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SOME EFFECTS OF TEMPERATURE ON THE GROWTH OF *CHARA ZEYLANICA* WILLD.

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Members of the Characeae have been used extensively in various investigations including ion accumulation, cyclosis, and cytoplasmic permeability, yet investigations on mineral nutrition and the effects of light and temperature have been meager. Concerning temperature effects Karling, 1924, using cultures of *Chara fragilis* Desvaux, concluded that formation of reproductive structures depended primarily on the length of day, and secondarily on temperature. The purpose of the present investigation was to determine certain growth responses of another species of *Chara* to the influence of temperature.

Fritsch, 1948, may be consulted for an account of the morphology of *Chara*. However, the nomenclature used in this paper for discussion of reproductive organs was used originally by Lindley, 1830, and adopted currently by Smith, 1955.

MATERIALS AND METHODS

In September 1954, specimens of *Chara* were collected from three areas in Nebraska: (1) a small pool near Arabia; (2) the Gretna Fish Hatchery; and (3) the Platteview Recreation Grounds in Louisville. The plants were identified by Mrs. Fay Kenoyer Daily of Butler University, Indianapolis, Indiana, as *Chara contraria* A.Br. (1 and 2 above), and *Chara zeylanica* Willd. (3 above). Herbarium specimens are filed at Butler University and at the University of Nebraska. The plants were established in aquaria containing tap water and approximately one inch of common garden soil covered with a half-inch surface layer of white quartz sand. Aquaria were located in a north window, and additional continuous light was furnisbed by a fluorescent lamp. One collection of *C. contraria* grew rapidly under these conditions but soon died. The other collection of *C. contraria* like *C. zeylanica*, showed a moderate rate of growth. Preliminary attempts to grow excised portions anchored only in quartz sand showed that *C. zeylancia* grew equally well in tap water or in nutrient solution, while the excised portions of *C. contraria* grew slowly or not at all. For this reason *C. zeylancia* was chosen as the experimental plant for this study.

One plant of *C. zeylanica* was selected from an aquarium and planted in a large battery jar containing soil covered by sand as mentioned above. It was kept in a windowless basement room in which the temperature was fairly uniform. An incandescent tungsten lamp burning 16 hours a day provided the only illumination and kept the temperature of the culture at about 27°C. As the plant developed, the lateral branches of unlimited growth were removed and replanted in the same battery jar where they were allowed to grow until the substrate was covered by many shoots. Thus the experimental plants comprised a single clone. After these shoots produced a luxuriant growth, branches were severed at internodes and planted in the experimental set-up with the lowest node buried in quartz sand. Measurements of growth included neither the buried node nor the internode above it. When the test shoots were planted the number of nodes and plant length were recorded.

Constant temperature tanks, located in a greenhouse, were maintained at 16°, 20°, 24°, 28°, and 32°C. These temperatures were maintained in a series of five tanks, each containing four stainless steel cans designated "A," "B," "C," and "D." Approximately one inch of white quartz sand was placed in each can which was then filled with tap water. Two clonal members of *C. zeylanica* (each bearing five nodes) were planted in can "A" in each of the five different temperature tanks. One clonal member was planted in each of the other three cans "B," "C," and "D" of each of the five tanks. All plants received daylight supplemented by a 100-watt bulb placed over each tank. A time clock regulated the light period at 16 hours per day The experiment began February 13, 1955, and the final observations were made April 1 of the same year.

RESULTS

Increments of growth are shown in Table I. Many of the main shoots ceased to grow during the course of the experiment and became covered with a layer of filamentous green and blue-green algae. A brown scum was also present on many plants. Only one plant died before the first growth increment was measured. On many plants held at 20° and 32°C. and on all plants at 28°C. the main shoots had stopped growing before the conclusion of the experiment. Of the 12 plants in which this occurred, seven plants (3 at 32°, 3 at 28°, and 1 at 24°C.) developed lateral branches whose lengths per plant totaled more than the growth of the main shoot. One of the seven showed the greatest total growth increment (46.9 cm.) with development of 37.2 cm. laterals, while the main shoot increased its length only 9.7 cm. The five remaining plants (2 at 28°, and 3 at 20°C.) developed laterals totaling less than the length of the main shoot before the latter ceased growing. The production of laterals also accounted for the largest sum of growth increments (154.4 cm.) for all five plants in any one temperature bath at 32°C. How-

TABLE 1

Growth increments of *Chara zeylanica* at various constant temperatures.

Temp. C.	Plant	Main shoot cm.	Lateral branches cm.	Total growth cm.	No. nodes main shoot	No. nodes lateral branches	Total No. nodes	Rhizoidal mass main shoot cm.
16	A_1	0.2	0.0	0.2	0	0	0	1.0
	A_2	0.0	0.0	0.0	0	0	0	0.9
	B	0.2	0.0	0.2	0	0	0	0.3
	C	0.1	0.0	0.1	0	0	0	0.5
	D	0.1	0.0	0.1	0	0	0	0.3
20	A_1	6.5*	0.0	6.5	4	0	4	4.0
	A_2	5.2*	5.1	10.3	3	5	8	2.0
	B	15.0	1.3	16.3	8	2	10	5.0
	C	9.3*	0.0	9.3	4	0	4	3.8
	D	—**	—	—	—	—	—	—
24	A_1	23.4	14.7	38.1	11	10	21	6.0
	A_2	12.7*	20.2	32.9	6	15	21	8.0
	B	21.3	15.6	36.9	12	14	26	7.0
	C	16.7	3.5	20.2	10	3	13	12.0
	D	15.3	5.5	20.8	9	4	13	8.0
28	A_1	9.7*	37.2	46.9	5	21	26	4.0
	A_2	5.8*	12.5	18.3	4	11	15	2.5
	B	5.1*	0.0	5.1	3	0	3	1.5
	C	6.6*	1.0	7.6	4	3	7	3.5
	D	7.8*	20.5	28.3	5	15	20	3.0
32	A_1	5.2*	25.5	30.7	3	19	22	4.0
	A_2	4.0*	15.5	19.5	4	21	25	3.2
	B	24.0	19.5	43.5	12	15	27	3.0
	C	6.8*	18.8	24.2	3	11	14	2.0
	D	26.0	10.5	36.5	13	12	25	3.0

* indicates those plants, the main shoots of which ceased development during the course of the experiment.
** indicates plant that died soon after planting.

ever, many of the lateral branches which developed on these plants consisted of small outgrowths that appeared to be stunted and did not develop "leaves." In all of the plants whose main shoots were actively growing at the end of the experiment, the growth of the main shoot exceeded the growth of its laterals. The greatest total growth of such a plant (43.5 cm.) occurred at 32°C. with the main shoot contributing 24.0 cm. and the laterals 19.5 cm. The largest increment of growth of the main shoot (26.0 cm.) also occurred at 32°C. The most consistent growth of main shoots, however, occurred at 24°C., at which temperature the increments of main-shoot growth for all 5 plants totaled 89.4 cm. in contrast with 66.0 cm. at 32°, 36.0 cm. at 20°, and 35.0 cm. at 28°C. This consistent growth of the main shoot at 24°C., combined with a sum of 59.5 cm. growth of laterals, accounted for the second largest sum of growth increments (148.9 cm.) for all five plants in one temperature bath. The only significant growth increment which increased with temperature was that of the sum of the laterals at one temperature. Laterals totaled 6.4 cm. at 20°, 59.5 cm. at 24°, 71.2 cm. at 28°, and 89.8 cm at 32°C.

A record was kept of the number of nodes produced by each plant in order to check the growth increments for evidence of excessive elongation. The total number of nodes developed by all five plants in a temperature bath were as follows: 113 at 32°, 94 at 24°, 71 at 28°, and 26 at 20°C. The total number of nodes which developed on laterals increased with a rise in the temperature of water baths: there were 7 nodes at 20°, 46 at 24°, 50 at 28°, and 78 at 32°C. This corresponds to the increased growth of laterals at the same temperatures. A similar relationship existed between the growth of main shoots and the number of nodes produced on the main shoots of all plants grown at a given temperature. Total new nodes on the main shoots were 48 at 24°, 35 at 32°, 21 at 28°, and 19 at 20°C. Although there were a few more nodes developed on the plants at 32°C. than at 24°C., a greater number of nodes developed on the main shoots at 24°C.

Development of the rhizoidal system was greatest at 24°C.; the plants, producing a heavy entangled mass, attained a total length of 41.0 cm. for the five plants. The shortest rhizoidal system on any plant at 24°C. (6 cm.) was longer than the maximum length attained at any other temperature. Total lengths for the remainder of the plants at the other temperatures were 15.2 cm. at 32°, 14.8 cm. at 20°, 14.5 cm. at 28°, and 3.0 cm. at 16°C. Rhizoidal development at 16°C., however, consisted of only one or two short filaments per plant.

Secondary protonemata which developed on the experimental plants had the form of lateral branches. They originated from the nodal region, but were soon distinguished as protonemata by their smaller "leaves," longer internodes, and their downward growth to the substratum. Measurements of secondary protonemata were included under lateral branch measurements in Table I. At 20°C. the plants produced secondary protonemata to the exclusion of lateral branches. At higher temperatures secondary protonemata as well as lateral branches were formed by the main shoot. At 28°C. all plants developed lateral branches which, in turn, produced secondary lateral branches.

At 16°C. the growth of shoots and the production of nodes and secondary protonemata was either absent or negligible, and was therefore not mentioned in the above paragraphs.

Of the five plants in each temperature bath, three plants each at 16°C., three at 32°C., one plant at 24°C., and one plant at 28°C. produced reproductive structures, but none were produced on plants in the 20°C. bath. These structures were horne on new growth of the main shoot of plants subjected to 24°C. and 32°C., as well as on branches of unlimited growth at 28°C. In the 16°C. bath, however, the plants failed to grow appreciably in length, and reproductive structures were produced on the main shoot of the original material.

Nucules developed beyond the yellow, linear stage, becoming spheroidal and white, only on plants grown at 32°C. At this temperature, however, fertilization apparently did not occur since the oogonial wall did not turn black (3).

DISCUSSION

The temperatures chosen were those which were easily maintained and which gave promise of vegetative growth. The low temperature of 16°C. was maintained by a flow of tap water through the tank; facilities for refrigeration were not available for maintaining lower temperatures. Copeland, 1936, reported the maximum temperature record for Characeae growing in hot springs of Yellowstone National Park to be 38.1°C. He noted that Prat, 1929, also working in Yellowstone, recorded epiphytes on *Chara* growing in tepid water. Karling, 1924, stated that cultures of *C. fragilis* soon deteriorated and died at constant temperatures above 32°C. which therefore was chosen as the maximum temperature in this study.

Although, as previously stated, the greatest total growth occurred at 32°C., the growth rate was irregular. The main shoots developed rapidly at the beginning of the experiment but ceased to grow after lateral branches were initiated. This may have been due in part to the relatively low nutritional status of the experimental plants which grew in tap water and were anchored in quartz sand. Algal contaminations of the experimental plants were also most severe at 32°C. The possibility that changes of other environmental conditions might permit maintenance of apical growth of the main shoot, development of a larger rhizoidal system, and still make use of the warmer temperature that stimulates lateral branch development was not investigated in this study. Development of the main shoot for all five plants at one temperature was most rapid and constant at 24°C. This tended to emphasize that main shoot growth at this level of nutrition progressed more rapidly at a lower temperature than that which gave maximum lateral branch development under the same nutritional conditions. However, high and low temperatures may not be the only factors which adversely affect the continuous growth of the apical cell of the main shoot. Even in *Chara,* a thallophyte, the activities of the apex may have an inhibitory effect on the development of laterals, since in all plants irrespective of temperature, in which the apical cell was growing at the end of the experiment, the growth of the main shoot exceeded the total growth of its laterals. The most striking feature shown by the data was increased lateral branch development with increase of temperature. Lateral branches and secondary protonemata were not developed in direct response to the growth of the main shoot as was clearly demonstrated by the lack of correlation between the number of nodes developed on the main shoot compared with those developed on laterals in any one temperature bath.

Ratios of growth in length to number of nodes produced for each plant varied widely for plants grown at 20°, 28°, and 32°C., but were more closely grouped at 24°C. This indicated a more consistent growth in length per number of nodes produced at 24°C. than at other temperatures. These ratios when determined for laterals and main shoots separately for each plant at each temperature showed that in general the laterals produced less growth per node while the main shoots produced more growth per node developed. This helped to establish that growth increments as measured for a small group of plants in this experiment were nearly as valid as an index of plant growth as was the number of nodes produced. Ordinarily the number of nodes produced would be considered more significant since they are produced with remarkable regularity by the activity of the apical cell which in *Chara* produced a cell that divides to form a node and an internode.

The rhizoidal development which occurred at 24°C. was nearly three times greater than the moderate growth at 20°, 28°, and 32°C. Importance of the rhizoidal system has been shown by Vouk and Benzinger, 1929, who concluded that the rhizoids were the main organs of absorption of nutritive materials and that the thallus surface had only a subordinate function in this respect. Rhizoids are, perhaps, a better index of vegetative growth than are shoots, for they are not directly affected by visible algal contaminations as are the shoots.

Under the conditions maintained in this experiment, 24°C. gave the greatest growth for the main shoots and rhizoids and the most consistent over-all growth of the main shoots and lateral branches.

The production of reproductive structures in only a few plants minimizes their value as indicators of growth rate in this experiment. Transeau, 1903, stated that in certain algae a vegetative period is necessary before reproduction occurs, and the approach of the reproductive period in *Spirogyra* sp. is accelerated or retarded by temperature, light intensity, and concentration of the mineral content of water. Although plants in the 16°C. bath showed little vegetative growth, indicating a retarding effect of temperature, reproductive structures were not prevented from developing. Karling, 1924, found that reproductive structures were borne on *C. fragilis at* 2°C. under constant illumination. He also found that nucules aborted at 32°C. This agrees with the findings of the present study. Since reproductive structures were formed during the experiment at most of the temperatures used, it would seem, as stated earlier by Karling, 1924, for *C. fragilis,* that temperature is not the primary factor in formation of reproductive structures.

SUMMARY

1. Clonal members of *Chara zeylanica* were grown at five different constant temperatures (16°, 20°, 24°, 28°, and 32°C.). Growth of the main shoots and lateral branches were recorded separately for increase in length and the number of nodes produced. The length of the rhizoidal mass was recorded for each plant at the end of the experiment and notations were made of the presence of reproductive structures.

2. Under the conditions of the experiment the most abundant growth of main shoots and of the rhizoidal system occurred at 24°C.

3. Growth at 16°C. was restricted, while at higher temperatures main shoots ceased to grow. Production of lateral branches increased with an increase of temperature but at 32°C. was limited, for the most part, to small outgrowths. Growth increments were nearly as valid as the number of nodes produced as an index of plant growth.

4. Reproductive structures developed on plants growing at all temperatures except 20°C.

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