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Volatile and semi-volatile terpenes impact leaf flammability: differences according to the level of terpene identification

Anne Ganteaume¹ · Bastien Romero¹ · Catherine Fernandez² · Elena Ormeño² · Caroline Lecareux²

Abstract

In flammability assessment, the terpene effect is usually studied using their total or subgroup content, missing, therefore, the information that could be provided by the molecules themselves. In this study, the specific role of terpenes on leaf flammability was sought comparing different levels of terpene identification—total, subgroup (i.e. mono-, sesqui-, and diterpene), and single compound—as well as their interactions with fuel moisture content (FMC) in four species common in Mediterranean Wildland–Urban Interfaces (*Pinus halepensis*, *Cupressocyparis leylandii*, *Hesperocyparis arizonica*, *Cupressus sempervirens*). *Pinus halepensis* was the most flammable species (low FMC and higher sesquiterpene content but low terpene diversity) while *Cupressocyparis leylandii* presented the highest terpene diversity and total terpene content (higher mono- and diterpene content). Flammability was differently affected according to the terpene identification level used in the models. The effects ranged from non-significant for most species studied, using subgroup or total terpene content, to mostly significant, using single compound content. Regarding the former, the lack of significant results could be due to opposite effects of different single compounds within a terpene subgroup. For the latter, terpene molecules driving flammability and their effects (positive or negative) differed among species. A cumulative effect with FMC was also highlighted in some cases but terpenes mostly remained the main flammability drivers regardless of the species. Using the refined terpene level in modelling allowed a better understanding of the compounds' role on flammability, which is useful in the identification of plant traits linked to flammability.

Keywords Volatile organic compounds · Fire-prone species · Fuel moisture content · Terpene content

Introduction

Most Mediterranean plant species are known to synthesize terpenes as secondary metabolites involved in the interactions of plants with their environment (Ormeño et al. 2007; Ciccioli et al. 2014; Karban et al. 2014). Some species exhibit specialized structures such as lenticular glands (e.g. in *Gossypium hirsutum* L.), trichomes (e.g. in *Cistus* sp.), cavities, ducts, canals, or resin ducts in conifers (Walter et al. 1989), where these compounds are stored in significant amounts (Loreto et al. 1996; Llusià and Peñuelas 2000; Castro and De Magistris 1999), in the form of liquid micelles (Fall 1999). Other species produce and emit volatile terpenes almost simultaneously without previous specific storage of these metabolites (i.e. non-storing species) (Loreto et al. 1996; Ormeño et al. 2011). The main secondary metabolites biosynthesized in conifers are terpenes and phenols (Langenheim 2003). In these plants, oleoresin is a mixture of terpenes including volatile monoterpenes and sesquiterpenes

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as well as semi-volatile diterpenes, both in similar proportions (Steele et al. 1998). These terpenes are stored in resin ducts in woody tissues and in secretory cavities in needles of the Pinaceae species (Phillips and Croteau 1999) as well as in the sub-epidermal resin glands of scale-leaves in the Cupressaceae species (Castro and De Magistris 1999). The quantity and composition of terpenes produced against a stressor (such as drought, herbivory, etc.) can be constrained by the plant's physiological status (Sampedro et al. 2011) and its genetics (Pausas et al. 2016), but also by the nature, severity, and time since the stress (Page et al. 2012).

Plant flammability is primarily controlled by different traits that can be structural (such as surface-to-volume ratio) or chemical (Scarff and Westoby 2006; Engber and Varner 2012; Schwilk and Caprio 2011; Clarke et al. 2014; Varner et al. 2015; Ganteaume 2018). Along with fuel moisture content (FMC), terpenes are among the chemical compounds associated with flammability (Ormeño et al. 2009; Pausas et al. 2016; Romero et al. 2019). Some of these molecules are volatile organic compounds which are naturally very flammable (Barboni et al. 2011) because of their high heating content value, relatively low flash point, and low flammability limit (See Sigma-Aldrich Data Sheets, for instance). They can, therefore, affect plant flammability and possibly fire regimes (White 1994; Cornelissen et al. 2003; Keeley et al. 2012). The positive impact of terpenes on plant flammability could be an issue in Mediterranean areas where summer drought induces a water stress that forces some species to increase their terpene production and possibly their storage (Llusà and Peñuelas 1998; Blanch et al 2009; Marchese et al 2010), thereby making them potentially more flammable. Moreover, as large amounts of these compounds can be emitted in response to high temperatures (Centritto et al. 2011), episodes of massive terpene emission can result from wildfire events (Chetehouna et al. 2009; Courty et al. 2012). The role of terpenes in flammability is, therefore, becoming even more important, now and in the near future, since their content could possibly increase in response to predicted warmer and drier climatic conditions in the context of the current climate change.

Previous studies attempted to highlight the effect of terpenes on live leaf or litter flammability of various species. Usually, they focused on the total amount of terpenes (e.g. De Lillis et al. 2009), on their main fractions, often formed by monoterpenes (White 1994; Alessio et al. 2008a, b), sometimes supplemented by the sesquiterpenes (Owens et al. 1998; Ormeño et al. 2009; Della Rocca et al. 2017). Only a few works tested the effect of individual terpene compounds on flammability (e.g. Pausas et al. 2016) and hardly any, as Romero et al. (2019), have taken into account the semi-volatile fraction represented by diterpenes.

In a previous paper (Romero et al. 2019), the role of the three main terpene subgroups on leaf and litter flammability

was compared to that of two other main flammability drivers, FMC and leaf thickness, taking into account different species commonly found in WUI (including those studied in the current work). However, the authors pooled the data of the different terpene-containing species together, therefore, failing to grasp the pattern of each species. Moreover, their models took into account only the terpene subgroups extracted from leaves sampled in summer; the effect of the terpene compounds themselves (or of their combinations) on flammability has yet to be tested, including data obtained on a larger time frame (e.g. throughout a year) than a single season. Testing flammability variability according to single terpene concentrations is an important step since different terpenes may differently influence flammability (Ormeño et al. 2009) and since terpene composition is species-specific. Moreover, comparing results obtained with different levels of terpene identification (from the least to the most refined: total amount, subgroup, or single compound) is still to be addressed.

Given these gaps, the first objective of the current study was to highlight differences in flammability that could be due to a difference in terpene content and FMC among species sampled throughout a year in order to take into account the variability in terms of weather conditions or leaf physiology (which was not addressed in Romero et al. 2019). Then we sought to evaluate and quantify, the effect (positive or negative) of terpenes on flammability, testing the content of each terpene compound, subgroup, as well as their total content to highlight any change according to the level of terpene identification as well as each species' pattern. Finally, it was important to quantify the role of FMC on flammability when combined with terpene content in the models run for each species studied.

Materials and methods

Species studied and sampling

The species studied in the current work (one Pinaceae: *Pinus halepensis* Mill. 1768 and three Cupressaceae: *Cupressus sempervirens* L. 1753, *Hesperocyparis arizonica* Greene 1882 Bartel (also known as *Cupressus arizonica*), and *Cupressocyparis leylandii* A. B. Jacks. and Dallim 1926) are common in the Wildland-Urban Interfaces (WUI) of the French Mediterranean region. *P. halepensis* is the only species native to the area while *H. arizonica* comes from the southwestern USA and *C. sempervirens* forms forest stands both as native or naturalized in the Mediterranean basin (in Tunisia, Italy, and Greece, for instance). *C. leylandii* is an intergeneric hybrid of the yellow cedar (*Xanthocyparis nootkatensis* D. Don 1824 Farjon & Harder) native to north-western America (USA and Canada) and of the Monterey

cypress (*Hesperocyparis macrocarpa* Hartw. 1847), a species endemic in Monterey Bay in California (USA); *C. leylandii* is used worldwide as ornamental and as screen. These species can be involved in the fire propagation from wild-land vegetation to nearby buildings (or the other way round), especially when they are commonly used in ornamental hedges that provide a strong horizontal fuel continuity.

Leaves from the different species were sampled in Le Tholonet (southeastern France) where the climate is typically Mediterranean (mild spring and autumn, cool winter, as well as hot and dry summer, often with high winds). Completing the data obtained in summer 2016 (August–September) used in Romero et al. (2019), leaf collection also occurred in January–February and April–May 2016; all the data were pooled in order to grasp the variability in terms of weather conditions and plant physiology that could affect terpene and moisture content. We chose to work only on green leaves as Romero et al. (2019) showed that the terpene content of these leaves did not differ from that of litter leaves (i.e. entire leaves undergoing the first stage of decomposition on soil). For each sampling date and each species, a maximum of 25 g of mature live leaves was collected at different locations (base, middle and top) in the canopy of five different grown and healthy plants (always the same trees, facing South, were sampled at each date) separated by, at least, 4 m. For each plant, 6 g was used for the burning experiments, 5 g for FMC measurements, and 1 g for terpene analysis. When necessary, sampling was conducted at least 48 h following a precipitation event to avoid any impact of recent rain on FMC. Collected leaves were placed in plastic bags that were stored in a cool box for immediate transportation to the laboratory, minimising changes in water content.

Terpene identification and quantification

After sampling, leaves were stored at $-80\text{ }^{\circ}\text{C}$ in order to completely stop their metabolism. For each species, terpene content was analyzed according to the sampling plan (samples were collected and analyzed at the three sampling dates), using 500 mg of fresh leaves randomly collected in the canopy of the five different plants, as presented in Romero et al. (2019). Terpenes were qualitatively and quantitatively analyzed using a gas chromatography coupled to a mass spectrometry (GC–MS, 7890B—Agilent Technologies®). One microliter was injected into a $30\text{ m}\times 0.25\text{ mm}\times 0.25\text{ }\mu\text{m}$ thickness capillary column (HP-5MS–Agilent J&W GC Columns), at a constant flow (1 ml min^{-1}) and in the splitless mode. The injection temperature was maintained at $250\text{ }^{\circ}\text{C}$ with Helium (99.99%) as the carrier gas. The initial temperature was $40\text{ }^{\circ}\text{C}$ and increased at $3\text{ }^{\circ}\text{C min}^{-1}$ up to $300\text{ }^{\circ}\text{C}$ during analysis. A 5-min solvent delay was respected and the total run time was 90 min. The very high temperatures reached in the GC oven

even allow the detection of compounds with high molecular weight, such as diterpenes (as a whole, the chromatographic run applied allowed the detection of molecular hydrocarbons with up to 34 carbon atoms).

Terpene identification was achieved based on the molecule retention time, which was compared to that of the pure standard then available, as well as on the molecule mass spectrum, which was compared to available libraries (Adams 2007; Nist 2011). To complete this identification, experimental retention indexes were calculated for each molecule identified and compared to the theoretical retention indexes of these libraries. The terpene content was calculated performing several dilutions of many authentic reference compounds (Aldrich–Firmenich) in order to establish the response factor of the terpene subgroup as compared to the internal standard (dodecane). Then, the integrated area of each peak was multiplied by the appropriate response factor and divided by the sample volume. The sample dry mass was previously calculated to obtain the mass of terpene compound per dry mass unit, as the terpene content is usually given in mg g^{-1} of dry matter (DM).

For each plant species, contribution of terpenes was investigated at three identification levels, from the least to the most refined: total terpene amount, subgroup (mono-, sesqui-, and diterpenes), and compound (belonging to each subgroup).

Flammability experiments

The samples were burned the same day they were collected on returning to the laboratory. For each species, thirty 1-g leaf samples collected on the five different trees (with six replicates per tree) were burned using a 500-W epiradiator composed of a 10-cm radiant disc according to the methodology presented in Romero et al. (2019). The variation in temperature (one record per second) was recorded during the burning using a thermocouple (chromel–alumel, k type, $30\text{ }\mu\text{m}$ diameter) positioned 1 cm above the disc center. As soon as the fuel was in contact with the epiradiator surface, time and temperature recordings were started. Five flammability variables were measured during the burning experiments: (i) time-to-ignition, (TTI, s), defined as the time necessary for the fuel to ignite once laid on the radiant disc; (ii) ignition temperature ($t\text{TTI}$, $^{\circ}\text{C}$), defined as the temperature recorded when the flame appeared; (iii) flaming duration (FD, s), time elapsed between the flame occurrence and its extinction; and (iv) the maximum temperature reached during the burning (T_{max} , $^{\circ}\text{C}$). Just before the burning experiments, three 5-g leaf subsamples of each plant were oven-dried for 48 h at $60\text{ }^{\circ}\text{C}$ in order to measure their moisture content (calculated on a dry mass basis) at the time of burning.

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Table 1 Content of the different terpene compounds belonging to the three subgroups

Terpene subgroups	Terpene compounds (mg g ⁻¹ DM)					Cupressocyparis leylandii					Hesperocyparis arizonica					Cupressus sempervirens					Pinus halepensis				
	Mean	SD	CV	%		Mean	SD	CV	%	Mean	SD	CV	%	Mean	SD	CV	%	Mean	SD	CV	%				
Monoterpenes	α _Pinene	0.423	0.129	30.4	9.81		0.099	0.052	52.0	9.14	0.315	0.160	50.9	21.22	0.164	0.081	49.2	6.85							
	α _Thujene	0.078	0.029	37.5	1.81						0.014	0.017	120.9	0.95	0.022	0.026		0.91							
	β _Pinene	0.472	0.192	40.7	10.95		0.040	0.031	79.0	3.65	0.028	0.017	60.2	1.91	0.103	0.097	93.7	4.32							
	δ _3-Carene	0.396	0.172	43.5	9.19		0.014	0.020	143.3	1.27	0.120	0.062	51.7	8.10	0.012	0.011	94.9	0.48							
	Fenchene	0.053	0.019	35.7	1.23																				
	γ _Terpinene	0.041	0.022	54.2	0.94																				
	Limonene	0.273	0.091	33.2	6.34		0.093	0.098	104.7	8.57	0.012	0.009	73.9	0.80	0.020	0.013	64.1	0.83							
	Myrcene	0.228	0.074	32.6	5.29						0.239	0.344		10.02											
	Sabinene-hydrate	0.187	0.077	41.2	4.35		0.018	0.013	71.5	1.64															
	Terpinene-4-ol	0.183	0.046	25.1	4.25		0.059	0.026	43.5	5.45															
	Terpinolene	0.046	0.014	30.0	1.07		0.011	0.009	85.6	0.74	0.066	0.064	98.2	2.74											
	<i>Monoterpenes</i>	2.379			55.23		0.323			29.71	0.496		33.71		0.625		26.16								
Sesquiterpenes	α _Aconerol					0.052	0.030	56.5	4.81																
	α _Humulene																								
	α _Murolene	0.013	0.005	40.5	0.31	0.022	0.010	45.0	2.06						0.161	0.048	29.8	6.73							
	α _Bisabolol	0.018	0.014	80.6	0.41																				
	Aromadendrene					0.110	0.049	44.2	10.11	0.016	0.010	62.4	1.07												
	β _Elemene	0.039	0.022	55.7	0.91																				
	β _Ylangene									0.022	0.010	46.5	1.46												
	Cadina-1(6)-4-diene-cis	0.296	0.098	33.0	6.88	0.283	0.148	52.2	26.00	0.008	0.003	33.6	0.54												
	Cadinene									0.010	0.001	10.5	0.69	0.019	0.008	40.3	0.78								
	Cadinol					0.015	0.010	64.7	1.37																
	Calamene					0.018	0.011	58.0	1.69																
	β -Caryophyllene	0.009	0.002	25.5	0.20					0.017	0.006	37.6	1.13	1.034	0.302	29.3	43.25								
	Cedrol	0.007	0.004	56.3	0.17	0.014	0.008	59.1	1.29	0.039	0.038	99.0	2.60												
	Copaene	0.010	0.004	43.0	0.24																				
	Cubebol														0.033	0.015	46.0	1.36							
	Cubebol-10-epi					0.026	0.016	61.5	2.40																
	Curcumen-12-ol-beta-(Z)	0.054	0.038	71.1	1.25																				
	Elemol	0.027	0.013	49.6	0.62										0.023	0.011	47.1	0.96							
γ _Elemene	0.015	0.006	39.7	0.34																					
γ _Murolene										0.138	0.057	40.9	9.33												

Table 1 (continued)

and longest FD, which made this species the most flammable species in contrast to *H. arizonica* (Fig. 2). Regardless of the species, the intra-individual variation of the flammability variables was low for T_{\max} ($CV < 20\%$) as well as for most TTI (but could be up to 44%) and tTTI (but could be up to 40%). In contrast, the variation was overall higher for FD (up to 79%). Overall, there was no inter-individual variation of the flammability variables ($CV < 20\%$) regardless of the species (except for FD in *H. arizonica*, mostly) (Suppl. Mat. 3).

Relationships between flammability, FMC, and terpene subgroup and total content

The significant correlations highlighted between flammability variables and terpene content or FMC (simple linear regression analyses; Suppl. Mat. 4) differed according to the species and significant results were more often highlighted for *C. leylandii* than for the other species (Fig. 3). Regarding the role of terpenes, total terpene content did not affect flammability regardless of the species and the variable considered (Suppl. Mat. 4). At subgroup level, diterpene content correlated to T_{\max} (negatively) and TTI (positively) in *C. leylandii*, the former presented the best correlation (Fig. 3a). Diterpenes also significantly affected FD (positively) in *C. sempervirens* (Fig. 3b), thus with an opposite effect on flammability compared to the former species. Instead, neither mono- nor sesquiterpene content affected flammability in these two plant species. The results of the analyses were not significant for *H. arizonica* and *P. halepensis*, regardless of the terpene subgroup (Suppl. Mat. 4). FMC was not significantly correlated with flammability regardless of the species, except in *C. sempervirens*. For this species, the only significant correlation highlighted positively related FMC and TTI (Fig. 3d).

Combining the content of the different terpene subgroups in the modelling (Fig. 4a), we found that, in *C. leylandii*, diterpene content was the main driver of the four flammability variables considered (and the only one regarding TTI), except for tTTI for which the test was not significant. This terpene subgroup always had a negative effect on flammability in contrast to sesqui- and monoterpene content, when significant. The subgroup content accounted for 45 to 75% (the latter regarding T_{\max}) of the flammability variation in this species (Table 2). When FMC was included in the model, the proportion of variation increased only for FD (R^2 increasing from 0.45 to 0.68) and TTI (R^2 increasing from 0.49 to 0.56), highlighting a combined effect of terpenes and FMC but the former remaining the main driver (Table 2; Fig. 4b). Regarding *C. sempervirens*, diterpene content was the only driver of FD (positive effect, contrary to the results obtained in the previous species) accounting only for 31% of flammability variation, the relationships were not significant for the other flammability variables (Table 2; Fig. 4a). When

used in the models, FMC presented the highest correlation coefficient regardless of the flammability variables (with a positive effect on TTI and a negative effect on tTTI and T_{\max}), except for FD (Fig. 4b). The effect of FMC combined with that of di- and sesquiterpene content explained 30% to 52% of the flammability variation (Table 2). Regarding *P. halepensis*, TTI was the only variable affected by terpene subgroup content (monoterpenes, and to a lesser extent diterpenes, enhanced TTI; Fig. 4a), explaining only 27% of *P. halepensis*' flammability (Table 2). In this species, FMC and terpene content combined their negative effects on flammability (thereby explaining 36% of its variation), the former presenting the highest correlation coefficient (Table 2, Fig. 4b). Regardless of the flammability variable, the analyses were not significant for *H. arizonica*.

Relationships between flammability, FMC, and single terpene compound content

Using this refined level of terpene identification, more significant correlations linking flammability to terpene compound content were obtained for the four plant species, improving the results compared to the previous approach that used the subgroup level in the linear regression analyses (Suppl. Mat. 4). For *C. leylandii*, several diterpene compounds negatively correlated with T_{\max} and FD (cembrene, isophyllocladene, manool-oxide, and manool-oxide-13-epi) while others positively affected TTI (cembrene A and abietal-4-epi). The best relationship was highlighted between T_{\max} and the diterpene manool-oxide-13-epi ($R^2 = 0.56$; Fig. 3c), confirming the results obtained at the subgroup level. In *C. sempervirens*, FD was positively related to the diterpene totarol but the best relationship for this species was obtained linking TTI and FMC (Fig. 3d) while, in *H. arizonica*, the only significant correlation positively related T_{\max} with the monoterpene α -pinene (Fig. 3e). In *P. halepensis*, the sesquiterpene germacrene and the monoterpene myrcene positively affected FD (thus enhancing flammability) and TTI (thus mitigating flammability), respectively. It is worth noting that, in the former case, the significance of the relationship was only due to an outlier (Fig. 3f).

When the content of all the significant single terpene compounds was taken into account in the PLS analyses (Table 3 and Fig. 5), the modelling was refined since the resulting relationships were stronger (more significant results and higher R^2) than previously noted. However, results remained not significant for T_{\max} and TTI in *H. arizonica*, TTI and tTTI in *C. sempervirens*, and tTTI in *P. halepensis*. The numbers of compounds resulting as main flammability drivers were 12 in *C. leylandii* (Fig. 5a), four in *C. sempervirens* (Fig. 5b) as well as two in *H. arizonica* (Fig. 5c) and eight in *P. halepensis* (Fig. 5d), and, most of the time, these drivers differed among species (none of them

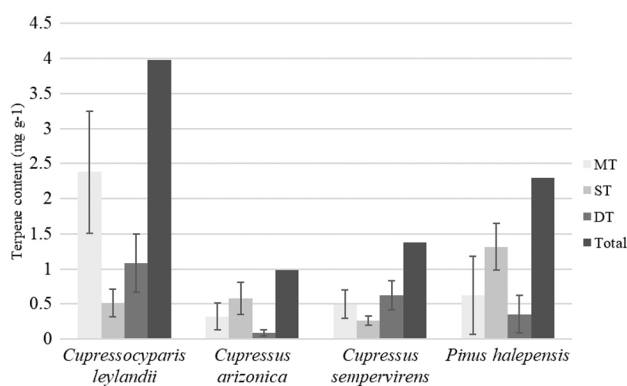


Fig. 1 Comparison of the content of the three terpene subgroups (MT monoterpene, ST sesquiterpene, DT diterpene) and of the total content (means \pm SD) among species. Means were calculated from the total terpene content of each individual. For each terpene subgroup, different letters indicate significant differences between species ($p < 0.05$). Lowercase letters indicate significant differences between species for each terpene subgroup

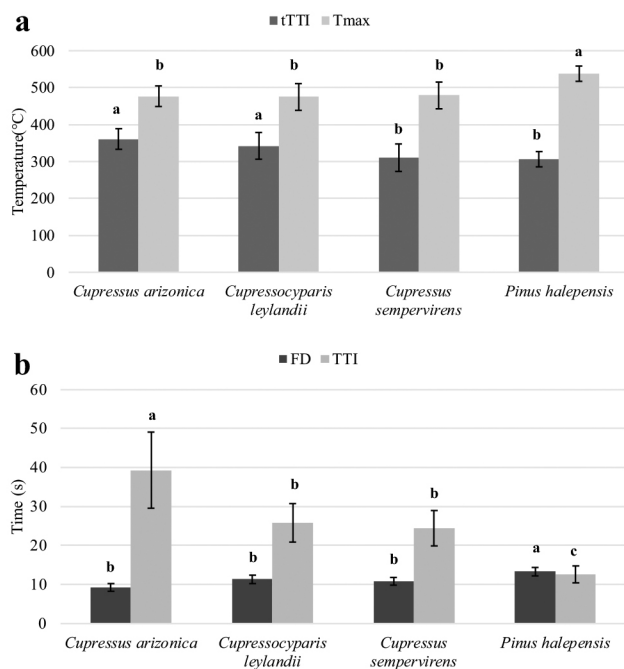


Fig. 2 Flammability variables (mean and standard deviation, $n=30$) recorded on the four species studied: **a** ignition temperature (tTTI) and maximum temperature (T_{\max}), **b** flaming duration (FD) and time-to-ignition (TTI)

being shared by the four species). The effect (positive or negative) of these significant compounds on flammability also varied among plant species (e.g. the monoterpene limonene enhanced FD in *C. leylandii* but mitigated this

variable in *C. sempervirens*). Similarly, a variation of the compound effect on flammability could be highlighted according to the flammability variable considered within a same species (e.g. the sesquiterpene germacrene enhanced FD and Tmax but also TTI in *P. halepensis*, thus enhancing and mitigating this species' flammability, respectively). It is worth noting that, for *C. leylandii*, most significant compounds mitigated flammability, mostly belonging to the diterpene subgroup. In contrast, the diterpene totarol positively correlated with FD in *H. arizonica*. The compound that best drove flammability (highest regression coefficients) could vary according to the variable within the same species (e.g. in *P. halepensis*, the sesquiterpene germacrene was the best driver of FD and α -humulene best drove TTI while the diterpene cembrene presented the highest regression coefficient for Tmax) and among species for a given variable (Fig. 5). The best explained flammability metrics were FD in *C. sempervirens* ($R^2=0.70$, combining four significant terpene compounds), Tmax in *C. leylandii* ($R^2=0.77$, combining four significant compounds), tTTI in *C. arizonica* ($R^2=0.45$, combining three significant compounds), and TTI in *P. halepensis* ($R^2=0.93$, combining six significant compounds) (Table 3 and Fig. 5). In several cases, the significant driving compounds did not belong to the subgroup highlighted as main flammability driver in the analyses performed at the subgroup level. For instance, in *P. halepensis*, the sesquiterpenes α -humulene and β -caryophyllene were identified as the most significant compounds driving TTI (Fig. 5d) while the subgroup of monoterpenes (and diterpenes, to a lesser extent) was the main driver of TTI in the analysis performed at the subgroup level (Fig. 4a). Moreover, several compounds of a same subgroup could present opposite effects on flammability; in *C. sempervirens*, for example, the two monoterpenes α -pinene and limonene, respectively, enhanced and mitigated FD, as well as the two sesquiterpenes aromadendrene and cadina-1(6)-4-diene-cis (Fig. 5b). Moreover, we found that the proportion of flammability variation related to terpene content varied among species (51 to 77% in *C. leylandii*, 57 to 70% in *C. sempervirens*, 29 to 45% in *H. arizonica*, and 43 to 93% in *P. halepensis*) (Table 3).

Including FMC in the models improved the results of three of the analyses that were previously not significant (TTI in *C. sempervirens* as well as T_{\max} and TTI in *H. arizonica*), this parameter thereby becoming the main driver of flammability (with a negative effect on flammability whether combined or not to the terpene effect) as shown in Fig. 5. However, in the majority of the analyses, flammability was partially or not driven by FMC, regardless of the variable and the species. In the former case, an increase in the value

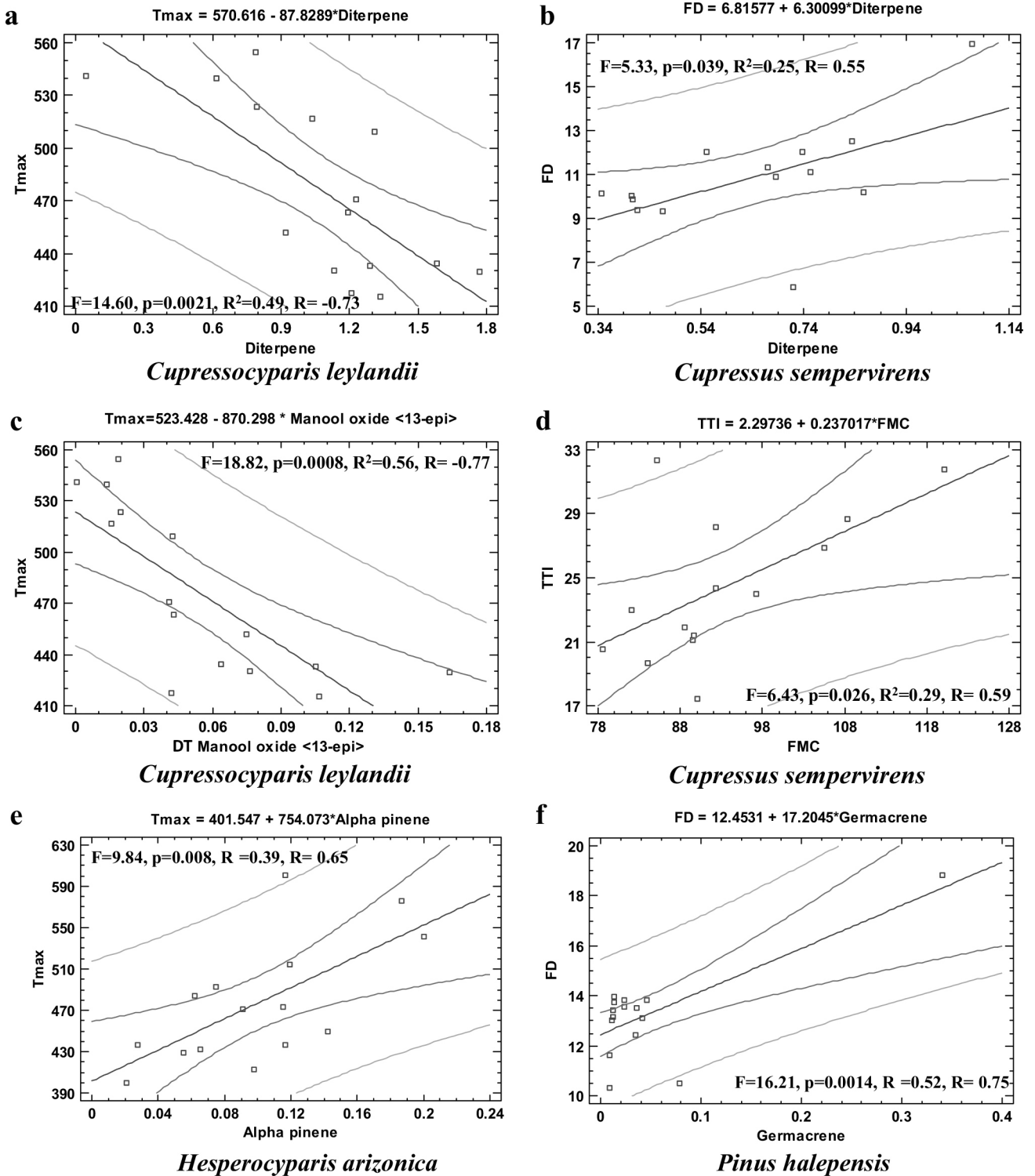


Fig. 3 Best correlations obtained between terpene content (subgroup or compound) or fuel moisture content (FMC) and flammability variables (TTI: time-to-ignition; FD: flaming duration; T_{max} : maximum

temperature) for the four species studied (simple regression analyses, F fisher test, p probability, R correlation coefficient, R^2 coefficient of determination; MT monoterpenes, ST sesquiterpenes, DT diterpenes)

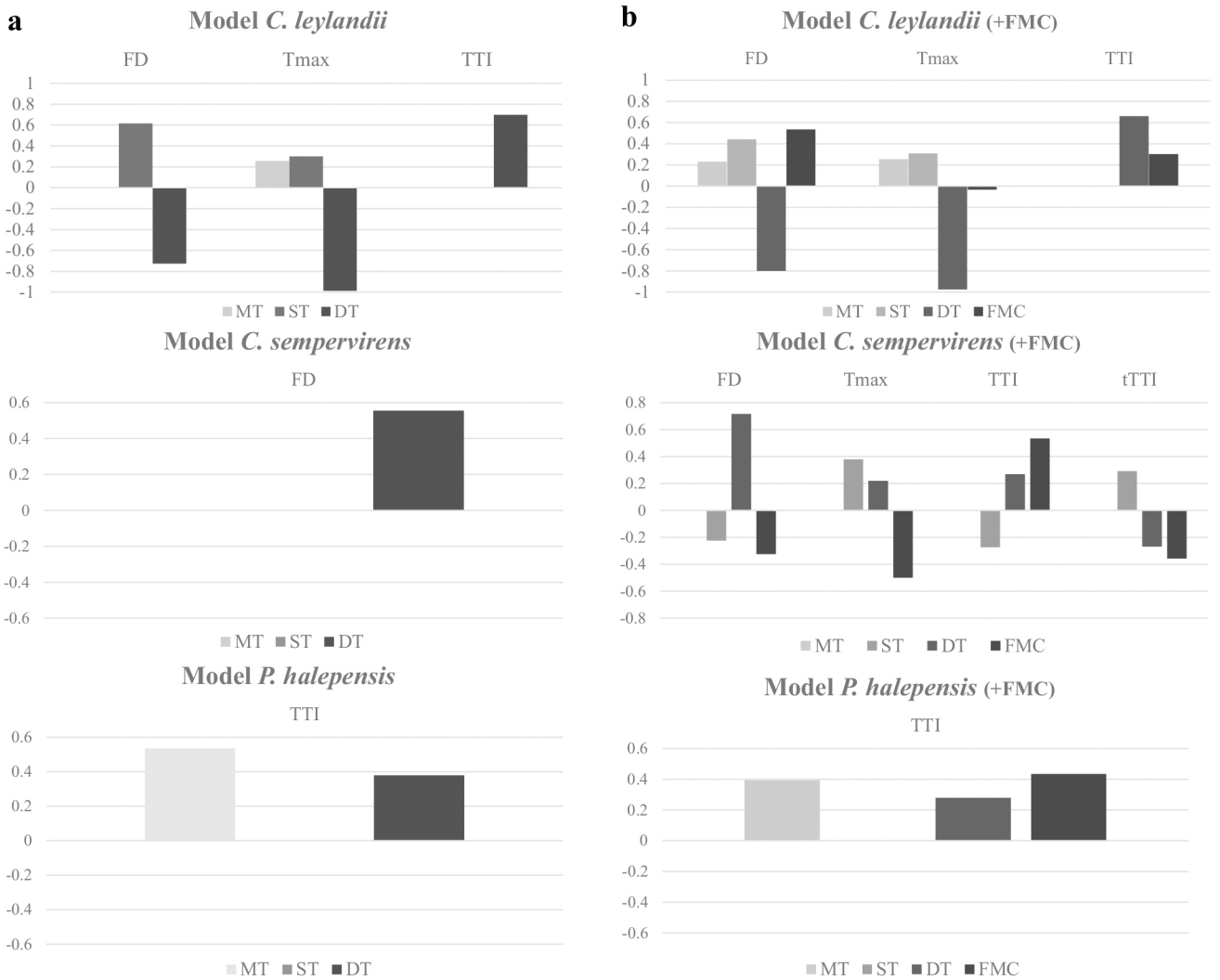


Fig. 4 Scaled and centered regression coefficients of the significant parameters (when $|value| > 0.2$) among terpene subgroups (*DT* diterpene, *ST* sesquiterpene, *MT* monoterpene) and fuel moisture content (FMC) highlighting the drivers of each flammability variable (FD: flaming duration; TTI: time-to-ignition; T_{max} : maximum temperature;

tTTI: ignition temperature). Partial least squares regression analyses were performed on the studied species' datasets excluding (a) or not (b) FMC as an independent variable along with terpenes in the models. Results were not significant for *Hesperocyparis arizonica*

of R^2 was observed when FMC was added in the models (Table 3; e.g. for T_{max} in *C. sempervirens*, FD and tTTI in *H. arizonica*, TTI and tTTI in *C. leylandii* and T_{max} in *P. halepensis*). In the latter, the coefficient of regression of FMC was lower than 0.2 (Fig. 5; e.g. for T_{max} and FD in *C. leylandii* as well as for FD in *P. halepensis*). However, combining both FMC and terpene content, we highlighted a rather small decrease in the ability of the model to explain TTI variation (from 93 to 90%) in *P. halepensis* and FD variation (from 70 to 57%) in *C. sempervirens* only (Table 3). In a few cases, considering FMC in the model involved a change in the best flammability driver (e.g. in *P. halepensis*, the best driver of TTI was α -humulene in the analysis considering only terpenes and became myrcene when FMC

was included in the model as shown in Fig. 5d). Integrating or not FMC in the models, the results of the analyses were not significant for tTTI in *C. sempervirens* and *P. halepensis*.

Discussion

Variation of leaf terpene content

Our results showed that the terpene content presented a strong intraspecific variation mainly due not only to an inter-individual variation but also to the variation between sampling dates that could be due to a seasonal pattern. Plant-to-plant variations in terpene concentration are

Table 2 Results of partial least squares regression analyses showing the proportion of flammability

	Terpene subgroups	Terpene subgroups + FMC
<i>C. leylandii</i>		
FD	$p=0.026$; $R^2=0.45$; 2 components	$p=0.0011$; $R^2=0.68$; 2 components
T_{\max}	$p=0.00025$; $R^2=0.75$; 2 components	$p=0.0003$; $R^2=0.74$; 2 components
TTI	$p=0.0039$; $R^2=0.49$; 1 component	$p=0.0014$; $R^2=0.56$; 1 component
tTTI	NS	NS
<i>C. sempervirens</i>		
FD	$p=0.040$; $R^2=0.31$, 1 component	$p=0.004$; $R^2=0.52$; 1 component
T_{\max}	NS	$p=0.023$; $R^2=0.36$; 1 component
TTI	NS	$p=0.031$; $R^2=0.47$; 2 components
tTTI	NS	$p=0.042$; $R^2=0.30$; 1 component
<i>H. arizonica</i>		
FD	NS	NS
T_{\max}	NS	NS
TTI	NS	NS
tTTI	NS	NS
<i>P. halepensis</i>		
FD	NS	NS
T_{\max}	NS	NS
TTI	$p=0.048$; $R^2=0.27$; 1 component	$p=0.019$; $R^2=0.36$; 1 component
tTTI	NS	NS

FD: flaming duration; TTI: time-to-ignition; T_{\max} : maximum temperature, tTTI: ignition temperature, explained by the different terpene subgroups including or not fuel moisture content (FMC) as independent variable in the models run on the four species' datasets

R^2 coefficient of determination, p probability, NS non-significant

known to be naturally high, even over short spatial distances, or when plants grow in the same soil in the same geographic area (Ormeño et al. 2008; Kännaste et al. 2013; Pausas et al. 2016). Terpene content also varied according to species in agreement with the results of Romero et al. (2019) obtained in summer only. *Cupressocyparis leylandii* presented the highest terpene diversity and content, excepting sesquiterpenes that exhibited the highest concentrations in *Pinus halepensis*, mostly due to one compound: β -caryophyllene. However, our results also differed from those of some previous studies. For instance, Cool (2001) identified the sesquiterpene (+)-dauca-5.8-diene as one of the main compounds in the foliage of *C. leylandii*, but this compound was not detected in our analyses; this could be explained by the difference in the samples' provenance (USA vs. Europe). Similarly, in *H. arizonica*, (Chéraif et al. 2007) identified the monoterpene umbellulone as the major compound (18.4% of the terpene content) instead of the sesquiterpene cadina-1(6)-4-diene-cis in our work; these authors also highlighted a strong variation according to the provenance of samples. Moreover, previous studies usually did not consider diterpenes (e.g. Mazari et al. 2010; Achotegui-Castells et al. 2015; Chéraif et al. 2007; Della Rocca et al. 2017) focusing only on volatile terpenes, dismissing the semi-volatile molecules

(assuming maybe that these compounds presented negligible effects on flammability because of their more complex structure, thus higher flash point). Without diterpenes in their analyses, these authors found that monoterpenes (α -pinene being the main compound) represented the highest proportion of *Cupressus sempervirens*' leaf terpene content while in our work monoterpenes were the second main terpene subgroup for this species (33%), diterpenes presenting the highest content (48%). Contrasting to our results, Della Rocca et al. (2017) showed that the proportion of monoterpenes was much higher in *C. sempervirens* (> 90%) than in *P. halepensis* (56%). These authors also found more monoterpenes than sesquiterpenes in *P. halepensis*' leaves. In this latter species, Valor et al. (2017) found that diterpenes represented 45% of the total terpene concentration (with thunbergol, not detected in *P. halepensis* in the current study, accounting for 22% of the total terpene content), while this subgroup accounted for only 18% in our study, sesquiterpenes being the dominant subgroup. A higher number of compounds identified by these authors (56 vs. 18) could explain this difference. In accordance with our results, Ioannou et al. (2014) as well as Ormeño et al. (2008) found that the sesquiterpene β -caryophyllene was the main compound in *P. halepensis*' leaves (44–55%) but the proportion of diterpenes reported

Table 3 Results of partial least squares regression analyses showing the proportion of flammability

	Terpene compounds	Terpene compounds + FMC
<i>C. leylandii</i>		
FD	$p=0.007$; $R^2=0.66$; 3 components	$p=0.005$; $R^2=0.67$; 3 components
T_{\max}	$p=0.0008$; $R^2=0.77$; 3 components	$p=0.0006$; $R^2=0.78$; 3 components
TTI	$p=0.044$; $R^2=0.51$; 3 components	$p=0.023$; $R^2=0.57$; 3 components
tTTI	$p=0.012$; $R^2=0.61$; 3 components	$p=0.0014$; $R^2=0.74$; 3 components
<i>C. sempervirens</i>		
FD	$p=0.010$; $R^2=0.70$; 4 components	$p=0.054$; $R^2=0.57$; 4 components
T_{\max}	$p=0.006$; $R^2=0.57$; 2 components	$p=0.0030$; $R^2=0.62$; 2 components
TTI	NS	$p=0.040$; $R^2=0.28$; 1 component
tTTI	NS	NS
<i>H. arizonica</i>		
FD	$p=0.045$; $R^2=0.29$; 1 component	$p=0.007$; $R^2=0.47$; 1 component
T_{\max}	NS	$p=0.032$; $R^2=0.33$; 1 component
TTI	NS	$p=0.018$; $R^2=0.38$; 1 component
tTTI	$p=0.037$; $R^2=0.45$; 2 components	$p=0.026$; $R^2=0.49$; 2 components
<i>P. halepensis</i>		
FD	$p=0.004$; $R^2=0.69$; 3 components	$p=0.003$; $R^2=0.71$; 3 components
T_{\max}	$p=0.034$; $R^2=0.43$; 2 components	$p=0.024$; $R^2=0.46$; 2 components
TTI	$p=0.00007$; $R^2=0.93$; 5 components	$p=0.0003$; $R^2=0.90$; 5 components
tTTI	NS	NS

FD: flaming duration; TTI: time-to-ignition; T_{\max} : maximum temperature; tTTI: ignition temperature, explained by the different terpene compounds including or not fuel moisture content (FMC) as independent variable in the models run on the four species' datasets

R^2 coefficient of determination, p probability, NS non-significant

by Ioannou et al. (2014) was higher than in our study (31 vs. 18%). These authors also acknowledged high variation in both terpene composition and content.

Effect of terpene subgroups on flammability

While total terpene content did not affect leaf flammability (in contrast to the results of Della Rocca et al. 2017), the effect of terpene subgroup content was significant, but mostly in *C. leylandii* and *C. sempervirens*. Results, however, differed according to the species and the flammability variable considered. *C. leylandii* was the only species in which the subgroup content (diterpenes) was the main driver of flammability regardless of the variable considered (although a higher correlation occurred with T_{\max} : $R^2=0.69$). In *C. sempervirens*, diterpene content was the main driver of FD (positive effect) but was overridden by FMC regarding the other variables (the same result was obtained for monoterpene content driving TTI in *P. halepensis*). A possible explanation could be that, in most species, the different compounds composing a subgroup could present opposite effects on a given flammability variable, resulting in a non-significant relationship at the subgroup level.

Our results mostly highlighted a predominant effect of diterpenes on flammability in contrast to those of Romero et al. (2019) mostly because, in their study, the data obtained on different species were pooled together. For their part, Della Rocca et al. (2017) found that sesquiterpenes best enhanced flammability of *P. halepensis* and *C. sempervirens* but without searching to detect the diterpenes. Other previous studies (Owens et al. 1998; Alessio et al. 2008a) highlighted the role of monoterpenes on flammability but this subgroup was the only one analyzed.

The content of the terpene subgroup could also present opposite effects on flammability from one species to another considering the same flammability variable (e.g. negative effect of diterpene content in *C. leylandii* but positive in *C. sempervirens*). Romero et al. (2019) also observed these contrasting effects when comparing flammability variables, as the species' data were pooled. Previous studies highlighted an important negative effect of monoterpene content on flammability in different species (Owens et al. 1998; Alessio et al. 2008a; Della Rocca et al. 2017) but we only found such a result for TTI in *P. halepensis*. This weaker contribution of monoterpenes to flammability could be due to their higher volatility (owing to their lower molecular weight) implying

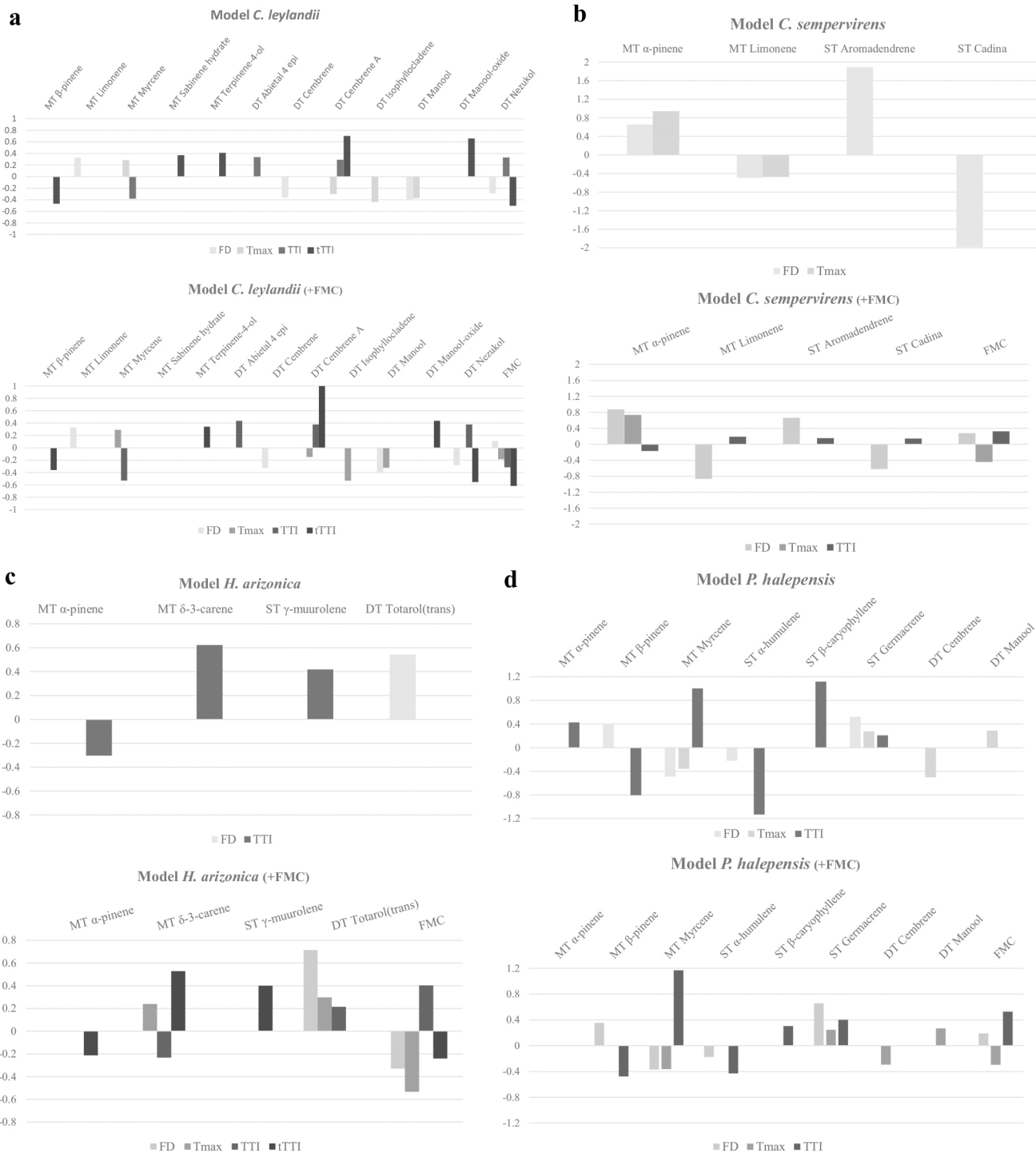


Fig.5 Scaled and centered regression coefficients of the significant parameters ($lvalue > 0.2$) among terpene compounds (*DT* diterpene, *ST* sesquiterpene, *MT* monoterpene) and fuel moisture content (FMC) highlighting the drivers of each flammability variable (FD: flaming duration; TTI: time-to-ignition; T_{max} : maximum temperature; tTTI:

ignition temperature). Partial least squares regression analyses were performed on the studied species' datasets (**a** *Cupressocyparis leylandii*, **b** *Cupressus sempervirens*, **c** *Hesperocyparis arizonica*, **d** *Pinus halepensis*) including or not FMC as an independent variable along with terpenes in the models (only the significant results are shown)

that they are released from leaves before ignition occurs (Ciccioli et al. 2014). This phenomenon could be enhanced

especially with the high radiant flux emitted by the burning device used in our experiments (direct contact between

fuel and the radiant disc heated to 300 °C). Contrary to the assumption of Kauf et al. (2014), heavier molecules (sesquiterpenes and diterpenes) still affect flammability, even under such high heat flux, given the frequent predominant role of diterpenes on flammability shown in the present study, the effect being positive or negative. Della Rocca et al. (2017) hypothesized that sesquiterpenes increased flammability in *P. halepensis* since these terpenes poorly escape from deeply buried terpene storage structures (Bernard-Degan 1988) in contrast to the subepidermal glands of cypresses closer to leaf surface (Castro and De Magistris 1999). However, our results did not support this hypothesis when we took into account terpenes at the subgroup level, since only mono- and diterpenes significantly correlated with TTI (the only significant variable) in *P. halepensis*.

Adding FMC to the models substantially improved the results with cumulative effects of terpene content and FMC on flammability. However, in most cases, this parameter failed to be the main driver of flammability, its effect being overridden by that of terpenes. FMC best drove TTI in *P. halepensis* (maybe because of the lower value of FMC in this species' leaves) and most flammability variables in *C. sempervirens* (T_{\max} , TTI, and tTTI), agreeing with Della Rocca et al. (2017) but did not drive any variable in the two other species. This result is not often presented since the predominant role of FMC (over terpenes) on flammability has been often highlighted in literature (monoterpenes being mainly considered, e.g. White 1994; Alessio et al. 2008a; De Lillis et al. 2009; Pausas et al. 2016), for different species. Indeed, FMC slows down heat transmission to the fuel and interacts with leaf terpenes (Alessio et al. 2008b; De Lillis et al. 2009). Moreover, we also highlighted an effect of diterpene content and FMC on *C. sempervirens*' flammability that can be the opposite depending on the variable considered (enhancing TTI and mitigating tTTI while the reverse was obtained with sesquiterpene content). Previous studies suggested that water played a role in carrying terpenes during the preheating phase, explaining the different contributions to flammability (Ciccioli et al. 2014).

Effect of single compound content on flammability

At the compound level, terpene content affected the different flammability variables in diverse ways according to the plant species, with more pronounced effects (i.e. more significant results) compared to analyses at the subgroup level. Single terpene content explained combustibility (T_{\max}) and sustainability (FD) more efficiently ($R^2=0.66$ and 0.77 , respectively) than ignitability (TTI, tTTI) in *C. leylandii*, sustainability ($R^2=0.70$) than combustibility in *H. arizonica*, ignitability (tTTI: $R^2=0.45$) than sustainability in *C. sempervirens*, and ignitability (TTI: $R^2=0.93$) than

combustibility and sustainability in *P. halepensis*. The latter results could possibly be due to this species' lower FMC.

Within a given species, the same compound had the same effect on flammability but this effect could differ from one species to another contrary to the results of Owens et al. (1998) and Della Rocca et al. (2017). We confirmed that the subgroup to which a flammability-driving compound belongs was not necessarily highlighted in the analyses at the subgroup level, since compounds within subgroups can have opposite effects on flammability. The magnitude of the compound content was often not responsible for the effect on flammability as, for instance, the sesquiterpene β -caryophyllene, which was the most concentrated terpene in *P. halepensis* (and above all other compounds in the other species studied) was not highlighted as the best driver of this species' flammability (except for TTI, along with α -humulene). Depending on the variable considered, we even highlighted opposite effects between sesquiterpene compounds (germacrene vs. α -humulene or β -caryophyllene vs. germacrene). Sesquiterpene compounds best drove flammability in *P. halepensis* (positive effect due to germacrene and α -humulene) and in *H. arizonica* (negative effect due to cadina-1(6)-4-diene-cis) while several diterpene compounds mainly mitigated flammability in *C. leylandii*. Monoterpene compounds could also display the opposite effect on a given flammability variable within a same species (α -pinene and limonene for T_{\max} and FD in *C. sempervirens*, β -pinene and terpinene-4-ol for tTTI in *C. leylandii*, α -pinene and δ -3-carene for tTTI in *H. arizonica*, and β -pinene and myrcene for FD in *P. halepensis*). This result agreed with those of Owens et al. (1998) who found that the monoterpene limonene enhanced juniper's flammability contrary to bornyl acetate. In contrast, most previous works did not find a variable effect of these compounds on flammability (Alessio et al. 2008a; Pausas et al. 2016). Moreover, monoterpene compounds could also present opposite effects on flammability from one species to another, depending on the variable considered (positive effect of myrcene in *C. leylandii* but negative in *P. halepensis*), revealing that the pattern could vary among species and suggesting some interactions between leaf morphology and chemistry on flammability.

FMC did not substantially change the relative effect of terpenes on flammability, except for TTI in *C. sempervirens* (as in the analyses at the subgroup level) as well as for TTI and T_{\max} in *H. arizonica*. In these cases, correlations between terpenes and flammability metrics were not significant unless FMC was combined to terpenes, showing the importance of this combination (also highlighted at a lesser refined terpene identification level, i.e. the terpene subgroup), given the fact that FMC was not significantly correlated with any flammability variable (except with TTI in *C. sempervirens*). Moreover, this parameter was not the best driver of TTI anymore in *P. halepensis* (combined or

not to terpenes) contrary to the analyses performed at the subgroup level. Della Rocca et al. (2017) obtained the same trend showing that, when FMC was included in the models, the effect of terpenes on flammability was substantially reduced only for FD in *C. sempervirens*. It is worth noting that, in *P. halepensis* and *H. arizonica*, the combination of FMC with terpenes could induce a change in the compound that best drove flammability.

Ecological role of terpenes in species' fire adaptation

Contrary to *C. leylandii*, *P. halepensis*, *Hesperocyparis arizonica*, and *Cupressus sempervirens* are three fire sensitive species coming from different fire-prone areas. Of these species, *P. halepensis* is the most flammable and is also the only one native to the study area while the others are strictly used as ornamental species and do not come from the natural areas nearby. Therefore, they have not been confronted to these areas' fire history, unlike in their respective native areas, contrary to *P. halepensis*. Given that secondary metabolites such as terpenes are, in general, considered among the most evolvable traits (Moore et al. 2014), this pine species should thus favor traits allowing for higher flammability that will trigger the cone opening and the seeds' dispersion (i.e. serotiny), according to its fire-adapted strategy (i.e. resilience to fire through post-fire regeneration). It should be the same for the two other species in their native areas as they also present serotiny as functional fire-adapted trait (Lev-Yadun 1995; De Magistris 2001). The adaptive importance of these chemical compounds in fire-prone ecosystems, in terms of flammability that was pointed out by Valor et al. (2017) for different pine species, was also hypothesized by Pausas et al. (2016) for *Rosmarinus officinalis*. Since terpenes were the best drivers of *P. halepensis*' flammability regardless of the variable considered, they may grant this species higher flammability. However, in the light of our results, this is not that simple given the antagonist effects of the most concentrated compounds driving *P. halepensis*' flammability (mostly sesquiterpenes). However, even if the terpene content takes part in plant flammability, other factors must be considered besides FMC, such as fuel thickness that could override the terpene content according to the flammability variable considered, as highlighted in Romero et al. (2019) working on the same species.

It is not easy to predict flammability at one scale (e.g. landscape level) with information from another scale (e.g. fuel particle level); however, in many ecosystems, there is a consistency across scales (Pausas et al. 2017). This is the case for most species from the Mediterranean Basin that are flammable from the smallest scale (flammability assessed directly by laboratory burning tests and investigation of the VOCs content) to individuals, communities, and landscapes

(Pausas et al. 2012, 2016). Indeed, the Mediterranean fire-prone species currently studied (i.e. *P. halepensis* and *C. sempervirens*) present flammable leaves but also accumulation of dead fuel in a dense canopy (Ganteaume et al. 2013), dead fuel retention on the trunk for *P. halepensis* as well as serotinous cones. All of these species' characteristics are beneficial for the "hot-flammable strategy" developed by Pausas et al. (2017), the species belonging to this strategy presenting post-fire recruitment as most obligate seeders. Other strategies correspond to a variation in flammability at the different fuel scales that can be modified by natural selection, as these strategies are selected under different evolutionary contexts that confer fitness benefits to plants living under recurrent fires (Pausas et al. 2017). In its native areas, *H. arizonica* is a fire-sensitive but fire-embracing species that depends on stand-replacing fire for regeneration (Barton and Poulos 2019). Arizona cypress is the least fire-tolerant of all trees and shrubs in the Arizona chaparral zone but fire regimes in this species' native communities (whose locations range from Arizona to southern California) vary greatly in frequency and severity, from surface fires to crown fires depending on the area (Wright and Bailey 1982). This implies that the Arizona cypress could benefit from the "fast-flammable strategy" (in the former fire regime) as well as from the "hot-flammable strategy" (in the latter). As an intergeneric artificial hybrid of *Xanthocyparis nootkatensis* and *Hesperocyparis macrocarpa*, *C. leylandii* could present characteristics of the parent species. However, previous studies showed that most sesquiterpenes were unidentified (Cool 2001; Kelsey et al. 2014) and not present in the parent species. Given that this species does not occur naturally but is strictly used as ornamental in different regions, it has not evolved according to a given pressure such as fire and does not present a specific strategy to fire. Given their different native locations, the parent species show different susceptibilities to fire. *X. nootkatensis*, native to NW USA, presents low resistance to fire (Sawyer and Thornburgh 1977) as it comes from an environment where fire is not a selecting pressure (old growth forests). In contrast, *H. macrocarpa*, native to SW USA, is a fire-adapted species that present serotinous cones (Vogl et al. 1977) so could benefit from Pausas et al.'s "hot-flammable strategy".

Conclusions

Our results showed that, regardless of the species, terpene content influence on flammability was more significant when using the most refined terpene identification level, i.e. the single terpene compound. At this level, the compounds that best drove flammability (usually differing among species and flammability metrics for a given species) presented the same effect (negative or positive)

on flammability within a same species but this effect could vary according to the species. Moreover, for a given species, different terpene compounds of a same subgroup could display opposite effects on a flammability entailing a difference in results between subgroup and compound levels. Using such a refined terpene identification level in the modelling, therefore, improved understanding of these molecules' effects on flammability, especially highlighting the variability of these effects. A cumulative effect of terpene content and FMC on flammability was also highlighted although, in most cases, the latter parameter was not the main driver of flammability. We also found that, regardless of the species, the terpene content could strongly vary, regardless the terpene identification level. For a better explanation of this intraspecific variation, besides the intra-individual variation that could have a genetic basis (Pausas et al. 2016), terpene content should be assessed according to the season and linked to seasonal variation in flammability; this will be the aim of a further work.

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