135. Seedling emergence in the endangered *Juniperus oxycedrus* subsp. *macrocarpa* (Sm.) Ball in southwest Spain. Juan, R., Pastor, J., Fernandez, I., and Diosdado, J. C. Acta Biologica Cracoviensia Series Botanica 48(2):49-58. 2006.

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 48/2: 49–58, 2006

SEEDLING EMERGENCE IN THE ENDANGERED *JUNIPERUS OXYCEDRUS* SUBSP. *MACROCARPA* (SM.) BALL IN SOUTHWEST SPAIN

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Received April 10, 2006; revision accepted July 20, 2006

Juniperus oxycedrus subsp. *macrocarpa* is an endangered species in southwest Spain, with seed dormancy as found in other species of the same genus. This study employed different experiments to determine a method to improve the seedling emergence in this species. Three types of seedling emergence trials were performed: (a) untreated seeds under greenhouse conditions, (b) untreated seeds under natural conditions, and (c) treated seeds under greenhouse conditions, with different acids (sulphuric, hydrochloric and nitric) for 10 and 30 min, followed or not by cold stratification for 3 months. In all trials, seeds derived from both mature and immature cones were used to verify which one produced higher seedling emergence. Previously, seed viability was verified and a proper substrate for greenhouse sowing was selected. The best percentage of seedling emergence was obtained in the "a" and "b" trials. In trial "a", seeds derived from immature cones germinated significantly better than mature ones. Chemical scarification of seeds with or without cold stratification yielded less seedling emergence than the other trials.

Key words: Cupressaceae, *Juniperus oxycedrus* subsp. *macrocarpa*, dormancy, seedling emergence, viability.

INTRODUCTION

Juniperus oxycedrus subsp. *macrocarpa* (Sm.) Ball is a shrub or small tree typical of coastal environments in the Mediterranean region (Jalas and Suominen, 1973; Castroviejo et al., 1986). Although this taxon, common to both dunes and cliffs, used to be spread along the seacoast, it has suffered severe regression due to human pressure, leaving reduced populations in small isolated patches, as for other species of the genus (Hajar, 1991; Clifton et al., 1997; Sharew et al., 1997). In southwest Spain in particular, it is common to find low-density populations of this taxon because of severe urban pressure along coastal areas, resulting in a discontinuous distribution (Pastor and Juan, 1999). The progressive destruction of its natural habitat in southwest Spain has caused coastal juniper to be classified as in danger of extinction (Hernández and Clemente, 1994).

Previous studies have shown that natural regeneration of *J. oxycedrus* subsp. *macrocarpa* and other taxa in this genus is not easy due to several factors, including slow growth (Ceballo and Ruíz de la Torre, 1979), low seed viability and/or difficulty in germinating (Pardo and Lazaro, 1983; Hajar, 1991; Lee et al., 1995; Owens and Schliesing, 1995; Jordan de Urries, 1997; Cantos et al., 1998; Ortiz et al., 1998; García, 2001; Juan et al., 2003).

Seed germination difficulties in this genus are high because of dormancy directly associated with the embryo, seed coat impermeability to water, and/or the presence of germination inhibitors (Hajar, 1991; Cregg et al., 1994; Lee et al., 1995; Ueckert, 1997; Cantos et al., 1998). Some trials suggest that it is due to physical dormancy directly related to the seed coat, because germination increases when the seed coat is removed (Cantos et al., 1998). However, Baskin and Baskin (1998) point out that seeds from *Juniperus* plants exhibit physiological dormancy in which the embryo is unable to develop a radicle due to an inhibition mechanism. Pardos and Lazaro (1983) suggested that seeds of *J. oxycedrus* have a double dormancy feature involving both endogenous and exogenous factors. Other authors have also acknowledged that *Juniperus* seeds show both seed coat and embryo dormancy (Herrero, 1959; Cregg et al., 1994; Johnson, 1995; Lee et al., 1995; Barbour, 1999).

A phenomenon which may occur in other *Juniperus* species is one first suggested by Herrero

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PL ISSN 0001-5296

(1959) in *J. thurifera* and confirmed later by other authors (Balboa-Zavala and Dennis, 1977; Pinfield et al., 1990; Pinfield and Gwarazimba, 1992; Jensen and Eriksen, 2001) for some angiosperm species belonging to *Acer*, *Prunus* or *Malus* genera. By this phenomenon, seeds become dormant as they mature, so that when they are fully mature the seeds lose their germination ability, while immature seeds are able to germinate. This feature could help explain the low percentage of germination found in some species within *Juniperus*, in studies always using seeds derived from mature cones in the experiments (Pardos and Lazaro, 1983; Rietveld, 1989; Cregg et al., 1994).

Seed dormancy can be overcome through different methods. Cold or hot stratification, or both methods applied in turn, are among the treatments most used to break physiological seed dormancy and therefore increase germination frequency in many conifers (Herrero, 1959; Johnsen and Alexander, 1947; Catalan, 1978; Gosling, 1988; Poulsen, 1996; Downie et al., 1998; Offord and Meagher, 2001). On the other hand, in *Juniperus* species the method most usually employed to overcome physical seed dormancy is pretreatment with different acids such as commercially pure (96–98%) sulphuric acid (Herrero, 1959; Pardo and Lazaro, 1983; Hajar, 1991).

Juniperus oxycedrus is of ecological interest because it is a pioneer of inhospitable habitats in nutrient-poor and unstable soil with strong sea spray, habitats which later can be colonized by other less tolerant species. Recently, Joy and Young (2002) suggested that *J. virgiana*, another pioneer species in coastal environments, can facilitate and accelerate primary succession, due to reduction of solar radiation and temperature fluctuations beneath its canopy, which help to retain soil moisture and organic matter levels. Previously, Cregg et al. (1994) and Lee et al. (1995) pointed out the high ecological value of some species of *Juniperus*, which act as natural windbreaks or shelterbelts.

The aim of the present study was to determine the effects of different variables (substrate, seed ripeness, cold stratification, chemical scarification, etc.) on seedling emergence in *J. oxycedrus* subsp. *macrocarpa*. Determining the most appropriate method to improve the germination rate should contribute to the conservation and recovery of an endangered species.

MATERIALS AND METHODS

SPECIES AND STUDY AREA

Juniperus oxycedrus subsp. *macrocarpa* is a dioecious species whose female individuals require two years to develop mature cones. During the first year the cones are small, hard and green-glaucous in color. During the second summer, these cones reach their final size; they are still hard but turn green-yellowish (immature cones). Finally, during the second autumn the cones are fleshy and show a reddish color (mature cones). It is possible to find cones at different stages of maturity on the same plant simultaneously.

Seed for these trials were harvested from late summer to autumn 1999. Mature and immature cones were collected from four populations in southwest Spain whose cone characteristics had been previously studied (Juan et al., 2003): Doñana (DO), Punta Umbría (PU), Caños de Meca (CM) and Punta Paloma (PP). The first three are protected areas under different categories (national park, natural site and natural park, respectively), while the last is in an area not under protection.

VIABILITY

All trials were carried out with sinking seeds, because floating ones are empty (Juan et al., 2003).

Before starting the germination trials, the viability of the seeds to be used in this work was checked. From 800 to 1200 randomly selected seeds from immature cones (~3 months from full maturity) were tested for each population, as seed viability derived from mature cones had been previously published (Juan et al., 2003). Seed viability of both mature and immature seeds was tested by both the cutting method and the tetrazolium chemical test (ISTA, 1976; 1999). In the first method, each seed was opened to determine the presence or absence of an embryo. In the second method, the seeds were immersed in 2,3,5-triphenyltetrazolium chloride solution to verify the condition of the embryo.

SUBSTRATE SELECTION

For selection of the substrate used in all trials, seedling emergence trials were carried out with mature seeds from six individuals randomly chosen from Punta Umbría (PU) and Caños de Meca (CM), two populations with high cone production. From each tree, 200 seeds were extracted and sown in three different substrates: (a) pine humus (PH), material derived from pine bark and needle compost, (b) a mixture of blonde and black peat in a 20:80 ratio (Blo/Bla 20:80), and (c) the same mixture but in an 80:20 ratio (Blo/Bla 80:20).

SEEDLING EMERGENCE

Untreated seeds in greenhouse trials

This trial started in November 1999, using 500 seeds (both mature and immature) per individual and population. For each individual, seeds were allocated to four replicates of 125 seeds each. Each was placed in a tray (40 × 25 × 10 cm) maintained under greenhouse conditions (25°C day/17–18°C night). The number of individuals used from each population was 22 in PU, 20 in CM, and 15 in DO and PP.

To monitor the timing of seedling emergence, during the first month the trays were observed daily, and thereafter weekly until September 2000.

Untreated seeds in natural habitat trials

This trial started in March 2000, using mature and immature seeds derived from 10 randomly selected individuals of each studied population. From each individual, 200 mature seeds were extracted and distributed as follows: 50 seeds exposed to sunlight without watering (full sun/not watered), 50 seeds exposed to sunlight with watering (full sun/watered), 50 seeds sunlight-protected without watering (shaded/not watered), and 50 seeds sunlight-protected with watering (shaded/watered). The same trial was repeated with the same number of immature seeds at the same time. These trials were made at the location of the Punta Umbria population due to its good conservation conditions and easier access. All seeds were protected from predators with mesh. To keep moisture in the substrate, trays were placed 15 cm beneath the seeds to be watered. Monitoring was done weekly for 18 weeks, during which 10 l/week of water was applied in both watered trials (full sun and shaded).

Pretreated seeds in greenhouse trials

This part of the study was carried out in a greenhouse kept at 25°C/day and 17–18°C/night in January 2000. As in the previous trials, mature and immature seeds were used, from 5 individuals per population (PU, DO and CM). In this case the Punta Paloma population was not used due to its lower seed production. The following trials were carried out: 100 seeds as control; 100 seeds scarified with sulphuric acid for 10 min and 100 more for 30 min; 100 seeds scarified with hydrochloric acid for 10 min and 100 more for 30 min; and 100 seeds scarified with nitric acid for 10 min and 100 more for 30 min. All groups of seeds were allocated to four replicates of 25 seeds each. After scarification these seven trials were carried out with stratified seeds for 3 months in moist sand at 4–6°C. This protocol produced 28 seed lots per individual and population, 14 of which derived from mature seeds and the other 14 from immature seeds. The seeds were observed each week until September 2000.

TABLE 1. Immature seed viability by cutting and tetrazolium methods

Population	Cutting method % seeds with embryo mean ± SD	Tetrazolium method % totally dyed mean ± SD	n
Punta Umbria	51.8 ± 19.1 (56.53 ± 13.67)	8.3 ± 6.0 (8.48 ±7.00)	1200
Doñana	37.0 ± 15.3 (64.81 ± 12.28)	9.0 ± 6.5 (11.92 ± 8.11)	1000
Caños de Meca	50.8 ± 18 (60.49 ± 16.59)	5.3 ± 4.6 (8.50 ± 6.56)	1000
Punta Paloma	59.5 ± 20 (55.85 ± 17.49)	8.0 ± 5.5 (6.43 ± 5.87)	800

Data in parentheses correspond to mature seed viability previously published (Juan et al., 2003)

STATISTICAL ANALYSIS

The variation of each response variable was analyzed by one-way or two-way ANOVA. The significance of differences between treatments was tested using Tukey's test. Prior to statistical analysis, the data were assessed for normal distributions. Arcsin transformation was applied for percentage data.

RESULTS AND DISCUSSION

IMMATURE SEED VIABILITY

The frequency of seeds containing an embryo ranged from 37.0% in Doñana up to 59.5% in Punta Paloma, but no significant differences between the four studied populations were found (Tab. 1). The tetrazolium test did not reveal differences between the four populations either, although the percentages of completely stained seeds were lower (from 5.3% in Caños de Meca to 9.0% in Doñana) (Tab. 1).

Seed viability of immature cones was similar to that found earlier for mature ones (Juan et al., 2003), except for the Doñana population, for which the cutting method gave 37.0% viability for immature seeds (Tab. 1) as opposed to the 64.81% previously found for mature seeds (Juan et al., 2003). With the tetrazolium test, however, the percentages were similar: 11.92% for mature seeds (Juan et al., 2003) and 9.0% for immature ones (Tab. 1).

The lower viability percentages that the chemical test gave for *J. oxycedrus* subsp. *macrocarpa* seem normal, having been reported by other authors in other taxa of *J. oxycedrus* such as subsp. *oxycedrus* and subsp. *badia* (Pardo and Lazaro, 1983; Jordan de Urries, 1997). It should also be noted that in our study the seeds were submerged in tetrazolium solution for 24 h after cutting 1/4 of the widest extreme, following the protocol recommend-

TABLE 2. Seedling emergence frequency for mature seeds from two *J. oxycedrus* subsp. *macrocarpa* populations with three different substrates. Different capital letters indicate significant differences between values in the same row (Tukey's test, $p < 0.05$), and different small letters indicate significant differences between values in the same column (Tukey's test, $p < 0.05$), $n = 1200$. Blo – Blonde peat; Bla – Black peat

Population	Pine humus	Blo/Bla 20:80	Blo/Bla 80:20
Punta Umbría	8.6 ± 7.2^{Aa}	31.5 ± 21.5^{Ba}	14.1 ± 10.2^{ABa}
Caños de Meca	34.5 ± 20.2^{Ab}	49.3 ± 10.7^{Aa}	47.6 ± 23.8^{Ab}

TABLE 3. Seedling emergence percentages in the greenhouse for untreated seeds, both mature and immature, for the four *J. oxycedrus* subsp. *macrocarpa* populations. Different capital letters indicate significant differences between values in same column, and different small letters indicate significant differences between values in the same row (Tukey's test)

Population	Mature seeds (%) *** (mean ± SD)	Immature seeds (%)* (mean ± SD)	n
Punta Umbría**	28.9 ± 20.9^{Aa}	49.3 ± 25.2^{Ba}	11000
Doñana**	6.6 ± 4.8^{Ab}	21.9 ± 18.1^{Bb}	7500
Caños de Meca**	5.0 ± 6.8^{Ab}	41.7 ± 18.7^{Ba}	10000
Punta Paloma*	1.3 ± 4.1^{Ab}	8.8 ± 12.3^{Bb}	7500

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

ed by ISTA (1976; 1999) for *Juniperus* genus. However, it has been shown that the percentage of tetrazolium-stained seeds can vary depending on the cutting area, temperature and infiltration time. Thus, Savonen (1999) showed in *Pinus sylvestris* that when staining time lasted 24 h, it was necessary to cut both seed ends to obtain more effective infiltration of the stained solution into seed tissues, so the proportion of embryo and endosperm staining will be higher. According to Buszewicz and Holmes (1957), an incompletely stained embryo of a conifer seed can still develop a seedling. Hence, the germination percentage of *J. oxycedrus* subsp. *macrocarpa* seeds may have been higher than shown in the tetrazolium test.

SUBSTRATE SELECTION

The responses obtained with the three different substrates differed between the two selected populations (PU from Huelva and CM from Cádiz) (Tab. 2). While the percentages from the Caños de Meca seeds are similar for the three different substrates, for Punta Umbría seeds the emergence percentages differed significantly between blonde/black 20:80 peat and pine humus ($F = 4.09$, Df_{2-15}, $p < 0.05$), with the other substrate (blonde/black 80:20 peat) yielding an intermediate percentage.

Because germination percentages were highest in Blo/Bla 20:80, we used this substrate for the remaining germination trials, except for those carried out in the natural habitat.

SEEDLING EMERGENCE STUDY

Germination response of untreated mature and immature seeds under greenhouse conditions

Generally these results were relatively low, especially those for mature seeds, which, except for the Punta Umbría population, had seedling emergence lower than the viability levels indicated by the tetrazolium test (Juan et al., 2003). This suggests that these seeds exhibit some dormancy.

With mature seeds, seedling emergence percentages varied from 1.3% for Punta Paloma up to 28.9% for Punta Umbría, the latter showing a significant difference from the others ($F = 15.95$, Df_{3-68}, $p < 0.001$) (Tab. 3). The differences in seedling emergence rates between populations could be related to seed size or weight, or to the number of seeds per cone in each population, since previous studies have shown the influence of these factors on seedling emergence rates (Piatt, 1973; Harrington et al., 1989; Vera, 1997). According to these studies, the largest seeds or those from cones that produce more seeds tend to show better results. In view of this, and a previous biometric study on cones and mature seeds of these four populations (Juan et al., 2003), we would expect the Doñana population to exhibit higher seedling emergence based on seed size and the greater number of seeds per cone, followed by the Punta Paloma population. However, the highest seedling emergence frequency was for Punta Umbría, which had the smallest and lightest seeds. Thus, as with other species (Larson, 1961; Vera, 1997; Eriksson, 1999), our results obtained do not establish any relationship between those characters and seedling emergence rates.

The frequencies of seedling emergence for seeds from immature cones ranged from 8.8% for Punta Paloma to 49.3% for Punta Umbría (Tab. 3). The Punta Umbría and Caños de Meca populations had seedling emergence percentages significantly higher than Punta Paloma and Doñana seeds ($F = 14.13$, Df_{3-68}, $p < 0.05$). As shown in Table 3, in all the studied populations the seedling emergence rates were significantly higher for seeds from immature cones ($F = 48.44$, Df_1, $p < 0.001$). For all four populations the seedling emergence levels were also

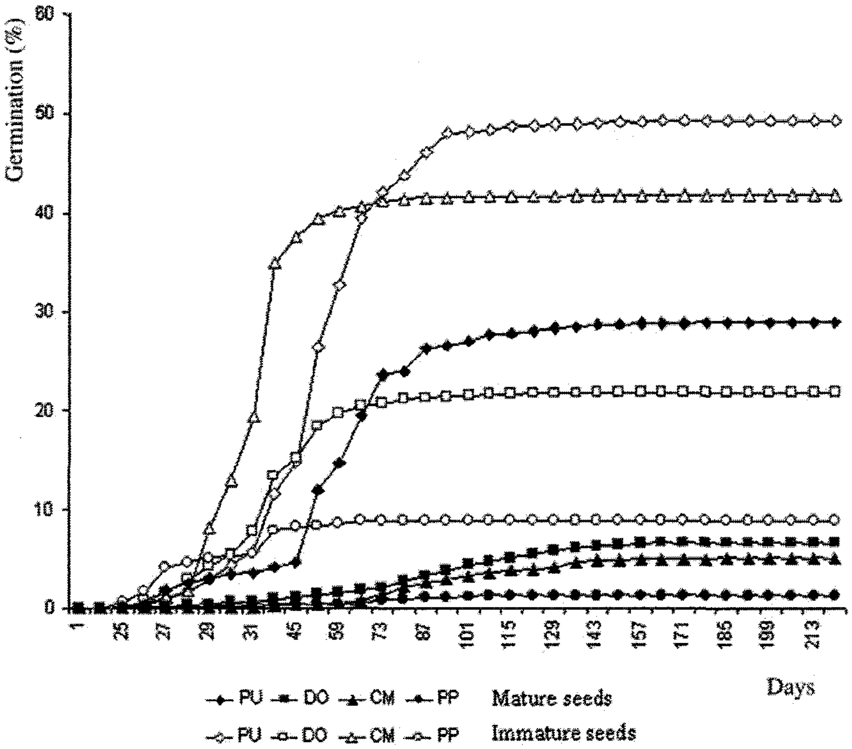

Fig. 1. Cumulative emergence percentages for untreated seeds, both mature and immature, of four *J. oxycedrus* subsp. *macrocarpa* populations under greenhouse conditions. PU – Punta Umbría; DO – Doñana; CM – Caños de Meca; PP – Punta Paloma.

higher than the viability levels indicated by the tetrazolium test (Tabs. 1, 3).

This significant increase in seedling emergence percentages for immature seeds of all the studied populations indicates that these seeds acquire some form of dormancy while maturing, as demonstrated by Balboa-Zavala and Dennis (1977), Pinfield et al. (1990) Pinfield and Gwarazimba (1992) or Jensen and Eriksen (2001) in some angiosperm species.

The timing of seedling emergence was similar for mature and immature seeds. Seedling emergence began between 25 and 28 days after sowing and ended after 150–200 days. Immature Punta Paloma seeds were the only exception; they completed seedling emergence after ~75 days (Fig. 1).

SEEDLING EMERGENCE RESPONSE IN UNTREATED MATURE AND IMMATURE SEEDS IN THEIR NATURAL HABITAT

Under all experimental conditions and for both mature and immature seeds, the Punta Umbría and Caños de Meca populations had the best seedling emergence, whereas Punta Paloma had the worst (Tabs. 4, 5).

Generally the seedling emergence percentages of mature seeds was higher in their natural habitat than in the greenhouse, except for the seeds placed in full sun and not watered; in Caños de Meca, however, in those conditions (full sun/not watered) seedling emergence was slightly higher than in the greenhouse (Tabs. 3, 4). The seedling emergence percentages were generally higher in shaded/watered conditions, although the differences were significant only in full sun/not watered conditions ($p < 0.05$), where the seedling emergence frequencies were lower in all the populations. The Punta Paloma population again had the lowest seedling emergence percentages, significantly different ($p < 0.001$) from the Punta Umbría and Caños de Meca ones except under full sun/not watered conditions.

For immature seeds (Tab. 5) the seedling emergence percentages were also highest in shaded/watered conditions, but significantly different only from the results under full sun/not watered conditions in the Punta Umbría ($F = 3.76$, $Df_{3\text{–}36}$, $p < 0.05$), Caños de Meca ($F = 13.3$, $Df3_{-36}$, $p < 0.01$) and Punta Paloma ($F = 14.5$, $Df_{3\text{–}36}$, $p < 0.01$) populations. Generally the seedling emergence percentages for immature seeds were slightly lower in their natural habitat than in the greenhouse, except for the Caños de Meca population, where in shaded conditions, both watered and not watered, the results were slightly higher (Tabs. 3, 5).

TABLE 4. Seedling emergence percentage in the natural habitat for untreated mature seeds from four *J. oxycedrus* subsp. *macrocarpa* populations. Different capital letters indicate significant differences between values in the same row, and different small letters indicate significant differences between values in the same column (Tukey's test), n = 500. SW – full sun/watered; SNW – full sun/not watered; ShW – shaded/watered; ShNW – shaded/not watered

Population	SW** (mean ± se)	SNW* (mean ± SE)	ShW** (mean ± SE)	ShNW** (mean ± SE)
Punta Umbria*	34.4 ± 16.1^{ABa}	14.6 ± 15.7^{Aa}	41.6 ± 27.2^{Ba}	35.6 ± 13.9^{ABa}
Doñana*	19.2 ± 13.8^{ABab}	0.4 ± 1.2^{Ab}	25.2 ± 21.7^{Bab}	12.4 ± 16.2^{ABb}
Caños de Meca*	33.8 ± 22.5^{Aa}	7.1 ± 12.0^{Bab}	48.0 ± 13.5^{Aa}	39.5 ± 18.8^{Aa}
Punta Paloma*	3.6 ± 2.4^{Ab}	0^{Bb}	4.8 ± 3.1^{Ab}	2.3 ± 2.1^{ABb}

*p < 0.05; **p < 0.001

TABLE 5. Seedling emergence percentage in natural habitat from untreated immature seeds from the four *J. oxycedrus* subsp. *macrocarpa* populations. Different capital letters indicate significant differences between values in same row, and different small letters indicate significant differences between values in the same column (Tukey's test), n = 500. SW – full sun/watered; SNW – full sun/not watered; ShW – shaded/watered; ShNW – shaded/not watered

Population	SW** (mean ± SE)	SNW* (mean ± SE)	ShW* (mean ± SE)	ShNW** (mean ± SE)
Punta Umbría*	41.0 ± 14.0^{ABa}	26.0 ± 13.1^{Aa}	46.7 ± 18.0^{Ba}	35.1 ± 10.9^{ABa}
Doñana*	14.4 ± 21.5^{Ab}	0^{Ab}	18.4 ± 25.0^{Ab}	9.2 ± 17.3^{Ab}
Caños de Meca**	28.0 ± 11.6^{ABab}	9.8 ± 11.0^{Ab}	48.9 ± 18.9^{Ba}	43.5 ± 15.7^{Ba}
Punta Paloma**	4.7 ± 3.3^{Ab}	0^{Bb}	5.8 ± 3.2^{Ab}	3.7 ± 2.6^{Ab}

*p < 0.05; **p < 0.01

In none of the four populations were there significant differences between the two seed types, since both mature and immature seeds had better seedling emergence in shaded places. This could be related to the fact that the shade provided by other plants, of *J. oxycedrus* subsp. *macrocarpa* or any other woody species, can favor the seedlings' development, providing a microclimate characterized by lower light level, lower air and soil temperatures, and therefore lower evaporation in the canopy layer (Callaway, 1992; Debussche and Isenmann, 1994; García, 2001; Joy and Young, 2002). However, the seeds sown in full sun/watered conditions tended to germinate at higher rates than those in shaded/not watered conditions (Tabs. 4, 5). This suggests that for this species the moisture of the substrate during seedling emergence is more important than the reduction of solar radiation by a shading woody plant, although this shading contributes to retention of soil moisture.

The weekly monitoring showed that the seeds sown in the natural habitat had slower seedling emergence than those in the greenhouse, starting in the 5th or 7th weeks except under full sun/not watered conditions, in which seedling emergence took up to 10 weeks. Seedling emergence frequencies declined drastically after the 13th or 14th weeks. Hence, seedling emergence in the natural habitat is quicker, since similar or slightly higher percentages were reached in a shorter period (90–100 days) than in the greenhouse trials.

From the beginning of the trial, the mean daytime temperature ranged from 19°C in March up to 35°C in July, and the nighttime temperature from 10°C to 18°C. Seedling emergence peaked in May, when the daytime temperature was 25°C and 15°C during the night, indicating the importance of temperature in the germination of this taxon. Smith et al. (1975) found the optimal soil temperature for *J. pinchoti* seedling emergence to be 18°C, whereas at 10°C the seeds did not germinate and at 27°C the seedling emergence rate was fairly low.

SEEDLING EMERGENCE RESPONSE IN TREATED MATURE AND IMMATURE SEEDS

As seen in Figure 2 and Table 6, the seedling emergence percentages for treated seeds (scarified and stratified, scarified and nonstratified), both mature and immature, were considerably lower than those for untreated seeds (Tabs. 3, 4, 5). There were significant differences between stratified and nonstratified seeds in all the studied populations ($p < 0.05$) (Tab. 6).

Seedling emergence was lowest among cold-stratified seeds, including mature seeds for which increased seedling emergence might be expected since stratification is an effective method for overcoming embryo dormancy, as found in other species of this genus (Herrero, 1959; Benson, 1976; Young et al., 1988). Thus, our results show that stratifica-

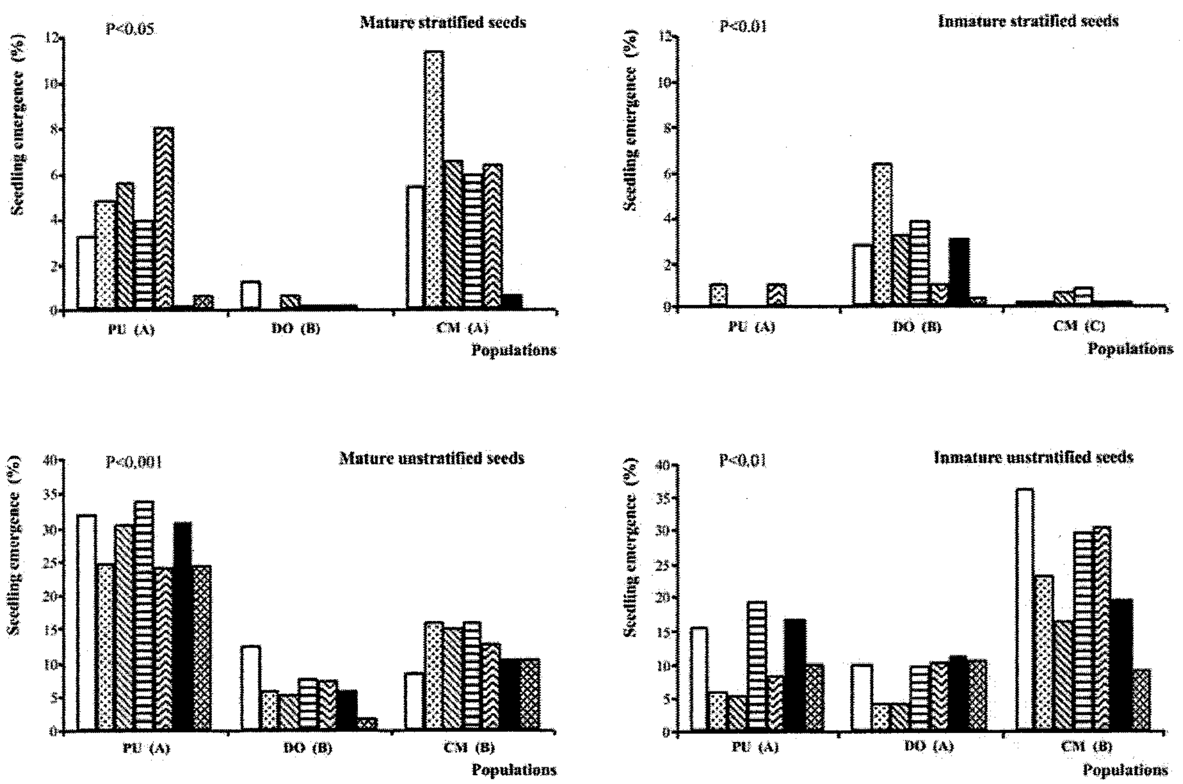

Fig. 2. Seedling emergence percentages for acid-treated stratified and nonstratified seeds, both mature and immature, of three *J. oxycedrus* subsp. *macrocarpa* populations under greenhouse conditions. Differences between the three populations are indicated in each figure. PU – Punta Umbría; DO – Doñana; CM – Caños de Meca.

TABLE 6. Seedling emergence frequency in the greenhouse for chemically scarified seeds, both stratified and nonstratified for three of the four *J. oxycedrus* subsp. *macrocarpa* populations. Different capital letters indicate significant differences between values in the same row, and different small letters indicate significant differences between values in the same column (Tukey's test), n = 500

Population	Mature scarified seeds		Immature scarified seeds	
	stratified (mean ± SD)*	nonstratified (mean ± SD)***	stratified (mean ± SD)**	nonstratified (mean ± SD)**
Punta Umbría*	3.7 ± 4.8^{ABa}	28.4 ± 19.6^{Ca}	0.2 ± 0.4^{Aa}	11.6 ± 13.1^{Ba}
Doñana*	0.3 ± 0.8^{Ab}	6.4 ± 5.7^{BCb}	2.9 ± 5.4^{ABb}	8.6 ± 11.4^{Ca}
Caños de Meca*	5.2 ± 7.1^{Aa}	12.6 ± 7.2^{Bb}	0.3 ± 0.6^{Aa}	23.4 ± 18.1^{Cb}

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

tion in *J. oxycedrus* subsp. *macrocarpa* seeds suppresses seedling emergence, as Rietveld (1989) found in *J. scopulorum*. For stratified seeds, both mature and immature, the Doñana population differed significantly from Punta Umbría and Caños de Meca ($F = 8.58$, Df_{2-102}, $p < 0.05$ and $F = 7.84$, Df_{2-102}, $p < 0.01$, respectively), although in the case of mature seeds the difference was due to poorer seedling emergence (0.3%), and in the case of immature seeds it was due to better performance (2.9%).

In none of the four chemical scarification treatments (stratified/nonstratified mature seeds, stratified/nonstratified immature seeds) was a pattern detected based on the acid used (sulphuric, hydrochloric, nitric), or on treatment time (10 min, 30 min). In all cases, however, the seedling emergence frequencies differed significantly between populations. For nonstratified seeds, Punta Umbría had the highest seedling emergence percentage for mature seeds ($F = 27.43$, Df_{2-102}, $p < 0.001$), whereas Caños de Meca had the highest seedling emergence rate for immature seeds ($F=10.26$, Df_{2-102}, $p<0.01$).

The result that the acid treatments did not increase any seedling emergence percentages versus previous trials with untreated seeds suggests that scarification is not an effective treatment to overcome seed coat dormancy in *J. oxycedrus* subsp. *macrocarpa*. In other studies, numerous angiosperm seeds had improved germination when they were treated with sulphuric acid (Sacheti and Al Rawahy, 1998; Tischler and Burson, 1999; Caloggero and Parera, 2000), and there was even a slight increase in *J. pinchoti* (Johnsen and Alexander, 1974). However, in agreement with Pardo and Lazaro (1983) and Hajar (1991), this study found that seeds treated with sulphuric acid had low seedling emergence percentages, even lower as the treatment time was increased (Herrero, 1959).

In general, acid-treated seeds began seedling emergence between the 5th and 7th weeks for stratified seeds and slightly earlier (4th to 6th weeks) for nonstratified ones, reaching top values of only 5.2% for stratified and 23.4% for nonstratified ones (Tab. 6). In other *Juniperus* species, after stratification the seeds began seedling emergence between the 6th and 10th days (Johnsen and Alexander, 1974).

The results of viability tests on this species showed relatively low seed viability relative to cone production. Tetrazolium tests indicated viability even lower than the seedling emergence percentages, suggesting that interpreting this test is problematic.

The origin of the seed population significantly influenced seedling emergence, with the best results in this trial originating from the populations of Punta Umbría and Caños de Meca. The optimal results obtained from the Punta Umbría population, in particular, could be related to features of the seeds, size being the most important.

Generally, seeds derived from immature cones germinated significantly better than those derived from mature ones, suggesting that they have lower levels of dormancy.

When the seeds were sown in their natural habitat, seedling emergence rates did not differ between mature and immature seeds. Substrate moisture level proved essential for seedling emergence, apparently even more important than light availability.

Acid-treated seeds had lower germination rates. On the one hand, stratification inhibited seedling emergence in both mature and immature seeds; on the other hand, acid treatments failed to increase seed seedling emergence, and no differences in seedling emergence between treatments were found. Moreover, when the seeds were treated with acid, the differences between mature and immature seeds in the greenhouse trials disappeared.

In light of these results, we can suggest that in future this taxon could be regenerated in degraded areas by two different methods. The first is to germinate mature and immature seeds in an accessible area of the natural habitat where watering is possible, as soil moisture is essential for optimal germination. The second and most reliable method lies in germinating immature seeds in a greenhouse.

ACKNOWLEDGEMENTS

This research was supported by the Consejería de Medio Ambiente de la Junta de Andalucía. The authors thank the personnel from Parque Natural de Doñana, and José Mª Higuera and Jesús V. García from the General Greenhouse Service of the University of Sevilla for help extended to us during the work.

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