

1 **Effects of soil abiotic factors and plant chemical defences on seed predation on sea**
2 **fennel (*Crithmum maritimum*)**

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4 Running title: **Soil and seed predation**

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26 **ABSTRACT**

27 • Aims: Soil abiotic factors commonly influence plant defensive traits by shaping the
28 costs of defence production and these bottom-up effects on plants can in turn affect
29 insect herbivory. However, few studies have disentangled direct and indirect effects of
30 soil abiotic factors on plant defences and insect herbivory.

31 • Methods: To address this gap, we tested the influence of soil abiotic factors on seed
32 predation via changes in plant defences for sea fennel (*Crithmum maritimum*), a
33 common coastal plant in southern Spain whose seeds are consumed by specialist
34 caterpillars (*Aethes* species). To this end, we estimated seed predation on plants across
35 several sea fennel populations, as well as measured different types of putative
36 chemical seed defences (phenolics, terpenes) and soil abiotic factors (macro- and
37 micro-elements, physiochemical variables).

38 • Results: We found a positive association between seed chemical defences (terpenes
39 such as α -thujene, α -pinene, β -pinene, β -myrcene, α -terpinene, γ -terpinene, and
40 thymol methylether) and seed predation. In addition, a few macro- and micro-
41 elements such as Ca, S and Sr negatively correlated with seed defences (terpenes);
42 other macro- and micro-elements or physiochemical variables had no detectable
43 association with defences. Despite observed effects of soil abiotic factors on defences
44 and of the latter on seed predation, there was no detectable indirect effect of soil
45 abiotic factors on seed attack.

46 • Conclusions: Our findings suggest that variation in a few key soil macro- and micro-
47 elements in these coastal environments can exert an important influence on seed
48 chemical defences in sea fennel, with potential consequences for interactions between
49 sea fennel and seed predators.

50

51 **Keywords:** *Aethes* species, coastal environment, herbivory, phenolics, sea fennel, soil
52 macro- and micro-elements, terpenes

53

54 **INTRODUCTION**

55 Insect herbivory is an ancient and widespread interaction (Labandeira 2007). Over
56 evolutionary time, a striking diversity of phytophagous insects has arisen and these fall
57 into different feeding guilds (e.g. leaf chewers and miners, cell-content feeders,
58 piercing-sucking herbivores, root feeders, gall-makers, and seed predators; Marquis
59 1992), which have varying negative consequences on plant growth and reproduction
60 (Maron 1998). Amongst these groups, pre-dispersal seed predators have particularly
61 negative consequences for plant reproductive output and population dynamics (Kolb et
62 al. 2007), and many of these herbivores exhibit high dietary specialization (Abdala-
63 Roberts and Mooney 2013; Chen and Moles 2018; Gripenberg et al. 2019).
64 Accordingly, pre-dispersal seed predators have life cycles that are highly synchronized
65 with plant reproductive phenology (Diniz and Morais 2002; Novotny and Basset 2015)
66 and often exhibit co-evolutionary arms races with their host plants (Berenbaum and
67 Zangerl 1998; Thompson 2005).

68 Research on plant-herbivore interactions has historically focused on plant
69 defences and the mechanisms by which the potency and diversity of defensive traits has
70 evolved as a means to fend off herbivores or reduce their consumption (Simms and Fritz
71 1990; Agrawal 2007; Futuyma and Agrawal 2009). In turn, studies have also shown that
72 quantitative and qualitative variation in plant physical and chemical defences play an
73 important role in determining the risk and intensity of herbivory (Feeny 1976; Marquis
74 1992), as well as in shaping insect herbivore communities (Richards et al. 2015; Salazar
75 et al. 2016). Understanding these bottom-up effects of plant defences on phytophagous

76 insects thus represents a fundamental challenge for explaining intra- and inter-specific
77 variability in herbivory, one that also requires embracing the complex nature of plant
78 defensive phenotypes by simultaneously studying multiple, often correlated, traits
79 (Moreira et al. 2020a; Quijano-Medina et al. 2021).

80 Other well-known sources of bottom-up control in herbivory are abiotic factors,
81 including soil macro- and micro-elements (Coley et al. 1985; Fine et al. 2004), and
82 water or light availability (Gutbrodt et al. 2011; Abdala-Roberts et al. 2014; Ballaré
83 2014). Plant defence theory postulates that, since the cost of replacement of damaged
84 tissues by herbivores is higher in resource-limited environments (e.g. low availability of
85 soil macro- and micro-elements, high soil salinity), plants should exhibit higher levels
86 of defences when resources are scarce (reviewed by Stamp 2003). By increasing plant
87 defences, soil abiotic factors can in turn negatively affect herbivore host plant choice or
88 feeding behaviour (Abdala-Roberts et al. 2016a; Moreira et al. 2018), leading to
89 decreased herbivore damage. To date, however, studies disentangling direct and indirect
90 effects of soil abiotic factors on plant defences and herbivory are scarce (but see Dalling
91 et al. 2011; Moreira et al. 2018).

92 Sea fennel (*Crithmum maritimum* L.) is a common halophyte herb in coastal
93 habitats throughout Western Europe, and is attacked by seed-eating specialist
94 caterpillars of the genus *Aethes* spp. These insects are highly common on sea fennel
95 plants, resulting in up to 80% of seeds attacked at some sites. In addition, the coastal
96 sites where sea fennel grows exhibit variable but generally low availability of macro-
97 and micro-elements, as well as high soil salinity, alkalinity, and physical stress (e.g.
98 wind exposure). In the present study, we sampled sea fennel plants from seven
99 populations located in the south of Iberian Peninsula, spanning a wide range of soil
100 conditions (from sandy beaches to steep cliffs). At each population, we estimated seed

101 predation, and measured several types of seed chemical defences (phenolics, terpenes)
102 as well as associated soil abiotic factors (macro- and micro-elements, physiochemical
103 variables). Phenolic compounds and terpenes are putative defensive compounds that are
104 toxic and deterrent to a broad range of phytophagous insects, and are also associated
105 with interactions involving specialist herbivores (Mithöfer and Boland 2012). We tested
106 whether chemical defences correlate with seed predation, if soil abiotic factors correlate
107 with seed defensive traits, and whether any such associations between soil abiotic
108 factors and seed defences indirectly affect seed predation. We expected that defence
109 levels would negatively correlate with herbivory (signalling resistance against seed
110 predation across populations), and that plants from sites with lower resource availability
111 and higher soil-related abiotic stress would be more chemically defended, and in turn
112 suffer lower seed predation. By addressing multiple plant defences simultaneously,
113 providing a comprehensive assessment of soil abiotic factors, and testing for both direct
114 and indirect soil effects on seed predation, this study contributes to a more nuanced
115 understanding of bottom-up effects of soil abiotic factors and plant defences on pre-
116 dispersal seed predation.

117

118 **MATERIAL AND METHODS**

119 *Natural history*

120 Sea fennel (*Crithmum maritimum* L.) is a perennial halophyte herb distributed
121 throughout the western coasts of Europe, North Africa and the Black Sea. It flowers
122 from June to September, and fruits mature from September to December. Plants
123 typically bear 100-200 inflorescences at the peak of the flowering season. Each fruit
124 contains a single dry seed from 4–10 mm long. Along its distribution, sea fennel is
125 found in both sandy and rocky beaches, as well as cliffs, and grows frequently in sandy

126 soils, with low availability of macro- and micro-elements and high alkalinity and
127 salinity (Meot-Duros and Magné 2009).

128 Some of the most important insect herbivores on this plant are pre-dispersal seed
129 predators of the genus *Aethes* species (Lepidoptera: Tortricidae) (e.g. *A. bilbaensis*, *A.*
130 *francillana*, *A. eichleri*), which specialize on several species of Apiaceae (mainly *C.*
131 *maritimum* and *Carum verticillatum*). The adult female moth bores a small hole through
132 the coat of immature fruits to lay eggs in June-July. Moth larvae then grow inside fruits
133 feeding on the seeds and leave the mature fruit by drilling an exit hole in September-
134 October. Then, larvae bore into the stems and hibernates until the following growing
135 season.

136

137 ***Field sampling***

138 In mid-September, when plants bear both immature and mature fruits, we surveyed
139 seven populations of sea fennel in southern Portugal and southern Spain (Fig. 1). The
140 sites sampled had different soil characteristics and habitat topographies, including sandy
141 or rocky beaches, and cliffs. Populations were separated by 13 to 785 km, and vary two-
142 fold in annual precipitation and 1.2 °C in annual mean temperature. Likewise, soil
143 abiotic factors, including macro- and micro-elements, also vary considerably across
144 these sites (Table S1 in the Supplementary Material). Each population was comprised of
145 at least 30 adult sea fennel plants.

146 At each site, we selected 11-12 adult (reproductive) plants ($n = 83$) of similar
147 height (range: 10-56 cm; 44.59 ± 1.62 cm [mean \pm SE]). Plants were separated by at
148 least 2 m, and displayed, on average, 186.3 ± 15.1 (SE) umbels and 351.6 ± 20.6 seeds
149 per umbel. For each plant, we collected seven umbels with immature fruits, placed them
150 in ice at -10°C, and transported them to the laboratory. Then, we opened the fruits to

151 obtain the seed, and ground the seeds with liquid nitrogen and stored them at -80°C for
152 chemical analyses. During sampling, we took special care to only collect undamaged
153 immature fruits such that chemical defence measurements represented a rough proxy of
154 constitutive seed defences (the influence of systemic induction if other umbels in the
155 same plant were attacked cannot be discarded). In addition, to assess fruit predation, we
156 collected from the same plants seven more umbels in which all or most fruits were
157 mature and were placed in paper envelopes and transported to the laboratory where
158 larvae continued feeding and completed their development. These samples were used to
159 estimate seed predation (see next).

160

161 *Estimation of seed predation*

162 For each plant, we counted the total number of mature fruits (i.e. seeds) in each umbel
163 and calculated the proportion of seeds attacked (number of attacked seeds / total number
164 of mature seeds collected per plant), i.e. “seed predation” hereafter. Seed predation was
165 overwhelmingly caused by larvae of *Aethes* species (> 95% of cases; J. Cambrollé, data
166 from this study).

167

168 *Quantification of seed chemical defences*

169 Phenolic compounds are feeding deterrents against insect seed predators found in many
170 plant taxa (Dalling et al. 2020), including Apiaceae species (Berenbaum 2001). Briefly,
171 we extracted phenolic compounds from immature seeds using 20 mg of dry material
172 (oven-dried for 48 h at 40°C) with 0.25 mL of 70% methanol in an ultrasonic bath for
173 15 min, followed by centrifugation (Moreira et al. 2014). We then transferred the
174 extracts to chromatographic vials to perform phenolic profiling. For phenolic compound
175 identification, we used an ultra-performance liquid chromatography coupled with

176 electrospray ionization quadrupole (Thermo Dionex Ultimate 3000 LC) time-of-flight
177 mass spectrometry (UPLC-Q-TOF-MS/MS) (Bruker Compact™) (Moreira et al.
178 2020b). We performed chromatographic separation in a Bruker UHPLC Intensity Solo 2
179 C18 2.1 × 100 mm 1.7 μm pore size column using a binary gradient solvent mode
180 consisting of 0.1% formic acid in water (solvent A) and acetonitrile (solvent B). We
181 used the following gradient: 3% B (0–3 min), from 3% to 25% B (3–10 min), from 25% to
182 80% B (10–18 min), from 80% to 100% B (18–22 min), and held at 100% B until 24 min.
183 The injection volume was 3 μL, the flow rate was established at 0.3 mL min⁻¹ and
184 column temperature was controlled at 35°C. We operated MS analysis in a spectra
185 acquisition range from 50 to 1200 m/z. We used negative (-) ESI modes under the
186 following specific conditions: gas flow 8 l/min, nebulizer pressure 38 psi, dry gas 7 L
187 min⁻¹, and dry temperature 220 °C. We set capillary and end plate offset to 4500 and
188 500 V, respectively. We performed Tandem Mass Spectrometry (MS/MS) analysis
189 based on the previously determined accurate mass and retention times and fragmented
190 by using different collision energy ramps to cover a range from 15 to 50 eV. We
191 recorded chromatograms at 330 nm. We identified individual compounds based on the
192 data obtained from the standard substances or published literature including retention
193 times, λ_{max}, ([M-H]⁻), and major fragment ions. We only identified phenolic
194 compounds from two groups: flavonoids (N = 7) and hydroxycinnamic acids (N = 3).
195 For phenolic compound quantification, we injected 10 μL of each sample (using the
196 same column and conditions mentioned above) in an UHPLC (Nexera LC-30AD;
197 Shimadzu) equipped with a Nexera SIL-30AC injector and one SPD-M20A UV/VIS
198 photodiode array detector (Moreira et al. 2018). We quantified flavonoids as rutin
199 equivalents and hydroxycinnamic acids as ferulic acid equivalents (Moreira et al. 2018).
200 We achieved the quantification of these phenolic compounds by external calibration

201 using calibration curves at 0.25, 0.5, 1, 2 and 5 $\mu\text{g mL}^{-1}$. We expressed phenolic
202 compound concentrations in mg g^{-1} tissue on a dry weight basis and analysed plant-level
203 data for total concentration of phenolics and by type of phenolic compounds (see
204 statistical analyses ahead).

205 We also quantified terpenes, which are similarly considered putative chemical
206 defences against insect seed predators in many plant taxa (Dalling et al. 2020),
207 including Apiaceae species (Berenbaum 2001). For this, we extracted compounds from
208 immature seeds using 300 mg of ground fresh material with 1 mL of 70% methanol in
209 an ultrasonic bath for 20 min and stored samples at 4°C for 24 h. We also added
210 dodecane (Merck, #1.09658.0005) as the internal standard solution (100 ppm of
211 dodecane in *n*-hexane). We injected the samples (1 μL) onto a gas chromatograph (GC,
212 Thermo Finnegan Trace GC Ultra, Waltham, MA, USA) with a mass spectrometer
213 (MS) detector that was fitted with a 30 m \times 0.25 mm \times 0.25 μ film thickness ZB-5MSi
214 (Phenomenex, UK) in single ion monitoring mode (SIM: m/z 68, 69, 77, 79, 92, 93, 94,
215 105, 119, 121, 136, 148, 161, 175) used to make visible known terpene fragments. The
216 GC was operated in split mode (50 mL min^{-1}) with helium as the carrier gas (flow rate 1
217 mL min^{-1}). The GC oven temperature program was: 2 min hold at 60°C, 10°C min^{-1}
218 ramp to 70°C, 15 min hold at 70°C, 5°C min^{-1} ramp to 130°C, 30°C min^{-1} ramp to
219 250°C, and 1 min hold at 250°C. We identified terpenes comparing their Kováts
220 indices, calculated relative to the retention times of a series of *n*-alkanes (C₈-C₂₀,
221 Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) analysed under the same
222 chromatographic conditions, with those reported in the literature (Tsoukatou et al. 2001;
223 Nabet et al. 2017). We only identified monoterpenes. For each plant, we estimated the
224 amount of terpenes by using normalized peak areas per dry weight. The normalized
225 peak area per dry weight of each compound was obtained by dividing their integrated

226 peak area by the integrated peak area of the internal standard and then dividing this
227 value by the leaf dry weight. Terpene concentration was expressed in mg g⁻¹ leaf dry
228 weight (d.w.), and we analysed plant-level data for both total concentration of terpenes
229 and by type of terpene compounds (see statistical analyses ahead).

230

231 *Measurement of soil abiotic factors*

232 In mid-September, for each plant we collected top soil samples (0-30 cm depth) at a
233 distance of 10 to 30 cm from the limit of the plant canopy projection. We measured nine
234 soil physiochemical properties, namely: pH, water content, carbon content, electrical
235 conductivity, and the percentage of clay, silt, gravel, and fine and gross sand. We
236 potentiometrically determined soil pH in a 1:2.5 soil:water suspension, estimated soil
237 water content as the proportion of mass loss after oven-drying at 50°C for 48 h, carbon
238 content by a muffle furnace calcination (450°C for 4 h), and electrical conductivity with
239 a conductivity meter after mixing the soil with distilled water (1:5). For texture analysis,
240 we removed coarse elements (> 2 mm) by sieving and estimated the percentage of
241 gravel. We then separated the soil fraction of particle size 2-0.5 mm by sieving to
242 differentiate between gross and fine sand, and determined the proportions of fine sand,
243 silt and clay in the < 0.5 mm fraction by the Bouyoucos hydrometer method
244 (Bouyoucos 1962). In addition, we measured the concentration of six soil macro-
245 elements (Ca, K, Mg, N, P, S) and 14 micro-elements (As, B, Ba, Cr, Cu, Fe, Li, Mn,
246 Na, Ni, Pb, Sr, V, Zn). To this end, we oven-dried soil samples at 40°C for 48 h,
247 homogenized them by sieving to < 2 mm in order to remove large stones and dead plant
248 material, and ground them to <1 mm. We digested samples with HNO₃ and HCl and
249 analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES
250 Varian ICP 720-ES). We estimated nitrogen concentration by the Kjeldahl method

251 (Kjeldahl 1883). We used individual values per plant for each soil variable for statistical
252 analyses.

253

254 *Statistical analyses*

255 We first assessed population variation in seed defences and seed predation using general
256 linear models with data at the plant level (PROC GLM in SAS 9.4 System, SAS, Cary,
257 NC) (Littell et al. 2006). Specifically, these models tested for an effect of population on
258 seed total terpenes and phenolics, and seed predation.

259 We then proceeded to assess and disentangle direct and indirect effects of
260 predictors on seed predation by using a piece-wise structural equation model (SEM)
261 using data at the plant level (Lefcheck 2016). To avoid inflating Type I error due to
262 multiple tests, rather than individually testing for the effects of soil abiotic factors on
263 seed defences and seed predation in the SEM, we previously summarized soil abiotic
264 factors with a principal component analysis (PCA) using PROC FACTOR (rotation =
265 varimax) in SAS ver. 9.4 (Moreira et al. 2015). Similarly, we also summarized seed
266 chemical defences with PCA. The standardized z -scores from these PCs were used to
267 test for soil abiotic factor effects on plant defences and herbivory in the SEM (see
268 ahead). It is important to note that conventional SEM simultaneously estimates the
269 relationships between all variables, while for piece-wise SEM the association network is
270 broken down into different independent linear regression models and then combined
271 (Lefcheck 2016). This approach allows to easily incorporate specific assumptions in
272 each of the regression models that were included in the SEM (Lefcheck 2016). The
273 SEM allowed us to test for direct associations among soil abiotic factors and seed
274 defences, and among seed defences and seed predation, as well as indirect associations
275 between soil abiotic factors and seed predation through seed defences. We ran two

276 SEMs, one estimating for direct effects and one for estimating indirect effects. For
277 direct effects, the SEM was composed by three different linear mixed models, two
278 modelling seed defences as a function of soil abiotic factors and one modelling seed
279 predation as a function of both soil abiotic factors and seed defences. For indirect
280 effects, the SEM was also composed by three different linear mixed models, two
281 modelling seed defences as a function of soil abiotic factors and one modelling seed
282 predation as a function of seed defences. We estimated direct associations between all
283 variables as standardized partial regression coefficients. Indirect associations were
284 calculated by fitting a multiple regression model between the two variables of interest
285 (soil abiotic factors and seed predation) with any conditioning variables included as
286 covariates (i.e. seed defences). All these models included plant population as a random
287 factor. We assessed the significance of direct and indirect coefficients with *t*-tests. The
288 goodness of fit of the general model was evaluated with a ‘test of direct separation’
289 based on the Fisher’s C-test (Lefcheck 2016). The SEM analysis was performed in R
290 ver. 4.0.3 (R Core Team 2020) using the *piecewiseSEM* package (Lefcheck 2016). We
291 used the *psem* function to obtain SEM fit parameters and the *partialResid* function to
292 extract the partial effects of significant predictors on seed defences or predation after
293 accounting locally for all other covariates in each piece-wise model (Lefcheck 2016).

294

295 **RESULTS**

296 We found significant variation among sea fennel populations for seed phenolics ($F_{6,76} =$
297 2.78 , $P = 0.017$) and seed predation ($F_{6,76} = 5.62$, $P < 0.001$), but not for seed terpenes
298 ($F_{6,76} = 1.80$, $P = 0.110$). Specifically, seed phenolics varied up to 1.9-fold (3.61 to 6.78
299 mg g^{-1} d.w.), whereas seed predation varied up to 2.2-fold (25.81 to 56.23%) (Table 1)
300 Terpene concentration varied up to 1.8-fold (2291.0 to 4234.8 mg g^{-1} d.w.), but this

301 heterogeneity was mainly due to variability among individuals rather than among
302 populations (Table 1).

303 In the case of soil abiotic factors, the first axis of PCA explained 36% of the
304 variation and was positively related to the concentration of Fe, Mg, Mn, Ni, and Zn
305 (hereafter "PC1 soil") (Table 2). The second axis of PCA explained 20% of the
306 variation in soil abiotic factors and was positively related to the concentration of Ca, S
307 and Sr (hereafter "PC2 soil") (Table 2). The third axis of PCA explained 10% of the
308 variation in soil abiotic factors and was positively related to water content and
309 percentage of clay (hereafter "PC3 soil") (Table 2). In the case of seed chemical
310 defences, the first axis of PCA (hereafter "PC1 defences") explained 32% of the
311 variation and was positively related to the concentration of terpenes such as α -thujene,
312 α -pinene, β -pinene, β -myrcene, α -terpinene, γ -terpinene, and thymol methylether (Table
313 3). The second axis of PCA (hereafter "PC2 defences") explained 23% of the variation
314 in seed chemical defences and was positively related to the concentration of phenolics
315 such as 3-caffeoyl quinic acid, 5-caffeoyl quinic acid, and ferulic acid (Table 3).

316 The piece-wise SEM indicated a significant negative association between PC2
317 soil and PC1 defences (Fig. 2), whereby plants growing in soils with higher
318 concentrations of some elements such as Ca, S and Sr had lower concentrations of
319 terpenes (α -thujene, α -pinene, β -pinene, β -myrcene, α -terpinene, γ -terpinene, and
320 thymol methylether) in their seeds. We found no significant associations between the
321 other two soil PCs and PC1 defences or between any of the soil PCs and PC2 defences
322 (Fig. 2). We did, however, find a significant positive association between PC1 defences
323 and seed predation (Fig. 2), indicating that plants with higher concentrations of some
324 seed monoterpenes such as α -thujene, α -pinene, β -pinene, β -myrcene, α -terpinene, γ -
325 terpinene, and thymol methylether were more attacked by seed predators. There was no

326 detectable association between PC2 defences and seed predation (Fig. 2). Finally,
327 despite the observed effect of soil abiotic factors on PC1 defences and the latter's
328 association with seed predation, there were no detectable indirect associations between
329 soil abiotic factors and seed predation (Fig. S1 in the Supplementary Material).

330

331 **DISCUSSION**

332 Results from this study indicate that coastal plant species such as sea fennel can exhibit
333 a large variability in seed chemical defences, which is partially explained by the large
334 heterogeneity in soil abiotic factors of the habitats where they naturally inhabit. In
335 particular, we found that the concentration of seed defences (terpenes such as α -thujene,
336 α -pinene, β -pinene, β -myrcene, α -terpinene, γ -terpinene, and thymol methylether) was
337 negatively correlated with a few soil elements such as Ca, S and Sr, whereas other
338 macro- and micro-elements or physiochemical variables had no detectable effects on
339 seed defences. This high population variability in seed defences exerted a direct effect
340 on seed predation, whereby we observed an unexpected positive association between
341 seed defences (terpenes) and seed predation. Finally, despite observed soil effects on
342 seed defences and of the latter on seed predation, there was no detectable indirect effect
343 of soil abiotic factors on seed predation. Overall, these findings suggest that variation in
344 a few key soil macro- and micro-elements (and the biotic or abiotic processes
345 underlying their variation) can exert an important influence on sea fennel chemical
346 defences, with potential consequences for seed predation.

347 There was a negative association between several soil macro- and micro-
348 elements such as calcium, sulphur, and strontium and the concentration of seed defences
349 (terpenes such as α -thujene, α -pinene, β -pinene, β -myrcene, α -terpinene, γ -terpinene,
350 and thymol methylether), suggesting that sea fennel plants growing in soils with lower

351 availability of these elements were more highly defended. Calcium (Ca) is known to be
352 a critical macro-element for the formation and development of new plant tissues
353 (reviewed by White and Broadley 2003) whereas sulphur (S) is essential for the
354 formation of chlorophyll (reviewed by Zhao et al. 2008), and both elements are
355 abundant in coastal soils (Phleger 1970; Howarth 1984). In the case of strontium (Sr),
356 this micro-element is found abundantly in soils in the form of sulphate and carbonate
357 minerals, and the uptake by roots appears to be related to mechanisms of mass-flow and
358 exchange diffusion (Sasmaza et al. 2020). It also displays complex interactions with
359 calcium, but usually cannot replace Ca in biochemical functions (Walsh 1945). Plant
360 defence theory holds that plants adapted to nutrient-poor environments invest more
361 resources in defences as the cost of replacement of herbivore-damaged tissues is higher
362 under stressful conditions (reviewed by Stamp 2003), which could explain the negative
363 association between seed defences and these soil variables. Likewise, it is also possible
364 that sea fennel plants growing at sites with lower amounts of these macro- and micro-
365 elements allocate less to growth and in turn exhibit higher terpene-based defences via
366 growth-defence trade-offs (Fine et al. 2006; Sampedro et al. 2011). Unfortunately, we
367 currently cannot differentiate these candidate mechanisms because this species is
368 perennial and in situ measurements of plant size at hand (e.g. height) do not separate
369 between effects of resources on growth and plant age. Follow-up work in situ or ex situ
370 (e.g. greenhouse, common gardens) with planted individuals of known age for which we
371 manipulate soil concentrations of these macro- and micro-elements and measure
372 defences are needed to understand the relationships between resources, plant growth,
373 and defences for sea fennel.

374 The positive association between seed predation and seed defences (terpenes)
375 mirrors findings from previous work on insect herbivory and plant chemical defences,

376 particularly for specialist herbivores (e.g. Castillo et al. 2014; Abdala-Roberts et al.
377 2016a; Ochoa-López et al. 2020). One possible explanation is that terpenes (including
378 volatile compounds) serve as host finding cues by *Aethes* ovipositing females, as shown
379 for other seed-eating species of Tortricidae for which specific monoterpenes have been
380 shown to attract ovipositing females (e.g. Wearing and Hutchins 1973; Sutherland et al.
381 1977), and similar findings have been reported for other groups of specialist insect
382 herbivores such as Coleoptera (e.g. bark beetles, Erbilgin and Raffa 2000; Seybold et al.
383 2006). In addition, these caterpillars may obtain physiological or immunological
384 benefits from feeding on host plant toxins (reviewed by Erb and Robert 2016),
385 including terpenes (e.g. Becerra 1997) as well as other types of compounds such
386 furanocoumarins which are also common in the case of Apiaceae (e.g. Carroll et al.
387 1997; Carroll and Berenbaum 2006). These benefits include boosted immunological
388 responses against pathogens (e.g. Singer et al. 2014; Barthel et al. 2016; Garvey et al.
389 2021) as well as defence against parasitoids and predators via metabolite sequestration
390 (Katsanis et al. 2016; Kelly and Bowers 2018; Ochoa-López et al. 2020). It should be
391 noted, however, that although we used only undamaged seeds for chemical analyses,
392 systemic induction due to attack on other umbels of the same plant could have resulted
393 in higher chemical defences in undamaged seeds (Abdala-Roberts et al. 2016b; Moreira
394 et al. 2018), thus explaining a positive correlation between seed predation and defences
395 (terpenes). Past studies have found terpenes to be induced upon feeding by other
396 Tortricidae species (e.g. Hern and Dorn 2002; Giacomuzzi et al. 2016) and spatial
397 variation in herbivore pressure could be an important driver of population variation in
398 sea fennel defences as shown in other plant taxa in Apiaceae (Berenbaum and Zangerl
399 1998) or other families (Züst et al. 2012; Abdala-Roberts et al. 2016b). Unfortunately,
400 the methodology used in the present study does not allow us to differentiate between

401 these two explanations to unambiguously assess bottom-up effects of plant defences.
402 Further work involving sampling methodologies in which the timing of seed sampling is
403 conducted prior to the onset of seed predation to gain insight into how pre-existing
404 (constitutive) defence levels affect (from the bottom-up) seed predation. At the same
405 time, experimental manipulation of herbivory in situ or in greenhouse conditions,
406 coupled with caterpillar bioassays (including sequestration of secondary metabolites)
407 are needed to understand the consequences of plant induced responses for this
408 interaction.

409 While phenolics and terpenes are commonly studied as defences in the context
410 of plant-herbivore interactions, they may also serve for other functions, particularly in
411 the context of abiotic or physical stress. For example, phenolics are expressed in higher
412 levels under conditions of high light availability to protect leaves from damaging effects
413 of excess light (Abdala-Roberts et al. 2014; reviewed by Ballaré 2014). Likewise,
414 terpenes have also been shown to be involved in plant protection against extreme abiotic
415 conditions such as drought or high temperatures (e.g. Llusia and Peñuelas 1998),
416 indicating also a role in abiotic tolerance and stress responses. In addition, recent work
417 shows that salt-stressed plants can prime physiological responses to better cope with
418 salinity stress in neighbouring plants via airborne terpenes (Caparrotta et al. 2018).
419 Based on data currently at hand in our study system, it is unclear whether they are
420 known to function as defences in this system and especially against the studied seed
421 predator. In this sense, manipulations of abiotic factors such as salinity, temperature, or
422 soil humidity could be coupled with manipulations of focal soil nutrients to understand
423 the joint influences and interactions between soil resources and other abiotic factors
424 potentially influencing sea fennel chemical defences.

425 Despite soil macro- and micro-elements negatively correlated with seed defences
426 (terpenes) and these metabolites positively correlated with seed predation, there was no
427 detectable indirect association between soil abiotic factors and seed predation. Recent
428 work of our investigating indirect effects of soil and climatic factors on herbivory via
429 plant defences in other plant taxa has shown mixed evidence. For example, in
430 agreement with present findings, precipitation negatively affected leaf pubescence and
431 positively affected leaf-chewer herbivory in wild cotton, but there was no indirect effect
432 of precipitation on herbivory (Abdala-Roberts et al. 2019). However, for English oak
433 (*Quercus robur*) we found that temperature and precipitation were negatively associated
434 with leaf and seed defences (respectively) and this led to positive indirect effect on seed
435 predation (Moreira et al. 2020b); likewise soil physical properties (e.g. porosity)
436 positively correlated with oak chemical defences and this led to an indirect negative
437 effect on leaf herbivory (Moreira et al. 2018). Results thus far appear to be system-
438 specific depending on the plant taxa studied or type of defences or abiotic factor looked
439 at, thus warranting more studies before general rules for the strength of indirect abiotic
440 effects on herbivory can be inferred. Identifying the most important abiotic factors
441 affecting plant defences in a given system (e.g. limiting macro- and micro-elements or
442 soil physical properties), as well as key physical or chemical defences with known
443 impacts on associated phytophagous insects (e.g. specialized chemical defences against
444 focal insect herbivores or broad-spectrum metabolites against diverse generalist insects)
445 are needed in order to achieve more focalized and robust tests of abiotic forcing indirect
446 effects on herbivory.

447 We envision a couple of avenues for future research on abiotic bottom-up effects
448 on herbivory in sea fennel as well as other plant taxa. First, expanding research to
449 include a greater number of populations replicated across different coastal habitats in

450 order to assess spatially varying abiotic factors that cause variation in herbivory.
451 Accounting for other abiotic factors such as climatic or mechanical damage due to wind
452 is also warranted to achieve a more comprehensive evaluation of abiotic sources of
453 spatial variation in herbivory. Second, conducting within- and among-population
454 manipulative assessments of macro- and micro-element effects (mimicking observed
455 variation in situ) on chemical defences, both constitutive and induced, under controlled
456 and/or in situ conditions. These tests would also involve measuring effects on
457 herbivory, namely identifying key metabolites (volatile or non-volatile) influencing
458 seed predator host plant preference and seed consumption, as well as testing for indirect
459 effects of abiotic manipulations on these herbivore responses.

460

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471

472 **AUTHOR CONTRIBUTIONS**

473 Formulated the idea of the manuscript: XM, JC, LAR. Designed the experiment: JC,
474 IMPR, LM. Performed the field sampling: JC, IMPR, LM. Performed plant and soil

475 measurements: XM, JC, IMPR, LM, RMN, AGG, MF. Contributed reagents/tools: XM,
476 JC. Analysed the data: XM, CVG. Wrote the first draft of the manuscript: XM.
477 Contributed critically to the writing: LAR, JC, IMPR, LM.

478

479 **ETHICS DECLARATION**

480 The authors declare that they have no known competing financial interests or personal
481 relationships that could have appeared to influence the work reported in this paper.

482

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695 **Table 1.** Descriptive statistics (population mean and standard error) of *Crithmum*
696 *maritimum* population variation in seed terpenes (in mg g⁻¹ d.w.), seed phenolics (in mg
697 g⁻¹ d.w.), and seed predation (in %). Data are from seven coastal populations sampled
698 along southern Portugal and southern Spain. Latitude and longitude (in decimal
699 degrees) are shown. Different letters indicate significant differences among plant
700 populations at $P < 0.05$ based on Tukey post hoc tests.

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| Population | Latitude | Longitude | Terpenes | Phenolics | Predation |
|-------------------|-----------------|------------------|-------------------|------------------|------------------|
| Bolonia | 36.088 | -5.785 | 2291.0 ± 555.2 b | 5.55 ± 0.77 ab | 46.10 ± 4.35 abc |
| Calblanque | 37.602 | -0.731 | 2502.4 ± 555.2 ab | 4.46 ± 0.77 b | 42.98 ± 4.35 bc |
| Conil | 36.314 | -6.154 | 4234.8 ± 579.8 a | 6.76 ± 0.81 a | 54.62 ± 4.55 ab |
| El Toyo | 36.836 | -2.326 | 2744.3 ± 555.2 ab | 4.52 ± 0.77 b | 56.23 ± 4.35 a |
| Falesia | 37.080 | -8.148 | 3615.6 ± 555.2 a | 6.78 ± 0.77 a | 36.80 ± 4.35 cd |
| Los Muertos | 36.956 | -1.900 | 4029.7 ± 555.2 ab | 3.62 ± 0.77 b | 43.25 ± 4.35 bc |
| Valdevaqueros | 36.067 | -5.695 | 2808.0 ± 555.2 ab | 6.64 ± 0.77 a | 25.82 ± 4.35 d |

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713 **Table 2.** Results of a Principal Component Analysis summarizing the information of nine
 714 soil physiochemical properties and concentration of six soil macro-elements and 14
 715 micro-elements. Data are from soil samples collected in the top soil (0-30 cm depth) at a
 716 distance of 10 to 30 cm from the limit of the plant canopy projection. Factor loadings,
 717 eigenvalues and % of variance explained of the three main principal components (PC1,
 718 PC2 and PC3) are shown. Values in bold show factor loadings greater than 0.80.
 719

| Variables | PC1 | PC2 | PC3 |
|----------------------------------|--------------|--------------|--------------|
| Physiochemical properties | | | |
| Water content | 0.046 | -0.039 | 0.802 |
| Carbon content | 0.159 | 0.513 | 0.196 |
| pH | 0.181 | 0.195 | 0.150 |
| Conductivity | 0.401 | -0.387 | 0.240 |
| Gross sand | 0.048 | 0.308 | -0.106 |
| Fine sand | 0.535 | -0.272 | 0.677 |
| Silt | 0.533 | -0.311 | 0.637 |
| Clay | 0.159 | -0.225 | 0.866 |
| Gravel | 0.067 | -0.513 | -0.115 |
| Macro-elements | | | |
| Ca | 0.375 | 0.859 | 0.148 |
| K | 0.693 | -0.512 | 0.037 |
| Mg | 0.846 | 0.369 | -0.006 |
| N | 0.443 | 0.309 | -0.010 |
| P | 0.768 | 0.122 | -0.007 |
| S | 0.435 | 0.820 | 0.058 |
| Micro-elements | | | |
| Al | 0.569 | -0.580 | -0.173 |
| As | 0.619 | 0.714 | -0.089 |
| B | 0.590 | 0.259 | 0.406 |
| Ba | 0.594 | -0.483 | -0.035 |
| Cr | 0.782 | -0.309 | -0.131 |
| Cu | 0.781 | -0.501 | -0.225 |
| Fe | 0.896 | 0.038 | -0.269 |
| Li | 0.735 | -0.480 | -0.027 |
| Mn | 0.908 | 0.047 | -0.206 |
| Na | 0.538 | -0.344 | -0.027 |
| Ni | 0.829 | -0.131 | -0.173 |
| Pb | 0.762 | 0.547 | -0.122 |
| Sr | 0.308 | 0.852 | 0.103 |
| V | 0.752 | -0.383 | -0.205 |
| Zn | 0.839 | 0.445 | -0.091 |
| Eigen value | 10.85 | 6.18 | 2.92 |
| % Variance Explained | 36.17 | 20.60 | 9.74 |

720 **Table 3.** Results of a Principal Component Analysis summarizing the information of
 721 concentration of 11 terpenes and 10 phenolic compounds. Factor loadings, eigenvalues
 722 and % of variance explained of the two main principal components (PC1 and PC2) are
 723 shown. Values in bold show factor loadings greater than 0.80.

724

| Variables | PC1 | PC2 |
|----------------------------------|--------------|--------------|
| Terpenes | | |
| α -thujene | 0.930 | -0.042 |
| α -pinene | 0.843 | 0.136 |
| Sabinene | 0.510 | 0.146 |
| β -pinene | 0.876 | 0.118 |
| β -myrcene | 0.987 | 0.041 |
| α -terpinene | 0.840 | 0.077 |
| p-cymene | 0.584 | -0.163 |
| limonene | 0.171 | -0.075 |
| cis-ocimene | 0.541 | 0.179 |
| γ -terpinene | 0.936 | -0.058 |
| Thymol methylether | 0.844 | -0.047 |
| Phenolic compounds | | |
| 3-caffeoyl quinic acid | -0.220 | 0.820 |
| 5-caffeoyl quinic acid | -0.077 | 0.897 |
| p-coumaroyl quinic acid | -0.097 | 0.679 |
| Feruloyl quinic acid | -0.167 | 0.674 |
| Ferulic acid | -0.107 | 0.892 |
| 3,5-di-caffeoyl quinic acid | 0.190 | 0.625 |
| 4,5-di-caffeoyl quinic acid | 0.247 | 0.721 |
| Quercetin-O-hexoside | -0.060 | 0.717 |
| Quercetin-7-xyloside | -0.025 | -0.145 |
| Chrysoeriol-7-O-neohesperidoside | 0.057 | 0.207 |
| Eigen value | 6.74 | 4.80 |
| % Variance Explained | 32.11 | 22.90 |

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732 **FIGURE LEGENDS**

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734 **Figure 1.** Map showing the location of the seven populations of *Crithmum maritimum*

735 sampled along the coasts of southern Portugal and southern Spain.

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737 **Figure 2.** Diagram showing results from a piece-wise structural equation model testing

738 for direct associations among soil abiotic factors and seed defences, and among seed

739 defences and seed predation on *Crithmum maritimum* individuals sampled from seven

740 populations. Soil abiotic factors represent z -score values from a principal component

741 analysis summarizing a suite of variables associated to soil macro- and micro-elements

742 and soil physico-chemical properties (PC1 soil, PC2 soil and PC3 soil, *see statistical*

743 *analyses*). Seed defences represent z -score values from a principal component analysis

744 summarizing a suite of compounds associated to seed terpenes and phenolics (PC1

745 defences and PC2 defences, *see statistical analyses*). Values next to each arrow are path

746 coefficients (i.e. standardized partial regression coefficients). Black arrows indicate

747 significant associations whereas grey arrows indicate non-significant associations.

748 Explained variance: PC1 defences (marginal = 0.15, conditional = 0.24); PC2 defences

749 = (marginal = 0.03, conditional = 0.28); seed predation = (marginal = 0.18, conditional

750 = 0.59). Fisher's $C = 0.074$, $P = 0.964$, $AICc = 40.07$.

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