Formaldehyde emission and uptake by Mediterranean trees 
Quercus ilex and Pinus halepensis

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A B S T R A C T

Formaldehyde (FA) is an ubiquitous gas in the atmosphere which reaches notable concentrations in polluted areas and can have great impact on human health. We studied FA exchange between air and two widespread Mediterranean tree species, Quercus ilex and Pinus halepensis. Experiments were conducted at the leaf level under laboratory conditions using air from outside the building. In both plant species FA exchange was mainly determined by the atmospheric mixing ratios, with a compensation point calculated around 20 ppbv. Higher values led to uptake and lower values to emission. The second factor that regulated FA exchange was stomatal conductance. FA exchange followed a diurnal cycle with the greatest exchange when stomatal conductance was at maximum. Such stomatal control is consistent with previous studies and is probably due to the high water solubility of FA, resulting in stomatal transpiration being its main exchange pathway. We also observed this relationship between stomatal conductance and FA exchange under conditions of drought and posterior rewatering, in which changes in stomatal conductance were paralleled by changes in FA exchange. Under projected future conditions of enhanced aridity in the Mediterranean, drought-driven limitations of FA exchange may be more relevant.

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1. Introduction

The emissions of volatile organic compounds (VOC) from plants have attracted great interest from the scientific community in recent years because they play an important role in atmospheric chemistry (Kavouras et al., 1998; Atkinson, 2000; Peñuelas and Llusia, 2003; Holzinger et al., 2005), in plant physiology (Singsaas and Sharkey, 1998; Peñuelas and Llusia, 2002; Velikova et al., 2005), and in plant–plant and plant–animal communication (Peñuelas et al., 1995; Pichersky and Gershenzon, 2002).

Among VOCs, a group of short-chained oxygenated compounds (oxVOCs) is being increasingly studied and, with the improvement of analytical techniques, more information is becoming available. This group includes C1-C3 VOCs like methanol, ethanol, formaldehyde, acetaldehyde, acetone, and formic and acetic acids. These oxVOCs reach considerable concentrations in the atmosphere, and are characterised by a high water solubility, and a long atmospheric lifespan; thus they can affect tropospheric chemistry far away from where they were emitted.

In this paper we focus on formaldehyde (FA). It is the most abundant carbonyl in the atmosphere (Muir and Shirazi, 1996) and also, after isoprene, the most abundant VOC in tropical forest air (Kesselmeier et al., 2002). Its concentrations in urban and polluted areas are notable (Granby et al., 1997), specially taking into account that it has been recognised as having a great impact on human health due to carcinogenic properties (Cogliano et al., 2004). FA emission from vegetation as well as deposition have been described in the literature (see Seco et al., 2007 for a review).
Our first aim was to monitor and describe the exchange of FA between the air and two widespread Mediterranean tree species, *Pinus halepensis* (aleppo pine) and *Quercus ilex* (holm oak), under controlled laboratory conditions. Our second aim was to test the effect of low water availability on FA exchange. Water is the most limiting factor for Mediterranean plants and aridity has increased in recent years (Piñol et al., 1998; Peñuelas et al., 2002) and is projected to increase even more in the coming decades in the Mediterranean basin (IPCC, 2007). This lack of water may affect FA exchange through direct effects on stomatal conductance. To see whether such influence exists, our experiment was designed to test the effect of decreased water availability and of the posterior rewatering on the FA exchange. Control well watered plants were left unwatered until soil water content reached 5% and these conditions were maintained until measurements started. After 1 day of measurements under these drought conditions plants were rewatered until soil water content reached 25% and then measurements were taken again.

Each single replication was started in the afternoon and left some time -normally until night- to adapt to the cuvette environment.

2. Materials and methods

2.1. Plant material and experimental design

For this study we used 2-year-old potted *Q. ilex* and *P. halepensis* plants grown in a nursery (Forestal Catalana, S.A., Breda, Catalonia, Spain), maintained under Mediterranean ambient conditions outdoors, in a semi-urban area near Barcelona (Catalonia, NE Spain, see Filella and Peñuelas, 2006b for a description of the site). They were grown in 2 L pots with a substrate composed of peat and sand (2:1), prior to being brought into the laboratory, where they were kept for some days to acclimatise to laboratory conditions before starting the experiment.

Experiments were conducted in leaf cuvettes in the laboratory at room temperature (21–34 °C) between July and November 2005, and in February 2006 (Table 1). A light diurnal cycle was programmed in the cuvette to simulate a typical sunny day, ranging from 0 to 1500 μmol m⁻² s⁻¹ of photosynthetic active radiation (PAR) flux. The air entering the leaf cuvette was taken from outside the building, filtered with glass wool to prevent dust intake and passed through a polyethylene terephthalate (PET) recipient to buffer exterior CO₂ and VOC fluctuations. All tubing used was made of inert polytetrafluoroethylene (PTFE).

The monitoring was done in three sets (*n* = 4, *n* = 3, *n* = 3) of plants for each species (Table 1). The last set of individuals was submitted to a drought treatment to study the effect of decreased water availability and of the posterior rewatering on the FA exchange. Control well watered plants were left unwatered until soil water content reached 5% and these conditions were maintained until measurements started. After 1 day of measurements under these drought conditions plants were rewatered until soil water content reached 25% and then measurements were taken again.

Each single replication was started in the afternoon and left some time -normally until night- to adapt to the cuvette environment.

2.2. Gas exchange measurements: CO₂, H₂O and FA

Leaves of the trees were enclosed in leaf cuvettes of either a LCpro-+ Photosynthesis System (ADC BioScientific Ltd., Hoddesdon, England) or a CIRAS-2 Photosynthesis System (PP Systems, Hitchin, UK). These instruments gathered photosynthesis (net CO₂ uptake), stomatal conductance, air humidity, and temperature data, while controlling the light radiation and the flow of air entering the leaf cuvette.

For VOC determination and quantification, both the air entering and exiting the leaf cuvette were monitored with flow meters and analysed with proton transfer reaction–mass spectrometry (PTR–MS from Ionicon Analytik, Innsbruck, Austria) at alternative intervals. The difference between the concentration of VOCs before and after passing through the cuvette, along with the flow rates, was used to calculate the VOC exchange.

Formaldehyde is a very soluble and sticky compound and thus blank levels measurements of the system were performed before conducting the experiment. Those blank measurements showed that the small variations in FA concentration between the air before and after passing the cuvette were insignificant compared with the higher variations observed during the experiments. Moreover, under atmospheric concentrations of FA where our experiments showed FA uptake, the blank measurements did never show FA uptake.

2.3. The PTR–MS technique

PTR–MS is based on chemical ionisation, specifically non-dissociative proton transfer from H₃O⁺ ions to most of the common VOCs, and has been fully described elsewhere (Lindinger et al., 1998).

In short, the H₃O⁺ ions produced by a hollow-cathode act as primary reactants and transfer a proton to every VOC with more proton affinity than that of H₂O molecules. Natural components of air do not suit this condition and

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Each single plant is identified with a reference number and is accompanied with information about the period of measurement, the external concentration of FA, and the treatment it received (if any). D & R means “drought and rewatering”.

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The table shows the sets of plants used in the experiment, including the species, period, reference number, external FA concentration, and treatment. The data are organized by set, with separate entries for each species and measurement period.
thus are not protonated, so the air itself is an adequate carrier gas. Once protonated, VOCs traverse a drift tube under the influence of an electric field and finally are detected by a quadrupole mass spectrometer. In our experiment the PTR–MS drift tube was operated at 2.1 mbar and 40 °C, with a E/N (electric field/molecule number density) of around 130 Td (townsend) (1 Td = 10⁻¹⁷ V cm²). The primary ion signal (H₃O⁺) was maintained at c. 4 x 10⁶ counts per second.

2.4. Correction of formaldehyde concentration for humidity

Formaldehyde is detected at the mass (m/z) 31 (Steinbacher et al., 2004). The measurement of FA by PTR–MS is affected by humidity due to its proton affinity similarity with that of water: protonated FA can give back the proton to a water molecule (what can be denominated “backwards reaction”) and in consequence can not be detected by PTR–MS (Hansel et al., 1997; Kato et al., 2004). Given that, due to transpiration, the air exiting the leaf cuvette was more humid than it was before entering the cuvette, we corrected the signal of m/z 31 for the humidity artefact. We considered the water vapour pressure of both the air before and the air after passing through the cuvette to correct the m/z 31 concentrations. The rate constant used for the forward reaction was 2 x 10⁻⁹ cm³ s⁻¹, and the rate constant for the backward reaction was 2 x 10⁻¹¹ cm³ s⁻¹ (T. Karl, personal communication).

In addition, we conducted measurements to test whether the humidity added by leaf transpiration at the gas stream flowing into the cuvette was affecting the stickiness of FA into the cuvette system or the sink of FA. The results showed no decrease in m/z 31 signal that could be attributed to this possible artefact: i.e. without leaves but with the same increase in atmospheric water vapour generated by adding it experimentally, there was no FA uptake.

2.5. Data treatment

Statistical analyses such as correlation and linear regression were performed with SPSS 13.0 for windows (SPSS Inc., Chicago, IL, USA). We conducted a step-wise regression to identify the main factors that regulate the exchange of formaldehyde. The factors included in the analysis were: photosynthesis, light, stomatal conductance, external concentration of FA, and leaf temperature.

3. Results

The air entering the leaf cuvette reached levels of FA ranging from 2 to 126 ppbv (1 ppbv = 1 part in 10⁹ by volume) during all the period, with mean values in the range of 4–68 ppbv and peaks at certain moments on some days (Table 1).

Fig. 1 shows a typical time series for P. halepensis of formaldehyde exchange, stomatal conductance, photosynthetic rate, light, temperature, and external concentration of FA. The same for Q. ilex is represented in Fig. 2. There was uptake of FA in both species, especially when mixing ratios outside the leaf were higher. When FA mixing ratios were lower, Q. ilex emitted FA.

Upon enclosure in the leaf cuvette, all plants showed a burst of photosynthetic assimilation, stomatal conductance, and formaldehyde uptake – the latter only if external concentrations were high (see Fig. 2A for an example).

Step-wise regression showed that, in the case of P. halepensis, FA exchange was primarily explained by stomatal conductance (change in r² = 0.46, p < 0.001), followed by external mixing ratios (change in r² = 0.25, p < 0.001). For Q. ilex this was different and the main regulating parameter was external concentration (change in r² = 0.75, p < 0.001), while stomatal conductance played a minor part (change in r² = 0.002, p < 0.001).

Figs. 3 and 4 plot FA exchange versus FA external concentration. Regression lines were calculated, and the equations reveal that for both species the compensation point (CP, the ambient concentration of FA that does not promote uptake or emission from the plant) for FA was around 20 ppbv.

Besides the influence of external concentrations, stomatal conductance correlated with the exchange of FA in some cases. This is clearly visible for P. halepensis in Fig. 3:

![Fig. 1. Uptake of FA by Pinus halepensis. One example is presented (P1, October 2005) out of seven replications.](image-url)
for a similar external concentration, the higher the conductance, the higher the uptake rate. For *Q. ilex* this relation is not clear (Fig. 4), although for some replicates under high external concentration such correlation was present (data not shown).

Pines submitted to drought took FA up at lower rates according to their lower stomatal conductance, while rewatering caused an increase in the uptake of FA, paralleled by stomatal conductance (Fig. 5). On the contrary, oak plants submitted to drought did not show a different FA exchange pattern (data not shown). Likewise, rewatered oaks showed no change in FA emission rates, although they had increased stomatal conductance (data not shown).

4. Discussion

Both species exchanged FA with the air, and the exchange generally showed a diurnal trend, being greater in the light than in the dark (Figs. 1 and 2). An analysis of the results indicates that the exchange was ruled by the external FA concentration and by stomatal conductance.

The influence of external concentration on FA exchange is not surprising since gas exchange depends on the gradient. External concentration has been highlighted to be one of the drivers of the emission or the uptake of short-chained oxVOCs in general, and of FA in particular (Kesselmeier and Staudt, 1999; Kesselmeier, 2001; Cojocariu et al., 2004; Rottenberger et al., 2004, 2005).

FA mixing ratios entering our leaf chamber were quite high. FA probably originated from the nearby highways by direct emission or as a product of the photochemical reactions of motor-produced VOCs (Grosjean et al., 2001). Although our laboratory is located in a semi-urban environment and thus may not be directly comparable with reported values for big cities, maximum peaks of FA were within the same range (Granby et al., 1997; Khare et al., 1997). Some of the replicates were in the same range of values as those reported inside motorways in Finland.
Formaldehyde exchange (nmol m$^{-2}$ s$^{-1}$)

Fig. 3. FA exchange ratios of Pinus halepensis plotted versus ambient mixing ratios, grouped according to stomatal conductance (gs, mmol m$^{-2}$ s$^{-1}$). Dashed lines are regressions for different stomatal conductance groups. Solid line and equation is the regression for all the data. Dotted lines indicate the compensation point, around 20 ppbv.

Formaldehyde exchange (nmol m$^{-2}$ s$^{-1}$)

Fig. 4. FA exchange ratios of Quercus ilex plotted versus ambient mixing ratios, grouped according to stomatal conductance (gs, mmol m$^{-2}$ s$^{-1}$). Solid line and equation is the regression for all the data. Dotted lines indicate the compensation point, around 20 ppbv.

(around 30 ppbv, Viskari et al., 2000). It is also possible that there were other FA sources in the surroundings of our laboratory of which we are unaware. Whatever the cause, the fact is that plants were submitted to a quite wide external concentration range, and this allowed us to see different patterns of FA exchange.

Our pines were always in ambient concentrations above the CP (≈20 ppbv) and thus in all cases they predominantly took FA up. Oaks, in contrast, had ambient levels of FA above and below the CP (≈20 ppbv) and thus they showed emission when mixing ratios were lower than the CP, and uptake when mixing ratios were above the CP.

CPs found in our study were very high in comparison to those in other reports. For instance, for Quercus pubescens in the field, the CP was calculated to be around 1 ppbv by Kesselmeier (2001). In forest canopies in Amazonia, the calculated CP was even lower, around 0.6 ppbv (Rottenberger et al., 2004). However, in canopies single plants are not solely responsible for the exchange of FA, therefore this data may not be comparable to our results.

We do not know the origin of these big differences in the magnitude of the CP, apart from the fact that we are comparing different species studied in distinct places. It has been reported that CP varies with physiological variables (temperature, light, stomatal conductance, etc.) (Cojocariu et al., 2004). Our CPs were calculated with a variety of stomatal conductances, temperatures, humidity, and light intensity values, reflecting what can be found throughout a 24-h day, including some drought-stressed plants, which may have influenced the result.

From the slopes of the equations in Figs. 3 and 4 we can deduce that the mean rates of FA uptake, normalised for the external concentration, were 0.10 nmol m$^{-2}$ s$^{-1}$ ppbv$^{-1}$ for pines and 0.05 nmol m$^{-2}$ s$^{-1}$ ppbv$^{-1}$ for oaks. These numbers are in the upper range of those described by Kondo and colleagues for oleander (Nerium indicum) (1995) and for various tree species (1996).

In the case of P. halepensis, our findings that FA exchange is influenced by stomatal conductance is in agreement with other authors’ conclusions, as far as that the exchange of FA between various plant species and the atmosphere takes place mainly through the stomata, either for uptake or for release (Kondo et al., 1995, 1996; Kreuzwieser et al., 2000; Schmitz et al., 2000; Rottenberger et al., 2004). Other studies on Pinus pinea reported in certain cases a relationship between stomatal conductance and FA exchange, but in general did not find a clear correlation with physiological variables (assimilation, transpiration and leaf conductance) (Kesselmeier et al., 1997).

A suggested reason for the stomatal control of FA exchange is its high solubility in water. FA has a Henry’s law constant (H, gas-aqueous phase partitioning coefficient) in the order of $10^{-2}$ Pa m$^3$, which is low if compared to those of highly volatile isoprenoids, which have H values in the order of $10^3$ Pa m$^3$ (Sander, 1999). This low H value partly links FA exchange to transpiration and makes it sensitive to changes in stomatal conductance (Niinemets and Reichstein, 2003). For this reason, the burst in stomatal conductance shown in Fig. 2A led to a burst in formaldehyde exchange. These bursts could be attributed to the response to the change in leaf conditions between before and after the act of enclosing the leaf. A sudden increase of the light and the flow of air reaching the leaf may have triggered stomatal opening and photosynthetic activity, as well as a high FA exchange through stomata due to the stomatic control of FA exchange.

In our Q. ilex measurements, a certain influence of stomatal conductance on FA exchange was seen for some plants. They showed uptake under high external concentrations, while the plants under drought treatment - which had lower external mixing ratios and presented mainly emission - were not influenced by stomatal conductance. This poor or lack of relationship between FA exchange and stomatal conductance is consistent with previous studies on Q. ilex, which reported that a relationship was occasionally observed but in general FA exchange was not clearly correlated to any of the physiological parameters (assimilation, transpiration and leaf conductance).
Further publications follow this trend: FA exchange did not correlate with stomatal conductance in *Picea abies* (Cojocariu et al., 2004) and *Fagus sylvatica* (Cojocariu et al., 2005). In tropical forests, while stating that the stomata were the main pathway for FA exchange, Rottenberger et al. (2004) also concluded that the influence of stomatal conductance on the exchange was not quantitatively reflected in diurnal courses of deposition rates. They suggested that for FA, mesophyll resistance may be in the same order of magnitude as stomatal resistance, and thus metabolic processes of FA consumption may also control the FA exchange, besides physical and chemical processes. It has been shown that glutathione–dependent formaldehyde dehydrogenase (FALDH) activity in *Arabidopsis* plants is proportionally related to the ability to detoxify FA (Achkor et al., 2003). Other studies also conclude that after a compound enters through the stomata, metabolism in leaf cells may be the limiting step for the absorption of FA (Filella et al., 2006) and other volatile compounds (Omasa et al., 2000). This could explain the weak relationship observed between stomatal conductance and FA exchange in our *Q. ilex* plants. In addition, adsorption to or absorption through the cuticle may have a role in deposition and these effects could be of more significance in *Q. ilex* than in *P. halepensis*, although we have not studied these aspects in the present work. However, in studies done with another oak species, bamboo-leafed oak (*Quercus myrsinaefolia*), Kondo et al. (1996) deemed the role of the cuticle in FA uptake to be insubstantial.

Projected future increases in the aridity of Mediterranean ecosystems (IPCC, 2007) may affect FA exchange, as a consequence of it being under stomatal control. Thus drought-driven limitations of FA exchange may become more relevant. Although the emission of many volatile organic compounds from vegetation is particularly sensitive to temperature (Guenther et al., 1993; Peñuelas and Llusia, 2001, 2003; Filella and Peñuelas, 2006a), in the conditions of this experiment the changes in stomatal conductance were of higher magnitude than the...

![Fig. 5. Exchange of FA by a Pinus halepensis plant (P5) submitted to drought and rewatering. Vertical line indicates the moment of watering. The increase in stomatal conductance is accompanied by an increase in the FA uptake rate.](image-url)
temperature changes, and the low range of temperatures studied did not significantly affect emissions. However, to estimate the consequences of Mediterranean summer drought on vegetation emissions, the effects of the high temperatures that accompany drought are to be also considered, especially because both these factors often have an antagonistic effect on VOC emissions.

5. Conclusions

We observed both emission and uptake of FA in Quercus ilex plants. In Pinus halepensis plants uptake was predominant and emission was not clearly observed, probably because the external FA concentration did not reach below the compensation point (CP). In both species the exchange of FA was under the rule of external FA mixing ratios, with a CP of approximately 20 ppbv. As expected, due to the high water solubility of FA, the exchange was also determined by stomatal conductance in *P. halepensis*. In *Q. ilex*, however, stomatal conductance seemed to play a secondary role, and mesophyll resistance to FA diffusion, along with adsorption to the cuticle, may have had more influence on the exchange of FA.

Under predicted future conditions of enhanced aridity in the Mediterranean, drought-driven limitations of FA exchange may be more relevant. In addition, the effect of the interaction between drought and high temperatures on FA emission and uptake deserves further study.

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