymus zygis* was the most productive species with a 26% and 148% more biomass than *T. vulgaris* and *T. hyemalis* respectively. Yield of essential oil showed a decrease between species inverse to biomass production, *T. hyemalis* produced 39% and 13% more essential oil than *T. zygis* and *T. vulgaris* respectively and only *T. hyemalis* produced a 17.54% more essential oil in intermediate CO₂ concentration than in ambient. CO₂ concentration did not affect the essential oil composition in the same way in all the species; β-myrcene in *T. hyemalis* and ocymene in *T. zygis* increased under the elevated CO₂ treatment but the carvacrol concentration in *T. hyemalis* was higher in ambient CO₂. In *T. hyemalis* the concentration of finals phenols (thymol and carvacrol) showed a positive tendency to CO₂ increase and this pattern is inverse to their precursors (γ-terpinene and ρ-cymene).

**Resumen**

Esquejes de *T. vulgaris*, *T. zygis* y *T. hyemalis* crecieron durante 17 semanas en invernadero bajo tres concentraciones diferentes de CO₂ gas: alta (750 ppm), intermedia (500 ppm) y ambiente (350 ppm). Se estudió la respuesta de cuatro variables: biomasa total, alocación de biomasa y cantidad y calidad del aceite esencial producido. La producción de biomasa fue diferente entre especies, pero no entre los diferentes tratamientos de CO₂, solo mostraron una respuesta positiva al incremento del CO₂ la biomasa de raíces que también incrementó. *T. zygis* fue el mas productivo con un 26% y un 148% mas de biomasa que *T. vulgaris* y *T. hyemalis* respectivamente. El rendimiento en aceite esencial entre las tres especies fue inverso a la producción de biomasa, *T. hyemalis* produjo un 39% y un 13% mas aceite esencial que *T. zygis* y *T. vulgaris* respectivamente y solo *T. hyemalis* respondió al CO₂ produciendo un 17.54% mas de aceite esencial en la concentración de 500 ppm de CO₂. La composición del aceite esencial bajo el efecto del CO₂ no respondió de igual manera en las tres especies; β-myrceno en *T. hyemalis* y ocymeno en *T. zygis* dieron una respuesta positiva en ambiente enriquecido con CO₂, pero la
concentración de carvacrol en *T. hyemalis* tuvo un comportamiento opuesta. En *T. hyemalis* la concentración de los compuestos fenólicos finales (tymol y carvacrol) mostró una tendencia positiva al incremento del CO₂ y esta fue inversa a la presencia de sus precursores (γ-terpineno y ρ-cymeno).

**INTRODUCTION**

The increase of atmospheric CO₂ due to global change and/or horticultural practices promotes direct effects on plant growth and development (Bazzaz, 1990). The responses observed in different species show a wide range of patterns either in the biomass production or in the composition of secondary metabolites. These responses may occur in natural ecosystems, but also can be used to increase the production of some plants and some secondary compounds (Llusia et al., 1996; Peñuelas et al., 1996; Marks et al., 2000; Tisserat and Vaughn, 2001).

The departments involved in this paper have a long tradition in the study of the effects of carbonic fertilization on plant growth (Biel, 2002), therefore we studied these effects on *Rosmarinus officinalis*, *Taxus baccata* and *Hypericum perforatum*. The present paper studies the effects of atmospheric CO₂ concentrations under greenhouse conditions on growth and in the composition of secondary metabolites of *Thymus vulgaris*, *T. zygis* and *T. hyemalis* in the Mediterranean area.

**MATERIALS AND METHODS**

**Plant Material**

Two month old seedlings of *T. vulgaris*, *T. zygis* and *T. hyemalis* got from commercial seeds, were grown for 17 weeks from mid January till May in a greenhouse in 3 L plastic pots filled with peat and perlite (2:1: v:v). The plots were watered and fertilized every day by drip irrigation system without restriction. The plastic greenhouse was divided in three compartments, each with a different CO₂ gas concentration and with a minimum temperature above 12°C. During all the time it was not modified the natural Mediterranean light conditions.

**CO₂ Gas Treatment**

The nursery experiment was developed in a plastic multi-tunnel greenhouse divided into three modules located in IRTA, Cabrils, Barcelona (2°30'E, 41°45'N). Each module had a different CO₂ concentration: high (750 ppm), intermediate (500 ppm) and ambient or control (350 ppm). In the 750 ppm and 500 ppm modules the air was enriched with a constant flow of pure industrial CO₂ (Carburos Metálicos, S.A.) through polythene vessels. CO₂ concentration was continuously measured by an infrared gas analyzer (IRGA) LIRA Model 3600 (MSA, Spain) and controlled by an automata PLC OMRON C20K connected to a personal computer. In each module 32 pots per species were placed.

**Biomass Measurement**

After 17 weeks growing, plant harvesting was carried. Eight plants of each CO₂ level were chosen randomly and their total biomass was separated into three fractions: leaves, stems and roots. Their dry weight was calculated after drying at 60°C (72 h) and leaf area was measured with a LI-COR area meter (LI-COR Model 3100, USA).

**Essential Oil Measurement**

Essential oil quantity and composition was studied for each species at the onset of flowering. Essential oil yield was obtained from dry aerial part of the plant (35°C, 72 h) by steam distillation, using a Clavenger apparatus. Qualitative analysis was carried out on a GC Hewlett Packard HP 6890 series II Plus data processor and a flame ionisation detector (FID), using an HP-5 capillary column (30 m x 0,32 mm x 0,25 um, 5% phenyl methyl silicone).
Experimental Design and Data Analysis

The experiment was designed as a double factorial with 3 species and 3 atmospheric CO2 treatments. The results were analyzed using the SAS system for the means analysis by GLM procedure.

RESULTS

Biomass

Differences in biomass production were significant between species, but not between the three CO2 treatments. The total biomass of *Thymus zygis* was 26% greater than *T. vulgaris* and 148% greater than *T. hyemalis* (Fig. 1). Above-ground biomass (stems and leaves) also was bigger in *Thymus zygis* than *T. vulgaris* and *T. hyemalis*, but not roots biomass, the roots of *T. hyemalis* were smaller than those to *T. zygis* and *T. vulgaris* which were similar (Fig. 2).

The plants grown under elevated CO2 gas concentration had the highest root production (Fig. 3), but stems and leaves production did not exhibit.

Essential Oil

The total amount of essential oil is different statistically between the three species: *T. hyemalis* produced 39.43% and 13.04% more essential oil than *T. zygis* and *T. vulgaris*, respectively (Fig. 4). Only *T. hyemalis* showed a positive response to CO2 treatment; the essential oil produced was 17.54% higher under elevated CO2 (500 CO2 ppm) than under the control treatment (350 CO2 ppm) (Fig. 5).

There were different responses to elevated CO2 in the composition of essential oils between species. Ocymene in *T. zygis* and β-myrcene in *T. hyemalis* had a positive response under the elevated CO2 treatment. (Fig. 6 and 7). Carvacrol in *T. hyemalis* had an inverse response under elevated CO2 (Fig. 8). In *T. hyemalis* the concentration of thymol and carvacrol (considered finals phenols) had a tendency to increase with the raise of CO2 gas concentration and this pattern is inverse to their precursors (γ-terpinene and ρ-cymene) (Fig. 9).

CONCLUSIONS

The elevated CO2 treatment had a clear effect on the allocation of root biomass in all the plants and in trans-β-ocymene in *T. zygis* and β-myrcene in *T. hyemalis*. It seems that the differences in the total production of essential oil and biomass were more due to species identity than to CO2 treatments. There is an inverse relationship between total biomass production and essential oil production. It is necessary to continue studying the CO2 effects on secondary metabolism. The response obtained was during the winter growth period; ideally, we should study effects of CO2 enrichment during the whole growing season.

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Literature Cited


**Figures**

Fig. 1. Total biomass of each species at the end of the experimental time.

Fig. 2. Differences between species in each part of biomass at the end of the experiment.

Fig. 3. Total biomass produced at the end of the experimental time in the three different CO2 treatments.

Fig. 4. Yield of essential oil production of each species at the onset of flowering period.
Fig. 5. Yield of essential oil in *T. hyemalis* in the CO$_2$ treatments at the onset of flowering.

Fig. 6. Total amount of ocymene in *T. zygis* between the three CO$_2$ treatments.

Fig. 7. Total amount of β-myrcene in *T. hyemalis* between the three CO$_2$ treatments.

Fig. 8. Total amount of carvacrol in *T. hyemalis* between the three CO$_2$ treatments.

Fig. 9. Tendency of final amount of precursors (γ-terpinene and ρ-cymene) and final phenols (thymol+carvacrol) in *T. hyemalis* in the different CO$_2$ treatments at the onset of flowering.