Emissió de Compostos Orgànics Volàtils en vegetació mediterrània

COVs oxigenats de cadena curta, interacció entre organismes i impacte atmosfèric

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Tesi doctoral

Emission of Volatile Organic Compounds by Mediterranean vegetation:

short-chain oxygenated VOCs, interaction between organisms and atmospheric impact

Ph.D. thesis

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Programa de Doctorat en Diversitat i Funció d'Ecosistemes Mediterranis
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Bellaterra, desembre de 2010

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Memòria presentada per

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per optar al grau de doctor

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Agraïments

Sigueu benvinguts a l'única part de la tesi que es llegeix tothom. Ja fa una pila –molts, qui sap si massa– d'anys que vaig començar aquest *viatge*, aquesta aventura d'escriure una tesi doctoral. Ha passat el temps, les experiències, les persones, els *papers*... i ja he arribat a la darrera *estació*.

Tot va començar quan en Josep Peñuelas va acceptar de vendre'm un bitllet pel *tren* de la recerca. Certament, hauria pogut vendre'l a algú altre, però va confiar en mi. Amb el bitllet a la mà vaig enfilar cap a l'andana i allà hi vaig trobar a la Iolanda Filella, que junt al Josep també m'acompanyaria durant tot el viatge. Vam pujar al vagó i el tren va arrencar. En aquell moment no n'era conscient, però a la part del darrere del vagó hi havia la resta de membres del grup d'Ecologia Global (que en aquest temps ha anat canviant de nom: primer *Ecofisiologia*, després *Ecofisiologia i Canvi Global*) liderat per en Josep Peñuelas. Tots ells m'han ajudat molt, ja sigui al laboratori, al despatx o al camp, en especial en Joan Llusià, en Jordi Sardans, en Marc Estiarte, en Romà Ogaya, la Sue Owen i l'Àngela Ribas.

Al vagó del costat hi havia uns passatgers que s'assemblaven molt a mi: els *be*caris i els *pre*caris. La llista és molt llarga (Loles A., Patricia P., Salva B., Giorgio A., Nacho B., Jara A., Belén S., Núria G., Imma O., Montse dC., Lucía G., Xavi A., Xavi D., Albert A., Miriam C., i tots els que em deixo) i seva va ser la culpa de passar molt bons moments, ja sigui tot dinant o en d'altres activitats dins i fora del CREAF. A cada nova parada, pujaven nous precaris (Miquel V., Martín G., Marta C., Maria D., Anna H., Albert R., etc.) alhora que d'altres acabaven el seu trajecte (Giorgio, Loles, Patricia, Salva, Marta D., etc.) o passaven a vagons amb millors seients (Xavi A., Xavi D., etc.).

Menció especial mereixen els ja doctors Loles i Salva (*¡hay que decirlo más!*), per les nostres aventures per mig món i els bons moments viscuts a tot arreu. Una menció especial no menys important també cal fer-la pels companys de *compartiment* (Marta D., Miquel V., Martín G., Rebeca I., amb curtes aparicions de la Marta P. i en Gorka M.) que han creat i mantingut un bon ambient de treball i de no-treball al llarg d'aquest viatge. Tan plàcida ha estat la convivència, que les decisions conjuntes més transcendentals a prendre han estat qui o quan s'obrien i tancaven la finestra, la persiana i la climatització. Sort que no ha passat cap revisor, oi, Miquel?

Uns vagons més enllà hi havia un vagó-gimnàs, on els *futbolerus* em van fer un racó i fins i tot em van deixar guanyar una lliga, després de la qual em vaig retirar quan encara estava al cim de la meva carrera esportiva. Van ser dels millors migdies que es poden passar al CREAF. La resta de gent del CREAF, repartida per tots els vagons, investigadors (Anna À., Daniel S., Jordi B., etc.), tècnics (Vicenç C., Helena B., etc), administratius (Magda P., Marta B., Cristina G., Charo dH., Sílvia Q.), informàtics (Carles C., Franc R.), els de la cuina dels migdies (Joan M., Arnald M., Agustí E., Víctor G., Ivette S., Lluís P., Edu L., Charo G., José Ángel B., Anna G., etc.) i tots els que ja no caben en aquest text, posen el seu granet de sorra per fer més fàcil la convivència i el treball al CREAF.

En un parell d'ocasions el tren va fer trajectes *transfronterers* i a cada estació em van acollir molt bé, tant en Jürgen W. i l'Einhard K. a Jülich com la gent de l'NCAR a Boulder (Alex G., Thomas K., Jim G., Tiffany D., Peter H., Andrew T., Romain B., Mónica M., Sasha M., Steve S., etc.). Ara fa poc, les dues campanyes de camp en què he participat m'han permès treballar

amb gent tan diversa i interessant com els investigadors de l'Institut Jaume Almera (Xavier Q., Andrés A., Jorge P., Marco P., etc.), els d'Innsbruck (Simon S., Axel M., etc.) i els italians de l'IBIMET de Bolonya (Francesca R., Osvaldo F. i Rita B.).

El meu tren particular va tenir algunes avaries, majoritàriament relacionades amb el *mystic* PTR-MS. En el seu moment em vaig arribar a preguntar si la R venia de Renfe... He d'agrair l'ajuda que m'han donat des de Ionicon per resoldre totes les incidències que anava tenint, en especial a n'Alfons J., Gernot H., Eugen H. i Lukas M.

Els amics, pocs però bons, m'han ajudat a esbargir-me de tant en tant. Un record especial pels amics de la carrera (Jordi S., Núria T., Cristina R., Martí B., Marc P., Gerard T., Dídac R., Sandra N., Laura R., Jordi M., Isis R., Jordi V., Eli S., etc.), i del CAU (Joan C., Dani B., Albert B., Linus M., Pere A., Xavi A., Carme F., Laura P., Enric F., etc).

A la família (pares, germans, avis, etc.) li pertoca també un bon agraïment, per haver-me *recollit* a l'estació cada cop que el tren parava a Granollers i haver escoltat amb atenció les meves poques paraules. Encara que no entenguin gaire bé el que faig, sempre m'han recolzat i sustentat quan ha calgut.

Last but not least, en una parada del Bages va pujar la Mònica, fa ja un grapat d'anys. Des de llavors, ha estat el meu millor i més sever *referee*, millorant el que ha pogut de les nostres vides. Ara el tren ha arribat a una estació de correspondència i hem de *triar* quin tren és el següent...

Com veieu, tot ha anat *sobre rodes*, en concret, rodes sobre rails: en tren. Què seria de tots aquests anys sense la participació de la RENFE? Durant la tesi, els trens (la RENFE fins i tot s'ha dividit en dues per embolicar encara més la troca) han canviat de logo –com el CREAF–. Però –com el CREAF– tot ha continuat igual. Sense la RENFE potser hauria trigat una mitjana de menys d'una hora en fer el trajecte entre Granollers i la UAB... inconcebible, oi? Gràcies als seus gestors i *al Estado* per haver-me donat l'oportunitat de conèixer en profunditat la bella *vila* de Montcada i Reixac, amb les seves boniques postes de sol i els bancs de metall gelat de les andanes.

Per últim, un recordatori per al finançament, perquè... com vaig *pagar* el bitllet del *tren*?

Gran part d'aquest treball (70% del temps) ha estat finançada amb una beca de Formació de Personal Investigador (FPI) del Ministeri d'Educació i Ciència del Govern Espanyol, amb referència BES-2005-6989, associada al projecte CGL2004-01402. Vull pensar que ha estat la meva modesta contribució per tractar d'equilibrar les tristament famoses balances fiscals *del Estado*. Aproximadament un 12% del temps de la tesi ha estat finançat per una prestació d'atur de nivell contributiu abonada per la Seguretat Social. El seu petit paper (7% del temps) en el finançament ha tingut també el projecte Consolider-Ingenio Montes CSD2008-00040 del Ministeri d'Innovació i Ciència del Govern Espanyol.

SUMARI

Resum de la tesi

Els compostos orgànics volàtils (COVs) presents a l'atmosfera poden tenir fonts naturals o antròpiques. Els COVs estan implicats en reaccions químiques que afecten la química atmosfèrica i poden tenir impactes indirectes sobre el clima a través de la generació d'aerosols i compostos oxidants. Globalment, la vegetació és la font principal de COVs naturals, mentre que els COVs antropogènics són la majoria de vegades producte d'emissions industrials i de motors de vehicles. D'entre els COVs biogènics, els isoprenoids són els més coneguts i estudiats.

La nostra recerca es va centrar en el grup de COVs de cadena curta oxigenats (oxCOVs), un grup poc conegut format per metanol, etanol, formaldehid, acetaldehid, acids fòrmic i acètic, i acetona. Els oxCOVs poden ser emesos directament tant per les plantes com per les activitats humanes, i també es poden formar per la degradació atmosfèrica d'altres COVs de major massa molecular. Entre d'altres raons, han estat menys estudiats degut a problemes analítics, que s'han resolt en part recentment amb l'aparició de noves tecnologies, especialment l'espectrometria de masses per transferència de protons. Els oxCOVs tenen diverses propietats que els diferencien d'altres COVs com els isoprenoids. Els oxCOVs són més solubles en aigua i això fa la seva emissió en plantes depenent dels estomes. També tenen una vida atmosfèrica relativament llarga i presenten concentracions considerables a l'atmosfera en el nivell de ppbv (1 part en 10^9 en volum).

Les emissions d'oxCOVs en plantes mediterrànies són molt poc conegudes, per això vam estudiar-ne l'intercanvi en els arbres típicament mediterranis *Quercus ilex* i *Pinus halepensis*. Es va confirmar el control estomàtic i de la concentració atmosfèrica del seu intercanvi. Per exemple, es va observar un punt de compensació de 20 ppbv pel formaldehid en les dues espècies d'arbre, mentre que per l'acetaldehid va ser de 6 ppbv en *P. halepensis*. Un tractament de sequera i posterior reg va mostrar que la sequera afecta l'intercanvi d'oxCOVs perquè afecta la conductància estomàtica. A més, es va confirmar que *Q. ilex* emet isoprè, encara que amb taxes petites, i que l'emissió d'isoprenoids en *Q. ilex* va disminuir per la sequera.

Els COVs poden tenir també un paper ecològic molt important: participar en les comunicacions entre organismes. En interaccions planta-planta la nostra hipòtesi era que el metanol podia jugar un paper senyalitzador, ja que el metanol s'emet en grans quantitats des de les fulles quan són atacades per un herbívor. Un experiment de fumigació amb metanol va mostrar que les respostes de *Q. ilex* al metanol arribat per l'aire eren similars a les respostes a l'herbivoria i també que l'emissió de monoterpens es va incrementar lleugerament. En la comunicació entre animals, vam identificar alguns components volàtils emesos pels capolls i els excrements d'una espècie d'abella solitària (*Osmia cornuta*), essent-ne l'àcid acètic un dels més abundants. Llavors vam provar l'atracció exercida per l'àcid acètic sobre femelles de

Monodontomerus aeneus, una vespa parasitoide d'*O. cornuta*, que busquen capolls per ovipositar-hi. Encara que l'àcid acètic sol atreia *M. aeneus*, probablement un senyal volàtil complex intervé en el procés d'atracció, ja que la ràtio àcid acètic-acetaldehid característica dels excrements va resultar més atractiva que altres ràtios.

L'objectiu final de la tesi va ser observar quin impacte tenen les emissions de COVs en l'atmosfera mediterrània, ja sigui pel nivell de concentració que atanyen els COVs a l'aire com també per la seva participació en la formació de compostos com l'ozó. Per això vam mesurar (hivern i estiu) les concentracions d'oxCOVs, isoprenoids i COVs aromàtics en una zona boscosa situada dins del parc natural del Montseny, a uns 60 km al NE de la ciutat de Barcelona. Mentre que els COVs aromàtics no varen presentar gairebé cap variació estacional, les concentracions dels oxCOVs van assolir nivells més alts a l'estiu degut a les majors emissions de la vegetació i a l'increment de les reaccions fotoquímiques resultants de les altes temperatures i radiació solar de l'estiu. Els COVs isoprenoids van mostrar el canvi entre estacions més gran: les concentracions van augmentar un ordre de magnitud a l'estiu, degut a l'increment de l'activitat fisiològica de la vegetació i de les seves taxes d'emissió. També les concentracions diürnes màximes d'ozó van augmentar a l'estiu, per la major activitat fotoquímica i la presència de més COVs a l'aire. La variació diària de concentracions de COVs seguia bàsicament el règim de vent de la muntanya. La brisa marina que es desenvolupa quan surt el sol transporta cap a la muntanya masses d'aire que prèviament han passat sobre la regió metropolitana de Barcelona, on han estat enriquides en NO_2 i en COVs d'origen biòtic i abiòtic. A més, aquestes masses d'aire contaminades poden reaccionar amb els COVs biogènics emesos de forma local per la vegetació, tot afavorint la formació d'O_3 en aquesta zona boscosa. L'únic tipus de COV que presentava un cicle diari diferent dels demés van ser els monoterpens, perquè tenien un origen biogènic local. L'isoprè també compartia parcialment el cicle diari dels monoterpens, però només a l'estiu quan les seves fonts biòtiques eren més fortes.

Thesis abstract

Volatile organic compounds (VOCs) present in the atmosphere can have natural or anthropic sources. VOCs are involved in atmospheric chemistry reactions that can have indirect climatic impacts through the generation of aerosols and oxidant compounds. Globally, vegetation is the main source of natural VOC emission, while anthropogenic VOCs are in most cases product of vehicle exhaust and industry emissions. Within biogenic VOCs, isoprenoids are the most studied and known group.

Our research was focused in a group of short-chain oxygenated VOCs (oxVOCs), a poorly known group composed by methanol, ethanol, formaldehyde, acetaldehyde, formic and acetic acids, and acetone. They can be emitted directly either by plants or by human activities, and they can also be formed through degradation in the air of other higher molecular mass VOCs. Among other reasons, they have been less studied due to analytical problems than have been partly solved in recent years with the advent of new technologies, specially proton transfer reaction mass spectrometry. OxVOCs have several properties that set them apart of other VOCs like isoprenoids. OxVOCs are more soluble in water and this fact makes their release from plants stomatal-dependent. In addition, these compounds have relatively long atmospheric half-lives and reach considerable concentrations in the atmosphere in the range of parts per billion in volume (ppbv).

Emissions of oxVOCs in Mediterranean plants are scarcely known, for this reason we studied oxVOCs exchange in the typically Mediterranean tree species *Quercus ilex* and *Pinus halepensis*. The stomatal and atmospheric mixing ratios control of their exchange was confirmed. For instance, a compensation point of 20 ppbv for formaldehyde was observed for both tree species, while for acetaldehyde it was of 6 ppbv in *P. halepensis*. A treatment of drought and posterior re-watering showed that drought affects oxVOC exchange because it affects stomatal conductance. In addition, it was confirmed that *Q. ilex* emits isoprene, although at small rates, and that isoprenoid emission in *Q. ilex* decreased due to drought.

VOCs can also have an important ecological role: taking part in the communication between organisms. In plant-plant interaction our hypothesis was that methanol could have a signalling role, given that methanol is emitted in big amounts from leaves upon herbivore attack. An experiment of fumigation with methanol showed that *Q. ilex* responses to airborne methanol were similar to responses to herbivory and also that monoterpene emission was slightly increased. In communication between animals, we identified some volatile components emitted by the cocoons and frass of a solitary bee species (*Osmia cornuta*), being acetic acid an abundant one among them. We then tested acetic acid attractiveness for *Monodontomerus aeneus* female wasps, parasitoids of *O. cornuta*, that look for cocoons to oviposit. Although

acetic acid alone attracted *M. aeneus*, a complex volatile signal is probably involved in the attraction process since the ratio acetic acid-acetaldehyde characteristic of the frass was more attractive than other ratios.

The last objective of this thesis was to observe the impact that VOC emissions have on the Mediterranean atmosphere, either by the mixing ratios that VOC reach in the air or also by their participation in the formation of compounds like ozone. To achieve this, the mixing ratios of oxVOCs, isoprenoids, and aromatic VOCs were measured (winter and summer) in a forest site located within a densely forested natural park (Montseny) about 60 km NE of the city of Barcelona. While aromatic VOCs showed almost no seasonal variability, short-chain oxygenated VOCs presented higher mixing ratios in summer due to the increased emission by vegetation and the increased photochemistry, both enhanced by the high temperatures and solar radiation in summer. Isoprenoid VOCs showed the biggest seasonal change in mixing ratios:they increased by one order of magnitude in summer, as a result of the increased vegetation physiological activity and emission rates. Ozone highest diurnal concentrations increased in summer too, due to more photochemical activity and the presence of more VOCs in the air. The daily variation of the VOC mixing ratios was mainly governed by the wind regime of the mountain. Sea breeze that develops after sunrise advects to the mountain polluted air masses that previously had passed over the Barcelona metropolitan region, where they were enriched in NO_2 and in VOCs of biotic and abiotic origin. Furthermore, these polluted air masses can react with biogenic VOCs emitted at the local valley by the vegetation, thus enhancing O_3 formation in this forest site. The only VOC species that showed a different daily pattern were monoterpenes because of their local biogenic emission. Isoprene also shared partially the daily pattern of monoterpenes, but only in summer when its biotic sources were stronger.

Introducció general

Introducció general

Els Compostos Orgànics Volàtils

- Què són els COVs?

La finalitat d'aquesta tesi doctoral ha estat investigar l'intercanvi de Compostos Orgànics Volàtils (COVs, *VOCs* en anglès) en plantes mediterrànies i el seu paper en la comunicació entre organismes i en la química atmosfèrica. Els COVs són molècules *orgàniques* –formades per una base estructural d'un o més àtoms de carboni (C)– i *volàtils* –que s'evaporen fàcilment–. Per tant, podríem dir que són compostos químics basats en carboni que s'acostumen a trobar principalment en forma gasosa a l'atmosfera. Tenint en compte que l'aire de l'atmosfera està format majoritàriament per nitrogen (N_2, 78% en volum) i oxigen (O_2, 21% en volum) que són molècules inorgàniques, els COVs només poden formar part de la petita fracció que resta (1%). Dins d'aquesta petita part, també trobem gasos com el diòxid de carboni (CO_2) i com el metà (CH_4), tots ells força coneguts per la seva relació amb l'efecte hivernacle i el canvi climàtic. La majoria de gasos orgànics que no són metà, es poden englobar sota la denominació COVs, fet que es reflecteix en els noms que varen rebre en un principi: *NonMetane Hidrocarbons* (NMHC) i *NonMetane Volatile Organic Compounds* (NMVOC) (Kesselmeier i Staudt, 1999).

Rera uns noms tan poc concrets s'amaguen milers de molècules diferents, ben diverses tant en estructura i propietats químiques com en fonts, embornals i interaccions amb la biosfera i l'atmosfera. Per això s'han subclassificat els COVs en grups, alguns dels quals són objecte d'aquesta tesi.

- Tipus de COVs

S'han descrit milers de COVs diferents al llarg dels anys, amb múltiples classificacions com per exemple la de COVs antropogènics i COVs biogènics, que no deixa de ser "imperfecta" ja que hi ha compostos que en cada cas concret podrien ser assignats tant en una categoria com en una altra segons si han estat generats per l'acció humana o per la vegetació. Classificats segons la seva estructura molecular i composició atòmica, obtenim altres categories. Dels anomenats antropogènics, els COVs aromàtics són dels

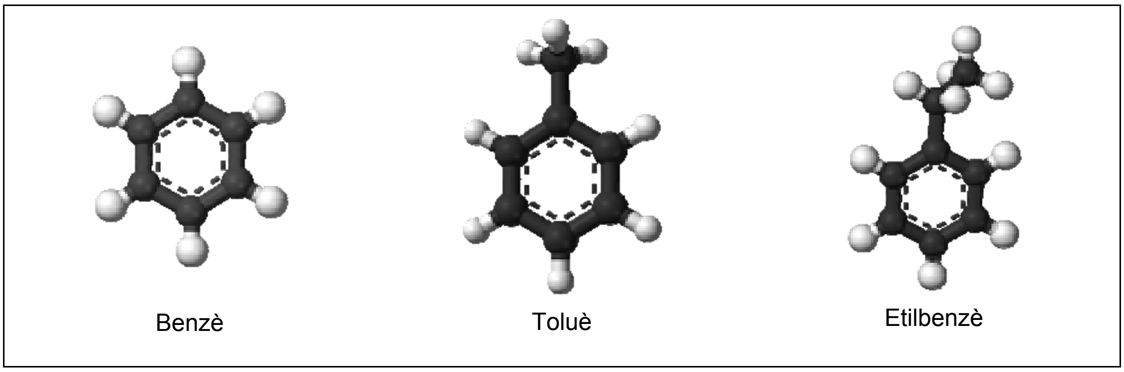


Fig. 1. Exemples de compostos orgànics volàtils aromàtics, típics COVs antropogènics.

Àtoms: ● Carboni ○ Hidrogen

més estudiats. Pertanyen a aquest grup compostos com el benzè, el toluè, l'etilbenzè i el xilè, tots ells formats a partir d'un anell benzènic (Fig. 1).

Centrant-nos en els COVs biogènics, els dominadors absoluts de l'escena científica són els isoprenoids. Tenen com a base l'isoprè (2-metil-1,3-butadiè), primer COV biogènic descrit als anys 60, que està format per una estructura atòmica de 5 carbonis (Fig. 2). Resumidament, podem dir que de la unió de dues molècules d'isoprè n'obtenim una de monoterpè (10 carbonis), i de tres n'obtenim un sesquiterpè (15 carbonis), que són els isoprenoids volàtils més habituals (Fig. 2). Els monoterpens i sesquiterpens, en sentit ampli, formen un conjunt de milers de compostos que poden presentar diferents estructures (amb anells o linials), poden contenir àtoms d'oxigen, etc. Especialment l'isoprè i els monoterpens han estat extensament descrits pel que fa a emissió (Kesselmeier and Staudt, 1999), efectes en la fisiologia vegetal

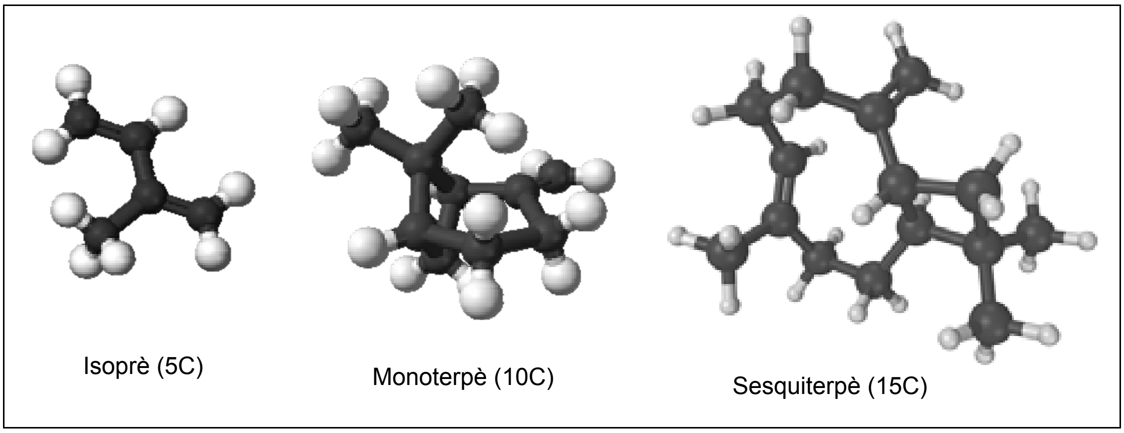


Fig. 2. Exemples d'isoprenoids. Entre parèntesi, el nombre d'àtoms de carboni que contenen.

Àtoms: ● Carboni ○ Hidrogen

(Peñuelas i Llusià, 2002; Loreto et al., 2004), interacció planta-planta (Godard et al., 2008) i planta-animal (Pinto et al., 2007; Halitschke et al., 2008), etc. Al mateix temps, són els COVs que compten amb un major treball de modelització de les seves emissions (Guenther et al., 1993; Keenan et al., 2009), arran de les seves implicacions en la química atmosfèrica i el clima (Tunved et al., 2006; Kiendler-Scharr et al., 2009; Pacifico et al., 2009).

Dins de la gran diversitat dels COVs biogènics, però, la nostra atenció inicial es va fixar en un grup de compostos de cadena curta oxigenats (oxCOVs, *oxVOCs* en anglès). Aquests oxCOVs tenen d'un a tres àtoms de carboni, i són els que es mostren a la Taula 1. Des del punt de vista de les emissions vegetals, aquests oxCOVs són pràcticament desconeguts, sobretot si es compara amb la ingent informació disponible sobre l'intercanvi atmosfera-biosfera d'altres COVs com els isoprenoids (Kesselmeier i Staudt, 1999). Els oxCOVs, com a grup, tenen certes característiques que els distingeixen dels isoprenoids, com per exemple que són molt menys volàtils, menys reactius, i molt més solubles en aigua. Aquestes característiques influeixen molt en com s'esdevé l'intercanvi entre l'aire i les plantes.

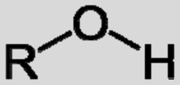
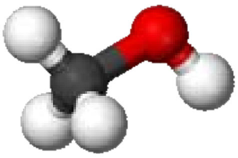
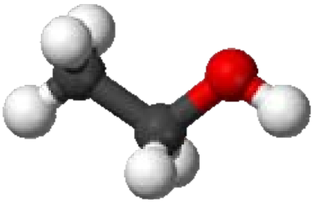
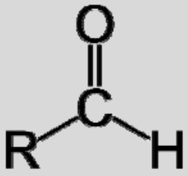
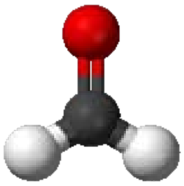
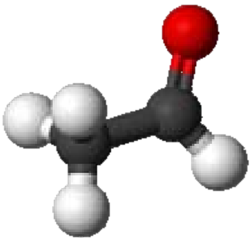
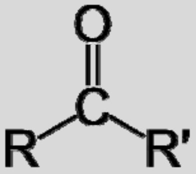
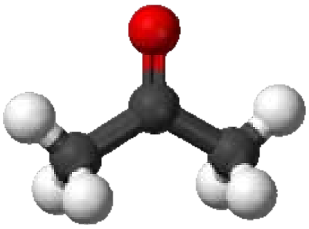
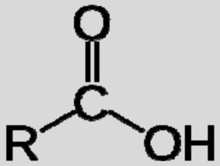
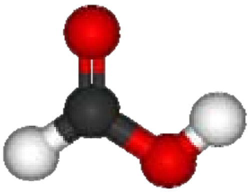
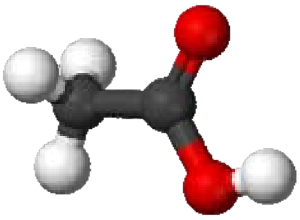
- Interès de l'estudi dels COVs

Amb una mirada antropocèntrica, els COVs que des de fa anys s'estudien són els gasos contaminants producte de la combustió dels motors (per exemple, de vehicles com cotxes o motos), dels processos industrials (evaporació de dissolvents, etc.), i en general de tota l'activitat humana. Per això aquests se solen anomenar *COVs antropogènics*. Els seus efectes perniciosos sobre la salut han contribuït indubtablement al foment del seu estudi i són la raó del seu monitoratge en ambients urbans i industrials per part de les agències governamentals de medi ambient a bona part del món.

Des d'un punt de vista més biològic, hi ha certs tipus de COVs que bàsicament són coneguts perquè són emesos per la vegetació i, per tant, han rebut el nom genèric de *COVs biogènics*. Ja a partir dels anys seixanta del segle passat es va assenyalar la vegetació com a font de COVs (Went, 1960; Rasmussen i Went, 1965), i des de llavors s'ha anat avançant –de la mà dels avenços tecnològics– cada cop més en el coneixement de l'intercanvi de COVs entre les plantes i l'aire, així com de les seves funcions fisiològiques i ecològiques. La importància d'estudiar-los rau en el fet que poden tenir, i de fet se sap que tenen, diversos papers en la fisiologia i l'ecologia de les plantes.

Taula 1. Els COVs de cadena curta oxigenats (oxCOVs) estudiats en aquesta tesi, mostrats segons el nombre d'àtoms de carboni que formen la molècula i segons els tipus de grup funcional: de menys oxidat (alcohols) a més oxidats (àcids), passant pels carbonils (aldehids i cetona).

Àtoms: ● Carboni ○ Hidrogen 🔴 Oxigen

Tipus compost / **num C**	**1 àtom de carboni**	**2 àtoms de carboni**	**3 àtoms de carboni**
 **Alcohol**	 Metanol	 Etanol	
 **Aldehid**	 Formaldehid	 Acetaldehid	
 **Cetona**	(inexistent)	(inexistent)	 Acetona
 **Àcid carboxílic**	 Àcid fòrmic	 Àcid acètic	

Per exemple, sembla que proporcionen protecció contra altes temperatures (Singsaas and Sharkey, 1998; Peñuelas et al., 2005), forta irradiació solar (Peñuelas and Munné-Bosch, 2005) i estrès oxidatiu (Peñuelas and Llusià, 2002; Velikova et al., 2005). També actuen com senyals de comunicació planta-planta (Peñuelas et al., 1995; Pierik et al., 2003; Baldwin et al., 2006; Heil and Kost, 2006; Kegge and Pierik, 2010) i com "vàlvules de seguretat" de les plantes (Rosenstiel et al., 2004). A més, també tenen un rol en les relacions planta-animal, que no només impliquen relacions planta-pol·linitzador (Wright i Schiestl, 2009) o defensa directa contra herbívors, sinó també defensa indirecta a través de l'atracció dels enemics naturals dels herbívors (Kessler i Baldwin, 2001; Llusià i Peñuelas, 2001; Pichersky i Gershenzon, 2002).

Lligant els punts de vista humà i ecològic ens trobem la biogeoquímica, que és l'àmbit on conflueixen tant els COVs antropogènics com els biogènics. D'una banda, els COVs tenen un paper en el cicle del carboni (Fig 3).

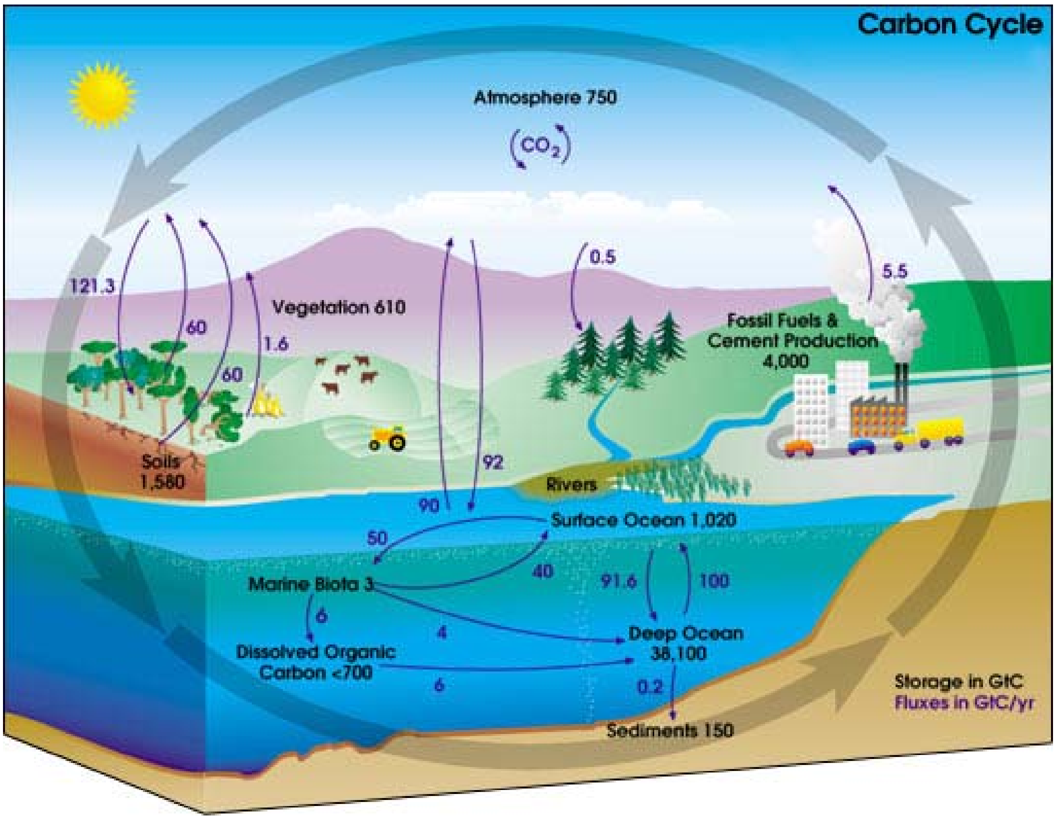


Fig. 3. El cicle global del carboni. Hi ha indicades les quantitats de carboni emmagatzemades en color negre, i els fluxos en color violeta, tot en gigatones de carboni (GtC =10^{15} gC). Font: NASA.

S'ha vist que la vegetació no només retorna carboni a l'atmosfera mitjançant el CO_2 de la respiració cel·lular sinó que també n'emet una bona part en forma de COVs, podent arribar a representar un 1-2% del carboni assimilat globalment pels ecosistemes terrestres (Peñuelas i Staudt, 2010). A més, després de la seva emissió els compostos passen a formar part de la barreja de gasos de l'aire, on participen en reaccions químiques i fotoquímiques (Kavouras et al., 1998; Atkinson, 2000; Peñuelas i Llusià, 2003; Holzinger et al., 2005). Aquestes reaccions propicien la formació i degradació d'altres compostos (ozó, òxids de nitrògen, altres COVs producte de la degradació dels COVs originals, etc) i partícules (Fig. 4). Tots ells tenen influència en les propietats atmosfèriques (nivell oxidatiu, albedo, formació de núvols, regulació tèrmica, etc.) i per tant poden acabar afectant la fisiologia dels éssers vius, la qualitat de l'aire i la salut humana, així com finalment el balanç energètic de la terra, que ja està actualment afectat pel canvi global (Fig. 4).

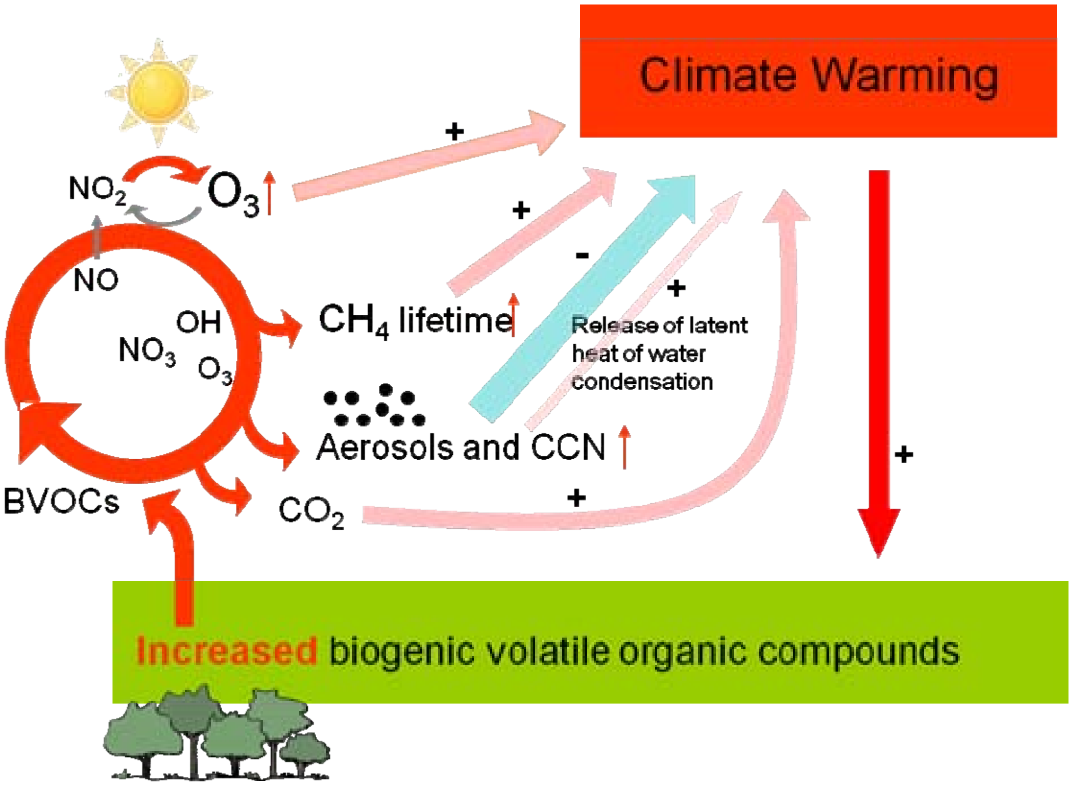


Fig. 4. Els COVs participen en múltiples reaccions a l'atmosfera, que poden comportar la generació de nous compostos com l'ozó troposfèric i la formació d'aerosols. Aquests fets interaccionen amb l'escalfament climàtic, que al seu torn també pot provocar un augment de les emissions biogèniques dels COVs. Imatge extreta de Peñuelas i Staudt (2010).

- Els COVs i el canvi global

Quan parlem del canvi global ens referim als canvis que s'han i s'estan produint en diferents processos bàsics de funcionament del planeta a causa de l'activitat humana. L'espècie humana ha estat vivint a la Terra durant centenars de milers d'anys, però durant el darrer segle les seves activitats han augmentat i així també els seus efectes sobre el medi ambient. Per bé que l'escalfament climàtic és el factor més conegut del canvi global (Fig. 5), no podem oblidar que n'hi ha d'altres com l'augment de CO_2 a l'atmosfera, l'eutrofització dels ecosistemes, els canvis en els usos del sòl i la fragmentació dels hàbitats, les invasions biològiques i la pèrdua de biodiversitat, la disminució de les precipitacions (Fig. 5) i l'augment de la desertització, etc.

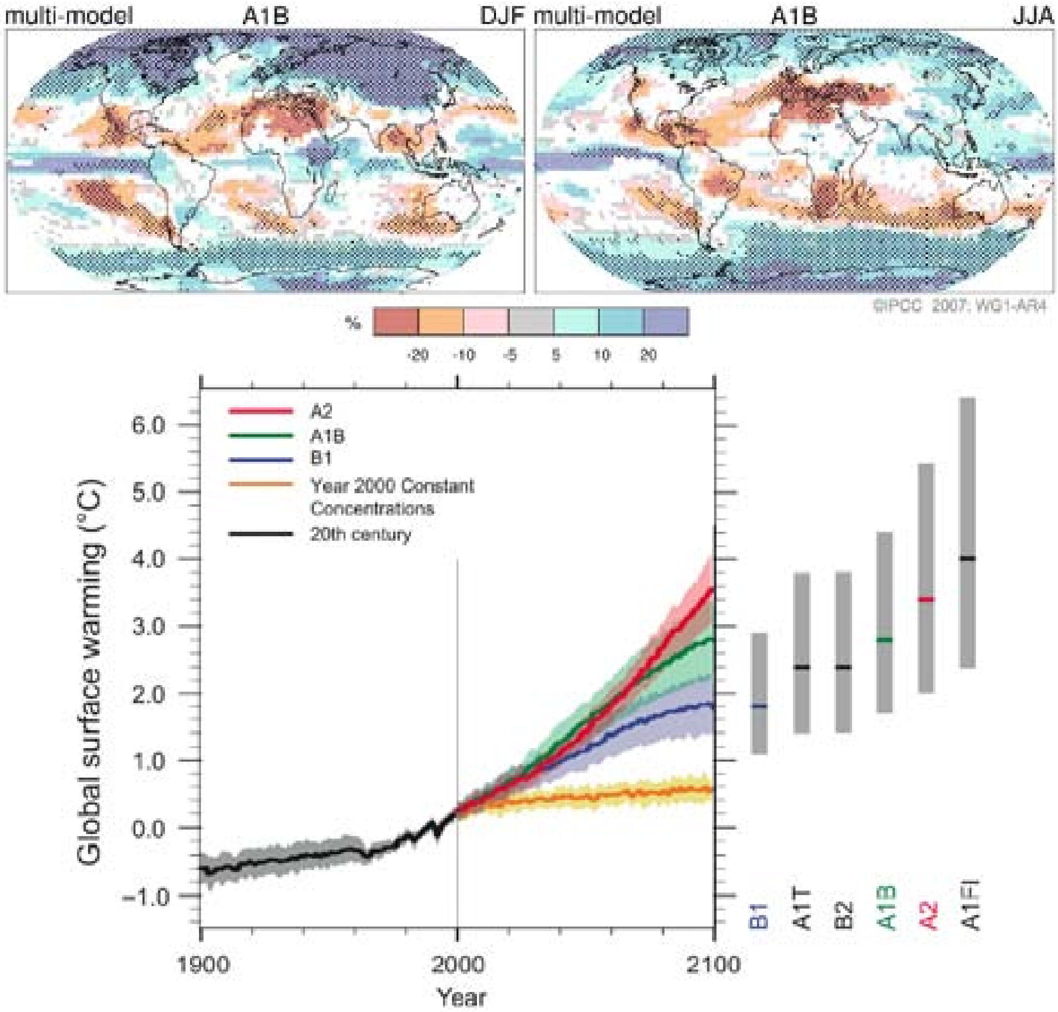


Fig. 5. Projeccions de l'IPCC de precipitació i temperatura per les properes dècades. A dalt, projecció del canvi en el règim de precipitacions pels mesos de desembre, gener i febrer (esquerra) i juny, juliol i agost (dreta) estimat per l'escenari A1B comparant el període 2080-2099 amb el 1980-1999. A baix, projecció de l'evolució de la temperatura de la superfície de la terra en diferents escenaris (B1, A1T, B2, etc). Font: IPCC (2007).

Les conseqüències del canvi global sobre les emissions de COVs biogènics són altament incertes, tot i que es preveu que en general les emissions s'incrementin en resposta a l'escalfament, i estan actualment al centre de l'atenció científica (Peñuelas i Staudt, 2010). A més a més, també es creu que els COVs emesos a l'aire poden afectar el clima, com ja hem dit mitjançant la seva interacció amb la química atmosfèrica (Fig. 4), participant en la regulació de la formació d'ozó, radicals oxidants i aerosols, entre d'altres (Kulmala et al., 2004; Fowler et al., 2009; Kiendler-Scharr et al., 2009; Peñuelas i Staudt, 2010). Tant els efectes sobre el clima dels COVs emesos com els efectes del clima sobre l'emissió de COVs es retroalimenten positivament o negativa (Peñuelas et al., 2009), segons els casos, i s'inscriuen en un marc més ampli on interactuen altres factors de canvi global (Fig. 6), tot configurant un complex conjunt de relacions entre els cicles biogeoquímics terrestres i el sistema climàtic del planeta (Arneth et al., 2010).

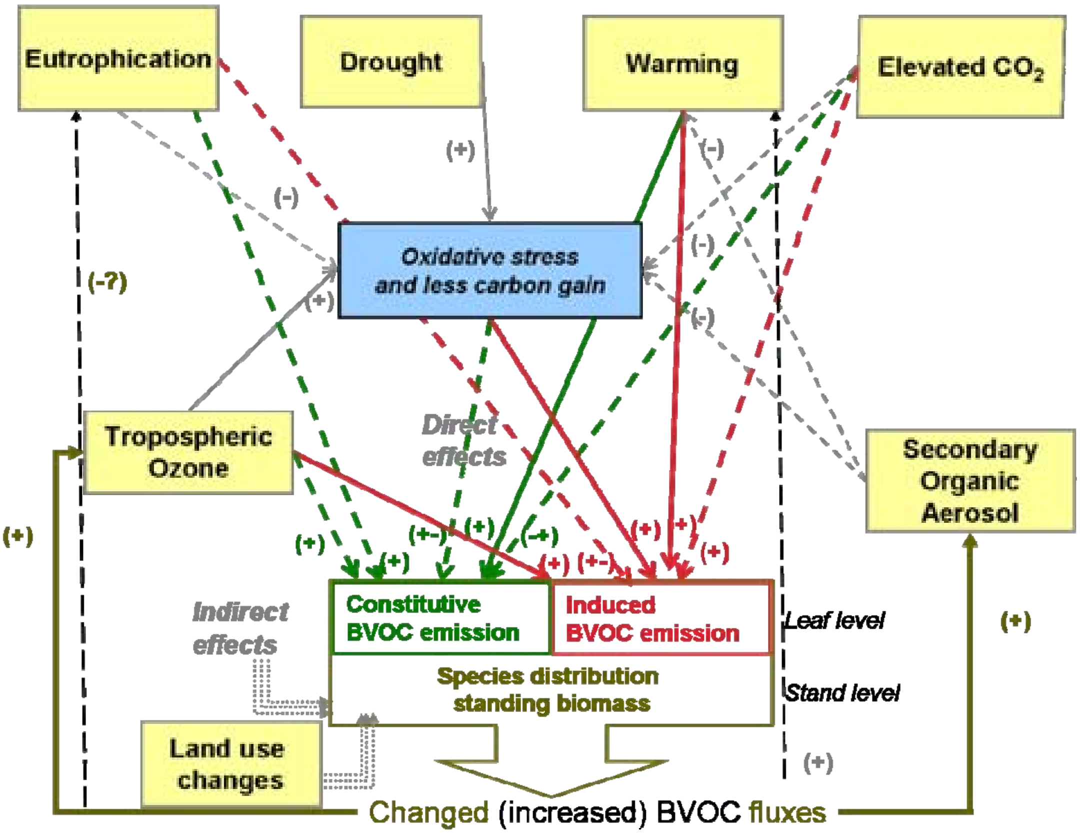


Fig. 6. Visió general del efectes i possibles interaccions dels canvis globals del clima i la composició atmosfèrica sobre/amb les emissions biogèniques de VOCs, distingint entre emissions constitutives (verd) i emissions induïdes per estrès (vermell). Les evidències experimentals suggereixen que molts canvis ambientals globals tenen efectes directes sobre l'emissió de COVs biogènics a nivell de fulla, ja siguin incrementant-la (línies contínues) o tenint efectes diversos (línies discontínues). (+) indica clars efectes globals positius, (+-) indica efectes tant positius com negatius, i (-?) possibles efectes negatius no totalment demostrats. Imatge extreta de Peñuelas i Staudt (2010).

Treball realitzat a la tesi

- Objectius de la tesi

Els objectius d'aquesta tesi doctoral es poden resumir en els següents punts.

Capítol 1

- Sumaritzar el coneixement actual sobre els oxCOVs pell que fa a:
  - a) característiques fisicoquímiques, fonts i embornals atmosfèrics, concentracions atmosfèriques registrades per tot el món
  - b) paper en el metabolisme vegetal, magnitud de les emissions descrites, tant a nivell de fulla com de comunitat, i quins factors les regulen

Capítol 2

- Conèixer les taxes d'emissió o absorció d'oxCOVs en plantes mediterrànies (*Quercus ilex* i *Pinus halepensis*)
- Veure què regula aquest intercanvi
- Observar l'afectació de la sequera i el posterior reg sobre l'intercanvi d'oxCOVs i també d'isoprenoids

Capítol 3

- Comprovar el possible paper dels oxCOVs en la comunicació entre plantes: respostes de *Quercus ilex* a la fumigació amb metanol
- Veure el rol dels COVs en les interaccions entre animals: la localització de l'hoste per la vespa parasitoide *Monodontomerus aeneus*

Capítol 4

- Conèixer quines concentracions de COVs es registraven en un bosc mediterrani al parc natural del Montseny, pel que fa a:
  - a) variacions diàries i estacionals (hivern i estiu)
  - b) relació amb les emissions vegetals i les concentracions d'ozó
  - c) magnitud en relació a d'altres ecosistemes boscosos i naturals del món

La primera cosa que vaig fer per estudiar l'intercanvi d'oxCOVs va ser cercar la informació disponible a la literatura. D'aquest treball bibliogràfic en va sortir un article de revisió que sumaritza i aglutina el saber actual sobre aquests compostos: característiques generals, origen i destí dins les plantes, fonts i embornals a l'atmosfera, factors que n'augmenten les emissions, magnitud de les emissions descrites per altres autors, incògnites, etc. En el ***capítol 1*** trobareu aquesta revisió, que serveix com a introducció als oxCOVs.

Un cop feta la revisió, va ser el torn d'entrar al laboratori per observar les emissions i/o absorcions d'oxCOVs en dues espècies arbòries típiques i àmpliament distribuïdes per la conca mediterrània: el pi blanc (*Pinus halepensis*) i l'alzina (*Quercus ilex*). Per a fer-ho vam usar unes cambres foliars i el PTR-MS (Fig. 7). El ***capítol 2*** recull els resultats de l'observació de l'intercanvi de oxVOCs en plançons de pi blanc i alzina, en condicions normals al laboratori, usant aire de l'exterior, sense filtrar ni modificar. Amb aquesta observació de l'intercanvi en condicions normals volíem veure quina pauta diària segueix l'intercanvi, quins factors fisiològics o ambientals el condicionen, i quina era la seva magnitud. Més enllà d'això, també vam fer un petit experiment que consistia en sotmetre els plançons a una certa privació d'aigua, per veure si la sequera afectava, i de quina manera, l'emissió o l'absorció d'oxCOVs. Seguidament vam regar les plantes per observar la reacció de l'intercanvi d'oxCOVs al reg després del tractament de sequera. El *subcapítol 2.1* tracta exclusivament de l'intercanvi de formaldehid en *Q. ilex* i *P. halepensis*, mentre que en el *subcapítol 2.2* es tracten la resta d'oxCOVs, en pi blanc només. El *subcapítol 2.3* presenta breument els resultats d'aquesta mateixa caracterització, però centrant-se en l'intercanvi d'isoprenoids en l'alzina.

El proper pas, un cop conegut una mica més com s'esdevé l'intercanvi d'oxCOVs en aquestes plantes mediterrànies, va ser intentar comprovar si podien tenir algun paper en la comunicació entre organismes. Aquesta idea dóna lloc al ***capítol 3***. Primer, sabent que les emissions de metanol augmenten en resposta a l'herbivoria, vam pensar que una planta envoltada d'altres plantes que estan essent atacades per herbívors rebria un senyal en forma de metanol i hi podria reaccionar. Amb aquesta premissa vam realitzar un experiment que consistia en fumigar plançons d'alzina amb metanol (*subcapítol 3.1*), tot introduint-los sencers dins d'una cambra de tefló transparent. Volíem observar les possibles reaccions fisiològiques de les alzines a la fumigació amb metanol, especialment pel que fa a l'intercanvi de COVs. Alhora vam comparar aquestes reaccions al metanol amb les reaccions al fet de tallar parts de les fulles, simulant herbivoria. A la segona part del capítol (*subcapítol 3.2*) es tracta la participació d'alguns oxCOVs a les relacions animal-animal. En col·laboració amb companys del CREAF que estudien insectes pol·linitzadors, vam estudiar el paper dels COVs en la localització del capoll d'una abella solitaria (*Osmia cornuta*, de la família dels megaquílids) per part d'un dels seus parasitoids (*Monodontomerus aeneus*, vespa de la família dels torímids).

Finalment, es va fer un exercici per abordar l'impacte dels COVs biogènics sobre la composició química atmosfèrica. El ***capítol 4*** mostra l'anàlisi de les mesures de COVs fetes al Parc Natural del Montseny durant l'hivern i l'estiu. El punt de mostreig al Montseny, la casa de La Castanya, està situat en una vall dominada per alzinar. Aquesta campanya va servir per estudiar-hi per primera vegada els nivells i variacions (diàries i estacionals) de les concentracions de COVs a l'aire, i veure com es relacionaven amb altres paràmetres meteorològics i ambientals, com ara les concentracions d'ozó.

- Material de laboratori i tècniques analítiques

En tots els treballs on volia estudiar l'intercanvi de COVs realitzats a nivell de fulla, vaig emprar unes cambres foliars, comercials, dissenyades específicament per a mesurar paràmetres fisiològics de fulles. Consisteixen bàsicament en una mena de pinça que enclou suaument la fulla o fulles estudiades, de manera que crea una cambra amb les condicions controlades, com per exemple la temperatura, la llum incident i el flux d'aire que hi circula. A més, un sensor d'infraroig (IRGA, Infra Red Gas Analyzer) quantifica la concentració de CO_2 i aigua de l'aire abans i després de passar per la cambra. Així, la fulla es mantenia en unes condicions ambientals definides prèviament alhora que se'n seguia contínuament l'intercanvi de CO_2, la transpiració i conductància estomàtica, la temperatura, la radiació incident, etc. D'aquestes cambres foliars se n'extreia una mostra d'aire, a intervals regulars, per comparar alternativament la diferència de concentració de COVs entre l'entrada de la cambra i la sortida, i deduir-ne l'emissió o absorció de la fulla o fulles (Fig. 7).

Per a mesurar la concentració de COVs al llarg de la tesi, ja sigui en mesures a nivell de planta o bé d'aire ambiental, vaig usar principalment un espectròmetre de masses per reacció de transferència de protons (PTR-MS, de l'anglès *Proton Transfer Reaction-Mass Spectrometry*), una tecnologia relativament nova (poc més d'una dècada d'existència) i que està revolucionant el coneixement dels oxCOVs perquè en facilita la identificació i, a més, en permet saber la concentració de forma gairebé instantània. El seu funcionament es basa en la ionització química, específicament en una transferència no dissociativa d'un protó des d'una molècula d'hidroni (H_3O^+) cap a la majoria dels COVs més comuns. Una font d'ions genera les molècules de H_3O^+ a partir de vapor d'aigua, i aquestes reaccionen amb les molècules de COV que hem fet entrar per a analitzar al PTR-MS (Fig. 7).

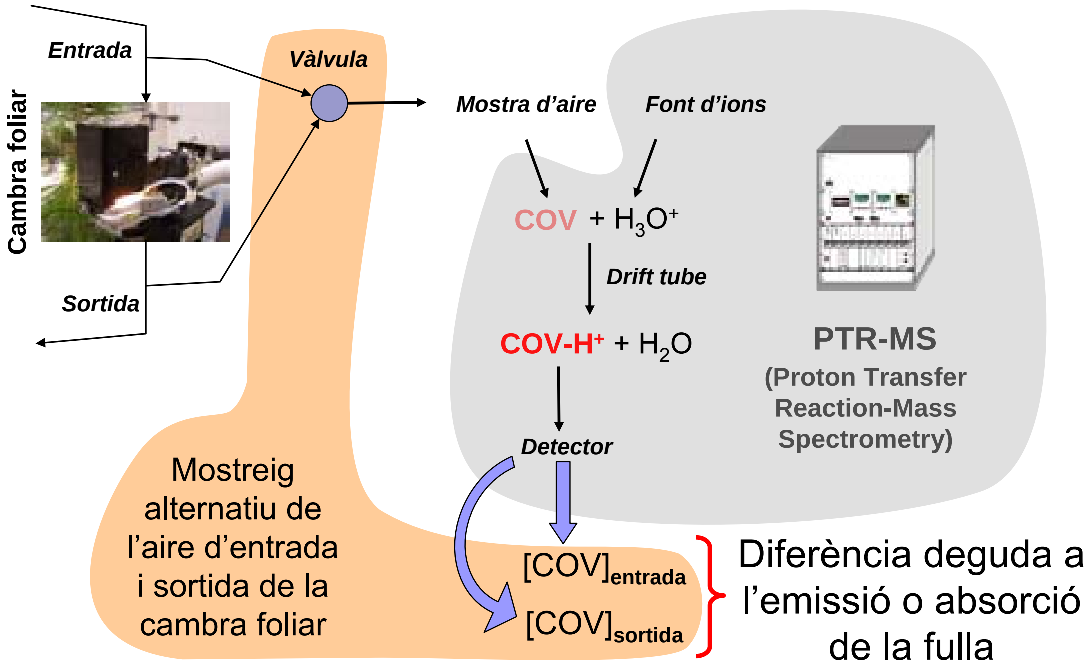


Fig. 7. Representació esquemàtica del sistema de mesura d'intercanvi de COVs mitjançant una cambra foliar. A través d'una vàlvula, se'n mostrejava alternativament l'aire d'entrada i de sortida. Aquest aire era utilitzat per detectar-ne la concentració de COVs ([COV]), que eren analitzats mitjançant un espectròmetre de masses per reacció de transferència de protons (PTR-MS, veure'n descripció al text).

La reacció té lloc dins d'un tub (*drift tube*), sotmesa a un camp elèctric, pressió i temperatura concrets, de manera que tots els gasos que tenen més afinitat protònica que l'aigua (que són la majoria de COVs però no els gasos majoritaris a l'aire, com el N_2, l'O_2 o el CO_2, etc.) reben un protó i passen a tenir càrrega elèctrica. Aquesta és la condició necessària per a poder seleccionar, tot seguit, les molècules amb un *quadrupol*, que el que fa és separar les molècules desitjades segons la seva ràtio $^{\text{Massa}}/_{\text{Càrrega}}$ (m/z). Finalment, un detector compta quantes molècules (prèviament seleccionades mitjançant el quadrupol) arriben en un temps determinat, i així sabem quina proporció d'aquell COV hi ha a l'aire. L'avantatge de la ionització química és que es tracta d'una reacció *suau*, en què les molècules de COV no es trenquen o ho fan molt poc, donant lloc a pocs fragments i facilitant la identificació dels COVs. Un altre dels punts forts de la tècnica PTR-MS és el fet que no calgui cap preparació prèvia de la mostra, simplement amb fer arribar una mica d'aire cap al sistema n'hi ha prou per analitzar-ne els COVs. Això, unit al fet que tant la reacció com el sistema de detecció són extremadament ràpids, fa que les mesures puguin realitzar-se en temps real de forma gairebé instantània i per tant puguem tenir una gran resolució temporal de les concentracions de COVs. Així, podríem dir que quan mesurem les emissions d'una fulla tenim una pel·lícula –contínua–

de l'evolució de les emissions, i no un seguit de fotografies –de moments concrets–, com ha estat habitual amb altres tècniques. El principal incovenient de la PTR-MS basada en un quadrupol és que separa les molècules segons la ràtio m/z però amb poca resolució màssica, de manera que diferents molècules amb la mateixa massa molecular nominal (en nombres enters) no es poden distingir. Per exemple, els monoterpens que tenen la mateixa composició atòmica ($C_{10}H_{16}$, massa molecular exacta de 136'132 g/mol) es detecten tots junts a la m/z 137, com correspon als COVs amb massa molecular nominal de 136 g/mol. Això també succeeix amb molècules amb diferent composició (i per tant, diferent massa molecular exacta) però mateixa massa molecular nominal, com per exemple l'àcid fòrmic (CH_2O_2, 46'03 g/mol) i l'etanol (C_2H_6O, 46'048 g/mol), que tenen la mateixa massa molecular nominal de 46 g/mol, i per tant es detecten junts a la m/z 47 sense poder-se destriar. Actualment s'està desenvolupant una important millora de la tècnica de PTR-MS, que consisteix en usar un espectròmetre basat en la tècnica anomenada *time-of-flight* en comptes d'un quadrupol. Aquesta tècnica permet diferenciar casos com el de l'àcid fòrmic i l'etanol: molècules amb diferent composició atòmica i per tant diferent massa molecular exacta. Tot i així, els compostos que tenen una mateixa composició atòmica (i mateixa massa exacta), com els monoterpens, segueixen sense poder ser individualitzables encara amb PTR-MS.

Per a la identificació de mono i sesquiterpens en alguns experiments també em vaig ajudar de la tècnica de cromatografia de gasos-espectrometria de masses (GC-MS, en anglès), més clàssica i coneguda que la PTR-MS. Aquesta fa una ionització d'alta energia mitjançant l'impacte d'electrons, fet que trenca la molècula de VOC en múltiples fragments, a més de requerir una preconcentració de la mostra d'aire que ens interessa en tubs omplerts de carbó actiu, que reté els COVs fins que són alliberats a l'hora de l'anàlisi. Per tant, a diferència de la PTR-MS no dóna resultats en temps real, però per altra banda sí que permet diferenciar, per exemple, entre diferents COVs amb el mateix pes molecular (per exemple, entre diferents monoterpens). Així doncs, per les seves característiques, l'anàlisi amb GC-MS complementa la feta amb PTR-MS, especialment pel que fa als monoterpens.

Estructura de la tesi

D'acord amb els objectius explicats, aquesta tesi doctoral presenta l'estructura de la Taula 2.

Tot i que podria semblar estructurada en format clàssic, la tesi en realitat és un compendi de publicacions on cada capítol o subcapítol és la base d'un article, ja publicat en alguna revista internacional (indicada a l'inici de cadascun si és el cas) o bé en procés de preparació per a ser enviat a publicar. Els capítols d'aquesta tesi estan escrits en anglès, llengua en què originalment es varen redactar tots ells, i s'acompanyen d'un resum en català a l'inici de cadascun, així com d'un recull de conclusions generals al final de la tesi.

Taula 2. Estructura dels capítols de la tesi.

Tipus	Capítol	Espècies	COVs
Coneixement dels oxCOVs: *revisió bibliogràfica*	**1**	Múltiples	oxCOVs
Taxes d'emissió i absorció: *caracterització al laboratori*	**2.1**	*Q. ilex* i *P. halepensis*	oxCOVs
	2.2	*P. halepensis*	oxCOVs
	2.3	*Q. ilex*	isoprenoids
Comunicació entre organismes: *experiment al laboratori*	**3.1**	*Q. ilex*	oxCOVs
	3.2	*M. aeneus*	oxCOVs
Concentracions atmosfèriques: *mesures de camp*	**4**	Aire mostrejat en boscos dominats per *Q. ilex*	oxCOVs, isoprenoids i aromàtics

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Capítol 1

COVs oxigenats de cadena curta: Emissió i absorció per les plantes i fonts, embornals i concentracions a l'atmosfera

Short-chain oxygenated VOCs: emission and uptake by plants and atmospheric sources, sinks, and concentrations

Una versió editada d'aquest capítol va ser publicada l'abril de 2007 a la revista

Atmospheric Environment 41(12), 2477-2499. doi: 10.1016/j.atmosenv.2006.11.029

Resum

Les emissions de Compostos Orgànics Volàtils (COVs) tenen múltiples implicacions atmosfèriques i juguen diversos papers en la fisiologia i l'ecologia vegetals. Entre aquests COVs, s'està dedicant un creixent interès a un grup de COVs oxigenats de cadena curta (oxCOVs). Millores tecnològiques com l'espectrometria de masses per transferència de protons estan facilitant l'estudi d'aquests hidrocarburs, de manera que noves dades sobre aquests compostos van apareixent continuament. Aquí repassem el que se sap actualment de les emissions d'aquests oxCOVs per part de les plantes i els factors que les controlen, i també donem una visió general de les seves fonts, embornals i concentracions que trobem a l'atmosfera.

Els oxCOVs que tractem aquí són els àcids fòrmic i acètic, l'acetona, el formaldehid, l'acetaldehid, el metanol i l'etanol. En general, per la seva alta solubilitat en aigua (baix coeficient de partició gas-líquid), l'intercanvi planta-atmosfera és depenent dels estomes, encara que també es pot donar a través de la cutícula. Aquest intercanvi també ve determinat per les concentracions atmosfèriques. Aquests compostos tenen una vida atmosfèrica relativament llarga i presenten concentracions considerables a l'atmosfera, a nivell de ppbv. Així mateix, en condicions de no-estrès, les plantes poden emetre tots aquests oxCOVs junts amb fluxos que van de 0,2 fins a 4,8 $\mu g(C)g^{-1}$(pes sec foliar)h^{-1} i amb taxes que s'incrementen diverses vegades amb l'estrès.

El coneixement incomplet dels processos relacionats amb la síntesi, emissió, absorció i reactivitat atmosfèrica dels oxCOVs impedeix de clarificar exactament què està condicionant l'intercanvi planta-atmosfera –i també quan, com i per què aquest ocorre– i per tant aquestes incògnites asseguren recerca en aquest camp en el futur.

Abstract

Emissions of Volatile Organic Compounds (VOCs) have multiple atmospheric implications and play many roles in plant physiology and ecology. Among these VOCs, growing interest is being devoted to a group of short-chain oxygenated VOCs (oxVOCs). Technology improvements such as proton transfer reaction-mass spectrometry are facilitating the study of these hydrocarbons and new data regarding these compounds is continuously appearing. Here we review current knowledge of the emissions of these oxVOCs by plants and the factors that control them, and also provide an overview of sources, sinks, and concentrations found in the atmosphere.

The oxVOCs reviewed here are formic and acetic acids, acetone, formaldehyde, acetaldehyde, methanol, and ethanol. In general, because of their water solubility (low gas-liquid partitioning coefficient), the plant-atmosphere exchange is stomatal-dependent, although it can also take place via the cuticle. This exchange is also determined by atmospheric mixing ratios. These compounds have relatively long atmospheric half-lives and reach considerable concentrations in the atmosphere in the range of ppbv. Likewise, under non-stressed conditions plants can emit all of these oxVOCs together at fluxes ranging from 0.2 up to 4.8 $\mu g(C)g^{-1}$(leaf dry weight)h^{-1} and at rates that increase several-fold when under stress.

Gaps in our knowledge regarding the processes involved in the synthesis, emission, uptake, and atmospheric reactivity of oxVOCs precludes the clarification of exactly what is conditioning plant-atmosphere exchange –and also when, how, and why this occurs– and these lacunae therefore warrant further research in this field.

Introduction

Over the last few years great emphasis has been placed on emissions of Volatile Organic Compounds (VOCs) by plants. Their presence in the air affects atmospheric chemistry: reactions with OH radicals and NO_x produce ozone (Atkinson, 2000), a secondary oxidant pollutant in the troposphere. Furthermore, VOCs also react with ozone to form OH radicals and can form aerosol particles (Matsunaga et al., 2003; Goldstein et al., 2004; Holzinger et al., 2005) that can act as cloud condensation nuclei (Kavouras et al., 1998) with climatic implications (Peñuelas and Llusià, 2003).

Emitted VOCs can represent up to 10% of the carbon fixed by plants (Llusià and Peñuelas, 2000; Peñuelas and Llusià, 2004) and their multiple roles in plant physiology have been widely studied. They seem to provide protection against high temperatures (Singsaas and Sharkey, 1998; Peñuelas and Llusià, 2002), high irradiation (Peñuelas and Munne-Bosch, 2005), and oxidation stress (Velikova et al., 2005). As well, they also act as herbivore deterrents, as attractants of pollinators and the enemies of herbivores (Pichersky and Gershenzon, 2002), as plant-plant communication cues (Peñuelas et al., 1995), and as plant ‘safety valves’ (Rosenstiel et al., 2004). The release of VOCs by plants after wounding or stress and, specially, the release of methanol and hexenals in connection with lipoxygenase activity (Fukui and Doskey, 1998; Fall et al., 1999; Heiden et al., 2003; Peñuelas et al., 2005) are currently widely under study.

Most of the interest in VOCs has to date been focused on isoprenoids (isoprene, monoterpenes, and sesquiterpenes), and less studies have dealt with short-chain oxygenated compounds (oxVOCs) such as formaldehyde, acetaldehyde, acetone, methanol, ethanol, and formic and acetic acids. These compounds were formerly grouped together under the generic name of 'other VOCs' and were thus split into two categories: (i) other reactive VOCs with lifetimes of less than one day (including formaldehyde and acetaldehyde) and (ii) other less reactive VOCs with lifetimes of more than one day (including methanol, ethanol, formic acid, acetic acid, and acetone) (Guenther et al., 1995).

Emissions of many of these oxygenated compounds, because there are analytical difficulties associated with their sampling and analysis, were identified only recently, and many of them were previously thought to have only a limited role in atmospheric chemistry (Fall, 1999). Nowadays, however, growing importance is being attached to the study of these compounds. A large amount of carbon (150-500 Tg C y^{-1}) fluxes from the Earth's surface into the atmosphere in the form of oxygenated species. There is evidence of the presence of a highly abundant global source of oxVOCs, although its origin is not as yet exactly clear (Singh et al., 2001, 2004). These sources are either direct –primary– or indirect –secondary– and can be either biogenic or anthropogenic.

Some analytical problems have been solved with the use of Proton Transfer Reaction – Mass Spectrometry (PTR-MS) (described by Lindinger et al., 1998). This technique allows for real-time or on-line (without preconcentration) monitorization of emissions at concentrations as low as pptv (1 pptv = 1 part in 10^{12} by volume) and so the responses of plant emissions to environmental changes can be detected almost instantaneously. It has been used in measures of direct leaf emissions (Fall et al., 1999; Karl et al., 2002a; Peñuelas et al., 2005) and atmospheric mixing ratios (Sanhueza et al., 2001; Warneke et al., 2003; Filella and Peñuelas, 2006), as well as coupled to micrometeorological techniques like eddy covariance (Karl et al., 2001a, b), proving to be a powerful tool.

In this paper we review current knowledge of emissions of oxVOCs by plants and the factors that control them (Fig. 1), and also provide an overview of sources, sinks, and concentrations found in the atmosphere. These are areas of stydy that are still not fully understood and are yet to be fully deciphered. The exchange of oxVOCs between plants and the atmosphere has been studied (i) in various plant species or communities, (ii) using different approaches and objectives, (iii) with different sampling and analytical techniques, and (iv) under wide-ranging field and laboratory conditions. The papers cited in our review reflect the whole spectrum of situations that have led authors to reach conclusions that in some cases may seem even to be contradictory. For instance, some authors have proposed that oxVOCs emissions are mainly physiologically controlled, while others consider that the principal control mechanisms are physicochemical. In fact, control by physicochemical processes does not preclude biochemical control (or vice versa) and it is very likely that both mechanisms are involved. However, the lack of knowledge regarding processes involved prevents us from clarifying exactly what is conditioning the exchange of a certain compound and when, how, and why it is doing so.

Short-chain oxVOCs can be emitted or taken up by plants. The direction of the exchange is thought to be at least partly determined by the atmospheric mixing ratios, since gases move along the concentration gradient between the inner part and the outside part of the leaf. Another physicochemical property that influences oxVOC atmospheric exchange is solubility. These compounds all have in common high water

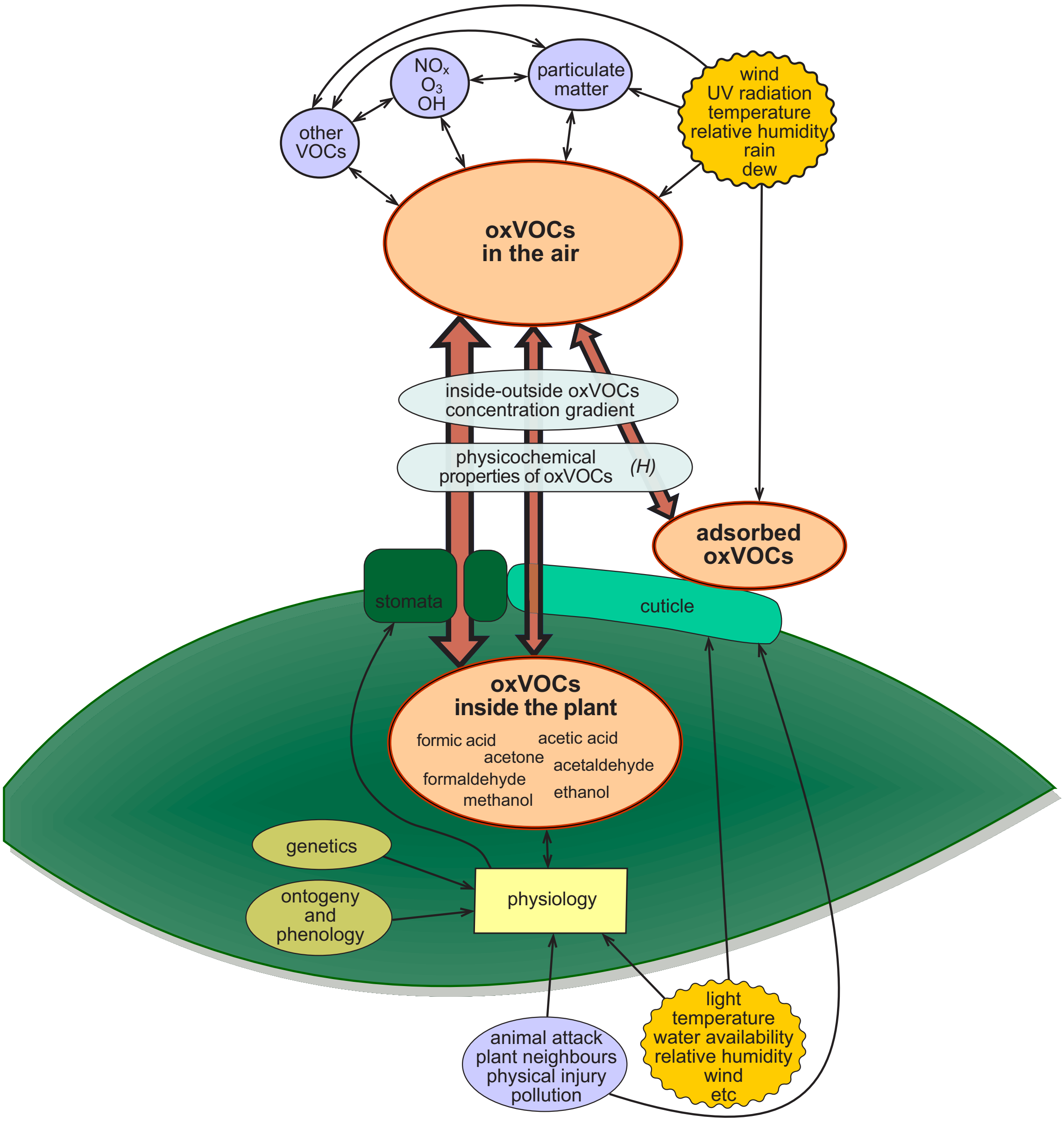

Fig. 1. Schematic diagram showing the factors driving the emission and uptake of short-chain oxVOCs.

solubility, as shown by their Henry's law constants (*H*) that range in magnitudes from 10^{-2} (formic acid) to 10^{1} (acetaldehyde) Pa m^3 mol^{-1} under standard conditions. These figures are low in comparison with those of highly volatile isoprene and monoterpenes, both of which have constants in the order of 10^3 Pa m^3 mol^{-1} (Sander, 1999). In highly water-soluble compounds exchange is more affected by stomatal conductance, with the degree of stomatal sensitivity varying with *H* (Niinemets and Reichstein, 2003b). Thus, physicochemical characteristics such as low volatility or diffusion of some VOCs may also control emissions and interact with physiological limitations (Niinemets and Reichstein, 2003a, b; Niinemets et al., 2004).

Aside from stomatal regulation, the exchange of oxVOCs can also be physiologically controlled by metabolic activity rates and both of these processes are affected by internal (genetic traits, developmental stage, phenology, water content, etc) and environmental (light, temperature, relative humidity, herbivory, and pollution stresses, wind speed, etc) conditions. The oxVOCs formation mechanisms within the plant are known in some cases, although often our knowledge of these processes is not as detailed as would be desirable. Furthermore, unknown processes contributing to the VOC pool within the plant might be at work. The metabolic pathways working in the opposite direction, that is, those that determine the fate of the uptaken oxVOCs within plants, also warrant further study.

Uptake can occur by means of the absorption of gas-phase oxVOCs through the stomata into the mesophyll or via adsorption into the cuticle as a result of dry (gas-phase) or wet (liquid-phase) deposition. Although entry through the stomata is the most commented upon, studied, and –possibly– important way of absorption of short-chained oxVOCs, the diffusion of adsorbed oxVOCs through aqueous pores of the cuticle may also play an important role in absorption, as has been described recently for inorganic and organic ions (Schreiber, 2005; Schönherr, 2006). This could happen, for example, after rain, on dew-wetted leaves, or by means of the transpired water vapour recondensed on the leaf surface (Burkhardt et al., 1999). On the other hand, if not absorbed, the oxVOCs adsorbed on the cuticle may be revolatilized, for example with the evaporation of dew in the morning, as a result of a decrease in atmospheric concentration caused by photochemical activity or through the action of air turbulence (wind). As well, oxVOCs may react with other atmospheric chemical species on the cuticle itself.

In addition to adsorbed and absorbed oxVOCs, in certain studies uptake estimates may include oxVOC scavenging resulting from the reactions of the oxVOCs with other reactive VOCs freshly emitted by plants. This would specially be the case in measurements carried out within canopies or above communities, although the low reactivity of the short-chained oxVOCs may render this process quantitatively unimportant and ozone chemistry may turn out to be the main factor responsible for scavenging newly emitted reactive VOCs such as isoprenoids (Goldstein et al., 2004; Holzinger et al., 2005).

The emission of oxVOCs from within the leaf may occur along the same pathways as the uptake, that is, via the stomata and the cuticle, the former being again the most studied pathway and probably the most important in quantitative terms. However, more research is needed to elucidate the importance of each pathway and their respective roles and mechanisms in both the release and the deposition of oxygenated VOCs.

Emitted and revolatilized oxVOCs enter the atmospheric compartment in which they can react with ozone molecules, OH radicals, other VOCs, and nitrogen oxides. Otherwise, they may be degraded by ultra-violet (UV) radiation. Such transformations, however, are slow due to the low reactivity of short-chain oxVOCs, a fact that is illustrated by their relatively long half-lives ranging from a few hours (aldehydes) to 15 days (acetone). A long-range transport of oxVOCs on the wind is thus likely to occur and hence influence the atmospheric chemistry of distant regions.

The low reactivity of these compounds is derived from the fact that short-chained oxVOCs are themselves products of photolysis and the chemical reactions of a vast diversity of other VOCs present in the atmosphere. Currently, this secondary photochemical production from VOCs is thought to be the main atmospheric source of oxVOCs. Another related issue that needs further investigation is the balance between biogenic emission and photochemical production: sometimes the maximum reported emissions of oxVOCs take place when secondary atmospheric production is likely also to be at a maximum, e.g. at midday or in the afternoon when stomatal conductance, photosynthetic rate, transpiration, irradiation, and temperatures are high, and thus plant metabolic activity and oxVOCs volatilization are also high. However, if atmospheric mixing ratios control the gas exchange, and photochemical production increases the mixing ratios, then oxVOCs emissions should be hindered. Photochemical destruction of oxVOCs may partly offset photochemical production, although the long lifespan and low reactivity of oxVOCs may in fact ensure that this effect is of little import. These processes are not well understood and future research may well unravel some of these uncertainties.

In general, plants synthesize C_1 oxVOCs during many growth and developmental processes such as seed maturation, cell expansion, cell wall degradation, leaf abscission, and senescence of plant tissues. C_2 oxVOCs seem mainly related to responses to changes in the environment, above all during periods of stress (Kreuzwieser et al., 1999b). In this detailed overview, we will group the short-chained oxVOCs into acids, acetone, aldehydes and alcohols. Usually, the compounds within each group –specially aldehydes and acids– have been studied together due to the similarities in the sampling and analytic techniques involved.

Acids

Formic ($HCOOH$) and acetic (CH_3COOH) acids are the most prominent organic acids emitted by vegetation (Kesselmeier and Staudt, 1999). In C_3 plant leaves in the light, formic acid can be generated in its deprotonated form –formate– by the non-enzymatic decarboxylation of glyoxylate formed in photorespiration (Igamberdiev et al., 1999; Kesselmeier and Staudt, 1999; Hanson and Roje, 2001). In the dark, in nonphotosynthetic tissues and in C_4 species its origin is not clear. In these cases, formic acid may be produced through the oxidation of methanol and formaldehyde, 10-formyl-tetrahydrofolate cleavage, or the direct reduction of CO_2 to formate, as reported in potato tubers (Igamberdiev et al., 1999; Hanson and Roje, 2001). Acetic acid is mainly found in its activated form acetyl-CoA, which participates in multiple metabolic processes, and is the main product of the decomposition of carbohydrates and fats (Kesselmeier and Staudt, 1999).

Formic and acetic acids have been reported to be both deposited and emitted. Studies in a remote tropical forest site in Amazonia detected predominantly the uptake of these acids at branch level as a function of the atmospheric mixing ratios (Kuhn et al., 2002) and in a tropical rain forest canopy in Costa Rica these acids were also mainly deposited (Karl et al., 2004). In the Valencia region, high concentrations in ambient air promoted acid deposition on orange trees (*Citrus sinensis*) (Staudt et al., 2000). These authors stated that both acetic and formic acids exhibited similar emission time-series, which suggests that the exchange of acids is mainly controlled more by physicochemical than biochemical processes, since these compounds do not usually share the same metabolic pathways.

After uptake, organic acids may be transferred from the apoplast to the symplast and thereby enter the plant metabolism, thereby maintaining the concentration gradient between the interior and the exterior of the plant that permits continuous uptake. Once in the symplast, acetic acid may be converted to acetyl-CoA and enter general metabolism pathways such as the TCA (TriCarboxylic Acids) cycle; formic acid may participate in the transfer of methyl and hydroximethyl groups for the synthesis of other molecules (see Kesselmeier and Staudt, 1999 and Kuhn et al., 2002, and references therein) or may be oxidized into CO_2 in mitochondria, specially when under stress (Igamberdiev et al., 1999; Hanson and Roje, 2001).

On the other hand, other studies have reported emission of these acids (Tables 1 and 2). Aside from physicochemical control, some authors have observed that short-chain acid emissions are controlled physiologically. Light triggers emission (Kesselmeier et al., 1997, 1998; Staudt et al., 2000; Kesselmeier, 2001) and release is generally correlated with transpiration (Kesselmeier et al., 1997, 1998; Gabriel et al., 1999) and temperature (Kesselmeier et al., 1997; Staudt et al., 2000), although Martin et al. (1999) found poor correlation with temperature in New Mexico. Stomatal conductance is a further important factor in the emission of acids (Kesselmeier et al., 1997, 1998; Kesselmeier, 2001; Niinemets and Reichstein, 2003b). The high solubility of these acids explains the correlation between emission and both transpiration and stomatal conductance, which allows for bursts of emissions after stomatal opening (Niinemets and Reichstein, 2003a, b).

Staudt et al. (2000) detected emission at noon and in the afternoon and deposition at night and morning in orange trees, observations that agree with the findings of Gabriel et al. (1999) in holm oak (*Quercus ilex*). Gabriel et al. (1999) also propose that the pH is a factor that controls the solubility of acids in the apoplastic solution. Turgor manteinance mechanisms in leaves lead to lower pH levels of the apoplast, which in turn helps acids to leave the aqueous phase and enter the gaseous phase. They also found that formic acid has more cuticular permeability than acetic acid so this acid escapes not only through stomata but also in an uncontrolled fashion through plant membranes and the cuticle.

Kesselmeier et al. (1997) found convincing evidence that acid emissions in *Q. ilex* follow the isoprene algorithm for the light-and-temperature-dependent release developed by Guenther et al. (1993, 1995). Filella et al. (2007) found that acid emission by the Norway spruce (*Picea abies*) increased with temperature, but reached saturation at 30 ºC.

In a study with various species of trees and crop plants, Kesselmeier et al. (1998) found that all tree species turned out to be acid emitters, while all crop plants showed uptake of acids but no emissions. These authors consider that forests should be seen as sources of acids and agricultural sites as sinks, although their use of purified air with trees could have interfered in their results and produced higher emissions than those expected using ambient air. This fits in well with the hypothesis of a compensation point concentration that determines the emission or the absorption of acids, as reported by Kuhn et al. (2002). Kuhn et al. (2002) also stated that tropical forests are more a sink than a source of formic and acetic acids.

Table 1. Foliar emissions and atmospheric mixing ratios of Formic Acid (HCOOH).

FORMIC ACID

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Quercus ilex		221–434[c]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Pinus pinea		320–345[c]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Populus fremontil		310 ± 300[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Pinus ponderosa		210 ± 210[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
diverse		15-916[d]	New Mexico (USA)	summer 1996	Martin et al. (1999)
Quercus ilex	0.03-0.86		Castelporziano (Italy)	May, Aug. 1994	Gabriel et al. (1999)
Quercus ilex	0.04[c]		Laboratory		Kesselmeier et al. (1998)
Citrus sinensis	0.3		Burriana (Spain)	June 1997	Staudt et al. (2000)
Citrus sinensis	0.08[c]		Laboratory	Nov-Dec. 1997	Staudt et al. (2000)
Fraxinus excelsior	0.06-0.49[c]		Laboratory		Kesselmeier et al. (1998)
Fagus sylvatica	0.02[c]		Laboratory		Kesselmeier et al. (1998)
Picea abies	0.02[c]		Laboratory		Kesselmeier et al. (1998)
Betula pendula	0.11[c]		Laboratory		Kesselmeier et al. (1998)
crop plants	(-4.25*)-(-0.01*)[d]		Laboratory		Kesselmeier et al. (1998)

ATMOSPHERIC MIXING RATIOS[b]

Site	Mixing ratio (ppbv)	Location	Period	Reference
rain forest	(0.75-2.5)	Amazonia (Rondonia, Brazil)	wet season 1999	Kesselmeier et al. (2002)
rain forest	(4-17)	Amazonia (Rondonia, Brazil)	dry season 1999	Kesselmeier et al. (2002)
tropical rain forest	0.51-1.3	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
savannah	2	Venezuela	Jan.-Feb. 2000	Sanhueza et al. (2001)
remote forested site	0.6 ± 0.5	Langmuir (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
forested site	1.05-2.5[d]	diverse (America)	diverse	Villanueva-Fierro et al. (2004)
rural ambient	1.7 ± 1.5 / 0.4-4.4	Gopalpura (India)	monsoon 1995, 1996	Khare et al. (1997)
rural ambient	0.7 ± 0.9	Socorro (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	0.2-5.6[d]	diverse (world)	diverse	Khare et al. (1997)
rural ambient	0.6-0.7	Germany	diverse	Granby et al. (1997)
semi-rural ambient	0.6 ± 0.3 / 0.2-1.5	Denmark	Feb. 1994	Granby et al. (1997)
semi-rural ambient	0.1-3.8	diverse (Europe, USA)	diverse	Granby et al. (1997)
urban ambient	0.7 ± 0.3 / 0.3-1.5	Copenhagen (Denmark)	Feb. 1994	Granby et al. (1997)
urban ambient	3.6 ± 1.6 / 0.9-8.5	Brussels (Belgium)	June 1993	Granby et al. (1997)
urban ambient	4.36 ± 2.70	São Paulo (Brazil)	July (winter) 1996	Souza et al. (1999)
urban ambient	1.7-9.3[d]	diverse (USA)	diverse	Villanueva-Fierro et al. (2004)
urban ambient	0.02-19.8	diverse (N.America, Japan)	diverse	Khare et al. (1997)
urban ambient	0.2-10.5	diverse (world)	diverse	Granby et al. (1997)
marine air	0.02-1.7	Pacific, Indian oceans	diverse	Khare et al. (1997)
diverse	0.03-40	diverse (world)	diverse	Chebbi and Carlier (1996)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).
[b] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.
[c] Normalized standard emission rate at 303 K and 1000 µmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) flux, fitted according to equations developed by Guenther et al. (1993, 1995).
[d] Range of means
* Negative values represent uptake

Similar disturbances could occur, for instance, in the emissions reported by Staudt et al. (2000) in orange trees in laboratory experiments using purified air and by Villanueva-Fierro et al. (2004) in tree experiments in New Mexico in the case not only of acids but also of other oxVOCs.

Table 2. Foliar emissions and atmospheric mixing ratios of Acetic Acid (CH_3COOH).

ACETIC ACID

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Quercus ilex		36.6–289[c]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Pinus pinea		182–190[c]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Populus fremontil		470 ± 540[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Pinus ponderosa		170 ± 180[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
diverse		52-1275[d]	New Mexico (USA)	summer 1996	Martin et al. (1999)
Quercus ilex	0.02-0.75		Castelporziano (Italy)	May, Aug. 1994	Gabriel et al. (1999)
Quercus ilex	0.33		Laboratory	June 2001	Holzinger et al. (2000)
Quercus ilex	0.03[c]		Laboratory		Kesselmeier et al. (1998)
Citrus sinensis	0.15		Burriana (Spain)	June 1997	Staudt et al. (2000)
Citrus sinensis	0.035[c]		Laboratory	Nov-Dec. 1997	Staudt et al. (2000)
Fraxinus excelsior	0.02-0.13[c]		Laboratory		Kesselmeier et al. (1998)
Fagus sylvatica	0.02[c]		Laboratory		Kesselmeier et al. (1998)
Picea abies	0.01[c]		Laboratory		Kesselmeier et al. (1998)
Betula pendula	0.03[c]		Laboratory		Kesselmeier et al. (1998)
crop plants	(-0.035*)-0.004[d]		Laboratory		Kesselmeier et al. (1998)

ATMOSPHERIC MIXING RATIOS[b]

Site	Mixing ratio (ppbv)	Location	Period	Reference
rain forest	(0.75-2.5)	Amazonia (Rondonia, Brazil)	wet season 1999	Kesselmeier et al. (2002)
rain forest	(2-12)	Amazonia (Rondonia, Brazil)	dry season 1999	Kesselmeier et al. (2002)
tropical rain forest	0.8-1.01	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
savannah	1	Venezuela	Jan.-Feb. 2000	Sanhueza et al. (2001)
remote forested site	1.7 ± 2.0	Langmuir (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
forested site	2.0-2.6[d]	diverse (America)	diverse	Villanueva-Fierro et al. (2004)
rural ambient	2.7 ± 3.8	Socorro (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	1.6 ± 1.6 / 0.2-4.0	Gopalpura (India)	monsoon 1995, 1996	Khare et al. (1997)
rural ambient	0.2-2.2[d]	diverse (world)	diverse	Khare et al. (1997)
rural ambient	1.2-7.5	Germany	diverse	Granby et al. (1997)
semi-rural ambient	1.0 ± 0.5 / 0.4-2.0	Denmark	Feb. 1994	Granby et al. (1997)
semi-rural ambient	0.1-3.4	diverse (Europe, USA)	diverse	Granby et al. (1997)
urban ambient	1.2 ± 0.5 / 0.4-2.8	Copenhagen (Denmark)	Feb. 1994	Granby et al. (1997)
urban ambient	4.0 ± 2.0 / 1.3-8.1	Brussels (Belgium)	June 1993	Granby et al. (1997)
urban ambient	3.66 ± 2.63	São Paulo (Brazil)	July (winter) 1996	Souza et al. (1999)
urban ambient	3.8-8.8[d]	diverse (USA)	diverse	Villanueva-Fierro et al. (2004)
urban ambient	0.2-17.8	diverse (N.America, Japan)	diverse	Khare et al. (1997)
urban ambient	0.1-9.5	diverse (world)	diverse	Granby et al. (1997)
marine air	0.05-1.9	Pacific, Indian oceans	diverse	Khare et al. (1997)
diverse	0.05-7.3	diverse (world)	diverse	Chebbi and Carlier (1996)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).
[b] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.
[c] Normalized standard emission rate at 303 K and 1000 μmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) flux, fitted according to equations developed by Guenther et al. (1993, 1995).
[d] Range of means
* Negative values represent uptake

Once released from the plant, carboxylic acids have low reactivity in the atmosphere because they are terminal products of the photooxidation of hydrocarbons so dry and wet deposition are their major sinks (Chebbi and Carlier, 1996; Sanhueza et al., 1996, 2001; Granby et al., 1997). Their tropospheric residence times are over a week and appear to be strongly controlled by precipitation events (Chebbi and Carlier, 1996). Formic acid mixing ratios measured in different sites around the world range from 0.02 to 40 ppbv (1 ppbv = 1 part in 10^9 by volume; Table 1) and acetic acid mixing ratios from 0.05 to 17.8 ppbv (Table 2). They can be responsible for up to 60% of the precipitation acidity in remote areas and up to 30% of the acidity of wet deposition in polluted areas (Kesselmeier et al., 1998; Peña et al., 2002).

VOC emission from vegetation is the main source of atmospheric formic and acetic acids and leads to a seasonal pattern of atmospheric concentrations (Granby et al., 1997; Peña et al., 2002), either by direct or indirect –in the form of precursor hydrocarbons-release (Glasius et al., 2001). Their major precursors in the gas phase are hydrocarbons (such as alkenes, aldehydes, isoprene, and terpenes), either from biogenic or anthropogenic origin, and their degradation products that mainly take place via photochemical oxidation (Sanhueza et al., 1996; Granby et al., 1997; Kesselmeier et al., 1998; Khare et al., 1999; Glasius et al., 2001; Peña et al., 2002; Pacheco and Sanhueza, 2004; Lee et al., 2006a, b). Soils and biomass burning are other direct sources (Chebbi and Carlier, 1996; Sanhueza et al., 1996; Khare et al., 1999; Kesselmeier et al., 2002). Their direct origin from fuel combustion and industry is minor in global terms but can be important in urban or industrialized areas (Chebbi and Carlier, 1996). Vehicle exhaust may also be locally significant (Chebbi and Carlier, 1996) although some authors report the opposite (Granby et al., 1997).

Different studies have reported lower emission rates in laboratory experiments than in field measurements, which have led some authors to postulate an indirect origin for formic and acetic acid in the field (Kesselmeier, 2001) and emphasize the importance of plant phenology (Staudt et al., 2000).

Acetone

Acetone (CH_3COCH_3) is the simplest existing ketone. Within plant tissues, acetone can be produced by the cyanogenic pathway (activated to deter herbivores), leading to the production of hydrogen cyanide (HCN) and –as a byproduct– acetone. Another way in which acetone may be formed is via acetoacetate decarboxylation, a well-known reaction that occurs in certain soil bacteria and animals. For a detailed description of these pathways see Fall (2003).

In direct plant emission measurements (Table 3), Janson and de Serves (2001) found acetone to be the main light carbonyl emitted by both Scots pines (*Pinus sylvestris*), accounting for between 64 and 94% of the carbon mass of emissions, and Norway spruce (*Picea abies*), with more than 80% of CH_3COCH_3 among its emitted carbonyls. Carbonyls are compounds –like ketones and aldehydes– that are characterized by a carbonyl functional group composed of a carbon atom double-bonded to an oxygen atom. These results contrast with those of Martin et al. (1999) who found levels of

Table 3. Foliar emissions, ecosystem fluxes and atmospheric mixing ratios of Acetone (CH_3COCH_3).

ACETONE

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Pinus sylvestris		1403 ± 774[e] / <32-2258	Sweden and Finland	spring/summer 1997, 1998, 1999	Janson and de Serves (2001)
Picea abies		427[e] / <32-468	Sweden	May-June 1997	Janson and de Serves (2001)
Populus fremontil		550 ± 430[d]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Pinus ponderosa		180 ± 90[d]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Quercus ilex	0.03-0.2		Tuscany (Italy)	June, July, Sep. 2000, 2001	Kreuzwieser et al. (2002)
Quercus ilex	0.71		Laboratory	June 2001	Holzinger et al. (2000)
Fagus sylvatica	0.66		Freising (Germany)	Aug. 2001, 2002	Cojocariu et al. (2005)

ECOSYSTEM FLUXES[b]

Ecosystem	Flux (mg m^{-2} h^{-1})	Location	Period	Reference
tropical rain forest	0.09 (0.36)	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
ponderosa pine plantation	0.35	Sierra Nevada (CA, USA)	July-Oct. 1998	Goldstein and Schade (2000)
ponderosa pine plantation	0.03-0.34[f] (0.4)	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
mixed hardwood forest	0.5 (1.6)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	0.3 (1.2)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
subalpine forest	(0.8)	Rocky Mountains (CO, USA)	July 2001	Karl et al. (2002b)
subalpine forest	(4)	Rocky Mountains (CO, USA)	summer 1999	Baker et al. (2001)

ATMOSPHERIC MIXING RATIOS[c]

Site	Mixing ratio (ppbv)	Location	Period	Reference
tropical rain forest	0.73-1.14	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
savannah	1.49	Venezuela	Jan.-Feb. 2000	Sanhueza et al. (2001)
mixed hardwood forest	1.2 (3.1)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	1.9 (5.6)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
ponderosa pine plantation	1.0-8.0	Sierra Nevada (CA, USA)	July 1997, 1998, 1999	Goldstein and Schade (2000)
ponderosa pine plantation	0.3-11	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
remote forested site	1.2 ± 1.2	Langmuir (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
hay field	1-5	Tirol (Austria)	May 2000	Karl et al. (2001b)
rural ambient	2.4 ± 2.4	Socorro (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	0.6-3.0	diverse (Germany, Canada)	diverse	Granby et al. (1997)
rural and semi-rural ambients	0.4-2.3[f]	diverse (Europe and N.America)	diverse	Christensen et al. (2000)
semi-rural ambient	0.9 ± 0.4 / 0.2-1.7	Denmark	Feb. 1994	Granby et al. (1997)
semi-rural ambient	1.9 / 0.4-6.1	Denmark	May-July 1995	Christensen et al. (2000)
semi-urban ambient	3.29-8.53[f]	Bellaterra, Catalonia (Spain)	Dec. 2003-Oct. 2004	Filella and Peñuelas (2006)
urban ambient	0.25-76.4	Athens (Greece)	June-Dec. 2000	Bakeas et al. (2003)
urban ambient	1.0 ± 0.5 / 0.2-2.2	Copenhagen (Denmark)	Feb. 1994	Granby et al. (1997)
urban ambient	1.6-16[f]	diverse (world)	diverse	Villanueva-Fierro et al. (2004)
urban ambient	0.6-8.4	diverse (world)	diverse	Granby et al. (1997)
atmosphere 0-6 km height	1.14 ± 0.41 / 0.36-2.31	Eastern Canada	July-Aug.	Singh et al. (1994)
atmosphere 0-12 km height	0.44-0.64[f]	tropical Pacific troposphere	Feb.-Apr. 2001	Singh et al. (2004)
atmosphere 0-12 km height	0.3-1.9[f]	diverse (world)	diverse	Jacob et al. (2002)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).

[b] Ecosystem fluxes measured with micrometeorological techniques. Displayed values are mean daytime emissions or range of observed values. Numbers between brackets represent daily maximum peaks of emission. Fluxes are referred to ground surface area (m^2).

[c] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.

[d] Normalized standard emission rate at 303 K and 1000 µmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) flux, fitted according to equations developed by Guenther et al. (1993, 1995).

[e] Normalized standard emission rate at 303 K, fitted according to equations developed by Guenther et al. (1993, 1995).

[f] Range of means

below 8% of acetone in emitted carbonyls, but agree with results from both a previous study by the same authors (Janson et al., 1999) who reported that acetone/acrolein make up 79 ± 13 % of carbonyls in *Pinus sylvestris* and 64 ± 20% in *Picea abies*, and a study by Filella et al. (2007) also on Norway spruce.

Janson and de Serves (2001) expected physiological factors to control the emissions of carbonyls, since these compounds are not stored in the needles of conifers. Nevertheless, acetone emission levels did not correlate with CO_2 assimilation or H_2O fluxes. The correlation between acetone emissions and relative humidity has been reported in *Picea abies* (Cojocariu et al., 2004); the same authors also report a diurnal pattern of emission under light conditions (maximal at midday) and uptake under dark conditions. Similar atmospheric diurnal patterns have been described for tropical rain forest canopies (Karl et al., 2004), with emission during the day and deposition at night, and for alfalfa (*Medicago sativa*) fields (Warneke et al., 2002) with release in the afternoon and dry deposition in the evening and morning. Villanueva-Fierro et al. (2004) also described a diurnal trend of acetone release in branch enclosures of ponderosa pine (*Pinus ponderosa*) and cottonwood (*Populus fremontil*) but found poor correlation with temperature. On the other hand, both Baker et al. (2001) in a subalpine forest in the Rocky Mountains and Schade and Goldstein (2001) in a ponderosa pine plantation found no distinct diurnal pattern of atmospheric fluxes, although the latter authors did find temperature to be the main regulating factor in CH_3COCH_3 emissions.

Acetone emission in alfalfa was found to be enhanced over a period of three days after cutting (Warneke et al., 2002). Similarly, de Gouw et al. (2000) observed increased emissions when wounding alfalfa and other crop species, and also when the harvested material was drying. Under stress caused by exposition to O_3, emission was enhanced in beech (*Fagus sylvatica*)(Cojocariu et al., 2005) and the diurnal pattern of maximum emissions at midday was also maintained. In flooded *Quercus ilex* plants in the laboratory, acetone release almost doubled (Holzinger et al., 2000).

The lifetime of acetone in the troposphere is estimated at 15 days (Singh et al., 2004), time enough for it to be transported to remote regions of the troposphere (Atkinson, 2000). Acetone is lost by photolysis (45-64%), through reaction with OH radicals (24-30%), ocean uptake (15%) and dry deposition (10-12%) (Singh et al., 2004).

Typical measured mixing ratios are shown in Table 3 and range from 0.3 to 76.4 ppbv. Borbon et al. (2004) describe a seasonal pattern of biogenic acetone at a rural site in France that in summer accounts for 37 ± 25% and in winter for 5 ± 4% of total mixing ratios. Bakeas et al. (2003) found acetone to be the third carbonyl in abundance in the urban air of Athens after formaldehyde and acetaldehyde.

Jacob et al. (2002) estimated the global source of acetone to be 95 Tg y^{-1}, in which the C_3-C_5 isoalkanes (propane, isobutane, isopentane), all of anthropogenic origin, are the major atmospheric precursors (~27 Tg y^{-1}). The oxidation of methylbutenol and terpenes of biogenic origin also contribute ~7 Tg y^{-1} to the secondary acetone pool (see also Lee et al., 2006a, b). Terrestrial vegetation (~33 Tg y^{-1}) was confirmed as a primary source along with biomass burning (~5 Tg y^{-1}) and dead plant matter (~2 Tg y^{-1}).

Warneke et al. (1999) described emissions from dead plant matter and proposed a potential source of 6-8 Tg y^{-1}, which Jacob et al. (2002) found to be inconsistent with the seasonal cycle observed at European sites and concluded that acetone could be consumed within the litter rather than emitted into the atmosphere. However, Karl et al. (2003) observed a fall peak in emissions and attributed this to senescing and decaying biomass in a hardwood forest in Michigan.

Soil emission has been reported by Schade and Goldstein (2001) in a ponderosa pine plantation with a Mediterranean climate in California, while Schade and Custer (2004) noted emissions from soil during a heat wave in Germany, attributing them to a biological production mechanism deep in the soil.

Calculations of absolute and relative contributions for each source and sink for acetone and all other compounds are continuously under review and greater importance now seems to be given to emission from plants. In line with the growing knowledge of the subject, the numbers reviewed here may underestimate biogenic emissions (Singh et al., 2004). For further information on acetone budget, sources, and sinks, see Singh et al. (1994, 2004) and Jacob et al. (2002).

Aldehydes

The short-chain aldehydes emitted by plants are the C_1 formaldehyde ($HCHO$) and the C_2 acetaldehyde (CH_3CHO). The formaldehyde origin within plants remains unclear. It seems to be a product of methanol oxidation, although its actual biochemical basis in plants is not known and other possible origins such as 5,10-methylene-tetrahydrofolate dissociation, glyoxylate decarboxylation or oxidative demethylation reactions have been proposed (Hanson and Roje, 2001). Acetaldehyde formation is known to be caused, at least, by root metabolism in flooded plants and by sunflecks and leaf pyruvic acid decarboxylation. On one hand, in root systems ethanol production is triggered by anoxic conditions: the plant uses the classic ethanolic fermentation pathway to obtain energy by converting glucose to ethanol. Once in the leaf, under aerobic conditions, ethanol can be oxidized into acetaldehyde and further into acetate. Ethanol oxidation is mediated by alcohol dehydrogenase (ADH) and acetaldehyde emission should be seen essentially as a leak between acetaldehyde production and metabolism (Kreuzwieser et al., 2001; reviewed by Fall, 2003). On the other hand, transient acetaldehyde releases have been observed during light-dark transitions in the leaves of various tree species (Holzinger et al., 2000; Karl et al., 2002a; Graus et al., 2004; Hayward et al., 2004). To explain this phenomenon Karl et al. (2002a) proposed a pyruvate overflow mechanism that acts when cytosolic pyruvate accumulates during light-dark transitions and leads to increases in pyruvate decarboxylase activity (see Fall, 2003). Graus et al. (2004) concluded that a different biochemical pathway, unconnected to cytosolic pyruvate, must be active during such transitions and suggested that it is related to leaf response to wounding. In addition, these authors demonstrated that CH_3CHO emissions due to light-dark changes are not of quantitative significance under field conditions.

Evidence exists of the absorption of these oxVOCs by plants. Giese et al. (1994) demonstrated that formaldehyde was detoxified and metabolized by the spider plant (*Chlorophytum comosum*) and Schmitz et al. (2000) concluded that all higher plants with normal green leaves can probably metabolize HCHO. For both aldehydes, Kondo et al. (1995, 1996, 1998) stated that trees could act as an important sink, with absorption rates increasing in the following order: coniferous < evergreen broad-leaved < deciduous broad-leaved trees. Furthermore, Rottenberger et al. (2004) proposed that forests act as a sink rather than as a source for aldehydes in Amazonia. All these studies regarding the absorption of aldehydes agree that this process occurs via the stomata. Once inside the leaves, HCHO would be converted enzymatically into CO_2 and thus enter the Calvin cycle (Giese et al., 1994; Schmitz et al., 2000). The mechanism for acetaldehyde metabolism has not been studied yet although there is evidence that enzymatic pathways take CH_3CHO into the general metabolism (Rottenberger et al., 2004). According to Rottenberger et al. (2004), such metabolic pathways or the transport within the leaf tissue determine the uptake of acetaldehyde more than water solubility does. On the contrary, formaldehyde is more soluble and this characteristic accounts for most of its exchange between air and the substomatal compartment. On the basis of such observations it has been concluded that for HCHO mesophyll resistance may be of the same order as stomatal resistance (Kondo et al., 1998; Rottenberger et al., 2004). Aside from stomatal uptake, adsorption to the cuticle may also be an important sink for these aldehydes (Rottenberger et al., 2004, 2005).

Regarding emission from vegetation (Tables 4 and 5), some authors found that most of the short-chained carbonyl emissions from conifers (Cojocariu et al., 2004) and from both deciduous and coniferous trees (Martin et al., 1999) consisted of aldehydes. As stated before, according to the measurements made by Janson et al. (1999), the main carbonyl emitted by *Picea abies* and *Pinus sylvestris* is acetone. Acetaldehyde and formaldehyde make up 26 ± 19% and 10 ± 16% of the carbonyl flux, respectively, in *Picea abies*. In *Pinus sylvestris*, the percentages are 11 ± 8% for acetaldehyde and 10 ± 12% for formaldehyde.

A number of studies of *Quercus ilex* and *Pinus pinea* (Kesselmeier et al., 1997) and trees in New Mexico (Martin et al., 1999) found no clear correlation between emission and any physiological parameters; other studies conclude that fluxes of aldehydes are mostly determined by ambient mixing ratios (Kesselmeier et al., 1997; Rottenberger et al., 2004). Stomata have been seen to be the pathway for the aldehyde release (Kreuzwieser et al., 2000; Rottenberger et al., 2004), which -along with their role in uptake- means that they are the major pathway for the bidirectional exchange of aldehydes between plants and atmosphere, a process that is determined by mixing ratios and stomatal conductance.

Diurnal patterns of emission with higher rates at noon and in the afternoon than at night, when even deposition was observed, have been described for both aldehydes and for several plant species and canopy measurements (Kreuzwieser et al., 2000; Karl et al., 2004; Villanueva-Fierro et al., 2004; Cojocariu et al., 2005). In an alfalfa field acetaldehyde emissions in the afternoon and deposition in the evening and morning have also been reported (Warneke et al., 2002).

Hayward et al. (2004) reported that acetaldehyde emissions showed exponential temperature dependence in Sitka spruce (*Picea sitchensis*) and suggested that emissions only originate from internal pools. Another suggested explanation for this behavior is that enzymatic reactions mediate the production and emission of carbonyls (Cojocariu et al., 2004).

In alfalfa and other hay-field experiments related to physical stress, enhanced emissions of CH_3CHO were reported in two phases: during the cutting of hay and while the

Table 4. Foliar emissions and atmospheric mixing ratios of Formaldehyde (HCHO).

FORMALDEHYDE

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Quercus ilex		382–520[d]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Pinus pinea		519–589[d]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Pinus ponderosa		500 ± 400[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Populus fremontil		4070 ± 3570[c]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
diverse		70-1658[e]	New Mexico (USA)	summer 1996	Martin et al. (1999)
Fagus sylvatica	0.66		Freising (Germany)	Aug. 2001, 2002	Cojocariu et al. (2005)

ATMOSPHERIC MIXING RATIOS[b]

Site	Mixing ratio (ppbv)	Location	Period	Reference
tropical forest	0.5-3	Amazonia (Brazil)	Mar.-Apr. (wet season) 1998	Kesselmeier et al. (2000)
rain forest	(1.5-4)	Amazonia (Rondonia, Brazil)	wet season 1999	Kesselmeier et al. (2002)
rain forest	(5-25)	Amazonia (Rondonia, Brazil)	dry season 1999	Kesselmeier et al. (2002)
remote forested site	2.3 ± 2.4	Langmuir (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	3.4 ± 1.4	Socorro (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	2.61 ± 1.94	Annaka (Japan)	June 1997-Mar. 2003	Tago et al. (2005)
rural ambient	1.4 ± 0.8 / 0.3-4.2	Gopalpura (India)	monsoon 1995, 1996	Khare et al. (1997)
rural ambient	0.03-6.5	diverse (Germany, N.America)	diverse	Khare et al. (1997)
rural ambient	0.4-5.0	diverse (Germany, Canada)	diverse	Granby et al. (1997)
rural and semi-rural ambients	0.4-13.4	diverse (Europe and N.America)	diverse	Christensen et al. (2000)
semi-rural ambient	0.9 ± 0.5 / 0.1-2.8	Denmark	Feb. 1994	Granby et al. (1997)
semi-rural ambient	1.2 / 0.1-4.7	Denmark	May-July 1995	Christensen et al. (2000)
semi-rural ambient	0.63-13.4	diverse (Austria, USA)	diverse	Granby et al. (1997)
urban ambient	7-28 / 18 ± 6	Rome (Italy)	summer 1994-96	Possanzini et al. (2002)
urban ambient	7-20 / 10 ± 4	Rome (Italy)	winter 1995-97	Possanzini et al. (2002)
urban ambient	2.6 ± 0.7 / 0.2-6.4	Copenhagen (Denmark)	Feb. 1994	Granby et al. (1997)
urban ambient	1.9 ± 0.9 / 0.1-4.3	Osaka (Japan)	May-Dec. 1997	Nguyen et al. (2001)
urban ambient	5.0 ± 2.8 / 1.4-9.7	São Paulo (Brazil)	Feb. (summer) 1998	Nguyen et al. (2001)
urban ambient	0.04-29	Athens (Greece)	June-Dec. 2000	Bakeas et al. (2003)
urban ambient	3.14 ± 1.87	Isesaki (Japan)	June 1997-Mar. 2003	Tago et al. (2005)
urban ambient	5.3-26.4[e]	diverse (world)	diverse	Villanueva-Fierro et al. (2004)
urban ambient	1.4-150[e]	diverse (America)	diverse	Khare et al. (1997)
urban ambient	4-176	diverse (world)	diverse	Granby et al. (1997)
atmosphere 0-12 km height	0.05-0.21[e]	tropical Pacific troposphere	Feb.-Apr. 2001	Singh et al. (2004)
marine air	0.12-1.0	diverse (world)	diverse	Khare et al. (1997)
diverse	0.94-17[e]	diverse (world)	diverse	Nguyen et al. (2001)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).
[b] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.
[c] Normalized standard emission rate at 303 K and 1000 μmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) flux, fitted according to equations developed by Guenther et al. (1993, 1995).
[d] Normalized standard emission rate at 303 K, fitted according to equations developed by Guenther et al. (1993, 1995).
[e] Range of means

harvested material was drying (de Gouw et al., 2000; Karl et al., 2001b; Warneke et al., 2002). These authors concluded that as a result crop harvesting may influence local air chemistry, since acetaldehyde has a short atmospheric life that does not allow for significant transportation by wind (Sanhueza et al., 2001). Similarly, in *Succisa pratensis*, acetaldehyde emissions occurred just after the plant was attacked by a herbivore (Peñuelas et al., 2005); likewise, acetaldehyde emissions of *Fagus sylvatica* increased after ozone exposition (Cojocariu et al., 2005). Stress by anoxia in the roots,

Table 5. Foliar emissions, ecosystem fluxes and atmospheric mixing ratios of Acetaldehyde (CH_3CHO)

ACETALDEHYDE

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Quercus ilex		691 ± 214[e]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Pinus pinea		773–1034[e]	Castelporziano (Italy)	May 1994	Kesselmeier et al. (1997)
Populus fremontil		1190 ± 1360[d]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
Pinus ponderosa		250 ± 190[d]	New Mexico (USA)	summer 1997	Villanueva-Fierro et al. (2004)
diverse		49-1122[f]	New Mexico (USA)	summer 1996	Martin et al. (1999)
Fagus sylvatica	0.75		Freising (Germany)	Aug. 2001, 2002	Cojocariu et al. (2005)
Picea abies	0.008-1.66		Bavaria (Germany)	July-Aug. 2001, 2002	Cojocariu et al. (2004)
Quercus ilex	0.23 – 0.75[f]		Tuscany (Italy)	June, July, Sep. 2000, 2001	Kreuzwieser et al. (2002)
Quercus ilex	0.3		Laboratory	June 2001	Holzinger et al. (2000)
Populus x canescens	0.33 – 0.83		Laboratory		Kreuzwieser et al. (2001)
Populus x canescens	0.45	2160	Laboratory		Kreuzwieser et al. (1999a)
Picea sitchensis		370 / 30-530	Laboratory		Hayward et al. (2004)

ECOSYSTEM FLUXES[b]

Ecosystem	Flux (mg m^{-2} h^{-1})	Location	Period	Reference
tropical rain forest	0.08 (0.2)	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
mixed hardwood forest	0.3 (1.0)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	0.2 (0.7)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
ponderosa pine plantation	0.03-0.26[f]	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
subalpine forest	0.4	Rocky Mountains (CO, USA)	July 2001	Karl et al. (2002b)

ATMOSPHERIC MIXING RATIOS[c]

Site	Mixing ratio (ppbv)	Location	Period	Reference
rain forest	<1	Amazonia (Rondonia, Brazil)	wet season 1999	Kesselmeier et al. (2002)
rain forest	(5)	Amazonia (Rondonia, Brazil)	dry season 1999	Kesselmeier et al. (2002)
tropical rain forest	0.26-0.44	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
savannah	0.85	Venezuela	Jan.-Feb. 2000	Sanhueza et al. (2001)
remote forested site	1.0 ± 0.7	Langmuir (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
mixed hardwood forest	0.4 (1.6)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	0.4 (2.7)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
rural ambient	1.4 ± 0.9	Socorro (NM, USA)	summer 1997	Villanueva-Fierro et al. (2004)
rural ambient	0.3-1.2	diverse (Germany, Canada)	diverse	Granby et al. (1997)
rural and semi-rural ambients	0.2-5.0	diverse (Europe and N.America)	diverse	Christensen et al. (2000)
semi-rural ambient	0.7 ± 0.4 / 0.2-1.4	Denmark	Feb. 1994	Granby et al. (1997)
semi-rural ambient	0.8 / 0.2-3.6	Denmark	May-July 1995	Christensen et al. (2000)
semi-urban ambient	1.56-3.29[f]	Bellaterra, Catalonia (Spain)	Dec. 2003-Oct. 2004	Filella and Peñuelas (2006)
urban ambient	2.19-24.95	Athens (Greece)	June-Dec. 2000	Bakeas et al. (2003)
urban ambient	3-17 / 10 ± 4	Rome (Italy)	summer 1994-96	Possanzini et al. (2002)
urban ambient	2-7 / 4 ± 2	Rome (Italy)	winter 1995-97	Possanzini et al. (2002)
urban ambient	1.0 ± 0.7 / 0.2-1.8	Copenhagen (Denmark)	Feb. 1994	Granby et al. (1997)
urban ambient	5.4 ± 2.8 /1.0-10.2	São Paulo (Brazil)	Feb. (summer) 1998	Nguyen et al. (2001)
urban ambient	1.5 ± 0.8 / 0.6-3.5	Osaka (Japan)	May-Dec. 1997	Nguyen et al. (2001)
urban ambient	3.8-37.1[f]	diverse (world)	diverse	Villanueva-Fierro et al. (2004)
urban ambient	0-35	diverse (world)	diverse	Granby et al. (1997)
atmosphere 0-12 km height	0.06-0.2[f]	tropical Pacific troposphere	Feb.-Apr. 2001	Singh et al. (2004)
diverse	0.77-17.3[f]	diverse (world)	diverse	Nguyen et al. (2001)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means and/or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).

[b] Ecosystem fluxes measured with micrometeorological techniques. Displayed values are mean daytime emissions or range of observed values. Numbers between brackets represent daily maximum peaks of emission. Fluxes are referred to ground surface area (m^2).

[c] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.

[d] Normalized standard emission rate at 303 K and 1000 µmol m^{-2} s^{-1} PAR (Photosynthetic Active Radiation) flux, fitted according to equations developed by Guenther et al. (1993, 1995).

[e] Normalized standard emission rate at 303 K, fitted according to equations developed by Guenther et al. (1993, 1995).

[f] Range of means

usually as a result of flooding, also produces enhanced emission of acetaldehyde (up to 10- to 37- fold [Kreuzwieser et al., 1999a; Holzinger et al., 2000]) and this type of CH_3CHO emission is controlled by production in the leaves, which in turn is at least in part controlled by the delivery of ethanol via the transpiration stream (Kreuzwieser et al., 1999a, b, 2000, 2001; Holzinger et al., 2000; Cojocariu et al., 2004).

The lifetime of these short-chained aldehydes in summer is estimated to be in the order of a few hours (Possanzini et al., 2002). They are removed from the air by photolysis and the reaction with OH radicals, leading to the formation of photochemical oxidants such as peroxyacetyl nitrate (PAN) and ozone (Atkinson, 2000). These and subsequent reactions result in the formation of more OH radicals, which ensures that formaldehyde and acetaldehyde are important sources of free radicals (Possanzini et al., 2002).

After isoprene, formaldehyde is the most abundant VOC in the tropical forest air (Kesselmeier et al., 2000, 2002). In rural and remote sites of New Mexico and Italy (Larsen et al., 1998; Villanueva-Fierro et al., 2004) and in the urban air of Rome and Athens (Possanzini et al., 2002; Bakeas et al., 2003) it is also the prevalent carbonyl compound, being followed by acetaldehyde. HCHO is the most abundant carbonyl in the atmosphere (Muir and Shirazi, 1996) and it is reasonable to assume that CH_3CHO is globally ubiquitous and its mixing ratios are substantial (Singh et al., 2004 and references therein). See Tables 4 and 5 for examples of the measurements of the mixing ratios of these carbonyl compounds in the range of 0.03-176 ppbv for formaldehyde and 0-37.1 ppbv for acetaldehyde.

These short-chained aldehydes have been recognised as having a great impact on human health given their potentially carcinogenic and mutagenic properties and their capacity for forming toxic and phytotoxic radical intermediates and stable species (Possanzini et al., 2002 and references therein). Regarding plants, although high atmospheric HCHO concentrations have been found to slightly delay budbreak and depress growth in Douglas fir (*Pseudotsuga menziesii*), the results were not statistically significant (Muir and Shirazi, 1996).

Formaldehyde and acetaldehyde are directly released into the atmosphere by vegetation and soils (specially CH_3CHO; Schade and Goldstein, 2001), by industrial processes and motor exhausts (Granby et al., 1997; Possanzini et al., 2002; Bakeas et al., 2003), and by biomass burning (Holzinger et al., 1999). In the future, if vehicles increasingly become powered by methanol and natural gas, formaldehyde emissions may predominate over secondary production (Altshuller, 1993). In Amazonia Kesselmeier et al. (2002) reported an increase up to 25 ppbv in the concentration of these aldehydes, which these authors link to the prevalence of forest fires. Emission from dead plant matter has also been proven to occur in the case of acetaldehyde (Warneke et al., 1999); Karl et al. (2003) attributed a fall peak of atmospheric CH_3CHO to this phenomenon, as in the case of acetone.

Nevertheless, both aldehydes have as a major atmospheric source the indirect origin through oxidation (via OH, NO_3 and O_3 radicals, for example) of other -biogenic or anthropogenic- hydrocarbons of higher molecular weight (Possanzini et al., 2002; Rottenberger et al., 2004; Lee et al., 2006a, b). For HCHO the main precursor hydrocarbon is thought to be isoprene, at least in the Amazon (Kesselmeier et al., 2000); other contributing hydrocarbons are methane, methanol, and acetaldehyde (Macdonald et al., 2001; Schade and Goldstein, 2001). For CH_3CHO, the largest source seems to be ethane and propene oxidation (Singh et al., 2004).

Alcohols

The short-chain alcohols emitted by plants are methanol (CH_3OH) and ethanol (CH_3CH_2OH). Methanol is a product of the demethylation of pectin during cell wall formation (biochemical details reviewed by Fall, 2003). Ethanol is produced in fermentative reactions when energy is obtained from glucose.

As in the case of acids, the high solubility of these alcohols ensures that their release is stomata-dependent (Macdonald and Fall, 1993; Nemecekmarshall et al., 1995; Schade and Goldstein, 2002) and also favors emission bursts after stomatal opening under the control of the gas-liquid phase equilibration of the alcohol within the leaf (Niinemets and Reichstein, 2003b). Similar bursts of methanol have been attributed to evaporation in the morning of the dew (Sanhueza et al., 2001; Warneke et al., 2002), given that at night methanol will probably be deposited on dew-wetted surfaces (Holzinger et al., 2001).

The emission of methanol (see Table 6) has been related to leaf development and generally declines with increasing leaf age after leaf expansion (Nemecekmarshall et al., 1995; Karl et al., 2003). A model designed by Galbally and Kirstine (2002) links plant cell growth to the global atmospheric concentration of methanol, with the ocean acting as a buffer for methanol concentration. As in the case of acetaldehyde, methanol

Table 6. Foliar emissions, ecosystem fluxes and atmospheric mixing ratios of Methanol (CH_3OH).

METHANOL

FOLIAR EMISSIONS[a]

Species	Emission rates		Location	Period	Reference
	nmol m^{-2} s^{-1}	ng g^{-1}dw h^{-1}			
Picea sitchensis		780 / 20-2390	Laboratory		Hayward et al. (2004)
Quercus ilex	2.03		Laboratory	June 2001	Holzinger et al. (2000)
Succisa pratensis	3.6		Laboratory		Peñuelas et al. (2005)

ECOSYSTEM FLUXES[b]

Ecosystem	Flux (mg m^{-2} h^{-1})	Location	Period	Reference
tropical rain forest	0.13 (0.5)	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
mixed hardwood forest	0.5 (1.5)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	0.9 (2.0)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
subalpine forest	(1)	Rocky Mountains (CO, USA)	July 2001	Karl et al. (2002b)
subalpine forest	(2.6)	Rocky Mountains (CO, USA)	summer 1999	Baker et al. (2001)
Ponderosa pine plantation	0.67-2.91[d] (4)	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
alfalfa field	(4-10)	Colorado (USA)	Aug. 2000	Warneke et al. (2002)
grassland	0.46 ± 0.07[e]	Midwestern USA	growing season (2-year period)	Fukui and Doskey (1998)

ATMOSPHERIC MIXING RATIOS[c]

Site	Mixing ratio (ppbv)	Location	Period	Reference
rain forest	1-6	Amazonia (Rondonia, Brazil)	dry season 1999	Kesselmeier et al. (2002)
tropical rain forest	1.53-2.78	Costa Rica	Apr. (dry season) 2003	Karl et al. (2004)
savannah	1.54	Venezuela	Jan.-Feb. 2000	Sanhueza et al. (2001)
mixed hardwood forest	4.2 (15.6)	N Michigan (USA)	Sep.-Nov 2001	Karl et al. (2003)
mixed hardwood forest	7.7 (21.0)	N Michigan (USA)	Apr.-Jul. 2002	Karl et al. (2003)
ponderosa pine plantation	1.1-41	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
semi-urban ambient	4.61-12.92[d]	Bellaterra, Catalonia (Spain)	Dec. 2003-Oct. 2004	Filella and Peñuelas (2006)
urban ambient	34.1 ± 9.2 / 20.8-51.1	São Paulo (Brazil)	Feb. (summer) 1998	Nguyen et al. (2001)
urban ambient	5.8 ± 3.8 / 0.3-17.3	Osaka (Japan)	May-Dec. 1997	Nguyen et al. (2001)
atmosphere 0-12 km height	0.51-0.86[d]	tropical Pacific troposphere	Feb.-Apr. 2001	Singh et al. (2004)
atmosphere (near surface)	0.2-195[d]	diverse (world)	diverse	Heikes et al. (2002)
atmosphere (troposphere)	0.6-20[d]	diverse (world)	diverse	Heikes et al. (2002)
diverse	3.6-19.6[d]	diverse (world)	diverse	Nguyen et al. (2001)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means and/or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).

[b] Ecosystem fluxes measured with micrometeorological techniques. Displayed values are mean daytime emissions or range of observed values. Numbers between brackets represent daily maximum peaks of emission. Fluxes are referred to ground surface area (m^2).

[c] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values. Numbers between brackets represent maximum mixing ratios.

[d] Range of means

[e] Static enclosure technique

emissions showed exponential temperature dependence in *Picea sitchensis* (Hayward et al., 2004); a similar exponential relationship between temperature and CH_3OH emissions has also been observed in *Picea abies* (Filella et al., 2007). This exponential relationship suggests that emissions may originate from an internal pool (Hayward et al., 2004) and/or that enzymatic mediation is involved, as has been suggested for carbonyl emission by Cojocariu et al. (2004).

Table 7. Foliar emissions, ecosystem fluxes and atmospheric mixing ratios of Ethanol (CH_3CH_2OH).

ETHANOL

FOLIAR EMISSIONS[a]

Species	Emission rates nmol m^{-2} s^{-1}	 ng g^{-1}dw h^{-1}	Location	Period	Reference
Quercus ilex	0.23[e]		Laboratory	June 2001	Holzinger et al. (2000)
Populus x canescens	0.33 – 0.83		Laboratory		Kreuzwieser et al. (2001)

ECOSYSTEM FLUXES[b]

Ecosystem	Flux ($mg\ m^{-2}\ h^{-1}$)	Location	Period	Reference
ponderosa pine plantation	0.19-0.52[d] (0.6)	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)

ATMOSPHERIC MIXING RATIOS[c]

Site	Mixing ratio (ppbv)	Location	Period	Reference
ponderosa pine plantation	0.1-9.6	Sierra Nevada (CA, USA)	July-Sep. 1999	Schade and Goldstein (2001)
urban ambient	176.3 ± 38.1 / 109.9-242.7	São Paulo (Brazil)	Feb. (summer) 1998	Nguyen et al. (2001)
urban ambient	8.2 ± 4.6 / 1.4-21.9	Osaka (Japan)	May-Dec. 1997	Nguyen et al. (2001)
atmosphere 0-12 km height	0.02-0.03[d]	tropical Pacific troposphere	Feb.-Apr. 2001	Singh et al. (2004)
diverse	0.25-310[d]	diverse (world)	diverse	Nguyen et al. (2001)

[a] Foliar emissions measured with enclosure chambers. Displayed values are means or range of observed emissions. Rates are referred to leaf area (m^2) or leaf dry weight (dw).

[b] Ecosystem fluxes measured with micrometeorological techniques. Displayed values are mean daytime emissions or range of observed values. Numbers between brackets represent daily maximum peaks of emission. Fluxes are referred to ground surface area (m^2).

[c] Atmospheric mixing ratios reported in the literature. Displayed values are mean mixing ratios and/or range of observed values.

[d] Range of means

[e] May be overestimated by a factor of 2.

In measurements taken in tropical rain forest canopies, methanol was emitted by day and deposited at night (Karl et al., 2004); measurements in different forests of the USA showed no distinct diurnal patterns (Baker et al., 2001; Schade and Goldstein, 2001). In an alfalfa field, a significant methanol flux was observed from undisturbed plants. After cutting, the emission of methanol was enhanced during the three days the alfalfa was drying (Warneke et al., 2002). In the same way, two studies on crops revealed increased emissions during cutting and, above all, during drying. Thus, hay harvesting may have an influence on local air chemistry (de Gouw et al., 2000; Karl et al., 2001b). Methanol emissions in other stress situations have been also described. Studying the reaction of *Succisa pratensis* to attacks by herbivore caterpillars, Peñuelas et al. (2005) noted a great release of methanol 24 hours after attack. The trigger for these caterpillar-elicited emissions seems to be the pH shift caused by the oral secretions of the larvae (von Dahl et al., 2006). In young holm oak trees subjected to flooding, emission also increased (Holzinger et al., 2000).

Methanol is the second most abundant organic gas in the atmosphere after methane (Jacob et al., 2005), with mixing ratios lying within the range 0.2-195 ppbv (Table 6). Its sources include emissions from the vegetation and soil (Fukui and Doskey, 1998;

Schade and Goldstein, 2001) possibly linked to the physico-chemical degradation of soil organic matter (Schade and Custer, 2004), biomass burning (Holzinger et al., 1999; Kesselmeier et al., 2002), and dead plant matter (Warneke et al., 1999). Methanol atmospheric sinks are oxidation into formaldehyde and formic acid, dry and wet deposition and uptake by ocean. Its lifetime in the atmosphere lasts for about 10 days (Jacob et al., 2005). For more extensive considerations about methanol budget see Heikes et al. (2002) and Jacob et al. (2005).

A number of papers exist that deal with the effects of methanol on plants. Experiments testing the reaction of *Arabidopsis thaliana* when exposed to methanol showed that CH_3OH affects the expression of hundreds of genes and activates multiple detoxification and signalling pathways (Downie et al., 2004). Furthermore, methanol emitted by *Nicotiana attenuata* after caterpillar attack has been reported to suppress plant defense responses to herbivores (von Dahl et al., 2006). Some authors have reported enhanced productivity in C_3 plants sprayed with methanol (see Fall and Benson, 1996, and the references therein). As has been reported for sycamore (*Acer pseudoplatanus*) cell cultures, plant tissues can metabolise CH_3OH at a rate of 0.2 μmol h^{-1} g^{-1} wet weight (Gout et al., 2000).

The relationship of ethanol with acetaldehyde and root hypoxia has been reviewed in the previous section on aldehydes. However, Kreuzwieser et al. (1999a) reported higher emissions of acetaldehyde than ethanol in leaves of *Populus x canescens,* while Holzinger et al. (2000) found the contrary in leaves of *Quercus ilex*. Schade and Goldstein (2002) found an increase in ethanol emissions after high ozone deposition fluxes in *Pinus ponderosa*, supporting the theory that ozone-induced stress may trigger fermentation processes in the leaves.

Ethanol is present in the atmosphere in mixing ratios generally below 10 ppbv (Table 7) and has an atmospheric lifetime of ~3.5 days (Singh et al., 2004). Biogenic emissions seem to be the most important source of ethanol, followed by biomass burning, the photochemical oxidation of hydrocarbons, and anthropogenic-related releases (Singh et al., 2004). This last type of emission includes motor exhausts in countries such as Brazil, where ethanol is mixed with fuel and thus reaches high concentrations (200-300 ppbv; see Table 7). Dead plant matter is also a source of ethanol (Warneke et al., 1999). Ethanol is removed from the atmosphere by oxidation into acetaldehyde (Atkinson, 2000) and other compounds. As a precursor of PAN, it may have a significant role in the long-range transportation of NO_x (Schade and Goldstein, 2001).

Final remarks

All these short-chain oxygenated VOCs have some common characteristics which set them apart from other widely studied VOCs such as isoprene and monoterpenes. For example, it is noticeable that their higher water solubility (low gas-aqueous phase partitioning coefficient, *H*) partly relates their emission to transpiration and makes it sensitive to changes in stomatal conductance. Compared to isoprenoids, they have a long atmospheric lifespan and so can affect tropospheric chemistry far from where they are released. These compounds build up considerable concentrations in the atmosphere, in the range of ppbv. Likewise, under non-stress conditions plants may emit all of these oxVOCs together (adding up minimum and maximum values reported for each compound, from Tables 1-7), at fluxes ranging from 0.2 up to 4.8 $\mu g(C)g^{-1}$(leaf dry weight)h^{-1}; these rates increase several fold when under stress. These values lie within the lower part of the reported range of monoterpene [0.02-41 $\mu g(C)g^{-1}$(leaf dry weight)h^{-1}] and isoprene [0.004-152 $\mu g(C)g^{-1}$(leaf dry weight)h^{-1}] emission fluxes in a number of species and are comparable to fluxes in many species (Kesselmeier and Staudt, 1999). More information on the emission/uptake of these compounds is needed to correctly quantify and model biosphere-atmosphere short-chain oxVOCs exchange. Gaps in our knowledge of the metabolic reactions responsible for compound synthesis and degradation, their localization inside the cells, and, above all, the biotic and abiotic factors controlling emission or uptake are issues that should be addressed as soon as possible. The relative importance and functioning of the cuticle and stomatic pathways in every particular situation a plant faces throughout its lifetime warrants future investigation, as does the possible role of the oxVOCs in the formation and/or growth of atmospheric particulate matter, and the balance between biogenic emission and photochemical production in certain situations. In the same way, further knowledge regarding these compounds may allow us to identify physiological and ecophysiological functions of which we are currently unaware.

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Capítol 2

Emissions de COVs de les espècies mediterrànies *Quercus ilex* i *Pinus halepensis*: cicle diari i influència de la disponibilitat hídrica

2.1 Formaldehyde emission and uptake by mediterranean trees *Quercus ilex* and *Pinus halepensis*

Una versió editada d'aquest capítol va ser publicada el novembre de 2008 a la revista Atmospheric Environment 42(34), 7907-7914. doi: 10.1016/j.atmosenv.2008.07.006

Resum

El formaldehid (FA) és un gas ubic a l'atmosfera que assoleix concentracions notables en àrees contaminades i que pot tenir un gran impacte en la salut humana. Hem estudiant l'intercanvi de FA entre l'aire i dos arbres mediterranis ben comuns, l'alzina (*Quercus ilex*) i el pi blanc (*Pinus halepensis*). Els experiments es van fer a nivell de fulla en condicions de laboratori, usant aire de l'exterior de l'edifici. Ambdues espècies varen presentar un intercanvi bàsicament determinat per les concentracions atmosfèriques, amb un punt de compensació calculat entorn de 20 ppbv. Valors més alts produïen absorció i valors inferiors, emissió. El segon factor que regulava l'intercanvi de FA era la conductància estomàtica. L'intercanvi de FA seguia un cicle diari, amb màxim intercanvi quan la conductància estomàtica era màxima. Aquest control per part dels estomes concorda amb estudis previs i probablement s'explica per la gran solubilitat en aigua del FA, que fa de la transpiració estomàtica la seva principal via d'intercanvi. Aquesta relació entre la conductància estomàtica i l'intercanvi de FA també es va veure en condicions de sequera i posterior reg, quan els canvis en conductància estomàtica van anar paral·lels a canvis en intercanvi de FA. En condicions futures, que les projeccions preveuen que seran més àrides al Mediterrani, les limitacions de l'intercanvi de FA induïdes per la sequera poden ser més rellevants.

Abstract

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.

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 Llusià, 2003; Holzinger et al., 2005), in plant physiology (Singsaas and Sharkey, 1998; Peñuelas and Llusià, 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 C_1-C_3 VOCs like methanol, ethanol, formaldehyde, acetaldehyde, acetone, and formic and acetic acids. These oxVOCs reach considerable concentrations in the atmosphere, and are characterized 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 *capítol 1* for a review; Seco et al., 2007).

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 influence FA exchange through direct effects on stomatal conductance. To see whether such influence exists, our plants were submitted to a drought treatment with posterior rewatering.

Materials and methods

- Plant material and experimental design

For this study we used two-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 ºC) 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 CO_2 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.

Table 1. Sets of plants used in the experiment. 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".

Set	Species	Period	ref #	External FA concentration (ppbv) Range	External FA concentration (ppbv) Average	Treatment
1	*P. halepensis*	July 2005	P1	39.4-67.3	52.37	
			P2	51.1-74.4	64.09	
			P3	56.1-84.2	68.17	
			P4	53.4-79	68.98	
	Q. ilex	September 2005	Q1	7.7-87	34.45	
			Q2	8-12	10.17	
			Q3	8.7-27.3	14.25	
			Q4	14.8-26.3	20.71	
2	*P. halepensis*	October 2005	P1	20.9-29.3	25.12	
			P3	24.4-55.3	30.99	
			P5	20.7-126.3	31.48	
	Q. ilex	October 2005	Q1	23.2-31.8	26.8	
			Q2	28-35.2	31.45	
			Q4	29.9-38.5	33.8	
3	*P. halepensis*	November 2005	P3	25.7-51	32.61	D & R
			P5	22.6-31.8	26.48	D & R
			P1	23.9-30.5	26.81	D & R
	Q. ilex	February 2006	Q4	3.3-12.3	5.7	D & R
			Q5	2.9-7.1	4.4	D & R
			Q1	2.7-6.5	4.25	D & R

- Gas exchange measurements: CO_2, H_2O 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 CO_2 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.

- The PTR-MS technique

PTR-MS is based on chemical ionisation, specifically non-dissociative proton transfer from H_3O^+ ions to most of the common VOCs, and has been fully described elsewhere (Lindinger et al., 1998).

In short, the H_3O^+ ions produced by a hollow-cathode act as primary reactants and transfer a proton to every VOC with more proton affinity than that of H_2O molecules. Natural components of air do not suit this condition and 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 40ºC, with a E/N (electric field/molecule number density) of around 130 Td (townsend) (1 Td = 10^{-17} V cm^2). The primary ion signal (H_3O^+) was maintained at c. 4 x 10^6 counts per second.

- 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 vapor 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 2×10^{-9} cm^3 s^{-1}, and the rate constant for the backward reaction was 2×10^{-11} cm^3 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 vapor generated by adding it experimentally, there was no FA uptake.

- 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.

Results

The air entering the leaf cuvette reached levels of FA ranging from 2 to 126 ppbv (1 ppbv = 1 part in 10^9 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).

Figure 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 where higher. When FA mixing ratios were lower, *Q. ilex* emitted FA .

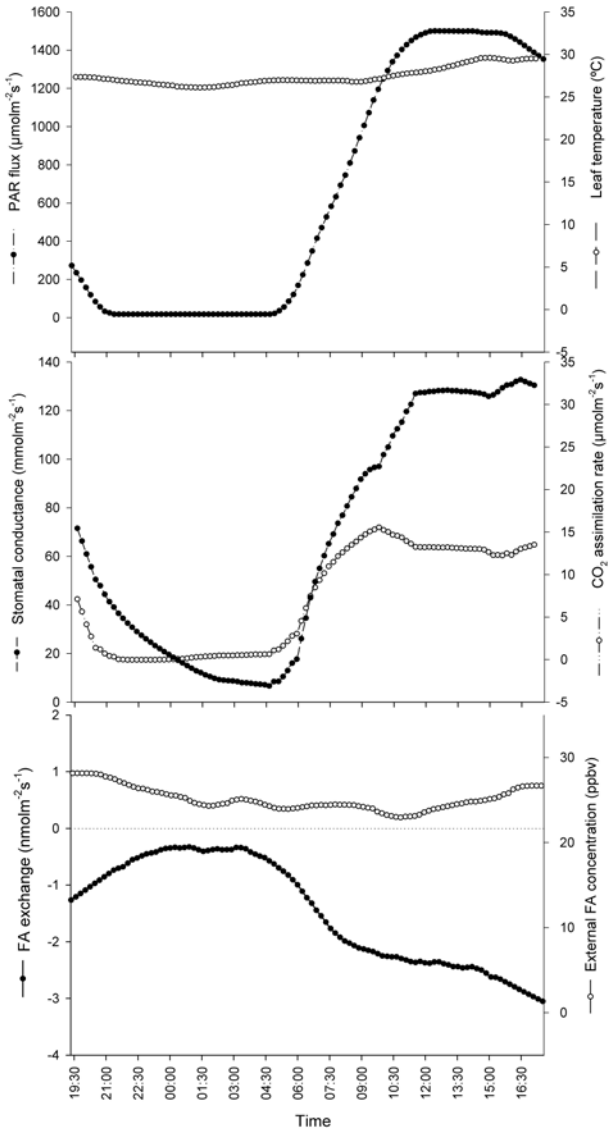

Fig. 1. Uptake of FA by *Pinus halepensis*. Represented one example (P1, october 2005) out of seven replications. PAR means Photosynthetic Active Radiation flux.

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 Figure 2A for an example).

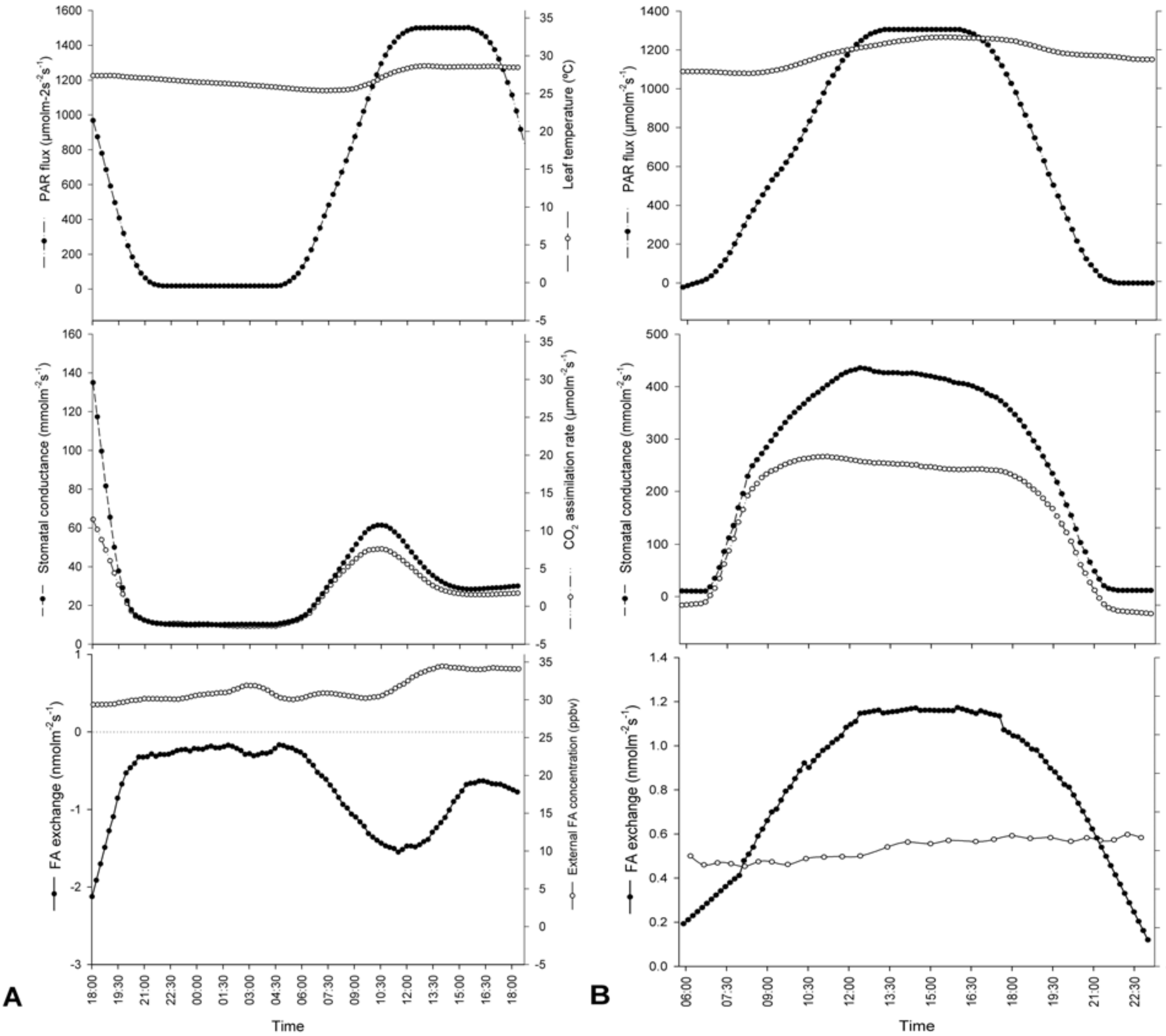

Fig. 2. FA exchange by *Quercus ilex*. Represented two examples (out of seven replications) corresponding to the same specimen (Q2). **A.** Uptake of FA (october 2005). A burst in stomatal conductance, assimilation and uptake can be appreciated during the first hours, just after the leaf was inserted into the chamber. **B.** Emission of FA (september 2005). External FA concentration was below the compensation point and thus the plant released FA. PAR means Photosynthetic Active Radiation flux.

Stepwise regression showed that, in the case of *P. halepensis*, FA exchange was primarily explained by stomatal conductance (change in r^2=0.46, p<0.001), followed by external mixing ratios (change in r^2=0.25, p<0.001). For *Q. ilex* this was different and the main regulating parameter was external concentration (change in r^2=0.75, p<0.001), while stomatal conductance played a minor part (change in r^2=0.002, p<0.001).

Fig 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.

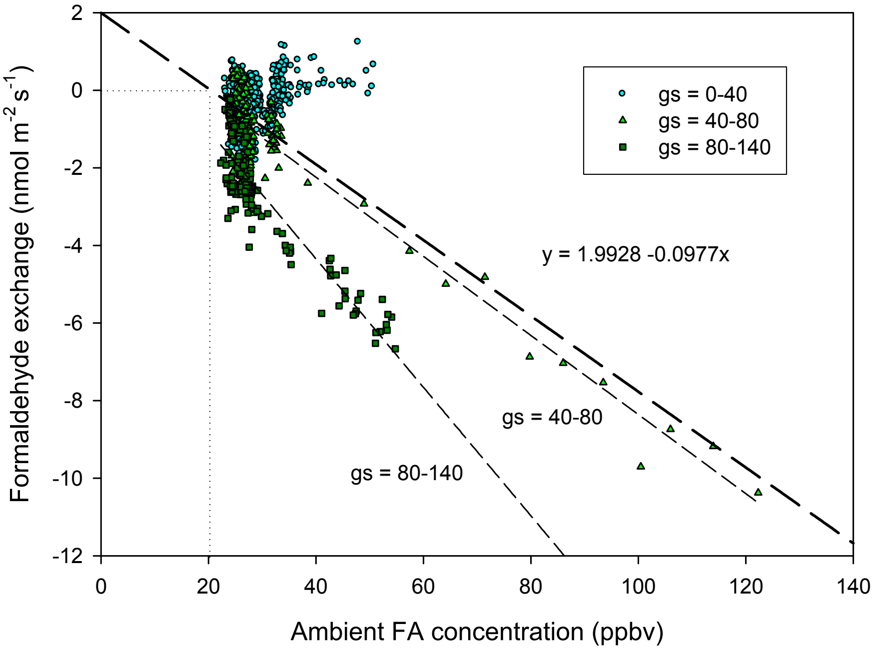

Fig. 3. FA exchange ratios of *Pinus halepensis* plotted versus ambient mixing ratios, grouped according to stomatal conductance (gs, mmol m^{-2} s^{-1}). Short-dashed lines are regressions for different stomatal conductance groups. Long-dashed line and equation are the regression for all the data. Dotted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

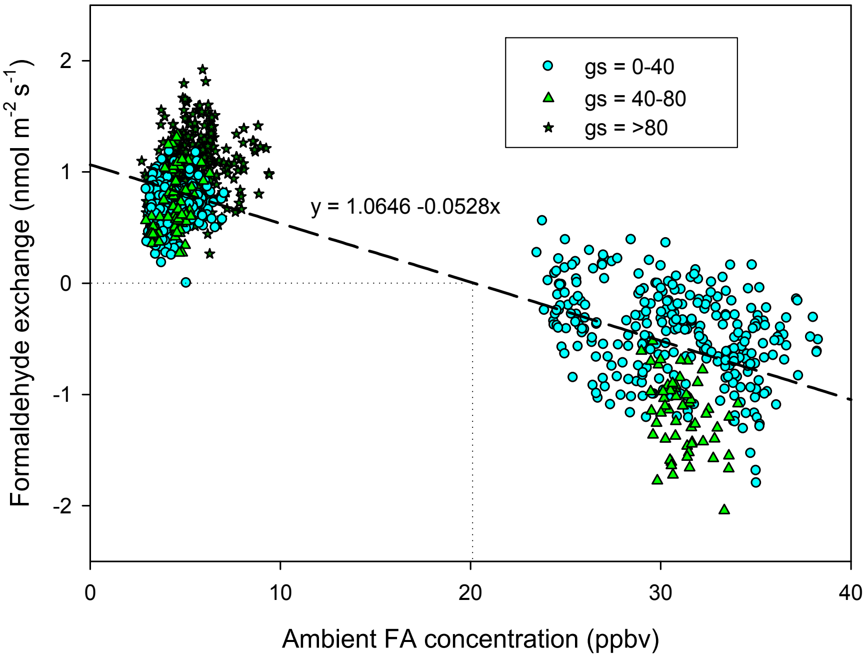

Fig. 4. FA exchange ratios of *Quercus ilex* plotted versus ambient mixing ratios, grouped according to stomatal conductance (gs, mmol m^{-2} s^{-1}). Long-dashed line and equation are the regression for all the data. Dotted lines indicate the compensation point, around 20 ppbv.

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: 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).

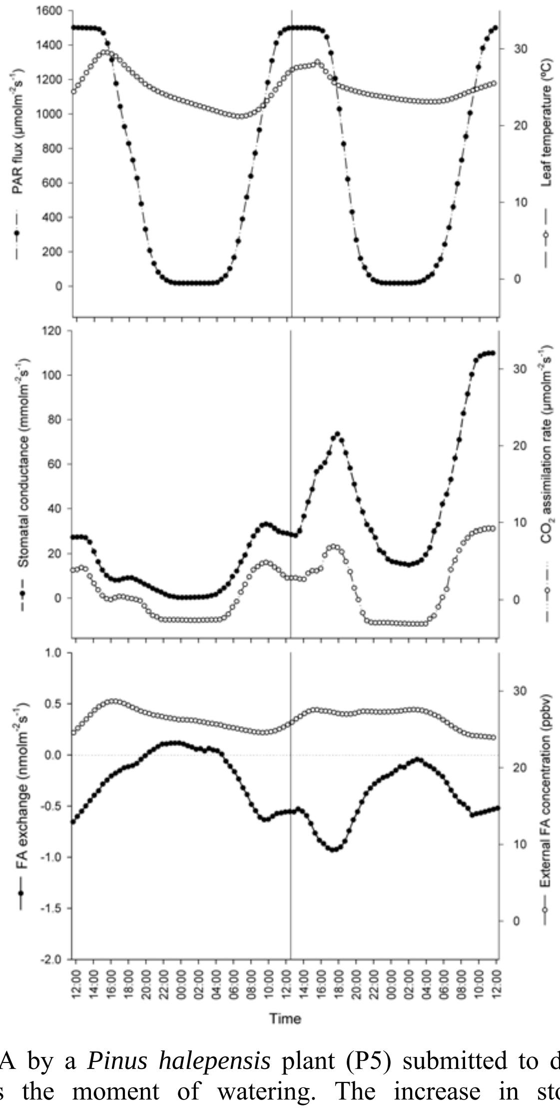

Fig. 5. Exchange of FA by a *Pinus halepensis* plant (P5) submitted to drought and rewatering. Vertical line indicates the moment of watering. The increase in stomatal conductance is accompanied by an increase of the FA uptake rate. PAR (Photosynthetic Active Radiation flux, µmolm^{-2}s^{-1}), A (assimilation rate, µmolm^{-2}s^{-1}), gs (stomatal conductance, mmolm^{-2}s^{-1}), Tl (Leaf temperature, º C), ext FA (external FA concentration, ppbv).

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 (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-hour day, including some drought-stressed plants, which may have influenced the result.

From the slopes of the equations in Fig. 3 and Fig. 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 Figure 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) (Kesselmeier et al., 1997). 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 glutatione-dependent formaldehyde deshydrogenase (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, metabolisation 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 Llusià, 2001, 2003; Filella and Peñuelas, 2006a), in the conditions of this experiment the changes in stomatal conductance were of higher magnitude than the 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.

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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2.2 Short-chained oxygenated VOC emissions in *Pinus halepensis* in response to changes in water availability

Una versió editada d'aquest capítol va ser publicada el març de 2009 a la revista
Acta Physiologiae Plantarum 31(2), 311-318. doi: 10.1007/s11738-008-0235-6

Resum

Es van observar les emissions de COVs oxigenats de cadena curta (oxCOVs) de plançons de *Pinus halepensis* en resposta a canvis en la disponibilitat hídrica. Les mesures en temps real es van realitzar amb un espectròmetre de masses per transferència de protons, en condicions controlades, junt amb mesures de l'intercanvi de CO_2 and H_2O. Les masses corresponents al metanol i l'acetona van ser les més emeses d'entre els oxCOVs. Tots els intercanvis d'oxCOVs, excepte el de l'acetona (M59) estaven significativament relacionades a la conductància estomàtica i la transpiració. A més, l'emissió d'acetaldehid (M45) va mostrar una forta dependència de la concentració d'acetaldehid a l'ambient: l'obertura estomàtica (conductància per sobre de 75 mmol m^{-2} s^{-1}) només permetia majors emissions quan les concentracions exteriors estaven per sota de 6 ppbv. L'acetona (M59) presentà un pic d'emissió important després de l'obertura dels estomes i de la llum al matí, quan les plantes estaven sota estrès hídric. Així doncs, les alteracions en les emissions d'oxCOVs en Pinus halepensis causades per dèficit d'aigua semblen regides, principalment, per l'efecte de l'estrès hídric en el tancament dels estomes i per les concentracions d'oxVOCs de l'aire.

Abstract

Short-chained oxygenated VOC (oxVOCs) emissions from *Pinus halepensis* saplings were monitored in response to changes in water availability. Online measurements were made with a proton transfer reaction – mass spectrometer under controlled conditions, together with CO_2 and H_2O exchange measurements. Masses corresponding to methanol and acetone were the most emitted oxVOCs. All the oxVOC exchanges, except that of acetone (M59), were significantly related to stomatal conductance and transpiration. Acetaldehyde (M45) emission showed, moreover, a strong dependence on the concentration of acetaldehyde in the ambient: stomatal opening (stomatal conductance above 75 mmol m^{-2} s^{-1}) only allowed increased emissions when external concentration were below 6 ppbv. Acetone (M59) presented an important peak of emission following light and stomatal opening in the morning when plants were water stressed. Thus, the alterations in oxVOC emissions in *Pinus halepensis* caused by the water deficit seem to be mainly driven by water stress effect on stomatal closure and oxVOC air concentrations.

Introduction

Variations in the emission of volatiles can be triggered by changing environmental conditions (Peñuelas and Llusià 2001a) such as water availability (Llusià and Peñuelas, 1998; Staudt et al., 2002) or temperature and humidity (Takabayashi et al., 1994; Gouinguené and Turlings, 2002). Relatively little information is available on how these abiotic conditions affect short-chain oxygenated VOC (oxVOCs), (such as formaldehyde, acetaldehyde, acetone, methanol, ethanol, and formic and acetic acids), a group with growing interest that reach considerable concentrations in the atmosphere, and characterized by a high water solubility and a long atmospheric lifespan (so they can affect tropospheric chemistry far away from where they were released) (*capítol 1*; Seco et al., 2007).

In the Mediterranean areas, one of the main ecological drivers is summer drought (di Castri, 1973), caused by high evaporative demand and low soil water content. Drought driven changes in emission may be even more relevant under the future climate since water availability in the Mediterranean region is likely to be reduced in the near future by the predicted increases of temperature and the consequent increases of evapotranspiration rates (Piñol et al., 1998; Sabaté et al., 2002; Peñuelas et al., 2005a). These changes in emissions may have important consequences in the formation of photochemical pollutants in this Mediterranean area (Filella and Peñuelas, 2006), a region that is recognized as one of the regions with highest tropospheric ozone concentrations in Europe (Lelieveld et al., 2002).

Pinus halepensis is one of the most abundant species in Mediterranean forests. Although terpenoid emissions in this species have already been studied (Peñuelas and Llusià, 1999; Simon et al., 2005), to our knowledge, emissions of short-chained oxVOCs have been scarcely studied (*capítol 1*; Seco et al., 2007). In other *Pinus* species, abundant emissions of oxygenated volatile organic compounds have been measured (Schade and Goldstein, 2001; Karl et al., 2005).

Proton-transfer-reaction mass spectrometry (PTR-MS) is a new technique that allows monitoring a large number of different VOC species with a fast time response (< 1 second), on-line and almost simultaneously and with a low detection limit (pptv). It has emerged as a useful tool in biological studies (Peñuelas et al., 2005b, Filella et al., 2006). Here, we studied oxVOC emissions from *Pinus halepensis* by using a proton transfer reaction-mass spectrometer (PTR-MS) system. Our aim was to describe the changes in oxVOC emission rates in this important tree species induced by drought conditions and posterior rewatering. The recovery process is biologically very important (Munné-Bosch and Peñuelas, 2003) and it is likely that affects also oxVOC emissions. In order to also study the implication of physiological variables such as CO_2 and water exchange in the response of these emissions to changing water conditions, net photosynthetic rates, stomatal conductance and transpiration rates were also measured simultaneously.

Materials and methods

- Plant material and experimental design

We used two-year-old *Pinus halepensis* plants grown in a nursery (Forestal Catalana, S.A., Breda, Spain) under the site typical Mediterranean environmental conditions (mean annual average of temperature 16ºC and mean annual precipitation 600 mm). 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 for an acclimation period of some days under laboratory conditions before starting the experiment. Three replicates were obtained by sampling three plants. In each of the three plants two leaves of similar characteristics (age, position, and illumination) were measured. Each single replication was left 1 h to adapt to the cuvette environment and, thus, to avoid the emissions caused by wounding after manipulation. The experiments were conducted at room temperature (28 ºC) in February 2005 and a light diurnal cycle was programmed 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 system was sampled at 4 m above ground from the outside of the building, filtered with glass wool to prevent the dust intake and passed through a polyethylene terephthalate (PET) recipient to buffer exterior CO_2 and VOC fluctuations. All tubing used (except that of the photosynthesis systems) was made of inert polytetrafluoroethylene (PTFE).

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 water stress conditions plants were rewatered until soil water content reached 25% and measured again. Measurements were taken continuously (every 2 minutes) from 12 h of the first day to 18 h of the second day.

- Gas exchange measurements: CO_2, H_2O and VOCs

Intact leaves were clamped in a 25mm x 25mm Parkinson leaf cuvette (Std Broad 2.5, PP Systems, Hitchin, Hertfordshire, England), the leaf temperature was maintained at 25ºC, and the flow at 400 ml min^{-1}. A calibrated Ciras-2 IRGA- porometer (PP Systems, Hitchin, Hertfordshire, England) was used for determining rates of CO_2 and H_2O exchange. Part of the air exiting the leaf cuvette flowed through a T-system to the PTR inlet.

- The PTR-MS technique

We used a highly sensitive Proton-Transfer-Reaction Mass Spectrometer (PTR-MS-FTD hs) from Ionicon Analytik, Innsbruck, Austria. The PTR-MS system and its use in VOC analysis has been described in detail elsewhere (Lindinger et al., 1998). The uncertainty of the system is currently assessed about 30% and the reproducibility for quantitative analysis is better than 10% (Hansel et al., 1999). The detection limit of he PTR model we used, a PTR-MS hs with a high sensitivity, is in the order of pptv (Hansel et al. 1998). The quantification of VOCs was based on the use of 3 times replicated calibration of individual standards of ethylene, methanol, isoprene, α-pinene, methyl salicylate and caryophylene (Sigma-Aldrich, Abelló- Linde). These standards were prepared in a desorption system connected to Tedlar bags. The standard error of the three calibrations was less than 10% for all the standards The PTR-MS drift tube was operated at 2.1 mbar and 40ºC, with a drift field of 600 V cm^{-1}. The parent ion signal was maintained at around $3x10^6$ counts per second during the measurements. We measured methanol (m33), acetaldehyde (m45), m47 (formic acid/ethanol), acetone (m59) and acetic acid (m61). It has been demonstrated that the ions associated with methanol (m33) and acetaldehyde (m45) are free from significant interferences of other VOC species in PTR-MS measurements (Warneke et al. 2003). A potential interference for acetone (m59) is propanal (de Gouw et al., 2003). Acetic acid is detected at m61 although peroxyacetic acid is a possible interference (de Gouw et al., 2003).

For VOC determination and quantification, both the air entering and exiting the leaf cuvette were analysed with PTR-MS, at alternative intervals, and monitored with flow meters. 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.

- Data treatment and statistical analyses

We used t-tests, one way ANOVAS, and post-hoc tests (Statistica, StatSoft Inc., Tulsa, USA) to compare the leaf emissions after the treatments. Regression and correlation analyses were also conducted for the studied variables.

Results

The relative water content of *Pinus halepensis* needles in the control, drought and rewatered treatments were 91.22±1.94, 87.31±1.09 and 92.34±1.46%, respectively. After rewatering soil water content increased from c. 5% under water stress conditions to 25 %, midday leaf temperatures decreased from 31ºC to 28ºC, stomatal conductance increased from 15 to 111 mmol m^{-2} s^{-1}, and photosynthetic rates increased from -0.2 to 12 μmol m^{-2} s^{-1} (Fig. 1).

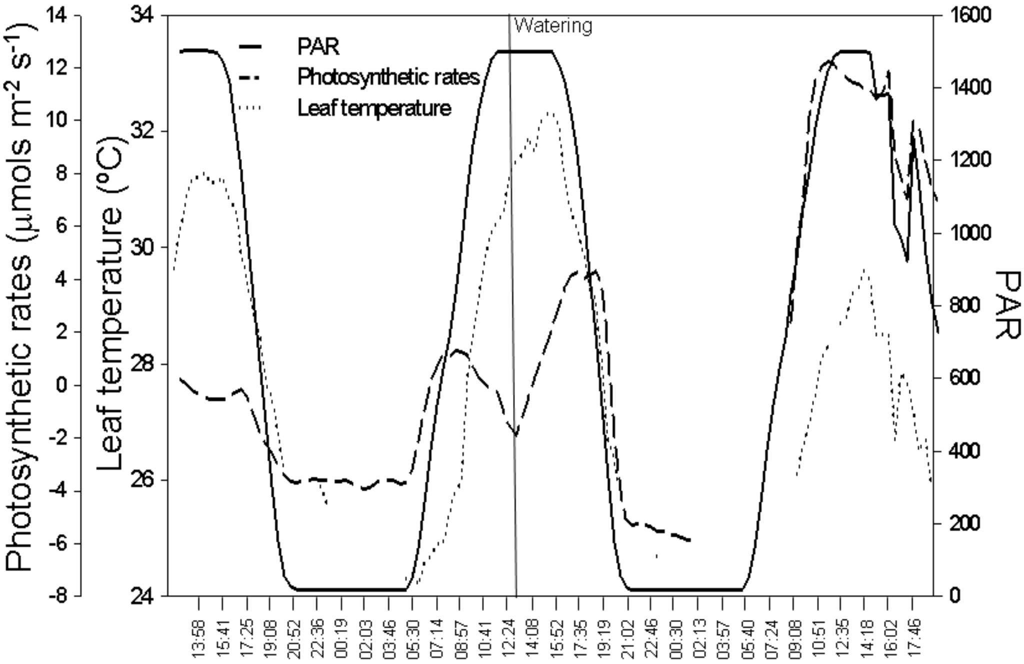

Fig. 1. Time course dynamics of PAR, leaf temperature and photosynthetic rates before and after watering. *Vertical line* indicates the moment of watering.

Table 1. Average emission rates of oxVOcs (nmol m^{-2} s^{-1}) at 10:30 am. Different letters indicates significant ($p<0.05$) differences between treatments (Fisher post-hoc test).

	Control	Drought	Rewatered
M33 (methanol)	2.9±0.43a	0.22±0.08b	2.79±0.25a
M61 (acetic acid)	0.12±0.13a	0.038±0.15a	-0.41±0.15b
M45 (acetaldehyde)	0.36±0.1a	0.25±0.05a	0.29±0.09a
M47 (formic	0.21±0.24a	0.31±0.03a	0.49±0.08a
M59 (acetone)	0.48±0.08a	0.41±0.16a	0.5±0.24a

Pinus halepensis non water stressed seedlings emitted mainly methanol (M33), and when drought-stressed they mainly emitted acetone (M59) (Table 1, Fig. 2, Fig. 3). The water availability of the plant significantly affected the emission rates of methanol (M33), which decreased when pine was drought-stressed and increased one order of magnitude after irrigation. The water status also affected the acetic acid (M61) uptake, with significantly higher uptake the higher the stomatal conductance after watering (Table 1). Acetaldehyde (M45), formic acid/ethanol (M47) and acetone (M59) emissions slightly increased after plant watering but changes were not significant (Table 1, Fig. 2).

The sequences of emission rates measured for a typical pine (Fig. 2) showed a rapid increase in acetaldehyde (M45) and formic acid/ethanol (M47) emissions after watering. The emissions of methanol (M33), acetaldehyde (M45) and formic acid/ethanol (M47) followed the changes in stomatal conductance, but acetone (M59) emissions did not. Acetone (M59) presented an important peak (burst) of emission following light and stomatal opening in the morning, when water-stressed but the emissions the day after irrigation were similar to the emissions before watering (Fig. 2). There was an increase in uptake of acetic acid (M61) after irrigation that was more important the day after irrigation coinciding with an increase in stomatal conductance (Fig. 2).

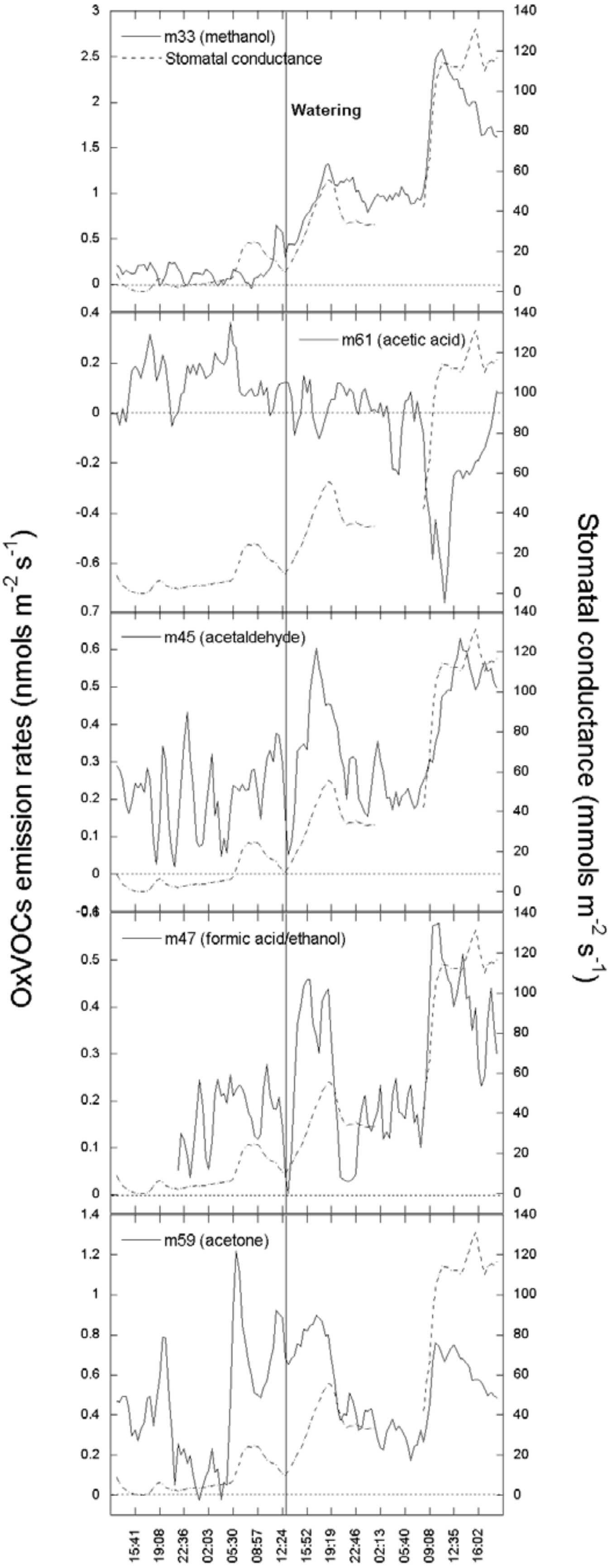

Fig. 2. Time course dynamics of stomatal conductance and m33 (methanol), m61 (acetic acid), m45 (acetaldehyde), m47 (formic acid/ethanol) and m59 (acetone) emissions, before and after watering. *Vertical line* indicates the moment of watering. The dynamics were similar for the three replicates.

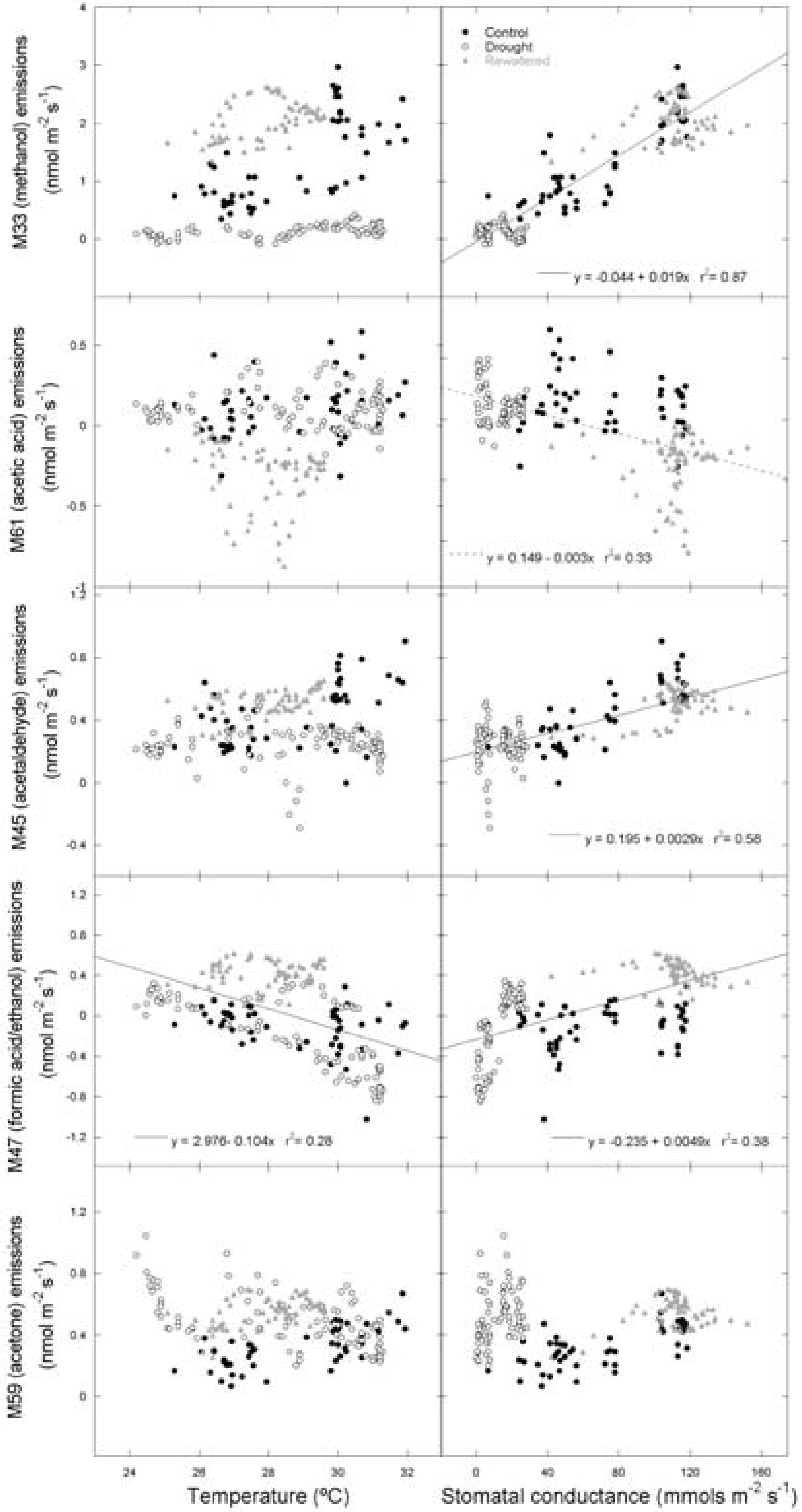

Fig. 3. Relationship of oxVOC emissions with leaf temperature and stomatal conductance for the three studied pines submitted to control, drought and rewatering treatments. Only significant relationships are depicted with the correspondent equation and r^2.

Stomatal conductance, transpiration and photosynthetic rates, followed rapidly the changes in water availability; they increased after watering (Fig. 1). All the oxVOC exchanges, except that of acetone (M59), were significantly related to stomatal conductance and transpiration, and were not related to temperature in the measured range (between 24ºC and 32ºC) (Fig. 3). However, acetaldehyde (M45) emission was also dependent on its concentration in the ambient. Stomatal opening (stomatal conductance above 75 mmol m^{-2} s^{-1}) allowed increased emissions only when external concentration were below 6 ppbv (Fig. 4). In drought stressed plants, formic acid/ethanol (M47) emissions decreased with increasing temperature (Fig. 3).

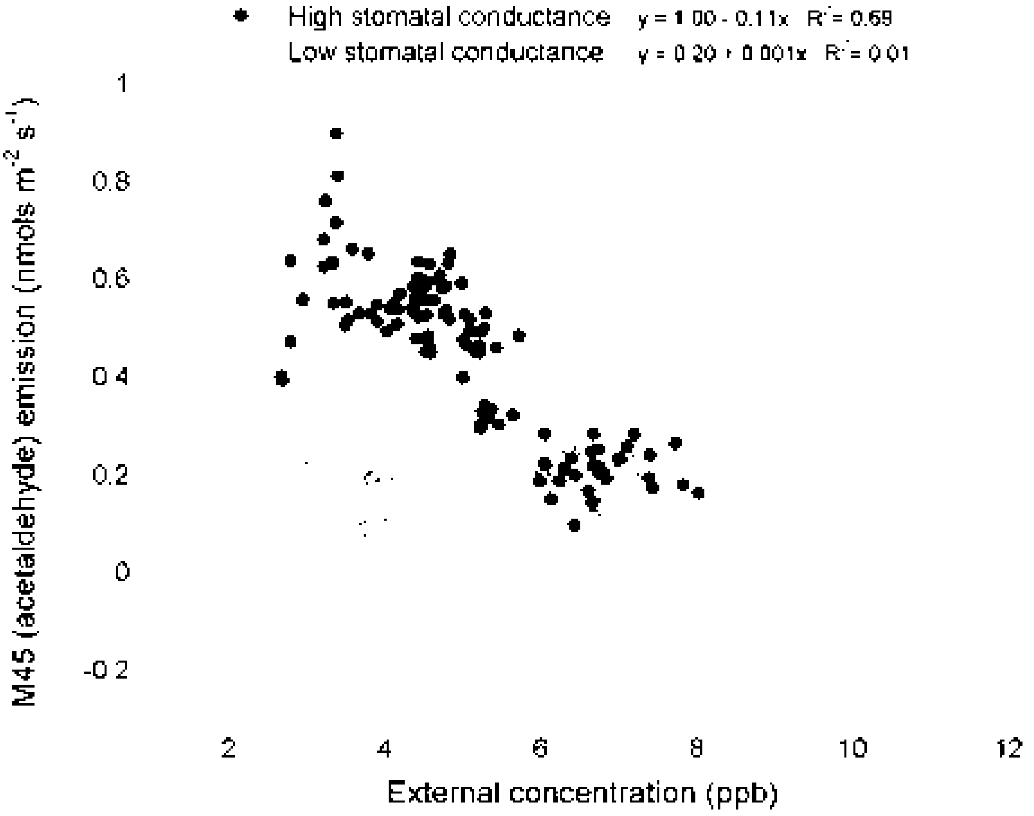

Fig. 4. M45 (acetaldehyde) emission as a function of acetaldehyde external concentration. Two levels of stomatal conductance were established, below and above 60 mmol m^{-2} s^{-1}.

Discussion

When not drought-stressed, *Pinus halepensis* emitted methanol, acetaldehyde, formic acid/ethanol and acetone in the higher range of emissions found in other species (*capítol 1*; Seco et al., 2007). The alterations in oxVOC emissions in *Pinus halepensis* caused by the water deficit seem to be mainly driven by water stress effect on stomatal closure (Fig. 3). Stomatal constraints on emissions may have also altered the diurnal dynamics of the studied oxVOC flux rate (Figs. 1 and 2). Methanol was the oxVOC with the emissions most correlated to stomatal conductance, although the response to stomatal opening was faster for acetaldehyde and formic acid emissions. The behaviour of acetone emissions, although did not parallel stomatal conductance, was also affected by stomatal changes (emission burst when stomata opened in the morning). And also acetic acid uptake was related to stomatal opening.

Methanol solubility makes its release stomata-dependent (Nemecek-Marshall et al., 1995; Schade and Goldstein, 2002). Methanol emissions followed stomatal conductance after plant was watered, but, there was a very low emission, that was not related to stomatal conductance, when plant was water stressed. In the plant, the major source of methanol is pectin demethylation, and methanol release seems linked to pectin methyl esterases (PME) activity (Fall, 2003). Thus, our results showing decreased methanol emission suggest that drought stress could reduce the levels of PME activity. Moreover, as plant growth is a known source of methanol (Nemecek-Marshall et al., 1995) the lower emissions in water stressed plants could also be due to lower growth.

Stomatal conductance is an important factor in the emission/uptake of acids (Kesselmeier et al., 1997, 1998; Kesselmeier, 2001; Niinemets and Reichstein, 2003; Filella et al., 2007). Formic and acetic acids have been reported to be both deposited and emitted (*capítol 1*; Seco et al., 2007). Studies in tropical forests in Amazonia and Costa Rica stated that tropical forests are more a sink than a source of formic and acetic acids (Kuhn et al., 2002; Karl et al., 2004). In the Valencia region, high concentrations in ambient air promoted acid deposition on orange trees (*Citrus sinensis*) (Staudt et al., 2000). Kuhn et al. (2002) reported the hypothesis of a compensation point concentration that determines the emission or the absorption of acids. They suggest bidirectional exchange behaviour of the plants and found that the uptake of organic acids was primarily a function of the ambient atmospheric mixing ratios. After uptake, acetic acid enters the plant metabolism, thereby maintaining the concentration gradient between the interior and the exterior of the plant that permits continuous uptake. Once in the symplast, acetic acid may be converted to acetyl-CoA and enter general metabolism pathways such as the TCA (TriCarboxylic Acids) cycle. In this experiment, acetic acid ambient concentration was above the compensation point. M47 (formic acid/ethanol) was emitted although emissions did not follow exactly stomatal conductance. Gabriel et al. (1999) found that formic acid has more cuticular permeability than acetic acid so this acid escapes not only through stomata but also in an uncontrolled fashion through plant membranes and the cuticle.

M45 (acetaldehyde) emissions showed a rapid response to stomatal opening, and after watering followed quite close stomatal conductance variations (Fig. 2). Acetaldehyde formation is known to be an intermediate step in the oxidation of ethanol into acetate. Ethanol delivery to the cell is also governed by stomatal conductance. Acetaldehyde emission should be seen essentially as a leak between acetaldehyde production and metabolism (Kreuzwieser et al., 2001; reviewed by Fall, 2003). Niinemets and Reichstein (2003) concluded that stomatal control of acetaldehyde emissions are the result of a complex interplay between the limitations caused by gas-phase diffusion conductance and stomatal effects on ethanol delivery rates, simultaneous processes of varying time-constant that are superimposed. Moreover, in this study the final driver of emissions was the external concentration of acetaldehyde

There were no differences in acetone emissions between drought-stressed and watered plants. Ebel et al. (1995) also found no changes in acetone emission between control and drought stressed apple trees. A weak relationship between acetone emissions and stomatal conductance has been previously found (Filella et al., 2007). Acetone can be produced within plant tissues by the cyanogenic pathway (a widespread mechanism in plants activated to deter herbivores), leading to the production of hydrogen cyanide (HCN) and –as a byproduct– acetone (Fall, 2003). There are evidences that cyanogenic capacity increases in response to water stress (Gleadow and Woodrow, 2002). The peak of emission of acetone observed after stomatal opening in water-stressed pines could be a consequence of this increase in cyanogenic capacity. VOCs may accumulate in the leaves during periods of stomatal closure, and release after the gas-phase conductance starts to rise, in particular volatiles with low *H*, and the amount of volatile emitted during the burst is directly proportional to the amount of volatile accumulated during the period of low gas-phase conductance (Niinements and Reichstein, 2003).

In highly water-soluble compounds the foliar-atmosphere exchange is more affected by stomatal conductance, with the degree of stomatal sensitivity varying with *H* (Henry's law constant) (Niinemets and Reichstein, 2003). Stomata may control effectively the emission rates of compounds with the lowest *H*, such as acetic acid, formic acid or methanol, (Niinemets and Reichstein, 2003; Filella et al., 2007). However, for compounds with intermediate H values, such as acetaldehyde or acetone, the role played by stomata in controlling emission rates is less clear. Here, partial pressures, and thus final emission rates, can be more affected by variations in compound synthesis rate, or metabolic activity rates. Finally, both emission rates and metabolic activities are affected by internal (genetic traits, developmental stage, phenology, water content, etc) and environmental (light, temperature, relative humidity, herbivory and pollution stresses, wind speed, etc) conditions. However, ambient concentration can be the main driver in oxVOC exchange since gases move along the concentration gradient between the inner part and the outside part of the leaf. When concentration inside the leaf is lower than the external concentration, there is not emission, independently of stomatal conductance.

Although the extrapolation of these results to field conditions is never straightforward, they show that the alterations in oxVOC emissions in *Pinus halepensis* caused by the water deficit seem to be mainly driven by water stress effect on stomatal closure and oxVOC air concentrations. However, drought-driven limitations in emissions during hot summers may be even more relevant under the future climate which is supposed to become more arid in the Mediterranean (IPCC, 2001; Sabaté et al., 2002; Peñuelas et al., 2005a). Although the emission of volatile organic compounds from vegetation is particularly sensitive to temperature (e.g. Peñuelas and Llusià, 2001a, b, 2003; Filella et al., 2006, Guenther et al., 1995), in the conditions of this experiment the changes in stomatal conductance were of higher magnitude than the temperature changes, and the low range of temperatures 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 of the antagonist effect of both factors on VOC emissions. Future work should investigate, thus, the interactive effect of both stresses on vegetation emissions.

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2.3 Increase in isoprene and monoterpene emissions after re-watering of droughted *Quercus ilex* seedlings

Una versió editada d'aquest capítol va ser publicada el juny de 2009 a la revista
Biologia Plantarum 53(2), 351-354. doi: 10.1007/s10535-009-0065-4

Resum

Es van seguir els cicles diaris d'emissions d'isoprenoids en plançons d'alzina (*Quercus ilex*) sotmesos a sequera i posteriorment regats. Hem vist que l'alzina, espècie generalment considerada no-emissora d'isoprè, també emetia isoprè encara que amb poca intensitat. Les taxes d'emissió d'isoprè varen arribar a 0.37 ± 0.02 nmol m^{-2} s^{-1} en plantes control, 0.15 ± 0.03 nmol m^{-2} s^{-1} sota sequera i 0.35 ± 0.04 nmol m^{-2} s^{-1} després de regar, mentre que les taxes d'emissió de monoterpens assoliren els 11.0 ± 3.0, 7.0 ± 1.0 i 23.0 ± 5.0 nmol m^{-2} s^{-1}, respectivament. Les taxes d'emissió es van recuperar abans que les taxes fotosintètiques després de regar, i van seguir els canvis diaris d'irradiància en les plantes control i sota sequera, però seguiren els canvis en temperatura foliar després del reg.

Abstract

We followed the diurnal cycles of isoprenoid emissions from *Quercus ilex* seedlings under drought and after re-watering. We found that *Quercus ilex*, generally considered a non-isoprene emitter, also emitted isoprene although at low rates. The emission rates of isoprene reached 0.37 ± 0.02 nmol m^{-2} s^{-1} in controls, 0.15 ± 0.03 nmol m^{-2} s^{-1} under drought and 0.35 ± 0.04 nmol m^{-2} s^{-1} after re-watering, while emission rates of monoterpenes reached 11.0 ± 3.0, 7.0 ± 1.0 and 23.0 ± 5.0 nmol m^{-2} s^{-1}, respectively. Emission rates recovered faster after re-watering than photosynthetic rate and followed diurnal changes in irradiance in controls and under drought, but in leaf temperature after re-watering.

Introduction

Plant volatile organic compounds (VOCs) include isoprene, terpenes, alkanes, alkenes, alcohols, esters, carbonyls and acids (Peñuelas and Llusià, 2003). VOCs have many protective and ecological functions for the plant species that produce them and have important effects on the photochemistry and radiative properties of the atmosphere (Zimmerman et al., 1978; Kavouras et al,. 1998; Peñuelas and Llusià, 2003; Owen and Peñuelas, 2005). Consequently there is great interest in determining the emission capacities of the different species and how environmental factors affect the volatile isoprenoid emissions (Peñuelas and Llusià, 2001).

Oaks belong to the greatest VOC emitters (Harley et al., 1999). The holm oak (*Quercus ilex* L.), a widely distributed species in the Mediterranean basin, is considered a monoterpene and non-isoprene emitter (Kesselmeier and Staudt, 1999; Loreto, 2002) although Owen et al. (1997) reported small emission rates of isoprene. Variations in the emission of volatiles can also be triggered by changing environmental conditions (Peñuelas and Llusià, 1999a, 2001; Niinemets et al., 2004).

No specialized storage structures for monoterpenes have been found in its leaves or bark, and emissions appear to be mainly influenced by temperature, irradiance and water availability (Loreto et al., 1998; Llusià and Peñuelas, 1998, 1999, 2000; Peñuelas and Llusià, 1999a,b; Staudt et al., 2002).

In Mediterranean areas, drought driven changes in emission may be even more relevant under the future climate, since water availability in the Mediterranean region is likely to be reduced in the near future by the predicted increases in temperature and the consequent increases in evapotranspiration rates (Sabaté et al., 2002; Peñuelas et al., 2005). These changes in emissions may have important consequences in the formation of photochemical pollutants in this Mediterranean area (Filella and Peñuelas, 2006).

The aim of this study was to describe the changes in isoprenoid emission rates induced by drought conditions and after subsequent re-watering together with physiological variables such as CO_2 and water exchange.

Materials and methods

We used two-year-old *Quercus ilex* L. plants grown in a nursery (Forestal Catalana, S.A., Breda, Spain) under typical Mediterranean environmental conditions (mean annual average temperature 16 ºC and mean annual precipitation 600 mm). They were grown in 2 dm^3 pots with a substrate composed of peat and sand (2:1), prior to being brought into the laboratory, where they were kept some days for an acclimation before starting the experiment. Control (well watered) plants were measured over one day at soil moisture of 25 % (volume of water per volume of dry soil; v/v), measured by time domain reflectometry (*Tektronix 1502C*, Beaverton, Oregon, USA) and left un-watered until soil water content reached 5 %. After one new day of measurements under these drought conditions, plants were re-watered until soil water content reached 25 % and measured again over another day. Three replicates were conducted, all at room temperature (25 ºC).

Intact leaves were clamped in a Parkinson leaf cuvette (*Std Broad 2.5*, *PP Systems*, Hitchin, England). The air entering the system was sampled at 4 m above ground from the outside of the building, filtered with glass wool to prevent any dust intake and passed through a polyethylene terephthalate (PET) recipient to buffer exterior CO_2 and VOC fluctuations. All tubing used was made of inert polytetrafluoroethylene (PTFE). A diurnal cycle of irradiance was programmed to simulate a typical sunny day, photosynthetic photon flux density (PPFD) ranging from 0 to 1500 μmol m^{-2} s^{-1}. The leaf temperature ranged between 25 and 32º C. A calibrated *Ciras-2* IRGA-porometer (*PP Systems*) was used for determining rates of CO_2 and H_2O exchange. Part of the air exiting the leaf cuvette flowed through a T-system to the PTR inlet.

We used a highly sensitive proton-transfer-reaction mass spectrometer (PTR-MS-FTD, *Ionicon Analytik*, Innsbruck, Austria) as described by Lindinger et al. (1998). The PTR-MS drift tube was operated at 0.21 kPa and 40 ºC, with a drift field of 600 V cm^{-1}. The parent ion signal was maintained at around 3×10^6 counts s^{-1} during the measurements. We measured isoprene (m69) and monoterpenes (m137). The quantification of isoprene and monoterpenes was based on the use of 3 times replicated calibration standards of isoprene and α-pinene (*Sigma-Aldrich*, Barcelona, Spain).

For volatile isoprenoid determination and quantification, both the air entering and exiting the leaf cuvette were analysed by (PTR-MS), at alternate 5-min intervals, and continuously monitored with flow meters. The difference between the concentration of volatile isoprenoids before and after passing through the cuvette (with leaves and without leaves), along with the flow rates, was used to calculate the volatile isoprenoid exchange (Peñuelas et al., 2007). Leaves were excised after emission sampling to measure leaf area (*LI 3100*, leaf area meter, *Li-Cor*, Lincoln, NE, USA). Leaves were then dried in an oven at 70 °C until constant weight (usually for 72 h) to determine dry mass.

We used one way *ANOVA*, and Bonferroni post-hoc tests to compare the leaf emissions in the three different treatments. Regression and correlation analyses were also conducted for the studied variables. In all cases we used *Statistica* (*Statsoft Inc.*, Tulsa, OK, USA) programme package.

Results and discussion

Q. ilex seedlings emitted isoprene in all three replicates both under control watered conditions and in the three replicate measurements of the diurnal cycle during drought/re-watering experiment (Fig. 1 shows one of those replicates). The emission rates of isoprene reached 0.37 ± 0.02 nmol m^{-2} s^{-1} in control conditions, 0.15 ± 0.03 nmol m^{-2} s^{-1} in drought conditions and 0.35 ± 0.04 nmol m^{-2} s^{-1} in re-watering conditions (significantly different at, $P < 0.01$, $n = 3$). Those of monoterpenes reached 11.0 ± 3.0, 7.0 ± 1.0, and 23.0 ± 5.0 nmol m^{-2} s^{-1}, respectively. *Q. ilex* was thus emitting low but significant amounts of isoprene, and the emissions ranged between 1.5 and 3.4 % of monoterpene emissions. These emissions of isoprene were slightly higher than those reported in the previous study (Owen et al., 1997).

There was a clear diurnal cycle in emissions of both isoprene and monoterpenes. Both emissions were stimulated during day and declined in the dark. As in recent physiological studies of oaks (Schnitzler et al., 2004) these isoprenoid emissions by *Q. ilex* were largely associated to net photosynthetic rate (P_N) and stomatal conductance (g_s) which presented two peaks, one in the morning and another one in the afternoon when irradiance and temperature started to decrease (Fig. 1). The concurrently performed measurements of photosynthesis revealed that *Q. ilex* leaves lost about 0.7 - 1.5 ‰ of the assimilated carbon *via* isoprenoid emission; lower than reported under field conditions in several other studies (Sharkey and Loreto, 1993; Kesselmeier and Staudt, 1999; Peñuelas and Llusià, 1999a,b, 2001; Llusià and Peñuelas, 2000).

The relative water content of *Q. ilex* leaves in the control, drought and re-watering treatments were 93.0 ± 2.2, 77.1 ± 4.0 and 91.8 ± 0.3 %, respectively (significantly different, $P < 0.01$). The early morning values of P_N and g_s were high even under drought since these plants take advantage of a short window of activity in the mornings after the night water status recovery. P_N and g_s in the afternoon followed the changes in water availability rapidly; they increased by more than twice the day after re-watering (Fig. 1). The emissions of isoprene and monoterpenes increased even more, *ca*. 3 times, after re-watering the droughted plants (Fig. 1). They responded immediately to changes in leaf temperature but not to changes in P_N or g_s as occurred in controls and under drought. This is another manifestation of the uncoupling between volatile isoprenoid emissions and photosynthesis described in several previous studies that showed that short term drought caused substantial reduction in photosynthesis, whereas isoprene emissions were either not inhibited or only slightly reduced (Tingey et al., 1981;

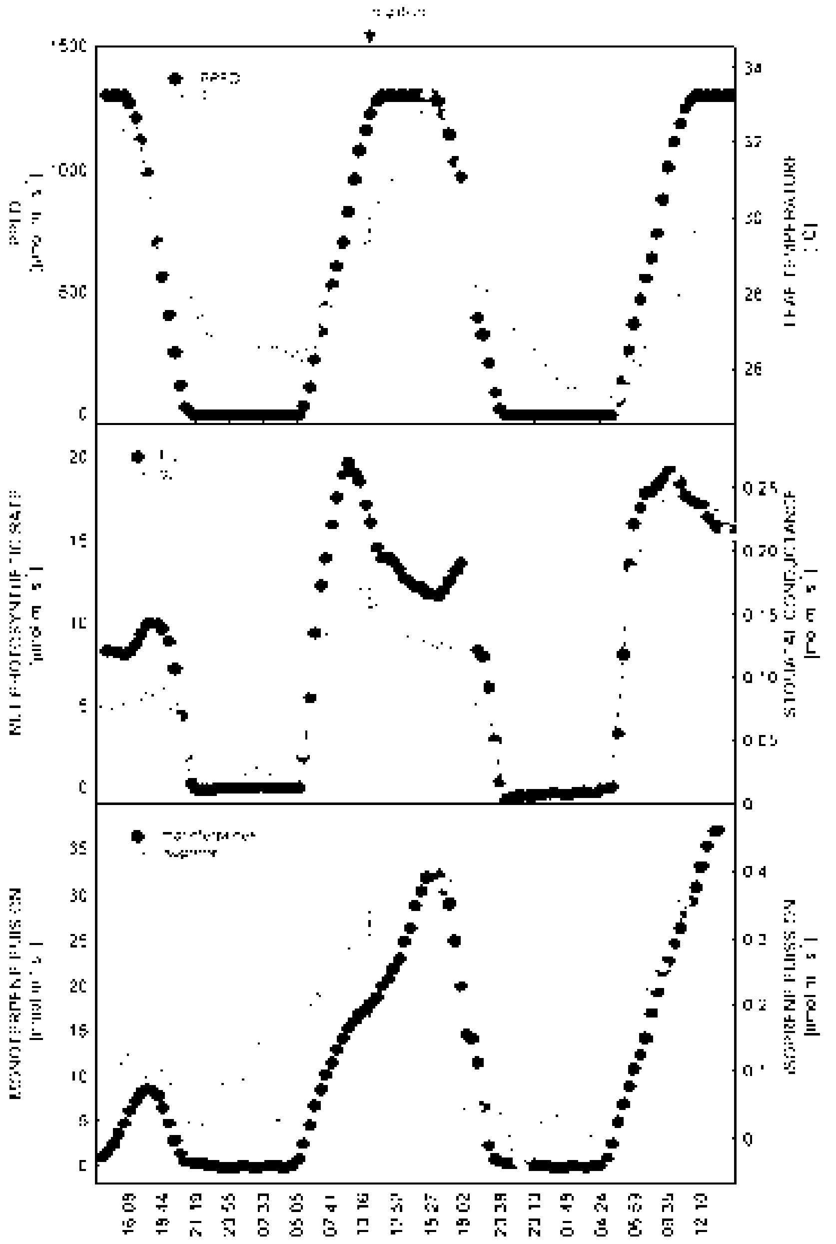

Fig. 1. Daily time course dynamics of PPFD, leaf temperature, net photosynthetic rates, stomatal conductance and isoprene and monoterpene emissions in a *Quercus ilex* potted seedling submitted to drought and re-watering. *Vertical line* indicates the moment of re-watering. Replicated three times (see text).

Sharkey and Loreto, 1993; Fang et al., 1996; Funk et al., 2005). The changes in the emissions' dependence on temperature after re-watering (Fig. 1) indicated that the temperature was the factor driving increases in the emission rates, probably as a result of enhanced isoprenoid synthesis, diffusivity and volatility (Peñuelas and Llusià, 2001). Our results also indicate that emissions recovered to previous unstressed values faster than P_N or g_s which is in agreement with the recent results reported for isoprene emissions in *Populus alba* by Brilli et al. (2007). In addition, our results show very high terpene emission rates just after the re-watering of plants. During the recovery, the emission rates reached values in the higher limit or above the normal range reported for this species in this Mediterranean region (Llusià and Peñuelas, 2000; Peñuelas et al., 2007). Further studies are necessary to figure out the mechanisms ruling this high emission rates temporally uncoupled of photosynthesis and highly responsive to temperature.

Although the extrapolation of these results to field conditions is never straightforward, they show that *Q. ilex* is able to emit isoprene and that there are complex responses of isoprene and monoterpene emissions to drought cycles. The emission of volatile organic compounds from vegetation is particularly sensitive to temperature (e.g. Peñuelas and Llusià, 2001, 2003; Guenther et al., 1995; Filella et al., 2007), but the effects of the drought that accompany high temperatures must also be considered to estimate the consequences of climate change on isoprenoid emissions, especially because of the antagonist effect of the two factors on VOC emissions. Future work should therefore investigate the interactive effect of both stresses on vegetation emissions in order to improve current generation of emissions models (Monson et al., 2007).

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Capítol 3

Efectes dels oxCOVs en les relacions entre organismes

3.1 Methanol as a signal triggering isoprenoid emissions and photosynthetic performance in *Quercus ilex*

Resum

S'han descrit diversos compostos orgànics volàtils (COVs) que tenen una funció de comunicació entre plantes i també entre plantes i animals. Volíem provar si el metanol, un COV oxigenat de cadena curta, podia tenir també un paper en la comunicació entre plantes. Vàrem seguir el rendiment fotosintètic i l'intercanvi de COVs en plançons de *Quercus ilex* L. abans i després de dos tractaments: a) tallar algunes fulles per simular un atac d'herbívors i b) fumigació amb metanol gasós durant 5 hores per simular la quantitat de metanol que una planta podria rebre de les plantes que l'envolten si aquestes haguessin estat ja atacades per herbívors. El tractament de tallar va afavorir les taxes fotosintètiques, la ràtio clorofil·la a/b, i la ràtio carotenoids/clorofil·la, suggerint una activació del metabolisme protector de la planta. A més, es va observar un petit però interessant increment sistèmic (en fulles no tallades) de la taxa d'emissió de metanol, que concorda amb la possibilitat que el metanol pugui actuar com a senyal de comunicació. El tractament de fumigació va induir una increment de l'eficiència fotoquímica del fotosistema II (PSII) i també de la ràtio carotenoids/clorofil·la. La fumigació amb el metanol també va provocar un increment del 14% de la taxa d'emissió de monoterpens, un dia després del tractament, resposta similar a les induïdes per d'altres COVs senyalitzadors. Les emissions incrementades de monoterpens podrien afegir-se a la barreja de COVs emesos després de patir estrès i formar part de següents vies de senyalització, propoagant així el missatge començat pel metanol. Aquest estudi mostra que tallar i fumigar amb metanol a concentracions naturals provoca respostes fisiològiques significatives en les plantes veïnes i més emissions de COVs biogènics.

Abstract

Several Volatile Organic Compounds (VOCs) have been reported as having a communication role between plants and also between plants and animals. We aimed to test whether methanol, a short-chain oxygenated VOC, could also have a signalling role between plants. We monitored photosynthetic performance and VOC exchange rates of *Quercus ilex* L. saplings before and after two different treatments: a) clipping of some leaves to simulate an attack by herbivores and b) fumigation with gaseous methanol during 5 hours to simulate the amount of methanol a plant could receive from surrounding plants if those had been already attacked by herbivores. The clipping treatment enhanced the photosynthetic rates, the chlorophyll a to b ratio, and the carotenoid to chlorophyll ratio, suggesting an activation of plant protective metabolism. Also, a small but interesting systemic (in non-clipped leaves) increase in methanol emission rates was observed, which agrees with the possibility that methanol may act as a signalling cue. The methanol fumigation treatment induced an increase in the actual photochemical efficiency of PSII and also in the carotenoid to chlorophyll ratio. Methanol fumigation also promoted a 14% increase in the monoterpene emission rate, one day after the treatment, a similar response to the ones induced by other signalling VOCs. The enhanced monoterpene emissions could add to the blend of VOCs emitted after stress and be part of further signalling pathways, thus forwarding the message started by methanol. This study shows that clipping and methanol fumigation at natural concentrations elicit significant neighbour plant physiological responses and further BVOC emissions.

Introduction

Biogenic Volatile Organic Compounds (BVOCs) are recognized as having many roles in plant physiology and ecology (Peñuelas and Llusià, 2002, Peñuelas and Staudt, 2010, Singsaas and Sharkey, 1998; Velikova et al., 2005) as well as in atmospheric chemistry (Atkinson, 2000; Holzinger et al., 2005; Kavouras et al., 1998; Peñuelas and Llusià, 2003), and consequently their emissions by plants have been measured and modeled extensively during last years (Guenther et al., 2006; Keenan et al., 2009; Kesselmeier and Staudt, 1999; Seco et al., 2007, *capítol 1*). In addition, BVOCs are implicated in plant-animal communication (Gershenzon, 2007; Peñuelas et al., 1995b). Plant-animal relationships mediated by BVOCs do not only involve plant-pollinator relationships (Wright and Schiestl, 2009) or direct defense against herbivores, but also indirect defense through the attraction of natural enemies of the herbivores (Kessler and Baldwin, 2001; Llusià and Peñuelas, 2001; Pichersky and Gershenzon, 2002).

BVOCs can also play an important role in plant-plant communication by transmiting information between plants (Peñuelas et al., 1995b). One example is the sensing of proximate neighbour plants mediated by the phytohormone ethylene, which has been reported for *Nicotiana tabacum* (Pierik et al., 2003). Most recent BVOC-mediated plant-plant communication studies have dealt with response to herbivory and priming. In this context, priming refers to the fact that a plant gets ready for the possible attack of a pathogen or an herbivore based on the BVOC signals that attacked plants send to the atmosphere (Heil and Kost, 2006), so the primed plant is then able to respond quicker and/or stronger to the herbivore when attacked. This phenomenon has been observed even between different parts of the same individual (Frost et al., 2007; Heil and Silva Bueno, 2007). Great attention has been focused on certain C6 compounds (aldehydes, alcohols, and their acetates) biosynthesized via the lipoxygenase/hydroperoxide lyase pathway, called green leaf volatiles (GLV, Matsui, 2006). GLV are emitted specially after herbivore attack and are responsible for priming effects on other plants (Engelberth et al., 2004; Frost et al., 2008; Heil and Kost, 2006; Kost and Heil, 2006). Other plant-emitted BVOCs such as methyl jasmonate (MeJA) and methyl salicylate (MeSA) are also involved in defense responses (Laothawornkitkul et al., 2009), at least partly in interaction with the aforementioned ethylene (Arimura et al., 2008; Ruther and Kleier, 2005; von Dahl and Baldwin, 2007).

Methanol is the smallest alcohol and is among a group of short-chain oxygenated BVOCs which are becoming more studied in recent years (*capítol 1*, Seco et al., 2007;

capítol 2.2, Filella et al., 2009). Under non-perturbed situations, it is released from plants to the atmosphere by demethylation of pectins during cell wall formation, e.g. during leaf growth (Harley et al., 2007; Hüve et al., 2007). Globally, it is the second most abundant organic gas in the atmosphere after methane (Jacob et al., 2005). But despite being somewhat abundant in the air, it can be emitted by plants in such high amounts during stress episodes –like herbivore attacks (Peñuelas et al., 2005a; von Dahl et al., 2006) and crop harvesting (Karl et al., 2001)– that it might play a communication role by being detectable as a signal by plants themselves or by other organisms in the near area. It has been suggested that plants may rely on more long-lived compounds such as methanol for long distance communication, since molecules like GLV are very reactive in the atmosphere and may be rapidly degraded by oxidants like ozone (Pinto et al., 2007a).

Previously published experiments dealing with methanol effects on plants mainly consisted in spraying liquid methanol solutions on the leaves of several plant species. Some reported an increase of photosynthesis (Faver and Gerik, 1996) and growth (Madhaiyan et al., 2006; Ramirez et al., 2006), as well as no effect on growth (Rajala et al., 1998). One experiment that used gaseous methanol, at a high concentration (1% in air), focused on the effect on the photosynthetic apparatus and found an inhibition of its biochemical reactions (Loreto et al., 1999). Sprayed methanol treatment also induced the expression of hundred of genes in *Arabidopsis thaliana* and activated multiple detoxification and signalling pathways (Downie et al., 2004), facts that agree with a possible signalling function of methanol.

We aimed to discern whether methanol in natural concentrations in the air could act as a signalling cue in plant-plant interactions, specially with regard to the triggering of further BVOC emissions by the receiving plant. We studied the widespread Mediterranean tree *Quercus ilex* L. (holm oak), one of the most abundant species in Mediterranean forests, and whose physiology has been broadly studied from different points of view, including BVOC exchange (Peñuelas and Llusià, 2002; Blanch et al., 2007; *capítol 2.1*, Seco et al., 2008; *capítol 2.3*, Peñuelas et al., 2009). This species has, moreover, been reported to suffer from episodic outbreaks of herbivores like *Lymantria dispar* L. (Staudt and Lhoutellier, 2007) which could potentially lead to high local methanol emissions and, consequently, to possible signalling to neighbour plants. We measured the physiological and BVOC emission responses of *Q. ilex* seedlings to i) a simulated herbivore attack by clipping leaves and ii) a fumigation with gaseous methanol during several hours.

Materials and methods

- Plant material

We used 3-year-old potted *Quercus ilex* L. plants grown in a nursery (Tres Turons S.C.P., Castellar del Vallès, Catalonia, Spain), maintained under Mediterranean ambient conditions outdoors. 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 allowed to acclimate to laboratory conditions for several days before starting the experiment.

- Plant and leaf chambers

We used two different chambers (Fig. 1). The whole aerial part of the monitored plant was enclosed in a 65 L cylindrical chamber made of transparent teflon film. This big plant chamber (PC) was illuminated from the top by fluorescent lights (Agrolite CFL, Barcelona) giving about 300 µmol m^{-2} s^{-1} of photosynthetic active radiation (PAR) flux inside the chamber, with a 12:12h light:dark photoperiod. Ambient air from outside the building was introduced into the PC at a rate of about 15 L min^{-1} by means of an oil-free pump. A fan stirred the air inside the PC, and there was always an excess of air escaping from the PC through the tiny space between the trunk of the tree and the PC teflon film, thus preventing external air to enter the PC. CO_2 was added from a bottle (Abelló Linde S.A., Barcelona, Catalunya) through a mass flow controller (Bronkhorst High-Tech B.V., Ruurlo, Netherlands) during high photosynthetic activity periods, to keep atmospheric CO_2 concentrations ca. 385 µmol mol^{-1}.

A second, small leaf chamber (LC) was introduced inside the PC and clamped a leaf. This leaf cuvette was part of a LCpro+ Photosynthesis System (ADC BioScientific Ltd., Herts, England), which recorded photosynthesis (net CO_2 uptake), stomatal conductance, air humidity, and temperature data, while controlling the light radiation and the flow of air entering the leaf cuvette. A light diurnal cycle was programmed in the LC to simulate a typical sunny day, ranging from 0 to 1500 µmol m^{-2} s^{-1} of PAR and with the same 12:12h photoperiod as the PC. The air entering the LC was taken from inside the PC: this way the air reaching all the leaves of the plant was the same.

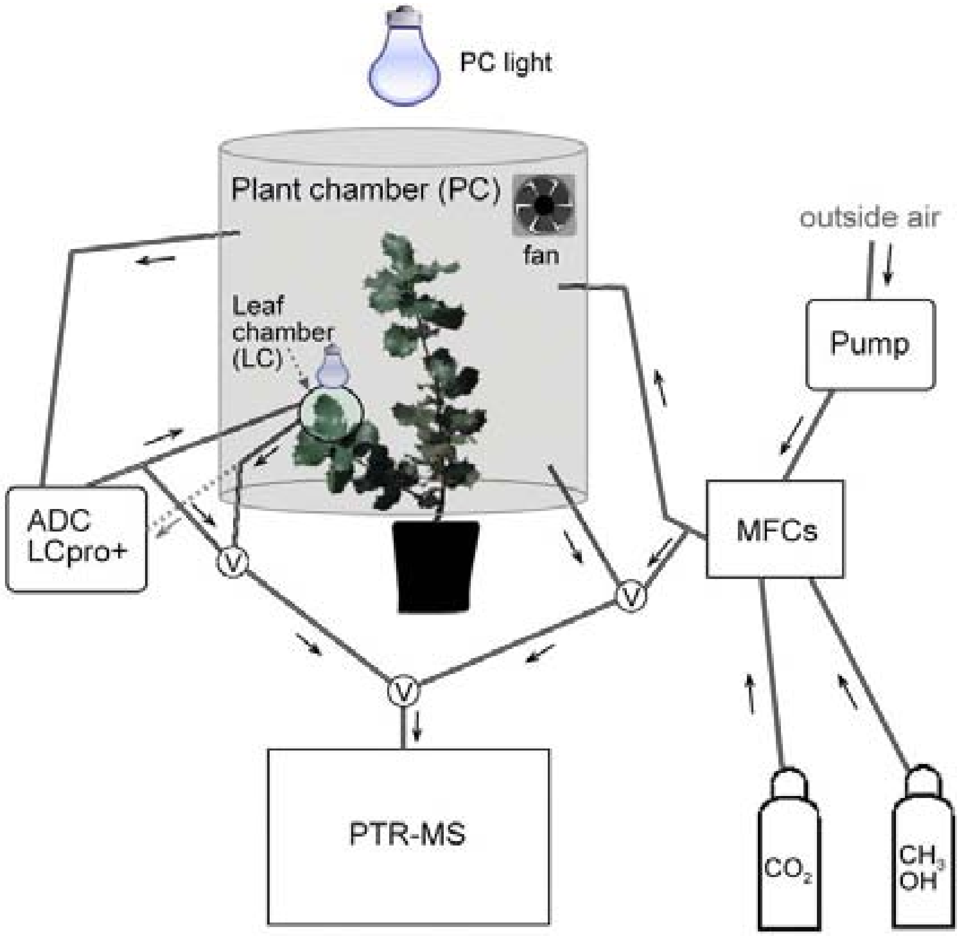


Fig. 1. Scheme showing the chamber setup for this experiment. *Quercus ilex* seedlings were inclosed in a 65L teflon chamber (Plant Chamber, PC). Outside air was flowing into the PC by means of an oil-free pump, at a flow regulated by a Mass Flow Controller (MFC). CO_2 from a bottle was used to keep concentration inside the PC at ca. 385 μmol mol^{-1}. An additional MFC regulated the flow of methanol (CH_3OH) coming from a bottle during fumigation episodes. A fan inside the PC assured that the air was well mixed. Inside the PC, a small Leaf Chamber (LC, part of an ADC LCpro+ photosynthesis system) enclosed one leaf, and controlled its illumination by means of a separate light source. It also gathered information on photosynthetic activity and, stomatal conductance. The air coming in and out from both the PC and the LC was sampled by a PTR-MS system through the use of teflon solenoid valves (V). The solid black arrows show the direction of the flow of gas inside the passivated stainless steel tubing.

- <u>Experimental design</u>

Two groups (*A* and *B*) of plants were used, corresponding to two experiments. In *A* plants (n=3) herbivory damage was simulated by clipping some leaves, leaving the petiole and a little part of the leaf still attached to the plant. *B* plants (n=3) were submitted to a methanol fumigation treatment.

This experimental setup resulted in two sampling and measuring times for *A* plants: Control1 (control before any treatment) and Cut (after cutting leaves). Each one of these measures was taken on different consecutive days. On day 1, the plant was left untouched to acclimate to the chamber conditions. On day 2, at 15h, the PC was open from the top and in vivo, non-destructive measurements of reflectance and fluorescence were taken (Control1 measurement). After these measurements, some leaves were clipped with scissors to simulate herbivory, and the cut fragments were stored in liquid N_2 as leaf samples for further processing (pigment analysis, see later). On day 3, the PC was opened again at 15h and the same measurements and leaf samples were taken, corresponding to the Cut measurement. Gas (BVOCs, H_2O and CO_2) exchange was monitored continuously during all the days, but for comparison between treatments we mostly focused on the average exchange rate from 14h to 15h, before the PC was opened (on day 2 for Control1, and day 3 for Cut). Additionally, *A* plants were further submitted to a methanol fumigation treatment the day after the clipping. The fumigation was identical to the one described for *B* plants.

B plants had also two measures: Control2 and Fumigated (fumigated with methanol). The treatment consisted of five hours of methanol fumigation, starting around 16h the day 2. On day 2, before fumigation, only non-destructive measurements were taken, and on day 3, the same measures and leaf samples were taken as for A plants.

- Fumigation with methanol

Gaseous methanol introduced into the chambers was obtained from a calibration mix (100 ppmv in N_2 from Abelló Linde S.A., Barcelona, Catalunya) and diluted into the PC inlet line by means of a mass flow controller (Environics 4000, Environics Inc., Tolland, CT, USA). Methanol fumigation lasted for five hours for each plant and the chamber methanol mixing ratio during such episodes reached values around 500-600 ppbv. This amount is in the range of what we calculated that a plant would receive, assuming that the surrounding plants would emit methanol at the 24 nmol m^{-2} s^{-1} high rates reported by Peñuelas et al. (2005a) for *Succisa pratensis* after being attacked by caterpillars of *Euphydryas aurinia*.

- Plant reflectance and fluorescence measurements

Leaf-based reflectance was measured with a UniSpec Spectral Analysis System/ Reflectometer (PP Systems, Haverhill, MA, USA) operated with a palmtop PC. Single leaves were sampled by clamping them into a leaf clip (adaxial side upwards) attached to a fibreoptic halogen light source and detector. Four scans per sample were integrated (integration time 10 ms). Reflectance measurements were preceded by a dark scan, and were compared with reflectance from a Spectralon (Labsphere Inc., North Sutton, NH, USA) white standard to obtain percent reflectance. From the reflectance data, SIPI was calculated as (R445-R800)/(R680-R800) (Peñuelas et al., 1995a; Peñuelas and Filella, 1998).

Chlorophyll fluorescence was determined using a portable modulated fluorometer PAM-2000, and a leaf clip holder 2030-B (Heinz Walz GmbH, Effeltrich, Germany). The actual photochemical efficiency of PSII in light adapted state was estimated as (F_m'-F_s):F_m' or ΔF:F_m' as defined by Genty et al. (1989), where F_s is the steady-state fluorescence yield under the given environmental conditions, and F_m' is the maximum level of fluorescence obtained during a saturating flash of light (when all the PSII traps are closed) under the same environmental conditions (Genty et al., 1989).

- Plant pigment analysis

Randomly collected cut leaves were immediately frozen in liquid nitrogen each sampling day. Samples were subsequently stored at –80 ºC until extraction. Chl a and total Cars were extracted in 80% acetone. The concentration was determined according to Lichtenthaler (1987)

- CO_2, H_2O and BVOC exchange measurements

CO_2 and H_2O exchange were measured in the LC connected to the LCpro+ Photosynthesis System (ADC BioScientific Ltd., Herts, England). For BVOC exchange determination and quantification, both the air entering and exiting the PC and LC 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 BVOCs before and after passing through the chambers, along with the flow rates, was used to calculate the BVOC exchange. The tubing used to connect PC and LC with the PTR–MS system was made of Siltek-passivated stainless steel (Restek, Bellefonte, PA, USA). In addition, the output and input air flowing from both chambers was sampled by means of glass tubes filled with terpene adsorbents, and thereafter analyzed by thermal desorption and gas chromatography–mass spectrometry.

The PTR–MS technique. PTR–MS is based on chemical ionisation, specifically non-dissociative proton transfer from H_3O^+ ions to most of the common BVOCs, and has been fully described elsewhere (Lindinger et al., 1998). In our experiment the PTR–MS drift tube was operated at 2.1 mbar and 60ºC, with a E/N (electric field/molecule number density) of around 130 Td (townsend) (1 Td = 10^{-17} V cm^2). The primary ion signal (H_3O^+) was maintained at c. 4 × 10^6 counts per second. The instrument was calibrated using an aromatic mix standard gas (TO-14A, Restek, Bellefonte, PA, USA).

Terpene sampling and analysis by GC–MS. Exhaust air of the chambers was pumped through a glass tube (8 cm long and 0.3 cm internal diameter) manually filled with terpene adsorbents Carbopack B, Carboxen 1003, and Carbopack Y (Supelco, Bellefonte, Pennsylvania) separated by plugs of quartz wool. Samples were taken using a Qmax air sampling pump (Supelco Inc., Bellefonte, PE, USA). For more details see Peñuelas et al. (2005b) The sampling time was 10 min, and the flow varied between 470 and 500 mL/min depending on the glass tube adsorbent and quartz wool packing. Glass tubes were stored at -28 ºC until the analysis.

Terpene analyses were performed by a GC-MS system (Hewlett Packard HP59822B, Palo Alto, CA, USA). The monoterpenes trapped on the tubes were processed with an automatic sample processor (Combi PAL, FOCUS-ATAS GL International BV 5500 AA Veldhoven, The Netherlands) and desorbed using an OPTIC3 injector (ATAS GL International BV 5500 AA Veldhoven, The Netherlands) into a 30m x 0.25mm x 0.25μm film thickness capillary column (SPB TM-5 Fused Silica Capillary column; Supelco Inc., Bellefonte, PE, USA). The injector temperature (60 ºC) was increased at 16 ºC s^{-1} to 300 ºC. The injected sample was cryofocused at -20 ºC for 2 min. After this time, the cryotrap was heated rapidly to 250 ºC. Helium flow was 0.7 ml min^{-1}. Total run time was 23 min and the solvent delay was 4 min. After the sample injection, the initial temperature (40 °C) was increased at 30 ºC min^{-1} up to 60 ºC, and thereafter at 10 ºC min^{-1} up to 150 ºC. This temperature was maintained for 3 min, and thereafter increased at 70 ºC min^{-1} up to 250 ºC, and maintained for another 5 min. Helium flow was 1 mL min^{-1}.

The identification of monoterpenes was conducted by comparing the retention times with standards from Fluka (Buchs, Switzerland), and the fractionation mass spectra with standards, literature spectra, and GCD Chemstation G1074A HP and the mass spectra library wiley7n. Terpene concentrations were determined from calibration curves. The calibration curves for common monoterpenes, α-pinene, Δ^3-carene, β-pinene, β-myrcene, *p*-cymene, limonene and sabinene, and common sesquiterpenes such as αhumulene were determined once every five analyses using four different terpene concentrations. The calibration curves were always highly significant ($r^2 > 0.99$ for the relationships between the signal and terpene concentration).

- Plant harvest

At the end of each individual experiment, the leaf inside the LC as well as all the leaves inside the PC were harvested, their area measured with a LI-3100 Area Meter (LI-COR, Lincoln, NE, USA), and dried at 70ºC until constant weight for dry weight calculation.

- Data treatment

Variables were measured on each individual plant before and after each treatment, and thus paired t-test were performed to minimise the effect of inter-individual variation in all the parameters. T-tests were conducted using Statistica 6.0 software (StatSoft Inc., Tulsa, OK; USA).

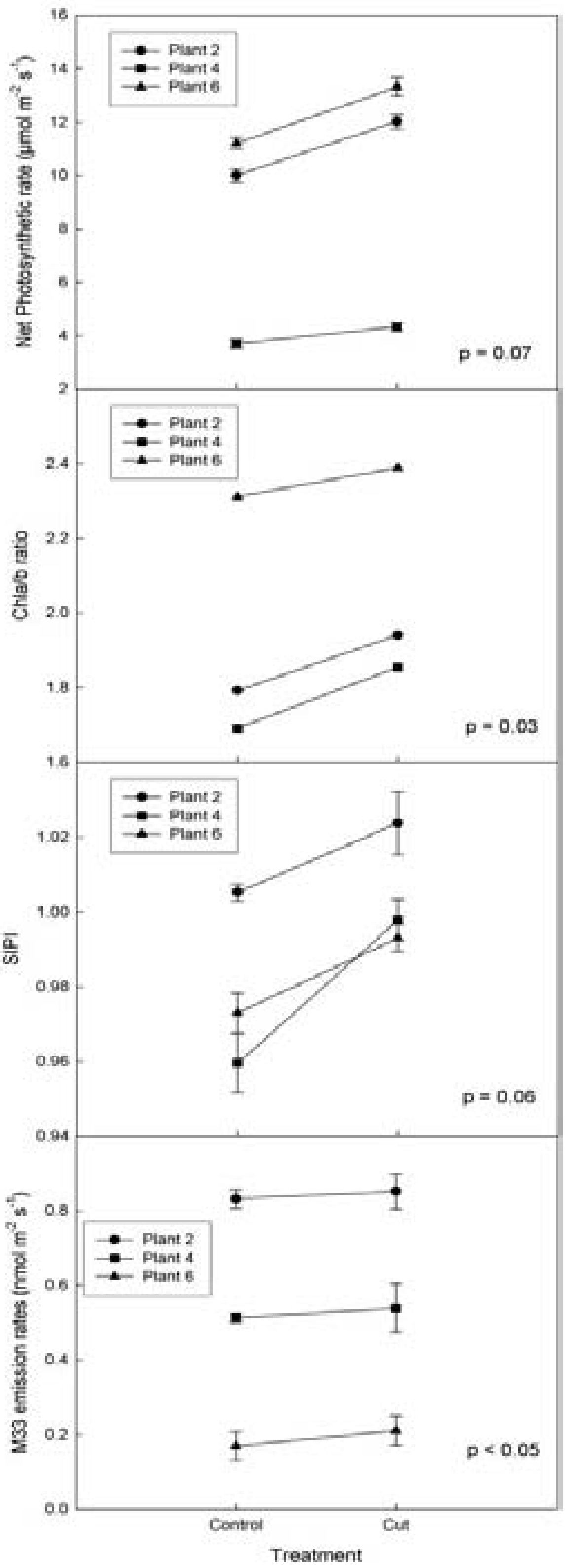
Results

- Clipping

Physiological status. The clipping (Cut) treatment increased, on average, 19% the photosynthetic assimilation rate (p=0.07), 7% the chlorophyll a to chlorophyll b (Chl a/b) ratio (p=0.03) and 3% the SIPI (index for carotenoid to chorophyll ratio, p=0.056) (Fig. 2). The different plants presented different values of these ecophysiological variables but in all cases they responded with similar increases after clipping (Fig. 2) Although not significantly, $\Delta F:F_m'$ also increased in all three tested plants (data not shown).

BVOC exchange. Plants submitted to the clipping treatment to simulate herbivory did not show detectable or different methanol emissions from the damaged leaves, maybe due to the low accuracy of the PC (plant chamber) BVOC flux measurements. However, an interesting increase in methanol emission was observed from the undamaged leaves enclosed in the LC (leaf cuvette). Control emissions averaged 0.5 nmol m^{-2} s^{-1} while Cut emissions averaged 0.53 nmol m^{-2} s^{-1} (Fig. 2), in what represents a small but significant increase in methanol emissions (paired t-test: t=4.45, p=0.046). Again, inter-individual variation was high but all plants showed an increase in emissions (Fig. 2). No other significant change in BVOC emissions was detected in Cut plants, including monoterpene emissions (data not shown).

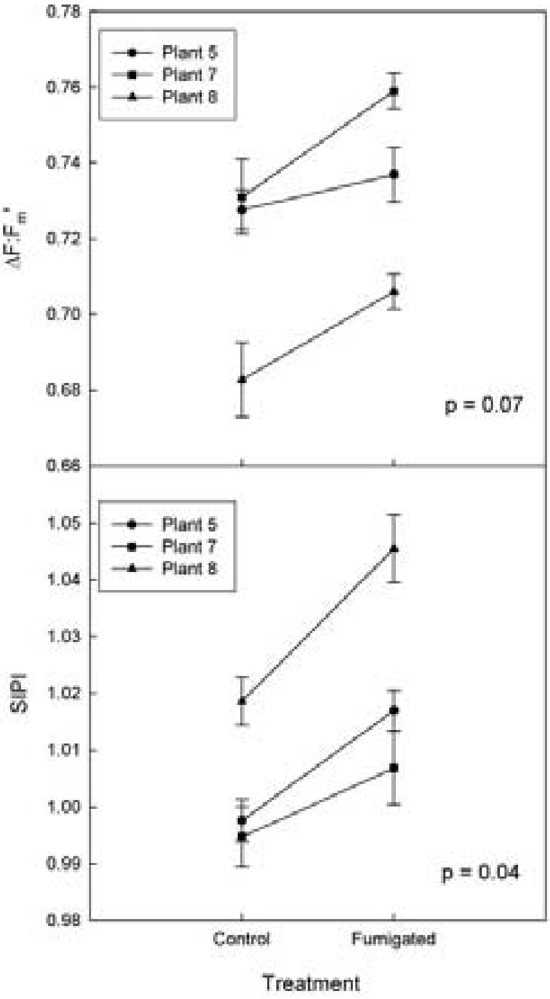
Fig. 2. Increases in the net photosynthetic rate, Chla/b ratio, SIPI (Car/Chl ratio), and M33 (methanol) emission, in response to clipping leaves. Values shown are the mean values ± standard error (n=10 measurements in the LC for photosynthetic rate; n=7 leaves from the PC for SIPI; n=4 measurements in the LC for methanol between 14:00h and 15:00h; for the Chla/b ratio, a pool of 4-5 leaves from the PC was used for the measurement). The p value is the significance level of the paired t-test for the 3 plants.



- Methanol fumigation

Physiological status. Methanol fumigation induced a mean 3% increase of the actual photochemical efficiency of PSII (p=0.07) and 2% in the SIPI (index for Car/Chl ratio, p=0.04) of fumigated plants (Fig. 3).

BVOC exchange. Uptake of methanol during fumigation episodes reached values up to 15 nmol m^{-2} s^{-1} in the LC. Methanol fumigation induced an increase in monoterpene emissions (Fig. 4), as measured by the PTR-MS signals of the protonated masses M81 and M137 which correspond to monoterpenes (de Gouw and Warneke, 2007). Fumigation of unattacked plants led to an average increase of about 1.8 nmol m^{-2} s^{-1}, from 12.2 to 14 nmol m^{-2} s^{-1} of monoterpene release (~14% more). Although monoterpene fluxes of the different plants were very different in magnitude, ranging from 0.25 to 22 nmol m^{-2} s^{-1}, all of them increased significantly (independent t-test for each plant, p<0.01). This increase in monoterpene emission after methanol fumigation in *B* –undamaged– plants was also found in *A* –previously clipped– plants when they were further submitted to an additional methanol fumigation treatment, and their monoterpene



◀ **Fig. 3**. Increases in actual photochemical efficiency of PSII ($\Delta F{:}F_m'$) and SIPI (Car/Chl ratio) after the methanol fumigation. Values shown are the mean values ± standard error (n=7 leaves from the PC for $\Delta F{:}F_m'$ and SIPI). The p value is the significance level of the paired t-test for the 3 plants.

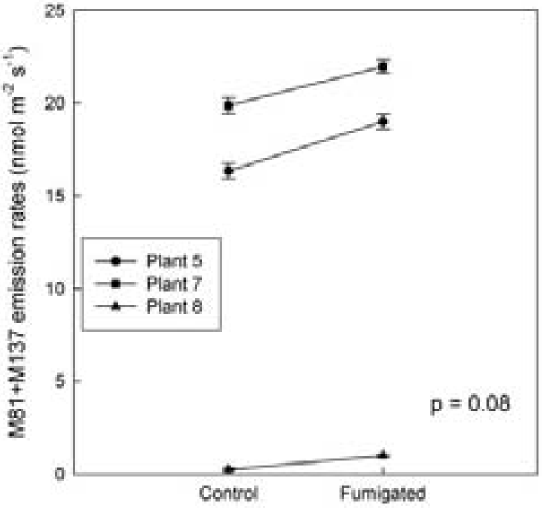


Fig. 4. Increases in the M81+M137 (monoterpenes) emisison rate in the leaf chamber (LC), after the methanol fumigation treatment. Values shown are the mean values for the 14:00h-15:00h of each day ± standard error (n=4). The p value is the significance level of the paired t-test for the 3 plants.

emission rate increased on average by 1.1 nmol m^{-2} s^{-1} (~8% increase, paired t-test p=0.02). Analysis by GC-MS revealed that the released monoterpenes were mainly composed of α-pinene and β-pinene, with minor contribution of sabinene, myrcene, and limonene. The relative contribution of each monoterpene to the sum of them was not changed substantially after fumigation.

Discussion

The clipping treatment increased the photosynthetic rates and the plant's chlorophyll a to chlorophyll b (Chl a/b) ratio, and also the SIPI (carotenoid to chorophyll ratio) (Fig.2), suggesting an activation of the physiological activity together with an activation of the plant protective metabolism (Young and Britton, 1990). These responses agree with the enhanced expression of genes related to wounding, hormones, secondary signalling messengers, and photosynthesis, reported by Arimura and colleagues (2000) for lima bean (*Phaseolus lunatus*) after herbivory. The increased methanol release after clipping agrees with previous studies showing local increased emissions after biotic and abiotic mechanical damage (Karl et al., 2001; Peñuelas et al., 2005a; von Dahl et al., 2006). But, furthermore, in this case the increased methanol emission after the clipping treatment, although only slight, was found in non-clipped leaves, indicating a systemic response of the plant to the herbivore attack, i.e. occurring in the whole plant –beyond the local emission at the wound–.These results thus could confer methanol the possibility of becoming a signal from attacked plants.

This possibility was tested with the methanol fumigation treatment. This fumigation induced an increase in the actual photochemical efficiency of PSII ($\Delta F{:}F_m'$) and the carotenoid to chlorophyll ratio (SIPI), similarly to what happened to the cut plants in the clipping treatment. This similarity suggests a possible signalling function of airborne methanol, priming the plant to be able to cope with a likely imminent attack or stress. Pointing in the same direction, the higher monoterpene release by either damaged and undamaged plants after methanol fumigation further supports that methanol could have a role in plant-plant interaction. A previous experiment that reported the effects of methanol fumigation on horticultural plants showed that the treatment inhibited the plant's photosynthetic reactions (Loreto et al., 1999). However, in that occasion the high concentration of methanol used (1% in air) was about four orders of magnitude bigger that what we used in this paper. This fact may have caused the negative effects on photosynthesis, opposite to what we report here. In fact, stimulatory effects on growth at low concentrations of methanol have been reported to become inhibitory effects as liquid methanol concentration was increased in spraying experiments with *Lemna gibba* (Dewez et al. 2003).

The increase in monoterpene emissions shown is not of a big magnitude (14%). However, it should be considered in the context of the complex mixtures of BVOCs released by plants, in which the effect of every individual BVOC could add to or complement the effects of other BVOCs. Similar increased monoterpene emissions by *Q. ilex* have also been reported as induced by other volatile signals like jasmonic acid (JA) (Filella et al., 2006) and MeSA (Peñuelas et al., 2007), with increases that were in the range of those described in this paper. Increases in terpenoid emissions induced by herbivore-induced plant volatiles (HIPV) have been reported in *Zea mays* (Ton et al., 2007). Likewise, terpenoid increased emissions have been reported also as a consequence of the concerted action of several other signalling BVOCs like JA, ethylene, and GLV (Arimura et al., 2008; Engelberth et al., 2004; Farag and Pare, 2002; Ruther and Kleier, 2005).

Monoterpenes emitted following a signalling cue (e.g. methanol or other BVOCs) have, in their turn, some possible functions in plant physiology and ecology. For example, it has been proposed that they may act as deterrents against herbivores (Raffa et al. 1985) in addition to become antioxidants (Loreto et al., 2004; Peñuelas and Llusià, 2002) or cell membrane stabilizers (Sharkey and Singsaas, 1995). Moreover, they may act as signals to parasitoids (Mumm et al., 2008; Pinto et al., 2007b), –or maybe, in the opposite sense, to attract more herbivores (Brilli et al., 2009; Halitschke et al., 2008). Hence this terpene signalling role in plant-animal communication, and also in plant-plant communication (Godard et al., 2008) forwards the message started by methanol.

The possible roles of each of these BVOCs found to be increasingly emitted in response to clipping or to methanol fumigation (methanol and monoterpenes) in the mixtures released by plants will be more clear when the roles of these and the other compounds of the BVOCs bouquet become gradually known and the modulation by biotic and abiotic factors are taken into account (Baldwin et al., 2006). Further research in this field should also address the mechanisms by which plants would distinguish between a "normal" and an "alarm" BVOC blend. Meanwhile, the results of this study show that clipping and methanol fumigation at natural concentrations elicit significant neighbour plant physiological responses and further BVOC emissions.

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3.2 The role of frass and cocoon volatiles in host location by *Monodontomerus aeneus*, a parasitoid of Megachilid solitary bees

Aquest capítol ha estat acceptat l'octubre de 2010 per a publicació a la revista Environmental Entomology.

Resum

Monodontomerus aeneus és una vespa paràsita que oviposita dins les prepupes i les pupes d'*Osmia cornuta* i d'altres espècies d'abelles solitàries. Es va utilitzar un olfactòmetre de dos braços per provar l'atracció exercida per la prepupa, el capoll i els excrements d'*O. cornuta* sobre les femelles de *M. aeneus*. Tant el capoll com els excrements van atraure les femelles del parasitoid, però els excrements sols van ser més atractius que el capoll, i el capoll junt als excrements, més atractius que els excrements sols. Les femelles de *M. aeneus* no varen ser atretes per les prepupes d'*O. cornuta*. La massa m33 (metanol) va ser el volàtil orgànic més emès pels capolls i la m61 (àcid acètic), el compost més emès pels excrements. No obstant, els capolls emetien més quantitat de la majoria dels compostos, incloent la m61 (àcid acètic). Encara que l'àcid acètic sol atreia les femelles de *M. aeneus*, probablement un senyal complex que inclou els diversos COVs està implicat en el procés d'atracció dels parasitoids, ja que la ràtio àcid acètic/acetaldehid característica dels excrements va resultar més atraient que altres ràtios.

Abstract

Monodontomerus aeneus is a parasitic wasp that oviposits on the prepupae and pupae of *Osmia cornuta* and other solitary bee species. A two-armed olfactometer was used to test the olfactory attractiveness of *O. cornuta* prepupae, coccon and larval frass to female *M. aeneus*. Both cocoon and frass attracted the female parasitoids, but frass alone was more attractive than the cocoon and the cocoon with frass more attractive than frass alone. Female parasitoids were not attracted by the host prepupa. M33 (methanol) was the organic volatile most emitted by cocoons and m61 (acetic acid) the compound most emitted by frass. However, cocoons showed higher emission for almost all compounds, including m61 (acetic acid). Although acetic acid alone attracted *M. aeneus*, a complex volatile signal is probably involved in the attraction process since the ratio acetic acid-acetaldehyde characteristic of the frass was more attractive than other ratios.

Introduction

Parasitoids locate and recognize their hosts through the use of stimuli that are highly detectable within the environment and also reliable as indicators of host presence (Vet and Dicke, 1992; Fischer et al., 2001). This includes visual and tactile cues, but, most importantly, chemical cues, which may be produced by the host itself, or by products derived from the host, and/or emitted by the host habitat (Vet et al., 1995). They are detected by olfactory receptor neurons located in antennal sensilla, involved in host location and host discrimination behaviors (Bleeker et al., 2004; de Bruyne and Baker, 2008). Several studies report on the attraction of parasitoids by the volatiles emitted by their host larvae (Chiu-Alvarado et al., 2009). Cocoon-derived semiochemicals have been identified as both contact host recognition cues (Bekkaoui and Thibout, 1993) and long range attractants (Jumean et al., 2005) for parasitoids. Frass has often been reported to provide (long-distance) cues during the host location process (Auger et al., 1990; Reddy et al., 2002; Weiss, 2006), also in parasitic wasps (Quicke, 1997; Mattiacci et al., 1999; Gandolfi et al., 2003; Chuche et al., 2006; Chiu-Alvarado et al., 2009).

The genus *Monodontomerus* (Hymenoptera; Torymidae) includes 32 described species, distributed mostly in the Holarctic (Grissell, 2000). About two thirds of the species are ectoparasitoids of solitary bees, mainly in the family Megachilidae (Grissell, 2007). Previous studies have investigated host acceptance in *Monodontomerus* (Eves, 1970; Tepedino, 1988). After antennal inspection of the host cocoon, females insert their ovipositor, through the cocoon and oviposit 10-20 eggs on the surface of the host prepupa or pupa. These studies show that the host cocoon is a key element to elicit ovipositor insertion. They also show that frequency of ovipositor insertion decreases in cocoons that have been washed with alcohol, suggesting that chemical cues play a role in host acceptance. Final acceptance, however, is dependent on cues from the host prepupa or pupa, sensed through the tip of the ovipositor (Bosch, unpublished). As far as we know, cues involved in host location have not been studied in *Monodontomerus*. Megachilid bees typically nest in small cavities in dead tress and clay embankments, and *Monodontomerus* females are sometimes seen flying in front of artificial holes in vertical structures (e.g. small holes in bricks, key-holes in doors) indicating that visual cues are involved in nest location. However, because cells containing potential hosts are usually located at some distance from the nest entrance, chemical cues are also likely to play an important role in host location. Some *Monodontomerus* species are common parasitoids of the genera *Osmia* and *Megachile* (Eves, 1970; Maeta, 1978; Bosch, 1992), which include several species used as commercial pollinators of crops in parts of Europe, North America and Eastern Asia (Richards, 1984; Bosch and Kemp, 2002). Control measures have been developed to protect commercial populations of these valuable pollinators against *Monodontomerus* (Richards, 1984; Bosch and Kemp, 2001).

In this study we address the role of volatile organic compounds (VOCs) emitted by *Osmia cornuta* on host location by females of *M. aeneus*. *Osmia cornuta* females build nests in pre-established cavities above ground (e.g. beetle burrows in dead trees, vacant bee or wasp nests). Each nest consists of a linear series of cells separated by mud partitions. In each cell, the nesting female stocks a pollen-nectar provision and lays one egg. The larva consumes the provision and then defecates and spins a cocoon (prepupal stage). Fecal pellets remain loosely attached to the cocoon. Each nest typically contains 3-5 cocoons (Bosch and Vicens, 2005). The prepupae of *O. cornuta* are surrounded by a thick multilayered cocoon (Bosch et al., 2001), which leads us to hypothesize a greater role of emissions from secondary host components (cocoon, frass) than from the host itself (prepupa). Our goal was to determine the relative attractiveness of larva, cocoon and frass emissions of *Osmia cornuta* to *M. aeneus* females, and to identify the volatiles potentially involved in this interaction.

Materials and methods

- The bee host and the parasitic wasp

To obtain *Osmia cornuta* nests we set up trap-nests in various locations near Barcelona. Trap nests consisted of solid wooden blocks with a number of drilled holes in which a paper straws (15 cm long, 8 mm inside diameter) were inserted. At the end of the *O. cornuta* nesting period, trap-nests were taken to the laboratory, and paper straws were pulled out and dissected to obtain individual cocoons. In some of the obtained cocoons, frass attached in the external part of the cocoon and the larvae inside the cocoon were collected to be tested in the olfactometer experiment.

Monodontomerus aeneus females flying about *O. cornuta* nesting sites were netted and placed in individual vials with two *O. cornuta* cocoons containing prepupae. These vials were left at 22-30 C for two days to allow females to oviposit. Parasitized cocoons were left at the same temperature to allow parasitoid immatures to complete development (20-30 days). Upon emergence, adults were left in the vials for an extra day to allow for mating. Mated females were isolated in vials and used in the bioassays described below.

- Analysis of VOCs

For VOC emission determination and quantification, both the air entering and exiting the cuvettes containing samples or blank controls (3 replicates per treatment) were analysed with a highly sensitive Proton-Transfer-Reaction Mass Spectrometer (PTR-MS-FTD hs) from Ionicon Analytik, Innsbruck, Austria. The PTR-MS system is based on chemical ionisation, specifically non-dissociative proton transfer from H_3O^+ ions to most of the common VOCs and the posterior determination of the masses of the product ions. Its use in VOC analysis has been described in detail elsewhere (Lindinger et al., 1998). The PTR-MS drift tube was operated at 2.1 mbar and 40ºC, with a drift field of 600 V cm^{-1}. The parent ion signal was maintained at around $3x10^6$ counts per second during the measurements. Flow was monitored with flow meters (Cole-Parmer, London). The detection limit of this PTR model is in the order of pptv (Hansel et al., 1998).

Concentrations of the different compounds were calculated from the PTR-MS counts of the protonated ion by using the transmission curve and calibrating with standards. The ion transmission curve was used to correct for biases associated with the transmission and detection of ions of different masses in the PTR-MS. This curve was used to "correct" all ion masses. The quantification of VOCs was based on the use of 3 times replicated calibration standards (ethylene, methanol, isoprene, α-pinene, methyl salicylate and caryophylene, from Sigma-Aldrich, Madrid, and Abelló-Linde, Barcelona, Spain). The difference between the concentrations of VOCs before and after passing through the chambers, along with the flow rates and the dry weight, were used to calculate the VOC emissions per dry weight. Samples were dried at 70ºC until constant weight for dry weight calculation.

These VOC analyses were replicated three times for each sample. We conducted scans of all masses between 22 and 205 to determine which compounds were emitted by the different samples (Peñuelas et al., 2005). In some cases different compounds can give the same ion masses. Because of that, we here have always referred to the masses first and indicated the possible compounds in parentheses.

- Olfactometer and experimental setup

We built a Y-shaped olfactometer with transparent glass (internal diameter = 0.5 cm, stem length = 5 cm, arms length = 5 cm) and Teflon tubing connectors. Samples (cocoons, frass, in amounts approximately corresponding to two cocoons) and controls (blanks) were placed in two cuvettes at the end of the olfactometer arms. A diaphragm pump (SKC Inc., Eighty Four, PA) pumped charcoal filtered air at a constant flux of 300 mL min^{-1} through Teflon tubing into the two cuvettes. In order to avoid a position effect, samples and controls were alternatively placed on the left and right sides of the olfactometer. Mated, naïve (with no ovipositing experience) *M. aeneus* females were placed at the stem entrance. Between 20 and 60 wasps were used in each test. Females were scored as responding positively to a sample or a control after they settled for at least 10 sec at the end of one of the olfactometer arms. Females that did not express a choice 5 min after entering the Y tube were discarded. Each female was used only once. To avoid females responding to possible chemical marking of the walking tracks, the glass Y tube was changed after each test. Used Y tubes were washed with soap, rinsed in purified water and dried in an oven at 60ºC. To avoid contact of the female with the samples or controls, the distal ends of the olfactometer arms were covered with a piece of cotton gauze, which was discarded after each test. All experiments were conducted in a laboratory with homogeneous light and environment. Light (ca. 300 mmol m^{-2} s^{-1} PAR) was supplied by fluorescent lights (Agrolite CF). Temperature was 25ºC and relative humidity was ca 70%.

- Bioassay with VOCs

After the identification of emitted VOCs and the olfactometer test, given the attractiveness of frass and since acetic acid was the main compound emitted by frass and the second-most emitted compound by the cocoon, we tested the attractiveness of acetic acid in another bioassay conducted with the olfactometer. The dose of acetic acid tested was in the order of the amounts measured in frass and cocoon olfactometer tubes (ca. 2 ppbv). The same bioassay was also conducted with other compounds not emitted by cocoon, prepupape or frass (linalool, cineol, α-pinene, β-pinene, limonene, α-myrcene), i.e. each one of these other compounds versus a blank (no odor), to test for the possibility that the results obtained for acetic acid were the response to odor versus no odor at all.

To test the importance of the ratio among compounds in the host location process, we measured the attractiveness of air with acetic acid (the main emitted compound in frass and cocoon) and acetaldehyde in a 2:1 ratio (the ratio measured in frass) against air with acetic acid in the same concentration but in a different ratio with acetaldehyde (1:1). We chose acetaldehyde as a reference because this compound presented the most contrasting ratio with acetic acid between frass and cocoons. The doses of acetic acid and acetaldehyde tested were in the order of the amounts measured in frass and cocoon olfactometer tubes (ca. 2 ppbv). Flow was monitored with flow meters (Cole-Parmer, London). We used mass flow controllers (Environics 4000, Environics Inc., Tolland, CT, USA) to mix clean air (obtained by flushing air through a catalytic converter consisting in a steel tube filled with platinum pellets that was heated at 350 ºC by a heating tape) with acetaldehyde and acetic acid gas standards (Abelló Linde S.A., Barcelona, Spain) whose concentrations were controlled with a needle valve. The mixed air was measured with the PTR and the acetaldehyde and acetic acid concentrations were adjusted until we obtained the wanted ratio of concentrations. The final concentrations applied in the test were confirmed with PTR-MS measurements.

- Statistical analysis

A χ^2-test was used to analyze choice frequencies in the olfactometer bioassays. We used one way ANOVA and the post-hoc Tukey test to compare the emissions from frass and cocoons.

Results

- Attractiveness of the different host components

Female *M. aeneus* were not attracted to *O. cornuta* prepupae, but were significantly attracted to cocoons and frass (Fig. 1). When given the choice between cocoon and frass, they were much more attracted by the odor of frass than by the odor of cocoons. However, they preferred cocoons with frass over frass alone (Fig. 1).

- Volatile emissions

The VOC spectra of cocoons, frass and prepupae are shown in Fig 2. The main compound emitted by the cocoons was m33 (methanol), which accounted for more than a half of total cocoon emissions. Conversely, methanol was hardly emitted by the frass. Cocoons also emitted m29 (ethylene), m31 (formaldehyde) and m47 (formic acid,

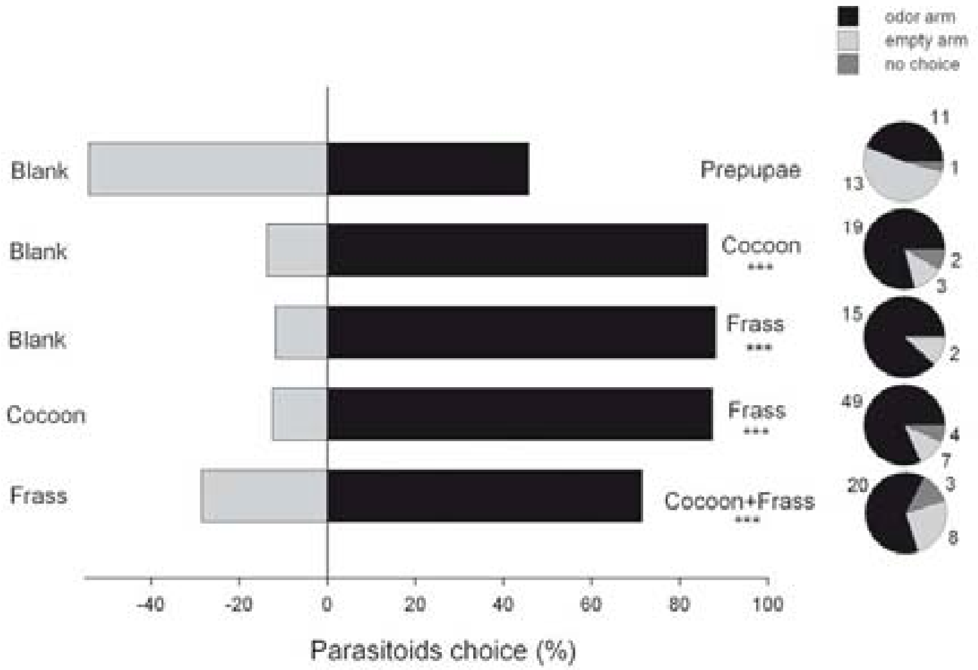

Fig. 1.Outcome of olfactometer tests in which *Monodontomerus aeneus* females were offered a choice between different host components or a host component and a blank. (*** $p<0.001$; χ^2; sample sizes indicated in the pie diagram).

ethanol) among others (Fig. 2). Frass mainly emitted m61 (acetic acid, propanol, 2-propanol,…), but also m45 (acetaldehyde), m31 (formaldehyde) and m47 (formic acid, ethanol) among others. There are strong reasons to interpret m61 as acetic acid: acetic acid has been found to be the most abundant acid in frass of diverse insects (McFarlane and Alli 1985). M61 (acetic acid) was the main compound emitted by the frass and the only compound whose emission percentage was significantly higher in the frass than in the cocoon (Fig. 2). Prepupae emitted m47 (formic acid, ethanol), m45 (acetaldehyde), and did not emit m61 (acetic acid) (Fig. 2).

Given the attractiveness of frass and since acetic acid was the main compound emitted by frass and the second-most emitted compound by the cocoon, we tested the attractiveness of acetic acid. Female *M. aeneus* were significantly attracted by acetic acid (Fig. 3). These females were not attracted by any of the other tested volatiles (linalool, cineol, α-pinene, β-pinene, limonene, α-myrcene) (data not shown).

The ratio between acetic acid and acetaldehyde emissions was markedly different between frass (2:1) and cocoons (19:1) (Fig. 2). We hypothesized that this ratio could be involved in the host location process. Since frass was more attractive than the cocoon we tested the attractiveness of air with acetic acid and acetaldehyde in a 2:1 ratio against air with acetic acid in the same concentration but in a 1:1 ratio with acetaldehyde. Even with a low number of replicates female *M. aeneus* showed a preference for the 2:1 blend (Fig. 3).

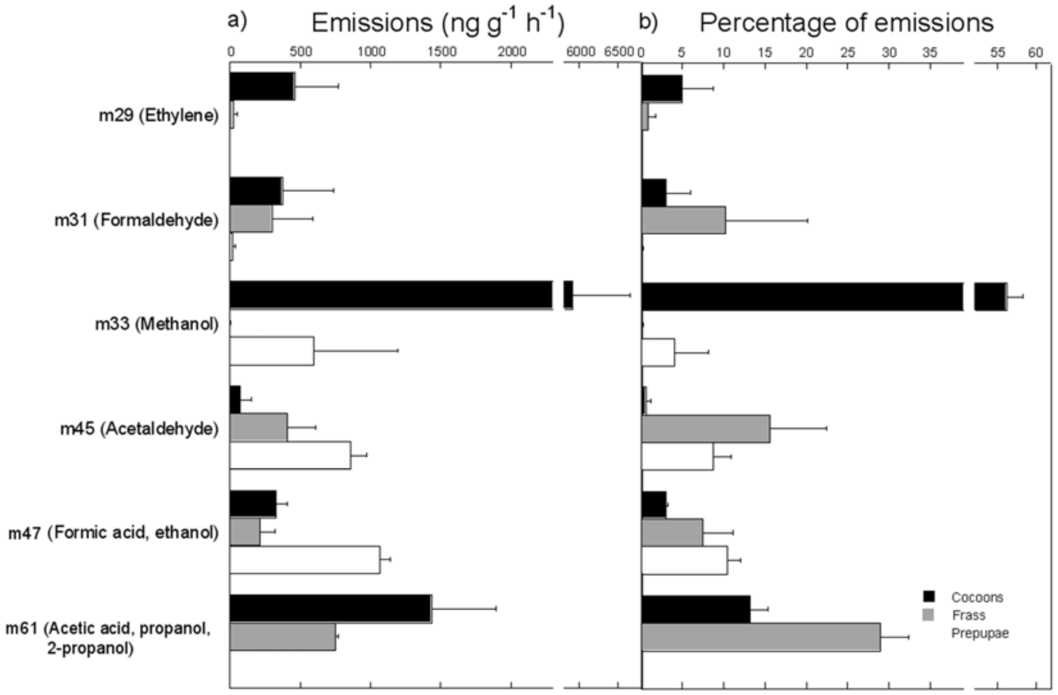

Fig. 2. Volatile organic compounds emitted by *Osmia cornuta* cocoons, frass and prepupae in a) absolute values, and b) percentage of emissions. The vertical axis shows the protonated molecular mass and the name of the most-probable assigned compound. Error bars indicate SE (n=3).

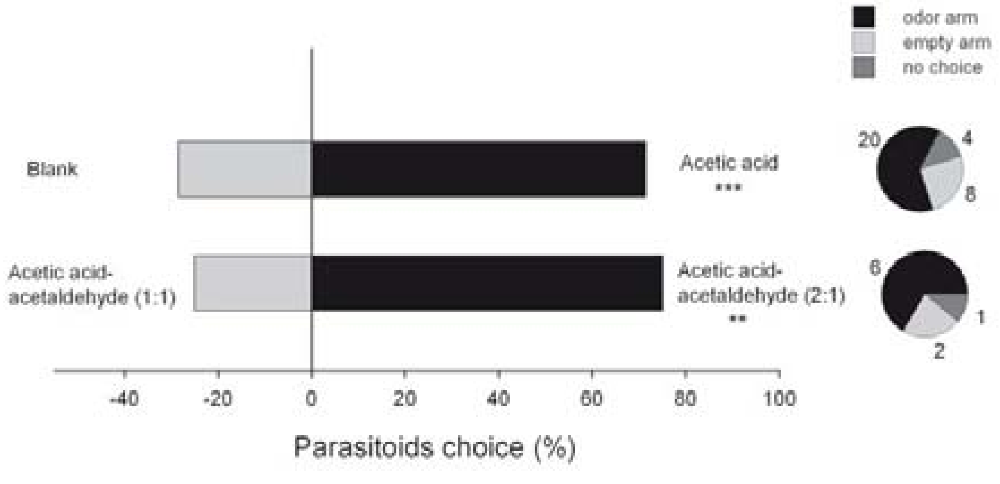

Fig. 3. Outcome of olfactometer tests in which *Monodontomerus aeneus* females were offered a choice between acetic acid and a blank, or different mixtures of acetic acid and acetaldehyde. (** $p<0.01$, *** $p<0.001$; χ^2, sample sizes indicated in the pie diagram).

Discussion

Our experiment demonstrates for the first time that *M. aeneus* females use volatiles emitted by frass and, to a lesser extent, the cocoon, to locate *O. cornuta* hosts, disregarding volatiles emitted by the host prepupa. Acetic acid, one of the compounds most emitted by the frass, appears to play a key role in host location. These results coincide with findings on other parasitoids that also use cues from waste materials to locate their hosts (Weiss, 2006).

M. aeneus females were not attracted to *O. cornuta* prepupae which is congruent with the finding that females *Mondontomerus* do not readily recognize naked prepupae as suitable hosts (Eves, 1970; Tepedino, 1988). This lack of attraction has also been reported in other cases (Chiu-Alvarado et al., 2009). Cueing on cocoon rather than pre-pupae volatiles has several advantages. First, the cocooned pre-pupa is less mobile than the feeding larva and therefore it is easier to paralize prior to oviposition. Second, the pre-pupa has acquired its maximum size and therefore can support a larger number of parasitoid offspring and/or offspring of larger size than the feeding larva. Third, the cocoon provides a protected environment for the parasitoid's offspring. When the larva is covered by the cocoon, the odor from cocoon and frass may be more easily perceived and, therefore, perform better as a host signal. Cocoon-derived VOCs have been measured in the cocoons of the codling moth *Cydia pomonella* L. (Lepidoptera:Tortricidae) that attract its parasitoid *Mastrus ridibundus* (Hymenoptera:Ichneumonidae). They included mainly monoterpenes, aldehydes and ketones (Jumean et al., 2005). Previous studies have also identified frass emissions of other species that are attractive to parasitic wasps (Auger et al., 1989; references in Quicke, 1997). In fact, parasitoids have been found to show a stronger response to cues produced by frass as a product of the host feeding activity than by the host itself (Rogers and Potter, 2002).

In this study we have found that m33 (methanol) was the most emitted compound in cocoons, and m61 (acetic acid) the most emitted compound in frass. As far as we know, acetic acid emissions from frass have not been previously considered in terms of chemical communication. Acetic acid is the most common acid produced in microbial fermentation, and has been found to be the most abundant acid in frass of diverse insects (McFarlane and Alli, 1985). Regarding its role in attraction, acetic acid has been shown to be attractive to some species of social wasps (Landolt et al., 1999; Landolt et al., 2005). Generalist parasitoids are expected to cue on non-specific cues (Vet and

Dicke, 1992; reformulated by Steidle and Van Loon, 2003). *Monodontomerus aeneus* has been reared from nests of 22 solitary bee species belonging to seven genera, including two genera of non-Megachilids (Bosch et al., 1993; Vicens et al., 1993; Grissell, 2007). Some of these hosts (*Osmia*) spin thick silk cocoons, while others (*Hoplitis, Megachile*) spin much thinner, translucent cocoons (Bosch et al., 2001). In some cases the cocoon is surrounded by plant material used by the nesting female to line the cell (leaf cuts in *Megachile* spp., cottony down of plant pubescence in *Anthidium florentinum*). Thus emissions from cocoons and cocoon-associated materials are expected to vary widely across the range of suitable hosts. Instead, frass emissions are a necessary by-product of any suitable host and therefore are much more reliable as a host location cue for a species with such a degree of generalization. Because acetic acid is found in the frass of many insects, *M. aeneus* is likely to cue on a complex volatile signal involving emissions more specific to solitary bee hosts, as supported by our finding fact that cocoon with frass is preferred over frass alone. The observation of female *Monodontomerus* inspecting artificial holes not housing bee nests in vertical structures (Bosch, unpublished), suggests that visual cues interact with olfactory cues in the host location process. We expect that females will enter those cavities having the visual appearance of a Megachilid bee nest and emitting a mixture of volatiles including acetic acid and other frass/cocoon compounds.

We conducted an additional test where we reproduced the relative concentrations of two volatiles likely involved in the attraction process, acetic acid and acetaldehyde, and the test corroborated our hypothesis. It revealed a preference of the parasitoids for the ratio 2:1. Most studies indicate that the recognition of suitable host by olfactory signals is dependent on species-specific ratios of ubiquitous compounds (Bruce et al., 2005).

This work is a first step towards understanding the host-finding behaviour of *M. aeneus*. Future studies are warranted to compare female responses to the different volatile emitted by frass and cocoon, e.g. to methanol, highly emitted by cocoon, and to different volatile combinations, both in the lab and in the field. The ability of *M. aeneus* to parasitize a relatively wide range of hosts differing in cocoon structure and in the nature of materials covering the cocoon affords an opportunity to study the potential role of associative learning of olfactory cues in host location (Turlings et al., 1993).

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Capítol 4

Impactes atmosfèrics dels COVs

Winter and summer VOC mixing ratios at a forest site in the Western Mediterranean Basin

Resum

Els compostos orgànics volàtils (COVs) atmosfèrics estan implicats en la generació d'ozó i aerosols, per això tenen afectacions climàtiques. Els COVs i les seves emissions per la vegetació també tenen funcions ecològiques importants ja que poden protegir a les plantes dels estressos i actuar com senyals de comunicació entre plantes i entre plantes i animals. Tot i aquest paper clau, tant ambiental com biològic, a la literatura les descripcions de les concentracions diàries i estacionals de COVs en ambients naturals no són abundants, i són encara més poques les que tracten sobre àrees mediterrànies.

Vàrem mesurar les concentracions estacionals (hivern i estiu) en un bosc mediterrani elevat (720 m.s.n.m.) situat prop de l'àrea metropolitana de Barcelona (NE de la península Ibèrica). El metanol va ser el compost més abundant d'entre tots els COVs mesurats. Mentre que els COVs aromàtics no varen presentar gairebé cap variació estacional, les concentracions dels COVs oxigenats de cadena curta van assolir nivells més alts a l'estiu degut a les majors emissions de la vegetació i a l'increment de les reaccions fotoquímiques resultants de les altes temperatures i radiació solar de l'estiu. Els COVs isoprenoids van mostrar el canvi entre estacions més gran: les concentracions van augmentar un ordre de magnitud a l'estiu, degut a l'increment de l'activitat fisiològica de la vegetació i de les seves taxes d'emissió. També les concentracions diürnes màximes d'ozó van augmentar a l'estiu, per la major activitat fotoquímica i la presència de més COVs a l'aire.

La variació diària de concentracions de COVs seguia bàsicament el règim de vent de la muntanya, ja que la majoria dels COVs analitzats van seguir un cicle diari molt semblant. La brisa marina que es desenvolupa quan surt el sol transporta cap a la muntanya masses d'aire que prèviament han passat sobre la regió metropolitana de Barcelona, on han estat enriquides en NO_2 i en COVs d'origen biòtic i abiòtic. A més, aquestes masses d'aire contaminades poden reaccionar amb els COVs biogènics emesos de forma local per la vegetació, tot afavorint la formació d'O_3 en aquesta zona boscosa. L'únic tipus de COV que presentava un cicle diari diferent dels demés van ser els monoterpens, perquè tenien un origen biogènic local. L'isoprè també compartia parcialment el cicle diari dels monoterpens, però només a l'estiu quan les seves fonts biòtiques eren més fortes.

Abstract

Atmospheric volatile organic compounds (VOCs) are involved in ozone and aerosol generation, thus having air quality and climatic implications. VOCs and their emissions by vegetation have also important ecological roles as they can protect plants from stresses and act as communication cues between plants and between plants and animals. In spite of this key environmental and biological role, the reports on seasonal and daily VOC mixing ratios in the literature for natural environments are not abundant and are even more scarce for Mediterranean areas.

We conducted seasonal (winter and summer) measurements of the VOC mixing ratios in an elevated (720 m.a.s.l.) Mediterranean forest site near the metropolitan area of Barcelona (NE Iberian peninsula). Methanol was the most abundant compound among all the measured VOCs in both seasons. While aromatic VOCs showed almost no seasonal variability, short-chain oxygenated VOCs presented higher mixing ratios in summer, presumably due to the increased emission by vegetation and the increased photochemistry, both enhanced by the high temperatures and solar radiation in summer. Isoprenoid VOCs showed the biggest seasonal change in mixing ratios: they increased by one order of magnitude in summer, as a result of the increased vegetation physiological activity and emission rates. Ozone highest diurnal concentrations increased in summer too, due to more photochemical activity and the presence of more VOCs in the air.

The daily variation of the VOC mixing ratios was mainly governed by the wind regime of the mountain, as most part of the VOC species analysed followed a very similar daily cycle. Sea breeze that develops after sunrise advects to the mountain polluted air masses that previously had passed over the Barcelona metropolitan region, where they were enriched in NO_2 and in VOCs of biotic and abiotic origin. Furthermore, these polluted air masses can react with biogenic VOCs emitted at the local valley by the vegetation, thus enhancing O_3 formation in this forested site. The only VOC species that showed a somewhat different daily pattern were monoterpenes because of their local biogenic emission. Isoprene also shared partially the daily pattern of monoterpenes, but only in summer when its biotic sources were stronger.

Introduction

Volatile organic compounds (VOCs) present in the atmosphere have both natural and anthropic sources. Among the natural ones, emission by vegetation is regarded as the greatest source (Guenther et al., 2006). VOCs play several roles in atmospheric chemistry that increase the interest in VOC study, for example their implication in tropospheric ozone and aerosol generation. VOCs, together with nitrogen oxides (NO_x) and solar radiation are the ingredients needed to promote the photochemical formation of tropospheric ozone (Sillman, 1999; Atkinson, 2000). Ozone is an oxidant pollutant that has negative effects on plants (Fowler et al., 2009), poses a threat to human health (Lippmann, 1993) and acts as a greenhouse gas. VOCs are also precursors of atmospheric aerosols (Kavouras et al., 1998; Tunved et al., 2006), which can have important implications for planetary climate (Kulmala et al., 2004; Pacifico et al., 2009). In a context of global change, the relationships between VOCs, particles and ozone are a hot topic in current scientific research (Peñuelas and Staudt, 2010) because they all are involved in biogeochemical cycles and take part with other global change drivers in complex feedbacks with the climate (Arneth et al., 2010). Atmospheric VOCs and their emissions by vegetation are also ecologically important because VOCs can protect plants against high temperatures (Singsaas and Sharkey, 1998; Peñuelas et al., 2005), high irradiance (Peñuelas and Munné-Bosch, 2005), and oxidative stress (Peñuelas and Llusià, 2002; Velikova et al., 2005). In addition, they also act as plant-plant communication signals (Peñuelas et al., 1995; Pierik et al., 2003; Baldwin et al., 2006; Heil and Kost, 2006; Kegge and Pierik, 2010). Likewise, VOCs play a role in plant-animal relationships, not only involving plant-pollinator relationships (Wright and Schiestl, 2009) or direct defense against herbivores, but also indirect defense through the attraction of the natural enemies of the herbivores (Kessler and Baldwin, 2001; Llusià and Peñuelas, 2001; Pichersky and Gershenzon, 2002).

In the Mediterranean area, where potential for biogenic VOC emission and photochemistry is high, the ozone and particle formation can be elevated and the ecological role can be very significant. However, there is scarce information on the tropospheric VOC mixing ratios in the Mediterranean region. Ozone has been measured in high concentrations in the Mediterranean area (Gimeno et al., 1995; Ziomas, 1998; Ribas and Peñuelas, 2004), and has already been reported to produce damage to plants in the Western Mediterranean Basin (Sanz et al., 2000; Ribas et al., 2005). Moreover, ozone concentrations have been found to increase with altitude (Ribas and Peñuelas, 2006; Diaz-de-Quijano et al., 2009; Evtyugina et al., 2009).

In the last years, proton transfer reaction mass spectrometry (PTR-MS) technology has enabled the simultaneous measuring of several VOCs, including some oxigenated VOCs that were difficult to measure with previous techniques (*capítol 1*, Seco et al., 2007). Also, PTR-MS has improved the temporal resolution of these measurements, to the point of making possible the use of micrometeorological techniques (Karl et al., 2001). Recent papers reporting together several surface-level VOC mixing ratios in natural areas taking advantage of the PTR-MS technology are few (e.g. Spirig et al., 2005; Jordan et al., 2009b; Lappalainen et al., 2009; Ruuskanen et al., 2009; Holst et al., 2010), and there are even less dealing with Mediterranean ecosystems (Davison et al., 2009).

In this paper we report VOC data gathered during 2009 in an elevated site highly representative of the montane Mediterranean forest (Terradas, 1999), located in a forested mountain slope, in the Western Mediterranean Basin. We compared the VOC mixing ratios between winter (February-March) and summer (July-August), and analysed their relationship with meteorological data, and with ozone (O_3) and nitrogen dioxide (NO_2) concentrations. We also compared the VOC mixing ratio values and daily trends at this Montseny (MSY) site with those reported in the existing literature for other natural areas worldwide.

Methods

- Measurement site

The Montseny site (MSY) was located within a densely forested natural park about 60 km to the NNE of the city of Barcelona (Catalonia, NE of the Iberian peninsula) and 25 km from the Mediterranean coast (41°46'45.63"N 02°21'28.92"E, 720 m a.s.l.; Fig. 1). The site is highly representative of the montane holm oak (*Quercus ilex* L.) forests in the Mediterranean regions of France, Italy, Greece and eastern Spain (Terradas, 1999). At Montseny, these are dense forests of resprout origin, which evolved from coppicing until the 1950s and selection thinning afterwards (Avila and Rodrigo, 2004).

To our knowledge, this was the first time that VOC mixing ratios were measured continuously for some days at this Mediterranean forest location, which has a long record of other ecological and biogeochemical parameters such as nutrient cycling (Avila et al., 2002), dry and wet deposition (Avila et al., 1998, 2007, 2010; Avila and

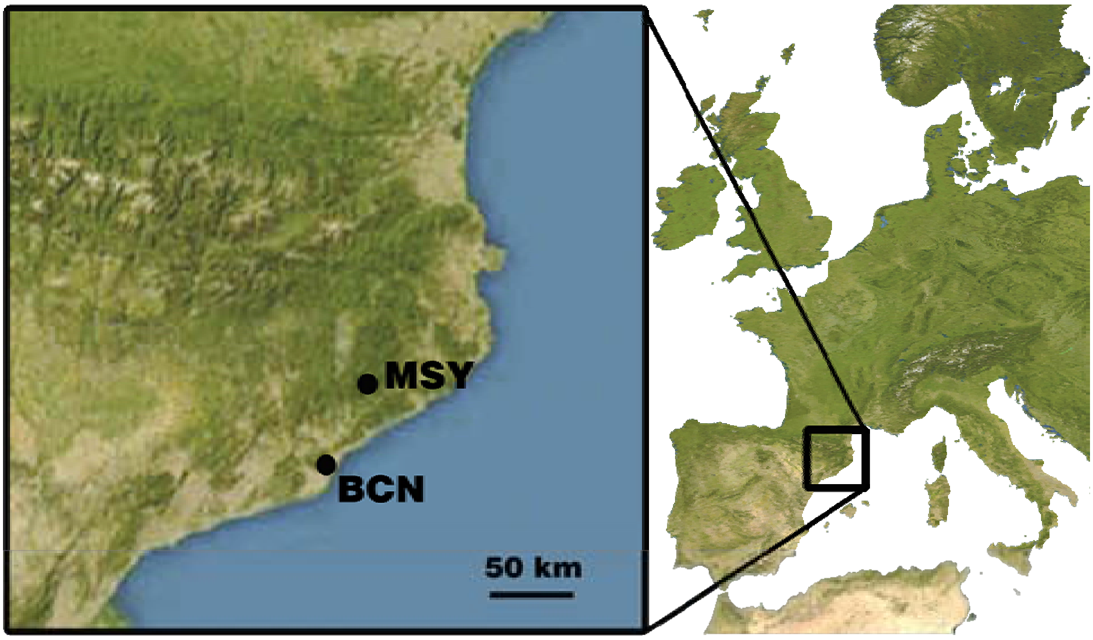

Fig 1. Location of the Montseny (MSY) measurement site, in relation to the city of Barcelona (BCN).

Rodà, 2002; Avila and Alarcon, 2003), hydrology (Piñol et al., 1992), and particulate matter (Perez et al., 2008; Pey et al., 2009), among others.

- <u>VOC measurements</u>

For VOC monitoring during the winter measurements, a newly developed Proton Transfer Reaction-Time of Flight-Mass Spectrometer (PTR-ToF-MS, see Jordan et al., 2009a) was deployed. It combined a Time-of-Flight MS detector (Tofwerk AG, Switzerland) with the characteristic chemical ionisation system that defines PTR systems. The PTR-ToF-MS drift tube was operated at 2.3 mbar, 600 V, and 50 ºC which corresponds to a E/N ratio of about 130 Td (E being the electric field strength and N the gas number density;1 Td = 10^{-17} V cm^2). During the summer measurements, the site was equipped with a High Sensitivity Proton Transfer Reaction Quadrupole Mass Spectrometer (PTR-Quad-MS, described by Lindinger et al., 1998). This instrument was operated at 2.3 mbar, 580V, and 50 ºC drift tube conditions, which corresponds to a E/N around 122 Td. System background for both instruments was measured every couple of hours by sampling zero air made by passing air through a catalytic converter, and instrument sensitivity was calculated by frequent calibrations with gas standards. Both set-ups sampled through a heated inlet line made either of deactivated Sulfinert or teflon tube.

These PTR systems have differences in the mass resolution of the analyzed VOCs, since the ToF MS is capable of separating nominally isobaric compounds that the PTR-Quad-MS detects at one single mass. Such differences are clear in Table 1, where the measured masses and the VOC identity assigned to each mass are displayed. In the case of the ToF, each VOC has a unique exact mass, while in the case of the Quadrupole the identification of VOCs can suffer from interferences, although it was based on calibrations, experience, and well-known and almost inteference-free VOC-mass correspondences (de Gouw and Warneke, 2007). For example, we report measurements of protonated mass 47 in summer and attribute them to formic acid/ethanol (Table 1), because the PTR-Quad-MS is not able to separate these two compounds. In the winter measurements, the PTR-ToF-MS could have identified each of them separately, and only detected ethanol (Table 1). In the case of terpenes, neither PTR-MS system could separate the different individual molecules. Thus, additionally, samples were taken for terpene determination via gas chromatography-mass spectrometry (GC-MS). By means of a pump, ambient air was drawn through a cartridge filled with terpene adsorbents, using a teflon line to sample from the same inlet point as the PTR-MS systems. The cartridges were then analysed in the laboratory as described in Llusià et al. (2010).

For clarity, we have divided the measured VOCs into three groups according to their structure and characteristics, as in previous literature reports (e.g. Jordan et al., 2009b). One group is composed by short-chain oxygenated compounds (oxVOCs): methanol,

Table 1. Masses measured with the PTR-MS systems and the corresponding VOC identity assigned to them.

Assigned VOC identity	PTR-Quad-MS measured nominal protonated mass	PTR-ToF-MS measured exact protonated mass
Methanol	33	33.033
Ethanol	-	47.048
Ethanol/formic acid	47	-
Acetone	59	59.049
Acetaldehyde	45	45.033
Acetic acid	61	61.028
Isoprene	69	69.069
MVK/MACR	71	71.09
Monoterpenes	81 and 137	137.132
Benzene	79	79.054
Toluene	93	93.069
C-8 aromatics	107	107.085
Acetonitrile	42	42.033

acetone, acetaldehyde, acetic acid, and formic acid/ethanol (see *capítol 1*, Seco et al., 2007 for a review). The second group is formed by the isoprenoids: isoprene and monoterpenes, and we additionally included here the compounds methyl vinyl ketone (MVK) and methacrolein (MACR), which are oxidation products of isoprene. The third group is composed by the aromatic volatiles: benzene, toluene, and C8-aromatics (term that includes compounds like xylenes). Finally, we also analysed acetonitrile.

- Meteorology and gas measurements

During both periods, meteorological data such as temperature, relative humidity, wind direction and speed, precipitation, and solar radiation was gathered by the CSIC team from a meteorological tower, at 10 m height (Pandolfi et al., in preparation).

Real time measurements of O_3 and NO_2 concentrations were provided by conventional gas phase air pollution monitors, maintained by the Department of the Environment of the catalan government (Generalitat de Catalunya).

- Statistics and data analysis

VOC mixing ratios calculations from the PTR-MS data, together with the treatment and graphication of the data time series, were done using Igor Pro (Wavemetrics Inc., Portland, OR, USA). The correlation coefficients between all the available data variables (VOC mixing ratios, wind, temperature, solar radiation, and NO_2 and O_3) were calculated, in order to conduct a preliminary screening of possible relationships. For this purpose the Statistica (StatSoft Inc., Tulsa, USA) package was used.

In addition, wavelet analysis was applied to analyse the relationship between VOC mixing ratios and ozone concentrations in summer. Wavelet analysis is best suited for studying transient signals (oscillatory or non-oscillatory), or signals whose amplitude vary in time and is widely used in the field of general signal processing, in cases where the signal is localized both in time and frequency (Torrence and Compo, 1998). Since we are interested in the relationship between transient signals that may be present in two different time series, we have calculated the crosswavelet transform and wavelet coherence pairwise to the dataset (for an introduction to this topic, see Grinsted et al., 2004). The wavelet coherence can be thought of as a "localized correlation coefficient in time frequency space" (Grinsted et al., 2004). The calculations have been performed in MATLAB R2009b (MathWorks, Natick, MA, USA) with the algorithms provided by these authors.

Results

- Meteorological conditions and atmospheric dynamics

The MSY site presents a typical Mediterranean climate with warm summers, temperate winters and irregular precipitation rates (Perez et al., 2008). In fact, precipitation was practically inexistent during the periods reported in this paper. Average maximum solar radiation reached around 600 W m^{-2} in winter and 800 W m^{-2} in summer (Fig. 2), and consequently the average temperatures differed 11-15 ºC between the two periods (7-13 ºC and 19-27 ºC daily averages in winter and summer, respectively), while relative humidity showed almost identical daily profiles (Fig. 2).

Air circulation in the MSY site is strongly determined by the topography of the valley, and two main wind directions are usually recorded (Fig. 3). The southerly direction is present when the mountain and sea breezes develop during daytime, entering the valley from the south, and when usually the highest wind speeds are recorded. The westerly direction corresponds to the intense advections from the north and north-west, channelled into the valley with a western direction (as was the case during the period 5-7 march), and to drainage flows during the night (Perez et al., 2008). The highest wind speeds for winter were measured during the Atlantic advection scenario (days 5-7 march) with values up to 10 m s^{-1}. The wind speeds for summer had some problems with the recording software and so the magnitudes displayed seem to be lower than real wind speeds of those days (Fig. 3).

- O_3 and NO_2 concentrations

Ozone concentrations showed similar values during the night and early morning both in winter and summer (average around 75 µg m^{-3}), and about a couple of hours after sunrise they increased up to an average of 128 µg m^{-3} (summer, around 14h GMT) or 95 µg m^{-3} (in winter, peaks around 11h and 15h GMT)(Fig. 4). NO_2 concentrations showed a similar daily profile, with very similar summer and winter values during night and morning (average around 2 µg m^{-3}), and then an increase -delayed around 2 hours with respect to the rise of O_3- reaching a first peak at 13h and a second peak around 17h (winter) or 19h (summer). Maximum average values were 11.6 µg m^{-3} for winter and 7.5 µg m^{-3} for summer (Fig. 4).

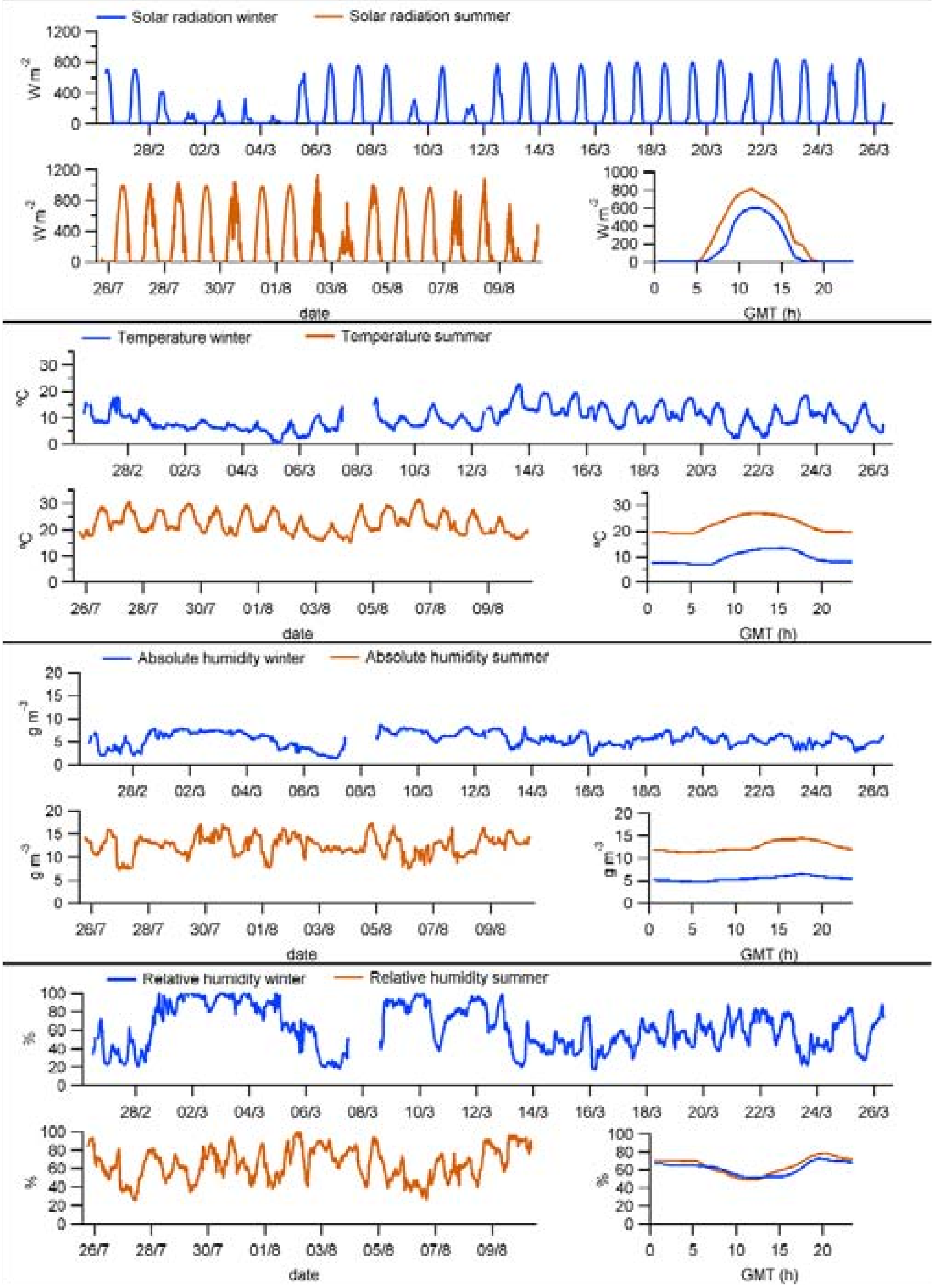

Fig. 2. Solar radiation, temperature, and absolute and relative humidity during the winter period (upper graph of each panel), during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Date labels indicate 0:00 GMT.

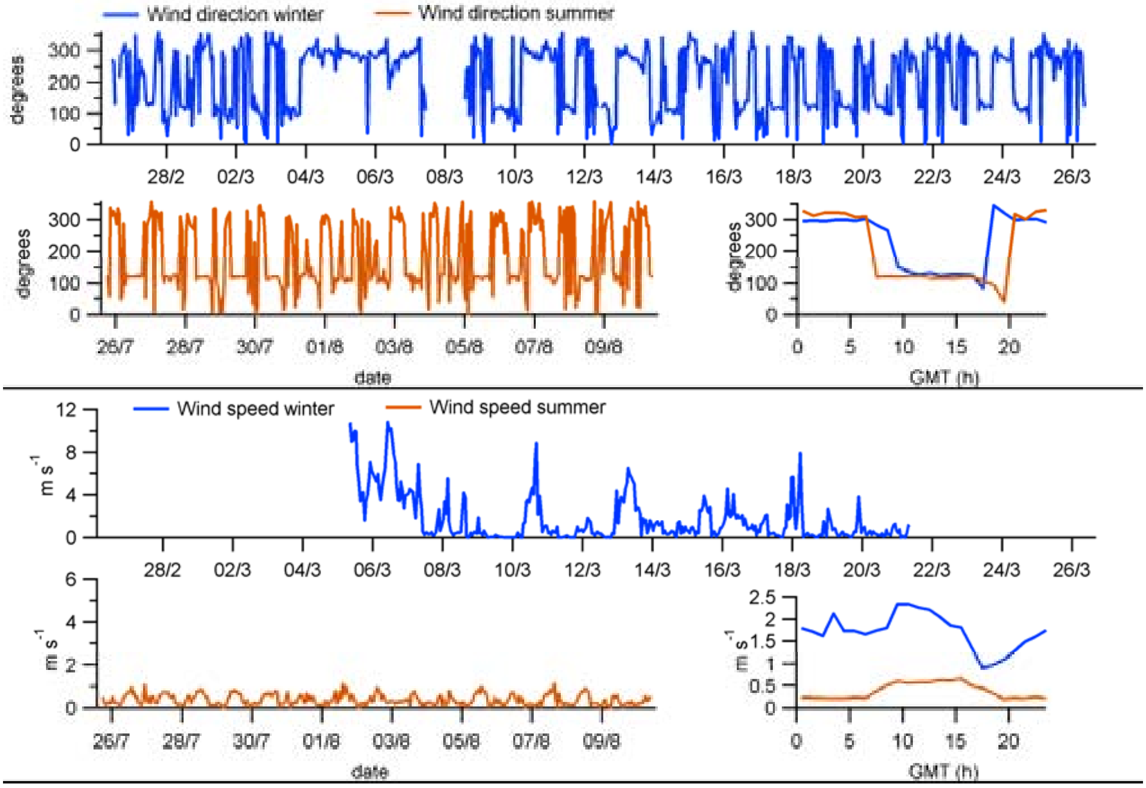

Fig. 3. Wind direction and speed during the winter period (upper graph of each panel), during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Note the different scaling for wind speed seasonal trend graphs, however, also note that the wind speeds for summer had some problems with the recording software and so the magnitudes displayed seem to be lower than real wind speeds of those days. Date labels indicate 0:00 GMT.

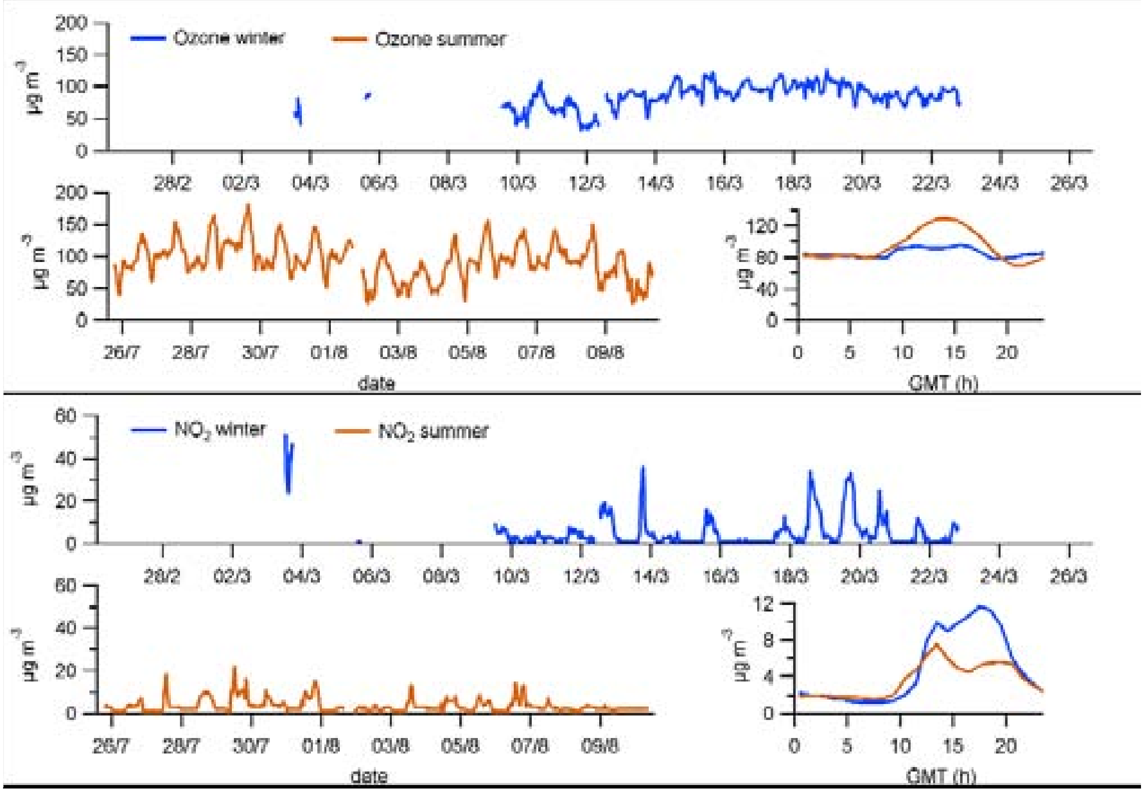

Fig. 4. Ozone and nitrogen dioxide concentrations during the winter period (upper graph of each panel), during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Date labels indicate 0:00 GMT.

- VOC mixing ratios

· *Seasonal differences*

All the measured VOC mixing ratios during the winter and summer campaigns as well as their mean daily cycles are displayed in Figs. 5, 6, and 7, while Table 2 lists maximum and minimum values of the same data. Almost all of them showed higher average mixing ratios during the summer period than in winter. Only some aromatic VOCs, normally linked to anthropogenic sources, had lower (benzene) or similar (toluene and C8-aromatics) mixing ratios in summer compared to winter (Fig. 7). The greatest difference in mixing ratios between seasons corresponded to the isoprenoids (Fig. 6), as seen by the 10-fold (isoprene and also its degradation products MVK/MACR) and 21-fold (monoterpenes) increases in their hourly average maxima (Table 2). In the case of monoterpenes, GC-MS analysis showed that the most abundant species were the same in winter and summer, being α-pinene the most abundant one among them (Table 3).

The VOC species with higher mixing ratios in both winter and summer was methanol (showing peaks of up to 9.7/13.4 ppbv and maximum hourly averages of 2.7/6 ppbv in winter/summer), followed in winter by ethanol (with peaks of 8.9 ppbv and maximum averages of 2 ppbv) and in summer by acetone (reaching up to 5.9 ppbv and maximum averages of 3.8 ppbv, Fig.5, Table 2). Regarding isoprenoids, monoterpenes were more abundant (reaching maximum averages of 1.4 ppbv) than isoprene in summer, whereas in winter both VOCs showed similar mixing ratios (around 0.7 ppbv

Table 2. Minimum and maximum VOC mixing ratios (ppbv) recorded at MSY station during the winter and summer campaigns.

	WINTER				SUMMER			
VOC	Minimum	Maximum	Hourly averages minimum	Hourly averages maximum	Minimum	Maximum	Hourly averages minimum	Hourly averages maximum
Methanol	0.256	9.761	1.279	2.704	1.410	13.400	4.142	6.053
Ethanol/formic acid	0.044	8.950	0.474	2.083	0.374	4.480	1.146	2.869
Acetone	0.257	3.897	0.787	1.547	0.967	5.950	2.260	3.834
Acetaldehyde	0.071	1.885	0.227	0.657	0.026	3.370	0.541	1.264
Isoprene	0.003	0.184	0.019	0.071	0	1.250	0.152	0.753
MVK/MACR	0.002	0.153	0.011	0.047	0.003	1.040	0.166	0.537
Monoterpenes	0.001	0.234	0.005	0.067	0.035	2.560	0.127	1.421
Acetic acid	0.026	5.348	0.393	1.428	0.270	5.640	1.317	2.940
Benzene	0.059	0.470	0.105	0.190	0.008	0.194	0.039	0.090
Toluene	0.001	1.903	0.057	0.408	0	1.340	0.083	0.467
C8-aromatics	0.003	1.328	0.031	0.249	0	0.821	0.091	0.272
Acetonitrile	0.027	0.258	0.075	0.101	0.037	0.588	0.159	0.216

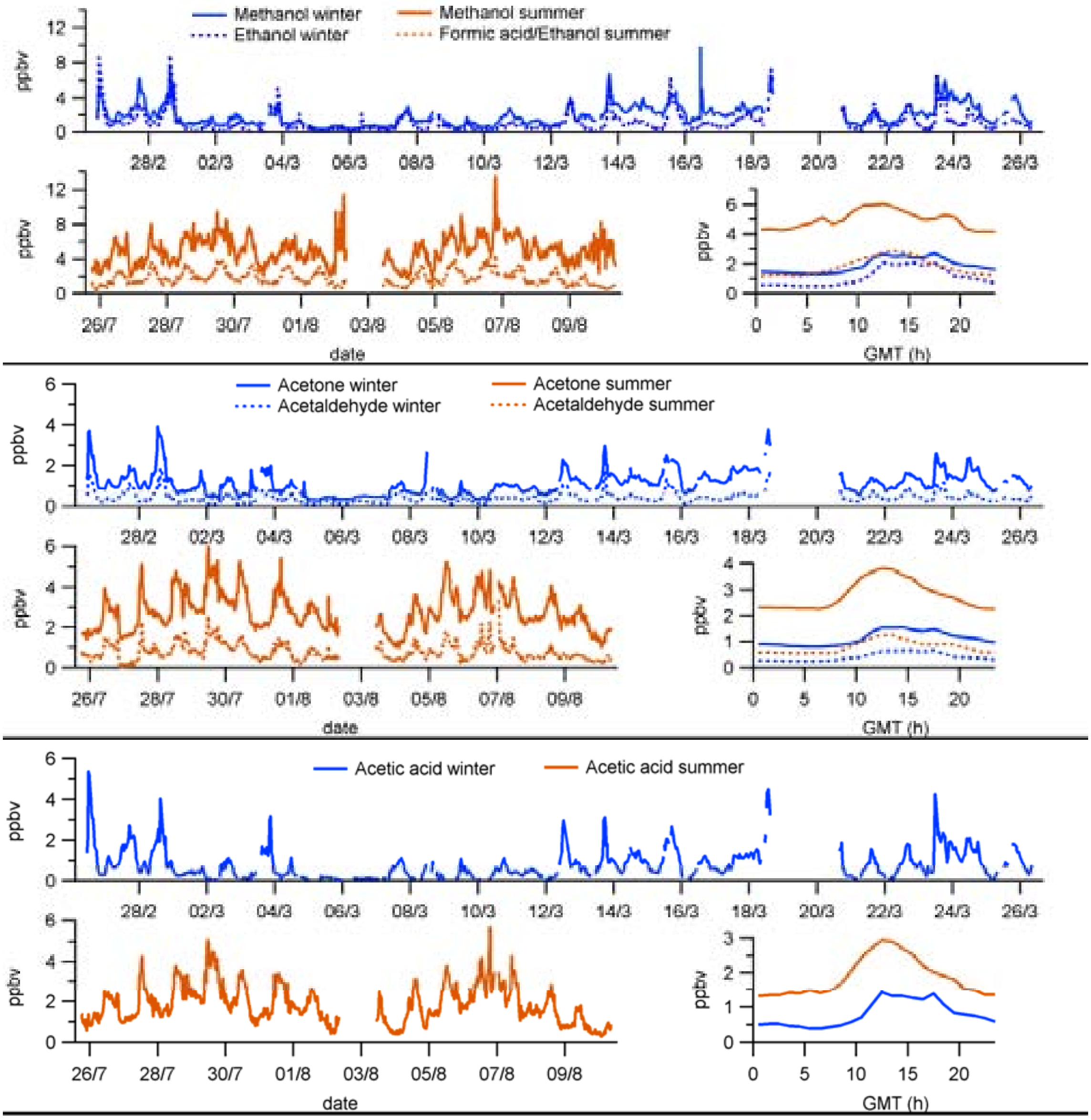

Fig. 5. Short-chain oxygenated VOCs measured at MSY, showing their mixing ratios during the winter period (upper graph of each panel), their mixing ratios during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Date labels indicate 0:00 GMT.

maximum averages). Among the aromatic VOCs, toluene presented the higher mixing ratios (with peaks below 2 ppbv and maximum averages around 0.4 ppbv) in both seasons (Fig.7).

Table 3. Most abundant monoterpenes in air as shown by GC-MS analysis. Units are percentage of each monoterpene out of total monoterpenes detected.

	WINTER	SUMMER
Monoterpene	%	%
α-pinene	51.4	31.9
sabinene + β-pinene	27.1	28.8
limonene	9.5	9.5

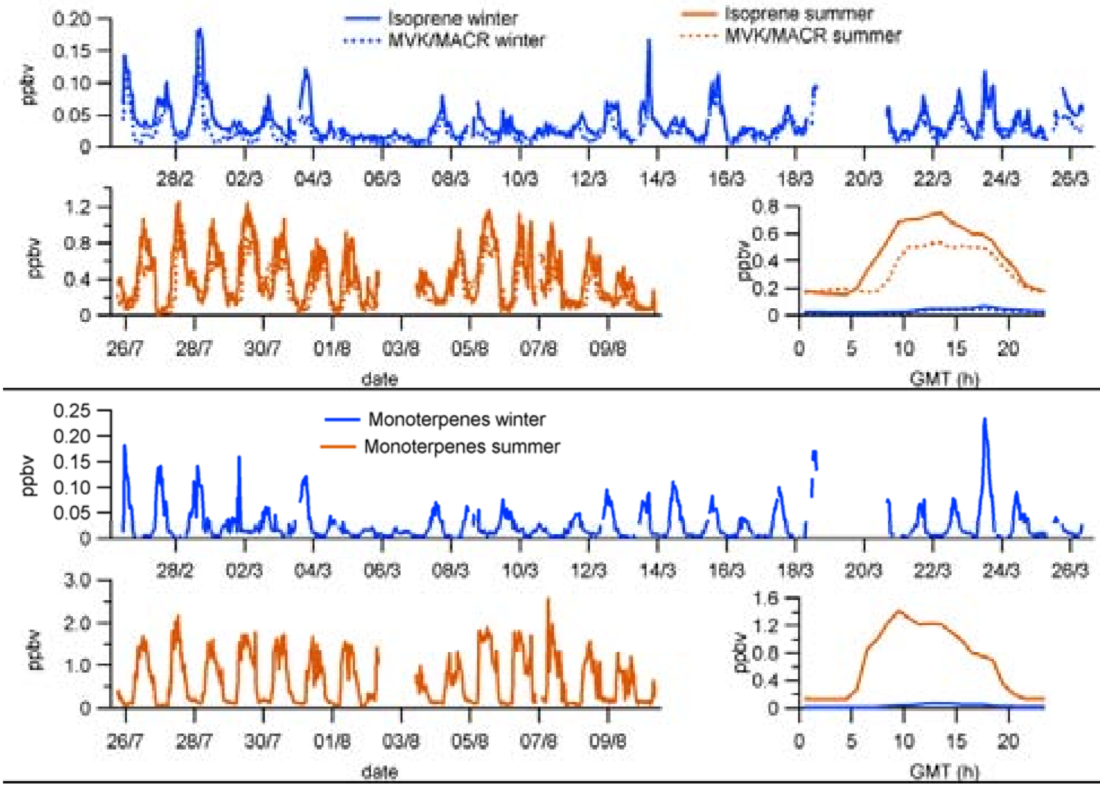

Fig. 6. Isoprenoid VOCs and MVK/MACR measured at MSY, showing their mixing ratios during the winter period (upper graph of each panel), their mixing ratios during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Note the different scaling for the seasonal trend graphs. Date labels indicate 0:00 GMT.

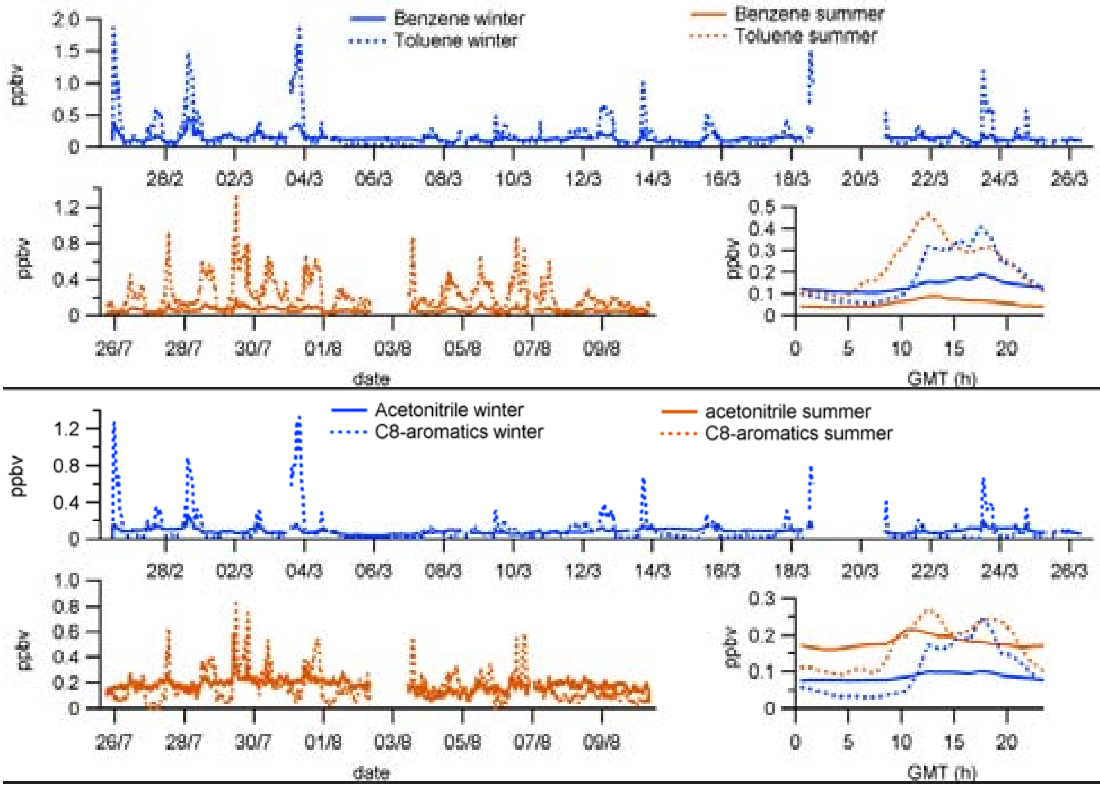

Fig. 7. Aromatic VOCs and acetonitrile showing their mixing ratios during the winter period (upper graph of each panel), their mixing ratios during the summer period (lower left graph) and their mean daily cycles (hourly averages) for both periods (lower right graph). Note the different scaling for the seasonal trend graphs. Date labels indicate 0:00 GMT.

· *Average daily cycles*

Looking at the daily average mixing ratios and in general terms, all VOCs presented very similar daily cycles with maximum values during daytime (Fig. 8). Linear correlations between them show variable Pearson's correlation coefficients (r), but always significant ($p < 0.01$) above 0.31 and with an average of 0.73 in winter and 0.68 in summer (Table 4).

When considering the different groups of VOCs separately, it is apparent that although their daily pattern is not exactly the same in summer than in winter all short-chain oxygenated VOCs (oxVOCs) have almost the same daily pattern in winter and all of them share the same daily cycle in summer. Their mixing ratios were highly correlated between them (Table 4), with $r > 0.77$ in both seasons, except for methanol in summer that presented a little bit lower r values ($0.65 \leq r \leq 0.73$). Aromatic VOCs also were highly correlated between them in both seasons, specially toluene and C8-aromatics ($r > 0.93$; Table 4). In addition, these two groups of compounds –oxVOCs and aromatics– showed very similar daily trends in winter ($0.34 \leq r \leq 0.85$) and in summer ($0.47 \leq r \leq 0.86$), being toluene (winter) and benzene (summer) the aromatic species with higher correlation to oxVOCs, and ethanol (winter) and acetaldehyde (summer) the oxygenated species more correlated to aromatics (Table 4). In both seasons, methanol showed the lowest correlation with aromatics among oxVOCs ($0.34 \leq r \leq 0.54$; Table 4). Acetonitrile presented the lowest correlation values with other VOCs, but always in the range between 0.31 and 0.82 (Table 4).

Relationship between isoprenoid VOCs (isoprene and monoterpenes) showed a mixed behaviour, depending on the season. In winter isoprene showed a daily cycle resembling those of aromatic or oxVOCs (Fig 8), which is ratified by its correlation with them ($r > 0.75$ for aromatic and $0.62 \leq r \leq 0.81$ for oxygenated VOCs; Table 4), while it had a still high but lower correlation with monoterpenes ($r = 0.66$). On the other hand, in summer isoprene's correlation with monoterpenes was much higher ($r = 0.88$) while maintaining high correlations with aromatic ($r >0.63$) and short-chain oxygenated VOCs ($0.54 \leq r \leq 0.8$; Table 4), and accordingly isoprene mean daily cycle was not exactly like the ones described by oxVOCs or aromatics nor the one of monoterpenes (Fig. 8). Isoprene correlation with MVK/MACR was invariably high: 0.88 in winter and 0.83 in summer (Table 4). Monoterpenes, in their turn, were less correlated than isoprene with oxVOCs ($0.56 \leq r \leq 0.73$ in winter and $0.51 \leq r \leq 0.72$ in summer) and aromatics ($0.54 \leq r \leq 0.63$ in winter and $0.4 \leq r \leq 0.61$ in summer; Table 4).

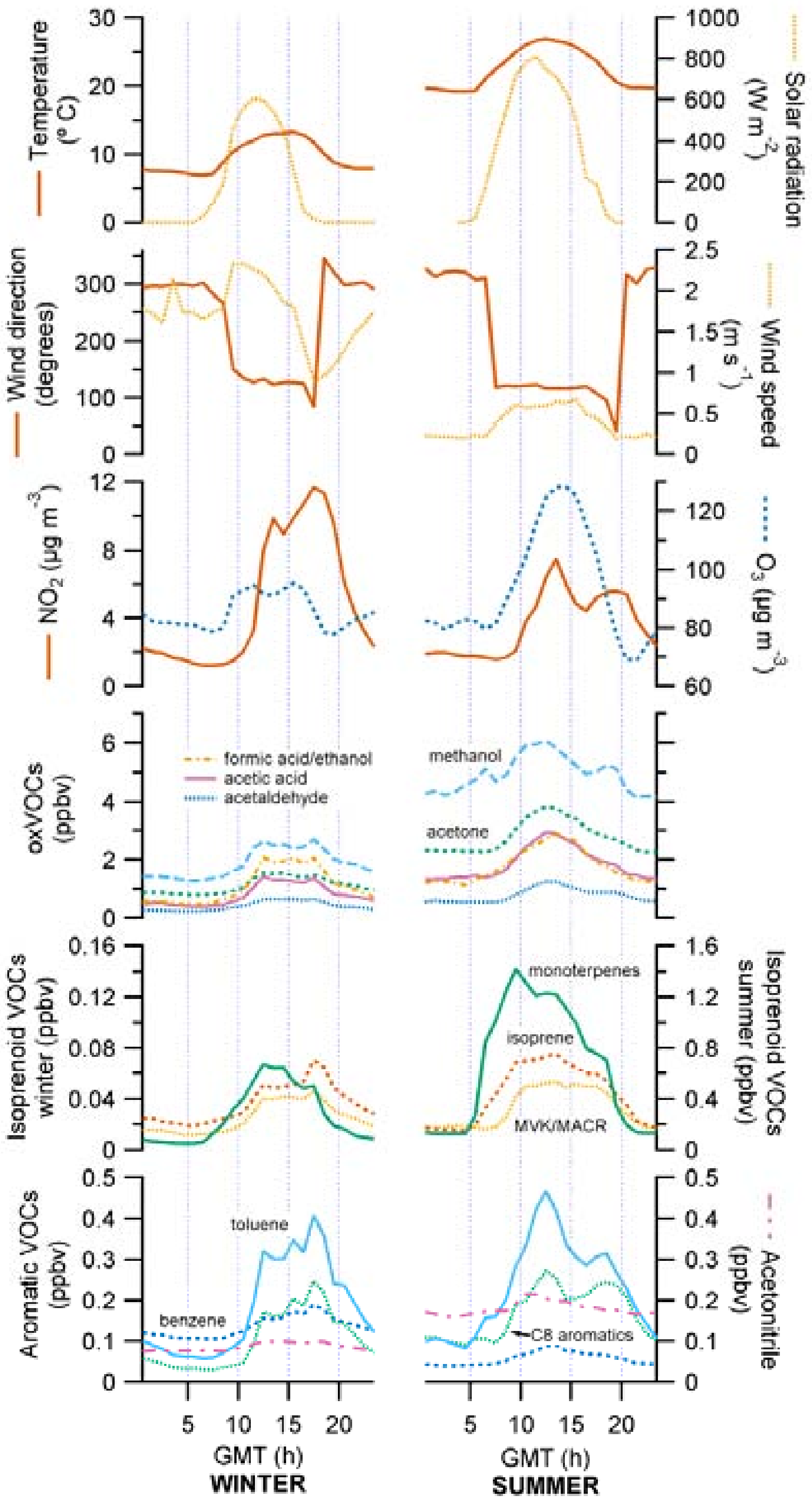

Fig. 8. Mean daily cycles of most part of the variables studied, comparing winter (left) with summer (right). VOC mixing ratios are split into three separate panels, corresponding to Figs 5, 6, and 7: (i) one for short-chain oxVOCs: methanol, acetone, formic acid/ethanol, acetic acid and acetaldehyde; (ii) the second one for isoprenoid VOCs: monoterpenes, and isoprene and its degradation products MVK/MACR; (iii) the last panel for acetonitrile and aromatic VOCs: benzene, toluene, and C8-aromatics. Note the different scaling for the isoprenoid VOCs between winter and summer (one order of magnitude higher in summer).

Table 4. Correlation coefficients between 60-minute averages of VOCs, ozone, nitrogen dioxide and meteorological variables for both periods of the campaign: winter (lower-left side of the table) and summer (upper-right part). All numers shown are significant ($p < 0.01$) Pearson's correlation coefficients ($353 \leq n \leq 608$ for winter; $343 \leq n \leq 348$ for summer). Wind data has been divided into its wind direction vectorial components (x and y) weighted by the wind speed. ns = not significant.

Sum / Win	Acetal-dehyde	Acetic acid	Acetone	Formic acid/ ethanol	Metha-nol	Isoprene	MVK/ MACR	Mono-terpenes	Benzene	Toluene	C8-aromatics	Aceto-nitrile	O_3	NO_2	Solar rad.	Temp.	Wind (x, y)
Acetaldehyde	-	0.86	0.88	0.77	0.65	0.72	0.86	0.51	0.86	0.83	0.80	0.41	0.55	0.76	0.47	0.58	0.46, -0.49
Acetic acid	0.91	-	0.93	0.92	0.72	0.72	0.80	0.58	0.80	0.77	0.67	0.50	0.80	0.66	0.56	0.78	0.48, -0.54
Acetone	0.89	0.91	-	0.88	0.73	0.75	0.83	0.60	0.83	0.77	0.67	0.52	0.72	0.63	0.55	0.73	0.51, -0.56
Formic acid/ ethanol	0.94	0.91	0.85	-	0.65	0.80	0.79	0.72	0.76	0.74	0.59	0.44	0.81	0.58	0.71	0.89	0.58, -0.65
Methanol	0.80	0.88	0.87	0.77	-	0.54	0.58	0.51	0.54	0.54	0.47	0.34	0.42	0.44	0.37	0.50	0.35, -0.38
Isoprene	0.81	0.76	0.72	0.81	0.62	-	0.83	0.88	0.76	0.77	0.63	0.37	0.61	0.56	0.75	0.80	0.73, -0.76
MVK/MACR	0.94	0.89	0.86	0.90	0.79	0.88	-	0.61	0.81	0.80	0.75	0.31	0.58	0.68	0.51	0.68	0.54, -0.57
Monoterpenes	0.72	0.70	0.63	0.73	0.56	0.66	0.66	-	0.57	0.61	0.40	0.32	0.53	0.31	0.84	0.79	0.72, -0.78
Benzene	0.68	0.58	0.58	0.70	0.34	0.75	0.69	0.54	-	0.89	0.83	0.51	0.60	0.80	0.53	0.63	0.52, -0.55
Toluene	0.76	0.74	0.67	0.85	0.52	0.81	0.75	0.68	0.83	-	0.93	0.46	0.49	0.86	0.54	0.63	0.49, -0.53
C8-aromatics	0.69	0.69	0.60	0.79	0.46	0.78	0.70	0.63	0.81	0.98	-	0.41	0.33	0.89	0.32	0.44	0.34, -0.36
Acetonitrile	0.72	0.76	0.82	0.68	0.80	0.65	0.71	0.55	0.43	0.57	0.52	-	0.51	0.37	0.35	0.42	0.29, -0.30
O_3	0.25	0.35	0.48	0.20	0.42	ns	0.14	0.16	ns	-0.16	-0.22	0.35	-	0.38	0.59	0.82	0.50, -0.57
NO_2	0.70	0.60	0.56	0.74	0.40	0.71	0.68	0.55	0.78	0.91	0.93	0.43	-0.16	-	0.28	0.42	0.32, -0.32
Solar radiation	0.14	0.13	0.12	0.11	0.14	0.13	0.15	0.22	ns	ns	ns	ns	0.19	ns	-	0.82	0.64, -0.76
Temperature	0.57	0.59	0.60	0.49	0.70	0.38	0.51	0.55	ns	0.23	0.17	0.60	0.47	0.21	0.28	-	0.57, -0.69
Wind (x, y)	0.28, -0.38	0.29, -0.40	0.32, -0.40	0.26, -0.36	0.28, -0.37	0.21, -0.24	0.22, -0.30	0.45, -0.52	0.16, -0.16	0.22, -0.23	0.17, -0.18	0.23, -0.28	0.17, -0.32	0.24, -0.22	ns, -0.22	0.35, -0.49	-

- Relationship between VOCs, meteorology, O_3, and NO_2

Linear correlations between VOC mixing ratios and the other available data variables (wind, temperature, solar radiation, and NO_2 and O_3) were calculated, in order to search for possible relationships, and the results are displayed in Table 4 too.

Wind direction presented its highest correlations with monoterpenes in both seasons, and also with isoprene in summer (Table 4). Temperature in winter correlated the most with some oxVOCs, specially methanol (r = 0.7), followed by acetonitrile (r = 0.6). In summer, however, temperature correlated less with methanol (r = 0.44) while correlation coefficients increased with other oxVOCs, and specially with isoprenoids (r > 0.79), ozone (r = 0.82) and solar radiation (r = 0.82; Table 4). Solar radiation, in turn, had low or even non-significant (with aromatic VOCs and acetonitrile) correlation with most variables in winter, being with monoterpenes (r = 0.22) and temperature (r = 0.28) the higher ones, while in summer all correlation coefficients increased (r > 0.28) but the same variables showed the maximum correlation: monoterpenes (r = 0.84) and temperature (r = 0.82; Table 4).

Finally, NO_2 concentrations were most correlated with aromatic VOCs (r > 0.78) in both seasons, followed by acetaldehyde (r = 0.76) in summer and ethanol and isoprene in winter (r > 0.71; Table 4). Ozone concentrations showed relatively low correlation coefficients with all the other gas mixing ratios in winter, whereas in summer their correlation coefficients were generally increased, specially with formic acid/ethanol and acetic acid (r > 0.8) and temperature (r = 0.82; Table 4). The wavelet analysis with ozone and VOC mixing ratios in summer showed that the maximum coherence between the two signals occurred with a period of one day (i.e. ozone was correlated with VOC data having a similar daily cicle). The phase between each VOCs and ozone was different however. OxVOCs and aromatics were in general in phase (i.e. their mixing ratios and ozone concentrations had a simultaneous trend), whereas monoterpenes were advanced with respect to ozone (i.e. their mixing ratios rised before ozone concentrations increased). Fig. 9 shows the cross wavelet analysis output graphs corresponding to acetic acid (representing oxVOCs), benzene (representing aromatics) and monoterpenes. Acetic acid showed an additional correlation with ozone, with a period of around half a day, in some days of the first half (up to the 2nd of August) of the measures (Fig. 9). This could be attributed to a small rise of both compounds taking place at night.

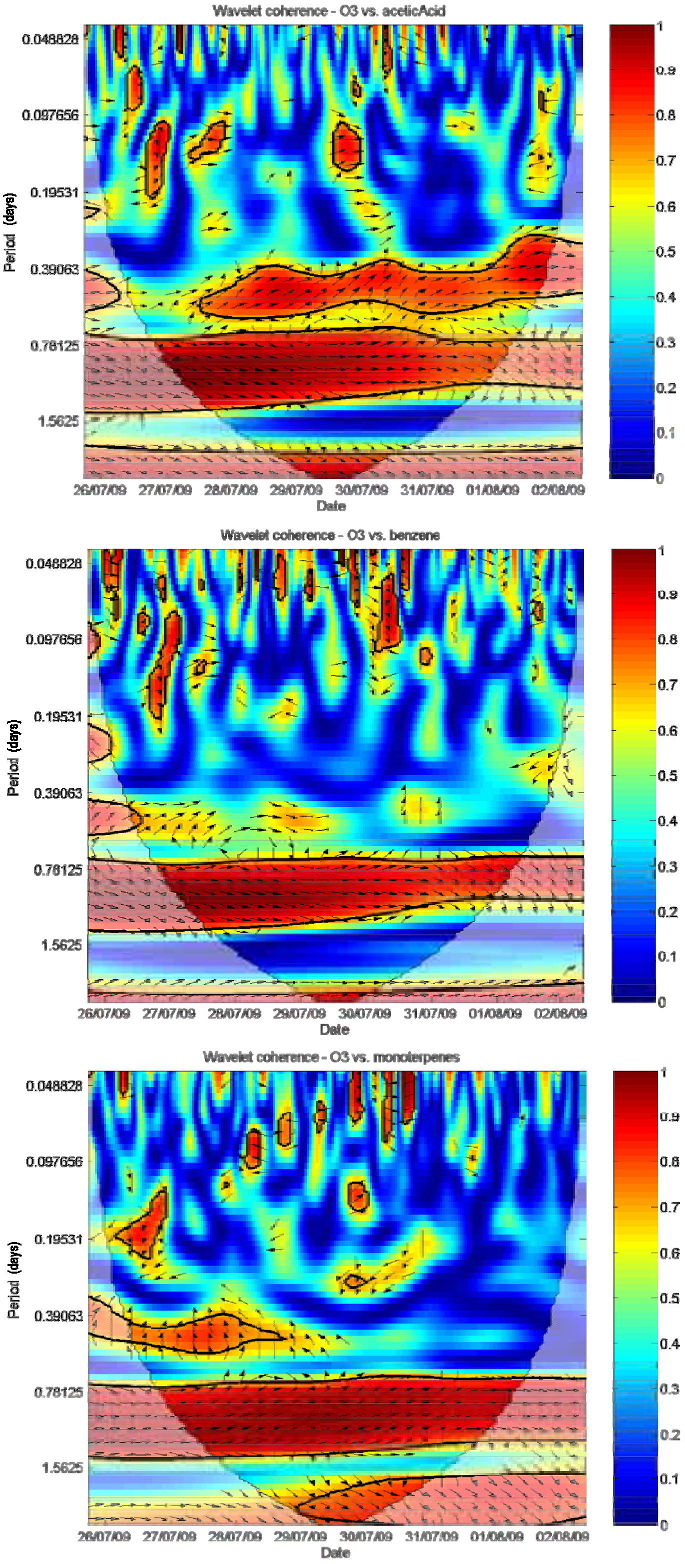

Fig. 9. Cross wavelet analysis output using ozone concentration data versus VOC mixing ratios: acetic acid (top panel), benzene (middle panel) and monoterpenes (bottom panel) mixing ratios. It shows the coherence in a scale from 0 to 1, the latter representing complete coherence between the two signals. In those areas enclosed by a thick line the coherence is significatively ($p<0.05$) different from zero, as calculated via 1000 Monte carlo simulations (Grinsted et al., 2004). Superimposed to the coloured diagrams there are arrows whose direction is related to the phase between the signals. An arrow pointing to the right corresponds to two signals which are in-phase. If the arrow points downward, ozone leads the VOC by 90º, whereas if it points upward it is the VOC that leads ozone concentration by 90º.

Discussion

The results show almost no seasonal variability for aromatic VOCs whereas short-chain oxygenated VOCs and specially isoprenoids presented much higher mixing ratios (one order of magnitude) in summer when the vegetation emission rates highly increased, also one order of magnitude (Llusià et al., in preparation). Ozone highest diurnal concentrations increased in summer too, due to more photochemical activity and the presence of more VOCs in the air.

The daily variations in the mixing ratios of most VOCs measured in this study show a very similar pattern. This fact, along with its correspondence in time with the wind direction and speed (Fig. 8), suggests that the changes in VOC mixing ratios are driven by the wind regime of the MSY site, that during daytime advects air masses from the metropolitan area of Barcelona, and during nighttime advects air masses from the north-western pastures and forests.

- <u>Seasonal VOC mixing ratios</u>

· *OxVOCs*

Methanol is the second most abundant organic gas in the atmosphere after methane (Jacob et al., 2005), with mixing ratios lying globally within the range 0.2–195 ppbv (*capítol 1*, Seco et al., 2007), so it is not strange it had the highest mixing ratios also at MSY in both seasons (Fig. 8; Table 2). Its direct sources, as well as those of the other short-chain oxygenated VOCs, include emissions from the vegetation and soil, biomass burning, and dead plant matter, as well as anthropic sources like fuel combustion and industrial processes. In addition, oxVOCs can be formed secondarily as products of reactions of other –biogenic or anthropogenic– hydrocarbons of higher molecular weight, and in fact this is the major atmospheric source of some of these oxVOCs, such as acetaldehyde (*capítol 1*, Seco et al., 2007). So, the difference in oxVOC mixing ratios between seasons may be attributed mainly to two phenomena: i) the summer larger biogenic emissions due to the increased physiological activity of vegetation, and ii) the increased secondary production in summer due to the higher photochemical reactivity as a result of the higher temperatures and insolation. These compounds are characterised by a variety of atmospheric lifetimes, which span from over 10 days for acetone and methanol (Singh et al., 2004; Jacob et al., 2005) to a few hours for acetaldehyde (Possanzini et al., 2002). This may be the reason why acetone and methanol had the highest mixing ratios in this group, even at night, and also why acetaldehyde showed the lowest ones of all oxVOCs measured (Fig. 8).

The comparison of these oxVOC mixing ratios with others reported in the literature shows that for example methanol, acetone and acetaldehyde mixing ratios in the boreal forest of Hyytiälä (Finland) were in the same order of magnitude but somewhat lower than at MSY, both in winter and summer, with only some occasional peaks higher than at MSY (Rinne et al., 2005; Lappalainen et al., 2009; Ruuskanen et al., 2009). In a sub-arctic wetland in Sweden, summertime values (Holst et al., 2010) were also lower in general and only the highest peaks were in the range reported here at MSY. These results are in accordance with the lower temperatures found in Scandinavia (which may determine lower photochemistry and also lower vegetation physiological activity and thus minor VOC emissions) compared to MSY and also to the less polluted nordic air given the lower population density of those countries.

Jordan et al. (2009b) have recently published a long-term study of VOCs at a rural area (Thompson Farm) near the coast in New Hampshire (East USA), and the oxVOC mixing ratios reported were lower than at MSY in both seasons, with the exception of acetaldehyde in winter that was within the lower range of the MSY values. Given the cold mean temperatures registered during winter at Thompson Farm (below 0 ºC), it could be reasonable to compare winter MSY VOC mixing ratios with Jordan et al. (2009b) data corresponding to spring or fall, when temperatures were similar to MSY winter. In this comparison methanol and acetone mixing ratios in Thompson Farm increased a little and reached similar values as in MSY winter, while acetaldehyde stayed similar to its winter values (in the range of MSY winter) and acetic acid keeped below MSY (as it did in all the seasons). A mixed deciduous forest in NW Germany showed similar minimum methanol averages but higher maximum methanol mixing ratios in summer compared to MSY (Spirig et al., 2005).

The only reports related to typical Mediterranean ecosystems are those from the Castelporziano site (Italy), surrounded by *Q. ilex* forests and macchia, and also influenced by the urban ambient of Rome. For the year 1994 and within the BEMA campaign, Kalabokas et al. (1997) described a lower daily mean acetone mixing ratio (1.74 ppbv) than in MSY summer. However, that campaign was held in May (when the authors considered that plants would have good temperature and water conditions for VOC emission) and such mixing ratio is, accordingly, between our MSY winter and summer values. By contrast, acetaldehyde daily average mixing ratios (1.8 ppbv) were above those registered at MSY. More recently in 2007, Davison et al. (2009) measured again at the same site and period (May-June). Reported mixing ratios of methanol (medians of 1.6 to 3.5 ppbv) and again acetone (0.96-2.1 ppbv) were in this case a little lower than those at MSY, while acetaldehyde showed similar values (medians of 0.44-1.3 ppbv). It can be seen that the Castelporziano results had almost the same relationship with MSY ones in both years. Finally, the MSY oxVOCs mixing ratios are generally in the range of values found in other rural areas of the world. To put MSY values in context with these other rural and also urban sites, please refer to the review by Seco et al. (2007, *capítol 1*).

· *Isoprenoids*

Isoprenoid VOCs (isoprene and monoterpenes) are considered to have their major atmospheric source in the direct emission from vegetation (Guenther et al., 1995). As in the case of oxVOCs, increased emission rates of plants with higher temperatures and radiation must be the reason for the great rise of their mixing ratios in summer compared to winter, which was the highest monitored in MSY (10- to 21-fold increase; Figs. 6 and 8; Table 2). Likewise, isoprenoid leaf-level emissions by *Q. ilex* measured during the campaign showed a similar increase of about one order of magnitude (Llusià et al., in preparation). In this case, however, increased photochemistry would avoid their mixing ratios increase, as these isoprenoids are far more reactive in the atmosphere than short-chain oxCOVs (Atkinson, 2000), so the vegetation source must have been stronger than the photochemical sink in summer.

Comparing isoprenoids in MSY with other site's measurements, in Hyytiälä (Finland) isoprene and monoterpene showed the same behaviour as oxVOCs: mixing ratios in winter were similar to those reported for MSY winter, but summer numbers were generally lower in Finland (Lappalainen et al., 2009; Ruuskanen et al., 2009) with only some peaks of monoterpenes (up to 2.5 ppbv) reaching values comparable to MSY in summer (Rinne et al., 2005). In a wetland in Sweden, also isoprene average summer mixing ratios were lower but some peaks (up to 1.2 ppbv) were in the range reported here for MSY summer (Holst et al., 2010). Again, in these cases the lower plant activity regarding emissions may be the reason for the lower summer mixing ratios found in Scandinavia. Measurements at the Mediterranean site of Castelporziano showed slightly lower mixing ratios of isoprene (medians of 0.1-0.37 ppbv) and MVK/MCAR (medians of 0.13-0.30 ppbv) and clearly lower monoterpene ones (medians of 0.13-0.3) in May than at MSY in summer (Kalabokas et al., 1997; Davison et al., 2009). As with oxVOCs, results in summer in Castelporziano may have been hypothetically closer to MSY ones, as these mixing ratios are somewhat in between the MSY winter and summer ones, although summer drought may exert a big influence in VOC emissions there and have the contrary effect, as the authors pointed out when choosing the period for the measurements.

An interesting detail is that maximum monoterpene mixing ratios in the 2007 Castelporziano campaign were detected at night, when the land breeze originated and air was coming from oak and pine forests, and those mixing ratios were higher (medians of 0.26 to 0.57 ppbv) than the night ones at MSY. The authors attributed this behaviour to the night temperature-dependent emissions of stored monoterpenes by the pines together with the ceased photochemical destruction of VOCs and the suppression of the vertical mixing at night (Davison et al., 2009). In MSY, vertical mixing and photochemistry were also suppressed at night, but the land breeze acted in the contrary sense, cleaning the air from VOCs (see discussion below). This may be in part due to the fact that there are no significant tree populations in the surroundings that could emit monoterpenes from storage pools at night, as holm oaks do not store monoterpenes and rather they emit them from de novo synthesis, influenced by temperature, irradiance and water availability (Loreto et al., 1998; Llusià and Peñuelas, 1999; Peñuelas and Llusià, 1999b; Staudt et al., 2002). It should be noted that forest measurements at Hyytiälä (Finland) by Rinne et al. (2005) and Ruuskanen et al. (2009) also reported higher monoterpene mixing ratios at night in summer. Likewise, in the report by Jordan et al. (2009b), monoterpenes at Thompson Farm show higher mixing ratios at night too. Their average mixing ratios were higher in winter (median of 0.1 ppbv) and also in spring (median of 0.12 ppbv) than at MSY in winter, while in summer where in the same range of values as MSY (median of 0.5 with peaks of 2.5 ppbv). Isoprene and MVK/MACR, in their turn, were at Thompson Farm in the same range of values as in MSY for both seasons (also in spring compared to MSY winter) but with somewhat higher mixing ratios (medians of 0.05 and 0.42 ppbv for isoprene and 0.04 and 0.33 ppbv for MVK/MACR in winter and summer, respectively). In addition, their daily cycle showed their maxima during daytime, as opposed to monoterpenes at the same location. Mielke et al. (2010) reported as well monoterpene mixing ratios that were highest during the night at a mixed forest in Michigan (USA) in summer, with values that were lower in average (mean of 0.25, at times reaching up to 0.97 ppbv) but higher at night than those at MSY. A different mixed forest in Jülich (NW Germany) presented lower values of monoterpenes (maximum at midday with average mixing ratios around 0.3 ppbv). Both mixed forests at Michigan and Germany had higher midday isoprene mixing ratios (around 2 ppbv) than MSY in summer, with spikes up to 9 ppbv in the case of Jülich (Spirig et al., 2005). For another german mixed forest near the city of Frankfurt isoprene summer mixing ratios reported were in the range of those at MSY (Steinbrecher et al., 2000).

· *Aromatic VOCs*

Aromatic VOCs did not change much in mixing ratios between winter and summer at MSY (Figs. 7 and 8; Table 2), suggesting a year-round similar strenght of their emission sources. Also, sources of these compounds –mainly tailpipe exhaust– are very few in the local environment of the MSY site, probably restricted to a little road in the valley and some small villages. Therefore their mixing ratios in MSY can be supposed to come from relatively distant sources outside the valley. The aromatic VOC with a biggest seasonal variation was benzene (was about double in winter compared to summer, see Table 2) but at the same time it was the aromatic VOC with less daily fluctuation (Fig. 7). The latter may be related to the fact that benzene has the longest atmospheric lifetime (in the order of days) among the measured aromatics (Atkinson, 2000) and for this reason it may be better mixed within the atmosphere.

Other studies also reported benzene mixing ratios to be lower in summer compared to winter, probably due to reduced removal by OH radicals. One of these studies found very similar benzene mixing ratios to MSY, both in winter and in summer in Hyytiälä, Finland (Ruuskanen et al., 2009). And another study reported benzene measurements in the range of MSY at Thompson Farm (New Hampshire, USA), but with higher medians (Jordan et al., 2009b) also in both seasons, together with toluene and C8-aromatics in the same range but generally lower medians. By contrast, in a urban impacted mixed forest near Frankfurt (Germany) aromatics were found to be higher in summer than at MSY, with the exception of toluene that was within the same range (Steinbrecher et al., 2000). Both reports from Thompson Farm and Frankfurt stated higher mixing ratios of aromatics at night, and the paper dealing with Finland data also shows that frequently its highest benzene mixing ratios are measured around 22h in the night.

Finally, acetonitrile is considered a biomass burning marker (Holzinger et al., 1999), given that it has biomass burning as major global source (94%), with only a minor part (less than 6%) coming from fossil fuel combustion (Holzinger et al., 2001b). Similarly to benzene, its relatively long lifetime (Sanhueza et al., 2004) in air may promote a good acetonitrile mixing in the atmosphere, and in consequence its mixing ratios showed small daily variations (Fig. 8). In contrast with benzene, in MSY acetonitrile doubled its mixing ratio in summer compared to winter (Table 2). This seasonal variation is very close to the 60% increase between these two seasons reported by Jordan et al. (2009b) at Thompson Farm (New Hampshire, USA). There, winter and spring mixing ratios of acetonitrile (medians of 0.08 ppbv) were similar to the MSY winter ones, while summer acetonitrile mixing ratios (median of 0.13 ppbv) were lower than at MSY. In the forest of Hyytiälä (Finland), reported values for acetonitrile were very low in summer (median of 0.05 ppbv), which is even less than those at MSY in winter (Ruuskanen et al., 2009).

- Daily cycles and wind regime

Mixing ratios of all VOCs at this forested site showed a marked daily cycle in both seasons, and a very similar pattern, in most cases, within one particular season. This is specially apparent in winter, when in average all VOC but monoterpenes –and to a lesser extent, acetonitrile– rised at the same time in morning, had almost the same mixing ratio peaks and decayed at the same moment in the evening (Fig. 8). Even the aromatic compounds followed this behaviour, despite their local sources at the MSY valley are few. These facts point to the idea that the VOC mixing ratios measured at the site are mainly governed by the wind regime of the mountain. In winter, after sunrise the sea breeze starts to blow mountain upwards and VOC mixing ratios slightly increase, and about 2 hours after that, they experiment a sharp increase. VOC levels are maintained more or less the same until the wind changes direction and reduces speed, when around 17h GMT the sea breeze stops and the mountain drainage flow towards the valley starts instead. This general pattern of VOC mixing ratios daily cycle driven by the wind is happening also in summertime. Such a behaviour of atmospheric pollutants has been described for this same campaign regarding aerosols, when comparing PM levels at the city of Barcelona and MSY (Pandolfi et al., in preparation). In that case, the levels of fine particulate matter (PM_1) increased in MSY when the Barcelona ones decreased and this was partly attibuted to the sea breeze transport toward the MSY site. The air masses from the Barcelona metropolitan area are advected to MSY by the sea-land breezes, passing over heavily industrialized areas, with some cities in the range of 50,000-100,000 inhabitants and many highways, and taking up more pollutants. At the same time, photochemical reactions take place within the air mass, so its composition is not the same when it arrives at MSY. Furthermore, this air mass with all the VOCs, PM, and gases transported inland from the polluted areas mixes with new biogenic VOCs at the forested site (Fig. 9), locally produced at the valley where MSY is located, and this enhances ozone production (Peñuelas and Staudt, 2010). This could be the reason for the higher ozone concentration found in MSY (means of 75 $\mu g\ m^{-3}$ at night and 90-125 $\mu g\ m^{-3}$ at day) compared to Barcelona (means of 40-50 $\mu g\ m^{-3}$ at night and 60-70 $\mu g\ m^{-3}$ at day; Pandolfi et al., in preparation), in both seasons, as several reports have highlighted in other areas (Millan et al., 1997; Evtyugina et al., 2006, 2009). Also, ozone levels measured in the metropolitan area of Barcelona, at a NO_x-rich semi-urban site (i.e. very similar to the areas upwind of MSY during daytime) with high influence of urban, industrial and highway pollution were lower (means of 10-20 $\mu g\ m^{-3}$ at night and 50-90 $\mu g\ m^{-3}$ at day) than at MSY in both seasons (Filella and Peñuelas, 2006b). Furthermore, other studies have reported that ozone concentrations increase with altitude at mountain sites too (Ribas and Peñuelas, 2006; Diaz-de-Quijano et al., 2009; Evtyugina et al., 2009). VOC mixing ratios in summer, specially those of isoprenoids, were much higher than in winter and this fact, together with a higher solar radiation and temperature promoting photochemical reactions, may explain the higher daytime ozone concentrations registered in summer (Fig. 9).

Monoterpenes constitute a special case regarding this airborne transport of the VOCs measured at MSY from polluted areas. In winter and summer, monoterpene mixing ratios started to rise earlier than the others, as soon as sun light started to increase too (Fig. 8), and this was probably due to the local origin of monoterpenes. Correlations between sun light and monoterpenes mixing ratios confirmed this, as they showed the highest correlations achieved by the solar radiation (Table 4). Also the wavelet analysis showed that in summer monoterpene mixing ratios were advanced in time to the ozone concentrations, while other VOCs were in phase (i.e. simultaneous) with ozone (Fig. 9). Monoterpenes were surely produced in the near surroundings of the MSY site and in the valley just beneath it, which is densely populated by holm oaks, species known for its capacity to emit monoterpenes (Kesselmeier and Staudt, 1999; Peñuelas and Llusià, 1999a; Llusià and Peñuelas, 2000), as confirmed by leaf-level measurements performed at MSY during this campaign (Llusià et al., in preparation). The case of isoprene was, however, not the same. Although it is normally considered a biogenic VOC, it has also known anthropogenic sources, like automobile exhaust (Borbon et al., 2001), while in the case of monoterpenes they are scarce. The difference in correlations of isoprene with monoterpenes between winter and summer, and its higher correlation with aromatic compounds in winter (Table 4), reflected in the mean wintertime daily cycle (Fig. 8), may be indicative that most of the isoprene detected in winter was also advected to MSY and maybe had a big contribution of anthropic origin. A previous study in an semi-urban area in the metropolitan region of Barcelona (upwind of MSY) described this dominance of the human-related sources of isoprene in winter (Filella and Peñuelas, 2006a). In addition, winter monoterpene emissions by the valley oaks were low (Llusià et al., in preparation), as reflected by the low mixing ratios reported, so isoprene emission must have been even lower because the emission of isoprene has been reported to be only below 5% of the emission of monoterpenes for this evergreen tree species (*capítol 2.3*, Peñuelas et al., 2009). Of course we can not rule out the possibility that different plant species of the vegetation found in the trajectory of the air masses advected by the sea breeze (i.e. before entering the MSY valley) were responsible for isoprene emission into those air masses. Following this reasoning, in summer part of the isoprene at MSY may have been also anthropogenic, however the biogenic contribution either from distant vegetation (advected, see Filella and Peñuelas, 2006a) and from valley (local) isoprene-emitting species other than *Q. ilex* would have been more important. Moreover, as correlations revealed (Table 4), isoprene in summer did not follow so closely the daily cycles of aromatics or oxVOCs as in winter, and showed an earlier rise in mixing ratios instead, at the same time as monoterpenes did (Fig. 8). This may clearly indicate its summertime biotic and local origin, at least in part. In contrast, most other VOCs including isopene's oxidation products MVK and MACR started to rise all together with a certain delay, when air masses advected by the sea breeze contributed to the VOC burden at MSY.

The only two other VOCs that also showed an early rise in the morning were methanol and toluene. On the one hand, methanol has been reported to build up inside the leaves of plants when stomata remain closed during the night, and then burst out when they open in the morning, under the control of the gas–liquid phase equilibration of the alcohol within the leaf and of the stomatal conductance (Niinemets and Reichstein, 2003; Filella and Peñuelas, 2006a; *capítol 2.2*, Filella et al., 2009). Some authors have seen this morning burst in mixing ratios, and attributed it to this stomata-related phenomenon; also others attributed it to the evaporation of the dew, supposing that methanol may be dissolved in it (Holzinger et al., 2001a; Sanhueza et al., 2001; Warneke et al., 2002; Filella and Peñuelas, 2006a). On the other hand, toluene has been reported to be emitted by plants (Heiden et al., 1999; White et al., 2009) and even emissions of m/z 93 have been reported for *Q. ilex* and attributed to toluene (Holzinger et al., 2000). Furthermore, the possibility that certain monoterpene fragments may contribute to m/z 93 and thus being mistaken for toluene has been suggested (Kim et al., 2010). However, Ambrose et al. (2010) has recently reported this interference to be practically insignificant.

Nevertheless, the high influence of the wind regime on VOC mixing ratios at MSY is illustrated by Fig. 10, which has the same information as Fig. 8 but corresponding only to the 6th of August, as a representation of atmospheric conditions that happened on some other days. That day all VOCs, including monoterpenes, suffered a reduction in mixing ratios when around 14-15h wind started to change speed and specially direction, turning from SE (around 120 degrees) to W (260-270 degrees). Solar radiation did not change (e.g. due to clouds), so it is probable that biogenic emissions did not decrease. This VOC mixing ratio reduction was paralleled by a big reduction in NO_2 levels and a small increase in O_3 concentration. At around 17h the wind went back to SE direction briefly to change then at 18h to N, and staying like that for the whole night. VOC and NO_2 concentrations showed an increase again when the wind direction returned to the SE direction, and when it changed to N, the gases experienced a sharp decrease. The SE wind direction corresponded to the usual sea breeze that occurs at MSY, with the wind coming valley upwards from the Barcelona metropolitan region. When the wind was blowing from the W, the air was coming from another valley, and although *Q. ilex* is the dominant plant species there too, before arriving at the MSY site the air was passing over Pla de la Calma (1100-1300 m a.s.l.), a plateau covered by heath communities of *Calluna vulgaris*, *Pteridium aquilinum*, *Festuca ovina*, and *Scleranthus annuus* (Bolòs, 1983). This different vegetation, much less dense and with less biomass, likely emits much less VOCs than the holm oak forests of the SE valley.

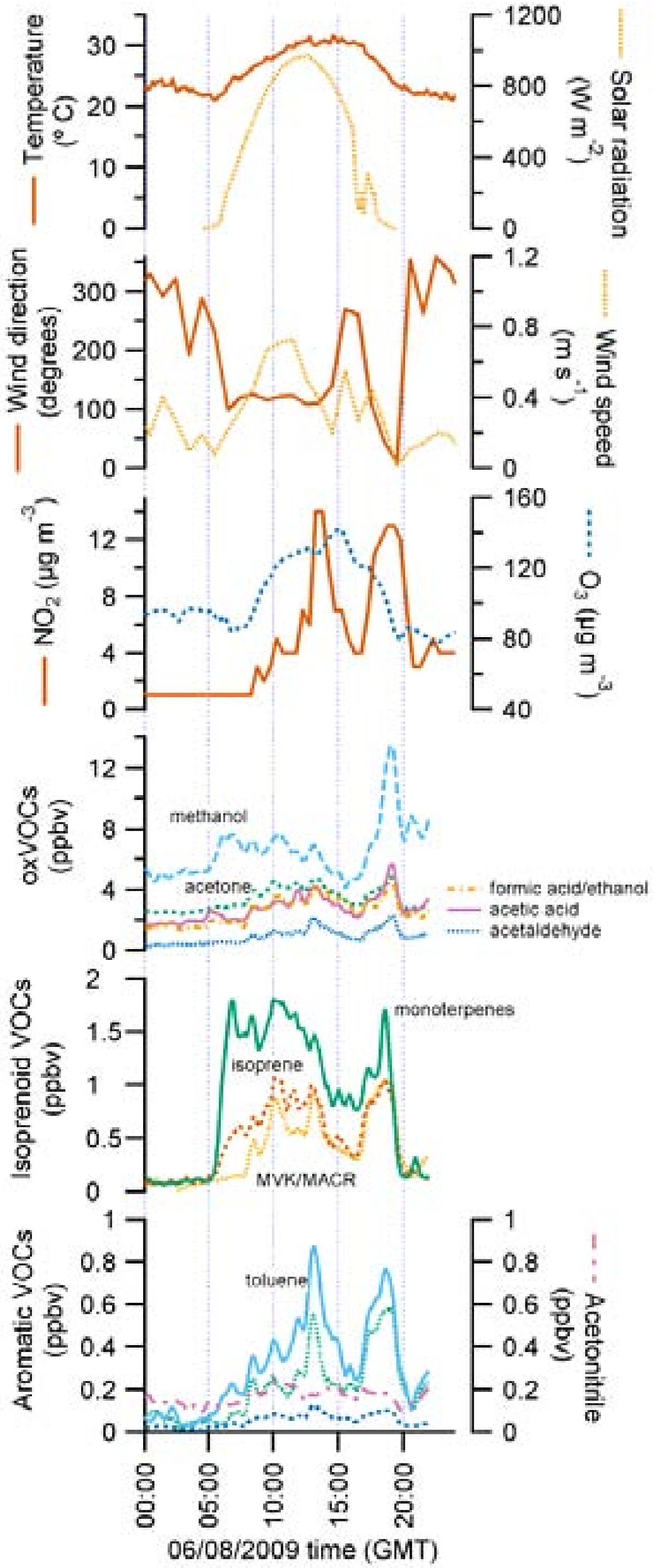

Fig. 10. Daily cycles of most part of the variables studied, corresponding to the 6th of august 2009. Panels and traces are the same as in Fig. 8.

Summary and conclusions

VOC mixing ratio measurements in an elevated Mediterranean forested site (MSY) with a dense vegetation dominated by *Quercus ilex* were performed. There were two sets of measures, one in winter (February-March) and another in summer (July-August). While aromatic VOCs showed almost no seasonal variability (only benzene had lower summer mixing ratios), short-chain oxygenated VOCs presented higher mixing ratios in summer, presumably due to the increased emission by vegetation and the increased photochemistry, both enhanced by the high temperatures and solar radiation in summer. Methanol was the most abundant compound among all the measured VOCs in both seasons.

Isoprenoid VOCs (monoterpenes and isoprene, and its oxidation products MVK/MACR) were involved in the biggest change in mixing ratios between winter and summer. That is, their mixing ratios increased by one order of magnitude, as a result of the increased vegetation physiological activity and emission rates. Ozone highest diurnal concentrations increased in summer too, due to more photochemical activity and the presence of more reactive VOCs in the air.

VOC mixing ratios analysis at MSY forest site in the Western Mediterranean Basin showed that their daily variation is mainly governed by the wind regime of the mountain, as most part of the VOC species analysed followed a very similar daily cycle. Sea breeze that develops after sunrise advects to the mountain polluted air masses that previously had passed over the Barcelona metropolitan region, where they were enriched in NO_2 and in VOCs of anthropic and biotic origin. Furthermore, these polluted air masses can react with biogenic VOCs emitted at the MSY valley by the vegetation, thus enhancing O_3 formation in this mountain site. The only VOC species that showed an own, different daily pattern –though still affected by the wind regime– were monoterpenes, because they were emitted by the local vegetation of the valley. Isoprene also shared partially the daily pattern of monoterpenes, but only in summer when its biotic sources were stronger.

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Conclusions generals

Conclusions generals

Dels capítols precedents se'n deriven les següents conclusions:

Capítol 1

- Els oxCOVs tenen moltes fonts, tant naturals com antropogèniques, i arriben a concentracions destacables a l'atmosfera, essent el metanol el més abundant a nivell global. Molts d'ells poden tenir implicacions per a la química atmosfèrica, en la formació de radicals reactius com peroxacetil nitrat (PAN), ozó i radicals OH.
- Els oxCOVs són espècies poc reactives, aquest fet condiciona que tinguin una vida atmosfèrica llarga i per tant puguin tenir efectes en llocs allunyats d'allà on van ser emesos (transport a llarga distància).
- L'intercanvi planta-atmosfera d'oxCOVs està regulat pel gradient de concentració entre l'interior de la planta i l'exterior. Això vol dir que, generalment, com més gran és la concentració ambiental d'un compost, menor serà la seva emissió, i fins i tot serà absorbit per la planta.
- La cutícula és una possible via d'intercanvi d'oxCOVs, però sens dubte la més important són els estomes. L'explicació d'aquest fet rau en la gran solubilitat en aigua que presenten aquests compostos, que lliga parcialment el seu intercanvi a la transpiració.
- Diferents tipus d'estrès, com atacs d'herbívors o sega, arrels negades en aigua, exposició a elevades concentracions d'ozó, etc., en general augmenten les emissions d'aquests compostos.
- Resten múltiples incògnites sobre les fonts, embornals i intercanvis planta-atmosfera d'oxCOVs, la qual cosa fa necessaris futurs experiments dedicats a esclarir-les, com els que es presenten en aquesta tesi.

Capítol 2

- L'intercanvi de formaldehid va ser regulat per la conductivitat estomàtica i pel gradient de concentracions.
- En pi blanc i alzina el punt de compensació que vàrem trobar pel formadehid va ser al voltant de 20 ppbv. Concentracions per sobre d'aquest valor resultarien en absorció de formaldehid.
- Els oxCOVs emesos en major quantitat per pi blanc van ser metanol (en condicions normals) i acetona (en condicions de sequera). Aquesta darrera era l'únic oxCOV que no seguia una clara relació amb la conductància estomàtica, mentre que el metanol va ser el que va disminuir més l'emissió durant la sequera. L'àcid acètic va passar de ser emès en condicions normals a ser absorbit després del reg.
- En el cas de l'intercanvi d'acetaldehid en el pi blanc, el punt de compensació que vam observar va ser de 6 ppbv.
- La sequera va afectar l'intercanvi d'oxCOVs en tant que afectava l'obertura estomàtica. Per això caldrà veure quin impacte sobre les emissions d'oxCOVs genera l'escalfament climàtic i la disminució de les precipitacions previstos per les properes dècades.
- L'alzina, tradicionalment considerada una espècie no emissora d'isoprè, es va veure en aquest i altres estudis que n'emet, encara que en quantitats petites.
- Les emissions d'isoprè i monoterpens en alzina es van reduir per la sequera, i després del reg van seguir els canvis en temperatura, mentre que en condicions control i de sequera seguien els canvis en llum i fotosíntesi.

Capítol 3

- En l'experiment de comunicació planta-planta, la fumigació amb metanol va fer augmentar l'eficiència fotosintètica i la ràtio carotenoids/clorofila a, de forma similar al que va provocar el fet de tallar parts de fulles per simular herbivoria.
- La fumigació amb metanol va fer augmentar, també, l'emissió de monoterpens el dia després del tractament. Altres VOCs s'havia vist anteriorment que també provocaven un augment de les emissions de terpenoids. L'augment no va ser de gran magnitud (14%), tot i això cal interpretar-lo en el marc d'unes emissions més complexes, on cada VOC individual pot afegir-se o complementar l'efecte d'altres.
- L'increment d'emissió de monoterpens pot esdevenir, al seu torn, un nou senyal, i així continuaria difonent el missatge començat pel metanol.
- En l'experiment de comunicació animal-animal, tant els volàtils del capoll com els dels excrements de prepupes de l'abella *Osmia cornuta* varen atraure les femelles de la vespa parasitoide *Monodontomerus aeneus*, tot i que els excrements sols van ser més atractius que el capoll, i els excrements amb el capoll més atraients que tan sols els excrements. Les femelles no van ser atretes per la prepupa d'*O. cornuta*.
- El capoll emetia majoritàriament metanol i els excrements, àcid acètic. No obstant, el capoll presentava una major emissió de tots els COVs, incloent l'àcid acètic.
- En les proves d'olfactòmetre, l'àcid acètic atreia les femelles de *M. aeneus*. Ara bé, la ràtio àcid acètic/acetaldehid característica dels excrements va resultar més atraient que altres ràtios.
- Probablement un senyal complex que inclou els diversos COVs està implicat en el procés d'atracció dels parasitoids.

Capítol 4

- El COV més abundant de tots els mesurats a la vall de la Castanya (sud del massís del Montseny) va ser el metanol, tant a l'hivern com a l'estiu.
- Els nivells de COVs aromàtics d'origen antròpic pràcticament no variaren entre hivern i estiu, només el benzè va disminuir una mica a l'estiu a la Castanya.
- Les concentracions d'oxCOVs hi van ser més altes a l'estiu que a l'hivern.
- Els isoprenoids van augmentar un ordre de magnitud les seves concentracions a l'estiu respecte a l'hivern, en el que representa el canvi estacional més espectacular de tots els COVs estudiats. Aquest fet es pot relacionar amb la major activitat fisiològica i d'emissions de la vegetació de la vall, dominada per alzinar.
- Les concentracions de COVs i les seves fluctuacions diaries i estacionals a la vall de la Castanya (Montseny) estaven fortament determinades pel règim de vents, caracteritzat per la brisa mar-terra durant el dia i per la brisa terra-mar a la nit. Això va fer que gairebé tots els COVs fossin transportats des de la regió metropolitana de Barcelona cap al Montseny de dia, registrant-hi les seves concentracions màximes, i retornats cap a la plana de nit, disminuint-ne les concentracions al Montseny.
- Les masses d'aire transportades al Montseny amb tots els COVs i demés contaminants antropogènics de la zona de Barcelona prenen part en reaccions fotoquímiques amb els COVs biogènics de la vall i generen altres contaminants com l'ozó, que es va trobar en més alta concentració a l'estiu que a l'hivern, i també més alta al Montseny que a la ciutat de Barcelona en totes dues estacions de l'any.