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THE ATTACHMENT AND EARLY DEVELOPMENT OF THE TETRASPORES OF SOME CORALLINE RED ALGAE

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INTRODUCTION

Corallina officinalis L. and *Jania rubens* (L.) Lamour, are both members of the Corallinaceae; until fairly recently their similarity led to their being placed in the same genus, as, for instance by Newton (1932) but the separation is now accepted and Johansen (1970) has shown that important differences exist, particularly in the reproductive structures. Ecologically there are additional differences: *Corallina officinalis* is a lithophyte occurring on a wide range of shores, both sheltered and exposed, throughout the cooler waters (0°C-20°C surface temperature) of the Northern Hemisphere. It is common on all coasts of the British Isles in littoral pools, on lower littoral rock and extends into the sub-littoral. *Jania rubens* is found in warmer waters (10°C-30°C) and in the British Isles is common only on the more temperate coasts, including the Channel Islands, Southern England west of the Isle of Wight, Ireland and northwards to Anglesey on the coast of Wales. The species is less common in Scotland and eastern England. Unlike *Corallina*, *Jania* is invariably epiphytic on other algae in pools or in the immediate sub-littoral on fairly sheltered shores. It has a restricted range of hosts; on Anglesey these are *Furcellaria fastigiata* (L.) Lamour., *Laurencia pinnatifida* (Huds.) Lamour, and most frequently, *Cladotephus verticillatus* (Lightf.) C. Ag. and *C. spongiosus* (Huds.) C. Ag.

Previous work on the morphology, life history and taxonomy of both species has appeared in publications by Rosenvinge (1917), Suneson (1943), Von Stosch (1969) and Johansen (1970). Cabioch (1966) has investigated the early development of some coralline spores but did not specify her culture conditions.

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Preliminary observations suggested that there might be differences in the settlement and rate of development of the spores which might be related to their habitats and a closer examination of these topics is the subject of the present paper. During the course of the work, observations were also made on a third coralline alga, *Melobesia minutula* Fosl. which occurs as a small epiphyte on *Jania rubens* and, to a lesser extent, on *Corallina*.

DEVELOPMENT RATE OF TETRASPORES

Materials and Methods. Fertile material of both species was collected during July and August 1970, at Rhosneigr and Treaddur Bay on the west coast of Anglesey and transferred without delay to the laboratory. Plants were maintained in a healthy condition in the re-circulating sea water system described by Jones and Dent (1970). Tetrasporic plants were more readily available than cystocarpic and tetraspores were therefore used exclusively. The spores are produced in conceptacles which were examined for the presence of mature spores before the material was used in experiments. In order to do this, selected conceptacles were first de-calcified in dilute hydrochloric acid.

To obtain spores, fertile plants or portions of plants were cleaned of obvious epiphytes and placed on glass microscope slides in shallow dishes containing a 2 cm depth of sea water. These were left undisturbed for 18 hours during which spores were shed naturally from conceptacles. The fertile fronds were then removed but, in order to allow the spores to attach themselves to the slides, the dishes were left undisturbed for a further period. This was 24 hours in the case of *Jania* but it was found that *Corallina* required longer and 48 hours was therefore allowed for this species. After attachment the slides could be handled for examination or placed in running sea water without risk of accidental detachment.

Three temperature regimes were employed :—

- 1) 10°C in a temperature - controlled tank of re-circulating sea water.
- 2) 15°C in a temperature-controlled cabinet in filtered sea water which was changed every other day.
- 3) 17-20°C in the laboratory's circulating sea water system. This temperature was not controlled and was subject to seasonal and some daily variation.

Illumination was provided for a daily period of 12 hours by means of Atlas 'Daylight' fluorescent tubes producing 1300-1500 lux at the position of the experimental material. This was the same for all temperature regimes.

Batches of 30 slides were used in each experiment, ten being placed in each temperature regime. A record was kept of the course of development and its

rate by examination of the slides under the microscope. Whilst the spores were being observed and photographed each of the slides bearing them was held in a shallow 'Perspex' tray into which it fitted closely and which contained sufficient sea water to cover the spores and prevent their desiccation.

In order to detect the onset of calcification, sodium alizarin sulphonate, which stains calcified tissue red, was employed. Dilute hydrochloric acid was also used, calcification being indicated by the appearance of a bubble of carbon dioxide which could be observed under the microscope.

Development of tetraspores of Jania rubens

In running sea water at a temperature of 17°–20°C the course of development was similar to that described by Cabioch (1966a). At the time of release the tetraspores were spherical; on becoming attached they flattened somewhat against the substratum with an increase of diameter of about 20%. Newly attached spores were found to measure from 35 μ m to 100 μ m in diameter with a modal value of 75 μ m. At this stage the light microscope revealed a halo of refractive material, presumably an attaching substance, around the spore. Division began within a few hours of attachment, the first wall being more or less upright but not apparently orientated in any particular direction related to external stimuli. A second division divided the spore into four approximately equal

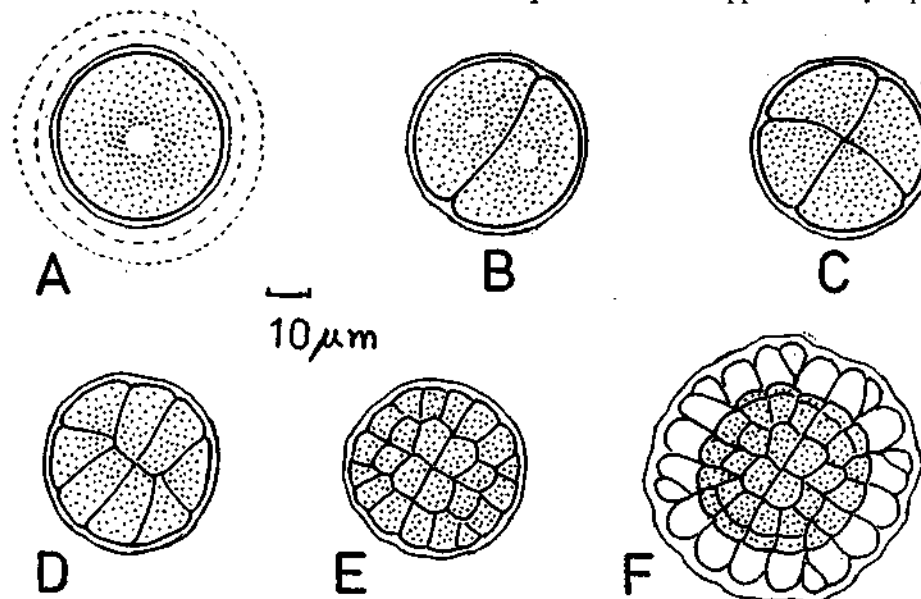


Fig. 1. Stages in the early development of tetraspores *Jania rubens* at 17°C - 20°C
 A, Newly attached spore showing 'Halo' of mucilaginous material. B, 2-celled stage, 9 hours after attachment. C, 4-celled stage. D, 8-celled stage. E, 24 cells visible at surface of spore, 1-2 days after attachment. F, Initiation of marginal growth, 2-3 days after attachment.

segments; these four cells were found, as Cabioch (1966a) reported, to behave as 'primordial cells'. Subsequent division (see Fig. 1) resulted in the formation of a 24-celled stage, as seen in surface view. Frequently additional divisions occurred in the peripheral cells to give a 36-celled stage. Up to this time no enlargement of the spore could be detected but subsequently growth began, appearing first in the marginal cells which acted as the primordial cells of the basal crust. Each of these apical cells cut off cells of the crust by tangential divisions, also undergoing frequent oblique divisions which served to increase the number of marginal apical cells and kept pace with the expanding circumference.

Calcification was first detected at the 24- or 36-celled stage when red staining could sometimes be obtained with sodium alizarin sulphonate; the production of a gas bubble by dilute hydrochloric acid could not be observed earlier than the initiation of the basal crust.

By the 6th day the basal discs had grown to a diameter of 70–120 μ m. Beyond this, there was little increase in diameter but the initiation of the upright axis was observed on the 7th day. Groups of elongated, colourless cells were cut off at the surface of the sporeling, either on the central portion formed by the original spore or on the marginal disc. These uncalcified cells constituted the basal geniculum which reached 40–50 μ m in height within 24–48 hours. In a further 24 hours the darker cells of the first intergeniculum had appeared and the cells of the second geniculum were being cut off at its distal end. Thus, within 10 days, the tetraspore had grown into a recognisable plant with a well marked upright axis and a fully formed basal attachment disc. By the 13th day the first intergeniculum was up to 200 μ m long and 50 μ m broad; its cells had become calcified very quickly.

Hairs of the type usually seen in the Rhodophyceae developed on the first intergeniculum of most sporelings in culture at 15°C. These hairs were unicellular, up to 200 μ m long and 10 μ m wide. They were deciduous and were shed after about three weeks.

In subsequent development, sporelings were often observed on which one or two additional axes developed from a single basal disc.

Development of tetraspores of Corallina officinalis

The tetraspores of *Corallina* were also spherical but smaller than those of *Jania*; newly attached spores had diameters ranging from 35 μ m to 100 μ m with a modal diameter of 58 μ m. Their development in running seawater at 17°C–20°C was as follows:

The halo surrounding the newly attached spore was considerably smaller and had a more distinct margin than in the case of *Jania*. *Corallina* spores

were more densely pigmented and this made cell divisions more difficult to observe. This difficulty was increased by the earlier calcification of the sporeling which could be detected at the 4-celled stage. Within 48 hours of attachment, a 24- or 36-celled sporeling similar to that of *Jania* was produced and growth of the basal crust began within the next 24 hours. The crust was organised in four segments, each originating from one of the four primordial cells. If any of these failed to develop, the crust would at first be deficient of the corresponding segment(s) but this became less obvious with the continuing spread of the crust. The expansion of the crust continued as in *Jania* and as described by Cabioch (1966a) but there was no early cessation of growth; the crust continued to grow to exceed 1.5 mm in some cases. Coalescence of sporeling crusts similar to that reported by Jones (1956) in *Gracilaria verrucosa* (Hudson) Papenfuss was often observed but the position of the original spore was marked by the presence of a dome of cells which reached an average height of 70 μ m and a diameter of 150 μ m in four weeks. The production of upright axes occurred much later than in *Jania*; only after 13 weeks was the first intergeniculum visible. The axes developed only from the sporeling domes and were never observed to do so from the crust in these cultures. If a dome was damaged, as sometimes occurred during the removal of epiphytic diatoms, no axis developed. However, in earlier experiments, when *Corallina* spores were grown for a long period at a lower light intensity, very extensive crusts were produced which covered the full width of a microscope slide and extended over the edges; these produced upright fronds at the edges after many months of growth.

As in *Jania*, hairs were observed. These grew from the basal crust in cultures at 15°C and 10°C.

Development of spores of Melobesia minutula

This species was identified growing epiphytically on *Corallina* and *Jania*. Spores were presumably shed by fertile plants on the experimental material since *Melobesia* sporelings appeared on the slides. The thallus of *M. minutula* is a thin fan-shaped encrustation which grows from spores similar to those of *Jania* and *Corallina*. *Melobesia* spores were measured as having diameters of 28—30 μ m. Development began very soon after attachment. The first two divisions gave rise to four primordial cells, which each divided tangentially to give rise directly to a crust initial or, sometimes, a second cell which again divided to give the crust initial. In either case a rapid development of the creeping thallus began. Fronds corresponding to both *f. typica* Fosl. and *f. lacunosa* Fosl. were observed, both were monostromatic, bright pink in colour and lightly calcified.

Effects of temperature: Results

1) *Corallina officinalis*. Growth was measured as the expansion of the basal crust taking the average of 10 sporelings in the 17°C—20°C regime and 5 each at 15°C and 10°C. The results are shown in Table 1. In this the maxi-

imum individual size attained at the end of 40 days at 17—20°C and 48 days in the others is shown together with the average growth of the 5 or 10 sporelings measured at each temperature.

TABLE 1. *Growth of Corallina sporelings measured as diameter of basal crust in the first 40-48 days*

Temperature	17°C — 20°C	15°C	10°C
Maximum size recorded	651 μ m	412 μ m	230 μ m
Average growth per day	13.6 μ m	3.7 μ m	3.0 μ m

The initiation of the upright axis showed differences of the same kind. At 17—20°C the first segment was formed in 12 weeks. At 15°C only the basal cellular dome was formed by this time and at 10°C no more than a slight rise could be seen at the position of the original spore.

2) *Jania rubens*. Growth in *Jania* was not measured by the expansion of the basal disc which was of limited growth. Table 2 shows the time taken to reach various stages at the two higher temperatures.

TABLE 2. *Time taken in days to reach successive stages in the development of Jania sporelings*

Temperature	17°C—20°C	15°C
24 cell stage	1-2	2-3
Crust initials	2-3	4
Basal geniculus	7	8
1st. segment	8-9	10

At 10°C few sporelings developed beyond the 24—36-celled stage; loss of pigmentation and death occurred at this stage or earlier.

3) *Melobesia minutula*. This species grew equally well under all three temperature regimes and development was considerably faster than that of *Jania*. Fronds, 2 or 3 cells broad, were formed within 24 hours and grew into well developed encrusting thalli recognisable as *Melobesia* plants in 3 days.

ATTACHMENT OF SPORES

Sinking Rate. The sinking rate of spores in still water is, to some extent, a measure of the time for which they can remain suspended. This rate was measured in *Jania* and *Corallina* by timing the sinking, through a measured distance (15 mm) of spores which had already been sinking long enough to reach their terminal velocity. Spores could be readily observed through a stereobinocular microscope when illuminated from the side by a beam of light at right angles to the line of vision.

The average sinking rates were 0.7 mm. sec⁻¹ for *Jania* tetraspores and 0.33 mm. sec⁻¹ in the case of *Corallina*. These differences are in accordance

with Stoke's law on the basis of the relative radii of the spores and providing that their densities are similar.

Time required for attachment. It has been mentioned that it was found necessary to allow *Corallina* spores a longer period for attachment. This is a matter with interesting ecological implications and it is also important to know if the process is a gradual one extending over hours or if it is a comparatively speedy process which is subject to a mechanism which delays its occurrence. It seemed advisable to investigate this more fully.

Method. The apparatus shown diagrammatically in Fig. 2 was designed to subject settled spores to an oblique force which could be duplicated exactly for each test.

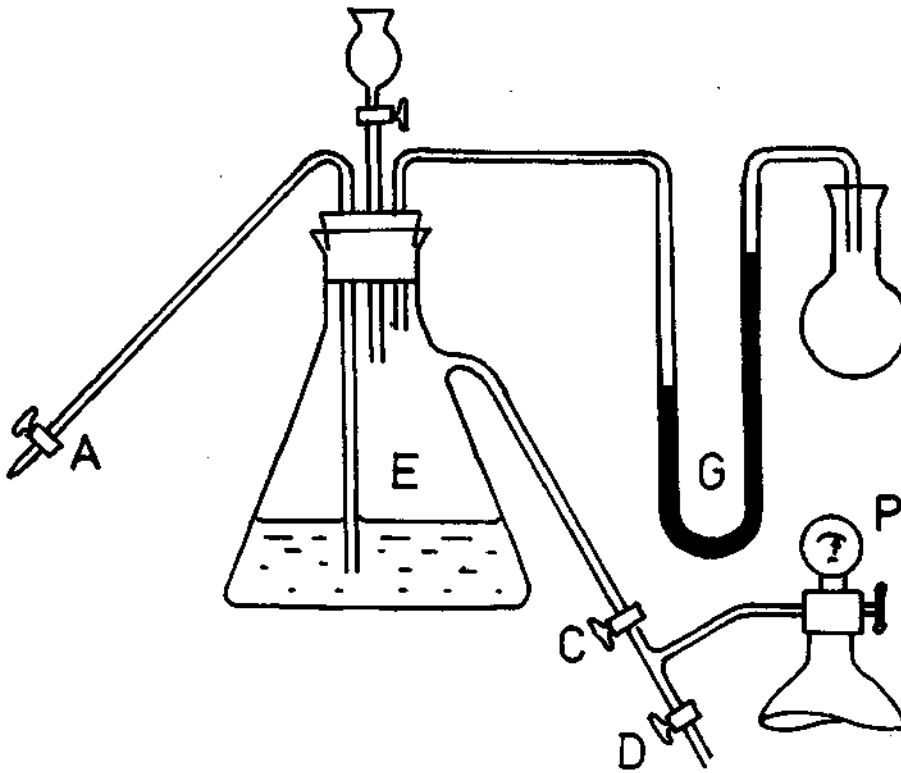


Fig. 2. Apparatus used to determine numbers of spores successfully attached. For explanation, see text.

A cylinder of compressed air was connected via a reducing valve at P. With taps C open and D closed, the pressure in the Buchnar flask E could be adjusted by the reducing valve and read on the mercury manometer G. On opening tap A, water was forced through the capillary tube. The pressure in E remained constant by the action of the reducing valve regardless of the changing volume.

In use the capillary tube was directed towards the spores on a slide held at 45° to the direction of the jet and 3 cm. away from it. The pressure was maintained at 122.4 gm. cm⁻². Fertile material was set to shed spores on to slides and was removed after one hour. Then at suitable intervals batches of three slides were removed from the dish. On each in turn the spores were counted, the slide subjected to the jet and the spores remaining counted again.

Results. Table 3 gives the percentage of spores of *Jania* and *Corallina* respectively which remained after treatment based on the totals from three slides in each case.

TABLE 3. *Percentage survival of spores under standard jet treatment*
Numbers given are totals from three slides

A. <i>Jania rubens</i> tetraspores						
Hours after shedding		Number of spores before test		Number of spores after test		% Attached
0-1	..	36	..	0	..	0
1-2	..	33	..	4	..	12.5
2-3	..	37	..	10	..	27
3-3.5	..	38	..	33	..	80
3.5-4	..	22	..	18	..	82
4-4.5	..	45	..	44	..	98
B. <i>Corallina officinalis</i> tetraspores						
Hours after shedding		Number of spores before test		Number of spores after test		% Attached
2-3	..	73	..	0	..	0
5-6	..	81	..	4	..	5
8-9	..	96	..	12	..	12
23-24	..	80	..	43	..	54
25-26	..	89	..	56	..	60
27-28	..	128	..	107	..	76
31-32	..	116	..	98	..	80
47-48	..	94	..	94	..	100

In the case of *Jania*, no attachment occurred in the first hour but subsequently the number surviving each successive test rose rapidly until after 4-4½ hours, 98% of the spores were attached. *Corallina* spores, on the other hand, attached themselves much less readily; only 5% had done so after 5-6 hours, 54% after a day and only after two days was 100% attachment observed.

Conclusions. Assuming that the attachment process is similar in all spores of a particular species, it appears that some spores can complete the process in under 2 hours in *Jania* and 5 hours in *Corallina*. However, some spores have not done so after 4 hours in *Jania* and 32 hours in *Corallina*. It seems more probable that, in these late attachments we are dealing not with a long drawn-

out cementing process but with a comparatively speedy one, the onset of which is delayed for a period which varies between individuals but is generally longer in *Corallina* than in *Jania*.

Examination of newly settled spores

Since the delay in attachment was so different in the two species and since differences could easily be seen in the appearance of newly settled spores, it was decided to investigate this aspect in more detail.

Methods. Observations by means of the light microscope was supplemented by examination under a Cambridge 'Stereoscan' electron microscope for which the spores were prepared as follows: Settlement of tetraspores was obtained onto cover glasses. Twelve hours were allowed for attachment in *Jania* and 48 hours in the case of *Corallina*. Fixation was effected in 4% glutaraldehyde solution buffered to pH7 with Cacodylate and with sucrose added to 0.25 M concentration. Overnight fixation was followed by three 30 minute washes in buffer with successively smaller sugar concentrations. The cover-glasses were then passed through liquid Arcton to liquid Nitrogen and freeze dried. A carbon film was then deposited followed by a film of 60:40 gold/paladium.

Observations. The attached spore of *Jania* was seen, under the light microscope, to be surrounded by a light halo of material spreading as much as 20 μ m from the spore and apparently of more than one concentric band of refractive material. Under the Stereoscan microscope the surface of the spore appeared to be covered by a substance which spread into a rim around the spore. Under a higher magnification, the rim appeared to consist of at least 3 layers, the lowermost spreading farthest outwards, which had a distinctly stranded appearance. The inference drawn was that a considerable quantity of some (? mucilaginous) substance had been produced in a series of discharges from the spore and spread outwards cementing it to the glass.

Under the light microscope, *Corallina* spores lacked the extensive halo and had a much more restricted and clearly defined rim resembling a cell wall. Under the Stereoscan, the exudation from the spore was much less copious than in *Jania* seemed less stranded and to be one layer only. The well defined outer edge of the rim suggested a material of greater viscosity than in *Jania*.

Chemical nature of the cementing substance

Methods. Some preliminary tests were made on the attached spores using the following stains and reagents: 1) Gurr's negative stain, the particles of which adhere to mucilage; 2) Methylene blue as a general stain for mucilage; 3) Alcian blue as a stain for acidic mucopolysaccharides; 4) Sodium alizarin sulphate as a stain for calcified material; 5) Dilute hydrochloric acid as an indicator of calcification.

Observations. Negative stain particles adhered to the surface of newly attached spores of both species and to the halo of *Jania* indicating the presence of mucilage. Blue coloration obtained with Methylene blue and Alcian blue, indicated that the attaching material was probably a mucopolysaccharide.

In *Jania* spores, sodium alizarin sulphonate produced no colour, indicating no calcification at this stage; hydrochloric acid had no obvious effect. In *Corallina* spores, however, the stain produced a red coloration, indicating some calcification and hydrochloric acid caused the spores to become detached. In view of the early calcification of the spore, it appears that this process is also involved in the attachment of the *Corallina* spore at an early stage but not in the case of *Jania*. Possibly the cementing mucilage is less important in *Corallina* and the cell wall itself plays a part in early attachment.

Other observations

1) *Tolerance of desiccation.* Both species required continuous immersion for successful attachment. After this, young, uncalcified spores of *Jania* could not survive 30 minutes out of water in the laboratory but spores of *Corallina* of the same age could survive a longer period of desiccation.

2) *Spore production.* When the rate of release of tetraspores from receptacles was investigated, it was found that *Jania* shed an average of 9 spores per receptacle (range 5-14) in 12 hours. In the same period, *Corallina* shed an average of 25 spores per receptacle (range 10-38).

GENERAL DISCUSSION

The differences described in this paper between the development patterns of *Jania rubens* and *Corallina officinalis* correlate well with the ecology of the species.

The more southerly distribution of *Jania* and its greater prominence in Anglesey shores in summer, together with its short, summer fruiting season, are reflected in its failure to develop beyond the 24-36 cell stage at 10°C. It is interesting to note that development to a similar stage, which involves cell division without growth, can take place readily in the dark in some red algae such as *Gracilaria verrucosa*, presumably at the expense of stored food reserves. The division of the *Jania* spores under unfavourable conditions which will not permit growth is analogous. *Corallina* has a more northerly distribution and a much longer fruiting season on Anglesey, reflected in its slow, but otherwise normal, development at 10°C.

Other characteristics may be related to the epiphytic habit of *Jania* contrasted to that of the lithophytic *Corallina*. Thus the more rapid development of *Jania* correlates with its growth on a living and, therefore, short-lived sub-

stratum. In this connection the still more rapid development of *Melobesia*, an epiphyte on *Jania*, seems entirely appropriate.

Jania's smaller spore output, faster sinking rate and short delay before attachment argues a reduced need for wide distribution. Where there is one suitable host plant, there will probably be another nearby and the chance of successful settlement is likely to diminish with increasing lapse of time. Suitable habitats for lithophytes are more widespread. Similarly the small basal attachment disc of *Jania* seems appropriate; an extensive basal crust as seen in *Corallina* would have no great survival value on a narrow cylindrical substratum.

In *Corallina* the long delay before attachment in the majority of its spores is arguably an advantage in a lithophyte. Such plants are more often found growing in cracks and depressions than on smooth rock; presumably initial fixation in such places has the advantages of protection against dislodgment by water movement, grazing by browsing molluscs and, in littoral situations, against desiccation. A spherical, non-motile spore is more likely to lodge in such a position if there is a period during which water currents can roll it across a rock surface. A short delay would be no disadvantage in *Jania* but here the limited range of its host plants suggests that there may be some kind of chemical stimulus triggering the attachment process when contact is made with the appropriate hosts. We have no evidence on this point, however, but have noted that, following the formation of its basal disc, *Jania* adheres much less securely to glass than does *Corallina*. Glass is much more similar to the natural substratum of *Corallina* and it may be that the attachment chemistry of *Jania* is better adapted to fixation to plants. Our observations suggest that the cell wall of *Corallina* is more directly concerned in the attachment of the plant than in the case of *Jania*.

Although we cannot conclude that the attachment process in *Jania* is essentially different from that of *Corallina*, it seems that there are at least differences of degree. A reasonable conclusion would be that there may be differences as large or greater in the attachment process of other Rhodophyceae and that the investigation of these mechanisms should be a fruitful field for study.

SUMMARY

The growth rate of tetraspores of *Jania rubens* (L.) Lamour and *Corallina officinalis* L. at different temperatures correlates well with their geographical distribution. *Corallina* grows slowly at 10°C whereas *Jania* will not develop beyond an early stage at this temperature. Subjection of settled spores to a standardised water jet showed 98% of *Jania* spores securely attached after 4½ hours, but the same result in *Corallina* was obtained only after 2 days. Examination of spores showed that *Jania* spores produced more mucilage than those of *Corallina*. Dilute acid caused the detachment of *Corallina* spores but not those of *Jania*. Development after attachment was faster in *Jania* where an axis grew from a basal disc of limited size in 8 days. In *Corallina* broad basal crusts

produced axes only after 12 weeks. *Corallina* sporelings became calcified at an early stage; *Jania* considerably later. It is suggested that *Jania's* more rapid development, its limited basal development and shorter delay before attachment are related to its epiphytic habit, while the longer delay in *Corallina's* attachment allows wider dispersal and its wide basal spread and slower development are not disadvantageous on a longer lasting stony substratum. It is also postulated that similar or greater differences may be found between the attachment processes of other red algae.

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