

14 **Abstract**

15 *Alnus* pollen has been frequently detected in the atmosphere of different airborne
16 sampling sites of Southern Spain. However, *Alnus* sp. populations are very scarce and
17 fragmented in the area, being restricted to a few river valleys in the southwest, and
18 other further away regions of the Iberian Peninsula. This leads to think that the airborne
19 pollen detected could be mainly the result of a medium- or long-distant transport. So,
20 the aim of this study was to characterize the annual patterns of airborne *Alnus* pollen
21 detected at three different locations of Malaga province, as well as to determine its
22 possible origin, the pollen dispersion potential of these *Alnus* isolated populations and
23 their possible reproductive connectivity. Pollen sampling was conducted by means of
24 three Hirst-type volumetric pollen traps. Samples were mounted and counted following
25 the recommendations of the Spanish Aerobiology Network and the European
26 Aeroallergen Society. The possible pollen sources were detected by means of a
27 combination of meteorological information and backward air trajectories analysis. A
28 high inter-annual variability in the annual pollen integrals was found in all the stations,
29 favouring certain meteorological conditions a long-range transport and, therefore,
30 causing the high concentrations detected in some specific days. *Alnus* pollen seems
31 to have an heterogeneous origin with prevalence of the long-distant transport, which
32 would suggest a possible reproductive connection among distant populations.

33 **Keywords**

34 aerobiology; alder; HYSPLIT; protected area; long-term transport; airborne pollen;

35 **1 Introduction**

36 *Alnus* Mill. pollen type has been reported to be one of the main causes of pollinosis in
37 central and northern Europe. It also constitutes a health risk for allergic people living
38 in the northwest part of the Iberian Peninsula, where these trees are very common
39 (Iglesias et al. 2003; Smith et al. 2007; D'Amato et al. 2007; Gehrig et al. 2015;
40 Ghasemifard et al. 2020). Due to its early pollination period and its cross-reactivity with
41 the pollen of other species of Fagales (such as *Betula* L. or *Quercus* L.), *Alnus* pollen
42 can set the start of the allergic sensitization in sensible population, increasing the
43 immunological response to pollen of other spring flowering Fagales (Smith et al. 2007;
44 Emberlin et al. 2007; Hauser et al. 2011; Gehrig et al. 2015; Skjøth et al. 2015;
45 Kasprzyk and Borycka 2019).

46 The genus *Alnus* is represented by three species in the Iberian Peninsula: *Alnus*
47 *glutinosa* (L.) Gaertn., *Alnus cordata* (Loisel.) Duby and *Alnus lusitanica* Vít, Doua &
48 Mandák, according to recent molecular studies (Castroviejo et al. 1990; López
49 González 2001; Vít et al. 2017). In the Iberian Peninsula, all these species are riverside
50 trees that prefer siliceous soils and abundant water availability, being mainly distributed
51 in the north, central and western areas (Castroviejo et al. 1990; Anthos. 2011; Vít et
52 al. 2017; GBIF.org. 2019). Their soil and water requirements make them not suitable
53 for being used as ornamental trees in the area. Nevertheless, there are some disperse
54 natural populations distributed in the south (Pérez Latorre and Cabezudo 2006; Blanca
55 et al. 2011; GBIF.org. 2019). Beside this, other previous aerobiology studies detected
56 *Alnus* pollen in the atmosphere of some localities in the south of the Iberian Peninsula
57 (Recio et al. 1998, 2006; Minero 1999; Docampo et al. 2007; Picornell et al. 2019b;
58 Velasco-Jiménez et al. 2020).

59 River ecosystems are deeply endangered in the Mediterranean region due to the
60 traditional exploitation of their water resources, and this anthropic pressure has led to
61 the fragmentation of their habitats (Skoulikidis et al. 2017; Degirmenci et al. 2019).
62 Moreover, small populations have problems related to low genetic diversity, being
63 pollen exchange among populations a solution that can smooth this effect. Therefore,
64 knowing the potential pollen connection among such fragmented populations could
65 provide useful information for stablishing conservation strategies (Sork and Smouse
66 2006; Lander et al. 2010; Morán-López et al. 2016; Zhang et al. 2019).

67 Since 1991, the Aerobiology research team of the University of Malaga has been
68 sampling the atmospheric pollen content at different points of Malaga province
69 (Southern Spain) (Picornell et al. 2019a). *Alnus* pollen has been frequently detected
70 during the winter season in low concentrations, according to the categories established
71 by the Spanish Aerobiology Network (REA) (Recio et al. 2000, 2006; Docampo et al.
72 2007; Galán et al. 2007; Picornell et al. 2019b). The lack of local *Alnus* pollen sources
73 near the stations and the distance existing to the nearest natural populations (i.e., the
74 nearest populations are 80-150 km away in the case of Malaga city) suggest a medium-
75 or even a long-term transport to explain *Alnus* pollen detection (Castroviejo et al. 1990;
76 Anthos. 2011; Blanca et al. 2011; Vít et al. 2017; GBIF.org. 2019). To search for
77 potential pollen sources and long distance transport events, many authors have
78 applied back trajectory analysis of air masses for different pollen types, including *Alnus*
79 (Šauliene and Veriankaite 2006; Hernández-Ceballos et al. 2011, 2014; Fernández-
80 Rodríguez et al. 2014; Izquierdo et al. 2015; Bilińska et al. 2017; Kasprzyk and Borycka
81 2019; Ghasemifard et al. 2020). Although many studies have been carried out on *Alnus*
82 pollen in northern and central Europe (Smith et al. 2007; Emberlin et al. 2007; Gehrig

83 et al. 2015; Kasprzyk and Borycka 2019; Ghasemifard et al. 2020), or even in the north
84 of Spain (Jato et al. 2000, 2013; Rodríguez-Rajo et al. 2004, 2011, 2017; González-
85 Parrado et al. 2006; Rodríguez-Rajo et al. 2006; González Parrado et al. 2009), we
86 can hardly find monographic studies regarding the presence of this pollen type in the
87 atmosphere of the western Mediterranean area.

88 The main aims of this study were to find if there is an annual pattern for detecting
89 airborne *Alnus* pollen at three different locations of the Malaga province, as well as to
90 determine the possible origins of the pollen detected at the sampling stations, the
91 pollen dispersion potential of these *Alnus* populations and their possible reproductive
92 connectivity.

93 **2 Material and methods**

94 2.1 Study area

95 The study was carried out in three different localities situated in the province of Malaga
96 (Southern Spain): Malaga capital, Ronda and Sierra de las Nieves (Parauta). Malaga
97 sampling station is located in the roof of the Faculty of Sciences in the university
98 campus (36°42'N, 4°28'W; 58 m a.s.l.; urban environment; average annual
99 temperature: 18.4 °C; average total annual rainfall: 540 mm); Ronda sampling station
100 is located in the roof of the “Pérez de Guzmán” public high school (36°44'N, 5°10'W;
101 768 m a.s.l.; semi-rural environment; 14.7 °C; 777 mm); and Sierra de las Nieves
102 sampling station is located in “Las Conejeras” recreational area (36°39'N, 5°5'W; 1070
103 m a.s.l.; natural environment; 13.2 °C; 831 mm), inside the domains of the
104 homonymous Natural Park and Biosphere Reserve. Malaga province has a
105 Mediterranean climate, with warm temperature during the whole year (hot summers

106 and mild winters), most rainfalls occurring mainly in autumn and winter, while summer
107 is the driest period (Recio et al. 1996, 2009; Fick and Hijmans 2017).

108 2.2 Pollen data

109 Airborne pollen were captured by means of three Hirst-type volumetric pollen traps
110 (Hirst 1952), one per sampling station. They were adjusted to aspirate a continuous
111 flow of 10 l/min. The methodology proposed by the Spanish Aerobiology Network
112 (REA) and the recommendations of the European Aeroallergen Society (EAS) were
113 followed in order to mount and count the samples (Galán et al. 2007, 2014). Four
114 longitudinal sweeps were read per slide at a magnification of 400X with the aid of a
115 light microscope.

116 Annual pollen integral (API_n) is defined as the sum of the average daily concentrations
117 over the whole year (Galán et al. 2017).

118 All the available pollen data for each station have been included in this study. These
119 dates were: 11th May 1991 to 30th June 2019 for Malaga; 16th December 2016 to 30th
120 June 2019 for Ronda; and 24th January 2018 to 30th June 2019 for Sierra de las Nieves.

121 2.3 Meteorological data

122 Meteorological data were obtained from the official stations of the Spanish National
123 Agency of Meteorology (AEMet). For that, the nearest meteorological stations to the
124 sampling points, considering only complete registers, were selected. Malaga-Airport
125 meteorological station was selected for Malaga (4.64 km away), and Ronda
126 meteorological station was used for both Ronda (0 km away) and Sierra de las Nieves
127 (11.72 km away) aerobiological stations. Daily values of maximum, mean and
128 minimum temperatures (°C), total rainfall (mm), average relative humidity (%), average

129 wind speed (km/h), calm frequency (%), and frequency of winds (%) coming from the
130 four quadrants, i.e. 1st (northeast, NE), 2nd (southeast, SE), 3rd (southwest, SW) and
131 4th (northwest, NW) were used.

132 2.4 Backward air trajectories and map elaboration

133 Only days with daily mean concentrations ≥ 10 *Alnus* pollen grains/m³ were used for
134 determining backward air trajectories in order to study the main pollination events
135 recorded during the whole studied period in each station. Backward air trajectories were
136 calculated according to Hybrid Single-Particle Lagrangian Integrated Trajectory model
137 (HYSPPLIT4), developed by the National Oceanic and Atmospheric Administration Air
138 Resources Laboratory (NOAA ARL) (Stein et al. 2015; Rolph et al. 2017). Trajectories
139 were studied at three different heights: 500, 750 and 1000 m AGL, as this range of
140 altitude is widely used in backward trajectories studies of air masses (Šauliėne and
141 Veriankaite 2006; Stach et al. 2007; Hernández-Ceballos et al. 2011, 2014; Bilińska et
142 al. 2017). Air masses below 500 m AGL were not considered to avoid interactions with
143 the orography and local climatic conditions. Meteorological data from 2005 to 2019 used
144 for calculating the trajectories were extracted from the global Global Data Analysis
145 System (GDAS) database with 1 degree of precision provided by the NOAA
146 (Environmental Modeling Center et al. 2001). Data prior to 2005 were obtained from
147 Archived Reanalysis Database provided by the NOAA (National Centers for
148 Environmental Prediction and National Center for Atmospheric Research 2003), which
149 was one of the few databases that had available data for this period. Isentropic vertical
150 motion was selected. For each target day, three new trajectories per hour were
151 calculated (one for each height; ending hours from 01:00 to 24:00 h at local time zone),

152 resulting in 72 trajectories per day. Each air mass movement was observed 48 hours
153 backwards in time from the target station.

154 Geographical information was managed by using QGIS software (QGIS Development
155 Team 2019). Trajectories were plotted over the political map of southwest Europe and
156 northern Africa, elaborated with data obtained from the Spatial Reference Data of
157 Andalusia (DERA) (Junta de Andalucía. 2019). Georeferenced information about
158 preserved specimens of *Alnus* were obtained from GBIF database (Global Biodiversity
159 Information Facility) (GBIF.org. 2019), which, in turn, integrates information from 58
160 different databases. GBIF database has the most extensive and detailed registers for
161 the studied area, due to the integration of all the main databases from herbariums,
162 universities and research centers (GBIF: The Global Biodiversity Information Facility.
163 2019). These occurrences were included in the maps in order to show the situation of
164 the main populations of *Alnus* in the area. Registers without preserved specimens were
165 not considered to avoid possible misidentifications or non-reliable sources.

166 2.5 Statistical studies

167 Pollen data were expressed as daily mean concentrations (pollen grains/m³). They
168 were analyzed and managed by using “AeRobiology” package, developed for R
169 software (Development-Core-Team 2017; Rojo et al. 2019). Statistical analysis were
170 performed by using R software too. A comparative statistical study, regarding the
171 meteorological parameters registered in Malaga, was carried out by comparing the days
172 with ≥ 10 *Alnus* pollen grains/m³ and the days of January-March from all the sampling
173 years. Since the meteorological data did not fit a normal distribution, according to a
174 Kolmogorov-Smirnov test with Lilliefors correction ($\alpha=0.05$), non-parametric statistics

175 were used. Therefore, comparisons were made by means of Mann-Whitney-Wilcoxon
176 tests.

177 Correlations analyses were performed between the daily mean concentrations of *Alnus*
178 pollen (pollen grains/m³) and the main meteorological variables. Due to the reduced
179 number of days per year in which this pollen type was detected (≥ 1 pollen grains/m³),
180 days without *Alnus* pollen detection were omitted to avoid statistical noise. Since data
181 did not fit a normal distribution, according to a Kolmogorov-Smirnov test with Lilliefors
182 correction ($\alpha=0.05$), Spearman correlation coefficients were calculated.

183 The high number of comparisons increased the risk of type I errors and a post-hoc
184 correction was advisable. Bonferroni correction was applied and the α level to check
185 each hypothesis was reduced in each case.

186 **3. Results**

187 3.1 Pollen registers

188 *Alnus* pollen was not an abundant pollen type in the atmosphere of Malaga province.
189 In fact, it only represented the $0.10\pm 0.05\%$ of the annual pollen integral of all pollen
190 types recorded in Malaga (1992-2018); the $0.10\pm 0.03\%$ in Ronda (2017-2018); and
191 the 0.06% in Sierra de las Nieves (2018).

192 The annual pollen integrals registered in the different sampling stations were, as
193 average, 35.82 ± 25.29 pollen*day/m³ in Malaga (1992-2019), 77.33 ± 46.06
194 pollen*day/m³ in Ronda (2017-2019) and 49.50 ± 12.02 pollen*day/m³ in Sierra de las
195 Nieves (2018-2019). For 2019, it only included data from January to June, but no pollen
196 was expected outside this period according to the historical records (Supplementary
197 material).

198 During the studied period, *Alnus* pollen was mainly detected during the period January-
199 March in all the stations (Fig. 1 and Supplementary material). However, the pollen
200 registers not only showed high fluctuations from one day to another but varied from
201 year to year. The average daily values of *Alnus* pollen concentration during the studied
202 period were normally lower in Malaga than in Ronda and Sierra de las Nieves. The
203 highest historical *Alnus* daily mean pollen concentration detected in Malaga was 26
204 pollen grains/m³, being registered on 28th January 2019. In Ronda the historical peak
205 was registered the same day with 33 pollen grains/m³, and in Sierra de las Nieves it
206 occurred on 3rd February 2019 with a daily mean concentration of 12 pollen grains/m³.

207 -FIG 1-

208 3.2 Backward air trajectories

209 In Malaga, backward air trajectories were studied for 12 different days (Fig. 2). Since
210 not a clear hourly pattern was observed during the chosen dates, i.e. pollen was
211 detected during almost the whole day, all trajectories were plotted to establish possible
212 *Alnus* pollen sources.

213 -FIG. 2-

214 Trajectories suggested a multiple origin of the *Alnus* pollen detected in Malaga. The
215 nearest *Alnus* populations, located southwest of Malaga (province of Cadiz and the
216 Genal river valley), may have contributed in some days, such as the 5th February 1996,
217 the 18th January 2004, the 14th February 2014, 27th January 2019, 28th January 2019
218 and 3rd February 2019 (Fig. 2). However, there are other 6 days with alder pollination
219 events in which air trajectories did not overpass these populations, but other larger
220 populations located in the north and northwest of Malaga.

221 The meteorological parameters registered in those 12 days were studied and
222 compared with those of January-March from the period 1991-2019 (Fig. 3).

223 -FIG. 3-

224 Bonferroni correction was applied and the α level was reduced to $5 \cdot 10^{-03}$. With this
225 significance level, only significant differences in relative humidity, wind speed,
226 frequencies of wind blowing from 2nd and 4th quadrants, as well as frequencies of calm
227 were not rejected. No rainfall was registered during the studied days.

228 In Ronda and Sierra de las Nieves, 4 and 2 days of alder events, respectively, were
229 studied (Fig. 4). As *Alnus* pollen were detected during the major part of the day, hourly
230 trajectories were also studied.

231 -FIG. 4-

232 Trajectories in these stations did not have a unique pattern either. Only on 28th January
233 2019 and 3rd February 2019 in Ronda, and on 3rd February in Sierra de las Nieves,
234 some trajectories overpassed the nearest *Alnus* populations situated in the southwest
235 of the aerobiological stations (province of Cadiz and the Genal river valley). During the
236 other three days, the air masses came from over the populations located north and
237 northeasterly of the study area (north and east of Andalusia).

238 The low number of *Alnus* pollination events in Ronda and Sierra de las Nieves did not
239 allow the use of statistics nor the boxplot representation. Therefore, the mean and
240 deviation values of the meteorological parameters during the days with *Alnus*
241 pollination events (≥ 10 pollen grains/m³) and during the period January-March for all
242 the years studied are shown in Table 1. As observed, predominant meteorological
243 conditions during the days of alder events were: lower temperatures, slightly lower

244 relative humidity, as well as a higher predominance of winds blowing from the first and
245 fourth quadrant (i.e. dominance of north direction). No rain was registered during those
246 days.

247 -TABLE 1-

248 3.3 Correlations with meteorological variables

249 The results of Spearman correlations carried out between daily values of *Alnus* pollen
250 concentration and the main meteorological variables, during all the days in which *Alnus*
251 was detected, are shown in Table 2.

252 -TABLE 2-

253 Bonferroni post-hoc correction was applied and the α level was reduced to $4.55 \cdot 10^{-03}$.
254 Significant negative correlation coefficients were obtained in Malaga between daily
255 values of airborne alder pollen concentrations and relative humidity, frequency of wind
256 blowing from 2nd quadrant (ES, coming from the Mediterranean sea) and frequency of
257 calm, while significant positive correlations coefficients were obtained with the wind
258 speed and frequency of wind blowing from the 4th quadrant (NW). In Ronda, significant
259 negative correlations were obtained with mean and minimum temperatures and
260 frequency of winds from 3rd quadrant (SW). In Sierra de las Nieves only a significant
261 negative correlation coefficient was detected between daily values of airborne alder
262 pollen concentration and frequency of wind from the 2nd quadrant (SE).

263 4 Discussion

264 *Alnus* pollen, when detected, is frequently found in low concentrations in the
265 atmosphere of the Malaga province, probably due to the distance existing between the
266 aerobiological sampling stations and the populations of this taxon, which, on the other

267 hand, makes it a perfect target for the study of medium and long distance pollen
268 transport. The high interannual variability of the annual *Alnus* pollen integral in Malaga,
269 together with the scarce sampling years in Ronda and Sierra de las Nieves
270 (Supplementary material), it does not make possible to conclude which station
271 registers the highest *Alnus* pollen values. The scarce number of years of sampling
272 carried out in Ronda and Sierra de las Nieves, are also reflected in the mean daily
273 values obtained (Fig. 1), which seems to be higher than those obtained in Malaga.
274 However, it is expected those values gradually decrease when more years of sampling
275 be added to the calculation of average values.

276 The 28 years of samplings carried out in Malaga, can be considered an acceptable
277 and sufficient number of years for establishing the general annual pattern of the *Alnus*
278 pollen in the atmosphere of the area (Martínez-Bracero et al. 2015; Galán et al. 2017).
279 The period in which *Alnus* pollen was mostly detected in the sampled locations
280 matched that of the flowering phenophase of *Alnus* populations from different and
281 distant parts of the Iberian Peninsula according to the results obtained by previous
282 researchers (Jato et al. 2013; Rodríguez-Rajo et al. 2017; Velasco-Jiménez et al.
283 2020). In this situation, not a clear origin of the *Alnus* pollen detected can be stated
284 from phenology timing. The small concentrations of pollen sporadically found outside
285 the period January-March may correspond to resuspension events.

286 As observed in the average daily values of Malaga (Supplementary material), there is
287 not a clear pollination peak nor a clear start or end of the pollination season either. The
288 detection of airborne *Alnus* pollen is sporadic and the few days with *Alnus* pollen did
289 not allow the calculation of a representative main pollen season. Once again, this may
290 be explained by the lack of a local source which could continuously contribute to the

291 *Alnus* pollen concentrations, as well as by possible long- or medium-distance transport
292 events. Additionally, it may also explain the variations observed from one year to
293 another in the dates of pollen detection (Sofiev et al. 2006). The low amount of pollen
294 registered and their interannual variability points to a dependence of wind direction and
295 speed more than of local sources, which is reinforced by the backward air trajectories
296 obtained and the meteorological conditions of the days with *Alnus* pollination events.
297 Furthermore, *Alnus* pollen has been previously reported to be involved in long range
298 transport events in the Iberian Peninsula (Izquierdo et al. 2015). As observed in Fig. 2
299 and 4, wind trajectories passed over the main *Alnus* populations situated northern and
300 southeastern of the sampling points (i.e., medium- or long-range transport) in the days
301 in which the highest *Alnus* pollen concentrations were detected in all the aerobiological
302 stations. Trajectories during these days did not present great variations among the
303 different heights. Therefore, most of the pollen detected during these high-
304 concentration days were likely to come from distant populations.

305 Meteorological conditions during these days were also favorable to pollen transport
306 (Fig. 3), i.e. absence of precipitation, low relative humidity and high wind speed
307 (Rodríguez-Rajo et al. 2004, 2017; Bonofiglio et al. 2008; Rojo et al. 2015; Recio et al.
308 2018). Despite high temperatures have been reported to be favorable for pollen
309 dispersion, favouring drying and dehiscence of anthers (Rojo et al. 2015; Recio et al.
310 2018), no significant differences were found in Malaga between the temperatures of
311 these days and the average temperature for the period. On the contrary, negative
312 correlation coefficients were obtained between temperatures and pollen
313 concentrations in all the studied stations (Table 2). This may be related to wind
314 direction, due to wind blowing from the North, when main pollination events occurred,

315 normally tend to lower temperatures. In fact, regarding wind conditions, the days with
316 *Alnus* pollination events had lower frequencies of wind in calm than average for this
317 period in all the stations (statistically significant in the case of Malaga). During these
318 events, the dominant winds in Malaga were those blowing from the 4th quadrant, which
319 suggests a main northwestern origin of the *Alnus* pollen detected. In Ronda and Sierra
320 de las Nieves, the 1st and 4th quadrants were the dominants (Table 1), which pinpoints
321 to a northern origin of the *Alnus* pollen. These results are also supported by the study
322 of backward air trajectories (Fig. 2 and 4). All this leads us to think that the highest
323 contributions of *Alnus* pollen detected in the atmosphere of Malaga province, come
324 from populations located in Sierra Morena (north of Andalusia; 160-200 km away), but
325 not discarding punctual contributions of the populations situated in the southwest
326 (Cadiz province and Genal river valley; 50-100 km away) and the east (Sierra Nevada,
327 Granada province; 130-160 km away) as observed in the backward air trajectories.
328 Contributions from the populations situated in the center and north of the Iberian
329 Peninsula are less likely but not disposable (> 300 km away).

330 The correlation study carried out between *Alnus* pollen concentrations and
331 meteorological variables (Table 2), did not show a clear pattern for all the
332 aerobiological stations, what can be explained by the smaller amount of available data
333 in Sierra de las Nieves and Ronda when compared to Malaga and also due to the very
334 restrictive post-hoc correction applied (i.e., the more data, the more significant
335 correlations are expected). However, it should be noted that, when significant, negative
336 coefficients were obtained in the case of relative humidity, temperatures, % of calms
337 and winds blowing from the 2nd and 3rd quadrants. On the contrary, the significant
338 coefficients were positive in the case of wind speed and winds blowing from the 4th

339 quadrant. This explains that the meteorological conditions commented above be the
340 most favorable for the capture of *Alnus* pollen. In general, in Malaga, which is a coastal
341 town, winds blowing from the 2nd and 3rd quadrants, coming from the sea, tend to
342 decrease atmospheric pollen concentrations, while those of northern component
343 increase the pollen concentrations of plants whose populations are situated inland
344 (Recio et al. 2018).

345 The highest concentrations detected in all the sampling locations are low when
346 compared with other European locations (Smith et al. 2007; Jantunen et al. 2012;
347 Ghasemifard et al. 2020) or other regions of the Iberian Peninsula (Rodríguez-Rajo et
348 al. 2017; Velasco-Jiménez et al. 2020). Most of the concentrations detected are not
349 expected to trigger allergic symptoms in sensible population by themselves, but there
350 were punctual concentrations that reached higher values which may detonate an
351 immunological response (Jantunen et al. 2012; Rodríguez-Rajo et al. 2017). The
352 cross-reactivity existing between *Alnus* pollen and other species within the Fagales
353 order has been unveiled by several authors (Smith et al. 2007; Emberlin et al. 2007;
354 Hauser et al. 2011; Gehrig et al. 2015; Skjøth et al. 2015; Kasprzyk and Borycka 2019).
355 Although the quantitative relevance of the cross-reaction between *Alnus* and *Betula*
356 pollen has been widely studied, this has not been the case of *Quercus* pollen, since
357 their species are not as abundant in central or northern Europe as they are in the south
358 (Hauser et al. 2011; Jantunen et al. 2012; Skjøth et al. 2015). Therefore, it would be
359 interesting for future research to study with clinical data if these sporadic peaks of
360 *Alnus* pollen concentrations in some years have a correlation with an increase of
361 *Quercus* allergic symptoms during spring.

362 Finally, we would like to comment that the long-range pollen transport studied here, it
363 could make reproductive contact between isolated *Alnus* populations possible. This is
364 because the distance between the sampling stations and the *Alnus* populations, are
365 similar to the distance between such populations.

366 **5. Conclusions**

367 *Alnus* pollen can be considered a minority pollen type in the atmosphere of the Malaga
368 province due to its low annual pollen integrals and relative abundance values.
369 Nevertheless, it has been detected days with higher airborne pollen concentrations
370 which notably contribute to the *Alnus* annual pollen content.

371 The irregularity of *Alnus* pollen occurrences, the prevailing meteorological conditions
372 during the events, as well as the backward air trajectories study, supported the
373 possibility of a long-term transport to the localities studied.

374 According to the backward air trajectories study, *Alnus* pollen concentrations detected
375 in the atmosphere of the province of Malaga may correspond to an heterogeneous
376 origin: a main pollen source from the *Alnus* populations situated northern the stations
377 (long-term transport), but also punctual contributions from the populations situated in
378 the province of Cadiz and Genal river valley (southwest; the nearest *Alnus* populations;
379 medium- or long-term transport depending of the pollen sampling station). In a lesser
380 extent, it is also possible a contribution of populations situated further north. This long-
381 range pollen transport detected would support the possible pollen exchange among
382 isolated populations in the area. To support this, the genetic characterization of the
383 pollen detected could be a very interesting topic for future research.

384

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611

612 **Fig. captions**

613 **Fig. 1.** Mean (black line) and maximum (upper limits of the colored areas) daily
614 concentrations of *Alnus* pollen detected in each aerobiological station during the
615 studied years. Areas of each color represent the daily pollen concentrations of a
616 year of study. Those days that exceeded the discontinuous red line (≥ 10 *Alnus*
617 pollen grains/m³) were studied as possible long transport events. Malaga data:
618 from 11th May 1991 to 30th June 2019; Ronda data: from 16th December 2016 to
619 30th June 2019; Sierra de las Nieves data: from 24th January 2018 to 30th June
620 2019.

621 **Fig. 2.** Hourly backward air trajectories during the 12 days with with *Alnus* pollen
622 concentrations in Malaga ≥ 10 pollen grains/m³, at different heights. Each line
623 represents a 48 hours length backward trajectory for each hour of the target day.
624 Trajectories in green arrived at 500 m AGL at the sampling station; red trajectories,
625 at 750 m AGL; and black trajectories, at 1000 m AGL. Blue dots represent
626 georeferenced preserved specimens of *Alnus* obtained from GBIF database.

627 **Fig. 3.** Boxplot comparison between the meteorological parameters prevailing
628 during the days with higher *Alnus* pollen concentrations in Malaga (≥ 10 pollen
629 grains/m³, *Alnus* event days), in orange and the days from January to March (in
630 blue) of the studied period (1991-2019). p-values were obtained from Mann-
631 Whitney-Wilcoxon tests. Tm: daily mean temperature; Tmax: daily maximum
632 temperature; Tmin: daily minimum temperature; Wind Q1, Q2, Q3 and Q4: daily
633 frequencies of winds (%) blowing from the 1st (NE), 2nd (ES), 3rd (SW) and 4th (NW)

634 quadrants; Wind Calm: daily frequencies of wind in calm. *Alnus* event days, n=12;
635 January-March days, n=2617.

636 **Fig. 4.** Hourly backward air trajectories during the days with high *Alnus* pollen
637 detection in Ronda and Sierra de las Nieves (≥ 10 pollen grains/m³) at different
638 heights. Each line represents a 48 hours length backward trajectory for each hour
639 of the target day. Trajectories in green arrived at 500 m AGL at the sampling station
640 in Ronda and Sierra de las Nieves respectively; red trajectories at 750m AGL and
641 black trajectories at 1000 m AGL Blue dots represent georeferenced preserved
642 specimens of *Alnus* obtained from GBIF database.

643 **Table 1.** Mean and standard deviations of meteorological parameters in the days
644 with high *Alnus* pollen detection in Ronda and Sierra de las Nieves (≥ 10 pollen
645 grains/m³; “*Alnus* events”), as well as in January-March days of the entire studied
646 period (2017-2019 in Ronda and 2018-2019 in Sierra de las Nieves). Rel. Hum.:
647 daily relative humidity; Tm: daily mean temperature; Tmax: daily maximum
648 temperature; Tmin: daily minimum temperature; Wind Q1, Q2, Q3 and Q4: daily
649 frequencies of winds blowing from the 1st (NE), 2nd (ES), 3rd (SW) and 4th (NW)
650 quadrants; Wind Calm: daily frequencies of wind in calm.

651 **Table 2.** Spearman correlation coefficients (Coef.) and p-values obtained between
652 daily values of *Alnus* pollen concentration (pollen grains/m³) and meteorological
653 variables in the days with any *Alnus* pollen detection (≥ 1 grain/m³) of the period
654 studied in each aerobiological station. Rel. Hum.: average relative humidity (%);
655 Tm: mean temperature (°C); Tmax: maximum temperature (°C); Tmin: minimum
656 temperature (°C); Wind Q1, Q2, Q3 and Q4: daily frequencies of winds blowing

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657 from the 1st (NE), 2nd (SE), 3rd (SW) and 4th (NW) quadrants (%); Wind Calm: daily
658 frequencies of wind in calm (%). (*) Significant correlations after Bonferroni post-
659 hoc correction.