GROWTH OF GAMETOPHYTES AT VARYING ANGLES ON CLINOSTATS AND LIGHT INTENSITIES

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ABSTRACT--Ten-day old Pteris longifolia L. gametophytes were grown on clinostats at a speed of 1 rph from 30 to 60 days at angles of 30°, 60°, and 90°. Zero degree or normal position was designated as the control. The prothallia exhibited progressively more lobulation as the dorsiventral axis approached 90°. The physiological and morphological changes were observed at approximately 10-day intervals by recording the length, width, number of rhizoids, and time of sex organ formation. Simultaneously, 10-day-old gametophytes were grown in the dark, at 15 ft-c, 95 ft-c, and 560 ft-c, in the normal position without the use of the clinostat. Optimal growth occurred at 95 ft-c. No growth took place in the dark on Zinzadzé's inorganic medium as expected.

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INTRODUCTION--Numerous species of fern plants in different stages of growth and development have been studied under various environmental, nutritional, and chemical conditions (Banbury, 1962). The fern gametophyte has been employed extensively since numerous aspects of growth may be conveniently observed with a simple organism. Reports on the gametophyte include those on the effect of gibberellin on antheridium formation (Voeller, 1964), relationship between sugar concentration and morphology (Kato, 1964), effects of auxin on cell enlargement (Miller, 1961, Miller and Miller, 1964b), effects of light on growth (Miller and Miller, 1961, 1963, 1964a), and certain chemicals on growth (Kelley and Postlethwait, 1962). Bussmann (1939, 1941) investigated dorsiventrality of fern gametophytes.

The objectives of the present investigation were to observe the effects of light and gravity on the morphology and physiology of fern gametophytes. Although emphasizing gravity effects, light effects were studied simultaneously for comparison.

MATERIALS AND METHODS--Spores of Pteris longifolia L. were inoculated on Zinzadzé's (1932) nutrient solution solidified with 2% agar in 100 mm Petri dishes. Light intensity of 200 ft-c was provided with daylight Sylvania fluorescent tubes for the initial 10-day period and a temperature of 25 C was maintained throughout the experiment. Germination occurred within 6 days and on the tenth day the developing prothallia were placed under the following experimental conditions:

1. Gametophytes were grown at angles of 30°, 60°, and 90° on clinostats that revolved at 1 rph. A position of 0°, normal position with a vertical dorsiventral axis, was designated as receiving

1 × g force. Light intensity between 73 ft-c and 95 ft-c was provided. In a later experiment the light intensity was increased and maintained at 100 ft-c for all angles on the clinostat.

2. Gametophytes were grown in continuous light, employing 40-WATT daylight fluorescent tubes at 15 ft-c, 95 ft-c, and 560 ft-c. A sample was placed in the dark to observe the effect of absence of light on gametophyte development.

Recording of length, width, and number of rhizoids was made 10 days after inoculation and subsequently at 21, 30, and 41 days.

Fig 1 here

Table 1 here

Figure 1 shows diagrammatically the appearance of gametophytes 10 days after inoculation of spores and 20 days after starting the experiment. The average dimension of 25 gametophytes (Table 1) for each experimental condition was obtained at intervals throughout the experimental period.

With the expression $G = \frac{Rw^2}{g}$, where G = gravitational force, R = radius in feet, w = radians/sec, and g = 32.2 ft/sec, the centrifugal force (gravity) imparted laterally on the gametophyte by the clinostat at 1 rph is 1.18×10^{-8} or less. The angles of growth of 30°, 60°, 90° were employed to obtain varying g forces on the dorsiventral axis of the developing gametophytes. With increase in angle from 0° to 90° the g force has been calculated to decrease. The g force imposed on the gametophyte by the variable clinostat was calculated by selection of an angle, the sine of which gave the approximate g force (Finn, Johnson, and Casey, 1964). Consequently the g force obtained was determined to be theoretically 1.0 × g for 0°, 0.87 × g for 30°, 0.5 × g for 60°, and 0 × g for 90°, using the sine of the angles of the dorsiventral axis of the inclining plants.

Fig. 2 here

OBSERVATIONS--Varying angles on the clinostats--Good growth was observed at all angles on the clinostat (Figs. 1 and 2). With greater inclination the growth pattern did not show the typical heart-shaped prothallus; instead it produced a random pattern of lobulation with an indistinguishable apical notch. There was progressively increased lobulation and fewer rhizoids as the angle of the clinostat was increased to 90°. The thickness of the thallus and the number of cells of gametophytes grown on the clinostat were comparable to those of controls. The antheridia appeared at 40 to 50 days in the normal position, whereas 50 to 60 days were required for their development on a 90° clinostat. There was no significant difference in the time of appearance or formation of archegonia. Inverting the gametophytes still subjects the prothallus to $1 \times g$ gravity, whereas plants on a clinostat receive a continuous change of the g force which provides a continually distributed geotropic influence on the behavior of the plant. Gravity force of more than $1 \times g$ can be imposed on a plant by employing a centrifuge; however, using a tilted clinostat instead of a centrifuge enables calculation and interpretation of g forces imparted on the dorsiventral axis of the plant to be less than $1 \times g$. Lobulation in gametophytes produced on the clinostat is a growth response rather than epinasty which is common in higher plants.

Fig. 3 here

Varying light intensities--At light intensities of 15 ft-c and 560 ft-c (Figs. 1 and 3) growth was slower than at 95 ft-c which was found to be optimum. The number of cells appeared to be the same at comparable stages in all cases; under 560 ft-c, however, the smaller size was due to compact cells. A morphological comparison of 20-day-old gametophytes grown under varying light intensities is presented

schematically in Fig. 1. Growth at 95 ft-c was optimum in terms of total size, number of cells, normal appearance of rhizoids, formation of gametes, and the general appearance of the thallus. Although the prothallus lagged in growth under 15 ft-c, its appearance was normal, nonchlorotic, with a normal number of rhizoids. Under the highest light intensity the prothallus had a dense growth of rhizoids and the cells were small and slightly chlorotic.

Figure 1 also shows the appearance of gametophytes placed in the dark. Total darkness did not promote growth and the size was essentially the same as that at the beginning of the experiment. After 10 days of continuous darkness, they became chlorotic and died. The comparatively few chloroplasts present were clustered toward the center of the gametophyte.

The actual measurements on the size of gametophytes under varying angles on the clinostats and light intensities are presented in Table 1. Each figure represents an average from a minimum of 25 gametophytes.

DISCUSSION--The response of a fern gametophyte to rotation on a clinostat differs from that of a higher plant which is rotating horizontally with respect to its stem axis. The higher plant develops in a distinct leaf epinasty or stem curvature. The gametophyte grows flat on the surface of the growth medium, hence, cannot respond similarly as in leaf epinasty; however, the frond of a sporophyte displays behavior similar to that of higher plants (Brain, 1935).

The fern gametophyte grown on the clinostat exhibits lobulation of the thallus margin, an effect probably due to a loss of the dominant influence of the apical notch on normal morphological appearance.

Although light intensity varied slightly from 73 to 95 ft-c, difference in size and morphology is not attributed to light intensity, but rather to the angle of the clinostat.

When a higher plant is rotated horizontally on the clinostat at 1 rph and simultaneously centrifuged at $1 \times g$ toward the roots, the growth pattern is normal as though the plants were grown in the usual upright position (Finn et al., 1964). However, when the clinostat is kept at a constant revolution of 1 rph and the centrifugal force reduced to less than $1 \times g$ in the direction of the root, plagiotropic responses are observed. Furthermore, varying the angle of the clinostat without horizontal centrifugal force results in a plagiotropic response similar to those observed for reduced centrifugal force (Finn and Brown, 1961). Tilting the clinostat produces a physiological effect similar to that of less than $1 \times g$ centrifugal force and results in epinasty of higher plants. The gametophyte response, however, is different owing to the thallus structure. The dorsiventral axis of the gametophyte corresponds to the shoot-root axis of the higher plants. Observations on the clinostat at different angles show progressive morphological changes from 0 to 90° and possibly reflect the effect of reduced gravity on the dorsiventral axis. On the clinostat the reduced gravitational effect on higher plants is actually due to equal distribution of gravity on the horizontal plant, whereas in gametophytes the effects are on the lateral margin of the prothallus. Bussmann (1939, 1941) observed the young sporophyte formation in order to determine the dorsiventrality of the apogamic gametophyte grown on a horizontal clinostat.

The effects of varied light intensities were investigated independently from the clinostat studies; however, the optimum light intensity for growth observed in this experiment was employed in the clinostat investigation. Since Zinzadzé's inorganic medium will not permit autotropic growth, a low light intensity was found to be essential. Organic supplements in the nutrient medium (Miller and Miller, 1961) are needed for growth in the dark. Kato (1963, 1964) obtained heterotrophic growth of gametophytes in the dark with a medium containing dextrose and yeast extract.

Optimal light intensities of 400 ft-c have been reported for Onoclea sensibilis (Miller and Miller, 1961); however, for Pteris longifolia on Zinzadzé's inorganic nutrient medium, the optimum growth in terms of gametophyte morphology and physiology was obtained at 100 ft-c ± 20%. The production of rhizoids was profuse at 560 ft-c. Further investigation undoubtedly will show optimal growth at a different light intensity with variations in the quality of light, nutrient, temperature, and humidity. At 15 ft-c gametophyte morphogenesis progressed normally but at a slower rate than at 95 ft-c.

SUMMARY--Simulated reduced gravity environment produces progressively more lobulation of the prothallus by increasing the angle of the plant toward 90° with the use of a tilting clinostat.

Optimal increase in growth was at 95 ft-c in normal position on Zinzadzé's inorganic nutrient solution. Lower or higher light intensities produced less growth and although the size (in mm^2) was similar, the morphology was distinctly different. With increasing light intensity the number of rhizoids increased.

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TABLE TITLE

Table 1.- Shows increase in growth for 31 days at varying angles of the clinostat and under varying light intensities.

FIGURE LEGENDS

Fig. 1.- Schematic illustration of growth of gametophytes at varying angles of the clinostat, 0° (normal position), 30°, 60°, and 90° and in darkness and under light intensities of 15 ft-c, 95 ft-c, and 540 ft-c for 20 days.

Fig. 2.- Growth rate at varying angles of the clinostat for 40 days.

Fig. 3.- Growth rate under varying light intensities for 40 days.

EXPERIMENTAL PERIOD

EXPERIMENTAL CONDITION	11 DAYS			20 DAYS			31 DAYS		
	LENGTH (MICRONS)	WIDTH (MICRONS)	AREA (mm^2)	LENGTH (MICRONS)	WIDTH (MICRONS)	AREA (mm^2)	LENGTH (MICRONS)	WIDTH (MICRONS)	AREA (mm^2)
DARK	267	80	0.021	266	74	0.019			
15 F.C.	615	451	0.277	1329	938	1.246	2244	1740	3.904
95 F.C.	1413	924	1.303	3521	3107	10.939	4678	3812	17.823
560 F.C.	551	371	0.204	1480	978	1.447	2585	2031	5.250
0°	1413	924	1.303	3521	3107	10.939	4678	3812	17.823
30°	1261	774	0.976	2602	2151	5.596	4724	3734	17.639
60°	1155	784	0.906	2077	1660	3.448	1955	1455	2.845
90°	652	486	0.317	1198	907	1.086	1406	1039	1.460

ANGLE ON CLINOSTAT LIGHT INTENSITY

Fig. 1

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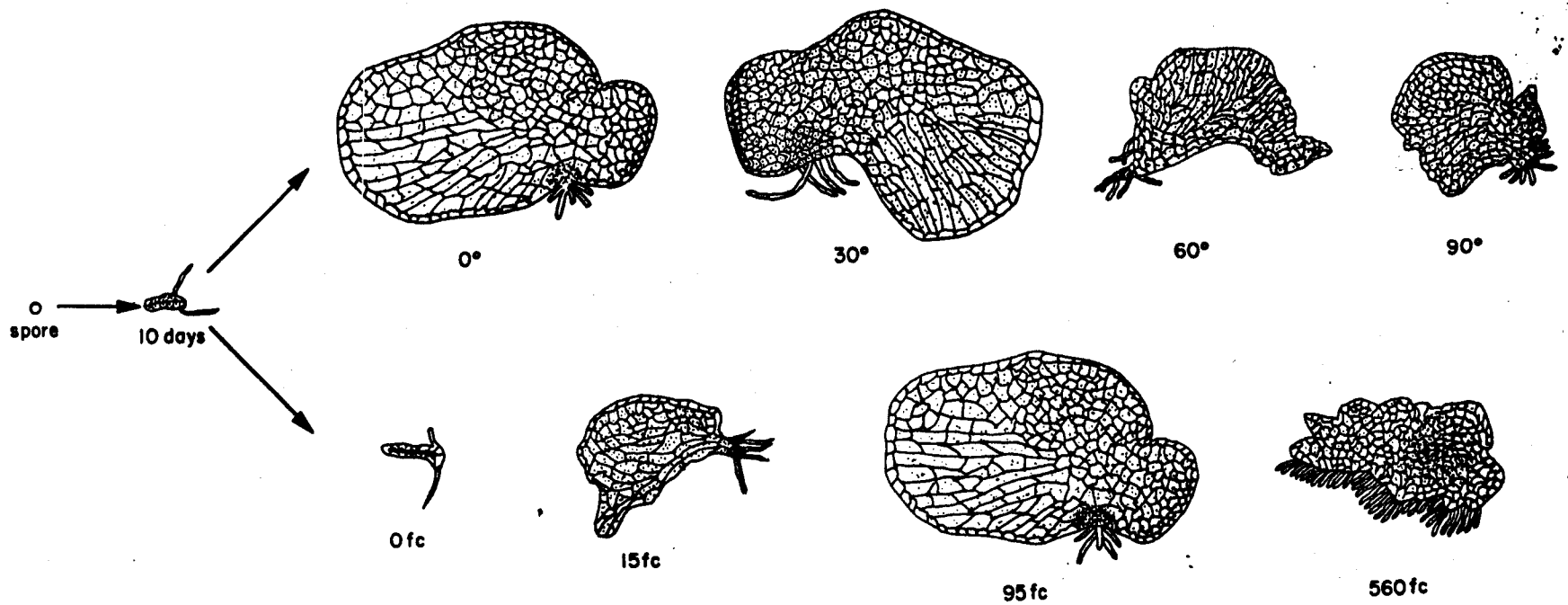

Fig. 2

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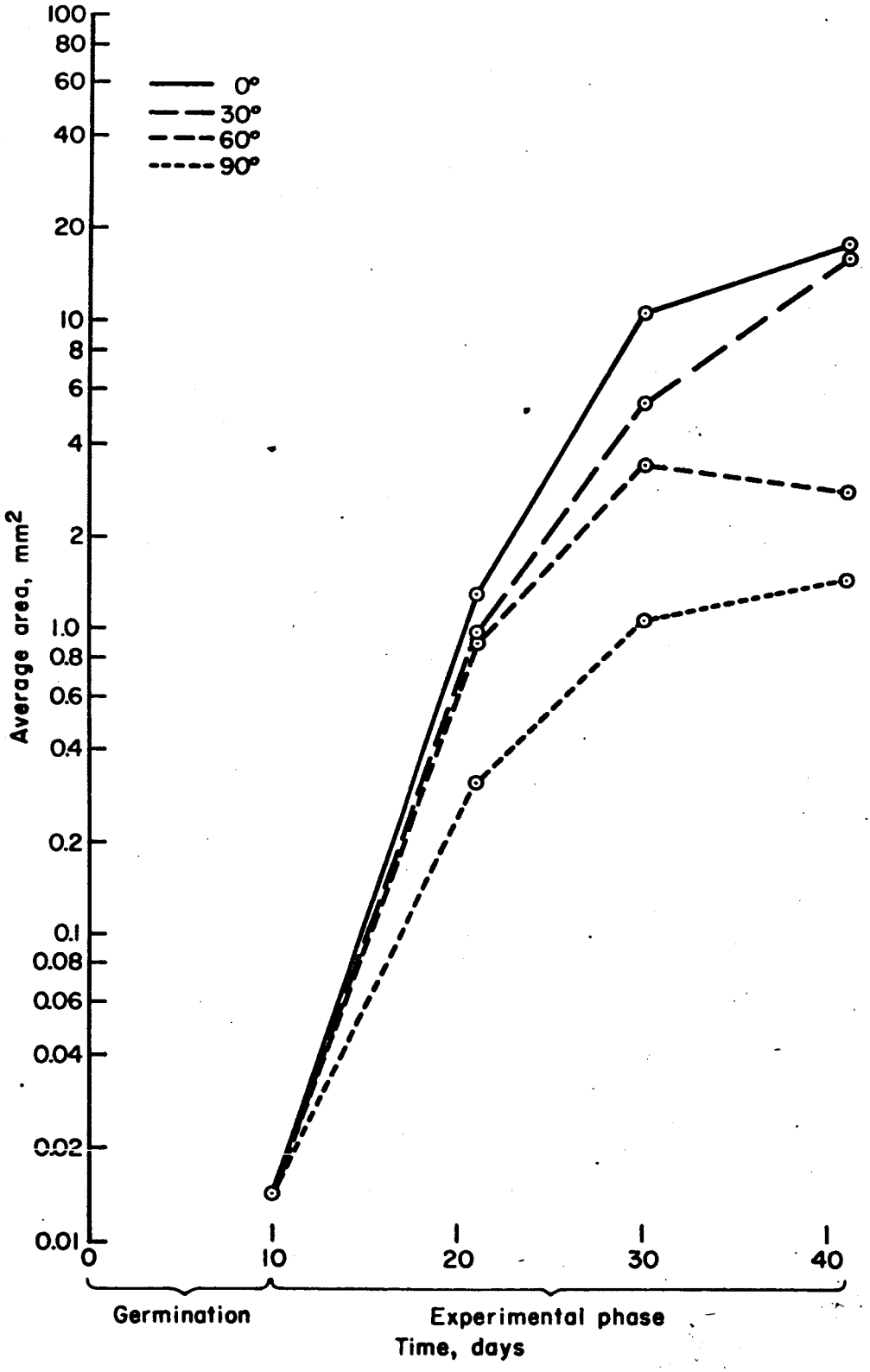

Fig. 3

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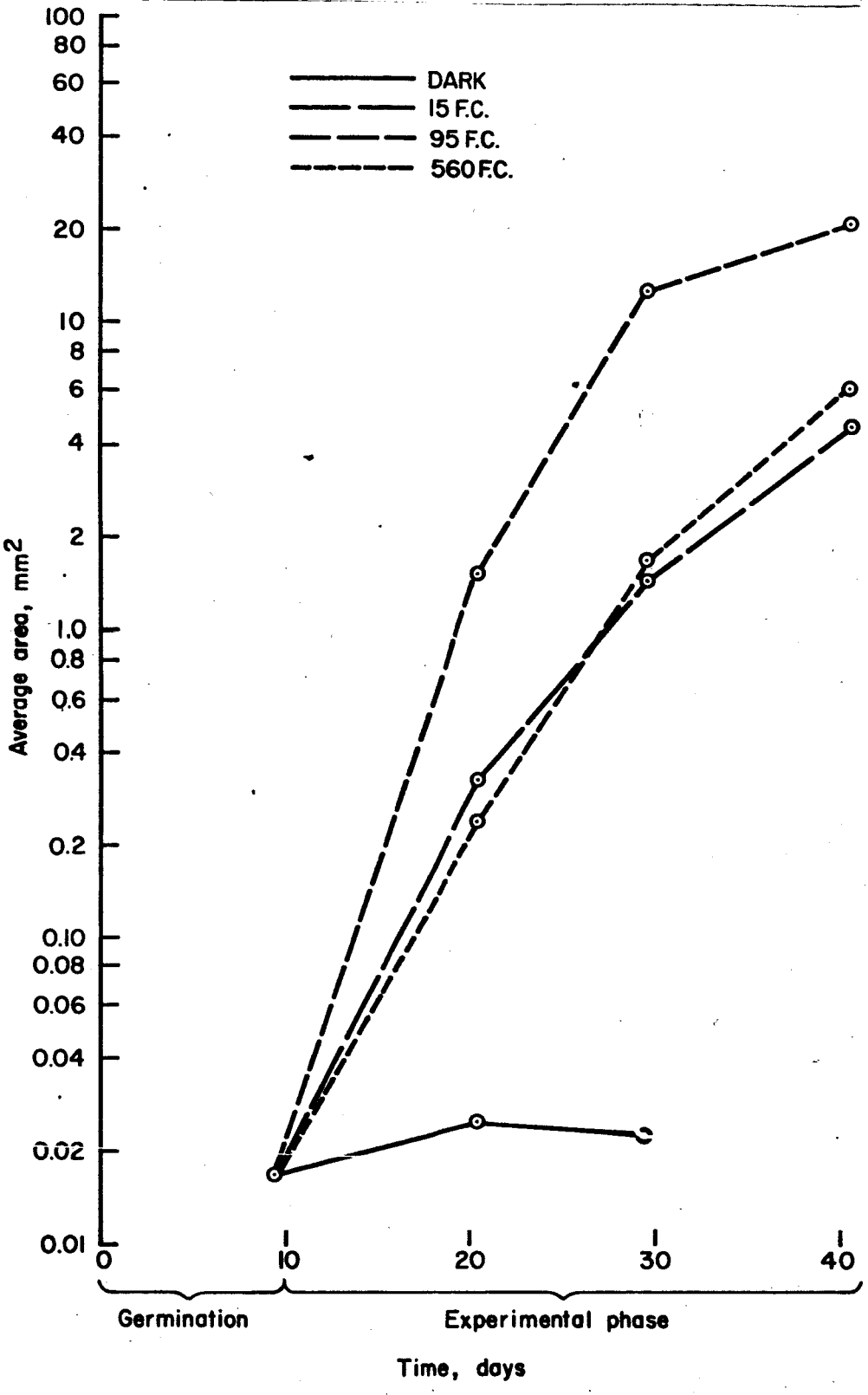

Fig. 4

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