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POPULATION ECOLOGY - ORIGINAL RESEARCH

Secondary compounds enhance flammability in a Mediterranean plant

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Abstract Some plant secondary compounds, such as terpenes, are very flammable; however, their role in enhancing plant flammability is poorly understood and often neglected in reviews on plant chemical ecology. This is relevant as there is growing evidence that flammability-enhancing traits are adaptive in fire-prone ecosystems. We analyzed the content of monoterpenes and sesquiterpenes, performed flammability tests and genotyped microsatellite markers, all in the same individuals of *Rosmarinus officinalis*, to evaluate the link between the content of terpenes, flammability and the genetic similarity among individuals. The results suggest that terpenes enhance flammability in *R. officinalis,* and that variability in flammability among individuals is likely to have a genetic basis. Overall our results suggest that the capacity to produce and store terpenes can be considered a flammability-enhancing trait and could have an adaptive value in fire-prone ecosystems.

Keywords Volatile organic compounds · Terpenes · Fire ecology · Secondary metabolism · *Rosmarinus officinalis*

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Introduction

Many plant volatile organic compounds (VOCs) are secondary metabolites that can act as signals between plants, within plants, and between plants and other organisms (e.g., animals, microorganisms) for a multitude of physiological and ecological functions (Gershenzon and Dudareva 2007; Heil and Karban 2010; Baluška 2013; Karban et al. 2014). Terpenes are the most widely distributed naturally occurring VOCs and are synthesized by many plants. Given that most terpenes ignite easily, it has been suggested that they may also play a role in enhancing plant flammability and affecting fire regimes (White 1994; Cornelissen et al. 2003; Keeley et al. 2012). However, the role of terpenes in plant flammability is still poorly understood and often neglected in reviews on plant chemical ecology (e.g., Baluška 2013; Moore et al. 2014). Understanding the mechanism that enhances plant flammability is especially important because of the emerging view suggesting a key role of flammability in plant evolution (Bond and Scott 2010; Belcher et al. 2010; He et al. 2011; Pausas et al. 2012; Pausas and Moreira 2012). There are different plant characteristics that can enhance flammability, such as retaining dead biomass or having small and thin leaves close to each other (Cornelissen et al. 2003; Pérez-Harguindeguy et al. 2013); storing terpenes is another of these characteristics that can be relevant for some species. The link between terpenes and flammability becomes even more relevant given the importance of changes in fire activity and VOC dynamics due to global change factors (drought, warming and CO_2 emissions).

Mediterranean ecosystems are probably the most aromatic and among the most flammable ecosystems in the world (Thompson 2005; Keeley et al. 2012), yet the link between terpenes and fire remains elusive. Terpenes ignite at relatively low temperatures (low flash point) and at low concentrations, and are produced and emitted by many plants, although only some species store them in specialized structures such as oil cavities (Myrtaceae), resin canals (Pinaceae), or glandular trichomes (Lamiaceae) (Boix et al. 2011). The main volatile compounds present in these structures are monoterpenes and sesquiterpenes. The content of these stored volatile compounds are in the order of micrograms per gram or milligrams per gram dry matter (DM), considerably higher than in species without storage structures in which these compounds can be only temporarily accumulated in the order of nanograms per gram DM (Ormeño et al. 2011). While the production and emission of these compounds varies largely with weather and environmental conditions (due to alterations in the C source-sink balance), storage capacity depends more on the genetic traits of the species (Peñuelas and Estiarte 1998; Peñuelas and Llusià 2001; Castells et al. 2002). Mutch (1970) already suggested that species that have acquired the capacity to persist after recurrent fires might have also evolved traits that enhance the flammability of their community. Mutch's (1970) hypothesis was later reformulated at the individual level (Bond and Midgley 1995). In this framework, storing flammable compounds to increase the chance of ignition could be beneficial in species that are large enough and their post-fire recruitment dense enough so that burning the neighbor is possible (White 1994; Bond and Midgley 1995). This is the case for many of the post-fire seeders found in Mediterranean ecosystems (Pausas et al. 2012; Keeley et al. 2012; Pausas and Keeley 2014). These species would benefit from being flammable because fire opens gaps and stimulates the germination of their soil-stored seeds, resulting in a high post-fire recruitment and an increase in population size. Consequently, flammability in Mediterranean plants provides a clear example of a niche-constructing trait (Laland et al. 1999; Schwilk 2003). Indeed, recent criticisms on the selection of flammability given by Midgley (2013) are relaxed for obligate seeders with soil-stored seed banks. In other species that do not depend on fire for recruitment, plant flammability could have other functions [e.g., fire protection (Gagnon et al. 2010)].

Previous research relating plant terpenes and flammability is very scarce. Owens et al. (1998) found a positive relationship between flammability (as the proportion of material burned) and limonene concentration—and a negative relationship with bornyl acetate in *Juniperus ashei*. Alessio et al. (2008) reported some limited evidence of a negative relationship between monoterpene content and 'ignitability' (time-to-ignition or ignition delay) in *Globularia alypum,* and De Lillis et al. (2009) studied the relation between terpenes and ignitability across five species and found no conclusive results. The accumulation of terpenes in the litter layer has also been related to flammability (Ormeño et al. 2009). That is, although the relationship between terpenes and flammability is often assumed in the ecological literature (Cornelissen et al. 2003; Pérez-Harguindeguy et al. 2013; Keeley et al. 2012) current knowledge regarding this relationship is very limited and poorly recognized in plant chemical ecology (Moore et al. 2014). One possible limitation of previous studies relating terpenes and flammability is that they were performed using a few leaves pooled from different individuals. If there is selection for flammability-enhancing traits, it should be detected when considering the variability at the individual level (Pausas et al. 2012). Studying both flammability and the content of terpenes at the individual level and in the same individuals within a population should provide a much clearer picture of the possible role of terpenes in explaining plant flammability. An individual-based study would also enable relating the variability in flammability with the genetic variability of the populations and thus, provide information on the possible heritability of the flammability variation (Moreira et al. 2014).

Our hypothesis is that stored volatile compounds have a flammability enhancing role in Mediterranean plants and thus, we predict a positive relationship between the content of terpenes and flammability. Moreover, because being flammable may confer a fitness benefit in fire-prone ecosystems (Bond and Midgley 1995) and terpene composition can have a genetic basis (Thompson et al. 2013; Pratt et al. 2014), we also expect a positive correlation between the relatedness of individuals (kinship) and their flammability. To test these predictions we quantified flammability (as the time-to-ignition given a standardized heat source), analyzed the content of monoterpenes and sesquiterpenes, and genotyped microsatellite markers in the same individuals of *Rosmarinus officinalis* L. We used this post-fire obligate seeder as a model species because it is a typical aromatic and terpene-storing species that is common in fire-prone shrublands of the Mediterranean Basin.

Materials and methods

Species

Rosmarinus officinalis (rosemary; Lamiaceae) is an aromatic evergreen shrub up to 1.5 m tall that is native and widespread in the west and central parts of the Mediterranean Basin. Its flowers are insect pollinated and its seeds very small, lacking any specialized dispersal mechanism. *R. officinalis* individuals do not resprout after severe disturbances such as fire (Paula et al. 2009). Its seeds are water permeable and germination is stimulated by fire-related cues such as heat and smoke (Moreira et al. 2010); in addition, there is abundant field evidence of post-fire seedling recruitment (Paula et al. 2009). Leaves of *R. officinalis* are rich in glandular trichomes where volatile terpenes are stored (Ormeño et al. 2007; Boix et al. 2011); these terpenes can influence fire behavior (Chetehouna et al. 2009, 2014).

Study area

The study was performed in a population located at 775 m a.s.l. in Sot de Chera (39°36′10.0″N 0°55′21.0″W, Valencia, eastern Spain, Iberian Peninsula). The site is a fire-prone shrubland growing on calcareous bedrock, dominated by *R. officinalis, Ulex parviflorus* and *Cistus albidus*. The mean annual precipitation is ca. 600 mm, with 78 mm falling in the summer months; the mean annual temperature is 14.2 °C, and mean summer temperature is 22 °C. The population has burned frequently, with the most recent fires recorded in 1978, 1986, and 1994 (Pausas and Fernández-Muñoz 2012).

Sampling

We selected and geolocalized 32 adult individuals separated by 7.8 ± 3.9 m (mean distance between individuals sampled). The sampling was performed mid-morning, under sunny conditions, in the 2011 fire season (4 and 5 July, i.e., mid-summer). For each individual we haphazardly collected ca. 50 healthy terminal leaves from all over the shrub, which were immediately frozen in liquid nitrogen. Leaves were then stored at −80 °C on arrival at the laboratory until the extraction of terpenes was performed (see below). In the field we also clipped the above-ground biomass of each individual and took it to the laboratory (avoiding exposure to high temperatures) where we performed the flammability experiments on live twigs with leaves (hereafter ‘twigs’).

Terpene content

Five frozen leaves from each individual were ground with a Teflon embolus head under liquid nitrogen. We then added 2 ml of pure pentane, centrifuged this mixture at 10,000 r.p.m. for 20 min and transferred the solution to a vial and stored it at −20 °C until the analysis was performed (24–48 h). The sediment was dried at 70 °C until maintaining a constant dry weight. The extract was analyzed using gas chromatography and mass spectrometry (GC–MS) at the Experimental Research Support Service Center of the University of Valencia. All analyses were carried out in the split mode; 2μL of the extract was injected into the GC (Agilent 6890 N) with a capillary column and compounds were detected by a mass spectrometer (Agilent 5973 N) with a quadrupole of low resolution. An apolar column (19091S-433) HP-5MS Agilent (30 m–0.25 mm–0.25 μm) was used. The split ratio and split flow were 50.1:1 and 60:1, respectively. The GC–MS analysis parameters followed Llusià and Peñuelas (2000). Terpenes were identified by comparing their retention times and mass spectra with those of pure standards [α-humulene, linalool, β-pinene, eucalyptol (=1,8 cineole), γ-terpinene, bornyl acetate, *cis*-3-hexen-1-ol] or with the library of the National Institute of Standards and Technology for the remaining compounds. Terpene content was reported in micrograms per gram DM of leaves and their quantification was based on the averaged calibration curves of the standards given above.

The analysis of terpenes was repeated twice for ca. 50 % of the individuals (i.e., on a different set of five leaves of the same individual that were collected at the same moment). The comparison of these two analyses suggested that compounds at concentrations below 50 $\mu g\ g^{-1}$ DM were poorly detected (i.e., detected in only one of the replicates). To reduce type-II error, all subsequent numerical analyses were performed with compounds that had concentrations above 50 $\mu g\ g^{-1}$ DM.

Flammability experiment (time-to-ignition)

Once the plants were in the laboratory (after the field sampling), we selected ten terminal live twigs (ca. 4–5 cm long) from each individual. To reduce the variability in moisture among samples (i.e., the variability related to microsite conditions), twigs were hydrated for 24 h at 5 °C in dark conditions and then allowed to equilibrate to controlled conditions for 24 h (24 °C in dark conditions and 60 % humidity in a conditioned growth chamber). Immediately before each flammability test, three of the ten twigs were weighed and then oven dried (24 h at 70 °C) to obtain dry matter in order to calculate twig moisture (%) at the moment of the flammability assay. The remaining seven twigs were individually weighed (overall mean = 0.36 g, SD = 0.08 g; fresh weight) and used for the flammability tests (224 tests).

Flammability experiments were carried out with a 500-W epiradiator (Helios, Italquartz) that reached a constant temperature of 660–700 °C. The flammability tests consisted of placing each twig in the center of the epiradiator and registering the time-to-ignition (i.e., to initiate a flame, often called ‘ignitability’ or ‘ignition delay,’ in seconds; Pérez-Harguindeguy et al. 2013). Time-to-ignition reflects how easily the sample should catch fire given an ignition source (e.g., a lightning strike) and how easily the fire should spread within and between individuals. Almost all (99 %) of the twigs ignited at some point. Some replicates were discarded due to methodological issues (e.g., the twig was not correctly placed in the center of the epiradiator) and thus the final number of replicates per individual was between six and seven.

Genetic analysis

For each individual, we extracted genomic DNA from ca. 30 mg DM of silica gel-dried leaves, ground using stainless steel beads on a MM 400 mixer mill (Retsch, Llanera, Spain). The DNA extraction was performed using a Speedtools plant DNA extraction kit (Biotools, Madrid), with small modifications to the manufacturer's protocol. Individuals were genotyped with 11 nuclear microsatellite loci (Segarra-Moragues and Gleiser 2009) and the polymerase chain reaction (PCR) protocols described therein. The single locus PCR products were pooled into two groups for electrophoresis on an ABI PRISM 3730 automated DNA sequencer (Applied Biosystems, Madrid). The first group included loci Roff101, Roff135, Roff203, Roff405, Roff424 and Roff438 and the second group included loci Roff237, Roff246, Roff335, Roff515 and Roff850 and used ROX-400HD and LIZ500, respectively, as the internal lane standard. The amplified fragments were assigned to alleles with GENEMARKER version 1.85 software (Softgenetics, State College, USA).

Data analysis

Time-to-ignition (in seconds) and moisture (%) of twigs were averaged for each individual. Because moisture explained a significant proportion of the variability in time-to-ignition (see "Results" section), we used the residuals of the regression as a moisture-corrected measure of time-to-ignition. This corrected time-to-ignition was regressed against the content of each of the terpenes, and the *p*-values were adjusted for multiple comparisons using the false discovery rate procedure (FDR). For the terpenes that were significantly related to time-to-ignition, we used a standard multiple regression to determine the proportion of variance of twig ignitability that is explained by these terpenes. Given that there were correlations between the content of different terpenes we performed a principal component analysis (PCA) of terpenes and regressed flammability against the first axis of the PCA; there was no need to standardize variables as all terpenes were in the same units. Because the different terpenes may contribute to flammability, and they were in the same units (micrograms per gram DM), we also regressed flammability against the sum of the terpenes.

Genetic diversity descriptors (number of alleles, observed and expected heterozygosities) were calculated with GENEPOP'007 (Rousset 2008); and polymorphism information content (PIC) was determined using MolKin version 3.0 (Gutiérrez et al. 2005) for the 11 microsatellite loci at the population level to have a general measure of the informativeness of the microsatellite loci. Relatedness among individuals was then estimated from the microsatellite data by calculating genetic distance (Nei et al. 1983) and kinship (Ritland 1996) pairwise matrices using POPULATIONS version 1.2.30 (Langella 2000) and SPAGeDi version 1.3d (Hardy and Vekemans 2002), respectively. The dissimilarity among individuals based on their ignitability was first correlated with the dissimilarity based on their terpene content, and then with the genetic distance and kinship among individuals, using Mantel tests (10,000 permutations; ADE4 software).

Results

Time-to-ignition varied among individuals, from 7 to 23 s (mean = 15.6, CV = 19 %), and it was positively related to the moisture of the samples ($R^2 = 0.26$, $F_{1,30} = 10.38$, $p = 0.0031$). We used the residuals of this regression as a moisture-corrected measure of flammability (time-to-ignition).

We found a total of 20 different terpenes (with concentrations above 50 $\mu g\ g^{-1}$ DM), mainly monoterpenes, with mean concentrations ranging from 54 to 1000 $\mu g\ g^{-1}$ DM (mean = 222 $\mu g\ g^{-1}$ DM, CV = 114 %). Only 12 of these compounds were present in more than ten individuals (Tables 1, 2), and these were the ones considered for further analyses. None of these terpenes were significantly correlated with leaf moisture (correlations ranged from −0.2 to 0.2 with *p*-values from 0.18 to 0.96). Of these 12 terpenes, 11 showed a negative relation between concentration and corrected time-to-ignition (i.e., a positive relation between terpene concentration and twig flammability), and for four terpenes (camphene, borneol, limonene, *para*-cymene) this relation was significant after FDR correction (Table 1; Fig. S1). That is, the greater the concentration of these terpenes, the more quickly the sample ignited. The sum of the terpenes was also significantly related to flammability, when considering all 20 ($F = 5.54$, $p = 0.025$), the 12 most abundant ($F = 5.77$, $p = 0.023$), or only the four major aforementioned terpenes ($F = 15.17$, $p = 0.0005$; Fig. 1). The compound that explained most variability was camphene (29 %, Table 1). The concentrations of the four major terpenes explained 34 % of the variability in twig (moisture-corrected) flammability (multiple regression); no significant interaction between terpenes was detected. The first axis of the PCA performed either with the 20 terpenes (which explained 35 % of the variability), with the 12 most abundant (47 %), or with the four major ones (74 %) were also significantly related to flammability ($F = 6.66$, $p = 0.015$;

Table 1 Main terpenes detected in *Rosmarinus officinalis*, their flash point (°C; the lowest temperature at which the compound can vaporize to form an ignitable mixture in air), and mean concentration [μg g^{-1} dry matter (DM)]; number of plants with concentrations above 50 μg g^{-1} DM; and the relationship with flammability [coefficient (*Coeff.*), R^2 and adjusted *p*-value (p_{adj})]

Compound	Group	Flash point	Mean concentration	*n* plants	Coeff.	R^2	$P_{\text{adj.}}$
Eucalyptol	MT	49	1007.98	32	−0.001	0.035	0.368
Camphor	MT	64	653.42	32	−0.003	0.12	0.125
α-Pinene	MT	32	633.25	32	−0.002	0.056	0.259
Camphene	MT	29.5	329.45	32	−0.012	0.289	0.015
β-Myrcene	MT	44	253.91	19	0.000	0.000	0.988
Verbenone	MTids	85	213.79	32	−0.005	0.083	0.195
Borneol	MT	65	151.98	31	−0.016	0.248	0.015
Limonene	MT	48	126.90	32	−0.027	0.196	0.033
Caryophyllene	ST	96	125.27	28	−0.004	0.013	0.587
α-Terpineol	MT	90	97.05	25	−0.011	0.059	0.259
Para-cymene	MT	47	83.95	30	−0.04	0.265	0.015
Terpinen-4-ol	MT	79	71.05	13	−0.02	0.081	0.195

Only compounds with concentrations above 50 μg g^{-1} DM and present in more than 30 % of the individuals are listed. Flash point from: Sigma-Aldrich (http://www.sigmaaldrich.com)

MT Monoterpenes, *MTids* monoterpenoids

* Statistically significant adjusted *p*-values

Table 2 Pairwise correlations between the content of the different terpenes in *R. officinalis*

	Eucalyptol	Camphor	α-Pinene	Camphene	β-Myrcene	Verbenone	Borneol	Limonene	Caryophyllene	α-Terpineol	*Para*-cymene
Camphor	0.110										
α-Pinene	0.364*	−0.04									
Camphene	0.160	0.622**	0.488**								
β-Myrcene	0.240	0.270	−0.030	0.220							
Verbenone	0.502**	−0.06	0.615**	0.290	−0.150						
Borneol	0.320	0.479**	0.417*	0.720**	0.070	0.446*					
Limonene	0.479**	0.34	0.779**	0.735**	0.290	0.620**	0.641**				
Caryophyllene	0.373*	0.300	0.390*	0.362*	0.554**	0.260	0.320	0.552**			
α-Terpineol	0.901**	0.120	0.518**	0.180	0.190	0.584**	0.443*	0.605**	0.416*		
Para-cymene	0.584**	0.160	0.498**	0.548**	0.210	0.613**	0.501**	0.755**	0.386*	0.588**	
Terpinen-4-ol	0.572**	0.200	0.190	0.280	0.250	0.559**	0.448*	0.539**	0.444*	0.594**	0.628**

p * <0.05, p ** <0.01

$F = 8.21$, $p = 0.0075$; $F = 15.03$, $p = 0.00053$, respectively; Fig. 1).

All 11 microsatellite loci were polymorphic and we detected a total of 120 alleles in the 32 genotyped individuals of *R. officinalis*. The number of alleles per locus ranged from two (Roff237) to 21 (Roff850) with an average of 10.91 ± 6.50 alleles per locus. Observed heterozygosities ranged from 0.406 (Roff203) to 1.000 (Roff850) and expected heterozygosities from 0.395 (Roff237) to 0.948 (Roff850) with overall values of 0.673 and 0.769, respectively. PIC values ranged from 31.40 % (Roff237) to 92.97 % (Roff850) and a population level value of 72.27 %. Six out of the 11 loci showed PIC values higher than 80 %, which together with the high heterozygosity levels supported the high information value of this set of microsatellite loci.

The dissimilarity between individuals based on the content of either the four terpenes (those related to flammability) or camphene only (the terpene that explains most variance), was significantly related with the dissimilarity between individuals based on time-to-ignition (Mantel test for the four terpenes, $r = 0.225$, $p = 0.006$; for camphene only, $r = 0.224$, $p = 0.005$). This latter dissimilarity of flammability was significantly related to both the molecular distance ($r = 0.17$, $p = 0.006$, Mantel test) and the kinship estimation ($r = 0.092$, $p = 0.011$, Mantel test).

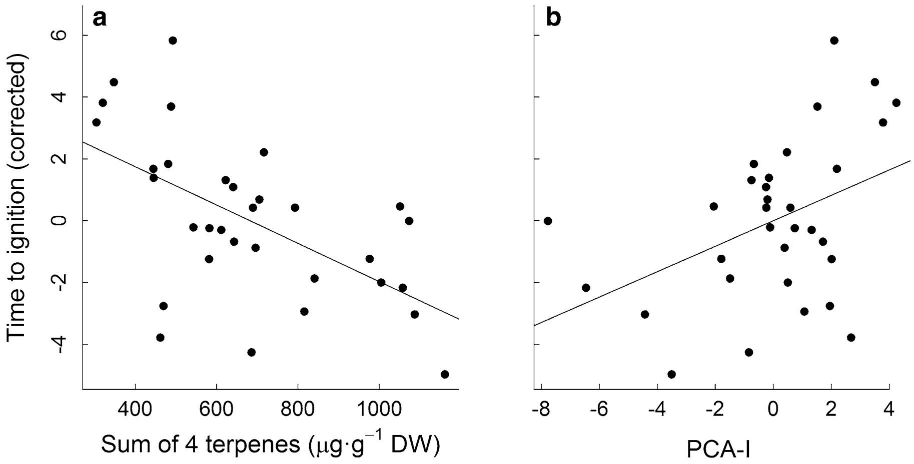

Fig. 1 Relationship between flammability (moisture-corrected time-to-ignition) and the content of the four major terpenes (camphene, *para*-cymene, borneol, limonene) in *Rosmarinus officinalis*. Terpene content is expressed as **a** the sum of the terpenes [μg g^{-1} dry weight (*DW*)] and **b** the first axis of the principal components analysis (*PCA-I*). *Lines* represent a significant fit ($F = 15.17$, $p = 0.0005$; $F = 15.03$ $p = 0.0005$). The relationship with individual compounds is shown in Appendix Fig. S1

Discussion

Given an ignition source and the right environmental conditions, all plants can potentially burn. However, some plants have characteristics that make them burn more easily; the capacity to store flammable compounds such as terpenes, can be considered one of these flammability-enhancing traits. In *R. officinalis*, we have shown that the content of stored terpenes (monoterpenes and sesquiterpenes) varies greatly among individuals of the same population, and that this variation contributes to their flammability, specifically to their ability to ignite. Despite the many other functions these compounds may have, such as signaling among plants and deterring herbivores, in fire-prone ecosystems their role in enhancing flammability needs to be fully considered. In fact, all major terpenes found in *R. officinalis* are highly flammable in such a way that, in the presence of an ignition source, they ignite at relatively low temperatures and at low concentrations (Table 1). All these compounds were also reported in previous studies for this species in different populations (Ormeño et al. 2007). The relationship between terpenes and plant flammability has often been assumed in the fire ecology literature, but evidence for this was almost anecdotal (Owens et al. 1998; Alessio et al. 2008), especially considering the abundance of aromatic plants in fire-prone ecosystems. This knowledge gap may be partially because terpenes have other functions and have mostly been studied in other contexts (Moore et al. 2014) with little attention given to their flammability-enhancing role. The fact that the few previous flammability studies were based on pooled samples from different individuals may have also contributed to this lack of evidence. By analyzing terpenes and flammability at the individual level and for the same individuals we were able to reveal a more proximal relationship.

The within-population variability in terpenes confers variability in flammability. Although there have been few studies that have examined individual-level variation in flammability, at least one previous study has shown differences among individuals [for a species that does not store terpenes (Pausas et al. 2012)]. There is increasing evidence that variability in flammability-enhancing traits can influence local fire behavior (Schwilk and Caprio 2011; Pausas and Moreira 2012). Specifically, high variability in ignitability among individuals should generate variability in the probability of burning and in how fire spreads within and between plants. By self-immolating and burning neighbors, obligate seeders ensure open spaces (niche construction) for the successful recruitment of their offspring from the seed bank (Bond and Midgley 1995). In such cases, flammability-enhancing traits such as the capacity to store terpenes can be selected, assuming that they are inheritable (Sampedro et al. 2010; Thompson et al. 2013; Pratt et al. 2014). In fact, secondary metabolites in general are considered among the most evolvable traits (Moore et al. 2014), and even small amounts of niche construction are assumed to significantly alter both ecological and evolutionary patterns (Laland et al. 1999). In the absence of better knowledge of the *R. officinalis* genome we cannot confirm a linkage of the microsatellite loci used here to loci encoding for flammability-enhancing traits; nevertheless, they still inform on the genetic relatedness among individuals. The finding that there is a positive correlation between genetic dissimilarity and dissimilarity in flammability provides further evidence about the possible heritability of flammability in *R. officinalis*, which is consistent with recent observations in another Mediterranean shrub (Moreira et al. 2014). However, further research in disentangling the genetic and adaptive basis of flammability, including the role of plasticity, is needed. The use of next-generation sequencing techniques that enable accurate estimation of trait heritability under field conditions (e.g., Brachi et al. 2011) could be a powerful tool to unravel the adaptive role of flammability-enhancing traits (Pausas 2015).

Despite the growing evidence of the importance of flammability in plant evolution (Bond and Scott 2010; Belcher et al. 2010; Gagnon et al. 2010; He et al. 2011; Pausas et al. 2012) the debate is still open (Midgley 2013) and requires further research. Our results suggest that terpenes, which may have a variety of functions, also enhance plant flammability, and this role should be fully considered at least in Mediterranean ecosystems. To what extent the evolutionary pressure exerted by fire could have contributed to the abundance of aromatic plants in many fire-prone ecosystems remains unknown. To fully understand the adaptive role of plant terpenes requires a multivariate evolutionary approach that considers fire together with other selective forces such as herbivory in a unified framework.

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Author contribution statement J. G. P. conceived the idea, performed the statistical analysis and wrote the first version of the manuscript. G. A. A. performed the flammability tests and analyzed the terpene contents. B. M. contributed to the design of the experiments and to the flammability tests. J. G. S.-M. performed the genetic analyses. All authors contributed to the final version of the manuscript.

Compliance with ethical standards

Conflict of interest We declare no conflict of interest.

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