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4 2 **Isoprenoid emission in hygrophyte and xerophyte European woody flora:**
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6 3 **ecological and evolutionary implications**
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4 30 **ABSTRACT**
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8 32 **Aim.** The relationship between isoprenoid emission and hygrophily was investigated in woody
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10 33 plants of the Italian flora, which is representative of European diversity.
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13 35 **Methods.** Volatile isoprenoids (isoprene and monoterpenes) were measured or data collected from
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15 36 the literature, on 154 species native or endemic in the Mediterranean. The Ellenberg indicator value
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17 37 for moisture (EIVM) was used to describe plant hygrophily. Phylogenetic analysis was carried out,
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19 38 at a broader taxonomic scale on 128 species, and then refined on strong isoprene emitters (Salix and
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21 39 Populus species) based on isoprene synthase gene sequences (IspS).
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24 41 **Results.** Isoprene emitters were significantly more common, and isoprene emission was higher in
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26 42 hygrophilous EIVM classes, whereas monoterpene emitters were more widespread, and
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28 43 monoterpene emission was higher, in xeric classes. However, when controlling for phylogeny,
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30 44 isoprene emission was not associated with EIVM, possibly due to the large presence of Salicaceae
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32 45 among hygrophilous isoprene emitters. Moreover, the distribution of isoprene emitters among
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34 46 EIVM classes was not related to IspS-based phylogenesis in Populus and Salix, suggesting that the
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36 47 gene has not undergone evolution linked to ecological pressure. In contrast, monoterpene emission
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38 48 pattern is independent of phylogeny, suggesting that the evolution of monoterpenes is associated
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40 49 with transitions to more xeric habitats.
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43 51 **Main conclusions.** Our results reveal an interesting ecological pattern linking isoprenoids and
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45 52 water availability. The idea is surmised that isoprene is a trait that i) evolved in plants adapted to
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47 53 high water availability; ii) is replaced by more effective protection mechanisms, e.g. more stable
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49 54 isoprenoids, in plants adapting to more xeric environments; iii) being strongly constrained by
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51 55 phylogeny, persists in Salicaceae adapted to more xeric environments.
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58 59 **Keywords:** Adaptation, Chemo-taxonomy, Hygrophytes, Isoprene, Monoterpenes, Phylogenies,
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60 60 Salicaceae, Xerophytes, Water stress.
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INTRODUCTION

Leaves of many woody and perennial plants constitutively emit volatile isoprenoids (isoprene and monoterpenes) to the atmosphere, at rates that often exceed 1-2% of the photosynthetic carbon fixation, especially in stressed leaves (Loreto & Schnitzler 2010). Isoprene and monoterpenes are formed from photosynthetic metabolism in the chloroplasts (Loreto & Schnitzler 2010). Generally, either isoprene or monoterpenes are emitted but not both (Harrison *et al.* 2013). However, some species (e.g. Myrtales) show significant storage of monoterpenes in specialized structures and these species can emit both isoprene and monoterpenes (Niinemets *et al.* 2004).

Isoprene is believed to play a role against thermal and oxidative stresses, possibly because of the capacity of this molecule to stabilize thylakoidal membranes (Singsaas *et al.* 1997, Velikova *et al.* 2011), or to remove reactive oxygen or nitrogen species within the mesophyll (Loreto & Velikova 2001; Vickers *et al.* 2009). Light-dependent monoterpenes may play similar roles, but they are also often involved in plant communication with other organisms, especially in multitrophic plant defense and pollination (Dicke & Baldwin 2010).

The emission of isoprene and monoterpenes is widespread across plant families (Harley *et al.* 1999). A recent study has indicated a strong phylogeographic signal for monoterpenes; the emission of monoterpenes is qualitatively different in cork oaks across their distribution range in Europe (Loreto *et al.* 2009). Alien species of Hawaii emit more monoterpenes than native ones, which is also suggested to be an indication of greater evolutionary success of alien species since monoterpene emission is associated with higher stress resistance (Llusiá *et al.* 2010).

However, there seems to be no straightforward relationship between isoprene emission and plant taxonomy or phylogeny. Isoprene emission is absent in herbaceous, annual vegetation, whereas it is widespread in trees and perennial plants (Kesselmeier and Staudt 1999). However, this robust trend may not be associated to phylogeny, as isoprene emission is limited to woody life-forms of families that also include herbaceous species (Fineschi *et al.* 2013). Hanson *et al.* (1999) reported that isoprene emission is more widespread in mosses than in all other taxa, and this is so far the only unambiguous phylogenetic pattern. This finding led to the suggestion that the isoprene emission trait evolved when plants conquered the land and started coping with more severe thermal extremes than in the water-buffered environment (Hanson *et al.* 1999). Similarly, Vickers *et al.* (2009) and Fineschi & Loreto (2012) commented that isoprene could have evolved as a first mechanism to cope with more recurrent and stronger oxidative stress in the terrestrial than in aquatic environments, being then replaced by more effective mechanisms when plants adapted to more

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3 97 xeric conditions. No other adaptive relationships are apparent when dealing with volatile
4 98 isoprenoids emitted from plants that do not have specialized structures to accumulate isoprenoids.
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6 99 We reasoned that, if the emission of isoprene has evolved in plants conquering the land, then the
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8 100 trait could still be more widespread in hygrophytes than in xerophytes. To test this idea, the
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10 101 emission of isoprene was assessed in the Italian woody flora, which is representative of the
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12 102 Mediterranean eco-region, one of the primary global biodiversity hotspots, and an area of
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14 103 exceptional biodiversity value exhibiting high endemism (Blondel & Aronson 1999; Médail &
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16 104 Quézel 1999; Comes 2004; Thompson 2005; Médail & Diadema 2009). Further, the vast majority
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18 105 of the tree genera of continental and northern-Europe (including Scandinavia and the British Isles)
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20 106 naturally occurs in Italy today, as the Italian peninsula was one of the main Quaternary glacial
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22 107 refugia (Bennet *et al.* 1991). Thus, the Italian woody species account for most of the total European
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24 108 diversity of trees and shrubs.

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26 109 The Ellenberg indicator values (EIV; Ellenberg 1974; Ellenberg *et al.* 1991) characterize the
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28 110 adaptation of a vascular plant species to edaphic and climatic conditions in comparison to other
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30 111 species: i.e., each plant species is given values denoting the position at which plants reach peak
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32 112 abundance along environmental gradients (Diekmann 2003; Godefroid & Dana 2007). A 9- or 12-
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34 113 point ordinal scale for each of the following parameters is used: moisture, soil nitrogen status, soil
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36 114 pH, soil chloride concentration, light, temperature and continentality. Although EIV were originally
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38 115 designed for Central Europe and assigned to the Central European flora only (Ellenberg 1974;
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40 116 Ellenberg *et al.* 1991), they have been subsequently redefined and calculated for other floras, such
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42 117 as Britain (Hill *et al.* 1999), Southern Greece (Böhling *et al.* 2002) and Italy (Pignatti *et al.* 2005).
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44 118 EIV have been widely used to interpret responses to environmental gradients (Diekmann 2003), and
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46 119 are now used also as an effective tool for applied purposes, such as remotely-sensed vegetation
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48 120 monitoring (Schmidtlein 2005), conservation strategies (Sullivan *et al.* 2010), ecological restoration
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50 121 (Krecek *et al.* 2010), and prediction of pollution effects (Jones *et al.* 2007 ; Dupré *et al.* 2010).

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52 122 Experimental studies found that EIV ranking within a given flora is a highly reliable indicator of
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54 123 adaptation to environmental conditions (Schaffers & Sýcora 2000; Diekmann 2003; Schmidtlein
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56 124 2005; Jones *et al.* 2007; Klaus *et al.* 2012): in particular, the index for soil moisture (EIVM) was
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58 125 found to perform the best (Schaffers & Sýcora 2000; Fanelli *et al.* 2007; Krecek *et al.* 2010). The
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60 126 EIVM was therefore used here to rank isoprenoid-emitting species of the Italian woody flora
127 according to an index of hygrophily.

128 Two phylogenetic analyses were carried out on this dataset at different taxonomic scales. The first
129 analysis was performed at a broad scale on woody species belonging to 31 different orders
130 representing main lineages among woody plant species, to assess whether isoprenoids emissions

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3 131 and EIVM show phylogenetic signal (i.e. whether phylogenetically related species tend to have
4 132 more similar EIVM and/or isoprenoid emission values than more distantly related species). The
5 133 second analysis was performed on a narrower range of taxa to assess whether changes of the coding
6 134 sequences of isoprene synthase (IspS), the enzyme responsible for isoprene production (Silver &
7 135 Fall 1995; Loreto & Schnitzler 2010), are associated with changes of EIVM. To perform the latter
8 136 test, poplars (*Populus* sp.) and willows (*Salix* sp.), two main genera of isoprene emitters in the
9 137 Mediterranean area and worldwide (Kesselmeier & Staudt 1999), with plant species spanning
10 138 several classes of EIVM, were studied in detail.
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21 142 **MATERIALS AND METHODS**

22 143 23 144 **Plant material**

24 145 Constitutive emissions of isoprene and monoterpenes from light-dependent pools that are not
25 146 concentrated in storage compartments, are found almost exclusively in perennial, woody plants
26 147 (Loreto & Schnitzler 2010), thus this survey was limited to these plant species. A check-list of
27 148 woody species (i.e., trees, shrubs and lignified lianas) of the flora of Italy, was compiled using, as a
28 149 first approximation, the life form assignments made by Pignatti (1982). This preliminary list, only
29 150 including Phanerophytes (P) and Nano-phanerophytes (NP) life forms, was then complemented
30 151 with some Chamaephyte (Ch) species that, based on field experience and on species description in
31 152 regional floras, are in fact lignified shrubs. Further refinement was done by deleting from the check-
32 153 list: i) all non-native species, as Ellenberg indices can be defined only in comparison to other
33 154 species growing in natural communities within an homogeneous biogeographical area. Exceptions
34 155 were possible for those species of very ancient or controversial introduction, such as *Castanea*
35 156 *sativa* and *Pinus pinea*, or for alien plants that are now widely naturalized in the Mediterranean
36 157 vegetation (e.g. *Robinia pseudoacacia*); ii) the micro-species of critical genera such as *Rosa* and
37 158 *Rubus* (which were then limited to ‘main’ species; cf. Diekmann 2003); iii) the hybrid taxa and the
38 159 species of controversial taxonomic value [i.e., those species listed in Pignatti (1982), but rejected or
39 160 doubtfully accepted in Conti *et al.* 2005]; iv) some species which had an obviously wrong life form
40 161 in Pignatti (1982). As a result, 323 plant species were considered in the check-list of the Italian
41 162 woody flora (Appendix S1).
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58 164 **The Ellenberg ecological indicator for moisture**

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3 165 We used the Ellenberg indicator value for moisture (EIVM) to formalize the ranking of the woody
4 166 species along a gradient of hygrophily. The ordinal scale defined by Ellenberg (1974) for EIVM is
5 167 composed of 12 classes; however no woody species of the Italian flora fall within classes 10-12 (i.e.
6 168 plants with permanently submerged roots and aquatic plants, see Pignatti *et al.* (2005)). Thus, the
7 169 EIVM of the species included in the present work range from class 1 (plants of extremely arid
8 170 habitats), to class 9 (species of marshy soils undergoing frequent root submersion).

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13 171 The EIVM were assigned to plants according to Pignatti *et al.* (2005). However, for 39 species the
14 172 original attribution by Pignatti *et al.* (2005) was either considered obviously wrong (see Fanelli *et*
15 173 *al.* 2007), or missing. In these cases the correct EIVM was attributed according to descriptive
16 174 vegetation papers and original field data, as recorded during field surveys to collect volatile
17 175 isoprenoids (Appendix S1).

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22 23 177 **Volatile isoprenoids**

24 178 The emission of volatile isoprenoids was reported for 149 species, i.e. about half of the total native
25 179 woody flora of Italy, and in five exotic species common in the Mediterranean vegetation, that we
26 180 had considered, without any relevant bias in terms of EIVM class (Appendix S1). No important
27 181 European tree species is missing from the emission database, whereas, for a small number of
28 182 common European shrubs or lianas (i.e. *Clematis vitalba*, *Cornus sanguinea*, *Crataegus*
29 183 *oxyacantha*, *Euonymus europaeus*, *Lonicera caprifolium*, *Rhododendron sp.pl.*, *Viburnum tinus*,
30 184 *Vinca sp.pl.*) it was not possible to obtain reliable emission data.

31 185 Species were assigned to two Boolean (0/1) categories, emitting or non-emitting, based on the
32 186 potential emission rate threshold of $1 \mu\text{g g}^{-1} \text{h}^{-1}$ for isoprene and $0.2 \mu\text{g g}^{-1} \text{h}^{-1}$ for monoterpenes,
33 187 which are known to be emitted 5-10 times less than isoprene. The emission rates actually measured
34 188 are also presented, to have a quantitative assessment of the relationship between emission and
35 189 EIVMs.

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44 190 Plant material was both collected and tested during the summer months (June-August) in a common
45 191 garden at CNR-Rome, or measurements of isoprenoid emission were made in situ across Italy in
46 192 periods (June or September) characterized by high temperatures and non-limiting conditions
47 193 (especially no drought) for the physiology of plants. In all cases, a LI-COR 6400 (LI-COR, Lincoln
48 194 Nebraska, USA) was used to standardize measurements in its 6 cm^2 gas-exchange cuvette. This leaf
49 195 area was exposed to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density, 30°C , and 50% relative
50 196 humidity, under a flux of 0.5 L min^{-1} of air that was passed through a catalytic converter (Parker
51 197 Hannifin Corp., ChromGas Zero Air Generator 1001) to filter contaminants and other volatile
52 198 organic compounds. The released isoprenoids were collected into a cartridge packed with adsorbent

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3 199 (200 mg of Tenax; SRA Instruments, Milan, Italy). Tenax has been used in many past experiments
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5 200 for isoprene measurements. Though unable to retain high isoprene concentrations without
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7 201 undergoing breakthrough, Tenax may reveal concentrations as low as < 1 ppb, thus fulfilling the
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9 202 scope of separating non-emitters when loading small volumes of air onto the adsorbent. Two to five
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11 203 L of air were trapped at a flow of 150 ml min^{-1} in the cartridge that was placed at the outlet of the
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13 204 cuvette. Measurements were made when the physiological parameters of the leaf (photosynthesis,
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15 205 transpiration, stomatal conductance; also monitored by the LI-COR 6400 instrument) were stable,
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17 206 and were repeated on at least three different leaves of different plants. The number of replications
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19 207 was increased in presence of large intraspecific variation of the emission, particularly in the case of
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21 208 low monoterpene emitters.

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23 209 The cartridges were kept refrigerated until desorbed and analyzed with a GC-MS (Agilent 6850;
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25 210 SRA Instruments) using a capillary column (DB-5, Agilent, $30 \text{ m} \times 0.25 \text{ mm}$ inner diameter and
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27 211 $0.25 \mu\text{m}$ film thickness). The actual emissions were positively quantified filling the cartridges with
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29 212 2 L of air in which 70 ppb of gaseous standards (Rivoira, Milan, Italy) of isoprene or main
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31 213 monoterpenes (α -pinene, β -pinene, sabinene, myrcene, limonene) were mixed.

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33 215 **Broad-scale phylogenetic analysis**

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35 216 We created a composite phylogenetic tree representing the relationships among the studied species
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37 217 (Fig. 1). The tree is based on the Angiosperm Phylogeny Website (Stevens, 2001 onwards) and was
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39 218 further refined based on published molecular phylogenies (Appendix S2). In this way, we could
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41 219 determine the phylogenetic position of 128 species. However, as some of these species tolerate a
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43 220 wide range of moisture conditions (see Appendix S1), the phylogenetic analyses involving EIVM
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45 221 were limited to 119 species.

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47 222 By using the phylogenetic tree in Fig. 1 we performed an Abouheif (1999) test to assess whether
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49 223 isoprenoids emitters and EIVM show phylogenetic signal at this taxonomic scale. To test the
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51 224 association between phylogenetic signal and hygrophily we used the Ellenberg indicator values for
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53 225 moisture. By contrast, for testing for phylogenetic signal in isoprene and monoterpene emission
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55 226 capability, we performed two distinct tests on the Boolean, emitting/non-emitting (0/1) classes of
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57 227 both isoprenoid emission types.

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59 228 Next, we used the phylogeny to analyze the relationship between Ellenberg indicator values and
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1 229 isoprenoid emission. Therefore, we built Bayesian Phylogenetic Mixed Models using the
2 230 MCMCglmm R package (Hadfield 2010, R Core Team 2012), with either isoprene (emitter/non-
3 231 emitter) or monoterpene (emitter/non-emitter) emission as binary dependent variables and EIVM as
4 232 explanatory variable. The mixed model implemented in MCMCglmm can incorporate the

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3 233 phylogenetic relationships among species as a random factor, thereby controlling for the non-
4 234 independence of data points due to shared ancestry.

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7 236 **Narrow-scale phylogenetic analysis on Salicaceae**

8 237 Leaf samples were collected from *Salix* and *Populus* species (Appendix S3) and stored at -80°C
9 238 until DNA extraction. We selected *Populus* and *Salix* because i) species of these two genera play an
10 239 important role in the woody Mediterranean and European flora, ii) all species emit isoprene, and iii)
11 240 species from both genera represent several EIVM classes, spanning from class 3 (e.g. *Salix alpina*)
12 241 or 5 (*Populus tremula*) to class 8 (e.g. *Salix viminalis* or *Populus nigra*).

13 242 Total DNA was extracted using Invitex Invisorb Spin Plant Mini Kit (Stratec GmbH, Berlin,
14 243 Germany) according to manufacturer's instructions, from approximately 100 mg of material,
15 244 ground in the automatic grinding mill MM200 (Retsch GmbH, Haan, Germany). Isoprene synthase
16 245 gene was amplified using PaIspS-Fw2 and PaIspS-Bw3 primers (Fortunati *et al.* 2008; Appendix
17 246 S4). Polymerase chain reactions (PCRs) were performed in 100 µl containing 30 ng of template
18 247 DNA, 5x PCR reaction buffer (Promega Corporation, Madison, Wisconsin, USA), 0.2 mM of each
19 248 dNTPs, 0.2 µM of each primer, 2.0 mM MgCl₂, 3.2 U Taq polymerase (GoTaq, Promega). All
20 249 samples were amplified on a Mastercycler thermal cycler (Eppendorf, Hamburg, Germany),
21 250 following two touchdown PCR profiles for *Populus* and *Salix* species, respectively: 1) 3 min at
22 251 95°C, 15 touchdown cycles of 95°C 30s, 70°C 1' (-1°C/cycle), 72°C 2'; 20 cycles of 95°C 30s,
23 252 55°C 1', 72°C 2' and final extension at 72°C 10 min; 2) 3 min at 95°C, 15 touchdown cycles of
24 253 95°C 30s, 65°C 1' (-1°C/cycle), 72°C 2'; 20 cycles of 95°C 30s, 50°C 1', 72°C 2' and final
25 254 extension at 72°C 10 min.

26 255 PCR products were purified using GFX PCR DNA and Gel Band Purification Kit (GE Healthcare,
27 256 Uppsala, Sweden), and directly sequenced on an ABI 3130 Avant automated sequencer (Life
28 257 Technologies Corporation, Carlsbad, California, USA) using PaIspS-Fw2 and PaIspS-Bw3 primers
29 258 and specific internal primers (Appendix S4). Purifications of sequencing reactions products
30 259 followed the ethanol-sodium acetate precipitation protocol provided with the sequencing kit.
31 260 Confirmation of sequence identity was performed by BLASTN search against the GenBank non-
32 261 redundant database using default parameters (Altschul *et al.* 1997). The resulting amino acid
33 262 sequences were screened for the presence of specific residues that appear to be implicated in
34 263 reducing active site volume in isoprene synthases relative to monoterpene synthases (Sharkey *et al.*
35 264 2013).

36 265 The eleven *IspS* coding sequences obtained from poplar and willow species where the EIVM was
37 266 also identified, together with sequences of the same gene from other plant species (Appendix S3)

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3 267 were aligned using ClustalX (Thompson *et al.* 1997). The phylogenetic analyses were conducted
4 268 using the software MEGA v.5.05 (Tamura *et al.* 2011). Maximum likelihood phylogenetic trees
5 269 (ML) were reconstructed and the reliability of tree branches was evaluated by using bootstrapping
6 270 with 9999 pseudo-replicates (Felsenstein 1985). Further, a ds/dn analysis using SNAP
7 271 (Synonymous (ds) vs Nonsynonymous (dn) Analysis Program) at
8 272 <http://www.hiv.lanl.gov/content/sequence/SNAP/SNAP.html>, which calculates the proportion of
9 273 synonymous substitutions per potential synonymous site and the proportion of nonsynonymous
10 274 substitutions per potential nonsynonymous site using the Nei and Gojobori method (Nei and
11 275 Gojobori 1986), was carried out.

12 276 By using the *IspS* phylogenetic tree, an Abouheif test of phylogenetic signal was performed to
13 277 evaluate whether the EIVM of the *Salix* and *Populus* species were related to changes in *IspS*
14 278 sequences at this taxonomic scale.
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18 282 **RESULTS**

19 283

20 284 Among the woody species tested for emission of volatile isoprenoids, the proportion of isoprene-
21 285 emitting species was clearly higher in more hygrophilous EIVM classes, especially in class 8, where
22 286 about 80% of the plants emit isoprene (Fig. 2a). The trend was opposite for the emission of
23 287 monoterpenes, with monoterpene-emitters being found more often in the more xeric Ellenberg
24 288 classes (Fig. 2b). The association between the two classes of volatile isoprenoids and the moisture
25 289 level that characterize the habitats of the Mediterranean woody species was confirmed by a non-
26 290 parametric Mann-Whitney Z-test. This test showed that the median EIVM is significantly higher in
27 291 isoprene-emitting than in non-emitting species, while the EIVM is significantly lower in
28 292 monoterpene emitters than in non-emitters (Fig. 3, $p < 0.001$ in both cases).

29 293 A trend was also found when isoprenoid emission rates were attributed to EIVM classes. Plant
30 294 species belonging to hygrophilous EIVM classes emitted more isoprene (Fig. 4a, $p = 0.028$),
31 295 whereas the emission of monoterpenes was generally higher in the xeric EIVM classes (Fig. 4b, $p =$
32 296 0.030). However, when differences of emission rates among EIVM classes were assessed
33 297 statistically, only isoprene was significantly different (Kruskal-Wallis non-parametric test, $p =$
34 298 0.0042 , followed by post-hoc Dunn's Multiple Comparison Test showing differences between
35 299 means of EIVM contrasting classes, e.g. 2-6 and 7-8). In the case of monoterpenes, the Kruskal-
36 300 Wallis test yielded non-significant differences ($p = 0.136$), possibly because of the higher variability
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3 301 classes. The presence of monoterpene emitters with and without storage organs among the sampled
4 302 plant species might have contributed to make more variable the emission. As we only assessed
5 303 emissions, not contents, we did not separate monoterpene emitters according to the presence of
6 304 storage organs. The different emission rates of isoprenoids, as highlighted above, were not
7 305 associated with differences in the rates of photosynthesis among EIVM classes (data not shown).
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13 307 Since isoprene-emitters of hygrophyte EIVM classes are dominated by Salicaceae, and
14 308 monoterpene-emitters of xeric EIVM classes mostly belong to Cistaceae and Pinaceae, a
15 309 phylogenetic analysis was carried out to understand how the phylogeny could have interacted with
16 310 the ecological signal.

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19 311 Evidence of evolutionary conservatism in Ellenberg's indicator values was already found by
20 312 Prinzing *et al.* (2001). In agreement with this former report, the Abouheif test showed significant
21 313 phylogenetic signal in EIVM in our data set of woody species ($C = 0.380$, $p = 0.001$; 999
22 314 permutations, 119 species). Likewise, the species also showed significant phylogenetic signal in
23 315 both isoprene and monoterpene emitting competence ($C = 0.547$, $p = 0.001$, and $C = 0.276$, $p =$
24 316 0.001 for isoprene and monoterpene, respectively; in both cases 999 permutations and 128 species
25 317 were used). Accordingly, we may hypothesize that, at this broader phylogenetic scale, the species'
26 318 capability to adapt to more or less xeric terrestrial environments and their isoprenoid emissions are
27 319 both related to the evolutionary history of plants. In this view, EIVM and isoprenoids emissions
28 320 refer to large-scale environmental gradients, *sensu* Silvertown *et al.* (2006, Figure 1).

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31 321 However, when controlling for phylogeny in the MCMCglmm analysis, we found that the presence
32 322 of isoprene emission is not associated with Ellenberg indicator values (binomial phylogenetic mixed
33 323 model: posterior mean 2.804, lower 95% credibility interval -2.236, upper 95% credibility interval
34 324 7.272, $p = 0.133$). This is most likely due to the overwhelming influence of closely related,
35 325 hygrophilous isoprene emitters (mainly Salicaceae) in the dataset. To the contrary, monoterpene
36 326 emission is significantly less frequent in hygrophytes (binomial phylogenetic mixed model:
37 327 posterior mean -0.465, lower 95% credibility interval -0.882, upper 95% credibility interval -0.124,
38 328 $p < 0.01$), suggesting that the evolution of monoterpene emission is associated with transitions to
39 329 more xeric habitats. The two traits (i.e. isoprene and monoterpene emission) were not significantly
40 330 related to each other (binomial phylogenetic mixed model with isoprene emission as dependent
41 331 variable: posterior mean: 3.146, lower 95% credibility interval -23.604, upper 95% credibility
42 332 interval 28.925, $p = 0.711$), which suggests that the two traits are not complementary and their
43 333 evolution is probably determined by separate ecological factors.
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3 334 Since isoprene emitters belonging to *Populus* and *Salix* genera were clearly distributed along the
4 335 gradient of hygrophily, we further explored whether this distribution was associated with
5 336 phylogenetic differences, as inferred from differences in the isoprene synthase gene. Eleven partial
6 337 genomic isoprene synthase sequences were identified in *Populus* and *Salix* species (Appendix S3).
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8 338 All sequences displayed high degree of homology (from 95% to 100%) with already available *IspS*
9 339 sequences. All the sequences were screened for the presence of two Phe residues that are involved
10 340 in reducing active site volume in isoprene synthases relative to monoterpene synthases (Sharkey *et al.*
11 341 *al.* 2013). In addition, SNAP analysis demonstrated higher ds than dn in all sequences (average
12 342 ds/dn pairwise comparison ratio = 8.29).

13 343 Phylogenetic analysis was carried out by using the coding sequences of *IspS* of *Populus* and *Salix*
14 344 species isolated in this study, together with poplar sequences available in GenBank
15 345 (<http://www.ncbi.nlm.nih.gov/genbank/>) (listed in Appendix S3). When using *Vitis vinifera* and
16 346 *Pueraria montana* as outgroups, the ingroup turned out to be monophyletic even if the relative
17 347 position of the two outgroups has low bootstrap support. Two main clades were identified within
18 348 the in-group, one clustering most *Populus* species and the other clustering *Salix* species (Fig. 5a, b).
19 349 Furthermore, within the *Populus* clade, the species grouped according to section classification based
20 350 on other markers (Eckenwalder 1996). An exception was represented by *P. nigra*, which was
21 351 grouped within the *Populus* section in spite of being classified as a member of the *Ageiros* section
22 352 (Eckenwalder, 1996). The ecological adaptation trait, as marked by the EIVM classes, and the
23 353 pattern of nucleotide changes in *IspS* were not associated in poplar and willow species (Fig. 5b).
24 354 This was further confirmed by the non-significant results of the Abouheif test (Abouheif C = 0.033;
25 355 p = 0.357).

26 356 27 357 28 358 **DISCUSSION** 29 359

30 360 An association between isoprene emission and hygrophily was suggested by several independent
31 361 observations: a) that isoprene is emitted at higher rates in hygrophyte forest plants than in more
32 362 xeric plants of transitional woodlands and savannahs, e.g. in central Africa (Greenberg *et al.* 1999);
33 363 (b) that isoprene emission is generally more common in fast-growing, water-spending species
34 364 (Vickers *et al.* 2009). Perhaps this is in turn related to the phloem-loading mechanism, because
35 365 isoprene emitters are characterized by symplastic phloem loading (Kerstiens & Possell 2001).
36 366 Whether this trait is also related to fast-growth and hygrophily should be investigated; c) that
37 367 isoprene emission is more common in mosses than in other clades of plants (Hanson *et al.* 1999).
38 368 Hanson *et al.* (1999) suggested that isoprene emission by plants could have been an important

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3 369 ancient mechanism of adaptation to terrestrial environment that increased tolerance to thermal
4 370 stresses in environments not buffered by water. Vickers *et al.* (2009) argued that isoprene could also
5 371 have helped early land plants cope with high oxidative stress in the atmosphere.

6 372 Indeed, isoprene emission is more common and the emission rates are higher in the hygrophytes of
7 373 the Italian flora that we have tested. Emission rates expressed on a leaf area basis are reported here,
8 374 but the trend would hold when expressing isoprene emission on a leaf mass basis, as leaves of
9 375 hygrophytes are generally thinner than in xerophytes. An exception to this trend was found in
10 376 EIVM class 1. However, this xeric class includes only three species, and only one isoprene-emitting
11 377 species, *Chamaerops humilis*, which is the sole representative of Arecaceae (the palm family) in
12 378 mainland-Europe. This taxon evolved in moist tropical climates of the rain-forest biome, where it
13 379 still harbors its highest diversity; most palms have a very low drought-tolerance, and the few
14 380 species adapted to dry habitats are probably the result of recent radiation (Eiserhardt *et al.* 2011).
15 381 Interestingly, a similar reasoning might apply to *Myrtus communis*, the only emitter found in EIVM
16 382 class 2 (out of 16 tested taxa in this class), as this is the only European member of the tropical
17 383 family Myrtaceae (Biffin *et al.* 2010).

18 384 However, our large-scale phylogenetic analysis does not support the ecological value of these
19 385 observations, because of the strong phylogenetic signal in isoprene emission; for instance, most
20 386 isoprene emitting species in the more hygrophilous Ellenberg categories belong to Salicaceae (see
21 387 Appendix S1). As the evolution of isoprene emission is not associated with evolutionary adaptation
22 388 to hygrophily, our data suggests that resistance to other environmental factors (such as coping with
23 389 thermal or oxidative stresses (Vickers *et al.* 2009)) might characterize isoprene emitters. On the
24 390 other hand, our analysis has shown that, when the phylogenetic relationships are taken into account,
25 391 monoterpene emission is more common in xeric species of the Italian woody flora, suggesting that
26 392 monoterpenes evolved in arid habitats, independently on whether the emission of monoterpenes
27 393 occurs from storage pools or directly from photosynthesis, in a light-dependent way. Thus, different
28 394 classes of isoprenoids might have evolved in response to different environmental factors, rather than
29 395 being complementary of each other.

30 396 It is unclear why the isoprene emission trait has been lost multiple times in terrestrial plants (Harley
31 397 *et al.* 1999, Sharkey *et al.* 2005, Sharkey *et al.* 2013). Monson *et al.* (2013) recently noted that the
32 398 high frequency of loss might indicate that isoprene emission is a favorable trait only in a limited
33 399 number of environments, or for few plants. As monoterpenes and non-volatile isoprenoids are
34 400 effective antioxidants protecting plants from many abiotic and biotic stressors (Vickers *et al.* 2009),
35 401 our observations suggest that isoprene is synthesized and emitted only when more effective
36 402 mechanisms of stress protection, especially regarding stress conditions associated with xerophily,

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3 403 are not active. In fact, as shown in Appendix S1, most plant species emit either isoprene or
4 404 monoterpenes, not both. The trade-off between isoprene and monoterpenes was also observed by
5 405 Harrison *et al.* (2013) in a survey that was carried out at worldwide level, and therefore emerges as
6 406 an important feature, not limited to Mediterranean conditions. The emission of monoterpenes seems
7 407 to be a successful trait in alien species invading new territories, possibly again due to the ability of
8 408 monoterpenes of conferring resistance against multiple stresses (Llusia *et al.* 2010).

9 409 At a finer taxonomic scale, we then explored whether the hygrophily of isoprene emitters, as
10 410 indicated by species assignment to the Ellenberg classes, showed phylogenetic signal within
11 411 Salicaceae. Specifically, we tested whether isoprene emitters, phylogenetically close with respect to
12 412 *IspS*, also shared similar EIVM classes. However, the distribution of EIVM classes was not
13 413 associated to the phylogenetic patterns of *IspS*. We therefore hypothesize that *IspS* has not
14 414 undergone convergent evolution linked to ecological pressure, namely to adaptation to xeric
15 415 environments. Perhaps genes at earlier stages of the chloroplastic isoprenoid pathway are more
16 416 pleiotropic and are therefore subjected to heavier selective pressure than *IspS* (Ramsay *et al.* 2009),
17 417 or regulation of gene expression or enzyme activation, rather than gene sequence, provides
18 418 sufficient response to changes in hygrophily.

19 419 On the other hand, the phylogeny based on *IspS* showed that poplar and willow species could be
20 420 properly separated, indicating a strong match with taxonomic information (Eckenwalder 1996), and
21 421 confirming the value of genes underlying volatile isoprenoid biosynthesis as chemo-taxonomical
22 422 markers (Loreto *et al.* 2009). A relevant exception to the clear match between *IspS* phylogeny and
23 423 taxonomy in Mediterranean poplar species is represented by *P. nigra*, which grouped within the
24 424 section *Populus* in spite of being a member of the section *Ageiros*, maybe as a consequence of its
25 425 hybrid origin (Smith & Sytsma 1990).

26 426 The public availability of *IspS* sequences in GenBank made it possible to match the phylogenies of
27 427 our Mediterranean poplars with those of non-European poplars. The resulting ML tree showed that
28 428 gene identity between poplars of different regions of the world is higher than the identity between
29 429 genera sharing the same ecological environment. Moreover, *P. euphratica*, a species adapted to
30 430 desert conditions (Qiu *et al.* 2011) was phylogenetically very distant from Mediterranean species
31 431 that are adapted to xeric conditions. Accordingly, changes in *IspS* sequences on poplars of different
32 432 habitats strongly reflect the species' phylogenetic relationships rather than ecological adaptation.
33 433 Therefore, the gene evolution and function (i.e. isoprene emission) appears to be a strong
34 434 phylogenetic trait that did not undergo adaptive modification in recent evolutionary time. This
35 435 observation is in good agreement with the outlier behavior of *Chamaerops humilis* (Arecaceae) and
36 436 *Myrtus communis* (Myrtaceae): in spite of their xeric nature, both these plants have retained their

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3 437 ancestral isoprene-emission character. A similar conclusion was reached when analyzing isoprenoid
4 438 emissions in oaks (Loreto *et al.* 1998; Loreto *et al.* 2009). Similarly, also monoterpene-emitting
5 439 taxa, like e.g. the few *Betula* species that can be found in the Mediterranean area, in clearly
6 440 hygrophytic habitats, may have retained this trait due to a strong phylogenetic signal rather than
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8 441 with their present-day ecological distribution.

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11 442 In conclusion, we surmise that biosynthesis and emission of different volatile isoprenoids have
12 443 likely evolved in response to different stimuli. Isoprene likely has evolved independently many
13 444 times, characterizing about all vascular plants, from ferns to angiosperms. It might be a primitive
14 445 adaptive trait to terrestrial life, which might not have further evolved in response to more recent
15 446 ecological pressures, being rather lost in favor of more effective protective mechanisms, in
16 447 agreement with the ‘opportunistic’ hypothesis put forward by Owen & Peñuelas (2005).
17 448 Monoterpenes might have evolved to adapt to xeric environments and might yet be an important
18 449 adaptive trait in response to drought in the Mediterranean flora. Further studies are needed to test
19 450 these conclusions, both completing the current survey of European flora, and, at an even wider
20 451 level, providing more data about vegetation worldwide.

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3 626 **BIOSKETCHES**

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7 629 Sciences at the Italian National Research Council. His work spans plant physiology and ecology
8
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10
11 631 FL conceived and designed the experiments. FB, DC, JT, GS and SF conducted the phylogenetic
12 632 analysis, CC and GG performed eco-physiological measurements. MDL prepared the first database
13
14 633 of isoprenoids emission by woody species, which was revised by GF, and used as a template for
15
16 634 ecological assignment of plants by GF and GG. CR performed biostatistics. FL and CR wrote the
17 635 paper, and all authors contributed to the editing.

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639 **FIGURE LEGENDS**

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641 **Figure 1.** Cladogram describing, for the 128 species subjected to broad-scale phylogenetic analysis,
642 phylogenetic position, isoprenoid emissions capability (black circles; i = isoprene emitter, m =
643 monoterpene emitter), and Ellenberg indicator values for moisture (EIVM).

644

645 **Figure 2.** Fraction of isoprene (A) and monoterpene (B) emitters in the different classes of the
646 woody plant species of the Italian flora as ranked for hygrophily according to the Ellenberg
647 indicator values for moisture (EIVM: 1 = driest; 12 = wettest). Main families of isoprene
648 (Salicaceae) and monoterpene (Pinaceae, Cistaceae and Betulaceae) emitters are shown with
649 different bar patterns, as indicated in the figure legend. Statistical analysis is shown in Figure 3.

650

651 **Figure 3.** Box plots of the distribution in classes of Ellenberg indicator values for moisture (EIVM)
652 of isoprene (grey) and monoterpene (white) emitters versus non-emitters of the woody Italian flora
653 (see Figure 1). Boxes indicate 25-75 percentiles of the collected data. The lines inside boxes
654 indicate the median values. Bars outside boxes indicate the 5-95 percentiles of data, and circles
655 indicate outlier data. A non-parametric Mann-Whitney Z-test was used for comparing median
656 EIVM between emitters and non-emitters. Significant differences with respect to non-emitters were
657 found for both isoprene-emitters ($Z = 3.403$; $p < 0.001$), and monoterpene-emitters ($Z = -4.125$; $p <$
658 0.001). The latter is significant also after phylogenetic control, confirming the ecological relevance
659 of this finding, while this is not the case for isoprene (see Results).

660

661 **Figure 4.** Emission rates of isoprene (A) and monoterpenes (B) by woody species of the flora of
662 Italy ranked according to the Ellenberg indicator values for moisture (EIVM). The means and
663 standard errors of data collected through field measurements ($n \geq 3$) and surveys of available data
664 sets are shown. Best fits based on linear regressions are shown, together with regression
665 coefficients. The best fit lines showed a statistically significant trend toward higher emission of
666 isoprene in hygrophytes ($p = 0.028$) and higher emission of monoterpenes in xerophytes ($p =$
667 0.030). Further statistical analysis confirmed isoprene emission rates to be higher in hygrophytes
668 (Kruskal-Wallis non-parametric test, $p = 0.0042$), and statistically significant among EIVM classes
669 (Dunn's Multiple Comparison Test, significantly different means are shown by different letters, $p =$
670 0.05 ; class 1 was not included in the post-hoc test due to the low sample size (only one emitting
671 species, as shown in the text)). The Kruskal-Wallis test yielded non-significant differences ($p =$

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3 672 0.136) for monoterpenes, and therefore no test was performed to separate EIVM classes of
4 673 monoterpene-emitters.
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8 675 **Figure 5.** Phylogenetic tree based on *IspS* coding sequences identified in this study for *Populus* and
9 676 *Salix* species of the Italian flora (A). The numbers close to each species name refer to Ellenberg
10 677 indicator values for moisture (n.a. = not available). The numbers next to each node are the bootstrap
11 678 percentages from 10000 pseudo-replicates. Only bootstrap values above 50 % are presented on the
12 679 tree. In (B) the phylogenetic tree based on available *IspS* coding sequences of *Populus* and *Salix*
13 680 species is widened to compare with non-European poplar species, and with two outgroup species
14 681 whose *IspS* sequence is also known. Black dots refer to sequences obtained in this research. The
15 682 sections *Populus* (P), *Aigeiros* (A), *Tacamahaca* (Ta) and *Turanga* (Tu) are also indicated in (B).
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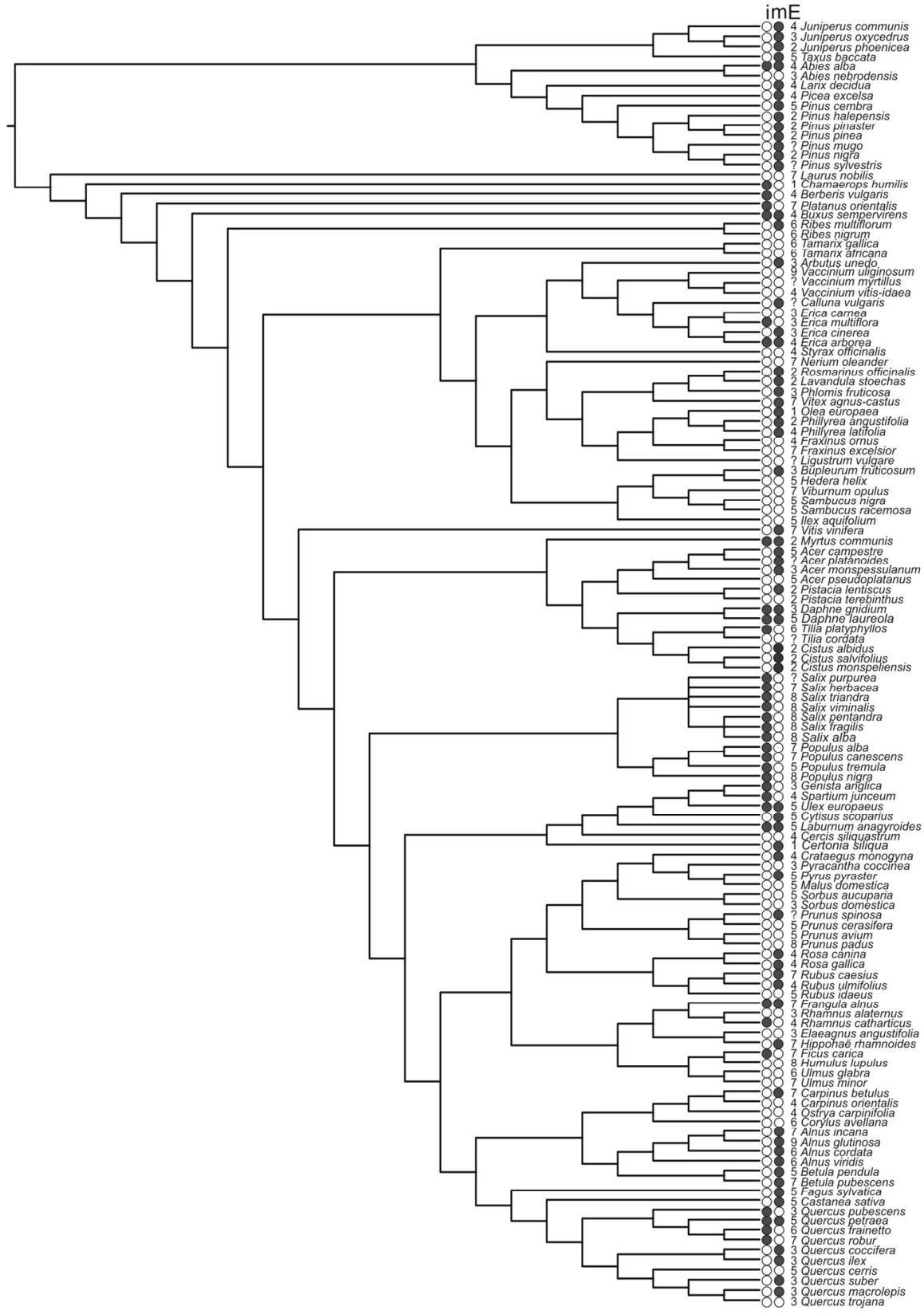
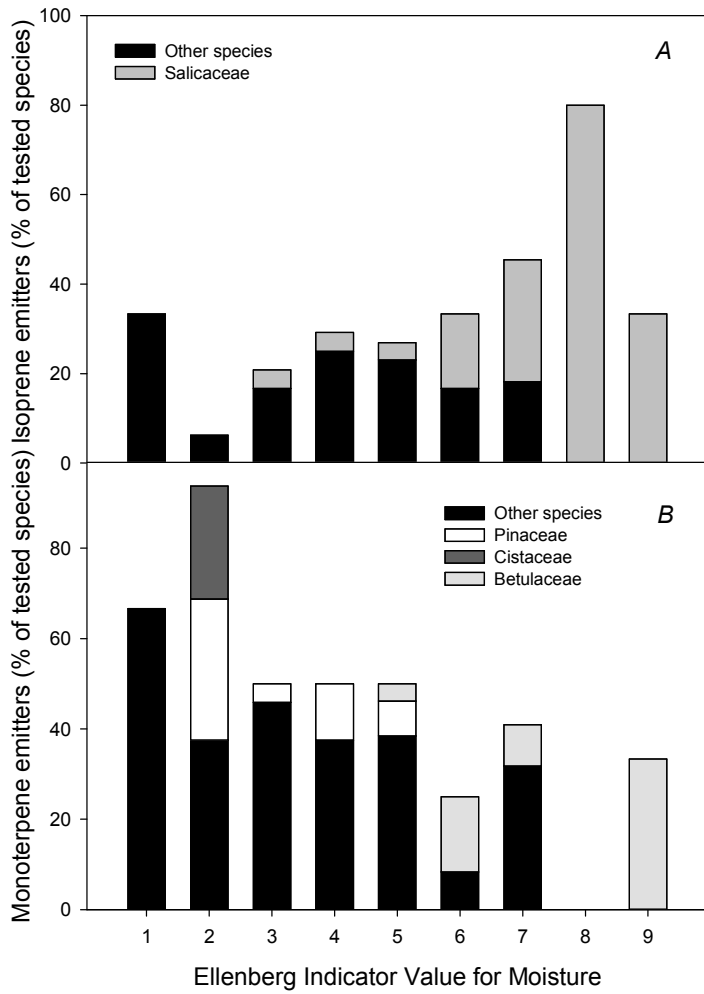


Figure 1

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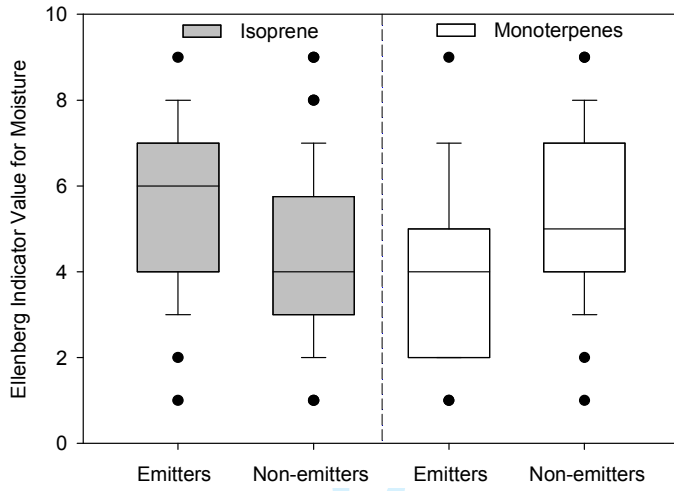


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Figure 2

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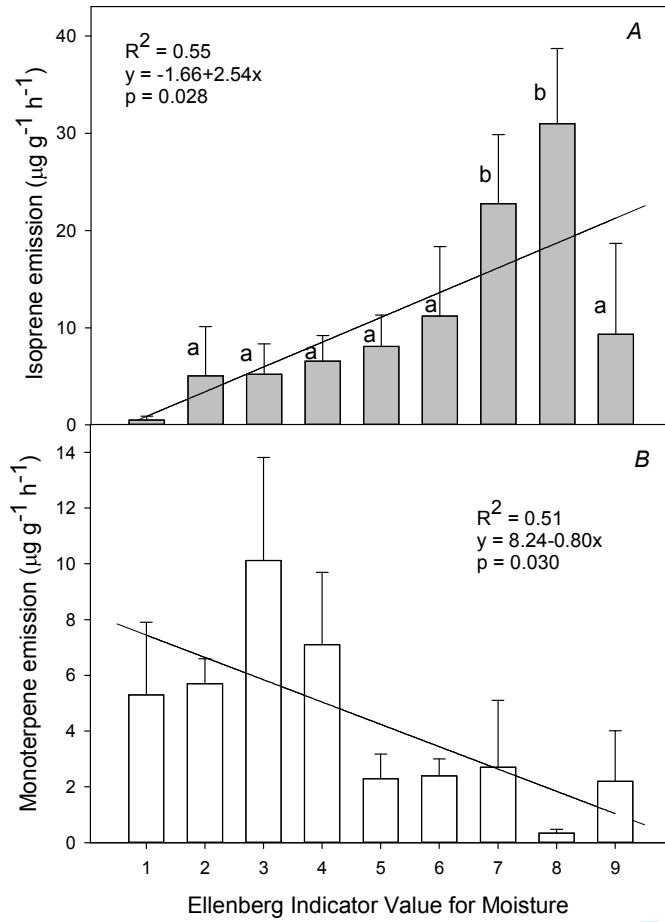


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Figure 3.

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Figure 4.

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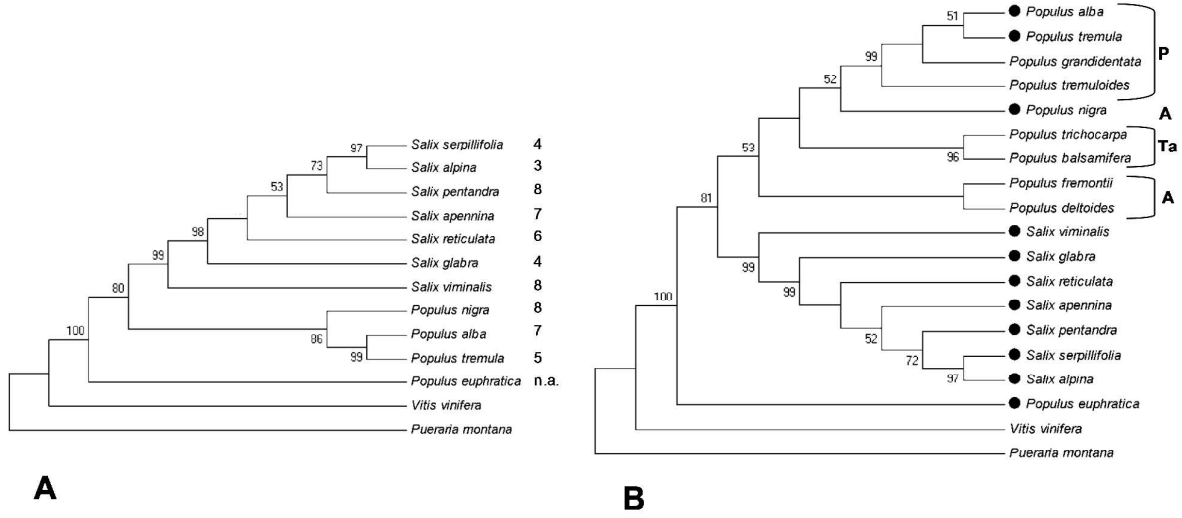


Figure 5.

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For Peer Review

Isoprenoid emission in hygrophyte and xerophyte European woody flora: ecological and evolutionary implications

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SUPPORTING INFORMATION

Appendix S1. Attribution of woody species of the flora of Italy to classes of Ellenberg Indicator Values for Moisture (EIVM; 1 to 9 is the incremental scale for moisture, ? indicates species that tolerate a wide range of moisture conditions), and to isoprenoid emission types (I = isoprene; M = monoterpenes; NE = non-emitter; NA = data not available). The superscripted number indicates references are available, as reported at the end of the table.

Family	Species	EIVM	Isoprenoid Emission
Pinaceae	<i>Abies alba</i> Miller	4	I+M ³
Pinaceae	<i>Abies nebrodensis</i> (Lojac.) Mattei	3	NE ²
Aceraceae	<i>Acer campestre</i> L.	5	M ³
Aceraceae	<i>Acer lobelii</i> Ten.	5	NA
Aceraceae	<i>Acer monspessulanum</i> L.	3	M ³
Aceraceae	<i>Acer obtusatum</i> W. et K.	4	M ¹
Aceraceae	<i>Acer platanoides</i> L.	2	M ³
Aceraceae	<i>Acer pseudoplatanus</i> L.	5	NE ⁴
Fabaceae	<i>Adenocarpus complicatus</i> (L.) Gay	3	NA
Betulaceae	<i>Alnus cordata</i> (Loisel.) Desf.	6	M ³
Betulaceae	<i>Alnus glutinosa</i> (L.) Gaertner	9	M ¹
Betulaceae	<i>Alnus incana</i> (L.) Moench	7	M ³
Betulaceae	<i>Alnus viridis</i> (Chaix) DC.	6	M ³
Rosaceae	<i>Amelanchier ovalis</i> Medicus	3	NA
Fabaceae	<i>Anagyris foetida</i> L.	2	NA
Fabaceae	<i>Anthyllis barba-jovis</i> L.	2	NA
Ericaceae	<i>Arbutus unedo</i> L.	3	M ⁵
Asteraceae	<i>Artemisia arborescens</i> L.	2	NA
Fabaceae	<i>Astragalus massiliensis</i> Lam.	2	NA
Fabaceae	<i>Astragalus sempervirens</i> Lam.	4	NA
Berberidaceae	<i>Berberis aetnensis</i> Presl	2	NA
Berberidaceae	<i>Berberis vulgaris</i> L.	4	I ¹
Betulaceae	<i>Betula nana</i> L.	9	NA
Betulaceae	<i>Betula pendula</i> Roth	5	M ³
Betulaceae	<i>Betula pubescens</i> Ehrh.	7	M ³
Apiaceae	<i>Bupleurum fruticosum</i> L.	3	M ⁷

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3	<u>Buxaceae</u>	<u>Buxus balearica Lam.</u>	<u>3</u>	<u>NA</u>
4	<u>Buxaceae</u>	<u>Buxus sempervirens L.</u>	<u>4</u>	<u>I+M³</u>
5	<u>Fabaceae</u>	<u>Calicotome spinosa (L.) Link</u>	<u>2</u>	<u>NA</u>
6	<u>Fabaceae</u>	<u>Calicotome villosa (Poiret) Link</u>	<u>2</u>	<u>NA</u>
7	<u>Ericaceae</u>	<u>Calluna vulgaris (L.) Hull</u>	<u>?</u>	<u>M⁸</u>
8	<u>Capparidaceae</u>	<u>Capparis ovata Desf.</u>	<u>2</u>	<u>NA</u>
9	<u>Capparidaceae</u>	<u>Capparis spinosa L.</u>	<u>2</u>	<u>NA</u>
10	<u>Corylaceae</u>	<u>Carpinus betulus L.</u>	<u>7</u>	<u>M¹</u>
11	<u>Corylaceae</u>	<u>Carpinus orientalis Miller</u>	<u>4</u>	<u>NE³</u>
12	<u>Fagaceae</u>	<u>Castanea sativa Miller</u>	<u>5</u>	<u>M³</u>
13	<u>Ulmaceae</u>	<u>Celtis aetnensis (Tornabene) Strobl</u>	<u>3</u>	<u>NA</u>
14	<u>Ulmaceae</u>	<u>Celtis australis L.</u>	<u>3</u>	<u>NA</u>
15	<u>Fabaceae</u>	<u>Ceratonia siliqua L.</u>	<u>1</u>	<u>M^{1,9}</u>
16	<u>Fabaceae</u>	<u>Cercis siliquastrum L.</u>	<u>4</u>	<u>NE³</u>
17	<u>Areaceae</u>	<u>Chamaerops humilis L.</u>	<u>1</u>	<u>I¹⁰</u>
18	<u>Cistaceae</u>	<u>Cistus albidus L.</u>	<u>2</u>	<u>M^{1,9}</u>
19	<u>Cistaceae</u>	<u>Cistus clusii Dunal</u>	<u>2</u>	<u>NA</u>
20	<u>Cistaceae</u>	<u>Cistus corsicus Loisel.</u>	<u>2</u>	<u>NA</u>
21	<u>Cistaceae</u>	<u>Cistus creticus L.</u>	<u>2</u>	<u>NA</u>
22	<u>Cistaceae</u>	<u>Cistus crispus L.</u>	<u>2</u>	<u>NA</u>
23	<u>Cistaceae</u>	<u>Cistus incanus L.</u>	<u>2</u>	<u>M¹</u>
24	<u>Cistaceae</u>	<u>Cistus laurifolius L.</u>	<u>2</u>	<u>NA</u>
25	<u>Cistaceae</u>	<u>Cistus monspeliensis L.</u>	<u>2</u>	<u>M¹</u>
26	<u>Cistaceae</u>	<u>Cistus parviflorus Lam.</u>	<u>2</u>	<u>NA</u>
27	<u>Cistaceae</u>	<u>Cistus salvifolius L.</u>	<u>2</u>	<u>M⁶</u>
28	<u>Ranunculaceae</u>	<u>Clematis alpina (L.) Miller</u>	<u>5</u>	<u>NA</u>
29	<u>Ranunculaceae</u>	<u>Clematis cirrhosa L.</u>	<u>2</u>	<u>NA</u>
30	<u>Ranunculaceae</u>	<u>Clematis flammula L.</u>	<u>3</u>	<u>NA</u>
31	<u>Ranunculaceae</u>	<u>Clematis vitalba L.</u>	<u>5</u>	<u>NA</u>
32	<u>Ranunculaceae</u>	<u>Clematis viticella L.</u>	<u>4</u>	<u>NA</u>
33	<u>Cneoraceae</u>	<u>Cneorum tricocon L.</u>	<u>2</u>	<u>NA</u>
34	<u>Fabaceae</u>	<u>Colutea arborescens L.</u>	<u>3</u>	<u>NA</u>
35	<u>Coriariaceae</u>	<u>Coriaria myrtifolia L.</u>	<u>3</u>	<u>NA</u>
36	<u>Cornaceae</u>	<u>Cornus mas L.</u>	<u>5</u>	<u>NA</u>
37	<u>Cornaceae</u>	<u>Cornus sanguinea L.</u>	<u>6</u>	<u>NA</u>
38	<u>Fabaceae</u>	<u>Coronilla emerus L.</u>	<u>4</u>	<u>NA</u>
39	<u>Fabaceae</u>	<u>Coronilla juncea L.</u>	<u>2</u>	<u>NA</u>
40	<u>Fabaceae</u>	<u>Coronilla valentina L.</u>	<u>2</u>	<u>NA</u>
41	<u>Corylaceae</u>	<u>Corylus avellana L.</u>	<u>6</u>	<u>NE³</u>
42	<u>Anacardiaceae</u>	<u>Cotinus coggygria Scop.</u>	<u>3</u>	<u>NA</u>
43	<u>Rosaceae</u>	<u>Cotoneaster integerrimus Medicus</u>	<u>3</u>	<u>NA</u>
44	<u>Rosaceae</u>	<u>Cotoneaster nebrodensis (Guss.) Koch</u>	<u>3</u>	<u>NA</u>
45	<u>Rosaceae</u>	<u>Crataegus laciniata Ucria</u>	<u>3</u>	<u>NA</u>
46	<u>Rosaceae</u>	<u>Crataegus monogyna Jacq.</u>	<u>4</u>	<u>M⁸</u>
47	<u>Rosaceae</u>	<u>Crataegus oxyacantha L.</u>	<u>5</u>	<u>NA</u>
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3	<u>Asclepiadaceae</u>	<u>Cynanchum acutum L.</u>	<u>7</u> <u>NA</u>
4	<u>Fabaceae</u>	<u>Cytisus aeolicus Guss.</u>	<u>3</u> <u>NA</u>
5	<u>Fabaceae</u>	<u>Cytisus scoparius (L.) Link</u>	<u>5</u> <u>I</u> ⁶
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7	<u>Fabaceae</u>	<u>Cytisus sessilifolius L.</u>	<u>5</u> <u>NA</u>
8	<u>Fabaceae</u>	<u>Cytisus villosus Pourret</u>	<u>4</u> <u>NA</u>
9	<u>Thymelaeaceae</u>	<u>Daphne alpina L.</u>	<u>3</u> <u>NA</u>
10	<u>Thymelaeaceae</u>	<u>Daphne gnidium L.</u>	<u>3</u> <u>I + M</u> ⁸
11	<u>Thymelaeaceae</u>	<u>Daphne laureola L.</u>	<u>5</u> <u>I + M</u> ¹¹
12	<u>Thymelaeaceae</u>	<u>Daphne mezereum L.</u>	<u>5</u> <u>NA</u>
13	<u>Thymelaeaceae</u>	<u>Daphne oleoides Schreber</u>	<u>2</u> <u>NA</u>
14	<u>Thymelaeaceae</u>	<u>Daphne sericea Vahl</u>	<u>3</u> <u>NA</u>
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16	<u>Elaeagnaceae</u>	<u>Elaeagnus angustifolia L.</u>	<u>3</u> <u>NE</u> ²
17	<u>Empetraceae</u>	<u>Empetrum hermaphroditum Hagerup</u>	<u>4</u> <u>NA</u>
18	<u>Ephedraceae</u>	<u>Ephedra distachya L.</u>	<u>3</u> <u>NA</u>
19	<u>Ephedraceae</u>	<u>Ephedra fragilis Desf.</u>	<u>3</u> <u>NA</u>
20	<u>Ephedraceae</u>	<u>Ephedra helvetica C.A. Meyer</u>	<u>3</u> <u>NA</u>
21	<u>Ephedraceae</u>	<u>Ephedra major Host</u>	<u>3</u> <u>NA</u>
22			
23	<u>Ericaceae</u>	<u>Erica arborea L.</u>	<u>4</u> <u>I+M</u> ³
24	<u>Ericaceae</u>	<u>Erica carnea L.</u>	<u>3</u> <u>NE</u> ²
25	<u>Ericaceae</u>	<u>Erica cinerea L.</u>	<u>3</u> <u>M</u> ⁸
26	<u>Ericaceae</u>	<u>Erica multiflora L.</u>	<u>3</u> <u>I</u> ⁵
27	<u>Ericaceae</u>	<u>Erica scoparia L.</u>	<u>3</u> <u>NE</u> ³
28	<u>Ericaceae</u>	<u>Erica sicula Guss.</u>	<u>2</u> <u>NA</u>
29	<u>Ericaceae</u>	<u>Erica terminalis Salisb.</u>	<u>2</u> <u>NA</u>
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31	<u>Celastraceae</u>	<u>Euonymus europaeus L.</u>	<u>5</u> <u>NA</u>
32	<u>Celastraceae</u>	<u>Euonymus latifolius (L.) Miller</u>	<u>5</u> <u>NA</u>
33	<u>Celastraceae</u>	<u>Euonymus verrucosus Scop.</u>	<u>5</u> <u>NA</u>
34	<u>Euphorbiaceae</u>	<u>Euphorbia dendroides L.</u>	<u>2</u> <u>NA</u>
35	<u>Fagaceae</u>	<u>Fagus sylvatica L.</u>	<u>5</u> <u>M</u> ³
36	<u>Moraceae</u>	<u>Ficus carica L.</u>	<u>7</u> <u>I</u> ⁹
37	<u>Rhamnaceae</u>	<u>Frangula alnus Miller</u>	<u>7</u> <u>I+M</u> ⁸
38	<u>Rhamnaceae</u>	<u>Frangula rupestris (Scop.) Schur</u>	<u>3</u> <u>NA</u>
39	<u>Oleaceae</u>	<u>Fraxinus excelsior L.</u>	<u>7</u> <u>NE</u> ³
40	<u>Oleaceae</u>	<u>Fraxinus ornus L.</u>	<u>4</u> <u>NE</u> ³
41	<u>Oleaceae</u>	<u>Fraxinus oxycarpa Bieb.</u>	<u>7</u> <u>NA</u>
42	<u>Fabaceae</u>	<u>Genista acanthoclada DC.</u>	<u>2</u> <u>NA</u>
43	<u>Fabaceae</u>	<u>Genista aetnensis (Biv.) DC.</u>	<u>3</u> <u>NA</u>
44	<u>Fabaceae</u>	<u>Genista anglica L.</u>	<u>3</u> <u>I</u> ¹¹
45	<u>Fabaceae</u>	<u>Genista aspalathoides Lam.</u>	<u>2</u> <u>NA</u>
46	<u>Fabaceae</u>	<u>Genista cinerea (Vill.) DC.</u>	<u>3</u> <u>NA</u>
47	<u>Fabaceae</u>	<u>Genista corsica (Loisel.) DC.</u>	<u>2</u> <u>NA</u>
48	<u>Fabaceae</u>	<u>Genista ephedroides DC.</u>	<u>2</u> <u>NA</u>
49	<u>Fabaceae</u>	<u>Genista morisii Colla</u>	<u>2</u> <u>NA</u>
50	<u>Fabaceae</u>	<u>Genista salzmannii DC.</u>	<u>2</u> <u>NA</u>
51	<u>Cistaceae</u>	<u>Halimium halimifolium (L.) Willk.</u>	<u>2</u> <u>NA</u>
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3	<u>Araliaceae</u>	<u>Hedera helix L.</u>	<u>5</u>	<u>NE</u> ⁴
4	<u>Elaeagnaceae</u>	<u>Hippophae rhamnoides L.</u>	<u>7</u>	<u>M</u> ¹
5	<u>Cannabaceae</u>	<u>Humulus lupulus L.</u>	<u>8</u>	<u>NE</u> ²
6				
7	<u>Aquifoliaceae</u>	<u>Ilex aquifolium L.</u>	<u>5</u>	<u>NE</u> ³
8	<u>Cupressaceae</u>	<u>Juniperus communis L.</u>	<u>4</u>	<u>M</u> ³
9	<u>Cupressaceae</u>	<u>Juniperus oxycedrus L.</u>	<u>3</u>	<u>M</u> ³
10	<u>Cupressaceae</u>	<u>Juniperus phoenicea L.</u>	<u>2</u>	<u>M</u> ¹²
11	<u>Cupressaceae</u>	<u>Juniperus sabina L.</u>	<u>3</u>	<u>NA</u>
12	<u>Cupressaceae</u>	<u>Juniperus thurifera L.</u>	<u>3</u>	<u>NA</u>
13				
14	<u>Chenopodiaceae</u>	<u>Kochia prostrata (L.) Schrader</u>	<u>3</u>	<u>NA</u>
15	<u>Fabaceae</u>	<u>Laburnum alpinum (Miller) B. et Presl</u>	<u>6</u>	<u>NA</u>
16	<u>Fabaceae</u>	<u>Laburnum anagyroides Medicus</u>	<u>5</u>	<u>I + M</u> ¹¹
17				
18	<u>Pinaceae</u>	<u>Larix decidua Miller</u>	<u>4</u>	<u>M</u> ³
19	<u>Lauraceae</u>	<u>Laurus nobilis L.</u>	<u>7</u>	<u>M</u> ²¹
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21	<u>Lamiaceae</u>	<u>Lavandula angustifolia Miller</u>	<u>3</u>	<u>NA</u>
22	<u>Lamiaceae</u>	<u>Lavandula latifolia Medicus</u>	<u>3</u>	<u>NA</u>
23	<u>Lamiaceae</u>	<u>Lavandula multifida L.</u>	<u>3</u>	<u>NA</u>
24	<u>Lamiaceae</u>	<u>Lavandula stoechas L.</u>	<u>2</u>	<u>M</u> ⁶
25	<u>Malvaceae</u>	<u>Lavatera agrifolia Tineo</u>	<u>2</u>	<u>NA</u>
26	<u>Malvaceae</u>	<u>Lavatera maritima Gouan</u>	<u>2</u>	<u>NA</u>
27	<u>Malvaceae</u>	<u>Lavatera olbia L.</u>	<u>2</u>	<u>NA</u>
28	<u>Malvaceae</u>	<u>Lavatera triloba L.</u>	<u>2</u>	<u>NA</u>
29	<u>Fabaceae</u>	<u>Lembotropis nigricans (L.) Griseb.</u>	<u>4</u>	<u>NA</u>
30	<u>Oleaceae</u>	<u>Ligustrum vulgare L.</u>	<u>?</u>	<u>NE</u> ²
31	<u>Caprifoliaceae</u>	<u>Lonicera alpigena L.</u>	<u>6</u>	<u>NA</u>
32	<u>Caprifoliaceae</u>	<u>Lonicera caprifolium L.</u>	<u>6</u>	<u>NA</u>
33	<u>Caprifoliaceae</u>	<u>Lonicera coerulea L.</u>	<u>8</u>	<u>NA</u>
34	<u>Caprifoliaceae</u>	<u>Lonicera etrusca Santi</u>	<u>3</u>	<u>NA</u>
35	<u>Caprifoliaceae</u>	<u>Lonicera implexa Aiton</u>	<u>3</u>	<u>NA</u>
36	<u>Caprifoliaceae</u>	<u>Lonicera nigra L.</u>	<u>5</u>	<u>NA</u>
37	<u>Caprifoliaceae</u>	<u>Lonicera periclymenum L.</u>	<u>?</u>	<u>NA</u>
38	<u>Caprifoliaceae</u>	<u>Lonicera stabiana Pasquale</u>	<u>2</u>	<u>NA</u>
39	<u>Caprifoliaceae</u>	<u>Lonicera xylosteum L.</u>	<u>5</u>	<u>NA</u>
40	<u>Rosaceae</u>	<u>Malus domestica Borkh.</u>	<u>5</u>	<u>NE</u> ³
41	<u>Rosaceae</u>	<u>Malus florentina (Zuccagni) Schneider</u>	<u>5</u>	<u>NA</u>
42	<u>Rosaceae</u>	<u>Malus sylvestris Miller</u>	<u>5</u>	<u>NA</u>
43	<u>Rosaceae</u>	<u>Mespilus germanica L.</u>	<u>4</u>	<u>NA</u>
44	<u>Myrtaceae</u>	<u>Myrtus communis L.</u>	<u>2</u>	<u>I+M</u> ¹³
45	<u>Apocynaceae</u>	<u>Nerium oleander L.</u>	<u>7</u>	<u>NE</u> ⁶
46	<u>Oleaceae</u>	<u>Olea europaea L. var. sylvestris Brot.</u>	<u>1</u>	<u>M</u> ^{1,9}
47	<u>Corylaceae</u>	<u>Ostrya carpinifolia Scop.</u>	<u>4</u>	<u>NE</u> ³
48	<u>Santalaceae</u>	<u>Osyris alba L.</u>	<u>3</u>	<u>NA</u>
49	<u>Rhamnaceae</u>	<u>Paliurus spina-christi Miller</u>	<u>3</u>	<u>NA</u>
50	<u>Asclepiadaceae</u>	<u>Periploca graeca L.</u>	<u>7</u>	<u>NA</u>
51	<u>Asclepiadaceae</u>	<u>Periploca laevigata Aiton</u>	<u>2</u>	<u>NA</u>
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3	Oleaceae	<u>Phillyrea angustifolia L.</u>	<u>2</u> <u>M</u> ¹²
4	Oleaceae	<u>Phillyrea latifolia L.</u>	<u>4</u> <u>M</u> ³
5	Lamiaceae	<u>Phlomis ferruginea Ten.</u>	<u>3</u> <u>NA</u>
6	Lamiaceae	<u>Phlomis fruticosa L.</u>	<u>3</u> <u>M</u> ¹
7	Pinaceae	<u>Picea excelsa (Lam.) Link</u>	<u>4</u> <u>M</u> ³
8	Pinaceae	<u>Pinus cembra L.</u>	<u>5</u> <u>M</u> ³
9	Pinaceae	<u>Pinus halepensis Miller</u>	<u>2</u> <u>M</u> ⁹
10	Pinaceae	<u>Pinus laricio Poiret</u>	<u>3</u> <u>M</u> ¹
11	Pinaceae	<u>Pinus leucodermis Antoine</u>	<u>2</u> <u>M</u> ³
12	Pinaceae	<u>Pinus mugo Turra</u>	<u>?</u> <u>M</u> ³
13	Pinaceae	<u>Pinus nigra Arnold</u>	<u>2</u> <u>M</u> ³
14	Pinaceae	<u>Pinus pinaster Aiton</u>	<u>2</u> <u>M</u> ⁸
15	Pinaceae	<u>Pinus pinea L.</u>	<u>2</u> <u>M</u> ³
16	Pinaceae	<u>Pinus sylvestris L.</u>	<u>?</u> <u>M</u> ³
17	Pinaceae	<u>Pinus uncinata Miller</u>	<u>5</u> <u>M</u> ³
18	Anacardiaceae	<u>Pistacia lentiscus L.</u>	<u>2</u> <u>M</u> ³
19	Anacardiaceae	<u>Pistacia terebinthus L.</u>	<u>2</u> <u>NE</u> ¹
20	Platanaceae	<u>Platanus orientalis L.</u>	<u>7</u> <u>I</u> ³
21	Salicaceae	<u>Populus alba L.</u>	<u>7</u> <u>I</u> ³
22	Salicaceae	<u>Populus canescens (Aiton) Sm.</u>	<u>7</u> <u>I</u> ³
23	Salicaceae	<u>Populus nigra L.</u>	<u>8</u> <u>I</u> ³
24	Salicaceae	<u>Populus tremula L.</u>	<u>5</u> <u>I</u> ³
25	Rosaceae	<u>Prunus avium L.</u>	<u>5</u> <u>NE</u> ³
26	Rosaceae	<u>Prunus brigantina Vill.</u>	<u>5</u> <u>NA</u>
27	Rosaceae	<u>Prunus cerasifera Ehrh.</u>	<u>5</u> <u>NE</u> ¹⁴
28	Rosaceae	<u>Prunus coccomilia Ten.</u>	<u>5</u> <u>NA</u>
29	Rosaceae	<u>Prunus fruticosa Pallas</u>	<u>3</u> <u>NA</u>
30	Rosaceae	<u>Prunus mahaleb L.</u>	<u>3</u> <u>NA</u>
31	Rosaceae	<u>Prunus padus L.</u>	<u>8</u> <u>NE</u> ³
32	Rosaceae	<u>Prunus prostrata Labill.</u>	<u>2</u> <u>NA</u>
33	Rosaceae	<u>Prunus spinosa L.</u>	<u>?</u> <u>M</u> ¹¹
34	Rosaceae	<u>Prunus webbii (Spach) Vierh.</u>	<u>2</u> <u>NA</u>
35	Rubiaceae	<u>Putoria calabrica (L.fil.) Pers.</u>	<u>2</u> <u>NA</u>
36	Rosaceae	<u>Pyracantha coccinea Roemer</u>	<u>3</u> <u>NE</u> ¹⁴
37	Rosaceae	<u>Pyrus amygdaliformis Vill.</u>	<u>4</u> <u>NA</u>
38	Rosaceae	<u>Pyrus pyraister Burgsd.</u>	<u>5</u> <u>M</u> ¹
39	Fagaceae	<u>Quercus cerris L.</u>	<u>5</u> <u>NE</u> ¹⁵
40	Fagaceae	<u>Quercus coccifera L.</u>	<u>3</u> <u>M</u> ³
41	Fagaceae	<u>Quercus frainetto Ten.</u>	<u>6</u> <u>I</u> ³
42	Fagaceae	<u>Quercus ilex L.</u>	<u>3</u> <u>M</u> ³
43	Fagaceae	<u>Quercus macrolepis Kotschy</u>	<u>3</u> <u>M</u> ³
44	Fagaceae	<u>Quercus petraea (Mattuschka) Liebl.</u>	<u>5</u> <u>I+M</u> ³
45	Fagaceae	<u>Quercus pubescens Willd.</u>	<u>3</u> <u>I</u> ³
46	Fagaceae	<u>Quercus pyrenaica Willd.</u>	<u>5</u> <u>I + M</u> ⁸
47	Fagaceae	<u>Quercus robur L.</u>	<u>7</u> <u>I</u> ¹
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3	Fagaceae	<u>Quercus suber L.</u>	<u>3</u> <u>M</u> ⁹
4	Fagaceae	<u>Quercus trojana Webb</u>	<u>3</u> <u>NE</u> ¹⁵
5	Fabaceae	<u>Retama raetam (Forsskal) Webb et Berth.</u>	<u>1</u> <u>NA</u>
6			
7	Rhamnaceae	<u>Rhamnus alaternus L.</u>	<u>3</u> <u>NE</u> ¹⁶
8	Rhamnaceae	<u>Rhamnus alpinus L.</u>	<u>5</u> <u>NA</u>
9	Rhamnaceae	<u>Rhamnus catharticus L.</u>	<u>4</u> <u>I</u> ¹¹
10	Rhamnaceae	<u>Rhamnus glaucophyllus Sommier</u>	<u>4</u> <u>NA</u>
11	Rhamnaceae	<u>Rhamnus lojaconoi Raimondo</u>	<u>4</u> <u>NA</u>
12	Rhamnaceae	<u>Rhamnus oleoides L.</u>	<u>2</u> <u>NA</u>
13	Rhamnaceae	<u>Rhamnus persicifolius Moris</u>	<u>3</u> <u>NA</u>
14	Rhamnaceae	<u>Rhamnus pumilus Turra</u>	<u>2</u> <u>NA</u>
15	Rhamnaceae	<u>Rhamnus saxatilis Jacq.</u>	<u>3</u> <u>NA</u>
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17	Ericaceae	<u>Rhododendron ferrugineum L.</u>	<u>6</u> <u>NA</u>
18	Ericaceae	<u>Rhododendron hirsutum L.</u>	<u>4</u> <u>NA</u>
19	Anacardiaceae	<u>Rhus pentaphylla (Jacq.) Desf.</u>	<u>3</u> <u>NA</u>
20	Anacardiaceae	<u>Rhus tripartita (Ucria) Grande</u>	<u>3</u> <u>NA</u>
21			
22	Saxifragaceae	<u>Ribes alpinum L.</u>	<u>?</u> <u>NA</u>
23	Saxifragaceae	<u>Ribes multiflorum Kit.</u>	<u>6</u> <u>M</u> ¹
24	Saxifragaceae	<u>Ribes nigrum L.</u>	<u>6</u> <u>NE</u> ⁴
25	Saxifragaceae	<u>Ribes petraeum Wulfen</u>	<u>4</u> <u>NA</u>
26	Saxifragaceae	<u>Ribes rubrum L.</u>	<u>8</u> <u>NA</u>
27	Saxifragaceae	<u>Ribes sardoum Martelli</u>	<u>3</u> <u>NA</u>
28	Saxifragaceae	<u>Ribes uva-crispa L.</u>	<u>?</u> <u>NA</u>
29			
30	Rosaceae	<u>Rosa agrestis Savi</u>	<u>3</u> <u>NA</u>
31	Rosaceae	<u>Rosa arvensis Hudson</u>	<u>5</u> <u>NA</u>
32	Rosaceae	<u>Rosa canina L.</u>	<u>4</u> <u>M</u> ^{1,17}
33	Rosaceae	<u>Rosa gallica L.</u>	<u>4</u> <u>M</u> ¹
34	Rosaceae	<u>Rosa micrantha Sm.</u>	<u>3</u> <u>NA</u>
35	Rosaceae	<u>Rosa pendulina L.</u>	<u>5</u> <u>NA</u>
36	Rosaceae	<u>Rosa pouzinii Tratt.</u>	<u>3</u> <u>NA</u>
37	Rosaceae	<u>Rosa sempervirens L.</u>	<u>3</u> <u>NA</u>
38	Lamiaceae	<u>Rosmarinus officinalis L.</u>	<u>2</u> <u>M</u> ^{1,13}
39	Rosaceae	<u>Rubus caesius L.</u>	<u>7</u> <u>M</u> ¹¹
40	Rosaceae	<u>Rubus canescens DC.</u>	<u>4</u> <u>NA</u>
41	Rosaceae	<u>Rubus hirtus W. et K.</u>	<u>4</u> <u>NA</u>
42	Rosaceae	<u>Rubus idaeus L.</u>	<u>5</u> <u>NE</u> ⁴
43	Rosaceae	<u>Rubus ulmifolius Schott</u>	<u>4</u> <u>M</u> ⁸
44	Salicaceae	<u>Salix alba L.</u>	<u>8</u> <u>I</u> ¹
45	Salicaceae	<u>Salix alpina Scop.</u>	<u>3</u> <u>I</u> ¹
46	Salicaceae	<u>Salix apennina Skvortsov</u>	<u>7</u> <u>I</u> ¹
47	Salicaceae	<u>Salix atrocinnerea Brot.</u>	<u>7</u> <u>I</u> ¹
48	Salicaceae	<u>Salix aurita L.</u>	<u>8</u> <u>I</u> ¹
49	Salicaceae	<u>Salix breviserrata Flod.</u>	<u>3</u> <u>NA</u>
50	Salicaceae	<u>Salix caesia Vill.</u>	<u>4</u> <u>NA</u>
51	Salicaceae	<u>Salix caprea L.</u>	<u>6</u> <u>I</u> ³
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3	<u>Salicaceae</u>	<u>Salix cinerea L.</u>	<u>9</u>	<u>I¹</u>
4	<u>Salicaceae</u>	<u>Salix crataegifolia Bertol.</u>	<u>6</u>	<u>NA</u>
5	<u>Salicaceae</u>	<u>Salix daphnoides Vill.</u>	<u>4</u>	<u>NA</u>
6				
7	<u>Salicaceae</u>	<u>Salix eleagnos Scop.</u>	<u>7</u>	<u>I¹</u>
8	<u>Salicaceae</u>	<u>Salix foetida Schleicher</u>	<u>4</u>	<u>NA</u>
9	<u>Salicaceae</u>	<u>Salix fragilis L.</u>	<u>8</u>	<u>I¹</u>
10	<u>Salicaceae</u>	<u>Salix glabra Scop.</u>	<u>4</u>	<u>I¹</u>
11	<u>Salicaceae</u>	<u>Salix glaucosericea Flod.</u>	<u>3</u>	<u>NA</u>
12	<u>Salicaceae</u>	<u>Salix hastata L.</u>	<u>6</u>	<u>NA</u>
13	<u>Salicaceae</u>	<u>Salix hegetschweileri Heer</u>	<u>3</u>	<u>NA</u>
14	<u>Salicaceae</u>	<u>Salix helvetica Vill.</u>	<u>4</u>	<u>NA</u>
15	<u>Salicaceae</u>	<u>Salix herbacea L.</u>	<u>7</u>	<u>I¹</u>
16	<u>Salicaceae</u>	<u>Salix myrsinifolia Salisb.</u>	<u>7</u>	<u>NA</u>
17	<u>Salicaceae</u>	<u>Salix pentandra L.</u>	<u>8</u>	<u>I¹</u>
18	<u>Salicaceae</u>	<u>Salix purpurea L.</u>	<u>?</u>	<u>I¹</u>
19	<u>Salicaceae</u>	<u>Salix repens L.</u>	<u>8</u>	<u>I¹</u>
20	<u>Salicaceae</u>	<u>Salix reticulata L.</u>	<u>6</u>	<u>I¹</u>
21	<u>Salicaceae</u>	<u>Salix retusa L.</u>	<u>6</u>	<u>NA</u>
22	<u>Salicaceae</u>	<u>Salix serpyllifolia Scop.</u>	<u>4</u>	<u>NA</u>
23	<u>Salicaceae</u>	<u>Salix triandra L.</u>	<u>8</u>	<u>I¹</u>
24	<u>Salicaceae</u>	<u>Salix viminalis L.</u>	<u>8</u>	<u>I¹⁹</u>
25	<u>Salicaceae</u>	<u>Salix waldsteiniana Willd.</u>	<u>6</u>	<u>NA</u>
26	<u>Caprifoliaceae</u>	<u>Sambucus nigra L.</u>	<u>5</u>	<u>NE¹¹</u>
27	<u>Caprifoliaceae</u>	<u>Sambucus racemosa L.</u>	<u>5</u>	<u>NE¹¹</u>
28	<u>Rosaceae</u>	<u>Sarcopoterium spinosum (L.) Spach</u>	<u>2</u>	<u>NA</u>
29	<u>Smilacaceae</u>	<u>Smilax aspera L.</u>	<u>3</u>	<u>NA</u>
30	<u>Rosaceae</u>	<u>Sorbus aria (L.) Crantz</u>	<u>4</u>	<u>NE³</u>
31	<u>Rosaceae</u>	<u>Sorbus aucuparia L.</u>	<u>5</u>	<u>NE³</u>
32	<u>Rosaceae</u>	<u>Sorbus chamaemespilus (L.) Crantz</u>	<u>4</u>	<u>NA</u>
33	<u>Rosaceae</u>	<u>Sorbus domestica L.</u>	<u>3</u>	<u>NE³</u>
34	<u>Rosaceae</u>	<u>Sorbus torminalis (L.) Crantz</u>	<u>4</u>	<u>NE³</u>
35	<u>Fabaceae</u>	<u>Spartium junceum L.</u>	<u>4</u>	<u>I⁹</u>
36	<u>Staphyleaceae</u>	<u>Staphylea pinnata L.</u>	<u>5</u>	<u>NA</u>
37	<u>Styracaceae</u>	<u>Styrax officinalis L.</u>	<u>4</u>	<u>NE²</u>
38	<u>Tamaricaceae</u>	<u>Tamarix africana Poiret</u>	<u>6</u>	<u>NE⁶</u>
39	<u>Tamaricaceae</u>	<u>Tamarix canariensis Willd.</u>	<u>6</u>	<u>NA</u>
40	<u>Tamaricaceae</u>	<u>Tamarix dalmatica Baum</u>	<u>6</u>	<u>NA</u>
41	<u>Tamaricaceae</u>	<u>Tamarix gallica L.</u>	<u>6</u>	<u>NE²¹</u>
42	<u>Taxaceae</u>	<u>Taxus baccata L.</u>	<u>5</u>	<u>M²¹</u>
43	<u>Fabaceae</u>	<u>Teline monspessulana (L.) Koch</u>	<u>4</u>	<u>NA</u>
44	<u>Lamiaceae</u>	<u>Teucrium fruticans L.</u>	<u>2</u>	<u>NA</u>
45	<u>Thymelaeaceae</u>	<u>Thymelaea dioica (Gouan) All.</u>	<u>3</u>	<u>NA</u>
46	<u>Thymelaeaceae</u>	<u>Thymelaea hirsuta (L.) Endl.</u>	<u>2</u>	<u>NA</u>
47	<u>Thymelaeaceae</u>	<u>Thymelaea tartonraira (L.) All.</u>	<u>2</u>	<u>NA</u>
48	<u>Lamiaceae</u>	<u>Thymus capitatus (L.) Hofm. et Lk.</u>	<u>2</u>	<u>NA</u>
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Tiliaceae	<u>Tilia cordata</u> Miller	<u>?</u>	<u>NE</u> ³
Tiliaceae	<u>Tilia platyphyllos</u> Scop.	<u>6</u>	<u>I</u> ¹¹
Fabaceae	<u>Ulex europaeus</u> L.	<u>5</u>	<u>I + M</u> ¹¹
Ulmaceae	<u>Ulmus canescens</u> Melville	<u>3</u>	<u>NA</u>
Ulmaceae	<u>Ulmus glabra</u> Hudson	<u>6</u>	<u>NE</u> ³
Ulmaceae	<u>Ulmus minor</u> Miller	<u>7</u>	<u>NE</u> ³
Ericaceae	<u>Vaccinium gaultherioides</u> Bigelow	<u>5</u>	<u>NA</u>
Ericaceae	<u>Vaccinium microcarpum</u> (Turcz.) H. Fil.	<u>9</u>	<u>NA</u>
Ericaceae	<u>Vaccinium myrtillus</u> L.	<u>?</u>	<u>NE</u> ¹⁹
Ericaceae	<u>Vaccinium oxycoccos</u> L.	<u>9</u>	<u>NA</u>
Ericaceae	<u>Vaccinium uliginosum</u> L.	<u>9</u>	<u>NE</u> ²⁰
Ericaceae	<u>Vaccinium vitis-idaea</u> L.	<u>4</u>	<u>NE</u> ¹⁹
Caprifoliaceae	<u>Viburnum lantana</u> L.	<u>4</u>	<u>NA</u>
Caprifoliaceae	<u>Viburnum opulus</u> L.	<u>7</u>	<u>NE</u> ¹¹
Caprifoliaceae	<u>Viburnum tinus</u> L.	<u>4</u>	<u>NA</u>
Apocynaceae	<u>Vinca difformis</u> Pourret	<u>3</u>	<u>NA</u>
Apocynaceae	<u>Vinca major</u> L.	<u>4</u>	<u>NA</u>
Apocynaceae	<u>Vinca minor</u> L.	<u>5</u>	<u>NA</u>
Apocynaceae	<u>Vinca sardoa</u> (Stearn) Pign.	<u>3</u>	<u>NA</u>
Verbenaceae	<u>Vitex agnus-castus</u> L.	<u>7</u>	<u>M</u> ³
Vitaceae	<u>Vitis vinifera</u> L.	<u>7</u>	<u>M</u> ⁸
Rhamnaceae	<u>Ziziphus lotus</u> (L.) Lam.	<u>1</u>	<u>NA</u>

Common exotic woody plants

Cupressaceae	<u>Cupressus sempervirens</u> L.	<u>3</u>	<u>M</u> ¹
Platanaceae	<u>Platanus x acerifolia</u> (Aiton) Wild.	<u>8</u>	<u>I</u> ¹
Salicaceae	<u>Populus canadensis</u> L.	<u>7</u>	<u>I</u> ¹
Fabaceae	<u>Robinia pseudoacacia</u> L.	<u>4</u>	<u>I</u> ¹
Oleaceae	<u>Syringa vulgaris</u> L.	<u>5</u>	<u>NE</u> ¹

¹ This study

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Appendix S3. IspS phylogenetic analysis on Mediterranean species of the genus *Populus* and *Salix* sampled for isoprene emission in this study and belonging to different classes of EIVM (Appendix S1), and on outgroups (North-American species) for which *IspS* accessions are available. *Populus euphratica* is denoted with *, as this species was sampled in this study but does not belong to the flora of Italy and is characterized by extreme adaptation to aridity and salinity (Ding *et al.* 2010).

Species	GenBank accessions	References
<i>Populus grandidentata</i>	JN173038	Gray <i>et al.</i> unpublished
<i>Populus fremontii</i>	JN173040	Gray <i>et al.</i> unpublished
<i>Populus deltoides</i>	JN173039	Gray <i>et al.</i> unpublished
<i>Populus trichocarpa</i>	EU693027	Calfapietra <i>et al.</i> (2007)
<i>Populus balsamifera</i>	JN173037	Gray <i>et al.</i> unpublished
<i>Populus tremuloides</i>	AY341431	Sharkey <i>et al.</i> (2005)
<i>Pueraria montana</i>	AY316691	Sharkey <i>et al.</i> (2005)
<i>Populus alba</i>	JQ943922	this study
<i>Populus euphratica</i> (*)	JQ943923	this study
<i>Populus nigra</i>	JQ943924	this study
<i>Populus tremula</i>	JQ943925	this study
<i>Salix apennina</i>	JQ943915	this study
<i>Salix serpyllifolia</i>	JQ943916	this study
<i>Salix alpina</i>	JQ943917	this study
<i>Salix glabra</i>	JQ943918	this study
<i>Salix pentandra</i>	JQ943919	this study
<i>Salix reticulata</i>	JQ943920	this study
<i>Salix viminalis</i>	JQ943921	this study

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Appendix S4. Primers used in amplification and sequencing of *IspS*. Primers used in initial PCR reactions are highlighted in bold.

PRIMER	SEQUENCE 5'-3'
PaISPS-Fw2	gtcgttggagcattgaagca
ISPS_Nested1-F	gttcgaacctcaatatagtg
ISPS_Nested2-F	gaggcgtgttggtcttgc
ISPS_Nested3_F	cggattatatgaagctctgc
ISPS_Nested4_F	gagttggagctattacaga
ISPS_Nested5_F	gataccatgtcaaggaacca
ISPS_Nested6_F	gtacagtataaattcatcag
PaISPS-Bw3	ttatctctcaaagggtagaat
ISPS_Nested1_R	acagaattcgcagttcacc
ISPS_Nested2_R	caggtttcgtctatcaaattc
ISPS_Nested3_R	ctgaggatgattccatgca
ISPS_Nested4_R	cttaacaagccctagaatag
ISPS_Nested5_R	gagtctcatcctcctcattc
ISPS_Nested6_R	gttggctcctaacaagccc