Revta brasil. Bot., São Paulo, V.22, n.3, p.385-390, dez. 1999

The survival and establishment potential of spores of *Cyathea delgadii* Sternb. in soils from Itirapina and Moji Guaçu (SP), Brazil

THAIS DE BEAUCLAIR GUIMARÃES[1,2] and GIL M. FELIPPE[1,3]

(recebido em 11/09/98; aceito em 16/06/99)

ABSTRACT - (The survival and establishment potential of spores of *Cyathea delgadii* Sternb. in soils from Itirapina and Moji Guaçu (SP), Brazil). Germination of spores of *Cyathea delgadii* was carried out in soils collected in March 1997 (rainy season) at three depths (0-5, 5-10 and 10-15 cm) from the cerrado, open-cerrado, gallery forest and marsh at Moji Guaçu and cerradão, cerrado and gallery forest at Pedregulho, in Itirapina, both in the state of São Paulo, Brazil. Viability of spores mixed with soil and buried in the cerrado at the Reserva in Moji Guaçu were conducted for up to 10 months. The spores germinated in all soils. Germination in the soils was significantly lower than in distilled water. Germination was higher in soils from the gallery forest and cerrado than from open cerrado, cerradão and marsh. The germination was the same in the soil samples from the three depths of gallery forest, cerrado and cerradão. Spores of *C. delgadii* maintained viability longer when buried in cerrado soil than when dry stored at 4°C. After 10 months germination was 50.5% in buried spores against 3.8% in spores of the same age and harvest, stored at 4°C.

RESUMO - (Sobrevivência e estabelecimento potencial de esporos de *Cyathea delgadii* Sternb. em solos de Itirapina e Moji Guaçu (SP), Brasil). Foi estudada a germinação de esporos de *Cyathea delgadii* em solos, coletados em março de 1997 (estação chuvosa) em três profundidades (0-5, 5-10 e 10-15cm), no cerrado, cerrado aberto, mata ciliar e brejo na Reserva Biológica e Estação Experimental de Moji Guaçu, em Moji Guaçu e no cerradão, cerrado e mata ciliar no Pedregulho, Estação Experimental de Itirapina, em Itirapina, estado de São Paulo, Brasil. A viabilidade foi estudada em esporos de *C. delgadii* armazenados, misturados com solo, no cerrado da Reserva em Moji Guaçu por 10 meses. Os esporos germinaram em todos os solos. A germinação em solos foi significativamente menor do que em água destilada, mas foi mais alta em solos da mata ciliar e cerrado do que nos solos de cerradão, cerrado aberto e brejo. A germinação foi semelhante para as três camadas de solo no caso da mata ciliar, cerrado e cerradão. A viabilidade foi mantida por mais tempo quando os esporos de *C. delgadii* foram armazenados em solo. Depois de 10 meses de armazenamento, a germinação foi de 50,5% em solo e de 3,8% para esporos, da mesma idade e coleta, armazenados a 4°C.

Key words - Fern spores, soil storage, cerrado, gallery forest, marsh

Introduction

The natural cover of about 25% of the land area of Brazil was a savannah vegetation given the generic name of cerrado, but a great part of this vegetation has been destroyed for agricultural purposes. Much of the cerrado "sensu lato" is subject to prolonged and often severe winter drought lasting for up to four months of the year. Cerrado vegetation is floristically and physiognomically diverse; the vegetation is predominantly sclerophyllous. Trees and shrubs show a characteristic tortuous and gnarled appearance, especially where they have been exposed to fire. Many areas are subject to regular burning as part of their management for cattle rearing (Eiten 1972, Coutinho et al. 1982).

When the cerrado presents a canopy cover of ca. 50% it is called cerradão and when presenting only scattered trees and shrubs to give a canopy cover of less than 2% it is called open-cerrado. In the cerrado region the presence of typical cerrado species in the vegetation bordering the rivers, the gallery forest, is very rare (Eiten 1972, Felippe & Dale 1990). Cerrado soil is very poor in nutrients like P, N, S, Ca, Mo and B but the aluminium availability is very high (Arens 1958, McClung et al. 1958, Alvim et al. 1968, Reatto et al. 1998).

The cerrado is very rich in species of angiosperms (Furley & Ratter 1988). In relation to pteridophytes, about 40 species were found in the cerrado, cerradão, marsh and gallery forest of a preserved area in Moji Guaçu, state of São Paulo (Esteves & Felippe 1985, Simabukuro et al. 1994). Of these, *Cyathea delgadii* is a very frequent arboreal fern species in the gallery forests in the cerrado region but it is absent in the cerrado (Simabukuro et al. 1994, 1998b). However *C. delgadii* spores could be retrieved in the spore rain and in the spore soil bank in the cerrado (open cerrado, cerrado and cerradão) as well as in gallery forests and marsh in Moji Guaçu and Itirapina (Simabukuro et al. 1996, 1998b, c, 1999), and it was

1. Instituto de Botânica, Caixa Postal 4005, 01061-970 São Paulo, SP, Brazil.
2. PIBIC, CNPq, Brazil.
3. Research-fellow CNPq, Brazil.

not sure whether this species could form a persistent spore bank as the species produces spores all year round. It seems that spores of pteridophytes can survive for long periods in hydrated state as they are viable in the soil many months after dispersal (Lindsay & Dyer 1990). As *C. delgadii* produces spores all year round we do not know if the spores found in the spore banks mentioned here have recently been introduced into the bank or not; also we do not know if they are viable in the soil or if they remain viable in a hydrated state. Also, unlike seed plants, homosporous ferns have two very different free-living stages (gametophyte and sporophyte), both of which have to succeed at the same site, and the dormant dispersal unit is not a relatively large seed containing an embryo within maternal tissue as in the flowering plants, but a single-celled spore (Dyer 1994). One of our objectives was to determine if the spore could germinate in the soil from four habitats in Moji Guaçu (cerrado, open cerrado, gallery forest and marsh) and from three in Itirapina (cerrado, cerradão and gallery forest). A second objective of this work was to find out if spores of *Cyathea delgadii* would remain viable when stored in cerrado soil, which would indicate if these spores could form a lasting spore bank.

Material and methods

Soil sampling - The soil samples used for testing the germination were collected in March 1997 (rainy season) with a 56.70 cm^3 metal cylinder with 5 cm bore diameter (Endecott - EFL1 mk3). Vertical cores were taken and subdivided into samples at 0-5, 5-10 and 10-15 cm depth. The samples of soils were collected from two different cerrado areas in the state of São Paulo, Brazil. The soil samples were collected from four sites in the Reserva Biológica e Estação Experimental de Moji Guaçu, located in Moji Guaçu (22°18'S and 47°11'W): gallery forest, marsh, cerrado and open cerrado and from three sites in Pedregulho, Estação Experimental de Itirapina (22°51'S and 47°52'W): gallery forest, cerrado and cerradão. The chemical and physical analyses of the soil samples from Moji Guaçu are presented in Sassaki et al. (1999) and from Itirapina in Simabukuro et al. (1999).

Spores - The spores of *Cyathea delgadii* Sternb. were collected from a population in the Reserva do Parque Estadual das Fontes do Ipiranga of the Instituto de Botânica, in the city of São Paulo (23°39'S and 46°37'W), SP, Brazil. Fertile leaves were placed with the sporangia facing down on absorbent paper at 25°C. After liberation the mature dry spores were separated from debris and stored dry in closed bottles in darkness at 4°C.

General procedures for germination studies - For the control, germination was carried out in distilled water. Germination of spores previously treated with 0.5% calcium hypochlorite for two minutes (Simabukuro et al. 1998a) was carried out in growth cabinets at 25 (±1°C) in continuous white fluorescent light at 437 $\mu W.cm^{-2}.s^{-1}$ at Petri dish level (Ruggiero & Zaidan 1997). Treatment with calcium hypochorite does not reduce *C. delgadii* spores viability (Simabukuro et al. 1998a). Germination was recorded as the emergence of the rhizoid, verified under an optical microscope (100x) by day 7 from sowing (Randi & Felippe 1988a). Three conical flasks were used per treatment and two microscope slides were prepared from each flask; a total of 100 spores were counted on each slide, giving a total of 600 spores for each treatment.

Germination on soil samples - When soil samples were used as substrates, they were autoclaved at 120°C and 1.2 atm during 40 minutes to kill all fern spores. No gametophytes appeared on these soils when they were put wet in the growth cabinets. A layer of 5 mm of autoclaved washed sand was first placed in an autoclaved Petri dish of 9 cm diameter and then a layer of 5 mm soil sample (Dyer 1995). The dishes were watered with autoclaved distilled water and placed in growth cabinets at 25°C under continuous white fluorescent light for seven days and then any growing fungi present were removed. The Petri dishes were then autoclaved and 5 ml Nystatin (2,500 $units.ml^{-1}$) added before the spores (previously treated with 0.5% calcium hypochlorite) were sown over the wet soil samples, because the spores of *C. delgadii* germinate only in light conditions (Simabukuro et al. 1993). The dishes were closed and transferred to growth cabinets at 25°C in continuous white fluorescent light. The dishes were checked seven, 14 and 21 days from sowing. Three Petri dishes were used per treatment and two microscope slides were prepared scraping the soil surface from each dish; a total of 100 spores were then counted in each slide, giving a total of 600 spores for each treatment and in the control (in distilled water) on days seven, 14 and 21.

Storage of spores in cerrado soil in the cerrado - In one experiment, spores of *C. delgadii* were mixed with soil from the cerrado of the Reserva Biológica e Estação Experimental de Moji Guaçu and stored in the same cerrado where the soil sample came from. Spores stored in closed bottles at 4°C for three months and showing 80% germination were used (germination tests done as shown for the control treatment). The spores were then divided in two lots: one remained stored in closed bottles at 4°C in the darkness (control treatment) and the second lot was used for storage in the soil. The soil from the superficial layer (0-5 cm) from the cerrado was autoclaved (120°C and 1.2 atm during 40 minutes). Small bags (7 x 15 cm) were prepared with nylon gauze with a 20 µm diameter pore (the trilete spores of *C. delgadii* measure 47.7 µm according to Simabukuro et al. 1998b). Each bag received 50 g of the soil sample mixed with 200 mg of *C. delgadii* spores; the bags were then buried at a depth of 10 to 15 cm deep beginning 4th of April 1997. The bags were removed at the end of one, four, seven and 10 months. Before the germination tests, the bags were opened and the spores were separated from the soil according to the method described by Pires et al. (1998). The 50 g of soil were thoroughly mixed with 200 ml of a solution of Tween 20 (0.223 mg of Tween 20 diluted in 100 ml of distilled water). Wet sieving was carried out with tap water (a total of 20 liters was needed) with 125 and 20 µm pore sieves mounted in a shaker (test sieve shaker EFL1 mk3, Endecott). The filtration lasted for one hour: a sequence of 10 min jet of water and 5 min pause. The material retained in the two sieves were mixed and centrifuged for 10 min at 482 *x*g. The spores thus separated were treated with 0.5% calcium hypochlorite for two minutes before germination in 25 ml of distilled water in three conical flasks as already described. The tests were always planned for comparing germination of spores stored in soil against spores kept at 4°C.

Statistical analysis - The germination percentage (at day 7, 14 and 21 from sowing) was subjected to angular transformation and treatments were compared by analysis of variance (Snedecor

1962). Germination values for the two types of storage (at 4°C and in the soil) were analysed by the Student's test and ANOVA (Snedecor 1962).

Results and Discussion

Cerrado soils are generally acid and minerals from the aluminium silicate clays are the main source of acidity, liberating aluminium ions according to the pH (Goodland 1971). The saturation of aluminium is higher than 50% in cerrado soils (Reatto et al. 1998). When in extreme acid conditions, the aluminium appears in solution as exchangeable cation (Al^{3+}) and this has deleterious effects upon plants (Raij 1983). In the case of the soil samples from the Reserva Biológica e Estação Experimental de Moji Guaçu (Sassaki et al. 1999) all of them, independent of the kind of vegetation (cerrado, open cerrado, gallery forest or marsh) were very acid, with very high saturation of aluminium and low values of base saturation. In Pedregulho, Estação Experimental de Itirapina (Simabukuro et al. 1999) the saturation of aluminium was also high, being only lower in the superficial layers from the cerradão and gallery forest. The high acidity and high aluminium concentration could be toxic to spores affecting their viability. However, these characteristics of the soil samples as well as the soil texture being either medium sandy or clayey (Sassaki et al. 1999, Simabukuro et al. 1999) did not inhibit the germination of *C. delgadii* spores although germination was low in some samples.

Germination of *C. delgadii* was delayed when the spores were sown on the soil samples from Reserva Biológica e Estação Experimental de Moji Guaçu (table 1) and from Pedregulho, Estação Experimental de Itirapina (table 2). Maximum germination was reached by day 14 from sowing (no significant differences between days 14 and 21). Germination on day 14 and day 21 in all soil samples was statistically lower than in distilled water (control). In Moji Guaçu germination was higher in all soil samples from the gallery forest than from soil samples from cerrado, open cerrado and marsh (table 1). In Itirapina germination was lower in soil samples from the cerradão than in the gallery forest and cerrado (table 2). The germination was the same in soil samples in the three layers in each habitat considered: gallery forest, marsh, cerrado and cerradão (tables 1 and 2).

Table 1. Germination of spores of *Cyathea delgadii* in samples of soils of cerrado, open cerrado, gallery forest and marsh collected in the Reserva Biológica e Estação Experimental de Moji Guaçu at three depths. Germination was checked 7, 14 and 21 days from the beginning of the experiment. The spores presented a germination of 72.5% on day 7 in distilled water.

Soil samples depth (cm)	Germination (%)		
	day 7	day 14	day 21
Cerrado			
0 - 5	23.8cA	65.3aB	63.3bB
5 - 10	26.0bA	66.3aB	63.6bB
10 - 15	24.0cA	63.1aB	63.8bB
Open cerrado			
0 - 5	23.6cA	62.6abB	63.6bB
5 - 10	23.6cA	63.8aB	63.3bB
10 - 15	22.5cA	62.0bB	62.5bB
Gallery forest			
0 - 5	27.6aA	66.6aB	67.5aB
5 - 10	27.6aA	65.3aB	67.3aB
10 - 15	27.0aA	64.6aB	65.1aB
Marsh			
0 - 5	22.3cA	62.3abB	62.5bB
5 - 10	23.0cA	63.1aB	62.6bB
10 - 15	22.0cA	62.0bB	63.1bB

Different letters mean significant differences at 5% level. Capital letters compare values in each row. Small letters compare values in each column. All values are statistically different from the control. C.V. = 6.5% (day 21).

Table 2. Germination of spores of *Cyathea delgadii* in samples of soils of cerradão, cerrado and gallery forest collected in Pedregulho, Estação Experimental de Itirapina, at three depths. Germination was checked 7, 14 and 21 days from the beginning of the experiment. The spores presented a germination of 71.3% on day 7 in distilled water.

Soil samples depth (cm)	Germination (%)		
	day 7	day 14	day 21
Cerradão			
0 - 5	20.3bA	62.8bB	62.8bB
5 - 10	20.0bA	63.0bB	62.6bB
10 - 15	20.3bA	62.8bB	62.7bB
Cerrado			
0 - 5	22.3aA	65.3aB	65.5aB
5 - 10	22.1aA	64.6aB	64.3aB
10 - 15	21.1bA	64.5aB	64.0aB
Gallery forest			
0 - 5	22.5aA	64.8aB	66.1aB
5 - 10	22.8aA	64.8aB	66.3aB
10 - 15	23.1aA	65.8aB	65.1aB

Different letters mean significant differences at 5% level. Capital letters compare values in each row. Small letters compare values in each column. All values were statistically different from the control. C.V. = 5.2%.

Thus, spores of *C. delgadii* can germinate on soils from the cerrado (open cerrado, cerrado and cerradão) and not only on the gallery forest and marsh soils. As mentioned before, the cerrado is subject to prolonged and often severe winter drought lasting for up to four months of the year (Eiten 1972) and this might be the reason for the absence of specimens of *C. delgadii* in the cerrado. *C. delgadii* produces viable spores all the year round (Randi & Felippe 1988b) and they can germinate during the rainy season but the gametophytes and young sporophytes will not survive the following dry season in the cerrado (but will survive in the gallery forest and marsh).

Viability of most pteridophyte spores was believed to be retained when stored in very dry and cold conditions (Dyer 1979, Scheuerlein et al. 1989, Lindsay et al. 1992). In our laboratory, the spores of *C. delgadii* are usually stored (after being air dried) in the dark in closed bottles at 4°C as they lose their viability very rapidly when stored in dry conditions at 25°C (Marcondes-Ferreira & Felippe 1984). The batch of spores used in this experiment presented 80% germination immediately after collection but germination was as low as 23.7% after four months storage in dry conditions at 4°C; by the end of the experiment germination was only 3.8%.

The results of the germination of *C. delgadii* spores stored in cerrado soil (Sassaki et al. 1999) in the cerrado of Moji Guaçu are shown in table 3. Storage in soil maintained spore viability and very high germination for at least four months (values statistically similar to the value of 80% when the batch of spores was newly collected) and was reduced to 50% by the end of the experiment (10 months storage in the soil). Thus it seems that for this species storage in the soil, in natural wet conditions and temperatures over 20°C for some time (figure 1) are better than at 4°C in dry conditions. Whittier (1990) showed that spores of *Psilotum nudum* (L.) P. Beauv. survived longer if stored on mineral agar. Lindsay et al. (1992) have clearly demonstrated that spores of *Athyrium filix-femina* (L.) Roth, *Blechnum spicant* (L.) Roth, *Polystichum setiferum* (Forsskal) Woynar and *Phyllitis scolopendrium* (L.) Newm. stored for two years and of *Todea barbara* (L.) T. Moore for five months on agar (fully hydrated) in the dark at 20°C showed complete ability to germinate; the spores of the same species stored in dry conditions at 20°C lost their viability.

Table 3. Germination of spores of *Cyathea delgadii* stored in closed bottles at 4ºC and in cerrado soil for 1, 4, 7 and 10 months in the cerrado at Reserva Biológica e Estação Experimental de Moji Guaçu. The spores used had been stored at 4°C for three months before transfer to soil storage. Germination checked on day 7 from the beginning of the germination experiment.

Storage at 4°C (months)	Germination %	Storage in cerrado soil (months)	Germination %
3	80.0a	0	80.0a
4	23.7bA	1	72.2aB
7	19.2bcA	4	71.7aB
10	12.2cA	7	51.3bB
13	3.8dA	10	50.5bB

Different letters mean significant differences at 5% level. Capital letters compare values in each row. Small letters compare values in each column.

In temperate regions, the presence of a spore bank just before the main spore release season, demonstrates the ability of spores of at least some

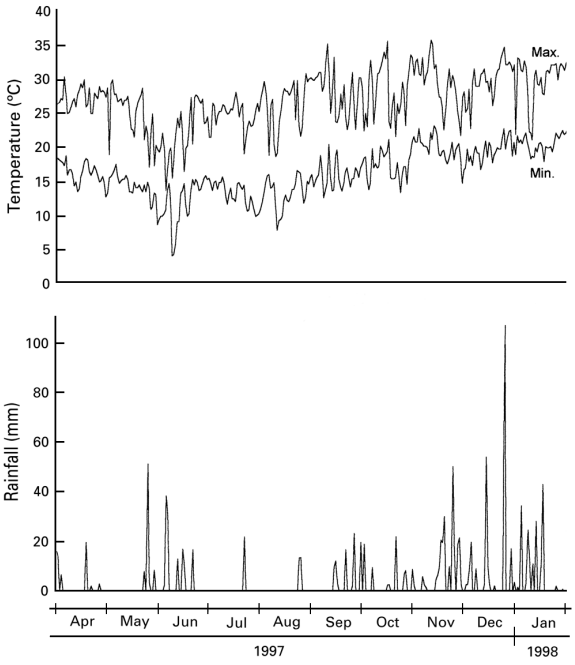

Figure 1. Temperature (ºC) and rainfall (mm) between April 1997 and January 1998. Reserva Biológica e Estação Experimental de Moji Guaçu, São Paulo, Brazil.

species to survive for nearly a year in the soil (Dyer & Lindsay 1992). As *C. delgadii*, at least in São Paulo, produces spores all the year round (Randi & Felippe 1988b), it is impossible to say how long spores of this species remain alive in soil spore banks. With the approach presented here we can say that *C. delgadii* spores have the potential to contribute to the formation of lasting fern spore bank in soils of the state of São Paulo. The demonstration of viable spores in several other persistent soil banks creates possibilities for new approaches to conservation, including the restoration of populations by promoting germination of spores stored in soil (Dyer & Lindsay 1996). The present results plus the findings of spores of this species in samples of soils in two different cerrado regions in the state of São Paulo (Simabukuro et al. 1998c, 1999) show the potential of storage in soils (or soil banks) to recover viable spores of *Cyathea delgadii*. But it must be remembered that much more information on the ecology of gametophytes and sporeling establishment is also needed (Dyer & Lindsay 1996).

Acknowledgements - Thanks are due for technical help from Dra Regina M. Sassaki. Thanks are also due to Dr Adrian F. Dyer (University of Edinburgh) for very helpful discussions and for revising the English in a draft version.

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