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# Biotic and abiotic degradation of $\Delta^5$ -sterols in senescent Mediterranean marine and terrestrial angiosperms

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8 2 **Biotic and abiotic degradation of  $\Delta^5$ -sterols in senescent**  
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11 3 **Mediterranean marine and terrestrial angiosperms**  
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62 19 **Abstract.** In this work,  $\Delta^5$ -sterols and their degradation products have been used to compare  
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64 20 the efficiency of biotic and abiotic degradation processes in senescent Mediterranean marine  
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66 21 (*Posidonia oceanica*) and terrestrial (*Quercus ilex* and *Smilax aspera*) angiosperms. Type II  
67  
68 22 photosensitized oxidation processes appeared to be more efficient in *P. oceanica* than in *Q. ilex*  
69  
70 23 and *S. aspera*. The low efficiency of these processes in senescent terrestrial angiosperms was  
71  
72 24 attributed to: (i) the fast degradation of the sensitizer (chlorophyll) in these organisms and (ii)  
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74 25 the relatively high temperatures observed on ground in Mediterranean regions favoring the  
75  
76 26 diffusion of singlet oxygen outside of the membranes. Senescent leaves of *P. oceanica*  
77  
78 27 contained highest proportions of photochemically-produced 6-hydroperoxysterols likely due to  
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80 28 the presence of trace amounts of metal ions in seawater catalyzing selective homolytic cleavage  
81  
82 29 of 5- and 7-hydroperoxysterols. Bacterial metabolites of sitosterol and of its photooxidation  
83  
84 30 products could be detected in senescent leaves of *P. oceanica* but not in *Q. ilex* and *S. aspera*.  
85  
86 31 These results confirmed that biotic and abiotic degradation processes may be intimately linked  
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88 32 in the environment.  
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33  
34 **Keywords:** *Posidonia oceanica*; *Quercus ilex*; *Smilax aspera*; Marine and terrestrial  
35 gymnosperms; Type II photosensitized oxidation; Bacterial degradation;  $\Delta^5$ -sterols;  
36 Hydroperoxides.

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121 **39 1. Introduction**  
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124 40 Senescence in plants is a complex deterioration process that can lead to the death of whole  
125  
126 41 organisms or a single organ. It is regulated by internal factors (age, reproductive development,  
127  
128 42 and phytohormone levels) and by environmental signals, including photoperiod, stresses such  
129  
130 43 as drought, ozone, nutrient deficiency, wounding, and shading (Gan et al., 1997). One of the  
131  
132 44 earliest responses of plant cells under abiotic stresses and senescence is the generation of  
133  
134 45 reactive oxygen species (ROS) and notably of singlet oxygen ( $^1\text{O}_2$ ) (Lee et al., 2012). The  
135  
136 46 chlorophyll pigments associated with the electron transport system are the primary source of  
137  
138 47  $^1\text{O}_2$ . Indeed, senescence extends the half-life of excited singlet chlorophyll ( $^1\text{Chl}$ ), thereby  
139  
140 48 increasing the likelihood that  $^1\text{Chl}$  will undergo intersystem crossing to form triplet chlorophyll  
141  
142 49 ( $^3\text{Chl}$ ).  $^3\text{Chl}$  is longer lived than  $^1\text{Chl}$  and reacts with ground state triplet oxygen ( $^3\text{O}_2$ ) to produce  
143  
144 50  $^1\text{O}_2$  (Karuppanapandian et al., 2011). The very high reactivity of  $^1\text{O}_2$  with numerous membrane  
145  
146 51 components (chlorophyll phytyl side-chain, mono- and polyunsaturated fatty acids, sterols,  
147  
148 52 tryptophan, tyrosine, histidine, methionine, cysteine and guanine bases of DNA) (Rontani,  
149  
150 53 2012; Devasagayam and Kamat, 2002) mainly results from the loss of the spin restriction that  
151  
152 54 normally hinders reaction of  $^3\text{O}_2$  with these biomolecules (Zolla and Rinalducci, 2002).  $^1\text{O}_2$ -  
153  
154 55 induced photoreactions (named Type II photosensitized oxidations) afford hydroperoxides,  
155  
156 56 which are prominent non-radical intermediates in lipid peroxidation (Halliwell and Gutteridge,  
157  
158 57 2000; Devasagayam and Kamat, 2002).  
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163 58 Due to its high reactivity and short lifetime (3.1 to 3.9  $\mu\text{s}$  in pure water) (Krasnovsky,  
164  
165 59 1998), it is generally considered that  $^1\text{O}_2$  is able to interact with molecules mostly in its nearest  
166  
167 60 environment. While its diffusion distance has been calculated to be up to 10 nm in a  
168  
169 61 physiologically relevant situation (Sies and Menck, 1992), it was also observed that  $^1\text{O}_2$   
170  
171 62 produced in the photosynthetic apparatus of *Chlamydomonas reinhardtii* under high light is  
172  
173 63 able of leaving the thylakoid membrane and reaching the cytoplasm or even the nucleus (Fisher  
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180 64 et al., 2007). The lifetime of  $^1\text{O}_2$  seems thus to vary significantly in membranes according to  
181  
182 65 physiological conditions. Interestingly, Ehrenberg et al. (1998) demonstrated that increasing  
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184 66 temperatures favored the diffusion of  $^1\text{O}_2$  from the membranes, where it is generated, into the  
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187 67 aqueous medium.  
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189 68 Autoxidation (free radical reaction of organic compounds with  $\text{O}_2$ ) can also act in  
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191 69 senescent plants (Rontani et al., 2017). These processes are not spontaneous but autocatalytic;  
192  
193 70 once started they are self-propagating and self-accelerating (Schaich, 2005). The mechanisms  
194  
195 71 of initiation of these processes in senescent plants seem to involve the homolytic cleavage of  
196  
197 72 photochemically produced hydroperoxides (Girotti, 1998; Rontani et al., 2003). This cleavage  
198  
199 73 may be induced by heat, light, metal ions and lipoxygenases (Schaich, 2005).  
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202

203 74 It was previously observed that solar irradiation can increase the bioavailability of the  
204  
205 75 detrital pieces of vascular plants (Vähätalo et al., 1998) by reducing structural barriers hindering  
206  
207 76 bacterial colonization and by degrading phenols (Opsahl and Benner, 1993), which can inhibit  
208  
209 77 bacterial growth (Harrison, 1982). It seems thus clear that in the environment biodegradative,  
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211 78 autoxidative and photooxidative degradation processes are inextricably linked, and that an  
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213 79 understanding of their interactions, although complex, is fundamental to the precise  
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215 80 identification of the balance between degradation and preservation of vascular plant material  
216  
217 81 (Rontani et al., 2017).  
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220 82 Recently, we could observed a very high efficiency of Type II photosensitized oxidation  
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222 83 processes in the seagrass *Zostera noltii* strongly contrasting with that observed in terrestrial  
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224 84 higher plants (Rontani et al., 2014). In the present work, we carried out analysis of senescent  
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226 85 leaves of two Mediterranean terrestrial higher plants (*Quercus ilex* and *Smilax aspera*) and a  
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228 86 marine seagrass (*Posidonia oceanica*) with the primary aim of confirming these preliminary  
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230 87 results and to study more closely the interactions between biotic and abiotic degradation  
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239 88 processes. For this purpose, we used oxidation products of  $\Delta^5$ -sterols known to be excellent  
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241 89 biomarkers for tracing diagenetic transformations (Mackenzie et al., 1982).  
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244 90

## 245 246 91 **2. Results and discussion**

249 92 During plant senescence, Type II photosensitized oxidation processes act on the  
250  
251 93 chlorophyll phytyl side-chain affording photoproducts quantifiable after  $\text{NaBH}_4$ -reduction and  
252  
253 94 alkaline hydrolysis in the form of 6,10,14-trimethylpentadecan-2-ol and 3-methylidene-  
254  
255 95 7,11,15-trimethylhexadecan-1,2-diol (phytyldiol) (Rontani et al., 1994). Phytyldiol is  
256  
257 96 ubiquitous in the environment and constitutes a stable and specific tracer for photodegradation  
258  
259 97 of chlorophyll phytyl side-chain (Rontani et al. 1996; Cuny and Rontani, 1999). The molar ratio  
260  
261 98 phytyldiol:phytol (Chlorophyll Phytyl side-chain Photodegradation Index, CPPI) was proposed  
262  
263 99 to estimate the extent of chlorophyll photodegraded in natural marine samples (Cuny et al.  
264  
265 100 2002). The CPPI values measured in lipid extracts resulting from the treatment of senescent  
266  
267 101 leaves of *Q. ilex* and *S. aspera* attested to a complete photooxidation of chlorophyll (Table 1).  
268  
269 102 In contrast, only 20% of chlorophyll seems to be photodegraded in detached leaves of *P.*  
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271 103 *oceanica*.  
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274  
275 104 As expected, the main sterol detected in lipid extracts of *Q. ilex*, *S. aspera* and *P. oceanica*  
276  
277 105 appeared to be 24-ethylcholest-5-en-3 $\beta$ -ol (sitosterol). Indeed, this  $\text{C}_{29}$  sterol constitutes the  
278  
279 106 major sterol of terrestrial vascular plants (Lütjohann, 2004) and seagrasses (Volkman et al.,  
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281 107 2008). As previously described (Iatrides et al., 1983), lipid extracts of *P. oceanica* also  
282  
283 108 contained lesser proportion of 24-ethylcholesta-5,22(*E*)-dien-3 $\beta$ -ol (stigmasterol). Type II  
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285 109 photosensitized oxidation of  $\Delta^5$ -sterols affords  $\Delta^6$ -5 $\alpha$ -hydroperoxysterols (which rearrange  
286  
287 110 quickly to  $\Delta^5$ -7 $\alpha/\beta$ -hydroperoxysterols, Smith, 1981) and  $\Delta^4$ -6 $\alpha/\beta$ -hydroperoxysterols (Kulig  
288  
289 111 and Smith, 1973) (Fig. 1). Although produced in lesser proportion than  $\Delta^6$ -5 $\alpha$ -  
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298 112 hydroperoxysterols,  $\Delta^4$ -6 $\alpha$ / $\beta$ -hydroperoxysterols were selected as tracers of  $\Delta^5$ -sterol  
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300 113 photooxidation due to their high specificity and relative stability (Rontani et al., 2009;  
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302 114 Christodoulou et al., 2009). These compounds were quantified after NaBH<sub>4</sub> reduction to the  
303  
304 115 corresponding diols and photooxidation percentage of the parent sterol was obtained from the  
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306 116 equation: photooxidation % = ( $\Delta^4$ -stera-6 $\alpha$ / $\beta$ -diols %  $\times$  (1+0.3)/0.3) (Christodoulou et al.,  
307  
308 117 2009). 24-Ethylcholest-4-en-3 $\beta$ ,6 $\alpha$ / $\beta$ -diols (arising from sitosterol photooxidation) could be  
309  
310 118 detected in NaBH<sub>4</sub>-reduced lipid extracts of the three gymnosperms investigated (Fig. 2). In the  
311  
312 119 case of *P. oceanica* lipid extracts, the presence of 24-ethylcholest-4,22(*E*)-dien-3 $\beta$ ,6 $\beta$ -diol  
313  
314 120 (arising from stigmasterol photooxidation) could be also noticed (Fig. 2A). On the basis of the  
315  
316 121 proportion of these diols relative to the parent  $\Delta^5$ -sterols, the photooxidation state of sitosterol  
317  
318 122 and stigmasterol could be estimated (Table 1). The strongest photooxidation of  $\Delta^5$ -sterols  
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320 123 observed in *P. oceanica* (Table 1) is in good agreement with previous results obtained in the  
321  
322 124 case of *Z. noltii* (Rontani et al., 2014). Type II photosensitized oxidation processes thus  
323  
324 125 appeared to be really more efficient on  $\Delta^5$ -sterols in seagrasses than in terrestrial angiosperms.  
325  
326 126 This highest efficiency may be attributed to: (i) the relative persistence of chlorophyll  
327  
328 127 (photosensitizer) in senescent seagrasses (Pellikaan, 1982; Aubby, 1991) allowing the  
329  
330 128 production of <sup>1</sup>O<sub>2</sub> during long periods or (ii) the temperature (Amiriaux et al., 2016). Indeed, it  
331  
332 129 was previously demonstrated that the diffusion rate of <sup>1</sup>O<sub>2</sub> in biological membranes increases  
333  
334 130 strongly with temperature (Ehrenberg et al., 1998). The high temperatures observed on land in  
335  
336 131 Mediterranean regions (notably in summer) could thus strongly favor the diffusion of <sup>1</sup>O<sub>2</sub>  
337  
338 132 outside of the chloroplasts of terrestrial higher plants decreasing thus strongly the efficiency of  
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340 133 photosensitized processes.  
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346 134 Autoxidation (free radical-induced oxidation) of  $\Delta^5$ -sterols mainly afford 7 $\alpha$ / $\beta$ -  
347  
348 135 hydroperoxysterols and to a lesser extent 5,6-epoxysterols and 3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -steratriols (Smith,  
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357 136 1981) (Fig. 1). Due to their production by allylic rearrangement of photochemically-produced  
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359 137  $5\alpha$ -hydroperoxysterols (Nickon and Bagli, 1961),  $7\alpha/\beta$ -hydroperoxysterols were discarded as  
360  
361 138 possible markers of autoxidation processes. During the treatment, 5,6-epoxysterols may be  
362  
363 139 hydrolyzed to  $3\beta,5\alpha,6\beta$ -trihydroxysterols. These triols were thus selected as specific and stable  
364  
365 140 tracers of autoxidation processes (Christodoulou et al., 2009). Only trace amounts of 24-  
366  
367 141 ethylcholestane- $3\beta,5\alpha,6\beta$ -triol (arising from sitosterol autoxidation) could be detected in the  
368  
369 142 lipid extracts of *Q. ilex*, *S. aspera* and *P. oceanica* attesting to a weak autoxidation of  $\Delta^5$ -sterols  
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371 143 in these senescent plants.  
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375 144 During singlet oxygen-mediated photooxidation of sterols in biological membranes  
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377 145 (Korytowski et al., 1992) and senescent phytoplanktonic cells (Rontani et al., 1997), the ratio  
378  
379 146  $\Delta^4$ -6-hydroperoxysterols /  $\Delta^5$ -7-hydroperoxysterols generally ranges between 0.30 and 0.35.  
380  
381 147 The lower values observed in lipid extracts of *Q. ilex* and *S. aspera* (Table 1) resulted likely  
382  
383 148 from a slight autoxidation of sitosterol mainly producing  $\Delta^5$ -7-hydroperoxysterols. In contrast,  
384  
385 149 in the case of *P. oceanica* this ratio appeared to be very high (Table 1). This high value was  
386  
387 150 attributed to the involvement of an intense and selective homolytic breakdown of  $\Delta^5$ -7-  
388  
389 151 hydroperoxysterols and  $\Delta^6$ -5-hydroperoxysterols catalyzed by metal ions (Schaich, 2005)  
390  
391 152 present in seawater. Indeed, according to the stability of the alkyl radicals formed during  $\beta$ -  
392  
393 153 scission of the corresponding alkoxy radicals, the following order of stability of  
394  
395 154 hydroperoxysterols was previously proposed:  $\Delta^4$ -6-hydroperoxysterols >  $\Delta^5$ -7-  
396  
397 155 hydroperoxysterols >  $\Delta^6$ -5-hydroperoxysterols (Christodoulou et al., 2009).  
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401 156 Treatment of senescent leaves of *Q. ilex*, *S. aspera* and *P. oceanica* (see Section 4.2)  
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403 157 involved  $\text{NaBH}_4$ -reduction (carried out in order to avoid thermal breakdown of hydroperoxides  
404  
405 158 during the subsequent alkaline hydrolysis). This treatment resulted to the reduction of  
406  
407 159 hydroperoxides and ketones to the corresponding alcohols. The sum of the corresponding  
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409 160 hydroperoxides, ketones and alcohols was thus quantified under the form of alcohols.  
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416 161 Application of a different treatment (see Section 4.3) allowed us to specifically quantify  
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418 162 hydroperoxides and their main degradation products: alcohols and ketones in these senescent  
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420 163 plants. The results obtained allowed to confirm the instability of  $\Delta^5$ -7-hydroperoxysterols in *P.*  
421  
422 164 *oceanica* (Fig. 3). In this senescent seagrass the formation of ketonic degradation products  
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425 165 appeared to be highly favored.

426  
427 166 Bacterial degradation of  $\Delta^5$ -sterols is initiated by oxidation of the 3 $\beta$ -hydroxyl moiety  
428  
429 167 and isomerization of the  $\Delta^5$  double bond to the  $\Delta^4$  position (Sojo et al., 1997). The resulting 4-  
430  
431 168 steren-3-ones are then degraded via hydroxylation at C<sub>26</sub> to initiate side-chain degradation, or  
432  
433 169 cleavage of the ring structure (9,10-seco-pathway; Philipp, 2011). It is interesting to note that  
434  
435 170 bacterial hydrogenation of  $\Delta^5$ -sterols is also often observed in oxic and anoxic environments,  
436  
437 171 this process involving the well-known sequence:  $\Delta^5$ -sterols  $\rightarrow$  ster-4-en-3-ones  $\rightarrow$  5 $\alpha$ (H)-  
438  
439 172 stanones  $\leftrightarrow$  5 $\alpha$ (H)-stanols (Gagosian et al., 1982; de Leeuw and Baas, 1986; Wakeham, 1989)  
440  
441 173 (Fig. 1). Interestingly, 24-ethylcholest-4-en-3 $\beta$ -ol and 24-ethylcholestan-3 $\beta$ -ol could be  
442  
443 174 detected in lipid extract of *P. oceanica* (Fig. 2A). Reduction with NaBD<sub>4</sub> instead of NaBH<sub>4</sub>  
444  
445 175 allowed us to demonstrate that 100% of 24-ethylcholest-4-en-3 $\beta$ -ol and 60% of 24-  
446  
447 176 ethylcholestan-3 $\beta$ -ol resulted from the reduction of the corresponding ster-4-en-3-one and  
448  
449 177 stanone, respectively. Bacterial conversion of  $\Delta^5$ -sterols to 5 $\alpha$ (H)-stanols seems thus to act in  
450  
451 178 senescent leaves of seagrasses, but not in terrestrial vascular plants (Fig. 2B).  
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457 179 24-Ethyl-5 $\alpha$ -cholestane-3 $\beta$ ,6 $\beta$ -diol could be detected in lipid extracts of *P. oceanica* (Fig.  
458  
459 180 2A). Reduction with NaBD<sub>4</sub> instead of NaBH<sub>4</sub> also allowed to demonstrate the additional  
460  
461 181 presence of: 24-ethylcholest-4-en-6 $\beta$ -ol-3-one, 24-ethylcholest-4-en-3,6-dione, 24-ethyl-5 $\alpha$ -  
462  
463 182 cholestan-3 $\beta$ -ol-6-one, 24-ethyl-5 $\alpha$ -cholestan-6 $\beta$ -ol-3-one and 24-ethyl-5 $\alpha$ -cholestane-3,6-  
464  
465 183 dione in this lipid extract (Fig. 4). The presence of these different compounds attests to the  
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475 184 bacterial use of the main photooxidation products of sitosterol (i.e. 24-ethylcholest-4-en-3 $\beta$ ,6 $\beta$ -  
476  
477  
478 185 diol and 24-ethylcholest-4-en-3 $\beta$ -ol-6-one) (Fig. 1).  
479

480 186

### 483 187 **3. Conclusions**

486 188 Degradation products of  $\Delta^5$ -sterols (mainly sitosterol) were characterized and quantified  
487  
488 189 in senescent leaves of Mediterranean terrestrial (*Q. ilex* and *S. aspera*) and marine (*P. oceanica*)  
489  
490 190 angiosperms. The results obtained allowed to confirm the highest efficiency of Type II  
491  
492 191 photosensitized processes in marine seagrasses. The fast degradation of chlorophyll (sensitizer)  
493  
494 192 during the senescence and the high temperatures observed on ground (favoring migration of  
495  
496  
497 193  $^1\text{O}_2$  outside of biological membranes) seem to be at the origin of the weak efficiency of  
498  
499 194 photosensitized processes in Mediterranean terrestrial angiosperms. Homolytic cleavage of  
500  
501 195 photochemically-produced hydroperoxides appeared to be highly favored in seawater likely due  
502  
503 196 to the presence of metal ions in trace amounts. Bacterial degradation processes, which are more  
504  
505 197 efficient in senescent leaves of *P. oceanica* than in these of *Q. ilex* and *S. aspera*, acted not only  
506  
507 198 on sitosterol but also on its photooxidation products. These observations confirm the  
508  
509 199 complexity of the interactions between biotic and abiotic degradation processes in the  
510  
511 200 environment.  
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### 517 202 **4. Experimental**

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#### 522 204 *4.1. Sampling*

525 205 Senescent leaves of *Q. ilex* and *S. aspera* (both widespread species in Mediterranean  
526  
527 206 ecosystems) were collected on the ground near Marseilles (France). Detached leaves of *P.*  
528

532  
533  
534 207 *oceanica* were collected on the Catalans beach in Marseilles (France). All the leaves were freeze  
535  
536 208 dried and then placed in a mortar and intensively ground.

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#### 542 210 4.2. Treatment

544 211 Freeze-dried leaves were reduced in methanol (25 ml) by excess NaBH<sub>4</sub> or NaBD<sub>4</sub> (70  
545 212 mg) at room temperature for 30 min (Rontani et al., 2009). This reduction was carried out to  
546 213 reduce labile hydroperoxides resulting from photooxidation to alcohols that are amenable to  
547 214 gas chromatography-mass spectrometry (GC-MS). During this treatment, ketones are also  
548 215 reduced and the possibility of some ester cleavage cannot be totally excluded. After reduction,  
549 216 25 ml of water and 2.8 g of potassium hydroxide were added and the mixture was directly  
550 217 saponified by refluxing for 2 h. After cooling, the content of the flask was filtered and extracted  
551 218 three times with hexane. The combined hexane extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>,  
552 219 filtered and concentrated by rotary evaporation at 40°C to give the neutral fraction.

553 220

#### 567 221 4.3. Estimation of hydroperoxysterols and their alcoholic and ketonic degradation product 568 222 contents

569 223 Freeze-dried leaves were extracted four times with chloroform-methanol-water (1:2:0.8,  
570 224 v/v/v) using ultrasonication (separation of leave debris and solvents by centrifugation at 3500  
571 225 G for 9 min). To initiate phase separation after ultrasonication, chloroform and purified water  
572 226 were added to the combined extracts to give a final volume ratio of 1:1:0.9 (v/v/v). The upper  
573 227 aqueous phase was extracted twice with chloroform and the combined extracts were dried over  
574 228 anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and the solvent removed via rotary evaporation. The residue  
575 229 obtained after extraction was dissolved in 4 ml of dichloromethane and separated in two equal  
576 230 subsamples. After evaporation of the solvent, degradation products were obtained for the first

591  
592  
593 231 subsample after acetylation (inducing complete conversion of hydroperoxides to the  
594  
595 232 corresponding ketones, Mihara and Tateba, 1986) and saponification and for the second after  
596  
597 233 reduction with NaBD<sub>4</sub> and saponification. Comparison of the amounts of alcohols present after  
598  
599 234 acetylation and after NaBD<sub>4</sub> reduction made it possible to estimate the proportion of  
600  
601 235 hydroperoxysterols and hydroxysterols present in the samples, while after NaBD<sub>4</sub>-reduction  
602  
603 236 deuterium labeling allowed to estimate the proportion of ketosterols really present in the  
604  
605 237 samples (Marchand and Rontani, 2003).  
606  
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609 238

#### 611 239 *4.4. Derivatization*

614 240 Residues were taken up in 300 µl of a mixture of pyridine and N,O-  
615  
616 241 bis(trimethylsilyl)trifluoroacetamide (BSTFA; Supelco) (2:1, v:v) and silylated for 1 h at 50°C  
617  
618 242 to convert OH-containing compounds to their TMSi-ether derivatives. After evaporation to  
619  
620 243 dryness under a stream of N<sub>2</sub>, the derivatized residues were taken up in a mixture of ethyl acetate  
621  
622 244 and BSTFA (to avoid desilylation of fatty acids) for analysis using GC-EIMS. It should be  
623  
624 245 noted that under these conditions 3β,5α,6β-trihydroxysterols were only silylated at the 3 and 6  
625  
626 246 positions and thus need to be analyzed with great care.  
627  
628  
629  
630 247

#### 632 248 *4.5. Gas chromatography-electron ionization mass spectrometry (GC-EIMS) analyses*

635 249 Lipid oxidation products were identified by comparison of retention times and mass  
636  
637 250 spectra with those of standards and quantified (calibration with external standards) using an  
638  
639 251 Agilent 7850-A gas chromatograph connected to an Agilent 7010-QQQ mass spectrometer. For  
640  
641 252 low concentrations, or in the case of co-elutions, quantification was achieved using selected ion  
642  
643 253 monitoring (SIM). The main characteristic mass fragment ions used to quantify degradation  
644  
645 254 products of sterols have been described previously (Christodoulou et al., 2009; Rontani et al.,  
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651  
652 255 2009). The following conditions were employed: 30 m x 0.25 mm (i.d.) fused silica column  
653  
654 256 coated with HP-5MS (Agilent; film thickness: 0.25  $\mu\text{m}$ ); oven programmed from 70 to 130  $^{\circ}\text{C}$   
655  
656 257 at 20  $^{\circ}\text{C min}^{-1}$ , then to 250  $^{\circ}\text{C}$  at 5  $^{\circ}\text{C min}^{-1}$  and then to 300  $^{\circ}\text{C}$  at 3  $^{\circ}\text{C min}^{-1}$ ; carrier gas (He),  
658  
659 258 1.0 bar; injector (splitless), 250  $^{\circ}\text{C}$ ; electron energy, 70 eV; source temperature, 230  $^{\circ}\text{C}$ ;  
660  
661 259 quadrupole temperature, 150  $^{\circ}\text{C}$ ; scan range  $m/z$  40-700; cycle time, 0.2 s.

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#### 666 261 4.6. Standard compounds

668  
669 262 Phytol, sitosterol and stigmasterol were obtained from Sigma-Aldrich. The synthesis of  
670  
671 263 3-methylidene-7,11,15-trimethylhexadecan-1,2-diol (phytyldiol) from phytol was described  
672  
673 264 previously by Rontani and Aubert (2005). 5 $\alpha$ - and 6 $\alpha/\beta$ -Hydroperoxides were obtained after  
674  
675 265 photosensitized oxidation of the corresponding  $\Delta^5$ -sterols in pyridine in the presence of  
676  
677 266 haematoporphyrin as sensitizer (Nickon and Bagli, 1961). Allylic rearrangement of 5 $\alpha$ -  
678  
679 267 hydroperoxides to 7 $\alpha$ -hydroperoxides and epimerization of the latter to 7 $\beta$ -hydroperoxides was  
680  
681 268 carried out at room temperature in chloroform (Teng et al., 1973). Subsequent reduction of  
682  
683 269 these different hydroperoxides in methanol with excess  $\text{NaBH}_4$  afforded the corresponding  
684  
685 270 diols. Hydrogenation of 24-ethylcholest-4-en-3 $\beta$ ,6 $\beta$ -diol in acetic acid solution in the presence  
686  
687 271 of  $\text{PtO}_2$  as catalyst yielded 24-ethyl-5 $\beta$ -cholestane-3 $\beta$ ,6 $\beta$ -diol and 24-ethyl-5 $\alpha$ -cholestane-  
688  
689 272 3 $\beta$ ,6 $\beta$ -diol (Nishimura and Mori, 1963). Oxidation of sitosterol in THF at room temperature  
690  
691 273 with  $\text{HIO}_4$  afforded 24-ethylcholestane-3 $\beta$ ,5 $\alpha$ ,6 $\beta$ -triol (Voisin et al., 2014).  
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## FIGURE CAPTIONS

**Figure 1.** Biotic and abiotic degradation of sitosterol in senescent leaves of *P. oceanica*.

(Bacterial metabolites are in blue).

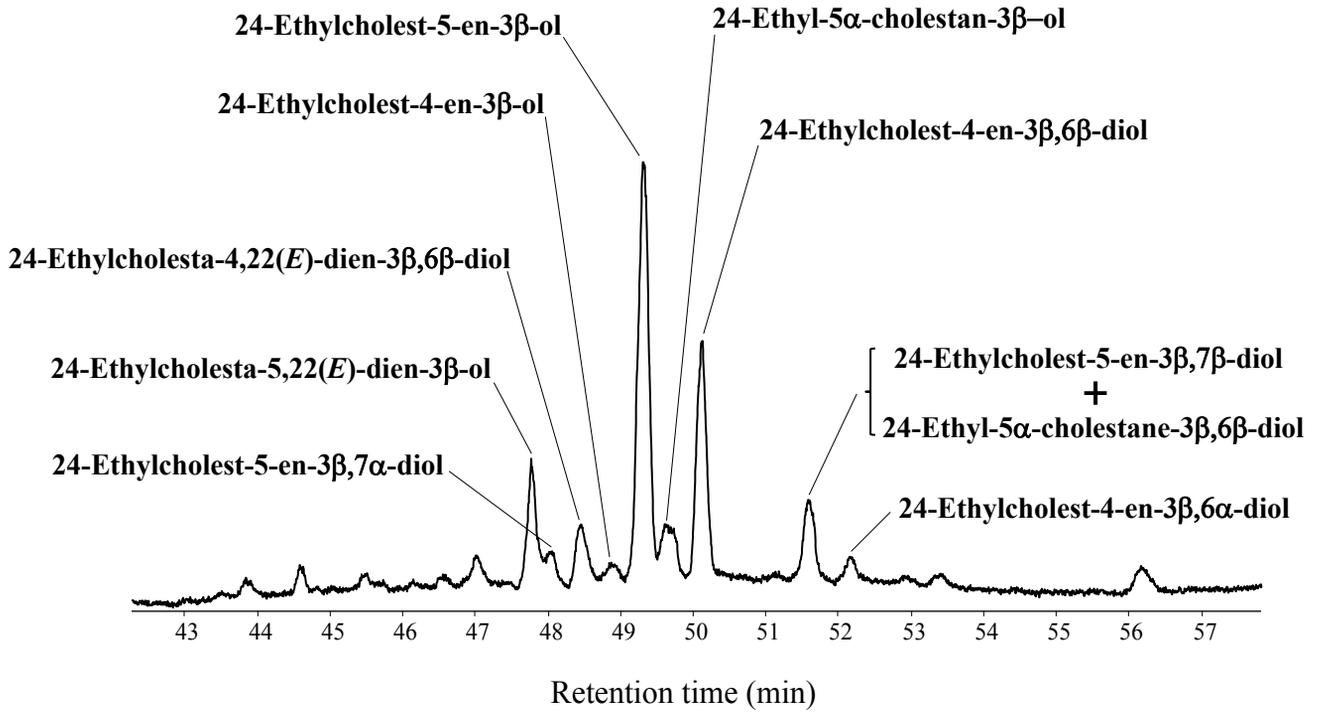
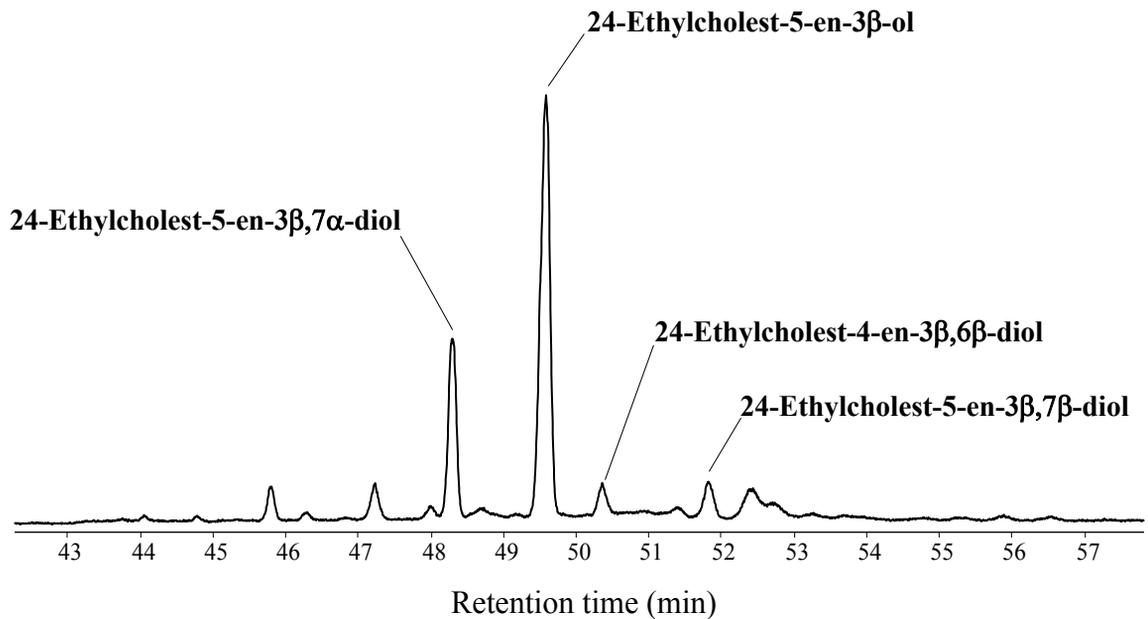
**Figure 2.** Partial total ion current (TIC) chromatograms of silylated NaBH<sub>4</sub>-reduced lipid extracts of senescent leaves of *P. oceanica* (A) and *S. aspera* (B).

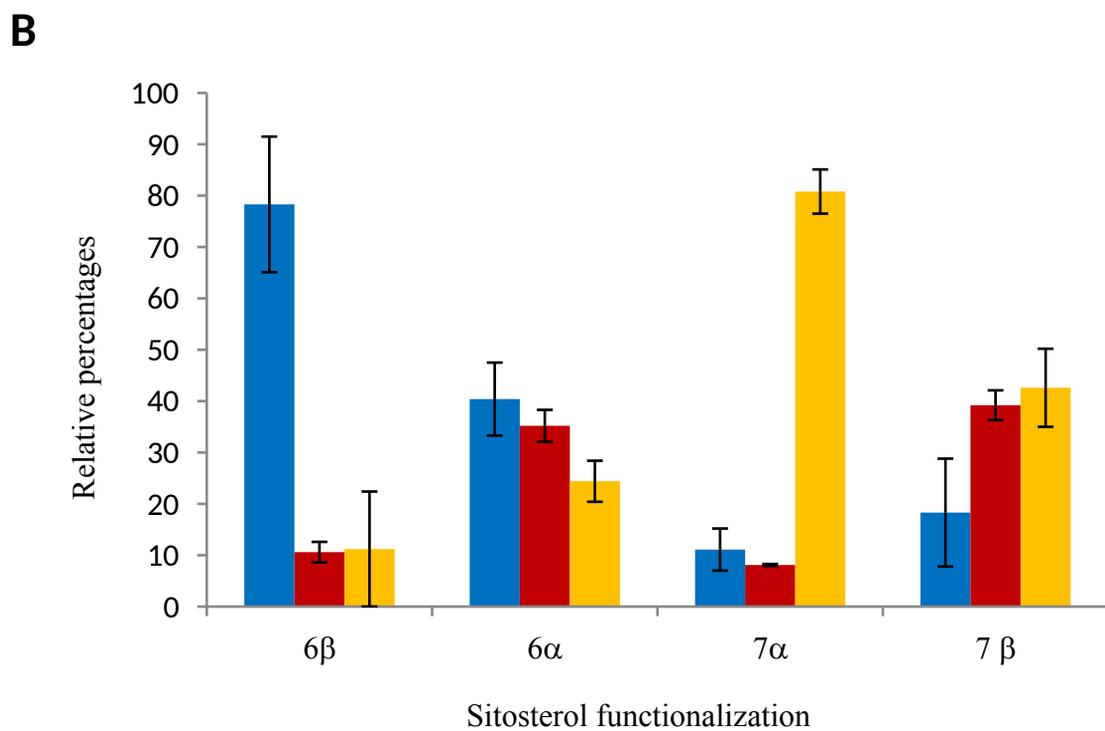
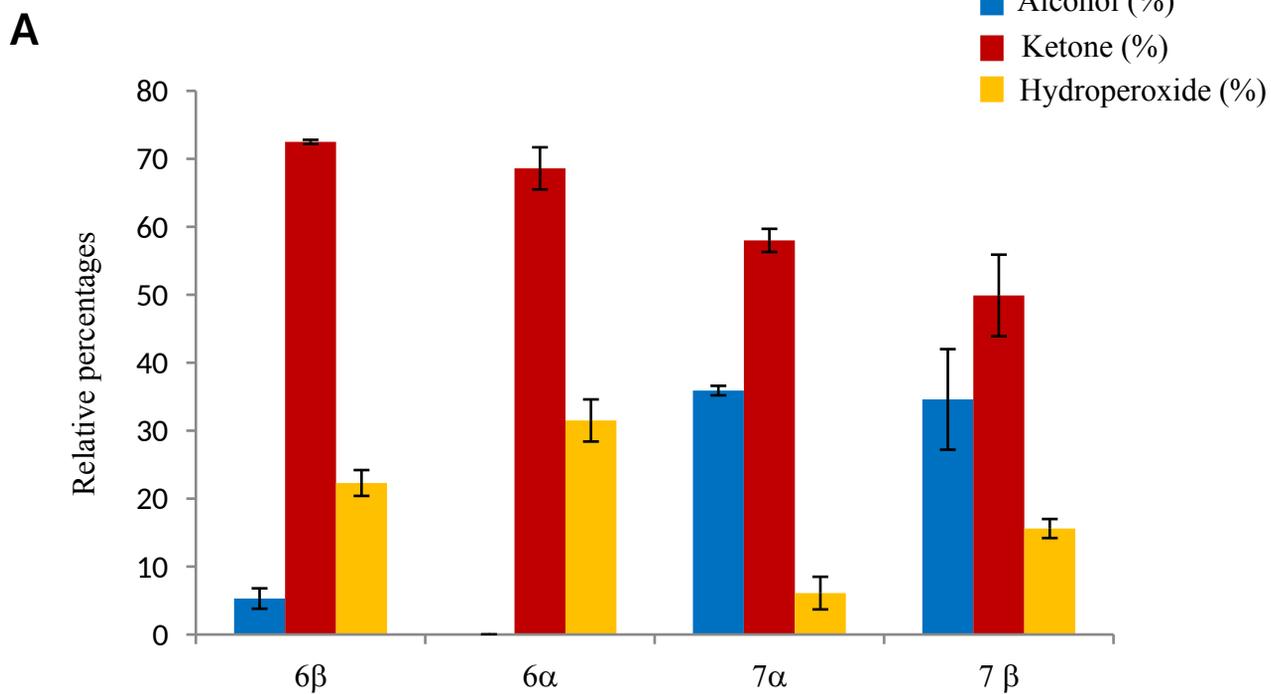
**Figure 3.** Relative percentages of intact 6- and 7-hydroperoxysitosterols and their ketonic and alcoholic degradation products measured in senescent leaves of *P. oceanica* (A) and *S. aspera* (B).

**Figure 4.** Partial EI mass spectra of silylated 24-ethylcholest-4-en-3 $\beta$ ,6 $\beta$ -diol (A) and 24-ethylcholestane-3 $\beta$ ,6 $\beta$ -diol (B) obtained after NaBD<sub>4</sub>-reduction of lipid extract of *P. oceanica*.





**A****B**



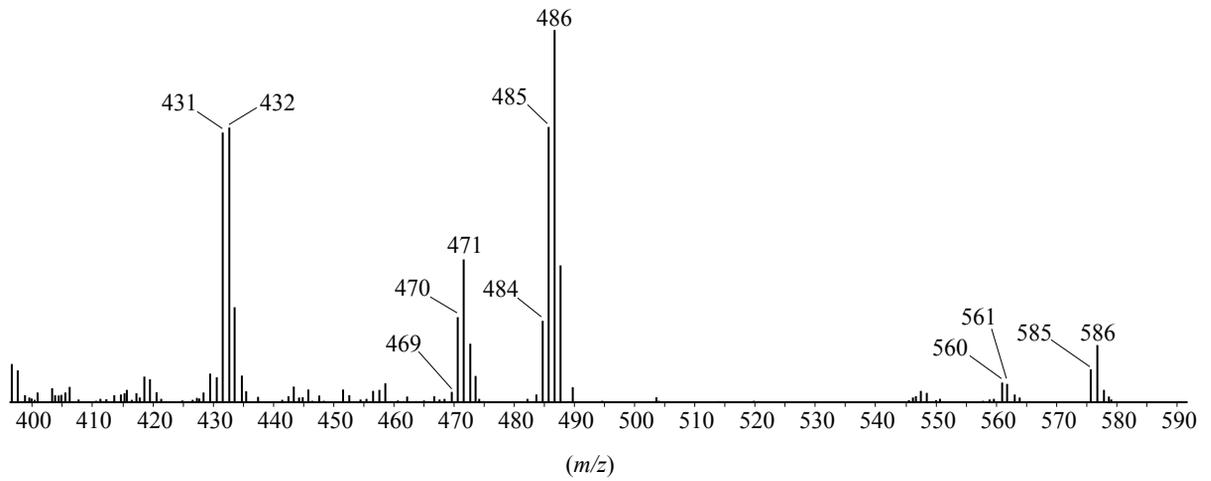
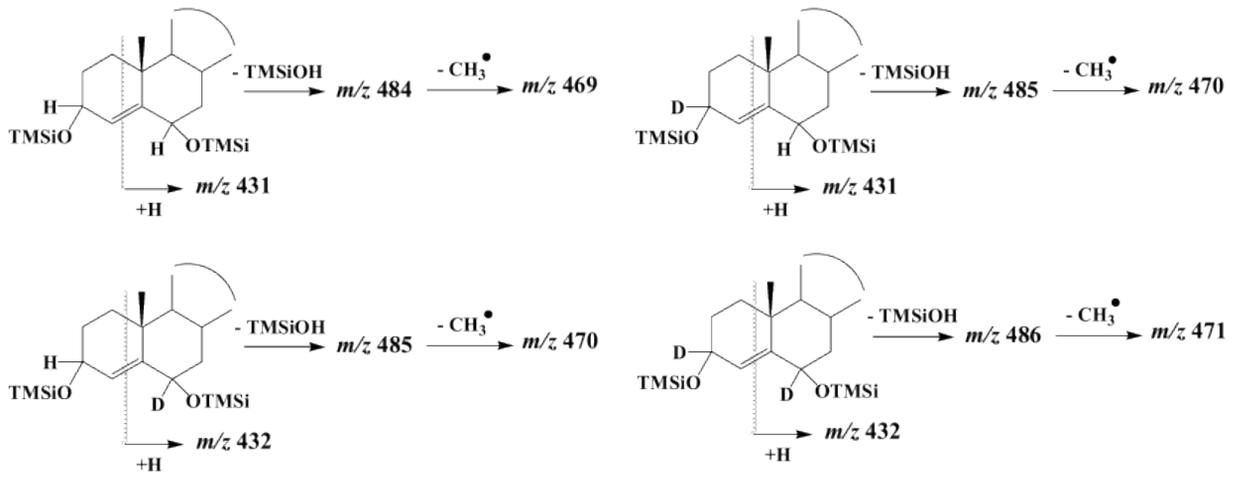
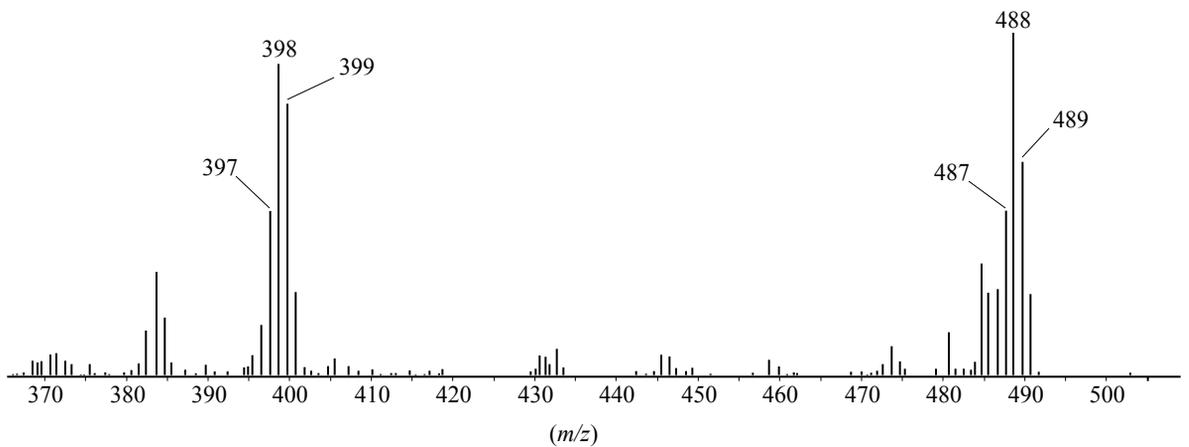
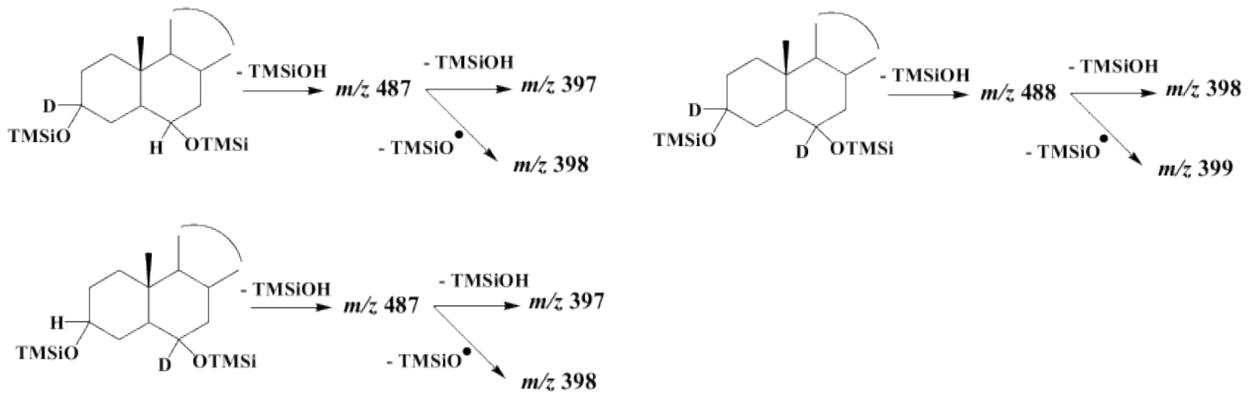
**A****B**

Table 1

Efficiency of photooxidation processes in senescent leaves of marine and terrestrial angiosperms

	<i>Posidonia oceanica</i>	<i>Quercus ilex</i>	<i>Smilax aspera</i> L.
CPPI <sup>a</sup>	0.012 ± 0.002	60 ± 46	5 ± 4
Chlorophyll photooxidation (%)	20 ± 3 <sup>b</sup>	100 ± 0	100 ± 0
Sitosterol photooxidation (%)	72 ± 17 <sup>c</sup>	49 ± 7	33 ± 5
Stigmasterol photooxidation (%)	69 ± 3 <sup>c</sup>	-	-
6 $\alpha$ / $\beta$ -diols/7 $\alpha$ / $\beta$ -diols <sup>d</sup>	6.28 ± 2.38	0.22 ± 0.02	0.09 ± 0.01

<sup>a</sup> Chlorophyll Phytyl side-chain Photodegradation Index (molar ratio phytyldiol/phytol) (Cuny et al., 1999)

<sup>b</sup> Estimated with the equation: Chlorophyll photodegradation percentage = ( 1 - (CPPI + 1)<sup>-18.5</sup>) x 100 (Cuny et al., 1999)

<sup>c</sup> Estimated with the equation: Sterol photooxidation % = ( $\Delta^4$ -3 $\beta$ ,6 $\alpha$ / $\beta$ -dihydroxysterol %) x (1 + 0.3) / 0.3 (Christodoulou et al., 2009)

<sup>d</sup> 24-Ethylcholest-4-en-3 $\beta$ ,6 $\alpha$ / $\beta$ -diols/24-ethylcholest-5-en-3 $\beta$ ,7 $\alpha$ / $\beta$ -diols