Effect of Temperature on Germination in Northernmost Populations of *Culcita macrocarpa* and *Woodwardia radicans*

L. G. Quintanilla [1,3], S. Pajarón [2], E. Pangua [2], and J. Amigo [1]

[1] Departamento de Bioloxía Vexetal, Facultade de Farmacia, Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[2] Departamento de Biología Vegetal I, Facultad de Biología, Universidad Complutense, Madrid, Spain
[3] *Present address:* Departamento de Biología Vegetal I, Facultad de Biología, Universidad Complutense, Madrid, Spain

Received: April 27, 2000; Accepted: October 11, 2000

Abstract: Spore germination of *Culcita macrocarpa* C. Presl and *Woodwardia radicans* (L.) Sm. from nine populations at the northern limit of their distribution, in the northwest Iberian Peninsula, was investigated. In a first experiment, population type and temperature (10, 15, 20, and 25 °C) were both found to affect germination percentage and germination time significantly in both species. There were also significant interactions between the two factors with respect to the percentage germination of *C. macrocarpa* and the germination time of *W. radicans*. In *C. macrocarpa* there was an outstanding increase in germination time at 15 °C and, above all, at 10 °C, whereas in *W. radicans* the most remarkable result was the existence of two populations with especially low germination percentages. In a second experiment, germination of 20 individuals from each population of *W. radicans* was compared with similar inter-population differentiation. Although its variability possibly has a genetic basis, these species are able to germinate successfully, and it seems probable that the season in which it occurs depends more on spore release than on thermal conditions in the populations. The effect of temperature on germination in both species does not explain their coastal distribution. Temperature is probably more important in limiting other stages of the life cycle.

Key words: Germination, temperature, population, *Culcita macrocarpa*, *Woodwardia radicans*, Iberian Peninsula.

Introduction

The northwest of the Iberian Peninsula is very rich in thermophilous ferns of a relict nature restricted to very moist habitats, including *Christella dentata, Culcita macrocarpa, Cystopteris diaphana, Davallia canariensis, Dryopteris aemula, D. guanchica, Stegnogramma pozoi, Trichomanes speciosum,* and *Woodwardia radicans.*

Culcita macrocarpa, the only Dicksoniaceae in Europe, and *Woodwardia radicans,* with *Blechnum spicant* the only Blechnaceae, have been named Macaronesian relicts (Pichi Sermolli, 1979[24]; Pichi Sermolli et al., 1988[25]). This name is applied to relicts of the tropical flora that covered the Mediterranean area during the Tertiary Period. Their present range extends discontinuously through Macaronesia (Azores, Madeira and Canary Islands), the Atlantic side of the Iberian Peninsula, and, in the case of *W. radicans,* some locations in the Mediterranean area and north Africa. Their northern distributional limit is in the northwestern Iberian Peninsula, where they always grow near the coast, usually at altitudes below 300 m. In more southern locations, especially in Macaronesia, they are associated with fog belts in high zones, even being found above 1000 m (Page, 1977[20]).

The forests of the northwest Iberian coast where these ferns live, often in mixed populations, have particular sheltered conditions, beside steep-sided streams, with high atmospheric and soil moisture, favoured by north-facing aspects (Amigo and Norman, 1995[1]). Temperatures are mild throughout the year, and frost is almost absent.

Besides their biogeographical affinities, *C. macrocarpa* and *W. radicans* share the same life-form, with large aboveground rhizomes and evergreen fronds up to 3 m long (together with *Pteridium aquilinum* they are the biggest ferns in Europe).

However, *C. macrocarpa* and *W. radicans* are included in several lists of threatened species (Ormonde, 1990[19]; Cellinese et al., 1996[5]; Quintanilla and Amigo, 1999[27]). The scarce knowledge of their life cycle is another disadvantage for their conservation. Due to the abundant spores produced by ferns that reach a wider area than that occupied by the sporophytes (Page, 1979[21]), germination may be the first stage limiting their distribution and abundance. Germination of *C. macrocarpa* spores was studied by Rezende Pinto (1943[30]) and Mukherjee and Sen (1986[16]). There are no studies of *W. radicans* spore germination. Nayar et al. (1966[17]) studied *W. unigemmata,* a very similar taxon that some authors regard as the same species. Nevertheless these are mainly morphological studies. Considering the thermophilous behaviour of *C. macrocarpa* and *W. radicans,* we have judged it to be of interest to study the effect of temperature on the germination of their spores, comparing it in several populations.

Plant biol. 2 (2000) 612 – 617

ISSN 1435-8603

Materials and Methods

Four populations of *C. macrocarpa* and six populations of *W. radicans,* representing the entire altitudinal range of both species in the northwest Iberian Peninsula, were selected for the study (Table **1**). All the populations are located along narrow and steep river valleys, protected by hazel *(Corylus avellana)* forest, except for population W2, which is located in a flat valley where the forest is dominated by alders *(Alnus glutinosa).* The conservation status of these forests is good except in population W3, where the natural vegetation has been almost entirely replaced by a *Eucalyptus globulus* plantation. The two most distant populations are separated by 40 km.

Spores were collected in mid-November 1998. Lamina fragments with sporangia from 20 plants were collected from each population, and kept in closed plastic bags. In order to avoid decreased viability with time and possible culture contamination, recent fronds were selected, i.e., those that developed during the same year, with mature sporangia protected by the closed indusia. To obtain the spores, the fragments were kept in a plant press at laboratory temperature for a week.

Spores were sown on mineral agar (Dyer, 1979[8]) in 5.5 cm diameter Petri dishes. To avoid contamination, the sori extract was filtered through two layers of lens tissue (Whatman International Ltd., Maidstone, n 2105841), and dishes were sealed with Parafilm (American National Can, Chicago). The spores were observed under a light microscope at 60 × magnification. The proportion of defective spores (i.e., with underdeveloped or unusually massive perispore, or collapsed, without protoplast) was evaluated, counting 50 randomly selected spores from 6 dishes (300 spores per population). This proportion was relatively low and homogeneous among the populations of *W. radicans* (Table **1**) and higher in *C. macrocarpa,* with values higher than 80% in two populations in which there were specimens with defective spores in all their sori. Thus, population C0 (89% defective spores) was eliminated in experiment 1 and all *C. macrocarpa* populations were eliminated in experiment 2.

The dishes were checked daily until germination started, then the control was done every four days. First cell emergence was taken as the criterion of germination. Two variables were measured: *germination percentage,* the number of germinated spores calculated among 50 non-defective randomly selected spores from each dish; and *germination time,* the number of days between sowing and germination of the first spore among the 50 randomly selected spores each day. Percentages were compared after 29 days, when germination became stable.

Analysis of variance (ANOVA) was carried out after arcsine transformation of the germination percentages, and log transformation of the germination times (Zar, 1996[33]). A Tukey test ($p < 0.05$) was performed to identify homogeneous groups of means.

Experiment 1

Population–temperature interaction and the effects of population and temperature variation on the germination response were analysed. Spores were incubated in four growth chambers with an identical 16 h light/8 h dark photoperiod (Osram fluorescent tubes L20 W/105, 30 – 45 Em^{-2} s^{-1}), but different constant temperatures: 10, 15, 20, and 25 °C. These temperatures were selected considering the range of monthly mean values of the meteorological stations nearest to the populations (Carballeira et al., 1983[4]). Temperature was considered as a fixed factor as was the population, since the small number of locations sampled and their proximity do not allow their consideration as representative of the species. For each temperature and each population, three replicates of mixed spores from 20 plants were sown. A two-factor ANOVA was subsequently performed.

Experiment 2

Once experiment 1 had been completed, variation within and among populations of the germination response of all individuals (20 per population) of *W. radicans* was studied. The spores were obtained from the same fragments of fronds kept on paper sheets in the laboratory for one month. In this experiment, we used one dish per individual and the cultures were kept in a controlled temperature room at 20 °C ± 2, with the same light conditions as used in experiment 1. Results were tested with a single-factor ANOVA.

Table 1 Spore sources. Acronym and situation of each population used as spore source. The percentage of defective spores for each population is included

Species	Pop.	Location (river basin)	Latitude	Altitude (m a.s.l.)	Distance to sea (km)	% defective spores (mean ± standard error)
Culcita macrocarpa	C0	Xubia	43°29′N	280	11	89 ± 2
	C1	Eume	43°25′N	160	3	24 ± 2
	C2	Seixo de Landoi	43°41′N	280	3	84 ± 3
	C3	Eume	43°24′N	90	8	41 ± 2
Woodwardia radicans	W1	Xubia	43°29′N	210	11	16 ± 1
	W2	Mera	43°32′N	370	15	37 ± 2
	W3	Morela	43°44′N	110	2	24 ± 1
	W4	Seixo de Landoi	43°41′N	280	3	20 ± 1
	W5	Eume	43°24′N	80	8	20 ± 1
	W6	Xubia	43°30′N	250	9	13 ± 2

Results

Fig. **1** shows the evolution of germination percentages of the first experiment over one month. The curves level out after two weeks in the case of the dishes of *W. radicans*, and after three weeks in *C. macrocarpa*, except at 10 °C in which the germination times were clearly longer.

Temperature and population type had a significant effect on percentages and times of germination in both species. Interaction between both factors was also significant on germination time in *W. radicans*, and on germination percentage in *C. macrocarpa* (Table **2**, Fig. **2**). Germination time was significantly longer in population C2 of the latter species compared with the other two populations (Table **3**). Statistical comparison of germination time at different temperatures confirms what was observed in the curves: they decreased from almost four weeks at 10 °C to only one above 20 °C. On the other hand, germination percentages in *C. macrocarpa* must be interpreted with caution due to the interaction between the considered factors. There are clear differences between populations C3 and C1, and among the four temperatures, with values ranging from only 7 % at 10 °C to 87 % at 20 °C (Table **3**).

In general, *W. radicans* had higher germination percentages and shorter germination times than *C. macrocarpa* (Table **3**, Fig. **2**). The highest germination rate was observed in population W6 (95 %) and the lowest were seen in populations W1 and W2 (62 and 67 %). The best temperature for germination was 25 °C (86 %) and the worst was 15 °C (79 %). Germination times by temperature ranged between 2.0 days (25 °C) and 8.2 days (10 °C), and the variation was smaller if populations were compared (Table **3**).

In the second experiment, with 20 plants of each population of *W. radicans*, percentage as well as time of germination differed significantly between the populations (Table **4**). The Tukey test revealed groups of means similar to those distinguished in the first experiment (Table **5**).

Discussion

The conservation of an organism requires knowledge of its genetic structure and breeding system (Falk and Holsinger, 1991[9]). At the moment there are few published studies dealing with the effect of temperature on germination of fern spores (Miller, 1968[15]; Dyer, 1979[8]; Raghavan, 1989[28]; Bennert and Danzebrink, 1996[3]), but enough to indicate that temperature is one of the most important factors affecting germination. Temperatures in the range of those of our experiments (10 – 25 °C) are adequate for most homosporous ferns, but the optimal values are known only in a small number of species. In the first experiment, *W. radicans* shows an optimum at 25 °C, although germination percentages are high and germination times short at all four chosen temperatures (Table **3**). The spores of *W. radicans* and *C. macrocarpa* are non-chlorophyllous. Ferns with this type of spore require longer periods for germination than ferns that produce chlorophyll-containing spores, with times of only 1.5 days on average (Lloyd and Kle-

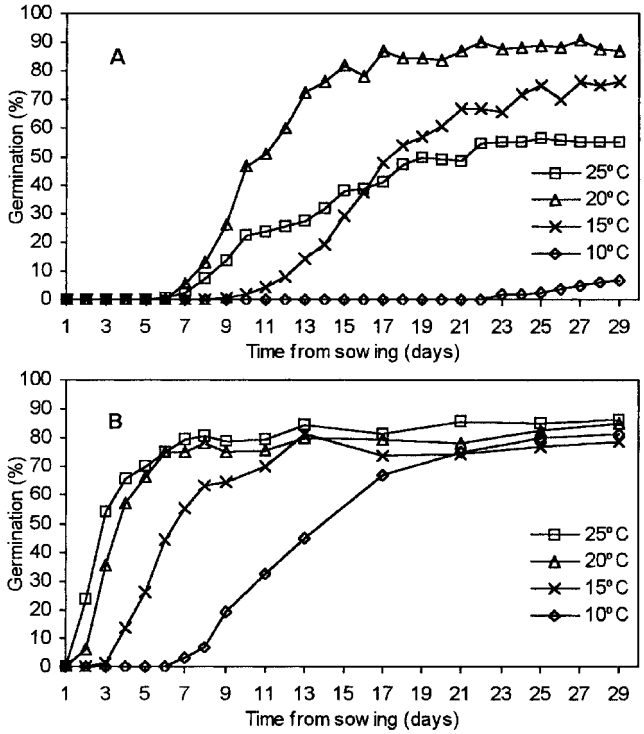

Fig. 1 Curves of germination percentage of *Culcita macrocarpa* (**A**) and *Woodwardia radicans* (**B**) at different temperatures (experiment 1).

Table 2 Results of two-factor ANOVA for spores of 9 populations incubated at 4 different temperatures. df, degrees of freedom; SS, sum of squares; *p*, significance level

Species	Source	df	Germination percentage		Germination time	
			SS	*p*	SS	*p*
Culcita macrocarpa	Population	2	304	0.023	0.061	0.000
	Temperature	3	16044	0.000	1.414	0.000
	Population × Temperature	6	1062	0.002	0.009	0.269
	Error	24	828		0.027	
Woodwardia radicans	Population	5	7722	0.000	0.086	0.000
	Temperature	3	344	0.009	2.599	0.000
	Population × Temperature	15	707	0.070	0.050	0.000
	Error	48	1284		0.035	

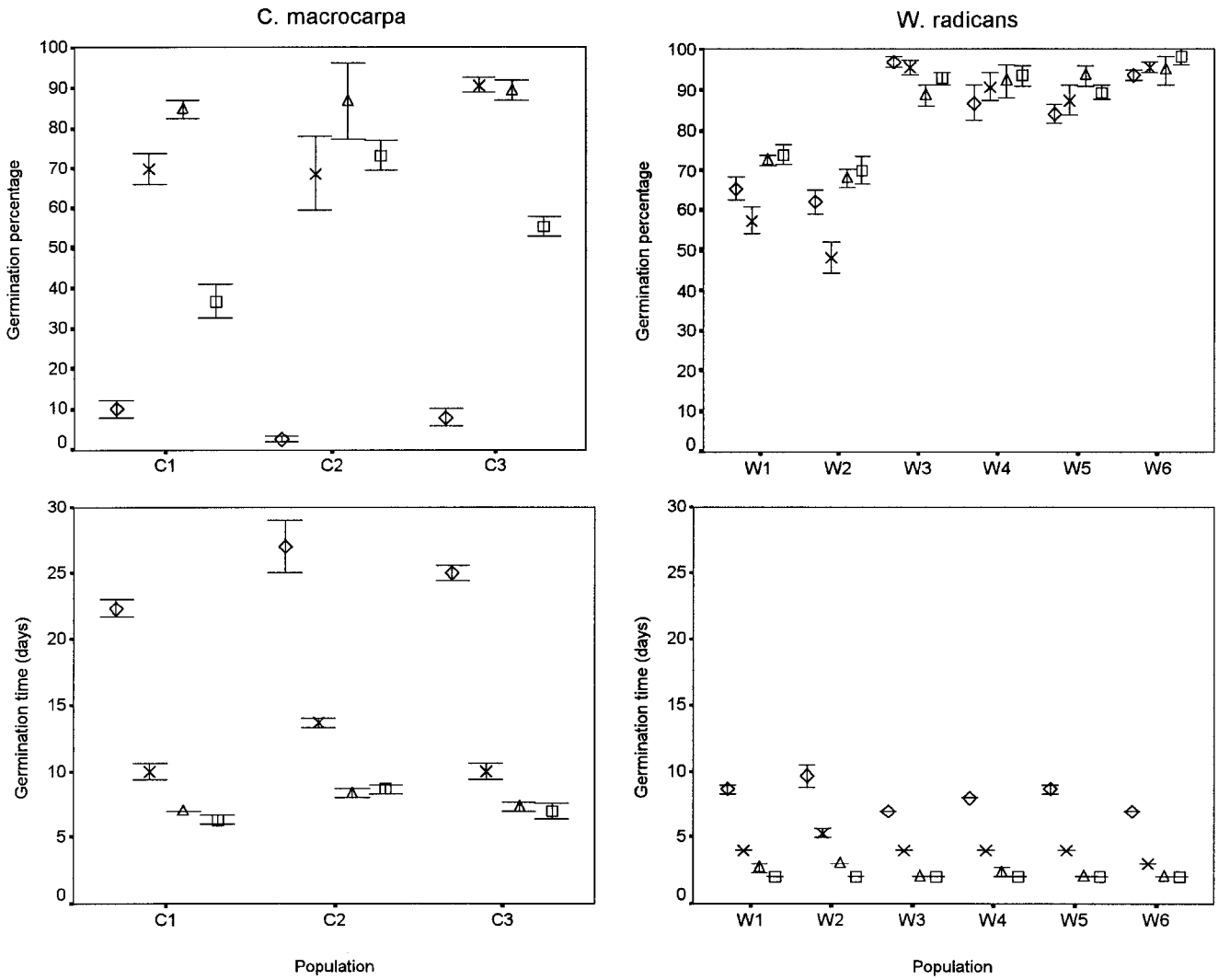

Fig. 2 Germination of 3 populations of *Culcita macrocarpa* and 6 populations of *Woodwardia radicans* incubated at different temperatures: 10 °C (◇), 15 °C (×), 20 °C (Δ), and 25 °C (□). Markers correspond to means of three replicates, bars indicate S.E.

kowski, 1970[12]; Bennert and Danzebrink, 1996[3]). Germination times of ferns with non-chlorophyllous spores can be as long as 210 days (Lloyd and Klekowski, 1970[12]) but are normally shorter than a week, when light, moisture, and temperture conditions are adequate (Page, 1979[21]). Nevertheless, *C. macrocarpa* triples this time at 10 °C, which indicates a stronger sensitivity to low temperatures, though a question remains about whether germination percentage would be as high as that at higher temperatures if incubation time was increased. At the other extreme, high temperatures (25 °C) seem to reduce germination percentage, but do not affect the germination time, that is similar to results obtained at 20 °C (Table **3**) and to those obtained by Mukherjee and Sen (1986[16]) at 24 °C.

According to our results and the available climatic data, germination of both species is feasible throughout the year. In the meteorological stations close to the studied locations (Carballeira et al., 1983[4]; Anon, 1995[2]) monthly mean temperature hardly drops below 10 °C during the coldest months, January and February, when even the mean minimum temperature is above 5 °C. In the warmest month, August, the mean temperature is between 15 and 20 °C, and the mean maximum temperature between 20 and 25 °C. Considering this oceanic climate, probably enhanced by the tempering capacity of the forest, we may conclude that the germination phenology of these ferns depends more on the time when the spores are released than on the thermal conditions in which they develop. Considering only germination, it is difficult to explain why these ferns grow above 300 m altitude in the northwest Iberian Peninsula. Although thermal amplitude increases with altitude and distance from the sea (Carballeira et al., 1983[4]), there are forests above that level where germination seems possible for most of the year. This coastal distribution may be explained with respect to temperature acting on other stages of the life cycle. Other physical factors, such as water accessibility, have been proposed as limitations to fern establishment (Kornás, 1985[11]; Ranal, 1999[29]), and some biological factors, such as competition, may also be important (Pangua et al., 1999[23]).

Table 3 Effects of population (bold, in brackets) and temperature (bold, in brackets) on germination (mean ± standard error) of *Culcita macrocarpa* and *Woodwardia radicans* in experiment 1. The lines group means that were not significantly different (Tukey test, $p < 0.05$)

	Culcita macrocarpa		*Woodwardia radicans*	
	Germination percentage	Germination time	Germination percentage	Germination time
Population	61 ± 10 (**C3**)	14.4 ± 2.3 (**C2**)	95 ± 1 (**W6**)	5.0 ± 0.9 (**W2**)
	58 ± 10 (**C2**)	12.3 ± 2.2 (**C3**)	93 ± 1 (**W3**)	4.3 ± 0.8 (**W1**)
	50 ± 9 (**C1**)	11.4 ± 2.0 (**C1**)	91 ± 2 (**W4**)	4.2 ± 0.8 (**W5**)
			88 ± 2 (**W5**)	4.1 ± 0.7 (**W4**)
			67 ± 2 (**W1**)	3.8 ± 0.6 (**W3**)
			62 ± 3 (**W2**)	3.5 ± 0.6 (**W6**)
Temperature	87 ± 3 (**20**)	24.8 ± 0.9 (**10**)	86 ± 3 (**25**)	8.2 ± 0.3 (**10**)
	76 ± 5 (**15**)	11.2 ± 0.7 (**15**)	85 ± 3 (**20**)	4.1 ± 0.2 (**15**)
	55 ± 6 (**25**)	7.6 ± 0.2 (**20**)	81 ± 3 (**10**)	2.3 ± 0.1 (**20**)
	7 ± 1 (**10**)	7.3 ± 0.4 (**25**)	79 ± 5 (**15**)	2.0 ± 0.0 (**25**)

Table 4 Results of single-factor ANOVA for spores of 20 sporophytes from 6 populations of *Woodwardia radicans* incubated at 20 °C. df, degrees of freedom; SS, sum of squares; *p*, significance level

Source	df	Germination percentage		Germination time	
		SS	*p*	SS	*p*
Population	5	7029	0.000	0.190	0.000
Error	114	4248		0.307	

Table 5 Effect of population on germination (mean ± standard error) of *Woodwardia radicans* in experiment 2. The lines group means that were not significantly different (Tukey test, $p < 0.05$)

	Germination percentage	Germination time
Population	95 ± 1 (**W4**)	3.1 ± 0.1 (**W4**)
	94 ± 1 (**W3**)	3.1 ± 0.1 (**W2**)
	94 ± 1 (**W6**)	3.0 ± 0.0 (**W1**)
	88 ± 2 (**W5**)	2.7 ± 0.1 (**W5**)
	76 ± 2 (**W1**)	2.6 ± 0.1 (**W3**)
	72 ± 2 (**W2**)	2.2 ± 0.1 (**W6**)

On the other hand, spore source population also affects germination in both species, as happens in other species of *Asplenium* (Pangua et al., 1994[22]; Prada et al., 1995[26]), in *Blechnum spicant* (Cousens, 1981[7]), and in *Cryptogramma crispa* (Pangua et al., 1999[23]). These differences may be due to genetic causes. Moreover, there is a significant interaction between population and temperature with respect to the germination time of *W. radicans,* and the germination percentage of *C. macrocarpa,* i.e., different populations are not affected in the same way at different temperatures. Interactions between populations and different treatments have also been found in seed germination experiments (Maruta, 1994[14]; Martin et al., 1995[13]; Nishitani and Masuzawa, 1996[18]); environmental differences among populations that would select different genotypes have been proposed as the cause.

As well as any genetic differences, environmental differences among populations may also have affected germination behaviour. The populations sampled are located at the northern limit of the distribution of both ferns, where conditions might be unfavourable for spore development (Wilce, 1965[32]; Wagner et al., 1986[31]), or for maintaining spore viability. This would explain why populations W1 and W2 of *W. radicans* have the lowest germination percentages in both experiments. Population W2 (Table **1**) is at the highest altitude and is farthest from the sea, and W1, although not very high, is located at the bottom of a valley with particularly cold microclimatic conditions, probably due to the scarce solar radiation received and thermal inversion phenomena (own unpublished data). The high percentages of *C. macrocarpa* spores considered to be defective on the basis of their morphology (Table **1**), especially those with an underdeveloped perispore, may also be due to lack of maturation. In the nearby populations of north Portugal development is completed in late September, with dehiscence in February or March (Rezende Pinto, 1943[30]). The phenology of *W. radicans* has not been studied but might be expected to be similar.

It is of particular biological interest that the fronds of both species last for more than a year. According to the classification of Chabot and Hicks (1982[6]) these ferns are evergreen (own unpublished data). Therefore, fertile fronds from different years can be found in all seasons. Our study focused on recently produced spores, but fronds from other growing periods can also act as spore sources (Farrar, 1976[10]).

Another consideration is that, while there are abundant young sporophytes originated by sexual reproduction in the populations of *C. macrocarpa,* there are none in the populations of *W. radicans,* and the vegetative production of plants by rooting of the buds at the distal part of each frond is very common (personal observation). Page (1977[20], 1979[21]) has argued that this reproductive mechanism supplements the slow gametophytic development.

In conclusion, we can highlight the good germination ability of the spores of *C. macrocarpa* and *W. radicans,* depending on the source population, in the range of temperatures occurring in their northern distributional limit. For the successful conser-

vation of these two rare species their whole life cycle requires urgent study. With respect to the germination stage, future investigation might focus on the phenology of spore release, the role of old fronds as spore sources, the characteristics of spore banks, and the relation between germination performance and gametophyte establishment.

Acknowledgements

The authors thank Adrián Escudero, Félix Pérez-García, and Felipa Fernández Llorente, of E.U.I.T. Agrícola (U.P.M.), and Isabel Iglesias, of Facultade de Farmacia (U.S.C.) for helpful advice and for letting us use the growth chambers in their laboratories; and also two anonymous reviewers for helpful comments on an earlier version of the manuscript. Part of the study was carried out during a visit by LGQ financed by the U.S.C. This research was supported by the project PGIDT99PXI20301A of the Xunta de Galicia and the project PB97-0307 of the Spanish Ministry of Education and Culture.

References

[1] Amigo, J. and Norman, G. (1995) Identification of site-types important for rare ferns in an area of deciduous woodland in northwest Spain. Vegetatio 116, 133 – 146.
[2] Anon, (1995) Resumo de datos climatolóxicos, período 1955 – 94. Xunta de Galicia: Santiago de Compostela.
[3] Bennert, H. W. and Danzebrink, B. (1996) Spore germination of endangered pteridophytes in Germany. Verhandlungen der Gesellschaft für Ökologie 26, 197 – 207.
[4] Carballeira, A., Devesa, C., Retuerto, R., Santillán, E. and Ucieda, F. (1983) Bioclimatología de Galicia (2 vols.). A Coruña: Fundación P. Barrié de la Maza.
[5] Cellinese, N., Jarvis, C. E., Pichi Sermolli, R. E. G., Press, J. R., Short, M. J., and Viciani, D. (1996) Threatened plants of Italy. Pteridophytes. Memorie della Academia Lunigianese di Scienze "Giovanni Capellini". La Spezia 66, 117 – 145.
[6] Chabot, B. F. and Hicks, D. J. (1982) The ecology of leaf life spans. Annual Review of Ecology and Systematics 13, 229 – 259.
[7] Cousens, M. I. (1981) *Blechnum spicant:* habitat and vigor of optimal, marginal, and disjunct populations, and field observations of gametophytes. Botanical Gazette 142 (2), 251 – 258.
[8] Dyer, A. F. (1979) The culture of fern gametophytes for experimental investigation. In The experimental biology of ferns (Dyer, A.F., ed.), London: Academic Press, pp. 254 – 305.
[9] Falk, D. A. and Holsinger, K. E. (1991) Genetics and conservation of rare plants. Oxford: Oxford University Press.
[10] Farrar, D. R. (1976) Spore retention and release from overwintering fern fronds. American Fern Journal 66 (2), 49 – 52.
[11] Kornás, J. (1985) Adaptive strategies of African pteridophytes to extreme environments. In Biology of pteridophytes (Dyer, A. F. and Page, C. N., eds.), Edinburgh 86 B: Proceedings Royal Society, pp. 391 – 396.
[12] Lloyd, E. M. and Klekowski, R. J. Jr. (1970) Spore germination and viability in pteridophyta: evolutionary significance of chlorophyllous spores. Biotropica 2, 129 – 137.
[13] Martin, A., Grzeskowiak, V., and Puech, S. (1995) Germination variability in three species in disturbed Mediterranean environments. Acta Oecologia 16 (4), 479 – 490.
[14] Maruta, E. (1994) Seedling establishment of *Polygonum cuspidatum* and *Polygonum weyrichii* var. *alpinum* at high altitudes of Mt. Fuji. Ecological Research 9, 205 – 213.
[15] Miller, J. H. (1968) Fern gametophytes as experimental material. The Botanical Review 34 (4), 361 – 440.
[16] Mukherjee, A. K. and Sen, T. (1986) Gametophytes of some treeferns and their impact on phylogenetic relationships. Indian Fern Journal 3, 70 – 81.
[17] Nayar, B. K., Bajpai, N., and Raza, F. (1966) Morphological studies on some species of *Blechnum, Doodia, Woodwardia* and *Stenochlaena*: I. The gametophytes and the juvenile sporophytes. Journal of the Linnean Society (Botany) 59, 405 – 423.
[18] Nishitani, S. and Masuzawa, T. (1996) Germination characteristics of two species of *Polygonum* in relation to their altitudinal distribution on Mt. Fuji, Japan. Arctic and Alpine Research 28 (1), 104 – 110.
[19] Ormonde, J. (1990) Pteridófitos endémicos, raros ou ameaçados das Ilhas Macaronésicas. Fontqueria 28, 5 – 12.
[20] Page, C. N. (1977) An ecological survey of the ferns of the Canary Islands. Fern Gazette. 11, 297 – 312.
[21] Page, C. N. (1979) Experimental aspects of fern ecology. In The experimental biology of ferns (Dyer, A. F., ed.), London: Academic Press, pp. 552 – 589.
[22] Pangua, E., Lindsay, S., and Dyer, A. (1994) Spore germination and gametophyte development in three species of *Asplenium.* Annals of Botany 73, 587 – 593.
[23] Pangua, E., García-Álvarez, L., and Pajarón, S. (1999) Studies on *Cryptogramma crispa* spore germination. American Fern Journal 89 (2), 159 – 170.
[24] Pichi Sermolli, R. E. G., (1979) A survey of the pteridological flora of the Mediterranean Region. Webbia 34 (1), 175 – 242.
[25] Pichi Sermolli, R. E. G., España, L., and Salvo, A. E. (1988) El valor biogeográfico de la pteridoflora ibérica. Lazaroa 10, 187 – 205.
[26] Prada, C., Pangua, E., Pajarón, S., Herrero, A., Escudero, A., and Rubio, A. (1995) A comparative study of gametophyte morphology, gametangial ontogeny, and sex expression in the *Asplenium adiantum-nigrum* complex (Aspleniaceae, Pteridophyta). Annales Botanici Fennici 32, 107 – 115.
[27] Quintanilla, L. G. and Amigo, J. (1999) Catálogos de las pteridofloras de los espacios naturales protegidos de Galicia. Botanica Complutensis 23, 99 – 110.
[28] Raghavan, V. (1989) Developmental biology of fern gametophytes. Cambridge, Great Britain: Cambridge University Press.
[29] Ranal, M. A. (1999) Effects of temperature on spore germination in some fern species from semideciduous mesophytic forest. American Fern Journal 89 (2), 149 – 158.
[30] Rezende Pinto, M. C. de. (1943) *Culcita macrocarpa* Pr. Contribuïção para o seu estudo monográfico. Boletim da Sociedade Broteriana. II 17, 94 – 146.
[31] Wagner, W. H. Jr., Wagner, F. S., and Taylor, W. C. (1986) Detecting abortive spores in herbarium specimens of sterile hybrids. American Fern Journal 76 (3), 129 – 140.
[32] Wilce, J. H. (1965) Section Complanata of the genus *Lycopodium*. Beihefte zur Nova Hedwigia 19, 1 – 233.
[33] Zar, J. H. (1996) Biostatistical analysis, 3rd ed. New Jersey: Prentice-Hall.

L. G. Quintanilla

Departamento de Biología Vegetal I
Facultad de Biología
Universidad Complutense
28040 Madrid
Spain

E-mail: lugarqui@eucmos.sim.ucm.es

Section Editor: U. Lüttge