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Biology and conservation status of *Juniperus macrocarpa* Sm. in Sardinia

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Presentata da: Maria Silvia Pinna

Coordinatore Dottorato: Prof. Gianluigi Bacchetta
Tutor/Relatore. Prof. Gianluigi Bacchetta
Co-Tutor: PhD. Eva Maria Cañadas Sánchez, PhD. Oscar Grillo, PhD. Efisio Mattana, PhD. Cristiano Pontecorvo

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“Germogli invisibili, infiniti, ben celati,

Sotto la neve e il ghiaccio, sotto la tenebra, in ogni pollice quadrato o cubico,

Germogli, raffinati, in delicati pezzi, microscopici, non ancora nati,

Come feti nell’utero, latenti, ripiegati, compatti, dormenti,

Bilioni di bilioni, trilioni di trilioni in attesa,

(Sulla terra e nel mare – l’universo – lassù le stelle nei cieli)

Urgono lenti e scuri,urgono verso il futuro, si formano all’infinito,

E molti più, sempre più in attesa”.

Walt Withman, “Germogli invisibili”, Foglie d’erba

“ALBERO

l'esplosione lentissima

di un seme”

Bruno Munari, "Fenomeni bifronti"

SUMMARY

ABSTRACT

The present Ph.D. thesis aimed to provide a better understanding of *Juniperus macrocarpa* and the habitat that it characterizes (*Juniperus* spp. habitat; European priority habitat) in Sardinian coastal dunes, as well as to achieve results useful to support *in situ* and *ex situ* conservation actions. In particular, the specific aims of the thesis were: (1) to analyse morphological variation in seeds of Mediterranean *Juniperus taxa* (at inter- and intraspecific level) and morphometric differences in *J. macrocarpa* seeds collected in different populations, seasons and sources; (2) to evaluate the effect of the collecting season, source, laboratory pre-treatments and temperatures on seed viability and germination of *J. macrocarpa*; (3) to investigate factors affecting seedling emergence, survival and growth of the species; (4) to explore the floristic variability of the habitat in Sardinia in relation to geographic, climatic and human variables, as well as the potential effect of human disturbance and sampling period on key plant parameters; and finally (5) to assess the conservation status of this habitat in Sardinia.

In the first chapter, a statistical classifier for Mediterranean *Juniperus taxa* based on seed morphometric parameters analysed by image analysis techniques, was tested at interspecific, specific and intraspecific levels. Analysed seeds came from galbules of *Juniperus taxa* collected from different regions of the Mediterranean Basin and galbules of *J. macrocarpa* collected in 2010 from four Sardinian populations, in two seasons and in plants and soil. Two taxonomic treatments for *Juniperus* genus (Flora Europaea and The Plant List) were compared and inter-population, seasonal and source variability in seed morphology were analysed. High percentages of correct identification were reached for both taxonomic treatments at specific and intraspecific level and from the comparison among *taxa* of the *J. oxycedrus, J. communis* and *J. phoenicea* complexes. Moreover, this statistical classifier discriminated *J. macrocarpa* seeds collected in spring better than those collected in autumn, but it seemed not to be able to discriminate those seeds collected from plants and soil, nor those ones collected in different populations from the same geographical region.

In the subsequent chapter, seed viability and germination phenology of *J. macrocarpa* were investigated. For this purpose, ripe galbules in four localities and in two seasons, both from plants and soil, were collected. In order to verify the presence of physiological dormancy, warm (W) and cold stratification (C), two combinations of them (W+C and C+W), and no pre-treatment (control) were applied. After pre-treatments, seeds were incubated in a range of

constant (10–25°C) and alternating (25⁄10°C) temperatures. Seed viability was low (ca. 40%) and the source (plant or soil) had not a significant effect on it, but it varied significantly according to season, showing lower percentages for seeds collected in autumn than in spring. Seed germination was low (ca. 10%), the control and W were the most effective on stimulating germination, while C negatively affected germination. The best temperatures for germination were 15 and 20°C and seeds collected in spring showed higher germination percentages (ca. 11%) than in autumn (ca. 7%). Seeds of *J. macrocarpa* are dormant and the results of this study suggested the presence of secondary dormancy induced by cold stratification. The germination phenology all along the dispersal season (from autumn to spring) was illustrated, as well as the potentiality of this *taxon* to create a soil seed bank.

In the third chapter, factors affecting emergence, survival and growth of *J. macrocarpa* seedlings, were investigated. For this study, permanent plots were placed and periodically monitored. Within them, besides seedling parameters (emergence, survival and growth), several biotic and abiotic variables (solar radiation, tree cover, herbaceous plus scrub cover, distance from the closer *J. macrocarpa* female, number of galbules on the soil and event number of herbivore trace) were measured. Linear mixed-effects models were used to test the relative importance of different groups of explanatory variables on seedling parameters. A total of 536 seedlings were marked, most of which emerged in winter. The "microclimate" was the best fit model explaining emergence, highlighting the positive relation between the number of emerged seedlings and tree cover. Survival was very low and most of the seedlings died in the first months from emergence, reaching the highest mortality rate in the first summer. High values of both herbivory and solar irradiation increased mortality risk. Our results confirmed that *J. macrocarpa* is a slow growing species, and no seedlings reached the subsequent size class. Moreover, growth depended on suitable microhabitats, and in particular it was positively related to tree cover, hours of sunlight, and herbaceous plus scrub cover. Despite these relevant results, long-term studies are needed to identify key issues in the life cycle of *J. macrocarpa* (e.g. germination, fitness, and recruitment).

In the last chapter, the floristic variability of *Juniperus* spp. habitat in Sardinia in relation to geographic, climatic and human variables was explored. Two data sets were created: the first one by inputting phytosociological relevés available in literature and our own relevés; the second one by including for each relevé geographic, climatic and floristic variables, as well as sampling period and human disturbance as categorical variables. The floristic composition differed among sites and this variation was mainly ruled by a latitudinal gradient linked to a climatic gradient. Regarding the results of key parameters in the evaluation of the habitat quality, floristic richness was positively influenced by low and medium level of human disturbance; the endemic *taxa* cover was positively related to a medium level of human disturbance, while the alien *taxa* cover was positively related to recent samplings. The conservation status of *Juniperus* spp. habitat in Sardinia following the IUCN protocol we also assessed. Preliminary results showed that this habitat should be considered as endangered (EN) at a regional level.

In conclusion, our results gave new findings for the recovery and conservation planning of the species and the habitat under study. Specifically, we found that spring was the best season for seed collection. We also suggest: autumn as the period for planting or sowing, with planting being preferable to sowing; shielding plants from solar radiation under canopy; the application of techniques such as organic blanket when sowing is the selected option. In addition, in order to improve the conservation status of *Juniperus* spp. habitat, we advise management measures such as the eradication of alien *taxa*, as well as interventions aimed to reduce human impact on the dune systems.

INTRODUCTION

The juniper vegetation in Sardinia has been described by various authors (De Marco *et al.*, 1985; Camarda *et al.*, 1995; Brullo *et al.*, 2001; Rivas-Martínez *et al.*, 2003; Bacchetta *et al.*, 2007, 2009). These woods are widespread in all lithological substrates, from the sea level to the highest altitudes of the Island (Bacchetta *et al.*, 2009). The vegetation of the cacuminal areas of the Gennargentu Massif (CE Sardinia) is characterized by the presence of the *Juniperus communis* L. subsp. *nana* Syme, ascribed to the *Pino-Juniperetea* Rivas-Martínez 1964 class, the *Juniperetalia hemisphaerica* Rivas-Martínez & J.A. Molina in Rivas-Martínez, Fernández-González & Loidi 1999 orden, the *Berberidion aetnensis* Brullo, Giusso & Guarino 2001 alliance, the endemic *Roso serafini-Juniperenion nanae* Brullo, Giusso & Guarino 2001 sub-alliance, the *Juniperetum nanae* Litar. & Malcuit 1926 association, and two exclusive sub-associations of Sardinia: *cerastietosum boissieriani* Brullo, Giusso & Guarino 2001 and *juniperetosum oxycedri* Brullo, Giusso & Guarino 2001 (Brullo *et al*., 2001).

Plant communities characterized by *J. communis* L. *subsp.* communis, present in many sites of central Sardinia, specifically in the “Tacchi” area (Ogliastra, CE Sardinia), have not been described to date (Bacchetta *et al.*, 2009). *J. oxycedrus* L. subsp. *oxycedrus* is present in southern Sardinia, and is referable to the *Quercetea ilicis* Br.-Bl. ex A. & O. Bolòs 1950 class, the *Pistacio lentisci-Rhamnetalia alaterni* Rivas-Martínez 1975 orden, the *Oleo sylvestris-Ceratonion siliquae* Br.-Bl. ex Guinochet & Drouineau 1944 em. Rivas-Martínez 1975 alliance, and the *Pistacio lentisci-Juniperetum oxycedri* Camarda, Lucchese, E. Pignatti & S. Pignatti 1995 association (Camarda *et al*., 1995).

The thermomediterranean juniper vegetation characterized by *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman, refers to four associations: *Oleo-Juniperetum turbinatae* Arrigoni, Bruno, De Marco & Veri in De Marco, Dinelli & Caneva 1985; *Erico-Juniperetum turbinatae* De Marco, Dinelli & Caneva 1985; *Chamaeropo-Juniperetum turbinatae* De Marco, Dinelli & Caneva 1985 and *Euphorbio characiae-Juniperetum turbinatae* Biondi, Filigheddu & Farris 2001, which belong to the *Juniperion turbinatae* Rivas-Martínez 1975 corr. 1987 alliance, of the *Pistacio lentisci-Rhamnetalia alaterni* orden (De Marco *et al.*, 1985; Biondi *et al.*, 2001; Biondi and Bagella, 2005). The Sardinian psammophilous formations with *J. macrocarpa* Sm. are included in the *Asparago acutifolii-Juniperetum macrocarpae* R & R. Molinier ex O. Bolòs 1962 (Géhu and Biondi, 1994) association. These latter types of vegetation are of particular phytogeographical interest and they characterize the stabilized dune systems of Sardinia, and are listed as priority habitat (2250***)** in the European Union Habitats Directive 92/43/EEC (European Commission, 2007).

The *Juniperus* spp. habitat (2250*)

The priority habitat 2250* (*Juniperus* spp. habitat, hereafter), is mainly distributed along the sandy coasts of southern and western Europe and secondly in northern Europe, on Mediterranean and Atlantic coasts. Italy hosts the most extended surface of this habitat at EU level (Picchi, 2008). The characteristic species of coastal dunes in northern Europe (Britain, Denmark and Germany) is mainly *J. communis*, while in southern (Greece, Italy) and western (Spain, Portugal and France) Europe the juniper species predominating are: *J. macrocarpa*, *J. phoenicea* L., *J. phoenicea* subsp. *turbinata*.

The vegetation that characterize the *Juniperus* spp. habitat in Sardinia (Figure 1), is generally dominated by micro-forests principally constituted by *J. macrocarpa* and referable to the *Pistacio lentisci*-*Juniperetum macrocarpae* association. They are dominated by sclerophyllous phanerophytes caespitose such *Pistacia lentiscus* L. and *Rhamnus alaternus* L.; the vines are frequent, in particular *Smilax aspera* L., *Rubia peregrina* L. subsp. *requienii* (Duby) Cardona *et* Sierra, *Clematis flammula* L., *Prasium majus* L., as well as the geophytes such as *Ruscus aculeatus* L. and *Asparagus acutifolius* L. (Bacchetta *et al*., 2007, 2008b). In the most exposed areas to erosion, this association has a scrub structure, while in the more sheltered areas evolves in woods, which often exceeds 5-6 m in height. In backdunes areas, protected from sea agents (wind and salt spray), *J. macrocarpa* is partially replaced by *J. phoenicea* subsp. *turbinata*, togheter with *Phillyrea latifolia* L. subsp. *rodriguezii* (P. Monts.) Romo, *Asparagus albus* L., *Olea europaea* L. var. *sylvestris*, *Osyris alba* L., *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter *et* Burdet (Bacchetta *et al*., 2007, 2008b).

Taxonomic treatments

The Cupressaceae family is mainly distributed in Northern Hemisphere, and more scattered in the south temperate regions and in the north east Africa, with singles genera present from either Northern or Southern Hemispheres (Stevens, 2001). The genus *Juniperus* L. comprises about 70 species and 27 varieties (Adams, 2008) most of which grow in the Northern hemisphere, except *J. procera* Hochst. ex Endl., that is present in the Great Rift Valley and Mountains (Adams *et al*., 1993). In the Mediterranean area, the genus *Juniperus* is mainly present in the mountains systems of Greece, Balkan Peninsula, France, Spain, Italy, Turkey, Algeria, Morocco, Portugal, Tunisia, as well as Corsica and Sardinia (Tutin *et al.*, 1993). The genus *Juniperus* can be divided into three monophyletic sections: *Caryocedrus* Endlicher, *Juniperus* L. (syn: *Oxycedrus* Spach), and *Sabina* (Mill.) Spach (Adams, 2008). In Sardinia seven *taxa* of *Juniperus* are present: 1) *J. communis* subsp. *communis*; 2) *J. communis* subsp. *nana*; 3) *J. macrocarpa*; 4) *J. oxycedrus* subsp. *oxycedrus*; 5) *J. oxycedrus* subsp. *badia* (H.Gay) Debeaux; 6) *J. phoenicea* L. subsp. *phoenicea*; 7) *J. phoenicea* subsp. *turbinata*.

The similarity within the *J. oxycedrus* complex is undoubted (Farjon, 1998; Adams, 2000). There are many different taxonomic treatments, such as The Plant List (2012), Flora Europaea (Tutin *et al*., 1993), the Cupressaceae monograph (Farjon, 2005) and The Annotated Checklist of the Italian Vascular Flora (Conti *et al*., 2005). According to The Plant List, there are two varieties belonging to the *J. oxycedrus* complex (*J. oxycedrus* var. *badia* H. Gay; *J. oxycedrus* var. *oxycedrus*), while *J. macrocarpa* is considered a different species. Conversely, Tutin *et al.* (1993) did not include *J. macrocarpa* as a species, but they proposed two subspecies of *J. oxycedrus* (*J. oxycedrus* subsp. *oxycedrus*; *J. oxycedrus* subsp. *macrocarpa*) and did not report *J. oxycedrus* var. *badia*. Farjon (2005) distinguished *J. oxycedrus* in four subspecies: *J. oxycedrus* subsp. *oxycedrus, J. oxycedrus* subsp. *macrocarpa*, *J. oxycedrus* subsp. *badia*, and *J. oxycedrus* subsp. *transtagana* Franco. Instead Conti *et al.* (2005) identified for *J. oxycedrus* complex only two subspecies: *J. oxycedrus* subsp. *oxycedrus* and *J. oxycedrus* subsp. *macrocarpa*. Adams (2000), on the basis of DNA and essential oils analysis, differentiated four *taxa*: *J. oxycedrus* L.; *J. badia* H. Gay [= *J. oxycedrus* subsp. *badia* (H. Gay) Debeaux], *J. navicularis* Grand. (= *J. oxycedrus* subsp. *transtagana* Franco) and *J. macrocarpa* Sm. [= *J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Neilr.]; meanwhile Farjon (1998) treated these *taxa* as a single species, *J. oxycedrus*. In this thesis treatment proposed by The Plant List w as used taxonomic, apart from chapter one, in which different *taxa* were analysed.

Study species

J. macrocarpa is a dioecious species, 1-5 m high, very branching, with large canopy and needle-like-lanceolate leaves 2.5 mm wide and 12-15 mm long, sharp-pointed. The galbules are globular or pear-shaped, with 8-15 mm diameter, and blue-green and brown-purple at ripening (Pignatti, 1982; Tutin *et al*., 1993; Arrigoni, 2006). The male cones are terminals, oval or subspherical, with rounded apex and placed in whorls of three cones.

The appearance of male and female cones occurs simultaneously in July. The differentiation of pollen sacs begins at late summer; microsporogenesis is completed in autumn with formation of pollen grains (Ciampi, 1958). The opening of male cones (dehiscence) and pollen dispersal begin in October (Figure 2) and continue through winter. The female cones appear in the leaf axil and ovules, three within each galbules, start differentiating in early autumn (Ciampi, 1958; Chambers *et al.*, 1999). In autumn the pollen sacs burst open and pollen dispersed by wind. Between pollination and fertilization, maturation of both gametophytes completes (Ciampi, 1958; Chambers *et al*., 1999). The development and maturation of female cones continues until July, when fertilization occurs and simultaneously also the pollen grain completed its development. The embryonic differentiation progresses until late summer of the second year, when embryo is mature at the same time galbules are ready for dispersal (Figure 3). *J. macrocarpa* galbules ripe at September-October (Ciampi, 1958) but do not simultaneously, their ripening and dispersal is distributed from autumn to spring (see Chapter 1). This is a strategy to facilitate predation by animals (foxes, badgers and wild boar) that ensures seed dispersal via excrements (Pacini and Piotto, 2004).

J. macrocarpa is a shrub or small tree typical of coastal environments, distributed in the Mediterranean region from southwestern Spain to western Turkey and from Morocco to Cyrenaica (Lybia), including Balearic Islands, Corsica, Sardinia, Sicily and Aegean Islands, also reaching the coasts of the Black Sea and Syria (Greuter *et al.*, 1984; Amaral Franco, 1986; Farjon, 2005).

Figure 1 – The *Juniperus* spp. habitat in Is Arenas dune system.

Figure 2 - 3: 2: Opening of male cones (dehiscence) and pollen dispersal; 3: Ripe galbules of *J. macrocarpa.*

Study area

Sardinia is situated in the western Mediterranean basin (38° 51’ and 41° 15’ latitude north, 8° 8’ and 9° 50’ east longitude), covering ca. 24.090 km^2, and the maximum altitude reaching 1.834 m a.s.l. (Punta La Marmora, Gennargentu Massif, CE-Sardinia).

Among the main dune systems of Sardinia we selected as study area (Figure 4) four Sites of Community Importance (SCI) in the southern of island:

1. “Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu”, ITB040020 (Villasimius), 39° 07' 16"N-9° 31' 22"E;
2. “Porto Campana”, ITB042230 (Domus de Maria), 38° 53' 04"N-8° 51' 43"E;
3. “Is Compinxius–Campo dunale di Buggerru-Portixeddu”, ITB042249 (Buggerru), 39° 26' 18"N-8° 25' 51"E;
4. “Da Piscinas a Riu Scivu”, ITB040071 (Arbus), 39° 31' 05"N-8° 25' 55"E.

Figure 4 - Study areas: 1 Villasimius; 2 Domus de Maria; 3 Buggerru; 4 Arbus.

Geological, geographical and climatic context of study areas

The Villasimius municipality is located at the southern end of Sardinian east coast, in Sarrabus territory. In this area, the quaternary alluvial detrital covers are constituted by conglomerates, sandstones and biocalcarenites of beach (Upper Pleistocene), along the coast, resting directly above an abrasion surface engraved on granite. There are also patchs of Quaternary deposits, marine and continental, dating back to last interglacial period (Palmerini, 1967; Orrù *et al*., 1994). The studied area comprises the beaches of Porto Giunco and Simius, separated by Serra 'e Morus promontory, Is Tarias and Punta Molentis beaches. The dune system has an medium height of 12 m which can reach 35 m (MATTM website; ftp://ftp.dpn.minambiente.it/Cartografie/Natura2000/).

Domus de Maria comprises the dune system and Chia beaches, located in Sulcis area and in particular in its south-western tip. The geological setting of area is characterized by the Palaeozoic basement, on which Quaternary deposits lie, that are formed from Holocenic and recent beach sands, ancient alluvial deposits related to the main rivers and colluvial deposits on the slope floors (De Muro *et al*., 2010). The dune system, situated 30-40 m from the shoreline, is fairly uniform and with a maximum height of 26 m (MATTM website; ftp://ftp.dpn.minambiente.it/Cartografie/Natura2000/). The continuity of these beaches is interrupted by two low rocky headlands which divide it into three areas from NE to SW, called Sa Colonia, Campana and Su Giudeu (De Muro *et al.*, 2010).

The dune system of Buggerru-Portixeddu extends over an area of ca. 4 km^2 and is composed of at least three generations of aeolian deposits, dating from the Middle Pleistocene to the Holocene (Cesaraccio *et al*., 1986; Arisci *et al*., 1999). From a morphological point of view, longitudinal, parabolic and transversal dunes can be distinguished. This dune field was stabilized through the plantation of many trees and shrubs over 40 years ago (Arisci *et al.*, 1999).

Is Arenas dune system (Arbus) represents one of the most important and well-preserved coastal system of the island, which spread to ca. 5 km inland. Geologically, the area mainly consists of Holocene sandstones and aeolian sands forms which present irregular heights ranging from 10 to 80-90 m (Annino *et al*., 2000).

From a climatic standpoint, all sites show a typical Mediterranean annual pattern of temperature and precipitation with a long-term dry summer. The mean annual temperature and annual precipitation are the following: 17.6 °C and 95.33 mm for Villasimius; 16.6°C and 168.33 mm for Chia; 16.2°C and 203.33 mm for Buggeru-Portixeddu; 16.4°C and 195.67 mm for Is Arenas, according to data downloaded from http://www.worldclim.org (Hijmans *et al.*, 2005).

Available data from the nearest weather stations (Montevecchio, Fluminimaggiore and Domus de Maria) allowed to classify bioclimatically Is Arenas, Buggeru-Portixeddu and Chia dune systems as Oceanic Pluviseasonal Mediterranean (MPO), with upper thermomediterranean thermotype and lower subhumid ombrotype (Bacchetta, 2006; Bacchetta *et al.*, 2008a). The dune system and the beaches of Villasimius, are classified as Oceanic Pluviseasonal Mediterranean (MPO), with lower thermomediterranean thermotype and upper dry ombrotype (Bacchetta *et al.*, 2010).

Threats and conservation

The coastal *Juniperus* spp. woods represent one of the most important psammophilous vegetation community in the Mediterranean area (Bacchetta *et al.*, 2007). The main threats affecting this habitat at European level are various: urban development, tourist pressure, forest fires, alien plant species, coastal erosion, overgrazing and habitat fragmentation (Picchi, 2008). In Sardinia, they face local critical conditions mainly due to human impact factors, such as the increasing of coastal urbanization and the transformation of dune systems in tourist recreational areas, in these latter the human trampling increase causes decrease or loss of characteristic species and starting of erosion processes (Bacchetta *et al*., 2007).

For the conservation of dunes with *Juniperus* woodland, measures for the recovery of coastal environmental conditions and the populations reinforcement should be implemented and the threat factors reduced, through an active management of coastal dune ecosystems (Muñoz and Gracía, 2009). Such measures could include: fire prevention, shrub clearance and controlled grazing, eradication of alien species, regulation and limitation of human presences and activities

(Picchi, 2008; Muñoz and García, 2009). Moreover, *in situ* conservation actions maybe be adopted, such as recovery interventions on degraded dunes, population reinforcement and regeneration of the juniper woodlands; as well as *ex situ* conservation measures, such as seed conservation in germplasm banks and/or plant propagation in nurseries for reintroduction purposes.

Plan of the work

The present Ph.D. thesis has been developed in the context of the LIFE PROVIDUNE project (LIFE07NAT/IT/000519), financed by LIFE + program "Nature and Biodiversity" for the period 2009-2013. The project aims at protecting th e priority habitat consisting of coastal dunes with *Juniperus* ssp. (2250*) *sensu* Dir. 92/43/CEE, which is one of the most endangered habitats in the EU, in five Italian SCI.

In particular, the present thesis deals with *J. macrocarpa* and the habitat that it characterizes. Besides habitat studied directly linked with LIFE project, we have analysed morphological variation in seeds of Mediterranean *Juniperus taxa*. In addition we have investigated the *J. macrocarpa* germination, fundamental for a better knowledge viability, actual reproductive capacity and some aspects on seedling dynamics of the *taxon*, essential for planning future conservation actions.

In the first chapter, we analysed the seeds to achieve a statistical classifier for Mediterranean *Juniperus taxa*, based on seed morphometric parameters, at specific and intraspecific level. We compared the achieved results with two different taxonomic treatments. Moreover, we tested if the classification system was able to discriminate seed lots of the same species (*J. macrocarpa*) collected in different populations, seasons and sources.

The subsequent chapter focused on a key stage in the life cycle of plants: seed germination. We verified the effect of the collecting season and source (plant and soil), as well as laboratory germination pre-treatments and temperatures on seed viability and germination of *J. macrocarpa*. The analysis performed aimed to maximize the effectiveness of conservation and regeneration activities of the fragile and threatened ecosystems where this species prevails.

The next phase of work concerned another critical stage of plant life-cycle, the transition period from seed germination to seedling establishment (Figure 5), particularly critical in environment as Mediterranean coastal dunes. Specifically, we evaluated factors affecting seedling emergence, survival and growth of *J. macrocarpa* in southern Sardinia, to determine seedling recruitment and its consequences for forest regeneration.

Finally, using the phytosociological relevés available in literature and our own relevés, we determined the floristic variability of 2250* habitat in Sardinia in relation to geographic, climatic and human variables; we analysed if tourism and period of samplings have an influence on the key parameters for habitat quality, as well as we evaluated the conservation status of 2250* habitat in Sardinia.

Figure 5 – Seedling of *J. macrocarpa*.

Aims

This thesis aims to better understanding of *J. macrocarpa* and the habitat that it characterizes, in Sardinian coastal dunes, as well as to achieve results useful to support *in situ* and *ex situ* conservation actions. The main specific aims of this work were:

- ✓ To analyse morphological variation in seeds of Mediterranean *Juniperus taxa* (at both inter- and intraspecific level);

- ✓ to test morphometric differences in *J. macrocarpa* seed collected in different populations, seasons and sources (plants or soil);
- ✓ to evaluate the effect of the collecting season, source, laboratory pre-treatments and temperatures, on seed viability and germination of *J. macrocarpa*;
- ✓ to investigate factors affecting seedling emergence, survival and growth of *J. macrocarpa*, to determine seedling recruitment and its consequences for forest regeneration;
- ✓ to explore the floristic variability of *Juniperus* spp. habitat in Sardinia in relation to geographic, climatic and human variables; to examine the potential effect of human disturbance and sampling period on key plant parameters for evaluating habitat quality;
- ✓ to assess the conservation status of *Juniperus* spp. habitat in Sardinia.

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CHAPTER 1

Inter and intraspecific morphometric variability in *Juniperus* L. seeds

Maria Silvia Pinna, Oscar Grillo, Efisio Mattana, Eva Cañadas, Gianluigi Bacchetta

Abstract

In this study, a statistical classifier for Mediterranean *Juniperus taxa* based on seed morphometric parameters analysed by image analysis techniques, was tested at interspecific, specific and intraspecific levels. Ripe galbules of eight or nine *Juniperus taxa*, were collected in different regions of the Mediterranean Basin. Moreover, ripe galbules of *J. macrocarpa* were collected in 2010 from four Sardinian populations, in two seasons, and in plant and soil, in order to analyse inter-population, seasonal and source variability in seed morphology. Furthermore, were compared two different taxonomic treatments proposed for this genus (by Flora Europaea and The Plant List). High percentages of correct identification were reached for both taxonomic treatments at specific and intraspecific level and from the comparison among *taxa* of the *J. oxycedrus*, *J. communis* and *J. phoenicea* complexes. Moreover, this statistical classifier discriminated *J. macrocarpa* seeds collected in spring better than those collected in autumn, but it seemed not to be able to discriminate those seeds collected from plants and soil, nor those ones collected in different populations from the same geographical region.

Keywords: Cupressaceae, EFDs, LDA, Mediterranean flora, morphometric seed analysis

Introduction

The family Cupressaceae shows great ecological diversity among its species (Farjon, 1999), mainly distributed in the Northern Hemisphere, and more scattered in south temperate regions and in the north east Africa, with singles genera present from either Northern or Southern

Hemispheres (Stevens, 2001). The genus *Juniperus* L. comprises about 70 species and 27 varieties (Adams, 2008), most of them growing in the Northern hemisphere, except *Juniperus procera* Hochst. *ex* Endl., that is present in the Great Rift Valley and Mountains (Adams *et al.*, 1993). In the Mediterranean area, the genus *Juniperus* is mainly present in the mountain systems of Greece, Balkan Peninsula, France, Spain, Italy, Turkey, Algeria, Morocco, Portugal, Tunisia, as well as Corsica and Sardinia (Tutin *et al.*, 1993). This genus can be divided into three monophyletic sections (Adams, 2008): *Caryocedrus* Endlicher, with only one species for the Mediterranean region (*J. drupacea* Labill.); *Juniperus* L. (syn: *Oxycedrus* Spach), consisting of ten species (nine in east Asia and the Mediterranean plus the circumboreal *J. communis* L.); and *Sabina* (Mill.) Spach, consisting of 56 species distributed in south-western regions of North America, Asia and Mediterranean Basin (Adams, 2008; Mao *et al.*, 2010; Silva *et al.*, 2011).

In *Juniperus* genus, genetic research at interspecific (Adams, 2008; Mao *et al*., 2010) and intra-specific (e.g. Opgenoorth *et al*., 2010; Douaihy *et al*., 2011) level has been published, highlighting high levels of genetic diversity. In particular, Jiménez *et al.* (2003) analysed genetic diversity and differentiation in Moroccan and Spanish *J. thurifera* L., showing that the Strait of Gibraltar acted as an efficient barrier against gene flow between the Moroccan and European populations of this species. Douaihy *et al.* (2011) revealed a high level of genetic diversity within *J. excelsa* M.Bieb. subsp. *excelsa*. Meloni *et al.* (2006) found genetic variability in five natural populations of *J. phoenicea*. In addition, Boratyński *et al.* (2009) compared natural populations of *J. phoenicea*, detecting two groups of populations, *J. phoenicea* L. subsp. *phoenicea* in the eastern Iberian Peninsula and southern France, and *J. phoenicea* subsp. *turbinata* (Guss.) Nyman from the Mediterranean and Atlantic shores and from the Atlas Mountains. High level of genetic differentiation for *J. communis* was found in populations sampled in Britain (Van Der Merwe *et al*., 2000) and throughout Europe (Michalczyk *et al*., 2010).

Some authors investigated interpopulation differences within the various species of the genus *Juniperus*, achieving different results depending on the *taxon*. Mazur *et al.* (2003) analysed biometrically (number, length, width of cones and seeds, features of shoots and leaves) the interpopulation variation of *J. phoenicea* from the Iberian Peninsula that was found to be much larger than that of *J. excelsa* (Mazur *et al.*, 2004). Klimko *et al.* (2007) examined the intra- and interpopulation variation of *J. oxycedrus* L. subsp. *oxycedrus* on the basis of morphological characters (length and width of cones, seeds and needles, seed number per cone). These authors found that the west-Mediterranean populations differed from the eastern ones, as well as intrapopulation differentiation of individuals.

The potentialities of biometric indices for seed studies are well known and proved by many authors, particularly regarding morpho-colorimetric evaluation (e.g. Liao *et al.*, 1994; Granitto *et al*., 2003; Shahin and Symons, 2003; Kiliç *et al.*, 2007; Bacchetta *et al*., 2008; Wiesnerová and Wiesner, 2008; Venora *et al*., 2009a; Grillo *et al*., 2010). Bacchetta *et al.* (2008), using digital images, characterized seeds of wild vascular plants of the Mediterranean Basin, implementing statistical classifiers able to discriminate seeds belonging to different genera and species. Grillo *et al.* (2010) developed 10 specific statistical classifiers at family level for Angiosperms and tested the system on the genus *Juniperus*, proving that the method is also reliable for Gymnosperms. Recently, Orrù *et al.* (2012a) confirmed the effectiveness of this identification method from a comparison between the reached results studying the discriminatory power of seeds biometric characters of *Vitis vinifiera* L. varieties and those achieved by De Mattia *et al.* (2007) during a study on genetic characterization of the same varieties. Afterwards, many authors used Elliptic Fourier Descriptors (EFDs) in seed studies (Terral *et al*., 2010; Mebatsion *et al*., 2012; Orrù *et al*., 2012b) achieving relevant results.

Since taxonomic controversies and different systematic treatments on *Juniperus* genus exist, we showed the perspective of morphometric seed analysis, which has proven to be a useful tool in taxonomy (e.g. Bacchetta *et al*., 2008). Specifically, the aims of this study were: (1) to validate and improve the statistical classifier, based on seed morphometric parameters, at specific and intraspecific level, previously implemented by Grillo *et al.* (2010) for the Mediterranean *Juniperus taxa*; (2) to compare the achieved results with the two different taxonomic treatments proposed by Flora Europaea (Tutin *et al.*, 1993) and The Plant List (2012); and (3) to test the capability of the classification system in the discrimination of seed lots of the same species (*J. macrocarpa* Sm.) collected in different populations, seasons and sources (plants or soil).

Materials and methods

Seed lot details

Ripe galbules of *Juniperus taxa* were collected in the field for a total of 43 seed lots from Algeria (Ag), Balearic Islands (Bl), Corsica (Co), Italy (It), Sardinia (Sa), Spain (Hs) and then stored at the Sardinian Germplasm Bank (BG-SAR) in Cagliari (Table 1). After collection, seeds were manually drawn out from the galbules and washed stirring them in water for 90 minutes. The cleaned seeds were dried at room temperature. Within these seed lots, 18 are accessions of *J.*

macrocarpa collect ed in Sardinia in 2010 in order to analyse inter-population and seasonal variability in seed morphology (Table 1).

Moreover, to analyse the intraspecific relationships among the studied *taxa*, comparisons at species, subspecies and variety level were implemented following the two different taxonomic treatments proposed by Flora Europaea (Tutin *et al.,* 1993; FE) and The Plant List (2012; PL).

Seed size and shape analysis

Digital images of seed samples were acquired using a flatbed scanner (Epson Perfection V600 Photo) with a digital resolution of 400 dpi and a scanning area not exceeding 2048×2048 pixel. Image acquisition was performed before drying the seeds at 15°C to 15% of R.H. to avoid spurious variation in dimension and shape. Samples consisting of 100 seeds, randomly chosen from the original seed lots and disposed on the flatbed tray, were used for the digital image analysis. When the original accession was numerically lower than 100 units, the analysis was executed on the whole seed batch. Digital images of seeds were processed and analysed using the software package KS-400 V.3.0 (Carl Zeiss, Vision, Oberkochen, Germany). A macro specifically developed for the characterization of wild seeds (Bacchetta *et al*., 2008), later modified to measure further 20 seed features (Mattana *et al*., 2008) and afterwards improved to perform automatically all the analysis procedures, reducing the execution time and contextually mistakes in the analysis process (Grillo *et al*., 2010), was used to analyse seeds images. This macro was further enhanced adding algorithms able to compute the EFDs for each analysed seed, increasing the number of discriminant parameters (Orrù *et al*., 2012b). The EFDs method allows describing the boundary of the seed projection, as an array of complex numbers which correspond to the pixels position of the seed boundary. So, from the seed apex, defined as the starting point in a Cartesian system, a chain codes are generated. A chain code is a lossless compression algorithm for binary images. The basic principle of chain codes is to separately encode each connected component (pixel) in the image. The encoder then moves along the boundary of the image and, at each step, transmits a symbol representing the direction of this movement. This continues until the encoder returns to the starting position. This method is based on the separate Fourier decompositions of the incremental changes of the X and Y coordinates as a function of the cumulative length along the boundary (Kuhl and Giardina, 1982). Each harmonic (*n*) corresponds to four coefficients (a*n*, b*n*, c*n* and d*n*) defining the ellipse in the XY-plane. The coefficients of the first harmonic, describing the best fitting ellipse of outlines are used to standardize size (surface area) and to orientate seeds (Terral *et al*., 2010). According to

Terral *et al*. (2010) findings, about the use of number of harmonics for an optimal description of seed outlines, in order to minimize the measurement errors and optimizes the efficiency of shape reconstruction, 20 harmonics were used to define the seed boundaries, obtaining further 80 parameters useful to discriminate among the studied *taxa*.

A total of 98 morphometric characters were measured on 2343 seeds (Table 2).

Table 1 - Location of studied *taxa* and populations of *Juniperus* genus (1 = seeds collected in spring; 2 = seeds collected in autumn; * = seeds collected from plant; ** = seeds collected from soil). Ag: Algeria; Bl: Balearic Islands; Co: Corsica; It: Italy; Sa: Sardinia; Hs: Spain.

Section (Adams, 2008)	***Taxon* according to**		**Locality**	**Region**	**Year**	**Number of sampled seeds**
	The Plant List (http://www.theplantlist.org/)	**Flora Europaea (Tutin *et al.*, 1993)**				
Juniperus	*J. communis* var. *saxatilis* Pall.	*J. communis* subsp. *nana* Syme	Desulo	Sa	2010	1813
			Albertacce-Evisa,	Co	2006	412
			Desulo,	Sa	2006	760
	J. communis L.	*J. communis* L. subsp. *communis*	Laconi,	Sa	2006	1221
		J. communis L. subsp. *hemisphaerica* (J.Presl & C.Presl) Nyman	Santiago de la Espada, Andalusia	Hs	2010	1728
			Cabañas, Andalusia	Hs	2010	273
	J. oxycedrus var. *badia* H.Gay	not reported	Buggerru	Sa	2012	836
			Huescar, Andalusia	Hs	2012	244
			Domus de Maria	Sa	2011	266
	J. oxycedrus L.	*J. oxycedrus* L. subsp. *oxycedrus*	Cuesta Carrascal, Andalusia	Hs	2010	1129
			Capoterra	Sa	2012	380
	J. macrocarpa Sm.	*J. oxycedrus* L. subsp. *macrocarpa* (Sibth. & Sm.) Neilr.	Domus de Maria	Sa	2007	3522
			Narbolia	Sa	2007	1409
			Cecina, Tuscany	It	2008	147
			Domus de Maria	Sa	2007	445
			Arbus	Sa	2010	46 ^{1*}
			Arbus	Sa	2010	137 ^{1**}
			Arbus	Sa	2010	2477 ^{1**}
			Arbus	Sa	2010	220 ^{2*}
			Arbus	Sa	2010	1010 ^{2**}
			Buggerru	Sa	2010	213 ^{1*}
			Buggerru	Sa	2010	273 ^{1**}
			Buggerru	Sa	2010	2414 ^{1**}
			Buggerru	Sa	2010	2465 ^{2*}
			Buggerru	Sa	2010	1984 ^{2**}
			Domus de Maria	Sa	2010	100 ^{1*}
			Domus de Maria	Sa	2010	430 ^{1**}
			Domus de Maria	Sa	2010	3527 ^{2*}
			Domus de Maria	Sa	2010	2087 ^{2**}
			Villasimius	Sa	2010	543 ^{1*}
			Villasimius	Sa	2010	269 ^{1**}
			Villasimius	Sa	2010	2210 ^{2*}
			Villasimius	Sa	2010	1763 ^{2**}
Sabina	*J. phoenicea* L.	*J. phoenicea* L.	Lula	Sa	2006	1200
			Aïn Sefra, wilaya de Naâma	Ag	2010	392
	J. phoenicea var. *turbinata* (Guss.) Parl.		Montagne des Lions, Oran	Ag	2010	317
			Villasimius,	Sa	2008	897
			Almerimar, Andalusia	Hs	2010	338
			Mallorca, Balearic Islands	Bl	2010	ND
	J. sabina L.	*J. sabina* L.	CIEF, Comunidad Valenciana	Hs	2007	1023
			Jerez del Marquesado, Andalusia	Hs	2007	843
	J. thurifera L.	*J. thurifera* L.	CIEF, Comunidad Valenciana	Hs	2007	1005
			Pedro Martinez, Andalusia	Hs	2010	554
Total amount of measured seeds						2343

Table 2 - List of 18 morphometric features measured on seeds, excluding the 80 Elliptic Fourier Descriptors (EFDs) calculated according to Hâruta (2011).

Feature		Description
$\boldsymbol{A}$	Area	Seed area (mm^2)
$\boldsymbol{P}$	Perimeter	Seed perimeter (mm)
$\boldsymbol{P}_{conv}$	Convex Perimeter	Convex perimeter of the seed (mm)
$\boldsymbol{P}_{Crof}$	Crofton Perimeter	Crofton perimeter of the seed (mm)
$\boldsymbol{P}_{conv} / \boldsymbol{P}_{Crof}$	Perimeter ratio	Ratio between $\boldsymbol{P}_{conv}$ and $\boldsymbol{P}_{Crof}$
$\boldsymbol{D}_{max}$	Max diameter	Maximum diameter of the seed (mm)
$\boldsymbol{D}_{min}$	Min diameter	Minimum diameter of the seed (mm)
$\boldsymbol{D}_{min} / \boldsymbol{D}_{max}$	Feret ratio	Ratio between $\boldsymbol{D}_{min}$ and $\boldsymbol{D}_{max}$
$\boldsymbol{EA}_{max}$	Maximum ellipse axis	Maximum axis of an ellipse with equivalent area (mm)
$\boldsymbol{EA}_{min}$	Minimum ellipse axis	Minimum axis of an ellipse with equivalent area (mm)
$\boldsymbol{Sf}$	Shape Factor	Seed shape descriptor = $(4 \cdot \pi \cdot \boldsymbol{A}) / \boldsymbol{P}^2$ (normalized value)
$\boldsymbol{Rf}$	Roundness Factor	Seed roundness descriptor = $(4 \cdot \boldsymbol{A})/(\pi \cdot \boldsymbol{D}_{max}^2)$ (normalized value)
$\boldsymbol{Ecd}$	Eq. circular diameter	Diameter of a circle with equivalent area (mm)
$\boldsymbol{F}$	Fiberlength	Seed length along the fiber axis
$\boldsymbol{C}$	Curl degree	Ratio between $\boldsymbol{D}_{max}$ and $\boldsymbol{F}$
$\boldsymbol{Conv}$	Convessity degree	Ratio between $\boldsymbol{P}_{Crof}$ and $\boldsymbol{P}$
$\boldsymbol{Sol}$	Solidity degree	Ratio between $\boldsymbol{A}$ and convex area
$\boldsymbol{Com}$	Compactness degree	Seed compactness descriptor = $[\sqrt{(4/\pi)}\, \boldsymbol{A}] / \boldsymbol{D}_{max}$

Statistical analysis

Morphometric and EFDs data were analysed applying the stepwise Linear Discriminant Analysis (LDA) method, in order to compare the *Juniperus* seeds at section level (Adams, 2008), as well as at specific and intraspecific level according to the taxonomic treatments proposed by PL and FE (Table 1). In particular, intraspecific analysis were performed for three species complexes (*J. communis, J. oxycedrus, J. phoenicea*). LDA was also used to assess seed morphological variability of *J. macrocarpa* collected in different populations, seasons and sources (plants and soil). To avoid the influence of the production year, only the *J. macrocarpa* seed lots collected in 2010 were considered.

LDA is commonly used to classify/identify unknown groups characterized by quantitative and qualitative variables (Fisher, 1936, 1940), finding the combination of predictor variables with the aim of minimizing the within-class distance and maximizing the between-class distance simultaneously, thus achieving maximum class discrimination (Hastie *et al.*, 2001;

Holden *et al*., 2011). The stepwise method identifies and selects the most statistically significant features among them to use for the seed sample identification, using three statistical variables: Tolerance, *F*-to-enter and *F*-to-remove. The Tolerance value indicates the proportion of a variable variance not accounted for by other independent variables in the equation. *F*-to-enter and *F*-to-remove values define the power of each variable in the model and they are useful to describe what happens if a variable is inserted and removed, respectively, from the current model. This method starts with a model that does not include any of the variables. At each step, the variable with the largest *F*-to-enter value that exceeds the entry criteria chosen ($F \geq 3.84$) is added to the model. The variables left out of the analysis at the last step have *F*-to-enter values smaller than 3.84, so no more are added. The process was automatically stopped when no remaining variables increased the discrimination ability (Venora *et al*., 2009b; Grillo *et al*., 2012). Finally, a cross-validation procedure was applied to verify the performance of the identification system, testing individual unknown cases and classifying them on the basis of all others (SPSS, 1999). Analyses were performed using the SPSS software package release 15 (SPSS, 1999).

To graphically highlight the differences among groups (species and populations), box plots were drawn using the Mahalanobis' square distance values (Mahalanobis, 1936). This measure of distance is defined by two or more discriminant functions and ranges from 0 to infinite. Samples are increasingly similar at values closer to zero. Higher values indicate that a particular case includes extreme values for one or more independent variables, and can be considered significantly different to other cases of the same group (Bacchetta *et al*., 2008).

Results

Comparing the seed lots belonging to the two *Juniperus* sections proposed by Adams (2008), an overall cross-validation percentage of correct identification was reached (86.8%), with performances of 81.6% and 73.4% for *Juniperus* and *Sabina* sections, respectively.

Following the PL taxonomic treatment at species level, an overall performance of correct identification of 73.8% was reached, ranging between 63.6% (*J. thurifera*) and 81.5% (*J. sabina* L.) (Table 3). In Table 3 are also resumed the performance of correct identification for the comparison according to the FE taxonomic treatment. An overall percentage of correct identification of 81.0% was achieved, showing values ranged between 63.6% (*J. thurifera*) and 88.0% (*J. oxycedrus*).

Table 3 - Percentage of correct identification at species level according to PL “The Plant List (http://www.theplantlist.org/; 24 Oct. 2012)” and “Flora Europaea”(Tutin *et al.*, 1993; FE) (in FE *J. macrocarpa* included in *J. oxycedrus*). In parenthesis, the number of analysed seeds.

***Taxon* according to PL**	*J. communis*	*J. oxycedrus*	*J. macrocarpa*	*J. phoenicea*	*J. sabina*	*J. thurifera*	**Total**
J. communis	**77.2 (447)**	1.2 (7)	0.0 (0)	14.2 (82)	4.3 (25)	3.1 (18)	100 (579)
J. oxycedrus	1.0 (5)	**66.7 (323)**	15.9 (77)	10.7 (52)	0.0 (0)	6.0 (27)	100 (484)
J. macrocarpa	0.0 (0)	29.8 (89)	**66.9 (200)**	2.0 (6)	0.0 (0)	1.3 (4)	100 (299)
J. phoenicea	10.6 (62)	7.0 (41)	0.5 (3)	**80.4 (471)**	0.2 (1)	1.4 (8)	100 (586)
J. sabina	13.0 (26)	0.0 (0)	0.0 (0)	0.0 (0)	**81.5 (163)**	5.5 (11)	100 (200)
J. thurifera	11.8 (23)	4.1 (8)	0.0 (0)	15.4 (30)	5.1 (10)	**63.6 (124)**	100 (195)
Overall							**73.8 (2343)**

***Taxon* according to FE**	*J. communis*	*J. oxycedrus*	*J. phoenicea*	*J. sabina*	*J. thurifera*	**Total**
J. communis	**76.9 (445)**	1.2 (7)	14.5 (84)	4.3 (25)	3.1 (18)	100 (579)
J. oxycedrus	0.8 (6)	**88.0 (689)**	7.0 (55)	0.0 (0)	4.2 (33)	100 (783)
J. phoenicea	9.6 (56)	7.3 (43)	**81.4 (477)**	0.2 (1)	1.5 (9)	100 (586)
J. sabina	12.5 (25)	0.0 (0)	0.0 (0)	**82.0 (164)**	5.5 (11)	100 (200)
J. thurifera	12.3 (24)	3.6 (7)	14.9 (29)	5.6 (11)	**63.6 (124)**	100 (195)
Overall						**81.0 (2343)**

At intraspecific level, in Table 4 the classification performance reached on the basis of the PL taxonomic treatment is showed. Percentages of correct identification ranged between 13.5% (*J. phoenicea* var. *phoenicea*) and 81.5% (*J. sabina*), with an overall performance of 60.6%. Regarding the performance according to FE (where *J. oxycedrus* subsp. *oxycedrus* included also *J. oxycedrus* var. *badia* H. Gay seed lots), an overall percentage of correct identification of 67.6% was reached, showing values ranged between 33.0% (*J. communis* subsp. *communis*) and 83.5% (*J. sabina*).

According to PL, the two varieties belonging to *J. communis* complex were compared, reaching performances of 76.2% and 86.5% for *J. communis* var. *saxatilis* Pall. and *J. communis* var. *communis*, respectively, giving an overall percentage of correct identification of 80.0%. In Figure 1, the scores of the only one implemented discriminant function are reported as box plots for both the *J. communis* varieties. *J. communis* complex was also analysed on the basis of the FE taxonomic treatment, achieving an overall identification performance of 71.8%, but correctly identifying only 33.0% of *J. communis* subsp. *communis*, misattributed in 51.0% of the cases to *J. communis* subsp. *nana* Syme, that reached 81.2% of correct identification, and in 16.0% of the cases to *J. communis* subsp. *hemisphaerica* (J.Presl & C.Presl) Nyman, that reached 78.2% of correct identification.

According to PL, the two varieties belonging to the *J. oxycedrus* complex (*J. oxycedrus* var. *badia*, *J. oxycedrus* var. *oxycedrus*) were also compared with *J. macrocarpa* (Figure 2), achieving an overall percentage of correct identification of 69.2%, with misattributions evenly distributed among the three *taxa*.

Discriminant analysis between the two varieties belonging to *J. phoenicea*, according to PL (Figure 3), showed an overall performance of 70.8%, with percentages of correct identification of 25.0% and 93.1% for *J. phoenicea* var. *phoenicea* and *J. phoenicea* var. *turbinata* (Guss.) Parl., respectively.

Table 4 - Percentage of correct identification between *taxa* (specific, subspecific and variety level), according to PL “The Plant List (http://www.theplantlist.org/; 24 Oct. 2012)” and “Flora Europaea”(Tutin *et al.*, 1993; FE) (in FE *J. oxycedrus* var. *badia* is included in *J. oxycedrus* subsp. *oxycedrus*). In parenthesis, the number of analysed seeds.

***Taxon* according to PL**	*J. communis* var. *saxatilis*	*J. communis* var. *communis*	*J. oxycedrus* var. *badia*	*J. oxycedrus* var. *oxycedrus*	*J. macrocarpa*	*J. phoenicea* var. *phoenicea*	*J phoenicea* var. *turbinata*	*J. sabina*	*J. thurifera*	**Total**
J. communis var. *saxatilis*	**52.5 (148)**	20.6 (58)	0.0 (0)	5.0 (14)	0.0 (0)	4.3 (12)	9.6 (27)	3.2 (9)	5.0 (14)	100 (282)
J. communis var. *communis*	16.8 (50)	**71.7 (213)**	0.0 (0)	0.0 (0)	0.0 (0)	1.3 (4)	3.4 (10)	6.7 (20)	0.0 (0)	100 (297)
J. oxycedrus var. *badia*	0.0 (0)	0.0 (0)	**59.4 (171)**	15.3 (44)	21.5 (62)	0.0 (0)	3.8 (11)	0.0 (0)	0.0 (0)	100 (288)
J. oxycedrus var. *oxycedrus*	3.6 (7)	0.0 (0)	19.4 (38)	**36.7 (72)**	11.2 (22)	0.5 (1)	13.8 (27)	0.0 (0)	14.8 (29)	100 (196)
J. macrocarpa	0.0 (0)	0.0 (0)	16.1 (48)	11.0 (33)	**69.9 (209)**	0.0 (0)	2.0 (6)	0.0 (0)	1.0 (3)	100 (299)
J. phoenicea var. *phoenicea*	14.1 (27)	6.3 (12)	0.0 (0)	3.6 (7)	0.0 (0)	**13.5 (26)**	59.9 (115)	1.0 (2)	1.6 (3)	100 (192)
J. phoenicea var. *turbinata*	5.3 (21)	4.1 (16)	3.0 (12)	6.9 (27)	0.8 (3)	3.8 (15)	**74.6 (294)**	0.0 (0)	1.5 (6)	100 (394)
J. sabina	3.0 (6)	10.5 (21)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	**81.5 (163)**	5.0 (10)	100 (200)
J. thurifera	12.8 (25)	1.0 (2)	0.0 (0)	7.7 (15)	0.0 (0)	2.1 (4)	8.2 (16)	5.1 (10)	**63.1 (123)**	100 (195)
Overall										**60.6 (2343)**

***Taxon* according to FE**	*J. communis* subsp. *nana*	*J. communis* subsp. *communis*	*J. communis* subsp. *hemisphaerica*	*J. oxycedrus* subsp. *oxycedrus*	*J. oxycedrus* subsp. *macrocarpa*	*J. phoenicea*	*J. sabina*	*J. thurifera*	**Total**
J. communis subsp. *nana*	**48.6 (137)**	7.4 (21)	11.3 (32)	2.5 (7)	0.0 (0)	20.9 (59)	2.5 (7)	6.7 (19)	100 (282)
J. communis subsp. *communis*	31.0 (31)	**33.0 (33)**	20.0 (20)	0.0 (0)	0.0 (0)	8.0 (8)	8.0 (8)	0.0 (0)	100 (100)
J. communis subsp. *hemisphaerica*	17.3 (34)	9.1 (18)	**62.9 (124)**	0.0 (0)	0.0 (0)	4.6 (9)	5.6 (11)	0.5 (1)	100 (197)
J. oxycedrus subsp. *oxycedrus*	1.0 (5)	0.0 (0)	0.0 (0)	**67.8 (328)**	15.1 (73)	10.1 (49)	0.0 (0)	6.0 (29)	100 (484)
J. oxycedrus subsp. *macrocarpa*	0.0 (0)	0.0 (0)	0.0 (0)	33.1 (99)	**64.5 (193)**	1.3 (4)	0.0 (0)	1.0 (3)	100 (299)
J. phoenicea	6.3 (37)	0.7 (4)	1.9 (11)	7.0 (41)	0.5 (3)	**81.4 (477)**	0.3 (2)	1.9 (11)	100 (586)
J. sabina	3.5 (7)	4.0 (8)	4.0 (8)	0.0 (0)	0.0 (0)	0.0 (0)	**83.5 (167)**	5.0 (10)	100 (200)
J. thurifera	10.3 (20)	0.0 (0)	1.0 (2)	4.1 (8)	0.0 (0)	14.4 (28)	5.6 (11)	**64.6 (126)**	100 (195)
Overall									**67.6 (2343)**

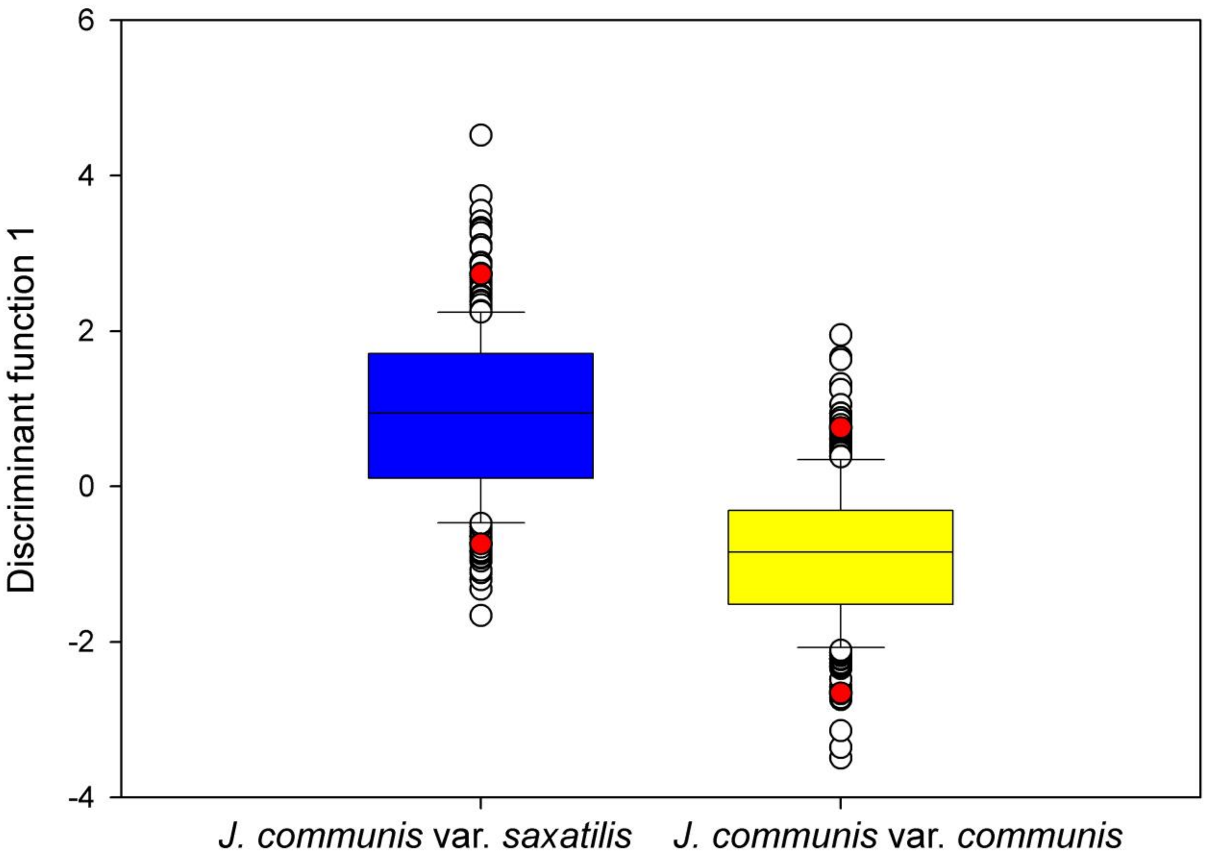

Figure 1 - Graphic representation of the discriminant function scores for both the *J. communis* varieties, according to PL "The Plant List (http://www.theplantlist.org/; 24 Oct. 2012)".

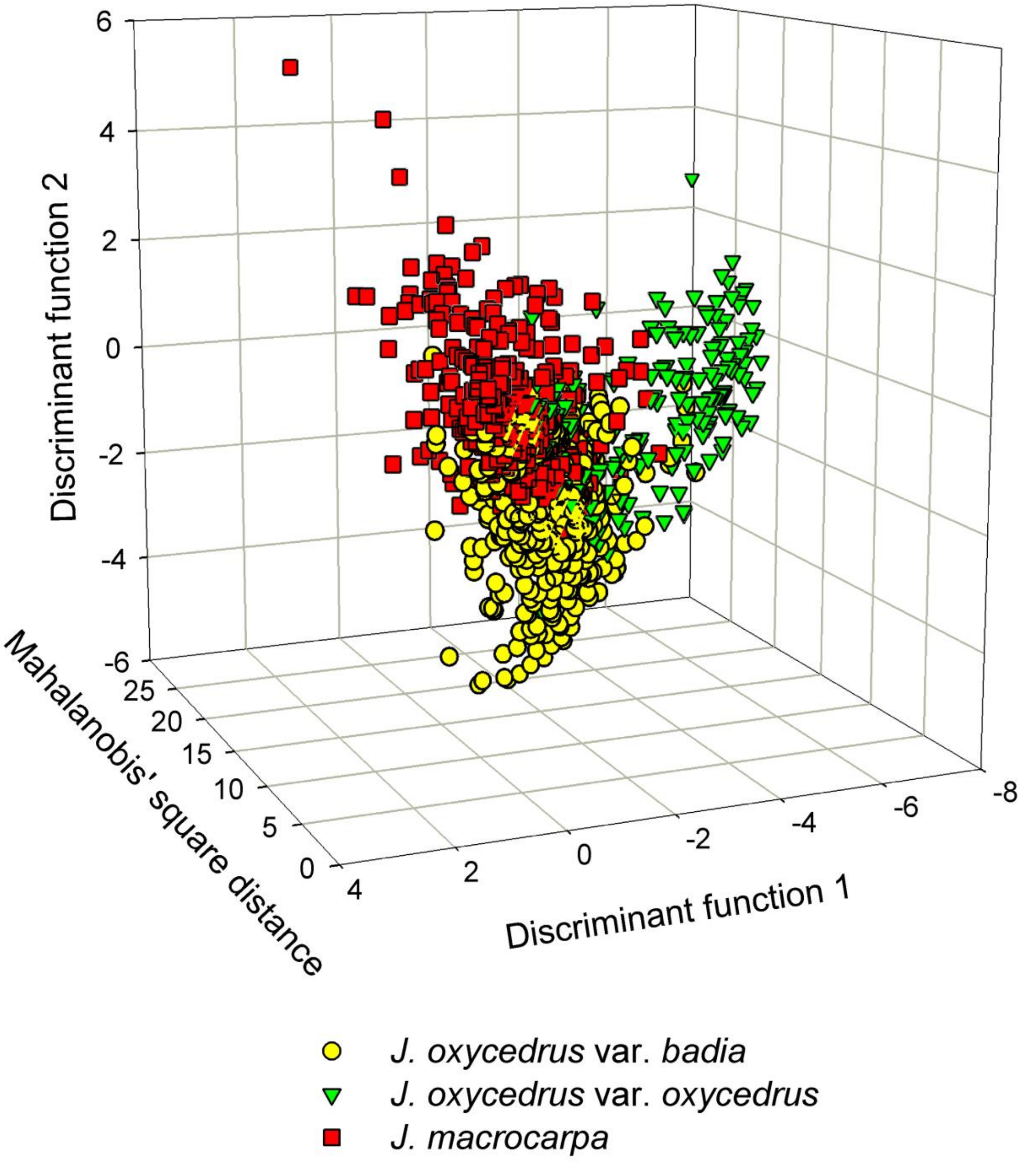

Figure 2 - Analysis discriminating of the varieties belonging to the *J. oxycedrus* complex, according to PL"The Plant List (http://www.theplantlist.org/; 24 Oct. 2012)", with *J. macrocarpa*.

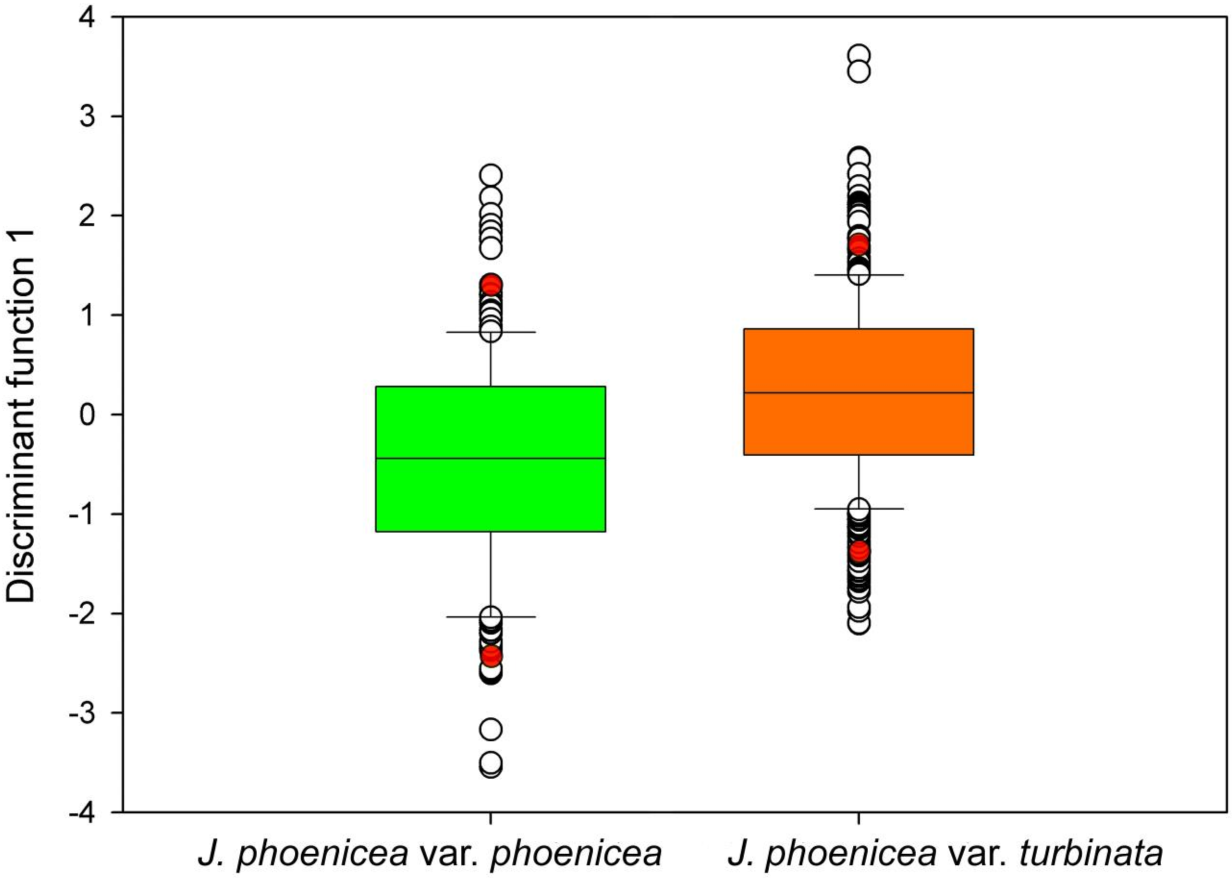

Figure 3 - Graphic representation of the discriminant function scores for the two varieties belonging to *J. phoenicea*, according to PL "The Plant List (http://www.theplantlist.org/; 24 Oct. 2012)".

The four *J. macrocarpa* populations highlighted percentages of right identification ranged between 22.3% (Domus de Maria) and 54.5% (Buggerru), with an overall performance of 37.9% (Table 5). Regarding the discriminant comparison between the *J. macrocarpa* seed lots collected in spring and autumn 2010, the overall percentages for population ranged between 66.6% (Villasimius) and 70.1% (Domus de Maria), with an overall identification performance of 63.5%. Similarly, the discriminant analyses conducted between the seed collected from plant and those collected from soil showed overall percentages of correct identification for population ranging from 61.5% (Buggerru) to 70.2% (Villasimius), with an overall identification performance of 59.2%.

Table 5 - Percentage of correct identification among populations of *J. macrocarpa* Sardinian. In parenthesis, the number of analysed seeds.

Locality	Arbus	Buggerru	Domus de Maria	Villasimius	**Total**
Arbus	**40.2 (194)**	30.2 (146)	12.8 (62)	16.8 (81)	100 (483)
Buggerru	21.8 (106)	**54.5 (265)**	14.8 (72)	8.8 (43)	100 (486)
Domus de Maria	29.9 (117)	38.1 (149)	**22.3 (87)**	9.7 (38)	100 (391)
Villasimius	36.5 (142)	23.4 (91)	10.3 (40)	**29.8 (116)**	100 (389)
Overall					**37.9 (1749)**

Evaluating the parameters influencing the discrimination process in the comparison between the two *Juniperus* sections the shape descriptive features resulted more powerful than the dimensional ones, showing high F-to-remove values, although many steps were necessary in the discrimination process. At specific and intraspecific level, both according to the PL and to the FE taxonomic treatments, parameters related to the seed size revealed to be more discriminant than the shape descriptive ones; in particular, seed area (A) and convex perimeter (P_{conv}) resulted mainly powerful. Also in these cases, between 19 and 26 steps were necessary for the *taxa* identification. The four comparisons among species aggregates at subspecies and varietal level, showed various size and shape descriptive features played a relevant role, with emphasis on the seed perimeter features (P, P_{conv}, P_{conv}/P_{Croft}).

Finally, regarding the comparison among the populations of *J. macrocarpa*, size descriptive features were principally used. In all these discriminant analysis, the EFDs resulted particularly powerful among the best five key parameters in spite of the reduced relative F-to-remove values both at section level and the *J. communis* and *J. phoenicea* aggregates, according to PL (Table 6).

Table 6 - The best five key parameters of correct classifications. The number of steps, the tolerance and F-to-remove values are reported in parenthesis.

Classifier	1	2	3	4	5
Sections	***Com*** (24; 0.074; 242.355)	EFD_{14} (24; 0.473; 137.652)	P_{conv}/P_{Crof} (24; 0.482; 53.325)	EFD_{22} (24; 0.675; 31.940)	***F*** (24; 0.760; 26.951)
Species according to PL	***A*** (23; 0.004; 45.394)	P_{conv} (23; 0.002; 30.524)	D_{max} (23; 0.004; 25.934)	***Ecd*** (23; 0.001; 24.011)	***Sf*** (23; 0.070; 19.493)
FE species *J. badia* in *oxycedrus*	***A*** (26; 0.003; 47.401)	***Com*** (26; 0.011; 24.077)	***Ecd*** (26; 0.001; 23.879)	D_{max} (26; 0.004; 22.983)	***Rf*** (26; 0.008; 18.341)
PL intraspecific	***A*** (19; 0.004; 41.734)	P_{conv} (19; 0.002; 25.568)	***Sf*** (19; 0.069; 24.239)	EFD_6 (19; 0.017; 22.642)	***Ecd*** (19; 0.001; 21.050)
FE intraspecific	***A*** (22; 0.004; 43.030)	P_{conv} (22; 0.002; 31.697)	D_{max} (22; 0.004; 17.652)	***Com*** (22; 0.013; 15.094)	***Rf*** (22; 0.009; 14.626)
PL *taxa J. communis* vs. *J. saxatile*	P_{conv} (12; 0.007; 112.470)	***P*** (12; 0.007; 99.101)	D_{min} (12; 0.244; 27.435)	EFD_{14} (12; 0.390; 25.428)	EFD_{11} (12; 0.717; 13.685)
FE *taxa J. communis* vs. *J. nana* vs. *J. hemisphaerica*	EA_{max} (13; 0.572; 183.525)	***Conv*** (13; 0.555; 58.720)	***Sf*** (13; 0.345; 49.828)	***Sol*** (13; 0.359; 17.433)	EFD_{12} (13; 0.945; 15.604)
PL *taxa J. oxycedrus* complex	***P*** (18; 0.009; 35.181)	P_{conv} (18; 0.002; 28.510)	***A*** (18; 0.009; 27.808)	EFD_{11} (18; 0.563; 22.905)	***Rf*** (18; 0.029; 18.992)
PL *taxa J. phoenicea* vs. *J. turbinata*	***P*** (8; 0.715; 11.558)	EFD_{50} (8; 0.933; 6.407)	EFD_{18} (8; 0.863; 5.105)	P_{conv}/P_{Crof} (8; 0.834; 4.981)	EFD_{12} (8; 0.940; 4.981)
Sardinian populations of *J. macrocarpa*	***A*** (5; 0.006; 15.832)	***Ecd*** (5; 0.004; 8.972)	***Sol*** (5; 0.475; 7.899)	P_{conv} (5; 0.025; 6.279)	D_{min} (5; 0.084; 5.946)

Discussion

The satisfactory discrimination achieved by the comparison between the seed morphometric data belonging to the *Juniperus* and *Sabina* sections, is in agreement with the results obtained by Adams (2008) and Mao *et al.* (2010) on the basis of cpDNA, nrITS and nrITS ⁄ cpDNA analysis, confirming the current taxonomic treatment at section level. These results illustrated that this method is effective also when the morphometric variability within each group is high.

The achievements obtained at species level reached good percentage of correct identification, for both the followed taxonomic treatments (PL and FE). *J. macrocarpa* reached almost 70% of correct identification according to PL, so proving a clear differentiation respect to *J. oxycedrus*, towards which gives almost all the misattributions, according to FE in which *J. macrocarpa* does not exist as a species. However, the performance of *J. oxycedrus* grows up to 88.0% following FE classification, proving that a certain similarity exists between the two species (Adams, 2000).

Instead, the two varieties of *J. oxycedrus* proposed by the PL reached lower identification percentages than the two subspecies proposed by FE, but it is important to note that FE does not report *J. oxycedrus* var. *badia* and does not consider *J. macrocarpa* as an independent species but as a subspecies of *J. oxycedrus*. However, *J. macrocarpa* seems to be fairly well identifiable in both cases, although, considering the misidentifications revealed, a certain similarity to the *J. oxycedrus taxa* is undoubted (Farjon, 1998; Adams, 2000).

Respect to the *J. oxycedrus* species complex according to the PL taxonomic treatment, also considering *J. macrocarpa*, a clear correlation among the three entities is evident, placing some legitimate doubt about the most appropriate taxonomic treatment. Anyway, the result reached from the comparison between *J. oxycedrus* var. *badia* and *J. oxycedrus* var. *oxycedrus* makes plausible a relationship at varietal level between these two *taxa*, as confirmed by several authors (Pignatti, 1982; do Amaral Franco, 1986; Farjon, 1998; Adams, 2000; Jeanmonod and Gamisans, 2007).

According to our results, the *J. communis taxa* seem to be more distinguishable following the taxonomic treatment proposed by the PL rather than the one by FE, although in both cases, considerable percentages of misattributions have been detected in relation to *J. phoenicea* species.

The results of the interactions between the *taxa* of the *J. communis* complex, according to the PL, confirmed the taxonomic distance between these *taxa*, although a varietal taxonomic rank is proposed. The performance achieved following the taxonomic treatment proposed by FE, shown that the three considered subspecific entities (*J. communis* subsp. *communis*, *J. communis* subsp. *nana* and *J. communis* subsp. *hemisphaerica*) are not easily sustainable on the basis of seed morphometric data. In a recent work, Grillo *et al.* (2010) reaching a rather high percentages of correct identification, confirmed the taxonomic distance between *J. communis* subsp. *communis* and *J. communis* subsp. *nana*, identified by several authors as two distinct subspecies (do Amaral Franco, 1980, 1986; Jeanmonod and Gamisans, 2007) or species (Pignatti, 1982; Lebreton *et al.*, 2000), but recently considered as unique *taxon* by Farjon (2001) and Adams (2008).

The results of correct classification obtained for the *J. phoenicea* complex indicate that, according to the PL classification, the two *taxa J. phoenicea* var. *phoenicea* and *J. phoenicea* var. *turbinata* are enough well distinguished, considering the taxonomic rank of variety as proposed by Adams *et al.* (1996, 2002), Farjon (2005) and Adams (2010). However, this result is in accordance with the achievements of Grillo *et al.* (2010) that, considering these *taxa* as two different subspecies, reached very high percentage of correct classification, according to many other authors (Lebreton, 1983; do Amaral Franco, 1986; Valdés *et al.*, 1987; Mazur *et al.*, 2003; Conti *et al.*, 2005; Farjon, 2005; Jeanmonod and Gamisans, 2007).

The comparison among the four populations of *J. macrocarpa* gave low performance of correct identification with misattributions evenly distributed, suggesting that seed morphometric data are not able to discriminate among different populations from the same geographical region, probably due to the low intrapopulation variability. These achievements are consistent with the results obtained by Juan *et al.* (2012), who investigated genetic structure of *J. macrocarpa* in three regions of Spain, founding only one meta-population without geographical structure. Also, Klimko *et al.* (2004) found a low genetic differentiation of *J. macrocarpa* Italian populations for most morphological features studied. However, some morphological variability for *J. macrocarpa* was found in south-western Spanish populations (Juan *et al*., 2003). Absence of geographic structure was also observed by Brus *et al.* (2011) in *J. oxycedrus* subsp. *oxycedrus* from the Balkan Peninsula. According to the results obtained in this study and the reported bibliographic data, it is possible to assume in the south sector of Sardinia, the presence of a single population of *J. macrocarpa* lacking of geographical differences. This hypothesis can be supported by the fact that the species studied was sampled in an area geographically restricted as it is Sardinia island.

Other works carried out on different species of the genus *Juniperus* showed contradictory and different results. Mazur *et al*. (2004), analysing biometrically the intra- and interpopulation variation in *J. excelsa* from Crimea and Balkan Peninsula, have found differences among particular individuals within the samples slight, as well as between populations compared. Even Dzialuk *et al.* (2011) have obtained low proportion of genetic variation contributed by the differences between populations of *J. phoenicea*. Conversely, high levels of intrapopulation genetic variability (Boratyński *et al.*, 2009) and between populations (Meloni *et al*., 2006) in *J. phoenicea* were found. On the basis of morphological and molecular results, high degree of genetic diversity in *J. excelsa* was detected at populations level (Douaihy *et al.*, 2011, 2012) and within populations (Yücedağ *et al.,* 2010). High levels of intrapopulation variability were also seen in genetic studies on *J. thurifera* (Jiménez *et al*., 2003; Terrab *et al*., 2008), *J. procera* (Sertse *et al*., 2011), *J. brevifolia* (Seub.) Antoine (Silva *et al*., 2011) and *J. communis* (Van Der Merwe *et al*., 2000; Oostermeijer and De Knegt, 2004; Michalczyk *et al.*, 2010; Vanden-Broeck *et al.,* 2011). Medini *et al*. (2010) have showed population variability, through the chemical composition of the essential oils extracted from the leaves of *J. oxycedrus*.

At specific and intraspecific level, parameters related to the seed size (i.e. morphometric) revealed to be more discriminant than the shape-descriptive ones. For the same taxonomic ranks Grillo *et al.* (2010) found that for the families Apiaceae, Brassicaceae and Fabaceae morphometric features were the first discriminant parameters. Also in Bacchetta *et al.* (2011a), regarding the *Lavatera triloba* aggregate, the first three parameters with the highest discriminatory power were of morphological type, although in this work colour evaluation was very important for correct seed identification. Instead in a previous contribution regarding *Astragalus* sect. *Melanocercis*, the only morphometric parameters taken into account were related to the seed length (Bacchetta *et al.*, 2011b).

The classification system identified *J. macrocarpa* seeds collected in spring, but it not allowed to identify between sources (plant and soil) of collection. The latter results appear particularly interesting because represent the first application of statistical classifier based on seed morphometric parameters to discriminate seed lots of the same species at season and source level.

The obtained results confirmed the validity of the proposed method for the Mediterranean *Juniperus* species, both at specific and intraspecific levels, and its identification capability after adding the EFDs among the measured features, incrementing number of accession of the database implemented by Grillo *et al*. (2010). Seed morphometric analysis did not discriminate among different populations, which could mean the presence of a single meta-population in the South of Sardinia. The classification system was able to discriminate seeds of *J. macrocarpa* collected in different seasons, being better identifiable those collected in spring, and could not identify seeds collected in different sources (plants and soil).

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CHAPTER 2

Seed viability and germination phenology in *Juniperus macrocarpa* Sm.

Maria Silvia Pinna, Efisio Mattana, Eva Cañadas, Gianluigi Bacchetta

Abstract

In this study, seed viability and germination phenology of *Juniperus macrocarpa* were investigated. Ripe galbules in four localities and in two different moments of the dispersal (i.e. autumn and spring), both from plant and soil, were collected. To verify the presence of physiological dormancy several pre-treatments were applied: warm (W: 3 months at 25°C) and cold stratification (C: 3 months at 5°C), two combinations of them (W+C and C+W), and no pre-treatment (control). After pre-treatments, seeds were incubated in a range of constant (10–25°C) and alternating (25⁄10°C) temperatures. Seed viability was low (ca. 40%) and the source (plant or soil) had not a significant effect on the viability of *J. macrocarpa* seeds, but it varied significantly according to the collecting season, showing lower percentages for seeds collected in autumn than in spring. Seed germination was low (overall mean value of ca. 10%), the control and W were the most effective on stimulating germination, while C negatively affected germination. The best temperatures for germination were 15 and 20°C and seeds collected in spring showed higher germination percentages (ca. 11%) than those collected in autumn (ca. 7%). Seeds of *J. macrocarpa* are dormant and the results of this study suggested the presence of secondary dormancy induced by cold stratification. The germination phenology all along the dispersal season (from autumn to spring) was illustrated, as well as the potentiality of this *taxon* to create a soil seed bank. Finally, spring was the best season for seed collecting whereas autumn for the sowing in the field, giving new findings for the recovery and conservation planning of this species.

Keywords: Cupressaceae, dormancy, galbules, *Juniperus*, Mediterranean flora

Introduction

The coastal dunes ecosystems are globally threatened by human activities (e.g. Brown and McLachlan, 2002; Defeo *et al*., 2009). In particular, dune vegetation is easily disturbed and susceptible to trampling by animals and humans and to crushing by vehicles (e.g. Williams *et al*., 1997). Furthermore, they are vulnerable ecosystems subjected to intense ecological stress caused by wind, drought, salt, erosion and pH (Crawford, 1989; Brown and McLachlan, 1990). Along Mediterranean coastal dune ecosystems, the characteristic woody vegetation is constituted by micro-forests of *Juniperus macrocarpa* Sm. These types of vegetation are listed as priority habitat 2250 "Coastal dunes with *Juniperus* spp." in the DIR. 92/43/EEC (European Commission, 2007).

In the Mediterranean area the populations of many species of *Juniperus* L. are formed principally from adults and senescent individuals, and their survival is therefore linked more to longevity of individuals than to emergence of new seedlings, indicating a difficult recruitment and a limited establishment of young individuals (García *et al.*, 1999). The causes of this low recruitment are attributed to several factors such as reproductive problems (e.g. Chambers *et al.*, 1999; García *et al.*, 2000), low germination capacity (Hajar, 1991), long reproductive cycle (Pacini and Piotto, 2004), summer aridity that may limit the survival of seedlings (García *et al.*, 1999), predation of galbules and seeds and presence of parasites (e.g. Roques *et al*., 1984; García, 1998).

A key stage in the life cycle of plants is seed germination. It is responsive to many environmental factors including temperature, light, time after dissemination, and soil moisture content (Bewley and Black, 1994; Cristaudo *et al*., 2007; Qu *et al.,* 2008). Among them, temperature is the major factor in regulating dormancy, the maximum germination percentage and rate of germination (Heydecker, 1977), and the success or failure of plant establishment (Kader and Jutzi, 2004). The optimal germination temperatures for Mediterranean species are typically within the range 5-15°C (Thanos *et al*., 1989, 1995) and are characterized by a low germination rate (Doussi and Thanos, 2002). This “delay mechanism”, with low germination rate and a narrow range of cool temperatures, is considered an advantageous ecological adaptation of Mediterranean species to the unpredictable rainfall pattern (Doussi and Thanos, 2002), optimising winter germination and therefore the duration of the growing season before the beginning of summer drought (Thanos *et al*., 1995).

Previous studies, carried out on some species of the genus *Juniperus*, showed a wide range of values in germination percentages (i.e. between 7% of *J. communis* L. and 87% of *J. virginiana* L.; Bonner, 2008). In particular, low values of germination detected for *J. oxycedrus*

L. seeds depended on the high proportion of non viable, empty, or immature seeds, and on seed dormancy (Tilki, 2007). The seeds of several *Juniperus* species have physiological dormancy (PD) (e.g. Young and Young, 1992; García-Fayos *et al.*, 2001), in which the embryo is unable to develop a radicle due to a physiological inhibition mechanism (Baskin and Baskin, 1998), while studies of physical dormancy (PY) have provided contradictory data (García-Fayos *et al.*, 2001; Flynn *et al.*, 2007). In particular, H_2SO_4 treated seeds of *J. oxycedrus* germinated at low germination percentages (< 20%; Pacini and Piotto, 2004; Tilki, 2007) and those of *J. excelsa* M. Bieb reached ca. 7-8% of final germination (Jones, 1989). Petersen *et al.* (2005) reported that the sulphuric acid treatment apparently damaged many of the embryos of *J. pinchotii* Sudw. (2.7% of germination). Conversely, Laurent and Chamshama (1987) highlighted a significant increases in germination of *J. procera* Hochst. *ex* Endl. seeds treated with this method, reaching germination percentages of ca. 78%. Indeed, there is wide variation among *Juniperus* species in the degree of dormancy (Chambers *et al.*, 1999), which can be also affected by ripeness of the seed, environmental factors during seed development and variations in genotype (Tilki, 2007). A substantial variation among seed sources, seed age, and individuals is also present (e.g. Van Haverbeke and Comer, 1985; Rietveld, 1989) to allow less competition and better distribution in time and space and increase the likelihood that some of the seeds may germinate and grow (Johnson, 1995; Pacini and Piotto, 2004).

Very few studies have been carried out on seed germination of *J. macrocarpa*. Pacini and Piotto (2004) reached a maximum germination of ca. 25% after warm followed by cold stratification, while cold stratification alone appeared to be totally ineffective for this species. Chemical scarification with sulphuric acid did not improve germination with percentages of ca. 20% (Pacini and Piotto, 2004). Cantos *et al.* (1998) found that intact seeds did not germinate in the greenhouse or in vitro conditions, while seeds without testa did not germinate under greenhouse conditions, and in vitro isolated embryos reached germination levels of about 50%. Juan *et al.* (2006) have found that seeds derived from immature cones of *J. macrocarpa* germinated under greenhouse conditions significantly better (i.e. 49.3%) than those derived from mature ones, suggesting lower levels of dormancy.

There is much to learn about stimulation of seed germination in junipers, and more research is called for (Bonner, 2008). In addition, considering the relatively low germination percentages achieved in the few previous studies on *J. macrocarpa* and the needs of conservation and recovery of this *taxon*, new approaches are needed to better understanding its reproductive cycle. Therefore, the aims of this work were to verify the effect of the collecting season, of the source (plant and soil), and laboratory germination pre-treatments and temperatures on seed viability and germination of seeds of this species. The achieved results may be helpful to enable regeneration activities of the fragile and threatened ecosystems of which *J. macrocarpa* is the cornerstone.

Materials and methods

Seed lot details

Ripe galbules of *J. macrocarpa* were collected in 2010 from four localities in two different moments of dispersal period: autumn (i.e. the beginning) and spring (i.e. the end; Table 1). Galbules were collected from plant and soil, leading to a total of 16 seed lots. Immediately after collection, seeds were manually drawn out from the galbules and washed by stirring them in water. Average seed mass was calculated for each seed lot by weighing 10 replicates of 20 seeds each (Table 1).

Table 1 - Population and seed lots details.

Locality	Coordinates (WGS 84)	Elevation (m a.s.l.)	Distance from the coastline (m)	N° of sampled individuals	Season	Source	Seed mass ± SE (mg)
Arbus, Medio Campidano, SW Sardinia	39° 31' 05"N 8° 25' 55"E	22	150	20	Spring	Plant	97.00 ± 3.80
						Soil	94.95 ± 2.10
				24	Autumn	Plant	90.25 ± 1.86
						Soil	92.40 ± 2.39
Buggerru, Carbonia-Iglesias, SW Sardinia	39° 26' 18"N 8° 25' 51"E	32	1650	20	Spring	Plant	83.50 ± 2.60
						Soil	82.80 ± 1.75
				30	Autumn	Plant	95.20 ± 1.58
						Soil	92.35 ± 2.79
Chia, Cagliari, SW Sardinia	38° 53' 04"N 8° 51' 43"E	5	200	20	Spring	Plant	88.35 ± 1.57
						Soil	91.75 ± 2.55
				20	Autumn	Plant	85.60 ± 1.15
						Soil	98.70 ± 1.98
Villasimius, Cagliari, SE Sardinia	39° 07' 16"N 9° 31' 22"E	15	62	20	Spring	Plant	111.75 ± 1.59
						Soil	127.55 ± 1.28
				20	Autumn	Plant	92.40 ± 6.15
						Soil	123.40 ± 4.31

Germination and viability tests

Besides factors related to seed lots, we included “pre-treatments” and “temperature” as factors to explain seed viability and germination. Specifically, to verify the presence of physiological dormancy (PD), the following pre-treatments were applied: warm (W: 3 months at 25°C), cold (C: 3 months at 5°C); as well as two combined warm and cold stratifications (W+C and C+W), and control (0), with no pre-treatment. After pre-treatments, seeds were incubated, with an irradiance of 12 h per day, at four constant temperatures: 10ºC, 15ºC, 20°C, 25°C as well as at alternating temperature regime 25⁄10°C. Three replicates of 30 seeds each were sowed in 90-mm diameter plastic Petri dishes with a substrate of 1% water agar. The experimental design was constituted by 3 replicates x 4 localities x 2 seasons x 2 sources x 5 pretreatments x 5 temperatures. However, due the low seed availability (autumn) only 3 pre-treatments (W, C and control) were carried out for seeds belonging to Arbus population (for plant and soil).

When no additional germination occurred for 15 days, tests were ended. The viability of the remaining seeds was assessed by a cut test (ISTA, 2006) and final number of germinated seeds calculated on the basis of the total number of filled seeds. Therefore seed viability was assessed as the sum of germinated and viable non germinated seeds.

Data analysis

Seed viability and germination percentages were modeled with Generalized Linear-Mixed Models (GLMM), using a binomial error distribution and logit link function. To estimate model parameters the Laplace approximation of likelihood was used (see Bolker *et al.*, 2009). In order to model seed viability, predictors included “population” as random factor, and “pre-treatment”, “temperature”, “season” and “source” as fixed factors. Seed germination was modeled including “source” within “population” as random factors, and “pre-treatment”, “temperature” and “season” as fixed factors. Germination models were performed using the overall data set, as well as from the data of each season separately in order to better understand effects of pre-treatments by season. Throughout the text, overall means are followed by standard error (± SE). All the statistical analyses were performed using the R 14.6 statistical package (R Development Core Team 2009).

Results

Viability

Seed viability was generally low, with seeds showing an overall mean viability of ca. 40%. Seed viability varied significantly according to the applied pre-treatments and the incubation temperatures as well as the season of collecting, while the source factor had not a significant effect (Table 2). In particular, the season factor showed the highest estimate, with seeds collected in autumn being less viable than those collected in spring, with mean values of 34.18 ± 0.62%

and 42.77 ± 0.52%, respectively (Figure 1). All pre-treatments had a negative effect on seed viability respect to the control which viability was 43.57 ± 0.85%.

Table 2 – Generalized Linear Mixed Model (GLMM) results, for the effect on seed viability of the following fixed factors: temperature, pre-treatment, season and source. Population was considered as random factor (Variance: 0.0767; SD: 0.2769). Akaike information criterion (AIC): 7595; Bayesian or Schwarz information criterion (BIC): 7656; logLik: -3786; deviance: 7571. NS: not significant; *:0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001).

Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-0.5249	0.1399	-3.752	0.0002 ***
T 15°C	-0.0054	0.0193	-0.280	0.7798 NS
T 20°C	-0.1304	0.0194	-6.725	$1.76e^{-11}$ ***
T 25°C	-0.3484	0.0197	-17.706	$< 2e^{-16}$ ***
T 25/10°C	-0.1892	0.0195	-9.723	$< 2e^{-16}$ ***
CW	-0.1630	0.0199	-8.185	$2.72e^{-16}$ ***
0	0.1916	0.0188	10.167	$< 2e^{-16}$ ***
W	-0.0509	0.0190	-2.675	0.0075 **
WC	-0.1522	0.0199	-7.647	$2.06e^{-14}$ ***
spring	0.3878	0.0125	30.938	$< 2e^{-16}$ ***
soil	0.0118	0.0124	0.956	0.3389 NS

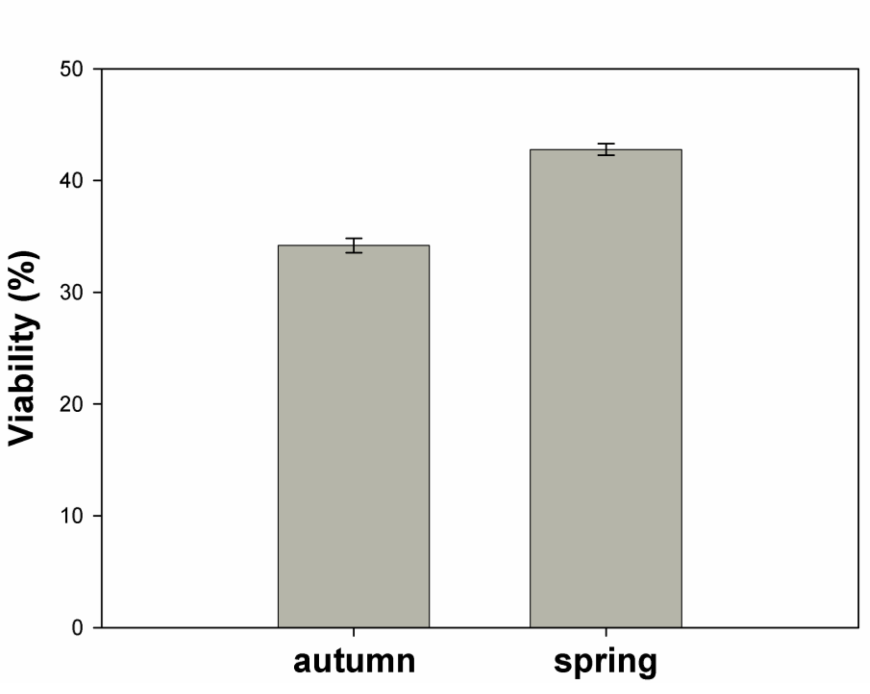

Figure 1 – Viability (mean ± SE) for seeds collected in the two seasons. P < 0.001 by GLMM (see Table 2).

Germination

Seed germination was low at all the tested condition, with an overall mean value of ca. 10% and never higher than ca. 50%, in accordance with the seed viability results. Due to the not statistically significant effect identified for the source factor in seed viability, this factor was considered as random in the analysis of the germination results (Table 3). All the fixed factors had a significant effect on seed germination, although the highest estimates were recorded for the applied pre-treatments, with the control (0) and the warm stratification (W), being the most effectives on stimulating germination, with mean percentages of 13.47 ± 0.74% and 13.61 ± 0.60%, respectively, while cold stratification (C) negatively affected germination (4.96 ± 0.46%). According to the statistical model, the best temperatures for germination were the constant 15 (11.35 ± 0.72%) and 20°C (10.72 ± 0.67%) and alternating temperature regime 25/10°C (9.95 ± 0.62%), while lower values were reached at the extreme constant temperatures of 10 (6.91 ± 0.50%) and 25°C (4.74 ± 0.38%). Regarding the season factor, the spring showed a positive significant effect on germination (Table 3), with mean values of 10.68 ± 0.41% and 6.57 ± 0.34% for spring and autumn, respectively.

Table 3 – Generalized Linear Mixed Model (GLMM) results, for the effect on seed germination of the following fixed factors: temperature, pre-treatment and season. Population (Variance: 0.0134; SD: 0.1157) and source nested within population (Variance: 0.2151; 0.4638) were considered as random factors. AIC: 7119; BIC: 7179; logLik: -3547; deviance: 7095.

Fixed effects	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Intercept	-3.6404	0.1788	20.36	$< 2e^{-16}$ ***
T 15°C	0.5725	0.0342	16.72	$< 2e^{-16}$ ***
T 20°C	0.5067	0.0346	14.66	$< 2e^{-16}$ ***
T 25°C	-0.4183	0.0414	-10.10	$< 2e^{-16}$ ***
T 25/10°C	0.4172	0.0350	11.91	$< 2e^{-16}$ ***
CW	-0.2358	0.0468	-5.04	$4.68e^{-07}$ ***
0	1.1290	0.0357	31.59	$< 2e^{-16}$ ***
W	1.1424	0.0357	32.01	$< 2e^{-16}$ ***
WC	0.3480	0.0411	8.48	$< 2e^{-16}$ ***
spring	0.5668	0.0225	25.18	$< 2e^{-16}$ ***

When analysing the results separately for season the same trend was detected, with all the fixed effects being statistically significant and the highest estimates recorded for 0 and W pretreatments and at the incubation temperatures of 15, 20 and 25/10°C, both in autumn and spring (Table 4).

Table 4 – Generalized Linear Mixed Model (GLMM) results on seeds collected in autumn and spring, respectively, for the effect on seed germination of the following fixed factors: temperature and pre-treatment. population (Variance: 5.1640^{-09} and SD: 7.1861^{-05}, Variance: 3.7511^{-11} and SD: 6.1247^{-06} for autumn and spring, respectively) and source (nested within population; Variance: 1.5084^{-01} and SD: 3.8838^{-01}, Variance: 3.9112^{-01} and SD: 6.2540^{-01} for autumn and spring, respectively) were considered as random factors. Autumn =AIC: 2747; BIC: 2794; logLik: -1362; deviance: 2725; spring = AIC: 3467; BIC: 3516; logLik: -1723; deviance: 3445.

Season	Fixed effects	Estimate	Std. Error	z value	Pr(>|z|)
autumn	Intercept	-4.1927	0.1570	-26.706	$< 2e^{-16}$ ***
	T 15°C	0.7855	0.0552	14.217	$< 2e^{-16}$ ***
	T 20°C	0.5008	0.0574	8.722	$< 2e^{-16}$ ***
	T 25°C	-0.7629	0.0764	-9.985	$< 2e^{-16}$ ***
	T 25/10°C	0.3583	0.0587	6.100	$1.06e^{-09}$ ***
	CW	-0.2604	0.1067	-2.440	0.0147 *
	0	1.8355	0.0699	26.245	$< 2e^{-16}$ ***
	W	1.8229	0.0700	26.044	$< 2e^{-16}$ ***
	WC	0.7854	0.0832	9.440	$< 2e^{-16}$ ***
spring	Intercept	-2.8891	0.2260	-12.782	$< 2e^{-16}$ ***
	T 15°C	0.4359	0.0440	9.913	$< 2e^{-16}$ ***
	T 20°C	0.5153	0.0435	11.848	$< 2e^{-16}$ ***
	T 25°C	-0.2674	0.0500	-5.351	$8.74e^{-08}$ ***
	T 25/10°C	0.4545	0.0439	10.364	$< 2e^{-16}$ ***
	CW	-0.3365	0.0527	-6.387	$1.69e^{-10}$ ***
	0	0.7951	0.0433	18.371	$< 2e^{-16}$ ***
	W	0.8291	0.0431	19.231	$< 2e^{-16}$ ***
	WC	0.1175	0.0478	2.458	0.014 *

In particular, while in autumn the highest germination percentages were 11.59 ± 0.97% and 11.44 ± 0.80%, for 0 and W pre-treatments, respectively, these values reached 15.36 ± 1.09% and 15.79 ± 0.85%, respectively, in spring (Figure 2). Seeds collected in spring were able to germinate at higher percentages respect to those collected in autumn also after pretreatments that negatively affected germination like CW and C (Table 3), with mean percentages increasing from ca. 2 to 8% (Figure 2).

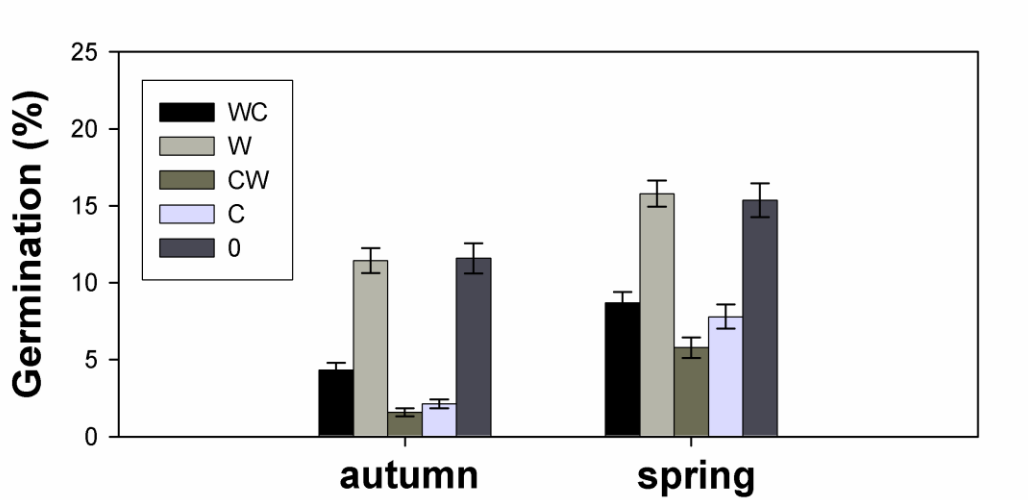

Figure 2 – Germination percentages (mean ± SE) after each pre-treatment for seeds collected in the two seasons (autumn and spring).

The effects of incubation temperatures on seed germination for each season are showed in Figure 3, with seeds germinating to ca. 20% at 10°C, irrespective of the season, while the positive effect of the season was more evident at 20 and 25/10°C reaching ca. 20% (Figure 3).

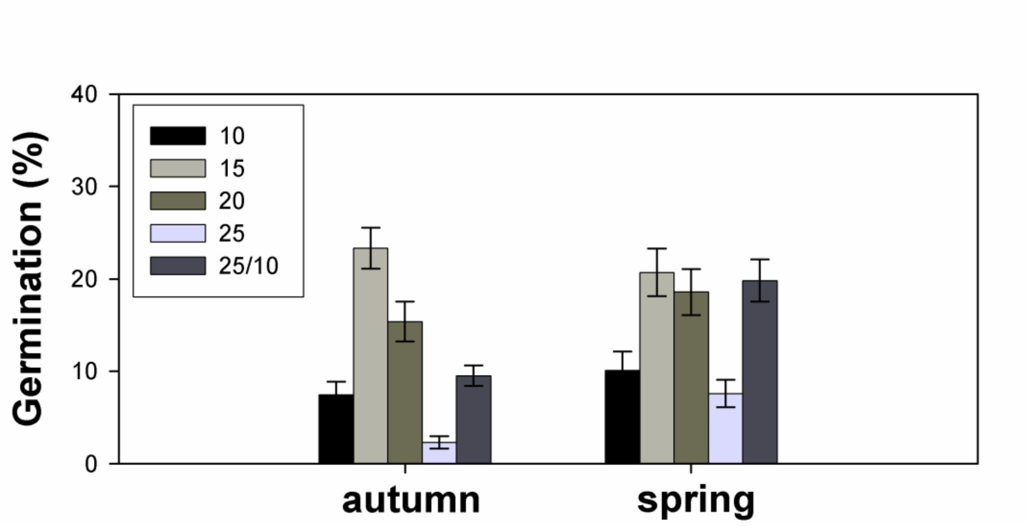

Figure 3 - Germination percentages (mean ± SE) at different temperatures for seeds collected in the two seasons (autumn and spring) and incubated without any pre-treatment (i.e. 0).

Discussion

The low viability of *J. macrocarpa* seeds highlighted in this work is in agreement with the values showed in other studies carried out on this species. In particular, Juan *et al.* (2003, 2006) indicated low values of viable seeds (< 12%), while Pacini and Piotto (2004) reported percentages of viable seeds ranging from 20% to 66.7%. Viability values comparable to *J. macrocarpa* seeds were also obtained for *J. oxycedrus* (< 50%; Pardo and Lázaro, 1983; Pacini and Piotto, 2004), and for *J. oxycedrus* var. *badia* H. Gay (2.5%; Jordan de Urriés, 1997). Moreover, other species such as *J. communis* presented low values of viability, both in the Mediterranean area (from 1.3% to 33.3%; Pacini and Piotto, 2004) and in northwestern European populations (from 0.10% to 5.49%; Vanden-Broeck *et al*., 2011). Rumeu *et al.* (2009), in a previous work on two Macaronesian endemic cedars, found low values in viable seeds of *J. cedrus* Webb & Berthel. (27%) and *J. brevifolia* Antoine (44.7%). In agreement with these results, also *J. thurifera* L. seeds showed low viability (9.5%; Montesinos *et al.*, 2010). Instead, higher average seed viability values (from 57% to 82%) were found in *J. scopulorum* Sarg. (Rietveld, 1989) and in *J. pinchotii* (100% viability in filled seeds; Petersen *et al.*, 2005). The results achieved in this study, as well as those reported in literature, confirmed that a low production of viable seeds is one of the principal ecological problems in Cupressaceae (Colangeli and Owens, 1990; Owens and Schliesing, 1995; Ortiz *et al*., 1998).

The mechanisms behind this low seed viability remain largely unclear (Gruwez *et al*., 2013). Fenner and Thompson (2005) considered the competition for nutrients among cones an explanation for low seeds viability. The higher number of filled non viable compared to empty seeds in *J. macrocarpa*, seems attributable to abortions after fecundation (Pacini and Piotto, 2004). In fact, junipers are pioneer plants that colonize environments subjected to stress and lack of resources, which can determine the death of developing zygotes during the long reproductive cycle of *J. macrocarpa* (Pacini and Piotto, 2004), and in particular the water stress can be an important limitation for seed viability (Montesinos *et al*., 2010). The low values of *J. macrocarpa* seeds viability, constitute a key factor limiting juniper recruitment (García, 2001), because dramatically reduces the potential number of diaspores capable of germinating (Wesche *et al.*, 2005). The results obtained in this work highlighted that the source factor had not a significant effect on the viability of *J. macrocarpa* seeds, while viability varied significantly according to the collection season, showing lower percentages for seeds collected in autumn than in spring. The differences of viability seeds at seasonal level might be caused by different temperature conditions and water availability that occur during the seeds development. This process begins in summer with the fertilization of female cones and ends in the next summer through the embryo maturation (Pacini and Piotto, 2004).

The results of this study, and in particular the gap between viability and germination percentages, suggest that seeds of *J. macrocarpa* are dormant. None of the applied pre-treatments improved germination or widened the range of germination temperatures. Several authors previously reported seeds of *Juniperus* as deeply dormant. In particular, Pacini and Piotto (2004) argued that the majority of the applied treatments to *J. macrocarpa* seeds did not remove the dormancy because these have conditions of very deep dormancy. Furthermore, Pardo and Lazaro (1983) suggested that *J. oxycedrus* seeds have a double dormancy feature involving both endogenous and exogenous factors. Further studies should be therefore carried out on *J. macrocarpa* seeds in order to detect the class, type and level of dormancy *sensu* Baskin and Baskin (2004).

Contradictory results are reported in literature on the effects of pre-treatments on seed germination for species belonging to the *Juniperus* genus (Mc Tavish and Shoplik, 1983) and in particular, few studies were carried out on *J. macrocarpa*. In this study the most effective pretreatment was the warm stratification (W) although the germination percentage was similar to seeds germinating without any pretreatment (control, 0). The high germination percentages, detected after W is in contrast with the findings of Livingston (1972) which found that warm stratification was totally ineffective for *J. virginiana,* a species growing in pasture areas of New England, characterized by stony ground and summer droughts. On the contrary, cold stratification (C) negatively affected germination also in combination with warm stratification (CW and WC treatments).

The use of cold stratification gave contradictory results in previous studies. Pacini and Piotto (2004) found that it was totally ineffective for *J. oxycedrus*, *J. macrocarpa* and *J. communis*, whereas increased germination of *J. phoenicea* L. (Al-Ramamneh *et al*., 2012). This treatment was effective also for seed germination of mountain *Juniperus* species such as *J. excelsa* from East Mediterranean and Caucasus area (Jones, 1989) and *J. ashei* Buchh., *J. deppeana* Steud., *J. monosperma* (Engelm.) Sarg. and *J. virginiana* (Taylor, 1941; Barton, 1951; Johnsen and Alexander, 1974; Benson, 1976), from Mexico and US. A detrimental effect of cold stratification was also detected for another typical Mediterranean conifer (*Pinus halepensis* Mill.), with seeds showing a slight but steady decline of germination after increased pre-chilling periods, due to a loss of viability (Skordilis and Thanos, 1995). However, a higher loss in viability after cold stratification than after the other pre-treatments was not detected in this study, suggesting the presence of secondary dormancy. This negative response to low temperatures is in agreement with the Mediterranean origin of the species, as seed stratification in a cold-moist medium at 5°C is especially recommended to overcome dormancy in species from temperate regions (Baskin and Baskin, 2004). Secondary dormancy was previously detected for other *Juniperus* species. In particular, Tylkowski (2009) suggested that in *J. communis* seeds a warm spring may induce secondary dormancy and Pack (1921) found that after stratification, dormancy was induced in seeds of mountain species of *Juniperus* (*J. virginiana*, *J. depressa* Raf., *J. communis* and *J. prostrata* Pers.) when they were kept at >12°C. Instead, Barbour and Carvaiho (2009) state that the *J. scopulorum* seeds go into secondary dormancy when dried out.

Germination in a narrow range of temperatures (i.e. 15-20°C) and at a very slow rate are features detected for *J. macrocarpa* seeds that in agreement with Doussi and Thanos (2002) could be associated with autumnal/wintry seed germination and seedling establishment. These authors considered that this strategy is ecologically advantageous and tuned to take place into the Mediterranean climate, characterized by a considerable unpredictability of precipitation. This temperature requirement evolved as an adaptation to autumnal temperatures of the Mediterranean climate. Seed germination occur during the rainy season so that the developing seedlings exploit moist of the mild winter and following spring prior to the harsh and water stressed conditions of summer (Thanos *et al.*,1991). These results are consistent with values obtained from Piotto and Di Noi (2001) for germination of *J. phoenicea*. Highest germination probability was recorded at 20°C also for *J. procera* and *J. communis* (Yirdaw and Leinonen, 2002; Bonner, 2008). Bonner (2008) indicated 15°C as recommended germination temperature for *J. virginiana* and *J. scopulorum*. While 18°C was found to be the most favorable temperature for *J. pinchotii* seeds germination (Smith *et al*., 1975).

According to the results achieved in laboratory conditions after the different applied pre-treatments, the phenology of *J. macrocarpa* germination may be graphically summarized as in Figure 4. The galbules of *J. macrocarpa* do not ripe simultaneously, but their ripening and dispersal is distributed from autumn to spring. This is in contrast with the statement of Pacini and Piotto (2004) who limited this period to October till January. The seed dormancy detected for this species is a strategy that increases the reproductive success of the species, allowing the occurrence of favourable conditions for germination (Pacini, 1995). In particular, it allows the formation of a soil seed bank, that represents a source of new individuals for potential colonization (Pacini and Piotto, 2004). *Juniperus macrocarpa* seed germination occurs all along the seed dispersal during the rainy season, from autumn (mean temperature of ca. 18°C and mean precipitations of ca. 59 mm; Figure 4) to the beginning of spring (mean temperature of ca. 15°C and mean precipitations of ca. 37 mm; Figure 4) so that the developing seedlings benefit of the moist conditions of the mild winter (mean temperature of ca. 11°C and mean precipitations of ca. 63 mm; Figure 4) and following spring. At the same time the gap between dispersed and germinated viable seeds (due to their dormancy) allows the soil seed bank to be established and improved, with germination being the sum of newly dispersed and buried seeds (Figure 4). Late spring germination is limited by the increasing temperatures and by the decreasing rainfall which precede the drought of the summer when no germination may occur due to the high temperatures and aridity (mean summer temperatures of ca. 23°C and summer precipitations of ca. 7 mm; Figure 4).

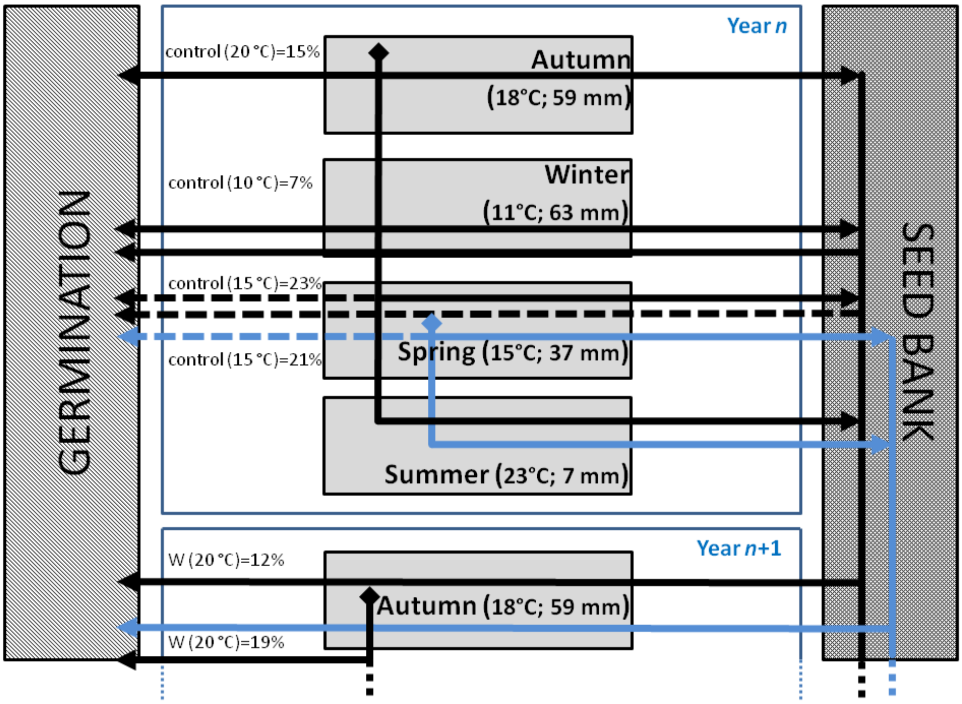

Figure 4 - Phenology of *J. macrocarpa* germination. For each season, mean values of temperatures and rainfall are reported. These mean values were obtained as an average of the climatic data of the four sites acquired using the GIS software from available data at a spatial resolution of one square kilometer, downloaded from the WorldClim website (http://www.worldclim.org) as documented by Hijmans *et al.* (2005). On the left, the mean germination percentages achieved in laboratory at different temperatures and after different pretreatments that mimic the correspondent seasons for both autumn and spring dispersed seeds are also reported. Germination event for which favourable conditions of both temperature and rainfall are indicated by continuous lines, whereas long dashed lines represent germination event that are limited by water availability (rainfall).

The results presented in this study could have direct implications for improving *in situ* conservation actions such as population reinforcement and regeneration of *J. macrocarpa*.

Moreover, they have implications for the planning, management and development of *ex situ* conservation and, consequently, of the fragile and threatened ecosystems of juniper woodlands. Considering the highest values of viability and germination obtained from seeds collected in spring, this *taxon* should be regenerated using galbules collected in this season to increase the chances of success in the reproduction of the species under controlled (greenhouse and nursery) or natural conditions. Mature galbules may be collected both from plant and soil as no differences on seed viability and germination were detected for the source of seeds. The sowing could be performed both with fresh seeds and after a warm treatment. Instead, the autumn should be privileged for sowing in field in agreement with Picchi (2008), and according to the results achieved in this study, as well as considering the higher success rate in function of seedlings survival and establishment (Piotto and Di Noi, 2001).

Seeds of *J. macrocarpa* are characterized by low values of viability and germination and high levels of dormancy. The applied pretreatments were not able to overcome the detected primary and secondary dormancy, highlighting the need of further studies. A germination phenology all along the dispersal season (from autumn to spring), as well as the potentiality of this species to create a soil seed bank as previously reported by Crosti and Piotto (2006), were illustrated. The narrow range of germination temperatures (15-20°C) and the slow germination rate detected for seeds of this species are ecologically advantageous and showed a good level of adaptation to the Mediterranean climate, characterized by a considerable unpredictability of precipitations (Doussi and Thanos, 2002). Spring was identified as the best season for seed collection whereas autumn for sowing in the field, giving new findings for the recovery and conservation planning of this species.

Acknowledgements

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CHAPTER 3

Factors affecting emergence, survival and growth of *Juniperus macrocarpa* Sm. seedlings

Maria Silvia Pinna, Eva Cañadas, Cristiano Ponetcorvo, Gianluigi Bacchetta

Abstract

The initial phases in the life cycle of *Juniperus macrocarpa* are still poorly understood. In this study factors affecting emergence, survival and growth of *J. macrocarpa* seedlings were investigated. Permanent plots were placed and periodically monitored from 2010 to 2012. Within them, seedling parameters such as emergence, survival and growth and several biotic and abiotic variables (solar radiation, tree cover, herbaceous plus scrub cover, distance from the closer *J. macrocarpa* female, number of galbules on the soil and event number of herbivore trace) were measured. Linear mixed-effects models were used to test the relative importance of different groups of explanatory variables on seedling parameters. A total of 536 seedlings were marked, most of which emerged in winter. The "microclimate" model was the best fit explaining emergence, highlighting the positive relation between number of emerged seedlings and tree cover. Survival was very low and most of the seedlings died in the first months from emergence, reaching the highest mortality rate in first summer. High values of herbivory and solar irradiation increased mortality. Our results confirmed that *J. macrocarpa* is a slow growing species and no seedling reached the subsequent size class after two years. Moreover, seedling growth depended on suitable microhabitats, and in particular it was positively related to tree cover, hours of sunlight, and herbaceous plus scrub cover. In conclusion, the recruitment of *J. macrocarpa* was highly limited in all initial phases of its life cycle. Therefore, the identification of the critical stages in the recruitment and factors influencing them have direct implications for improving recovery and *in situ* conservation actions, such as methods for introducing seeds or plants (sowing/planting), suitable period to do so, or the use of complementary techniques.

Key words: life cycle, Linear mixed-effects models, Mediterranean area, recruitment, sandy coastal ecosystems, Sardinia.

Introduction

The transition period from seed germination to seedling establishment is the most critical stage in the life cycle of vascular plants, consequently a large number of studies have dealt with this issue (e.g. Harper, 1977; Kitajima and Fenner, 2000; Castro *et al*., 2005; Barberá *et al*., 2006; Mendoza *et al.*, 2009). Numerous abiotic and biotic constrains affect plant emergence, seedling survival and establishment, such as litter (Facelli and Pickett, 1991; Molofsky and Augspurger, 1992), light (Augspurger, 1984), temperature (Callaway, 1995), soil moisture (Herrera *et al*., 1994; Kobe *et al*., 1995; Nicotra *et al*., 1999), microhabitat (Russell and Schupp, 1998; Gómez-Aparicio *et al*., 2005), microtopography (Huenneke and Sharitz, 1986), seed arrival (Dalling *et al*., 2002; Russo and Augspurger, 2004), herbivory (Ostfeld and Canham, 1993; Gómez *et al.*, 2003), pathogens (Augspurger, 1984; Packer and Clay, 2000) and competition with herbs (De Steven, 1991). Seedling dynamics in Mediterranean ecosystems are largely conditioned by the particularities of the Mediterranean climate, such as the strong seasonal alternation of favourable and unfavourable conditions (Debussche and Isenmann, 1994), that reduces efficiency of several processes involved in plant regeneration (Gulias *et al*., 2004). Nonetheless, the main abiotic factor constraining establishment of woody species in Mediterranean-type ecosystems is usually summer drought (Dunne and Parker, 1999; Rey and Alcántara, 2000; Castro *et al*., 2002a, b), together with the short duration of periods in which temperature and humidity are suitable for plant growth (Gulias *et al*., 2004). This is why, under the Mediterranean climate, the presence of vegetation, may increase seedling emergence and survival, defending against high radiation, temperatures and loss of soil moisture (Callaway, 1995; Castro *et al*., 2002a; Gómez *et al*., 2004).

Limitations for seedling establishment increase in special environments such as coastal sand dunes, where water and nutrient stress, lack of moisture, sand accretion, and salt spray are additional constraints. Moreover, the spatial and temporal variation in the dune substrate, together with micro-environmental variability mediated by wind and wave action, create rather harsh and uncertain conditions (Maun, 1994).

Besides environmental limitations for regeneration, coastal dune ecosystems are subjected to numerous human impacts such us off-road vehicles circulation, trampling or beach cleaning. In addition, the presence of recreational structures or touristic activities that limit and/or impede natural sand transport or alter the sand budget, lead to severe erosion often permanent (Brown and McLachlan, 2002; Defeo *et al.*, 2009). In fact, the coastal ecosystems are among those most threatened in the Mediterranean Basin (UNEP, 2003). Specifically, the micro-forests dominated by *Juniperus macrocarpa* Sm. are in these circumstances, and they have been listed as priority habitat (2250 "Coastal dunes with *Juniperus* spp.") in the DIR. 92/43/CEE (European Commission, 2007). This habitat is often dominated by adult and senescent individuals of *J. macrocarpa* (Muñoz Reinoso, 2003), consistently with the results showed by other *Juniperus* species such as *J. communis* L. by García *et al*. (1999), who pointed out that the conservation of this species in Mediterranean populations is just due to great individual longevity.

The regeneration problems for *J. macrocarpa* are showed from seed stage, since we found a low viability and germination rate (see Chapter 2), consistently with other studies (Cantos *et al*., 1998; Pacini and Piotto, 2004; Juan *et al*., 2006). This low success in germination stage is shared with other *Juniperus taxa* such us *J. oxycedrus* L. (Ortiz *et al*., 1998) and *J. communis* (García *et al.*, 2001). Germination can be delayed for several years due to impermeable seed coats, embryo dormancy, or the presence of inhibitors (Chambers *et al*., 1999). In addition, predation of galbules and seeds and presence of parasites limited seeds availability (e.g. Roques *et al*., 1984; García, 1998).

Successive stage, from emergence to seedling establishment, has been studied for some species of *Juniperus* genus (García *et al*., 1999; Joy and Young, 2002; Montesinos *et al*., 2007; Armas and Pugnaire, 2009; Jovellar *et al*., 2013). Generally, it has been found a low recruitment, that can be attributed to reproductive problems (e.g. Chambers *et al*., 1999; García *et al*., 2000), and to summer aridity that may limit the survival of seedlings (García *et al*., 1999), among other factors. In particular, in coastal areas, heat and water stress were the most common causes of seedling mortality, as demonstrated for *J. virginiana* L. by Joy and Young (2002). Therefore, seedling establishment probabilities of *Juniperus* depend on seeds being dispersed to suitable microhabitats (Chambers *et al*., 1999). For example, Armas and Pugnaire (2009) recorded that survival of *J. phoenicea* L. seedlings was enhanced under canopy in dune habitats. However, cover showed positive effect on growth in different environments (Miller and Rose, 1995; Callaway *et al*., 1996). Indeed, the light-demanding character of *J. oxycedrus* seems to be especially important during the early stages of growth (Jovellar *et al*., 2013). Also radiation was one of the most important variables predicting *J. oxycedrus* distribution according to Rupprecht *et al.* (2011). In addition, Jovellar *et al.* (2013) found limitations to *J. oxycedrus* seedlings due of resource competition, in agreement with Sevilla-Martínez (2008).

Hence, few studies explored the influence of environmental factors on seedling growth and mortality in *Juniperus* woodlands in dune ecosystems, and their role in community replacement or succession (Mckinley and Van Auken, 2005). Likewise, little is known about the initial phase in the life cycle of *J. macrocarpa*. In particular, Juan *et al*. (2006) performed a study using seeds from both mature and immature *J. macrocarpa* galbules, both under greenhouse and in natural conditions. These authors obtained the higher percentage of seedling emergence with the untreated seeds, and found that substrate moisture was essential during seedling emergence.

In this study, we evaluated factors affecting critical stages in the recruitment process of *Juniperus macrocarpa* seedlings, in southern Sardinia. The specific aims of this study were: (1) to investigate factors affecting emergence, survival, and growth of *J. macrocarpa* seedlings; (2) to provide tools for conservation and recovery of the fragile and threatened ecosystems characterised by this *taxon*.

Materials and methods

Study species and area

Juniperus macrocarpa is a dioecious species whose female individuals require two years to develop mature galbules. It is possible to find galbules at different stages of maturity on the same plant simultaneously (Juan *et al*., 2006) and their ripening and dispersal is distributed to October till January (Pacini and Piotto, 2004). It is a small tree or shrub typical of coastal environments in the Mediterranean region (Jalas and Suominen, 1973, Amaral Franco, 1986). *J. macrocarpa* woodlands are undergoing severe regression due to human pressure, remaining reduced populations in small isolated patches (Juan *et al*., 2006). Among the characteristics of this *taxon* may be noted that it is resistant to salt and sand-laden marine winds (Géhu *et al.*, 1990), it is adapted to substrate movement (García Novo and Merino, 1993) and it is related to a stabilized dunes (Allier, 1975).

Sardinia is situated in the western Mediterranean basin (38° 51’ and 41° 15’ latitude north, 8° 8’ and 9° 50’ east longitude), covering ca. 24.090 km^2. The total coastal length of the Island is 1.896 km, 24% of this (458 km) consist of low, sandy or pebbly shores (Atzeni *et al.*, 2000). We selected for the study four populations where the species is well represented, they correspond to four Sites of Community Importance (SCI) of southern Sardinia [Porto Campana, ITB042230 (Domus de Maria); Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu, ITB040020 (Villasimius); Is Compinxius-Campo dunale di Buggerru-Portixeddu, ITB042249 (Buggerru) and Da Piscinas Riu Scivu, ITB040071 (Arbus)].

Sampling design and data collection

In the study areas 44 random permanent plots of 1 x 1 m were placed (11 plots/population). For each plot, the distance from the closer *J. macrocarpa* female was measured. Furthermore, the global solar irradiation, including both annual hours (sunlight, hereafter) and annual Watts per square meter (solar irradiation, hereafter), was calculated on the basis of the data of exposure, slopes, shadow cones, coordinates, and considering the mean transparencies of the atmosphere, recorded by the nearest weather stations in different periods of the year (Gautam and Kaushika, 2002).

The plots were periodically monitored every three months, during 11 events from 2010 to 2012. All *J. macrocarpa* seedlings emerged inside the plots were marked to record their emergence, survival and growth. To calculate growth, we measured seedling height. We also measured the following parameters for each plot: cover percentage of plant (tree and shrub plus herbaceous cover), number of galbules on the soil, event number of herbivore trace (including excrements, trampling, and cut plants). For the analysis, we estimated mean values per plot of all these factors.

Data analysis

We used Linear Mixed-effects Model (LMM) to test the relative importance of different groups of explanatory variables on emergence and seedling growth, using “lme” function, which is included in the R nlme package (Pinheiro *et al*., 2011), meanwhile seedling survival was modeled by means of “lmer” function, included in the R lme4 package (Bates *et al*., 2012). Both functions fit linear mixed-effects models with specified mixtures of fixed effects and random effects. In particular, “lmer” allows for non-normal errors and non-constant variance, then, since survival is a binary response variable (dead or alive), we specified binomial error distribution and logit link function. Analysis were performed using the R 14.6 statistical package (R Development Core Team, 2009).

Firstly, we compared the following five basic models to study factors affecting seedling emergence: (1) a “null” model only with random effects (locality); (2) a “seed source” model, in which the fixed effects of distance from the closer *J. macrocarpa* female and number of galbules were added to the null model; (3) a “microclimate” model, in which the fixed effects of tree cover, solar irradiation (including both number of hours/year and W/m^2/year) were added to the null model; (4) a “competence” model, in which the fixed effect of shrub plus herbaceous cover was added to the null model; (5) a “herbivory” model, in which the fixed effect of herbivore trace was added to the null model. Secondly, we added to the best candidate model the other basic models or single variable, in order to select the most parsimonious models explaining seedling emergence on the basis of the explanatory variables acquired. The total number of new seedlings recorded in each plot from July 2010 to October 2012 was used as response variable.

Survival analysis was performed from two data sets: initially, from all new seedlings emerged since July 2010 and, secondly, for the specific cohort of seedling emerged at January 2011, which was the larger group of new seedling after the first year. Then, mixed-effects model were fitted to test the relative importance of different groups of explanatory variables on seedling survival. In a first step, we compared the following four basic models: (1) a “null” model only with random effects (locality); (2) a “microclimate” model; (3) a “competence” model; (4) a “herbivory” model, including in each one the fixed effect explain above. In a second step, similarly, we added progressively the other basic models or variables to select the best candidate model explaining *J. macrocarpa* survival.

In order to evaluate factors influencing seedling growth, in a similar way, we compared the following three basic models: (1) “null” model only with random effects (locality); (2) “microclimate” model; (3) “competence” model, as well as more complex models. As data set to model seedling growth we used the height growth of the survived seedlings emerged in January 2011

For statistical inferences, we employed model selection using Akaike’s information criterion (AIC), a likelihood based measure of model plausibility that penalizes more complex models, i.e. those with a higher number of parameters (Burnham and Anderson, 2002). Models were ranked using AIC, in which the best model, the one that sacrifices the least information when it is used to approximate the truth, had the lowest AIC value (Hobbs and Hilborn, 2006). The differences between the AIC value of the best model and the values of each model ranked below it (ΔAIC) provide information for evaluating which models in a set are as plausible as the best model. Values of ΔAIC between 0 and 2 indicate similar support (Burnham and Anderson, 2002).

Interaction terms were included in the models whenever significant interactions between variables were detected. Throughout the text, means are followed by Standard Error.

Results

Emergence

A total of 536 seedlings were marked from April 2010 to October 2012, over eleven monitoring events. Seedling emergence was not evenly distributed neither space nor time. Seedlings per plot ranged from 1 to 91 (13.4 ± 2.56). A total of 306 seedlings emerged from the second monitoring event, most of them emerged in winter (see Figure 1), with winter 2010 being particularly favorable for emergence (131 seedlings). The 230 remaining seedlings were in the first monitoring event, therefore it was not possible to know exactly the emergence time.

Regarding the factors explaining seedling emergence, there were significant differences in the explanatory power of each of the basic models performed, with the microclimate model being the best fit one (AIC = 362.6; Table 1). The microclimate model highlighted the significant positive relation between number of emerged seedlings and tree cover, as well as the significance of some interactions terms (Table 2).

However, the best basic model improved adding some other terms, with the optimal model predicting seedling emergence (AIC = 296.2) including all the variables of the three better basic models (microclimate + competence + herbivory; Table 2). Also the model composed by microclimate and competence variables showed a low AIC (312.3).

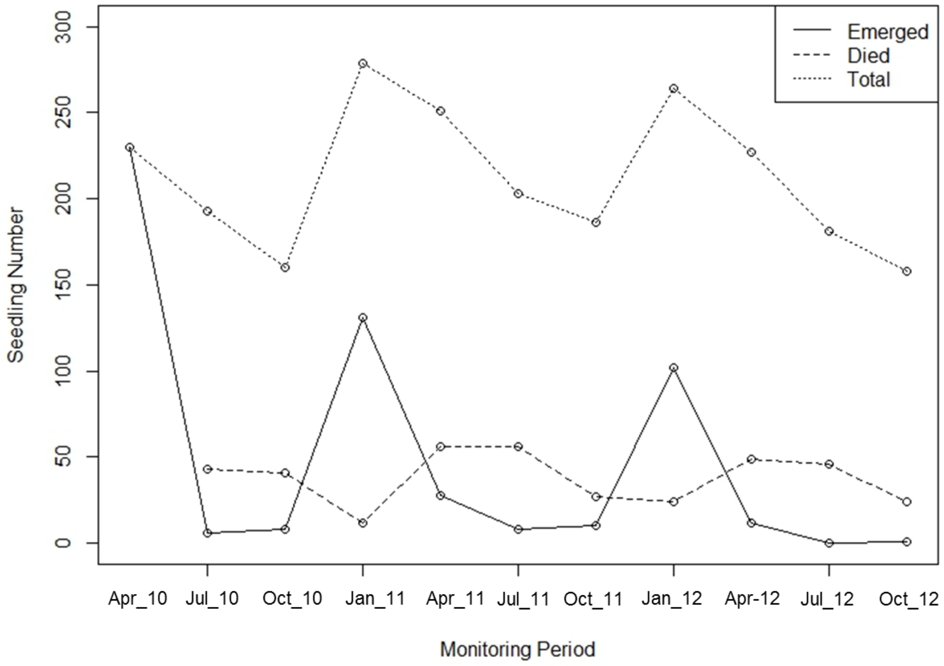

Figure 1 - Number of seedlings over the monitoring events.

Table 1 - AIC values of seedling emergence models.

Candidate models	Df	AIC	BIC	logLik
Null	3	369.4806	374.8331	-181.7403
Seed source (SS)	6	374.9297	385.6349	-181.4649
Microclimate (MC)	10	362.6171	380.4590	-171.3086
Competition (C)	4	369.3693	376.5060	-180.6846
Herbivory (H)	4	370.5820	377.7187	-181.2910
MC + SS	18	367.9423	400.0577	-165.9711
MC + C	18	312.3201	344.4356	-138.1601
MC + H	18	346.9576	379.0730	-155.4788
C+H	6	371.3266	382.0317	-179.6633
MC + C+H	34	296.1664	356.8289	-114.0832

Table 2 - Results from linear mixed-effects models on seedling emergence, including basic models and the best complex model. Final linear mixed-effects model fit by REML. Population was considered as random factor, StdDev: 1) Intercept: 8.1585, Residual: 14.2258; 2) Intercept: 7.8740, Residual: 14.7247; 3) Intercept: 0.0027, Residual: 13.1283; 4) Intercept: 7.4353, Residual: 14.1366; 5) Intercept: 7.7140, Residual: 14.3190; 6) Intercept: 0.0023, Residual: 6.1935. Female dist: distance from female individual; seedling N: seedling number; Galbules: number of galbules; Sun Watts: solar irradiation; Sun hours: sunlight; HS cover: herbaceous plus shrub cover.

	Value	Std. Error	DF	t-value	p-value
1) Null model					
Intercept	12.1818	4.6086	40	2.6432	0.0117
2) Seed source, Fixed effects: seedling N ~ Female dist * Galbules					
Intercept	13.4057	5.3383	37	2.5112	0.0165
Female dist	-0.4248	0.8510	37	-0.4992	0.6206
Galbules	-0.0113	0.0399	37	-0.2820	0.7795
Female dist : Galbules	-0.0464	0.1491	37	-0.31101	0.7575
3) Microclimate, Fixed effects: seedling N ~ Tree cover* Sun Watts * Sun hours					
Intercept	-22.7447	19.4743	33	-1.1679	0.2512
Tree cover	0.7848	0.2825	33	2.7777	0.0090
Sun Watts	$3e^{-6}$	$2\ e^{-5}$	33	0.1289	0.8982
Sun hours	0.0505	0.0275	33	1.8385	0.0750
Tree cover : Sun Watts	0.000000	0.000000	33	-0.9366	0.3557
Tree cover : Sun hours	-0.0010	0.0004	33	-2.4629	0.0192
Sun Watts : Sun hours	0.000000	0.000000	33	-2.2460	0.0315
Tree cover : Sun Watts: Sun hours	0.000000	0.000000	33	3.1179	0.0038
4) Competition, Fixed effects: seedling N ~ HS cover					
Intercept	8.6281	4.9752	39	1.73422	0.0908
HS cover	0.2872	0.2043	39	1.4059	0.1677
5) Herbivory, Fixed effects: seedling N ~ Herb					
Intercept	13.8450	4.8232	39	2.8705	0.0066
Herb	-0.8222	0.9544	39	-0.8615	0.3942
6) Best model, Fixed effects: seedling N ~ Tree cover* Sun Watts * Sun hours* HS cover* Herb					
Intercept	-31.2262	74.2384	9	-0.4206	0.6839
Tree cover	0.5910	0.9430	9	0.6267	0.5464
Sun Watts	$4\ e^{-5}$	$5\ e^{-5}$	9	0.8072	0.4403
Sun hours	0.0600	0.0808	9	0.7421	0.4769
HS cover	-0.3923	4.5895	9	-0.0855	0.9337
Herb	-11.8486	56.5396	9	-0.2096	0.8387

Survival

Seedling dynamic over the 11 monitoring events are showed in Figure 1. The three minima in the cumulative curve of seedlings were recorded in October, with the number of plants progressively decreasing from the emergence time to late summer.

Specifically, for the 306 new seedlings emerged since July 2010, 220 seedlings died by October 2012, with the survival being 27.21%. Among the basic models explaining seedling survival for this plant set (Table 3), the herbivory model showed higher explanatory power (AIC = 118.94), followed by the competence model (AIC = 121.45). Herbivory was positively related to seedling mortality (Estimate = 0.8312, p = 0.0082; Table 4), while herbaceous plus scrub cover was negatively related to mortality (Estimate = -0.0714, p = 0.0032). Regarding microclimate factors, high solar irradiation increase mortality risk, but sunlight were negatively related to mortality. In addition, the herbivory model improved adding other variables, with the optimal model including competence + herbivory variables (AIC = 113.27).

For the specific cohort of the 131 seedlings emerged at January 2011, 105 died by October 2012 (Figure 2), with the survival being 19.84%. Most of the seedlings died in the first months from emergence, reaching the highest mortality rate in first summer (Figure 3). After this season, mortality rate dropped, but it picked up in the second summer. First data on the cohort emerged at January 2012 showed a similar early trend.

Regarding factors influencing seedling survival of the January 2011 cohort, model comparison showed similar results to those performed on all seedlings monitored from July 2010. Specifically, the herbivory model (AIC = 118.9; Table 3) showed the highest explanatory power, followed by competence model (AIC = 121.4). Also more complex models improved these basic ones, with herbivory + competence model showing the lowest AIC (113.27).

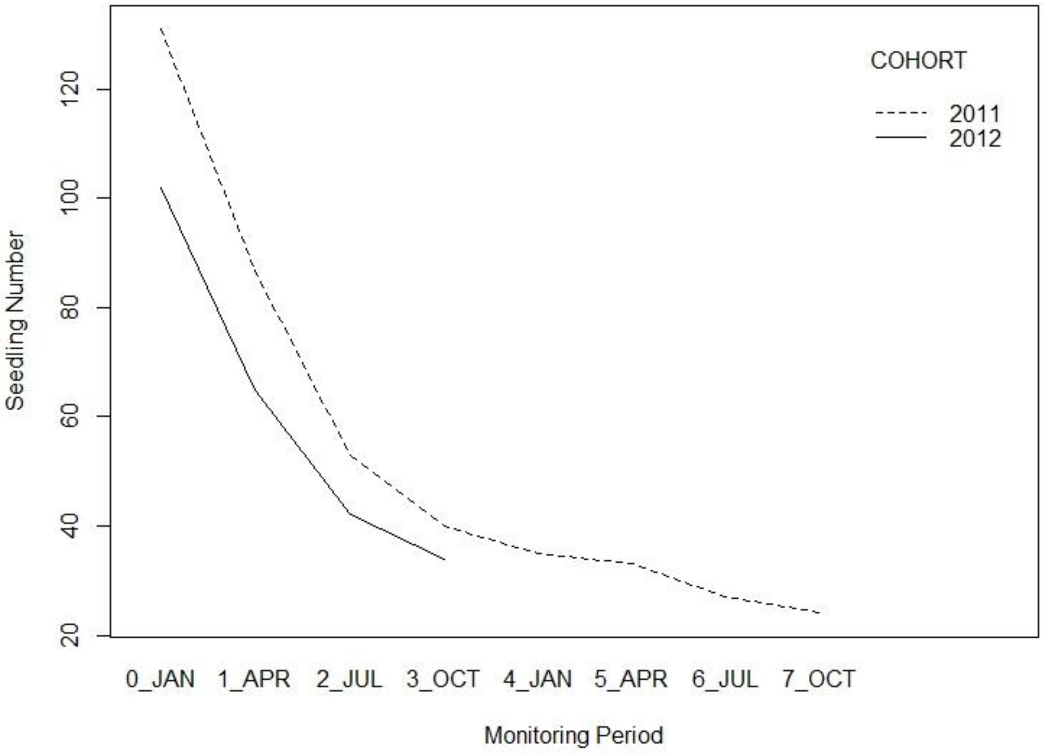

Figure 2 - Seedling number over the monitoring events for January 2011 cohort.

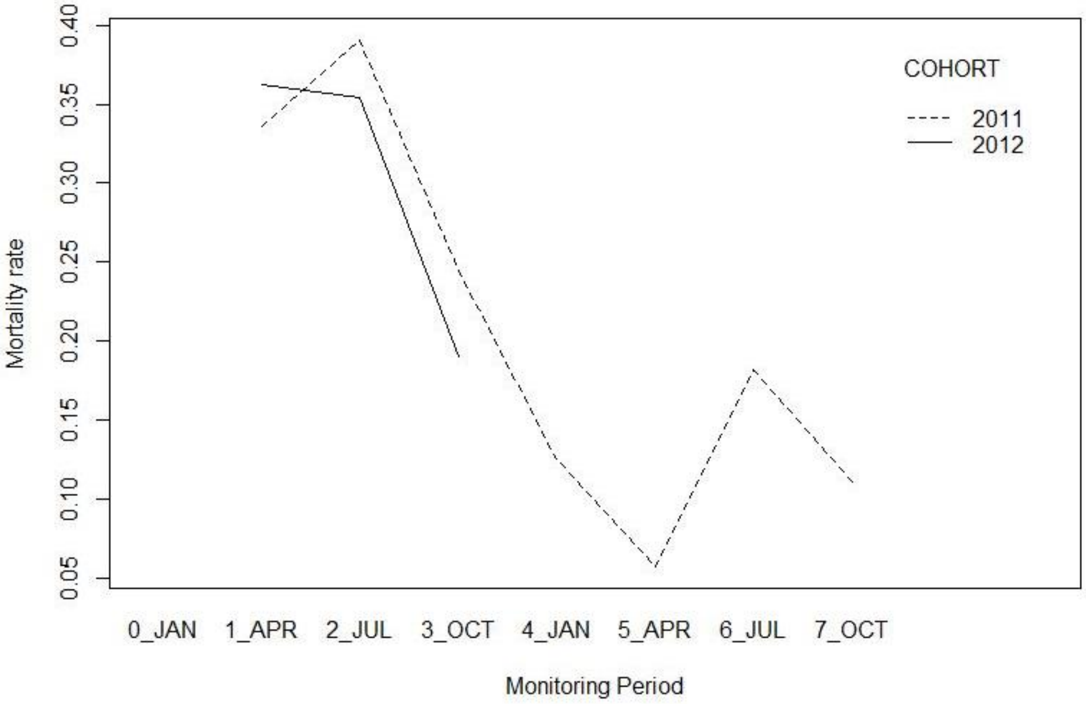

Figure 3 - Mortality rate over the monitoring events for January 2011 cohort.

Table 3 - AIC values of seedling survival models. Linear mixed-effects model fit by maximum likelihood. AIC: Akaike information criterion; BIC: Bayesian or Schwarz information criterion.

Candidate models	**Df**	**AIC**	**BIC**	**logLik**
Null	2	128.30	134.05	-62.15
Microclimate (MC)	3	128.85	137.47	-61.42
Competence (C)	3	121.45	130.07	-57.72
Herbivory (H)	3	118.94	127.57	-56.47
H+C	5	113.27	127.64	-51.63
H+MC	4	121.31	132.82	-56.66
C+MC	4	123.80	135.30	-57.90
H+C+MC	5	119.61	133.99	-54.81

Table 4 - Results from linear mixed-effects models on seedling survival, including basic models and the best complex model. Generalized linear mixed model fit by the Laplace approximation. Population was considered as random factor, 1) Variance: 0.1451; SD: 0.309; 2) Variance: 0.0814; SD: 0.2853; 3) Variance: 0; SD: 0; 4) Variance: 1.189e^{-15}; SD: 3.4482e^{-08}; 5) Variance: 0; SD: 0. Sun Watts: solar irradiation; HS cover: herbaceous and shrub cover; Herb: herbivory. NS: not significant; *:0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001. Status: died/alive.

	Estimate	**Std. Error**	**z value**	**Pr(>|z|)**
1) Null model				
Intercept	1.7132	0.3295	5.2	p < 0.001 ***
2) Microclimate, Fixed effects: Status ~ Sun Watts				
Intercept	1.204	3.678e-^{01}	3.273	0.0011 **
Sun Watts	7.459e-^{07}	5.259e-^{07}	1.418	0.1561 NS
3) Competition, Fixed effects: Status ~ HS cover				
Intercept	2.7641	0.5243	5.272	p < 0.001 ***
HS cover	-0.0714	0.0242	-2.952	0.0032 **
4) Herbivory, Fixed effects: Status ~ Herb				
Intercept	0.9612	0.2596	3.703	0.0002 ***
Herb	0.8312	0.3146	2.642	0.0082 **
5) Best model, Fixed effects: Status ~ HS cover + Herb				
Intercept	1.8813	0.6008	3.131	0.0017 **
HS cover	-0.0451	0.0254	-1.779	0.0752 .
Herb	0.6739	0.3292	2.047	0.0407 *

Growth

At October 2012, the mean height growth for the survival seedlings emerged in January 2011 was 4.27 ± 0.31 cm (maximum 7.0, minimum 1.5 cm).

Regarding variables influencing seedling growth, microclimate models showed lower AIC than competence model (AIC = 95.992; Table 5). In particular, the best microclimatic model (AIC = 92.189) included tree cover and sunlight, with tree cover positively related to growth (Value = 0.039, p = 0.043; Table 6). The sum of microclimatic and competence variables significantly improved the fit of the model (AIC = 83.838, p = 0.0004). The best composed model highlighted the significant positive relation between seedling growth and the three explicative variables (tree cover, sunlight, and cover of herbaceous plus scrub).

Table 5 - AIC values of seedling growth models. Linear mixed-effects model fit by maximum likelihood. AIC: Akaike information criterion; BIC: Bayesian or Schwarz information criterion.

Candidate models	Df	AIC	BIC	logLik
Null	3	93.996	97.770	-43.998
Microclimate (MC)	5	92.189	98.480	-41.095
Competence (C)	4	95.992	101.025	-43.996
MC+C	9	83.838	95.161	-32.919

Table 6 - Results from the linear mixed-effects models on seedling growth, including basic models and the best complex model. Final linear mixed-effects model fit by REML. Population was considered as random factor, StdDev: 1) Intercept: 1.4727, Residual: 1.1719; 2) Intercept: 1.2935; Residual: 1.1423; 3) Intercept: 1.4902; Residual: 1.1965; 4) Intercept: 0.0003; Residual: 0.9618. Sun hours: sunlight; HS cover: herbaceous and shrub cover.

	Value	Std. Error	DF	t-value	p-value
1) Null model					
Intercept	3.3682	0.9035	23	3.7278	0.0011
2) Microclimate, Fixed effects: Growth ~ Tree cover * Sun hours					
Intercept	0.6778	1.6401	20	0.4133	0.6838
Tree cover	0.0392	0.01820	20	2.1550	0.0435
Sun hours	0.0012	0.0007	20	1.6021	0.1248
Tree cover : Sun hours	-1.53e^{-5}	1.36 e^{-5}	20	-1.1203	0.2759
3) Competition, Fixed effects: Growth ~ HS cover					
Intercept	3.4220	1.0116	22	3.3828	0.0027
HS cover	-0.0029	0.0237	22	-0.1217	0.9043
4) Best model, Fixed effects: Growth ~ Tree cover * Sun hours * HS cover					
Intercept	8.9540	4.9508	16	-1.8086	0.0893
Tree cover	0.2022	0.0625	16	3.2357	0.0052
HS cover	0.5557	0.2590	16	2.1458	0.0476
Sun hours	0.0055	0.0021	16	2.6408	0.0178
Tree cover : HS cover	-0.0081	0.0032	16	-2.5082	0.0233
Tree cover : Sun hours	0.0002	3.5e^{-4}	16	-4.6524	0.0003
HS cover : Sun hours	-0.0002	0.0001	16	-2.2337	0.0401
Tree cover : HS cover: Sun hours	0.7 e^{-5}	0.2 e^{-5}	16	4.4628	0.0004

Discussion

Emergence

Our results suggest that seedling emergence of *Juniperus macrocarpa* depended on both temporal and environmental factors. The timing of seedling emergence is one of the key events in the life cycle of plants because it determines plant performance and success (Harper, 1977; Weiner, 1988). Specifically, we found that most of the seedlings emerged in winter, although we recorded seedling emergence in all seasons. According to local climatic conditions and our own results achieved in laboratory (see Chapter 2), *J. macrocarpa* seeds are able to germinate all along the seed dispersal during the rainy seasons, from autumn to the beginning of spring. In fact, a high number of new seedlings recorded in January probably germinated in autumn and in the early winter. Winter emergence represents an advantage, since the seedlings benefit of the moist conditions of the mild winter and the following spring (Thanos and Skordilis, 1987; Skordilis and Thanos, 1995; Thanos, 2000). In contrast, we found a very low number of new seedlings in summer and in the early autumn, as expected, but also in spring. However, in all seasons, a higher number of *J. macrocarpa* seeds probably germinated although the potential seedlings could die before be recorded as emerged. Low seedling emergence observed in spring, when the temperature is optimum for germination but water availability is declining, suggests that drought is the most limiting factor in seedling emergence, which agrees with the results obtained by Gulias *et al*. (2004).

This finding is also consistent with our results about the models performed to explain seedling emergence, since microclimate model was the best fit one. In particular, the positive relation found between number of emerged seedlings and tree cover, as in other studies about *Juniperus* (e.g. Van Auken *et al*., 2004; Jovellar *et al*., 2013), suggest that areas under the tree's protection favored emergence due to positive microsite effects. Tree canopy creates shaded habitats where the seedlings undergo more favorable water relations, lower evaporative demand and physical disturbance, and lower temperature, thus creating more favorable water relations between the seedling and the substrate (McLeod and Murphy, 1977; De Jong and Klinkhamer, 1988). Specifically, we found that tree cover was more significant than solar irradiation variables in the microclimate model explaining emergence. Actually, greater moisture availability under canopy may be more important for seedling establishment than reduced light levels when considering the xeric nature of the dune environment (Joy and Young, 2002).

Our results are in agreement with Juan *et al.* (2006) who in a previous work obtained higher seedling emergence of *J. macrocarpa* in southern Spain in shaded/watered conditions, being the moisture of the substrate during seedling emergence more important than the reduction of solar radiation. Also higher number of woody seedlings under other species of *Juniperus* trees has been found in dune systems of North America (Joy and Young, 2002). In particular, this study showed that radiation was reduced and soil temperature fluctuations were moderated under *J. virginiana*, while values of edaphic variables favoring seedling development, such us moisture content and organic matter, were higher under tree than in exposed sites. The positive effect of canopy for seedling has been reiteratedly showed in other Mediterranean environments (e.g. Castro *et al*., 2005; Lloret *et al*., 2005).

In contrast, and contrary to what it was to be expected, more galbules, and therefore more seeds, does not guarantee higher seedling number. This fact, may be related to the low germination and viability rate found for this species (see Chapter 2), but also to the importance of microclimate conditions, since seeds should be under appropriate tree cover.

Survival

Survival of seedlings in this study resulted very low, less than 20% for the cohort specifically evaluated; therefore seedling survival could be the most limiting factor in the recruitment process. Consistently with our results, low survival rate have been related to both other species of *Juniperus* genus (García *et al*., 1999; Van Auken *et al*., 2004) and other species of dune environments (Maun, 1994; Cogoni *et al*., 2013a).

The higher emergence is reached in January and the most of the seedlings died in the first months from emergence, reaching the highest mortality rate in the first summer. The highest summer mortality detected of seedlings is in agreement with what observed for *J. ashei* J. Buchholz (Van Auken *et al*., 2004) and *J. communis* (García *et al*., 1999), as well as for *J. phoenicea* in coastal dune environment (Armas and Pugnaire, 2009). Overcome first summer does not guarantee survival, indeed seedlings continue dying, although progressively in lower proportion, showing a new peak of mortality in the second summer, as has been also found for other species in Mediterranean environments (Herrera *et al*., 1994; Mendoza *et al*., 2009). Indeed, summer drought is one of the main causes of seedling mortality in Mediterranean ecosystems (Manzaneda *et al*., 2005; Garrido *et al*., 2007; Giménez-Benavides *et al*., 2007; Rodríguez-Pérez and Traveset, 2007).

Furthermore, we found that survival of *J. macrocarpa* seedlings was related to herbivory and competence factors. Specifically, herbivory was positively related to seedling mortality, in agreement with the results obtained by García *et al.* (1999) who showed that herbivores negatively affected *J. communis* recruitment by eating and trampling seedlings. Also, animal predation on seedlings decreased early survival greatly in *J. scopulorum* Sarg. (Fisher *et al*., 1990), conversely, herbivory did not showed significant effect on seedling survival of some *Juniperus taxa* (Jackson and Van Auken, 1997; Cadenasso *et al*., 2002; Joy and Young, 2002; Montesinos *et al.*, 2007), suggesting a limit palatable quality of junipers that may restrict herbivory. In fact, we did not found many cut seedlings, but we mainly found trace of trampling and excrements.

Herbaceous plus scrub cover was negatively related with *J. macrocarpa* seedling mortality, therefore no competence effects were found. In this sense, Armas and Pugnaire (2009) also found that survival of *J. phoenicea* seedlings was enhanced beneath shrubs, especially under clumps, in coastal sand dune system. Similarly *J. occidentalis* Hook. seedlings became established most readily on areas with well-developed herbaceous and shrubby vegetation (Burkhardt and Tisdale, 1976). Therefore, shrubs are more a microclimatic benefit than a competition constraint, since shading by shrubs reduces soil water evaporation and decreases thermal stress and transpiration in understory plants (Moro *et al*., 1997; Domingo *et al*., 1999; Pugnaire *et al*., 2004). This issue is related with results obtained by microclimate factors, we found that high values of solar irradiation increased seedling mortality risk, as expected, since higher radiation involve high temperatures and loss of soil moisture.

Growth

Our results confirmed that *J. macrocarpa* is a slow growing species, at least in the seedling stage, as it has been verified for other species of this genus (e.g. Ortiz *et al*. 1998; Rupprecht *et al.*, 2011; Jovellar *et al*., 2013). Specifically, we found that the growth of surviving plants did not overcome in any case the 7.2 cm, after two years monitoring this variable. Therefore, as expected and according to Bacchetta *et al.* (2008), no seedling reached the subsequent size class, defined for individuals over 0.25 m (Ward, 1973; Gatsuk *et al*., 1980; Ward, 1981; Clifton *et al*., 1997; García *et al*., 1999). In fact, also from an age standpoint, all individual remained as seedlings, since young plants (next age class), has been defined in this genus for individual between 6 and 20 years (Ward, 1982; Clifton *et al*., 1997).

The growth depended on seedling that had emerged in suitable microhabitats, resulting particularly relevant the positive relation between tree cover and growth. According to these results, Miller and Rose (1995) found that *J. occidentalis* enhanced growth rates beneath canopy, suggesting microclimates beneath shrub canopies are more beneficial than conditions in the interspace. Conversely, McKinley and Van Auken (2005) obtained lower growth rates for *J. ashei* seedling below adult canopies.

The seedling growth was also positively related to solar irradiation in accordance with other authors (Paleg and Aspinall, 1964; Friend *et al*., 1977; Ryle, 1996). Indeed, in natural conditions, the amount of light intercepted for photosynthesis is one of the most important environmental factors affecting seedling growth (Poorter, 2001; Quero *et al*. 2008).

Herbaceous and scrub cover did not show a clear competence effect on seedling growth, but on the contrary, this factor was positively related with *J. macrocarpa* seedling growth. This is in agreement with the results showed by Burkhardt and Tisdale (1976) in a study on *J. occidentalis*, as well as with the results showed on seedlings of other tree species (e.g. De Steven, 1991; Curt *et al*., 2005). Conversely, Fisher *et al.* (1990) found all growth parameters inversely correlated with shrub cover in *J. scopulorum* seedlings. Nevertheless, the generalizations obtained from our results should be considered with caution due to most of the seedlings died in the first months since emergence and, therefore, few seedling remained to evaluated factors influencing growth.

Implication for recovery and conservation

In conclusion, the recruitment of *J. macorcarpa* was highly limited in all initial phases of its the life cycle. Seed viability and germination are very low (Juan *et al*., 2003; Pacini and Piotto, 2004; Juan *et al*., 2006, see Chapter 2). Although the ripening and dispersal period of galbules are long (from autumn to spring), as it is also long the potential period for germination regarding temperature, emergence season is limited almost exclusively to winter. In addition, emergence is limited to particular microclimate conditions. Besides limitation in emergence, a very low survival rate was found, with the highest mortality rate recorded in the first summer. In addition, the few survival seedlings grew slowly, and after two years all individual remained as seedlings, as expected.

The identification of critical stages in the recruitment process of *J. macrocarpa*, together with factors influencing them, showed direct implications for *in situ* conservation actions, such as population reinforcement or recovery of the fragile and threatened habitat of which this *taxa* is an essential part. Regarding recovery, plantation could be a more effective option than sowing, due to limitations for both germination and emergence that could be surpass in greenhouse conditions. Anyhow, if sowing is the option, it should be performed applying techniques such as organic blanket, which reduced evaporation, and favored higher seedling density in harsh environments (Ballesteros *et al*., 2012). In addition, the results of both methods for introducing *J. macrocarpa* could be improved under tree canopy, in order to favor microclimate conditions protecting seedling against direct radiation, evapotranspiration, etc. Both plantation and sowing should be applied in autumn, which has been showed an advantageous period for plant reintroduction in Mediterranean dunes (Cogoni *et al.*, 2013b), since seeds and seedlings could benefit of the moist conditions from autumn to the beginning of spring, before summer drought. It could be advisable to place a protections against herbivore whenever this could be a problem in the area. Moreover, we did not found relevant competence problems, therefore removing herbaceous or scrub cover could not be a necessary technique.

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CHAPTER 4

Juniperus spp. habitat in coastal dunes: approach for conservation.

Maria Silvia Pinna, Eva Cañadas, Giuseppe Fenu, Gianluigi Bacchetta

Abstract

In this study, the floristic variability of *Juniperus* spp. habitat in Sardinia in relation to geographic, climatic and human variables was explored and moreover its conservation status was assessed. Two data sets were created: the first one by inputting phytosociological relevés available in literature and our own relevés; the second one by including, for each relevé, geographic, climatic and floristic variables, as well as sampling period and human disturbance as categorical variables. We assessed the conservation status of *Juniperus* spp. habitat in Sardinia following the IUCN protocol. The floristic composition differed among sites, with a significant role of all explicative variables analysed. This variation was mainly ruled by a latitudinal gradient, linked to a climatic gradient, which varied from north (where *J. phoenicea* subsp. *turbinata* prevails) to south (where *J. macrocarpa* predominates). Regarding the results of key parameters in the evaluation of the habitat quality, the floristic richness was positively influenced by low and medium level of human disturbance. In a similar way, the endemic *taxa* cover was positively related to medium level of human disturbance; while the alien *taxa* cover was positively related to recent samplings. Finally, the IUCN assessment indicated that *Juniperus* spp. habitat should be considered as endangered (EN) at a regional level. In order to improve conservation status of this habitat we suggest management measures as eradication of alien *taxa*, as well as interventions that aim to reduce human impact on dune systems.

Key words: Habitats Directive; human disturbance; *J. macrocarpa; J. phoenicea* subsp. *turbinata;* phytosociological relevés; Sardinia**.**

Introduction

The European Union Habitats Directive (Council Directive 92/43/EEC) aims to contribute towards ensuring biodiversity through the conservation of natural habitats and of wild fauna and flora in the member states. For this purpose, the Directive listed natural habitat types of community interest that require the designation of special areas of conservation; among these habitat is included the priority habitat “Coastal dunes with *Juniperus* spp.” (code 2250) object of this study (*Juniperu*s spp. habitat, hereafter). This habitat comprises juniper scrubs or micro-forests on coastal sand dunes in a variety of situations. It is mainly characterized by *Juniperus communis* L. in thermo-Atlantic coastal dunes of central/northern Europe (Britain, Denmark), while in southern Europe the juniper species predominating are: *J. macrocarpa* Sm., *J. navicularis* Gand. (= *J. transtagana, J. oxycedrus* subsp. *transtagana*), *J. phoenicea* L. subsp. *turbinata* (Guss.) Nyman. It is mainly distributed along the sandy coasts of southern and western Europe and secondly in central and northern Europe, on Mediterranean and Atlantic coasts (European Commission, 2007; Biondi *et al.*, 2009). Italy hosts the main area of this habitat at EU level (39% of the total habitat surface area with in Natura 2000 network; Picchi, 2008).

Coastal dune ecosystems show strongly dynamic interactions between abiotic and biotic factors, hosting a high biodiversity, compared with other natural ecosystems, and show an extremely specialized flora and fauna (Carranza *et al.*, 2008). Dune systems present a complex sea-to-inland environmental gradient (e.g. Wilson and Sykes, 1999; Frederiksen *et al.*, 2006; Acosta *et al.*, 2006, 2007; Miller *et al.*, 2010; Fenu *et al.*, 2013a). According to this gradient, structurally and floristically different vegetation types develop 17 habitat types in annex I of the 92/43/EEC directive describe the important environmental heterogeneity of coastal sand dune habitats in Europe (European Commission, 2007).

Coastal dunes are recognized as frequently disturbed and vulnerable ecosystems. In these environments, the structure and composition of plant communities are mainly affected by several factors such as incoherence of the substrate, impact of high wind, salt spray, or sand accretion (Maun, 2009). In addition, coastal dune systems have been subjected to high human disturbance for several thousands of years and the human pressure increasing remarkably in the last two centuries (Acosta *et. al.*, 2000). These ecosystems have been severely fragmented or destroyed primarily as a result of urbanization, industrialization, and tourism activities (EEA, 1999). As a consequence, sandy coasts in the Mediterranean Basin are highly modified by human impacts, being considered among the most endangered environments in Europe (van der Meulen and

Salman, 1996; van der Meulen *et al.*, 2004; Carboni *et al.*, 2009; Feola *et al.*, 2011), consequently the habitat under study has been designed priority in the Habitats Directive.

However, for this particular habitat, as for many other European habitats, information on distribution, species composition, and conservation status is lacking (Lengyel *et al*., 2008a, b). Efforts to characterize and classify plant communities on the basis of the Interpretation Manual of European Union Habitats (European Commission, 2007), taking into account species composition, have been undertaken only for few habitat types (e. g. Galán de Mera *et al*., 2000; Muller, 2002; Peco *et al*. 2005; Farris *et al*., 2010, 2012, 2013). Moreover, regarding human disturbance, many studies about alien species invasion have been published (Rejmánek *et al*., 2005; Chytrý *et al*., 2005, 2009; Hejda *et al*., 2009; Kalusová *et al*., 2013). Indeed, human disturbance is a relevant factor in coastal dunes environments, where a strong conflict between human activities and the preservation of native plant diversity have been identified, with native plants being more affected by human factors than alien species (Carboni *et al*, 2010a).

Although several ecological studies have been carried out on the Mediterranean coastal vegetation (Acosta *et al*., 2003, 2005, 2007; Carranza *et al*., 2008; Carboni *et al*., 2010b; Fenu *et al*., 2012, 2013a; Angiolini *et al*., 2013), as far as we know, no conservation studies are available on coastal habitats. In Sardinia, where coastal habitats are widespread, only phytosociological studies have been conducted. In particular, Farris *et al*. (2007), studying coastal and littoral habitats and plant communities in northern Sardinia, verified the attribution of plant communities in each habitat type. Meanwhile, Bacchetta *et al*. (2009) described micro-forest geo-series that characterise this habitat in Sardinia. More recently, Gianguzzi *et al*. (2012) studying the *J. phoenicea* subsp. *turbinata* coenosis in the Italo-Tyrrhenian biogeographic Province, characterized the floristic and structural composition of this habitat also in Sardinia. Nevertheless, little is known about factors influencing *Juniperus* spp. habitat composition and, in particular, about factors related to human disturbance. In this regard, it should be noted the study performed by De Luca *et al*. (2011), who investigated the relationships between disturbance factors and composition of several dune habitats to verify the applicability of numerous indices, to monitor the species richness, and ultimately to define the conservation status of dune systems. Actually, despite being the *Juniperus* spp a priority habitat type, little is known about its current conservation status, not for nothing, the assessment of the conservation status for habitat is a recent and emerging topic (Rodríguez *et al*., 2007, 2011). The World Conservation Union's (IUCN) proposed a procedure, analogous to that developed for the species, for assessing the extinction risk of terrestrial ecosystems, which may complement traditional species-specific risk assessments, or may provide an alternative when only landscape-level data are available (Rodríguez *et al*., 2007, 2011).

The aims of this work were: (1) to explore the floristic variability of *Juniperus* spp. habitat in Sardinia in relation to geographic, climatic and human variables; (2) to analyse the potential effect of human disturbance and sampling period on key plant parameters for evaluating habitat quality; (3) to assess the conservation status of *Juniperus* spp. habitat in Sardinia.

Materials and methods

Data collections

We analysed the phytosociological relevés carried out on this habitat in Sardinia according to the Sigmatist School of Zurich-Montpellier (Braun-Blanquet, 1965), available in literature (see Appendix 1), as well as relevés performed in 2011 during this study. Taxonomic treatment of each *taxa* reported in the relevés was updated to current taxonomy, according to Conti *et al.* (2005). The final floristic matrix consisted of 154 relevés x 167 species. In this matrix, the Braun-Blanquet values were transformed into the quantitative scale according to van der Maarel (1979) and Noest *et al.* (1989).

Subsequently, we created another matrix inputting, for each relevé, several groups of factors. Firstly, geographic variables were assigned (X and Y coordinates). Floristic variables included: plant cover, species number (richness), as well as number and cover of endemic and alien *taxa*. As endemics, Sardo-Corso-Tuscan Archipelago elements showed in Bacchetta *et al*. (2012a, b) were considered, while for the alien *taxa* Podda *et al*. (2010) was followed. In addition, we added the following climate variables downloaded from the WorldClim database version 1.4 (http://www.worldclim.org, Hijmans *et al.*, 2005): annual mean temperature, maximum temperature of the warmest month, minimum temperature of the coldest month, annual precipitation, and precipitation of the driest quarter. Two categorical variables, human disturbance and sampling period, were also assigned to each relevé. Three levels of human disturbance were established (low, medium and high) based on the tourists visiting each locality in summer. Finally, sampling period was grouped in two categories: old samplings, including the relevés carried out before 1996 (n = 94), and recent samplings for those subsequent to 2000 (n = 60).

Conservation status assessment

A working group established by the IUCN has begun formulating a system of quantitative categories and criteria, analogous to those used for species, for assigning levels of threat to ecosystems at local, regional, and global levels (Rodríguez *et al.*, 2011). This system was applied following the procedure for regional assessment (IUCN, 2003). Extent of occurrence and area of occupancy were measured following the last version of IUCN guidelines (IUCN, 2011a). We checked the presence of the *Juniperus* spp. habitat in all the standards forms regarding Sardinian SCI (Sites of Community Importance) available on the MATTM website (ftp://ftp.dpn.minambiente.it/Cartografie/Natura2000/), reporting habitat cover. We calculated the regional Area Of Occupancy (AOO), by counting the number of cells occupied by the habitat in each SCI in a grid with 2x2 km cells. Successively, we categorized the major threats affecting the habitat, following the IUCN Threats Authority File (Version 3.1; IUCN, 2011b).

Data Analysis

We tested the differences in *Juniperus spp.* habitat composition, as well as the influence of the explanatory variables, using ordinations and permutational multivariate analysis of variance (PERMANOVA; Anderson, 2001). The PERMANOVA used the ‘‘adonis’’ procedure. Ordinations were fitted and plotted with non-metric multidimensional scaling (NMDS) using ‘‘metaMDS’’ procedure; only significant variables were projected onto the ordination diagram. Both procedures are included in the R vegan package (Oksanen *et al.*, 2012). In addition, we used General Linear Model (GLM) to test the effect of tourist intensity and sampling period on plant richness, as well as on cover of endemic and alien *taxa*. GLMs were fitted specifying a Poisson error distribution and log as a link function. All the statistical analyses were performed using the R statistical package (R Development Core Team, 2011).

Results

Factors influencing floristic composition

The mean total cover of the *Juniperus* spp. habitat in Sardinia was 84.7%, being the mean cover of the two *Juniperus* species 53.32%. Habitat composition differed among sites; the most frequent *taxa* were *Pistacia lentiscus* (133 relevés, mean cover: 9.96%), *Juniperus macrocarpa* (104 relevés, mean cover: 47.38%), *J. phoenicea* (100 relevés, mean cover: 37.91%), *Rubia* *peregrina* (95 relevés, mean cover: 1.70%) and *Phillyrea angustifolia* (88 relevés, mean cover: 4.49%).

Floristic composition was significantly influenced by all the evaluated variables, according to PERMANOVA results (Table 1). In particular, floristic composition was mainly explained by geographic variables (Y: $r^2 = 0.1059$; X: $r^2 = 0.0525$). Among climatic variables, the variability explained by maximum temperatures highlighted ($r^2 = 0.0497$). Also human disturbance and sampling period were significant ($r^2 = 0.0255$ and $r^2 = 0.0381$, respectively). However, these variables explained ca. one-third of the total variability (38.74%), and the 61.26% was not explained by the used variables.

Table 1 - PERMANOVA test results for factors influencing habitat composition. Df: degrees of freedom; X: latitude; Y: longitude; Tma: annual mean temperature; Tmax: maximum temperature of the warmest month; Tmin: minimum temperature of the coldest month; Pa: annual precipitation; Pdq: precipitation of the driest quarter. **: 0.01 > p > 0.001; ***: p < 0.001.

Variables	Df	Sums of squares	Mean squares	F. Model	R^2	Pr(>F)
X	1	2.030	2.0305	12.2470	0.0525	0.0010 ***
Y	1	4.098	4.0983	24.7196	0.1059	0.0010 ***
Tma	1	1.331	1.3310	8.0282	0.0344	0.0010 ***
Tmax	1	1.924	1.9240	11.6046	0.0497	0.0010 ***
Tmin	1	1.167	1.1675	7.0417	0.0302	0.0010 ***
Pa	1	1.170	1.1699	7.0562	0.0302	0.0010 ***
Pdq	1	0.809	0.8091	4.8803	0.0209	0.0020 **
Human disturbance	2	0.985	0.4927	2.9716	0.0255	0.0020 **
Sampling period	1	1.474	1.4737	8.8891	0.0381	0.0010 ***

The Figure 1 provided a visual representation of these patterns, in which proximity among points means similarity. The relevés are differentiated into two main groups: in the right side of the graph (positive values of NMDS1) relevés are related to *J. phoenicea*, meanwhile the relevés on the left side (negative values of NMDS1) are related to *J. macrocarpa,* with the first group showing lower variability. This group included the relevés from La Maddalena and Nurra localities, mainly characterized by the presence of *taxa* such as *Chamaerops humilis, Hypochoeris achyrophorus, Arbutus unedo, Carex flacca* sl., *Genista corsica, Myrtus communis, Euphorbia characias,* as well as the relevés from Capo Comino and Maria Pia localities characterized by the presence of *Clematis cirrhosa*. The group connected to *J. macrocarpa* showed greater variability, in which are found *taxa* such as *Vulpia fasciculata, Polycarpon tetraphyllum, Cyperus capitatus, Crucianella maritima, Malcolmia ramosissima, Rumex bucephalophorus, Pancratium maritimum, Scrophularia ramosissima,* indicating other vegetation types (i.e. psammophylous habitats such as "*Crucianellion maritimae* fixed beach dunes”, “*Malcolmietalia* dune grasslands” etc.). This group are mainly constituted by the relevés performed in Chia, Villasimius, Piscinas, Buggerru, Portixeddu and Cala Domestica localities.

The variables most correlated with NMDS1 were precipitation of the driest quarter and Y coordinate (positively correlated), as well as maximum temperature of the warmest month and annual mean temperature (negatively correlated), while those variables most correlated with NMDS2 were X coordinate and annual precipitation (Figure 1, 2; Table 2). The length of the arrows showed strength of the gradient, with arrows pointing to the direction of most rapid change in the variable (direction of the gradient).

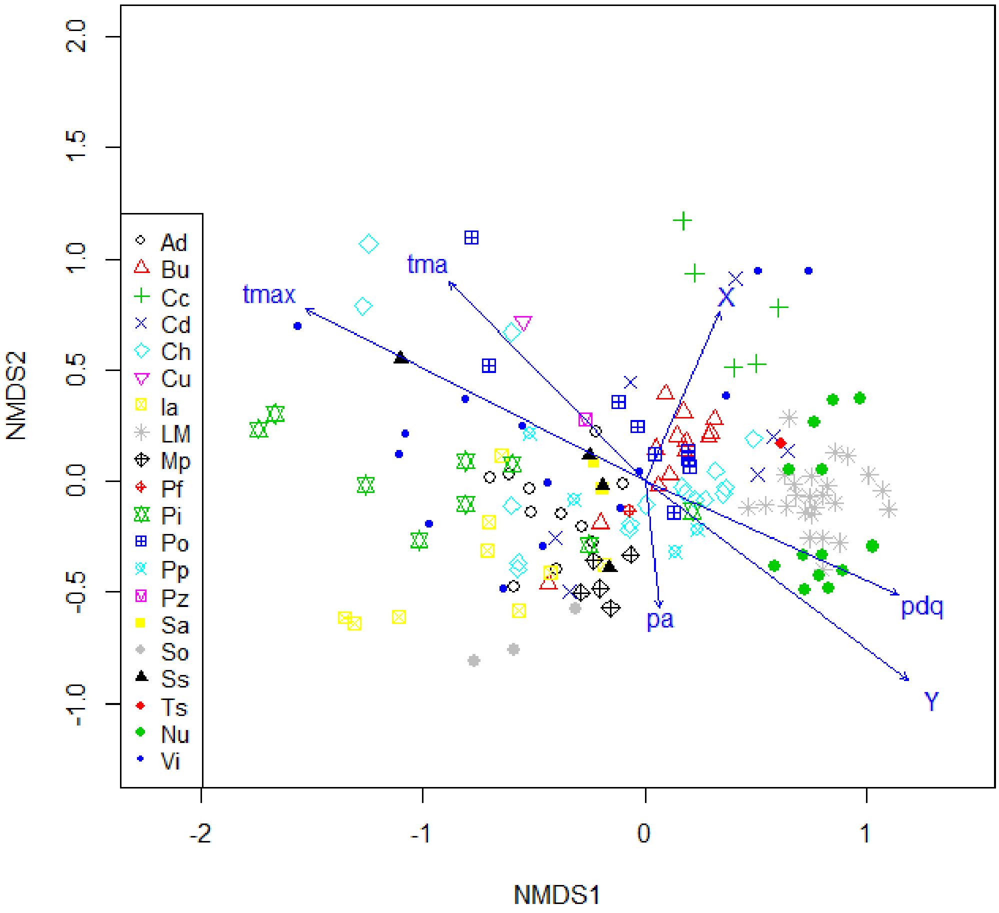

Figure 1 - NMDS ordination of 154 relevés showing distribution fo the main site groups and vectors of the most significant explanatory factors fitted. Explanatory factors: X: latitude; Y: longitude; Tma: annual mean temperature; Tmax: maximum temperature of the warmest month; Tmin: minimum temperature of the coldest month; Pa: annual precipitation; Pdq: precipitation of the driest quarter. For sites abbreviation see Appendix 2.

Table 2 - Vector results of explanatory factors fitted onto NMDS. P values based on 1000 permutations. NS: not significant; *:0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001. Abbreviations of variables are given in Table 1.

Variables	**NMDS1**	**NMDS2**	**r^2**	**Pr(>r)**
X	0.4074	0.9133	0.1139	0.0010 ***
Y	0.7979	-0.6028	0.3591	0.0010 ***
Tma	-0.7019	0.7122	0.2584	0.0010 ***
Tmax	-0.8922	0.4517	0.4802	0.0010 ***
Tmin	-0.2734	0.9619	0.0237	0.1548 NS
Pa	0.1174	-0.9931	0.0539	0.0160 *
Pdq	0.9126	-0.4089	0.2546	0.0010 ***
Human disturbance	0.3375	0.9413	0.0163	0.2717 NS
Sampling period	-0.6660	-0.7459	0.0067	0.5794 NS

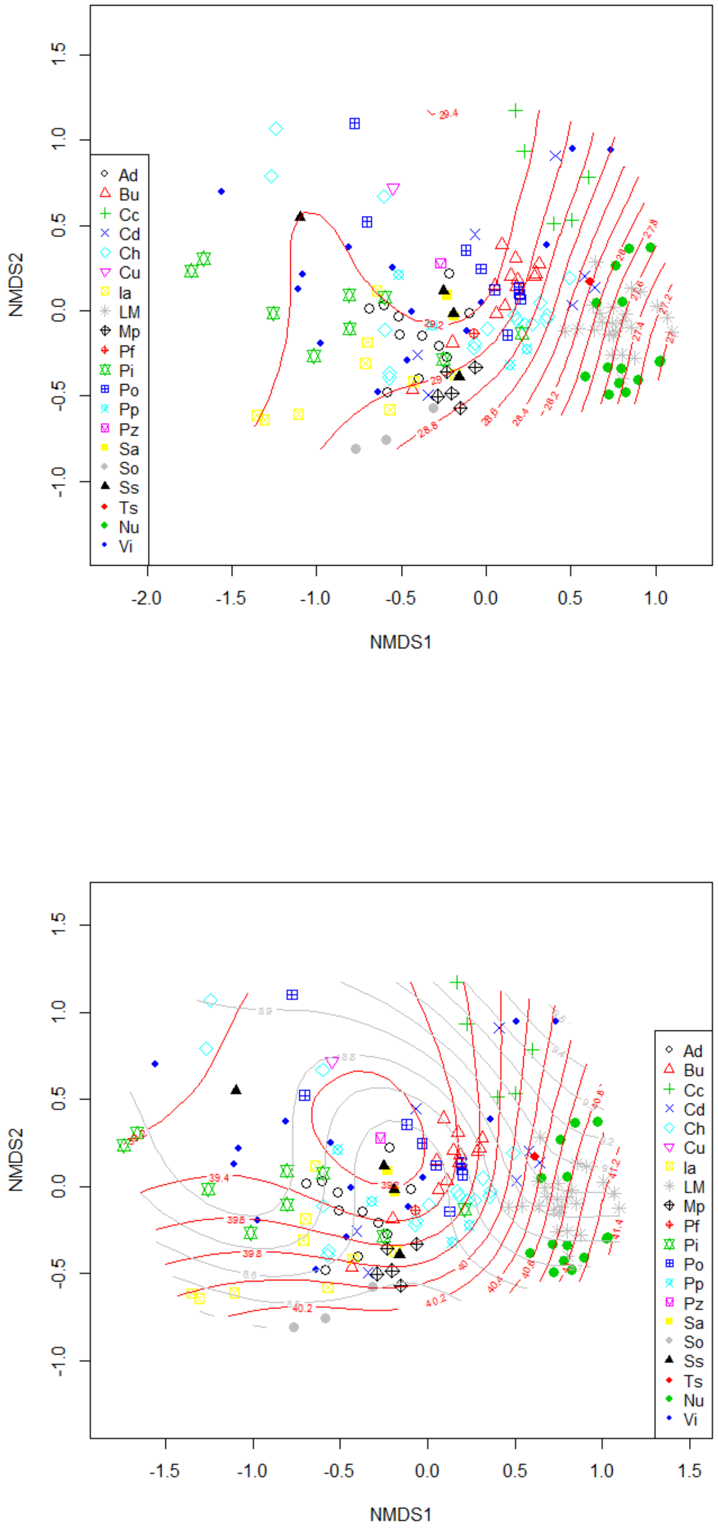

Figure 2 - Habitat relevès in ordination space (1st 2 axes) overlaid on a fitted geographic (2.A.) and climatic (2.B.) surface (contour lines). Fitted surface: 2.A. Latitude (red line) and Longitude (grey line). 2.B. Maximum temperature of the warmest month (red line).

Species richness, endemic and alien species: human disturbance and period effect

Total species number ranged from 4 to 27 *taxa* per relevé. The relevés richest in species were carried out in Sa Salina (27 and 26 *taxa*, for Ss3 and Ss2 relevés, respectively), Punta Cristallo, Porto Pino and Sant' Antioco (Nu8, Pp4, Sa2; 25 *taxa*) localities.

Among the recorded 167 *taxa*, 14 are endemics. The site richest in endemic species was Acqua Durci (4), and the maximum cover was found at La Maddalena (38.5%).

Moreover, a total of 6 alien *taxa* was recorded, with the site richest in alien *taxa* being Marina di Sorso (2), where also the maximum cover (39.75%) was recorded.

Human disturbance significantly influenced species richness, while sampling period was not significant (Table 3). In particular, a significant positive effect of low and medium human disturbance on species richness was found. A significant positive effect of medium human disturbance and recent samplings on endemic *taxa* cover was obtained, while low level of human disturbance was not significant. Finally, regarding alien *taxa*, in recent samplings a significant positive cover on alien *taxa* was found.

Table 3 - Generalized Linear Model (GLM) results examining the effect of human disturbance and sampling period on plant richness, as well as on cover of endemic and alien *taxa*. NS: not significant; *:0.05 > p > 0.01; **: 0.01 > p > 0.001; ***: p < 0.001.

	Estimate	**Standard Error**	**z value**	**Pr(>|z|)**
Richness				
Intercept	2.3119	0.0436	53.053	< 0.001 ***
Low disturbance	0.3189	0.0586	5.441	< 0.001 ***
Medium disturbance	0.4365	0.0561	7.783	< 0.001 ***
Recent samplings	-0.0331	0.0501	-0.659	0.51 NS
Endemic *taxa* cover				
Intercept	0.0123	0.1254	0.098	0.9220 NS
Low disturbance	0.0744	0.1889	0.394	0.6935 NS
Medium disturbance	0.8931	0.1447	6.172	< 0.001 ***
Recent samplings	0.3596	0.1343	2.677	0.0074 **
Alien *taxa* cover				
Intercept	-1.9694	0.4082	-4.824	< 0.001 ***
Low disturbance	-18.0651	1439	-0.013	0.990 NS
Medium disturbance	-17.7868	1311	-0.014	0.989 NS
Recent samplings	1.8394	0.4410	4.171	< 0.001 ***

Conservation status

Juniperus spp. habitat in Sardinia was reported on 35 of 91 SCI of the Island and it is spread over a total area of 1290.57 ha. This habitat was found in 117 cells of 2x2 km that constitute an AOO of 468 km^2.

According to the IUCN threats classification scheme (IUCN, 2011b), the major threats for the *Juniperus* spp. habitat in Sardinia are “Residential & Commercial Development” (“Tourism & Recreation Areas”, code 1.3) followed by “Pollution” (“Garbage & Solid Waste”, code 9.4), “Invasive & Other Problematic Species”, “Genes & Diseases” (in particular: “Invasive Non-Native/Alien Species/Diseases”, code 8.1; “Named Species”, code 8.1.2) and “Natural System Modifications” (“Fire & Fire Suppression”, code 7.1).

Therefore, based on our data on area of occupancy value, the criterion C in Rodríguez *et al.* (2011) and the estimated current decline, this habitat is endangered (EN) at regional level, following the formula EN = C2a.

Discussion

The *Juniperus* spp. habitat in Sardinia is well represented being present in many localities along coasts of the Island (reaching ca. 5% of the total European surface) and showed a relevant variability in floristic composition among sites, being significantly influenced by the studied variables. We found that variation in habitat composition was ruled by a dominant underlying gradient. It is mainly a latitudinal gradient, which, in turn, implies changes in climatic factors, also noted by some other authors (e.g. Miller *et al.*, 2010) as drivers of habitat dune composition. In particular, we found that floristic composition varied gradually from north (where habitat is characterized by the lack of *J. macrocarpa* and the dominance of *J. phoenicea* subsp. *turbinata*) to south (where *J. macrocarpa* is characteristic), with the habitat progressively more exposed to the extreme summer drought (higher temperature and lower precipitations, in particular in the driest quarter) as latitude decreases. Furthermore, it may be also interpreted a secondary longitudinal gradient, with precipitations increasing westward, where prevailing winds and Atlantic perturbations are always westerly, and coastal dune systems are also generally deep and well-structured than in the east part of the island. However, the variables evaluated can only partially explain changes in habitat composition, and other local environmental factors, such as dune morphology and topography, hydrography, and soil nutrients, may also determine floristic variability (e.g. Frederiksen *et al*., 2006; Li *et al.*, 2007; Zuo *et al*., 2009, Fenu *et al*., 2013a).

In addition, we found that floristic composition in *Juniperus* spp. habitat was also influenced by human disturbance and sampling period. Studying these factors in Mediterranean coastal dune systems is especially relevant, since they have been subjected to high human disturbance, mainly from the 20th (Acosta *et al*., 2000); as a consequence, sandy coast habitats are highly modified. Indeed, both human disturbance and sampling period, significantly influenced richness species, as well as cover of endemic and alien *taxa*, which are key factors to evaluate habitat conservation. A decrease in number of species was observed in areas with high level of human disturbance; while low and medium human disturbance intensity determined an increase in the floristic richness, consistently to previous studies about coastal vegetation (e.g. Kutiel *et al*., 1999; Kerbiriou *et al*., 2008; Attorre *et al*., 2012). In fact, an intermediate degree of disturbance can be beneficial to certain habitats (Huston, 1979) by acting to promote species diversity (Coombes *et al*., 2008). In this sense, a limiting human trampling appears to produce positive effect of vegetation dune systems (Santoro *et al*., 2012).

A similar effect to that described for floristic richness was found for the endemics cover, which was positively related with medium level of human disturbance. However, Otto *et al.* (2012) highlighted a negative effect of human disturbance on endemic species richness in juniper woodlands in Tenerife Island. In a similar way, a negative effect on endemic species conservation was found by Fenu *et al*. (2013b).

Regarding alien *taxa,* only the sampling period had a significant effect on the alien species cover. The increment of alien *taxa* in the last years appears to be related to recent urban development in coastal areas (Carboni *et al*., 2010a). In addition, the increase of alien species cover is also due to the expansion related to pine plantation performed during the last century for afforestation in the Mediterranean area (Court-Picon *et al*., 2004).

The *Juniperus* spp. habitat, together with others coastal habitat, has been considered among the most threatened in the Mediterranean basin (van der Meulen and Salman, 1996; Carboni *et al.,* 2009; Feola *et al.,* 2011), and therefore listed in the Habitats Directive as priority. In fact, several LIFE projects (financial instrument supporting environmental and nature conservation projects throughout the European Union) finance studies and actions promoting its conservation (PROVIDUNE, JUNICOAST, LIFE DUNA, LIFE ENEBRO, etc.).

The IUCN assessment procedure confirmed that this habitat should be considered as endangered (EN) at regional level. This represent the first approach to assign a threat category to a habitat following the IUCN methodology, consequently, further tests we would need to evaluate if this expeditious method can be useful to assess habitats conservation status.

In conclusion, *Juniperus* spp. habitat is endangered in Sardinia, according to our results. In fact, a decrease in species richness and endemic species, due to human disturbance, as well as an expansion of alien *taxa* in the last ten years we recorded. In order to improve the conservation status of *Juniperus* ssp. habitat, management measures such as eradication or control of alien *taxa*, as well as interventions aimed to reduce the human impact on dune systems are therefore necessary.

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APPENDIX 1 - Literature used to build the data set of phytosociological relevés

Authors	**Year of publication**	**Number of relevés**	**Title**	**Source**
Valsecchi F.	1976	14	Sui principali aspetti della vegetazione costiera della Nurra nord-occidentale (Sardegna settentrionale).	Giornale Botanico Italiano, 110 (1-2):21-63
Caneva G., De Marco G. and Mossa L.	1981	5	Analisi fitosociologica e cartografia della vegetazione (1:25.000) dell'Isola di S. Antioco (Sardegna sud-occidentale).	Quaderni C.N.R., AQ/1/124: 1-59.
Brambilla C., Caneva G., De Marco G., Mossa L.	1982	25	Analisi fitosociologica della seriazione psammofila costiera nella Sardegna meridionale	Annali di Botanica, 40: 69-96.
Mossa L. and Biondi E.	1989	5	Resoconto delle escursioni sul litorale sud-occidentale della Sardegna (27 e 28 ottobre 1989).	Colloques Phytosociologiques, 19: 739-760.
Mossa L.	1990	7	La vegetazione forestale del campo dunale di Buggerru-Portixeddu (Sardegna occidentale).	Annali di Botanica, 48: 69-96
Bartolo G., Brullo S., De Marco G., Dinelli A., Signorello P. and Spampinato G.	1992	33	Studio fitosociologico sulla vegetazione psammofila della Sardegna meridionale.	Colloques Phytosociologiques, 19: 251-273.
Arrigoni P.V.	1996	5	La vegetazione del complesso dunale di Capo Comino (Sardegna Nord- Orientale).	Parlatorea, 1: 35-45
Mossa L., Curreli F., Fogu M. C.	2000	10	La vegetazione degli habitat terrestri della riserva marina protetta di Capo Carbonara (Sardegna sud-orientale).	Rendiconti del Seminario della Facoltà di Scienze, Università di Cagliari, Suppl. vol. 70.
Biondi E., Filigheddu R., Farris E.	2001	6	Il paesaggio vegetale della Nurra (Sardegna nord-occidentale).	Fitosociologia, 38 (2) suppl. 2: 3-105
Biondi E., Bagella S.	2005	24	Vegetazione e paesaggio vegetale dell'arcipelago di La Maddalena (Sardegna nord-orientale).	Fitosociologia, 42 (2) suppl. 1.
Vagge I., Corradi N., Ferrari M., Balduzzi I., Mariotti L. M.	2007	3	Aspetti vegetazionali e morfo-sedimentologici dei campi dunari di Platamona-Marritiza con particolare riguardo all'area di Marina di Sorso (Sardegna settentrionale).	Fitosociologia, 44 (1): 33-48
Fenu G., Cogoni D., Ferrara C., Pinna M. S., Bacchetta G.	2012	5	Relationships between sandy dunes properties and plant community distribution in Mediterranean area: a case of Is Arenas dunal systems (Sardinia).	Plant Biosystems, 146:3, 586-602.

APPENDIX 2 - List of sampling localities and they abbreviations

Ad: Acqua Durci (CA); **Bu**: Buggerru (CI); **Cc**: Capo Comino (NU) **Cd**: Tra Capo Pecora e Torre di Cala Domestica (CA); **Ch**: Chia (CA); **Cu**: Cussorgia; **Ia**: Is Arenas; **LM**: La Maddalena (SS); **Mp**: Litorale Maria Pia - Alghero (SS); **Nu**: Porticciolo Alghero (SS); **Pf**: Porto Ferro (SS); **Pi**: Piscinas; **Po**: Portixeddu; **Pp**: Porto Pino; **Pz**: Porto Zafferano; **Sa**: Sant'Antioco; **So**: Marina di Sorso (SS); **Ss**: Sa salina; **Ts**: Torre salinas; **Vi:** Villasimius (CA)

APPENDIX 3 - List of species included in the matrix

Acacia saligna (Labill.) H. L. Wendl.
Achillea maritima (L.) Ehrend. & Y.P. Guo subsp. *maritima* [= *Otanthus maritimus* (L.) Hoffmanns. & Link subsp. *maritimus*]
Allium chamaemoly L. subsp. *chamaemoly*
Allium subhirsutum L.
Allium triquetrum L.
Ambrosina bassii L.
Ammophila arenaria (L.) Link subsp. *australis* (Mabille) Laínz
Anagyris foetida L.
Anthemis maritima L.
Arbutus unedo L.
Arisarum vulgare Targ. Tozz.
Artemisia arborescens L.
Arum pictum L. f.
Asparagus acutifolius L.
Asparagus albus L.
Asparagus aphyllus L.
Asparagus stipularis Forssk.
Asphodelus ramosus L. subsp. *ramosus*
Astragalus thermensis Vals.
Avena barbata Pott *ex* Link
Bellardia trixago (L.) All.
Brachypodium ramosum Roem. & Schult.
Brassica tournefortii Gouan
Briza maxima L.
Bromus rigidus Roth
Cakile maritima Scop. subsp. *maritima*
Carduus fasciculiflorus Viv.
Carex distachya Desf.
Carex flacca Schreb. s.l.
Carex halleriana Asso
Carpobrotus acinaciformis (L.) L. Bolus
Carpobrotus edulis (L.) N.E. Br.
Catapodium balearicum (Willk.) H. Scholz
Cistus albidus L.
Cistus creticus L. subsp. *eriocephalus* (Viv.) Greuter & Burdet
Cistus monspeliensis L.
Cistus salviifolius L.
Clematis cirrhosa L.
Clematis flammula L.
Convolvulus altheoides L.

Corynephorus divaricatus (Pourr.) Breistr.
Crithmum maritimum L.
Crucianella maritima L.
Cutandia maritima (L.) Barbey
Cyperus capitatus Vand.
Cytisus laniger (Desf.) DC. [= *Calicotome villosa* (Poir.) Link]
Cytisus spinosus Lam.[= *Calicotome spinosa* (L.) Link]
Chamaerops humilis L.
Charybdis maritima (L.) Speta
Dactylis glomerata L. s.l.
Daphne gnidium L.
Daucus carota L. s.l.
Daucus pumilus (L.) Hoffmanns. & Link
Dianthus morisianus Vals.
Elymus farctus (Viv.) Runemark *ex* Melderis subsp. *farctus*
Ephedra distachya L. subsp. *distachya*
Erica arborea L.
Eryngium maritimum L.
Euphorbia characias L.
Euphorbia dendroides L.
Euphorbia paralias L.
Euphorbia terracina L.
Ferula communis L.
Fumaria capreolata L. subsp. *capreolata*
Genista corsica (Loisel.) DC.
Gennaria diphylla (Link) Parl.
Geranium molle L.
Gladiolus italicus Mill..
Halimium halimifolium (L.) Willk. subsp. *halimifolium*
Helichrysum microphyllum Cambess. subsp. *tyrrhenicum* Bacch. Brullo & Giusso
Hyparrhenia hirta (L.) Stapf subsp. *hirta*
Hypochaeris aetnensis (L.) Benth. & Hook.
Hypochoeris achyrophorus L.
Juncus acutus L. subsp. *acutus*
Juniperus macrocarpa Sm.
Juniperus phoenicea L. subsp. *turbinata* (Guss.) Nyman
Lagurus ovatus L. subsp. *ovatus*
Lagurus ovatus L. subsp. *vestitus* (Messeri) H. Scholz
Lavandula stoechas L. subsp. *stoechas*
Limonium divaricatum (Rouy) Brullo
Limonium sulcitanum Arrigoni
Linaria flava (Poir.) Desf. subsp. *sadoa* (Sommier) A. Terracc.
Linum corymbulosum Rchb.
Lobularia maritima (L.) Desv. subsp. *maritima*

Lonicera implexa Aiton subsp. *implexa*
Lophochloa pubescens (Lam.) Scholz
Lotus cytisoides L. subsp. *conradiae* Gamisans
Malcolmia ramosissima (Desf.) Gennari
Matthiola incana (L.) R. Br.
Medicago litoralis Rohde
Medicago marina L.
Melica minuta L.
Minuartia geniculata (Poiret) Thell.
Misopates orontium (L.) Raf. subsp. *orontium*
Myoporum tenuifolium G. Forst.
Myrtus communis L. subsp. *communis*
Olea europaea L.
Ononis natrix L. s.l.
Ononis variegata L.
Orobanche artemisiae-campestris Gaudin
Osyris alba L.
Pancratium maritimum L.
Phillyrea angustifolia L.
Phillyrea latifolia L.
Phillyrea latifolia L. subsp. *rodriguezii* (P. Monts.) Romo
Phleum arenarium L. subsp. *caesium* H. Scholz
Phleum sardoum (Hacck.) Hack.
Pinus halepensis Mill.
Pinus pinaster Aiton s.l.
Pinus pinea L.
Piptatherum miliaceum (L.) Coss.
Pistacia lentiscus L.
Plantago coronopus L. s.l.
Plantago macrorrhiza Poir.
Polycarpon tetraphyllum (L.) L. subsp. *alsinifolium* (Biv.) Ball
Polygonum maritimum L.
Prasium majus L.
Pulicaria odora (L.) Rchb.
Quercus calliprinos Webb
Quercus ilex L. subsp. *ilex*
Reichardia picroides (L.) Roth
Reseda alba L. s.l.
Rhamnus alaternus L. subsp. *alaternus*
Rhamnus oleoides L.
Rosmarinus officinalis L.
Rostraria litorea (All.) Holub
Rouya polygama (Desf.) Coincy
Rubia peregrina L. s.l.

Rumex bucephalophorus L.
Ruscus aculeatus L.
Ruscus hypoglossum L.
Ruta chalepensis L.
Scrophularia ramosissima Loisel.
Scrophularia trifoliata L.
Schoenus nigricans L.
Senecio gibbosus (Guss.) DC. subsp. *cineraria* (DC.) Peruzzi, N.G. Passal. & Soldano
Senecio leucanthemifolius Poir. subsp. *leucanthemifolius*
Senecio transiens (Rouy) Jeanm.
Silene arghireica Vals.
Silene coelirosa (L.) Godr.
Silene colorata Poir.
Silene niceensis All.
Silene succulenta Forssk. subsp. *corsica* (DC.) Nyman
Sixalix atropurpurea (L.) Greuter & Burdet subsp. *grandiflora* (Scop.) Soldano & F. Conti
Smilax aspera L.
Solanum sodomaeum L.
Sonchus bulbosus (L.) N. kilian & Greuter subsp. *bulbosus*
Sonchus oleraceus L.
Spergularia rubra (L.) J. & C. Presl
Sporobolus virginicus Kunth
Stachys glutinosa L.
Stellaria media (L.) Vill. s.l.
Succowia balearica (L.) Medik.
Teucrium fruticans L. subsp. *fruticans*
Teucrium marum L.
Teucrium polium L. subsp. *polium*
Theligonum cynocrambe L.
Thymbra capitata (L.) Cav.
Thymelaea tartonraira (L.) All. subsp. *tartonraira*
Tolpis virgata (Desf.) Bertol. s.l.
Torilis arvensis (Huds.) Link subsp. *arvensis*
Torilis nodosa (L.) Gaertn.
Trifolium glomeratum L.
Umbilicus rupestris (Salisb.) Dandy
Vulpia fasciculata (Forssk.) Fritsch
Vulpia ligustica (All.) Link
Vulpia fasciculata (Forssk.) Fritsch

CONCLUSIONS

This thesis has contributed to a better understanding of *Juniperus macrocarpa* and the habitat that it characterizes (*Juniperus* spp. habitat). In particular, morphological variation in seeds of Mediterranean *Juniperus taxa*, germination of *J. macrocarpa* seeds, and some aspects on seedling dynamics of the *taxon*, essential for planning future conservation actions, were studied. In addition, factors affecting floristic composition and evaluation of conservation status of the habitat in Sardinia were investigated.

The results presented in Chapter 1 confirmed the validity of the statistical classifier for Mediterranean *Juniperus taxa*, based on seed morphometric parameters analysed by image analysis techniques, both at specific and intraspecific level. Seed morphometric analysis did not manage to discriminate seeds collected among different *J. macrocarpa* populations from the same geographical region, suggesting the possibility that a unique meta-population is present in the South of Sardinia. By contrast, the classification system was able to discriminate seeds of *J. macrocarpa* collected in different seasons and could not identify seeds collected in different sources (plants or soil).

Seeds of *J. macrocarpa* were characterized by low values of viability and germination and high levels of dormancy, confirming previous results reported in literature (see Chapter 2). The applied pretreatments were not able to overcome the detected primary and secondary dormancy, highlighting the need for further studies. A germination phenology all along the dispersal season (from autumn to spring), as well as the potentiality of this species to create a soil seed bank were illustrated. The narrow range of germination temperatures (15-20°C) and the slow germination detected for seeds of this species are ecologically advantageous and showed a good level of adaptation to the Mediterranean climate, characterized by a considerable unpredictability of precipitations. Spring was the best season for seed collection, whereas autumn for sowing in the field, giving new findings for the recovery and conservation planning of this species.

The recruitment of *J. macrocarpa,* as showed in Chapter 3, was highly limited in all initial phases of its life cycle, even after seed dispersal and germination (see Chapter 2). Emergence season was almost exclusively limited to winter, since the seedlings benefit of the moist conditions of the mild winter and spring, confirming the findings in Chapter 2. Besides limitation in emergence, a very low survival rate was found, with the highest mortality rate recorded in the first summer. *J. macrocarpa* is a slow growing species; the few survival seedlings grew slowly and after two years all individuals remained as seedlings, as expected. The growth depended on seedling which had emerged in suitable microhabitats and it was positively related to tree cover, hours of sunlight, and herbaceous plus scrub cover. The identification of critical stages in the recruitment process of *J. macrocarpa*, together with factors influencing them, showed direct implications for recovery and *in situ* conservation actions. Specifically, we suggest planting instead of sowing; autumn as the period for seed or plant introduction; shielding plants from solar radiation under canopy; and the application of techniques such as organic blanket when sowing is the selected option. Despite these relevant results, long-term studies are needed to identify key issues in the life cycle of *J. macrocarpa* (e.g. germination, fitness, and recruitment).

The floristic composition changes in *Juniperus* spp. habitat were mainly ruled by a latitudinal gradient, linked to a climatic gradient. As highlighted in Chapter 4, according to preliminary results on the IUCN assessment procedure, the habitat is endangered (EN) in Sardinia. In addition, a decrease in species richness and endemic species, due to human disturbance, as well as an expansion of alien *taxa* in the last ten years was recorded. Moreover, to improve the conservation status of the habitat, management measures such as the eradication of alien *taxa* and intervention aimed to reduce the human impact on dune systems are necessary.

APPENDIX 4 - Atti del IV Simposio Internazionale "Il Monitoraggio Costiero Mediterraneo, problematiche e tecniche di misura" Livorno, 2012

ANALISI DELL'IMPATTO ANTROPICO SULLE FORMAZIONI A *JUNIPERUS MACROCARPA* DELLA SARDEGNA MERIDIONALE

Maria Silvia Pinna, Cristiano Pontecorvo, Eva Cañadas, Gianluigi Bacchetta
Centro Conservazione Biodiversità (CCB) – Dipartimento di Scienze della Vita e dell'Ambiente.
Viale S. Ignazio da Laconi, 11-13- 09123 Cagliari. Tel.: 070 6753509,
e-mail: ccb@unica.it

Riassunto – Si presentano i primi risultati relativi all'analisi dell'impatto antropico sulle formazioni a *Juniperus macrocarpa* Sm. in quattro siti d'importanza comunitaria (SIC) della Sardegna. La scelta delle aree è stata compiuta in considerazione del grado di disturbo antropico: due siti sono interessati da un intenso turismo balneare, mentre gli altri presentano dei sistemi dunali tra i meglio conservati della Sardegna. L'obiettivo principale del confronto tra aree con differenti gradi di disturbo è stato quello di capire se la pressione antropica, dovuta soprattutto al turismo balneare, incida sullo sviluppo di *J. macrocarpa*. A questo fine in ogni SIC sono stati collocati 3 *plots* di 15x5 m, al cui interno sono stati effettuati dei periodici rilievi sugli individui di *J. macrocarpa* presenti. I risultati del monitoraggio nei 12 plots, per il periodo 2009-2011, hanno indicato un numero di plantule significativamente superiore nella stagioni primaverile rispetto a quella autunnale, oltre a differenze statisticamente significative tra località e annate. Questi dati confermano che l'estate rappresenta la stagione più critica per la sopravvivenza delle plantule. Le analisi condotte evidenziano altresì il pesante impatto antropico nelle località di Domus de Maria e Villasimius, ma mostrano che questo fenomeno influisce marginalmente sul ciclo di vita di *J. macrocarpa*, secondo i risultati preliminari.

***Abstract** – We present the first results of the analysis of human impact on the Juniperus macrocarpa Sm. populations in four Sites of Community Importance (SCI) in Sardinia. The choice of areas was made in consideration of the human disturbance: two sites are subjects by a heavy sea tourism while others have dune systems among the best preserved of Sardinia. The main objective of the comparison between areas with different degrees of disturbance has been to understand whether the anthropic pressure, mainly due to tourism resort, has a significant effect on J. macrocarpa. For this purpose in each SCI were placed three plots of 15x5 m, inside were performed periodic monitoring on J. macrocarpa plants. The results of monitoring in the 12 plots, for the period 2009-11, showed a significantly higher total number of seedlings in spring than in autumn, as well as significant differences among the localities and years. These data confirmed that the summer season represents the most critical for the survival of seedlings. The analyzes conducted confirming the heavy human impact in the Domus de Maria and Villasimius localities, but this phenomenon has only a marginal impact on the life cycle of J. macrocarpa, according to preliminary results.*

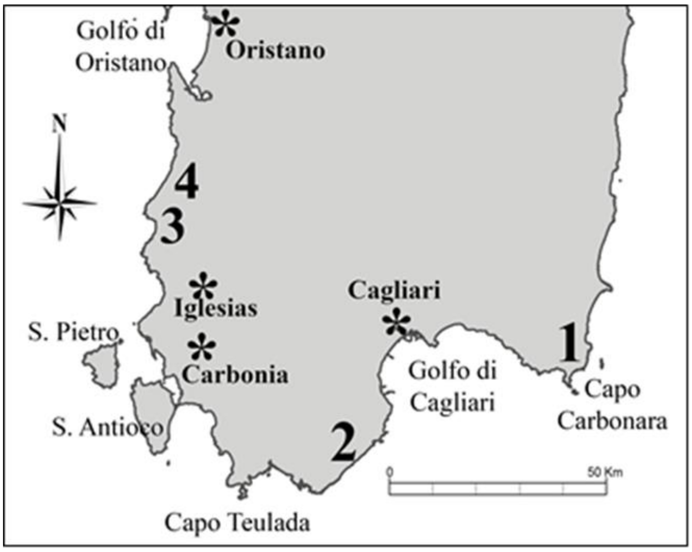

Figura 1 - Aree di studio: 1 Villasimius; 2 Domus de Maria; 3 Buggerru; 4 Arbus.
Figure 1 – Study areas: 1 Villasimius; 2 Domus de Maria; 3 Buggerru; 4 Arbus.

Introduzione

I sistemi dunali rappresentano delicati ecosistemi minacciati principalmente dall'urbanizzazione, dal transito di mezzi motorizzati o semplicemente pedonale, da fenomeni di erosione costiera oltre che dalla presenza di specie alloctone invasive [1; 2; 3; 4]. Tali pressioni costituiscono dei fattori di pressione che minacciano la loro stessa sopravvivenza.

La Sardegna è la quarta tra le regioni italiane per sviluppo complessivo delle dune costiere [5] e in particolare i sistemi dunali centro-meridionali rappresentano quelli più estesi e meglio conservati di tutta l'Isola. Dal punto di vista vegetazionale, queste aree sono caratterizzate dalla presenza del geosigmeto psammofilo sardo [6], i cui aspetti più maturi sono costituiti da microboschi a *Juniperus macrocarpa* Sm., inseriti come habitat prioritario (Dune costiere con *Juniperus* spp., codice 2250*) nell'allegato II della DIR. 92/43/CEE. Al fine di effettuare l'analisi dell'impatto antropico su tali formazioni, sono state individuate 4 aree di studio con differente grado di disturbo antropico (Fig. 1), scelte tra i principali sistemi dunali della Sardegna meridionale e corrispondenti ad altrettanti Siti di Importanza Comunitaria (SIC). In particolare, due appaiono interessati da un intenso turismo balneare [Porto Campana, ITB042230 (Domus de Maria); Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu, ITB040020 (Villasimius)], mentre quelli di Is Compinxius–Campo dunale di Buggerru-Portixeddu, ITB042249 (Buggerru) e Da Piscinas

Riu Scivu, ITB040071 (Arbus) presentano un elevato grado di naturalità e un buono stato di conservazione.

Il confronto tra aree con differente grado di disturbo antropico è stato effettuato allo scopo di valutare l'importanza di questo fattore in relazione alla mortalità delle plantule dovuta allo stress idrico estivo.

Materiali e metodi

Per quantificare l'impatto dei fattori di pressione sulle formazioni a *J. macrocarpa*, e in particolare la mortalità estiva delle plantule, tra la primavera 2009 e la primavera 2010, in ciascuna delle aree di studio sono state definite 3 aree permanenti delle dimensioni di 15x5 m, delimitate da picchetti metallici e georeferenziate attraverso il rilevamento delle coordinate. All'interno di ciascuna area sono stati rilevati i dati stazionali ed ecologici e inoltre sono stati realizzati inventari floristici e rilievi della vegetazione, secondo il metodo fitosociologico della scuola sigmatista di Zurich-Montpellier [7].

Per il monitoraggio all'interno dei *plots* sono stati marcati, mappati e misurati (altezza massima, diametro massimo della chioma e diametro del tronco) tutti gli individui, riproduttori e non, di *J. macrocarpa*, suddivisi secondo le classi dimensionali individuate da Muñoz-Reinoso [8] parzialmente modificate sulla base di quelle utilizzate da Bacchetta *et al*. [9]. Le classi dimensionali utilizzate sono state le seguenti: plantule (<0,1 m); giovani (≥0,1; <1 m); adulti (≥1 m). Per rilevare e quantificare il numero delle plantule il monitoraggio è stato effettuato con cadenza semestrale, prima e dopo la stagione turistica estiva. I monitoraggi semestrali nei *plots* sono stati effettuati a partire dalla primavera del 2009 fino all'autunno 2011, per un totale di 3 anni e di 6 monitoraggi per ciascun *plot* (a eccezione dei *plots* di Domus de Maria, monitorati a partire dalla primavera del 2010 e del *plot* P4 di Buggerru, monitorato a partire dall'autunno del 2009).

La valutazione del disturbo antropico sui siti è stata effettuata mediante l'individuazione dei fattori di pressione e la quantificazione degli effetti da essi causati. E' stata realizzata, inoltre, una stima dei possibili effetti provocati dal permanere dei fattori di pressione osservati. Le categorie dei fattori di pressione e delle minacce rilevati hanno seguito le indicazioni previste per i Formulari Standard della Rete Natura 2000 [10] e sono state rilevate mediante la compilazione di apposite schede di campo.

Per analizzare le differenze nel numero delle plantule tra diversi anni, stagioni, popolazioni e uso del territorio, nonché l'interazione tra anni e popolazioni, è stato utilizzato il GLMM con una distribuzione di errori tipo "*poison*" e una funzione *log link*. I fattori fissi e casuali cambiano in accordo al modello utilizzato (vedi Tabella 1). L'analisi statistica è stata realizzata attraverso l'utilizzo del software R [11].

Risultati

Nel corso dei monitoraggi effettuati sugli individui di *J. macrocarpa* all'interno dei *plots*, è stato registrato il numero totale di ginepri con un *range* che è variato tra 2 (P2, Buggerru, primavera 2010) e 103 individui (P4, Buggerru, primavera 2011). Il numero medio di individui totali per *plot* è risultato variabile in funzione della località da un minimo di 9,1 ± 4,9 ginepri (Domus de Maria) a un massimo di 17,9 ± 26,4 (Buggerru). Il numero di individui appartenenti alla classe dimensionale delle plantule, in particolare, è variato da 0 a 101 individui.

E' stato calcolato il dato relativo alla variazione percentuale nel numero di plantule dei *plots* tra le stagioni di monitoraggio, sia considerando la diminuzione percentuale osservata nel periodo autunnale rispetto a quello primaverile per il totale delle plantule (-31,6%), sia considerando tale variazione nelle diverse località (Buggerru: -44,9%, Villasimius: -27,9%, Domus de Maria: -20%, Arbus: +28,6%).

L'analisi statistica effettuata sulle plantule evidenzia un effetto significativo per tutti i fattori considerati ad esclusione del fattore uso del territorio (turistico o non turistico) (tabella 1). In particolare (figura 2), a Buggerru il numero medio di plantule conteggiate (19,2 ±16,6) è risultato significativamente superiore rispetto a quello di Domus de Maria (stima: -1,42, p: $2e^{-16}$), Arbus (stima: -1,75, p: $2e^{-16}$) e Villasimius (stima: -0,39, p: $3{,}93e^{-05}$). Si è rilevato un numero medio di plantule (5,7 ±11,4) significativamente più basso nel 2010 (stima: -0,34, p: 0,035). Il confronto tra i monitoraggi stagionali (figura 3) ha evidenziato come nella stagione primaverile fosse presente un valore medio di plantule (9,5 ±20,3) significativamente superiore (stima: 0,49, p: 0,028) a quella autunnale (7,5 ±11,7).

L'analisi statistica effettuata sugli individui passati alla classe dimensionale adulta nel periodo 2009-2011, non ha evidenziato nessun effetto significativo per tutti i fattori considerati; solamente nel sito di Arbus, nei *plots* A3 ed A4, si è riscontrato l'incremento rispettivamente di 1 e 2 individui della classe adulti nel corso dei sei monitoraggi effettuati. Infine, solo nel *plot* A2 di Arbus è stato registrato nell'autunno 2011 il passaggio di 1 individuo dalla classe dimensionale delle plantule a quella dei giovani.

L'analisi degli impatti della fruizione sugli habitat ha mostrato che nei siti di Villasimius e Domus de Maria i fattori di pressione più frequentemente osservati (sul totale dei rilievi effettuati in ogni sito) sono: erosione prodotta da calpestio (37% Domus de Maria, 33% Villasimius), rifiuti (27% Domus de Maria, 19% Villasimius) e deiezioni umane (14% Villasimius, 9% Domus de Maria). La presenza di specie aliene è stata riscontrata in entrambi siti, con una maggiore intensità a Villasimius (24%) dove sono stati osservati 11 *taxa* esotici.

L'analisi dei fattori di pressione ha indicato che nei siti di Villasimius e Domus de Maria gli habitat maggiormente disturbati sono il 2210 (Dune fisse del litorale del *Crucianellion maritimae*) con 7 tipologie di fattori di pressione per Domus de Maria e 5 per Villasimius, ed il 2250* (con 5 tipologie di fattori di pressione per entrambi i siti).

Nell'habitat 2250*, in particolare nelle zone in cui sono stati posizionati i *plots*, i disturbi più frequentemente rilevati sono stati: presenza di specie aliene [*Acacia saligna* (Labill.) Wendl., *Carpobrotus acinaciformis* (L.) L. Bolus], erosione da calpestio, presenza di rifiuti e deiezioni umane (Villasimius); erosione da calpestio, presenza di rifiuti, taglio di branche e di esemplari, vandalismo a carico di alcuni esemplari di *J. macrocarpa*, deiezioni umane (Domus de Maria).

Tabella 1 – Coefficienti degli effetti fissi del GLMM eseguito sul numero di plantule (NS: non significativo;*:0.05 >p>0.01; **:0.01 > p> 0.001; ***:p<0.001).

*± 1 – Coefficients for the fixed effects of the GLMM performed to explain seedling number (NS: not significant; *:0.05 >p>0.01; **:0.01 > p> 0.001; ***:p<0.001).*

	Stima	**Errore Standard**	**Valore z**	**Pr(>|z|)**
Località				
Intercept	2,71	0,14	18,94	< 2e^{-16} ***
Domus de Maria	-1,42	0,16	-8,69	< 2e^{-16} ***
Arbus	-1,75	0,15	-11,25	< 2e^{-16} ***
Villasimius	-0,39	0,09	-4,11	3,93e^{-05} ***
Uso del territorio				
Intercept	1,72	0,46	3,70	0,00022 ***
Turismo	0,12	0,66	0,18	0,86 NS
Anno x Stagione				
Intercept	0,13	0,78	0,17	0,87 NS
2010	-0,34	0,16	-2,11	0,035 *
2011	-0,09	0,15	-0,59	0,55 NS
primavera	0,49	0,16	2,99	0,0028 **
2010-primavera	-0,52	0,24	-2,20	0,028 *
2011-primavera	0,09	0,21	0,43	0,67 NS

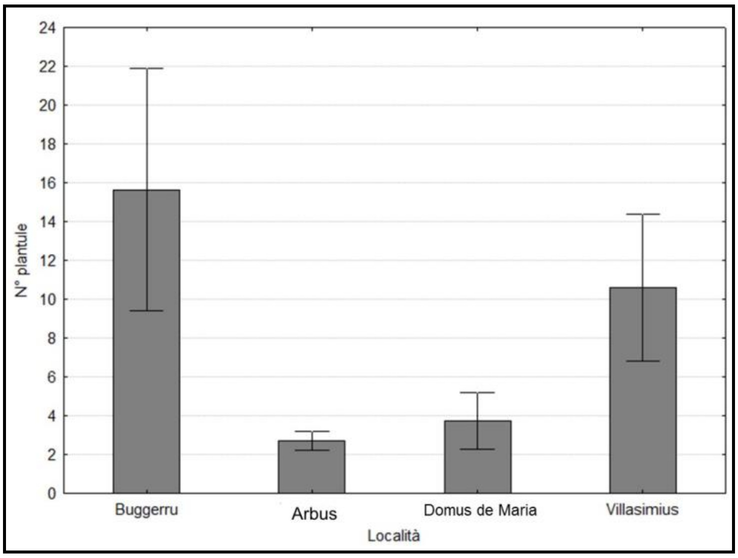

Figura 2 – Distribuzione del numero medio di plantule per località.
Figure 2 – Distribution of the seedlings mean number for locality.

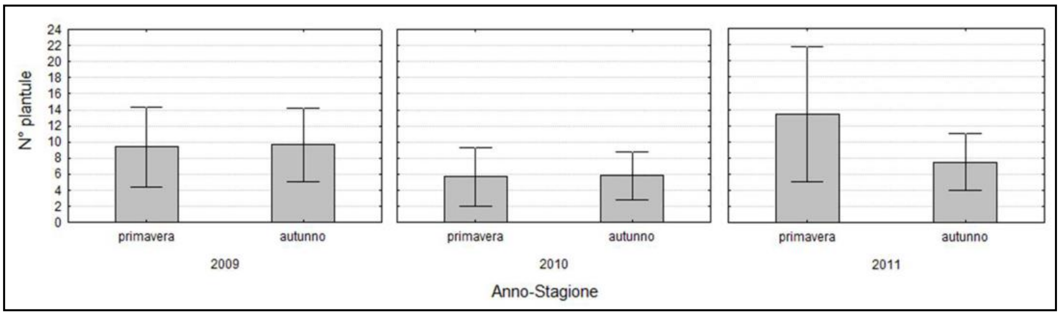

Figura 3 – Distribuzione del numero medio di plantule per anno e stagione.
Figure 3 – Distribution of the seedlings mean number per year and season.

Interessante notare come a Villasimius, nel *plot* che è risultato maggiormente sottoposto ai fattori di pressione osservati (Q2), nel corso dei tre anni di osservazione sono stati costantemente registrati valori medi nel numero di plantule (31,7 ± 8,7) nettamente più elevati rispetto agli altri 2 *plots* ubicati nella medesima località ad alcune decine di metri di distanza (Q 1: 0 ± 0; Q 3: 0,2 ± 0,4).

Nei siti di Arbus e di Buggerru non sono stati invece rilevati significativi fattori di pressione, in particolare di origine antropica, trattandosi di aree non direttamente interessate dal turismo balneare, né da altre attività umane rilevanti.

Discussione

I risultati dei monitoraggi effettuati per questo studio allo scopo di comprendere gli effetti dell'impatto antropico sulle formazioni a *J. macrocarpa*, hanno mostrato una rilevante variabilità nel numero di individui rilevati tra le diverse classi dimensionali, tra località e tra stagioni di rilevamento. In particolare, la variazione stagionale nel numero di plantule tra autunno e primavera concorda con i risultati già ottenuti da Bacchetta *et al.* [9] e conferma che l'estate rappresenta la stagione più critica per la sopravvivenza delle plantule. Questo può essere imputato allo stress idrico che caratterizza la stagione estiva in ambito mediterraneo [12] al quale sono particolarmente vulnerabili le plantule. Occorre precisare, tuttavia, che il significativo dato relativo alla diminuzione delle plantule nel periodo estivo riportato deve essere considerato indicativo in quanto, considerata l'elevata variabilità riscontrata tra diversi *plots*, anche nell'ambito della stessa località e tra diverse annualità, per una valutazione più attendibile sarebbe necessario analizzare i dati di un maggior numero di *plots* per località e relativi a un periodo di osservazione più lungo. Questo aspetto è tra gli obiettivi di uno studio attualmente in corso.

Il dato relativo a Buggerru, risultata la località con il maggior numero medio e totale di individui per *plots*, può essere, almeno in parte, spiegato sulla base delle differenze ecologiche di questo sito, costituito da dune stabilizzate, coperte da una fitta boscaglia e relativamente lontane dalla linea di costa, rispetto agli altri tre.

L'elevata variazione stagionale nel numero delle plantule, congiuntamente al basso numero di giovani e adulti presenti, evidenziano una forte selezione delle stesse, che ne spiega la bassa probabilità di passare alle successive classi dimensionali. Tra i fattori che causano il decesso delle plantule ha sicuramente una notevole importanza lo stress idrico nella stagione estiva. Questo incide in particolare nei *plots* in ambiente maggiormente aperto, dove manca l'effetto protettivo esercitato dalle chiome, che permettono una minore incidenza della radiazione solare al suolo, con conseguente minore evaporazione nel livello superficiale del substrato e minore evapotraspirazione da parte degli esemplari. Tuttavia, anche nelle aree più stabili e protette, dove un elevato numero di individui riesce a sopravvivere alla stagione estiva, la possibilità delle plantule di divenire adulte appare comunque limitata dalla copertura costituita dai ginepri adulti, che lasciano poco spazio alla colonizzazione vegetale, come dimostra la bassa incidenza degli individui della classe dei giovani [8].

I risultati di questo monitoraggio non hanno permesso di evidenziare una rispondenza significativa tra numero e/o mortalità di plantule e uso del territorio. L'impatto antropico dato dall'attività turistica nei siti analizzati non ha quindi avuto degli effetti osservabili sulle formazioni a *J. macrocarpa*, nello specifico sulle plantule, nell'arco del periodo di osservazione e in particolare delle singole annualità, nell'ambito delle quali la mortalità appare imputabile soprattutto alle condizioni climatiche stagionali. Questo non consente di affermare, tuttavia, che la pressione antropica non abbia nel lungo termine una incidenza sui ginepreti e sui sistemi dunali più in generale. La stessa risulta evidente dagli impatti dovuti ai fattori di pressione osservati, in particolare dai segni di erosione del sistema dunale presenti nelle due località interessate dal turismo estivo.

Per quanto riguarda l'origine di tale disturbo, è indicativo il fatto che nei siti indagati l'habitat sul quale è stato osservato il maggior numero di fattori di pressione sia il 2210. Questo, infatti, è costituito da formazioni appartenenti all'alleanza *Crucianellion maritimae* che si trovano più prossime al litorale rispetto all'habitat 2250* e sono pertanto più esposte agli effetti del calpestio e ad altri fattori di disturbo da parte dei fruitori delle spiagge.

I risultati ottenuti fanno ritenere utile proseguire e implementare gli studi sulle popolazioni della specie e sull'habitat, al fine di acquisire dati relativi all'ecologia del *taxon* e ai fattori di pressione che ne minacciano le popolazioni, indispensabili per pianificare future azioni di conservazione *in situ* ed *ex situ*.

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APPENDIX 5 - Altre pubblicazioni prodotte durante il dottorato di ricerca

1. Articoli scientifici su riviste indicizzate:
    - a Fenu G., Cogoni D., Ferrara C., Pinna M.S., Bacchetta G. (2012) Relationships between coastal sand dune properties and plant community distribution: The case of Is Arenas (Sardinia). Plant Biosyst. 146(3): 586-602.
2. Articoli scientifici su riviste non indicizzate:
    - a Pinna M. S., Pontecorvo C., Bacchetta G. (2010) Il progetto Life Providune per la valutazione delle minacce, ripristino e conservazione degli habitat dunali. Atti del Terzo Simposio CNR-Ibimet: "Il monitoraggio costiero mediterraneo: problematiche e tecniche di misura". 131-138.
    - b Pinna M.S., Pontecorvo C., Cañadas E.M., Bacchetta G. (2012) Analisi dell’impatto antropico sulle formazioni a *Juniperus macrocarpa* della Sardegna meridionale. Atti del IV Simposio Internazionale "Il Monitoraggio Costiero Mediterraneo, problematiche e tecniche di misura" (in stampa).
    - c Fenu G., Pinna M.S., Cogoni D., Bacchetta G. (2012) Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: Astragalus tegulensis Bacch. & Brullo. Informatore Botanico Italiano 44(1): 207-209.
    - d Pinna M.S., Fenu G., Farris E., Fois M., Pisanu S., Cogoni D., Calvia G., Bacchetta G. (2012) Schede per una Lista Rossa della Flora vascolare e crittogamica Italiana: Linaria flava (Poir.) Desf. subsp. sardoa (Sommier) A. Terrac. Informatore Botanico Italiano 44(2): 449-452.

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