Eocronartium muscicola: a basidiomycetous moss parasite exploiting gametophytic transfer cells

E. W. A. Boehm and D. J. McLaughlin

Department of Botany, University of Minnesota, 220 Biological Science Center, 1445 Gortner Avenue, St. Paul, MN 55108, U.S.A.

Received June 3, 1987

Boehm, E. W. A., and McLaughlin, D. J. 1988. *Eocronartium muscicola*: a basidiomycetous moss parasite exploiting gametophytic transfer cells. Can. J. Bot. **66**: 762–770.

The host–parasite interface in *Eocronartium muscicola*, Auriculariales *sensu lato*, was examined histologically for 6 of the 21 reported moss hosts, using light microscopy, scanning electron microscopy, and transmission electron microscopy. A unique mode of fungal biotrophy was encountered in 5 of the 6 mosses analyzed, in which *E. muscicola* exploits gametophytic host transfer cells concomitant with varying degrees of supplantation of the moss sporophyte. Basidiocarps are restricted in these mosses to postfertilized archegonia, in which they are seen to associate with the sporophyte foot region, where they gain access to the host transfer cell nutritional interface. Basidiocarp ontogeny is presented as it relates to the development of the host–parasite interface. The relationship of *E. muscicola* to other simple-septate auricularioid taxa and the the Uredinales is discussed.

Boehm, E. W. A., et McLaughlin, D. J. 1988. *Eocronartium muscicola*: a basidiomycetous moss parasite exploiting gametophytic transfer cells. Can. J. Bot. **66** : 762–770.

À l'aide de la microscopie photonique et électronique par transmission et par balayage, les auteurs ont examiné l'histologie de l'interface hôte–parasite chez l'*Eocronartium muscicola* Auriculariales *sensu lato* et ceci sur 6 des 21 mousses hôtes connues. Chez 5 des 6 mousses analysées, un mode unique de biotrophie fongique a été mis en évidence; les cellules de transfert du gamétophyte sont exploitées de façon concomittante avec divers degrés de supplantation du sporophyte de la mousse. Chez ces mousses, les basidiocarpes sont restreints aux archégones fertilisés dans lesquels on les retrouve associés à la région du pied du sporophyte et où ils réussissent à gagner l'interface nutritionnel des cellules de transfert. Les auteurs présentent l'ontogénie du basidiocarpe en relation avec le développement de l'interface hôte–parasite et ils discutent finalement les relations de *E. muscicola* avec d'autres taxa auricularioïdes à septations simples ainsi qu'avec les Urédinales.

[Traduit par la revue]

Introduction

Eocronartium muscicola (Fr.) Fitz., Auriculariales *sensu lato*, is a small heterobasidiomycete found parasitizing numerous temperate moss genera. The genus was established to accommodate a gelatinous, clavarioid hymenomycete bearing transversely septate metabasidia superficially reminiscent of the telial horn found in the uredinalean genus *Cronartium* (Atkinson 1902). Stanley (1940) described a thin-walled probasidium, carrying the fusion nucleus, as well as basidiospore germination by the production of secondary ballistospores. Septal ultrastructural studies have revealed a simple septum as in the Uredinales (Khan and Kimbrough 1980) and differing significantly from that found in *Auricularia* (McLaughlin 1980). Because of the presumed phylogenetic antiquity of the host group, the presence of thin-walled probasidia germinating without dormancy to yield rust-like metabasidia, and the simple septa, *E. muscicola* occupies a pivotal position in the phylogenies of the lower basidiomycetes proposed by numerous authors (Bandoni 1984; Donk 1972; Hennen and Buritica 1980; Khan and Kimbrough 1982; Leppik 1955; Oberwinkler 1982).

The nutritional mode used by *E. muscicola* was first described by Fitzpatrick (1918*a*, 1918*b*). In *Clinmacium americanum* Brid., Fitzpatrick determined the host–parasite interface to be entirely intracellular and concluded that *E. muscicola* was restricted to the gametophytic tissues of its host, eventually forming clavate basidiocarps at the apices of moss branches. In rare instances he did note that both the gametophyte and the sporophyte were parasitized, the fungus inhibiting the latter's development. Ulvinen (1981) noted that in *Fissidens viridulus* (Sw.) Warnst. the fungus, in a single instance, parasitized both the gametophyte and the sporophyte. Olive (1948) also collected *E. muscicola* on the sporophyte of *Amblystegium*. Aside from these singular observations, subsequent authors have considered *E. muscicola* to be wholly restricted to the gametophyte and to behave as an intracellular parasite (Bessey 1950; Julich 1982; Khan and Kimbrough 1980, 1982; Stanley 1940).

The present study was initiated to determine whether biotrophy as described in *C. americanum* (Fitzpatrick 1918*a*, 1918*b*) was operative among the other reported moss hosts. Specifically, the host–parasite interface was examined to clarify the relationship between those fungal hyphae comprising the bulbous basidiocarp base and the surrounding gametophytic and (or) sporophytic host tissue. Basidiocarp ontogeny is also presented as it relates to the development of the host–parasite interface.

Materials and methods

Six of the 21 reported moss hosts were analyzed histologically (see Table 1) using light microscopy (LM), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). Living *Pylaisiella polyantha* (Hedw.) Grout (McLaughlin 334, 5 July 1985, host det. J. A. Janssens, MIN) and *Haplocladium microphyllum* (Hedw.) Broth. (McLaughlin 339, 18 July 1985, host det. J. A. Janssens, MIN), bearing basidiocarps of *Eocronartium muscicola*, were collected near Dam Lake, Aitkin Co., MN. Infected herbarium specimens of *Drepanocladus uncinatus* (Hedw.) Warnst. (Heikkila 81-51; Joensuu, Finland, 8 Aug. 1981, OULU), *Fissidens viridulus* (Sw.) Wahl. (Saarenoksa/Ulvinen; Helsinki, Finland, 28 July 1979, OULU), *Pylaisiella polyantha* (Hedw.) Grout (Ohenoja; Oulunsalo, Finland, 8 Sept. 1982, OULU), *Eurhynchium hians* (Hedw.) Sande Lac. (Saarenoksa 20981/Korhonen 3901; Helsinki, Finland, 20 July

Printed in Canada / Imprimé au Canada

1981, OULU), and *Climacium dendroides* (Hedw.) Web. & Mohr. (Koponen 27642; Varsinais-Suomi, Finland, 21 July 1980, OULU) were kindly supplied to the senior author (E.W.A.B.) by Dr. T. Ulvinen (Botanical Museum, University of Oulu, Finland). Vouchers are deposited in MIN.

The living material was processed for LM, SEM, and TEM while the dried specimens were first rehydrated in aqueous 5% KOH and analyzed primarily by LM and SEM. Living *P. polyantha* and *H. microphyllum*, bearing *E. muscicola* at various stages of development, were fixed overnight with 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2 at 4°C, and rinsed in buffer. TEM material was postfixed for 4 h with 1% OsO_4 in the same buffer at room temperature, and *en bloc* stained overnight in an aqueous solution of 0.5% uranyl acetate at room temperature in the dark. After acetone dehydration the material was embedded in soft Spurr's resin (Spurr 1969) and thin sectioned on a Reichert Jung Ultracut-E. Serial thin sections were harvested only 2 × 1 mm slot grids (as in Rowley and Moran, 1975) and then stained for 50 min in 5% aqueous uranyl acetate followed by 45 min in lead citrate, and examined with a Hitachi H-600 transmission electron microscope operated at 75 kV. Material for LM, after primary fixation, was dehydrated as above and embedded in glycol methacrylate (Polysciences, Inc., Warrington, PA). Sections were cut at 4–7 μm and stained in toluidine blue at 60°C.

Material for SEM was critical point dried, sputter coated with gold, and examined with a Hitachi S-450 scanning electron microscope operated at 15 kV. The SEM samples, first photographed in their ıtirety, were then cryofractured to reveal internal structures. Specimens were immersed in liquid propane (−185°C), surrounded by a liquid nitrogen bath, and cryofractured using precooled razor blades. Liquid propane boiled less vigorously than liquid nitrogen at room temperature and so permitted a more accurate orientation of the sample before cryofracture. Cryofractured specimens were recoated with gold and examined with the scanning electron microscope.

The rehydrated herbarium specimens were fixed overnight in 3% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.0, at 4°C, and processed as above for LM or SEM.

Results

In analyzing 6 of the 21 reported moss hosts for *Eocronartium muscicola* (Table 1) we were able to substantiate Fitzpatrick's (1918*a*, 1918*b*) findings in only one of them, *C. dendroides*. Intracellular biotrophic hyphae were seen to pass from cell to cell (not illustrated) in a manner similar to that described for *C. americanum* (Fitzpatrick 1918*a*, 1918*b*).

The other five moss hosts analyzed display a remarkably different mode of fungal biotrophy, which as far as is known, has not previously been described. In *P. polyantha, H. microphyllum, D. uncinatus, F. viridulus*, and *Eurohynchium hians, E. muscicola* is not seen to fruit at the vegetative branch apices of the gametophyte, as in *C. dendroides* and *C. americanum*; instead the basidiocarp is restricted to determinate gametophytic branch apices bearing postfertilized archegonia (Figs. 1, 2, and 3). Nonfertilized archegonia, identified as such by their reduced size and lack of a developing sporophyte, did not bear basidiocarps of *E. muscicola* (Fig. 2). Sexual, as distinct from vegetative, moss branch apices bearing archegonia are recognized by the occurrence of somewhat modified leaves, perichaetial bracts, which envelope the archegonial region and are collectively termed the perichaetium (Hebant 1977). Acrocarpous mosses (*F. viridulis*, in this study) bear perichaetia at the apices of determinate leafy shoots, while pleurocarpous mosses (*P. polyantha, H. microphyllum, D. uncinatus*, and *Eurohynchium hians*) bear perichaetia at the tips of determinate lateral branches. Basidiocarps of *E. muscicola* appear to be restricted specifically to gametophytic perichaetia bearing postfertilized archegonia in these acrocarpous and pleurocarpous mosses.

TABLE 1. Reported moss hosts for *Eocronartium muscicola*

Order Bryales[a]
- Fissidentineae (Schimp.) Vitt
  - Fissidentaceae Schimp.
    - *Fissidens taxifolius* Hedw.[b]
    - **F. viridulus* (Sw.) Wahl.[b]
- Pottiineae Fleisch.
  - Pottiaceae Schimp.
    - *Weissia controversa* Hedw.[c]
- Leucodontineae Fleisch. (Isobryales)
  - Leskeaceae Schimp.
    - *Leskea obscura* Hedw.[d]
    - *Anomadon rostratus* (Hedw.) Schimp.[d]
  - Climaciaceae Kindb.
    - *Climacium americanum* Brid.[d]
    - **C. dendroides* (Hedw.) Web. & Mohr.[b,d]
- Hypnineae Fleisch. (Hypnobryales)
  - Thuidiaceae Schimp.
    - **Haplocladium microphyllum* (Hedw.) Broth.[c]
    - *Thuidium delicatulum* (Hedw.) BSG[d]
    - *T. minutulum* (Hedw.) BSG[d]
  - Amblystegiaceae G. Roth
    - *Amblystegium serpens* (Hedw.) BSG[d]
    - *A. varium* (Hedw.) Lindb.[d]
    - *A. riparium* (Hedw.) BSG[d]
    - *Campylium chrysophyllum* (Brid.) J. Lange[e]
    - **Drepanocladus uncinatus* (Hedw.) Warnst.[b]
  - Brachytheciaceae G. Roth
    - *Brachythecium oxycladon* (Brid.) Jaeg. & Sauerb.[d]
    - **Eurhynchium hians* (Hedw.) Sande-Lac.[b]
  - Entodontaceae Kindb.
    - *Entodon seductrix* (Hedw.) C.M.[d]
  - Plagiotheciaceae (Broth.) Fleisch.
    - *Isopterygiopsis muelleriana* (Schimp.) Iwats.[d]
  - Hypnaceae Schimp.
    - **Pylaisiella polyantha* (Hedw.) Grout[b,d]
  - Hylocomiaceae (Broth.) Fleisch.
    - *Rhytidiadelphus triquetrus* (Hedw.) Warnst.[b]

NOTE: Moss hosts analyzed in this study are indicated by an asterisk (*).
[a]Taxonomic arrangement follows Vitt (1984).
[b]Ulvinen (1981).
[c]Kahn and Kimbrough (1980).
[d]Fitzpatrick (1918*a*).
[e]Stanley (1940).

When young, postfertilized perichaetia of *D. uncinatus*, showing no external evidence of infection (Fig. 4), are cryofractured for SEM (Fig. 5), early stages of basidiocarp ontogeny are revealed. The perichaetial bracts encase a single postfertilized archegonium. The neck canal region and the archegonial venter are exposed in near-medial fracture as are the basidiocarp initials situated above the neck canal (Fig. 5). In a later developmental stage the basidiocarp initials have expanded the archegonial neck canal region in *D. uncinatus*, fully occupying the vaginular pocket (Fig. 7), and emerge as a loose weft of fungal hyphae enveloping the apex of the perichactium (Fig. 6).

The relationship between the developing sporophyte in the mosses analyzed and the basidiocarp initials of *E. muscicola* shows a certain amount of variability. In *D. uncinatus* (Figs. 4–7), *F. viridulus*, and *Eurhynchium hians* (the latter two not illustrated) the sporophytic generation is arrested early

in its development by *E. muscicola*. Rarely does the sporophyte develop beyond the apex of the perichaetium. *Pylaisiella polyantha* and *H. microphyllum* differ in that the sporophyte does develop beyond the perichaetial apex but is then progressively deterred from further growth (Figs. 1–3 and 8–10). In young nonsporulating basidiocarps of *E. muscicola* the sporophyte is encased within the developing fungal fruit body (Fig. 2), and even in mature fruit bodies the arrested sporophyte may remain encased (Fig. 3). Thus, macroscopically the sporophyte–fungus associations are cryptic in *D. uncinatus*, *F. virulens*, and *Eurhynchium hians*, unless examined in the earliest stages of development, while in *P. polyantha* and *H. microphyllum* the sporophyte ofter persists to varying degrees during the establishment of the fungal fruit body.

When *H. microphyllum* bearing an arrested sporophyte is examined with LM (Fig. 8), a necrotrophic association is revealed in which fungal hyphae ramify and disrupt the developing sporophyte. The sporophyte, having just emerged from the surrounding perichaetial bracts, has not yet ruptured the calyptra. Figures 9–11 illustrate *H. microphyllum* at a later developmental stage, in which the sporophyte is not completely ensheathed by fungal hyphae and protrudes beyond the perichaetial bracts. A near-medial fracture reveals fungal hyphae within the central core of the sporophyte (Fig. 10).

Hyphae of basidiocarp initials of *E. muscicola* infiltrate the sporophytic tissue and eventually supplant it completely so that very little, if any, remains at maturity (Figs. 11 and 12). However, as mentioned before, remnants of the sporophyte often may persist in *H. microphyllum* and *P. polyantha*. A traverse fracture through the basal region of a mature basidiocarp reveals a central core of gelatinized hyphae, constituting the central axis of the fruit body, surrounded by perichaetial bracts, some of which have been penetrated by fungal hyphae, and enveloped in a loose external hyphal weft (Fig. 11).

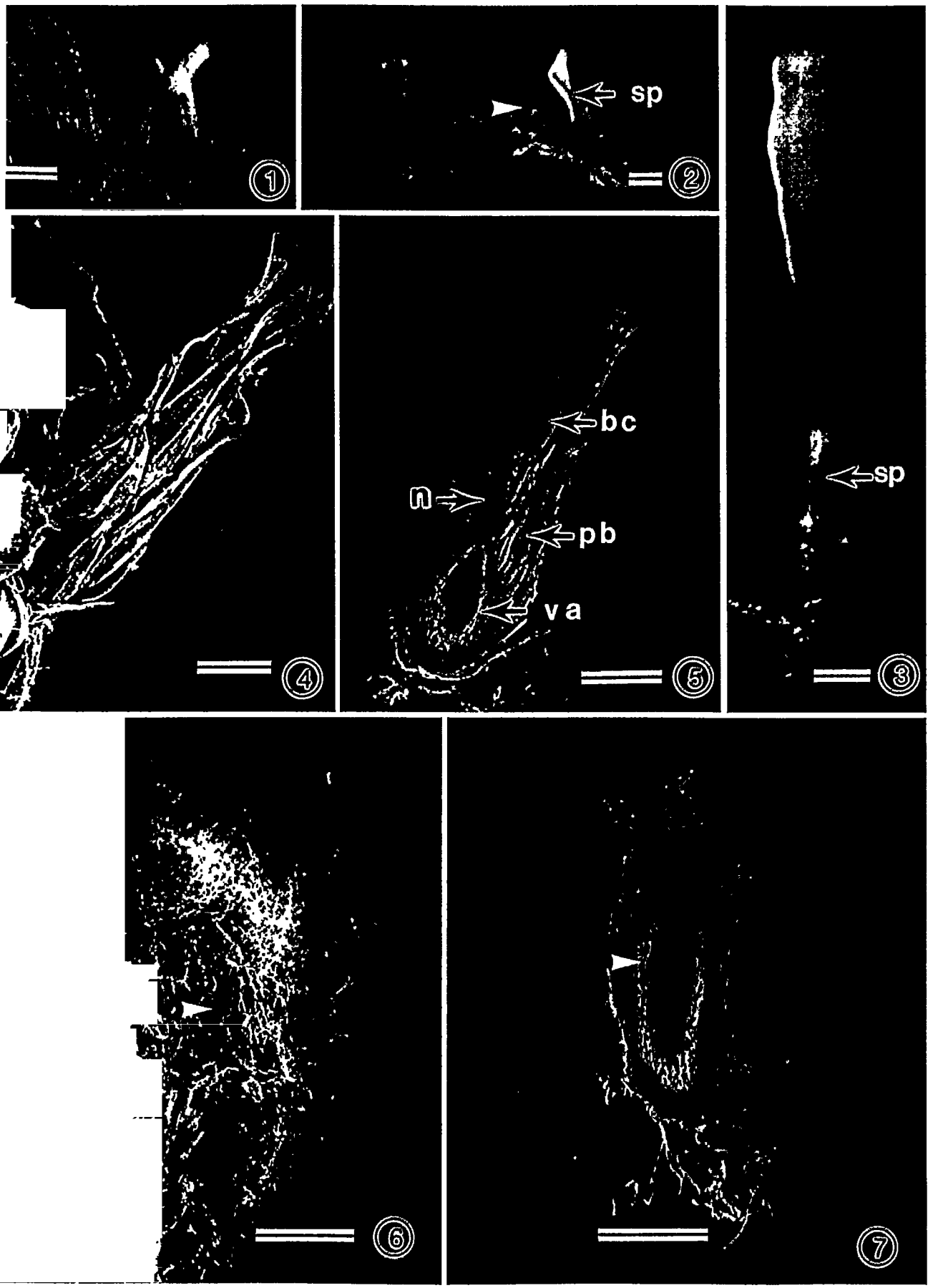
The mature basidiocarp sits in a pocket of gametophytic tissue termed the vaginula (Fig. 12), which remains chlorophyllous and apparently unaffected by the presence of the biotroph. In uninfected mosses the sporophyte foot is situated within the vaginula (Hebant 1977). In this particular longitudinal section of *P. polyantha* (Fig. 12) no remnants of the sporophyte are discernable, but in other sections of the same moss they were (not illustrated). In no instance were hyphae associated with the bulbous fruit-body base seen to penetrate adjacent host cells in *P. polyantha, H. microphyllum, D. uncinatus*, *F. viridulus*, and *Eurhynchium hians*, as has been reported and confirmed for the *Climacium* species. Instead, interfacial phenomena reported as commonly occurring in uninfected gametophyte–sporophyte interactions are encountered.

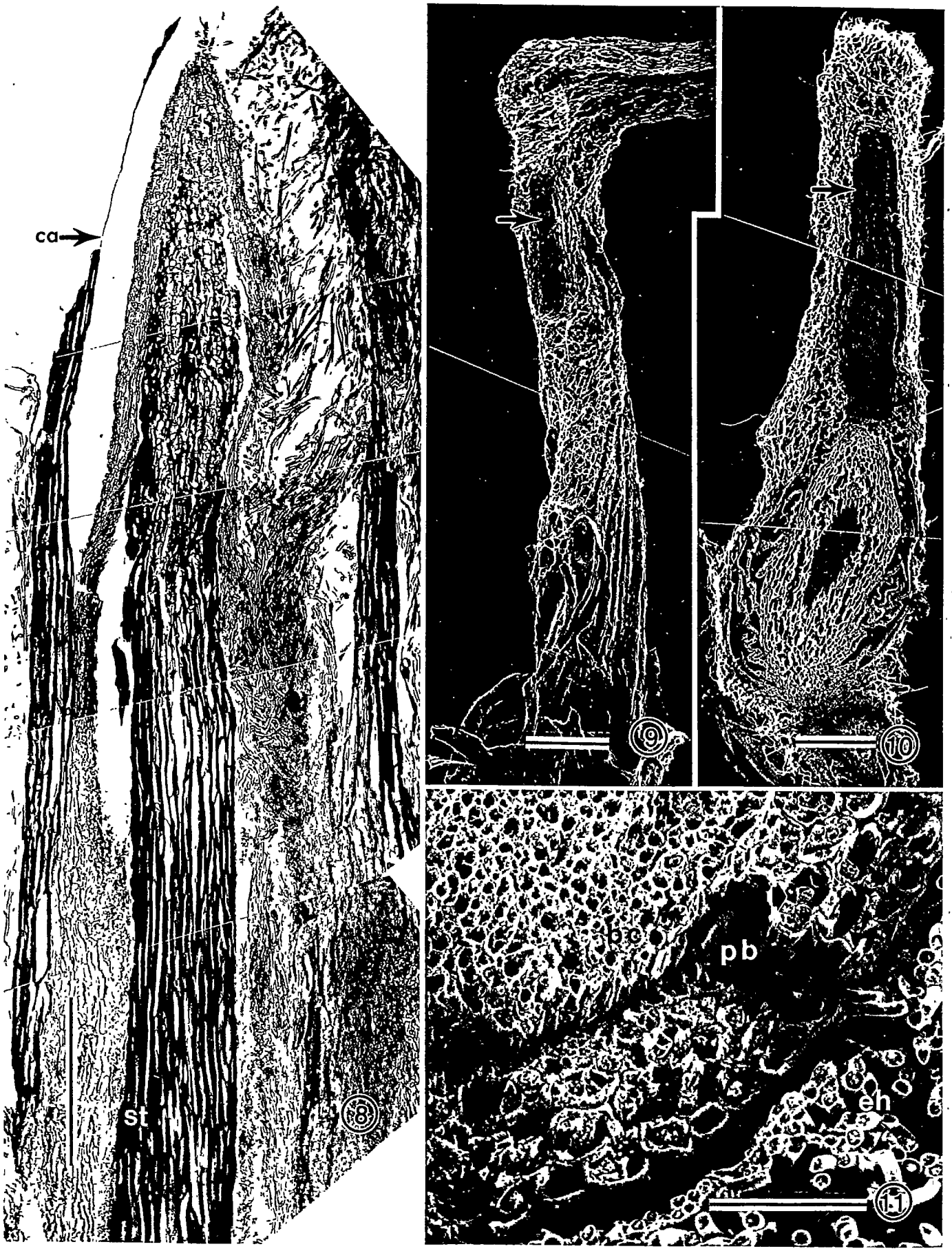
The fruit-body base of *E. muscicola* is surrounded by host gametophytic transfer cells (Figs. 12–17). The transfer cells are characterized by secondary wall appositions forming invaginations along the inner wall adjacent to the biotrophic hyphae (Figs. 13, 14, and 17). The far wall of the host transfer cells bears no invaginations (Fig. 13). The presence of nuclei and nucleoli in the transfer cells attests to their viability (Fig. 14). Interestingly, where fungal hyphae deviate intercellularly from the main axis of the fruit body, host transfer cells are seen to differentiate (Fig. 13). The secondary wall of the transfer cell can clearly be distinguished from the primary wall (Figs. 16 and 17) and the invaginations of the former often

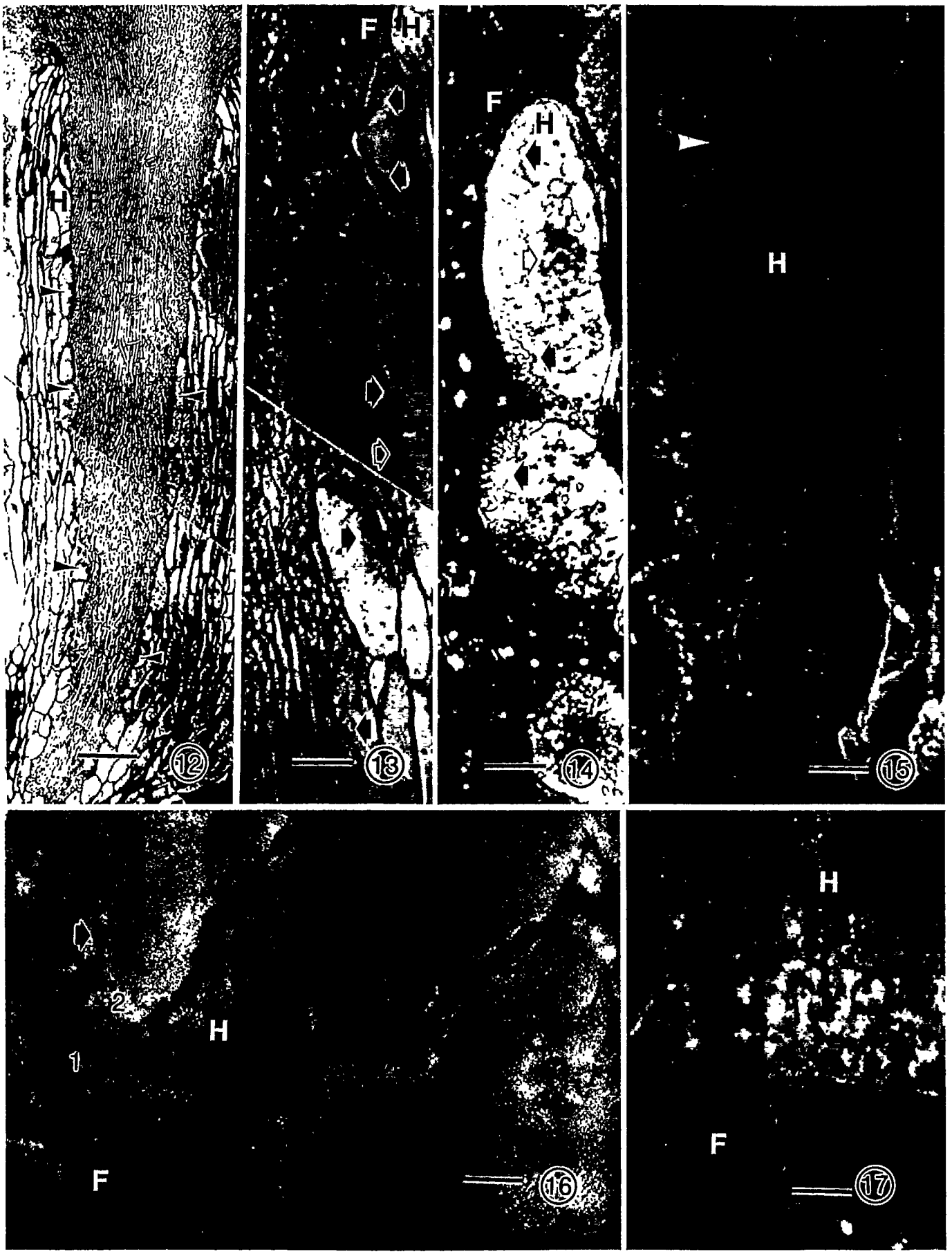
FIGS. 1 and 2. *Haplocladium microphyllum* with developing basidiocarps of *Eocronartium muscicola* associated with postfertilized perichaetia bearing arrested sporophytes (*sp*). Note the diminished size of unfertilized perichaetia (white arrowhead) in Fig. 2. Scales, 1 mm. FIG. 3. *Pylaisiella polyantha* with mature basidiocarp of *E. muscicola* encasing an arrested sporophyte (*sp*). *Haplocladium microphyllum* and *P. polyantha* tend to retain the sporophyte to varying degrees during the biotrophy, while in the other mosses analyzed it is rapidly displaced by the fungus. Scale, 1 mm. FIGS. 4–7. SEM of *Drepanocladus uncinatus* and *E. muscicola*. Figs. 4 and 5. Surface view and cryofracture of the same specimen. Fig. 4. Young postfertilized perichaetium showing no external evidence of infection. Scale, 250 μm. Fig. 5. Cryofracture revealing the vaginula (*va*), perichaetial bracts (*pb*), and developing basidiocarp (*bc*) situated above the archegonial neck canal region (*n*). Scale, 250 μm. Figs. 6 and 7. Surface view and cryofracture of the same specimen at a later stage of development. Fig. 6. Basidiocarpic initials emerging from the perichaetial bracts (arrowhead) in loose hyphal wefts. Scale, 500 μm. Fig. 7. Cryofracture revealing the expanded archegonium (arrowhead) in which no trace of the sporophyte is discernable, having been completely supplanted by biotrophic hyphae. In no instance are hyphae seen to diverge from the vaginular pocket into the surrounding gametophytic tissue. Scale, 500 μm.

FIGS. 8–11. *Haplocladium microphyllum* with *Eocronartium muscicola*. Fig. 8. LM illustrating the necrotrophic association in which hyphae of *E. muscicola* ramify and disrupt the developing seta (*st*) of the host sporophyte. This necrotrophic association ceases when hyphae encounter gametophytic tissue. The seta (*st*) has not emerged from the calyptera (*ca*). This is a similar developmental stage as that illustrated in Fig. 2. Scale, 250 μm. Figs. 9 and 10. SEM of the surface view and cryofracture of the same specimen. Fig. 9. The sporophyte (arrow), having emerged from the surrounding perichaetial bracts, is arrested and encased in basidiocarpic hyphae. Scale, 250 μm. Fig. 10. A near-medial fracture reveals fungal hyphae within the central core of the seta and occupying the vaginular pocket. Scale, 250 μm. Fig. 11. A transverse fracture through the basal region of a mature basidiocarp showing a central core of thick-walled, gelatinized hyphae comprising the central axis of the fruit body (*bc*), surrounded by periarchaetial bracts (*pb*), some of which have been penetrated by fungal hyphae (arrow), and enveloped in a loose external hyphae weft (*eh*). Scale, 50 μm.

FIGS. 12 and 13. *Pylaisiella polyantha* with *Eocronartium muscicola*. Fig. 12. LM of a medial longitundinal section through a mature basidiocarp. The fruit-body base is situated within the vaginula (VA), which remains chlorophyllous. In uninfected mosses the sporophyte foot occupies this region. Remnants of the sporophyte were detected in adjoining sections (not illustrated). The fruit-body base is surrounded by gametophytic host transfer cells (arrowheads). Scale, 250 μm. Fig. 13. LM on a longitudinal section illustrating the host–parasite interface. Note that the secondary wall appositions (arrows), characterizing transfer cells, are differentiated only along the abutting host wall. Where fungal hyphae deviate laterally from the main axis of the fruit body, transfer cells are differentiated (open arrow). Scale, 50 μm. FIG. 14. LM of *Haplocladium microphyllum* and *E. muscicola*. Host–parasite interface illustrating secondary wall appositions (solid arrows) along the host wall directly abutting fungal hyphae. Note host cell nucleus and nucleolus (open arrow). Scale, 25 μm. FIG. 15. SEM of *Pylaisiella polyantha* and *E. muscicola* cryofracture revealing secondary wall appositions, characterizing gametophytic transfer cells. Note some wall protrusions are branched (arrowhead). Scale, 5 μm. FIGS. 16 and 17. TEM of *Haplocladium microphyllum* and *E. muscicola*. Fig. 16. The fungus – gametophytic host nutritional interface. The secondary (2) wall appositions, characterizing the host transfer cell, can clearly be distinguished from the primary (*1*) wall. Note the plasma membrane (arrow) continuity over the secondary wall appositions. Scale, 0.5 μm. Fig. 17. Note the labyrinthine nature of the secondary wall appositions. Scale, 1.5 μm. Host (H) and fungus (F).


sp
bc
n
pb
va
sp


ca
st
8
9
10
pc
pb
eh
11


H
F
VA
12
F
H
13
F
H
14
H
15
2
H
1
F
16
H
F
17

anastomose to form labyrinths (Fig. 17) or dichotomous branches (Fig. 15). The host plasma membrane forms a continuum around the wall appositions (Fig. 16). The host transfer cells do not appear to form a distinct band or collar around the fruit-body base (Fig. 12), as has been reported in uninfected gametophyte–sporophyte associations (Wiencke and Schultz 1975), but instead are randomly distributed. In the host, hydroids, water-conducting elements, have been observed subtending the fruit-body base (not illustrated).

Discussion

Eocronartium muscicola establishes a unique biotrophic relationship, characterized by a host transfer cell nutritional interface, with a diverse assemblage of moss hosts. Fungal hyphae are seen to attack and eventually supplant entirely the developing sporophyte generation in the mosses analyzed in this study, with the sole exception of *C. dendroides*. Interestingly, this necrotrophic association ceases when host gametophytic tissue is encountered. If the mature basidiocarp–moss association is analyzed histologically without first studying the earlier developmental stages, the disintegration of the moss sporophyte and, hence, the nature of the fertilized perichaetial region as the site of fungal sporulation may remain undetected.

Transfer cells are induced to differentiate in response to fertilization in many bryophytes (Hebant 1975; Kelly 1969; Wiencke and Schultz 1975). Transfer cells may exist in both the gametophyte and the sporophyte (Eyme and Suire 1967) or they may be present solely on the sporophytic (Maire 1967) or gametophytic (Gunning and Pate 1969) side of the interface. Since no plasmodesmatal connection links the two generations (Hebant 1977), the increased surface-to-volume ratios afforded by wall ingrowths greatly increase the surface area of the plasma membrane and, therefore, augment transport of solutes between generations. Histochemical studies have shown that transfer cells are the site of intense enzymatic activities, especially of phosphatases and respiratory enzymes (Hebant and Suire 1974). High mitochondrial populations also characterize these zones (Hebant 1977). In certain Polytrichales (Maire and Maire 1972; Hebant 1975) extensive systems of endoplasmic reticulum have been reported. Specialized ER arrays have similarly been observed in various other cell types implicated in transport phenomena (Hebant 1977). Although structurally well characterized, bryophillous transfer cells are less well understood physiologically. Nevertheless, the gametophyte–sporophyte interface must represent a demanding nutritional sink, at least during the early stages of sporophyte development. According to Wiencke and Schultz (1975) the transport of nutrients to the sporophyte ceases after two-thirds of its development. Such a nutritional sink offers a unique niche for a biotrophic fungus capable of securing nutrients without undue damage to the host. Occupying the position of the sporophyte enables the biotroph to have direct access to the gametophytic transfer cell apparatus.

Amplification of interfacial membrane systems is not restricted to the bryophytes, having been well documented in a wide variety of anatomical situations within the higher plants (Gunning and Pate 1969; Browning and Gunning 1977). Transfer cells have also been recorded from interfaces involving dissimilar organisms including examples of basidiomycetous fungi involved in higher plant transfer cell associations. Monotropoid ectomycorrhizal associations apparently involve the inducement by the mycobiont of localized host transfer cells (Duddridge and Read 1982; Robertson and Robertson 1982) in which host wall appositions form in response to determinate hyphal protrusions into adjacent epidermal cells. Ashford and Allaway (1982) have found, in *Pisonia grandis* R. Br., that a wall labyrinth is differentiated along host walls directly abutting sheathing ectomycorrhizal hyphae. The ectomycorrhizal association is unusual in that, in place of the Hartig net, fungal-induced host transfer cells serve a similar function to augment the surface-to-volume ratio (Allaway et al. 1985). Transfer cells have also been induced *in vitro* between ectomycorrhizal associates that *in vivo* do not normally induce such cells (Duddridge and Read 1984). Transfer cells have also been reported in the *Alnus crispa* – *Alpova diplophloeus* ectomycorrhizal association (Massicotte et al. 1986).

Roth (1969) has demonstrated that a conducting strand linking the archegonium with the central axial strand of the gametophyte is established only by the presence of a developing embryo and that nonfertilized archegonia degenerate by the failure to establish this vascular connection. Similar findings have been recorded for other mosses (Hebant 1977). *Eocronartium muscicola* seems to establish basidiocarpic initials only in postfertilized archegonial regions, i.e., those bearing developing embryos. This fact suggests that these regions, in contrast to nonfertilized archegonia, apparently provide the necessary vascular connection to establish the perichaetium as a nutritional sink, capable of supporting a sporulating fungal fruit body.

Is *E. muscicola* actually inducing the transfer cells so as to facilitate nutrient uptake or is the fungus simply utilizing preformed transfer cells? It seems that transfer cells, for the most part, are exploited as preformed structures, under the inducement of the developing sporophyte prior to its supplantation. The mosses *H. microphyllum* and *P. polyantha*, which retain sporophytes for longer periods, tend to display a greater number of transfer cells than do those mosses in which sporophytes are rapidly displaced by the developing fungus, i.e., *D. uncinatus*, *F. viridulus*, and *Eurhynchium hians*. Clearly, a nutritional advantage is gained in those associations where *E. muscicola* is capable of a prolonged biotrophy without rapid degeneration of the sporophyte. *Eocronartium muscicola*, however, appears to possess a limited capacity for inducing host transfer cells. When basidiocarpic hyphae penetrate between cells of the host gametophyte, transfer cells are seen to differentiate.

Growing apices also are believed to represent nutrient sinks to which photosynthates are transported (Richardson 1981). *Climacium* species rarely produce sporophytes (Fitzpatrick 1918*a*), which may explain the association of *E. muscicola* as an intracellular fungal parasite in branch apices of *C. americanum* and *C. dendroides*. Unfortunately, Fitzpatrick (1918*a*) concentrated his histological analysis primarily on *C. americanum* and, thus, erroneously concluded that *E. muscicola* behaved solely as an intracellular parasite among all of its moss hosts. The present study clarifies the heterogeneity observed in the nutritional modes among the various hosts. *Eocronartium muscicola* may represent a diverse assemblage of physiologic races capable of different degrees of fungal biotrophy, and (or) genetic differences in the hosts may account for the diversity of biotrophic responses. The fungus is capable of (*i*) a mild biotrophy, as in *H. microphyllum* and *P. polyantha*, in which the sporophyte persists and hence a greater number of transfer cells may be exploited or (*ii*) a more aggres-

sive biotrophy, where the host sporophyte is arrested early in its development, as in *D. uncinatus, Eurhynchium hians,* and *F. viridulus,* or (*iii*), where sporophytes are infrequently produced, as in *Climacium* species, exploitation of another available nutritional sink, the gametophytic branch apices, occurs.

Despite Fitzpatrick's (1918*a*) detailed efforts to elucidate the life cycle of *E. muscicola*, including basidiospore inoculation on a wide variety of susceptible mosses, in no instance was successful infection realized. Suspecting an earlier stage in the life cycle of the host to be susceptible, Fitzpatrick (1918*a*) cultured protonema, derived from *Brachythecium oxycladon* (Brid.) Jaeg. & Sauerb., with germinating basidiospores, again without success. This latter failure, however, may have been because the dual cultures were retained for only 1 week. The current study did not attempt to examine the life cycle of *E. muscicola*. Presently, therefore, the ecology and manner by which the fungus becomes established remain unknown. However, numerous moss archegonia produce a simple sugar acting as a sperm attractant (Machlis and Rawitscher-Kunkel 1967). Germinating basidiospores on the moss surface may grow unidirectionally towards the archegonium following a sugar gradient through the water film, although this has not been substantiated.

Eocronartium muscicola is taxonomically related to species of *Jola* (Gaumann 1922; Khan and Kimbrough 1980, 1982; Lowy 1971; Martin 1939; Oberwinkler and Bandoni 1982, 1984; Stanley 1940), also members of the simple-septate Auriculariales *sensu lato* parasitizing mosses. *Jola* fructifications, however, are restricted to the apex of the sporophytes of the tropical mosses it parasitizes. It also likely exploits transfer cells but indirectly via the sporophyte, which serves as a nutritional bridge, unlike *E. muscicola*, in which transfer cells are exploited directly by the supplantation of the host sporophyte. Both *Eocronartium* and *Jola* parasitize moss sporophytes, the latter being restricted to the capsule while the former supplanting it to varying degrees.

The simple-septate auricularioid taxa encompass many nutritional modes ranging from biotrophy to saprotrophy. *Eocronartium muscicola*, unlike the rusts, does not produce haustoria but rather makes use of gametophytic nutritional sinks often via host transfer cell exploitation. The host–parasite interface in *Jola* has not been analyzed, but it is assumed to be restricted to diploid tissue (Gaumann 1922). *Herpobasidium* and *Platycarpa*, auricularioid fern parasites, produce elongate, coiled haustoria (Oberwinkler and Bandoni 1984). Haustoria within the Pucciniastraceae, Uredinales, show a trend towards an economy of surface area, with large, digitate and lobed haustoria in primitive rusts parasitizing ferns to smaller haustoria in more advanced groups (Moss 1926). The auricularioid fern parasites show some haustorial similarity with the rusts, but the phylogenetic significance of the host–parasite interface in *E. muscicola* remains unclear. It appears to be strikingly different from the host–parasite interface in the uredinalian and auricularioid fern parasites. It may reflect the distinctive nature of the bryophytic host group. The nonhaustorial intracellular hyphae of *E. muscicola* in *Climacium* species are comparable with monokaryotic hyphae in some rusts (Harder and Chong 1984), but they are dikaryotic in *E. muscicola.*

Eocronartium and *Jola* have been proposed as extant representatives of the ancestral complex postulated to have given rise to the Uredinales (Hennan and Buritica 1980; Khan and Kimbrough 1980, 1982; Oberwinkler 1982). Hennen and Buritica (1980) have suggested that simple "autoecious auricularioid rusts," parasitic on mosses and ferns (*Eocronartium, Jola, Herpobasidium*, and *Platycarpa*), represent archaic rust lineages with unexpanded life cycles, having never acquired the auxilliary spore states characteristic of the primitive heteromacrocyclic fern–fir rusts. Julich (1982) has even established the Eocronartiaceae in the Uredinales to accommodate *E. muscicola*; however, this proposal has been criticized (Bandoni 1984). The authors are inclined to agree with Oberwinkler and Bandoni (1982), who suggest the Cystobasidiaceae, Auriculariales *sensu lato*, as a temporary repository for simple-septate auricularioid taxa until a time when sufficient evidence has been accumulated to unite or segregate these organisms into a septate order or orders distinct from the Auriculariales *sensu strictu.* Towards this end the authors are currently engaged in an ultrastructural analysis of basidial development and the associated nuclear cycles to characterize further the simple-septate auricularioid taxa.

Acknowledgements

The authors express their gratitude to Dr. Tauno Ulvinen (Botanical Museum, University of Oulu, Finland) for his insightful correspondence and for the infected moss specimens from Fennoscandia. The authors thank Dr. Jan Janssens (Department of Ecology and Behavioral Biology, University of Minnesota), for his assistance in identifying the Minnesota moss hosts and for helpful suggestions, and Dr. R. J. Bandoni (Department of Botany, University of British Columbia), for reviewing the manuscript. This study was supported by National Science Foundation grants BSR-8307131 to D. J. McLaughlin and K. L. O'Donnell and BSR-8516895 to D. J. McLaughlin.

ALLAWAY, W. G., CARPENTER, J. L., and ASHFORD, A. E. 1985. Amplification of inter-symbiont surface by root epidermal transfer cells in the *Pisonia* mycorrhiza. Protoplasma, **128**: 227–231.

ASHFORD, A. E., and ALLAWAY, W. G. 1982. A sheathing mycorrhiza on *Pisonia grandis* R. Br. (Nyctaginaceae) with development of transfer cells rather than a Hartig net. New Phytol. **90**: 511–519.

ATKINSON, G. J. 1902. Preliminary note on two new genera of Basidiomycetes. J. Mycol. **8**: 106–107.

BANDONI, R. J. 1984. The Tremellales and Auriculariales: an alternative classification. Trans. Mycol. Soc. Jpn. **25**: 489–530.

BESSEY, E. A. 1950. Morphology and taxonomy of fungi. Hafner Press, New York.

BROWNING, A. J., and GUNNING, B. E. S. 1977. An ultrastructural and cytochemical study of the wall-membrane apparatus of transfer cells using freeze-substitution. Protoplasma, **93**: 7–26.

DONK, M. A. 1972. The Heterobasidiomycetes: a reconnaissance. II. Some problems connected with the restricted emendation. Proc. K. Ned. Akad. Wet. Ser. C, **75**: 376–390.

DUDDRIDGE, J. A., and READ, D. J. 1982. An ultrastructural analysis of the development of mycorrhizas in *Monotropa hypopitys* L. New Phytol. **92**: 203–214.

——— 1984. Modification of the host–fungus interface in mycorrhizas synthesized between *Suillus bovinus* (Fr.) O. Kuntz and *Pinus sylvestris* L. New Phytol. **96**: 583–588.

EYME, J., and SUIRE, CL. 1967. Au sujet de l'infrastructure de la zone placentaire de *Mnium cuspidatum* Hedw. (mousse bryale acrocarpe). C.R. Hebd. Seances Acad. Sci. Ser. D, **265**: 1788–1791.

FITZPATRICK, H. M. 1918*a*. The life history and parasitism of *Eocronartium muscicola*. Phytopathology, **8**: 197–219.

——— 1918*b*. The cytology of *Eocronartium muscicola*. Am. J.

Bot. 5: 397–418.
GAUMANN, E. 1922. Uber die Entwicklungsgeschichte von *Jola javensis* Pat. Ann. Mycol. **20**: 272–289.
GUNNING, B. E. S., and PATE, J. S. 1969. Transfer cells: plant cells with wall ingrowths, specialized in relation to short distance transport of solutes—their occurrence, structure and development. Protoplasma, **68**: 107–133.
HARDER, D. E., and CHONG, J. 1984. Structure and physiology of haustoria. *In* The cereal rusts: origins, specificity, structure, and physiology. Vol. I. *Edited by* W. B. Bushnell and A. P. Roelfs. Academic Press, Inc., New York. pp. 431–473.
HEBANT, C. 1975. Organization of the conducting tissue-system in the sporophytes of *Dawsonia* and *Dendroligotrichum*. J. Hattori Bot. Lab. **39**: 235–254.
——— 1977. The conducting tissues of bryophytes. J. Cramer, Vaduz.
HEBANT, C., and SUIRE, CL. 1974. Mise en évidence d'activités enzymatiques au niveau de la zone de transfert gamétophyte–sporophyte chez quelques bryophytes. Rev. Bryol. Lichenol. **40**: 171–181.
HENNEN, J. F., and BURITICA, P. 1980. A brief summary of modern rust taxonomy and evolutionary theory. Rep. Tottori Mycol. Inst. **18**: 243–256.
JULICH, W. 1982. Notes on some basidiomycetes (Aphyllophorales and Heterobasidiomycetes). Persoonia, **11**: 421–428.
KELLY, C. 1969. Wall projections in the sporophyte and gametophyte of *Sphaerocarpos*. J. Cell Biol. **41**: 910–914.
KHAN, S. R., and KIMBROUGH, J. W. 1980. Ultrastructure and the taxonomy of *Eocronartium*. Can. J. Bot. **58**: 642–647.
——— 1982. A reevaluation of the Basidiomycetes based upon septal and basidial structures. Mycotaxon, **15**: 103–120.
LEPPIK, E. E. 1955. Evolution of angiosperms as mirrored in the phylogeny of rust fungi. Arch. Soc. Zool. Bot. Finn. Vanamo, 9(Suppl.): 149–160.
LOWY, B. 1971. Tremallales. Flora Neotropica Monograph No. 6. Hafner Publ., New York.
MACHLIS, L., and RAWITSCHER-KUNKEL, E. 1967. Mechanisms of gametic approach in plants. *In* Fertilization. *Edited by* C. B. Metz and A. Monroy. Academic Press, New York. pp. 117–161.
MAIRE, K. 1967. Wandlabyrinthe im sporophyten von *Polytrichum*. Planta, **77**: 108–126.
MAIRE, K., and MAIRE, U. 1972. Localization of beta-glycerophosphatase and Mg-activated adenosine triphosphate in a moss haustorium, and the relation of these enzymes to the cell-wall labyrinth. Protoplasma, **75**: 91–112.
MARTIN, G. W. 1939. New and noteworthy fungi from Panama and Columbia. III. Mycologia, **31**: 239–249.
MASSICOTTE, H. B., PETERSON, R. L., ACKERLEY, C. A., and PICHÉ, Y. 1986. Structure and ontogeny of *Alnus crispa – Alpova diplophloeus ectomycorrhizae*. Can. J. Bot. **64**: 177–192.
MCLAUGHLIN, D. J. 1980. Ultrastructure of the metabasidium of *Auricularia fuscosuccinea*. Am. J. Bot. **67**: 1225–1235.
MOSS, E. H. 1926. The uredo stage of the Pucciniastreae. Ann. Bot. London, **40**: 813–847.
OBERWINKLER, F. 1982. The significance of the morphology of the basidium in the phylogeny of Basidiomycetes. *In* Basidium and basidiocarp. *Edited by* K. Wells and E. K. Wells. Springer-Verlag, New York. pp. 9–34.
OBERWINKLER, F., and BANDONI, R. J. 1982. A taxonomic survey of the gastroid, auricularioid Heterobasidiomycetes. Can. J. Bot. **60**: 1726–1750.
——— 1984. *Herpobasidium* and allied genera. Trans. Br. Mycol. Soc. **83**: 639–658.
OLIVE, L. S. 1948. Taxonomic notes on Louisiana fungi. II. Tremellales. Mycologia, **40**: 586–603.
RICHARDSON, D. H. S. 1981. The biology of mosses. John Wiley and Sons Inc., New York, Toronto.
ROBERTSON, D. C., and ROBERTSON, J. A. 1982. Ultrastructure of *Pterospora andromedea* Nuttall and *Sarcodes sanguinea* Torrey mycorrhiza. New Phytol. **92**: 539–551.
ROTH, D. 1969. Embryo und embryotheca bei den laubmoosen. Eine histogenetische und morphologische untersuchung. Bib. Botanica, **129**: 1–49.
ROWLEY, J. C., and MORAN, D. T. 1975. A single procedure for mounting wrinkle free sections or Formvar coated slot grids. Ultramicroscopy, **1**: 151–144.
SPURR, A. R. 1969. A low-viscosity resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**: 31–43.
STANLEY, I. N. 1940. Development of the basidium of *Eocronartium muscicola*. Trans. Am. Microsc. Soc. **59**: 407–413.
ULVINEN, T. 1981. *Eocronartium muscicola* (Fungi, Auriculariales) in Fennoscandia. Mem. Soc. Fauna Flora Fenn. **57**: 81–89.
VITT, D. H. 1984. Classification of the Bryopsida. *In* The new manual of bryology. Vol. 1. *Edited by* R. M. Schuster. Hattori Botanical Laboratories, Nichnian, Miyazaki, Japan. pp. 696–759.
WIENCKE, C., and SCHULTZ, D. 1975. Sporophytenentwicklung von *Funaria hygrometrica* Sibth. I. Strukturelle grundlagen der wasser-und nahrstoffaufnahme im haustorium. Protoplasma, **86**: 107–117.