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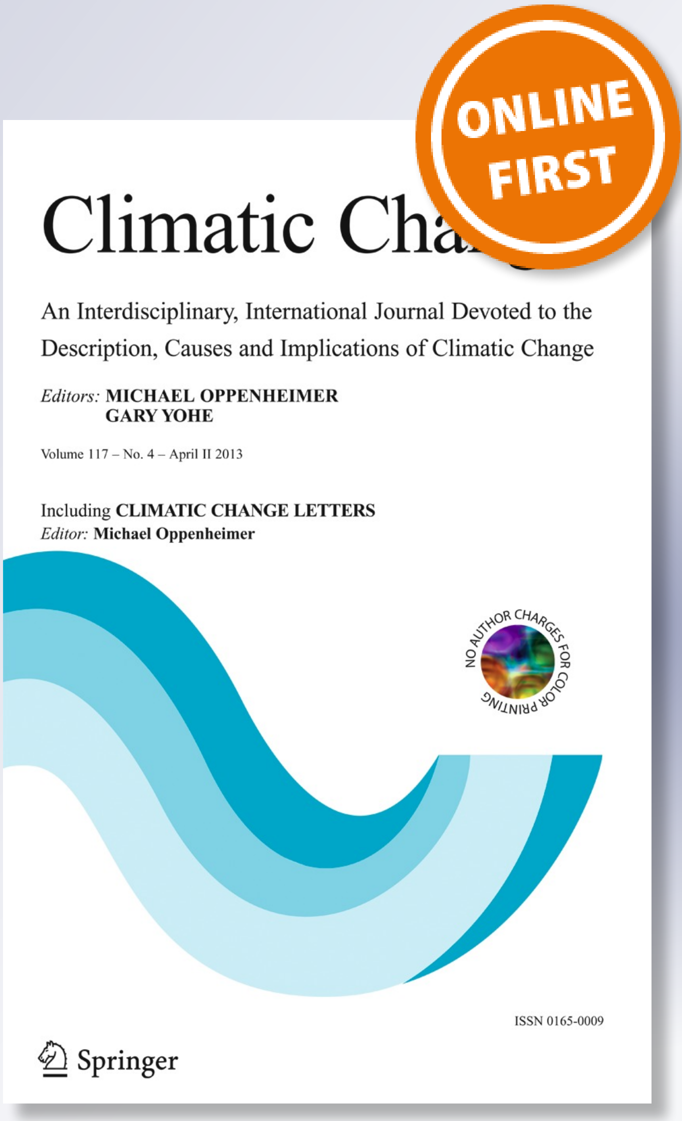

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Abstract This study sought to analyse the effects of climate change on *Artemisia* species growing in Sierra Nevada, a high mountain range in the south-east of the Iberian Peninsula, using pollen counts as a bioindicator. The study also examined the impact of Recovery Programmes implemented for the most endangered of these species. Analysis of historical *Artemisia* pollen-data series from 1992 to 2011 showed that flowering took place between late July and late September, but the trend towards higher summer temperatures detected over the series as a whole appeared to have delayed the start of flowering and brought forward the end of flowering, thus prompting a shortening of the season. A trend was also observed towards a delayed peak pollen period, together with a significant decline in the Annual Pollen Index, which was significantly influenced by rainfall over the months immediately prior to flowering. Recovery Programmes implemented for three species—*Artemisia granatensis, A. alba* subsp. *nevadensis* and *A. umbelliformis*—involved conservation measures including direct seeding and planting of seedlings. From the outset, these programmes led to a recovery of the Pollen Index, especially when using germinated seedlings, which adapted better than seeds to environmental conditions. In conclusion, pollen records proved to be a useful tool for assessing the status of endangered species.

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1 Introduction

Artemisia is one of the largest genera in the *Asteraceae* family, comprising almost 500 species and subspecies (Wright 2001), which grow from Arctic alpine or mountainous areas to dry deserts, and dominate the steppe communities of Asia, South Africa and the New World (Mucciarelli and Maffei 2002). Many species are anemophilous (Watson et al. 2002), and release considerable amounts of pollen into the air (Wahl and Puls 1989; Munuera et al. 1999). Monitoring of pollen production and subsequent release in wind-pollinated plants provides a clear indicator of the biological status of an individual or a whole plant population, not only because seed set and the production of viable seeds are functions of pollen intensity (McClanahan 1986), but also because variations in pollen release may be indicative of problems either inherent in the species themselves (Vinod 2005; Eckardt 2006) or prompted by environmental stress conditions and climate change (Garcia-Mozo et al. 2010; Cariñanos et al. 2010; Teranishi et al. 2000).

Of the 19 species and 7 subspecies of *Artemisia* reported as growing in Spain (Tutin et al. 1980), eight are to be found in the Sierra Nevada (south-eastern Spain): *A. campestris* subsp. *glutinosa* (Besser) Batt., *A. barrelieri* Besser, *A. herba-alba* Asso, *A. absinthium* L., *A. alba* Turra subsp. *nevadensis* (Willk.) Blanca & C. Morales, *A. chamaemelifolia* Vill., *A. umbelliformis* Lam. and *A. granatensis* Boiss (Blanca 2009). Some of these are thought to be virtually endemic to the Sierra Nevada, due to its highly-specific habitat and the isolated nature of the populations concerned. The risk posed by this situation is aggravated by a number of other real and potential threats including herbivore pressure, inappropriate forestry practices, fires, tourist activities, and the over-collection of certain species traditionally prized for medicinal purposes (Blanca et al. 1998). It is hardly surprising, therefore, that some of these *Artemisia* species are at serious risk of extinction (Blanca et al. 1999; Bañarés et al. 2004). A further factor to be borne in mind is the recent impact of climate change, both in general terms and more particularly in areas which are especially vulnerable due to their great dependence on strict, stable, environmental conditions (Gottfried et al. 2012; Pauli et al. 2012).

Among the more effective measures taken to prevent the extinction of such species is the design and implementation of Endangered Species Preservation and Recovery Programmes, that comprise a number of conservation measures involving both in situ and ex situ techniques (Blanca and Algarra 2011). Sierra Nevada is the highest mountain chain in southern Europe, and the second highest in Europe as a whole, after the Alps. Because of its unique biogeographical, bioclimatic, orographical and ecological properties, the local flora is not only largely endemic but also includes numerous endangered species. Work on the recovery of selected species started in 2000, focussing on species included in the Red Books and Atlas of Endangered Flora (Blanca et al. 1999; Bañarés et al. 2004), and belonging to one of the Red List categories established for threatened species by the IUCN (IUCN 2001). Three *Artemisia* species have been the object of conservation and recovery programmes in Sierra Nevada: *A. granatensis* (IUCN Critically Endangered—CR), *A. alba* subsp. *nevadensis* (IUCN Vulnerable-VU), and *A. umbelliformis* (IUCN Endangered—EN).

The present study sought to analyse the effects of temperature and precipitations on *Artemisia* species growing in Sierra Nevada, some of which are critically endangered, using pollen counts as a bioindicator. A historical data series was used to chart changes in airborne pollen dynamics over the last 20 years as a function of changing environmental conditions. The study also examined the impact of the Endangered Flora Recovery Programmes implemented for some of these species over the last 10 years.

2 Material and methods

2.1 Area description

Sierra Nevada (37°N, 3°W) is a mountain chain in south-eastern Spain belonging to the Sierras Béticas system. The Sierra Nevada chain runs east–west for almost 90 km, and contains a number of peaks over 2,500 m. high. It is remarkable for its silica central core, its complex relief, and a biogeographical range containing 5 of the 6 bioclimatic storeys comprising the Mediterranean region (Rivas Martínez 1990). Over 2,100 floral taxa have been catalogued, of which 80 are endemic to the Sierra Nevada (Blanca et al. 1998; Molero-Mesa and Pérez-Raya 1987); 30–40 % of the species growing on the peaks are exclusive to the massif. As a result, this is one the major focuses of plant diversity in the Western Mediterranean (Molero-Mesa 1994), and a biodiversity "hotspot" (Médail and Diadema 2009). The climate conditions are typical of high mountain environments. According to data logged at the Prado Llano weather station (2,500 m.a.s.l., north slope), the mean annual temperature for the period from 1975 to 1989 was 3.9 °C, with means dropping to below 0 °C during the winter months and maxima exceeding 20 °C in summer. Mean annual rainfall over the same period was 712.6 mm.

2.2 *Artemisia* species in Sierra Nevada

The key characteristics of the *Artemisia* species growing in Sierra Nevada are outlined in Table 1. The present study focussed on summer-flowering species: *A. absinthium, A. alba* subsp. *nevadensis*, *A. chamaemelifolia, A. granatensis, A. umbelliformis* and *A. campestris* subsp. *glutinosa*. With the exception of *A. campestris*, which is common in the Eastern Mediterranean and may grow from sea level upwards, these species are found in the Supramediterranean storey upwards, and are rare (ra) or very rare (rr) in this area. Some of these species display a narrow geographical distribution, broad habitat specificity and small population size, being found either only in Sierra Nevada (*A. granatensis*, *A. alba* subsp. *nevadensis*) or so distant from other populations that genetic flow is impossible (*A. absinthium, A. umbelliformis*). Although all species are chamaephytes, *A. granatensis* and *A. umbelliformis* are caespitose, which confers a certain advantage in dry periods.

2.3 Pollen records

Artemisia pollen data were obtained from the Eastern Andalusia Aerobiological Monitoring Unit, located at the University of Granada Science Faculty (37º 11′N, 3º 57′W, 685 m a.s.l., 30 km W of Sierra Nevada), which has a continuous series from 1992 to 2011. Airborne pollen sampling was carried out using the normalized, standardized procedure for Sampling Stations belonging to the Spanish Aerobiology Network (Galan et al. 2007). In order to chart local annual airborne *Artemisia* pollen dynamics, a pollen curve was constructed using mean daily counts for all the years in the series (Fig. 1). Since all *Artemisia* species share the same pollen type, and since pollen grains cannot be differentiated under light microscopy (Ghahreman et al. 2007; Rowley et al. 1981), this study focussed on records for the period from mid-July to early October, this being the flowering period for Sierra Nevada, once the snow has melted. For each year in the series, the following descriptive parameters were defined for the flowering season: start date, i.e. the date from which 1 pollen grain/m3/day was recorded for at least 5 consecutive days (Garcia-Mozo et al. 1999); end date; length of pollen season (in days); peak pollen count and date recorded; and summer Pollen Index (suPI), i.e. the sum of daily counts for each study year from mid-July to early October.

Table 1 Main characteristics of the *Artemisia* species in Sierra Nevada (Spain)

TAXON	Lifeform	Flowering period (Months)	Habitat	Bio-climatic belt	Distribution	N° Ind.	Rarity-type	IUCN category (Mean threats)
Artemisia absinthium L.	Ch.sf.	VII-VIII	Ruderal en zonas montañosas (1700–2600 m)	Supra-Oro	Europa, SW Asia, N Africa. Sierra Nevada (ra)	NK	WBL	LC
Artemisia alba Turra subsp. *nevadensis* (Willk.)Blanca & C. Morales	Ch.sf.	VII-IX (X)	Matorral almohadillado y canchales (1800–2300 m)	Supra-Oro	Sierra Nevada, Sierra de Baza (rr)	<200	NBS	EN (1,2)
Aremisia chamaemelifolia Vill.	Ch.sf.	VIII-X	Matorral almohadillado (1800–2500 m)	Supra-Oro	Submediterranea. Sierra Nevada Oriental (rr)	<200	WBS	VU (1,2)
Artemisia granatensis Boiss.	Ch.caesp.	VII-IX	Roquedos y pedregales (2700–3400 m)	Oro-Crioro	Sierra Nevada (rr)	<3000	NBS	CR (2,4)
Artemisia umbelliformis Lam.	Ch.caesp.	VII-VIII	Gleras de zonas húmedas (2800–300 m)	Oro-Crioro	Alps, Apennines, Pyrenees, Sierra Nevada (rr)	<500	WBS	EN (1,2.4)
Artemisia campestris L. subsp. *glutinosa* (Besser) Batt.	Ch.sf.	VII-I	Matorral nitrificado (0–2300 m)	Thermo-Oro	W Mediterranean Region (fr)	NK	WBL	LC
Artemisia barrelieri Besser	Ch.sf.	III-V	Matorral nitrificado (50–1300 m)	Thermo-Meso	S, E Iberian Peninsula (fr.)	NK	WBL	LC
Artemisia herba-alba Asso	Ch.sf.	IX-III	Matorral nitrificado (0–1500 m)	Thermo-Meso	S, W Mediterranean Region (fr)	NK	WBL	LC

Life-form: *Ch.sf.* chamaephyte suffruiticose, *Ch.caesp.* chamaephyte caespitose. Distribution: *ra* rare, *rr* very rare, *fr* frequent. Rarity type: Geographic distribution *N* narrow, *W* wide, Habitat specifity: *B* broad, *R* restricted, Local polulation size: *L* somewhere large, *S* everywhere small. IUCN Categories: *LC* low concern, *VU* vulnerable, *EN* endangered, *CR* critically endangered. Main threats: 1: natural causes; 2: overgrazing; 3: fire; 4: collecting

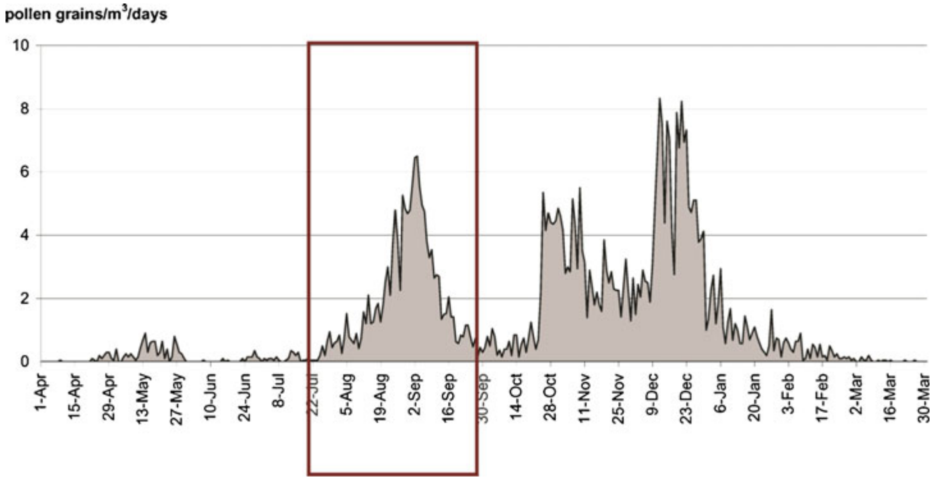

Fig. 1 Daily mean airborne *Artemisia* pollen counts for the city Granada, Spain, over the period 1992–2011

2.4 Weather-related parameters

Weather data were supplied by the Spanish Meteorological Agency (AEMET), from the weather station located at La Arquilla (2,000 m a.s.l., western slope of Sierra Nevada), this being the only station possessing data for the whole study period (1992 to 2011), and also the closest to the area where *Artemisia* populations are to be found. The mean annual temperature at this station over the study period was 9.30 °C, and mean annual rainfall was 515.35 mm. To chart the impact of weather conditions on *Artemisia* airborne pollen dynamics over the period studied, the following parameters were analysed: maximum, mean and minimum temperatures, relative humidity, hours of sunlight, and wind speed and direction.

2.5 Statistical analysis

In order to analyse trends in pollen-season start date, end date, length and duration, peak pollen count, date of peak count, Pollen Index, temperatures and rainfall, all these variables were fitted to simple linear regression models; slopes of regression equations and determination coefficients (R^2) were then examined. To ascertain the impact of weather-related parameters on airborne *Artemisia* pollen counts over the times series, the Spearman non-parametric correlation test was applied to the *Artemisia* Pollen Index and to fortnightly data for maximum and minimum temperature and rainfall. The influence of daily weather conditions (maximum, minimum and average temperature, rainfall, wind speed and calms, wind direction, humidity and hours of sunlight) on daily pollen counts was examined for each individual year in the series, in order to identify behaviour patterns. The IBM SPSS Statistics 20.0 software package was used in both cases.

2.6 Endangered species recovery programmes

Conservation and Recovery Programmes for endangered species in Sierra Nevada were launched in 2001, through a *LIFE* project funded by the EU and the Andalusian Regional Government Department of the Environment. The first phase of the High Peaks Flora Recovery Programme, implemented from 2004 onwards, considered among other techniques the reinforcement of the plant populations by direct sowing and planting and subsequent monitoring of populations with a view to recording data on the reproductive biology of the species concerned (Blanca et al. 2002; Lorite et al. 2002; Algarra et al. 2004). A summary of the sowing and planting operations undertaken for some populations of endangered *Artemisia* species in Sierra Nevada is provided in Table 2, since these operations were assumed to have the greatest impact on the Pollen Index.

3 Results

3.1 Pollen records

Analysis of local annual airborne *Artemisia* pollen dynamics (Fig. 1) revealed two periods of high pollen counts in the course of the year: of these, counts were rather lower in the first period, between July and September, coinciding with the flowering of species growing at high altitudes in Sierra Nevada (*A. granatensis, A. alba subsp. nevadensis, A. absinthium, A. chamaemelifolia,* and *A. umbelliformis*, in chronological order) than in the second period, from October to February, linked to pollen release from species growing at lower altitudes.

Pollen-season characteristics for *Artemisia* species growing in Sierra Nevada are shown in Fig. 2. Although in most years flowering started in late July/early August, pollen was often not detected until the second half of August or even early September. The difference between the earliest and latest start dates was 43 days. The pollen season end-date, however, proved to be more uniform, generally coinciding with the first snowfalls in the last ten days of September. The mean duration of the flowering season was 39 days (maximum 67 days, minimum 7 days). Data for the historical series confirmed a very slight trend towards later start-dates (s=0.206, R^2=0.015, p=0.06), earlier end-dates (s=−0.203, R^2=0.014, p=0.40),

Table 2 Re-introduction actions of plant material (seeds and germinated-plants) of Endangered *Artemisia* species in Sierra Nevada (Granada, Spain)

Year	Taxon	N° of Seeds	N° of Plants	Program
2001	*A. granatensis*		150	LIFE Sierra Nevada
2002	*A. granatensis*		247	LIFE Sierra Nevada
	A. alba. subsp. *nevadensis*	25.000		
2005	*A. alba.* subsp. *nevadensis*	36.940	323	Program of recover of high peaks flora in Andalucia. Phase 1
	A. granatensis		991	
2006	*A. alba.* subsp. *nevadensis*	2.291		Program of recover of high peaks flora in Andalucia. Phase 1
	A. granatensis		46	
	A. umbelliformis		110	
2007	*A. alba.* subsp. *nevadensis*		24	Program of recover of high peaks Flora in Andalucia. Phase 1
	A. granatensis		314	
	A. umbelliformis	200	30	
2010	*A.umbelliformis*		8	Program of Recover of High Peaks Flora in Andalucia. Phase 2

Program of recovery of endangered and critically endangered flora of Western Andalusia Mountains (2008, 2011)

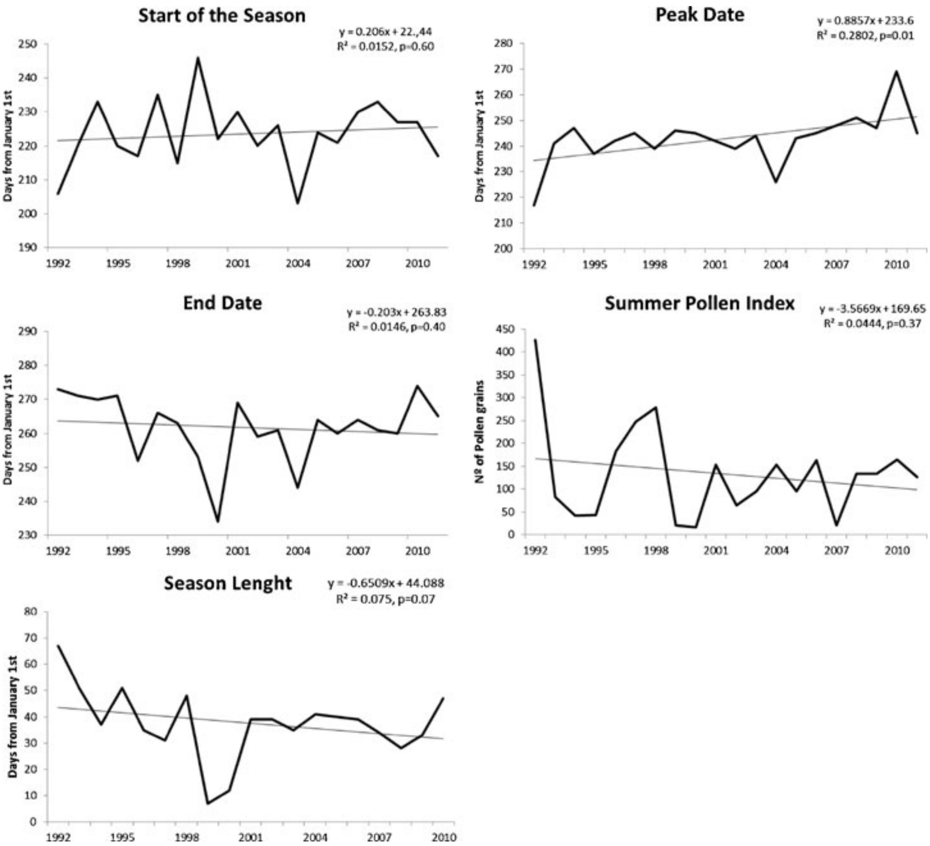

Fig. 2 Variations in pollen-season start-date, end-date and duration, pollen peak date and Pollen Index over the period 1992–2011

and thus an appreciably shorter season (s=−0.650, R^2=0.075, p=0.41). These trends were no significant (p>0.05) but the analysis of data revealed a significant trend towards later peak pollen dates (s=0.885, R^2=0.2802, p=0.01), and a marked decline in the summer Pollen Index (s=−3.556, R^2=0.0444, p=0.37). Also noteworthy was the year-on-year variation in values, ranging from a maximum 426 in 1992 to a minimum 16 in 2000.

3.2 Weather-related parameters

Analysis of seasonal weather data recorded at the La Arquilla station (Fig. 3) showed a gradual, though statistically insignificant, increase in maximum summer temperatures over the study period (s=0.10, R^2=0.009, p>0.05); whilst mean maximum temperatures in autumn, winter and spring tended to decline. Similarly, minimum summer temperatures displayed a significant increase over the period (s=0.11, R^2=0.09, p>0.05), whereas minima for the other three seasons recorded a declining trend; the decrease in minimum winter temperatures was particularly significant (s=−0.16, R^2=0.11, p<0.05).

A marked year-on-year variation in annual rainfall was observed: very wet years (1996, 2003, 2004, 2010) contrasted with years of severe drought (1994, 1995, 1998, 1999 and 2005). Inter-annual differences in seasonal patterns were also apparent (Fig. 3), particularly

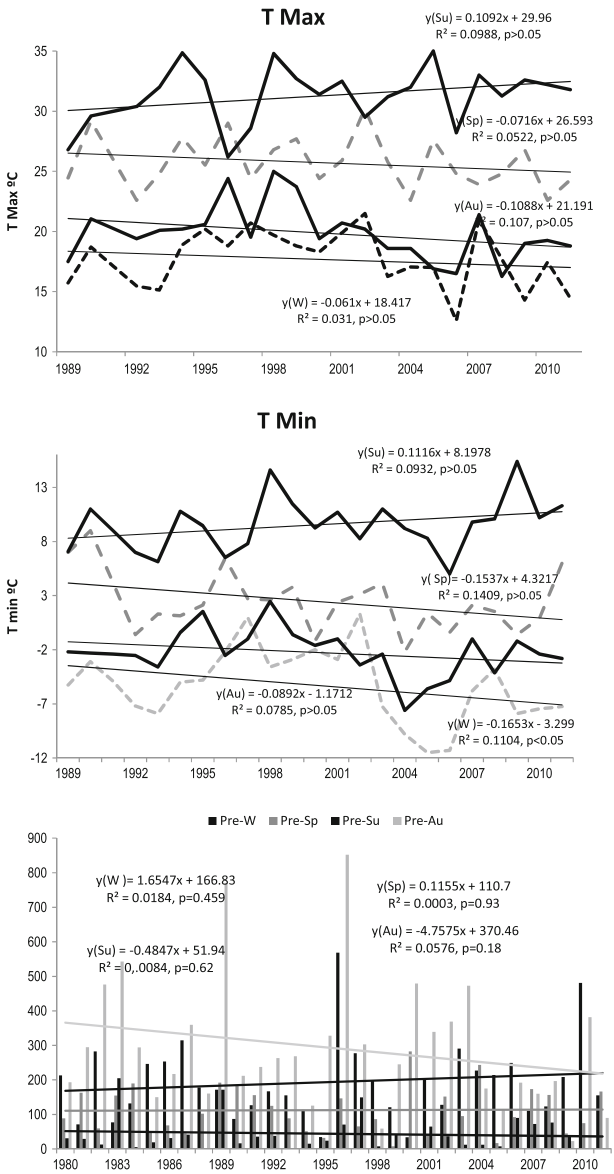

Fig. 3 Linear trends for seasonal maximum and minimum temperatures and rainfall in La Arquilla, (Granada, Spain), over the period 1989–2011 (only data available) for Temperatures (in °C) and 1980–2011 for Precipitations (in mm). (**Su**): Summer; (**Sp**): Spring; (**Au**): Autumn; (**Wi**): Winter

for spring (s=0.11, R^2=0.00, p>0.05) and summer rainfall (s=−0.48, R^2=0.00, p>0.05), which most influences *Artemisia* flowering.

3.3 Correlation between pollen counts and weather-related parameters

Spearman's correlation test confirmed a significant correlation between daily pollen counts and weather-related parameters for the series as a whole (Table 3). The correlation with temperature, wind speed and wind direction proved particularly strong. No significant

Table 3 Spearman Correlation Coefficient between daily meteorological parameters and daily *Artemisia* pollen records during the series of years, 1992–2011 (from mid-July to early October) in Granada city. Significant values in bold

Year	Tmax.	Taver.	Tmin.	Precip.	Wind vel.	Wind Dir.	WDir. 1°Q	WDir. 2°Q	WDir. 3°Q	WDir. 4°Q	Humidity	Sunsh. H.	Calms
1992	–.039	–.071	–.058	–.205	–.202	.231	.347	**.507****	**.443***	.327	.080	.108	.169
1993	.164	.153	.133	.167	**–.371****	–.106	–.172	–.053	–.210	**.306***	.049	.020	–.052
1994	.119	.093	.037	–.164	–.145	–.030	.063	–.137	.231	–.237	.079	–.047	.049
1995	**.375****	**.346****	**.303***	.116	.067	.028	.158	.196	–.238	**.310***	–.252	.216	–.084
1996	**.399****	**.418****	**.316***	–.081	.192	**–.433****	.142	.084	**.388****	–.096	–.167	.266	**–.310***
1997	**–.584****	**.557****	**.381***	**–.526****	**.335***	–.145	.056	**.473****	–.025	–.163	**–.631****	**.530****	–.229
1998	**.388****	**.345***	.213	.077	.096	**–.344****	.244	**.483****	**–.503****	–.180	–.101	–.181	.114
1999	–.388	**–.561***	–.390	.065	.017	.405	–.526	–.224	.043	.293	–.086	–.356	.004
2000	.160	–.090	**.184***	–.307	–.245	–.065	.076	**.324***	.175	.019*	–.240	.202	.167
2001	**.362****	**.353***	.203	–.287	**.382***	**–.335***	**.461****	.282	**.320***	**–.433****	**–.320***	.198	–.214
2002	**.320****	.140	.252	.–250	.126	–.166	–.156	–.041	.126	.–169	**–.357***	.393	.110
2003	**–.642****	**–.504****	**–.631****	.125	.026	.066	–.044	.061	.005	.048	**.495****	**–.439****	.085
2004	–.019	**–.286***	–.141	–.012	.048	–.078	.055	**.449****	**.479****	.050	.242	**–.496****	**–.276***
2005	.170	–.049	.101	.034	.253	.038	–.011	**.327***	**–.201***	–.201	.084	–.014	–.044
2006	.068	–.012	.006	.161	.079	**–.490****	**–.319***	–.081	–.192	**.368***	.258	**–.353***	.487
2007	.148	**.402***	.284	.219	.052	**–.378***	.–125	.147	.147	–.207	.006	–.226	–.520
2008	.090	.301	.175	.075	**.482****	**–.664****	–.279	**.363****	–.057	–.088	–.235	–.167	.025
2009	.062	.270	.171	–.260	–.368	**–.534****	**.568***	**.689****	–.413	**–.578***	–.062	–.160	.429
2010	**.517****	**.537****	**.364****	.156	**.332***	**–.283***	**–.313***	.093	–.030	**.327***	.089	**–.459***	.244
2011	.299	.190	.108	.225	–.119	.237	–.144	.025	**–.294***	–.128	–.398	**–.435***	–.076
All years	**.151****	**.137****	**.071***	–.052	**–.102****	**–.184****	**–.245****	**.237****	**–.137****	**–.129***	–.253	–.070	**–.304***

*=significant values at $p \leq 0.05$. **=significant values at $p \leq 0.1$

correlation was noted with either rainfall or relative humidity, although both had a negative effect on pollen counts. Separate analysis of data for each year revealed a stronger correlation with maximum temperatures and certain wind directions (E, SE, S, SW), and in some years with relative humidity.

With regard to the correlation between the values of summer *Artemisia* Pollen Index and weather-related parameters prior to flowering, expressed as fortnightly means, maximum June temperature and June and July rainfall were the parameters most influencing the summer PI, the correlation being significant in all cases (Table 4).

3.4 Recovery programmes

Application of Spearman's correlation test to data for the summer Pollen Index, following the introduction of measures under the endangered species Recovery Programme, which included the direct sowing of seeds and planting of one-year-old seedlings, revealed a significant correlation between annual Pollen Index and the use of germinated seedlings (0.0623, $p<0.05$). However, no significant correlation was observed with the number of seeds sown (−0.400, $p>0.05$).

4 Discussion

Analysis of a 20-year historical series of *Artemisia* pollen counts registered in Granada city provided useful information on pollen dynamics and on the factors influencing the biological status of *Artemisia sp*. populations growing in the Sierra Nevada.

The flowering period generally lasted from late July to mid-to-late September. Similar findings have been reported for other *Artemisia* species both in Spain (Munuera et al. 1999; Cariñanos et al. 2004, 2010) and elsewhere in Europe (Spieksma et al. 2000; Stach et al. 2007; Peternel et al. 2006). However, given that some *Artemisia* are highly endemic to the Sierra Nevada (*A. granatensis; A. alba* subsp. *nevadensis*), flowering was strongly dependent on local environmental conditions. A mild increase in maximum and minimum summer temperatures over the study period, together with changes in seasonal rainfall patterns, prompted an increasing delay in the start of flowering. The flowering-season end-date also occurred at an increasingly early date. As a result, the flowering season grew shorter over the series. This contrasts with the recent increase in flowering duration reported elsewhere in Europe, where *Artemisia* is represented by other more common species, most of which are early colonisers and better adapted to changing environmental conditions (Stach et al. 2007). By contrast, the present findings are consistent with a possible response to climate change by high-

Table 4 Spearman correlation coefficient between fortnight meteorological parameters previous (May–June) and during the pollen season (July–August) and summer Pollen Index (PI)

Forthnight	Tmax	Tmin	Precipit.
1st-May	–.371	–.282	.042
2nd-May	–.331	–.346	.123
1st-Jun.	–.645**	–.060	.300*
2nd-Jun.	–.449*	–.454*	.236**
1st-Jul.	–.306	–.205	.455**
2nd-Jul.	–.307	–.056	.299**
1st-Aug.	–.121	–.015	–.481*
2nd-Aug.	–.162	–.281	.280

*=significant values at $p\leq0.05$.
**=significant values at $p\leq0.1$

mountain vegetation; a recent study reports a trend towards increasing thermophilization, i.e. an increase in warm-adapted species and a decline in species more dependent on stable cold conditions (Gottfried et al. 2012).

A significant delay in the peak pollen date was also observed: in the early years of the study, the peak pollen count was recorded in August, whilst in the latter years of the series it occurred in September. The pollen peak date indicates the time of optimal flowering for local populations; therefore, a later peak date may be indicative of changes in those climate conditions most influencing flowering, among them temperature and rainfall (Gehrig 2006; Cariñanos et al. 2004; Stach et al. 2007). Unlike other studies, in which various *Artemisia* species have been found to contribute to the airborne pollen spectrum, giving rise to various peaks in the course of the flowering season (Munuera et al. 1999; Kapyla 1981; Cariñanos et al. 2000), the pollen curve in the present study displayed only one season peak, suggesting both a similar environmental response by the various species and a fair degree of phenological homogeneity. After the peak pollen date, the season varied in length from year to year, lasting barely a week in some years and almost a month in others. This generally-short reproductive period is shared by most species in the High Peaks, since it is limited by the summer thaw; for insect-pollinated species, this is the period of greatest pollinator abundance and diversity (Gómez et al. 2007).

Variations in the summer PI over the series are shown in Fig. 2, which illustrates a sharp drop in overall values together with considerable year-on-year variations. Analysis of the series as a whole enabled identification of fairly regular drought cycles: 1994–1995 (a particularly severe drought), 1999–2000, 2003 and 2005–2006. Summer PI values in these drought years were the lowest for the whole series, with total counts as low as 21 and 16 grains in 1999 and 2000, respectively, when no rain fell over the 8 weeks prior to the start of flowering (Fig. 3). The situation in 2003 is especially noteworthy: a record-breaking heat wave over most of Europe prompted major changes in habitual pollen seasons (Gehrig 2006). This gave rise to very low daily *Artemisia* counts over most of the season, and to a delayed peak pollen date (10 September). At the same time, however, it led to a significant correlation between daily pollen counts and maximum, mean and minimum temperature (Table 3), thus confirming the major influence of temperature on the flowering of *Artemisia* species in the cryo-Mediterranean belt of Sierra Nevada.

Another weather-related parameter affecting daily *Artemisia* pollen counts was wind direction, particularly given the location of the sampling station 30 km west of the study populations. The wind regime in the city of Granada is marked by a high percentage of calms (over 50 % of the year) and predominantly south-westerly winds, veering south in July and August (Viedma Muñoz 1998). The valley-mountain breeze flows west–east during the daytime, but is reversed at night. Some authors argue that during the time that the *Artemisia* pollen grain is airborne, estimated at 14 h on the basis of Stokes' Law (Fuchs 1964), pollen may be transported no further than 600 m from its source, thus hindering pollen transfer between isolated, distant populations (Peñas et al. 2011). However, other researchers have highlighted the strong influence of wind on airborne *Artemisia* counts, and noted that a certain amount of pollen may be transported long distances from the source (Stach et al. 2007; Wahl and Puls 1989; Spieksma et al. 2000). This is borne out by a statistically-significant correlation between *Artemisia* pollen counts and both wind speed and predominant wind direction in summer, and also by the recording of airborne pollen types from other summer-flowering species growing in the Sierra Nevada (Alba 1997).

Spearman correlation coefficients for the annual values of summer Pollen Index and fortnightly means for the weather-related parameters most influencing pollen counts (Table 4) highlight the major role of rainfall. Like other herbaceous species, *Artemisia* species respond rapidly to water input immediately prior to flowering (Cariñanos et al. 2004; Recio et al. 2009; Garcia-Mozo et al. 2010). For that reason, the highest summer PIs were recorded in years marked by heavy rainfall in June and July: 1992 (PI=426; June rainfall: 106.5 mm; July rainfall: 38 mm), 1997 (247; 37.4 mm; 97.5 mm), 1998 (279: 37.6 mm; 5 mm) and 2007 (201; 156.1 mm; 62 mm). This may also be linked to the chamaephyte and caespitose life form of *A. granatensis* and *A. umbelliformis*, which favours storage of rainwater for subsequent use during flowering. In some years, despite sufficient rainfall in late spring and early summer (e.g. 2004, with June rainfall: 243 mm; July rainfall: 10 mm), a high PI was not recorded (summer PI 2004: 153) due to the exceptional number of frosts recorded in May, which delayed snowmelt and thus prompted water stress, since available water remained frozen (Vostral and Boyce 2000).

Analysis carried out prior to the implementation of High Peaks Endangered Flora Recovery Programmes highlighted the elevated germination rate recorded for *Artemisia alba* subsp. *nevadensis* and *A. umbelliformis* seeds, which in both cases exceeded 20 % and for *A. umbelliformis* sometimes approached 90 % (Lorite et al. 2007). At the same time, *A. granatensis* seeds were classified into three types, as a function of weight and turgidity, each type displaying a different germination response (Clemente et al. 1991). An earlier study had noted that 1 year after the introduction of *A. granatensis* plants at two sites, mortality in one of them was as low as 30 % (Saenz-Ollero and Hernández-Bermejo 1979). These data provide a framework of reference for interpreting the findings of the present study, in which analysis of non-parametric correlations between the Pollen Index and reintroduced plant material (seeds and seedlings) revealed a significant correlation between PI and seedlings, even though the number of seeds was considerably higher. This may be because, although a high seed-germination rate was recorded, trials were carried out in the laboratory under controlled propagation conditions, thus minimising the impact of environmental factors under natural conditions (Lorite et al. 2007). For example, two of the years in which larger numbers of seeds were introduced (2005 and 2006) were declared drought years, with rainfall considerably below average. Moreover, in 2006 maximum and minimum summer temperatures were the lowest for the historical series, which may also have constrained germination. One striking finding was that following the introduction of recovery measures for endangered species, including the strengthening of existing populations, there was a slight upturn in the annual PI, which also displayed greater stability (Fig. 2). Coupled with this, the progressive reduction in nitrophilous *Artemisia* species (*A. campestris, A. absinthium*) in areas where land had been recovered and original species reintroduced (Diaz-Rodriguez 2012), probably led to a decline in the contribution of nitrophilous species to the *Artemisia* pollen spectrum and thus to a greater proportion of pollen from endangered endemic species.

The planting of germinated seedlings may have had advantages over direct seeding, and may also have had a more marked effect on the Pollen Index. Most *Artemisia* species are perennial or biannual (Vallés et al. 2003), which would favour at least 2 or 3 flowering seasons after reintroduction. Analysis of pollen counts for the year seedlings were introduced and the two following years revealed an increase in counts around 2 years after seedling introduction (Table 2, Fig. 2), i.e. in 2004 for *A. granatensis* reintroduced in 2001 and 2002; in 2007 for all three species reintroduced between 2005 and 2006, and in later years for those reintroduced in 2007. It is observed that summer PI in 2007 it was very under if is compared with the number of seedlings that were introduced in the previous years (2005 and 2006). This is due to have used pollen counts registered in Granada city, which causes that the wind direction is a factor very important to consider for the airborne pollen coming from the mountain, which has been verified by means of correlation of daily values

(Table 3): there was no significant correlation with the favourable wind (second quadrant) in 2007 whereas it was positive and significant in 2004, 2008 and 2009. It may also be significant that the seedlings used to strengthen existing populations were produced from material drawn from different populations, ensuring greater genetic variability and greater reproductive vigour, this being essential for the survival of critically-endangered populations (Martín et al. 1998; McClanahan 1986).

5 Conclusions

The following conclusions may be drawn from the findings of this study:

1- Pollen counts provide a useful biological indicator of the reproductive status of the most endangered *Artemisia* populations in Sierra Nevada.
2- Weather conditions prior to flowering, particularly temperature and rainfall, should be borne in mind, since they strongly influence pollen counts and hence population growth of these species.
3- The reintroduction of germinated seedlings is one of the most effective measures implemented under the Endangered Species Recovery Programme, since the perennial nature of most of the populations concerned, and the better adaptation of plants than seeds to environmental conditions, favour flowering in the years following reintroduction, increasing pollen release and thus enhancing seed production.

The results obtained here, in short, highlight the value of pollen counts as a means of evaluating the status of endangered species, and as the basis for specific measures aimed at conservation: directed pollination, reinforcement of male plants in dioecious populations, and the detection of sterility issues.

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