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Influence of combined hydric and thermal stresses on *Rosmarinus officinalis* and *Cistus albidus*

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ABSTRACT

Background. Wildfires are a growing threat, especially in Mediterranean climate areas during periods of drought. The wildfire research community continues to investigate propagation mechanisms considering thermal transfer and fluid mechanics and sometimes a simplified chemistry taking into account the main combustion products such as CO, CO₂, H₂O, H₂ and CH₄. However, volatile organic compounds (VOCs) cannot be neglected and have a major role in vegetation ignition. Despite this, knowledge on the influence of wildfire on plant emissions remains lacking. **Aims.** This work addresses the effect of combined hydric and thermal stresses on the physiology and emissions of *Rosmarinus officinalis* and *Cistus albidus*, which are widely consumed in Mediterranean wildfires. **Methods.** Plants were submitted to hydric stress inside a greenhouse, then to thermal stress inside a heated hermetic enclosure while collecting emissions with adsorbent elements for analysis. Simultaneously, heating experiments were carried out with leaves sampled to analyse their emissions. **Key results.** The heating tests showed significant variations in monoterpene, fatty acid and alkane emissions between stressed and unstressed plants with different results between *Rosmarinus officinalis* and *Cistus albidus*. **Conclusion/implication.** Further experiments with other plant species and quantification of emissions may help to better know why Mediterranean wildfires are a particular threat and how to improve firefighting methods.

Keywords: alkanes, chlorophyll, combined hydric and thermal stresses, fatty acids, hydrocarbons, leaf and plant emissions, microscopy, physiology analyses, plant heating, Py-GC-MS, radiant panel, *Rosmarinus officinalis*, *Cistus albidus*, solid-phase extraction elements (SPEE), TD-GC-MS, terpenes, thermodesorption, water content.

Context and objectives

Wildfires are a growing issue, especially in Mediterranean climate areas during periods of drought where more and more uncontrollable fires are observed, such as megafires, too large and strong to be confined, and eruptive fires, too fast and unpredictable to be fought. These last years have been particularly disastrous with megafires widely reported in the worldwide media (Silveira *et al.* 2022). As for eruptive fires, these are sudden and impressive wildfire accelerations generally occurring with moderate wind or even without wind. They have caused more than 150 fatalities in Europe and the USA so far (Carbonell *et al.* 2005; Viegas 2009; Chetehouna 2014; Chatelon *et al.* 2014; Coudour *et al.* 2016b; Lahaye 2018), mainly among firefighters involved in extinguishing operations.

According to Schneider *et al.* (2021) 'there is a strong need to increase the basic understanding of the propagation mechanisms of wildfires to improve the scientific tools needed for firefighting and fire prevention'. Classical propagation of a wildfire is mainly due to two correlated mechanisms: large heat transfers (energy) and formation of flammable mixtures ahead of the fire front (gases). Heat is transmitted principally by thermal radiation and convection. Flammable mixtures are formed from the only available fuel (vegetation) while it is heated, pyrolysed and burnt. The wildfire research community continues to investigate propagation mechanisms of fire fronts such as thermal radiation

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(Frangieh *et al.* 2020) or buoyant flame dynamics (Finney *et al.* 2015). Research on eruptive fires is also focusing on airflow considerations (Viegas and Caballero 2009; Chatelon *et al.* 2014) such as induced wind, flame attachment and inclination angle. The mentioned studies on wildfire propagation take into account large-scale mechanisms such as thermal and fluid mechanics ones, or take into account the main chemical compounds resulting from degradation and combustion (CO, CO₂, H₂O, H₂ and CH₄). However, there is a lack of investigation on the role of plants (Silveira *et al.* 2022) and the large range of molecules involved in wildfire propagation at the fire front, given that Mediterranean plants are particularly rich in volatile essences (Owen *et al.* 2001).

During the day, vegetation emits O₂, water, CO₂ and also volatile essences (Owen *et al.* 2001). Volatile essences contain mainly isoprenoids (Ormeño *et al.* 2007; Lavoit 2010; Ciccioli *et al.* 2014; Courty *et al.* 2014), which are produced by biosynthesis (Nagegowda and Gupta 2020) and emitted essentially by plant leaves (Lavoit 2010). Emitted isoprenoids include isoprene (C₅H₈), monoterpenes (C₁₀H₁₆), sesquiterpenes (C₁₅H₂₄) and their oxides, e.g. verbenone (C₁₀H₁₄O). These are very flammable compounds (Courty *et al.* 2012; Rudz *et al.* 2014) and their density, up to seven times higher than air for sesquiterpenes (C₁₅H₂₄), favours their accumulation at ground level. For a given plant species, emissions strongly depend on the season, time of day, meteorological conditions, plant history, drought, light, etc. (Lavoit 2010).

Among different plant stresses (Lemoine *et al.* 2013), thermal and hydric stresses are the main ones affecting vegetation during wildfires. Research has been carried out to estimate the main gases emitted by vegetation; studies have been carried out *in situ* with ordinary temperatures (Owen *et al.* 2001) or in laboratory conditions for higher temperatures (Chetehouna 2014). Results show that emissions increase quantitatively when the vegetation is heated, with a maximum around 180°C (Barboni 2006; Chetehouna 2014; Courty *et al.* 2014) corresponding to the boiling point of the main monoterpenes. Plant emissions have also been studied under hydric stress with conflicting results depending on plants and methodologies (Lavoit 2010). Hydric stress affects many functioning parameters of plant emission zones such as leaf temperature, stomatal conductance, photosynthesis and leaf surface (Lemoine *et al.* 2013). With hydric stress, stomata generally close in order to limit transpiration, thus inducing a decrease in absorption of carbon dioxide from the air, which involves a decrease in photosynthesis, but as far as we know, no explanation has been found concerning its influence on isoprenoid biosynthesis. According to experimental protocols and studied plant species, an increase or a decrease in volatile organic compounds emitted by plants can be seen under hydric stress, e.g. a decrease in sesquiterpenes emissions for *Rosmarinus officinalis* and an increase for *Cistus albidus* (Ormeño *et al.* 2007). Isoprenoid content generally increases in plant leaves with hydric stress for conifers and aromatic plants (Llusà and

Peñuelas *et al.* 1998), which makes them more flammable. Indeed, even if emissions do not increase, once exposed to a fire front, a plant will necessarily release its isoprenoid reserves owing to thermal degradation.

Thermal stress also affects functioning parameters of plant emission zones such as stomata. As mentioned above, maximum emission of isoprenoid is generally found at ~180°C. At temperatures between 200 and 280°C, hemicellulose is degraded, which leads to an increase in emissions of CO and CO₂ (Barboni 2006). Emission of acetic acid and methanol and dehydration of cellulose are also observed. Then, an exothermic phase occurs between 280 and 320°C with the emission of the aforementioned gases in addition to low molecular weight hydrocarbons like methane, ethane or ethylene. From 240 to 360°C, dehydrocellulose (Yang *et al.* 2018) is degraded to char and volatile material. Finally, lignin is degraded from 280 to 500°C with high emissions of char and phenolic compounds. Concerning plant emission flow rates of volatile organic compounds (VOCs), for Mediterranean vegetation emissions, data are principally available for seasonal temperatures, e.g. 50 kg/km² h for Valencia (Spain) (Owen *et al.* 2001). Very few authors have quantified emissions from heated plants. We found data for pine twigs (Zhao *et al.* 2012) with emissions of 16.314 µg/g dry weight (DW) of terpenoids after heating for 15 min at 200°C versus 0.042 at normal temperature. We observed that emissions may be multiplied ~400 times when a plant is heated. Depending on the species and measurement methodology, emissions are multiplied by eight for *Rosmarinus officinalis* (Chetehouna 2014) and more than 4000 times for *Cistus monspeliensis* (Barboni 2006) when the plant is heated. Regarding the flow rate of fire front emissions, presenting heterogeneous temperatures between 40 and 1000°C, interesting values of emission factors (EF) are available in the literature (Akagi *et al.* 2011; Simpson *et al.* 2011), e.g. for boreal wildfires: 4.9 g of aromatics by kg of burnt vegetation (phenols 60%, other aromatics 40%) and 3.24 g of isoprenoids (Akagi *et al.* 2011).

Thus, in the literature, the influences of hydric and thermal stresses on plant emissions have been studied independently but, to our knowledge, no one has studied them in combination despite their major influence during wildfires. For a better understanding of wildfire propagation from biological and thermochemical points of view, this project studied the influence of combined hydric and thermal stresses on plant physiology and emissions at the leaf and plant scales.

The objective of this study was to understand the effect of combined hydric and thermal stresses on the emission behaviours of two Mediterranean species particularly affected by wildfires, *Rosmarinus officinalis* and *Cistus albidus*. To this end, the two species were subjected to hydric stress in a first phase with a well-watered (control) and a water-deficit group. Both were then subjected to thermal stress in a second phase by lateral thermal radiation to approximate the conditions of the arrival of a distant wildfire. During these tests, we studied plant behaviour by analysing their physiology and the

composition of their emissions. The experiments were carried out at the plant scale for real conditions and at the leaf scale for more controlled conditions.

Material and methods

Plant growth conditions

Rosmarinus officinalis (Lamiaceae) and *Cistus albidus* (Cistaceae) (Fig. 1) were chosen as Mediterranean plants typically affected by documented eruptive fires (Courty et al. 2014) but also as physiologically different plants. The plants were grown in a mixture of potting soil and vermiculite (3/1, v/v) in pot of diameter 20 cm and height 16 cm for *R. officinalis* and of diameter 16 cm and height 12 cm for *C. albidus*. Twenty plants of each species grown under well-watered condition and 20 others were submitted to hydric stress by stopping watering for 16 days: water-deprived conditions. All the cultures were placed in a greenhouse whose average temperature during the period of the experiments was $22.5 \pm 5^\circ\text{C}$, and a photoperiod of 16 h day/8 h night.

Leaf water and chlorophyll content

The fresh leaf fragment was weighed (fresh weight, FW), then dried for 24 h at 80°C to determine its dry weight (DW). Based on these measurements, the leaf water content (LWC) was calculated: $\text{LWC} = (\text{FW} - \text{DW})/\text{FW}$. Approximately 0.5 g of fresh leaf was used to extract chlorophyll using acetone, and the total chlorophyll, chlorophyll *a* (Ca) and chlorophyll *b* (Cb) of the centrifuged solution were measured using the classical spectrophotometric method (Mackinney 1941). To estimate the available water for the plant in the pot, the soil volumetric water content was measured with a portable Fieldscout TDR 150 soil moisture instrument (Spectrum Technologies Inc.) with 12-cm long rods.

Microscopic analyses

Leaf samples were immediately fixed for 1 h at 4°C in a mixture of 2% paraformaldehyde and 0.5% glutaraldehyde in 0.05 M phosphate buffer (v/v v), pH 7.2. After three

washes for 20 min each in 0.2 M phosphate buffer, pH 7.2, supplemented with 7.5% sucrose, a post-fixation step in 1% osmium tetroxide for 5 min followed. The dehydrated samples were embedded in LR White resin according to Fleurat-Lessard et al. (2016). Cross sections were made using a microtome (EMUC6, Leica, Wetzlar, Germany), stained with toluidine blue and observed with light microscopy (Zeiss Axioplan, Berlin, Germany).

Heating tests at leaf scale

During the plant hydric stress conditioning, leaf samples were cut from both well-watered and water-deficient plants, and heated using a Multi-shot Pyrolyzer EGA/PY-3030-D (Frontier lab) coupled with a QP2010 Ultra (Shimadzu) gas chromatograph-mass spectrometer (GC-MS). The heating products were carried by helium gas flow at 1 mL/min to the GC and separated on a TR5-MS capillary column (30 m, 0.25 mm internal diameter, 0.25 μm phase thickness). The split/splitless injector was set to 250°C with a 50/1 split ratio. The temperature of the GC oven was programmed from 60°C (1 min isotherm) to 300°C (11 min isotherm) at a $5^\circ\text{C}/\text{min}$ rate. The interface temperature was set to 280°C . The MS was a quadrupole analyser, the ionisation mode was electron impact (70 eV) and the temperature of the source was set to 200°C . Acquisition was carried out in full scan mode from m/z 50 to 600 at 0.2 amu/s.

Fifteen milligrams of leaves (equivalent to one rosemary leaf or a piece of cistus leaf) was placed into an stainless steel cup and heating was carried out in isothermal mode at 180°C for 1 min. This temperature was chosen as it produced the highest VOC emission yield (Courty et al. 2014). Leaves were stored inside glass tubes covered with aluminium foil at $-21 \pm 1^\circ\text{C}$ between experiments.

Heating tests at plant scale

Material

For this part, we used a 1 m^3 compartment equipped with a radiant panel (Fig. 2a). The latter can provide a maximum of 84 kW m^{-2} of radiative flux and is has a fire-resistant glazed window for plant observation. It was instrumented with

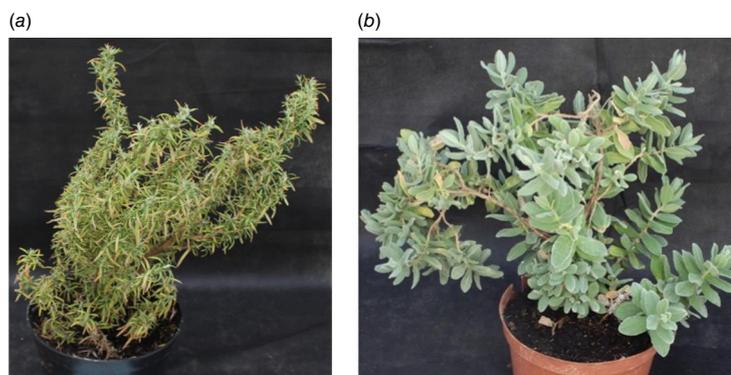


Fig. 1. *Rosmarinus officinalis* (a), and *Cistus albidus* (b) conditioned to hydric stress.

thermocouples, fluxmeters and a cooled scale. Adsorbent tubes were used during heating to trap VOCs, which were thermally desorbed (TD), separated by GC and identified using mass spectrometry using TD-GC-MS (Fig. 2b). The thermodesorption products were identified by comparison of their mass spectra with the NIST (National Institute of Standards and Technology) mass spectral library by their retention times. The adsorbent tubes, known as SPEE (solid-phase extraction elements), were titanium tubes (30 mm × 1.2 mm i.d. × 1.6 mm o.d.) coated with a 500 µm thick phase of polydimethylsiloxane (PDMS). During the tests, the SPEE were placed at the bottom inside (Fig. 3a), at the top inside (Fig. 3b), and at the top outside (Fig. 3c) of the heating compartment. In the cases of 'top' and 'outside', we tried vacuum extraction for active sampling.

Protocol

After hydric stress conditioning, both well-watered and water-deficit plants were retrieved from the greenhouse and introduced one by one into the heating compartment (Fig. 2a). Plants were cut at their bases and, depending on their size, one or two superimposed plants were attached to a stand at 50 cm

from the radiant panel (Fig. 2a). From preliminary tests, we chose to impose a temperature/heat flux ramp of 30 min not to exceed 200°C at the plant stem level in order to avoid hemi-cellulose degradation and study only plant emissions. The temperature ramp rate was ~6°C/min. During each heating test, we monitored the temperature, heat flux and light gases concentration with a continuous analyser. Thermocouples were placed at the same distance from the radiant panel as the stem of the plants (Fig. 2a). SPEE were introduced in the TD-GC-MS device to desorb, separate, identify and quantify VOC emissions. The thermodesorption of adsorbed compounds was performed from 150 to 280°C with a heating rate of 500°C/min and 1 min at the final temperature. Reproducibility was checked with triplicates of SPEE thermodesorption.

Results

Physiological analyses

In the first step, all cistus and rosemary plants were well watered, then at the beginning of the experiment, half of the plants for each species were subjected to water stress by totally

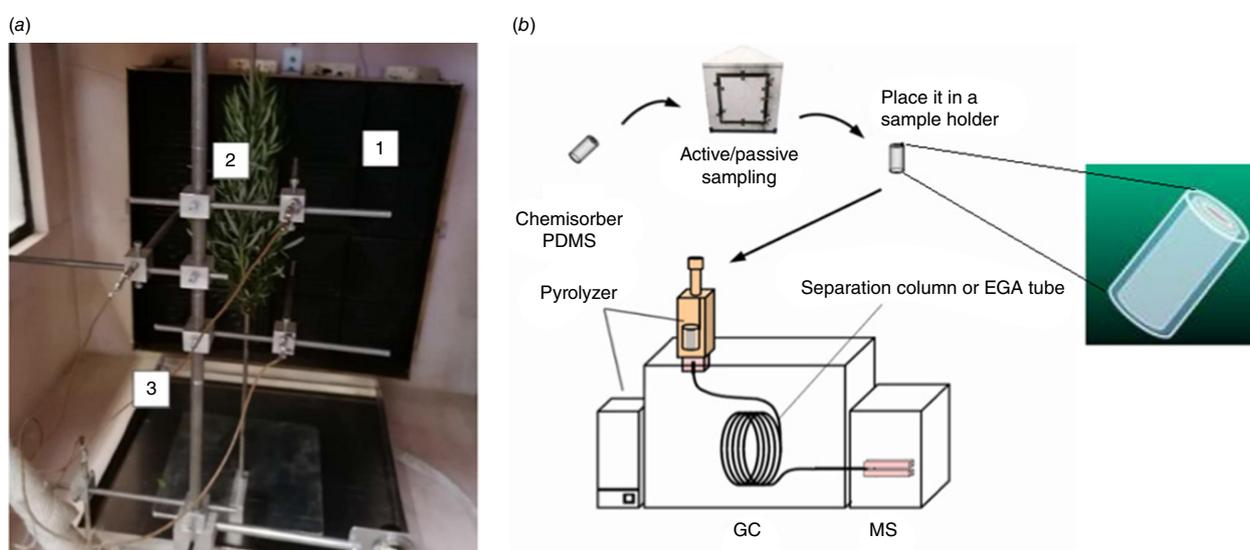


Fig. 2. *Rosmarinus officinalis* plant (2) submitted to thermal stress inside a 1 m³ compartment equipped with radiant panel (1) as well as fluxmeters and thermocouples (3), lent by PRISME laboratory (Bourges) (a). Method of analysis with adsorbent tubes (b).

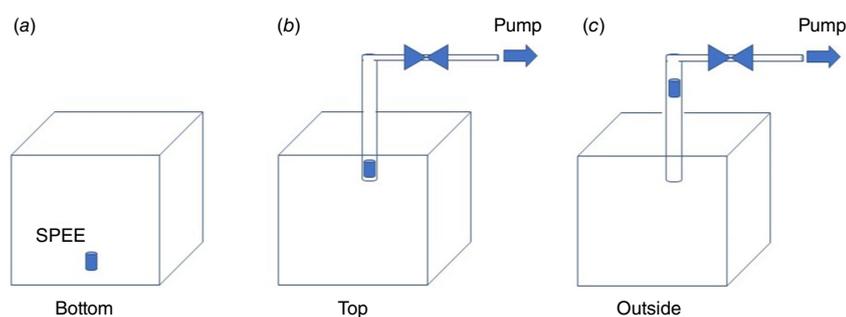


Fig. 3. Three locations were compared for the SPEE: at the bottom inside the heating compartment (a); at the top inside the heating compartment (b); at the top outside the heating compartment (c). In the cases of 'top' and 'outside', we tried vacuum extraction for active sampling.

Table 1. Leaf water content (LWC) in *Cistus albidus* and *Rosmarinus officinalis* leaves under well-watered growing conditions and after 16 days without water supply.

Leaf water content (LWC, %)	<i>Cistus albidus</i>	<i>Rosmarinus officinalis</i>
Well-watered plant	73 ± 7	70 ± 5
Water-deficit plant	62 ± 4	59 ± 3

stopping water supply for 16 days. In parallel, control plants were watered normally for the same amount of time. Physiological analyses were made on 15 leaves by species and by culture conditions to get consistent results. The volumetric water content was 90% for well-watered soil and 30% without water supply. The LWC was 73% ± 7 and 70% ± 5 respectively in cistus and rosemary leaves under well-watered growing conditions. After 16 days without water supply, the LWC of leaves was reduced by 15% and 16% in cistus and rosemary respectively (Table 1). The total chlorophyll content (Ca + Cb) was 4.84 mg/g DW ± 0.72 and 5.64 mg/g DW ± 1.38 respectively in cistus and rosemary leaves under well-watered growing conditions (Fig. 4). After 16 days without water, the total chlorophyll content (Ca + Cb) of leaves was reduced by 52 and 51% (56 and 44% for total chlorophyll content by direct measurement at 652 nm) in cistus and rosemary respectively. The decrease in total chlorophyll content is due to both lower chlorophyll *a* and chlorophyll *b*.

Light microscopy of cistus leaf sections under well-watered growing conditions shows the presence of an upper epidermis (adaxial) composed of bulky cells with thick cell walls (Fig. 5a, b). The palisade parenchyma and the lacunar parenchyma are composed of cells whose contents have a homogeneous aspect. The lower epidermis (abaxial) is composed of cells with a thicker cell wall than parenchyma cells. For both upper and lower epidermis, the cell walls are lignified (Fig. 5a, b). The presence of stomata is visible only on the lower surface of the leaves. After 16 days of water deprivation, the content of the cells appears denser and more heterogeneous, especially in the cells of the palisade parenchyma, which are plasmolysed (Fig. 5c, d). The volume of the palisade parenchyma cells is reduced. The presence of stomata is visible on the lower surface of the leaves (Fig. 5b, d). The different tissues are much more compact compared with the leaves under well-watered conditions (Fig. 5a, c). In the case of rosemary under well-watered conditions, there is a visible cuticular layer (Fig. 5e, f). The adaxial epidermis consists of a thin cell layer. The sclerenchyma is composed of large cells with thick cell walls and with visible pits. The palisade parenchyma and lacunar parenchyma show turgid cells where chloroplasts are pushed against the cell wall by the vacuole. After 16 days without water supply, the cell wall of the cells forming the adaxial epidermis are considerably thickened. Moreover, the sclerenchyma tissue is reduced and the cell wall is further stained by toluidine blue (Fig. 5g, h). Some cells of the palisade parenchyma show a

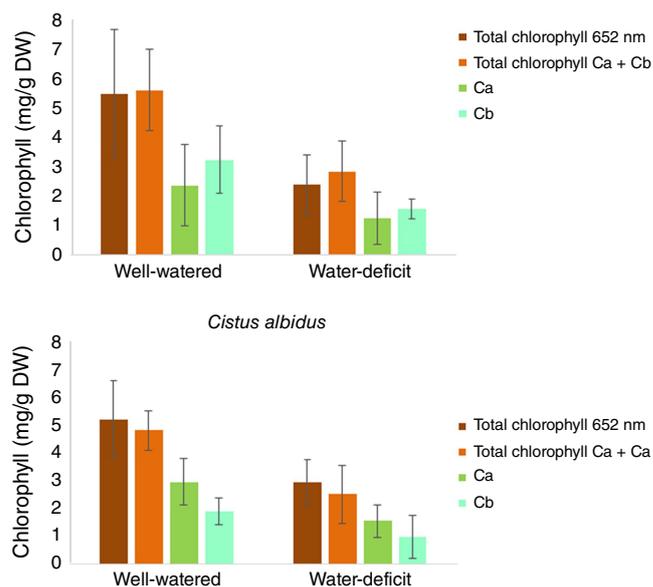


Fig. 4. Chlorophyll content per gram dry weight of *Rosmarinus officinalis* and *Cistus albidus* leaves. Total chlorophyll content by direct measurement at 652 nm wavelength: Chlorophyll *a* content (Ca); Chlorophyll *b* content (Cb); Total chlorophyll content calculated: Ca + Cb.

turgid protoplast while others are empty with only the cell wall visible. The thickness of the leaves is reduced compared with the leaves under well-watered conditions and they also appear more compact (Fig. 5e, g).

Emitted VOCs from heated sampled leaves

In this section, we heated the leaves sampled on both well-watered plants (control group) and water-deficit plants. The pyrolysis of cistus and rosemary samples was carried out in triplicate and showed good reproducibility (Fig. 6). During heating tests, *C. albidus* mainly emitted long chain alkanes, which accounted for 85% of the total emissions for the well-watered group leaves and 68% for the water-deficit plant ones (Fig. 7). Long C₂₃ to C₃₂ alkanes were observed for both well-watered and water-deficit group leaves with odd compounds being predominant. This alkane distribution, characteristic of plant origin, is generally associated with the epicuticular waxes and protective layer in vascular plants (Pascaud et al. 2017). Shorter C₂₁ and C₂₂ alkanes were only emitted by the stressed plant. Short fatty acids from C₈ to C₁₈, which represented 5% of the emissions for the well-watered group, increased to 27% for the stressed leaves while their distribution remained similar (Fig. 7). Terpenes were only emitted by well-watered group leaves, and accounted for 3% of the total emissions.

Rosemary mainly emitted hydrocarbons, terpenes, fatty acids and acetates (Figs 8 and 9). Alkanes accounted for respectively 69 and 34% and terpenes respectively 25 and 58% of the total emissions of well-watered and water-deficit rosemary. The main monoterpenes were α -pinene, eucalyptol,

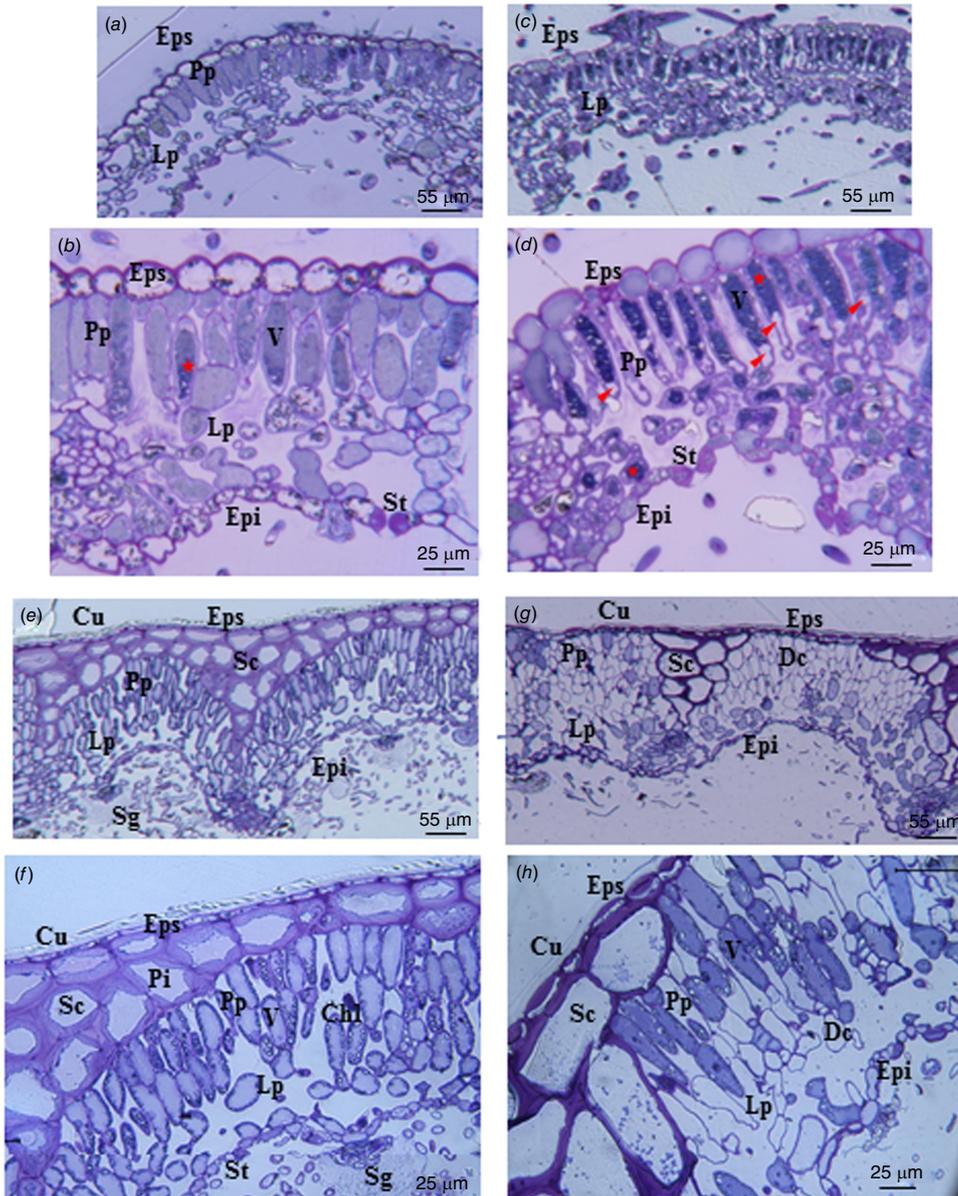


Fig. 5. Cross section of *Cistus albidus* (a–d) and *Rosmarinus officinalis* (e–h) leaves; plant growing conditions: well-watered (a, b, e, f), and without water supply for 16 days (c, d, g, h). Cu, cuticle; Eps, superior epidermis (adaxial); Epi, inferior epidermis (abaxial); Sc, Sclerenchyma; Pi, pit; Pp, Palisade parenchyma; Lp, lacunar parenchyma; Chl, Chloroplast; V, vacuolar; St, stomata; Sg, Secretory gland; Dc, Dead cell. Tannins, asterisk; cell plasmolysis, arrow. Semi-thin section, toluidine blue staining, optical microscopy.

linalool, camphor, borneol and verbenone. Long-chain alkanes ranged from C₂₇ to C₃₃ and their distribution was not affected by hydric stress (Fig. 8). Short even-chained fatty acids were observed from 12 to 18 carbon atoms and slightly increased from 4 to 7% with hydric stress. Tetramethoxyflavone and ferruginol, which were observed as traces in the well-watered group, accounted respectively for 1 and 3.5% in water-deficit rosemary.

Emitted VOCs from heated plants

Influence of SPEE location

In order to optimise the adsorption of emitted VOCs, three locations were compared for the SPEE: at the bottom inside the heating compartment (bottom, Fig. 3a); at the top

inside the heating compartment (top, Fig. 3b); at the top outside the heating compartment (outside, Fig. 3c). In the cases of ‘top’ and ‘outside’, we used active (vacuum extraction) or passive sampling. Fig. 10 compares the results of the three SPEE locations for heating tests on water-deficit *Rosmarinus officinalis*.

Active sampling with the adsorbent tube placed outside the heating compartment demonstrated the most representative results as the alkanes obtained were long odd-chained, which is characteristic of a plant distribution (Kolattukudy 1976).

Influence of the sampling delay

For some tests, incandescence was observed for the leaf closest to the radiant panel. This seemed to have little

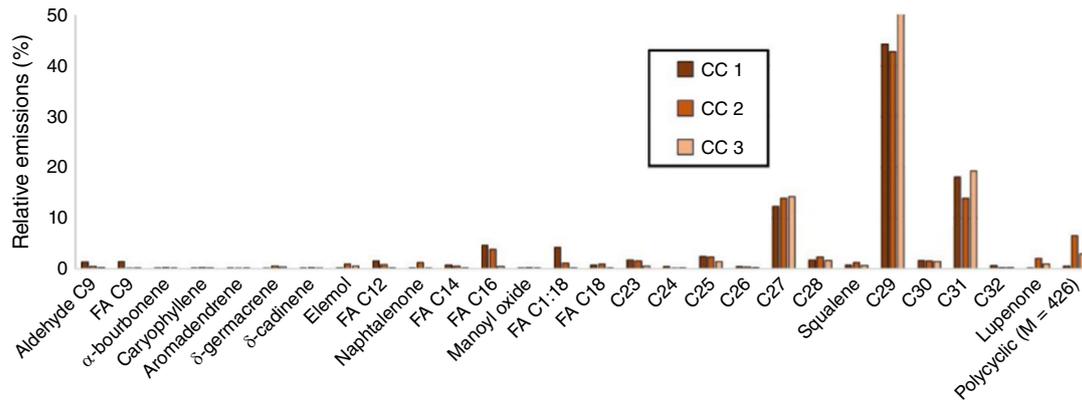


Fig. 6. Reproducibility of relative abundance of products from leaf heating of well-watered *Cistus albidus* (180°C for 1 min). M is for molar mass (g/mol), FA is for fatty acid and Cn is for alkane, n corresponding to the number of carbon atoms.

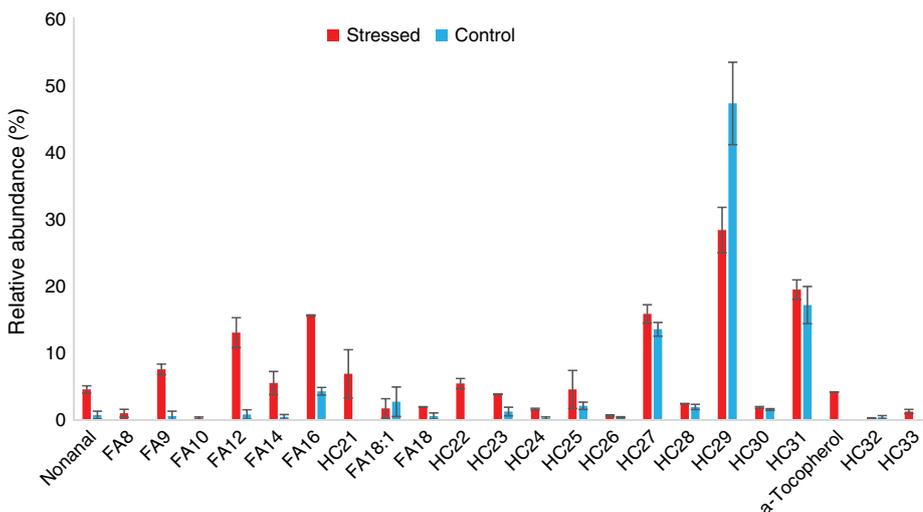


Fig. 7. Relative abundance of products from leaf heating of well-watered and water-deficit *Cistus albidus* (180°C for 1 min). HCn, alkane; FAn, fatty acid; n, number of carbons.

impact on results because the results obtained while activating the pump at different moments before and after glowing were similar (Fig. 11).

Long alcohol chained esters (octanoates, decanoates and dodecanoates) were observed amongst the heating products of *R. officinalis* at the plant scale. Long-chain esters can originate from the cutin, a biopolymer part of the cuticle of plants. These hydrophobic polyesters give their glossy aspect to leaves or non-woody stems and protect the plant from water loss, among other functions (Heredia 2003).

Discussion

The LWC is a good drought indicator because it is intimately connected with atmospheric aridity, soil water availability and plant drought tolerance (Zhou et al. 2021). In our greenhouse conditions, under well-watered growing conditions, we found that the LWC value of $70\% \pm 5$ for rosemary

was comparable with the literature (73% ; Szumny et al. 2010) whereas for cistus, the mean value of $73\% \pm 7$ was low compared with other studies (*C. creticus* 80.4% ; Stępień et al. 2019). Also, we hypothesise that our cistus well-watered plants were probably under slight water stress. Otherwise, compared with the LWC of *Arabidopsis thaliana* in well-watered growth conditions ($93.3 \pm 1.1\%$; Slawinski et al. 2021), a species with no particular adaptation to drought and which is not a so-called Mediterranean plant, the LWC values of rosemary and cistus are low.

The leaves of rosemary and cistus are typical of Mediterranean species adapted to drought and they are characterised by the presence of a thick cuticle in the case of rosemary and a dense abaxial indumentum for cistus. In our experimental conditions, in cistus, the heterogeneous aspect in the vacuole is probably due to the presence of tannin, these cells exhibiting a blue–black colour with toluidine blue (Robil and Tolentino 2015). Indeed, tannins in vacuoles generally have polymorphic aspects that have been

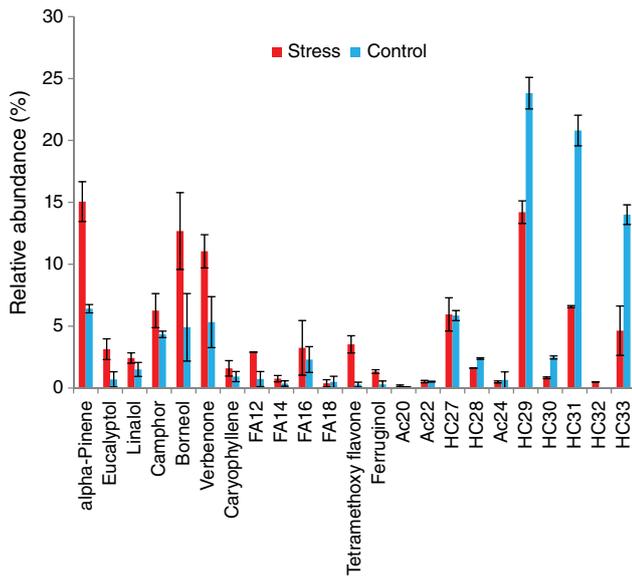


Fig. 8. Relative abundance of products from leaf heating of well-watered and water-deficit *Rosmarinus officinalis* (180°C for 1 min). HCn, alkane; FAn, fatty acid; Acn, acetates, n, number of carbons.

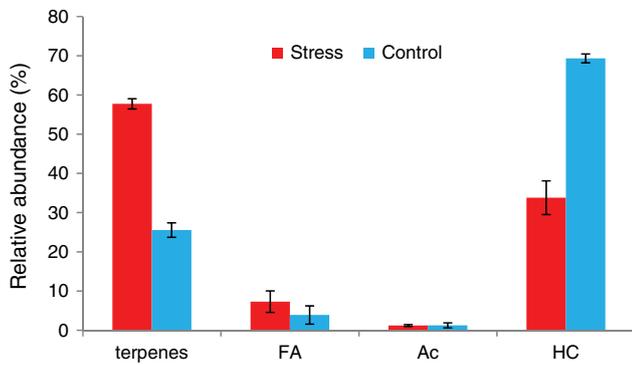


Fig. 9. Relative abundance of products from leaf heating of well-watered and water-deficit *Rosmarinus officinalis* (180°C for 1 min). HC, alkanes; FA, fatty acids; Ac, acetates.

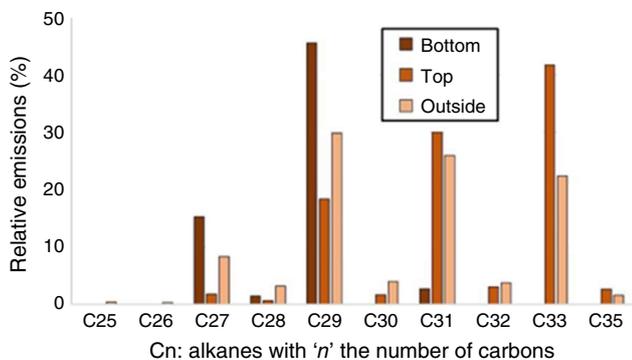


Fig. 10. Alkanes distribution for different locations of SPEE during heating tests at the plant scale for water-deficit *Rosmarinus officinalis*.

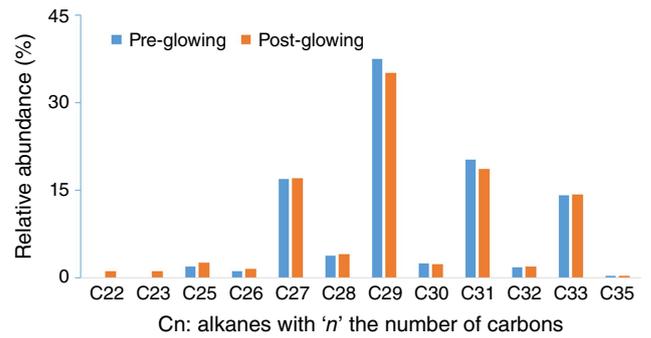


Fig. 11. Alkanes distribution trapped in SPEE placed outside the heating compartment during the tests at the plant scale for well-watered *Rosmarinus officinalis* with active sampling.

described as semifluid, with a granular, filamentous, or reticular network or as solid, with dense corpuscles with an amorphous arrangement (Fleurat-Lessard *et al.* 2016). The presence of polyphenols such as tannins in cistus under normal developmental conditions has been widely reported (Nicoletti *et al.* 2014) and, more generally, the positive effect of water deficit on these secondary metabolites (Munné-Bosch *et al.* 2003; Bucchetti *et al.* 2011).

In our experimental conditions, the anatomic studies of rosemary demonstrated that a water loss in the leaves of 15.5% modified the structure of the latter, with a thickening of the wall of the cells of the upper epidermis and modification of the cell wall of the sclerenchyma cells. The cell wall staining suggests an increase of lignification, an adaptation to drought that limits non-stomatal transpiration (O'Brien *et al.* 1964; Olmos *et al.* 2006; Herrera-Ubaldo and de Folter 2018). The lower thickness of the leaf and the density of the tissues is also a consequence of the loss of water in the cells (which reduces gas exchange). These changes in leaf anatomy affect CO₂ diffusion (Chartzoulakis *et al.* 2002). Also, we observed a high amount of dead cells compared with rosemary grown in well-watered conditions. Our experimental conditions in terms of water deficit were quite drastic owing to the low volumetric soil water content (30%). All the dead cells present in the parenchyma no longer contained chlorophyll, which explains the sharp decrease in chlorophyll content in rosemary (56%). These cells no longer participate in the metabolism and thus in the synthesis of terpenes.

The rosemary and cistus responded in a similar way to water deficit with a decrease in total chlorophyll content (Fig. 4) due to the increased degradation of photosynthetic pigments (Enneb *et al.* 2020) and to the death of some cells (Fig. 5h) due to the low plant LWC (Table 1: 59% ± 3 and 62% ± 4 respectively for rosemary and cistus leaves). Water deficit affected chlorophyll *a* and chlorophyll *b* content in the same way and had an impact on photosynthesis and secondary metabolism (Azhar *et al.* 2011).

However, the relative percentage of terpenes and fatty acids emissions increased with hydric stress whereas the relative percentage of alkanes decreased for rosemary. The

Table 2. Properties related to vegetation auto-ignition of the main compounds detected during a wildland fire.

Molecule	LFL% vol at 25°C (NF EN 1839 2017)	AIT (°C)	Density/air	Boiling point (°C)	Emission factor (EF, g/kg burnt) pine + oak (Akagi et al. 2013)
Benzene C ₆ H ₆	1.4	555	2.7	80	1.7
Toluene C ₇ H ₈	1.2	480	3.1	111	0.9
Phenol C ₆ H ₆ O	1.7	715	3.2	181	?
Isoprene C ₅ H ₈	1.5	220	2.3	34	?
Monoterpenes C ₁₀ H ₁₆	0.7	255	4.7	156	13.2
Sesquiterpenes C ₁₅ H ₂₄	0.5	?	7	245	?
Alpha-kaurene C ₂₀ H ₃₂	0.4	?	9.5	344	?
Tetradecane C ₁₄ H ₃₀	0.5	200	6.83	253	?
Lauric acid C ₁₂ H ₂₄ O ₂	0.6	261	6.91	225	?
Pentacosane C ₂₅ H ₅₂	?	?	12	403	?
Carbon monoxide CO	12.5	609	0.98	-191	222
Carbon dioxide CO ₂	-	-	1.5	-78.5	1305
Methane CH ₄	4.4	537	0.6	-161	10.3
Total hydrocarbons	-	-	-	-	57 (NMOC)

Values are taken from safety data sheets except for LFL and Emission Factors.

increase in relative percentage of fatty acids emission was observed for both plant species. Fatty acids are part of the cutin that acts as a barrier for water loss and pathogen protection (Cahoon and Li-Beisson 2020), which could explain their relative increase with hydric stress. Changes in relative percentages of the emitted compounds with hydric stress could impact plant flammability. Indeed, monoterpenes emitted by rosemary are the most flammable compounds, with a lower flammability limit (LFL) of ~0.7% by volume and an auto-ignition temperature (AIT) of ~250°C. There are few data for fatty acids about LFL or AIT because they have a high boiling point, e.g. 232°C for α -linolenic acid; they are considered less flammable than hydrocarbons with similar carbon numbers, e.g. the flash point of hexadecanoic acid is 206°C and for hexadecane, it is 135°C with an AIT of 201°C, although we found an AIT of 261°C \pm 5°C in the safety data sheet of fatty acids for C10–18 and C12–22-unsaturated, C14–18 and C16–18-unsaturated alkyl esters (Mercuria 2015).

The compounds responsible for plant ignition must be emitted by the plants in quantities of the same order of magnitude as their LFL to compose a flammable mixture with the other compounds. The LFL of gaseous mixtures can be approximately estimated thanks to Le Chatelier's mixing rule (Mashuga and Crowl 2004). To favour plant ignition, gaseous compounds also must have a low AIT. Finally, density may play a role, knowing that a density higher than air will favour accumulation at ground level during heating while the fire is approaching. As for the boiling point, the lower it is, the more easily a compound is emitted by the plant. These different properties are presented in Table 2 for the main compounds detected during wildland

fires. Monoterpenes, long-chain alkanes and fatty acids have properties more favourable than the other molecules to trigger auto-ignition, with lower LFLs and AITs and higher densities. Sesquiterpenes may play a role too but we do not have enough data on AITs and emission factors. We also note that aromatics formed at higher temperatures – with vegetation degradation and combustion, and dispersed by fire smoke – have a higher AIT than the abovementioned molecules but can play a role in the stability of the flame because they have a low LFL (Coudour et al. 2015, 2016a).

Conclusion

Cistus albidus and *Rosmarinus officinalis* reaction to hydric and thermal stresses was monitored through biological, chemical and physical parameters. The physiological analyses showed that *C. albidus*, with its large leaves covered by trichomes, faced hydric stress better than *R. officinalis*, whose chlorophyll content decreased and whose cells began to die rapidly. The VOC emissions determined by pyrolysis coupled to GC-MS at the leaf scale and TD-GC-MS showed differences between the two species. Both emitted mainly long odd-chained alkanes and short fatty acids but *R. officinalis* also emitted monoterpenes, which increased strongly with hydric stress. For both species, fatty acids emissions increased relatively to alkanes, which could be aimed at limiting evapotranspiration. Tests at the leaf scale showed good reproducibility and the increase of monoterpene emissions when *R. officinalis* was hydrically stressed tends to make it more flammable. Alkane emissions of well-watered and water-deficit *R. officinalis* monitored at the plant scale

using SPEE showed similar distribution to those observed at the leaf scale. Results of the composition of emissions at plant scale were in agreement with the ones at leaf scale. This work showed that plant species do not react equally with hydric stress or with combined hydric and thermal stresses. Several experiments were conducted in order to improve emission sampling with SPEE method in the compartment configuration. The active sampling from the outside of the compartment gave the best results. Concerning the influence of combined hydric and thermal stresses on fire propagation, these results suggest that both a lower content of water and a higher content of monoterpenes and fatty acids could induce an increase in plant flammability. Tests will be done in further work to determine experimentally the LFL of gaseous mixtures from wildfires to check the influence of the aforementioned compounds on flammability, theoretical LFL can be estimated thanks to Le Chatelier's mixing rule but it is not accurate enough with these kinds of compounds. The resulting protocol will be used to measure emissions at the plant scale in compartment conditions in further study, to which we plan to add quantitative measurement of emissions. These experiments could be carried out with other plant species to help better know why Mediterranean wildfires are a particular threat and how to improve firefighting methods.

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Data availability. Data used to generate the results in the paper are available on demand.

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