


Regeneration of submediterranean species *Euonymus latifolius* (L.) Mill. at its southernmost limit in Europe

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Abstract. Due to the ongoing effects of climate change in the Mediterranean (increasing temperature and decreasing rainfall), conditions for Submediterranean species are disappearing as their habitats are dwindling. We have focused on *Euonymus latifolius* (L.) Mill., a nemoral-Submediterranean species, at its southernmost populations of Europe. The aim was to evaluate the population structure and regeneration niche of the species at microhabitat scale. We selected five larger populations among the 13 existing ones, marking 25-30 individuals per population. We measured twice: height, width, vegetation cover, survival, soil moisture and temperature.

As result, we provided data of the 13 existing populations, containing 93 adults and 350 juveniles. Moreover, we have obtained a very skew population structure with a low number of recruits for five selected populations, especially at smaller populations. Most *E. latifolius* juveniles were encountered under dense tree canopy (more than 80 % in cover) formed by a mixture of Submediterranean and Mediterranean species. Biovolume per population showed significant differences among the main populations CP and CV, presenting this last a higher recruitment, while recruitment was very low in general. For soil parameters, we found a critical role of canopy, which showed a positive effect on juvenile microhabitat (higher moisture and lower soil temperature).

The results showed us the critical situation of the species, with very fragmented populations, low number of individuals, and scattered spatial patterns of individuals within the populations. Also, survival problems, a non-balance demographic structure, and regeneration problems were detected. Finally, we propose a serie of conservation measures, from monitoring to active measures (key tree species plantation, reintroduction, reinforcements), combined with threat control (herbivory, pests, and impact from outdoor activities). All combined may help to preserve this species at its southernmost populations.

Keywords: Submediterranean; climatic change; *Euonymus latifolius*; regeneration; survival; Cazorla-Segura massif.

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Introduction

Geographic distribution of plant species is strongly conditioned by different climatic features such as rainfall, soil moisture, temperature, solar radiation, etc. (Blasi *et al.*, 1999). Mediterranean is a bi-seasonal climate type in terms of temperature and precipitation, characterize by cool-wet winters with low solar irradiance and hot-dry summers with high solar irradiance. It may occur on the west side of continents between 30° and 40° latitude, affected by a large water mass (i.e., Mediterranean Sea) (Lionello *et al.*, 2006). Hence the typical Mediterranean plant species have to cope with these particular features (Davis & Richardson, 1995).

Many authors have recognized the so-called “nemoral-submediterranean floristic subregion” within the Mediterranean region. It is transitional between the Mediterranean and Eurosiberian regions of Europe (Sánchez de Dios *et al.*, 2009). It covers northern parts of peninsulas or other areas with high mountains (Meusel & Jäger, 1989; Larcher, 2000). Therefore, climatic characteristics are intermediate between those typical to Mediterranean and Temperate zones, which

determine this subregion (Ozenda, 1994). Accordingly, the characteristic flora is an admixture of species coming from the two climatic-phytogeographical areas and some endemic species (Bolòs, 1985; Morla & Pineda, 1985; Moreno *et al.*, 1990). Comparatively cooler and wetter conditions characterize typical summer to this sub-region. Therefore, broad-leaved deciduous species are characteristic of this transitional area (Bolòs, 1985; Ozenda, 1994). These nemoral-submediterranean plant species are also remnants from forest vegetation of warmer and wetter periods of the Middle and Late Tertiary, becoming relict niches in the last millennia (Meusel & Jäger, 1989). Currently, there are good examples of this mixture of species, such as *Euonymus latifolius* (L.) Mill., *Acer monpessulanum* L., *Fagus sylvatica* L., *Castanea sativa* Mill., or *Corylus avellana* L.

Global climate is changing worldwide and it is projected that continuously change along this century by means of anthropogenic increases in greenhouse gases, increasing global mean temperature, changing the world's hydrological cycle, increasing in frequency and severity of extreme droughts, hot extremes and

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heatwaves (Sterl *et al.*, 2008; Hardwick *et al.*, 2010). Under this global climate change scenario, models predict especially severe changes in Mediterranean mountains (Nogués-Bravo *et al.*, 2007), causing an outstanding increase in temperatures and decreased rainfall. Therefore, the suitable niches for this submediterranean species (Gosz, 1992; Lionello *et al.*, 2006) and their habitats (Río & Penas, 2006) are shrinking. Also, the synergistic effects of this climate change with other anthropogenic threats factors caused by the land use changes could jeopardize these species (Allen *et al.*, 2010; McIntyre *et al.*, 1999) and could lead to the loss of many taxa and plant communities (Petit *et al.*, 2005). An outstanding threat factor is overgrazing by domestic wild herbivores or livestock, affecting vegetation, soil, and hydrology (Ibáñez *et al.*, 2007; Kairis *et al.*, 2015). The excessive stocking rates combined with unfavorable rainfall conditions could reduce plant cover and a decrease in plant diversity (Heady & Child, 1994).

For these reasons, it is of paramount importance to understand the conservation and regeneration of these submediterranean plant species. Managing and monitoring selected populations in their natural habitats can help maintain evolutionary processes, thus allowing new variation to be generated in the local gene pool to allow the species to adapt to changing environmental conditions (Heywood, 2014). *Euonymus latifolius* (L.) Mill. constitutes a good example of these Submediterranean species and their conservation issues. To address this issue, we study this Submediterranean-relict species at its southernmost limit in Europe (Cazorla-Segura, Baetic mountain range). Therefore, the aim is to evaluate the population structure and regeneration niche of the species at microhabitat scale.

Material and Methods

Study site

The Sierras de Cazorla-Segura massif are located in the northern part of the Baetic Mountain System, in the southeastern Iberian Peninsula (37°52'1.32" N, 3°2'12.35" W). It constitutes the largest protected area in Spain, covering 209921 ha. The climatic regime is typically Mediterranean characterized by a hard summer drought, changing precipitation among years and within them. The average rainfall is about 1100 mm/year (ranging from 400 to 1900 mm), November and April being the wettest months, and July and August the driest ones. Average temperature is 11.7°C, with minima in January (4°C) and maxima in August (21°C). Lithology consists mainly of limestone and dolomites (Vera, 1994). A craggy topography characterizes these mountains, ranging from 500 to 2107 m asl at the highest point (Empanadas peak). The vegetation is composed of a mixture of pine forests (*Pinus halepensis* Mill., *P. pinaster* Ait., and *P. nigra* subsp. *salzmannii* (Dunal) Franco) with broad lived perennial or deciduous oaks such as *Quercus ilex* subsp. *ballota* (Desf.) Samp. and

Q. faginea Lam. The total amount of vascular plants accounted for the area is 2,200, with 360 endemics to the Baetic-Rifan complex, 35 of them being narrow endemics of these mountains (Benavente, 2005). This mountain range has typically been overgrazed by domestic and wild ungulates (García-González & Cuartas, 1989; Soriguer *et al.*, 2003), affecting significantly the structure, composition, and regeneration of the vegetation (Tíscar-Oliver, 2015). It is the main threat factor in the area acting synergistically with other threat factors such as climatic change, land use changes, or forestry (Lorite *et al.*, 2007; Heywood, 2014).

Studied species

Euonymus latifolius (L.) Mill. (*Celastraceae*), locally named as “Bonetero de hoja ancha” or “Bonetero de Cazorla”, is a deciduous shrub about 2-3 m high (rarely reaching 7 m) (García & Sánchez, 2007). Leaves are opposite, varying from obovate to elliptic, acuminate and serrulate. Flowers whitish to greenish with 5(4) petals arranged in a cymose inflorescence 5-10(15) flowered. Capsular fruit is bright-red when ripe, forming five lobes and provided with a wing on the back; seeds surrounded by reddish aril (Gutiérrez *et al.*, 2003). Flowers are pollinated by dipterous (*Calliphoridae*, *Muscidae*, and *Tachinidae* families) from May to June and fruit ripening occurs in September (Blanca *et al.*, 1999).

It is distributed throughout the European continent (Figure 1), from the Iberian Peninsula to the Caucasus, reaching the Irano-Turanian region and North Africa, in the Middle Atlas, Tell Atlas and Kabila (Benedí, 1997). Despite its wide distribution, it is very rare in Iberian Peninsula. It appears in only two relatively distant metapopulations: 1) Cazorla-Segura metapopulation (here studied, Table 1): Consisting of 13 population, with 93 mature individuals (modified from Nieto-Ojeda & Benavente, 1992), and 2) Eastern Spain metapopulation: Consisting of 10 populations along the Eastern mountain ranges of Spain: 5 populations in Cuenca (González *et al.*, 2001; Gómez-Serrano & Mayoral, 2005; Pinedo *et al.*, 2004; García & Sánchez, 2007), 2 populations in Teruel (Mateo & Lozano, 2005; Mateo *et al.*, 2013), one in Guadalajara (García-Muñoz & Martínez, 2018), 1 population in Castellón, and 1 population in Valencia (Fabregat *et al.*, 2013). All of them accounting some 220 mature individuals.

Species inhabit shady areas between 720-1900 m asl, close to mountain water-courses and usually associated with rocky walls and cracks on limestone with permanent moisture (Gutiérrez *et al.*, 2003).

The species has been evaluated as Critically Endangered (CR) in Spain (Gutiérrez *et al.*, 2003), being overgrazing, forest fires, drought, and climate change. Therefore, it increases in average temperatures as the main threat factors. They act synergistically with inherent problems to existing populations, such as reduced geographical distribution, small occupation area, population isolation, and a low number of sparse adult-reproductive individuals (Peña *et al.*, 2018).

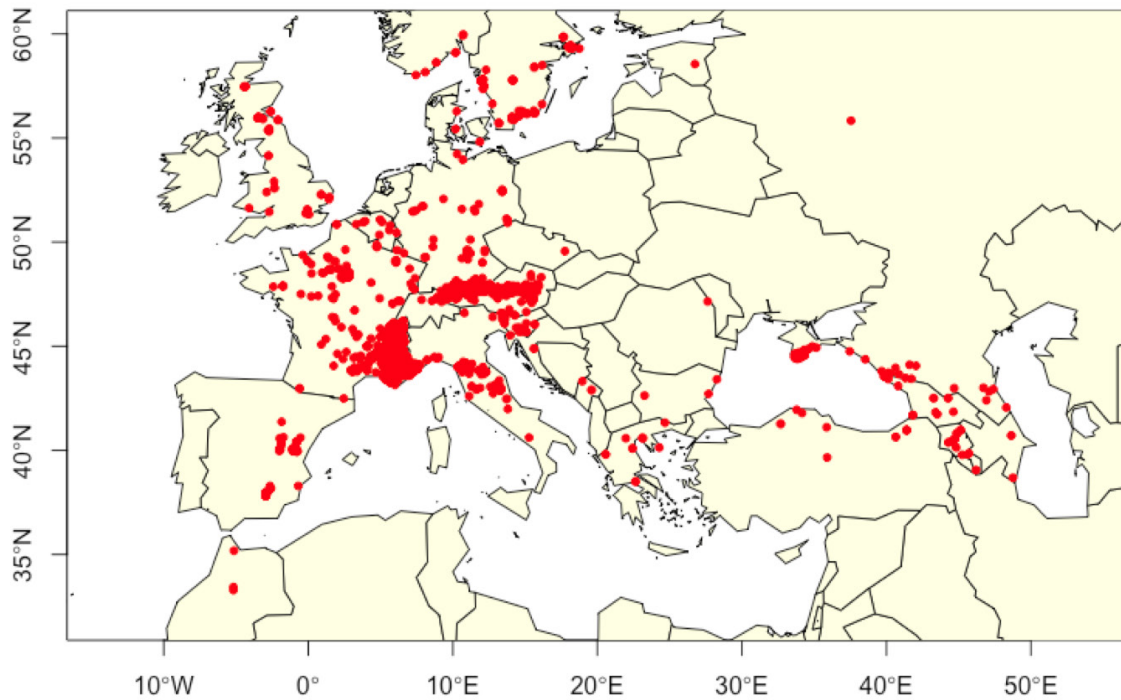


Figure 1. Distribution of *Euonymus latifolius* (L.) Mill. (Occurrences from GBIF, accessed 30/07/2020. Map was performed using R “maptools” library; Bivand & Lewin-Koh, 2020). Note: Map includes introduced populations (both as ornamental and invasive) in Centre and Northern Europe.

Table 1. Main features of *Euonymus latifolius* populations in Sierras de Cazorla-Segura massif (partially taken from FAME; Mateos *et al.*, 2010). Ab., Abbreviations used in the text for studied populations; Alt., altitude; Ad., Jv, Number of adult and juvenile individuals per population (Source: FAME, except for populations here studied); Mk., Marked individuals per 5 studied populations; Manag., Management measures taken: a, monitoring, b, fencing, c, reintroduction in suitable habitat.

Population	Ab.	Habitat	Alt.	Ad.	Jv.	Mk.	Species threats	Habitat threats	Manag.
Arroyo Gualay		<i>P. nigra</i> forest	1479	2	4		Low ecological plasticity	Grazing (wild)	a,b
Arroyo Sabuco		<i>P. nigra</i> forest	720	7	6		Parasitism, pests, diseases	Grazing (domestic)	a,b
Tornillos de Gualay		Grikes on limestone pavement (=alvar)	1100	3	0		Grazing (wild)	Outdoor recreation activities	a
Barranco de las Iglesias		<i>P. nigra</i> forest	1344	2	2		Landslide	Landslide	a,b
Cabañas	PC	<i>P. nigra</i> forest	1840	19	15	6	Natural competition	Trampling and artificialization	a,b
Cerrada del Pintor	CP	Willow and poplar forest	1496	5	73	69	Grazing (wild)	Trampling and artificialization	a,b
Coto del Valle	CV	Willow and poplar forest	870	21	73	73	Poor reproductive capacity	Grazing (wild)	a
Despierna Caballos		<i>P. nigra</i> forest	1565	27	0		Poor reproductive capacity	Landslide	a
Estrecho de los Perales	EP	<i>P. nigra</i> forest	1344	1	15	13	Low ecological plasticity	Grazing (wild)	a,b

Population	Ab.	Habitat	Alt.	Ad.	Jv.	Mk.	Species threats	Habitat threats	Manag.
Hoya del Tostón_1	HT	<i>P. pinaster</i> - <i>Juniperus sabina</i> forest	1740	1	5	5	Poor reproductive capacity	Trampling and artificialization	a,b
Hoya del Tostón_2		<i>P. nigra</i> forest	1740	2	0		Grazing (wild)	Natural competition	a,b
Linarejos		Willow and poplar forest	1062	2	1		Outdoor recreation activities	Outdoor recreation activities	a
Cueva del Horno		<i>P. nigra</i> forest	1556	0	160		Grazing (wild)	Grazing (wild)	a,b,c
Tejerina		Holm-oak (<i>Quercus ilex</i>) forest	730	1	0		Grazing (wild)	Grazing (wild)	a,b

One of the main intrinsic problems is the low reproductive success due to a low production of flowers and fruits (around 20% of the flowers), a low natural production of seeds and dispersive limitations (for frugivores, mainly the *Erithacus rubecula*, Blanca *et al.*, 1999). However, the seeds have proved to be viable even in populations with only one mature individual (Blanca *et al.*, 1999). Juveniles and seedlings are very vulnerable to browsing by domestic and wild herbivores, which also affects seedlings. There have also been attacks of some lepidopteran pests, in the populations of Cuenca (Gómez-Serrano & Mayoral, 2005), in addition to the infection by the *Euonymus* scale (*Unaspis euonymi* Comstock, *Diaspididae*; Salisbury *et al.*, 2013), typical of *Euonymus japonicus* Thunb. (cultivated as ornamental) in the study area (García *et al.*, 2017). Other threat factors are the reduced geographical distribution and population isolation (which affects their genetic diversity), together with the reduction of suitable habitats (Rivera *et al.*, 2018). An increasing outdoor recreation activity pressure would also be detrimental for the species in some populations (Gutiérrez *et al.*, 2003; García *et al.*, 2017).

Data collection

Data collection was carried out during summer 2019. Five larger populations were selected among the 13 existing in Cazorla-Segura massif, trying to get more than 25–30 individuals (adults, juveniles, and saplings) whenever possible (Table 1). Juvenile (including seedlings and saplings) individuals were tagged under the canopy or in the vicinity of 5 adults (i.e., reproductive) individuals in each population (whenever possible). For each of the tagged individuals, we measure twice (July and September 2019) the following features: 1) height, 2) width (mean diameter), 3) vegetation cover: recorded in percentage, as a fraction of canopy cover of 4 transects of 1 m (N, S, E, and W placed) around each marked individuals (saplings and juveniles), 4) Soil moisture and temperature: volumetric water content and temperature were taken by means of a TDR (HH2 Moisture Meter, Delta-T Devices) for each marked plant.

Data analysis

Statistical analyses were performed using R version 3.4.3, R Core Development Team, 2017. To address differences in cover, biovolume, temperature, and moisture, we performed permutational ANOVAs through the *lmer* R package (Wheeler *et al.*, 2016), a flexible and robust analysis that could cope with heteroscedasticity and a wide variety of statistical distributions. Pairwise multiple comparisons were made by applying the posthoc Tukey test after the permutational ANOVAs, using the R “multcomp” package (Hothorn *et al.*, 2008), to estimate differences among the studied populations for assessed variables (cover, biovolume, temperature, and moisture). For graphs included, we used *ggplot2* package (Wickham, 2009).

Results

Population features

According to the available data, we obtained 13 populations (12 spontaneous and one from a reintroduction) containing 93 adults and 350 juvenile (including seedlings and saplings; Table 1). The main populations were CV (21 adult and 73 juvenile), Despierna Caballos (27 adults) and Cabañas (19 adult and 15 juvenile). CP population presented only five adult individuals, though it showed the highest regeneration (73 juvenile). The altitude of populations ranged from 720 (Arroyo Sabuco population) to 1840 m asl (Cabañas), being the majority at 1200 m asl. Individuals occupied shady habitats mainly cover by riverside woodlands (especially larger populations), *Pinus nigra* forest, or mixed *Quercus faginea* and *Q. ilex* forests (Table 1). All the populations were small in terms of Occupation Area, being Despierna Caballos population the larger with some 4.6 ha, and always with very sparse individual distribution within the population patches (Author’s unpublished data). The main threats for species were grazing, usually due to wild ungulates (mountain goat, deer, and European mouflon), poor reproductive capacity, or low ecological plasticity.

Meanwhile, the *Euonymus* scale (*Unaspis euonymi*) and outdoor recreation activities were the main threats for the species in particular populations (Arroyo Sabuco and Linarejos, respectively). Main management activities with the species were monitoring, fencing (10 patches) and reintroduction (160 individuals in suitable habitat; Cueva del Horno population).

For the five populations studied, we tagged 166 individuals (juvenile, sapling, and seedling, Table 1). We obtained the following results:

Population structure and survival

Population structure (in biovolume classes) markedly varied among populations (Figure 2), presenting CP

and CV a clearly, higher recruitment and thus showing more individuals in the first two biovolume classes (0-2500 cm³). The rest of the scarce individuals were placed predominantly in upper biovolume classes (>2500 cm³).

Overall survival of marked individuals was 15.97 % after the summer, being evenly distributed among the populations (Figure 3) and accounting for no dead individuals in the smaller populations (HT and PC). We have taken into account the identity of species that formed the canopy of the individual (Figure 4). We did not find differences for most species in terms of dead vs. alive individuals. Except for *Pinus nigra* (significantly higher survival under its canopy) and *Viburnum tinus* (significantly lower survival).

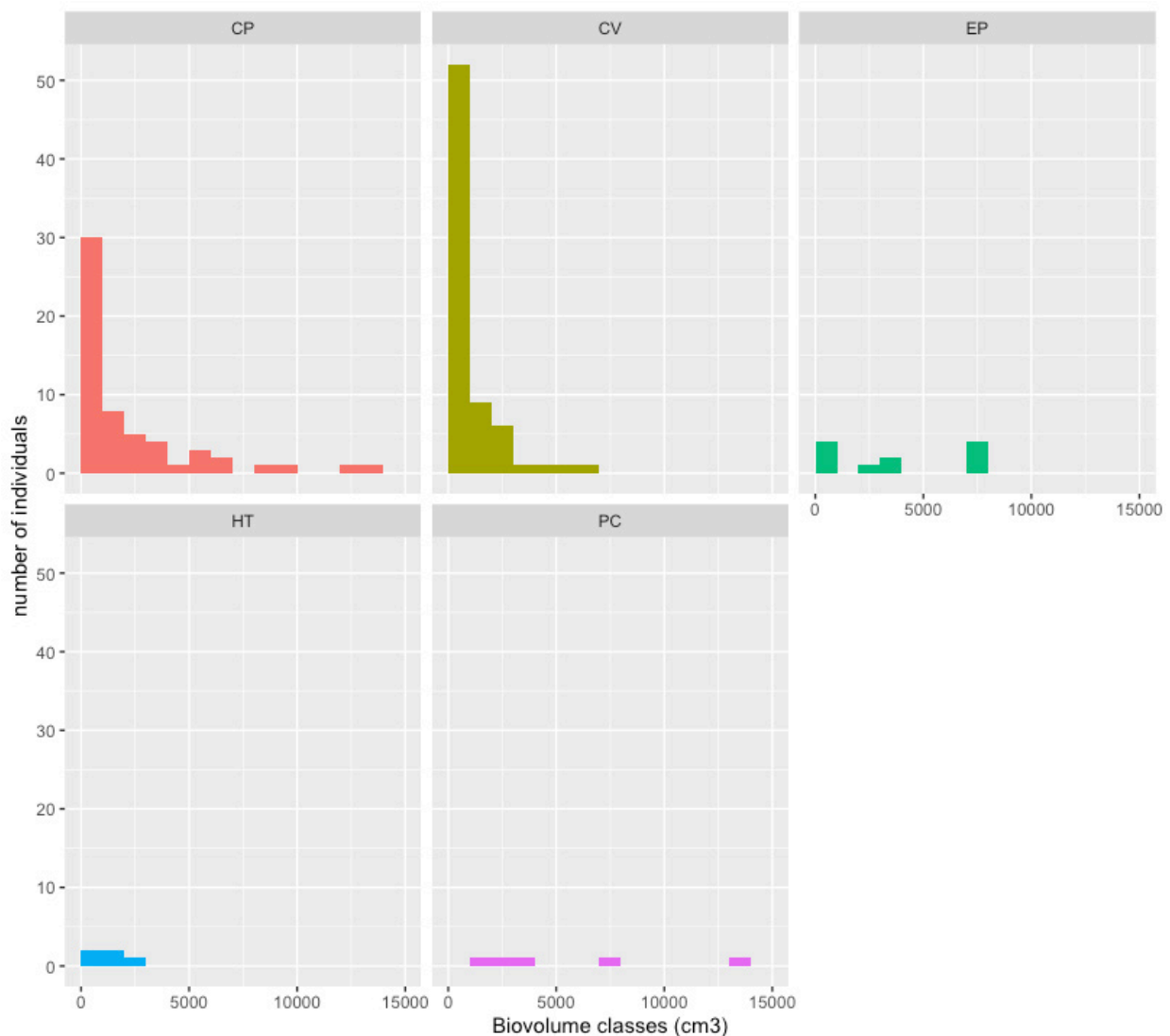


Figure 2. Structure of the studied population in biovolume classes (cm³). Populations: CP, Cerrada del pintor; CV, Coto del Valle; EP, Estrecho de los Perales; HT, Hoya del Tostón; PC, Pico Cabañas.

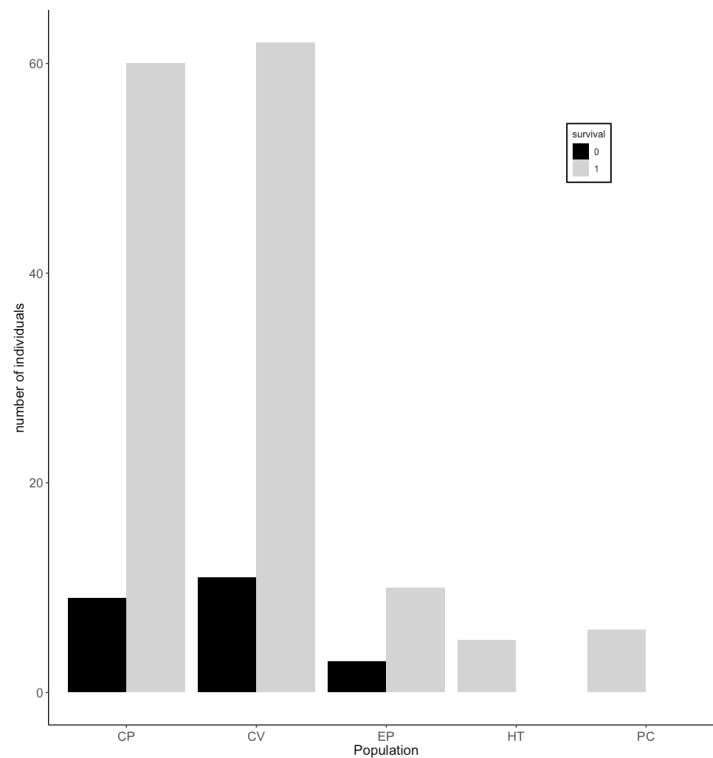


Figure 3. Survival of studied individual per population after summer 2019 (survival individuals in grey, dead individuals in black). Populations: CP, Cerrada del pintor; CV, Coto del Valle; EP, Estrecho de los Perales; HT, Hoya del Tostón; PC, Pico Cabañas.

Population features (vegetation cover, biovolume, moisture, and soil temperature)

Most *E. latifolius* juveniles were encountered in the microhabitat formed by the canopy of *E. latifolius* adult individual, *Acer opalus* Mill. subsp. *granatense* (Boiss.) FQ & Rothm., *Crataegus monogyna* Jacq., *Daphne laureola* L., or *Quercus faginea* Lam (Figure 4). Vegetation cover (Figure 5A) was very high in most of the populations, ranging from 90.96 ± 1.67 (CV) and 50.83 ± 9.70 (PC). Two different groups were obtained for this variable, CV, CP and EP with more than 80% and HT and PC with around 50%. As a surrogate of population structure, biovolume per population (Figure 5B) showed only significant differences among the main populations, CP and CV, presenting this last higher recruitment. The rest of the populations showed intermediate values.

Soil moisture (Figure 4C) was low in general, ranging from 5.88 ± 1.33 % (PC population) to 2.91 ± 0.19 % (CP). Being these differences significantly higher in CV (larger population) and PC (the highest in altitude) in comparison with the other 3 studied populations (CP, EP, and HT). Soil temperature in each population (Figure 4D) was the highest in CV (25.15 ± 2.38 °C) and lowest in PC (19.22 ± 0.33 °C). It was significantly higher for two populations (CV and EP) to PC, while CP and HT showed intermediate values. These values were related to the altitude and the canopy cover, which positively affected juvenile microhabitat (higher moisture and lower soil temperature).

Discussion

Under the present climatic change scenario, submediterranean conditions become rarer within Mediterranean mountains (Sánchez de Dios *et al.*, 2009). Consequently, the suitable habitat for typical submediterranean species is becoming scarcer as well, and *E. latifolius* is a paradigmatic example of this phenomenon. Hence, species present a critical situation in the area (Gutiérrez *et al.*, 2003) with very fragmented populations, a low number of individuals per population, and a sparse spatial pattern of the individuals within the populations. Also, most of the population have a low number of reproductive individuals, clearly under the Minimum Viable Population (MVP, an estimate of the minimum number of individuals needed for the population to survive for a given period of time with a specified probability of persistence; Flather *et al.*, 2011) even in data-deficient situations, has prompted researchers to ask whether general guidelines could replace individual estimates of extinction risk. To inform conservation policy, recent studies have revived the concept of the minimum viable population (MVP). Therefore, most of the populations could go extinct in the near future, unless they increase in the number of individuals via natural regeneration or population reinforcement. In this sense, even in the smaller populations, we have encountered some recruits. Although, these recruits may suffer an inbreeding depression problem (Ellstrand & Elam, 1993).

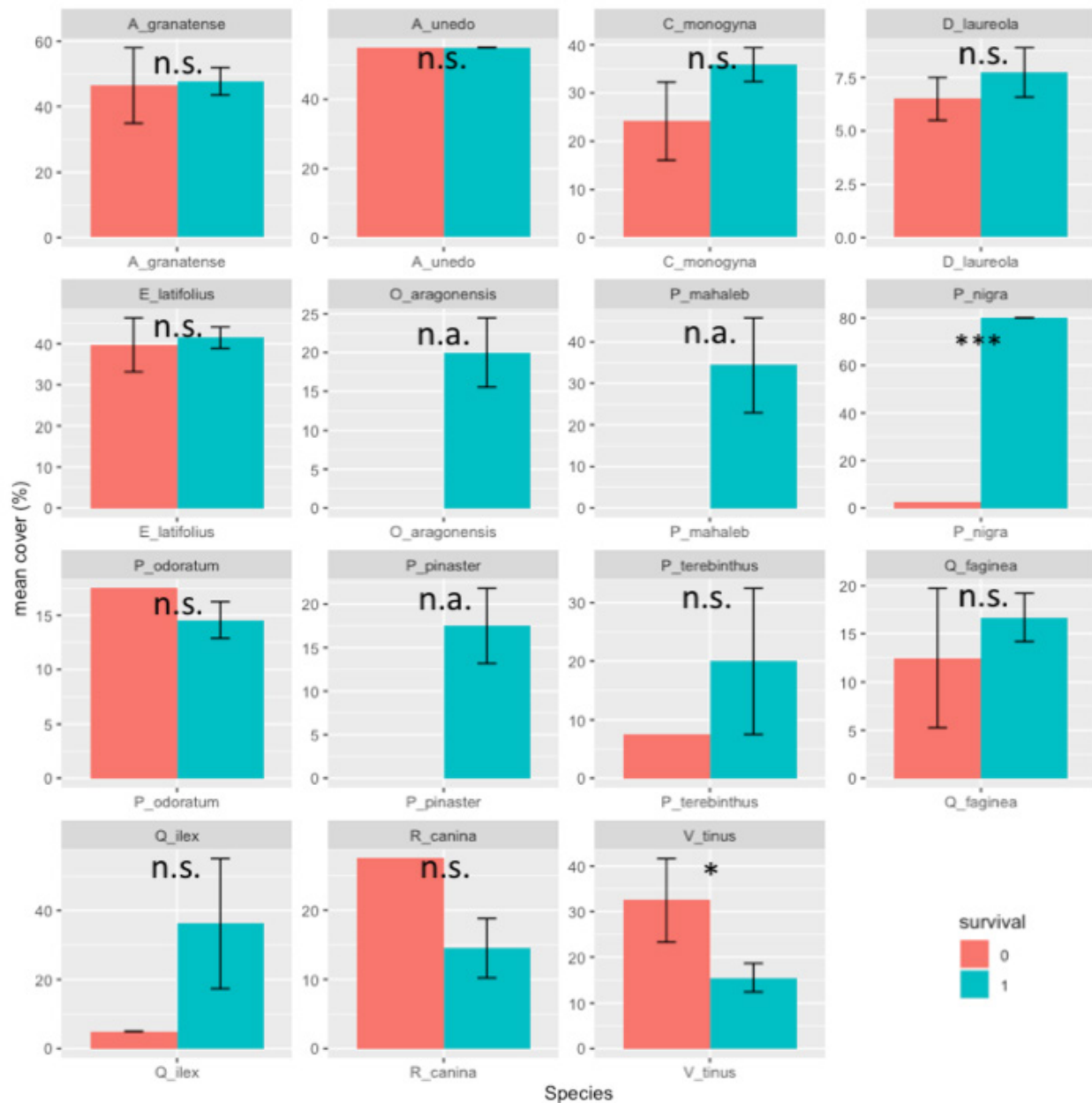


Figure 4. Comparison of canopy cover per species (mean±SE) for dead (0) and surviving (1) *E. latifolius* individuals. Only 11 most frequent species included. Abbreviations: n.s., no significant; n.a., not accounted; *, significant differences $p < 0.05$; ***, significant differences $p < 0.0001$. Species: A_granatense, *Acer opalus* subsp. *granatense*; A_unedo, *Arbutus unedo*; C_monogyna, *Crataegus monogyna*; D_laureola, *Daphne laureola*; E_latifolius, *Euonymus latifolius*; O_aragonensis, *Ononis aragonensis*; P_mahaleb, *Prunus mahaleb*; P_nigra, *Pinus nigra*; P_odoratum, *Polygonatum odoratum*; P_pinaster, *Pinus pinaster*; P_terebinthus, *Pistacia terebinthus*; Q_faginea, *Quercus faginea*; Q_ilex, *Quercus ilex* subsp. *ballota*; R_canina, *Rosa canina*; V_tinus, *Viburnum tinus*.

According to our results, a dense canopy cover is crucial for the species recruitment, coinciding with the findings in the very closely related *Euonymus europaeus* (Thomas *et al.*, 2011) habitat, communities, responses to biotic factors, responses to environment, structure and physiology, phenology, floral and seed characters, herbivores and disease, history, and conservation. *Euonymus europaeus* (Spindle tree, and usually associated with other deciduous or semideciduous species, such as *Acer opalus* subsp. *granatense* or *Quercus faginea* and willow and poplar riverside woodlands, where the species find suitable

environmental conditions. Some small populations with no recruitment showed a canopy cover of around 50 %, suggesting this could be a limiting factor for species' recruitment.

Grazing, especially from wild ungulates, is among the main threats for most populations, both for species and habitat. Overgrazing by domestic and wild ungulates (García-González & Cuartas, 1989; Soriguer *et al.*, 2003), affecting significantly the structure, composition and regeneration of the vegetation (Tíscar-Oliver, 2015). It was also the main threat factor in many endangered species and acted synergistically with other threat factors

(Lorite *et al.*, 2007). Another anthropogenic threat factor detected in some populations was outdoor recreation activities impact. This effect produces trampling and artificialization. It is a growing problem worldwide affecting many ecosystems (Liddle, 1991).

Euonymus scale (*Unaspis euonymi*) was localized at one population at c. 720 m asl. It could be related

to the higher environmental stress in the species' lower limit. This pest has proved to be very detrimental in many *Euonymus latifolius* communities across its distribution area (Salisbury *et al.*, 2013). Hence, this can act as an emergent disease in the study area, though the fragmented and sparse distribution of the species is not favorable for its spreading.

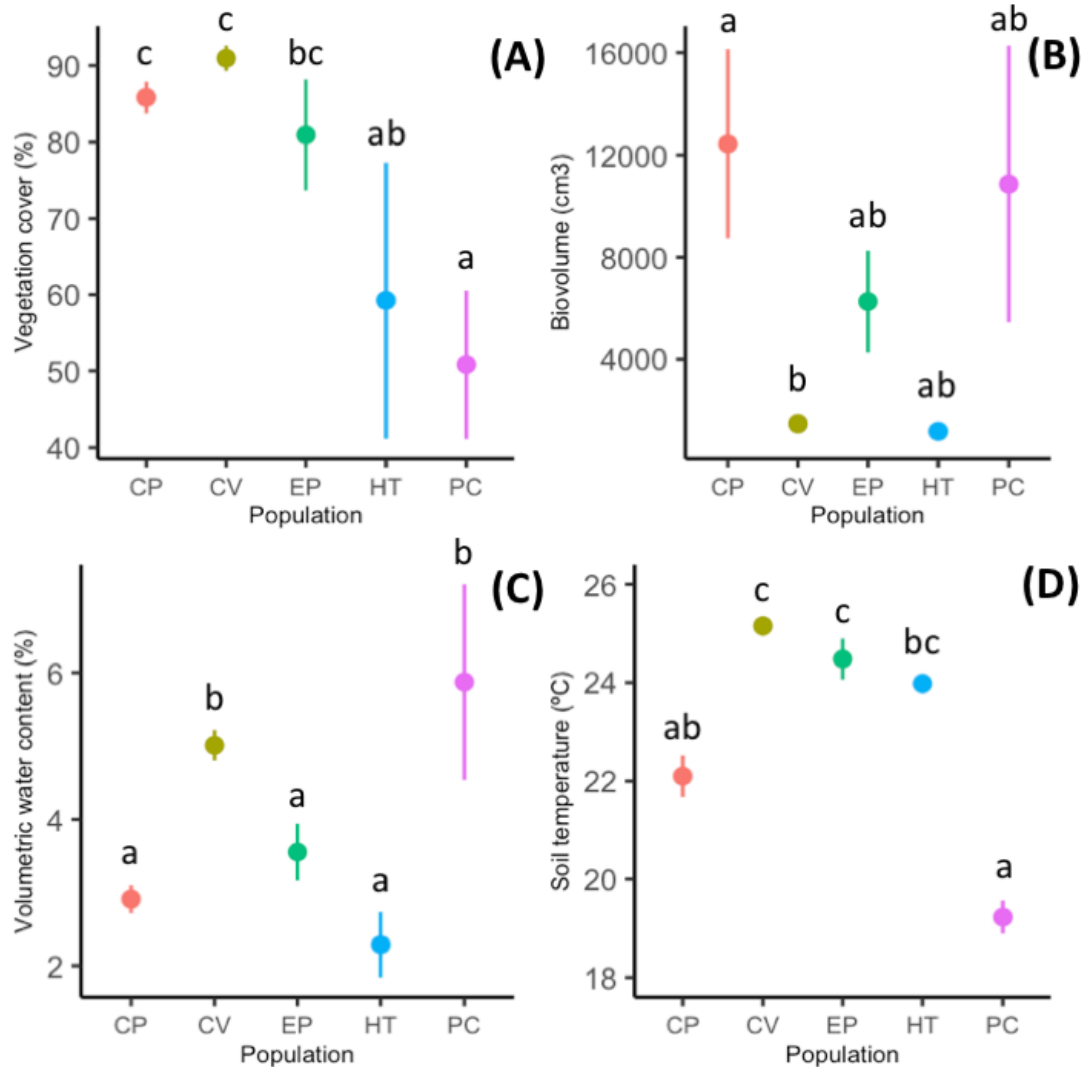


Figure 5. Mean values (mean±SE) per studied population for: A) Vegetation cover (%), B) Biovolume (cm³), C) Volumetric water content (%) and D) Soil temperature (°C). Different letters over the populations in a graph indicate significant differences ($p < 0.05$) for the post hoc Tukey tests performed after the permutational ANOVA. Populations: CP, Cerrada del pintor; CV, Coto del Valle; EP, Estrecho de los Perales; HT, Hoya del Tostón; PC, Pico Cabañas.

Fencing has been the conservation in situ measures applied for the species. The idea is very simple; the separation of biodiversity (plants in this case) from the processes threatening them (overgrazing) (Somers & Hayward, 2012). Fencing has a clear positive effect by avoiding herbivore grazing and trampling, also in some cases for human trampling and collection, which are the main threats for the species at most of the populations. This measure usually increases seed production and seedling survival. However, fencing may have negative aspects such as high costs for construction and maintenance, call effect for illegal collectors, limitation of seed dispersal for some species (zoochorous) increasing intraspecific competition. Fencing also induces a drastic change in the

habitat, increasing interspecific competition. Also, they produce aesthetic problems and management problems with stakeholders (e.g., farmers or hunters). Thus, fences might be temporary, and a monitoring program is also needed to address the possible negative outcomes.

Population features

Only two populations (Coto del Valle and Cerrada del Pintor) showed individuals in different biovolume classes regarding population structure. For the rest of the populations, the scarce individuals were placed predominantly in upper biovolume classes (>2500 cm³),

showing a typical regressive population structure (Brys *et al.*, 2003) with a poor or negligible regeneration.

After the harsh typical Mediterranean summer, a high mortality rate was accounted for. Especially taking into account, the species is a long-lived perennial bush. However, the regeneration process can be linked to pulses in very favorable years (Castro *et al.*, 2004) by monitoring emergence, survival and growth for up to 4 years in the microhabitats to which seeds are dispersed. Naturally established seedlings were monitored in two mountain ranges, and experimental sowings were performed both in woodlands and in adjacent successional shrublands into which the forest could expand. 2 Emergence was high in all microhabitats, although it was highest under the canopy of shrubs. Overall survival was low, with c. 90% of seedlings dying in the first growing season (c. 98% after several growing seasons, so this mortality could be typical of dry summers. Thus, it would not have very detrimental demographic negative consequences.

Submediterranean species are linked to moisture and a cool summer season, as pointed in the introduction section. However, climate change trends in the area (increasing temperatures and decreasing precipitation) may accelerate the loss of this submediterranean species (Allen *et al.*, 2010; McIntyre *et al.*, 1999), even at the most favourable microhabitats. Soil moisture after the summer was very low and soil temperature relatively high for most of the populations, especially those at lower altitudes. Thus, results showed an altitudinal gradient, tending to decrease in higher altitude populations and suggesting suitable habitats are migrating upwards. Also, populations close to riverbanks may be favored by the buffer effect generated by this microhabitat. Still, if temperatures continue to increase, the buffer effect may be reduced and these populations are likely to dwindle and disappear (Río & Penas, 2006).

Conclusion and conservation remarks

After evaluating the species population structure and regeneration niche, we conclude that species presents a critical situation in the area, suffering serious regeneration problems caused by both climatic and intrinsic characteristics of the species and human-mediated factors. To control threat factors, i.e., specially human-mediated ones, a series of conservation measures can be taken, such as i) Preserve vegetation structure (high vegetation cover) in suitable habitats, avoiding forest clearances. Also, promoting this dense structure by different management practices, such as key tree-species plantation; ii) Threat control, especially human-mediated, can need adequate management. Ungulate population control is a pending task in this natural area. Fencing could act as an emergency measure for some populations, but at medium or long term only a herbivore control can assure the correct conservation of the populations; iii) Monitoring *Euonymus* scale and controlling this pest if necessary; iv) Population reinforcement and reintroduction that guarantee

the Minimum Viable Population, especially for the larger populations; v) Finally, a long-term monitoring and management program for the species might be implemented. All these measures may help to guarantee species conservation at its southernmost limit in Europe.

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