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The effect of elevated CO_2, soil and atmospheric water deficit and seasonal phenology on leaf and ecosystem isoprene emission

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Abstract. Two cottonwood plantations were grown at different CO_2 concentrations at the Biosphere 2 Laboratory in Arizona to investigate the response of isoprene emission to elevated [CO_2] and its interaction with water deficits. We focused on responses due to seasonal variation and variation in the mean climate from one year to the next. In fall and in spring, isoprene emission rate showed a similar inhibition by elevated [CO_2], despite an 8–10°C seasonal difference in mean air temperature. The overall response of isoprene emission to drought was also similar for observations conducted during the spring or fall, and during the fall of two different years with an approximate 5°C difference in mean air temperature. In general, leaf-level isoprene emission rates, measured at constant temperature and photon-flux density, decreased slightly, or remained constant during drought, whereas ecosystem-level isoprene emission rates increased. The uncoupling of ecosystem- and leaf-scale responses is not due to differential dependence on leaf area index (LAI) as LAI increased only slightly, or decreased, during the drought treatments at the same time that ecosystem isoprene emission rate increased greatly. Nor does the difference in isoprene emission rate between leaves and ecosystems appear to be due solely to increases in canopy surface temperature during the drought, though some increase in temperature was observed. It is possible that still further factors, such as increased penetration of PPFD into the canopy as a result of changes in leaf angle, reduced sink strength of the soil for atmospheric isoprene, and decreases in the mean C_i of leaves, combined with the small increases in canopy surface temperature, increased the ecosystem isoprene emission rate. Whatever the causes of the differences in the leaf and ecosystem responses, we conclude that the overall shape of the leaf and ecosystem responses to drought was constant irrespective of season or year.

Additional keywords: Biosphere 2, climate, drought, interannual, leaf area index, NEE, photosynthesis, *Populus deltoides*, soil, temperature.

Introduction

Phytogenic isoprene plays a key role in atmospheric chemistry because it rapidly reacts with the hydroxyl radical (OH) (Fehsenfeld *et al*. 1992; Monson and Holland 2001). The reaction of forest-emitted isoprene with OH affects the radiative properties of the atmosphere by influencing the residence time of greenhouse gases, such as methane (Crutzen and Zimmermann 1991) and, in the presence of sufficient amounts of anthropogenic nitrogen oxides, affects atmospheric quality by forming tropospheric ozone, organic nitrates and organic acids (Fuentes *et al*. 2000; Monson 2002). Although the control that leaf temperature and incident light exert on isoprene emission

*Emiliano Pegoraro passed away on 22 April 2007, marking the premature end to a productive and influential scientific career. Emiliano was a friend to many in the isoprene emission research community. His seminal studies on the patterns and mechanisms underlying the responses of leaf and canopy isoprene emissions to drought, temperature and atmospheric CO_2 concentration should stand for a long time as exemplars for how to experimentally dissect the mechanisms underlying complex physiological processes. Emiliano's infectious love for life and science will be remembered and missed by his wife Ana, his family and many friends.

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is well known (Guenther *et al*. 1993; Harley *et al*. 1999; Lerdau and Gray 2003), the effect of other environmental variables has been less studied. In the last few years, there has been increased interest in improving our understanding of how forest isoprene emissions will respond to global change, including changes in elevated atmospheric CO_2 concentration and the frequency of drought (Monson *et al*. 2007).

Isoprene production inside the chloroplasts is controlled by the enzyme isoprene synthase (Silver and Fall 1991; Wildermuth and Fall 1996), which follows immediately from the methylerythritol 4-phosphate (MEP) pathway, and the availability of dimethylallyl diphosphate (DMAPP), the primary substrate of the MEP pathway (Lichtenthaler 1999; Sharkey *et al*. 2001). Isoprene is produced only in the presence of light and its emission responds to leaf temperature exponentially, which is typical of enzyme-controlled responses described by the Arrhenius model of temperature dependency (Monson and Fall 1989; Loreto and Sharkey 1990; Monson *et al*. 1992; Guenther *et al*. 1993; Singsaas and Sharkey 2000). Although isoprene is predominantly emitted through stomata, steady-state isoprene emission rates are independent of changes in the steady-state stomatal conductance (Fall and Monson 1992; Niinemets and Reichstein 2003). Although it has been shown that elevated atmospheric [CO_2] inhibits isoprene emission rate (Monson and Fall 1989; Sharkey *et al*. 1991; Rosenstiel *et al*. 2003; Scholefield *et al*. 2004; Pegoraro *et al*. 2005*b*), recently we have demonstrated that this effect may be compensated by water stress, which causes a decrease in intercellular CO_2 concentration (C_i) (Pegoraro *et al*. 2004*a*, 2005*b*). Furthermore, although the adaptive reasons for the production of isoprene in higher plants continues to be debated (Loreto and Velikova 2001; Sharkey and Yeh 2001; Rosenstiel *et al*. 2004; Owen and Peñuelas 2005), it is clear that emissions are stimulated by some stresses. During a mild drought, plants tend to maintain high isoprene fluxes despite large reductions in photosynthesis (Tingey *et al*. 1981; Sharkey and Loreto 1993; Fang *et al*. 1996; Pegoraro *et al*. 2004*b*), and isoprene emission may be stimulated upon recovery from water stress (Sharkey and Loreto 1993; although also see Monson *et al*. 2007 in which isoprene emission rate decreased after recovery from drought).

In temperate broadleaf tree species, seasonal variation of isoprene emission rate is controlled by both climate and phenology (Monson *et al*. 1994; Schnitzler *et al*. 1997; Fuentes and Wang 1999; Boissard *et al*. 2001; Kuhn *et al*. 2004). In general, isoprene emission is not detected in young leaves that emerge early in the spring. This is likely a result of control by springtime temperatures, rather than leaf developmental state (Wiberley *et al.* 2005). It is, therefore, crucial to take into account the seasonal and phenological changes in the activity of isoprene emission when developing models to obtain reliable estimates of regional and global isoprene emissions (Monson *et al*. 1995; Sharkey *et al*. 1999).

In order to better understand the interactions among atmospheric [CO_2], soil and atmospheric water deficits, and seasonal influences on forest isoprene emission, we conducted several experiments inside the controlled environment research facility of the Intensive Forestry Management (IFM) section of Columbia University's Biosphere 2 Laboratory near Tucson, Arizona. Through the use of this facility we were able to study emissions from model forest ecosystems under highly controlled environmental conditions. This allowed us to go beyond the typical type of study that has been conducted in the past using isolated leaves and potted plants. Our experiments were conducted over time intervals of several weeks and included forest plantations early in their seasonal development, and later in the growing season. By reproducing a drought experiment over non-consecutive years on plants grown for several years at ambient and elevated atmospheric [CO_2], we were able to understand whether the effect of drought and its interaction with elevated CO_2 was consistent in the face of changing phenology and seasonal and interannual changes in climate. Furthermore, we assessed these responses at both leaf and ecosystem scales.

Materials and methods

The Intensive Forestry Management (IFM) facility

Experiments were conducted in the IFM facility at the Biosphere 2 Laboratory, Oracle, AZ, USA (1130 m elevation, 32°35′N latitude, 110°51′W longitude), where three agriforest Cottonwood plantations (*Populus deltoides* Bartr.) were grown in three separate experimental bays (of ~12 000 m^3 volume and 550 m^2 soil surface). Each bay had independent control of atmospheric CO_2 concentration: 430, 800 and 1200 μmol mol^{-1} CO_2, air exchange, temperature, vapour pressure and precipitation (Osmond *et al*. 2004; Walter and Lambrecht 2004). The soil in the IFM facility has been previously described by Torbert and Johnson (2001). The cottonwood trees were planted from cuttings in 1998, coppiced at the end of each growing season through 2002, and exposed to controlled atmospheric CO_2 conditions during each growing season between the years 1999 and 2003 [for more details see Murthy *et al*. (2005)].

Experimental design

We focused on results from the 430 and 1200 CO_2 μmol mol^{-1} bays to simplify the study, and because the 430 and 1200 μmol mol^{-1} mesocosms have the most comparable diurnal patterns and intensities of photosynthetic active radiation; the 430 μmol mol^{-1} bay lies between the other two bays, and is therefore more affected in its diurnal light reception. Additionally, our past research has shown that the trees in the 800 μmol mol^{-1} growth treatment exhibit growth and physiological traits intermediate to those in the 400 and 1200 μmol mol^{-1} treatments (Rosenstiel *et al*. 2003; Barron-Gafford *et al*. 2005; Pegoraro *et al*. 2005*a*). In one detailed study of isoprene emission from poplar canopies in these same three bays with the same three CO_2 concentrations, Rosenstiel *et al*. (2003) showed that the response of emission rate to growth [CO_2] is approximately linear among all three concentrations. Thus, although 1200 μmol mol^{-1} [CO_2] may not be relevant to the immediate future of global atmospheric [CO_2], it represents one extreme of a linear response, and, thus, relevant to an analysis of the physiological response of the canopies to [CO_2]. During the course of the fall of 2000, fall of 2002 and spring of 2003, three drought experiments were carried out. In both experiments carried out during fall (2000 and 2002), soil water deficit and leaf-to-air vapour pressure deficit (VPD) were imposed on

the trees inside the bays, previously growing in non-stressed conditions. In spring 2003, only soil water deficit was imposed on the trees. Three experimental treatments were involved in this study:

(1) Water treatment: before all experiments, the two bays were watered to obtain field capacity. From 8 November 2000, 25 October 2002 and 14 May 2003 no water was added and the soil was left to dry naturally. The length of the water stress was variable between experiments. Drought was ended on 28 November 2000 (after 20 days), on 29 November 2002 (after 36 days) and on 6 June 2003 (after 22 days).

(2) VPD treatment: during the experiment in 2000 the two bays were subjected to two cycles of low and high vapour pressure deficit (VPD) treatments (1.0 and 2.5 kPa, respectively) each lasting seven days and starting on 25 October. The first cycle occurred when soil moisture remained at field capacity, and the second cycle was applied after irrigation had ceased. In 2002, the two bays were subjected to four cycles (of 3 days each) of low (~1 kPa) and high (~3 kPa) VPD starting on 24 October until 15 November when the high VPD level was imposed for the remainder of the experiment (this accelerated drying of the soil and accentuated the stress level on the plants). More details of treatments during the 2002 experiment can be found in a previous paper (Pegoraro *et al*. 2005*b*).

(3) CO_2 treatment: the treatments of 430 and 1200 μmol mol^{-1} of CO_2 for the east and west bays, respectively, were maintained for the duration of all experiments. In 2000, the bays were operated as open systems at night to maintain CO_2 values, and during the day, the bays were closed. Exchange rates at night were 68 and 40 m^3 min^{-1} for the 430 and 1200 μmol mol^{-1} CO_2 bays respectively, corresponding to approximate turnover times of 3 and 5 hours, with one hour of transition between 0600–0700 and 1700–1800 h. The exception was during the dry-down period of days 313–320 (9–16 November) when the bays were operated in flow-through mode during the day to dry out the soil. During the course of the 2002 experiment, the bays were closed for most of the day, allowing little exchange with outside air, with the exception of a period of 2–4 h at dawn, to facilitate the expulsion of night time respired CO_2; this period of dawn ventilation was necessary only if CO_2 rose inside the bay to such high levels that the next day trees were unable to bring down the CO_2 levels to the desired concentration.

Growth conditions

Incoming photosynthetic photon flux density (PPFD), reached ~2000 μmol m^{-2} s^{-1} at midday, air temperature was set at 27°C (day and night) for the experiment of fall 2000 until 3 November, when the temperature set-point was increased to 29°C. The set-point was 32/26°C day/night for the experiment of fall 2002 and 30/24°C for the experiment of spring 2003, and relative humidity ~75 and 30% for the low and high VPD settings, respectively, for both experiments of fall 2000 and fall 2002. Actual daytime air temperature tended to increase slightly above the set-points, as discussed below. More details on measurements of environmental variables can be found in a previous paper (Pegoraro *et al*. 2005*b*).

Ecosystem isoprene flux and net ecosystem exchange of CO_2 (NEE) measurements

Methods for measurements of isoprene concentration in the air of each bay in 2002–03 are described in previous papers (Pegoraro *et al*. 2005*a*, 2005*b*). Net exchange of isoprene in 2000 was calculated every 15 min with the following equation:

$$\text{Net exchange} = \underbrace{\text{Mixing}}_{(1)} - \underbrace{\text{Leaks}}_{(2)} - \underbrace{\frac{\Delta C}{\Delta t}}_{(3)} \qquad (1)$$

where isoprene production by the ecosystem is considered positive. 'Mixing' (Term 1) describes the flow of isoprene into and out of the system due to the mixing of outside air into the ecosystem. 'Leaks' (Term 2) describes the leak rates from one bay to the other bays and to the outside. Term (3) is similar to the calculation for a 'closed' system, and considers the time-dependent change in isoprene storage. Because in 2002 and 2003 the system worked as a closed system for most of the time, isoprene flux was calculated in the same way as in 2000 but without Term (1). Data from the periods when the system was open were excluded.

Net ecosystem exchange of CO_2 (NEE) was calculated continuously at 15 min intervals for each bay as μmol of CO_2 exchange per m^2 of ground area per second for the entire experimental period [for a more detailed description see Murthy *et al*. (2005)]. Data for the NEE calculation for each sampling period were collected at the end of the sampling period.

LAI was measured at the beginning and end of each drought treatment during all three drought experiments according to the methods described by Murthy *et al*. (2005).

Leaf gas-exchange measurements

We measured gas-exchange rates of leaves in the upper, sunlit portions of the cottonwood canopy. Photosynthetic rate, stomatal conductance and intercellular CO_2 concentration (C_i) were measured using a LI-6400 open path gas-exchange system (Li-Cor, Lincoln, NE). During the fall of 2000 experiment, isoprene was sampled with a glass syringe collecting 60 mL of the outgoing gas stream from the leaf cuvette, cryrofocused and then analysed with Gow-Mac gas chromatograph (Bethlehem, PA) using a column packed with 3S Unibeads from Alltech (Deerfield, IL) and a photo-ionisation detector from HNU (Newton, MA). Methods for measurements of isoprene emission rates during the experiments of fall 2002 are described in detail in a previous paper (Pegoraro *et al*. 2004*a*). During the experiment in spring of 2003, changes in isoprene concentration inside the leaf cuvette were measured by proton-transfer-reaction mass-spectrometry (PTR–MS). The cuvette exhaust was connected by 9-m long Teflon tubing (1.6-mm internal diameter) to a PTR–MS, Ionicon GmbH, Innsbruck, Austria) via a T junction in the tubing. Operational details of PTR–MS are described elsewhere (Lindinger *et al*. 1998; Warneke *et al*. 2001; Hayward *et al*. 2002). The air sample for isoprene concentration determination was pulled by the PTR–MS at a constant flow rate of ~12 μmol s^{-1}. Protonated isoprene (isoprene PA, 198.9 kcal mol^{-1}) was detected by the mass spectrometer as its molecular mass plus one (i.e. $M + H^+ = 69$) using a dwell time of 2 s (Hayward *et al*. 2002).

The instrument was calibrated before and after experiments by a three point calibration curve: pure certified standard (50 nmol mol^{-1}, Praxair Technology, San Ramon, CA), a dilution of the standard (25 nmol mol^{-1}) and zero air from a compressed air cylinder.

Over each experiment all measurements were made under the same cuvette standard conditions: air temperature of 30°C and PPFD of 1000 µmol m^{-2} s^{-1} in fall 2000; air temperature of 32°C and PPFD of 1500 µmol m^{-1} s^{-1} in fall 2002 and in spring 2003. In 2000, the relative humidity within the leaf cuvette was controlled to obtain a VPD of less than 2.0 kPa, whereas in 2002 and 2003 relative humidity was controlled to obtain a VPD similar to the growth conditions in the bays.

Data analysis

All statistical analyses were performed using SAS software (SAS Institute Inc., Cary, NC). Data were first analysed with a two-way ANOVA with repeated-measures with drought, treatment, and time and their interactions as factors. When this test was significant for treatment at the 5% level of probability, a single ANOVA was used to test differences on each date to understand how and when the CO_2 treatment affected the specific variables under study. When analysing the interactions of VPD and elevated [CO_2] on isoprene emission rates during the drought, data were first tested for significance using a three-way ANOVA. Variation around the mean ($n = 5$ or 6) is reported as 1 s.e.

Results

Environmental conditions

Over the course of the three month period during the fall experiments of 2000 and 2002, the mean midday photosynthetic photon flux density (PPFD) (measured between 1045 and 1545 h) decreased by 38 and 32%, respectively (Fig. 1). Days with rainstorms and heavy cloudiness caused a significant drop in the available light. Although these drops in solar radiation caused the short-term air temperature inside the bays to deviate from the set point (Fig. 1), mean daily air temperature remained essentially constant, rising only slightly (1–2°C) above the set-point during the drought period. In the fall of 2000 the deviation from the set point over the drought was more pronounced and midday mean air temperature increased from ~27°C at the beginning of the VPD experiment to ~30°C at the end of the drought experiment.

Over the course of the spring experiment of 2003, midday mean PPFD increased by 12% (Fig. 1). Similar to observations

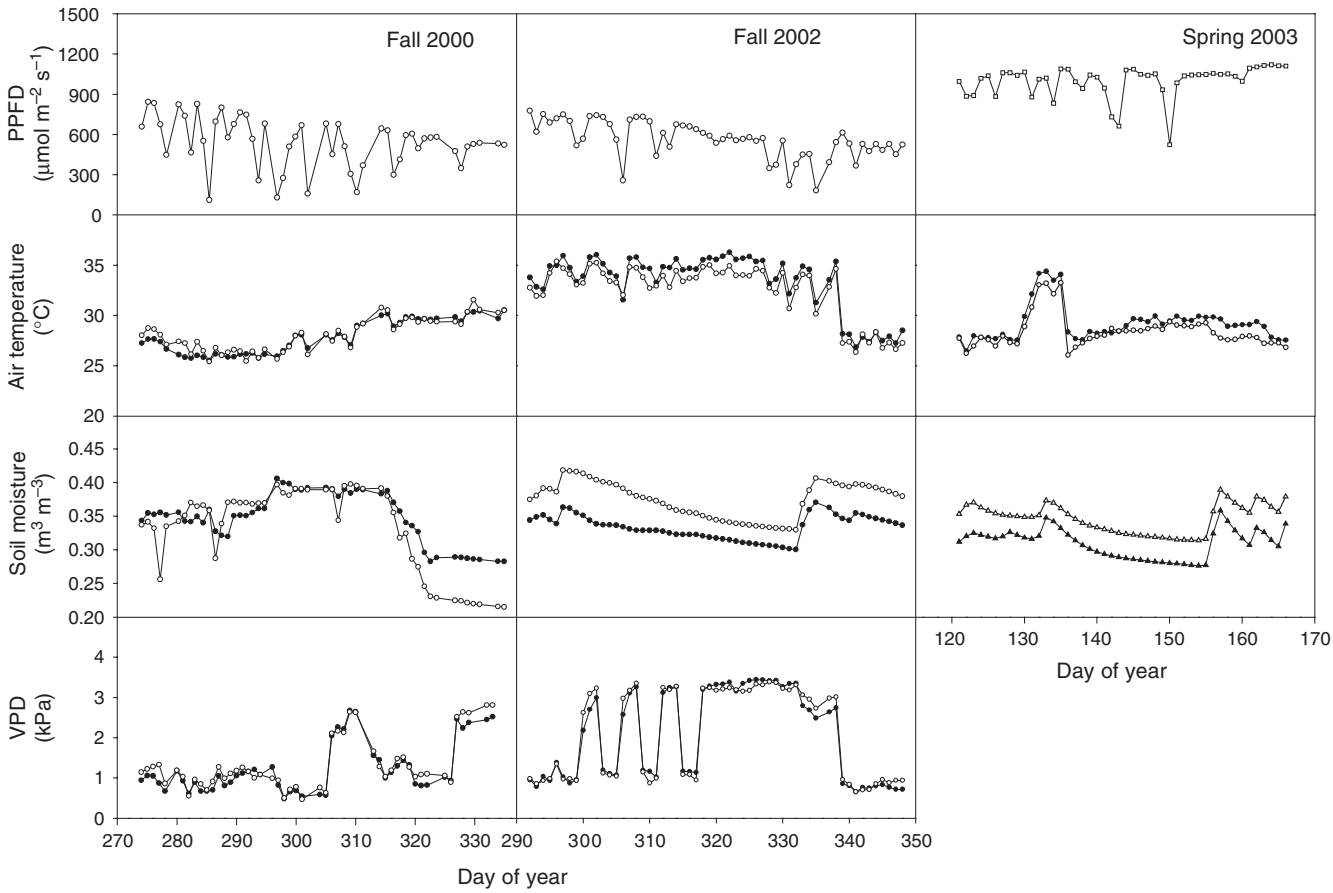

Fig. 1. Growth conditions inside the experimental growth bays of the Biosphere 2 facility during the drought experiments of fall 2000 and fall 2002: photosynthetic photon flux density (PPFD), air temperature (T_{air}), vapour pressure deficit (VPD) and soil moisture, for the ambient (white symbols) and elevated (black symbols) CO_2 treatments.

in the fall of 2002, the period of the spring 2003 experiment was characterised by the presence of days during which rainstorms and heavy cloudiness caused significant short-term variability in the mean air temperature. However, with the exception of a failure in the temperature control system on the day of the start of the experiment (12 May), which caused an increase in midday mean air temperatures from 28 to 34°C and lasted until Day 3 of the experiment (15 May), air temperatures remained essentially constant, rising only 2°C during the drought period.

Leaf gas exchange

Interannual variation in the response to water stress and [CO_2]

Leaf isoprene emission rates and photosynthesis rates measured in trees grown at ambient and elevated [CO_2] were consistently higher in the fall of 2002 than in the fall of 2000 ($P < 0.05$ for both fluxes), although the difference was more pronounced for isoprene emission rate (Fig. 2); these differences occurred concomitantly with higher temperatures in 2002. Despite interannual differences in the absolute magnitude of the isoprene and photosynthetic fluxes, both the isoprene emission rate and photosynthesis rate responded similarly to progressive drought in the two different years [for a more detailed description of the leaf level results in fall 2002 see Pegoraro *et al*. (2004*a*)]. In 2002, more frequent sampling of leaf gas-exchange rates provided us with a more detailed picture of dynamics in the responses of isoprene emission rate and photosynthesis rate, and the relationship between the two, compared with the experiment in 2000. In 2002, water limitation significantly reduced photosynthesis ($P < 0.05$ in both CO_2 treatments) rapidly, whereas the decline in isoprene emission rate was observed only when the water stress was severe. In the fall of 2000, isoprene emission rates exhibited significant reductions by the end of the 19-day drought experiment, but not as great as those exhibited for photosynthesis rate. In the fall of 2002, the warmer year, isoprene emission rates actually increased during the initial phase of the drought, at the same time that photosynthesis rates were decreasing. One consistent trend we observed for the 2 years is that isoprene emission rate decreased relatively less during the period of the drought in trees grown at elevated [CO_2] ($P < 0.05$), compared with trees grown at ambient [CO_2], despite similar reductions in photosynthesis rate.

Seasonal variation in the response to water stress and [CO_2]

Isoprene emission rates tended to be higher at the beginning of the drought experiment in spring 2003, compared with both fall experiments (Fig. 3). Similar to what we observed in the fall of 2002, leaf photosynthetic rates decreased rapidly from the beginning of the drought in both the elevated and ambient [CO_2] treatments. Photosynthetic rates also tended to recover quickly over the recovery period at the end of the drought, and on Day 5, after the first rain, rates of photosynthesis were not significantly different from pre-drought rates ($P > 0.05$). Similar to the fall experiments, isoprene emission rates from the trees grown at elevated [CO_2] did not show a significant ($P > 0.05$) decrease during the drought treatment, whereas emissions from trees grown in ambient [CO_2] did show a decrease as the drought progressed ($P < 0.05$). In the recovery period, isoprene emission rates quickly recovered and on Day 5, after the first rain, emission rates were not significantly different from pre-drought rates ($P > 0.05$).

Interactions among water stress, VPD and CO_2 treatments

Isoprene emission rate and photosynthesis rate responded similarly in trees exposed to elevated atmospheric [CO_2] in the fall experiments from the two different years (Fig. 2). In both years, in well-watered conditions and at low VPD, isoprene emission rates measured in elevated atmospheric [CO_2] were significantly ($P < 0.05$ in all cases) reduced (48 and 47% in fall 2000 and fall 2002, respectively). However, during the drought experiment in the fall of 2002, high VPD conditions always

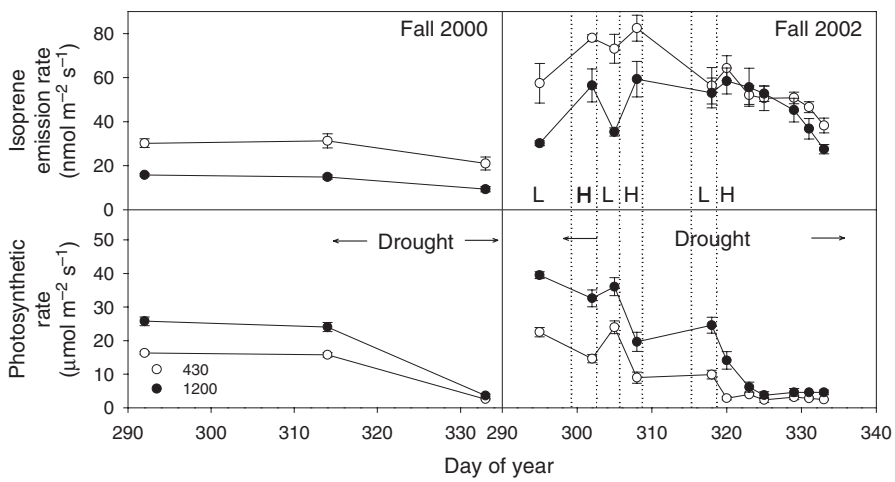

Fig. 2. Time course of leaf isoprene emission rate and net photosynthetic rate for the ambient (white symbols) and elevated (black symbols) CO_2 treatments during the course of the drought experiments of fall 2000 (measured at low VPD) and fall 2002 [measured at low (L) and high (H) VPD]. Values are means ± s.e. ($n = 5$).

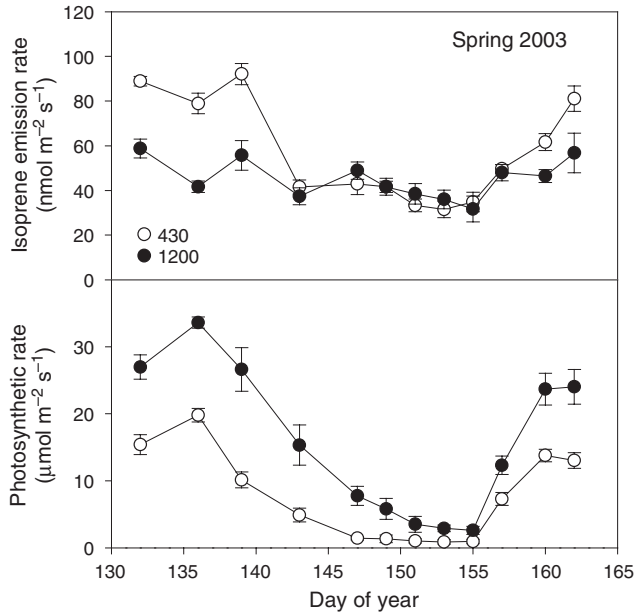

Fig. 3. Time course of leaf isoprene emission rate and photosynthetic rate for the ambient (white symbols) and elevated (black symbols) CO_2 treatments during the course of the drought experiment of spring 2003. The drought treatment was initiated on Day 131. Values are means ± s.e. ($n = 6$).

stimulated isoprene emission, especially in the elevated CO_2 treatment. Furthermore, the effect of VPD clearly interacted with the progressively increased water stress, and both treatments in turn interacted with the CO_2 effect [for a more detailed description of the 2002 results see Pegoraro *et al*. (2004*a*)]. As a result, in the second VPD cycle, the stimulation by high VPD tended to decrease while isoprene emission rates at low VPD were significantly higher ($P < 0.05$) (27 and 17% in the ambient and elevated CO_2 treatments, respectively). By the fourth VPD cycle, when the water stress was severe, isoprene emission rates were not significantly different between CO_2 treatments within or between both VPD treatments ($P > 0.05$ in all cases).

As expected and opposite to the response of isoprene emission, when measured in non-stressed conditions and at low VPD, photosynthesis was enhanced by elevated [CO_2], by 58 and 75% in the fall of 2000 and the fall of 2002 experiments, respectively. During the drought experiment of fall 2002, photosynthesis rate showed a response to both elevated VPD and water stress and their interaction was opposite to that observed for isoprene emission. Photosynthetic rates were always significantly reduced by high VPD compared with those measured at low VPD ($P < 0.05$). Furthermore, they tended always to decrease both at low and high VPD with the progression of the drought, further increasing the reduction in photosynthesis rate under high VPD conditions. However, similar to the response observed for isoprene emission, photosynthetic rates in the two CO_2 treatments responded differently to high VPD and drought. Photosynthesis was less sensitive to both high VPD and drought in the elevated CO_2 treatment. As a result, the difference in photosynthesis rate between CO_2 treatments disappeared later in the drought treatment than for isoprene emission rates, and photosynthetic rates were not significantly different from Day 26 onward. In the year 2000, the CO_2 suppression of isoprene emission was still apparent, even when the drought treatment had caused the photosynthesis rate to approach zero.

Ecosystem gas exchange

Interannual patterns in the response to CO_2, VPD and drought

Over the course of the fall 2000 drought experiment, NEE was higher for trees grown in the elevated [CO_2] treatment, but it declined as the drought progressed in both treatments (Fig. 4). Unlike the case for individual leaves, however, the ecosystem-level gross isoprene production (GIP) increased over

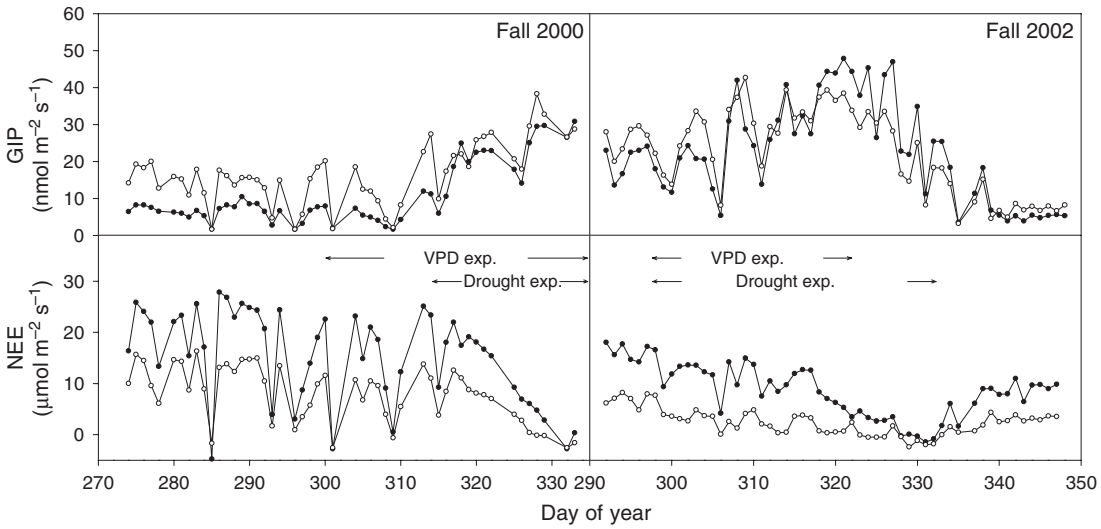

Fig. 4. Central daytime averages (1045–1545 h) of gross isoprene production (GIP) and net ecosystem exchange (NEE) for the ambient (white symbols) and elevated (black symbols) CO_2 treatments during the drought experiments of fall 2000 and fall 2002. Data for 2002 has already been published by Pegoraro *et al*. (2005*b*).

the entire drought treatment, even when NEE reached extremely low values. During the drought experiment of 2002, which lasted 36 days compared with the 20-day experiment of 2000, and was characterised by warmer temperatures, NEE also decreased to relatively low values, and GIP increased, at least during the initial ⅔ of the experiment; during the final ⅓ of the experiment, however, GIP decreased, in a pattern similar to what we observed for the leaf-level measurements.

In the fall of 2000, GIP from trees grown at elevated [CO_2] tended to remain lower than that from trees grown at ambient [CO_2] throughout the entire drought experiment. However, during the longer and warmer drought in the fall of 2002, the GIP from trees in the elevated [CO_2] treatment increased more during the initial ⅔ of the experiment, and decreased less during the final ⅓ of the experiment, compared with the trees from the ambient [CO_2] treatment ($P < 0.05$ for paired measurements from different treatments on the same date).

Over the drought period, GIP generally increased in both years and in both CO_2 treatments as a result of the interaction with soil drought and VPD. In the fall of 2000, GIP did not significantly increase over the first high VPD period ($P > 0.05$), perhaps because the high VPD treatment coincided with a period of heavy cloudiness. Compared with the average of GIP (calculated for days with full radiation) before the drought, in the second low VPD period during 2002, beginning after a week of drought, the average of GIP increased by 46 and 171% in the ambient and elevated CO_2 treatments, respectively. Towards the end of the drought experiment, when the water stress treatment interacted with high VPD, the average GIP further increased by 18 and 41% in the ambient and elevated CO_2 treatments, respectively.

LAI for the canopy during the drought experiment of fall 2000 increased slightly for trees in both the ambient and elevated [CO_2] treatments; from 2.1 to 2.5 m^2 (leaf area) m^{-2} (ground area) for the ambient [CO_2] treatment and from 4.1 to 4.5 for the elevated [CO_2] treatment (data not shown in graphs). The LAI for the canopies decreased slightly during the longer and more severe drought experiment of fall 2002 for trees in both the ambient and elevated [CO_2] treatments; from 2.0 to 1.4 for the ambient [CO_2] treatment and from 2.9 to 2.0 for the elevated [CO_2] treatment.

Representative diurnal courses of GIP for selected days at the beginning and end of the drought treatment in the fall of 2000 and the fall of 2002 are shown in Fig. 5. In non-limiting soil water conditions, and under low VPD, elevated [CO_2] resulted in a reduction of the average daily GIP (average calculated for both days between 0930 and 1430 h) of 61 and 55% for the fall of 2000 and the fall of 2002, respectively. When the water stress was severe, and under high VPD conditions, the reduction in GIP in the elevated [CO_2] treatment, compared with the GIP in the ambient [CO_2] treatment, decreased to 22% in the fall of 2000. In the drier days in the fall of 2002, the trees in the elevated [CO_2] treatment showed a GIP slightly higher (5%) but not significantly different ($P > 0.05$), than in the ambient [CO_2] treatment. As reported in detail in a previous paper (Pegoraro *et al*. 2005*b*), in the fall of 2002, the interaction of high VPD with soil water stress had a similar effect on GIP to that observed at the leaf level. High VPD always stimulated isoprene emission especially in the elevated [CO_2] treatment. Although this stimulation tended to decrease with the progression of the drought, in the last VPD cycle, when soil water stress was severe, GIP was stimulated also under low VPD conditions. As a result, the difference in GIP between the two CO_2 treatments tended to decrease.

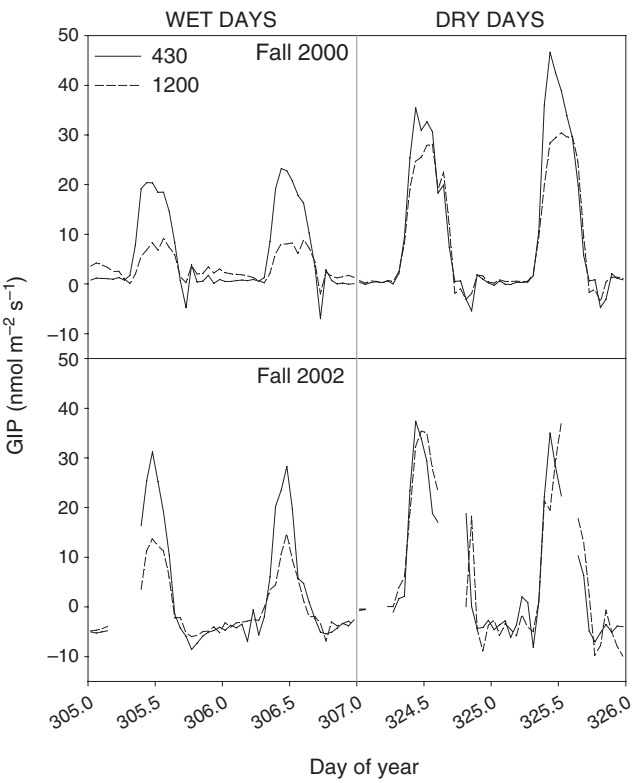

Fig. 5. Instantaneous gross isoprene production (GIP) over two wet and two dry days during the drought experiments of fall 2000 and fall 2002, for the ambient (solid line) and elevated (dashed line) CO_2 treatment.

Seasonal patterns in the response to CO_2, VPD and drought

In the drought experiment that was conducted during the spring of 2003, GIP increased throughout the progressive imposition of drought ($P < 0.05$) (Fig. 6), despite decreases in the leaf-level photosynthetic rate, and despite either constant or slightly decreasing rates of isoprene emission measured at the leaf level (Fig. 3). The increase in GIP during this springtime drought occurred in both the ambient and elevated [CO_2] treatments. There was sharp decrease in GIP on Day 150 because of a disturbance of chamber air flow patterns during the watering event. However, shortly thereafter, GIP decreased in response to the watering treatment. Once again, this pattern was opposite to the patterns observed in the leaf-level responses, in which isoprene emission rate increased in response to the re-watering event (see Fig. 3).

Similar to the fall 2000 and 2002 experiments, in the spring of 2003, GIP was affected by a 39% decrease due to growth at elevated [CO_2] ($P < 0.05$). Also, similar to the fall experiments, GIP showed a stronger increase in the elevated [CO_2] treatment (230%) than in the ambient CO_2 treatment (111%) ($P < 0.05$). As a result, between Days 11 and 14 of drought, when GIP

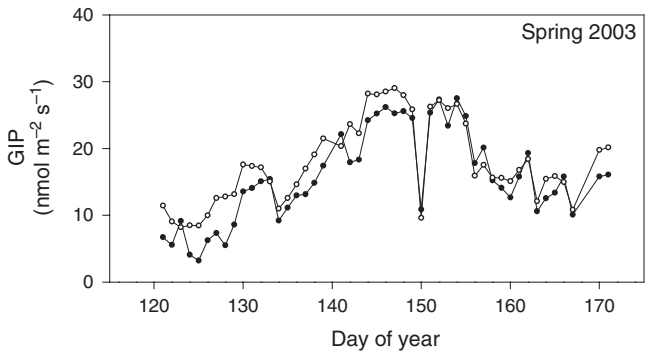

Fig. 6. Central daytime averages (0945–1545 h) of gross isoprene production (GIP) for the ambient (white symbols) and elevated (black symbols) CO_2 treatments during the drought experiment of spring 2003. The drought treatment was initiated on Day 131.

reached its maximum in the ambient [CO_2] treatment, the difference between CO_2 treatments was reduced to only 5% and not significantly different ($P > 0.05$). From Day 14, GIP in the ambient [CO_2] treatment tended to decrease, whereas in the elevated [CO_2] treatment it kept increasing, and over the last couple of days of the drought period the GIP in the elevated [CO_2] treatment was 14% higher than in the ambient CO_2 treatment. Similar to observations made in the fall of 2002, as soon as 'rainfall' was resumed, GIP decreased rapidly. However, at this time it tended to stabilise at values higher than in the pre-drought period, probably because of the higher light levels that occurred as the growing season progressed. Additionally, the difference between GIP in the CO_2 treatments was less than observed before the drought.

The LAI for the canopies were less than those during the fall experiments and decreased slightly during the drought experiment of spring 2003 for trees in both the ambient and elevated [CO_2] treatments; from 0.9 to 0.5 for the ambient [CO_2] treatment and from 2.2 to 1.6 for the elevated [CO_2] treatment (data not shown in graphs).

Discussion

Estimates of leaf isoprene emission rate still suffer from great uncertainty because of our poor understanding of the controlling patterns and processes that underlie variability in isoprene emission capacity. Funk *et al*. (2005) identified the main sources of variation in the isoprene standardised emission rate (SER, isoprene emission rate at a standard light and temperature) from *Quercus rubra* across broad spatial and temporal scales. The main conclusion from this work was that environmental drivers represent the dominant control over variability in isoprene SER, with inherent plant-to-plant variation making smaller contributions. Taking these conclusions a step further, future efforts to develop models aimed at understanding future and past trends in surface isoprene emissions and its effect on atmospheric chemistry should continue to focus on variation in the environment, including the climate, as primary determinants of SER. Our studies build on the insight provided in the study by Funk *et al*. (2005) by examining the potential for modest to moderate variation in the seasonal or interannual climate to cause variation in isoprene emission rate. These relationships are difficult to detect when working with field-grown plants and natural climatic variability. In our controlled-growth system, however, at the Biosphere 2 facility, we were able to manipulate the conditions of soil and atmospheric drought, as well as to examine time of year and interannual climate variation, to better elucidate the relevant responses in both the overall isoprene emission rate, the component processes that contribute to variation in emission rate, and the potential response of these processes to changes in the dominant seasonal and interannual forcing climate variables.

The responses of leaf isoprene emission rates to drought and elevated CO_2 have been observed previously in several studies. However, most of these studies concern leaf-level experiments carried out on potted seedlings or saplings (Tingey *et al*. 1981; Monson and Fall 1989; Guenther *et al*. 1991; Sharkey *et al*. 1991; Sharkey and Loreto 1993; Fang *et al*. 1996; Rosenstiel *et al*. 2003; Centritto *et al*. 2004; Pegoraro *et al*. 2004*b*; Rapparini *et al*. 2004); studies on whole isoprene-emitting ecosystems are more rare (Guenther and Hills 1998, 1999; Rosenstiel *et al*. 2003; Pegoraro *et al*. 2005*a*, 2005*b*, 2006). In this study, we observed differences in the responses of the leaf-level SER and ecosystem-level GIP to drought. As in previous studies, the decrease in isoprene SER that we observed in response to progressive drought was less than that observed for net photosynthesis rate or stomatal conductance. In contrast, we observed an increase in GIP at the ecosystem scale in response to drought. Our initial hypothesis to explain these results was that the increase in GIP was the result of an increase in LAI during the drought experiment, which would not be a factor in the leaf-level measurements, as these are conducted on a standardised amount of leaf area; this could explain the observed differences between the responses to drought at the leaf or ecosystem scales. However, the increase in LAI during the fall 2000 drought experiment was small compared with the increase in GIP, and there was a decrease in LAI during the fall 2002 and spring 2003 drought experiments. Thus, dynamics in LAI cannot provide a consistent explanation for the drought-induced increase in GIP, despite no increase in SER. Our second hypothesis was that increases in canopy surface temperature during the drought caused higher GIP, compared with no increase in temperature for the SER measurements (by definition, the SER is measured at constant temperature). For example, during the drought 2000 experiment, mean daytime surface canopy temperatures, measured with an infrared thermometer increased from 27 to 29°C in the ambient [CO_2] treatment, and from 27.5 to 30°C in the elevated [CO_2] treatment (data not shown). However, the GIP increased by 3–4 fold during this same period, which cannot be explained by the temperature increase, unless Q_{10} responses were in the range 9–32, which is much higher than the range typically reported for isoprene emission (Monson *et al*. 1992). We have no adequate explanation at the current time for the large increases in GIP at the ecosystem scale during drought, given no increase, or a slight decrease, in SER. It is possible that still further factors, such as increased penetration of PPFD into the canopy as a result of changes in leaf angle, and decreases in the mean C_i of leaves, combined with the small increases in canopy surface temperature, contribute to the increase in GIP. It is also possible

that there are yet to determine effects of changes in leaf age during the drought treatments; if the age profile of leaves in the canopy shifted from younger (with lower emission rates) to older (with higher emission rates) during the experiment, this may have contributed to the increase in GIP. Such an effect would probably have been greatest during the spring experiment. However, we note that the poplars were indeterminate in their growth, such that new leaves were continuously produced during both the fall and spring, and although the drought treatments did affect LAI, this was more the result of an increase in the abscission of mature leaves, than an inhibition in the initiation of new leaves (Murthy *et al*. 2005). Thus, the expected pattern would be one of loss of isoprene emitting potential of the canopy due to leaf age effects as the experiment proceeded. Finally, we note that during the same drought experiments reported in this paper (2002 and 2003), Pegoraro *et al*. (2005*a*) observed reductions in the soil sink strength for isoprene uptake. Although soil isoprene uptake tends to be modest in magnitude at natural ambient atmospheric isoprene concentrations, it can be significant under the mesocosm conditions used in this study, principally as a result of no UV radiation to drive the photooxidation of isoprene and reduced mixing of emitted isoprene into the above-canopy atmosphere. It is possible that decreases in soil isoprene uptake contribute to increases in the observed GIP during drought. Improvements in process models may provide a means of evaluating the complex interactions of numerous environmental factors in causing divergent patterns in GIP and SER during drought.

Whatever the causes of these divergent observations, our experiments show that they are constant irrespective of seasonal or interannual climate variation; in general, we observed an increase in ecosystem-level GIP during the initial phases of a progressive drought, irrespective as to whether it occurred in the spring or fall, or between different years (Figs 4, 6). Decreases in GIP occurred only in the late phases of the longer, more severe drought of 2002. Clearly, there must be extreme fluctuations in climate that would disrupt these fundamental responses, but within the scope of fluctuations imposed in this study, such responses were not evident.

The increase in GIP observed during the drought from the two CO_2 treatments was also reproduced by the high VPD treatments both at leaf and ecosystem level. Although isoprene emission showed always lower rates in the elevated CO_2 treatment, the stimulating effect of high VPD on isoprene emission in this treatment was stronger compared with the ambient CO_2 treatment. This was probably because the decrease in C_i caused by stomatal closure led to a stronger decrease of the inhibition effect of the elevated CO_2 in this treatment (Pegoraro *et al*. 2004*a*). However, this was partly compensated by the difference in the response of photosynthesis between CO_2 treatments. Because of the higher water-use efficiency of plants grown in elevated CO_2 than in ambient [CO_2] (Rey and Jarvis 1998), photosynthesis of leaves grown in elevated CO_2 was less sensitive to both water stress and high VPD than leaves grown in ambient CO_2. This may have caused a stronger inhibition of isoprene production in the elevated CO_2 treatment than in the ambient CO_2 treatment.

Our results in fall 2002 showed both, at leaf and ecosystem level, that isoprene emission started to decline only when water stress was severe (i.e. net assimilation was approaching zero). As shown in a previous paper (Pegoraro *et al*. 2004*a*) the depression of photosynthesis and the increase in isoprene emission caused by water stress and high VPD led to a dramatic increase in the ratio of fixed carbon lost as isoprene. The carbon loss observed in these drought experiments was similar to that observed for other species during drought, and carbon losses exceeding 50% have been observed when photosynthesis is reduced to almost zero (Tingey *et al*. 1981; Sharkey and Loreto 1993; Fang *et al*. 1996; Harley *et al*. 1996; Pegoraro *et al*. 2004*b*). Although most of the carbon in the isoprene molecule comes from recently-fixed photosynthate (Sharkey *et al*. 1991; Delwiche and Sharkey 1993; Karl *et al*. 2002), under stress conditions plants may use slow turnover, alternative carbon sources which may facilitate isoprene biosynthesis in the face of declining photosynthesis rates (Kreuzwieser *et al*. 2002; Funk *et al*. 2004; Schnitzler *et al*. 2004; Brilli *et al*. 2007). When water stress is severe, and causes prolonged depression of carbon assimilation, it is possible that the decline in isoprene emission reflects the depletion of these alternative carbon pools, or processes associated with posttranscriptional control of the expression of the enzyme isoprene synthase (Brilli *et al*. 2007).

In conclusion, we used the results from three different drought experiments conducted at two different times of the year, and across different years, and with whole-ecosystem isoprene fluxes as well as leaf-level fluxes, to show that while there are differences in the response to drought at different spatial scales (leaf *v.* ecosystem), these differences are consistent across seasons and in response to interannual climate variation. Although there were some differences among years and seasons, most of the responses to drought were similar; an increase in ecosystem-scale emission rate during the initial stages of the drought, despite decreases in leaf-level emission rates, and a decrease in ecosystem-scale emission rate during the late stages of the drought, despite increases in leaf-level emission rate seemed to be a constant observation. Thus, although seasonal and interannual effects are relatively small, ecosystem *v.* leaf effects are relatively large. Further clarification of the interactions causing these differences in spatial scale should help also clarify some of the temporal- and scale-dependent issues involved in construction of isoprene emission models.

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