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Light dependency of VOC emissions from selected Mediterranean plant species

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Abstract

The light, temperature and stomatal conductance dependencies of volatile organic compound (VOC) emissions from ten plant species commonly found in the Mediterranean region were studied using a fully controlled leaf cuvette in the laboratory. At standard conditions of temperature and light (30 °C and 1000 μmol m⁻² s⁻¹ PAR), low emitting species (Arbutus unedo, Pinus halepensis, Cistus incanus, Cistus salvifolius, Rosmarinus officinalis and Thymus vulgaris) emitted between 0.1 and 5.0 μg C (total VOCs) g⁻¹ dwh⁻¹, a medium emitter (Pinus pinea) emitted between 5 and 10 μg C (g⁻¹ dwh⁻¹) and high emitters (Cistus monspeliensis, Lavendula stoechas and Quercus sp.) emitted more than 10 μg C (g⁻¹ dwh⁻¹). VOC emissions from all of the plant species investigated showed some degree of light dependency, which was distinguishable from temperature dependency. Emissions of all compounds from Quercus sp. were light dependent. Ocimene was one of several monoterpene compounds emitted by P. pinea and was strongly correlated to light. Only a fraction of monoterpene emissions from C. incanus exhibited apparent weak light dependency but emissions from this plant species were strongly correlated to temperature. Data presented here are consistent with past studies, which show that emissions are independent of stomatal conductance. These results may allow more accurate predictions of monoterpene emission fluxes from the Mediterranean region to be made. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Biogenic VOC emissions; Mediterranean vegetation; Leaf cuvette; Environmental control of emissions

1. Introduction

Many volatile organic compounds (VOCs) are emitted from vegetation. Emissions of isoprene and monoterpenes have been studied extensively because of their role in atmospheric photochemistry and the formation of tropospheric ozone, and their indirect contribution to global warming (Fehsenfeld et al., 1992). Emissions of isoprene from plants are known to be both light and temperature dependent. Until recently it was thought that monoterpene emissions were dependent on temperature, but independent of light intensity (Guenther et al., 1995). However, several studies have reported light-dependent monoterpene emissions from different plant species (e.g. Yokouchi and Ambe, 1984: Pinus densiflora; Steinbrecher et al., 1991: Picea abies; Janson, 1993: Pinus sylvestris and P. abies; Hansted et al., 1994: Ribes nigrum flowers; Simon et al., 1994: Pinus pinaster; Staudt and Seufert, 1995: Quercus ilex; Schuh et al., 1997: Helianthus annuus and Fagus sylvatica; Staudt et al., 1997: Pinus pinea; and Shao et al., 2001: P. sylvestris).

Unless environmental controls of VOC emissions are understood, it is impossible to estimate even base emission factors (i.e. those measured at standard conditions of 30 °C and 1000 μmol m⁻² s⁻¹ PAR). The growth environment of a plant affects light dependency of emissions: for example, exposing a shade leaf of an
isoprene emitting plant to 1000 μmol m\(^{-2}\) s\(^{-1}\) PAR does not necessarily produce emissions of isoprene from that leaf equivalent to sun exposed leaves of the same plant (e.g. Harley et al., 1996).

Understanding the role of light intensity in regulating monoterpene fluxes from plants is important because of the part VOCs play in ozone formation in the troposphere. The construction of global VOC emission inventories depends upon the use of algorithms describing VOC emission rates under differing environmental conditions. If these algorithms and their underlying assumptions are incorrect, estimates of VOC fluxes, resultant ozone concentrations and other model outputs will also be incorrect. In this study, the effects of light and temperature on VOC emissions from ten common Mediterranean plant species were investigated in the laboratory under controlled conditions.

2. Materials and methods

2.1. Plant material

A total of ten Mediterranean plant species were studied in an initial investigation of light and temperature dependency of monoterpene emissions. Cistus incanus, Cistus monspeliensis, Cistus salviifolius, P. pinea, Pinus halepensis, Arbustus unedo. Thymus vulgaris, Lavandula stoechas and Rosmarinus officinalis and a Quercus sp., identified by the supplier as Quercus suber (but see discussion below), were obtained from commercial US nurseries. They were grown in a temperature-controlled greenhouse and watered daily. Because emission measurements from Q. suber in this study did not agree with values reported in the literature, a sample of this species was sent to Mediterranean Quercus experts in Montpellier. Identification of the specimen could not be confirmed, but it was reported that it was not a species native to French Mediterranean habitats (Trabaud, personal communication). In view of these uncertainties, the plants supplied as Q. suber will henceforth be referred to as “Quercus sp”.

2.2. Sampling device and analytical method

A dynamic flow-through, fan stirred leaf cuvette (nickel-plated brass, glass and a Delrin extension to accommodate pine twigs, Campbell Scientific Inc., Logan, UT, USA) with temperature control was used to sample emissions from individual leaves of each species. The cuvette was coupled to a capillary GC-FID analytical system (Greenberg et al., 1994). The humidity and carbon dioxide inside the leaf cuvette were controlled using a MPH-1000 plant gas exchange system (Campbell Scientific Inc.). Details of the gas exchange system are described elsewhere (Harley et al., 1998). Light was provided by a quartz halogen lamp (1000-W HID, Philips MS1000/BU) and varied by inserting neutral density filters of blackened window screen in the light path. Photosynthesis, transpiration and stomatal conductance (Gs) were calculated for each sample (von Caemmerer and Farquhar, 1981).

Samples from the leaf cuvette were transferred via a 1/16” o.d. stainless steel heated line (100°C) and preconcentrated in a stainless steel cold trap filled with glass beads cooled to −196°C by liquid nitrogen. The cold trap was then flash heated by plunging into a sand bath at 150°C to inject the sample directly to a Hewlett Packard 5880A gas chromatograph with FID detector, where separation was achieved on a DB1-fused silica capillary column (30 m × 0.32 mm i.d. × 0.3 μm). The detection limit for this system was 5 pg. Identification was by retention indices referenced to an alkane standard mix (Van den Dool and Kratz, 1963), and by GC-MS analysis of samples taken from each plant species onto 80 mg of 70/80 glass beads, 200 mg of 20/40 Carbotrap B and 350 mg of 60/80 Carbosieve III adsorbents in stainless steel cartridges (Supelco). Compounds were identified by MS in scan mode (HP MSD 5970) (Helmig and Greenberg, 1994).

2.3. Sampling artifacts

Loss of compounds to the leaf cuvette was investigated. A mixture of terpenes (~50 ppb p-cymene, ~7 ppb limonene, ~8 ppb ocimene, ~3 ppb γ-terpinene and ~3 ppb terpinolene) was introduced to the leaf cuvette air supply from a diffusion tube at concentrations similar to emissions from vegetation. Samples were analysed on the GC-FID on-line from the inflow to the leaf cuvette and from the sample outflow. There appeared to be no loss of compounds to the sampling system within the limits of standard deviation from the mean.

Some of the plant species studied possessed external glands that are very sensitive to mechanical damage, possibly releasing increased amounts of monoterpenes. In all cases, the leaf was inserted into the cuvette with extreme care and sensitive plants were allowed to equilibrate until emissions were constant. Projected leaf area was measured with a leaf area meter (Model LA-201, CID, Boise, ID). Plant material was then dried at 60°C for 48 h and weighed.

2.4. Calculation of emission rates

Emission rates (E), expressed as microgram (carbon) per gram leaf dry weight per hour (μg (C) g\(^{-1}\) dw h\(^{-1}\)), were calculated using

\[
E = FC(1 - MW)_{r}\times 36 \times 12/(1 - MW)\times W, \quad (1)
\]

where F is the air flow rate through cuvette (mol s\(^{-1}\)), C the concentration of emitted compound in cuvette (ppb),
MFWₐ, the mole fraction of water in air entering cuvette, MFWₖ, the mole fraction of water in air leaving cuvette, and W the dry leaf biomass (g). The constants 3.6 and 12 allow conversion of the emission rate unit to h⁻¹ and μg C, respectively.

Reproducibility of emission measurements at representative PAR and temperature values was checked at the beginning of the experiments (viz., Section 3.3), and was <5% (n = 3) RSD for ∑VOCs at constant temperature (PAR dependency experiments), and ~10% (n = 3) RSD for ∑VOCs at constant PAR (temperature dependency experiments). RSD (n = 3) varied from 0.2% to 24.6% for emissions of the individual compounds that showed light dependency. At least 30 min equilibration time was allowed at each PAR or temperature value before measurements were made.

2.5. Modelling emission rates

The relationship defined by the isoprene emission algorithm of Guenther et al. (1995, “G95”) was used to fit the observed data:

Emission rate = EₛCᵣCₔ, (2)

Cₔ = (zCₛₐL)(1 + x²L²)⁻⁰·₅, (3)

Cᵣ = {exp[Cₛₜₙ(T - Tₛ)(RTₛT)⁻¹]}

{1 + exp[Cₛₜₙ(T - Tₘ)(RTₛT)⁻¹]⁻¹}, (4)

where Eₛ is the emission rate at 30°C and 1000 μmol m⁻² s⁻¹ PAR. Empirically derived constants are z = 0.0027 m² s⁻¹ μmol⁻¹, CₛₐL = 1.0666 units, Cₛₜₙ=95,000 mol⁻¹, Cₛₜₙ=230,000 mol⁻¹, Tₘ=314 K and R = 8.314 J K⁻¹ mol⁻¹. L is the flux of PAR (μmol m⁻² s⁻¹) and Tₛ is the leaf temperature at standard condition (303 K for this study). At standard conditions of 1000 μmol m⁻² s⁻¹ PAR and 303 K, (CₛₐL Cₛₜₙ)ad = 1.

The G95 monoterpane algorithm of Guenther et al. (1995) describes monoterpane emissions as an exponential function of temperature:

M = exp(β(T - Tₛ))Mₚₛ, (5)

where M is the monoterpane emission rate (μg g⁻¹ dw h⁻¹) predicted at temperature T (K), Mₚₛ is a base emission at standard temperature, Tₛ is a standard temperature (303 K) and β is an empirical coefficient. The value of β is normally taken to be 0.09 K⁻¹, but in fact it can vary according to species and environmental conditions (e.g. Owen et al., 1997; Street et al., 1997). To improve the fit of the G95 algorithms to some of the experimental data, parameter values were generated using non-linear regression with quasi-Newton estimation of loss function in a statistical package (Statistica, Statsoft Inc, Benelux BV).

3. Results

3.1. Preliminary laboratory screening for PAR dependency

Table 1 lists ten plant species that were screened for PAR dependency at 30°C with their emitted compounds, and with total emission rates for those species whose leaf area could be measured expressed as both μg (C) g⁻¹ dw h⁻¹ and as ng (C) m⁻² leaf s⁻¹. At least one emitted compound from each of the species screened showed evidence of some light dependency. Emissions of ocimene from P. pinea showed particularly strong light dependency, with no emissions in the dark.

Emissions from Quercus sp. showed the strongest light dependency. At 30°C, seven compounds (2-methyl-1-propene, isoprene, x-pinene, camphene, sabinene, β-pinene and limonene) were emitted only minimally from Quercus sp. in the dark, compared with emissions of up to 56 μg (C) g⁻¹ dw h⁻¹ at 1000 μmol m⁻² s⁻¹ (Table 1). A. uedo and P. halepensis emitted the least ∑VOC (0.1 and 0.1 μg (C) g⁻¹ dw h⁻¹, respectively) in the light. However, even these very low emitting species demonstrated light dependency of a compound tentatively identified as p-cymene (Table 1). Of the remaining plant species, four emitted 0.5–5.0 μg (C) g⁻¹ dw h⁻¹ (“low” emitters: C. incanus, C. salvifolius, R. officinalis and T. vulgaris), one emitted 5–10 μg (C) g⁻¹ dw h⁻¹ (“medium” emitter: P. pinea) and three species emitted >10 μg (C) g⁻¹ dw h⁻¹ (“high” emitters: C. monspeliensis, L. stoechas and Quercus sp.).

Because PAR affects stomatal conductance (Gs), it was difficult to determine whether the changes in emission rates observed in this preliminary screening experiment were caused by changes in PAR alone or were due simply to changes in Gs. A plot of the light:dark ratio of emission rates against the light:dark ratio of Gₛ (Fig. 1) showed that for most species, the light:dark emission rate ratio is around 1.5–2.5. Exceptions are A. uedo, P. halepensis and Quercus sp., the first two of which had very low emission rates with associated larger errors in emission calculations and are omitted from Fig. 1. Although the light:dark emission ratios are very similar for most plant species, the light:dark Gₛ ratios vary between 2 and 14 (Fig. 1).

Three plant species were selected for further detailed investigation of PAR dependency of monoterpane emissions: Quercus sp. as a strong light-dependent “high” emitting species, P. pinea as a highest “medium” emitter with strong light-dependent ocimene emissions, and C. incanus as a representative of the “low” emitting group, but with the greatest number of compounds exhibiting apparent light dependency.

Two sets of experiments were performed on each species. A different plant was used for each experiment and a different leaf was used for replicates of each
| Retention time | Compound          | Quercus sp. | P. halepensis | C. monspeliensis | P. pinea | C. incanus | C. salviolus | A. unedo | T. vulgaris | R. officinalis | L. stoechas |
|----------------|------------------|-------------|---------------|-----------------|-----------|------------|-------------|---------|------------|---------------|------------|
|                | Light Light      | 0.16 0.08   | 0.07 0.06     | 1.70 0.82       | 0.19 0.08 | 0.40 0.23  | 0.48 0.22    | 0.09 0.01 | 0.43 0.20   | 0.96 0.48     | 3.12 1.65   |
| 6:03           | 0.00 1.33        |             |               |                 |           |            |             |         |            |               |            |
| 6:46           | 0.03 0.00        | 1.03 0.00   |               |                 |           |            |             | 0.90 1.64|             |               |            |
| 7:40           | 3-Hexene 0.00 0.09 |            |               |                 |           |            |             |         |            |               |            |
| 10:26          | 0.00 0.06        | 0.27 0.25   |               |                 |           |            |             |         |            |               |            |
| 10:38          | 0.00 0.09        |             |               |                 |           |            |             |         |            |               |            |
| 12:26          | Benzene 0.19 0.00 | 1.33 1.42   |               |                 |           |            |             |         |            |               |            |
| 15:62          | Toluene 0.13 0.05 |            |               |                 |           |            |             |         |            |               |            |
| 16:23          | 0.00 0.05 1.03 0.01 | 0.09 0.00 | 0.05 0.02 0.30 0.13 |            |         |            |             |         |            |               |            |
| 19:43          | Styrene 0.00 0.00 | 0.00 0.00   |               |                 |           |            |             |         |            |               |            |
| 20:04          | 0.05 0.04        |             |               |                 |           |            |             |         |            |               |            |
| 21:21          | α-Pinene 2.32 0.04 | 10.30 7.50 |               |                 |           |            |             |         |            |               |            |
| 21:72          | Camphene 1.67 0 0.03 0.04 | 1.60 1.80 |            |               | 0.38 0.27 | 0.27 0.08 | 0.07 0.07 |         |            |               |            |
| 22:40          | Sabinene 5.76 0 0.04 0 | 0.51 0.24 |               | 0.40 0.11 0.18 0.12 | 0.07 0 | 0.84 1.36 | 0.20 0.06 | 0.47 0.78 |            |               |            |
| 22:55          | β-Pinene 1.70 0 0.06 0.04 | 0.40 0.28 | 0.37 0.31 | 0.40 0.29 | 0.29 0.10 | 0.38 0.19 | 0.19 0.00 | 0.47 0.78 |            |               |            |
| 23:16          | β-Mycene 2.32 0.04 | 10.30 7.50 |               |                 |           |            |             |         |            |               |            |
| 23:55          | p-Cymene 0.05 0.04 | 0.05 0.02 | 0.05 0.03 | 0.15 0.05 | 0.14 0.04 | 0.08 0.00 | 0.07 0 | 0.12 0.08 |            |               |            |
| 23:88          | Limonene 56.60 2.37 | 0.03 0.05 | 0.53 0.45 |            | 3.80 3.16 | 0.32 0.26 | 0.10 0.04 | 0.03 0.02 |            |               |            |
| 23:99          | Ocimene 2.73 0 | 0.00 0.00 | 0.05 0.03 | 0.15 0.05 | 0.14 0.04 | 0.08 0.00 | 0.07 0 | 0.12 0.08 |            |               |            |
| 24:62          | γ-Terpine 0.05 0.03 | 0.15 0.05 | 0.14 0.04 | 0.08 0.00 | 0.07 0 | 0.12 0.08 |            |               |               |            |
| 25:39          | p-Cymenene 0.13 0 | 0.05 0.03 | 0.15 0.05 | 0.14 0.04 | 0.08 0.00 | 0.07 0 | 0.12 0.08 |            |               |            |
| 25:51          | Terpinolene 0.03 0.02 | 0.15 0.05 | 0.14 0.04 | 0.08 0.00 | 0.07 0 | 0.12 0.08 |            |               |               |            |
|                | Total light dependent | 68.1 2.41 | 0.13 0.00 | 11.7 8.4 | 7.6 3.9 | 2.6 1.9 | 1.5 0.9 | 0.1 0.0 | 0.5 0.2 | 4.8 2.9 | 12.8 3.1 |
|                | (μg (C) g⁻¹ dw h⁻¹) |            |               |             |         |         |         |         |         |         |         |
|                | Total light dependent | 1580 58 | n/a n/a | 76 59 | n/a n/a | 41 30 | 21 12 | 5 0 5 | 2 n/a | n/a n/a | n/a n/a |
|                | (ng (C) m⁻² leaf s⁻¹) |            |               |             |         |         |         |         |         |         |         |

Bold type indicates monoterpene emission rate values in light and dark, indicative of light dependency; blank indicates below detection limit; and n/a indicates leaf area measurements that were not available to express emission units on leaf area basis.
experiment. In the first set of experiments ("PAR dependency"), leaf temperature was kept constant at around 30°C (typically 29.8 ± 0.08°C), and CO₂ concentration and dew point around the leaf were maintained at 365 ppm (typically 362.4 ± 2.3 ppm) and 14°C (typically 13.8 ± 0.7°C), respectively. Emission rate measurements were made over a range of PAR values between ~1300 and 0 μmol m⁻² s⁻¹, ramping down from high to low PAR, then increasing PAR again. In the second set of experiments ("temperature dependency"), CO₂ concentration around the leaf was maintained at ~365 ppm, PAR was kept at around 1300 μmol m⁻² s⁻¹ and emission rate measurements were made at leaf temperatures between 20°C and 40°C, first increasing temperature from low to high values, then decreasing again. When leaf temperature was 30°C, dew point around the leaves was set to ~11.5°C (typically 11.5 ± 0.7°C) and allowed to decrease with decreasing temperature. For example, the actual dew point values achieved at the lowest experimental temperature (20°C) with *C. incanus* was 7.6 ± 0.8°C. Due to time constraints, only one temperature dependency experiment was performed for *P. pinea*, as temperature-dependent emissions from *Pinus* spp. are well characterized (e.g. Staudt et al., 1997).

### 3.2. PAR dependency

Emissions of monoterpenes from the *Quercus* sp. were strongly dependent on PAR when the PAR flux was varied stepwise down from ~1000 to 0 μmol m⁻² s⁻¹, then increased back to ~1000 μmol m⁻² s⁻¹. At PAR values > ~850 μmol m⁻² s⁻¹, emission rates continued to increase with PAR, while *Gₜ* values decreased slightly (Fig. 2). An overall 97% reduction in ∑VOC emission rate was observed in the dark. Total emissions from *Quercus* sp. were very well described by the G95 isoprene algorithm (Fig. 3).

Most compounds emitted from *P. pinea*, although demonstrating some degree of light dependency in the initial screening exercise, showed inconsistent and variable response to light intensity in subsequent experiments and are not discussed further here. However, ocimene emissions from this species showed consistently strong light dependency, disappearing in the dark in the initial screening experiment (Table 1). Two subsequent PAR dependency experiments showed that ocimene emissions were strongly correlated with light intensity with no emissions in the dark (Fig. 2). In the first PAR dependency experiment, as light intensity was decreased from 850 to 0 μmol m⁻² s⁻¹, ocimene emissions decreased from 2.1 to 0.02 μg (C) g⁻¹ dwh⁻¹ and *Gₜ* decreased from 192 to 39 μmol m⁻² s⁻¹ (Fig. 2). When light intensity was subsequently increased from 0 to 720 μmol m⁻² s⁻¹, ocimene emission rates increased to 3.7 μg (C) g⁻¹ dwh⁻¹, a much higher value than previously recorded at this PAR value, when light intensity was decreasing. *Gₜ* also increased to a higher value (260 μmol m⁻² s⁻¹) as PAR was increased. Similar observations were made in the second PAR dependency experiment with *P. pinea*; however, *Gₜ* did not recover to starting values on re-illumination (Fig. 2).

Ocimene emissions from *P. pinea* were also well predicted by the G95 isoprene algorithm. To improve the fit of the G95 isoprene algorithm, parameter values were generated using non-linear regression with quasi-Newton estimation of loss function in a statistical package (Statistica). For decreasing and increasing light intensity, *x* = 0.0032 and 0.0029 m²μmol⁻¹, respectively, and *Cₜₙ* = 1.265 and 1.924 units, respectively. Emission data modelled with this parameterization of the G95 algorithm correlated well (Pearson product moment linear correlation) with the observed ocimene emission measurements (e.g. Fig. 3; PAR decreasing, *r*² = 0.99, *n* = 7; PAR increasing: *r*² = 0.98, *n* = 9).

Some monoterpene emissions from *C. incanus* continued at lower concentrations in the dark (cf. *P. pinea*), so the differences between observed emissions in the light and the mean of emission rates in the dark ("residual emissions") were considered to be the fraction of total emissions directly or indirectly influenced by light ("light-dependent emissions"). Light dependency of this light-influenced fraction of ∑VOC emissions from *C. incanus* was linear and did not achieve saturation within the range of PAR values used in two replicate experiments (e.g. *r*² = 0.92 as PAR was changed stepwise from ~1400 to 0 μmol m⁻² s⁻¹ and...
In the first experiment, as PAR was reduced from 1400 to 0 μmol m$^{-2}$ s$^{-1}$, the light-dependent emissions decreased from 0.56 to 0 μg (C) g$^{-1}$ dw h$^{-1}$ and $G_s$ from 251 to 47 mmol m$^{-2}$ s$^{-1}$. However, when the leaves were re-illuminated to 1400 μmol m$^{-2}$ s$^{-1}$ PAR, the light-dependent emission rate returned to 80% of its starting value at this PAR (0.46 μg (C) g$^{-1}$ dw h$^{-1}$), whereas $G_s$ increased to only 43% of its starting value (108 mmol m$^{-2}$ s$^{-1}$, Fig. 2). Similar observations were made in the second replicate experiment; but here, when the leaves were re-illuminated to 1000 μmol m$^{-2}$ s$^{-1}$ PAR, $G_s$ increased to 154% of its previous value at 1000 μmol m$^{-2}$ s$^{-1}$ PAR (206 mmol m$^{-2}$ s$^{-1}$, Fig. 2). Correlations of measured VOC emission rates under varying light intensity with G95 modelled data ranged from $r^2 = 0.38$ to 0.80 (experiment 2, PAR decreasing and increasing, respectively, not shown). This suggests that the light control of VOC emissions from this plant species are different than that described by the G95 isoprene algorithm, and requires further investigation.

3.3. Temperature dependency

Monoterpene emissions from the Quercus sp. increased exponentially with temperatures up to ~32°C.
and ~30°C (experiments 1 and 2, respectively) followed by a rapid decline in emission rate as temperature was increased further (Fig. 4). In both replicate experiments, the pattern of emissions was reflected by the calculated $C_T C_L$ values (Fig. 5). Again, parameter values were generated to improve the fit of the G95 isoprene algorithm; $C_T 1 = 67000$ and 120,000; $C_T 2 = 870,000$ and 500,000 for experiment 1 and 2, respectively ($r^2 = 0.98$ and 0.93; $n = 8$ and 9, experiments 1 and 2, respectively). Although it is not clear why the two different plants of this species exhibited different activation and deactivation energies (represented by $C_T 1$ and $C_T 2$), it is possible that each plant had experienced different conditions of environmental variables, e.g. water stress, exposure to high PAR, etc., prior to the experimental measurements. $G_s$ values and photosynthetic C assimilation rates decreased steadily in both experiments to values indicating total stomatal closure and cessation of photosynthesis as temperature was increased from ~20°C to ~40°C. This suggests that the observed decline in emission rates at higher temperatures may be due to the lack of photosynthesized substrate. On reducing temperature from ~40°C to ~30°C, $G_s$ and photosynthetic C assimilation rates increased to values similar to those measured during the temperature increase. Thus at temperatures below 30°C, there appeared to be no correlation between $G_s$ and emission rate.
Emissions of ∑monoterpenes from *P. pinea* showed temperature dependency more typical of isoprene emissions than of monoterpene emissions (Fig. 4). The G95 isoprene algorithm for temperature dependency (Eq. (4)) fitted the measured data well ($C_{T1} = 50,000$, $C_{T2} = 400,000$ $r^2 = 0.97$, $n = 7$, Fig. 5). Emission rates from *P. pinea* modelled by the G95 monoterpene algorithm (Eq. (5)) were poorly correlated with measured VOC emission rates ($E = 23.14 \mu g (C) g^{-1} dw h^{-1}$, $\beta = 0.025$, $r^2 = 0.3$, $n = 7$). There was no emission of ocimene in the temperature dependency experiment; this can possibly be explained by the fact that monoterpene emissions from *Pinus* species are well known for their variability in magnitude and composition (e.g. Staudt et al., 1997). It is also possible that ocimene is emitted from *P. pinea* only as a response to changing light stimulus. Carbon assimilation rate and $G_s$ decreased steadily as temperature increased (Fig. 4).

In the first of two replicate experiments to investigate temperature dependency of VOC emissions from *C. incanus*, duplicate measurements were made at three temperature values to estimate uncertainty (viz., Section 2.4). In this experiment, $\ln(\sum$VOC emissions) was well correlated with temperature as temperature was reduced from 30°C to 21°C then increased again to 30°C ($r^2 = 0.96$, $n = 7$, Fig. 4). In the second replicate experiment, while emission rates increased exponentially with temperature up to a maximum of 6.8 $\mu g (C) g^{-1} h^{-1}$ at 33°C, they declined above this temperature to 1.8 $\mu g (C) g^{-1} h^{-1}$ at 38°C. When the temperature was reduced from 38°C to 26°C, emission rates continued to decline exponentially, and were therefore lower than when temperature was increasing (Fig. 4).

Using the G95 isoprene algorithm and parameter values $C_{T1} = 50,000$ and $C_{T2} = 400,000$ (derived from non-linear regression), $C_{T}C_{L}$ correlated well with
measured VOC emission rates from *C. incanus* in experiment 1 ($r^2 = 0.97$, $n = 7$) but there was poor correlation of $C_1C_1$ with VOC emission rates in experiment 2 ($r^2 = 0.26$, $n = 8$; Fig. 5). Using the G95 monoterpene algorithm, a $\beta$ value of 0.11 K$^{-1}$ ($r^2 = 0.97$, $n = 7$) was obtained for the first temperature dependency experiment. In the second replicate experiment, the G95 monoterpene algorithm predicted the increase in VOC emission rates as temperature was increased from 20°C to 34°C ($\beta = 0.21$, $r^2 = 0.99$, $n = 4$) and as temperature was decreased from 38°C to 28°C ($\beta = 0.15$, $r^2 = 0.99$, $n = 3$), but did not predict the sharp decrease in emission rate as the temperature was increased from 34°C to 38°C (Fig. 5). Carbon assimilation was reduced to cessation at this temperature, indicating that a supply of photosynthesized substrate may be necessary to sustain VOC emission rates, even though the mechanism of VOC emission from *C. incanus* seems to be typical of monoterpene emissions, i.e. volatilization from a pool. On cooling the leaf again to ~26°C, the rate of photosynthesis increased to rates much higher than those measured as temperature was increasing.

4. Discussion

4.1. Amount and composition of emissions from the plant species

Emission rates of $\Sigma$VOCs estimated using a leaf cuvette at conditions of standard temperature and PAR
(30°C and 1000 μmol m⁻² s⁻¹, respectively) for \textit{P. pinea}, \textit{A. unedo}, \textit{T. vulgaris}, \textit{P. halepensis} and \textit{Lavandula stoechas}, were equivalent to field measurements of \( \Sigma \text{VOC} \) emissions from these species in Mediterranean habitats using the branch enclosure sampling technique (Owen et al., 1997, 2000). The range of compounds constituting the major part of \( \Sigma \text{VOC} \) emissions from each plant species agreed with the expected speciation of emitted monoterpenes from these plant species (Owen et al., 1997, 2000). However, Owen et al. (2000) reported emissions of cineole and camphor from \textit{L. stoechas} in field conditions whereas these compounds were not detected as emissions from \textit{L. stoechas} in the present study. It is possible that (i) either the field measurements were made on plants that had been disturbed, and were thus emitting a different range of compounds, or that (ii) the young plants investigated in the present laboratory studies were emitting different compounds compared with older plants.

There are conflicting reports of monoterpenes emission rates from certain \textit{Quercus} species. Although Pio et al. (1993) measured emissions from \textit{Q. suber} of 7–37 μg g⁻¹ dw h⁻¹ at 25°C and 500 μmol m⁻² s⁻¹ PAR, Steinbrecher et al. (1997) reported negligible emissions from this species. Oak species frequently hybrized, and the major compound reported from \textit{Quercus} sp. in this study was limonene, which is also the major emission from \textit{Quercus rotonidifolia}, a species widely distributed throughout Spain and Portugal. This raises the possibility that measurements were made on hybrids of \textit{Q. suber} and \textit{Q. rotonidifolia}. However, the findings of Pio et al. (1993) and Steinbrecher et al. (1997) may suggest the possibility of regional differences in emission characteristics, or the operation of some sort of environmental controls that may inhibit emissions under certain conditions. In any case, it is likely that \textit{Quercus} sp., whether or not it is a hybrid, belongs to section Cerris of genus \textit{Quercus}, which has been shown to contain several species which fail to emit significant amounts of isoprene, but which emit monoterpenes in a light-dependent fashion, a trait thus far found in no other section of the genus (Harley et al., 1999).

In the present investigation, laboratory measurements of VOC emission rates from \textit{Quercus} sp. and from \textit{C. incanus} (68 and 2.6 μg (C) g⁻¹ h⁻¹, respectively) were greater than field measurements of reported emission rates from various \textit{Quercus} spp. and from \textit{C. incanus} normalized to standard conditions (e.g. 10 and 0.3 μg (C) g⁻¹ h⁻¹, respectively, Owen et al., 1997; ~22 μg g⁻¹ dw h⁻¹ for sun-exposed branches and ~2.3 μg g⁻¹ dw h⁻¹ for shade-adapted branches of \textit{Q. ilex}, Bertin et al., 1997.). Despite the difference in magnitude of emissions, the speciation of emitted monoterpenes compounds from \textit{C. incanus} was similar in this study to the findings of Owen et al. (1997).

Llusia and Penuelas (1998) studied the effect of water stress on VOC emissions from a range of Mediterranean plant species, approximately 2 years old, growing in polythene tunnels. They found no detectable emissions from \textit{A. unedo}. Indeed, very low emissions (~0.1 μg (C) g⁻¹ h⁻¹) from this species are reported here, and by Owen et al. (1997) using a branch enclosure in field conditions. The same authors reported a daily average total emission rate of 3.6 μg g⁻¹ h⁻¹ from \textit{C. monspeliensis}, dominated by α-phellandrene. In contrast, α-pinene was found to be the dominant emitted compound from \textit{C. monspeliensis} in the present study, with base emission rate of 10.3 μg (C) g⁻¹ h⁻¹. They also reported very large emissions of VOCs from \textit{P. halepensis}, dominated by α-pinene (85 μg g⁻¹ h⁻¹), whereas total emissions of 0.13 μg (C) g⁻¹ wh⁻¹, dominated by p-cymenene, are reported here. These differences could be attributed to different age and condition of the plants investigated, or to natural variation in magnitude and speciation of monoterpenes emissions from some plant species.

4.2. Pathways of monoterpenes emissions

Monoterpenes usually accumulate in stored pools in specialized structures, e.g. resin ducts (pines), resin blisters (firs), glandular trichomes (mint) or leaf storage cavities (eucalyptus). Four main emission routes are described by Fall (1999) for monoterpane compounds: (1) diffusion across the leaf epidermis cuticle (2) conductance through stomata (3) release from leaf air space following wounding and (4) evaporation from surfaces following wounding. In addition, the documented light-dependent emissions of monoterpenes from some Mediterranean \textit{Quercus} species may leave the plant from a small transient pool soon after synthesis (Fall, 1999). Temperature may affect monoterpane synthesis by enzyme activation or deactivation, shown here in the emission–temperature curves for \textit{Quercus} sp. and \textit{P. pinea}. Temperature may also affect any of the emission pathways by increasing monoterpane volatilization, as shown in the temperature dependency of emissions from \textit{C. incanus}. Monoterpane synthesis may also be affected by light if (1) the synthase enzymes are light sensitive or (2) photosynthesite is required for enzyme or monoterpane synthesis. Light may also affect emission of monoterpenes compounds by influencing stomatal aperture. The common method of using abscissic acid to control \( G_s \) was not used here, because this method requires excision of the shoot and emissions of VOCs are known to respond to stress (e.g. Litvak and Monson, 1998). The use of coloured light to control \( G_s \) was also considered unsuitable in view of the lack of knowledge of the effect of light on monoterpane emissions.

Although the experiments described in this study were not designed to isolate \( G_s \) as a single variable affecting
light-dependent VOC emissions, the results presented here support the widely accepted view that VOC emission rates are not affected by stomatal control. For example, in a review of the emission, physiology and ecology of biogenic VOCs, Kesselmeier and Staudt (1999) concluded that stomatal control has no effect on emissions of compounds that diffuse through the leaf cuticle, or on emissions from external glands and hairs. However, they stated that there could be some stomatal control of emissions of newly synthesized compounds, e.g. isoprene, and of compounds stored in internal pools. But although a number of studies demonstrated stomatal emissions of isoprene (e.g. Tingey et al., 1981) and monoterpenes (Loreto et al., 1996), further studies have shown that $G_s$ had no effect on isoprene emissions (e.g. Tingey et al., 1981; Monson and Fall, 1989; Sharkey and Loreto, 1993), nor on monoterpeno emissions (e.g. Tingey et al., 1980; Loreto et al., 1996). The apparent non-dependence of VOC emissions on $G_s$ has been explained by the low concentrations of these compounds in the leaf tissue, which increase when $G_s$ decreases and which compensate for stomatal closure. Kesselmeier and Staudt (1999) also suggested that increased internal VOC concentrations could increase diffusion of monoterpeno compounds through the leaf cuticular membrane.

4.3. Observed light and temperature responses of emissions compared with past studies

The initial laboratory screening for light-dependent monoterpeno emissions suggested that all the studied species emitted one or more compound with some degree of light dependency. Emissions of monoterpeno from Quercus sp. demonstrated temperature and light dependencies very similar to those of isoprene emissions (e.g. Guenther et al., 1995), and to those of monoterpeno emissions from Quercus ilex (Staudt and Seufert, 1995). The Mediterranean evergreen oaks are now well established as monoterpeno emitters, with a large number of studies reporting light-dependent monoterpeno emissions from Quercus sp. (e.g. Street et al., 1997; Bertin et al., 1997). The data for the Quercus sp. presented here supports previous reports of light-dependent emissions from other evergreen oak species (e.g. Hansen and Seufert, 1997), both the temperature and PAR dependencies indicating emissions from an enzymatic process.

Emissions of $\sum$VOCs from P. pinea were possibly from two sources. Temperature dependency of $\sum$VOC emissions from P. pinea was observed, with a decrease in emission rates at temperatures higher than an optimum of $\sim$37°C, which was reflected by modelled data using the G95 isoprene algorithm. This would imply that monoterpeno emissions from this species are from direct synthesis (i.e. are similar to isoprene emissions). Indeed, the G95 modelled values closely followed the observed emissions at all temperatures.

Light-dependent emissions of ocimene from P. pinea showed an interesting hysteresis, with higher emission rates occurring when the plant was re-illuminated compared with emission rates measured in the first part of the experiment while PAR was reduced. This effect could be attributable to increased availability of substrate for ocimene synthesis during the period of darkening, or to the light activation mechanisms of the synthase enzymes (Fall and Wildermuth, 1998). Staudt et al. (1997) also reported light-dependent emissions of ocimene from P. pinea in field conditions. A similar hysteresis was shown by the weak light-dependent monoterpeno emissions from C. incamus.

Temperature maxima for monoterpeno emissions were rather low, especially for Quercus sp. (see above), compared with published data for isoprene. Low values of $G_s$ and photosynthesis rates may indicate that low emission temperature maxima could be attributed to lack of photosynthesized substrate. Observed monoterpeno emission maxima here ranged from 30°C to 40°C compared with reports of 40–45°C for isoprene emissions (Fall and Wildermuth, 1998). This could be explained by differences in temperature optima for monoterpeno synthases compared with isoprene synthases. Staudt and Bertin (1998) found that emissions of $\alpha$-pinene, sabine and $\beta$-pinene from Q. ilex also had lower temperature optima of 35–40°C; however, ocimene emissions from this species increased greatly between 35°C and 45°C. It is clear that light and temperature dependencies of monoterpeno emissions are variable, and may depend on different properties of the synthase enzymes, or on substrate requirements and availability. There is also evidence that the temperature maximum for emission of a specific VOC is dependent on the temperature history of the plant’s growth (Guenther et al., 2000).

4.4. Implications for emission modelling

Identifying plant species that have light-dependent monoterpeno emissions can have a significant impact on regional emission model results. The emission factors used in regional models are often based on emission rates measured at various environmental conditions and by assuming a certain light and temperature dependence. If an emission rate is measured under low light conditions then the emission factor will be greatly underestimated by incorrectly assuming there is no light dependence. The need to identify the correct emission activity algorithm, for the purpose of developing emission factors, may be eliminated by always making emission measurements at a standard set of light and temperature conditions. This does not reduce the need to identify the emission activity algorithm needed to
predict regional emissions. The errors associated with mis-identifying the light dependence of monoterpenoid emissions from a plant species can be estimated by comparing predictions of the light emission activity algorithms described by Guenther et al. (1995). There are relatively small errors (<15%) associated with high light levels (PAR > 900 \( \mu \)mol m\(^{-2}\) s\(^{-1}\)) but large errors at low light levels: about a factor of two for PAR between 300 and 500 \( \mu \)mol m\(^{-2}\) s\(^{-1}\) and more than a factor of five for PAR < 100 \( \mu \)mol m\(^{-2}\) s\(^{-1}\). The error associated with the daily total emissions, for environmental conditions representative of the Mediterranean summer, is about a factor of two.

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