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ORIGINAL ARTICLE

Ex situ conservation of dwarf junipers (*Juniperus hemisphaerica* Presl, *Juniperus nana* Willd.) in Pollino National Park, southern Italy

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Abstract
Dwarf junipers (family Cupressaceae), dioecious and wind-pollinated coniferous shrubs with a low tolerance for shade and a slow growth rate, can be an important biological indicator of climate changes in a mountain environment at a Mediterranean latitude. In the southern Italian mountains they grow at altitudes above 1500 m a.s.l. in wide grazed clearings or over the timberline, and are characteristic of xeric communities, rich in flora and fauna. Nowadays, the conservation of these species is threatened because of the dynamism in the patterns of vegetation and the extremely scarce natural regeneration. This paper presents the initial results of ongoing nursery and laboratory experiments carried out on seed germination and vegetative propagation of autochthonous dwarf junipers (*Juniperus hemisphaerica* Presl, *Juniperus nana* Willd.) of the high-mountain areas of Pollino National Park (southern Italy) to promote *ex situ* conservation. Specific protocols were prepared to break seed dormancy and develop best practices for propagation using cuttings.

Keywords: *conservation, dwarf junipers, Mediterranean high mountains, pregermination treatments.*

Introduction

One of the effects of global warming in the European mountains is that orophilous species are seriously endangered by the advance of lowland plants (Grabherr et al., 1994; Penuelas et al., 2002). Although Mediterranean mountains, a biodiversity hotspot for conservation priorities, are considered one of the most threatened systems in the European Union, there is almost no information available for the conservation of high-level plants (Myers et al., 2000; Thuiller et al., 2005).

Pollino National Park mountains, in southern Italy, are considered "high mountains" of Europe (Grabherr et al., 1994), with interesting vegetal communities and floristic elements still worthy of scientific research.

The National Park is the largest natural park in Italy, spreading over more than 200,000 ha of wild areas and cultural landscapes in the southern Apennine, where timberline-forming tree and shrub species, Bosnian pines (*Pinus leucodermis* Antoine) and dwarf junipers (*Juniperus hemisphaerica* Presl (=*J. communis* L. subsp. *hemisphaerica* (J.Presl) Nyman; *J. communis* L. var. *communis* L.) and *Juniperus nana* Willd. (=*J. communis* L. subsp. *alpina* (Suter) Celak., *J. communis* L. var. *saxatilis* Pall.)) are characteristic of a relict habitat of ancient tundra.

Juniperus nana is quite common in low-altitude areas of northern Europe and in the high mountains of central and southern Europe. It is a prostrate dioecious shrub, less than 50 cm tall and up to 3 m in diameter. Galbulae are small ((2) 4–5 mm diameter) and leaves are short (1–1.5 × 9–10 mm), closely set, ascending appressed, abruptly tapered to a short and sometimes blunt tip such that the branchlets are scarcely sharp to the touch.

Juniperus hemisphaerica is found mainly in the mountainous areas of southern Europe and north-west Africa. It forms a low, compact, somewhat round shrub with larger galbulae (6–7 (10) mm) and longer leaves (1–1.5 × 15–17 mm) than those of *J. nana*. The leaves are closely set, patent, gradually

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tapering to a sharp tip so that branchlets are prickly to the touch. Dwarf junipers are considered as a critical taxonomic group related to *Juniperus communis* s.l. (Tutin et al., 1993; Farjon, 2001; Adams, 2004). In this paper, however, the authors follow *Flora d'Italia* (Pignatti, 1982) in referring to them as species.

Dwarf junipers in Pollino National Park form xeric shrublands with high ecological value in relation to their associated flora and fauna. In the lowest areas of the park, between 1500 and 2000 m a.s.l., they typically grow together with shrub and tree taxa, thus forming typical biogroups (*Daphno oleoidis–Juniperion alpinae* Blasi, Gigli, Abbate et Stanisci 1989; *Pino leucodermis–Juniperetum alpinae* Stanisci 1997).

On Mount Pollino (2248 m a.s.l.) and Serra del Prete (2181 m a.s.l.), the shrub and tree taxa become increasingly scarce and, in the highest zones, dwarf junipers coexist with perennial herbaceous species or small chamaephytes. These remnant communities are part of the habitat 4060 Alpine and boreal health and 95A0 High oro-Mediterranean pine forests (Dir. Habitat 92/43/EEC), which has an important biogeographical significance in southern Italy.

Dwarf junipers are biological indicators of climate-induced changes in mountain environments at Mediterranean latitudes (Grabherr et al., 2003; Nagy, 2006) and it is very important to investigate their reproductive biology to increase their chances of conservation. These species are characterized by high and variable complex exogenous and endogenous seed dormancy and low production of vital seeds (Broome, 2003; Mugnaini et al., 2004), so that specific germination methods are essential for successful propagation. Seed storage in germplasm bank is considered the best way to maintain large pools of genetic diversity in plants and, therefore, the reinforcement of wild populations using stored seed material may be a valuable tool to conserve endemic and threatened species (Cerabolini et al., 2004).

This paper presents the first attempt to promote *ex situ* conservation of autochthonous dwarf junipers of the high-mountain areas of Pollino National Park. Initial results of ongoing nursery and laboratory experiments focusing on seed germination and vegetative propagation are also reported.

Materials and methods

Collecting sites

Surrounded by Quaternary basins, where sediments of continental and marine origin accumulated, Pollino's mountains consist primarily of limestone rocks of the Triassic, Jurassic and Cretaceous periods, and represent the largest limestone mountain area of southern Italy. The highest mountain in the park is Serra Dolcedorme (2267 m a.s.l.). Other mountains that are more than 2000 m high are Monte Pollino (2248 m a.s.l.), Serra del Prete (2281 m a.s.l.), Serra delle Ciavole (2127 m a.s.l.) and Serra di Crispo (2053 m a.s.l.). The bioclime is Temperate Oceanic Submediterranean and the area belongs to the Mediterranean Italo-Tirrenian biogeographical region (Rivaz-Martinez, 1997, 2004).

Research on the presence and distribution of upper level junipers in Pollino National Park has led to the distinction of several areas of distribution, some with a few isolated individuals, others with more consistent groups both of *J. nana* and *J. hemisphaerica*, where 10 collecting sites were chosen at different altitudes, from 1450 m at Piano Visitone to 2148 m at Monte Pollino (Figure 1, Table I). The site were chosen in different habitats: the high mountain plains at Piano Visitone, Piano Ruggio, Vacquarro, Piano Jannace, Monte Zaccana and Piani del Pollino, and the rocky areas at Madonna del Pollino, Serra di Crispo, Serra delle Ciavole and Monte Pollino.

Data analysis

Eight morphological traits of 10 individuals of *J. nana* and 10 individuals of *J. hemisphaerica* were considered to characterize the morphological differences associated with the two species present in the collecting sites (Table II). Measurements were made by means of an electronic digital calliper, with an LCD, 150–0 mm on fresh needles, berries (galbulae) and seeds.

The number of empty seeds in a sample of 100 seeds for each species was checked.

Figure 1. Pollino National Park: Bosnian pine (*Pinus leucodermis* Antoine) and dwarf junipers (*Juniperus nana* Willd.) at Serra di Crispo site (2050 m a.s.l.). (Photograph by M.R. Lapenna.)

Table I. Pollino National Park collecting sites.

Sites	WGS84 geographical coordinates	Altitude (m a.s.l.)	Species	Samples
Piano Visitone	39°56′27″ N, 16°08′44″ E	1450	Jh	Seeds
Piano Ruggio	39°54′44″ N, 16°07′34″ E	1510	Jh	Seeds
Vacquarro	39°55′49″ N, 16°10′22″ E	1525	Jh	Seeds
Madonna del Pollino	39°57′11″ N, 16°10′39″ E	1537	Jh	Seeds
Monte Zaccana	40°02′21″ N, 15°56′43″ E	1580	Jh	Seeds/cuttings
Piano Jannace	39°56′22″ N, 16°11′44″ E	1676	Jh	Seeds
Piani del Pollino	39°54′48″ N, 16°12′29″ E	1803	Jh	Seeds
Serra di Crispo	39°55′52″ N, 16°12′39″ E	2050	Jn/Jh	Seeds/cuttings
Serra delle Ciavole	39°54′40″ N, 16°13′00″ E	2127	Jn/Jh	Seeds
Monte Pollino	39°54′31″ N, 16°11′18″ E	2248	Jn/Jh	Seeds

Note: Jh =Juniperus hemisphaerica Presl; Jn =Juniperus nana Willd.

Univariate statistical analysis and analysis of variance (generalized linear models GLM) (McCullagh & Nelder, 1989) between and within plants were performed.

SAS statistical software (version 9.1.3) was used in the analysis.

Propagation from cuttings

Fifteen branchlets from different individuals of *J. nana* and *J. hemisphaerica* each were collected in March 2008 (Table I), just before the winter dormancy began to break (Broome, 2003). Cuttings were only taken from the tips of the branches which included the previous year's growth and were inserted into trays of compost (equal mix of peat, bark and perlite) with a hormone rooting powder.

Propagation from seeds

In November 2007, branchlets with mature galbulae were randomly collected from three individuals on each collecting site (Table I). The collected mature galbulae were soaked in water containing 2% sodium hydroxide (NaOH) to soften the pulp for 24 h and then opened to take out 2800 seeds, equally subdivided into three groups. Three specific protocols, one for each group of seeds, were prepared to break seed dormancy. They provided a series of pregermination treatments: seed scarification by means of citric acid or sulphuric acid and seed stratification for variable periods (Table III). The treatments were applied to germination of *J. communis* L. and North American junipers (*J. scopulorum* Sarg., *J. virginiana* L. and *J. monosperma* (Engelm.) Sarg.). Seeds were first placed in Petri dishes on sterile absorbent cotton soaked in distilled water and fungicide during the pregermination period. Then they were sown in potting compost of grit and peat. Ninety seeds (30 for each protocol) were used for *in vitro* culture. *In vitro* culture was done using $2\,g\,l^{-1}$ Murashige and Skoog (MS) basal medium, $0.5\,g\,l^{-1}$ MS vitamin, $4\,g\,l^{-1}$ agar and $5\,g\,l^{-1}$ sucrose. The seeds were first soaked for 24 h in distilled water containing plant hormones: auxins ($0.1\,g\,l^{-1}$ indole-3-acetic acid (IAA), $0.1\,g\,l^{-1}$ α-napthalenacetic acid (NAA)) and cytokinins ($0.1\,g\,l^{-1}$ 6-benzylaminopurine). They were sterilized by immersion in a 75% ethanol for 3 min and then in 2% sodium hypochlorite for 10 min. They were washed three times with sterile distilled water and then put in glass culture tubes containing sterile medium under a horizontal laminar flow bench.

All of the sown seeds were maintained at a constant temperature of +15°C to prevent development of secondary dormancy (Piotto et al., 2003; USDA Forest Service Data and Information Systems). The sowings were carried out between March and July 2008. Part of the seeds (120) were sown in natural conditions in November 2007, without any pregermination treatment.

In July 2008, more mature galbulae of *J. nana* and *J. hemisphaerica* were collected at Serra di Crispo. The galbulae were stored at ambient temperature to favour possible maturation of the embryos. In April 2009, after 1 week of water maceration, 200 seeds were extracted from galbulae, washed in 80% ethanol and water, mechanically scarified and sown in potting compost without covering the seeds.

Results

The analysis of morphological traits confirms the presence of *J. nana* and *J. hemisphaerica* in Pollino's mountains. The values of the length of the needles and the width of the galbulae are in agreement with those reported in *Flora d'Italia* (Pignatti, 1982) and *Flora Europaea* (Tutin et al., 1993) (Table II).

The percentage of empty seeds of *J. nana* and *J. hemisphaerica* collected in Pollino National Park was 75% and 60%, respectively.

At the end of November 2009 only two seeds of *J. nana* had germinated (Figure 2). The third protocol was followed to break the dormancy of the group of

Table II. (a) Univariate statistical analysis for *Juniperus* species in Pollino National Park; (b) analysis of variance.(a) Univariate statistical analysis

Species	*n*	Source of variation		No. of observations	Mean	SD	Min.	Max.
J. nana	10	Galbulae	Length	79	6.34	0.88	4.70	7.91
			Width	79	6.41	0.64	5.14	7.93
		Leaves	Length	100	7.73	1.61	3.58	11.03
			Width	100	1.29	0.18	0.93	1.72
		Seeds	Thickness	83	0.45	0.14	0.18	0.86
			Length	83	4.44	0.60	3.12	5.86
			Width	83	2.90	0.47	1.69	3.74
			No. per galbulae	78	2.23	0.84	1	3
J. hemisphaerica	10	Galbulae	Length	90	8.58	0.96	6.84	11.80
			Width	90	8.93	1.36	6.90	11.32
		Leaves	Length	100	12.76	1.89	9.17	17.98
			Width	100	1.44	0.23	0.90	2.09
		Seeds	Thickness	97	0.45	0.12	0.18	0.75
			Length	97	5.62	0.56	4.37	7.09
			Width	97	3.35	0.55	2.29	5.06
			No. per galbulae	90	2.74	0.51	1	3

(b) ANOVA

		Galbulae				Needles				Seeds							
		Length		Width		Length		Width		Thickness		Length		Width		No. per Galbula	
Source of variation		df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS	df	MS
J. nana	Between plants	9	3.70*	9	1.17*	9	9.38*	9	0.08	9	0.06	9	2.00*	9	1.03*	9	3.74*
	Within plants	69	0.40	69	0.31	90	1.92	90	0.02	73	0.01	73	0.15	73 ×	0.12	68	0.30
J. hemisphaerica	Between plants	9	6.41*	9	8.42*	9	24	9	0.21*	9	0.03	9	1.90*	9	1.97*	9	1.41*
	Within plants	80	0.30	80	0.41	90	1.53	90	0.03	87	0.01	87	0.15	87	0.16	80	0.13

Note: df = degrees of freedom; MS = Mean Square.
*$p < 0.01$.

Table III. Pregermination treatments.

	Protocol 1 [a]	Protocol 2 [b]	Protocol 3 [c]
Scarification	24 h distilled water soak	24 h distilled water soak	24 h hydrogen peroxide soak (5%) + 24 h Genial GA3 soak
	96 h citric acid (1%) soak	15 min in H_2SO_4 soak Rinse and use baking soda to neutralize acid; rinse again Soak in water overnight	
Stratification	6 weeks warm moist (24°C) 10 weeks cold moist (5°C)		30 days cold moist (5°C)
Sowing			

Note: [a] *Juniperus virginiana*; [b] *Juniperus communis*; [c] *Juniperus monosperma* (USDA National Tree Seed Laboratory).

seeds (Table III). The seeds were scarified by immersion for 24 h in hydrogen peroxide soak (5%) and then soaked for 24 h in Genial GA3. They were maintained for 30 days at 5°C and then sown in a compost of peat and sand. The results of germination tests of the other seeds are still expected.

Propagation by cuttings was successful. Twenty cuttings have survived and show new roots and leaf buds (Figure 3).

Discussion

Propagating high mountain Mediterranean juniper from seeds is a difficult task to accomplish. The proportion of viable seeds is generally low and it exhibits considerable variation across Europe (Garcia et al., 2000). Junipers do not produce a persistent seed bank and it is estimated that first year seedling mortality is 75–80% and that only six out of 10,000 seeds are able to produce a seedling surviving during their first year (Garcia, 2001). Moreover, their first reproduction takes place only after approximately 20 years (Ward, 1982), and the variability in germination time is high, ranging between 2 and 5 years (Broome, 2003). The number of empty and not fully developed seeds is also very high (Piotto et al., 2003).

The natural reproduction of upper level junipers in Mediterranean mountains can also be impeded by climatic stress due to summer drought. The fact that adult and old individuals are predominant in the juniper populations of the circum-Mediterranean mountains in general, and the Pollino's mountains in particular, is symptomatic of a slow natural renovation: as a consequence, the population stability is often due to the longevity of the plants and not to the birth of new ones (Garcia et al., 1999).

It is important to consider that although junipers are pioneer species, capable of colonizing of extreme habitats, they can play an important role as forest species suitable for environmental restoration. Improving *ex situ* conservation of junipers is thus crucial to obtain plant from seed. Using cuttings is a valuable and rapid method for propagating junipers, but plants grown from cuttings have a genetic make-up that is identical to the parent plant. Seed propagation is the best way to promote biodiversity.

The procumbent juniper populations of Pollino National Park are in no immediate danger of rarefaction, but the conservation of these species

Figure 2. Seed of *Juniperus nana* during the first step of germination. (Photograph by M.R. Lapenna.)

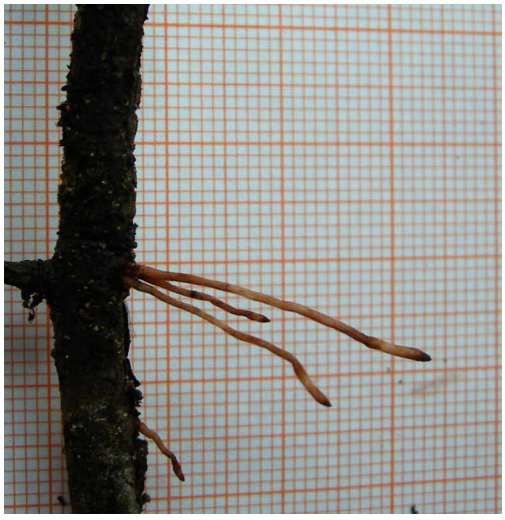

Figure 3. Cutting of *Juniperus hemisphaerica* with new roots. (Photograph by M.R. Lapenna.)

may be threatened by the dynamism in patterns of vegetation induced by global climate change, the impact of socioeconomic transformations on land use, and the extremely scarce natural regeneration due to high and variable seed dormancy and low production of vital seeds.

There are very few studies on the propagation of dwarf junipers, and this is the first attempt to carry out research on the propagation of these species in the Pollino National Park.

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