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THE LIFE HISTORY OF ACROSYMPHYTON PURPURIFERUM (J. AG.) SJÖST. (RHODOPHYCEAE, CRYPTONEMIALES). ISOLATION OF TETRASPOROPHYTES. With some remarks on the tetrasporophyte of Bonnemaisonia asparagoides (Woodw.) C. Ag. (Nemalionales)

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SUMMARY

The crustose tetrasporophyte of *Acrosymphyton purpuriferum* was isolated from nature. Growth patterns of germinating carpospores of *Acrosymphyton* were compared with growth patterns of regenerating fragments of two different types of creeping plants isolated from nature. Carpospores grow into serrate compact crusts, with a distromatic centre and monostromatic margins, and so do regenerating fragments of one type of creeping plants isolated from nature. In short day conditions these compact crusts form tetrahedral tetrasporangia in the uppermost cell layer. Tetraspores grow into *Acrosymphyton* gametophytes. Fragments of the second type of creeping plants isolated from nature grow into loosely constructed monostromatic crusts which are tetrasporophytes of *Bonnemaisonia asparagoides*. The life cycle of *Bonnemaisonia* could be completed in culture starting with these tetrasporophytes.

1. INTRODUCTION

In two previous papers the life history of *Acrosymphyton purpuriferum* (J. Ag.) Sjöst. *(Cryptonemiales, Dumontiaceae)* was shown to be heteromorphic and probably diplohaplontic (VAN DEN HOEK & CORTEL-BREEMAN 1969, CORTEL-BREEMAN & VAN DEN HOEK 1970). Carpospores grow into creeping plants, closely adhering to the substratum and much resembling the species *Hymenoclonium serpens* (Crn.) Batt. The creeping plants prove to be tetrasporophytes, because in short day conditions tetrahedral tetrasporangia are formed. Released tetraspores germinate to give rise to gametophytes with *Acrosymphyton* morphology.

Recently, creeping phases growing from carpospores have been shown to occur in the life histories of a number of *Cryptonemiales* (for a review see DIXON et al. 1972). For some species such phases were previously known as separate species. In the following species the creeping phase is a tetrasporophyte: *Acrosymphyton purpuriferum* (tetrasporophyte previously known as *Hymenoclonium serpens*) (VAN DEN HOEK & CORTEL-BREEMAN 1969, CORTEL-BREEMAN & VAN DEN HOEK 1970), *Gloiosiphonia capillaris* (tetrasporophyte previously

known as *Cruoriopsis hauckii*) (Edelstein 1970, EDELSTEIN & MCLACHLAN 1971), *Pikea californica* (Scott & Dixon 1971) and *Thuretellopsis peggiana* (tetrasporophyte previously known as *Erythrodermis allenii*) (DIXON & RICHARDSON 1969, RICHARDSON & DIXON 1960). In some other species the gametophyte apparently develops directly on the creeping phase. This occurs in *Pikea californica* (CHIHARA 1972) and *Schimmelmania plumosa* (creeping phase previously known as *Hymenoclonium serpens*) (CHIHARA 1972).

In all species listed above, life history studies were started with fertile gametophytes the carpospores of which were cultured. Isolation of the creeping phase from nature was never attempted, not even for those species whose creeping phases could be expected to be recognizable in the field. Since *Hymenoclonium serpens* has been reported from two different stations in the Mediterranean (FELDMANN 1942, FUNK 1923), it seemed worthwile to search for it in order to try and complete the life history of *Acrosymphyton purpuriferum* in culture starting with tetrasporophytes isolated from nature. This research seemed particularly interesting, because a second species occurring in the Mediterranean: *Bonnemaisonia asparagoides* (Woodw.) C. Ag. (*Nemalionales, Bonnemaisoniaceae*) has a *Hymenoclonium* phase in its life history too (J. & G. FELDMANN 1939, 1942).

2. MATERIAL AND METHODS

2.1 Material

Fertile *Acrosymphyton purpuriferum* plants were found growing on an irregular rocky substrate at a depth of 16 m near Le Troc (Banyuls, Pyrenées Orientales, France) by a scuba diver on 30 August 1973. The plants were brought up together with as much of the underlying substratum as could be collected. On these stones and coralline rubble small red patches occurred, consisting of branched creeping plants of two different types. The smaller crusts (diameter 1–2 mm) were of a very compact structure, whereas the larger crusts (diameter up to 1 cm) were more loosely constructed. In the laboratory unialgal cultures were started from:

1. Vegetative fragments consisting of determinate laterals of *Acrosymphyton* (isolate 1).
2. Carpospores from *Acrosymphyton* (isolate 2).
3. Vegetative fragments of compact crusts (isolate 3).
4. Vegetative fragments of loose crusts (isolate 4).

Unialgal cultures of tetrasporophytes grown from carpospores of *Acrosymphyton* in 1967 were used for comparison (isolate 5) (see CORTEL-BREEMAN & VAN DEN HOEK 1970).

2.2 Isolation techniques

In general the isolation techniques were the same as described before (CORTEL-BREEMAN & VAN DEN HOEK 1970). In addition germination of carpospores and regeneration of fragments of the crusts was followed in cultures, kept at 16° C in

a 16 hours daily photoperiod at a light intensity of 2000 lux. Observations were made with a high power microscope, using a water immersion objective (25 × or 50 ×).

a. Observation of germination of carpospores. Small fragments of *Acrosymphyton*, bearing mature gonimocarps, were washed several times in sterilized seawater and incubated in deep 50 ml petridishes that were each glued on to a slide. Individual carpospores could be mapped with the aid of the cross table gradients of the microscope and a drawing tube. As soon as a sufficient number of carpospores had been released from the gonimocarp, the initial *Acrosymphyton* fragment was removed. The development of ten individual carpospores was followed and drawings were made every second or third day.

b. Observation of the regeneration of fragments of the crusts. Ten to twenty celled fragments of both types of crusts were cut off with the sharp edge of a broken capillary pipette. The fragments were washed in sterilized seawater several times and dragged through sterilized 2% seawater agar which contained 1% diatomaceous earth to remove epiphytic algae (KOEMAN & CORTEL-BREEMAN 1975). Each fragment was cultured separately in a deep 50 ml petridish which was glued on to a slide. A drawing of the regenerating fragments was made every second or third day. Altogether, the regeneration of ten fragments of the compact crusts and of ten fragments of the loose crusts was followed.

2.3 Culture techniques

Throughout the experiments an enriched seawater medium (Provasoli 1968) was used which was changed every two weeks. During the initial growth period of the plants only, GeO_2 was added to the medium to suppress contaminating diatoms (LEWIN 1966). Stock cultures were maintained at 16°C (± 1°C) in a 16 hours daily photoperiod provided by 40W cool white fluorescent tubes (Philips TL34) at an intensity of about 2000 lux. Light intensity was measured with an AEG lux meter.

2.4 Experimental procedure

In order to examine the influence of different combinations of daylength and temperature on the isolates 2, 3, 4 and 5, cultures were established with fragments of the crustose plants from stock cultures. A suspension containing numerous ten to twenty celled fragments was obtained by fragmenting crustose plants with a sharp needle. Two ml of this suspension was inoculated into each 100 ml petridish, containing 40 ml of culture fluid. The fragments rapidly got attached to the bottom of the petridish and grew into new crusts. The influence of four different temperatures, 4°C, 12°C, 16°C and 20°C and two different photoregimes, 16 hours light per day ($16/\overline{8}$) and 8 hours light per day (8/16) was tested. To simulate a change from "summer" to "winter" and from "winter" to "summer" conditions, after sixty days cultures from 16°C and 20°C long day ($16/\overline{8}$) were transferred to 12°C short day ($8/\overline{16}$) and cultures from 12°C short day ($8/\overline{16}$) were transferred to 16°C long day ($16/\overline{8}$). In all these experiments the light intensity given was 2000 lux. Three replicate cultures were

incubated under each set of experimental conditions. Observations and growth measurements were made once every two weeks.

3. RESULTS

3.1 The isolates

3.1.1 Vegetative isolate of Acrosymphyton (isolate 1) (see 2.1)

Excised determinate laterals of *Acrosymphyton* grew into pale red pompons, consisting of branched hypha-like filaments. After four months small organised *Acrosymphyton* plants were observed. These results support earlier findings (CORTEL BREEMAN & VAN DEN HOEK 1970). These vegetative isolates were not subjected to further experiments.

3.1.2 Carpospores from *Acrosymphyton* (isolate 2) (see 2.1)

The carpospores of *Acrosymphyton* are spherical, 14–18 μm in diameter and contain some large reticulate chloroplasts and numerous floridean starch grains. Soon after a carpospore has become attached to the substratum it begins to germinate. In most cases the first division is perpendicular to the plane of the substratum, resulting in two hemispherical cells (*figs. 1*a, *2*g, *2*k). From one or both of these cells a protuberance arises, growing into a branched or unbranched filament (*figs. 1, 2*). In some cases the protuberance arises from the undivided carpospore (*fig. 1*e). Sometimes a further protuberance is formed later from one of the original hemispherical cells (*figs. 1*f, *1*h, *1*i). When they are not in

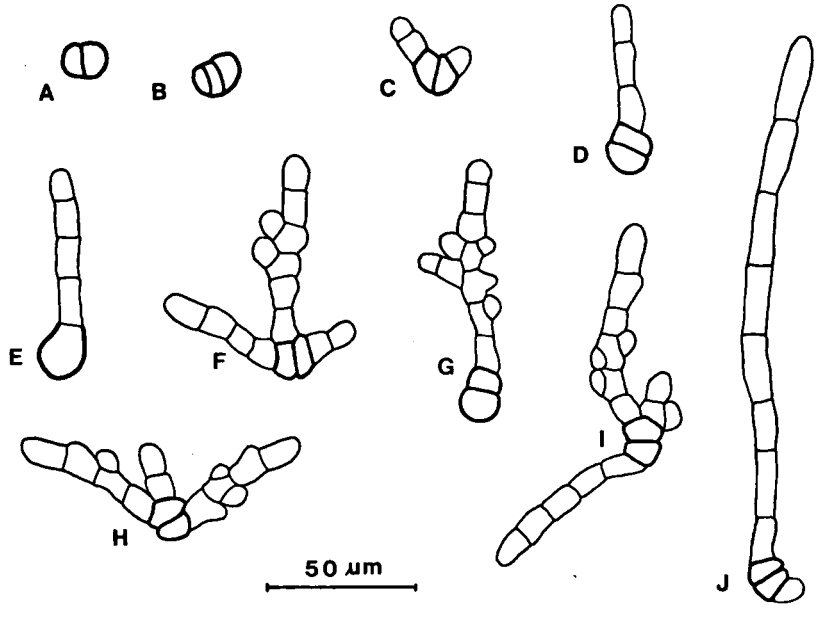

Fig. 1. Germinating carpospores of *Acrosymphyton*, 1–4 days after sporulation.

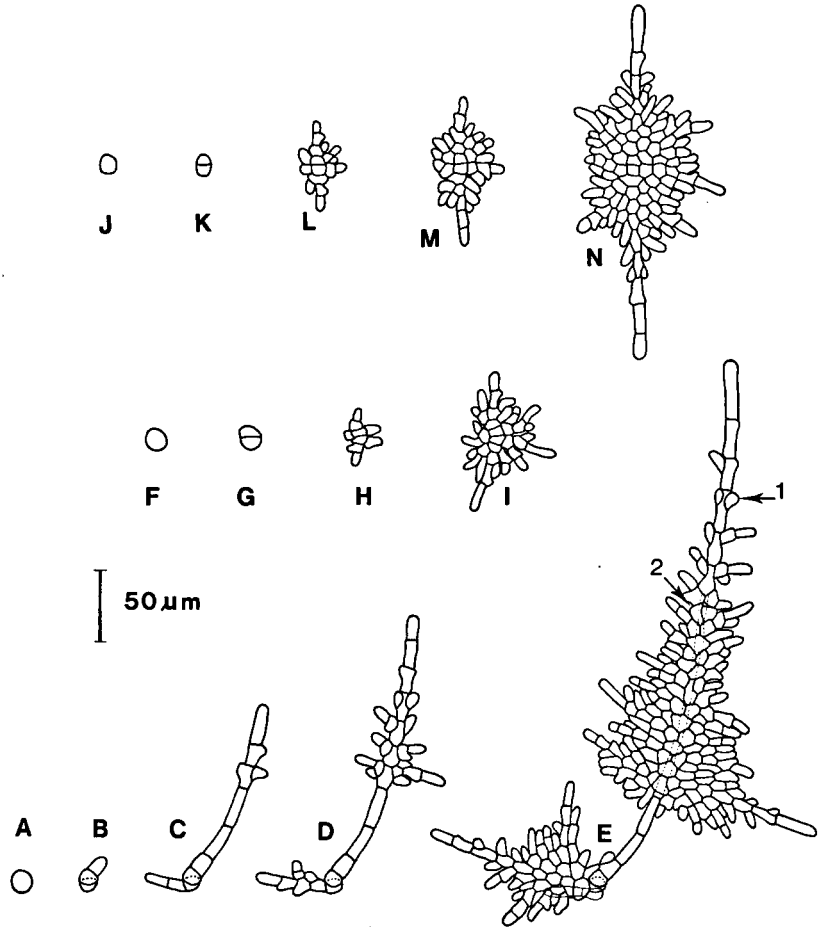

Fig. 2. Development of compact crustose plants from carpospores of *Acrosymphyton* after 0 (A, F, J), 1 (B, G, K), 3 (C, H, L), 5 (D, M), 6 (I), 8 (N) and 9 (E) days respectively. Note lenticular cells are cut off on apical (arrow 1) and antapical pole (arrow 2) of the cells of the main filament.

contact with the substratum, the filaments remain unbranched at first (*figs. 1*e, *1*j, *2*c). If a filament touches the substratum, it starts branching to form a creeping system (*fig. 2*). The branching pattern is as follows: the subapical cell, or a cell at a greater distance below the apex of the main filament, cuts off a lenticular cell on both sides of its apical pole (*fig. 2*e, arrow 1). These lenticular cells are the initials of lateral filaments. Also at the antapical pole of the filamental cells lenticular cells can be cut off on one or both sides. But these cells will develop only after the apical lenticular cells have started to grow out (*fig. 2*e, arrow 2). Both apical and antapical lenticular cells will develop in principle in the same way as cells of the main filament, but their development will cease as

soon as they touch neighbouring cells. In this way a compact pseudoparenchymatous crust is formed which is surrounded by a gelatinous layer and adheres closely to the substratum with its entire underside. The margin of the crust is serrate and the main and lateral axes remain well recognizable (*fig.* 2). Soon the crust becomes pluristromatic. One-month-old crusts are composed of two cell layers. The multi-layered nature of the crusts is difficult to distinguish in living or fixed material, the cells of both layers being mostly of exactly the same size and shape (*fig.* 7e). The margins of the crusts remain monostromatic (*fig.* 7d). Five-month-old crusts may have a central part which is four to six cells thick.

Proliferations, consisting of long erect filaments bearing callus-like clusters of cells, may arise from the centre or the margin of a crust. When such filaments make contact with the substratum, new crusts develop, so that they act as stolons (*fig.* 7c, arrow a).

Comparison of the morphology of isolate 2 with isolate 5 shows that they are identical (see CORTEL-BREEMAN & VAN DEN HOEK 1970).

3.1.3 Vegetative isolates of compact crusts (isolate 3) (see 2.1)

Vegetative fragments start their regeneration immediately. Each marginal cell of a fragment can grow into a filament which remains unbranched when not in contact with the substratum (*figs.* 3a, 3d). When touching the substratum, a filament starts branching, cutting off small lenticular cells on both sides at the apical pole of the subapical filament cell or of a filament cell at a greater distance below the apex (*fig.* 3c, arrow 1). Later a lenticular cell can be cut off on one or both sides at the antapical pole of a filament cell too (*fig.* 3c, arrow 2). These lenticular cells will develop in principle in the same way as the filament cells, but will cease growing when they touch neighbouring cells. The creeping branch system formed in this way is a pseudoparenchymatous crust within a gelatinous layer (*figs.* 3, 8a). Older crusts prove to be pluristromatic, with monostromatic margins. The structure of the compact crustose plants isolated from nature is exactly the same as the structure of the crustose plants grown from carpospores in culture (compare *fig.* 2 with *fig.* 3).

3.1.4 Vegetative isolates of loose crusts (isolate 4) (see 2.1)

Regeneration of the vegetative fragments starts after three or four days. Marginal cells of a fragment grow into branched filaments which mostly creep over the substratum. The subapical filament cell, or a filament cell at a greater distance below the apex, forms lens shaped cells at its apical pole on both sides (*fig.* 4d, arrow 1). These lenticular cells grow into laterals that branch in the same way as the main filaments (*fig.* 4). Some of these laterals are of limited growth. Thus a rather loose monostromatic creeping system develops, with only some of the cells adhering closely to the substratum (*figs.* 4, 8b, 8d). Secretory cells, recognizable by large vacuoles, arise on top of older cells of main and lateral axes at their apical ends (*figs.* 4c, 4d).

Comparison of *fig.* 4 with *figs.* 2 and 3 and of *fig.* 8b with *fig.* 8a clearly shows the distincty different morphology of isolate 4.

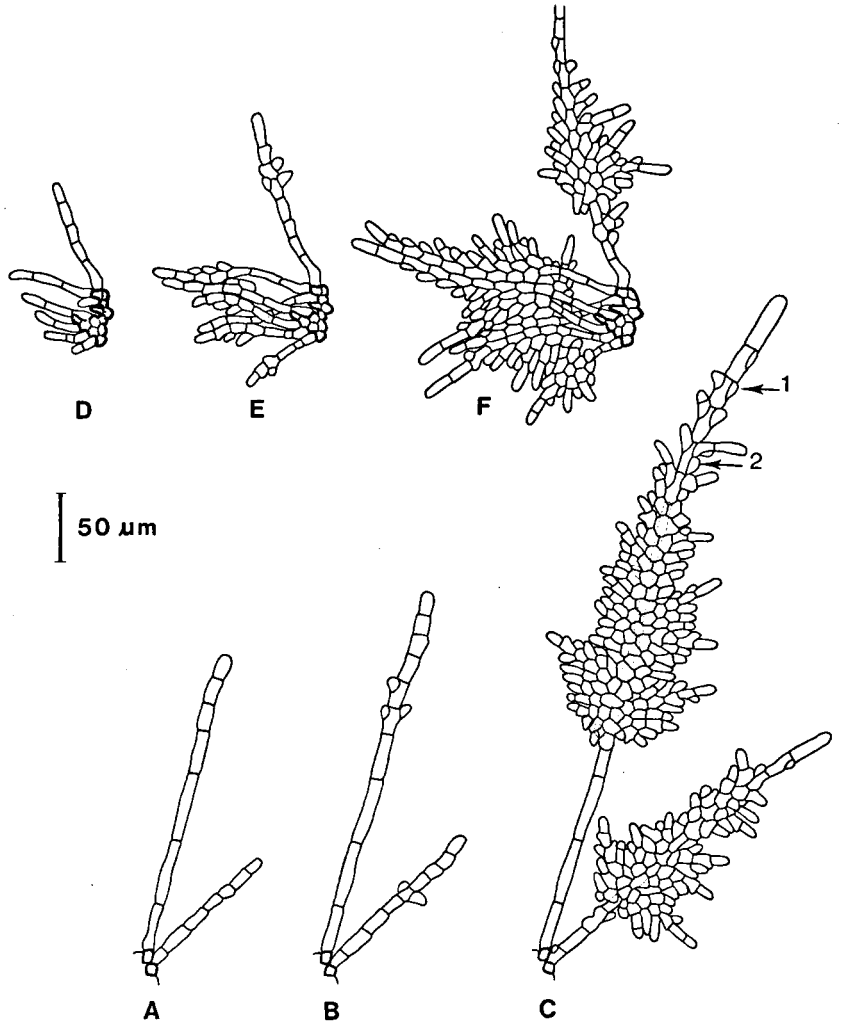

Fig. 3. Regeneration of fragments of compact crusts isolated from nature after 4 (A, D), 6 (B), 7 (E) and 11 (C, F) days respectively. Note lenticular cells are cut off on apical (arrow 1) and antapical pole (arrow 2) of the cells of the main filament.

3.2 The effect of different combinations of daylength and temperature on the crustose plants

Growth and reproduction of the isolates 2, 3, 4 and 5 (see 2.1) was studied at four different temperatures, 4° C, 12° C, 16° C and 20° C and two different photoregimes, long day (16/$\overline{8}$) and short day (8/16). Because the influence of daylength and temperature on growth and reproduction of tetrasporophytes of *Acrosymphyton* proved to be more important than the influence of light intensity

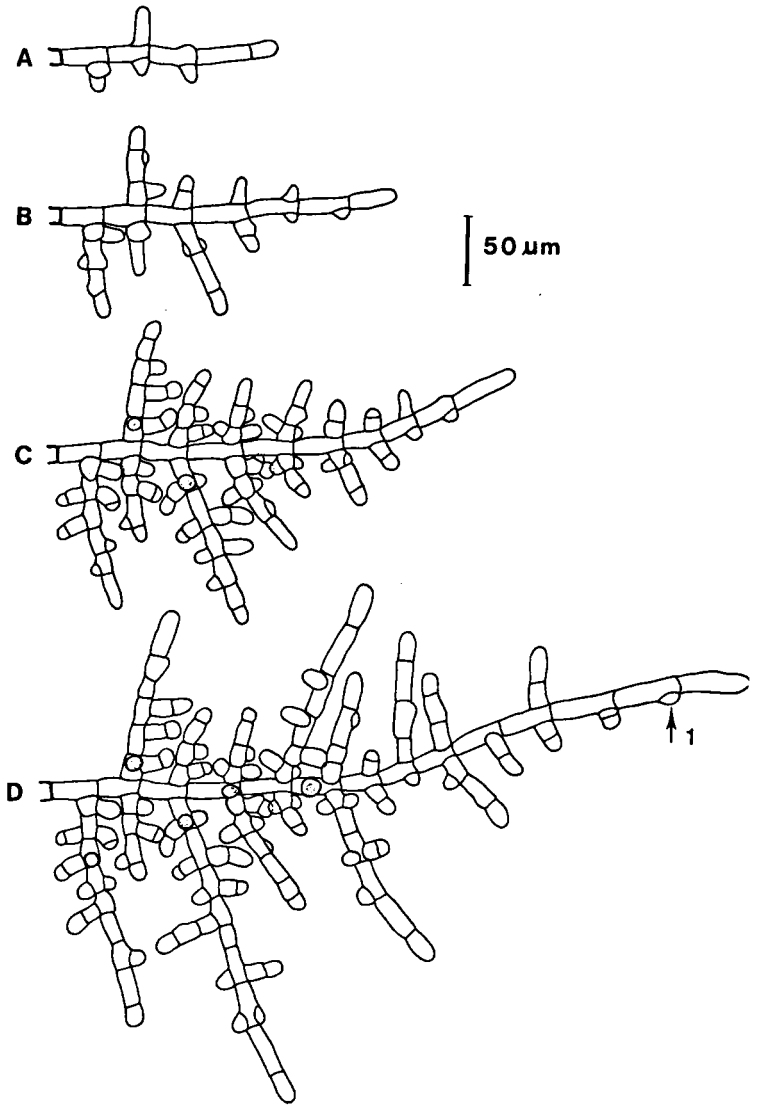

Fig. 4. Regeneration of a fragment of a loose crust isolated from nature after 7 (A), 9 (B), 12 (C) and 16 (D) days respectively. Note a lenticular cell cut off only at the apical pole of the cells of the main filament (arrow 1).

(CORTEL-BREEMAN & VAN DEN HOEK 1970), all cultures were kept at 2000 lux. After sixty days cultures from 12°C short day were transferred to 16°C long day and cultures from 16°C and 20°C long day were transferred to 12°C short day.

Vegetative growth of the crustose plants was measured as the diameter in mm of the largest plant in a culture dish. The results are presented in *fig. 6*. Each value is the average of three replicate cultures.

Reproduction was measured by estimating the quantity of tetraspores formed.

For this purpose the number of young gametophytes per culture dish was counted. The results are presented in table I and II. Each estimate is the average of three replicate cultures.

3.2.1 Crustose plants grown from carpospores in culture (isolates 2 and 5)
For isolate 2 and isolate 5, essentially the same results were obtained. In *fig. 6* (2) the results for isolate 2 are shown.

Temperature is the most important factor controlling vegetative growth. In 4°C short day as well as long day conditions the plants die within three weeks. In temperatures between 12°C and 20°C growthrate increases with increasing temperature. Plants kept at 16°C were influenced most by the length of the daily photoperiod; in long day the plants grow markedly faster. Plants kept at

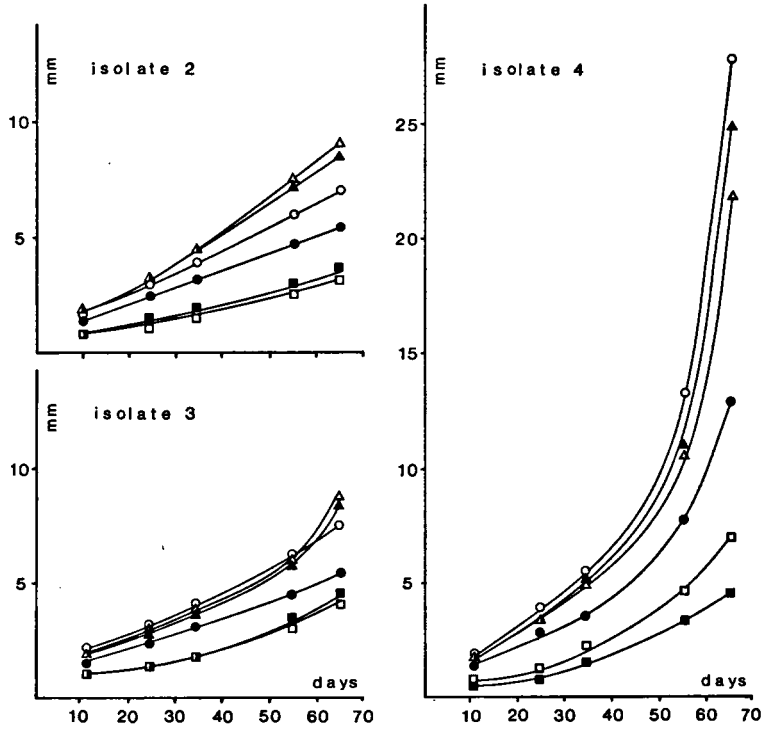

Fig. 6. The influence of daylength and temperature on vegetative growth of the isolates 2, 3 and 4. Meaning of symbols: ■ 12°C (8/$\overline{16}$), □ 12°C (16/$\overline{8}$), ● 16°C (8/$\overline{16}$), ○ 16°C (16/$\overline{8}$), ▲ 20°C (8/$\overline{16}$), △ 20°C (16/$\overline{8}$). Vegetative growth of the crusts was measured as the diameter in mm of the largest plant in a culture dish. Each value is the average of three replicate cultures.

12°C grow slightly faster in short day and plants kept at 20°C grow slightly faster in long day.

In short photoperiods at 12°C and at 16°C tetrasporangia develop on the crustose plants within three weeks (*table I*). The tetrahedral tetrasporangia are situated in the uppermost cell layer of the crusts on top of the main axes (*fig.* 7a) and in the callus-like cell clusters of the erect filaments (*fig.* 7b). Maturation and sporulation of the tetrasporangia proceeds rapidly and each crustose plant becomes surrounded by numerous germinating and ungerminated tetraspores (*fig.* 7c, arrow b). Developing germlings are soon clearly recognizable as *Acrosymphyton* gametophytes. After sixty days at 16°C the gametophytes are up to 1 cm high and fertile, at 12°C they are up to 0.5 cm high and sterile.

Tables I and II. Meaning of symbols. +++: more than 100 young gametophytes per culture dish. ++: 10–100 young gametophytes per culture dish. +: 1–10 young gametophytes per culture dish. Each estimation is the average of three replicate cultures.

Table I. The influence of daylength and temperature on the formation of tetrasporangia on the crusts.

temperature	photoregime	duration of experiment in days	isolate 2	isolate 3	isolate 4	isolate 5
			Acrosymphyton germlings	*Acrosymphyton* germlings	*Bonnemaisonia* germlings	*Acrosymphyton* germlings
12°C	8/$\overline{16}$	30	++	++	−	+++
		60	+++	+++	−	+++
12°C	16/$\overline{8}$	30	−	−	−	−
		60	+	+	−	+
16°C	$\overline{8/16}$	30	+	++	−	+++
		60	++	+++	++	+++
16°C	16/$\overline{8}$	30	−	−	−	−
		60	−	−	−	+
20°C	8/16	30	−	−	−	−
		60	−	−	−	−
20°C	16/$\overline{8}$	30	−	−	−	−
		60	−	−	−	−

Table II. The influence of a change of daylength and temperature on the formation of tetrasporangia on the crusts after thirty days.

cultures from		transferred to		isolate 2	isolate 3	isolate 4	isolate 5
temperature	photoregime	temperature	photoregime	*Acrosymphyton* germlings	*Acrosymphyton* germlings	*Bonnemaisonia* germlings	*Acrosymphyton* germlings
12°C	8/$\overline{16}$	16°C	16/$\overline{8}$	−	−	−	−
16°C	16/$\overline{8}$	12°C	8/$\overline{16}$	+++	+++	−	+++
20°C	16/$\overline{8}$	12°C	8/16	+++	+++	−	+++

The formation of tetrasporangia in short day conditions goes on infinitely. After five months numerous *Acrosymphyton* germlings are still present in the cultures. Formation of tetrasporangia is not entirely restricted to short day conditions. At 12°C long day, a few tetrasporangia had developed after sixty days. (*table I*).

In cultures transferred after 60 days from 12°C short day to 16°C long day, formation of tetrasporangia stopped within three weeks (*table II*). Abundant formation of tetrasporangia occurred within three weeks in cultures transferred from 16°C or 20°C long day to 12°C short day (*table II*).

For isolate 5 the same results were obtained as for isolate 2, both concerning vegetative growth and induction of tetrasporangia. Evidently the maintenance in culture of the tetrasporophyte of *Acrosymphyton* for five years has no effect on growth and reproduction.

3.2.2 Compact crusts from nature (isolate 3)

The influence of daylength and temperature on vegetative growth of the compact crustose plants isolated from nature (isolate 3) is about the same as on the crustose plants grown from carpospores in culture (isolate 2) (compare *fig. 6*(3) and *fig. 6*(2)). Here too, temperature appears to be the most important factor controlling growth rate, whereas the length of the photoperiod has the greatest influence at a temperature of 16°C. The only noticable difference between the two isolates is a slightly lower growth rate of isolate 3 at 20°C during the first 50 days of the experiment.

Formation of tetrasporangia in isolate 3 occurs under the same conditions as in isolate 2 (*table I* and *II*). Tetraspores grow into *Acrosymphyton* gametophytes. Evidently the compact crustose plants isolated from nature are tetrasporophytes of *Acrosymphyton purpuriferum*.

3.2.3 Loose crusts from nature (isolate 4)

The influence of daylength and temperature on vegetative growth of the loose crustose plants isolated from nature (isolate 4) is shown in *fig. 6*(4). Compared with the isolates 2 and 3, growth rates are much higher in this case (compare *fig. 6*(4) with *figs. 6*(2) and *6*(3)). Another important difference is that in isolate 4 growth rates at 16°C long day exceed growth rates at 20°C. Apparently, in long days, the optimal temperature for vegetative growth lies below 20°C.

No specialized structures for vegetative reproduction were observed on the loose crustose plants, but small fragments grew into new plants rapidly.

After thirty days no reproduction of the loose crusts was noticed, but after fifty days young sporelings had developed in the cultures kept at 16°C short day conditions (*figs, 5*a, *5*b). These plants soon showed the typical morphology of *Bonnemaisonia asparagoides*. The *Bonnemaisonia* plants did not grow on the crusts, but they had their own few-celled base (*figs. 5*a, *5*b, *8*c). Later on the plants developed a characteristically branched creeping holdfast, on which additional *Bonnemaisonia* shoots arose (*figs. 5*c, *5*d, *5*e). The loose crusts were regularly searched for tetrasporangia, but their presence could not be demon-

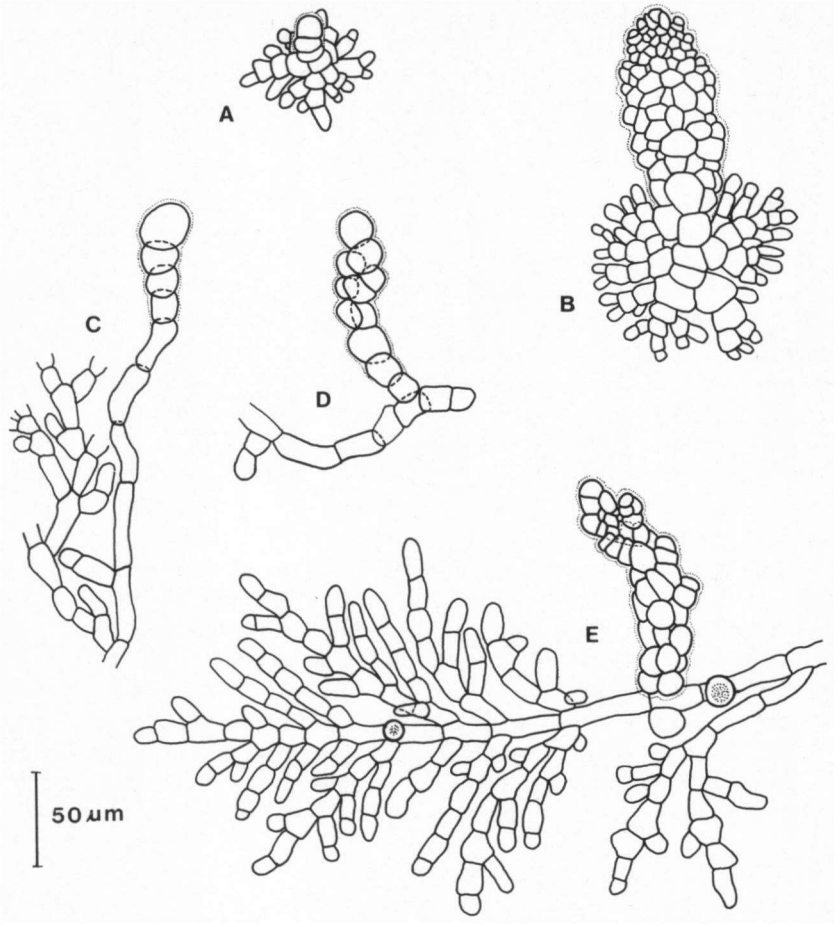

Fig. 5. A, B: Young *Bonnemaisonia asparagoides* plants. C, D: Early stages in the development of upright *Bonnemaisonia* shoots from the branched creeping holdfast. E: Branched creeping holdfast with young upright *Bonnemaisonia* shoot.

strated indisputably, though structures resembling tetrahedral tetrasporangia were observed occasionally. Since only ten to twenty young *Bonnemaisonia* plants grew in each culture dish, an abundant presence of tetrasporangia on the crusts would have been unlikely. Nevertheless the *Bonnemaisonia* plants must have been grown from some kind of spores, because they had their own few-celled base.

After sixty days at 16°C short day some of the *Bonnemaisonia* plants bore spermatangia. Two weeks later gonimocarps were observed. Fertile *Bonnemaisonia* plants were cultured separately and after some weeks fertilization occurred. The carpospores grew into crustose plants of exactly the same morphology as the loose crustose plants isolated from nature. So we succeeded in completing the

life cycle of *Bonnemaisonia asparagoides* in culture starting with a tetrasporophyte isolated from nature.

4. CONCLUSIONS AND DISCUSSION

The life history of *Acrosymphyton purpuriferum* is now completely known. In an earlier paper we showed the life history of *Acrosymphyton* to be heteromorphic (CORTEL-BREEMAN & VAN DEN HOEK 1970). Carpospores were shown to grow into small crustose tetrasporophytes strongly resembling the species *Hymenoclonium serpens* as figured in BATTERS (1895) and NEWTON (1931). We have now succeeded in isolating the tetrasporophyte of *Acrosymphyton* from nature. Earlier observations on all stages of the life history were confirmed and extended. Germination of carpospores was studied closely and our observations confirm the results of FELDMANN (1955). Tetrasporophytes isolated from nature prove to be identical with tetrasporophytes grown in culture from carpospores, concerning both morphology and the influence of daylength and temperature on vegetative growth and formation of tetrasporangia.

One question that remains to be answered, is what is the sequence of karyological events in the life history of *Acrosymphyton purpuriferum*. So far attempts to count chromosome numbers in both phases have been unsuccessful, the nuclei being very small.

Another interesting step will be to try and reach a full understanding of all factors regulating the progress of the life cycle in nature. One regulating factor undoubtedly is the induction of tetrasporangia in the tetrasporophyte in short day conditions. In nature formation of tetrasporangia could be expected as early as autumn. In culture at 12° C short day conditions, which simulate "winter" conditions in the Mediterranean, tetraspores germinate readily and young gametophytes grow quite well though rather slowly. Why gametophytes do not reappear in nature before April cannot be understood with our present knowledge. Experiments are now being carried out in which an attempt will be made to solve this problem.

Whether the formation of tetrasporangia in short day conditions is a true photoperiodic response as defined by TERBORGH & THIMANN (1964), is doubtful. Preliminary investigations showed that a thirty minutes or one hour lightbreak in the middle of a sixteen hours dark period has no effect. Similar results were obtained by WEST (1968) for *Acrochaetium pectinatum*. In *Acrochaetium* tetrasporangia were formed in short day conditions and a lightbreak of fifteen minutes in the middle of the dark period had no effect. So far no true photoperiodic responses have been demonstrated for the induction of reproductive structures in the *Florideophycidae*.

The tetrasporophyte of *Acrosymphyton* can reproduce independently of the gametophyte. Vegetative reproduction of the tetrasporophyte occurs in various ways. Four-celled structures terminating erect filaments can break off and function as propagula (CORTEL-BREEMAN & VAN DEN HOEK 1970). The erect filaments can bear callus-like cell clusters. The same phenomenon was reported in

other members of the *Cryptonemiales* such as *Dudresnaya japonica* (UMEZAKI 1968), *Pikea californica* (SCOTT & DIXON 1971) and *Thuretellopsis peggiana* (RICHARDSON & DIXON 1970). The erect filaments act as stolons; when they touch the substratum, crustose plants will develop. Regeneration capacity in the tetrasporophyte is high, even very small fragments will grow into new plants.

We were able to isolate from nature a second type of creeping plant, having a looser structure than the tetrasporophyte of *Acrosymphyton*. These loose-structured, creeping plants closely resemble the original pictures of *Hymenoclonium serpens* given by CROUAN (1859) (as *Callithamnion serpens* Crn.). These plants turned out to be tetrasporophytes of *Bonnemaisonia asparagoides*. These findings support the results of J.&G. FELDMANN (1939, 1942), who showed that carpospores of *Bonnemaisonia asparagoides* grow into *Hymenoclonium serpens* plants. Initially, J.&G. FELDMANN (1942, 1946) thought that the *Bonnemaisonia* phase grew from tetraspores, because they observed tetrasporangia on *Hymenoclonium* plants collected from nature. But KYLIN (1954) and later FELDMANN (1966) found that *Bonnemaisonia* gametophytes could develop directly on *Hymenoclonium* plants grown from carpospores in culture. In our cultures *Bonnemaisonia* definitely did not grow directly on the *Hymenoclonium* phase, but arose from some kind of spores, most probably tetraspores, because each young gametophyte had its own few-celled base. Perhaps in *Bonnemaisonia asparagoides* both modes of development can occur as has been suggested for *Pikea californica* (SCOTT & DIXON 1971). However, one can not rule out the possibility that germination of tetraspores inside a tetrasporangium was interpreted as direct development, or that both authors interpreted the basal holdfast of a *Bonnemaisonia* plant as part of the *Hymenoclonium* phase. At present experiments are being conducted to obtain more detailed information on the formation of tetrasporangia in the *Hymenoclonium* phase of *Bonnemaisonia*.

The environmental factors regulating the progress of the life cycle of *Bonnemaisonia asparagoides* in nature are still poorly understood. The occurence of young gametophytes in cultures kept at 16°C short day conditions suggests that both daylength and temperature are factors controlling the formation of tetrasporangia.

Whether in *Bonnemaisonia* as in *Acrosymphyton* the tetrasporophyte is independent of the gametophyte for its reproduction is uncertain. Special structures for vegetative reproduction were not observed, but small fragments grew into new plants rapidly.

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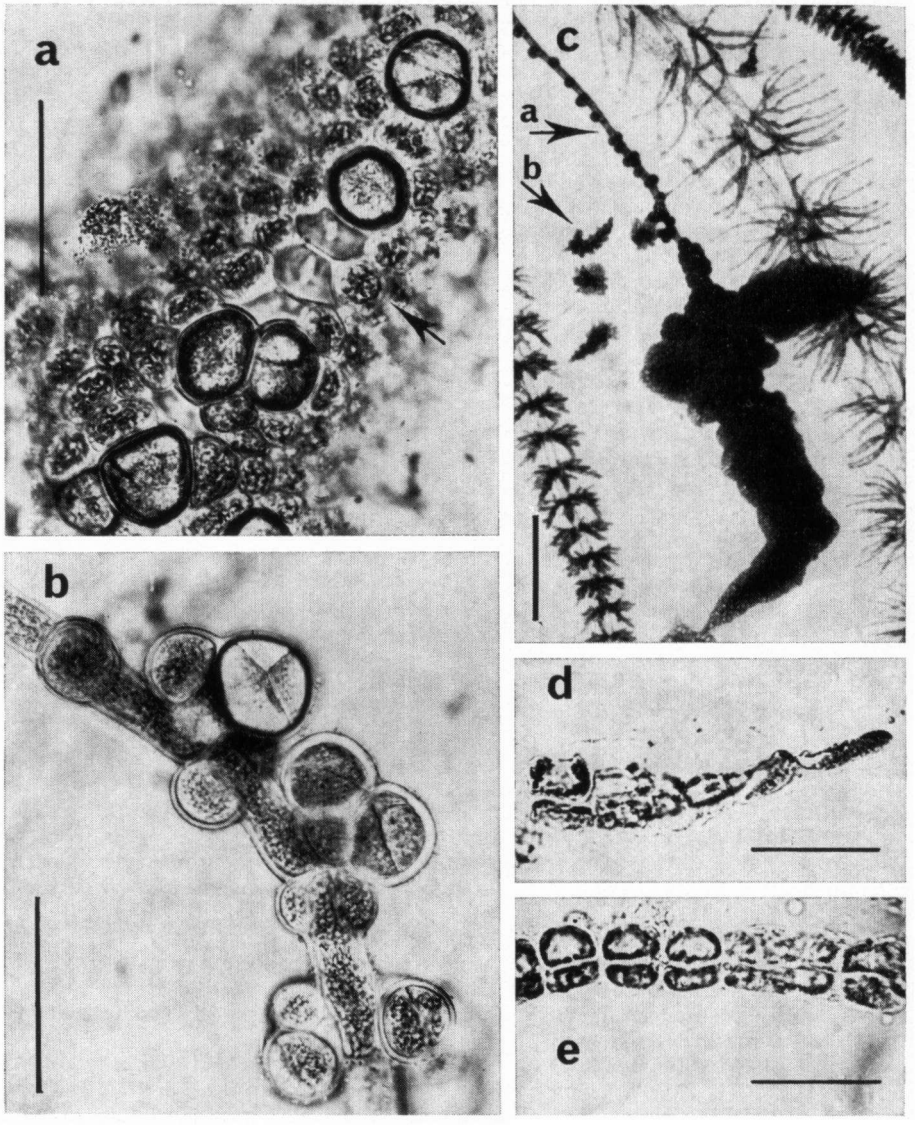

Fig. 7. Compact crustose tetrasporophytes of *Acrosymphyton purpuriferum* (isolate 2) grown at 12 °C (8/$\overline{16}$); a: scale 50 μm, tetrasporangia on a crust, note empty tetrasporangia (arrow); b: scale 50 μm, tetrasporangia on a stolon of a crust; c: scale 1 mm, compact crustose tetrasporophyte with stolon (arrow a) surrounded by young *Acrosymphyton* gamethophytes (arrow b); d, e: scale 20 μm, seven μm thick transverse section of a crust; d: margin; e: centre.

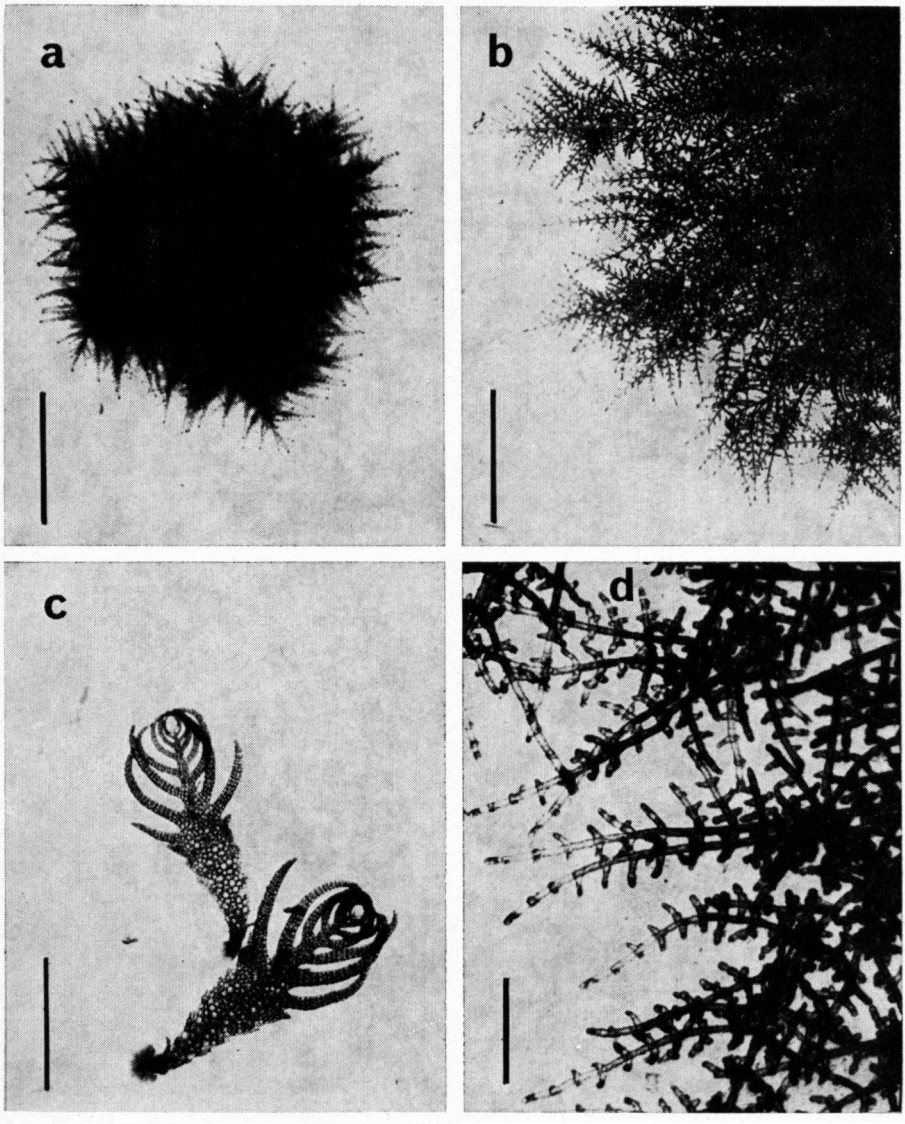

Fig. 8. a: scale 1 mm, Compact crustose tetrasporophyte of *Acrosymphyton* isolated from nature (isolate 3) grown at 16°C (16/$\bar{8}$). b, d: Loosely constructed crustose tetrasporophyte of *Bonnemaisonia* isolated from nature (isolate 4) grown at 16°C (16/$\bar{8}$); b: scale 1 mm; d: scale 200 μm. c: scale 1 mm, *Bonnemaisonia asparagoides* plants obtained in culture at 16°C (8/$\overline{16}$).