Ascophyllum and Its Symbionts. VIII. Interactions Among *Ascophyllum nodosum* (Phaeophyceae), *Mycophycias ascophylli* (Ascomycetes) and *Elachista fucicola* (Phaeophyceae)

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The brown alga *Ascophyllum nodosum* and its mutualistic, ascomycete symbiont, *Mycophycias ascophylli*, form a complex 'organism' or symbiotum. Here we show the interaction of the symbiotum to the abundant brown algal epiphyte, *Elachista fucicola*. Microscopy of field-collected plants shows morphological responses of *A. nodosum* to the common epiphyte *E. fucicola*. When *E. fucicola* attaches to *A. nodosum* a bundle of several to dozens of rhizoids penetrates into the host. On the surface of the host, the cells proliferate to form a donut-shaped ring, 100-200 μm in height that surrounds the thallus of *E. fucicola*. A pit forms in advance of the rhizoids and the cells of *A. nodosum* break down. This leaves the network of fungal hyphae partially intact and intermingling with the epiphyte rhizoids and its lowermost cells. After the pit is formed, the cells of *A. nodosum* bordering the infection chamber redifferentiate an epidermal layer. Neither the host nor its mutualistic fungus, *M. ascophylli* appears to recognize *E. fucicola* as an invader and to prevent the attachment and growth of this epiphyte. Based on the physical damage to the host caused by invading rhizoids, we conclude that the relationship of *E. fucicola* to *A. nodosum* is that of a parasite and its host.

Key Words: *Ascophyllum nodosum*, *Elachista fucicola*, epiphytism, *Mycophycias ascophylli*, parasitism, symbiosis

INTRODUCTION

Ascophyllum nodosum (L.) Le Jolis is among the most successful intertidal seaweeds in the North Atlantic. This success is reflected in the extreme longevity of individual fronds and whole plants and the ability of the species to dominate large expanses of the intertidal zone in marine habitats except in extremes of high wave exposure and low salinities (Baardseth 1970). *Ascophyllum* forms the basis of a complex community of associated organisms that includes red and brown epiphytic seaweeds (e.g., Lobban and Baxter 1983; Pearson and Evans 1990; Cardinal and Lesage 1992; Garbary and Deckert 2001), and a variety of invertebrates that associate directly with *Ascophyllum* or with its epiflora (e.g., Jarvis and Seed 1996; Pavia *et al*. 1999). Among the most intriguing aspects of the biology of *Ascophyllum* is the obligate symbiosis with the fungus *Mycophycias ascophylli* (Weber 1967; Kohlmeyer and Kohlmeyer 1972; Kohlmeyer and

Volkmann-Kohlmeyer 1998; Garbary and Deckert 2001). Experimental studies have demonstrated that this is a mutualistic symbiosis (Garbary and London 1995; Garbary and MacDonald 1995). This association was previously designated as a mycophycobiosis by Kohlmeyer and Kohlmeyer (1972); however, based on similarities with the mutualistic symbioses between some grass species and their fungal endophytes (e.g., Schardl *et al*. 1991; Bacon and Hill 1996) we prefer to designated this as a 'symbiotum' (Garbary and Deckert 2001; Deckert and Garbary 2005). Another brown alga, *Petroderma maculiforme* (Wollny) Kuck. also has a similar association with a fungal endophyte and has been designated as the lichen *Verrucaria tavaresiae* Moe (Moe 1997; Sanders *et al.* 2004).

Elsewhere, the basic structure of the *Ascophyllum* symbiotum is described in the absence of other associated algae (Deckert and Garbary 2005). The three way interactions among *Ascophyllum* and *Mycophycias* and the obligate red algal epiphyte *Vertebrata lanosa* (L.) Christensen are also well described. The latter species is typically a host specific epiphyte whose biology is highly
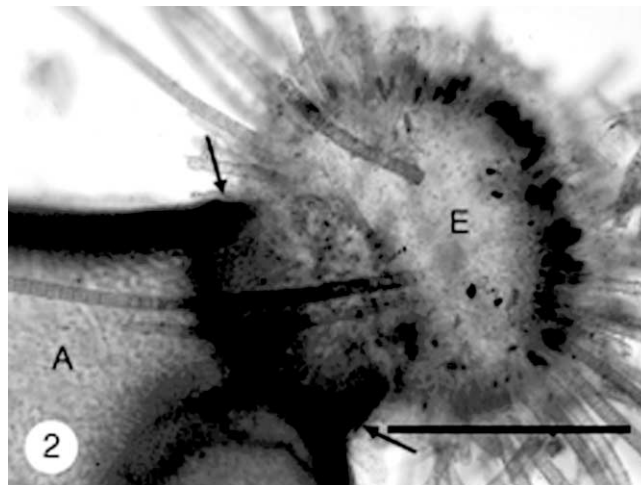
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Fig. 1. *Ascophyllum nodosum* and *Elachista fucicola*. Portions of two fronds from herbarium specimens of *A. nodosum* with numerous thalli of epiphytic *E. fucicola*. Frond on left was collected in early summer and *E. fucicola* has long assimilatory filaments; frond on right was collected in late summer and assimilatory filaments of *E. fucicola* have largely gone.


Fig. 2. Transverse section through base of *Elachista fucicola* (E) attached to host *Ascophyllum nodosum* (A). Note development of the *A. nodosum* collar (between arrows) forming a ring around the *E. fucicola* thallus where it attaches to the host. Scale bar = 500 μm.

integrated with that of its host (Garbary *et al.* 1991; Garbary and Deckert 2001; Garbary *et al.* 2005), although it is occasionally found on other fucoids where it might even be common (e.g., Rindi and Guiry 2004). Here the morphological and cellular interactions of *Elachista fucicola* (Velley) Aresch. are described when it is associated with *Ascophyllum* in nature. *Elachista fucicola* is a non host-specific epiphyte that is known from a variety of fucoid algae and commonly found with *Pilayella littoralis* (L.) Kjellm. In Nova Scotia *E. fucicola* is commonly found on *A. nodosum* and *Fucus vesiculosus* (Johnson and Scheibling 1987), whereas in Europe it is generally referred to as an epiphyte of *Fucus* spp. (Fritsch 1945; Rindi and Guiry 2004). As a common epiphyte on *A. nodosum*, *Elachista fucicola* provides a model system for investigating attachment mechanisms and interactions with the *A. nodosum* symbiotum. Here the interactions among the members of the symbiotum and *Elachista fucicola* are examined, and compared with previous observations of the symbiotum and *V. lanosa* (Garbary *et al.* 2005).

MATERIALS AND METHODS

Ascophyllum nodosum (L.) Le Jolis with endophytic *Mycophycias ascophylli* (Cotton) Kohlmeyer et Volkmann-

Kohlmeyer and epiphytic *Elachista fucicola* (Velley) Areschoug were collected at Tor Bay Provincial Park, Guysborough Co., Nova Scotia (49.19°N 61.34°W) in August, 2000. Many additional collections of *E. fucicola* were made at numerous sites along the Atlantic coast of Nova Scotia between 2000 and 2005. All plants were from the midintertidal zone where *A. nodosum* is abundant. Thalli were hand sectioned and stained with trypan blue (0.05 %) in lactoglycerol (1:1:1, lactic acid:glycerol:H$_2$O) for 0.5 to 12 h prior to observation. In addition, portions of plants were cleared with 1 M KOH changed daily in an oven at 60°C for 48-96 h prior to staining with trypan blue (Deckert and Garbary 2005). Hand sections or whole mounts of fresh or cleared material were observed using bright field or phase contrast optics on Zeiss Photomicroscope III or Leica M420 microscopes. Photomicrographs were captured with a Snap2 low-light digital camera (Diagnostic Instruments Inc., Sterling Heights, MI, USA) and processed in Adobe Photoshop.

RESULTS

Elachista fucicola is a common epiphyte on *Fucus vesiculosus* and *Ascophyllum nodosum* on rocky intertidal shores of Nova Scotia where the host species are abundant. The epiphyte can occur in high densities with thousands of thalli per m^2 and with many dozens of plants per host frond (Fig. 1). Individual thalli can be up to 1 cm wide and assimilatory filaments can reach to 2-3
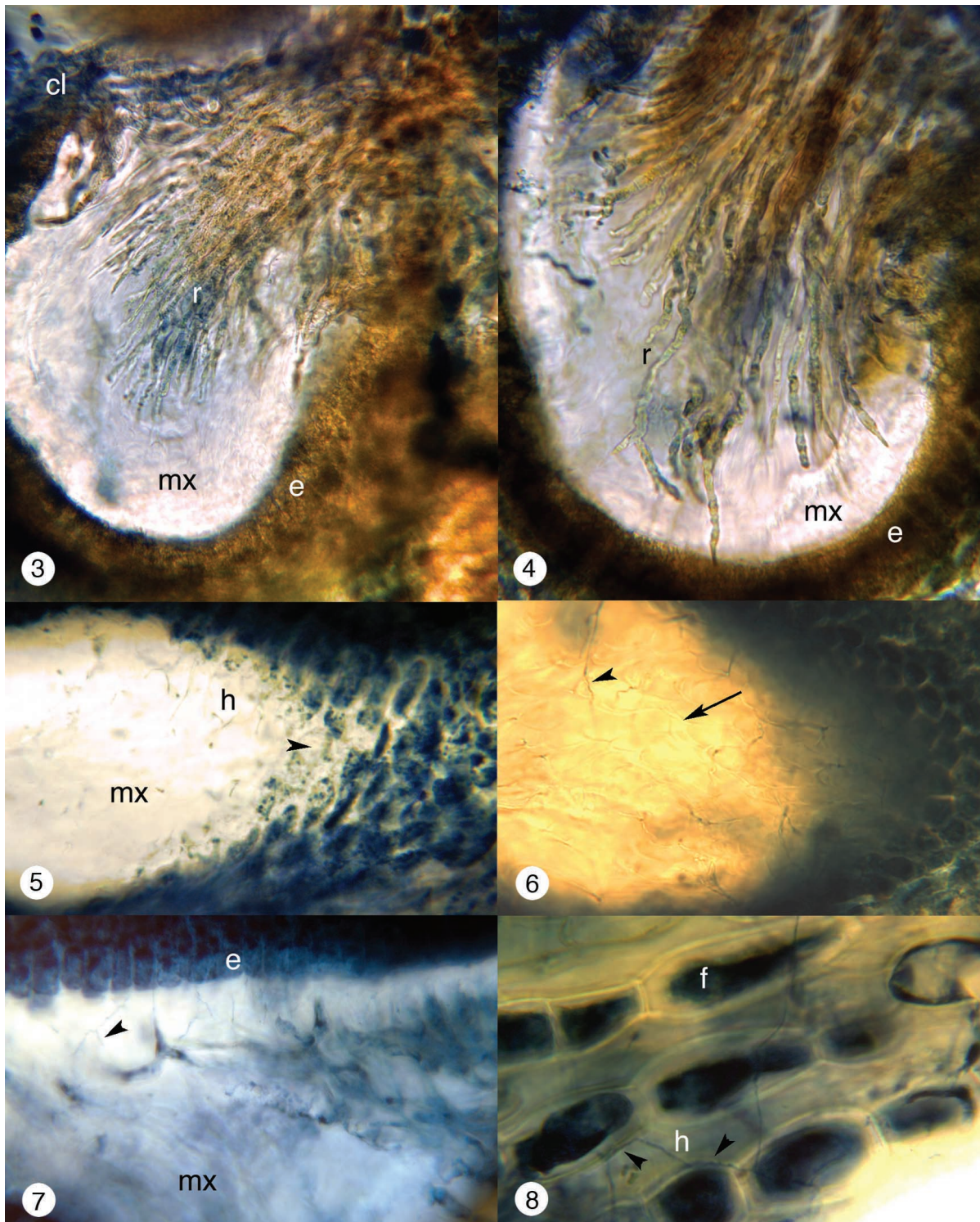
Fig. 3-8. Interactions of *Ascophyllum nodosum*, *Mycophycias ascophylli* and *Elachista fucicola*. **Fig 3.** Rhizoid system (r) of *E. fucicola* in penetration chamber. Note collar (cl) which is raised above thallus surface, chamber matrix (mx) and redifferentiated epidermis (e) and cortex of *A. nodosum* forming chamber wall. × 150. **Fig 4.** Rhizoid system (r) of *E. fucicola* penetrating through matrix (mx) to chamber wall. Note differentiated cortex of *A. nodosum*. × 225. **Fig 5.** Base of developing penetration chamber of *E. fucicola* showing remnant matrix (mx), hyphae (h) of *M. ascophylli* and cortical cells being degraded (arrow head) at pit base on right. × 300. **Fig 6.** Base of penetration chamber showing hyphae (arrow head) penetrating through matrix and region where cells of *A. nodosum* have been broken down (arrow). × 350. **Fig 7.** Hyphae (arrow head) emerging from redifferentiated host epidermis (e) and mingling with rhizoids of *E. fucicola*. × 350. **Fig 8.** Hyphae of *M. ascophylli* growing in filamentous system (f) of *E. fucicola*. Note attachment of hyphae (h) to cell walls of host (arrow heads). × 1000.

cm in height. When *E. fucicola* occurs in high densities plants can merge into one another forming a more-or-less continuous cover stretching over several cm of host. Unlike the red algal epiphyte *V. lanosa* that typically occurs only on scar tissue following receptacle shedding, damaged surfaces of fronds or in the axils of branches, *E. fucicola* can colonize all vegetative parts of the plant, including air bladders. When fronds of *E. fucicola* are removed from the host or longitudinal sections are made through the thallus of *E. fucicola*, a raised ring of tissue 100-200 μm in height is often apparent on the *A. nodosum* frond that tightly surrounds the thallus of *E. fucicola* (Fig. 2). In large thalli of *E. fucicola*, the epiphyte can overgrow the ring and spread out further onto the host thallus.

Early infection stages were not observed; however, thalli of *E. fucicola* have secondary rhizoid development that penetrates through the epidermis into the cortical cells. A primary morphological feature of *E. fucicola* with the *Ascophyllum* symbiotum is a chamber that forms in the *A. nodosum* frond by necrosis of *A. nodosum* cells (Figs 3, 4). This chamber initially forms as a narrow pit in advance of rhizoid growth of the epiphyte (Figs 5, 6). There is no apparent change in host cell cytology prior to necrosis, and the transition from healthy to necrotic cells is very sudden. The advancing rhizoid front may consist of 1-3 rhizoids with the infection pit only ca. 100 μm diameter. As rhizoids proliferate dozens may be associated with a single well-developed pit that has a rounded base when fully developed and can be ca. 1 mm diameter. Once the pit is fully formed, the living marginal cells of *A. nodosum* redifferentiate an apparently normal meristoderm and cortex (Fig. 4). Between the new meristoderm and a dense region of *E. fucicola* rhizoids is a translucent matrix (Figs 3, 4, 5, 7) comprised of the cell wall remnants of *A. nodosum* cells and mucilaginous materials. Some rhizoids penetrate this matrix material and come into virtual contact with host epidermis (Fig. 4); however, most rhizoids terminate before penetrating the chamber matrix (Figs 4, 5). The degradation of the host cells leaves the *M. ascophylli* hyphae morphologically outside its host organism, albeit in a chamber that is plugged from the outside environment by the thallus of the epiphyte.

As the necrosis proceeds, the hyphae of *M. ascophylli* remain roughly in place and become intermingled with the developing rhizoids of *E. fucicola* (Figs 6, 7). Some additional hyphal growth may also occur through the newly formed epidermis into the pit space (Fig. 6). There is no apparent negative reaction of the epiphyte rhizoids

to hyphae. Hyphae also proliferate among the lowermost cells of the nonrhizoidal portion of the *E. fucicola* thallus. Here they seem to proliferate in the intercellular spaces and occasionally become attached to the cell walls of their new host (Fig. 8).

DISCUSSION

The *Ascophyllum* symbiotum shows a major difference in response to their two major epiphytes in eastern Canada. In the case of *Vertebrata lanosa* (Garbary *et al.* 2005) the host symbiosis shows little apparent reaction, and the fungal partner effects the principal cytological changes. A hypersensitive reaction develops in which there is limited cell death in cells of *A. nodosum* surrounding the rhizoid and the *Mycophycias* shows extensive penetration of the *V. lanosa* cell walls. In the case of *E. fucicola*, the fungus appears oblivious to the epiphytizing algae, and it is *A. nodosum* that undergoes the most dramatic modifications from the invading rhizoids.

Elachista fucicola is terminal on many broken axes of *A. nodosum* (Garbary, unpublished observations), suggesting that the formation of the infections pits causes weak points in the fronds that make them liable to breakage. In addition to the induction of physical breakage, the presence of large populations of *E. fucicola* (Fig. 1) would cause extensive light and nutrient shadows for the underlying host tissue. These impacts on the *Ascophyllum* thallus suggest that *E. fucicola* is a parasite. Experimental studies would be useful to determine the extent of these negative interactions. Parasitic interactions between brown algae have been described. A variety of galls on fucoids and kelps have been associated with filamentous brown algae (e.g., Andrews 1977). Apt (1988) used Koch's postulates to show that *Streblonema* sp. caused tumor-like growths on *Laminaria japonica* Aresch. that formed gall-like protrusions based on a hyperplasic reaction. In this parasitic interaction the *Streblonema* is entirely within the host. In the *Ascophyllum-Elachista* interaction hyperplasia occurs to form the ring of tissue on the thallus surface surrounding the invading parasite.

The *Ascophyllum* symbiotum presents a different syndrome of interactions with *Elachista fucicola* than it does with *V. lanosa*. The parasitism induced by *E. fucicola* is very extensive. The rhizoids are much smaller than in *V. lanosa* (Rawlence 1972; Rawlence and Taylor 1970, 1972), and are able to penetrate between the host cells

rather than crushing them as they grow. The primary feature of rhizoid growth in *E. fucicola* is a necrosis of *A. nodosum* cells that forms in advance of the rhizoid front. This leaves the hyphal network largely intact, although certainly much less regular that in intact symbiotum tissue. Subsequent growth of the hyphae into the pit space from the surrounding cells suggests that *M. ascophylli* may take advantage of the resulting nutrients caused by breakdown of the host cells or leaching of other cell exudates. The ability of *M. ascophylli* to penetrate among the nonrhizoidal cells of *E. fucicola* is similar to the penetration of hyphae into *Fucus* holdfasts epiphytic on *A. nodosum* (Kingham and Evans 1986). Given the exchange of nutrients between the symbiotum and *V. lanosa* (e.g., Penot and Penot 1974; Penot *et al*. 1993; Cicciote and Thomas 1997), we speculate that the infection pit might function as mechanism of nutrient transport into *E. fucicola.*

One of the primary features of *Elachista* is the fact that it is an obligate epiphyte with the various species showing limited or host-specific associations with other algae. Indeed, the species of *Elachista* are typically distinguished based on the identity of the host. This is the case in eastern North America where the three species are identified based on their occurrence on fucoids (*E. fucicola*), *Chondrus crispus* Stackhouse (*E. chondrii* Aresch.) and other algae (*E. stellaris*) (Sears 1998). Similarly, in the British Isles four species can be distinguished based on their host identities (Fletcher 1987). The association of *E. fucicola* in Nova Scotia is with two primary hosts, *Fucus vesiculosus* and *A. nodosum*; however, the apparent parasitism (i.e. pit formation) only occurs when *E. fucicola* is associated with *Ascophyllum*. Russell and Veltkamp (1984) showed that zoospores of *E. fucicola* settle preferentially adjacent to the cryptostomata of *F. vesiculosus*. This differs from *A. nodosum* that does not have cryptostomata, pointing to the requirement for producing the infection pit described here. These observations raise the possibility that there are two forms of *Elachista* in eastern North America associated with fucoids.

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