BIOLOGIA PLANTARUM 41 (1): 139-143, 1998

BRIEF COMMUNICATION

Influence of intra- and inter-specific interference on terpene emission by *Pinus halepensis* and *Quercus ilex* seedlings

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Abstract

Seedlings of two common Mediterranean trees, *Pinus halepensis* L. and *Quercus ilex* L., were grown alone and together with seedlings of the same or of the other species in the same pot during one year to test the effects of intra and inter-specific interference on terpene emission. Light, nutrients and water were amply supplied. There were higher emission rates in *P. halepensis* than in *Q. ilex*. The emission increased when the neighbour was a pine and decreased when the neighbour was a holm oak. Volatile organic compound and terpene emission rates followed inverse trends to foliar biomass or growth, which decreased when the neighbour was a pine.

Additional key words: allelopathy, α-pinene, autopathy, camphene, volatile organic compounds.

Plants emit volatile organic compounds (ethylene, isoprene, mono and sesquiterpenes, alkanes, alcohols, carbonyls, aldehydes, organic acids, ketones, *etc.*) in response to abiotic and/or biotic factors (Tingey *et al.* 1991, Harborne 1993, Langenheim 1994, Takabayashi *et al.* 1994). Some species could exert allelopathic effects on other species or over themselves (self-toxicity, autopathy, self-inhibition or autotoxicity) by releasing or emitting terpenes and other volatiles (Vokou 1992, Langenheim 1994).

Volatiles may flow from a donor plant through the atmosphere to a receiving species or may be adsorbed on soil particles and solubilized in the soil solution.

Received 21 September 1997, *accepted* 10 February 1998.

Acknowledgments: This research was made possible by grants CICYT-CLI97-0334, CIRIT, and "Caixa de Catalunya". We gratefully acknowledge an F.P.I. (Spain) fellowship to J.LL. We also thank CARBUROS METÁLICOS, SA for continuous support to this research, and Dr. J. Sardans for technical help in growing seedlings.

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Major volatile monoterpenes of some species inhibit seed germination and growth of annual plants (Vokou 1992, Tarayre *et al.* 1995), and they have even been proposed as herbicides (Chou 1995). The suppression of respiration and oxygen uptake (Lorber and Muller 1980) or the activation of respiration alternative pathway have also been reported (Peñuelas *et al.* 1996). The phytotoxicity is dependent on the concentration, on the plant species and environmental conditions (Langenheim 1994).

In Mediterranean forest, *Q. ilex* and *P. halepensis* are the dominant trees and they both emit terpenes (Llusià and Peñuelas 1998). However, whereas *P. halepensis* contains (stores) considerable amounts of terpenes, *Q. ilex* does not (Llusià and Peñuelas 1998). We hypothesized that in case of existence of allelopathic-like effects, they would be stronger in *P. halepensis* than in *Q. ilex* because plants containing terpenes such as conifers have been suggested as able of having a strong allelopathic effect on non-containing species or on themselves (Jobidon 1986, Vokou 1992, Langenheim 1994).

In this study, we present relationships between terpene emission rates and growth of potted seedlings of *P. halepensis* and *Q. ilex* in different intra- and interspecific competition treatments.

Two year old *Pinus halepensis* L. and *Quercus ilex* L. seedlings of similar initial biomass growing in 3 dm^3 *WM* pots (Riedacker 1978) with a sandy-soil were used. The interference treatments were established as follows: P (one *P. halepensis* seedling per pot), PP (two *P. halepensis* seedlings per pot), PQ (one *P. halepensis* seedling and one *Q. ilex* seedling per pot), Q (one *Q. ilex* seedling per pot) and QQ (two *Q. ilex* seedlings per pot). All plants were irrigated twice a week to maximum soil capacity and were fertilized with P corresponding to 250 kg ha^{-1} in order to avoid its possible limitation in sandy soil. After one year terpene emission was measured on 16th June 1995 in the morning (8.30 - 11.30 solar time). Afterwards, seedling biomass was sampled and dried at 60 °C until constant mass.

The seedlings were closed in a 2 dm^3 cylindrical polyethylene chamber connected to a peristaltic pump (*A.P. Buck*, Orlando, USA). Air coming out of the chamber flowed to a glass tube (11.5 cm long and 0.4 cm internal diameter) filled with *Carbotrap C* (300 mg), *Carbotrap B* (200 mg) and *Carbosieve S-III* (125 mg) adsorbents (*Supelco*, Bellefonte, USA) separated by plugs of quartz wool. The tubes were previously conditioned for 3 min at 350 °C with a stream of purified helium. The sampling time was 3 - 5 min and the flow varied between 100 to 200 cm^3 min^{-1} depending on the glass tube. The trapping efficiency was practically 100 %. In order to eliminate the problem of "sticky" terpenes adsorbed by the surfaces and materials used in the gas exchange system, blanks consisting on 15 min free air sampling without enclosing seedlings were carried out immediately before and after each measure. The glass tubes (with trapped terpenes) were sealed with silicon caps, and stored at -30 °C before analysis (no longer than 24 - 48 h later).

For terpene and other volatile organic compound (VOC) analysis, a flame ionization detector (*GC-FID, HP5890 Series II, Hewlett Packard,* Palo Alto, USA) was used. Trapped emitted monoterpenes were desorbed (thermal desorption unit, *Model 890/891, Supelco,* Bellefonte, USA) at 220 °C during 8 min and injected into a 30 m × 0.25 mm × 0.25 µm (film thickness) capillary column (*Supelco SPB-1*).

After sample injection, the initial temperature (40 °C) was increased at 4 °C min^{-1} up to 85 °C, it was kept at 85 °C for 5 min, and there after it was again increased at 20 °C min^{-1} up to 300 °C, temperature that was maintained for other 5 min. Helium flow was 1 cm^3 min^{-1}. The identity of terpenes was confirmed by comparison with standards from *Fluka* (*Chemie AG, Buchs,* Switzerland).

Statistical analyses were conducted using *SYSTAT 5.2* (*SYSTAT* Inc., Evanston, USA) and *StatView 4.5* (*Abacus Concepts,* Berkeley, USA) statistical program packages.

Higher terpene emission rates were found in *P. halepensis* seedlings than in *Q. ilex* seedlings (Fig. 1). As it has been previously reported for the studied Mediterranean species (Peñuelas and Llusià 1998, Llusià and Peñuelas 1998), α-pinene, β-pinene and camphene were the most abundant terpenes.

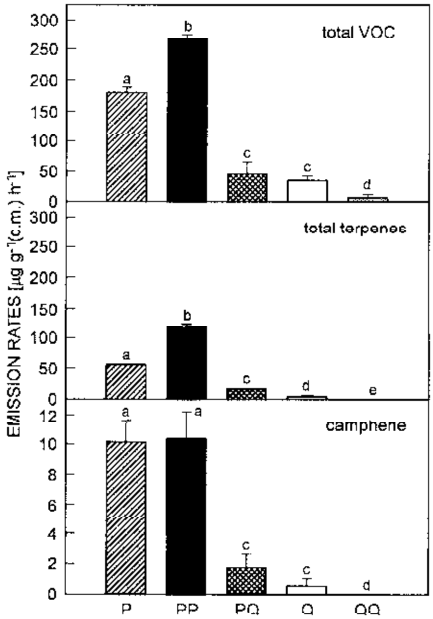

Fig. 1. Total volatile organic compounds (VOC), terpenes, and camphene emitted by P (*P. halepensis* seedling growing alone), PP (*P. halepensis* seedling growing together with another *P. halepensis* seedling), PQ (*P. halepensis* seedling growing together with a *Q. ilex* seedling), Q (*Q. ilex* seedling growing alone) and QQ (*Q. ilex* seedling growing together with another *Q. ilex* seedling). Different letters indicate statistically significant differences.

P. halepensis seedlings emitted more VOC and terpenes when accompanied by another *P. halepensis* seedling than when grown alone and, on the contrary, emitted less when accompanied by a holm oak seedling (Fig. 1). *Q. ilex* seedlings emitted more VOC and terpenes when growing alone than when growing accompanied by another holm oak seedling (Fig. 1). Most individual terpenes also followed those general trends, but others, such as camphene, did not differ between *P. halepensis* alone or accompanied by another pine (Fig. 1).

P. halepensis seedling biomass presented an inverse trend to their emission rates. Lower growth was found in pines when growing together with other pine but not when growing together with *Q. ilex* (Fig. 2). Holm oaks growing with a pine were smaller than growing alone or with other holm oak (Fig. 2).

Thus, although seedlings were provided with plenty of resources, such as water, nutrients and light, it still was found a decreased growth and an increased terpene emission in pines and holm oaks living together with other pines (only slightly smaller decreases in growth and decreased emission were found in pines and holm oaks growing with other holm oaks), indicating either better competitive ability of pine or possible autopathic and allelopathic effects on holm oak. These autopathic and allelopathic effects would be produced by the terpene storing species, *P. halepensis*, while the non storing species *Q. ilex* had not such effects. Terpenes have the capacity (in similar quantities as those of *P. halepensis*) to inhibit plant growth (Vokou 1992, Langenheim 1994).

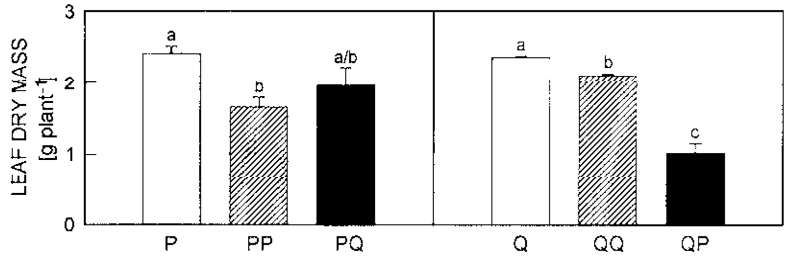

Fig. 2. Effect of inter- and intra-specific interference on leaf dry mass. Notation P, PP, PQ, Q, QQ and QP like in Fig. 1.

Although classical allelopathy experiments have found plant growth reductions (Hejl *et al.* 1993, Pellesier 1993, Einhellig 1995), there remains considerable controversy as to the ecological relevance of allelopathy in plant communities (Harper 1977, Harborne 1993). This study was not aimed to solve this controversy but only to study the inter- and intra-specific interference on terpene emission rates. It shows that a neighbour presence was translated in lower seedling biomass, especially when the neighbour was a pine, and that these reductions in growth were accompanied by higher terpene emission rates, but it is not possible with the present results to conclude whether the emitted terpenes were the cause or not. In fact, the biomass results have been also explained just as a result of different competitive ability for nutrients and water because of the different root system (*e.g.*, greater number of fine roots in *P. halepensis* L.) (Sardans 1997). In any case, the main conclusion here was that both intra- and inter-specific interference affected VOC and terpene emission of pines and holm oak.

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