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Viability of Dried Vegetative Cells and the Formation and Germination of Reproductive Structures in *Pithophora œdogonia*, *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* under Water Stress

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ABSTRACT. All dried vegetative cells of *Pithophora œdogonia* died within 1 h, while those of *Cladophora glomerata* and *Rhizoclonium hieroglyphicum* retain viability to some extent for 1 and 8 d, respectively, under similar storage conditions. The viability of dried vegetative cells of either *C. glomerata* or *R. hieroglyphicum* decreased more or less equally when stored either at 20 °C in light or dark or at 12 °C in dark, but was lost rapidly and drastically when stored at 0 °C in dark. Both dried and wet akinetes of *P. œdogonia* were equally more viable when stored at 20 °C in dark than in light, but they lost germination ability when stored either at 12 or 0 °C in dark; this might be either due to loss of viability or dormancy induction at low temperatures. The water stress imposed by growing vegetative filaments either on highly agarized media, in NaCl-supplemented liquid media or in media undergoing progressive air-drying to complete dryness did not induce, but reduced akinete formation in *P. œdogonia*, decreased zoosporangium formation in *C. glomerata* and *R. hieroglyphicum*, decreased or totally suppressed akinete germination in *P. œdogonia* and zoospore germination in *C. glomerata* and *R. hieroglyphicum*. Akinetes of *P. œdogonia* formed under water stress were equally viable, while zoosporangia of *C. glomerata* and *R. hieroglyphicum* formed under water stress were comparatively less viable than those formed without any water stress. Akinete germination in *P. œdogonia* and zoospore germination in *C. glomerata* and *R. hieroglyphicum* were comparatively more sensitive to water stress than the formation of akinetes and zoosporangia. The akinete germination in *P. œdogonia* was more sensitive to water stress than zoospore germination in *C. glomerata* and *R. hieroglyphicum* and it might be either due to their large size, thick wall or dense content.

Experimental papers on viability of vegetative cells, induction of spore formation and germination of spores in fresh-water algae under water stress are scant. Some earlier work showed that dryness can induce formation of cysts in *Vaucheria* (Stahl 1879) and *Protosiphon* (Moewus 1935) and akinetes in *Zygnema* (Fritsch 1916). Certain strains of blue-green algae were found to retain viability when stored in lyophilized state (Watanabe 1959; Whitton 1962; Holm-Hansen 1964). Belcher (1970) observed that desiccated cysts of fresh-water members of *Prasinophyceae* remain viable after exposure to 100°C for 1 h. Sili *et al.* (1994) observed that viability of *Cyanospira* akinetes was not affected by long storage or by desiccation. The present study will examine the viability of dried vegetative cells and formation and germination of reproductive structures in selected algae under water stress.

MATERIAL AND METHODS

All three algal materials used in the present study were collected from the University campus. *P. œdogonia* grew attached to cement walls and formed tangled mats in a quiet water pond, while *C. glomerata* and *R. hieroglyphicum* were collected while growing firmly attached to cement walls in separate running-water shallow tanks. Clonal cultures of *P. œdogonia* were raised through germinating akinetes, while those of *C. glomerata* and *R. hieroglyphicum* through germinating zoospores and were maintained under culture conditions in liquid Bold's basal medium (BBM) (Nichols and Bold 1965) adjusted at pH 7.5, at 22 ± 1 °C and light intensity of *c.* 2 klx from daylight fluorescent tubes for 16 h a day.

P. œdogonia reproduced by formation of akinetes, which started to appear 3 d after inoculation of vegetative filaments in liquid medium and it was evident by the contraction of the greater part of the protoplasm of a cell toward one end and separation of this part by a septum and subsequent development of a thick wall upon maturation. The process of akinete formation was not synchronous and required about 3 weeks or more for most of the vegetative cells to differentiate into akinetes in liquid BBM (Fig. 1A). The akinetes harvested from basal medium when transferred to fresh medium germinated directly into new filaments (Fig. 1B).

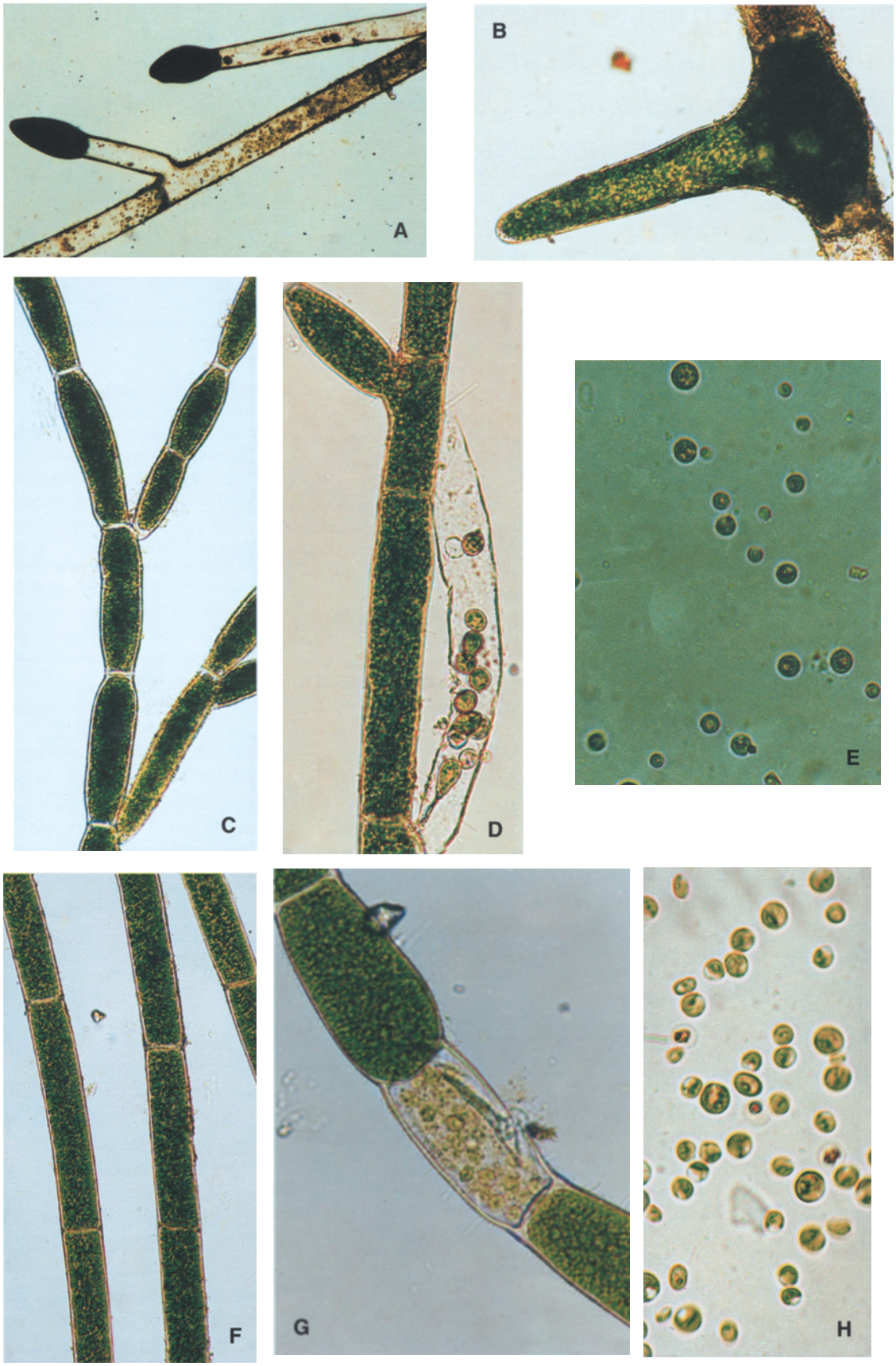

Fig. 1. A: *P. œdogonia* filament with akinetes; **B**: *P. œdogonia* akinete germination; **C**: *C. glomerata* filament; **D**: *C. glomerata* zoosporangium with zoospores; **E**: *C. glomerata* zoospores; **F**: *R. hieroglyphicum* filaments; **G**: *R. hieroglyphicum* zoosporangium with zoospores; **H**: *R. hieroglyphicum* zoospores.

About 5–10 % of vegetative cells of *C. glomerata* and *R. hieroglyphicum* differentiated into zoosporangia in liquid BBM 15 d after inoculation and the values continuously increased to about 20–30 % after 45 d. Entire vegetative cells of *C. glomerata* differentiated into zoosporangia, while those of *R. hieroglyphicum* divided once or twice before a zoosporangium developed. In both algae, a zoosporangium had a dark green content and was up to 1.5 times as broad as a vegetative cell. The zoospores were formed in large numbers and moved fast inside the zoosporangium just before escaping into the medium through a lateral pore one by one (Fig. 1 C–H). The released zoospore swam in the medium for about 10 m and then settled to the walls and bottom of the flask in *C. glomerata* and to cell walls of the parent filaments in *R. hieroglyphicum*. The zoospores of *C. glomerata* took more than 15 d to start germination, while those of *R. hieroglyphicum* started germination within 5 d of attachment.

Viability of dried vegetative cells and akinetes. Similarly blot-dried 5-d-old, actively growing, mature vegetative filaments of *P. œdogonia* obtained from liquid BBM containing a 5-fold greater $NaNO_3$ concentration than in BBM (presence of high $NaNO_3$ delayed akinete formation; Agrawal 1983), 7-d-old, actively growing, mature vegetative filaments of *C. glomerata* and *R. hieroglyphicum* and freshly formed akinetes of *P. œdogonia* obtained from liquid BBM were separately placed on a filter paper and kept in desiccators over fused calcium chloride at 20 °C in light (10 h duration at *c.* 1.4 klx intensity), 20 °C in dark, 12 °C in dark and 0 °C in dark for various time periods from 1 h to 10 d for vegetative filaments and to 2 months for akinetes. The vegetative filaments and akinetes suspended in distilled water and placed under similar storage conditions served as controls. The viability of dried vegetative cells was determined by counting the number of surviving vegetative cells on 3 d of inoculation in liquid BBM placed under normal culture conditions while those of akinetes was estimated by measuring the percentage of germinated akinetes 15 d after inoculation in liquid BBM kept under normal culture conditions.

Formation of akinetes and zoosporangia under water stress. Actively growing vegetative filaments of all algae used were separately spread on solid BBM containing 2–10 % agar or inoculated in liquid BBM containing 0.1–0.8 mol/L NaCl and kept in culture chamber under normal culture conditions. The controls were maintained in liquid BBM. Formation of akinetes in *P. œdogonia* and zoosporangia in *C. glomerata* and *R. hieroglyphicum* was estimated by counting their percentage of total number of vegetative cells. To assess the viability of zoosporangia formed, the percentage of empty zoosporangia which have already released zoospores of total zoosporangia with respect to total number of vegetative cells was counted on 45 d of inoculation, although it was not necessary that the rest of all zoosporangia were dead. The percentage of dead vegetative cells having shrinkage and discoloration of protoplasm was also counted with respect to the total number of vegetative cells.

In a separate set of experiments, actively growing vegetative filaments of *P. œdogonia* were inoculated into different amounts of liquid BBM contained in open Petri dishes and placed in culture chamber under normal culture conditions. The inoculated liquid BBM evaporated to complete dryness in varying time periods of 1–15 d. In the control vegetative filaments were always submerged in the medium. The percentage akinete formation was counted periodically to assess the influence of progressive air drying on akinete formation.

Viability of akinetes formed under water stress. The akinetes of *P. œdogonia* formed on 8 % agarized medium and in 0.25 mol/L NaCl-supplemented BBM were harvested, washed with distilled water and inoculated into liquid BBM and placed under normal culture conditions. The akinetes harvested from liquid BBM and inoculated similarly served as control. The percentage germination of akinetes was determined periodically in each case.

Akinete and zoospore germination under water stress. The freshly formed akinetes of *P. œdogonia* and zoospores of *C. glomerata* and *R. hieroglyphicum* in liquid BBM were transferred to 1–8 % agarized BBM and to 0.1–0.8 mol/L NaCl supplemented BBM and placed in a culture chamber under normal culture conditions. The controls were maintained in liquid BBM. They were examined at intervals from the start of the experiment so as to determine the percentage germination of akinetes and zoospores. The emergence of protuberances originating from them which subsequently developed into a germling was taken as a criterion for germination.

RESULTS AND DISCUSSION

Viability of dried vegetative cells and akinetes. All dried vegetative cells of *P. œdogonia* died within 1 h of storage, while those of *C. glomerata* and *R. hieroglyphicum* retained viability to some

extent for 1 and 8 d, respectively, under similar storage conditions (Table I). In *R. hieroglyphicum*, the viability of dried vegetative cells decreased progressively from 1 to 8 d and was lost totally within 10 d of storage. Desiccated vegetative cells of blue-green alga *Anabæna cylindrica* also failed to grow after 15 d of storage (Yamamoto 1975).

Table I. Percentage survival of dried vegetative cells[a] of *P. œdogonia*, *C. glomerata* and *R. hieroglyphicum* and dried akinetes[b] of *P. œdogonia* stored in desiccators over fused calcium chloride for different time periods at different light conditions and temperatures. Controls were maintained with vegetative filaments and akinetes suspended in distilled water and kept under similar conditions[c]

Storage time h, d, mon	Control (C), dried stage (D)	Storage conditions			
		light[d] 20 °C	dark 20 °C	dark 12 °C	dark 0 °C
		P. œdogonia, vegetative cells			
1 h	C	100	100	100	0
	D	0	0	0	0
		P. œdogonia, akinetes			
1 mon	C	55	70	1	0
	D	50	68	0	0
2 mon	C	56	71	0	0
	D	58	75	0	0
		C. glomerata, vegetative cells			
1 d	C	96	95	75	10
	D	30	29	41	0
2 d	C	90	80	55	0
	D	0	0	0	0
		R. hieroglyphicum, vegetative cells			
1 d	C	100	99	86	67
	D	60	62	50	34
2 d	C	99	99	80	55
	D	58	55	45	20
3 d	C	99	95	76	38
	D	50	43	30	0
5 d	C	95	86	75	32
	D	46	39	30	0
8 d	C	80	75	70	0
	D	8	12	5	0
10 d	C	70	50	18	0
	D	0	0	0	0

[a]Measurement 3 d after inoculation in liquid BBM. [b]Measured 15 d after inoculation in liquid BBM.
[c]All values represent rounded means of three replicates. [d]Storage under 10-h illumination with fluorescent light of 1.4 klx.

The dried vegetative cells of either *C. glomerata* or *R. hieroglyphicum* were equally viable when stored either at 20 °C in light or dark or at 12 °C in dark but they lost viability rapidly and drastically when stored at 0 °C in dark (Table I). The vegetative cells of certain algae can be successfully preserved for five years at liquid nitrogen temperature (Hwang and Horneland 1965).

Both dried and wet akinetes of *P. œdogonia* were equally more viable when stored for 1 or 2 months at 20 °C in dark than in light, but they did not germinate after storage for similar time periods either at 12 or 0 °C in dark (Table I). This might be either due to loss of viability or dormancy induction at low temperatures. However, in *Chara*, storage of dried oospores at 3 °C provided the most satis-

factory means for long-term preservation of viable disseminules (Proctor 1967). In *Volvox*, only wet oospores were capable of germination (Metzner 1945), but in *Closterium*, drying of zygospores was prerequisite for germination (Lippert 1967). In the present study with *P. œdogonia*, both dried and wet akinetes were equally effective in germination.

Table II. Influence of agarized BBM and NaCl-supplemented liquid BBM on percentage akinete formation (A) in *P. œdogonia*, zoosporangia formation (Z) in *C. glomerata* and *R. hieroglyphicum* and dead-cell formation (D) in all of them[a]

Days after inoculation	A, Z, D, %	Agar, %						NaCl, mol/L					
		0	2	4	6	8	10	0	0.10	0.15	0.20	0.25	0.50
						P. œdogonia							
3	A	30	25	–	–	27	–	39	36	32	35	38	0
	D	0	0	–	–	0	–	0	10	25	28	36	100
6	A	53	48	–	–	59	–	50	46	43	41	45	–
	D	0	0	–	–	2	–	0	35	45	40	55	–
9	A	72	68	–	–	87	–	75	52	48	53	45	–
	D	1	3	–	–	10	–	2	48	52	47	55	–
15	A	75	76	–	–	87	–	–	–	–	–	–	–
	D	1.5	12	–	–	13	–	–	–	–	–	–	–
21	A	79	82	–	–	–	–	–	–	–	–	–	–
	D	2	18	–	–	–	–	–	–	–	–	–	–
						C. glomerata							
15	Z	5	15	5	5	11	5	10	9	4	6	5	0
	D	0	10	15	23	28	30	0	3	11	15	20	100
30	Z	17	20	16	10	14	5	14	10	10	8	5	–
	D	5	20	27	42	43	60	5	15	20	26	33	–
45	Z	30	27	22	10	15	5[b]	20	18	15	12	5[b]	–
		(25)	(22)	(20)	(5)	(5)	(0)	(15)	(5)	(4)	(5)	(0)	
	D	5	34	48	80	85	95	6	20	28	40	65	–
						R. hieroglyphicum							
15	Z	10	24	22	28	23	8	10	0	0	0	0	0
	D	5	11	12	20	21	26	3.5	5	6	20	28	93
30	Z	28	27	22	28	23	8	20	0	0	4	0	0
	D	8	22	16	22	31	50	6	7	13	26	45	100
45	Z	30	27	22	30	26	8[b]	34	21	15	19	12	–
		(20)	(20)	(10)	(20)	(18)	(0)	(20)	(21)	(15)	(4)	(2)	
	D	10	26	40	50	58	68	8	9	15	36	60	–

[a]Values in parentheses show the percentage of empty zoosporangia which have released zoospores, of total zoosporangia with respect to total number of vegetative cells. All values represent rounded means of three replicates.
[b]All zoosporangia died.

Formation of akinetes and zoosporangia under water stress. The percentage akinete formation in *P. œdogonia* did not change much on agarized media (Table II) or in media progressively air-drying to complete dryness (Table III) as compared to controls, but it was decreased in NaCl-supplemented media (Table II); hence water stress was not helpful in inducing akinete formation, but decreased it and induced cell death in *P. œdogonia*. Natural population of *P. œdogonia* has been observed to form abundant akinetes under submerged conditions. Evans (1958) had shown that survival of desiccation by pond algae has no relation to the production of spores. Similarly, spore-forming blue-green algae are generally absent from desert floras (Cameron and Blank 1966).

The percentage zoosporangium formation in *C. glomerata* did not change much on all agarized media, as observed initially on 15 d after inoculation but thereafter the rate of zoosporangium formation slowed down as compared to the control. The percentage of viable zoosporangia which have released any zoospore also decreased on highly agarized media. There was also a progressive decrease

in percentage zoosporangium formation along with their viability with increasing concentration of NaCl-supplement media; all vegetative cells died without any zoosporangium formation at 0.5 mol/L NaCl or more (Table II).

Table III. Influence of progressive air drying of liquid BBM on percentage akinete formation in *P. œdogonia*[a]

Days after inoculation	No air drying of medium	Progressive air drying of medium completed in days					
		1	2	3	5	10	15
1	0	0	0	0	0	0	0
2	0	–	0	0	0	0	0
3	30	–	–	4	31	35	30
5	39	–	–	–	38	49	45
10	50	–	–	–	–	61	59
15	65	–	–	–	–	–	64

[a]All values represent rounded means of three replicates.

The percentage zoosporangium formation in *R. hieroglyphicum* appeared to be more on 2–8 % agarized media than in the control, as observed initially 15 d after inoculation but the values remained more or less the same with increasing the time after inoculation to 45 d. The percentage zoosporangium formation remained very low on 10 % agarized medium from the beginning. Most of the zoosporangia formed on highly agarized media died without any zoospore release (Table II). Zoosporangium formation in *R. hieroglyphicum* was delayed and decreased in all NaCl-supplemented media, ranging from 0.1 to 0.25 mol/L and no zoosporangium was formed above 0.5 mol/L NaCl. Most zoosporangia formed in 0.20 and 0.25 mol/L NaCl-supplemented media died without any zoospore release (Table II). Starr (1954) stimulated zoospore production in *Tetraedron* by transferring cells which had been growing on agar medium to distilled water after the agar medium had begun to dry noticeably. The percentage of dead-cell formation in all algae used in the present study increased progressively with an increase in concentration of agarized media and NaCl-supplemented media and along with the time of inoculation (Table II).

Table IV. Percentage germination of akinetes of *P. œdogonia* formed in liquid BBM, on 8 % agarized BBM and in 0.25 mol/L NaCl-supplemented medium, in liquid BBM[a]

Days after inoculation	Akinetes harvested from		
	liquid medium	8 % agarized medium	0.25 mol/L NaCl medium
5	24	32	35
10	35	40	43
15	56	65	68
20	80	90	96
25	90	99	99

[a]Values represent rounded means of three replicates.

Viability of akinetes formed under water stress. P. œdogonia akinetes formed under water stress were equally viable as those formed under no water stress (Table IV) but such was not the case with zoosporangia in *C. glomerata* and *R. hieroglyphicum*, where most zoosporangia formed under water stress did not release any zoospore (Table II). Lembi and Spencer (1981) proposed that akinetes of *P. œdogonia* insured survival during periods of desiccation caused by fluctuating water level.

Germination of akinetes and zoospores under water stress. The percentage akinete germination in *P. œdogonia* was stimulated on 1 % agarized medium more than in liquid medium, but decreased or was altogether suppressed on 2 % or more of agarized media and in 0.15 mol/L or more of NaCl-sup-

plemented media (Table V). In *C. glomerata*, zoospore germination was totally suppressed on 6 % or more of agarized media and in 0.20 mol/L or more of NaCl-supplemented media; while in *R. hieroglyphicum*, it was totally suppressed on 10 % agarized medium and in 0.20 mol/L or more of NaCl-supplemented media (Table V). The akinete germination in *P. œdogonia* and zoospore germination in

Table V. Percentage germination of akinetes of *P. œdogonia* and zoospores of *C. glomerata* and *R. hieroglyphicum* on agarized BBM and in NaCl-supplemented liquid BBM[a]

Days after inoculation	Agar, %							NaCl, mol/L			
	0	1	2	4	6	8	10	0	0.10	0.15	0.20
P. œdogonia, akinetes											
5	10	35	0	0	0	0	0	6	7	0	0
10	25	75	5	1	0	0	0	15	10	1	0
15	30	94	5	1	0	0	0	26	21	5	0
20	40	100	5	1	0	0	0	32	30	5	0
25	42	100	5	1	0	0	0	40	35	8	0
C. glomerata, zoospores											
20	25	-	10	5	0	0	0	28	15	20	0
25	50	-	50	15	0	0	0	60	50	55	0
28	100	-	99	50[b]	0	0	0	100	99	80	0
R. hieroglyphicum, zoospores											
5	5	-	10	5	4	2	0	5	5	8	0
10	85	-	80	65	60	12	0	50	50	50	0
15	90	-	82	65	60	12	0	95	80	80	0

[a]All values represent rounded means of three replicates. [b]All germlings died.

C. glomerata and *R. hieroglyphicum* appeared to be comparatively more sensitive to water stress than the formation of akinetes and zoosporangia (Table V and II). The akinete germination in *P. œdogonia* was more sensitive to water stress than zoospore germination in *C. glomerata* and *R. hieroglyphicum*; this may be due either to their large size, thick wall or dense content compared with zoospores.

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