REPRODUCTIVE POTENTIALS OF A TROPICAL FERN *CYCLOSORUS AFER* (CHRIST.) CHING (THELYPTERIDACEAE: PTERIDOPYHTE) AT OBAFEMI AWOLOWO UNIVERSITY, ILE IFE, NIGERIA

F. A. Oloyede [1a], B. Aponjolosun [1] & A. A. Ogunwole [1]
[1]Botany Department, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria.

(Received: September, 2010; Accepted: January, 2011)

ABSTRACT

Reroductive potentials of *Cyclosorus afer* (Christ.) Ching a tropical fern was studied. Propagation by the spores of this plant was therefore investigated. The aim was to determine the ability of early germination of these spores using nutrient (growth) agar. The experimental site was the Reforestation unit of the Department of Botany, Obafemi Awolowo University, Ile-Ife. Fresh matured spores collected from this site were cultured in a prepared nutrient medium inside the Petri dishes labeled A, B, C, D, and E. The rapid rate of germination was observed at day 10 and day 14. The experiment was replicated thrice. Statistical analysis was carried out using Analysis of Variance (ANOVA). The result showed that maximum germination occurred around the second week. This is because about three-quarters of the spores germinated between 10^{th} and 14th day of planting during which 80%, 50%, 90% and 60% germination was recorded in each of the Petri dishes B, C, D and E respectively while no germination was observed in the control experiment in Petri dish A. This indicates that the spores have potentials to raise this plant within a short period.

Keywords: *Cyclosorus afer*, Croziers, Sporangia, Spores, Sori,

INTRODUCTION

The genus *Cyclosorus* (H. Christ.) belongs to the family Thelypteridaceae (Agnew, 1974) of the Order Filicales, Class Pteropsida and the Division Pteridophyta (Sporne, 1975; Berrie, *et al.,* 1987). Pteridophytes are vascular (tracheophytes) cryptogamic plants (Kartesz, 1994). In Nigeria, fern species occur abundantly in the high rainfall belt of the south (Odu and Opapeju, 1986). *Cyclosorus afer* (Christ.) Ching occurs mostly in marshy to waterlogged habitats in Southwestern rainfall belt (Oloyede, 2008). Crozier unfolding establishes new fronds with the compound leaf formation. Sori are arranged on the abaxial surface of the pinnules. Each sorus contains many sporangia covered with indusia (Alston, 1959). Inside each sporangium lays a mass of small sized light numerous spores (Dutta, 2005). The spores seldomly germinate under favorable conditions of the temperature and moisture to prothalli. The prothallus gives rise to gametophyte.

The continuity and distribution of this plant are enhanced by the spore production and dispersal. Spore germination requires proper nutrition, protection, free dispersal at maturity and suitable substrate. Although spore longevity has a major impact on fern species migration and distribution, long distance dispersal may expose the spores to unfavourable conditions for germination (Barrington *et al.,* 1986). This plant possesses high aesthetic values and spreads rapidly. It has rapid growth even in poor soil and capable of colonizing bare land easily. The objective of this work is to study the ability of the spores of *C. afer* to germinate within few days as against prolonged delay under natural conditions.

MATERIALS AND METHODS

Counting of sori and sporangia was done in order to estimate the potentials of this plant to produce spores. Five matured fertile fronds of *C. afer* were collected from the Reforestation unit of the Department of Botany, Obafemi Awolowo University, Ile Ife. The plant was identified using fern floras (Alston, 1959; Agnew, 1974) and IFE herbarium specimens with number 14392 at the Department of Botany, Obafemi Awolowo University, Ile Ife. The numbers of pinnae with and without sori were counted on each collected frond. The numbers of sori on each pinna were similarly counted and the total number of sori on each frond was estimated. Five sori were randomly selected from each pinna and each sorus was put on a slide, little amount of water was added, covered with a slip, viewed and counted under a dissecting microscope using a counting chamber. The mean number of sporangia per sorus was estimated and analysis of variance (ANOVA) was carried out for both sori and sporangia counts. A nutrient medium (Parker's medium) was prepared as shown in Table 1. Sterilized filter paper was put into each of the sterilized six Petri dishes in a micro flow chamber (sterile chamber). The Petri dishes were labeled A, B, C, D, E & F. Under a working micro flow chamber, each pinna was surface sterilized in 1.5% sodium hypochlorite for 1-2 minutes. They were laid in sterilized Petri dishes to dry out. Each selected pinna was rubbed between the palms to release the spores into their respective Petri dishes. 4 ml distilled water was added to the Petri dish F, serving as the control experiment, while 4 ml of the prepared nutrient was added to each of the remaining five Petri dishes. The Petri dishes were covered and placed on the window sill in the laboratory. The cultures were frequently monitored for nutrient replacement and spore germination. All the cultures were viewed daily for up to 10 and 14 days through the lid or base of those closed Petri dishes by a dissecting microscope to avoid contamination. The experiment was replicated trice. The mean percentage germination of the spores was calculated for each of the Petri dishes after viewing and counting have been repeated ten times. The formula used was thus;

$$\frac{\text{Total number of germinated spores in a field}}{\text{Total number of spores in that field.}} \times 100\%$$

RESULTS

Observation shows that the fronds (leaves) of *Cyclosorus afer* have pinnae (leaflets) with serrated margins. Sori are found only on the abaxial surface of the pinnae and there are no sori at the extreme parts of the frond base and apex. Matured sori (dark brown) are also restricted to the middle part of the fronds, arranged on the margins of the pinnules. The longer the pinnule, the more the number of sori found on it. The croziers unfold; produce aerial leaves and sori throughout the year during favourable climatic conditions. Tables 2-4 show the observations and the statistical analyses for the sori and the sporangia counts in each of the collected fronds. Figure 1 shows the graphical representation of the mean percentage germination of spores from each of the Petri dishes. Plate 1 is the abaxial surface of a fertile frond showing the arrangement of mature sori on the pinnae (leaflets).

In the Petri dish A there was no germination at all because it is the control. Germination started within one week of planting in the Petri dishes B, C, D and E. The brownish coloration of the spores changed to green as an indication that germination has started. This was followed by the breakage of spore walls to produce the prothallial filament indicating successful germination of the spores.

Table 1: The Composition of Parker's Medium (Klekowski, 1969).

CODE	Macro-elements, stock solution (Parker nutrients)	Wgt (g), Vol. (ml). Dw
A	Ammonium Nitrate: NH_4NO_3	2.5g/100 ml
B	Potassium Hydrogen Phosphate: KH_2PO_4	2.0g/100 ml
C	Magnesium Sulphate: $MgSO_4.7H_2O$	1.0g/100 ml
D	Calcium Chloride: $CaCl_2$	1.0g/100 ml
	Micro elements, Stock solution (Thompson nutrients)	
E	Manganese Sulphate	0.22/L
	Copper Sulphate	0.24/L
	Zinc Sulphate	0.29/L
	Boric Acid	0.186/L
	Ammonium Molybdate	0.0035/L
F	Ferrous Sulphate	2.5/L
	Sodium EDTA	3.7/L

Wgt**:** Weight (g), Vol: Volume (ml). Dw: Distilled water

Ingredients of growth medium

1. 500 ml of distilled water
2. Macro-elements stock solution: A. 5 ml. B. 25 ml. C. 12 ml. D. 2 ml.
3. Micro-elements stock solution: E. 10 ml. F. 10 ml.

Table 2: Morphological and Reproductive Characters.

Observable features	**Fronds**				
	A	**B**	**C**	**D**	**E**
Approximate length of the frond (cm)	77	82	71	89	75
Total number of pinnae without sori	15	31	27	12	23
Total number of pinnae with sori	64	72	55	84	61
Total number of pinnules without sori	814	1380	1021	623	928
Total number of pinnules with sori	1980	2077	1240	2911	1618
Total number of sori	23441	44520	13061	99048	17841
The mean number of sporangia per sorus	10.16	10.33	9.48	11..51	10.12

Table 3 A: The Distribution of Son on the Pinnae of the Five Selected Fronds.

Pinna number	**Fronds**				
	A	**B**	**C**	**D**	**E**
1	115	520	208	549	144
2	316	190	132	428	301
3	189	116	131	217	241
4	210	413	297	579	118
5	250	322	116	615	146
6	241	119	145	300	188
7	116	511	168	225	262
8	182	391	189	611	163
9	223	871	222	720	189
10	306	188	169	516	175
11	119	620	288	572	223
12	397	514	172	720	111
13	440	588	166	193	377
14	332	777	158	101	169
15	250	645	110	815	8816
16	401	503	195	744	187
17	421	260	234	701	195
18	219	416	155	898	223
19	414	201	176	457	118
20	339	716	191	150	183

Table 3 B: Summary of the Distribution of Sori on the Pinnae of the Five Selected Fronds.

	Leaves				
	A	**B**	**C**	**D**	**E**
N	20	20	20	20	20
X	5480	8881	3622	10111	3963
Mean	274	444.05	181.1	505.55	198.15
X^2	1713842	4877173	703740	6183691	866937
Variance	11174.8421	49134.9974	2515.5684	56424.9974	4298.3447
Std. Dev.	105.7111	221.6642	50.1554	237.5395	65.5618
Std. Err.	23.6377	49.5656	11.2151	53.1154	14.6601

ANOVA summary for the Distribution of sori on the pinnae

Variance component	SS	df	MS	F	P
Between group	1721444.26	4	430361.07	17.42	<.0001
Within group	2347426.25	95	24709.75		

The tabulated T P.05. df 4 and 95 is 2.45. Since the F calculated (17.42) is greater than the *F* tabulated (2.45) it means that there are significant differences between the mean numbers of sori in the five groups of the leaves. The probability is less than 0.0001 in the F calculated: this shows that there is no relationship in the distribution of the sori on the pinnae of the selected five fronds.

Table 4 A: The distribution of Sporangia in the Selected Sori from each Frond.

Selectedd	A	B	C	D	E
1	13	17	9	18	8
2	15	16	12	12	15
3	7	9	11	14	10
4	10	12	15	9	16
5	17	17	10	16	7

Table 4 B: Data Summary for the Distribution of Sporangia in the Selected Sori.

	Leaves				
	A	**B**	**C**	**D**	**E**
N	5	5	5	5	5
X	62	71	57	69	56
Mean	12.4	14.2	11.4	13.8	11.2
X^2	832	1059	671	1001	694
Variance	15.8	12.7	5.3	12.2	16.7
Std.	3.9749	3.5637	2.3022	3.4928	4.0866

ANOVA Summary for the Distribution of sporangia on the pinnae

Variance component	SS	df	MS	F	P
Between group	37.2	4	9.3	0.7416	0.574797
Within group	250.8	20	12.54		

The tabulated F at P.05, df 4, 20 is 2.87 and since the F calculated (0.74) is less than the F tabulated (2.87) it means there is no significant difference in the mean number of sporangia per sorus in the five groups of the leaves. The probability is 0.57 in the F calculated. This shows that there is a close relationship between sporangia distribution and the pinnae of each leaf.

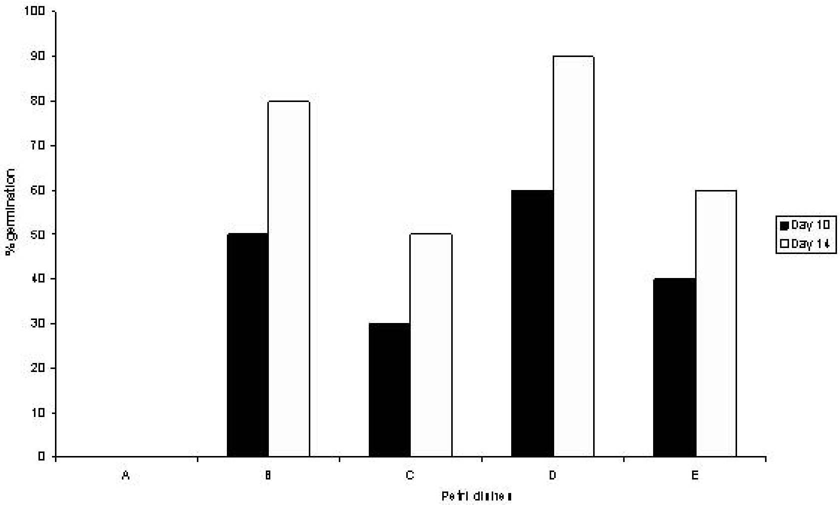

Figure 1: The Percentage Germination of Spores of *Cyclosorus afer*.

Plate 1. The Abaxial Surface of a Fertile Frond Showing the Arrangement of Mature Sori on the Pinnae

DISCUSSION

The maximum germination of spores took place after 14 days because there were observable differences in the percentage germination of spores between day 10 and day 14 (Fig.1). Plants generally perform well in their natural habitats. The length and the number of fronds, the number of sori, sporangia and spores produced by a plant determine the reproductive capacity of the plant in its habitat (Aponjolosun, 2006). Studies of primitive vascular lower plants show that the establishment of new individuals and populations is largely dependent on the dispersal, transportation and distribution of spores by winds (Tryon, 1970; Wagner, 1972). The differences observed were estimated by statistical test using analysis of variance (ANOVA). *Cyclosorus afer* produces many spores because its pinnae (leaflets) are sub-divided into pinnules which create large surface areas for sori arrangement and accommodation. The sori and sporangia maturation in this plant proceed from the basal part of the frond towards the apex. This indicates that spore dispersal begins from the lower pinnae while the sori on the upper pinnae are still maturing. This is in line with Wardlaw and Sharma (1963) who stated that the formation of sori is a gradual and progressive process in younger fronds. Reproduction by the spore is highly significant in the life cycle of a fern because gametic fusion from the recombination of genetic material produces variation and improved viability of the offspring. This is an advantage over vegetative means of reproduction. Rapid vegetative growth through the development of crozier from the underground rhizome compliments sexual reproduction by the spores. The reproductive ability of *C. afer* is therefore enhanced through high production of sporangia, sori, spores and successful germination of the spores. In conclusion, this study shows that the high rate of germination of fresh spores of *C. afer* enhances raising this plant from the spores as the occasion demands.

REFERENCES

Agnew, A. D. Q. 1974. A Flora of the ferns and herbaceous flowering plants of upland Kenya. Oxford University Press, London.

Alston, A. H. G. 1959. *The ferns and fern-allies of West Tropical Africa,* 2nd ed. London, pp. 61-63.

Aponjolosun, B. 2006. Reproductive capability of *Cyclosorus stiatus.* B. Sc. Thesis submitted to the Department of Botany, Obafemi Awolowo University, Ile-Ife.

Barrington, D., C. Paris, and T. Ranker, 1986. Systematic inferences from spore and stomata size in the ferns. *American Fern Journal,* 76: 149-159.

Berrie G. K., A. Berrie and J. M. O. Eze 1987. *Tropical Plant Sci.* Longman. U. K. pp. 280

Dutta, A. C. 2005. *Botany for Degree students*, 19th ed. Oxford University press, India.

Kartesz, J. T. 1994. A synonymized checklist of the vascular flora of the United State, Canada and Greenland. A product of the Biota of North America. *Botany* 620 Home

Klekowski, E. J. 1969. Reproductive Biology of the pteridophya III. A study of the Blechnaceae. *Bot. J. Linn. Soc.* 62: 361-377.

Odu, E. A. and C. O. Opapeju 1986. Reproductive phenology in the homosporous leptosporangiate ferns. *Nigeria Journal of Biological Sciences*, 1: 12-18.

Oloyede F. A. 2008.Taxonomic Evaluation of Homosporous leptosporangiate ferns in Southwestern Nigeria. A Ph. D. Thesis submitted to the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

Tryon A. R. And A. Tryon, 1982. Ferns and allied plants. New York: Springer-Verlag. In: Windham D. M. And C. H. Haufler 1986. Biosystematic uses of fern gametophytes derived from Herbarium Specimens. *American Fern Journal*, 76: 114-128.

Wagner, W. Jr. 1972. Disjunctions in homosporous vascular plants. *Annals Missouri Botanical Garden,* 59: 203-217.

Wardlaw, C.W and Sharma, D. N. 1963. Experimental and analytical studies of the Pteridophytes. Factors in the formation and distribution of sori in Leptopporangiate fern. *Annals of Botany,*

Ife Journal of Science
Tables of Contents: June Edition 2011; Vol. 13, No. 1