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DOMINGOS XAVIER VIEGAS
LUÍS MÁRIO RIBEIRO

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

Rawaa Jamaladdeen [1]; Bruno Coudour *[1]; Fabienne Dédaldéchamp [2]; Laurent Lemée [3]; Jean-Pierre Garo[1]; Hui-Ying Wang [1]

[1]*Institut P' - UPR 3346 CNRS, ENSMA, Univ. Poitiers, Futuroscope Chasseneuil, France, {rawaa.jamaladdeen, bruno.coudour, jean-pierre.garo, hui-ying.wang}@ensma.fr*
[2] *Unités Mixtes de Recherche, Ecologie et Biologie des Interactions, Université of Poitiers/Centre National de la Recherche Scientifique, Poitiers, France, {fabienne.dedaldechamp@univ-poitiers.fr}*
[3] *IC2MP - UMR CNRS 7285, Universite´ de Poitiers,*
Poitiers Cedex, France, {laurent.lemee@univ-poitiers.fr}

**Corresponding author*

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Abstract

Wildfires are a growing threat, especially in Mediterranean climate areas during periods of drought. Wildfire research community continues to investigate propagation mechanisms on a large scale considering the thermal and fluid mechanics effects, or the main fire emissions (CO, CO2, H2O, H2, CH4). However, research on the effect of abiotic stresses on the plant emission during wildfires remains lacking, despite the fact that Mediterranean are considered important volatile essence emitting and storing species. This article addresses the effect of combined hydric and thermal stresses on physiology and emissions of two important Mediterranean shrub species; Rosmarinus officinalis and Cistus albidus that are largely consumed in wildfires. Different levels of hydric stress were applied on plants of the two species in a greenhouse. Thermal stress was executed by placing the water stressed plants inside a hermetic enclosure equipped with a radiant panel of maximal radiant heat flux of 84kW.m-2 and a fire-resistant glazed window for visualisation. The gaseous emissions of the plants under thermal stresses were collected and analysed by two complementary devices: an instantaneous gas analyser for CO, CO2, H2 and CH4, and adsorbent tubes by using the techniques of adsorption and desorption (by pyrolysis) for emission collection and analyses, respectively. Simultaneous pyrolyses experiments were realised on a foliar scale of the water stressed plants. Gases of pyrolyses separated with gas chromatography and identified using mass spectrometry. The heating tests showed a good reproducibility for pyrolyses of leaf samples and interesting variations between the monoterpene emissions of stressed and unstressed plants. At plant scale, number of tests for each plant species at a given hydric stress level were insufficient to give trends and strong results because of some imposed technical problems and the constraints of public health crisis. However, these tests allowed us to adapt experimental protocols and devices for further testing such as: plant location and fixation, heat flux ramp, sampling location, use of adsorbent tubes, hydric stress duration and normalisation of measured concentrations according to the plant size.

1. Context and objectives

1.1. Context

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 able to fight. These last years have been particularly disastrous with worldwide mediatised megafires (Fernando et al. 2022). As to eruptive fires, these are sudden and impressive wildfire accelerations occurring without wind, or moderate one, which have already caused more than 150 fatalities so far [2-8], mainly among firefighters involved in the 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: important heat transfers

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(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. Wildfire research community continues investigating propagation mechanisms of the fire front such as thermal radiation (Frangieh et al. 2020) or buoyant flame dynamics (Finney et al. 2015). Research on eruptive fires issue are also focusing on airflow considerations (Viegas et al. 2009, Chatelon et al. 2014) such as induced wind, flame attachment, inclination angle, etc. 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, CO_2, H_2O, H_2, CH_4). However, there is a lack of investigation on the role of plants (Fernando et al. 2022) and the large range of molecules released during wildfires at the fire front knowing that Mediterranean plants are particularly rich in volatile essences (Owen et al. 2001). Hydric and thermal stresses have been studied independently showing influence on plant emissions (Barboni 2006, Chetehouna et al. 2014, Ormeño et al. 2007, Lavoir 2010, Ciccioli et al. 2014, Courty et al. 2014, Lemoine et al. 2013) but to our knowledge there is no study on combination of these wildfire stresses. This project intends to better understand the biological and thermochemical aspects of wildfire propagation taking into account a wider range of molecules involved in it and the role of two combined wildfire stresses.

1.2. Objectives

Experiments consisted of submitting two plant species (*Rosmarinus officinalis* and *Cistus albidus*) to different levels of hydric stress first, and then to a thermal stress similar than the one of the arrival of a wildfire. During these tests, we studied plant behaviour by analysing their physiology and their emissions (composition and concentration) at leaf and plant scale.

1.3. Team and funding

This study was conducted in the mark of Rawaa Jamaladdeen's thesis from P' Institute and in collaboration with Associate Professor Fabienne Dédaldéchamp from the laboratory EBI (Ecology and Biology of Interactions, SEVE team) and Engineer Researcher Laurent Lemée from IC2MP (Institute of Chemistry of Environments and Materials of Poitiers), as part of two Master internship grants from Labex Interactifs (Ms. Kenza Ait Ali Yahia) and EUR-InTREE which is a new training about Interfaces from a physico-chemical aspect in Poitiers (M. Axel Rigoulet), and a grant from P' Institute.

Associate Professor Fabienne Dédaldéchamp brought her expertise in biology [17] during plant conditioning to hydric stress and plant physiology analyses and issues. We co-supervised with her and Rawaa Jamaladdeen Ms. Kenza Ait Ali Yahia, a second year Master student in Green Chemistry, Catalysis and Environment (CVCE, University of Poitiers). She helped us during hydric stress conditioning, physiology analyses at EBI laboratory and thermal stress tests at P' Institute. Engineer Researcher Laurent Lemée brought his chemistry expertise for accurate chemical analyses by thermodesorption of adsorbent tubes. We co-supervised Mrs. Axel Rigoulet a first year Master student (EUR-CVCE). He helped us on leaf pyrolyses and adsorbent tubes analyses at IC2MP.

2. Material and methods

2.1. Plant conditioning to hydric stress

For this part, we benefitted from the greenhouse equipment of EBI laboratory. *Rosmarinus officinalis* and *Cistus albidus* were chosen to be Mediterranean plants typically concerned by documented eruptive fires (Courty et al. 2014) but also to be physiologically different (Ormeño et al 2007). 20 plants of each species were correctly watered, 20 others were submitted to hydric stress. The end of the hydric stress was decided according to physiology analyses made in EBI laboratory to keep plants alive for heating tests: plant soil moisture, leaf water amount and chlorophyll concentration. Hydric stress conditioning required about two to three weeks depending on the weather (sunny/cloudy, humid/dry). Other microscopic and macroscopic analyses were done to get more data on the influence of hydric stress. Analysed parameters included stomatal conductance, osmotic pressure and water status of leaves which are directly related to the plant emissions (Ormeño et al 2007, Lavoir 2010). Pictures of the plants were done and analysed with image based software in order to normalise emission quantification according to plant sizes during thermal tests. For each of the two species, we got physiological observations during hydric stress and a normalisation method for emission quantification according to plant size.

Watered and non-watered plants were then submitted to thermal stress (cf. below).

2.2. Heating tests at plant scale

2.2.1. Material

For this part, we benefitted from a compartment of 1 m^3 equipped with a radiant panel lent by PRISME laboratory (Fig. 1). The latter can provide a maximum of 84 $kW.m^{-2}$ of radiative flux and is made with a fire resistant glazed window for plant surveillance. It was instrumented with thermocouples, fluxmeters and a cooled scale (Fig. 1 left).

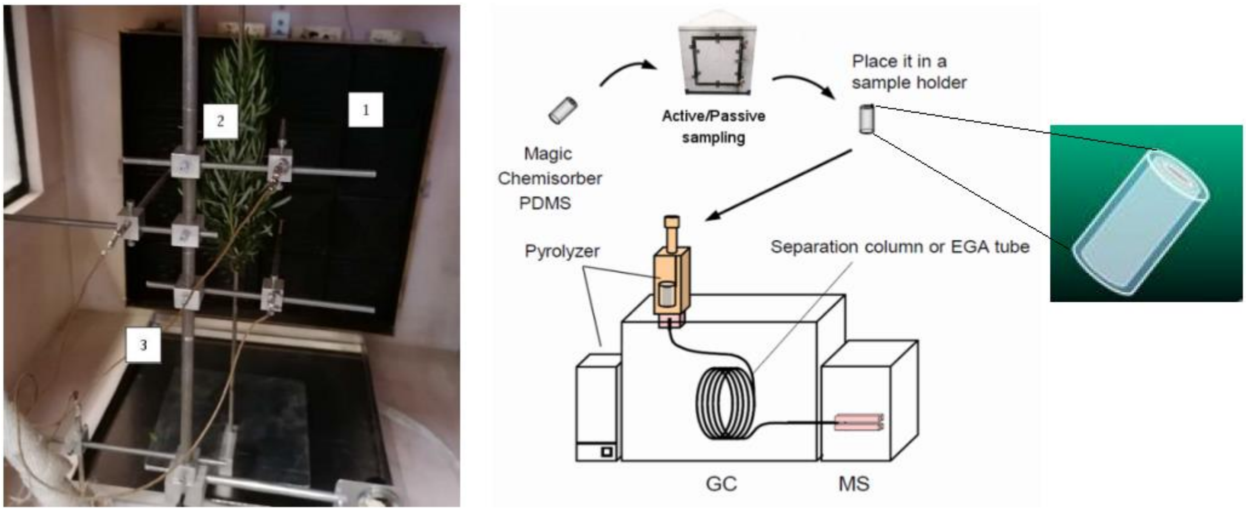


Fig. 1. On the left: photograph of Rosmarinus plant (2) submitted to thermal stress inside a 1m^3 compartment equipped with radiant panel (1) and lent by PRISME laboratory (Bourges). On the right: method of analysis with adsorbent tubes

Two complementary gas analysis devices were used during tests: a mobile analyser introduced from the top of the compartment to quantify continuously O_2, CO, CO_2, H_2 and CH_4; and adsorbent tubes to identify VOCs (Volatile Organic Compounds) by trapping them *in-situ*. Trapped compounds were identified by TD-GC-MS (Fig. 1 right), i.e. they were thermally desorbed (TD), separated with gas chromatography (GC) and identified using mass spectrometry (MS). The pyrolysis products were identified by comparison of their mass spectra with NIST (National Institute of Standards and Technology) mass spectral library and thanks to their retention times. The adsorbent tubes, also known as SPEE (solid phase extraction elements), are titanium tubes coated with polydimethylsiloxane (PDMS). For each test, an adsorbent tube was placed into the heating compartment at the ground level and another one was placed inside an active sampling composed from a pump and a flexible connecting the pump to the compartment. It allowed us to compare the gas composition at different heights knowing that density is very variable between e.g. isoprene and sesquiterpenes.

2.2.2. Protocol

At the end of hydric stress conditioning, plants were retrieved from the greenhouse and introduced one by one inside the compartment. Plants were cut at their basis and depending on their size, one, or two superimposed plants, were attached to a stand close to the radiant panel (see Fig. 1 left). Consequently to preliminary tests, we chose to impose a temperature/heat flux ramp of 30 minutes not to exceed 200°C at the plant level in order to avoid hemicellulose degradation and study only vegetation emissions. After each heating test, we retrieved data of temperature, heat flux, light gases concentration over time (continuous analyser), and the two adsorbent tubes for analyses by TD-GC-MS desorb, separate, identify and quantify the VOC emission. Thermodesorption of of adsorbed compounds was performed from 150°C to 280°C with a heating rate of 500°C/min and 1 min at final temperature.

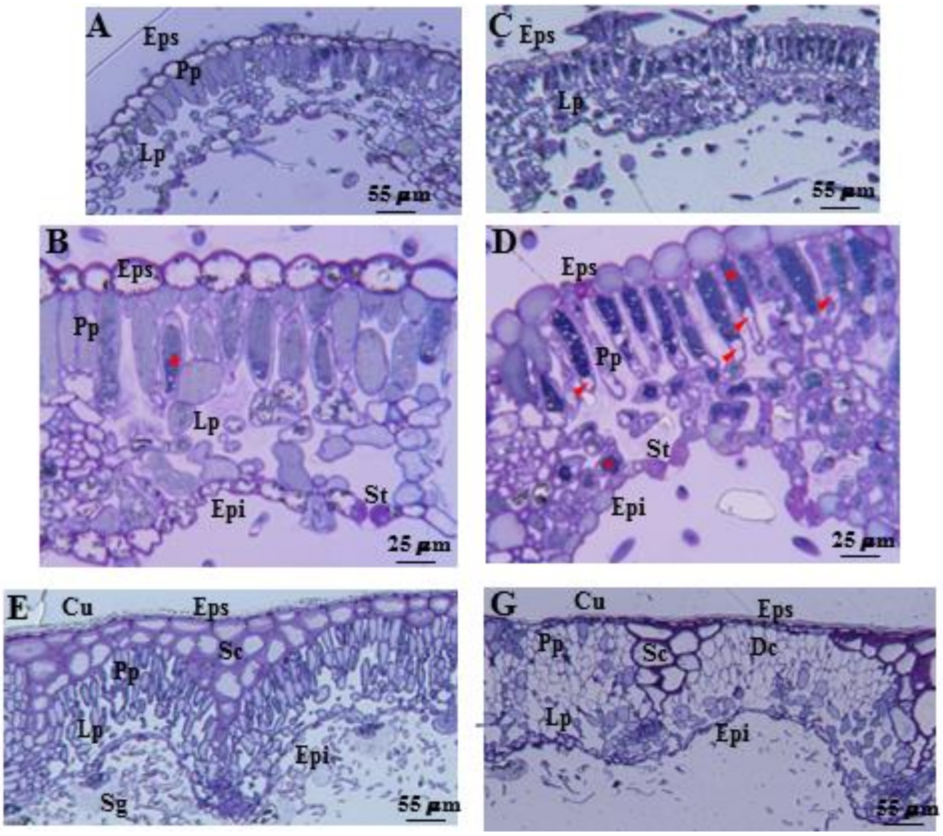
2.3. Heating tests at leaf scale

Leaf samples were cut from plants during the hydric stress conditioning and pyrolysed at IC2MP. The pyrolyser was a Multi-shot Pyrolyzer EGA/PY-3030-D which was coupled with a GCMS-QP2010 Ultra Gas Chromatograph-Mass spectrometer. The pyrolysis products were carried by a helium gas flow to the GC column and separated on a TR5-MS capillary column (dimensions: 30.0 m, 0.25 mm i.D, 0.25 µm phase thickness). The injector was a split/splitless. The MS device was a quadrupole analyser, the ionization mode was electron impact (70 eV).

Around 15 mg of not lyophilized leaves (equivalent to one rosemary leaf or a piece of cistus leaf) were placed into an inox cup and the pyrolysis was carried out in the isothermal mode at 180°C (temperature of maximal emissions of plants (Barboni 2006, Chetehouna et al. 2014, Courty et al. 2014) for 1 minute. When cut leaves needed to be stored for later experiments, they were conserved inside glass tubes covered with aluminium foil in a freezer at a temperature of -21±1°C.

3. Results

The physiological analyses show clearly the influence of the hydric stress at microscopic scale (Fig. 2) with a decrease in vacuole volume and an increase in secondary metabolite synthesis for stressed plants. For *Rosmarinus officinalis*, we also observed dead cells (Dc) (Fig. 2.d) indicating that hydric stress was worse than for *Cistus albidus*. The heating tests showed a good reproducibility for pyrolyses of leaf samples and interesting variations between stressed and unstressed plants regarding monoterpenes and fatty acids (Fig. 3). At plant scale, number of tests for each plant species at a given hydric stress level were insufficient to give trends and strong results because of technical problems, sanitary restrictions and specificity of each plant. However these tests allowed us to adapt experimental protocols and devices for further testing such as: plant location and fixation, heat flux ramp, sampling location, use of adsorbent tubes, hydric stress duration and normalisation of measured concentrations according to the plant size.



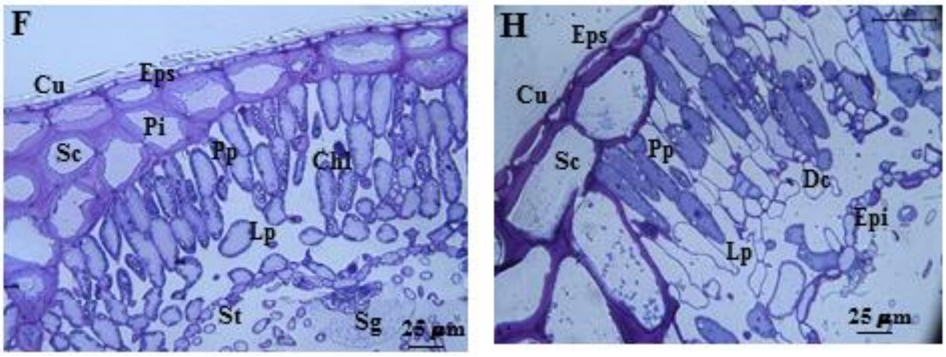


Figure 2: Cross section of Cistus albidus (A,B,C,D) and Rosmarinus officinalis (E,F,G,H) leaves, Plants growing condition: well-watered (A,B,E,F) and without water supply for 16 days (C,D,G,H). Cu, cuticle; Eps, superior epidermis (adaxial); Epi, inferior epidermis (abaxial); Sc, Sclerenchyma; Pi, pit; Pp,: Palisade parenchyma; Lp, lacunar parenchyma; Chl, Chloroplast; Sg, Secretory gland; Dc: Dead cell; St: Stomata. Tannins (asterisk): Cell plasmolysis (arrow). Semithin section, toluidine blue staining, optic microscopy.

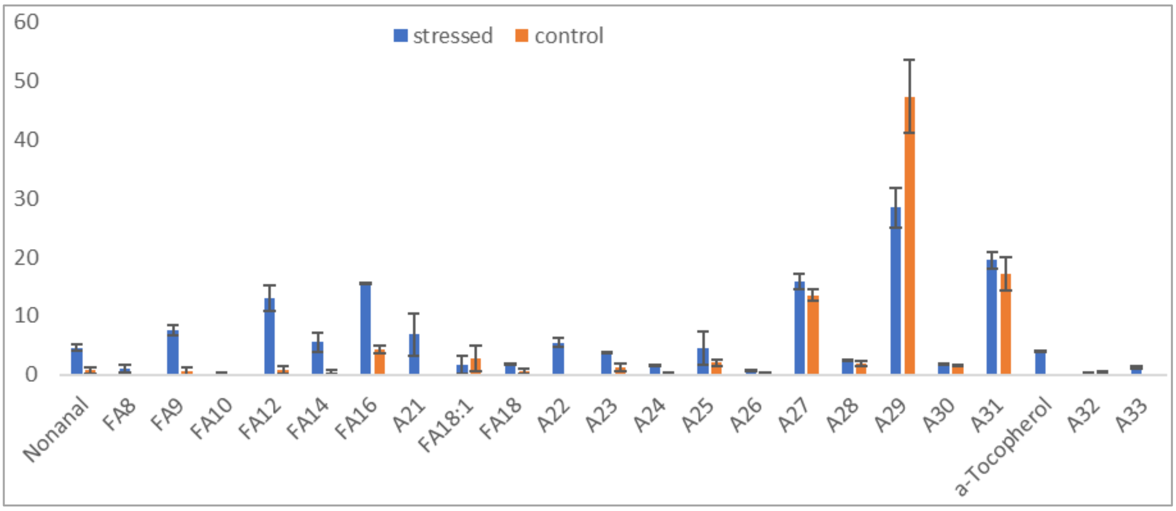


Fig. 3. Pyrolysis products (180°C for 1 minute) of control and hydrically stressed Cistus albidus (A_n: alcane C_n ; Fa_n : fatty acid C_n)

4. Acknowledgement

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