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Potential for Spore Germination, Sporophyte Formation and Growth of Young Sporophytes of Four Fern Species from the Atlantic Forest (Brazil)

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ABSTRACT.—The aim of this paper was to study spore germination and growth of young sporophytes of four ferns from Atlantic Forest (Brazil). *Blechnum brasiliense* is a subarborescent fern, *Saccoloma inaequale* is an herbaceous species, while *Cyathea corcovadensis* and *Cyathea delgadii* are tree ferns. The percentages of spore germination under experimental conditions were 65.3 ± 3.6% (*Blechnum brasiliense*), 31.7 ± 2.5% (*C. corcovadensis*), 77.5 ± 5.2% (*C. delgadii*) and 60.5 ± 2.0 (*Saccoloma inaequale*). The highest percentage of sporophytes produced from spore germination was 91.5 ± 2.5% for *B. brasiliense* and the lowest was 35.8 ± 16.2% for *C. corcovadensis*. The relative growth rate (RGR) ranged from 0.17 ± 0.02 cm cm^{-1} $month^{-1}$ (*C. corcovadensis*) to 0.25 ± 0.06 cm cm^{-1} $month^{-1}$ (*S. inaequale*). Spores stored at 7 ± 1 °C for 6 to 8 months were able to germinate and to produce mature gametophyte and sporophytes. The herbaceous species showed greater RGR than the tree species.

KEY WORDS.—germination, relative growth, spore, storage, viability

The world fern flora contains 9,000 to 12,000 species; about 3,250 are found in the American continent (Tryon and Tryon, 1982; Windisch, 2002) and 1,200 to 1,300 species are found in Brazil (Prado, 1997). Sehnem (1977) listed 493 species in the South of Brazil, the majority native to the Atlantic Forest.

According to "Conservation International" the Atlantic Forest is the third diversity "hotspot" in the world and only 8% of its original area remains preserved. In the last few decades, several ferns with ornamental or medicinal proprieties have been indiscriminately exploited and several of them are considered to be endangered species. Examples include some tree ferns belonging to the Cyatheaceae and Dicksoniaceae ("xaxins" or "samambaiaçus"), which have been exploited for commercial purposes, especially in the South of Brazil (Windisch, 2002). *Dicksonia sellowiana* Hook. (Dicksoniaceae) is an endangered tree fern in Brazil due to the extensive harvesting in its habitat (Sehnem, 1978, IBAMA, 1997) and *Rumohra adiantiformis* (Forst.) Ching., (Dryopteridaceae) *Sphaeropteris gardneri* (Hook.) R.M. Tryon, *Cyathea atrovirens* (Langsd. & Fisch.) Domin, *Alsophila setosa* Kaulf, some species of *Adiantum, Asplenium, Blechnum, Selaginella, Marsilea, Adiantopsis* and *Lycopodium* are extracted for ornamental arrangements (Milton & Moll 1988; Windisch, 2002).

According to Pence (2000), the *ex situ* preservation of spores, particularly of rare species from threatened habitats, can be an important supplement to the maintenance of pteridophyte species in the wild. The conditions under which spores are stored have a notable impact on their viability. Generally, to avoid

deterioration, they are stored in dry, ambient or low temperatures, although in some cases this has resulted in loss of viability (Beri and Bir, 1993; Camloh, 1999).

The aim of the present study was to verify the potential of germination of spores stored under refrigeration, the percentage of sporophyte formation after *in vitro* germination, and the relative growth rate (RGR) of young sporophytes cultivated in a growth room of four terrestrial species native from the Atlantic Forest: *Blechnum brasiliense* Desv. (Blechnaceae) an herbaceous species, *Cyathea corcovadensis* (Raddi) Domin and *Cyathea delgadii* Sternb. (Cyatheaceae) both tree ferns and *Saccoloma inaequale* (Kze.) Mett. (Dennstaedtiaceae) an herbaceous fern. These species, with exception of *Saccoloma inaequale,* are routinely used in Brazil as ornamental plants and are exploited indiscriminately from their habitats. *Saccoloma inaequale* is a rare species, which also has potential as ornamental plant. Such information will be useful for management and conservation programs in the future.

Materials and Methods

Blechnum brasiliense and *Cyathea corcovadensis* sporophylls were harvested from several plants on January 12th of 2003; sporophylls of *Cyathea delgadii* were harvested on April 25th of 2002 and of *Saccoloma inaequale* on March 29th of 2003. The collections were carried out in Natural City Park São Francisco de Assis, situated in Blumenau, Santa Catarina State, South Brazil, at 26°55′15″ S and 49°05′30″ W. The study site is a 22.29 ha natural Atlantic forest fragment.

Sporophylls were air-dried in an oven at 30°C for three days on filter paper in order to induce dehiscence. The spores were removed and separated from debris by filtering through lens paper, and stored in glass jars under refrigeration at 7 ± 1°C. Spores of *Blechnum brasiliense* and *Cyathea corcovadensis* were kept under refrigeration for eight months before the germination test; spores of *Cyathea delgadii* and *Saccoloma inaequale* were kept under refrigeration for six months before the germination test. Spores were surface sterilized using a 10% (v/v) solution of commercial bleach (2% of active chlorine) for 20 min. before filtering through sterile filter paper and washing several times in sterile distilled water. Spores of each species were sown in four conical flasks containing 20 mL of Mohr's mineral solution as modified by Dyer (1979) supplemented with Benomyl 0.01%. About 10 mg of spores were inoculated in each flask, which was plugged with two layers of autoclaved transparent commercial polypropylene film (7 × 7 cm) fixed with a rubber band.

All tests were done in a completely randomized design. All procedures were carried out in a laminar hood. Flasks were kept in a growth chamber (25 ± 2°C) under a 16-hour photoperiod and photon flux density of 30 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes. The irradiance was analyzed by a quantameter LICOR 250, of PAR sensor (400 to 700 nm). The percentage of abnormal spores and germination were scored daily or every two days. One

TABLE 1. Analysis of substrate composition (typic hapludult soil with the addition of thermophilic compost in the proportion of 3:1).

Soil composition	
pH	5.2
P (ppm)	+50
K (ppm)	450
Organic matter %	0.9
Al (cmolc/l)	traces
Ca (cmolc/l)	4.8
H^+, Al (cmolc/l)	3.90
N Total %	0.26
CEC (cmolc/l)	12.88

slide from each flask was prepared, with 100 spores counted on each slide. Germinated and abnormal spores were recorded in optical microscopy (40×). Spores were considered to be abnormal when they were totally empty or when they presented a few storage grains between empty spaces. The mean germination time was calculated for each replication per treatment according to the equation:

$$\bar{t} = \sum_{i=1}^{k} n_i t_i \Big/ \sum_{i=1}^{k} n_i$$

where t_i is the time in days starting from day 0 and n_i is the number of spores completing germination on day t_i (Labouriau, 1983; Santana and Ranal, 2004). After one month of cultivation in mineral solution, young filamentous gametophytes were transferred to trays containing sterilized typic hapludult soil (3 parts) with addition of thermophilic organic compost (1 part). The thermophilic compost was obtained from decomposition of vegetable and fruit wastes at the University of Santa Catarina. The substrate (soil with thermophilic compost) analysis was carried out in CIDASC (Companhia Integrada de Desenvolvimento Agrícola de Santa Catarina). The substrate presented a low pH and high levels of P, K, Ca and N (Table 1). The trays were covered with transparent film to avoid excessive water evaporation and plant dehydration. Substrate sterilization was carried out in a high power microwave oven for 20 minutes to avoid contamination with other ferns from the soil spore bank. To analyze the percentage of sporophyte originated from mature gametophytes and to obtain plants that were used later in growth analysis, 200 mature cordate gametophytes were transferred to four trays (50 gametophytes in each tray) containing the same substrate. Gametophytes were separated from each other by 1.0 cm. During the test, gametophytes were watered with sterile distilled water three times a week. The presence of sporophytes was recorded once a week. Plants were kept in a growth room as described above.

When the sporophytes of the four species were big enough to be manipulated (*ca* 3.0 cm tall), they were individually transplanted to small pots containing

TABLE 2. The period of cultivation after spore inoculation for the calculation of RGR of *B. brasiliense, C. corcovadensis, C. delgadii* and *S. inaequale* in typic hapludult soil with the addition of termophylic compost (3:1) in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30μmolm^{-2}s^{-}1. T1 is the day of transplantation to individual pots; T2 is 60 days after transplantation.

	Period of cultivation after spore inoculation (days)	
	T1	T2
B. brasiliense	270	330
C. corcovadensis	337	397
C. delgadii	386	446
S. inaequale	252	312

the same substrate used for gametophyte development and were kept in plastic trays covered with transparent film, in a completely randomized design (Time 1= T_1). For the growth analysis of *Blechnum brasiliense, Cyathea delgadii* and *Saccoloma inaequale,* we utilized 20 sporophytes and for the growth analysis of *Cyathea corcovadensis* we utilized 15 sporophytes. After 60 days of individual transplantation (Time 2= T_2), sporophytes were collected to count the number of fronds and to measure the longest frond's length. Table 2 shows the period of growth after spore inoculation at time 1 and time 2. The relative growth rate (RGR) was estimated according to Bernabe *et al.* (1999), as (Log_{10} L_2 − Log_{10} L_1) / T_2 −T_1 where Log is the base −10 logarithm, L_1 is the initial leaf length when the sporophytes were individually transplanted to the pots in time 1, and L_2 is the leaf length at time 2 (after 60 days of transplantation to the individual pots).

Data were analyzed by Excel for Windows (Microsoft), Minitab for Windows and Statgraphics software. Means and standard deviation were calculated. The Kolmogorov-Smirnov test for goodness of fit for normality and the Bartlett's test for the homogeneity of variance (0.05) were applied before the analysis. The parametric pairwise comparison test among the number of fronds of the four species, were done by the Duncan (5%) Multiple Range Test. The non-parametric Kruskal-Wallis test followed by the Dunn test was employed to analyze the RGR (relative growth rate) because these data did not present residual normality or did not present homogeneity of variance (Santana and Ranal 2004; Zar, 1996). The Student "t" test was applied to compare the number of fronds and the longest frond's length between T1 and T2 for each species,

RESULTS

The mean germination time for the four species studied in this work ranged from 9.9 ± 0.1 days for *Blechnum brasiliense* to 17.8 ± 0.1 days for *Sacolloma inaequale* spores (Table 3). The percentage of germination varied from 32.0 ± 2.5% for *Cyathea corcovadensis* to 77.0 ± 5.0% for *Cyathea delgadii* (Table 3 and Fig. 1a–1d) and the percentage of abnormal spores varied from 5.6 ±

TABLE 3. Days to reach maximum germination (DMG), mean germination time (MGT), maximum percentage of germination (MG), percentage of abnormal spores (AS) and time of spore storage (TS) of *Blechnum brasiliense, Cyathea corcovadensis, Cyathea delgadii,* and *Saccoloma inaequale Cyathea delgadii* Sternb. Spores were sown in liquid Dyer medium in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 μmol m^{-2} s^{-1} The table presents data from the literature for comparison. *Germination of unsterilized spore was carried out under continuous white light.

	DMG (Days)	MGT (Days)	MG (%)	AS (%)	TS (months)
		Mean ± standard deviation			
B. brasiliense	15	9.9 ± 0.1	66.0 ± 2.0	29.0 ± 0.7	8
C. corcovadensis	20	15.2 ± 0.5	32.0 ± 2,5	27.1 ± 5.5	8
C. delgadii	18	13.3 ± 0.1	77.0 ± 5.0	14.0 ±1.8	6
S. inaequale	24	17.8 ± 0.1	60.0 ± 1.0	5.6 ± 0.7	6

Data from literature

	DMG (Days)	MGT (Days)	MG (%)	AS (%)	ST (month)	
B. brasiliense	* 5	2,6	60.0	Not analyzed	Fresh spores	Simabukuro *et al.* (1993)
C. corcova-densis	*14	Not analyzed	20.0	Not analyzed	Not informed	Felippe *et al.* (1989)
C. delgadii	*10	Not analyzed	76.0	Not analyzed	Fresh spores	Randi & Felippe (1988 a,b)
C. delgadii	*10	Not analyzed	60.0	Not analyzed	6	Randi & Felippe (1988 a,b)
C. delgadii	*10	5.7	85.0	Not analyzed	Fresh spores	Simabukuro *et al.* (1993)
C. delgadii	*7	Not analyzed	74.0	Not analyzed	12 (at −12 °C)	Simabukuro *et al.* (1998)

0.7% for *Sacolloma inaequale* to 29.0 ± 0.7% for *Blechnum brasiliense* (Table 3).

All species produced sporophytes during the period of analysis, but the percentage of mature gametophytes that produced sporophytes and the time for sporophyte formation were variable among species. *Blechnum brasiliense* presented the first sporophytes after 86 days of cultivation and 91.5 ± 2.5% of gametophytes produced sporophytes after 303 days (Fig. 2a). The first sporophytes appeared in *Cyathea corcovadensis* after 117 days of cultivation and after 264 days of cultivation, 35.8 ± 16.2% of gametophytes produced sporophytes (Fig. 2b). *Cyathea delgadii* presented the first sporophytes after 109 days of cultivation and after 249 days of cultivation, 79.2 ± 13.5% of gametophytes produced sporophytes (Fig. 2c). The first sporophytes of *Saccoloma inaequale* appeared after 78 days of cultivation and after 295 days, 48.4 ± 24.4% of gametophytes produced sporophytes (Fig. 2d).

The number of fronds produced ranged from 4.5 to 7.0 for *Blechnum brasiliense,* from 4.7 to 5.0 for *Cyathea corcovadensis,* from 5.8 to 8.5 for *Cyathea delgadii* and from 6.0 to 9.0 for *Saccoloma inaequale* (Fig. 3a to 3d).

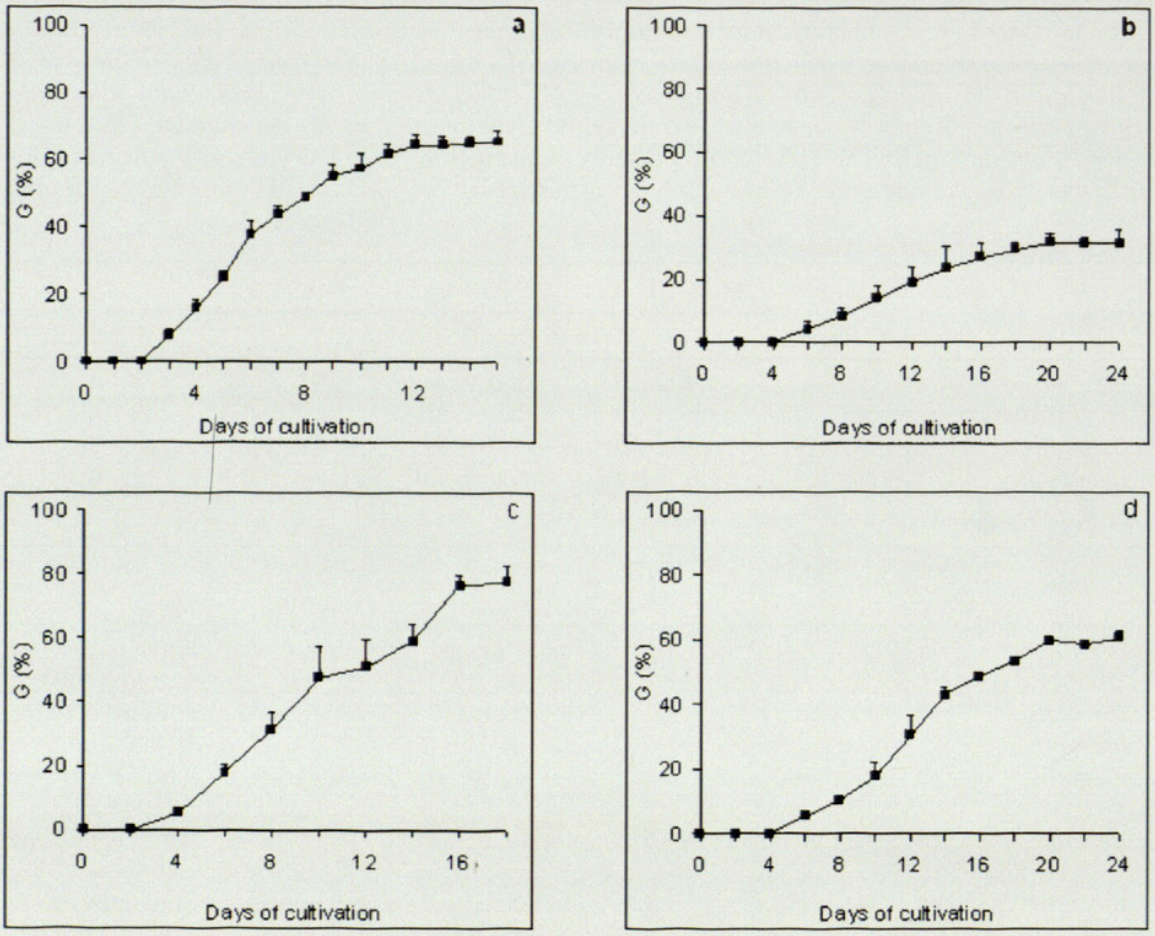

FIG. 1. Germination of spores of *Blechnum brasiliense* (a), *Cyathea corcovadensis* (b), *Cyathea delgadii* (c) and *Saccoloma inaequale* (d) cultivated in liquid Dyer medium in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 μmol m^{-2} s^{-1}. Bars are standard deviation. G =germinability.

The longest frond length ranged from 3.0 to 6.9 cm for *Blechnum brasiliense,* from 2.7 to 6.1cm for *Cyathea corcovadensis,* from 4.0 to 10cm for *Cyathea delgadii* and from 3.3 to 9.5cm for *Saccoloma inaequale* (Fig. 4a to 4d). The RGR of *Saccoloma inaequale,* an herbaceous fern, was statistically greater than the RGR of *Cyathea corcovadensis,* a tree fern, but was similar to the other species (Table 4). The number of fronds produced after two months was statistically smaller for *Cyathea corcovadensis* but did not differ among the other species (Table 4).

DISCUSSION

Preservation under refrigeration is a common method of spore storage. According to Pence (2000), the ex situ preservation of spores can be an important supplement to the maintenance of pteridophyte species in the wild. Pence (2000) stored spores of 33 species at 4°C, −20°C, or in liquid nitrogen and observed that spores of three species did not lose viability after more than

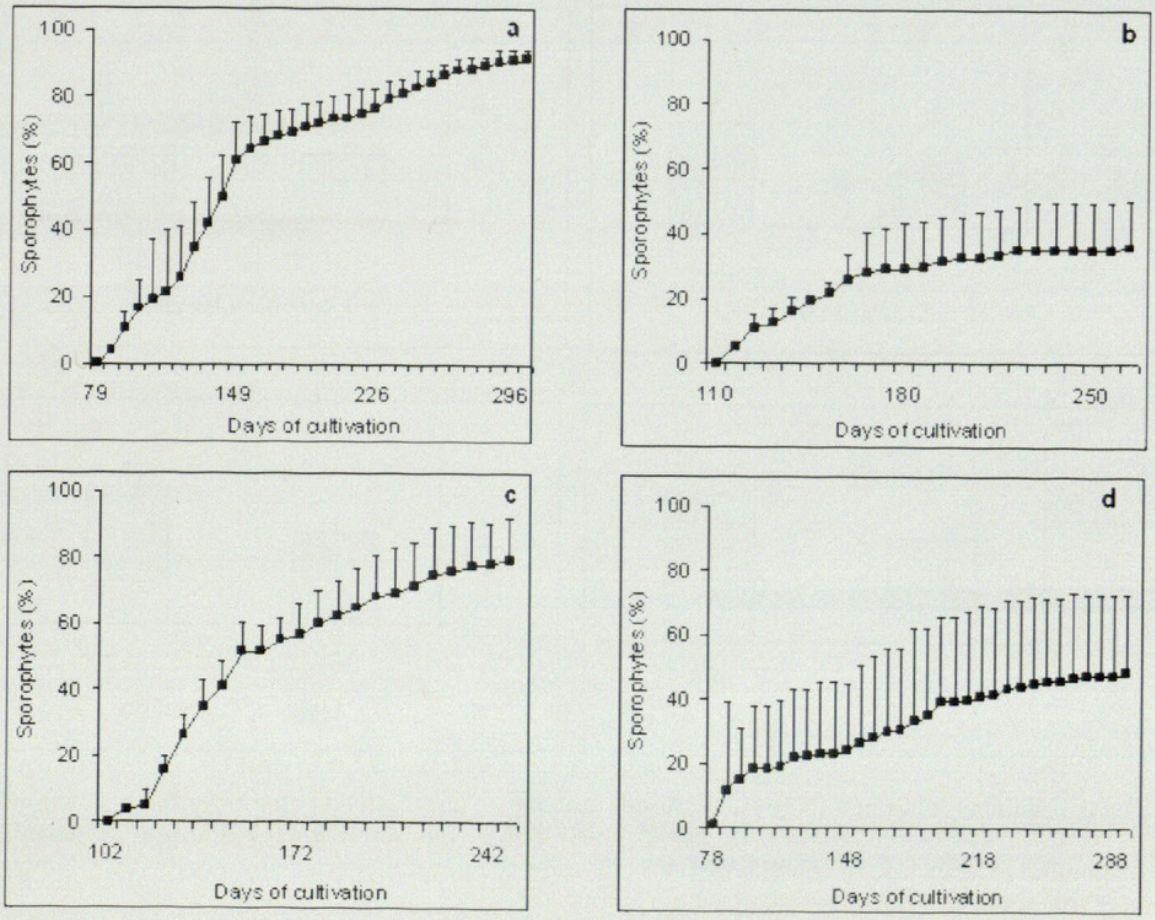

FIG. 2. Sporophyte formation of *Blechnum brasiliense* (a), *Cyathea corcovadensis* (b), *Cyathea delgadii* (c) and *Saccoloma inaequale* (d) cultivated in red soil with the addition of organic compound (3:1) in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 µmol m^{-2} s^{-1}. Bars are standard deviation.

75 months. Eighty-two percent of *Dicksonia sellowiana* Hook. spores germinated after more than 2 years under refrigeration at 10 °C (Filippini *et al.*, 1999). Previously sterilized spores of *D. sellowiana* remained viable after cryopreservation in liquid nitrogen and cryopreservation seems to improve the germination of the spores (Rogge *et al.*, 2000).

Aragon and Pangua (2004) analyzed the spore germination of four rupicolous taxa of *Asplenium* (*A. adiantum-nigrum* L. var. *adiantum-nigrum*, *A. adiantum.nigrum* L. var. *silesiacum*, *A. septentrionale* (L.) Hoffm subsp. *septentrionale* and A. *ruta-muraria* L. subsp. *ruta-muraria*) after 1, 6, and 12 months of storage in Eppendorf tubes (dry storage) or on agar plates (wet storage) at −20°C, 5°C and 20°C. In all cases, except for *A. ruta-muraria*, germination percentage was maintained in wet and dry storage, but in the dry storage method percent germination was higher. Though the authors did not carry out a thorough post-germination study, the plates used in the various experiments remained in culture chambers at 20°C for 6 months and the gametophytes appeared to develop normally. They suggested that storage time might not affect the subsequent development of the prothalli.

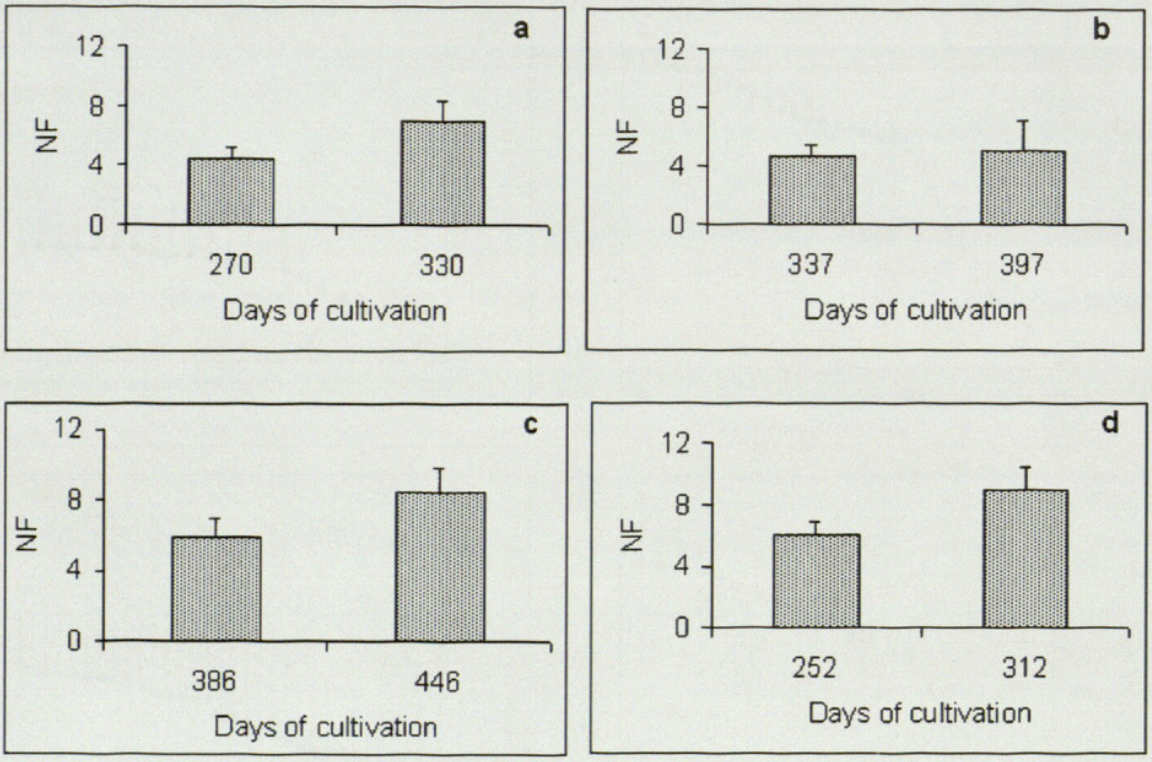

FIG. 3. Number of fronds (NF) of *Blechnum brasiliense* (a), *Cyathea corcovadensis* (b), *Cyathea delgadii* (c) and *Saccoloma inaequale* (d) cultivated in red soil with the addition of organic compound (3:1) in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 μmol m^{-2} s^{-1}. Bars are standard deviation.

In comparison to studies done by Simabukuro *et al.* (1993) the mean germination time for spores of *Blechnum brasiliense* and *Cyathea delgadii* were delayed in the present study. However, Simabukuro *et al.* (1993) inoculated fresh unsterilized spores at 25 °C under continuous white light. The surface sterilization could delay or reduce spore germination but is necessary in order to obtain higher percentages of gametophytes. Indeed, Camloh (1993, 1999) reported that the best germination of *Platycerium bifurcatum* (Cav.) C. Chr. occurred when unsterilized spores were used, but that contamination always occurred after 10 days of culture. Simabukuro *et al.* (1998) indicated that before the germination of dry-stored spores, in order to avoid the incidence of fungal growth, there is the need to sterilize them. The differences observed in the present paper for the mean germination time of *B. brasiliense* and *C. delgadii* could be an effect of the time and location of collection, sterilization method for spore germination and time of storage under refrigeration. For *Blechnum chilense* (Kaulf.) Mett. and *Blechnum cycadifolium* (Colla) Sturm, the germination of spores in Thompson agar-gelled medium started after 6–10 days of spore soaking (Pérez-García *et al.*, 1996).

The germination results of stored spores of *Blechnum brasiliense* are similar to the results of Simabukuro *et al.* (1993), who worked with sporophytes of *B. brasiliense* collected in the Biological Reserve of Moji-Guaçu localized in S. Paulo State (southeast region of Brazil). The data presented in this paper are in

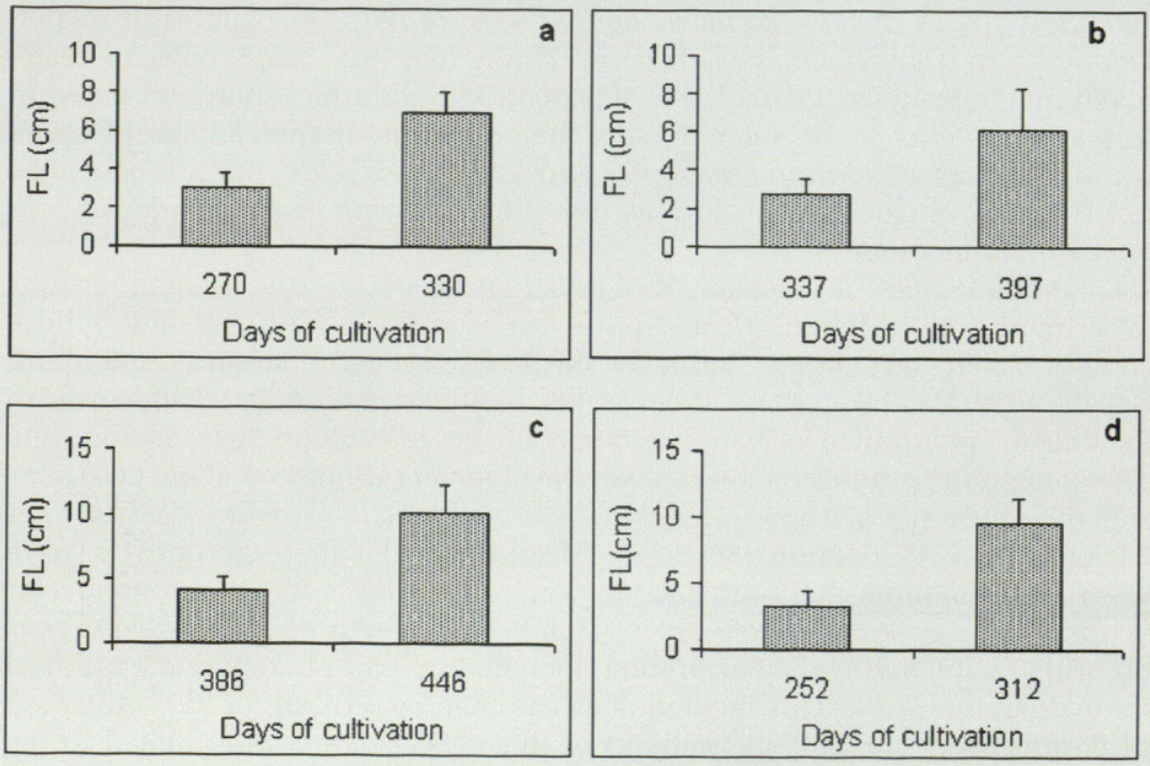

FIG. 4. Longest frond length (FL) of T1 and T2 of *Blechnum brasiliense* (a), *Cyathea corcovadensis* (b), *Cyathea delgadii* (c) and *Saccoloma inaequale* (d) cultivated in red soil with the addition of organic compound (3:1) in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 µmol m^{-2} s^{-1}. Bars are standard deviation.

accordance with similar studies in *Cyathea corcovadensis* (Felippe *et al.*, 1989) and *C. delgadii* (Randi and Felippe, 1988 a, b). Simabukuro *et al.* (1998) stored previously sterilized spores of *C. delgadii* for 12 months at −12 °C and observed 74% germination after 7 days from sowing. The germination of

TABLE 4. Relative growth rate (RGR) and mean number fronds per month (NF) of *Blechnum brasiliense, Cyathea corcovadensis, Cyathea delgadii,* and *Saccoloma inaequale* cultivated in red soil with the addition of organic compound (3:1) in growth chamber (25 ± 2 °C) under a 16-h photoperiod and photon flux density of 30 µmol m^{-2} s^{-1}. χ^2 is the statistic for the Bartlett test; D_{max} is the statistic for the Kolmogorov-Smirnov test, H is the statistic for the Kruskal-Wallis test; sd is the standard deviation. Letters denote statistical differences. * Data did not show normality.

	B. brasiliense	*C. corcovadensis*	*C.delgadii*	*S. inaequale*
RGR ($cm.cm^{-1}.month^{-1}$) (Mean ± sd)				
	0.18 ± 0.03 ab	0.17 ± 0.02 a	0.19 ± 0.028ab	0.25 ± 0.06 b
χ^2				4.087
D_{max}				0.21*
H				7.12
NF.$month^{-1}$ (Mean ± sd)				
	1.2 ± 0.7b	0.3 ± 0.9a	1.3 ± 0.7b	1.50 ± 0.8b
χ^2				1.259
D_{max}				0.029

sterilized spores of *Saccoloma inaequale* took 24 days. For spores of related *Odontosoria* (Dennstaedtiaceae), germination took six to 15 days to reach maximum percentage (Granados *et al.*, 2003). The abnormal spores observed in this study seems to be related with the reduction in percentage of spore germination for *Blechnum brasiliense* and *Cyathea delgadii*, but it seems to be not the only reason for the low germination of *Cyathea corcovadensis* and *Sacolloma inaequale*.

Large differences in percentages of germination are the rule in simultaneous experiments on different taxa. Spore age, storage conditions and culture conditions, all have a great influence on fern spore germination (Camloh and Gogala, 1992; Camloh, 1993; Camloh, 1999; Sheffield *et al.*, 2001). Differences in the time for sporophyte formation and percentages of sporophyte formation were also common in studies on sporophyte development (Sheffield *et al.*, 2001).

In this study, the first sporophytes of *Blechnum brasiliense* were observed after 2.9 months of cultivation (86 days). Meanwhile, the first sporophytes were observed 4.5 months after spore inoculation for *B. chilense* and after 7 months for *B. cycadifolium* (Pérez-García *et al.*, 1996). The growth of *B. brasiliense* was probably facilitated by the substratum, temperature and photoperiod employed throughout this work. This methodology can then be utilized for the cultivation of *B. brasiliense*. In fact, sporophytes of this species were transplanted to the garden of the Department of Botany and showed a normal development. However, the sporophytes of *C. corcovadensis*, *C. delgadii* and *S. inaequale* were not transplanted to the garden because they showed sensitivity to desiccation and require special cares during acclimatization, needing high humidity, shadow and mild temperatures during this period. *Blechnum brasiliense* appears to be a plant adapted to regimes of oscillating humidity and greater light intensity, being more easily acclimatized.

The gametophytes of *Sacolloma inaequale* that did not produce sporophytes grew successively until the end of this study. A similar observation was found by Sakamaki and Ino (1999) working with *Thelypteris palustris* Schott (Thelypteridaceae). They concluded that sporophytes appeared on most gametophytes that reached a critical size for sporophyte formation, but some gametophytes that did not produce sporophytes became very large and lived for a long time without producing sporophytes.

Our data for frond production by young sporophytes of *Blechnum brasiliense* and *Cyathea delgadii* are similar for the data found for *Acrostichum daneaefolium* Langsd. & Fisch. (Pteridaceae) which were 1.0 ± 0.03 to 1.3 ± 0.04 fronds per month (Mehltreter and Palacios-Rios, 2003; Mehltreter *et al.*, 2003). However, for adult sporophytes of several Cyatheaceae, the number of fronds produced each year varied from 3 to 14 (Tanner, 1983; Ash, 1987; Arens, 2001).

In this study, the RGR of *Sacolloma inaequale* was greater than the RGR of *Cyathea corcovadensis*. Statistically significant differences were not observed among the other species. Data for the RGR of some angiosperms from the Atlantic Forest, presented by Paulilo *et al.* (1993), showed that herbaceous species showed greater RGR than tree species. Concerning RGR for fern

species, data from the literature are very scarce. The RGR for young sporophytes of *Dicksonia sellowiana* cultivated under 10% of natural light was 0.22cm/cm/month (Suzuki *et al.*, 2005), which was similar than data found in the present paper. Bernabe *et al.* (1999) transplanted young sporophytes of *Alsophila firma* (Baker) D.S.Conant (3cm of length) into the interior and at the edge of the forest in Mexico and observed a RGR of 2.42 cm/cm/yr for the first six months of growth in the interior of the forest and 3.53 cm/cm/yr at the forest edge. They concluded that the forest edge where the photosynthetic active radiation (PAR) was nine times higher than the interior of forest was a better place for regeneration of tree ferns. In the present work, PAR at the growth room was very low (30 $\mu mol\ m^{-2}\ s^{-1}$), which could explain the low RGR found, even for the herbaceous fern.

The results of this study showed that spores stored at 7 ± 1°C for 6 to 8 months, of four species from the Atlantic Forest, were able to germinate *in vitro*, producing mature gametophytes and sporophytes after being transferred to appropriate substrate. The method of spore storage, the germination protocol, and the methods for gametophyte and sporothyte growth employed in this paper can be used by conservation programs for sustainable management and regeneration of these species native for the Brazilian Atlantic Forest.

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Literature Cited

Ash, J. 1987. Demography of *Cyathea hornei* (Cyatheaceae) a tropical tree-fern in Fiji. Aust. J. Bot. 35:331–341.

Arens, N. C. 2001. Variation in performance of the tree fern *Cyathea caracasana* (Cyatheaceae) across a sucessional mosaic in an Andean cloud forest. Amer. J. Bot. 88:545–551.

Aragon, C. F. and E. Pangua. 2004. Spore viability under different storage conditions in four rupicolous *Asplenium* L. Taxa. American Fern Journal 94:28–38.

Beri, A. and S. S. Bir. 1993. Germination of stored spores of *Pteris vittata* L. Amer. Fern J. 83:73–78.

Bernabe, N., G. Williams-Linera and M. Palacios-Rios. 1999. Tree ferns in the interior and at the edge of a Mexican Cloud Forest remnant: spore germination and sporophyte survival and establishment. Biotropica 31:83–88.

Camloh, M. and N. Gogala. 1992. In vitro culture of *Platycerium bifurcatum* gametophytes. Sci. Horticult. 51:343–346.

Camloh, M. 1993. Spore germination and early gametophyte development of *Platycerium bifurcatum*. Amer. Fern J. 83:79–85.

Camloh, M. 1999. Spore age and sterilization affect germination and early gametophyte development of *Platycerium bifurcatum*. Amer. Fern J. 89:124–132.

Conservation International. Biodiversity Hotspots. Atlantic Forest. http://www.biodiversity hotspots.org/xp/hotspots/atlantic _forest.

Dyer, A. F. 1979. The culture of fern gametophytes for experimental investigation. Pp. 253–305, *in*: A. F. Dyer, ed. *The experimental biology of ferns*. Academic Press, New York.

Felippe, G. M., L. M. Esteves and A. M. Randi. 1989. Lipids, proteins and sugars in spores of *Cyathea delgadii* Sternb., *Polypodium latipes* Langs & Fisch and *Trichipteris corcovadensis* (Raddi) Copel. Insula 19:3–12.

FILIPPINI, E. C. P., S. R. DUZ and A. M. RANDI. 1999. Light and storage in the germination of spores of *Dicksonia sellowiana* (Presl.) Hook., Dicksoniaceae. Rev. Brasil. Bot. 22:21–26.

GRANADOS, B., PÉREZ-GARCÍA, B. and A. MENDOZA. 2003. Fase sexual de los helechos *Odontosoria schlechtendalii* y *Odontosoria scandens* (Dennstaedtiaceae). Rev. Biol. Trop. 51:675–682.

IBAMA. 1997. *Workshop sobre Conversação e Manejo de Dicksonia sellowiana (Xaxim).* Relatório. IBAMA, Urubici, SC, Brazil.

LABOURIAU, L. G. 1983. A germinação das sementes. *Série de Biologia, Monografia 24.* Programa Regional de Desenvolvimento Científico e Tecnológico. Washington.

MEHLTRETER, K. and M. PALACIOS-RIOS. 2003. Phenological studies of *Acrostichum danaeifolium* (Pteridaceae, Pteridophyta) at a mangrove site on the Gulf of Mexico. J. Trop. Ecol. 19:155–162.

MEHLTRETER, K., P. ROJAS and M. PALACIOS-RIOS. 2003. Moth larvae-damaged giant leather-fern *Acrostichum danaeifolium* as host for secondary colonization by ants. Amer. Fern J. 93:49–55.

MILTON, S. J. and E. J. MOLL. 1988. Effects of harvesting on frond production of *Rumohra adiantiformis* (Pteridophyta: Aspidiaceae) in South Africa. J. Appl. Ecol. 25:725–43.

PRADO, J. 1997. Flora da Serra do Cipó, Minas Gerais: Pteridaceae - Adiantoideae e Taenitidoideae. Boletim de Botânica (USP), Brasil 16:115–118.

PAULILO, M. T. S., G. M. FELIPPE and J. E. DALE. 1993. Crescimento inicial de *Qualea grandiflora.* Rev. Brasil. Bot. 16:37–46.

PENCE, V. 2000. Survival of chlorophyllous and nonchlorophyllous fern spores through exposure to liquid nitrogen. Amer. Fern J. 90:119–126.

PÉREZ-GARCIA, B., M. E. FRAYLE and A. MENDONZA. 1995. Desarrollo del gametofito de *Lophosoria quadripinnata* (Filicales: Lophosoriaceae). Rev. Biol. Trop. 43:55–60.

PÉREZ-GARCÍA, B., A. MENDONZA and M. RICCI. 1996. Morfogénesis de la fase sexual de *Blechnum chilense* & *Blechnum cycadifolium* (Pterophyta: Blechnaceae). Rev. Biol. Trop 44:491–497.

RANDI, A. M. and G. M. FELIPPE. 1988a. Efeito do armazenamento de esporos, da aplicação de DCMU e da pré-embebição em PEG na germinação de *Cyathea delgadii* Sternb. Ciência e Cultura 40:484–489.

RANDI, A. M. and G. M. FELIPPE. 1988b. Efeitos da temperatura durante a pré e a pós-indução e do período de armazenamento na germinação de *Cyathea delgadii* Sternb. Hoehnea 15:10–19.

ROGGE, G. D., A. M. VIANA and A. M. RANDI. 2000. Cryopreservation of spores of *Dicksonia sellowiana.* An endangered tree fern indigenous to South and Central America. Cryoletters 21:223–230.

SAKAMAKI, Y. and Y. INO. 1999. Contribution of fern gametophytes to the growth of produced sporophytes on the basis of carbon gain. Ecol. Research 14:59–69.

SANTANA, D. G. and M. A. RANAL. 2004. *Análise da germinação: um enfoque estatístico.* Editora UNB, Brasília.

SEHNEM, A. 1977. As filicíneas do Sul do Brasil, sua distribuição geográfica, sua ecologia e suas rotas de migração. Pesquisas Botânicas 31:1–108.

SEHNEM, A. 1978. *Ciateáceas. Flora Ilustrada Catarinense.* Herbário Barbosa Rodrigues, Itajaí.

SHEFFIELD, E., G. E. DOUGLAS, S. J. HEARNE, S. HUXHAM and J. M. WYNN. 2001. Enhancement of fern spore germination and gametophyte growth in artificial media. Amer Fern J. 91:179–186.

SIMABUKURO, E. A., L. M. ESTEVES and G. M. FELIPPE. 1993. Light sensitivity of ferns from a gallery Forest. Insula 22:177–186.

SIMABUKURO, E. A., A. F. DYER and G. M. FELIPPE. 1998. The effect of sterilization and storage conditions on the viability of spores of *Cyathea delgadii.* Amer. Fern J. 88:124–132.

SUZUKI, C. C. L. F., M. T. S. PAULILO and A. M. RANDI. 2005. Substrate and irradiance affect the early growth of the endangered tropical tree fern *Dicksonia sellowiana* Hook. (Dicksoniaceae). Amer. Fern J. 95:115–125.

TANNER, E. V. J. 1983. Leaf demography and growth of tree-fern *Cyathea pubescens* Mett. ex Kuhn in Jamaica. Bot. J. Linnean Soc 87:213–227.

TRYON, R. and A. F. TRYON. 1982. *Ferns and allied plants with special reference to Tropical America.* Springer-Verlag, New York.

WINDISCH, P. G. 2002. Fern conservation in Brazil. Fern Gaz 16:295–300.

ZAR, J. H. 1996. *Biostatistical Analysis,* Prentice Hall, New Jersey.