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Variability in the Germination of Spores Among and Within Natural Populations of the Endangered Tree Fern *Dicksonia sellowiana* Hook. (Xaxim)

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ABSTRACT

The objective of this study was to analyze the existing variability in the germination of individuals' spores collected from two natural populations of Dicksonia sellowiana in southern Brazil. The largest intrapopulational variation was observed for the spores germination. These results suggested the existence of adaptative strategies that favored the gradual and continuous entrance of new individuals into the gametophytic population. The behavior of the individuals of the species within each population followed a similar pattern of initial development, in spite of conspicuous differences in the population structure. This showed a low differentiation between the populations and the existence of adaptative strategies which were common to both communities.

Key words: Arborescent fern, *Dicksonia sellowiana*, spore germination, intraspecific variability, adaptative strategies.

INTRODUCTION

Xaxim (*Dicksonia sellowiana* Hook. is a tree fern which belongs to the Dicksoniaceae family, and i is widely distributed in Latin America (Sehnem, 1978; Tryon and Tryon, 1982). Its trunk can reach up to 5m in height and 50cm in diameter (Pio Corrêa, 1931) and it is totally surrounded by a wide hem constituted by entangled foreign roots (Fernandes, 1997). This trunk is used as raw material for vases, and the substratum is used in the production of ornamental plants. The intense harvesting of its trunks has caused the inclusion of this species in Appendix II of CITES (MMA, 1998) such as endangered species. In spite of this, few studies have actually been carried out since studies that could provide information about conservation strategies and management in Pteridophytes are more complex and difficult to interpret than those regarding flowering plants. This is due to the fact that these plants have in their cycles two free life phases: a gametophytic phase and a sporophytic phase. These phases are very distinct with regard to their morphology, physiology, and biotic and abiotic relationships. They have different ecological requirements, because a specific environmental situation that is favorable to the gametophytes can be inappropriate for the sporophytes. This environmental situation cannot persist for the

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gametophitic and sporophytic phases since it propitiates the action of selective forces separately in each phase (Dyer, 1979; Soltis and Soltis, 1989; Ranal, 1999).

Many Pteridophytes, such as Xaxim, can present the capacity of formation of spore banks, as well as the dormancy of some spores (Schneller, 1998; Ranal, 1999; Filippini et al., 1999; Rooge et al., 2000) that can guarantee the individuals' germination at different times, making possible the decrease of the competition in the initial phases of development and increasing the chances of reproductive success of parental genotypes. Therefore, the recognition of variations in the behavior patterns between individuals and natural populations is important because it can indicate the existence of adaptative strategies in the initial phase of development and fixation of the gametophytes, which are reflected directly in the ability to colonize, the reproductive success, and the maintenance of the parental genotypes.

Although this kind of information involving the autoecology of tropical Pteridophytes and their intra and interspecific interactions in natural habitats is extremely important, it is practically not available. In this context, the objective of this study was to analyze the existing variability in the germination of individuals' spores collected from two natural populations of Xaxim (*Dicksonia sellowiana*) in southern Brazil.

MATERIAL AND METHODS

Description of the study locations

Two natural populations of Xaxim (*Dicksonia sellowiana*) were located in areas of the Floresta Ombrófila Mista in Santa Catarina State, southern Brazil (Figure 1). The first population was in an area of 10 hectares inside the limits of the National Forest (FLONA) of Três Barras, latitude 26°06'23" south and longitude 59°19'20" west. This area was exploited intensely until 50 years ago, but since then, it has not suffered anthropic action. The mean annual temperature of this area has been 21.4°C and the mean annual rainfall has been 1.170mm (Pandolfo et al., 2002). Xaxim occurs in the area with a density of 114 trunks per hectare (Gomes, 2001). Fifty-two percent of these trunks were in the class of 1.0m up to 4.5m of total height, and about 30% of these (34) were in the reproductive phase and were distributed in the larger height classes which were above 0.80m (Gomes, 2001).

The second natural population that was studied was in a forest fragment with approximately four hectares, on private property in Urupema, latitude 27°57'25" south and longitude 49°53'33" west. The mean annual temperature of this area is 13.38°C and the mean annual rainfall is 1,370mm (Pandolfo et al., 2002). A forest survey carried out in this area revealed the existence of 1925 trunks per hectare, and among them only 247 trunks per hectare had total heights between 1.0m and 4.0m, which represented 13% of the existing total (data not published). The apparent total number of individuals (or clusters composed of several trunks) was 1366 trunks per hectare and about 67% of these were in the reproductive phase (Gomes, 2001).

Germination and viability of the spores

The sporophylls were collected between May and August of 1999, in mature fronds of 30 reproductive *Dicksonia sellowiana* individuals' of the Três Barras (TB) population and 25 individuals of the Urupema (UR) population. After the collection, the protocol developed by Rooge and Randi (1999), Rooge (1999) and Fillipini et al. (1999) was followed.

Spore sterilization was achieved with a 20% (v/v) solution of commercial bleach (2% of active chlorine) and 1 µL of commercial detergent for 20 minutes. Then the spores were washed with sterile distilled water. Samples with 20mg of sterilized spores (approximately 80,000 spores) were placed to germinate inside 50ml conical flasks with 20ml of mineral Dyer medium (Dyer, 1979). Two flasks were used separately by individuals and they were incubated in a culture room at 25ºC and 74% relative humidity under a 16 photoperiod (31.68 µmoles quanta.$m^{-2}.s^{-1}$) provided by cool white fluorescent tubes.

The germination was assessed every four days until the 16th day and two slides were analyzed from each flask (four from each individual) with 100 spores counted on each slide. The percentages of viable, unviable and germinated spores were assessed on each slide with an optical microscope (40x dry objective). The spores were considered as unviable when they were translucent, as viable when they presented yellow coloration, and as germinated when they presented rhizoid emergence.

This experiment used Completely Randomized Design (CRD), with two populations and individuals within populations (30 and 25, respectively) and four repetitions. The experimental data was analyzed with the SAS program (S.A.S, 1986) as a nested design (Sokal and Rohlf, 1979), and the percentage data was transformed into $\arcsin(x+0,5)^{½}$. The germination behaviors of each individual's spores were adjusted by means of regression models. These models were compared through their estimated coefficients and the mean confidence interval of these coefficients (Steel and Torrie, 1980).

Figure 1 - Location of the studied areas in Santa Catarina State. southern Brazil. Núcleo de Pesquisas em Florestas Tropicais / UFSC. Florianópolis. 2004.

The individuals that had presented low germination in relation to the mean were assessed again on the 20th day to increase the precision of the germination regression models. The obtained regression models were used to estimate the minimum time when 50% of germination had been achieved (Laboriau, 1983; Simabukuro et al., 1993).

RESULTS

The spores germination was observed only on the 8th day after the beginning of the experiment. Tables 1 and 2 present the similar mean values of spore germination in the two populations. The final germination percentages evaluated were 89% in Urupema and 87% in Três Barras populations (Tables 1 and 2). In Urupema's population, the germination percentage assessed on the 8th day ranged from 29 to 99%, with a mean of 76% (s.d.=18.8), while in the last evaluation it ranged from 46 to 100%, with a mean of 89% (s.d.=13.8) (Table 1).

Table 1 - Mean values of germination and unviable spores obtained from individuals of a natural population of Xaxim (*Dicksonia sellowiana*) in Urupema – SC, in the 8th and in the 16th day after the beginning of the experiment. Núcleo de Pesquisas em Florestas Tropicais / UFSC, Florianópolis, 2004.

Individuals	**Germinated spores (%)**			**Unviable spores (%)**		
	8th	**16th**	**Final**	**8th**	**16th**	**Final**
P01	74	88	88	9	5	5
P02	49	73	73	16	12	12
P03	86	97	97	3	1	1
P04[1]	29	64	49	22	14	18
P05	93	96	96	1	2	2
P06[1]	71	86	87	2	2	2
P07[1]	70	89	91	3	1	0
P08	87	97	97	1	1	1
P09	89	98	98	0	0	0
P10	87	90	90	5	4	4
P11	94	97	97	1	1	1
P12	70	94	94	1	0	0
P13	45	98	98	4	1	1
P14	78	95	95	2	2	2
P15	99	100	100	0	0	0
P16	41	93	93	10	2	2
P17	69	89	89	9	5	5
P18	93	98	98	2	1	1
P19	96	98	98	0	1	1
P20	82	92	92	1	3	3
P21	95	99	99	2	0	0
P22[1]	70	76	90	2	5	4
P23	83	87	87	3	5	5
P24	59	47	46	1	2	5
P25	84	91	91	7	4	4
Mean	75.7	89.3	89.4	4.3	3.0	3.2
S.D.	18.8	12.4	13.8	5.4	3.5	4.1

[1] Individuals evaluated until the 20th day in bold.

The Três Barras population of individuals presented more contrasting behaviors than those of the Urupema population. The germination percentage assessed on the 8th day ranged from 6 to 98%, with a mean of 75% (s.d.=18.5), while in the last evaluation it ranged from 24 to 100%, with a mean of 88% (s.d.=15.2) (Table 2). Except for a few individuals that presented higher values of unviable spores (P4, P2, P30, P41, P51), most of these individuals had an unviable spore percentage that was smaller than 5% in both populations (Tables 1 and 2).

The analyses of variance of germination and unviable spore percentage of *Dicksonia sellowiana* (Table 3) indicated no differences between the spore's collection areas. The largest variation of germination spores was found within the collection sites and it was highly significant on the evaluation dates of this experiment. This revealed differences among the individuals within the locations assessed.

The obtained model, with a better adjustment to explain the individual germination behavior, was the linear model without constant (y = bx). This model had an adjustment of approximately 90% for most of the individuals (Table 4). The comparison of the regression coefficients through the confidence interval of the average permitted the detection of different behaviors. This suggested a variability among the reproductive individuals with to regard to the spores germination time (Table 4).

Table 2 - Mean values of germination and unviable spores obtained from individuals of a natural population of Xaxim (*Dicksonia sellowiana*) in National Forest (FLONA) of Três Barras – SC, in the 8th and in the 16th day after the beginning of the experiment. Núcleo de Pesquisas em Florestas Tropicais / UFSC, Florianópolis, 2004.

Individuals	Germinated spores (%)			Unviable spores (%)		
	8th	16th	Final	8th	16th	Final
P26	91	98	98	1	1	1
P27	86	94	94	2	1	1
P28	80	94	94	3	1	1
P29	71	97	97	8	1	1
P30[1]	78	81	70	4	9	15
P31[1]	66	80	89	1	3	1
P32[1]	76	89	83	2	0	2
P33[1]	82	95	97	2	0	0
P34[1]	69	86	87	0	1	0
P35[1]	66	91	93	0	3	2
P36[1]	76	84	89	4	5	3
P37[1]	76	88	87	5	4	4
P38	80	92	92	1	3	3
P39	98	97	97	0	1	1
P40[1]	69	89	93	1	1	4
P41[1]	6	33	24	2	30	38
P42	88	98	98	1	0	0
P43	92	96	96	1	1	1
P44	92	100	100	2	0	0
P45	80	90	90	1	4	4
P46[1]	57	94	79	2	1	5
P47[1]	54	89	81	1	4	5
P48	79	86	86	5	6	6
P49	86	96	96	5	1	1
P50	89	97	97	2	1	1
P51[1]	38	65	54	8	12	24
P52[1]	81	90	91	1	2	1
P53[1]	60	86	91	8	6	4
P54	90	92	92	2	3	3
P55	90	97	97	1	0	0
Mean	74.9	88.8	87.7	2.6	3.6	4.5
S.D.	18.5	12.6	15.2	2.4	5.7	8.1

[1] Individuals evaluated until the 20th day in bold.

Table 3 - Summary of the analysis of variance for the germination and unviable spores of Xaxim (*Dicksonia sellowiana*). Data from two natural populations of this species in Santa Catarina State, southern Brazil, evaluated on the 8th and 16th days of the culture. Núcleo de Pesquisas em Florestas Tropicais / UFSC, Florianópolis, 2004.

Sources of Variation	DF	Mean Squares [1]			
		Germinated spores		Unviable spores	
		8th	16th	8th	16th
Population	1	0.029	0.012	0.051	0.006
Indiv./Pop.	53	0.215	0.137	0.029	0.033
Residual	165	0.032	0.019	0.003	0.006
Total	219	0.076	0.047	0.009	0.013
C.V.(%)		16.0	11.0	30.0	48.0

[1] Significant F-test(5%) values are in bold.

This comparison detected a first group with values lower than the mean (9% of the individuals), a second group with values close to the mean (75% of the individuals), and a third group with values higher than the mean (16% of the individuals). The estimated time that 50% of the final germination (t) took place ranged from 7 to 27 days, with a mean of eight days (s.d.=2.7) (Table 4). The calculated values of regression coefficients for each individual ranged from 1.85 to 7.45, with a mean of 6.33 (s.d.=2.19) (Table 4). The individuals behavior is represented in Figure 2. This figure shows an example of three contrasting behaviors in relationship to the spores germination time.

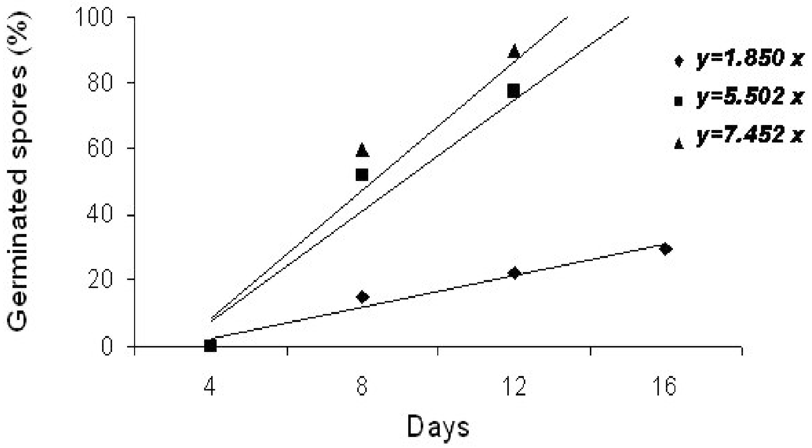

Figure 2 - Three contrasting behaviors with relationship to spore's germination time represented by three individuals of Xaxim (*Dicksonia sellowiana*). Núcleo de Pesquisas em Florestas Tropicais / UFSC. Florianópolis. 2004.

DISCUSSION

The final germination values of the spores obtained for both populations (UR=89% and TB=88%) were similar to the values that were found by Filippini et al. (1999). These authors analyzed spores of Xaxim (*Dicksonia sellowiana*) collected in the "Reserva Biologica de Caraguatá – SC" and obtained approximately 90% of germination after 15 days of spore storage. Also in Santa Catarina State, Rooge et al. (2000) obtained approximately 90% of germination for cryopreserved spores of this species, while for fresh spores the germination mean was 80% in spores evaluated on the 30th day of culture. Other tree ferns such as *Alsophila firma*, *Lophosoria quadripinnata* and *Sphaeropteris horrida*, collected in a forest in Mexico, had inferior values of spore germination and these values, according to the species, ranged from 16 to 86% (Bernabe et al. 1999). With relationship to the germination time, the mean time of eight days found for 50% of final germination was five days superior to the germination time obtained by Simabukuro et al. (1993) in *Cyathea delgadii*. For other creeping Pteridophytes, the same authors observed values of germination for a period of 2 to 9 days.

Tables 1, 2 and 3 present the differences among the individuals in each population. They indicate the existence of a larger intrapopulational variation for germination percentage as well as for unviable spore percentage in the analyzed populations. These results were in agreement with electrophoretic data of homosporous Pteridophytes compiled by Soltis and Soltis (1989), which revealed a lower interpopulational divergence caused by a high gene flow through spores due to their long dispersion distance. Kiss and Kiss (1998) in a study with *Schizaea pusilla* also found larger differences in the germination between individuals within populations and lower differences between geographically-separate populations. These results referred to the spores' storage conditions as well as to their culture. However, Pteridophytes studies with this approach are still scarce. In Angiosperms, studies have registered the occurrence of differentiated answers to germination and viability of seeds among individuals within populations (Cony and Trione, 1998; Qaderi and Cavers, 2000; Peters et al., 2000; El-Siddig et al., 2000).

Table 4 - Regression models and final germination values for Xaxim (*Dicksonia sellowiana*) individuals' behavior in two natural populations in Santa Catarina State, southern Brazil. Núcleo de Pesquisas em Florestas Tropicais / UFSC, Florianópolis, 2004.

Individuals[1]	Regression models	r^2 (%)[2]	Final germination (%)	t [3] (days)	Homogeneous groups[4]
UR- P01	y=6.5515x	91.72	88.16	8	M
UR- P02	y=5.1071x	90.13	72.57	10	I
UR- P03	y=7.0944x	91.86	97.50	7	S
UR- P04	y=4.0981x	88.44	48.85	12	I
UR- P05	y=6.9921x	90.26	95.87	7	S
UR- P06	y=6.0835x	89.58	87.32	8	M
UR- P07	y=6.3829x	91.38	90.53	8	M
UR- P08	y=7.0469x	91.54	96.80	7	S
UR- P09	y=7.1235x	91.44	98.25	7	S
UR- P10	y=6.8694x	90.28	90.47	7	S
UR- P11	y=7.2315x	90.66	97.45	7	S
UR- P12	y=6.4388x	91.27	93.77	8	M
UR- P13	y=5.8915x	94.57	97.68	8	I
UR- P14	y=6.7371x	91.24	94.83	7	S
UR- P15	y=7.4523x	90.13	100.00	7	S
UR- P16	y=5.5023x	87.43	93.32	9	I
UR- P17	y=5.6233x	91.13	88.74	9	I
UR- P18	y=7.2129x	90.95	97.53	7	S
UR- P19	y=7.3556x	90.39	98.27	7	S
UR- P20	y=6.6825x	91.53	91.90	7	S
UR- P21	y=7.2846x	90.71	98.80	7	S
UR- P22	y=5.6856x	89.36	90.25	9	I
UR- P23	y=6.5840x	90.28	87.38	8	M
UR- P24	y=3.8054x	83.25	46.30	13	I
UR- P25	y=6.4152x	90.64	91.25	8	M
TB- P26	y=7.1435x	91.12	97.53	7	S
TB- P27	y=6.8481x	90.93	93.62	7	S
TB- P28	y=6.5560x	92.3	93.96	8	M
TB- P29	y=6.7615x	94.27	97.10	7	S
TB- P30	y=5.9350x	87.86	70.48	8	I
TB- P31	y=5.7742x	90.43	89.18	9	I
TB- P32	y=6.3029x	90.33	83.46	8	M
TB- P33	y=6.8494x	92.00	96.75	7	S
TB- P34	y=5.7704x	86.65	87.03	9	I
TB- P35	y=6.2608x	93.01	92.55	8	M
TB- P36	y=6.1781x	90.42	89.02	8	M
TB- P37	y=6.4206x	92.18	86.87	8	M
TB- P38	y=6.6956x	91.30	91.64	7	S
TB- P39	y=7.1867x	89.72	96.97	7	S
TB- P40	y=6.0352x	87.27	93.10	8	I
TB- P41	y=1.8498x	81.73	24.23	27	I
TB- P42	y=7.0313x	91.76	97.56	7	S
TB- P43	y=7.1794x	90.69	96.11	7	S
TB- P44	y=7.3263x	91.52	99.75	7	S

(Cont.)

(Cont. Table 4)

TB- P45	y=6.4417x	91.77	89.88	8	M
TB- P46	y=5.7369x	91.58	94.47	9	I
TB- P47	y=5.3606x	87.01	88.61	9	I
TB- P48	y=6.3767x	90.58	86.03	8	M
TB- P49	y=6.8996x	91.97	96.29	7	S
TB- P50	y=7.1021x	91.57	97.06	7	S
TB- P51	y=4.4171x	91.74	53.56	11	I
TB- P52	y=6.4725x	91.07	91.06	8	M
TB- P53	y=6.1717x	92.19	90.97	8	M
TB- P54	y=6.8933x	90.18	92.34	7	S
TB- P55	y=7.1315x	91.09	96.73	7	S
Mean	y=6.3338x	90.48	88.87	8.29	
S.D.	1.0071			28.8	

[1] UR = Urupema and TB = Três Barras; [2] Determination coefficient; [3] Estimated times in days for 50% of final germination: [4] Homogeneous groups for the mean Confidence Interval: I=lower than the mean; M=mean; S= larger than the mean.

The behavior differences with relationship to the final germination and germination time existing among individuals of analyzed Xaxim populations suggested the operation of adaptative strategies for this species. These strategies allowed the species to get round a momentarily unfavorable situation, e.g. the intra and interspecific competition for water and light and/or microclimatic changes. These variations were observed among individuals in dense populations such as that of Urupema (1,366 individuals per hectare) and in less dense populations like that of Três Barras (114 individuals per hectare).

Another important fact is that several Pteridophytes and Angiosperms present a leptokurtic distribution of their units of dispersion (Raynor, 1976; Conant, 1978), with a larger density near the reproductive plant. This provides an increase in the intraspecific competition in these initial phases of development.

Since this initial phase of development constitutes a crucial stage for the individual's survival, plants that possess the strategy of forming propagule banks with seeds or spores can develop adaptative strategies such as spore dormancy or individual asynchrony germination. Therefore, the variations in the germination time and the presence of spore dormancy in some individuals suggest the existence of mechanisms that favor the gradual entrance and exit of individuals into/out of the bank of spores, allowing a gradual and continuous supply for the gametophyte bank. This can happen even in periods that spore dispersion does not exist, in a similar way to the results obtained by Kageyama and Viana (1989) for banks of seeds.

CONCLUSIONS

Our results revealed the existence of a large intrapopulational variation for the spore germination and viability in the analyzed populations. The Xaxim individuals behavior within each population maintained a similar pattern of initial development speed in spite of the conspicuous differences in the population structure. This suggested a low differentiation between the populations and the existence of adaptative strategies common to both populations in relation to the studied characteristics. Results suggested the existence of adaptative strategies that favored the new individuals gradual and continuous entrance into the gametophytic population, as well as a gradual and continuous sporophyte formation.

However, the results obtained in this study should be seen as preliminary, because besides the individuals genotypes, maternal effects, physiologic state of the reproductive plant and the microhabitats should also be considered. Factors such as these could also influence the reproductive vigor in the initial stages of development. Precise estimations of genetic variation require further studies based on progeny tests. Moreover, data provided by molecular markers could supply a great amount of information about the genetic variability in natural populations and especially about the reproductive system.

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RESUMO

O Xaxim (*Dicksonia sellowiana* Hook. (Dicksoniaceae) é uma samambaia arborescente que se encontra principalmente sob o domínio da Floresta Ombrófila Mista. A exploração intensiva de seus indivíduos para retirada do caule, empregado na fabricação de vasos e substrato para o cultivo de plantas ornamentais, e a crescente antropização das áreas florestadas, tem degradado suas populações naturais. Este estudo teve como objetivo analisar a variabilidade existente na germinação de esporos de Xaxim. Os esporos foram coletados das frondes de indivíduos de Xaxim procedentes de duas populações naturais do Sul do Brasil. Foi observada uma maior variação intrapopulacional para a germinação e porcentagem de esporos inviáveis. Estes resultados sugerem a existência de estratégias adaptativas que favorecem a entrada gradual e contínua de novos indivíduos na população de gametófitos. O comportamento dos indivíduos da espécie dentro de cada população segue padrão semelhante de velocidade de desenvolvimento inicial, a despeito das diferenças conspícuas na estrutura populacional, sugerindo baixa diferenciação entre as populações e a existência de estratégias adaptativas comuns a ambas comunidades, para as características estudadas.

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