TEMPERATURE GRADIENT EXPERIMENTS ON *GIRAUDIA SPHACELARIOIDES* (ALGAE: FUCOPHYCEAE) FROM LANZAROTE, CANARY ISLANDS

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With 4 figures

ABSTRACT. Temperature plays an important rôle in the growth and reproductive pattern in the heteromorphic brown alga *Giraudia sphacelarioides* from Lanzarote. Our experiments show that the lower lethal boundary is between 7.6 and 10.8°C and the upper lethal boundary is between 26.5 and 31.1°C. The upper lethal boundary may explain the absence of the species in the eastern part of the Mediterranean, at least in a recognizable form. Formation of macrothalli is also temperature regulated. Their formation is inhibited at 26.5°C and growth is very slow at the lower end of the temperature scale. Also with regard to formation of plurilocular sporangia there is an attenuation of the response, the optimum temperatures being between 16.1 and 20.1°C if microthalli and macrothalli are treated together.

It is suggested that the collar-like structures ("sporanges en manchons") is a developmental stage of sporangia in sori ("sporanges en pustules") and therefore they do not deserve a special term. The geographic distribution is discussed and it can not be fully explained unless we assume that temperature ecotypes have developed.

INTRODUCTION

The genus *Giraudia* DERBÈS & SOLIER was monotypic (*G. sphacelarioides* DERBÈS & SOLIER, type species) until 10 years ago when SKINNER & WOMERSLEY (1984) described *G. robusta.* They furthermore added the genus *Flabellonema* to the Giraudiaceae. *G. sphacelarioides* is characterized by its parenchymatous macrothallus with a distinct basal meristem. At fertility the macrothalli carry two types of plurilocular sporangia. In some cases they cover the entire circumference of the thallus forming a collar-like structure ("manchon" in French literature, SAUVAGEAU 1927) or they develop into a sorus ("sore en pustule", SAUVAGEAU 1927). The microthallus may also carry plurilocular sporangia and

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such can be formed later on hypomeristematic laterals from the macrothallus. The life history is always of the direct type as shown by previous investigators (SAUVAGEAU 1927, SKINNER & WOMERSLEY 1984). The swarmers from the plurilocular sporangia of each type develop into new microthalli later producing macrothalli under favourable conditions. There are numerous reports on the effect of environmental conditions on the life-history patterns of members of the Tilopteridales (= Dictyosiphonales s.l.), especially the temperature seems to play an important rôle in the shift between macro- and microthalli as well as in the formation of reproductive structures (PEDERSEN 1984). *G. sphacelarioides* was reported from the island of Madeira by LEVRING (1972) and the first report from the Canary Islands is the material under consideration (KRISTIANSEN et al. 1993). The aim of the present study is to elucidate the effect of temperature on formation of macrothalli, reproductive patterns of micro- and macrothalli in an isolate from Lanzarote. The results are used for biogeographic discussions and conclusions.

MATERIAL AND METHODS

G. sphacelarioides appeared in a crude culture of *Ectocarpus virescens* THURET which was collected in a littoral pool north of Arrieta, Lanzarote, Canary Islands on 31 January 1986. To prevent growth of diatoms in the crude culture germanium dioxide was added. One unialgal culture was obtained by pipetting germlings. The stock culture was maintained at 30‰ salinity (MV 30 growth medium, CHRISTENSEN 1993) and 15°C, and illuminated by Philips fluorescent tubes, colour 29 at a photon fluence rate of 18 μmol m^{-2} s^{-1} in a 16:8 h light:dark cycle.

Five few-celled germlings of almost equal size were inoculated in 100 ml of growth medium (MV30) with a salinity of 30‰ in crystallizing dishes, and placed on a temperature gradient plate (SIVER 1983) in 10 different temperature regimes as follows: 4.3, 7.6, 10.8, 13.7, 16.1, 18.6, 20.1, 23.2, 26.5 and 31.1°C. During the experiments the cultures were illuminated in a 16:8 h light:dark cycle, photon fluence rate 55-65 μmol m^{-2} s^{-1} provided by Philips fluorescent tubes, colour 29.

An experimental period of 30 days was used in four different experiments to test reproducibility. In the fifth experiment the final set-up was inspected after 7, 9, 11, 14, 17, 25 and 32 days, and morphological variation and presence of reproductive structures were recorded and documented by photomicrographs.

RESULTS

Growth

The inoculum died at the low temperatures of 4.3 and 7.6°C, but also at 31.1°C. It means that growth takes place in the temperature interval 7.6-10.8°C up to 26.5-31.1°C (Fig. 1).

Microthalli were observed already after 7 days of incubation, but only at temperatures of 18.6, 20.1, 23.2 and 26.5°C. At the lower temperatures of 16.1-13.7°C, 9 days were necessary before microthalli were visible and 17 days at 10.8°C (Fig. 1). Macrothalli appeared later and were recorded after 9 days, but in a much narrower temperature interval from 16.1 to 18.6°C. At 20.1°C as well as at 13.7°C it took 14 days before macrothalli appeared. The period was prolonged to 25 days at 10.8°C as well as at 23.2°C, and at 26.5°C macrothalli were not developed at all.

Germlings were observed in the temperature interval from 10.8 to 26.5°C. After 14 days at 23.2 and 26.5°C the germlings originated from microthalli due to absence of macrothalli at these temperatures. At 13.7 and 10.8°C it took 25-32 days before germlings developed.

REPRODUCTION AND MORPHOLOGY

Swarmers from plurilocular sporangia on both macro- and microthallus developed into more or less disc-shaped germlings (Fig. 2A), from which macrothalli developed in the entire temperature regime for growth except at 26.5°C. The macrothalli were initially uniseriate (Fig. 2B) with or without terminal true hairs. Later longitudinal divisions were formed in the apical part of the uniseriate filament while the proximal part was restricted to the differentiation of the meristems (Fig. 2C, arrow). A few hypomeristematic cells did not participate in the formation of the meristem, but they subsequently underwent longitudinal divisions and this proximal parenchymatous part of the macrothallus contributed later by branching to further formation of new macrothalli (Fig. 4A, arrow). Copulation and unilocular sporangia were never observed.

Plurilocular sporangia developed very quickly on the microthalli. They were observed after 7 days at 20.1, 23.2 and 26.5°C. Liberation of swarmers (recorded as presence of germlings) occurred after 8 days at 23.2°C and after 11-14 days at the other temperatures mentioned. At 18.6 and 16.1°C immature plurilocular sporangia were observed after 9 days. At still lower temperatures of 13.7 and 10.8°C it took 14 and 25 days, respectively, before fertility was introduced. The sporangia ripened after 14-27, 32 days, respectively (Fig. 1). The plurilocular sporangia on the microthalli were ovate-lanceolate structures (Fig. 2D, 4B). They resembled those on the macrothalli and occurred in immense number. Fertility in the macrothalli was delayed seen in relation to the microthalli. Plurilocular sporangia occurred after 14 days at the temperatures of 16.1, 18.6 and 20.1°C. At 13.7°C and 10.8°C the macrothalli produced plurilocular sporangia after 25 days, which also was the time consumption for their formation at 23.2°C (Fig. 1). The formation of the plurilocular sporangia followed a characteristic pattern. They developed in the apical part of the macrothallus from all cortical cells and fully developed sporangia formed a crown-like structure (Fig. 2D). The process continued basipetally and the initial stages in the genesis of the sporangia could be observed.

The cortical cells in some segments of the macrothallus underwent anticlinal divisions and formed a number of sporangial mother cells in a collar-like structure ("manchon") (Fig. 2D, 3B, arrows). In culture these structures always developed further into ovate-lanceolate plurilocular sporangia. There was no difference in the reproductive pattern through the entire temperature range.

MORPHOLOGICAL VARIATION

We have found only limited morphological variation of the macrothalli developed under the different temperatures. Macrothalli developed at 23.2°C seem however to be more slender, i.e. with fewer longitudinal walls in each segment than the comparable stage developed at 13.7°C (compare Fig. 3A and 3B).

DISCUSSION

The life history of *G. sphacelarioides* from Lanzarote is of the direct type as previously reported (SAUVAGEAU 1927, SKINNER & WOMERSLEY 1984). The swarmers from the plurilocular sporangia on either micro- or macrothalli develop into new microthalli, which later are physically connected with macrothalli. Previous authors (SAUVAGEAU 1927, SKINNER & WOMERSLEY 1984) distinguish between two types of plurilocular sporangia on the macrothalli. Our results show that the structures forming collars ("manchons") are initial stages of plurilocular sporangia in sori ("sore en pustule") as these stages in culture always come to maturity as plurilocular sporangia with a characteristic ovate-lanceolate outline. In nature it is likely that environmental conditions may close their development at an earlier stage of differentiation. Therefore, we see no reason to consider them being something different.

The gradient experiments show that temperature plays an important rôle for the development and life history of this species. The optimal temperature for growth and reproduction seems to be between 16.1 and 20.1°C where microthalli appear after 7-9 days and become fertile and macrothalli are visible after 9 days with ongoing fertility after 14 days. A dense layer of germlings can be observed after 17 days, which is the minimum generation time under the present culture conditions. Temperatures between 23.2 and 26.5°C inhibit the formation of macrothalli, but the species is still able to survive in this temperature interval as microthalli with plurilocular sporangia. The lethal temperature is between 26.5 and 31.1°C. The distribution in the Mediterranean can be explained by these results. *G. sphacelarioides* has been recorded in all parts of the Mediterranean except at the eastern part (RIBERA et al. 1992). Here mean August temperature is between 26 and 28°C, which corresponds to experimental lethal temperatures of 29 to 31°C (HOEK 1982) and this is in agreement with our observations. At experimental temperatures between 23.2 and 26.5°C,

which correspond to mean temperatures between 20.2 and 23.5°C, the species only exists as unidentifiable microthalli. Therefore, identifiable thalli should only occur in late autumn and in the spring, which is also in agreement with information in the literature, f.ex. FUNK (1955) reported *G. sphacelarioides* from December to May at low depths and only at greater depths (30-40 m) in July and August.

The northern boundary in the eastern part of the Atlantic is difficult to explain based on the results from the Lanzarote strain. According to SOUTH & TITTLEY (1986) the northern boundary is Norway (Trondhjemsfjorden, see PRINTZ 1926) with a mean February temperature of 6°C. This corresponds to an experimental temperature of 5°C, which is well below the lethal temperature in this experiment. It becomes even more difficult to explain its occurrence in Danish waters (ROSENVINGE & LUND 1947) with much lower mean February temperatures. We find that at least two solutions exist to explain its distribution in Scandinavia. Either temperature ecotypes have developed in *G. sphacelarioides* like in some other marine brown algae, f.ex. *Scytosiphon lomentaria* (DIECK 1987, KRISTIANSEN et al. 1991) or if it is assumed that only one ecotype exists, the experimental results show that it must die back to the English Channel (lethal boundary) during winter and then recolonize the rather large area further north during summer. The last possibility cannot be excluded due to the high reproductive potential and a short generation time for this marine alga with a relatively complex thallus construction. However, final conclusions must wait for studies of a strain from the northern part of its distribution area.

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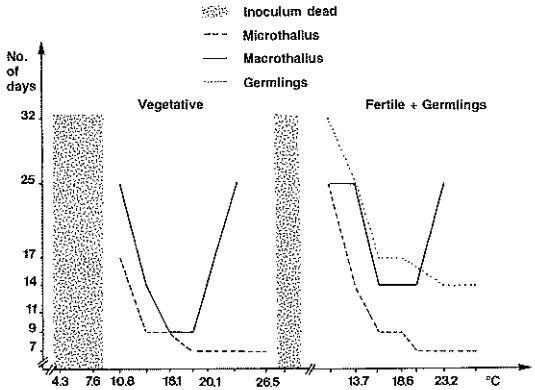

Figure 1 - Relationship between temperature and formation and fertility of micro-and macrothalli and formation of germlings.

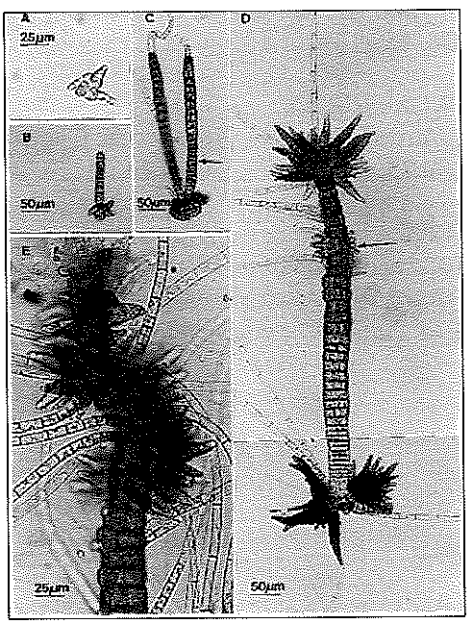

Figure 2 - Temperature gradient experiments. A: Germling. B: Microthallus with young, uniseriate macrothallus (23.2°C). C: Later developmental stage of macrothalli with apical true hairs and formation of longitudinal walls (23.2°C), (arrow indicates initial meristem). D: Well-developed macrothallus with apical plurilocular sporangia, young subapical sporangia (arrow = "manchon" sporangia) and plurilocular sporangia on microthallus (18.6°C). E: Detail of apical sorus of plurilocular sporangia (23.2°C).

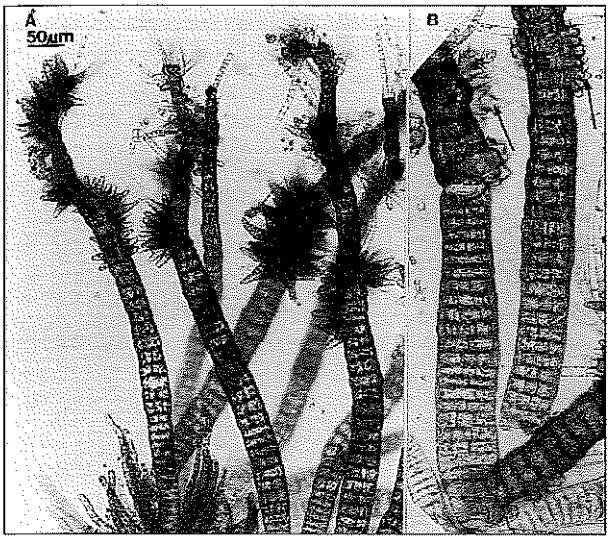

Figure 3 - Temperature gradient experiments. A: Fertile macrothalli with relatively few longitudinal walls (23.2°C). B: Comparable stage with several longitudinal walls in each segment (13.7°C); arrows show stages in the development of plurilocular sporangia.

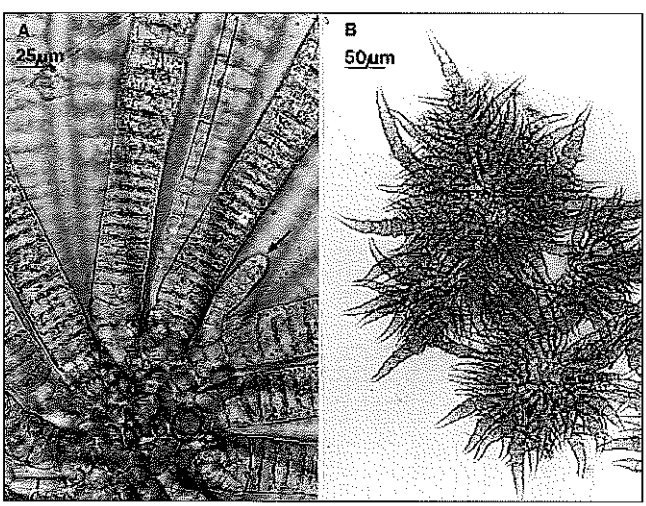

Figure 4 - Temperature gradient experiments. A: Branching (arrow) from hypomeristematic parenchymatous part of the macrothallus (18.6°C). B: Microthalli with plurilocular sporangia (26.5°C) - no macrothalli at this temperature.