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Ecological remarks on *Astragalus maritimus* and *A. verrucosus*, two threatened exclusive endemic species of Sardinia

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Abstract.- Astragalus maritimus Moris and *A. verrucosus* Moris are two endemic threatened species exclusive of South West Sardinia. In this study the populations of both species were characterized and their seed germination investigated. The *in situ* results allowed reviewing their conservation status in compliance with the IUCN criteria, confirming the CR category by new subcriteria for both species. The germination study allowed identifying as well their dormancy breaking requirements.

Key words : *Astragalus* - autoecology - endemics - conservation status - germination.

Résumé.- Astragalus maritimus Moris et *A. verrucosus* Moris sont deux espèces endémiques menacées exclusives du sud-ouest de la Sardaigne. Cette étude a permis la caractérisation des populations ainsi que l'étude de leur biologie de germination. Les analyses *in situ* ont permis de réexaminer le statut de la conservation selon les critères de l'IUCN et de confirmer la catégorie CR avec des nouveaux sous-critères. Les études sur la germination ont abouti à l'identification de l'exigence de la rupture de la dormance.

Mots clés : *Astragalus* - autoécologie - endémiques - état de conservation - germination - Sardaigne.

I. INTRODUCTION

This study aims to analyze the ecology of two threatened endemic taxa growing exclusively in the Sulcis-Iglesiente area (SW Sardinia): *Astragalus maritimus* Moris and *A. verrucosus* Moris. These two species, described by Moris (1827, 1837), were doubtingly regarded until De Marco *et al.* (1977) that produced a complete description of *A. maritimus*; the autonomy of the taxon was later confirmed by Corrias (1978), Pignatti (1982) and Conti *et al.* (2005).

These two taxa show a narrow distribution (Fig. 1 to 3) and are included in the Bern Convention annexes (CEE, 1982) as priority species, in Annex II of the 92/43/CEE 'Habitat' DIR. (EC, 1992) and in its updated version (CE, 1997). According to the IUCN risk categories (2001), they were classified as vulnerable (VU) by Conti *et al.* (1992) and as critically endangered (CR) by Conti *et al.* (1997) and Bacchetta (2001).

Only in recent years conservation biology studies were started in Sardinia, with the purpose to know, to conserve and to manage threatened and rare species. To date, only on some endemic taxa of Sardinia, ecological and genetic studies have been made, such as *Anchusa* sp. pl. (Quilichini & Debussche, 2000; Quilichini, 2001; Quilichini *et al.*, 2004; Coppi *et al.*, 2008) and *Centaurea horrida* Badarò (Mameli *et al.*, 2008). Other studies were carried out on the conservation status of threatened taxa such as *Lamyropsis microcephala* (Moris)

Fig. 1.- Localization of *A. maritimus* and *A. verrucosus*.
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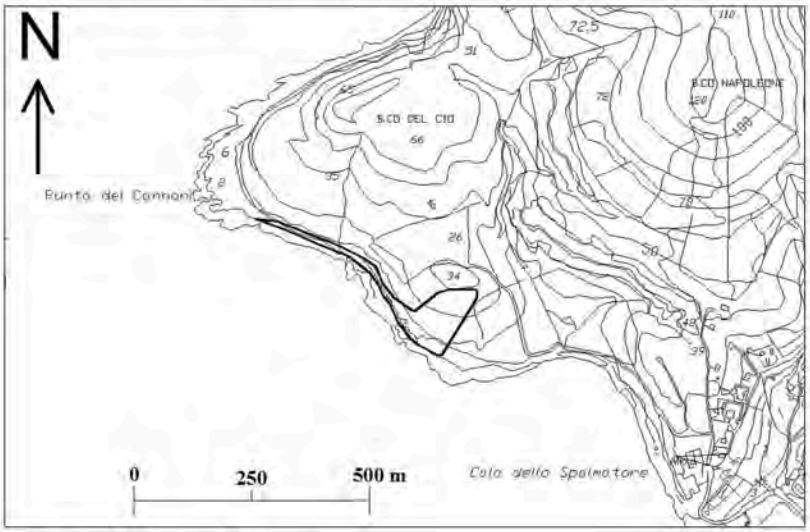

Fig. 2.- Population area of *A. maritimus*.
Fig. 2.- Zone de la population de *A. maritimus*.

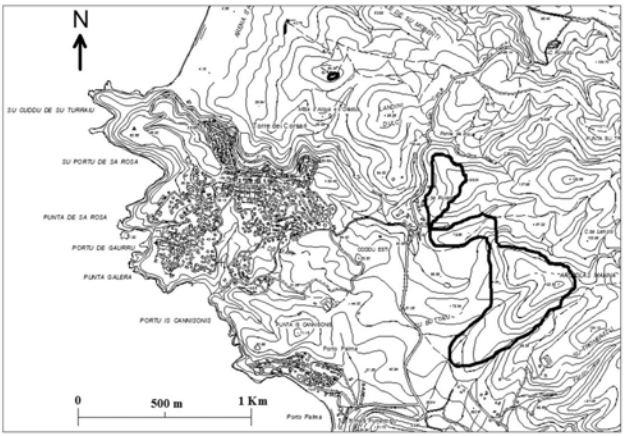

Fig. 3.- Population area of *A. verrucosus*.
Fig. 3.- Zone de la population de *A. verrucosus*.

Dittrich & Greuter (Bacchetta *et al*., 2007), *Sarcopoterium spinosum* (L.) Spach (Gargano *et al*., 2007) and *Centaurea horrida* (Farris *et al*., 2008; Pisanu *et al*., 2009).

A first integrated approach to conservation biology studies, also from a methodological point of view, on *A. maritimus* and *A. verrucosus* was made within the Interreg IIIB project « Genmedoc » (2004-2006) that allowed starting the research into sixteen endemic taxa placed in SACs of Nature 2000 network of Sardinia and their conservation at the Sardinian Germplasm Bank (BG-SAR) (Bacchetta *et al*., 2008).

This study introduces the results of the ecological research carried out over the last five years analyzing the auto-ecology, the population features, the reproductive biology and the threats on the populations of these two species, with the main aims to 1) characterize their populations, 2) update their risk categories applying the IUCN criteria and 3) investigate their germination biology.

Fig. 4.- *A. maritimus* flowered.
Fig. 4.- *A. maritimus* en fleurs.

II. MATERIALS AND METHODS

A. Plants description

The genus *Astragalus* L. (Fabaceae) has ca. 2,500 species, included in some 245 taxonomic sections (Lewis *et al*., 2005). *A. maritimus* and *A. verrucosus*, inside the subgenus *Trimeniaeus* Bunge, were assigned to the *Drepanodes* Bunge and *Platyglottis* Bunge sections, respectively (Podlech, 1990, 2008).

Fig. 5.- *A. verrucosus* flowered.
Fig. 5.- *A. verrucosus* en fleurs.

A. maritimus (Fig. 4) is a hemicryptophyte cespitose or, more rarely, a therophyte plant. It has prostrate-ascending stems [20-40(50) cm] with whitish hairs. Leaves are paripinnate (6)8-15 cm long, with pubescent stalk and rachis because of white patent hairs. Leaflets are (5)7-14 pairs [length: (6)7-16(23) mm, width: 4-10 mm], with short stalks and an obovate to elliptical shape, truncated and trimmed on the apex. Inflorescences are almost as long as the leaves, or even more, with slightly costated and scored footstalks, often burgundy-sprayed, with white patent hairs. The pod is lanceolate and elongate, (20)25-30 mm long, with a twisted and acuminate apex; it is dorsally and ventrally compressed, with a cordate section because of a deeply-introflexed suture; it has a membranous body, coriaceous when ripe (5)6-7(8) reniform seeds are available in each of two perfectly detached loculi. *A. maritimus* blooms from early April until the first half of May and fructifies between April and May (Moris, 1837; Chater, 1968; De Marco *et al.*, 1977; Corrias, 1978; Pignatti, 1982; Bacchetta, 2001).

A. verrucosus (Fig. 5) is a prostrate hemicryptophyte caespitose plant with tap-roots, often swollen, with yearly stems, woody at the base, 3-10 dm long, with long thin patent petals. Leaves are imparipinnate, (6)8-12(15) cm long. The plant has 7-15 leaflet pairs (4-8 x 8-15 mm) with short stalks, an ovate-oblanceolate shape and a truncate-trimmed apex. Inflorescences are almost equal to the leaves or a little longer, generally lax, in form of racemes with (4)5-8(10) flowers. Fresh pods are acuminate, ovate, falciform, wine-red (ochre when ripe), swollen and turgid because of a thick and spongy aqueous parenchyma, tuberculated and rugose with sparse hairs, compressed and remarkably furrowed on the dorsum, coriaceous when ripe, 10-15 mm wide and 20-25(30) mm long. Irregular reniform seeds are 4-5 mm wide, from ochre to orange-yellow. *A. verrucosus* generally blooms from late March until late June and fructifies between May and August (Moris, 1827, 1837; Chater, 1968; Corrias, 1978; Pignatti, 1982; Bacchetta, 2001).

B. *In situ* studies

A preliminary bibliographic study and an analysis on the exsiccata available in the main Sardinian herbaria (CAG, SS, SASSA) were carried out. The information collected in literature and referring to the herbarium samples were verified and integrated with the data gathered *in situ* during the whole period of the study.

The population studies were carried out according to the methodologies set out in the framework of the Interreg IIIB project Genmedoc and quoted in Bacchetta *et al.* (2006, 2008a), applying the criteria set out for the *Atlas Flora Amenazada* project in Spain (Albert *et al.*, 2003). The applied methodology included: population census, population location by plotting perimeter coordinates using a GPS device (datum WGS84), qualitative and quantitative gathering of stationary data, studies on the autoecology of both species and on the synecology of the communities they participate, identification of the current and potential threats for such populations, also with reference to the availability or unavailability of legal protection measures.

Populations were monitored every six months between 2002 and 2008 by dedicated survey sheets created in the framework of the project (Bacchetta *et al.*, 2006, 2008a), counting separately reproductive and non reproductive plants. In particular, with reference to the population censuses, these could not be carried out on *A. verrucosus* because of the abundant population, while they were conducted on *A. maritimus* in spring (2002 and 2005) and summer (2004, 2007 and 2008).

The major threats affecting the populations have been categorized following IUCN Threats Authority File (version 2.1; *www.iucnredlist.org/static/major_threats*). The IUCN risk categories for the two taxa were assessed according to the IUCN criteria, version 3.1 (2001), following the procedure for regional assessment (IUCN, 2003) and to the *Guidelines for Using the IUCN Red List Categories and Criteria, Version 6.2* (IUCN, 2006).

C. Seed germination tests

Seed lots stored in BG-SAR (Bacchetta *et al.*, 2008b; see Table I for seed lots details) were tested by applying different experimental pretreatments, in order to break physical dormancy. In particular, seeds were scarified by using sand paper, by immersion in H_2SO_4 at 96% for 5, 15 and 30 minutes and in boiling water (100 °C) after removing them from the heat source (Piotto *et al.*, 2001). After each preliminary treatment, seeds were left in immersion in distilled water for 24 hours. A control test was carried out exclusively by immersion for 24 hours and without preliminary treatments. Germination conditions were tested at the constant temperatures of 15, 20 and 25 °C with a 12/12 photo-period. For each condition, four replicates of 25 seeds each were prepared. Seeds were sown in autoclaved Petri glass dishes, with three filter-paper sheets humidified with distilled water. Germination was defined as visible radicle protrusion. Germination scores were carried out every two or three days and the total duration of the test was equal to two weeks, when a cut-test was carried out to determine the viability of any remaining seeds. The final germination percentages were calculated as the mean of the replicates (± 1 standard deviation) on the basis of the total number of filled seeds. T50, time to reach 50% of the maximum germination in one replicate, was also calculated.

D. Data analysis

Germination tests results were expressed as a final average percentage ± standard deviation and T50 values, the time to reach the 50% of the final cumulative germination in days

Table I.- Seed lot details.
Table I.- Details des accessions.

Taxon	Population	Code	Data	Sampled Area (m^2)	N. individuals	N. seed collected	Medium seed weight (g)
A. maritimus	San Pietro Island	312/05	04/07/2005	1000	105	36.881	0.00425 ± 0.00020
A. verrucosus	Arbus (Case Puxeddu)	305/05	30/06/2005	5000	63	26.704	0.01041 ± 0.00055

(Thanos & Doussi, 1995). Final germination percentages and T50 values were statistically analysed by the non parametric Kruskal-Wallis test. Statistical analyses were carried out by using *R package* (v. 2.4.1) for Windows.

III. RESULTS AND DISCUSSION

A. *In situ* analysis

The conducted study allowed assessing the autoecology and synecology of both species. *A. maritimus* is a heliophilous, thermo-xerophilous and moderately halophilous species, colonizing the hemicryptophyte meadows and the coastal chamephytic garigues located on the northern end of Cala dello Spalmatore, on San Pietro Island (Fig. 1 and 2). It can be found at altitudes from 2 to 35 m a.s.l., in a narrow coastal stripe with a width ranging from a few meters up to approximately 170 m and with a maximum length of 500 m, at an average distance from the foreshore of approximately 20 m, in locations characterized by low steepness, included between 5° and 10°, with prevailing S-SW exposure. The pedogenetic substrate on which it grows is made of liparitic tuffs and vitrophyric liparites with different liparite grains (De Marco *et al.*, 1977; Bacchetta, 2001). According to the data recorded by the thermopluviometric station of Carloforte and processed by Bacchetta (2000), the *A. maritimus* population can be found under Mediterranean pluviseasonal-oceanic bioclimatic conditions with lower thermomediterranean thermotype and lower dry ombrotype.

The literature provides only two phytosociological surveys for *A. maritimus* (De Marco *et al.*, 1977); the former is referable to *Oleo europaeae-Juniperetum turbinatae* Arrigoni, Bruno, De Marco & Veri *corr.* and the latter to biocoenoses not described but identifiable in *Thero-Brachypodion ramosi* Br.-Bl. 1925. *A. maritimus* is involved in hemicryptophyte communities concatenated with formations of *Crithmo maritimi-Limonion* Molinier 1934 and serially linked with the garigues of *Cistion ladaniferi* Br.-Bl. *ex* A. & O Bolòs 1950, evolving in under-growths referable to the linkage *Oleo europaeae-Ceratonion siliquae* Br.-Bl. *ex* Guinochet & Drouineau 1944 *em.* Rivas-Mart. 1975 and in scrubs dominated by *Juniperus phoenicea* L. subsp. *turbinata* (Guss.) Nyman. The vegetation series is therefore represented by micro-woods of *Oleo europaeae-Juniperetum turbinatae* Arrigoni, Bruno, De Marco & Veri 1985 *corr.* which, in the territories subject matter of the study, represents the climacic vegetation rather than the edaphoxerophil one.

A. verrucosus is a heliophilous and thermo-xerophilous species, colonizing the primary and secondary hemicryptophyte meadows originating on abandoned cultivated fields (Bacchetta, 2001). The only population of this species can be found on the Arbus munici-

pality coastal hills (Fig. 1 and 3), at altitudes included between 60 and 120 m a.s.l., in the highest internal areas with reference to the dune area of Pistis-Torre dei Corsari, at an average distance from the sea of 800 m, in locations characterized by moderate steepness, included between 0° and 25°, with prevailing W-NW exposure. It mainly grows on alluvial substrata, on which A-R soils develop, rich in clay and calcareous skeletons, mixed with trachitic stones (Corrias, 1978; Bacchetta, 2001). According to the data recorded by the thermopluviometric station of Capo Frasca and processed by Bacchetta (2000), the *A. verrucosus* population can be found under Mediterranean pluviseasonal-oceanic bioclimatic conditions with upper thermomediterranean thermotype and upper dry ombrotype.

Under the phytosociological point of view, *A. verrucosus* is involved in the linkage *Stipo bromoidis-Astragaletum verrucosi* Bacch., Brullo, Giusso & Guarino 2005; these are discontinuous herbaceous biocoenoses of hemicryptophyte type made up of cespitose graminoid species like *Stipa bromoides* (L.) Doerfl., *Dactylis hispanica* Roth and *Brachypodium retusum* (Pers.) P. Beauv. (Bacchetta *et al.*, 2005). Under the serial point of view, this association settles on the therophytic meadows referable to the linkage *Tuberarion guttatae* Br.-Bl. 1931 and precedes the garigues of *Cistion ladaniferi* Br.-Bl. *ex* A. & O Bolòs 1950, subsequently generating undergrowths of *Oleo europaeae-Ceratonion siliquae* Br.-Bl. *ex* Guinochet & Drouineau 1944 *em.* Rivas-Mart. 1975 and of *Ericion arboreae* (Rivas-Mart. *ex* Rivas-Mart., Costa & Izco 1986) Riv.-Mart. 1987. The climacic association of the series can be identified in the woods of *Quercus ilex* L., referable to the *Prasio majoris-Quercetum ilicis* Bacch., Bagella, Biondi, Farris, Filigheddu & Mossa 2004 (Bacchetta *et al.*, 2004).

The studies carried out allowed us to characterize the extent of the two taxa populations (Table II). The existence of one single population of *A. maritimus* located between the northern end of Cala dello Spalmatore and Punta dei Cannoni, on San Pietro Island (Fig. 2) is confirmed. At the beginning of the seventies, De Marco *et al.* (1977) estimated that this species included approximately 400 plants; later on, the data recorded by Bacchetta *et al.* (2001) indicated a reduction in population equal to approximately 300 individuals. According to the observations carried out at the end of summer 2000 (Bacchetta, 2001),

Table II.- Populations data.
Table II.- Données sur les populations.

	A. maritimus	*A. verrucosus*
Locality	Punta dei Cannoni - Carloforte (CI)	Case Puxeddu – Arbus (VS)
Altitude	2-35 m	55-194 m
Slope	10° (0-30°)	15° (0-25°)
Exposition	ENE-SW	E-WNW
Bedrock	Volcanic	Metamorphic
Substrata	Liparitic tuffs and liparites	Alluvial
Rockyness	25%	Absent
Stonyness	20-90%	10%
Thermotype	Thermomediterranean lower	Thermomediterranean upper
Ombrotype	Dry lower	Dry upper
Syntaxon	*Lygeo-Stipetea*	*Lygeo-Stipetea*
Vegetation series	*Oleo-Junipero turbinatae sigmetum*	*Prasio-Querco ilicis sigmetum*
Habitat Natura 2000	6220*	6220*
Habitat CORINE	3.2.1	3.2.1
SIC	San Pietro Island (ITB040027)	Is Arenas S'Acqua e S'Ollastu (ITB032229)

Table III.- Censuses of *A. maritimus*.
Table III.- Recensements de *A. maritimus*.

Data	Total number of plants	Non-reproductive plants	Reproductive plants
08/05/2002	2633	2339	294
02/08/2004	376	113	263
01/04/2005	1208	722	486
31/08/2007	321	172	149
05/09/2008	436	281	155

the population underwent a further decrease because of a fire involving it in the garigue behind Cala dello Spalmatore, only 150-200 plants were identified.

A. maritimus population occupies an area of approximately 1.8 ha, with remarkable seasonal variations and yearly changes. The censuses conducted (Table III) show remarkable differences in the number of total plants, particularly between spring and summer. Spring censuses also took into account many non-reproductive individuals that were no more present at the end of summer. For instance, 2633 individuals were identified in May 2002, of which only 294 were reproductive, while during the following spring census (April 2005) a total amount of 1208 individuals was recorded, of which 486 were reproductive (Table III). During the summer censuses, 376 (263 reproductive), 321 (149 reproductive) and 436 (155 reproductive) plants were counted in 2004, 2007 and 2008 respectively. It can be argued that the less-developed ones, located on superficial soils, are not always able to complete their life-cycle and, in any case, they are unable to survive summer, contrarily to the others, more developed and growing on deeper soils in locations protected by bushes. These plants are able to survive summer, thus indicating that the species can survive for several years.

A distribution limited to the coastal hills of Arbus municipality is confirmed for *A. verrucosus* but, unlike the information provided by Bacchetta (2001), confirming the presence of this taxon in three coastal areas quite close one to another, we see that these communities actually make up one single population. Currently, this population occupies a total area of approximately 70 ha (Fig. 3); the anthropization of the area lead to fragmentation into a main core and a secondary smaller core, which is approximately 750 m away from the first one as the crow flies.

As far as the extent of the population of *A. verrucosus* is concerned, Bacchetta (2001) estimated the presence of approximately 50 plants in the first location, of 100 in the second one and 70 in the third one. The surveys carried out in the framework of the present study allowed us to establish that the population is made up of an estimated number of individuals equal to approximately 10000 units.

For both taxa, the main threat is represented by the development of tourist activities (Threat 10.1. Recreation/tourism). These activities lead to a gradual urbanization (Threat 1.4.3. Tourism/recreation and 1.4.2. Human settlement), change of use of the territories (Threat 1.2.2. Change of management regime) and to an alternation of the natural habitats hosting the species. In addition, both species are subject to biotic threats, like low density of reproductive plants for *A. maritimus* (Threat 9.5. Low densities) and increasing competition due to vegetation developments towards the garigues and to serial undergrowths (Threat 1.6. Change in native species dynamics - directly impacting habitat). An additional threat for *A. maritimus* is represented by the passage of off-highway vehicles in the settlement area (Threat 10.6. Other: trampling, soil compaction and deterioration of soil structure).

Table IV.- IUCN categories assessment.
Table IV.- Évaluation de la catégorie UICN.

	A. maritimus	*A. verrucosus*
Location	1	1
Area of occupancy (AOO) with 1x1 km grid	1 km^2	4 km^2
Extent of occurrence (EOO)	0,018 km^2	0,70 km^2
Population area	1.8 ha	35.6 ha
Number of populations	1	1
Number of subpopulations	1	2
Number of individuals	321-436	10.000
An observed, estimated, inferred or suspected population size reduction over the last 10 years or three generations	n.d.	n.d.
A decline in area of occupancy, extent of occurrence and/or quality of habitat	presumed	presumed
Extreme fluctuations in number of mature individuals	no	no
Quantitative analysis showing the probability of extinction in the wild within 10 years or three generations, whichever is the longer (up to a maximum of 100 years)	n.d.	n.d.
Criteria and subcriteria	CR = B1ab(i, ii, iii, v) + 2ab(i, ii, iii, v)	CR = B1ab(i, ii, iii, v) + 2ab(i, ii, iii, v)

With reference to cultivation practices, these do not seem to seriously damage the plants, at least as far as *A. verrucosus* is concerned, this species appears to be well settled on ploughed fields.

B. IUCN categories assessment

The assessment of the IUCN risk categories for the two species was carried out mainly referring to the criterion B (Table IV). Both taxa are found to be organized in one single population occupying one location, which exposes them to remarkable risks, both due to anthropic activities and to stochastic events, with an extent of occurrence (EOO) not exceeding 100 km^2 and an area of occupancy (AOO) not exceeding 10 km^2. The reviewed risk categories, confirm a high risk of extinction for both taxa; consequently the implementation of conservation policies appears to be an urgent issue. In order to conduct a comprehensive assessment, additional analyses identifying population trends and reviewing habitat developments are needed.

The *A. verrucosus* population is fragmented into two cores separated by roads, because the coastal area is being subject to increasing urbanization; the same threat applies to the *A. maritimus* population, subject to motorcycle and off-highway vehicle passage. Moreover, the above mentioned activities, as confirmed by the observations carried out over the last few years, lead to an increasing deterioration of the quality of the habitat which, also for *A. maritimus*, was due to a fire involving part of the population (Bacchetta, 2001).

With reference to *A. maritimus*, we would also like to point out the availability of an extremely small quantity of mature individuals (Tables III and IV) not ensuring independent population reproduction over the long run (Reed, 2005). According to the gathered data, it was possible to recalculate the risk category for both species, and to confirm the critically endangered (CR) category, modifying the subcriteria proposed for *A. verrucosus* by Bacchetta & Pontecorvo (2005).

Table V.- Final imbibition and germination percentages and T50 values after each tested pretreatment and germination temperature for *A. maritimus*. *** Significantly different at $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant by non parametric Kruskal-Wallis test.
Table V.- Pourcentage de germination et T50 pour *A. maritimus*.

Pretreatment	Imbibition (%) (***)	Germination (%) (***)				T50 (days) (***)			
		15 °C	20 °C	25 °C	p	15 °C	20 °C	25 °C	p
Control	2.50 ± 0.55	0.00 ± 0.00	3.17 ± 3.58	1.00 ± 1.73	ns	_	2.50 ± 3.14	1.37 ± 2.75	ns
H_2SO_4 (5')	27.90 ± 9.40	15.35 ± 3.47	6.00 ± 3.46	8.00 ± 4.90	*	8.32 ± 3.12	8.19 ± 2.98	7.75 ± 4.48	ns
H_2SO_4 (15')	76.00 ± 4.34	61.00 ± 12.12	61.58 ± 7.36	65.00 ± 7.19	ns	6.11 ± 1.86	3.76 ± 0.21	3.55 ± 0.05	*
H_2SO_4 (30')	81.50 ± 1.87	81.88 ± 4.31	72.67 ± 3.83	79.00 ± 3.32	*	3.74 ± 0.43	4.52 ± 1.98	3.52 ± 0.10	ns
H_2O (100 °C)	34.50 ± 3.51	23.58 ± 7.94	30.00 ± 12.81	6.00 ± 3.46	ns	6.48 ± 1.19	7.93 ± 2.48	9.75 ± 4.79	ns
Scarification	89.50 ± 2.66	85.96 ± 5.93	90.00 ± 2.00	88.88 ± 1.82	ns	3.93 ± 0.54	3.19 ± 1.18	2.45 ± 1.37	ns

Table VI.- Final imbibition and germination percentages and T50 values after each tested pretreatment and germination temperature for *A. verrucosus*. *** Significantly different at $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ns, not significant by non parametric Kruskal-Wallis test.
Table VI.- Pourcentage de germination et T50 pour *A. verrucosus*.

Pretreatment	Imbibition (%) (***)	Germination (%) (***)				T50 (days) (***)			
		15 °C	20 °C	25 °C	p	15 °C	20 °C	25 °C	p
Control	8.46 ± 1.76	6.00 ±3.46	4.00 ± 4.00	0.00 ± 0.00	*	8.19 ± 3.94	2.12 ± 2.53	-	ns
H_2SO_4 (5')	33.66 ± 4.97	29.00 ± 1.73	27.00 ± 1.73	33.33 ± 1.77	ns	4.17 ± 0.68	4.37 ± 1.16	6.18 ± 2.21	ns
H_2SO_4 (15')	76.67 ± 3.61	78.00 ± 2.00	77.79 ± 4.39	62.00 ± 16.61	ns	3.76 ± 0.18	4.37 ± 0.84	5.61 ± 2.92	ns
H_2SO_4 (30')	88.58 ± 7.63	88.00 ± 0.00	85.00 ± 14.58	76.03 ± 4.09	ns	4.24 ± 0.96	4.00 ± 0.17	4.04 ± 0.76	ns
H_2O (100 °C)	59.04 ± 2.66	46.00 ± 3.46	50.67 ± 4.99	43.00 ± 4.36	ns	3.57 ± 0.08	4.34 ± 1.00	4.93 ± 1.43	ns
Scarification	64.91 ± 8.65	56.00 ± 2.83	64.87 ± 7.18	53.26 ± 8.35	ns	3.80 ± 0.35	3.86 ± 0.30	5.12 ± 1.21	ns

C. Germination

The application of pretreatments showed that seeds of both species feature a germination inhibition due to tegument impermeability (physical dormancy, *sensu* Baskin & Baskin 1998, 2004), as already reported for other species of this genus (Baskin & Quarterman, 1969; Kaye, 1999; ISTA, 2006) and more generally for species belonging to the Fabaceae family (Rolston, 1978; Grime *et al.*, 1981; Baskin & Baskin, 1988; ISTA, 2006) and as already observed in *A. maritimus* by Bocchieri *et al.* (2000) and for both species by Bacchetta *et al.* (2008b).

The applied pre-treatments had a significant effect on the seed imbibition of both species ($p < 0.001$, by non parametric Kruskal-Wallis test; Tables V and VI). The percentage of imbibed seeds not subject to pretreatments was extremely low (2.50% ± 0.55% and 8.46% ± 1.76%, for *A. maritimus* and *A. verrucosus* respectively) even after immersion in distilled water for 24 hours. The most effective scarification methods were mechanical scarification (89.50% ± 2.66%) for *A. maritimus* and H_2SO_4 bath for 30 minutes (88.58% ± 7.63%) for *A. verrucosus*.

As a consequence, final seed germination percentages and T50 values (Tables V and VI) strongly depended on the imbibition percentage and, therefore, on the effectiveness of the applied pretreatment. On the contrary, tested germination temperatures do not remarkably influence neither final germination percentage (maximum germination percentage 90.00% ± 2.00 at 20 °C for *A. maritimus* and 88.00 ± 0.00 at 15 °C for *A. verrucosus*) or its speed (T50) ($p > 0.05$ within each pretreatment, by non parametric Kruskal-Wallis test),

thus confirming that the two taxa do not show a physiological component of dormancy and that seeds germinate without problems after imbibition. The cut-test carried out at the end of the germination test showed as non germinated seeds were still viable.

IV. CONCLUSIONS

This study aimed at enriching the knowledge on the ecology of both species in order to create adequate strategies for *in situ* and *ex situ* conservation. According to our results, protection measures for *A. maritimus* appear to be a priority choice because of the reduced number of individuals building up the population, and because of the small area occupied by this species due to the anthropic pressure insisting on the territory. If not properly regulated, said pressure might lead to a further reduction in the number of individuals of this species. For the *A. verrucosus* population, observations showed that it includes a higher number of individuals and that it occupies an area greater than the one provided in literature; notwithstanding this, this species also needs an adequate *in situ* conservation strategy.

In addition, the availability of a germination protocol and the high germination percentages achieved in this study, suggest that *ex situ* conservation and plant multiplication by seed germination could be useful measures to assess further *in situ* population reinforcements for both species.

In conclusion, for both taxa, periodical monitoring activities will be fundamental and in-depth studies on population dynamics allowing us to understand the evolution of these populations and to implement management and conservation measures both *in situ* and *ex situ*. The initiated ecological analysis about two threatened species will consent the configuration of efficacious strategies for their conservation *in situ* and *ex situ*.

Moreover, this approach if applied to other endemic or/and to threatened taxa of the Island, may consent the individuation of the conservation priority, consenting an efficacious strategy.

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