

BRIEF COMMUNICATION

## ***Pinus halepensis* and *Quercus ilex* terpene emission as affected by temperature and humidity**

J. LLUSIÀ and J. PEÑUELAS

CREAF, Facultat de Ciències, Universitat Autònoma de Barcelona,  
E-08193 Bellaterra, Barcelona, Catalonia, Spain

### **Abstract**

The short-term relationships of monoterpene emission with temperature and relative humidity were studied in *Pinus halepensis* L. and *Quercus ilex* L. seedlings grown in air-conditioned chamber. In *P. halepensis* terpene emission rate increased with temperature (from 15 to 35 °C) and relative humidity (from 40 - 60 to 65 - 95 %). In *Q. ilex*, a terpene non-storing species, it increased with temperature only at high relative humidities but not at relative humidities lower than 60 %.

*Additional key words:* limonene, myrcene,  $\alpha$ -pinene, terpene non-storing species, terpene storing species.

---

Among the abiotic factors affecting plant terpene emission rates, temperature (Tingey *et al.* 1980, Lamb *et al.* 1985, Guenther *et al.* 1993, Staudt and Seufert 1995, Loreto *et al.* 1996, Llusia and Peñuelas 1999, Peñuelas and Llusia 1999), and relative humidity (Dement *et al.* 1975, Guenther *et al.* 1991, Tingey *et al.* 1991, Loreto *et al.* 1996) are outstanding. Existing models for prediction of emission rates are normally based only on temperature and photon flux density (PFD) (Guenther *et al.* 1991). They do not consider relative humidity and even less the possible interaction between temperature and relative humidity. We here approach the study on this possible interaction that may be different in terpene storing and non-storing species in *Pinus halepensis* L. and *Quercus ilex* L.

---

Received 14 December 1998, accepted 22 February 1999.

*Acknowledgements:* This research was made possible by grants CLI97-0344 from the CICYT (Spain), CIRIT-DMA-98 from the CIRIT and Departament de Medi Ambient (Catalonia), and "Fundació Territori i Paisatge 1998". We gratefully acknowledge an F.P.I. (Spain) fellowship to J.L. We also thank Carburos Metálicos, SA for continuous support to this research.

Fax: (+34) 3 5811312, e-mail: j\_llusia@creaf.uab.es or penuelas@uab.es

Two-year-old well-irrigated seedlings of *Pinus halepensis* L. and *Quercus ilex* L. were grown in 5 dm<sup>3</sup> pots filled with peat, perlite, vermiculite and sand (2:1:1:1) in a growth chamber (*AIR-BLUE H-1*, *Termiafrigo*, Barcelona, Spain) under a factorial combination of three temperatures (15, 25 and 35 °C) and two ranges of relative humidities (40 - 60 and 65 - 95 %). PFD was 150  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for 14-h photoperiod. Five plants per species and treatment were sampled.

Sampling of terpene emission was conducted for the main apical branch. We used a dynamic branch enclosure system consisting of a 540 cm<sup>3</sup> PET chamber and a fan that created a constant flux of air across the branch enclosed in the PET chamber. Air coming out from the PET chamber was moved by a peristaltic pump (*A.P. Buck*, Orlando, USA) and flowed through the exit hole by a *Teflon* tube towards a glass tube (11.5 cm long and 0.4 cm internal diameter). This glass tube was filled with terpene adsorbents *Carbotrap C* (300 mg), *Carbotrap B* (200 mg) and *Carbosieve S-III* (125 mg) from *Supelco* (Bellefonte, USA) separated by plugs of quartz wool. A blank with no branch in the gas exchange system was sampled immediately before each measure. Terpene analyses were conducted in a *GC-MS* (*Hewlett Packard HP59822B*, Palo Alto, USA). Trapped emitted monoterpenes were desorbed (*Thermal Desorption Unit, Model 890/891*; *Supelco*, Bellefonte, USA) at 320 °C during 3 min and injected into a 30 m  $\times$  0.25 mm  $\times$  0.25 mm film thickness capillary column (*Supelco HP-5*, crosslinked 5 % *pH Me Silicone*). After sample injection, the initial temperature (46 °C) was increased at 30 °C min<sup>-1</sup> up to 70 °C, and thereafter at 10 °C min<sup>-1</sup> up to 150 °C, which was maintained for other 5 min. Helium flow was 1 cm<sup>3</sup> min<sup>-1</sup>. The identification of monoterpenes was confirmed with GC-mass spectroscopy, by comparison with standards from *Fluka* (Buchs, Switzerland), literature spectra and *GCD Chemstation G1074A HP*. Detection limit was about 0.6 ng. Calibration curves were always highly significant ( $r^2 > 0.97$ ). All statistical analyses were conducted using *Statistica ver 5.0 for Windows* (*StatSoft*, Tulsa, USA). Five plants per treatment and species were measured. Data were log-transformed to accomplish normality requirements.

In *P. halepensis*, total terpene emission rates significantly increased with increasing temperatures in both high and low relative humidity (Fig. 1). Greater emission rates were found at higher than at lower relative humidities (Fig. 1). The main emitted individual terpenes were  $\beta$ -myrcene,  $\alpha$ -pinene, D-limonene,  $\Delta^3$ -carene and  $\beta$ -pinene (in decreasing order of emission rate).

The strong effect of temperature and humidity on the terpene emission rates is in agreement to previous reports (Tingey *et al.* 1980, Lamb *et al.* 1985, Guenther *et al.* 1993, Staudt and Seufert 1995, Loreto *et al.* 1996, Llusia and Peñuelas 1999). Among others, Croteau (1977), Lerdau (1991), and Tingey *et al.* (1991) have reported that terpene storing plants present a rapid increase in emission of monoterpenes when they are submitted to a high humidity environment. It may be because cuticular hydration results in an increase in its permeability to the monoterpenes (Croteau 1977). In our study, we found overall 78 % greater emission rates by *P. halepensis* under high than under low relative humidity in agreement with the 70 - 80 % increase reported by Croteau (1977) in *Mentha piperita*. The emission

rates of  $\alpha$ -pinene in a Douglas fir forest have also been reported to be closely related to relative humidity (Lamb *et al.* 1985). These effects of humidity on monoterpene volatilization fit well with the theory that cuticular transport is passive and occurs through cuticular pores formed by hydration of polar functional groups in the presence of water (Schönherr 1982). It seems worth noting that this plausible influence of water on the diffusive pathway of monoterpenes has been observed both in species of family *Lamiaceae* with external glandular trichomes and in *Coniferae* like the studied *Pinus halepensis* with internal resin ducts.

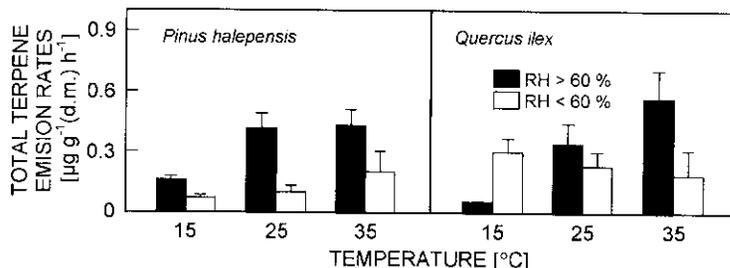


Fig. 1. Total terpene emission rates as a function of temperature and relative humidity for *Pinus halepensis* and *Quercus ilex* seedlings. Black bars represent total terpene emission rates at relative humidity ranging between 65 and 95 %, and white bars represent terpene emission rates at relative humidity ranging between 40 and 60 %. Means  $\pm$  SE ( $n = 5$ ).

*Q. ilex* emission rates also increased with temperature at high relative humidity, but they did not at low relative humidity (Fig. 1). Its emission rates were 67 % greater at high relative humidity only at the highest tested temperature (35 °C) (Fig. 1). The main emitted individual terpenes were  $\Delta^3$ -carene and D-limonene.

There was thus a significant interactive effect of temperature and relative humidity in this non-storing species that was absent in the storing species *P. halepensis*. It may be related to the difficulties of *Q. ilex* in producing terpenes at stressful high temperature and low humidity. On the contrary, *P. halepensis* emission rates seem to only depend on physical processes for volatilisation of their stored terpenes. We have thus a new indication that terpene emission control may differ between storing and non-storing species (Lerdau 1991, Llusià and Peñuelas 1999, Peñuelas and Llusià 1999).

In a recent annual field study we also found that monoterpene emission rates by several Mediterranean species tended to decrease in summer with increasing vapour pressure deficit (Llusià and Peñuelas 1999). The decreased permeability of the cuticle to gas exchange and the lack of carbon substrate and/or ATP under water limitation may explain this effect of low humidity (Bertin and Staudt 1996).

The interactive effects of micrometeorological factors such as temperature and relative humidity on terpene emission rates might improve existing terpene emission models.

## References

- Bertin, N., Staudt, M.: Effect of water stress on monoterpene emissions from young potted holm oak (*Quercus ilex* L.) trees. - *Oecologia* **107**: 456-462, 1996.
- Croteau, R.: Effect of irrigation method on essential oil yield and rate of oil evaporation in mint grown under controlled conditions. - *HortScience* **12**: 563-565, 1977.
- Dement, W.A., Tyson, B.J., Money, H.A.: Mechanism of monoterpene volatilization in *Salvia mellifera*. - *Phytochemistry* **14**: 2555-2557, 1975.
- Guenther, A., Zimmerman, P.R., Harley, P.C., Monson, R.K., Fall, R.: Isoprene and monoterpene emission rate variability: model evaluations and sensitivity analyses. - *J. geophys. Res.* **93**: 12609-12617, 1993.
- Guenther, A.B., Monson, R.K., Fall, R.: Isoprene and monoterpene emission rate variability: observations with eucalyptus and emission rate algorithm development. - *J. geophys. Res.* **96**: 10799-10808, 1991.
- Lamb, B., Westberg, H.H., Allwine, G.: Biogenic hydrocarbon emissions from deciduous and coniferous trees in the United States. - *J. geophys. Res.* **90**: 2380-2390, 1985.
- Lerdau, M.: Plant function and biogenic emission. - In: Sharkey, T.D., Holland, E.A., Mooney, H.A. (ed.): *Trace Gas Emission by Plants*. Pp. 121-134. Academic Press, San Diego 1991.
- Llusià, J., Peñuelas, J.: Seasonal patterns of terpene content and emission from seven Mediterranean woody species in field conditions. - *Amer. J. Bot.* (in press), 1999.
- Loreto, F., Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., Tricoli, D.: Influence of environmental factors and air composition on the emission of  $\alpha$ -pinene from *Quercus ilex* leaves. - *Plant Physiol.* **110**: 267-275, 1996.
- Peñuelas, J., Llusià, J.: Seasonal emission of monoterpenes by the Mediterranean tree *Quercus ilex* L. in field conditions. Relations with photosynthetic rates, temperature and volatility. - *Physiol. Plant.* (in press), 1999.
- Staudt, M., Seufert, G.: Light-dependent emission of monoterpenes by holm oak (*Quercus ilex* L.). - *Naturwissenschaften* **82**: 89-92, 1995.
- Schönherr, J.: Resistance of plant surfaces to water loss: transport properties of cutin, suberin and associated lipids. - In: Lange, O., Nobel, P., Osmond, C., Ziegler, H. (ed.): *Encyclopedia of Plant Physiology*. New Series Vol. 12B. Pp. 153-179. Springer-Verlag, Berlin - Heidelberg - New York 1982.
- Staudt, M., Seufert, G.: Light-dependent emission of monoterpenes by holm oak (*Quercus ilex* L.). - *Naturwissenschaften* **82**: 89-92, 1995.
- Tingey, D.T., Manning, M., Grothaus, L.C., Burns, W.F.: Influence of light and temperature on monoterpene emission rates from slash pine. - *Plant Physiol.* **65**: 797-801, 1980.
- Tingey, D.T., Turner, D.P., Weber, J.A.: Factors controlling the emission of monoterpenes and other volatiles compounds. - In: Sharkey, T.D., Holland, E.A., Mooney, H.A. (ed.): *Trace Gas Emission by Plants*. Pp. 93-120. Academic Press, San Diego 1991.