

**Effect of drought on isoprene emission rates from leaves of  
*Quercus virginiana* Mill. seedlings**

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## Abstract

Isoprene emissions were studied in two-year old seedlings of live oak (*Quercus virginiana* Mill.) during two drying-rewatering cycles. Photosynthesis and stomatal conductance were inhibited since the early stages of the dry treatments. During the first drying-rewatering cycle, when water was withheld over a period of 8 days photosynthesis and leaf conductance decreased  $92 \% \pm 6.6$  (n=6) and  $91 \% \pm 8.4$  (n=6) respectively, while isoprene emissions remained essentially constant. After 12 days under severe drought conditions, isoprene emission rates were also inhibited by  $64 \% \pm 6.1$  (n=6). Similar values were found during the second drying-rewatering cycle with a reduction after 8 days of withholding water of  $91 \% \pm 4.8$  (n=6) and  $86 \% \pm 6.4$  (n=6) for photosynthesis and stomatal conductance, respectively, while isoprene was reduced by only  $27 \% \pm 4.3$  (n=6) after 10 days. During the recovery phase of both cycles, isoprene emission recovered more quickly than photosynthesis and stomatal conductance. The lower drought sensitivity of isoprene emission compared with photosynthesis resulted in a higher percentage of fixed carbon lost as isoprene as seedlings became more stressed, reaching peaks of 50% when photosynthetic rates had been depressed to almost zero. We concluded that these results generally support our hypothesis that during the early stages of drought isoprene biosynthesis could draw on the carbon pool from recently fixed carbon; isoprene emissions fall only when production is limited because of the high demand of carbon compared to the limited supply from the depressed

photosynthetic process. Isoprene emission rate showed a strong negative linear correlation to leaf water potential that could be a useful parameter to include in isoprene emission models to account for effects of drought stress on leaf isoprene emission rates.

## **Keyword index**

Isoprene, photosynthesis, stomatal conductance, oak seedlings, drought cycles, leaf water potential, model parameterisation, carbon loss.

## Introduction

Isoprene is the most abundant hydrocarbon emitted by many tree species with an annual global flux estimated at  $5 \times 10^{14}$  g year<sup>-1</sup> (Guenther 1995). Because its high reactivity, isoprene exerts profound effects on tropospheric chemistry with the production of ozone and other oxidants, and contributing to increase the lifetimes of greenhouse gases such as methane. Several factors are known to affect the emission of isoprene by plants. It is known that isoprene biosynthesis occurs within chloroplasts, and early experiments with labelled carbon dioxide (CO<sub>2</sub>) have shown that carbon is incorporated into isoprene within minutes (Sanadze, Dzhaini & Tevzadze 1972, Mgaloblishvili *et al.* 1979). Typically in non stressed condition around 2 % of the assimilated carbon is emitted as isoprene, which represents a non-trivial loss of carbon to the plant (Sharkey, Loreto & Delwiche 1991, Baldocchi *et al.* 1995, Monson and Fall 1989, Harley *et al.* 1994, Fang, Monson & Cowling 1996). It has been shown that isoprene emission is related to photosynthesis (e.g. Jones and Rasmussen 1975, Tingey, Evans & Gumpertz 1981, Monson and Fall 1989, Loreto and Sharkey 1990): both photosynthesis and isoprene emission increase with photon flux and have similar saturation levels (Sanadze and Kalandadze 1966, Harley, Monson & Lerdau 1999) although their response to other environmental factors differ. It has been shown that isoprene emission is highly temperature sensitive (Loreto and Sharkey 1990, Monson *et al.* 1992, Guenther *et al.* 1993, Sharkey and Loreto 1993, Harley, Guenther & Zimmerman 1997), more than photosynthesis. Furthermore, the responses of

photosynthesis and isoprene emission to water stress seem to differ. Whereas photosynthesis is clearly suppressed, isoprene emission can be stimulated under water stress conditions (Tingey *et al.* 1981, Sharkey and Loreto 1993, Fang *et al.* 1996). Although little work has been done on the effect of elevated CO<sub>2</sub> concentration on isoprene emissions, in most studies it has been observed that high CO<sub>2</sub> concentrations tend to reduce isoprene production by plants (Monson and Fall 1989, Loreto and Sharkey 1990). Recently, new evidence showed that isoprene biosynthesis depends on the chloroplastic production of its immediate precursor dimethylallyl diphosphate (DMAPP) (Rosenstiel *et al.* 2002), and that the decrease in DMAPP synthesis caused by increased intercellular CO<sub>2</sub> concentration ( $C_i$ ) is the mechanism that underlie the suppression of isoprene emission rates at elevated atmospheric CO<sub>2</sub> concentrations (Rosenstiel *et al.* 2003).

Although the purpose of isoprene emissions by plants remains unclear, studies by Sharkey and Loreto (1993) and Sharkey and Singsaas (1995) have suggested that isoprene emissions may be a protective mechanism by plants against stress. The authors hypothesise that isoprene is a membrane-soluble compound capable of quenching photooxidative products of photosynthesis and/or protecting membranes from detrimental phase changes caused at high temperature.

Future climate scenarios suggests increases in global mean temperature, lower precipitation in some regions of the world and increases in atmospheric CO<sub>2</sub> concentrations (IPCC 2001, Cox *et al.* 2000). As a result, there is increasing

concern that future increases in global temperature and drought could result in enhanced isoprene fluxes that could have profound effects on atmospheric chemistry. Therefore, further studies that can further our understanding of how temperature and drought affect isoprene emissions are clearly needed.

Although little work has been done on the effect of water stress on isoprene emissions, there is evidence that the overall effect of a decline in water availability is, in the short term, often an increase in isoprene emissions (Tingey *et al.* 1981, Sharkey and Loreto 1993, Fang *et al.* 1996). This could be the result of an increase in leaf temperature induced by reduced stomatal conductance and evapotranspiration, but none of the studies actually report any measurement of leaf temperature. Furthermore, upon relief of water stress, plants can exhibit higher rates of isoprene emission than in pre-stress conditions (Sharkey and Loreto 1993). Tingey *et al.* (1981) found that isoprene emission from live oak (*Quercus virginiana* Mill.) was not inhibited during short-term drought despite large reductions in photosynthesis. Sharkey and Loreto (1993) found a similar response in *Pueraria lobata* (Willd) Ohwi.

In order to improve our understanding of the climatological and physiological control on isoprene emissions from temperate tree species, a controlled laboratory experiment was carried out. *The overall objective of this investigation was to elucidate the effect of water stress on leaf isoprene emission, and to test the hypothesis that during a drought the depletion of the carbon pool available for isoprene biosynthesis is responsible for the decrease in isoprene emission rates.*

The specific **objectives** were:

- (a) To investigate the effect of water stress on isoprene emission, specifically the short (days) and long term (more than one week) response of isoprene emission to drought stress.
- (b) To explore the relationship between isoprene emission and photosynthetic rates.
- (c) To calculate the isoprene: carbon dioxide flux ratio, important in the closure of the global carbon cycle.

It was hypothesised that:

- (1) In the short term, the decrease in leaf stomatal conductance leads to an increase in leaf temperature that in turn induce the plant to maintain high rates of isoprene emission;
- (2) Isoprene emission is less sensitive to water stress than photosynthesis and high emission levels can be maintained until the depression of the assimilation process leads to the depletion of the carbon pool used for isoprene production.

## Materials and Methods

### *Plant material*

In January 2002, 16 two year old seedlings of Live oak (*Quercus virginiana* Mill.) were obtained from the Camellia Forest Nursery (Chapel Hill, NC, USA). The seedlings were transplanted to 6 litre plastic pots (30 cm high and 16 cm wide) containing commercial potting soil (Miracle Grow) and placed in the NCAR Phytotron (a temperature-controlled greenhouse with supplemental lighting) for 70 days until the end of March when the experiment started.

### *Growth conditions*

The environmental conditions in the phytotron were: daily average photosynthetic photon flux density (PPFD) of *ca* 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  at the canopy level, day length of 12 hours, air temperature range of 23 °C/19 °C day/night, and relative humidity of *ca* 40 %.

### *Experimental design*

Six of the seedlings in the phytotron were randomly chosen as 'control' plants (C) (well watered plants) and six as 'treatment' plants (T) (subject to drought stress). Of the remaining four seedlings, two were grown in well-watered conditions and two were subject to the same dry regime as the 'treatment'

plants ('mirror' seedlings), and were monitored continuously for leaf temperature and soil moisture with leaf thermocouples and delta T probes (manufacturer) respectively, connected to a datalogger (CR10, Campbell Scientific, USA). In order to take into account the influence of growing conditions inside the phytotron on isoprene emission rates, air temperature, relative humidity, and photosynthetic active radiation were continuously measured and stored on an hourly basis in a CR10 datalogger.

During the course of the experiment, water stress was imposed on eight previously non-stressed plants (six 'treatment' plus two 'mirror' treatment plants) by withholding water during two successive drying-rewatering cycles. In the first cycle water was withheld from March 21 to April 2 (12 days) and in the second cycle from April 18 to April 28 (10 days). For both cycles treatment seedling were watered to field capacity approximately 12 hours before the start of the drought phase of each cycle. Initially, photosynthesis and isoprene emission rates were measured every three days and then daily when isoprene rates started to change more dramatically after treatment. The drying cycle was ended when isoprene emission rates of the treatment plants were reduced to less than 50 % of the control plants' emissions. At the end of each drought period treatment seedlings were watered daily over the following recovery period (the first from April 2 to April 18, the second from April 28 to May 8) to allow plants to recover before starting the following cycle. The end of the recovery period in the first cycle was established as the time when the isoprene emission rates from the treatment plants reached the

same mean emission rate as that of the control plants. During the first cycle, control plants were watered every three days, whereas during the second cycle they were watered every other day. The watering regime was changed to every other day because during the first cycle the control plants suffered a slight water stress that affected mainly photosynthetic rates and stomatal conductance.

#### *Water status*

In order to monitor water stress the following parameters were measured: soil moisture, pot weight and pre-dawn leaf water potential. Soil moisture was continuously monitored over the duration of the entire experiment using two soil moisture sensors (ML2 Theta Probe, Delta-T Devices, Cambridge, UK). The two sensors were inserted to 10 cm depth into the pot of a 'treatment mirror' plant and a 'control mirror' plant, and hourly data were collected with a CR10 data logger. In addition, soil moisture was measured with a portable sensor (ML2x Theta Probe, Delta-T Devices, Cambridge, UK), inserted into the pot at the time of each gas exchange measurement. Pot weight and soil moisture were measured at the time of each gas exchange measurement just before putting the leaf into the leaf cuvette.

Pre-dawn leaf water potential was measured using detached leaves with a Scholander pressure chamber (Model 610 Pressure Chamber, PMS Instrument Co., Corvallis OR, USA). Because measurements with the pressure bomb are

destructive, leaves used for these measurements were collected from the treatment and control 'mirror' plants. However, during the drying period of the second cycle leaf water potential was also measured in leaves from the 12 treatment and control seedlings to have a larger number of replicates.

### *Sampling protocol*

All gas exchange, soil moisture, pot weight, and pre-dawn leaf water potential measurements were performed within one single day using one leaf per plant for the gas exchange measurements. Two different leaves on each plant were used for measurements in the two cycles. At the beginning of the experiment, fully expanded leaves from the top of the canopy were randomly chosen and tagged for subsequent measurements. Prior to measurement, every plant was taken from the greenhouse to an adjacent laboratory where a gas exchange measurement system was set up. Inside the laboratory, before the leaf was measured, the plant was left 15 min. to adapt to a higher light environment under a light providing a photosynthetically photon flux density of *ca* 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and was kept under the same light while measurements were taken.

### *Leaf gas exchange measurements*

Photosynthetic rate, stomatal conductance and intercellular CO<sub>2</sub> concentration were measured using a LI 6400 open path gas exchange measurement system (Li-Cor, Lincoln, NE, USA). This system measures water vapour (H<sub>2</sub>O) and carbon dioxide (CO<sub>2</sub>) exchange from a leaf surface with an infra-red gas analyser and allows the control of PAR, leaf and air temperature, humidity, CO<sub>2</sub> concentration and air flow inside the cuvette. To avoid strong oscillations in the CO<sub>2</sub> level of the air supply the LI 6400 inlet drew air from outside the laboratory and an empty volume? canister was placed in line before the instrument to buffer against short-term variations. An in-line bubbler was added to the system to humidify the air that was entering the LI 6400, because of the very low relative humidity of the external air.

For measurements of isoprene emission rates, a gas sample was withdrawn through a "T" junction from the outlet of the leaf cuvette and injected into a gas chromatograph used for isoprene concentration determination. For each measurement, an air sample of around 300 ml was pulled at a constant flow of approximately 80 ml min<sup>-1</sup> through a Teflon tube into a custom-made inlet system capable of vacuum sample collection and cryogenic isoprene enrichment.

Here it first was passed through a stainless steel loop packed with 60/80 mesh Tenax TA (Sigma Aldrich, Dorset, UK) kept at around 4 °C with Peltier coolers, where the isoprene was trapped while most of the water was

removed. The gas path was then changed and a flow of helium was passed through the trap while it was rapidly heated to around 220 °C using heating wire wrapped around the trap. The gas sample was pre-concentrated on a cryogenically (liquid N<sub>2</sub>) cooled stainless steel loop packed with Unibeads 3S, 60/80 mesh (Alltech Assoc., Deerfield, Illinois). The transfer time from the Tenax trap was 6 min. and the transfer flow 12 ml min<sup>-1</sup>. The concentrated sample was then desorbed by rapidly heating the preconcentrator to around 200 °C and transferred to a portable gas chromatograph (SRI 310, Buck Scientific, East Norwalk, CT, USA) by a flow of high purity He that was used as carrier gas. Isoprene was separated on a 0.25 mm ID x 30 m MXT-624 capillary column (RESTEK Corporation, Bellefonte, PA, USA) with a carrier (He) flow of 3 ml min<sup>-1</sup> and with a temperature program from 40 to 200 °C at 10 °C min<sup>-1</sup>. Isoprene eluting from the column was measured using a flame ionisation detector (FID), and the peak was integrated using PeakSimple-32 integrator (SRI, Buck Scientific, East Norwalk, CT, USA). Additional details of the analytical system can be found in Greenberg et al. (2003).

Before the start of the experiment the FID-gas chromatograph was tested for a few days for linear response and detection stability. The linearity and the good stability (around 2%) of the results suggested that a single injection of a known isoprene standard (25.5 ppb in N<sub>2</sub> mix) on each measurement day was sufficient to calibrate the system. The best peak definition was obtained with a transfer flow of 12 ml min<sup>-1</sup>, a transfer time of 6 min and a carrier flow of 3 ml min<sup>-1</sup>.

Measurements on each leaf were made at the same time each day (+/- one hour). All measurements were made under the same standard conditions: leaf temperature of 28°C, PPFD of 800  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and air flow of 400  $\mu\text{mol s}^{-1}$ . After a leaf was placed in the cuvette, a minimum of 10 min. was allowed for equilibration, and all measurements were made after steady-state conditions were realised, as indicated by continuous monitoring of CO<sub>2</sub> and H<sub>2</sub>O fluxes.

### *Statistical analyses*

In order to analyse the data, we separated the experiment in two cycles: cycle I (21 March-2 April) and cycle II (18 April-8 May) and each cycle in two phases: drought phase and recovery phase. For each one of these phases, the overall mean comparison of photosynthesis, stomatal conductance, isoprene emission rate, soil moisture and leaf water potential between treatment and control was analysed with a one-way analysis of variance (ANOVA) model I (Sokal and Rohlf, 1995). For photosynthesis and stomatal conductance, data were transformed into logarithms to satisfy the assumption of homogeneity of variance. The experimental unit was the individual seedling (n= 6). To take into account the overall effect during the course of the experiment, data were first analysed using a two-way ANOVA with repeated measures on drought as a factor (SAS software, proc GLM) where treatment, time and their interaction were considered. When this test was significant for treatment at a 5 % level of probability, a single ANOVA was used to test differences on each

date to understand how and when the treatment affected the specific variable under study. Differences were considered to be significant at a 5 % level of probability in all cases.

Linear and non-linear regressions (SAS software, Proc NLIN) were used to determine the relationships between different physiological variables. All statistical analyses were done with SAS software (SAS Institute Inc., Cary, NC, 1995).

## Results

### *Growth conditions*

Inside the phytotrone, plants were grown with an average daylight (7am to 19 pm) photosynthetic active radiation of  $296 \pm 3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$  ( $n = 49$ ) over the duration of the whole experiment (**Figure 1a**).

Leaf temperatures for treatment and control seedlings were on average  $23 \pm 0.1 \text{ }^\circ\text{C}$  ( $n = 48$ ). During the two periods of drought stress, leaf temperature of the treatment seedlings tended to be higher than leaf temperature of the control seedlings as a consequence of the reduced cooling effect of transpiration (**Figure 1b**).

From early March, there were two strong temperature declines caused by the external extreme weather (snowstorms). These temperature drops affected the phytotron internal temperature and clearly influenced leaf temperature as shown in **Figure 1b**. Towards the end of March, temperatures increased and remained stable in the phytotron during the whole period of the experiment.

Soil water content for the control seedlings was on average  $0.5 \pm 0.005 \text{ m}^3 \text{ m}^{-3}$  ( $n = 38$ ). During the two drought periods, soil water content decreased to less than  $0.1 \text{ m}^3 \text{ m}^{-3}$  in the first cycle, and to *ca*  $0.2 \text{ m}^3 \text{ m}^{-3}$  in the second cycle in the treatment seedlings (**Figure 1c**).

*Drought effect on leaf isoprene emission and gas-exchange parameters*

At the beginning of the experiment (March 21), there was no significant difference (all  $P > 0.01$ ) in soil water content (*ca*  $0.45 \text{ m}^3 \text{ m}^{-3}$ ), isoprene emission (*ca*  $17 \text{ nmol m}^{-2} \text{ s}^{-1}$ ) and gas exchange parameters (photosynthetic rate:  $5.56 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ ; stomatal conductance:  $0.048 \text{ mol m}^{-1} \text{ s}^{-1}$ ) between treatment and control seedlings (**Figure 2**).

In the treatment plants, after six days of treatment (March 21 to March 27) soil water content was reduced to *ca*  $0.1 \text{ m}^3 \text{ m}^{-3}$ . Drought strongly decreased the net photosynthetic rate and stomatal conductance to  $1.94 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$  and  $0.013 \text{ mol m}^{-2} \text{ s}^{-1}$  respectively on day 6 (March 27), while the isoprene emission rate remained essentially constant at approximately  $16 \text{ nmol m}^{-2} \text{ s}^{-1}$ .

Isoprene emissions started to decline daily from day 8 of the experiment (March 29) when soil water content was less than  $0.1 \text{ m}^3 \text{ m}^{-3}$  and leaf water potential was *ca*  $-1 \text{ MPa}$ . The isoprene emission rate decreased to  $6.23 \text{ nmol m}^{-2} \text{ s}^{-1}$  at the end of the first drying cycle on day 12 (April 2), with a leaf water potential of *ca*  $-2 \text{ MPa}$ . In contrast to the slow decline in the isoprene emission rate over time, the net photosynthetic rate and leaf conductance decreased very rapidly from day 2 to almost zero on day 8, and remained essentially constant until day 14. Both net photosynthetic rate and leaf conductance showed similar decreases as the decline in soil water content (although with a one day lag period)

In the control plants isoprene emission rates remained essentially constant at *ca* 18 nmol m<sup>-2</sup> s<sup>-1</sup>. Photosynthetic rate and stomatal conductance dropped from 5.8 μmol m<sup>-2</sup> s<sup>-1</sup> and 0.04 mol m<sup>-2</sup> s<sup>-1</sup> respectively on day 8 to 1.85 μmol m<sup>-2</sup> s<sup>-1</sup> and 0.013 mol m<sup>-2</sup> s<sup>-1</sup> on day 10, as a consequence of a small drought stress. Soil water content decreased from 0.42 m<sup>3</sup> m<sup>-3</sup> to 0.2 m<sup>3</sup> m<sup>-3</sup> during the three day interval between March 27 and March 30 during which plants were not watered

In the recovery period (from April 2 to April 18, 16 days), isoprene emission rates recovered quickly upon re-watering, reaching the control emission rate (*ca* 15 nmol m<sup>-2</sup> s<sup>-1</sup>) on day 13 (April 15). Photosynthesis and stomatal conductance still showed a slight sign of water stress at the start of the second cycle, but the differences between treatment and control plants on day 16 were not significant ( $P > 0.01$ ).

Similarly to the first cycle, in the second cycle (**Figure 3**) isoprene emission rates declined later than and decreased at a slower rate than photosynthetic rates and stomatal conductance, reaching values of 11.12 nmol m<sup>-2</sup> s<sup>-1</sup>, 0.1 μmol m<sup>-2</sup> s<sup>-1</sup>, and 0.002 mol m<sup>-2</sup> s<sup>-1</sup> respectively by day 10. From day 1 to day 10, soil moisture content decreased from 0.45 to 0.045 m<sup>3</sup> m<sup>-3</sup>.

In the second recovery period isoprene emission rates from treatment plants reached control emission rates on day 6 (May 4) at 20.5 nmol m<sup>-2</sup> s<sup>-1</sup>, with soil moisture having recovered to field capacity values (*ca* 0.5 m<sup>3</sup> m<sup>-3</sup>). The photosynthetic rate and stomatal conductance from the treatment plants

recovered to control plant values by day 10 (May 8) at  $5.74 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0.043 \text{ mol m}^{-2} \text{s}^{-1}$  respectively.

In the control plants, isoprene emission, photosynthetic rate and stomatal conductance remained essentially stable at around  $19.5 \text{ nmol m}^{-2} \text{s}^{-1}$ ,  $5.9 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $0.05 \text{ mol m}^{-2} \text{s}^{-1}$  respectively. Soil water content remained essentially constant at  $0.48 \text{ m}^3 \text{ m}^{-3}$ .

During the two consecutive cycles there was a significant difference between the response of isoprene rate and that of photosynthesis to changes in soil water content, leaf water potential and stomatal conductance ( $P > 0.01$  in all cases).

**Figure 4** shows the relationship between isoprene flux and soil water content (a) and between photosynthetic rate and soil moisture content (b) over the duration of the whole experiment, for the drought and recovery (i.e. initial well watered plus recovery) periods. As previously mentioned, photosynthetic rate responded to water stress much more rapidly than isoprene emission rate, with a distinctive response to soil water content during the drought period and the recovery period. For equivalent soil moisture status, both isoprene emission and photosynthetic rate showed higher rates during the drought phase than during the recovery phase.

The relationship between stomatal conductance, photosynthetic and isoprene emission rate and leaf water potential is shown in **Figure 5**. The effect of decreasing leaf water potential on photosynthetic rate (b) was very strong,

with photosynthetic rates immediately reduced to zero when leaf water potential reached *ca* -1 Mpa. This was probably a result of stomata closure and it is reflected by the decrease in stomatal conductance with the decrease in leaf water potential (a), which followed the same pattern.

Isoprene emission rate and leaf water potential showed a well correlated ( $R^2 = 0.64$ ) linear negative trend. Isoprene emission rate decreased with decreasing leaf water potential (b). However, the negative effect was not as pronounced as for photosynthetic rates, probably as a result of the little effect stomata have in controlling isoprene emissions from leaves (reference).

As expected, photosynthetic rate showed a strong linear correlation with stomatal conductance ( $R^2 = 0.93$ ) (**Figure 6a**) for both treatment and control plants. Isoprene emission rate did not show a linear correlation to stomatal conductance, varying between *ca* 0.02 and its maximum values of *ca* 0.06 mol m<sup>-2</sup> s<sup>-1</sup>. When stomatal conductance decreased below 0.02 mol m<sup>-2</sup> s<sup>-1</sup> in the treatment plants isoprene emission rate dropped dramatically.

The relationship between isoprene emission rate and photosynthetic rate for the treatment plants over the duration of the experiment, divided in drought period and watered period, is shown in **Figure 7**. Isoprene emission was substantially less sensitive to drought, beginning later and showing a slower decrease than photosynthesis, and was characterised by a faster recovery during the watered period.

In the treatment plants, water stress strongly affected the percentage of carbon fixed in photosynthesis and immediately lost as isoprene (**Figure 8**). At the beginning and the end of the experiment and during the recovery period, only around 2% of the fixed carbon was lost as isoprene. During the two severe water stress periods, on average around 30 % of photosynthetically assimilated carbon was emitted as isoprene, reaching a maximum of *ca* 50 % during the first period. **Figure 9** shows that although isoprene emission was placing a heavy demand on the pool of photosynthesised carbon mostly during the most severe period of the drought when photosynthesis rate was reduced almost to zero, the isoprene flux appear to be limited by the recently fixed carbon pool availability even when the demand is around only the 10%.

In the control plants the percentage of carbon fixed by photosynthesis and lost as isoprene remained essentially constant at *ca* 2% (**Figure 10**), with the exception of a rapid increase to values of *ca* 5% reached in correspondence of the slight water stress that the control plants suffered during the first cycle.

## **Discussion**

Tingey et al. (1981) conducted the first short term study (less than two months) of the impact of drought on isoprene emission. In this study they used potted seedlings of live oak. Another short term study was conducted later by Sharkey and Loreto (1993) using potted plants of Kudzu (*Pueraria*

*lobata* (Willd) Ohwi). Fang et al. (1996) conducted a long term study (more than four months) exposing potted seedlings of sweetgum (*Liquidambar styraciflua* L.) to nine successive drought and recovery cycles. Guenther et al. (1999) conducted the first study observing the effects of drought on isoprene emissions from field grown plants under natural drought conditions. They compared gas exchange and water relations from plants of *Berberis trifoliolata* and *Condalia hookeri* that had been growing in plots receiving supplemental water to that of plants receiving only natural rainfall.

The results of the present study are in general agreement with the results of these previous studies. In the long-term, isoprene emission was affected by drought but its short-term response differed from that of photosynthesis. Although isoprene emission rate decreased when the water stress was severe, it was considerably less sensitive to drought than photosynthesis and stomatal conductance. It was also apparent that isoprene emission could recover much more quickly than photosynthesis and stomatal conductance. Furthermore, we observed for the first time that for equivalent soil moisture status both isoprene emission and photosynthetic rates appear to have higher rates during the drying phase than during the recovering phase.

As found in previous studies (Tingey *et al.* 1981, Fall and Monson 1992, Fang *et al.* 1996), our results also indicate that unlike photosynthesis, the response of the isoprene emission rate to drought appears to be independent of stomatal dynamics. In the short-term, during the drought phase, even though stomatal conductance was drastically reduced over an eight day period, the

isoprene emission rates remained essentially constant. Only when stomatal conductance decreased below *ca*  $0.01 \text{ mol m}^{-2} \text{ s}^{-1}$  were isoprene emission rates drastically reduced. This finding suggests that stomatal conductance did not control isoprene emission rates and that an internal factor controlled the decrease in the emission rate when the water stress was severe.

Although the exact physiological role of isoprene emission is not yet clear, recent evidence indicates that isoprene may help to protect against rapid and frequent high temperature episodes (Sharkey and Loreto 1993, Sharkey and Singsaas 1995, Singsaas *et al.* 1997, Singsaas and Sharkey 1998, Singsaas and Sharkey 2000).

The maintenance of high emission rates during the drought periods could support the hypothesis of a thermo-protective role of isoprene emission. As suggested in our first hypothesis, during the drought periods the leaf temperature of the treatment plants was higher than the leaf temperature of the control plants as a consequence of the strongly reduced cooling effect of transpiration. Higher leaf temperatures could then induce higher rates of isoprene biosynthesis increasing its intercellular concentration and consequently increasing the driving force for isoprene emission that will compensate for the effect of a reduction in leaf conductance under drought conditions.

In a similar way isoprene emission appeared not to be directly dependent on the photosynthetic process. This result is in agreement with previous studies

that provided evidence that these two processes respond independently to changes in several environmental conditions (Monson *et al.* 1992, Kuzma and Fall 1993, Sharkey and Loreto 1993, Monson *et al.* 1994).

Sharkey *et al.* (1991) used labelled  $^{13}\text{CO}_2$  in oak leaves to demonstrate that isoprene is mainly produced from recently fixed carbon through photosynthesis. Our results indicate that isoprene emission is not closely linked to current net photosynthesis but it is likely that the decline in isoprene emission after a period of severe water stress may be the result of the depletion of the available carbon pool for its biosynthesis.

Furthermore, drought stress appeared to have a profound influence on the percentage of readily fixed carbon that is immediately lost as isoprene emission. Previous studies indicated that in non drought stressed plants approximately 1-2% of the photosynthetically fixed carbon was emitted as isoprene (Sharkey *et al.* 1991, Baldocchi *et al.* 1995, Monson and Fall 1989, Harley *et al.* 1994, Fang *et al.* 1996). During the current study, similar carbon loss ratios were found for the control plants (*ca* 2%). However, this percentage was calculated for measurements in the light and the percentage loss will be higher over an entire day when including nighttime respiration. In the treatment plants the proportion of carbon lost as isoprene increased dramatically during water stress periods, with peak values that exceeded values of 50%. These peaks were mainly caused by the major reduction of photosynthetic rates while isoprene emission remained at sustained levels. These results are in agreement with previous studies where it has been

observed that instantaneous losses of fixed carbon may exceed 10-20% under conditions of high temperature or drought (i.e., conditions under which net photosynthesis falls to low levels) (Tingey *et al.* 1981, Sharkey and Loreto 1993, Harley, Guenther & Zimmerman 1996, Fang *et al.* 1996).

The lower drought sensitivity of isoprene emission compared to photosynthesis generally resulted in a higher percentage of fixed carbon lost as isoprene as seedlings became more stressed. Our results seem to indicate that during the drought isoprene biosynthesis could continue to rely on the carbon pool from recently fixed carbon, and was only reduced when it became limited because of the high demand of carbon compared to the limited supply from the photosynthetic process. Therefore, these results generally support our second hypothesis that during a drought in the long-term (weeks) the maintenance of high isoprene emission rates and the depression of the assimilation process may lead to the depletion of the carbon pool available for isoprene production.

Previous research into the relationship between isoprene emission rates and atmospheric CO<sub>2</sub> concentration concluded that elevated CO<sub>2</sub> depresses isoprene production (Monson and Fall 1989, Sharkey *et al.* 1991, Guenther, Monson & Fall 1991, Rosenstiel *et al.* 2003). The competition for cytosolic phosphoenolpyruvate (PEP) being converted to pyruvate as necessary substrate for increased mitochondrial day respiration might be the cause of reduced availability of cytosolic PEP necessary for the synthesis in the

chloroplast of dimethylallyl diphosphate (DMAPP), the immediate precursor for isoprene biosynthesis (Rosenstiel *et al.* 2003).

The results of the present experiment carry further those conclusions with the observation that this limitation appears to be present also at ambient CO<sub>2</sub> concentrations and that a mild drought may work in favor of isoprene emissions. As shown, isoprene fluxes appear to be limited also at low carbon demand rate indicating that the pool of available carbon for isoprene synthesis might be limited by the partitioning of PEP in favor of mitochondrial respiration also in non stressed conditions.

In a climate change scenario with higher temperatures and prolonged droughts, the carbon lost as isoprene emission could dramatically increase with profound impact on the global carbon balance, especially in regions such as the tropics which are estimated to contribute more than 80% of the annual isoprene flux (Jacob and Wofsy 1988, Zimmerman, Greenberg & Westberg 1988, Guenther *et al.* 1995).

Although a substantial volume of literature has been published over the past ten years on isoprene emission by plants, there is a dearth of additional detailed drought studies with more species in order to be able to accurately model plant isoprene emissions to the atmosphere in different climate scenarios. It is also desirable to relate biogenic emissions to measurable physiological parameters that control emission variations. Leaf water potential is the most likely candidate for describing the role of water

limitations on biogenic emissions from leaves and there are several land surface models (LSMs) that can be used to predict it. This parameter also may be needed for estimating accurate leaf temperatures during drought conditions. As our results suggest, there may exist a tight relationship between isoprene emission and leaf water potential during water stress episodes, and we believe that leaf water potential could be a useful parameter to include in isoprene emission models to account for effects of drought stress. In **Figure 5** we reported the coefficients for the best fit of the relationship between isoprene emission rates and leaf water potential so that modellers could set up a first model parameterization that could be improved upon in the future.

Finally, investigations using potted plants and laboratory measurements provide valuable information from which to build hypotheses, but these studies often lead to results that differ from those observed using field grown adult plants in their natural environment. There is therefore a strong need for further field studies; from the few existing studies is not yet possible to produce any definitive model for the isoprene emission response to water stress in a natural ecosystem.

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Figure 1.

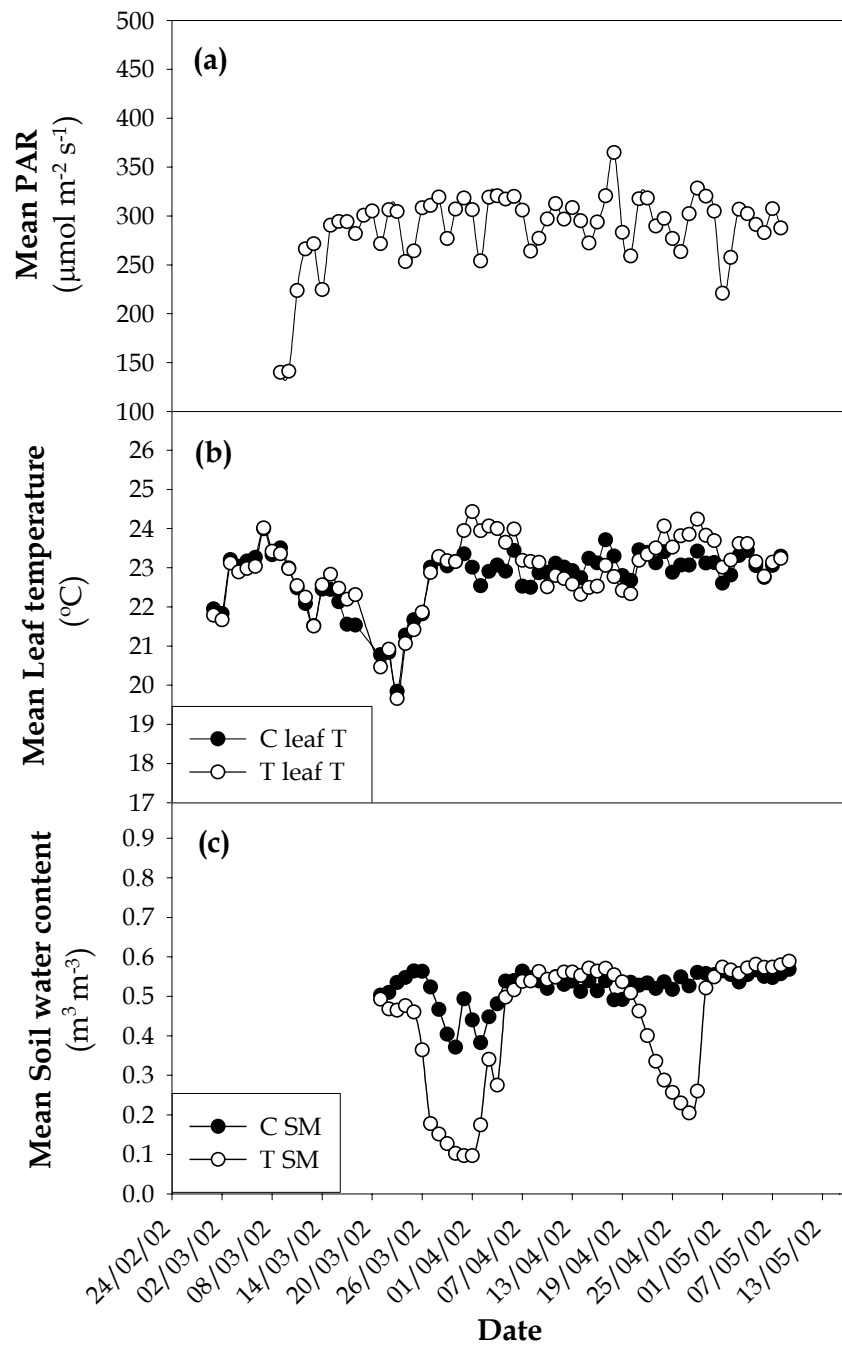


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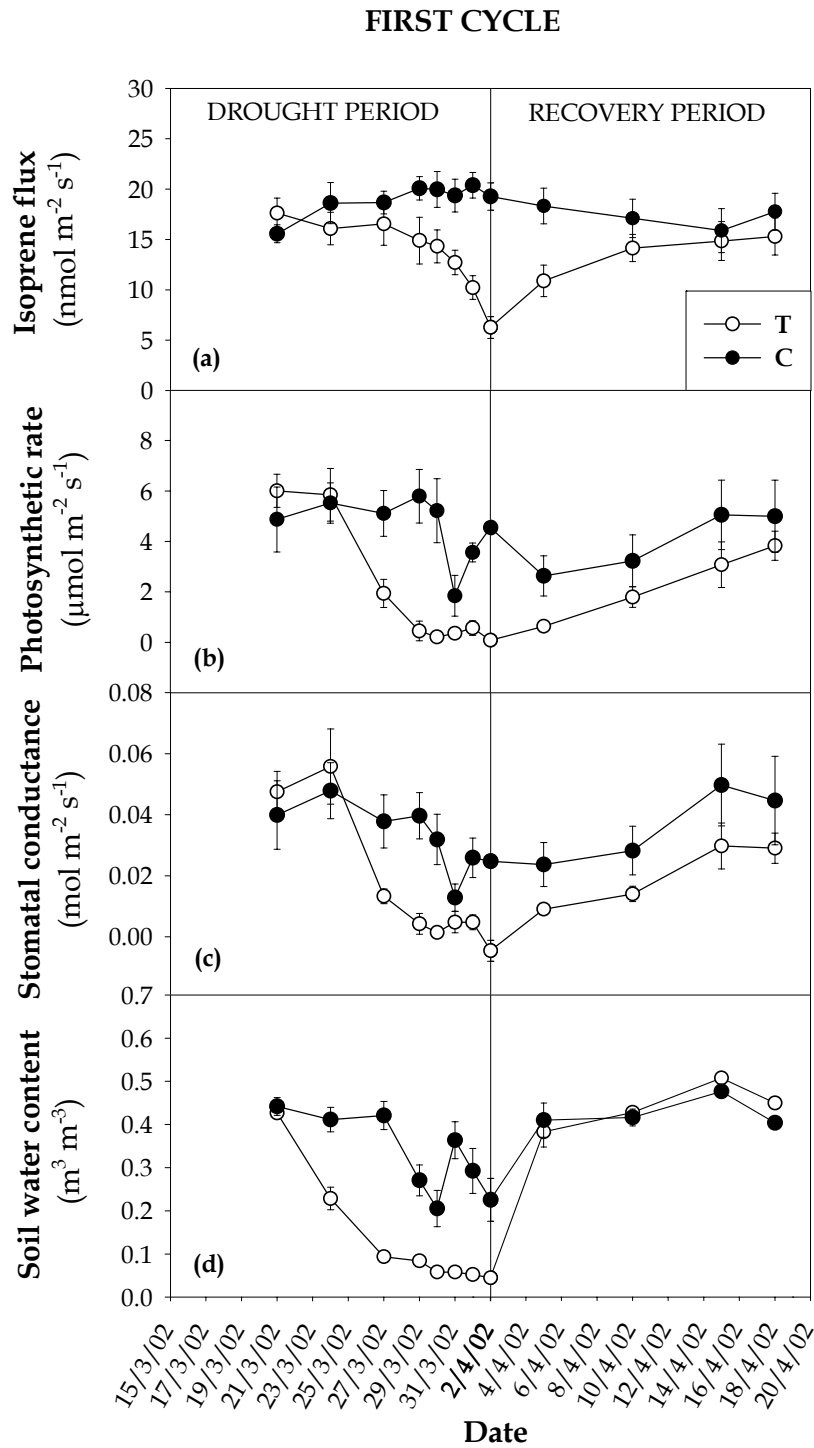


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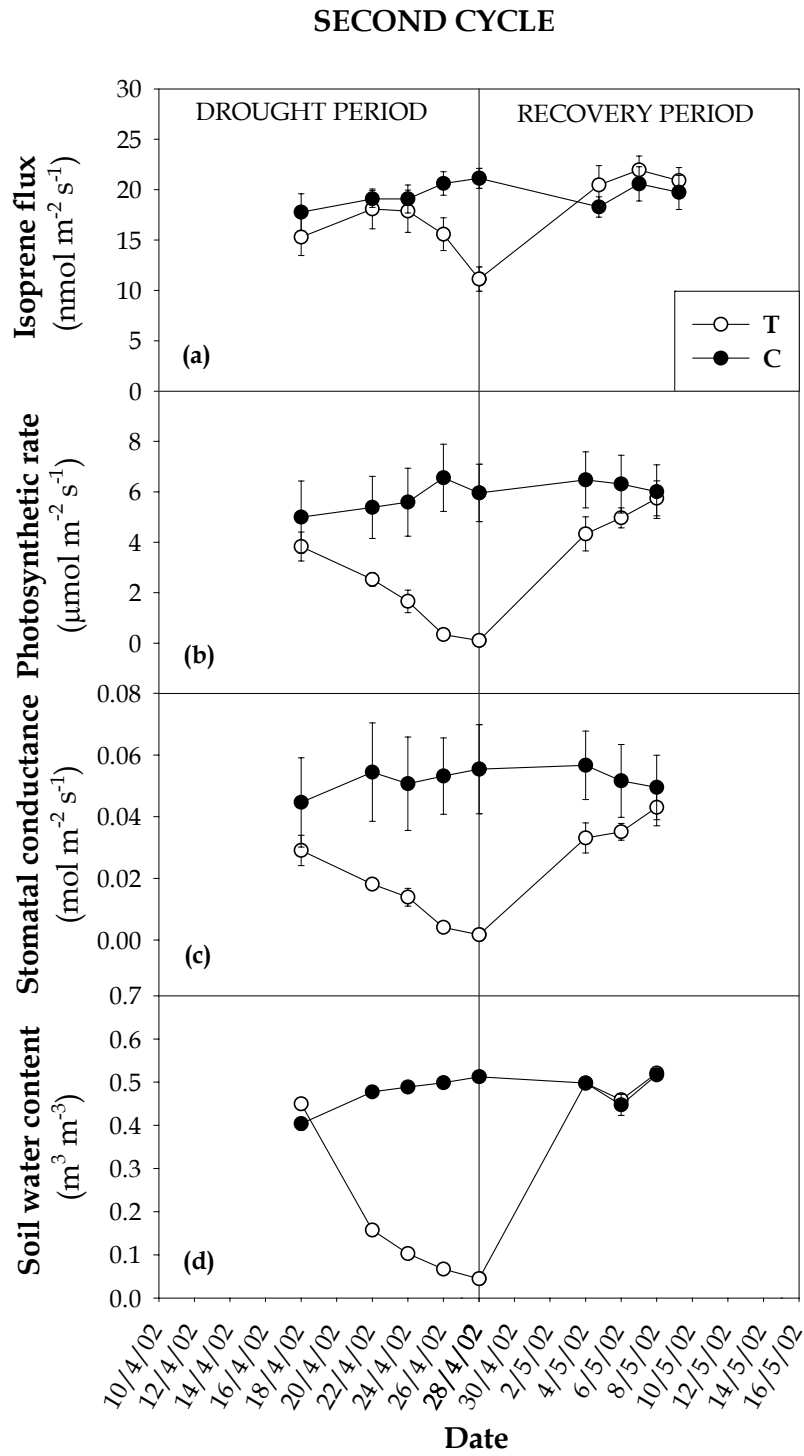


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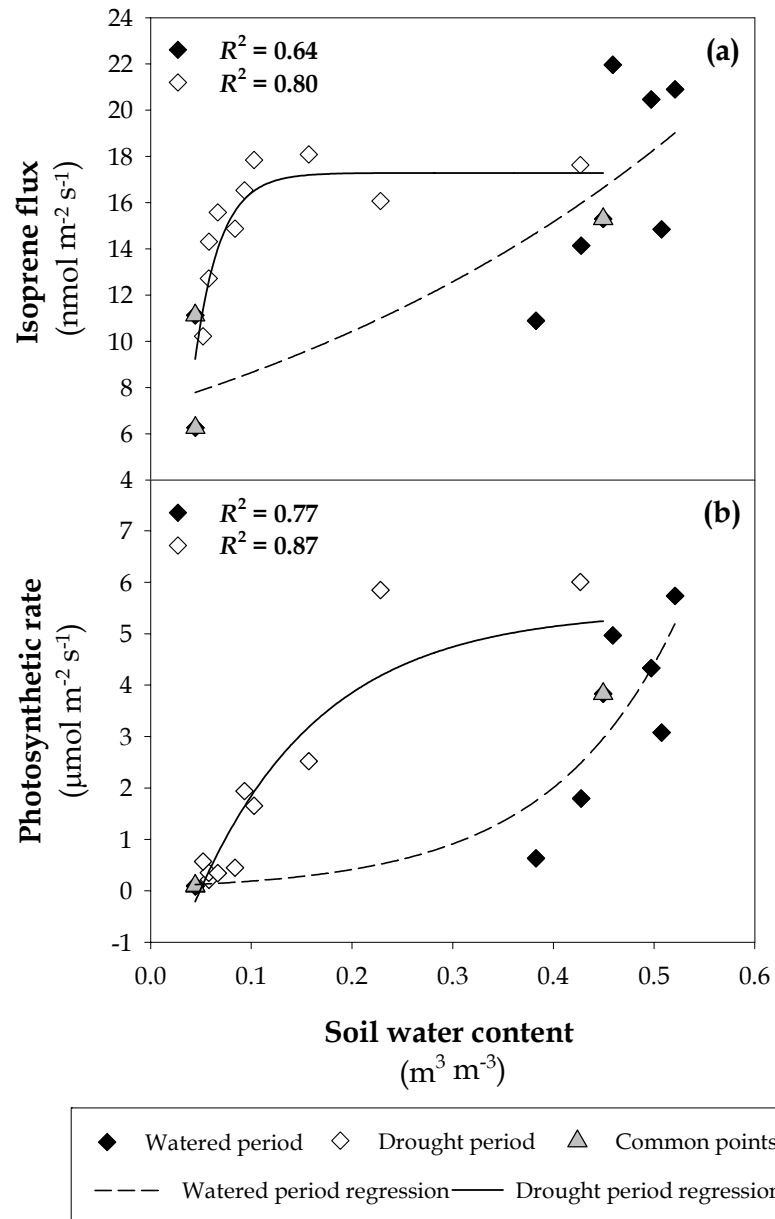


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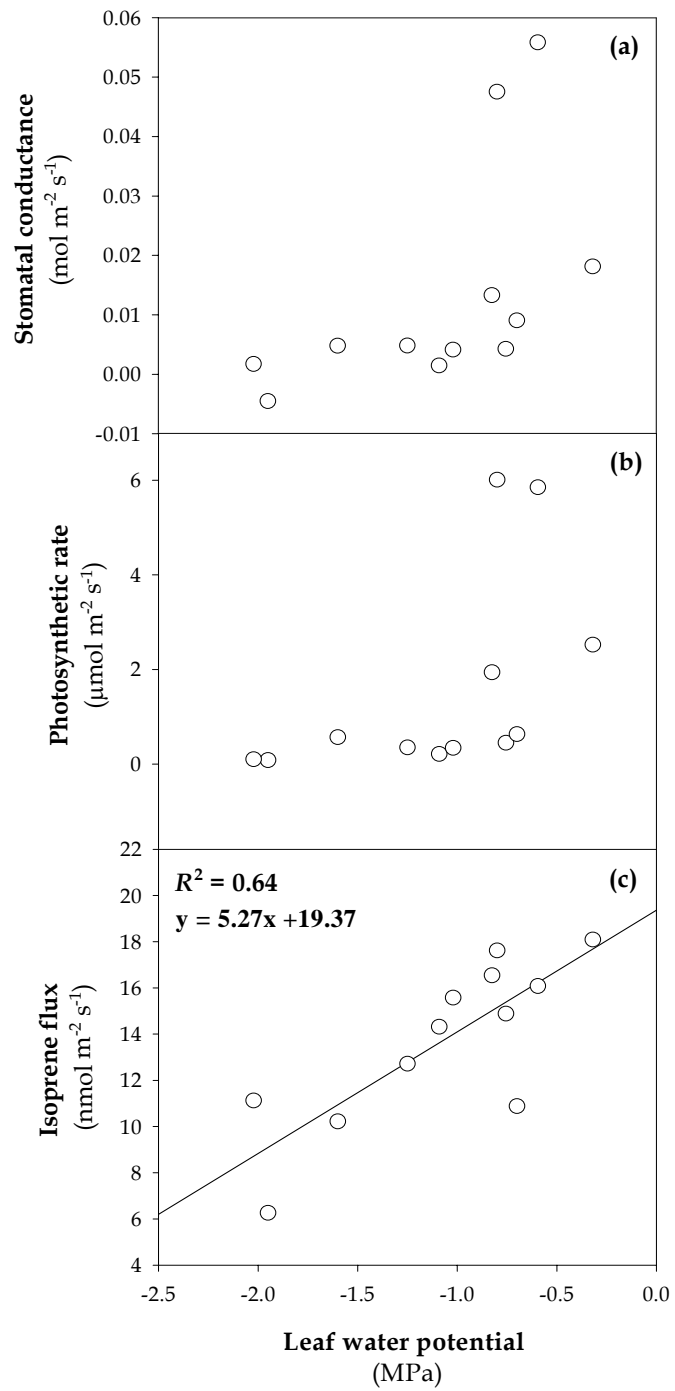


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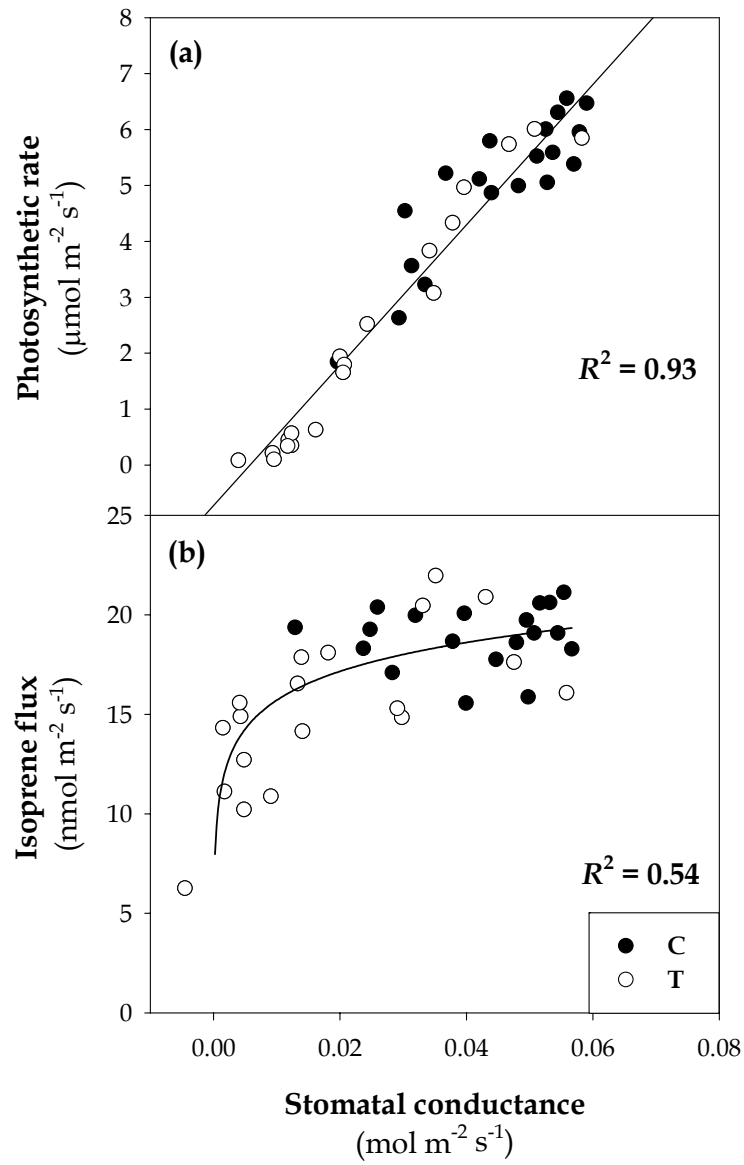


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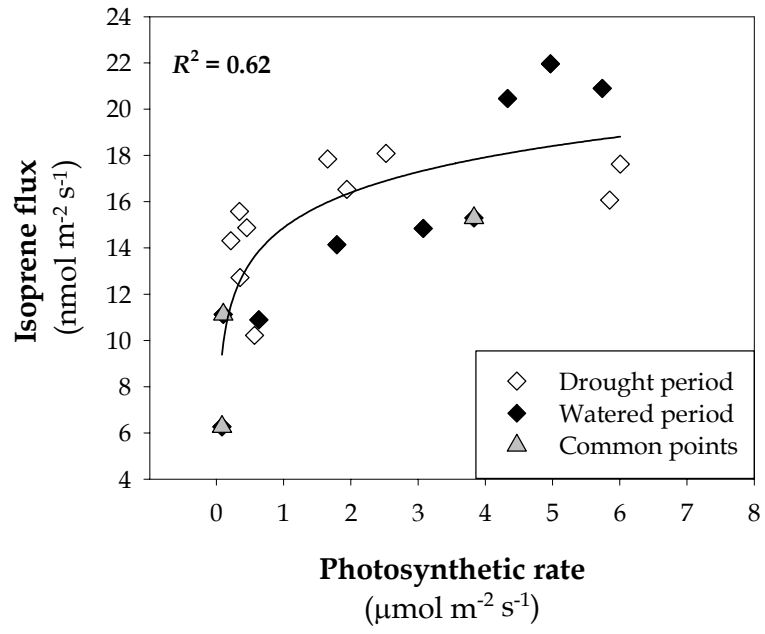


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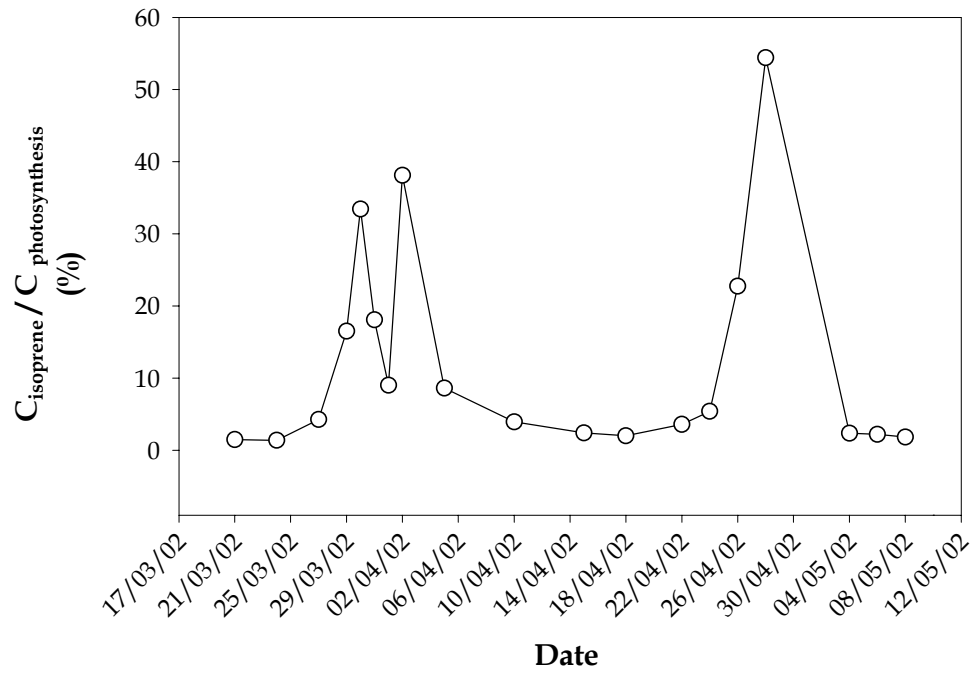


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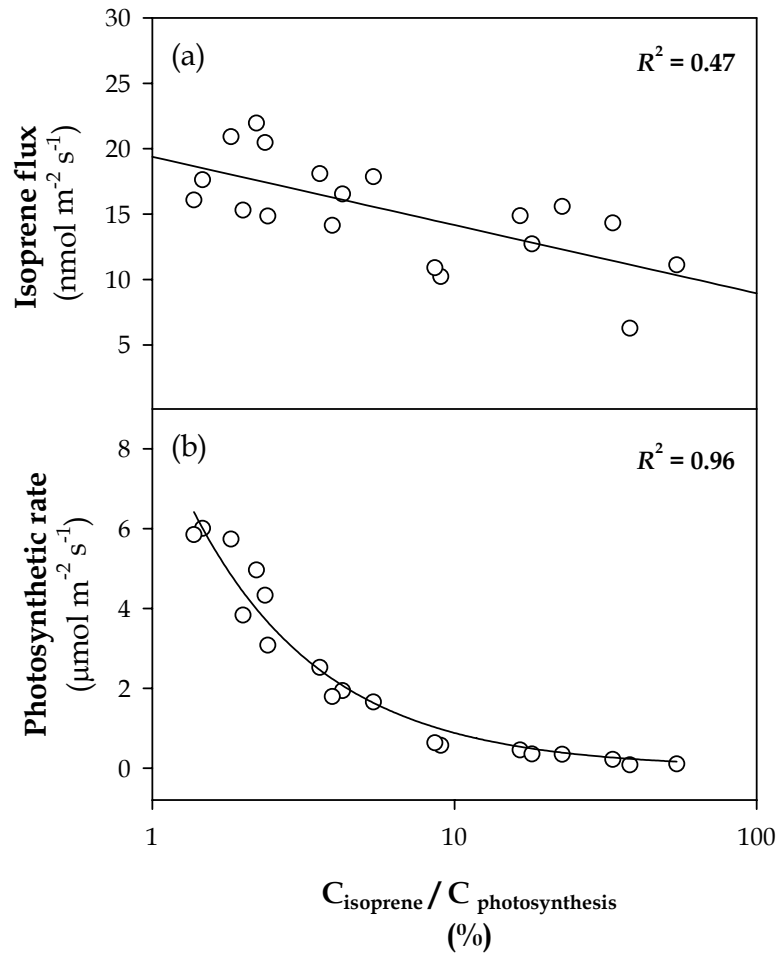
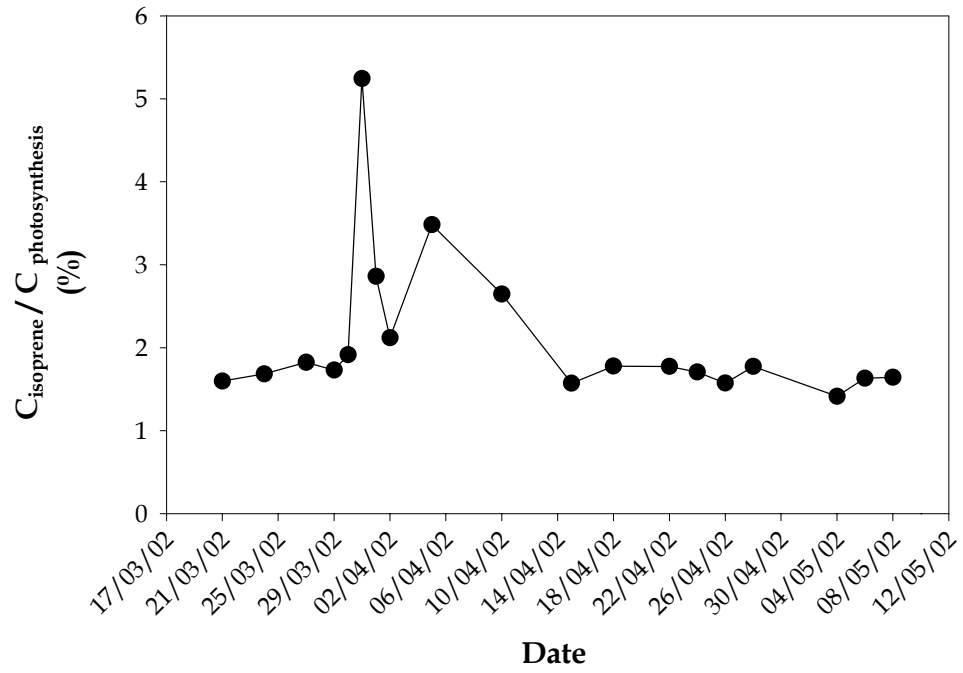


Figure 10.



## Figure Legends

**Figure 1.** Growth conditions inside the phytotrone during the experiment: daylight average photosynthetic active radiation (PAR) (a) and leaf temperature (b), and 24 hours average of soil water content (c) for treatment and control seedlings.

**Figure 2.** Time course of leaf isoprene emission rate (a), photosynthesis (b), stomatal conductance (c) and soil water content (d) during the first drying-rewatering cycle. Each point is the mean  $\pm$  SE (n = 6).

**Figure 3.** Time course of isoprene emission rate (a), photosynthesis (b), stomatal conductance (c) and soil water content (d) during the second drying-rewatering cycle. Each point is the mean  $\pm$  SE (n = 6).

**Figure 4.** Relationships between soil water content and isoprene emission (a) and soil water content and photosynthesis (b) for the treatment plants during the drought period and the recovery (initial well watered plus recovery) periods over the whole experiment (two cycles). Curves have been drawn only for a better illustration of the trend. Each point represents the average of six replicates.

**Figure 5.** Relationship between stomatal conductance (a), photosynthesis (b) and isoprene emission (b), and leaf water potential for the treatment plants during the whole experiment (two cycles).

**Figure 6.** Relationship between stomatal conductance and photosynthesis (a) and stomatal conductance and isoprene emission (b) for the treatment (T) and control (C) plants over the whole experiment (two cycles). Each point represents the average of six replicates.

**Figure 7.** Relationship between isoprene emission rate and photosynthesis for the treatment plants during the whole experiment (two cycles). Each point represents the average of six replicates.

**Figure 8.** Time course of the percentage of fixed carbon immediately lost as isoprene in the treatment plants. Each point represents the average of six replicates.

**Figure 9.** Relationship between the percentage of fixed carbon immediately lost as isoprene and isoprene emission rates (a) and the percentage of fixed carbon immediately lost as isoprene and photosynthesis (b) for the treatment plants during the whole experiment (two cycles). Note that the abscissa scale is logarithmic.

**Figure 10.** Time course of the percentage of fixed carbon immediately lost as isoprene in the control plants. Each point represents the average of six replicates.