OBSERVATIONS ON THE STRUCTURE, LIFE HISTORY AND BIOLOGY OF *MYCOSPHAERELLA ASCOPHYLLI*

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(With 5 Text-figures)

Pseudothecial development is initiated by the growth of a weft of hyphae in the cortex of the host receptacle. This weft enlarges to form a solid plectenchymatous stroma in which (1)3–5(7) ascogonia arise. Evidence available indicates that spermatization of the ascogonia occurs by means of filiform spermatia produced in flask-shaped spermogonia which also develop in the host receptacle simultaneously with the pseudothecial stromata, from similar hyphal wefts. Further development leads to the production of bitunicate asci which discharge their ascospores forcibly in water and in air. No paraphyses or pseudoparaphyses occur. Pseudothecial development of *Mycosphaerella ascophylli* Cotton differs little from that of known terrestrial species of *Mycosphaerella*. Infection of the host appears to occur by egg-borne ascospores and egg-borne hyphae. A comparison of *M. ascophylli* and *M. pelvetiae* Sutherland indicates that these species do not differ sufficiently to be regarded as distinct.

The genus *Mycosphaerella* includes many terrestrial species which cause common leaf- and stem-spot diseases in angiosperms. Many of them are parasitic on economically important crop plants and extensive studies have been made on these terrestrial representatives of the genus. Only two species of *Mycosphaerella* have been recorded from marine environments, namely *M. ascophylli* Cotton, which is endophytic in *Ascophyllum nodosum* (L.) Le Jol. and *M. pelvetiae* Sutherland, an endophyte of *Pelvetia canaliculata* (L.) Dcne. & Thur. The observations presented are concerned with *M. ascophylli*.

Church (1893) first drew attention to the fungus when he noted the presence of minute perithecia in the 'pods' of *A. nodosum* examined in early spring. Later, Cotton (1908) described and named the fungus *M. ascophylli*. Cotton stated that the perithecia were restricted to the receptacles of the host, and that if the receptacles were examined from December to June they would almost invariably be found to be infected with the fungus. On sectioning the receptacles he found a large quantity of slender mycelium traversing the tissue in all directions and penetrating the main fronds of the alga. The perithecia were described as being completely immersed in the host tissue, with a scarcely protruding ostiole, and containing very few asci. Almost ripe spores were found by Cotton in most perithecia from February to May.

Cotton pointed out that although the mycelium was very abundant, the

host remained uninjured and suggested that the endophyte was perennial. The fact that 'quite young plants' of *Ascophyllum* were found to contain mycelium suggested to Cotton that the infection of the host took place at a very early stage, and since the ascospores of the fungus and oospores of the seaweed were released at the same time the possibility of the ascospores being caught in the mucilaginous substance surrounding the egg and infection taking place in the early stages of segmentation of the egg was suggested.

David (1941, 1943), in a study of the autecology of *A. nodosum*, mentioned briefly the occurrence of *M. ascophylli* in his discussion of the seasonal decay and exfoliation of the *Ascophyllum* receptacles. It was noted that the decay of the host receptacles was 'coincident with the appearance of the perithecia' of the fungus but left unanswered the question whether the decay of the receptacles and their stalks could be attributed either wholly or partially to the activity of the endophyte.

Wilson (1951), following a survey of the marine pyrenomycetes occurring on seaweeds, rope and wood on parts of the coast of Wales (Cardigan Bay and Dale) and Northern Ireland (Newcastle, Co. Down), recorded the occurrence of *Mycosphaerella* on all plants of *Ascophyllum* investigated in these localities.

MATERIAL AND METHODS

The material used during this investigation was collected from the 'College Rocks', Aberystwyth, Cards., and gave the following measurements: pseudothecia, $85–155 \times 75–115\,\mu$; asci, $45\cdot5–64 \times 17\cdot5–21\cdot5\,\mu$; ascospores, $17\cdot5–22\cdot5 \times 4–5\cdot5\,\mu$. These are larger than Cotton's (1908) figures which fall within the range.

Collections of *Ascophyllum* receptacles were made at weekly intervals from September 1954 to September 1955 to secure material showing a complete annual cycle of development of *M. ascophylli*. Material to be used for permanent preparations was fixed at the time of collection in either chromacetic mixture or Flemming's fluid (weak) and later embedded in paraffin wax.

Microtome sections of embedded material were cut from 6 to 15 μ, stained in Heidenhain's haematoxylin and counterstained with erythrosin, light green or orange G. When Flemming's fluid was used as a fixative the sections were bleached in hydrogen peroxide before staining. Haematoxylin provided a satisfactory stain for most stages of development of the pseudothecium, but some difficulty was experienced in following the course of ascogonial development in the young pseudothecium due to the extremely small size of the structures and the consequent difficulty in proper differentiation during destaining. Further, the coiling of the ascogonia which takes place during their development made their interpretation difficult in cases where sections contained several incomplete ascogonial coils. In view of these difficulties squash methods were used in addition to the paraffin method during the examination of the younger stages. In this method young pseudothecia were dissected from thick sections of fresh host receptacles, mounted and stained in one of the later mentioned reagents, and squashed out between the slide and the cover-glass. This

technique provided a useful method for investigating the nuclear condition of the ascogonia and their derivatives. The reagents and methods used included Heidenhain's haematoxylin (Barrett, 1932), acid fuchsin (Barghoorn & Linder, 1944), basic fuchsin (de Lamater, 1948), iron alum acetocarmine (Godward, 1948), iron acetate acetocarmine (Evans, 1948) and Azure 'A' (Huebschman, 1952). The cell walls of the ascogonia and their derivatives however seemed to be only slightly permeable to each of these stains and it was only with great difficulty that the stains could be induced to penetrate the cell walls to stain the nuclei and cytoplasm within. An attempt was made to render the cell walls more permeable by the use of enzyme extracts obtained from the stomach of *Patella* sp. (Emsweller & Stewart, 1944; Fabergé, 1945), but with little success. Of the squash methods used, Godward's (1948) iron alum acetocarmine technique gave the best results in staining the nuclei of the ascogonial stage of perithecial development but even this method was not completely satisfactory.

<div align="center">STRUCTURE AND DEVELOPMENT</div>

<div align="center">*The mycelium*</div>

The association of the fungus with the alga is extremely widespread. Church (1893), Cotton (1908) and Wilson (1951) never found *Ascophyllum* free from the fungus. During the present investigation a large number of plants of *Ascophyllum* were examined from the Aberystwyth district and in every case the fungus was present. This was true also for a small sample of Swedish material and for a sample of *A. nodosum* var. *mackaii* which was examined. It seems likely that every plant of *Ascophyllum* is infected with *M. ascophylli*.

The mycelium of the fungus is composed of very fine septate hyphae, 1–1·5 (2) μ diam, which present a characteristic glistening appearance when viewed microscopically. Branching occurs regularly though not profusely and the mycelium forms a network traversing the mucilage-filled spaces between the host cells. The mycelium has been traced throughout the whole of the plant of *Ascophyllum* and has been found to be present in an intercellular position in the cortex and medulla of the hapteron and all parts of the vegetative thallus and its branches. During the fruiting season of *Ascophyllum*, mycelium is present in all parts of the receptacles and their stalks and within the cavity of the conceptacles of the host, where branches of the mycelium either lie freely in the cavity, or run for part of their length along the surfaces of the host paraphyses or oogonia. Although the mycelium is abundant throughout the tissue of the host, no haustorial structures have been observed, and the host plant displays no apparent injury or ill-effect as a result of the presence of the fungus in its tissues.

At the apical growing points of the vegetative branches of the thallus and of young receptacles, mycelium of the fungus has been found to be present at a distance of six to ten cells behind the apical cell (Fig. 1A). It appears that the growth of the fungus keeps pace with that of the alga, ensuring that the mycelium is distributed throughout the whole of the host plant. The infection is therefore systemic.
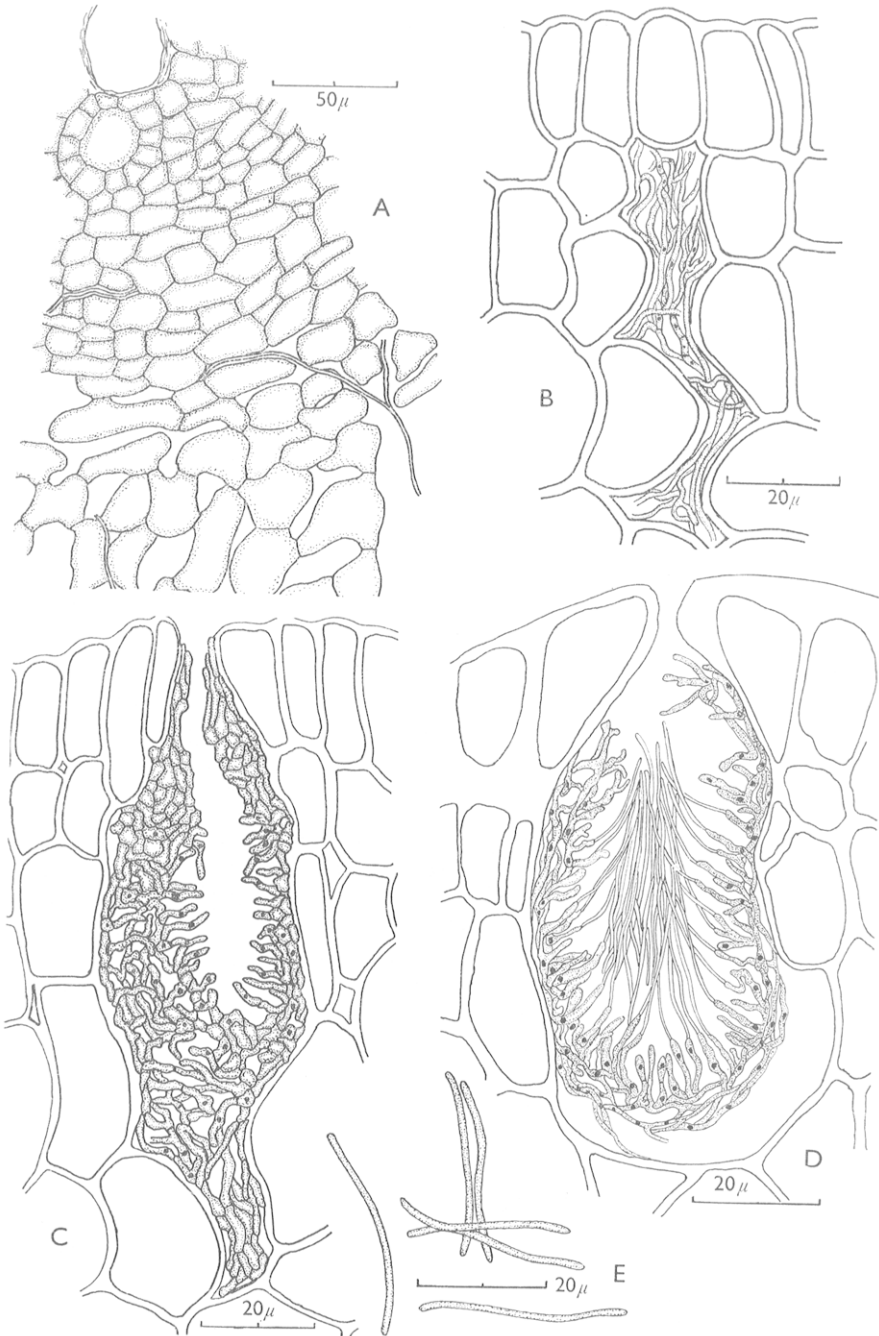
Fig. 1. *Mycosphaerella ascophylli*. A, Mycelium near apical cell of thallus of *A. nodosum*; B, fruit body initial; C, immature pycnidium; D, mature pycnidium; E, pycnidiospores.

The initiation of fruiting bodies

Although the mycelium of the fungus extends to all parts of the host plant the reproductive phase is restricted to the receptacles.

Young receptacles of *A. nodosum* appear in May and June as lateral outgrowths of the thallus of the alga. Sections of these lateral outgrowths from May onwards reveal that mycelium of *M. ascophylli* is present and keeps pace with the growth of the receptacles, the mycelium ramifying between the cells of the cortex and of the medulla.

The fructification initial (Fig. 1 B) consists of a small knot of hyphae. The hyphae branch abundantly and collect below the surface of the host receptacle, between the cortical cells. During November the hyphae increase in amount of branching and occupy an increasingly larger space between the cortical cells of the receptacle.

Two types of fruit body develop in the receptacles of *A. nodosum*, the pseudothecial state and a previously undescribed pycnidial state. During their early development the initials of these two types of fruit body are indistinguishable.

The pycnidial state

The development of the pycnidial state from the rudimentary knot of hyphae occurs by a continued increase in growth and branching until it finally extends to the surface of the host. As further growth proceeds the hyphae become more compact at the periphery of the young fruit body (Fig. 1 C) except for a small apical region which remains open as the ostiole. Simultaneously the hyphae towards the centre of the fructification become less evident and finally disappear from this position.

This course of development produces a flask-shaped fruit body (Fig. 1 D), $60-100 \times 40-60 \mu$, bounded by a thin-walled layer of colourless hyphae forming a compact plectenchyma. Within the wall layer at the base and sides of the fruit body is a lining of thin-walled plectenchyma from which short hyphae arise and project into the cavity of the fruit body. These short erect hyphae produce the pycnidiospores.

The formation of pycnidiospores begins by the growth in length of the short bearing hyphae. No branching occurs and when growth in length is completed each hypha becomes abstricted at the base by the ingrowth of the wall. This leaves a long, aseptate filament lying free in the cavity of the pycnidium and a short hypha projecting from the inner plectenchyma which presumably can resume growth and produce a succession of pycnidiospores.

The pycnidiospores (Fig. 1 E) are colourless, filiform, straight, curved or slightly flexuous structures, $20-30 \times 0 \cdot 5-1 \cdot 0 \mu$. Staining with acid fuchsin in lactophenol, and other stains such as iodine or cotton blue, failed to reveal the presence of septa.

The earliest record for the appearance of mature pycnidiospores in plants on the shore is late November, but by January all pycnidia are mature. Ripe pycnidia may be found in the receptacles from January until May, when the host receptacles are exfoliated.

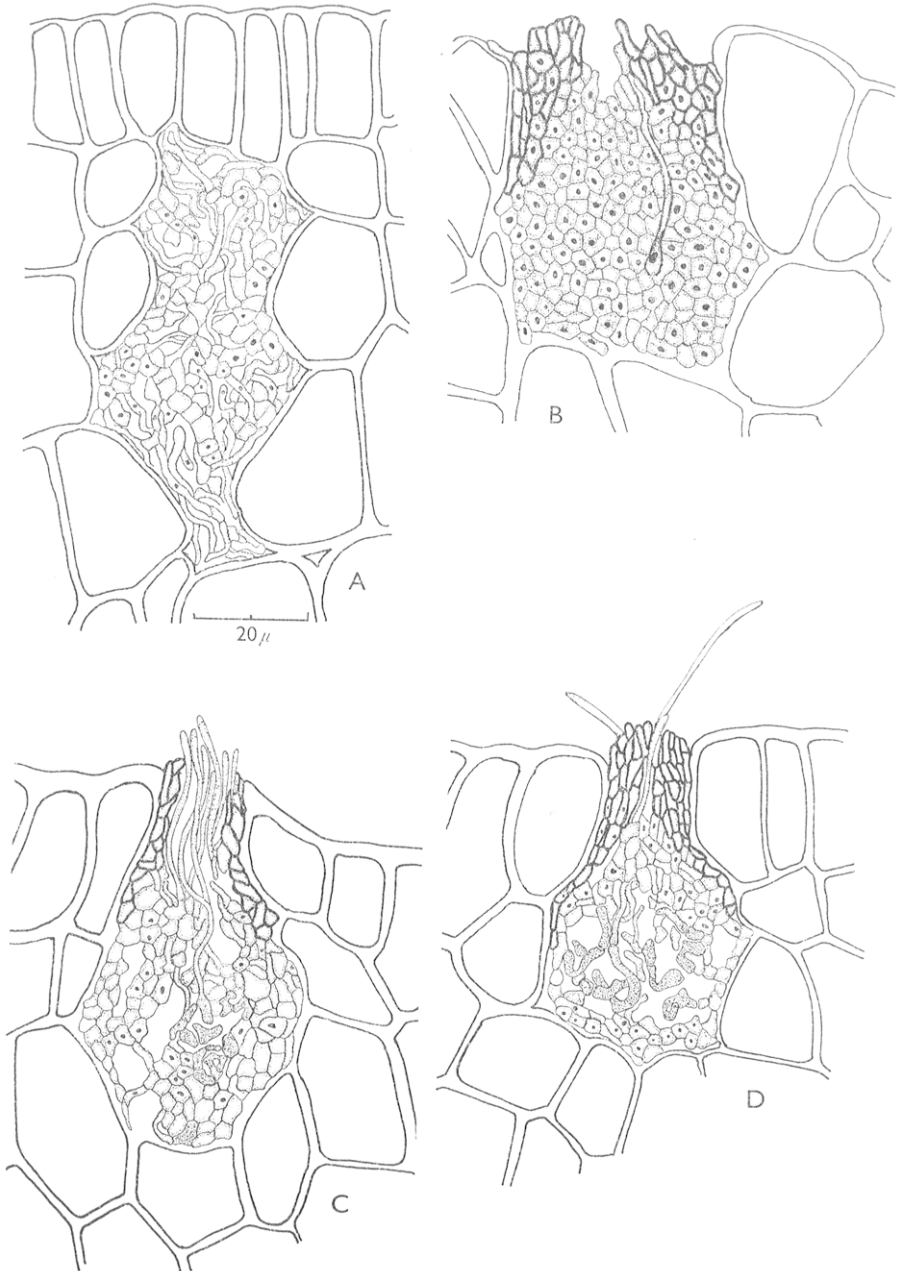Discharge of the products of the pycnidia probably occurs by the oozing

Fig. 2. *Mycosphaerella ascophylli*. A, Developing pseudothecium; B, young pseudothecium with single ascogonium in a solid stroma; C, young pseudothecium with seven emergent trichogynes and central stromal tissue breaking down; D, young pseudothecium with two emergent trichogynes, to one of which is attached a pycnidiospore.
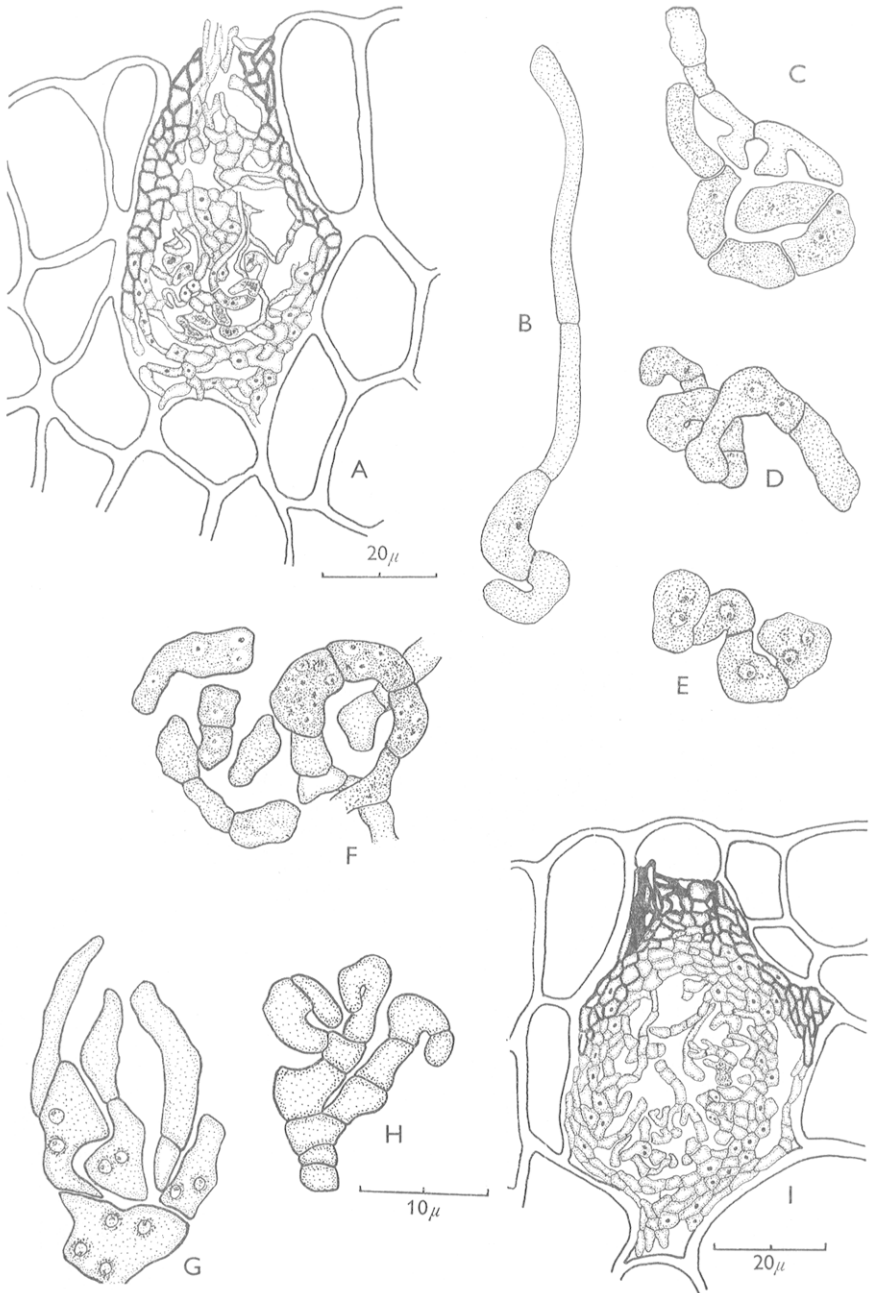
Fig. 3. *Mycosphaerella ascophylli*. A, young pseudothecium with loosely coiled ascogonium of two binucleate portions; B, complete mature archicarp with two celled ascogonium; C, early stage in division and coiling of ascogonium, ascogonial portions binucleate; D, E, later stage, tightly coiled ascogonia; F, coiled ascogonium with multinucleate portions; G, binucleate ascogenous hyphae arising from multinucleate section of an ascogonium; H, croziers formed distally on ascogenous hyphae; I, young pseudothecium showing some binucleate portions of ascogonial coils and breakdown of central stromal tissue. B–H, same scale.

of mucilage and pycnidiospores through the ostiole. Attempts to germinate the pycnidiospores on sea water agar, and in hanging drops of sea water failed.

The pseudothecial state

Further development of the pseudothecial state from the initial knot of hyphae (Fig. 2 A) proceeds by an increased branching of the hyphae of the initial accompanied by an increase in the diameter of the individual hyphae. Despite the increasing size of the pseudothecium the surrounding host cortical cells do not appear to be affected by pressure.

As a result of the increase in size and branching of the hyphae of the initial, the young pseudothecium takes on the appearance of a stroma (Fig. 2 B) consisting of a solid plectenchymatous mass. During late November and December young ascogonia become distinguishable in the lower part of this stroma. Usually three to five ascogonia arise within each stroma but up to seven (Fig. 2 C) have been observed occasionally. Each young ascogonium is a single uninucleate cell producing a long, filamentous trichogyne that grows towards the apex of the stroma.

While the ascogonia are developing, the stroma continues to enlarge and eventually the apex reaches the surface of the host receptacle and is exposed. At about this time, the cell walls in the apical region begin to thicken and become pigmented. Browning, and later blackening of the walls of the outermost cells of the stroma occurs from this time onwards in a progression downwards from the apical region to the sides and eventually to the base of the fruit body.

At approximately the time the stroma has enlarged sufficiently to reach the surface of the host receptacle, the trichogynes reach the apex of the stroma and project at the surface of the host receptacle. The mature ascogonium consists of an enlarged basal portion of one or two uninucleate cells (Fig. 3 B) bearing an emergent trichogyne. Young pseudothecia at this state of development are present during late December and January.

The occurrence of emergent and presumably receptive trichogynes in pseudothecial stromata coincides in time with the maturation of the pycnidiospores. An empty, colourless, filiform structure corresponding in size and form to a pycnidiospore has been seen attached to an emergent trichogyne (Fig. 2 D). This suggests that the pycnidiospores could be functional as male elements, although the actual passage of a nucleus from the pycnidiospore into the trichogyne has not been observed.

From January onwards, presumably after spermatization has occurred, the ascogonia begin a course of development leading to the production of asci. During this time the ascogonium first enlarges, becomes distinctly coiled (Fig. 3 A, C–E), and then becomes septate into three to five sections. These ascogonial sections are initially binucleate (Fig. 3 D, E, I) but later become multinucleate (Fig. 3 F). While the ascogonial portion is developing in this manner, the trichogyne gradually degenerates and finally disappears.

The multinucleate sections of the ascogonial coil, which may now be regarded as a primary ascogenous hypha, then begin to produce short, thick, secondary ascogenous hyphae which may branch. These hyphae are

septate and all cells in which nuclei could be distinguished were binucleate (Fig. 3 G). These ascogenous hyphae appear to produce croziers (Fig. 3 H). Proliferation of asci from croziers and ascogenous hyphae has been observed. In some microtomed sections asci appear to arise directly from the ascogenous hyphae (Fig. 4 A).

Fig. 4. *Mycosphaerella ascophylli*. A, Young asci which appear to have arisen directly from the ascogenous hyphae; B, mature pseudothecium with asci of different ages; C, mature ascus; D, dehiscing ascus showing inner wall projecting through ruptured outer wall.

While the asci are developing, changes occur in the appearance of the stromatal tissue in which the asci and their derivatives are embedded. The cell walls of the outermost layers of the stroma continue to become thicker and more darkly pigmented, this process being particularly evident in the apical region, and progressively less so downwards along the sides of the fruit body. In this way the outer stroma tissue forms the wall of the fruit body.

In the apical region, this process of thickening and pigmentation is incomplete since a small mass of tissue, surrounding the emergent trichogynes and continuous with the central stroma tissue, remains colourless and unthickened. This region is the incipient pore, and the actual pore appears to be formed by the gradual dissolution of the stromatic tissue in this region. Cells in this region begin to break down soon after the emergence of the trichogynes and simultaneously with the dissolution of the central stroma tissue. The process is gradual, however, and the ostiole is not fully formed until young asci appear in the pseudothecium. Periphyses may be weakly developed in the neck region towards maturity, but if produced they do not persist in the mature pseudothecium.

The mature pseudothecium (Fig. 4B) is completely sunken within the host tissue and is a globose or flask-shaped fruit body which opens to the exterior by a pore. The pseudothecial wall is heavily thickened and pigmented, particularly in the upper part, and the sunken pseudothecia are just visible to the naked eye as black spots on the host receptacles. Within the thickened and pigmented outer layer of the wall, at the base and sides of the fruit body is a layer of colourless, thin-walled tissue with which, at the base, is mixed the fertile tissue. The central cavity of the pseudothecium is occupied entirely by asci at various stages of development; there is no evidence of paraphyses or pseudoparaphyses and no interthecial tissue remains at maturity.

The earliest record for the presence of mature ascospores is mid-January, but by February the pseudothecia become generally mature. From February onwards to May, when the receptacles are exfoliated, mature pseudothecia produce a succession of ripening asci and during this period sections of pseudothecia invariably reveal a few ripe asci only with numerous younger asci at various stages of development.

Asci and ascospores

The ascus of *M. ascophylli* (Fig. 4D) is very distinctive in shape being clavate with a very heavily thickened apex. Staining with congo red revealed that the ascus is bitunicate in structure, the inner wall only taking up the stain. This method of staining also shows that the apical thickening is formed from the inner wall, the outer wall being uniformly thin throughout its length. The apex of the mature ascus is heavily thickened and is pierced by a narrow canal. This canal is filled with cytoplasm continuous with that in the main body of the ascus. Near the tip of the ascus, this canal opens out slightly and ends bluntly. This region marks the position of the opening which later develops in the inner wall through which the ascospores are discharged.

Each ascus contains eight ascospores which are fusiform in shape and are unequally bicellular, the larger cell always being uppermost in the ascus. As well as differing in size, the two cells of each spore differ in shape, the smaller one being cylindrical with parallel sides and a rounded apex, the larger cell having a maximum diameter at a relatively short distance from the septum and becoming gradually narrow towards the bluntly rounded apex. The ascospores are colourless or slightly greenish, and each cell of the spore contains numerous small oil globules.

Ascus dehiscence and ascospore discharge

The discharge of ascospores from the ascus in *M. ascophylli* is forcible and was observed by mounting, in sea water, thick sections of a living host receptacle bearing whole ripe pseudothecia of the fungus, and observing with a 4 mm objective. Since only that part of the ascus which projected beyond the ostiole could be seen by this method, observations were also made of ripe asci squashed from pseudothecia.

In squash preparations asci were found in which the outer wall had ruptured at the apex and the inner wall had elongated beyond it (Fig. 4D). It appears that it is the thickened apical portion of the inner wall which elongates during the early part of ascus dehiscence and during this process of elongation the inner wall becomes distinctly thinner. At the apex of the elongated inner wall the position of the apical pore is marked by a highly refractive spot. In all the observed cases the outer wall had ruptured apically and no 'thimble' structure was present over the apex of the extended inner wall.

As the inner wall of the ascus extends it projects through the ostiole for about 40 μ. An ascospore is usually visible in the projecting portion of the ascus. At first the single visible ascospore is situated near the apex of the elongated ascus tip, but later becomes more distant from the apex. When the inner wall of the ascus has elongated to its full length, tiny globules, which are in continuous movement, appear near the apex, between the uppermost ascospore and the ascus tip. These globules gradually take up a position at the extreme tip of the ascus, some of them coalescing. These globules are then extruded from the tip of the ascus to form a large droplet outside, but still in contact with, the ascus tip. The droplet increases in size until, suddenly, it is shot away and simultaneously the uppermost ascospore moves forward to fill the pore which is now present at the apex of the elongated inner wall of the ascus. The ascospore then moves slowly through the pore, until it reaches the widest part of the larger (uppermost) cell, then suddenly it is shot away at high speed. At the same time the second ascospore moves up, also at high speed, to fill the pore. The process is repeated until every ascospore has been discharged.

After the last ascospore has been ejected the diameter of the ascus is reduced and the ascus recoils, exuding a cloud of globules similar to those present at the apex immediately prior to ascospore discharge. The empty ascus may be withdrawn into the pseudothecium or it may remain partly projecting through the ostiole. Under these conditions the distance to which ascospores were shot in sea water varied from 70–90 μ. The time

lapse between the discharge of the first and last ascospore of an ascus has varied from 3–4 s to 19 min.

Asci also discharge their ascospores in air and this can be demonstrated by suspending a receptacle of *Ascophyllum* bearing ripe pseudothecia of *Mycosphaerella* over agar from the lid of a Petri dish. Ascospores will appear on the surface of the agar within 12–24 h. On inverting a series of dishes prepared in this way it was found that ascospores could be shot upwards into air to a distance of approximately 5 mm.

Ascospores germinated readily on sea water agar immediately after discharge from the asci. Short hyphae which were produced apically or laterally from the ascospores reached a length of about 5 μ. These remained unbranched. Growth then ceased and further development could not be induced.

<div align="center">INFECTION OF HOST</div>

The means by which *M. ascophylli* infects its host presents an interesting problem and one which is beset with considerable difficulties for the investigator. In the first place *Ascophyllum nodosum* has never been found in nature free of its endophyte so that controlled infection experiments on fungus-free plants cannot be carried out. Secondly, the association of the fungus and the alga appears to take place at a very early stage in the life of the host since naturally occurring sporelings approximately 1 month old or more of *A. nodosum* were always found to be associated with the fungus.

It is relevant to mention the mode of formation and release of eggs in *Ascophyllum* in considering the way in which the fungus and the alga become associated. The alga is dioecious, and the oogonia arise on short stalk cells developed from the wall of the female conceptacle. Paraphyses are interspersed amongst the numerous oogonia. The oogonial wall is three-layered and contains four functional eggs in a tetrad. At maturity the outer oogonial wall ruptures, releasing the tetrad, still enclosed within the middle and inner walls of the oogonium, into the cavity of the conceptacle. When the *Ascophyllum* plant is exposed at low tide, mucilage, together with the packets of eggs, is forced through the ostioles of the conceptacles and the egg tetrads remain on the surface of the receptacle until the incoming tide washes over them. In immersion in sea water the two remaining walls of the oogonium rupture and gelatinize to release the four naked eggs into the water.

Cotton (1908) suggested that since the ascospores of the fungus and the eggs of the seaweed are released at the same time the ascospores may be caught in the mucilage surrounding the eggs and that infection may take place during the early stages of segmentation.

Sutherland (1915) in his description of *Mycosphaerella pelvetiae* stated without any confirmatory evidence:

...the ascospores are set free from the perithecia at about the same time as the oospores are being liberated. They become entangled in the mucilaginous inner persistent oogonial wall surrounding the latter. There they germinate directly or sometimes a short mycelium is formed which penetrates slightly later...infection is rendered doubly sure by a vegetative process. Mixed with the paraphyses in the conceptacles are loosely

coiled, much branched hyphae. These frequently become entangled in the mucilaginous coating enveloping the oospheres, and are torn off as the latter are ejected. This mycelium is also capable of growing and gaining entrance to the developing oospore, when it again lodges between the young cells.

The following observations have provided some additional information relevant to this problem.

Examination of microtomed sections

Examination of permanent preparations of sections of *Ascophyllum* receptacles and of freezing microtome sections, showed that branches of the mycelium of *Mycosphaerella* were always present in the cavities of the host conceptacles. Often these were seen to run along the surface of the host paraphyses and oogonia for considerable distances. This finding agrees with that of Sutherland (1915) for *M. pelvetiae*.

Examination of Ascophyllum eggs

To determine whether the mycelium of *Mycosphaerella* can remain in association with the egg tetrads when the latter are discharged, a number of recently discharged *Ascophyllum* egg tetrads were collected from the shore and examined microscopically. Of 784 tetrads examined, thirty-seven (4·8%) were found to have mycelium on their surfaces, or ramifying through the mucilaginous middle and inner walls of the oogonium (Fig. 5B). An ascospore was found embedded in the mucilage around the egg in one case only (Fig. 5A).

Examination of naturally occurring Ascophyllum sporelings

David (1941) showed that sporelings of *Ascophyllum* are absent from the greater part of the *Ascophyllum* zone of the 'College Rocks' at Aberystwyth, but occur occasionally at the top and bottom of the *Ascophyllum* zone, and in the *Fucus spiralis* zone. Search in these regions at different times of the year produced a small collection of sporelings varying in size from 2–40 mm, and ranging in age from approximately 1–10 months. When examined, mycelium of *M. ascophylli* was found to be present in every case.

Observations on sporelings in culture

In order to increase the number of sporelings of *A. nodosum* available for examination and to investigate stages in development earlier than those found on the shore, attempts were made to culture sporelings in the laboratory. Initially this was done quite simply by pipetting freshly discharged eggs of *Ascophyllum* on to slides together with a few drops of a suspension of sperms. It could not be assumed that these eggs would be free of infection since it has been shown that a small percentage would be already infected with mycelium at discharge. The slides were then placed in Petri dishes and after a few hours, during which the fertilized eggs became attached to the slides, filtered sea water (which was subsequently renewed every 3 days) was added to the slides. Under these conditions the fertilized eggs developed rapidly during the first few days but then the growth rate

Fig. 5. *Mycosphaerella ascophylli*. A, Ascospore (*a*) embedded in mucilage surrounding discharged egg of *A. nodosum*; B, mycelium (*m*) on the surface of an egg tetrad of *Ascophyllum*; C, a sporeling of *A. nodosum* at the eight-cell stage with intercellular mycelium.

fell, the sporelings reaching the 32–64-cell stage in 5–6 weeks. Later, the procedure was improved by suspending the slides of sporelings in 4 litre glass tanks of filtered sea water to which was added a culture solution formulated by Provasoli, McLaughlin & Droop (1957). The medium was aerated and the sporelings provided with controlled illumination. As a result, the sporelings grew more rapidly in the early stages and were kept alive for almost a year.

Sporelings cultured in this way were examined at intervals for the presence of mycelium, taking random samples of fifty sporelings at a time. In one sample, examined 5 days after fertilization, a sporeling at the three-cell-stage was found with mycelium on its surface and one at the eight-cell stage was found to contain mycelium in an intercellular position. All other sporelings examined in this and other samples were free of the fungus.

In another series of preparations of *Ascophyllum* sporelings, the eggs were exposed immediately after fertilization to ascospores of the fungus by suspending whole receptacles of *Ascophyllum* bearing ripe pseudothecia of the fungus over the fertilized eggs. Within a few hours ascospores were seen to have been discharged on or near the fertilized eggs and were kept under observation. After 7 days none of the ascospores had germinated although the oospores were actively dividing. In a repeat of this experiment, it was found that a number of ascospores germinated in the sea water on the slide, within a few hours of being discharged over the fertilized eggs but none were seen to grow towards the developing sporelings. Of those ascospores which were discharged on to the surface of the sporelings, none were seen to germinate although the sporelings were segmenting.

In a further attempt to investigate the method and conditions necessary for the infection of *Ascophyllum* by *Mycosphaerella*, fertilized eggs of the alga were allowed to settle on concrete blocks (24 × 15 × 5 cm) which were then placed in various positions on the shore. Examination of the blocks after 6 weeks showed that a large number of sporelings had survived and had grown to lengths varying from 0·35–1·2 mm. During the period when these blocks were submerged (Dec. to Feb.) pycnidiospore discharge was occurring naturally from pycnidia, but pseudothecia were still immature generally in plants on the shore. A small number of sporelings from each block were detached and examined for the fungus but none was found to be infected. Each block was returned after examination to its original position on the shore for a further 4 weeks before re-examination. This time, 10 weeks after the eggs had been fertilized, no sporelings of *Ascophyllum* could be found on the blocks, although sporelings of *Fucus* spp. which had settled naturally on the blocks grew abundantly.

THE ROLE OF THE FUNGUS IN THE DECAY AND EXFOLIATION OF THE ALGAL RECEPTACLES

David (1941, 1943) described the phenomenon of the seasonal decay and exfoliation of the receptacles of *A. nodosum* which occurs during May and June. He stated that 'it would appear that this decay was coincident with the appearance of perithecia in the limiting layer of the receptacle,...but

whether this decay can be attributed directly to *Mycosphaerella*, or whether it is in part bacterial, can only be ascertained by more extensive research'.

Healthy receptacles and their stalks, and those in all stages of decay, were sectioned and examined. On sectioning, there was no apparent difference in amount, distribution or appearance of the mycelium of *Mycosphaerella* at any stage. However, when the receptacle stalks begin to discolour externally, thick, colourless septate hyphae of an undetermined fungus other than *Mycosphaerella* appear in the decaying tissue of the stalks. These hyphae ramify through the cortex and medulla of the receptacle stalks and are probably saprophytic.

COMPARISON OF *M. ASCOPHYLLI* AND *M. PELVETIAE*

Sutherland (1915) separated the two fungi as distinct species on the basis of smaller size of perithecia, asci and ascospores in *M. pelvetiae*. Meyers (1957) did not regard the asci and spores as being significantly different in size and doubted whether the possession of larger perithecia by *M. ascophylli* was sufficient to separate that species from *M. pelvetiae*.

Measurements of the relevant structures for the two species as given by Cotton (1908) and Sutherland (1915) together with those made during the present work are summarized in Table 1.

Table 1. *Comparison of morphological characters of*
Mycosphaerella ascophylli *and* M. pelvetiae

	M. ascophylli (μ)		M. pelvetiae (μ)	
	Cotton (1908)	Webber	Sutherland (1915)	Webber
Perithecia	100–130 × 80–90	85–155 × 75–115	65–85 (spherical)	65–103 × 85–125
Asci	50–60 × 18–20	45·5–64 × 17·5–21·5	45–55 × 15–20	48–62 × 12–15
Ascospores	18–21 × 4–5	17·5–22·5 × 4–5·5	19–25 × 4·5–5·5	16·5–23 × 4–5·5

Figures obtained for *M. pelvetiae* during the present work show a wider range of sizes for all three structures than those given by Sutherland (1915) indicating a greater coincidence in the sizes of the structures than when the figures of Cotton and Sutherland were considered. These results support the doubts expressed by Meyers (1957).

DISCUSSION

The structure and development of the perithecium of *M. ascophylli* in most essentials agrees with that described for other species of the genus, and is of the *Dothidea*-type (Luttrell, 1951). In this type the perithecium (in the wide sense) is developed from a pseudoparenchymatous stroma within which the archicarps develop; the central stroma tissue disappears completely at maturity and no paraphyses, pseudoparaphyses or inter-

thecial tissue remains; the ostiole develops lysigenously (though exceptions have been reported in a few *Mycosphaerella* spp.) and the asci are bitunicate.

A number of descriptions of *Mycosphaerella* spp. state that the asci are fasciculate, arising in a bundle from a small area in the base of the perithecium. Considerable importance has been attached to this character in taxonomic keys. Munk (1953) states that such a conception of the characteristic structure of *Mycosphaerella* is incorrect and points out that since the majority of the species in the genus have obclavate asci, it is impossible for such asci to arise from a limited area. He adds that the obclavate asci are generally distinctly converging but spring from a large area in the pseudothecial wall. Munk suggests that the earlier authors' idea of 'fasciculate' asci has arisen from the examination of squash mounts. In such mounts the asci usually take up a stellate arrangement because the rudimentary character of the interascal tissue does not allow the asci to retain their normal arrangement. During the present work, micro-tomed sections have shown that the asci can arise from a small area in the base of the perithecium in a fasciculate manner but can also arise from the whole of the base of the fructification. In species of *Mycosphaerella* in which only one, or rarely, two ascogonia are produced, the asci are figured as arising from a small area in the base of the perithecium, e.g. *M. tulipiferae* (Higgins, 1936). Where many ascogonia develop in the young stroma, e.g. *M. berkeleyi* (Jenkins, 1939) the asci are illustrated arising from a wide area in the perithecium. It seems likely that this character may be largely determined by the number of ascogonia which arise in the young stroma, and where several occur, the number which undergo further development.

In many species of *Mycosphaerella* the conidia are functional asexual bodies, e.g. the *Cercospora* state of *M. tulipiferae* (Higgins, 1936) and the *Septoria* state of *M. sentina* (Klebahn, 1908). In others the conidia of the *Phyllosticta* state have been considered to function as spermatia and Wolf & Wolf (1957) list twenty species where this is the case.

In *M. ascophylli* a previously undescribed *Septoria*-like state has been found. The following evidence suggests that in this case the pycnidio-spores function only as spermatia:

(1) The pycnidiospores fail to germinate in sea water and in air (on sea water agar).

(2) *Ascophyllum* sporelings without internal mycelium do not become infected when exposed only to pycnidiospores.

(3) The time of occurrence of pycnidiospores coincides with the time of appearance of trichogynes.

(4) A colourless filiform structure corresponding in size and shape to a pycnidiospore has been observed attached to an emergent trichogyne.

A similar pycnidial state has been observed in *M. pelvetiae* (Wilson, unpublished) but its function has not been studied.

Although it is unusual for a spore of this size and shape to function as a spermatium it is possible that such structures might be more suitable in the marine environment.

During the investigation of the processes of infection the evidence which has accumulated has been rather fragmentary, but it does indicate the

possible ways in which the association of the alga and the fungus may be brought about.

The observations clearly support Cotton's (1908) suggestion that infection by the fungus occurs early in the life of the alga. It would also appear, as Sutherland (1915) found for *M. pelvetiae*, that infection of the host may be brought about by ascospores or by fragments of vegetative mycelium which become associated with the eggs of the host while the latter are still enclosed within the oogonium or in the cavity of the conceptacle. The earliest and most probable time at which the ascospores can gain contact with the eggs is during the period when the discharged tetrads lie on the surface of the receptacle at low tide. The groups of tetrads, following discharge through the ostiole of the algal conceptacle, spread laterally over the surface of the receptacle and each group of tetrads must invariably lie over one or more ostioles of perithecia of the fungus. Any ascospores which may be discharged from asci in these pseudothecia will therefore be shot forcibly into the group of host egg tetrads where they may be trapped in the mucilaginous middle and inner oogonial walls which surround the tetrads. Although the observations confirm the views of Cotton (1908) and Sutherland (1915) that either mycelium or ascospores can become associated with the egg of *Ascophyllum*, this occurs only in a very small percentage of cases.

The failure of sporelings to survive when placed on the shore on blocks soon after fertilization, might indicate that the association of the alga with the fungus may be necessary for the growth and survival of the sporelings. This suggestion is supported by the fact that naturally occurring sporelings of *Ascophyllum* have never been found lacking the endophyte. While attempts to infect sporelings experimentally were unsuccessful there is strong evidence that only those sporelings naturally infected on the shore will survive. Uninfected sporelings may remain alive for several months in culture in the laboratory but these do not grow so well nor are they as well differentiated as naturally occurring sporelings of approximately the same age.

David (1941) failed to find sporelings of *Ascophyllum* in the middle of the 'Ascophyllum' zone on the College Rocks at Aberystwyth. Where sporelings survived they did so only at the top and bottom of the zone, and even here the numbers were small. David found that *A. nodosum* is capable of regenerating new plants from the hapteron and thallus of existing adult plants to a high degree, and it is probable that the majority of new plants which develop in the main part of the 'Ascophyllum' zone on the shore do so as a result of regenerative processes. In this case there is no difficulty in maintaining the association between the alga and the fungus since the fungus is systemic in the alga and the fungus can migrate from the parent alga into the regenerated thalli. Since the zone of *Ascophyllum* plants is maintained primarily by regeneration it is likely that the association between fungus and alga is maintained by vegetative means.

In considering the relationship between the fungus and the alga it is apparent that *M. ascophylli* is universally associated with its host and that the association is initiated at an early stage in the life of the alga. The fungus is systemic and perennial in the alga. Despite the widespread occurrence,

there is no evidence that the presence of the endophyte causes damage or destruction of host tissues even at the time of reproduction.

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