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LOW REPRODUCTIVE SUCCESS IN TWO SUBSPECIES OF *JUNIPERUS OXYCEDRUS* L.

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Mature seed cone and seed production were studied in six populations of *Juniperus oxycedrus* (three of subspecies *oxycedrus* and three of subspecies *macrocarpa*). In both subspecies, seed cone abortion took place mainly just after the pollination period, and most of the remaining seed cones reached full size. Percentage of mature cones was significantly higher in subspecies *oxycedrus* than in *macrocarpa*. In both taxa, seed cones developed fully in the absence of filled seeds. Both the number of full-sized seeds per cone and the seed viability were low, and they were similar in both subspecies. However, seed potential was markedly higher in subsp. *macrocarpa* (mean 4.1 ovules/cone) than in subspecies *oxycedrus* (mean 2.8 ovules/cone), indicating a lower seed efficiency in subspecies *macrocarpa*. Results indicate that deficient pollination and site quality can be causes of low seed cone production and low seed viability in both subspecies of *J. oxycedrus*.

Introduction

One of the most common ecological problems in conifers is the reduction in viable seed crops (Colangeli and Owens 1990; Owens et al. 1990; Arista and Talavera 1996). Lack of pollination, low pollen viability, or embryo degeneration (as a consequence of selfing) have all been implicated as causes of reduced seed production (Owens et al. 1991; Arista and Talavera 1996). Among conifers, monoecy is the predominant breeding system; the presence of male and female structures in the same plant often leads to self-pollination and consequently endogamy. However, in some monoecious conifers the spatial separation of female and male structures (Kuittinen and Savolainen 1992), the temporal difference between receptivity of female cones and shedding of pollen in the same plant (dichogamy, Sarvas 1962), or a subdioecious condition (Jordano 1991; Wilson et al. 1996; Arista and Talavera 1997) reduce the rate of selfing.

One of the most effective alternatives for preventing inbreeding is dioecy; however, because male and female strobili are on separate trees, dioecy can lead to a lack of pollination. Among conifers, dioecy is a rare breeding system, with only a few genera, e.g., *Juniperus* and *Taxus,* having dioecious species. *Juniperus* is the second largest genus of the conifers, with ca. 75–80 taxa (Franco 1964). *Juniperus oxycedrus* L. is a dioecious species widely distributed in the Mediterranean region (Franco 1964). There is little information about seed crops in dioecious junipers (Houle and Babeux 1994), and no data about cone or seed production are available for *J. oxycedrus.* The aim of this study was to determine interpopulation and intersubspecies variations in mature seed cone and seed production in *J. oxycedrus.*

Material and Methods

Juniperus oxycedrus has two common subspecies in southwestern Spain, *J. oxycedrus* subspecies *oxycedrus* and *J. oxycedrus* subsp. *macrocarpa* (Sibth. & Sm.) Ball, which grow on mountainous and on sandy coastal zones, respectively. Both subspecies of *J. oxycedrus* are shrubs—or sometimes small trees—that have a 2-yr reproductive cycle. Pollination occurs in October–November in a given year, fertilization takes place in May–June of the following year, and seed cones are ripe in September–October of the second year (M. Arista et al., unpublished data). Ripe seed cones are axillary, dark red, fleshy, and globose and are bigger in subspecies *macrocarpa* (about 16 mm vs. 10 mm in subsp. *oxycedrus*).

The study was carried out from 1994 to 1996 in six populations of *J. oxycedrus:* three of subsp. *oxycedrus* and three of subsp. *macrocarpa* (table 1). During October–November, the sexual ratio (number of male plants/number of female plants) was determined in each population. Production of seed cones was studied using five to six randomly selected branches per plant on ca. 10 individuals in each population. These branches were marked and their seed cone production, the number of seed cones in development after pollination, and the number of seed cones that ripened were counted. Mature seed cones were collected in October just before dispersion and the total number of full-sized seeds per cone was determined. Flat seeds were not counted. Each full-sized seed (hereafter, called simply "seed") was cut lengthwise to determine embryo presence (seeds with embryo are denoted as "filled seeds," and those without it are denoted as "empty seeds"). These data were used to estimate the percentage of seed viability per plant and per population.

Data were analyzed using one-factor factorial design analyses of variance (ANOVA). When significant differences between populations were presented ($P < 0.05$), these were located with Tukey tests. Prior to any statistical analyses, variables were checked for normality and transformed as necessary. Deviation from unity for the sex ratio of the six populations was verified with the G test (Sokal and Rohlf 1981).

Results

Interplant and Interpopulation Variation

Cone abortion was absent or rare during postpollination developmental in all populations. Percentage of mature cones was markedly variable between plants within each population and between populations (table 2). The Punta Umbría population had the lowest percentage of cone maturation (19.23%) and that of Puerto Saucillo had the highest (43.9%; table 2).

No external differences were observed between

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Table 1 Characteristics of the Six Studied Populations of *Juniperus oxycedrus* in Southern Spain

Subspecies and site	Province	Protection level	Elevation	Soils	Situation
macrocarpa:					
Punta Umbría	Huelva	Nature site (Enebrales de Punta Umbría)	0 m	Coastal sandy	Dunes beside sea
Pinar de Barbate	Cádiz	Nature park (Acantilado y Pinar de Barbate)	100 m	Coastal sandy	Plain on sea cliff
Punta Paloma	Cádiz	Unprotected	0 m	Coastal sandy	Dunes beside sea
oxycedrus:					
Puerto Saucillo	Málaga	Nature park (Sierra de las Nieves)	1300 m	Calcareous	Small valley
Puerto del Boyar	Cádiz	Nature park (Sierra de Grazalema)	1100 m	Calcareous	Rocky slope facing south
Sierra de Zafalgar ...	Cádiz	Nature reserve (Sierra de Grazalema)	900 m	Calcareous	Small valley

filled and empty seeds. Filled seeds per cone ranged from zero to three, the modal value of the distribution being zero in every population except Punta Paloma, where it was unity (fig. 1). The mean number of filled seeds per cone varied between populations. That of Puerto del Boyar had the lowest value (0.33; table 2) and presented, at most, one filled seed per cone (fig. 1). In contrast, the Punta Paloma population presented the highest mean number of filled seeds per cone (1.11; table 2).

Plants of each studied population displayed wide differences in seed viability. Thus, in Puerto del Boyar one individual had 0% seed viability while another reached 60%. Among populations, the highest seed viability was found in Punta Paloma (mean 52.58%) and the lowest were those of Puerto del Boyar and Punta Umbría (20.24% and 20.26%, respectively; table 2).

The proportions of male and female individuals did not differ from unity ($P > 0.05$) except in the Punta Umbría population, which had a very low proportion of male individuals (M/F = 0.4, $P < 0.05$; table 2). Cone production was independent of sex ratio ($R = 0.3$, $P = 0.55$, $n = 6$) as were mean number of filled seed per cone ($R = 0.248$, $P = 0.635$, $n = 6$) and mean seed viability ($R = 0.32$, $P = 0.536$, $n = 6$).

Intersubspecies Variation

In *Juniperus oxycedrus* subspecies, the average percentage of mature seed cones was significantly higher in the subsp. *oxycedrus* ($F = 9.04$, $P < 0.01$; table 3), while the number of ovules per cone was significantly higher in subsp. *macrocarpa* than in the subsp. *oxycedrus* ($F = 16.96$, $P = 0.0001$, 1 df; table 3). Nevertheless, the number of seeds in a mature cone was not significantly different in the two subspecies (means of 2.3 and 1.97 in subsp. *macrocarpa* and subsp. *oxycedrus,* respectively). Likewise, the mean number of filled seeds per cone was similar among subspecies, so that the percentage of seed viability was also similar (table 3). In subspecies *macrocarpa,* about 47% of mature cones had zero filled seeds, and in subspecies *oxycedrus* this proportion reached 56% (fig. 2). The seed-set (ovules that develop into filled seeds) was higher in subspecies *oxycedrus* (with 21.7%) than in *macrocarpa* (17.4%).

The presence of two embryos within one seed (polyembryony) was found on two occasions: the first in subsp. *oxycedrus* (Sierra de Zafalgar population) and the second in subsp. *macrocarpa* (Punta Paloma population).

Discussion

Mature cone production in *Juniperus oxycedrus* depends on the number of cones that develop after pollination. Because the time between pollination and fertilization is long, in this case ca. 6–7 mo (M. Arista et al., unpublished data), cone development is independent of ovule fertilization. In *J. oxycedrus,* pollination takes place in October–November. Seed cones are receptive during the whole pollination period, but unpollinated seed cones continue secreting pollen drops for several weeks, and receptivity may extend even until February. The bulk of seed cone abortion in this species took place during this stage, indicating that pollen is essential for seed cone development, as occurs in other conifers (Sarvas 1962; Dogra 1967; Owens and Blake 1984; Colangeli and Owens 1990). Thus, the percentage of mature cones reflects the de-

Table 2 Mature Cone Production (%) and Seed Characters of Six Populations of *Juniperus oxycedrus*

Subspecies and population	Mature cone production (%)			Filled seeds			Seed viability (%)			Sex ratio
	Mean ± SE	Range	n	Mean ± SE	Range	n	Mean ± SE	Range	n	
macrocarpa:										
Punta Umbría	19.23 ± 4.14[a]	1.7–48.8	14	0.60 ± 0.09[a]	0.2–1.4	13	20.26 ± 2.91[a]	8–45.3	13	0.4
Pinar de Barbate ...	29.39 ± 4.78[ab]	0.7–46.6	10	0.45 ± 0.11[a]	0.1–1.3	10	28.99 ± 6.41[ab]	5.9–60	10	1.16
Punta Paloma	38.32 ± 4.01[ab]	29.8–49.1	4	1.11 ± 0.13[b]	0.1–1.5	10	52.58 ± 6.46[b]	6.4–72.2	10	1
oxycedrus:										
Puerto Saucillo	43.9 ± 5.64[b]	15.5–71.4	13	0.74 ± 0.17[ab]	0.05–1.6	8	40.74 ± 6.96[ab]	2–66.6	8	1.08
Puerto del Boyar ...	28.69 ± 3.45[ab]	7.9–39.6	10	0.33 ± 0.13[a]	0–1	7	20.24 ± 7.83[a]	0–60	7	1.3
Sierra de Zafalgar ..	39.86 ± 3.72[b]	26.7–64	10	0.73 ± 0.21[ab]	0.05–1.9	7	29.99 ± 7.79[ab]	2.4–70.3	7	0.65

Note. Entries sharing the same superscript do not differ significantly (Tukey test, $P < 0.01$). Range refers to mean values per plant.

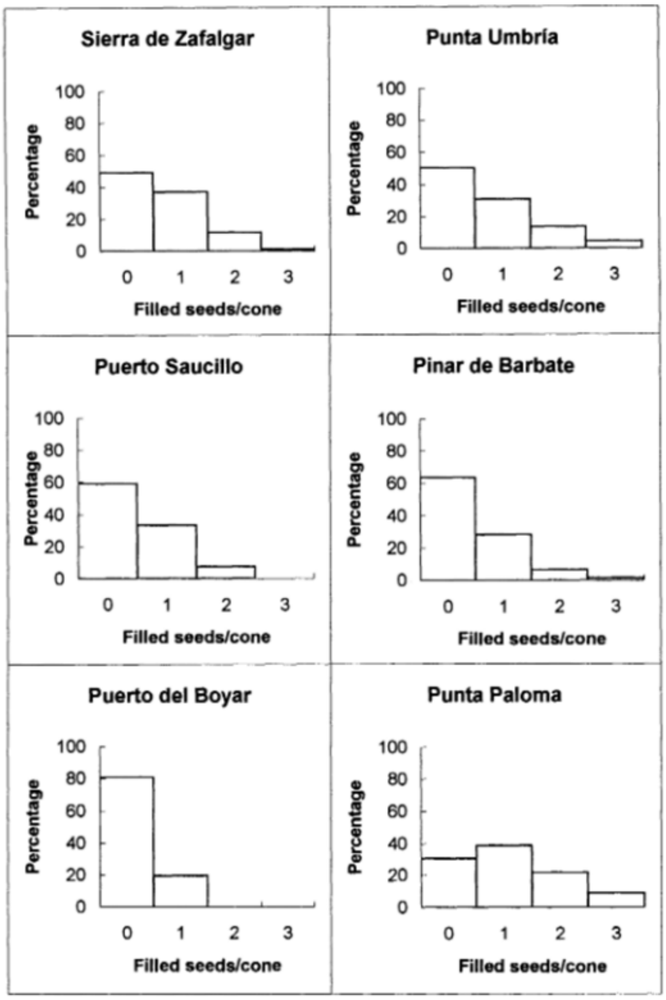

Fig. 1 Frequency distribution of the number of filled seeds per cone in six populations of *Juniperus oxycedrus.*

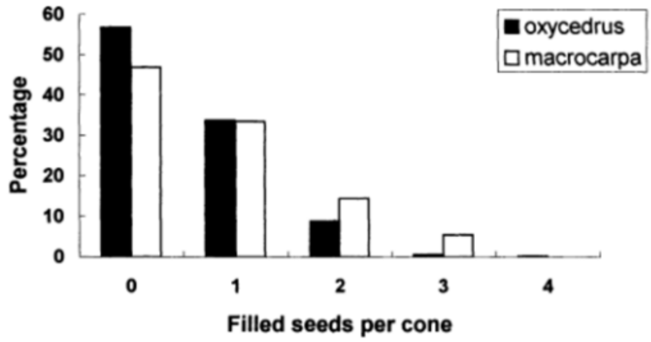

Fig. 2 Frequency distribution of the number of filled seeds per cone in *Juniperus oxycedrus* subsp. *oxycedrus* and in *J. oxycedrus* subsp. *macrocarpa.*

gree of pollination and differs markedly among individuals. In fact, pollen dispersal in wind-pollinated species is highly leptokurtic (Farris and Mitton 1984; Proctor et al. 1996); consequently, in dioecious species, large variances in reproductive success could result from proximity of mates (Freeman et al. 1997).

The proportion of aborted seed cones was quite high in all of the populations. The highest rate was found in Punta Umbría, where ca. 80% of seed cones aborted. This was the only population in which the sex ratio differed significantly from unity. The low proportion of male individuals, and consequently of pollen, could be the main cause of this abortion rate. However, the population of Puerto del Boyar presented the second highest rate of seed cone abortion and the highest sex ratio (M/F = 1.3). This population was situated on a calcareous rocky slope facing south, a site where summer drought was severe. In xeric sites, the greater cost associated with the production of fruits as opposed to pollen can give rise to a male-biased sex ratio in dioecious species (Freeman et al. 1976; Willson 1979). Moreover, in this population male and female individuals were clearly segregated by niches. Female individuals were distributed along a temporal watercourse that was probably the most favorable microsite. This kind of spatial segregation of sexes is common in dioecious wind-pollinated species (Bierzchudek and Eckart 1993; Freeman et al. 1997). In this situation, females could be pollen-limited in the more favorable microhabitats that they dominate (Freeman et al. 1993). This could be the reason of the low percentage of mature seed cones found in Puerto del Boyar population. Thus, spatial location of mates could be more important than sex ratio, if the latter is not extremely low.

In *J. oxycedrus,* the number of seeds per cone reflects the number of pollinated ovules (M. Arista et al., unpublished data). It is remarkable that the number of ovules per cone in *J. oxycedrus,* although different between subspecies, is lower than those in other Cupressaceae (Owens et al. 1990; Briand et al. 1992) and in most conifers. With only two to eight ovules, cones of *J. oxycedrus* would need only a few pollen grains to transform all their ovules into seeds. However, only some of these ovules were transformed into seeds, in-

Table 3 Characters of the Two Subspecies of *Juniperus oxycedrus*

	macrocarpa			*oxycedrus*		
Character	Mean ± SE	Range	n	Mean ± SE	Range	n
Mature cone production (%)	25.59 ± 2.98^{a}	0.7–49.1	28	38.07 ± 2.86^{b}	7.9–71.4	33
Ovules/cone	4.1 ± 0.25^{a}	3–8	30	2.8 ± 0.12^{b}	2–4	30
Seeds/cone	2.31 ± 0.12^{a}	1.0–3.3	33	1.97 ± 0.12^{a}	1–2.8	22
Filled seeds/cone	0.71 ± 0.08^{a}	0.1–1.5	33	0.61 ± 0.1^{a}	0–1.9	22
Seed viability (%)	32.7 ± 3.75^{a}	5.9–72.2	33	30.8 ± 4.51^{a}	0–70.3	22

Note. Range refers to mean values per plant. Entries sharing the same superscript do not differ significantly (ANOVA, $P < 0.01$).

dicating a deficient pollination. Pollination problems are usual in conifers (Owens and Molder 1977, 1979; Singh and Owens 1981; Colangeli and Owens 1990, 1991; Arista and Talavera 1994). Deficient pollination is more critical in the subspecies *macrocarpa*, because the two subspecies had a similar number of seeds per cone, but cones in subspecies *macrocarpa* had a higher number of ovules.

Of the seeds produced by *J. oxycedrus*, only ca. 31% had an embryo, leading to a similar mean number of 0.6 and 0.7 filled seeds per cone in subsp. *oxycedrus* and *macrocarpa*, respectively. In the dioecious *Juniperus communis*, the proportion of empty seeds is also markedly high, ranging from 40% to 96% in different populations (Houle and Babeux 1994). Owens et al. (1990) reported that if abortion takes place after fertilization period in *Thuja plicata*, the empty seeds would be externally indistinguishable from ripe filled seeds. This situation is similar to *J. oxycedrus*. Main causes of seed abortion at this stage are poor pollen viability or vigor (Owens et al. 1990), or endogamy (Sorensen 1982; Owens et al. 1991; Arista and Talavera 1996). As *J. oxycedrus* is a dioecious species, selfing is ruled out as a cause of abortion. Because of the nature of our work, it is not possible to know the effect of endogamy (crosses between siblings) and pollen vigor or viability as sources of empty seeds, but they should not be ruled out. The fact that the Puerto del Boyar population, situated in the most xeric site, showed the lowest number of filled seeds per cone indicates that resource limitation could be partly responsible for seed abortion at this stage. In fact, the investment in the maturing embryos may be very large (Willson 1983). Moreover, in *J. oxycedrus* the two reproductive cycles overlap, and two seed cone crops cooccur in an individual. Thus, female individuals require an investment of considerable magnitude for reproduction. In consequence, if resources are limited, abortion of developing embryos is likely to occur in *J. oxycedrus*. Decrease in reproductive female success in xeric sites has been reported previously (Freeman and Vitale 1985), and xeric conditions are usual in the Mediterranean region where *J. oxycedrus* is distributed.

On the other hand, the distribution area of *J. oxycedrus* subsp. *macrocarpa* in southwestern Spain has decreased recently as the consequence of urbanistic pressures on the coast. As a result, this taxon has become vulnerable in the zone, and restoration programs have been set in motion. The results of our study have several implications for the production and collection of juniper seeds. It would be appropriate to collect seeds from female individuals that have a very close mate and are placed in favorable sites. Of the studied populations, Punta Paloma had the highest number of filled seeds per cone, and this population seems to be the most suitable for seed collection.

In conclusion, both subspecies of *J. oxycedrus* display high percentages of empty seeds per cone. Populations and plants differ in their seed viability, and thus preliminary screening is necessary to optimize seed production for restoration purposes. Moreover, dioecy makes this species more vulnerable to any management-altering sex ratio or mate distances.

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