NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Pigmented algal endophytes of *Chondrus crispus* Stackhouse: Host specificity, fine structure, and effects on host performance in infections by *Acrochaete operculata* Correa & Nielsen and *A. heteroclada* Correa & Nielsen

by

Juan A. Correa

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

at

Dalhousie University
Halifax, Nova Scotia
October, 1990

ISBN 0-315-64503-2

Canada

DALHOUSIE UNIVERSITY

FACULTY OF GRADUATE STUDIES

The undersigned hereby certify that they have read and recommend to the Faculty of Graduate Studies for acceptance a thesis entitled " Pigmented Algal Endophytes of *Chondrus crispus* Stackhouse: Host Specificity, Fine Structure, and Effects on Host Performance in Iffections by *Acrochaete operculata* Correa & Nielsen and *A. heteroclada* Correa & Nielsen

by Juan Alberto Correa

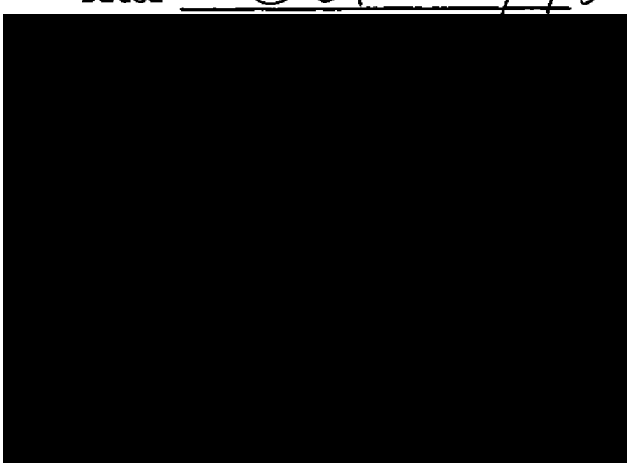
in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Dated Oct. 22/90

External Examiner

Research Supervisor

Examining Committee



DALHOUSIE UNIVERSITY

DATE: October 26, 1990

AUTHOR: Juan A. Correa

TITLE: Pigmented algal endophytes of *Chondrus crispus* Stackhouse: Host specificity, fine structure, and effects on host performance in infections by *Acrochaete operculata* Correa & Nielsen and *A. heteroclada* Correa & Nielsen

Department or School: Biology

Degree Ph.D. Convocation: Spring Year 1991

Permission is herewith granted to Dalhousie University to circulate and to have copied for non-commercial purposes, at its discretion, the above title upon the request of individuals or institutions.

Signature of Author

To my parents, my wife Patricia, my daughter María José, and sons Juan Eduardo and Cristián Andrés.

TABLE OF CONTENTS

Table of contents	v
List of illustrations and tables	vi
Abstract	viii
List of abbreviations	ix
Acknowledgments	xi
General Introduction	1
Chapter 1. Host Specificity	7
Introduction	8
Materials and Methods	14
Results	18
Discussion	24
Figures	32
Tables	47
Chapter 2. Fine structural aspects of the infections	56
Introduction	57
Materials and Methods	63
Results	68
Discussion	87
Figures	104
Chapter 3. Effect of infections on performance of the host	154
Introduction	155
Materials and Methods	158
Results	167
Discussion	172
Figures	182
Tables	223
Final Remarks	226
Bibliography	231

LIST OF ILLUSTRATIONS AND TABLES

Chapter 1. Host specificity.

Figs 1-12	35
Figs 13-21	38
Figs 22-23	40
Figs 24-25	42
Figs 26-27	44
Figs 28-33	46
Table 1	48
Table 2	49
Table 3	52
Table 4	54
Table 5	55

Chapter 2. Fine structural aspects of the infections.

Figs 1-6	106
Fig. 7	108
Figs 8-12	110
Figs 13-21	112
Figs 22-33	115
Fig. 34	117
Figs 35-42	119
Figs 43-50	122
Figs 51-57	124
Figs 58-64	126
Figs 65-72	129
Figs 73-81	131
Figs 82-90	134
Figs 91-96	136
Figs 97-103	138
Figs 104-107	140
Figs 108-109	142
Fig. 110	144
Fig. 111	146
Figs 112-115	148

Figs 116-122 151
Fig. 123 153

Chapter 3. Effects of infections on performance of the host
Fig. 1 184
Figs 2-3 186
Figs 4-5 188
Fig. 6 190
Figs 7-8 192
Figs 9-10 194
Figs 11-12 196
Fig. 13 198
Figs 14-15 200
Fig. 16 202
Figs 17-18 204
Fig. 19 206
Figs 20-21 208
Figs 22-23 210
Figs 24-25 212
Figs 26-27 214
Figs 28-29 216
Figs 30-31 218
Figs 32-33 220
Figs 34-35 222
Table 1 224
Table 2 225

ABSTRACT

The present study was undertaken to elucidate various aspects of the symbiosis established between the rhodophyte *Chondrus crispus* and its green algal endophytes *Acrochaete operculata* and *A. heteroclada*. The three main aspects investigated were (a) host specificity, (b) fine structure of the infections and (c) effects of the infections on the performance of *C. crispus* in culture.

Acrochaete operculata is a highly selective endophyte whose specificity is determined by the polysaccharide fraction of the host cell wall. It was demonstrated that only hosts producing carrageenan of the lambda family were susceptible, regardless of species. Sporophytic fronds of *Iridaea cordata* and *C. crispus*, both λ-carrageenophytes, displayed similar patterns of infections. The, gametophytic, κ-carrageenophytes, fronds of *I. cordata* were not infected and those of *C. crispus* displayed light infection which did not spread throughout the frond. Fine structural observations indicate that *A. operculata* breaches the outer cell wall of *C. crispus*, apparently by enzymatic means, reaches the cortex and spreads throughout the frond embedded in the intercellular matrix. Only minor host-cell damage occurs at this stage, whereas subsequent maturation of the endophyte, followed by zoospore release, causes massive host-cell destruction accompanied by disruption of the outer cell wall. This permits secondary bacterial infection, which accelerates the decay of the host tissue. Cellular destruction in infected fronds results in low biomass accumulation, poor or suppressed regenerative capacity and tissue softening. Furthermore, infected *C. crispus* becomes a preferred item when offered to crustacean grazers.

Acrochaete heteroclada was not host specific and infected various carrageenophytic, agarophytic and other hosts. Penetration of the host outer cell wall, which appears to be mechanical, is followed by development of endophytic filaments within the inner portion of the outer cell wall. No host-cell damage was observed at this stage, while subsequent penetration of the cortex of *C. crispus* resulted in massive cell destruction. Effects of infection on host performance were similar to those observed after infection by *A. operculata*, although *A. heteroclada* affected the two life-history phases of *C. crispus*.

The results of this study lead to the conclusion that *A. operculata* and *A. heteroclada* are pathogens, with potentially negative effects on both wild and cultivated *C. crispus*.

ABBREVIATIONS

Chapter 1.

Ah: *Acrochaete heteroclada*
Br: bromine
EN: endophytic in experimental plants
ENc: endophytic in control
EP: epiphytic on experimental plants
EPc: epiphytic on control
G: penetrating germling
Ga: gametophyte
H: host tissue
IF: inner face of cuticular lamellae
N.S.: Nova Scotia
OF: outer face of cuticular lamellae
PE: penetration of experimental plants
PEc: penetration of controls
P.E.I.: Prince Edward Island
PH: life history phase of the experimental plant
S: sporophyte
ZW: zoospore wall

Chapter 2.

AC: apical cell
B: bacteria
Cl: chloroplast
Cu: cuticle
E: invasive filament or endophytic cell
ER: endoplasmic reticulum
EW: endophyte cell wall
G: Golgi bodies
Gt: germ tube

H: host or host cells
ICW: immediate cell wall
IM: intercellular matrix
La: lamella
m: mitochondria
N: nucleus
Nu: nucleolus
OC: outer cortex
OCW: outer cell wall
Pp: pit-plug
Py: pyrenoid
S: sporangia
Se: septum
SP: starch plates
St: starch granules
V: vacuole
VC: vegetative cell
Ve: vesicles
ZW: zoospore cell wall

Chapter 3.

Ah: *Acrochaete heteroclada*
Ao: *Acrochaete operculata*
G: gametophyte
INF: infected
M: mandibles
NON INF: non infected
S: sporophyte

ACKNOWLEDGMENTS

I would like to thank J. McLachlan for his support and guidance during all these long and sometimes rough years. I specially appreciate the freedom he gave me to develop my research, and his endless patience to review early drafts of this thesis.

In a retrospective way, I want to thank my professor and friend B. Santelices, who was determinant in bringing about the conditions which allowed me to pursue graduate studies in Canada. Several other professors at the Pontificia Universidad Católica de Chile, including P. Sanchez, J. Lewin, and R. Albertini, also contributed with their effort and support not only before and during my graduate studies, but also did their best to facilitate my return to Chile.

Laboratory and many other facilities were provided by the Institute of Marine Biosciences, National Research Council of Canada. This institution also provided a stipend for my last three months in Canada. The Pontificia Universidad Católica de Chile granted a leave of absence for which I am thankful.

Special recognition and gratitude to Dave O'Neil and Paul Odense for their readiness to facilitate the use of the electron microscopy facilities at the IMB.

I greatly appreciate algal material provided by the following phycologists: C. J. Bird, J. McLachlan, J. S. Craigie, L. C-M. Chen, J. van der Meer, M. D. Guiry, C. Maggs, J. A. West, I. Novaczeck, G. Hannach and C. Luxoro.

Recognition also goes to R. Nielsen and J. S. Craigie for sharing unpublished information with me and for long and stimulating discussions regarding the phenomenon of endophytism.

Special thanks to M. Greenwell, who was always ready to help me and my family, and gave us happiness and friendship in a way that we will never forget.

GENERAL INTRODUCTION

Biologists have recognized that intimate associations among individuals of different taxa are the norm more than the exception, and marine multicellular algae do not escape this generalization. Indeed, finding multicellular algae living on other organisms, or serving as substratum for other algae and sessile invertebrates, is a phenomenon of common occurrence particularly in intertidal communities. For example, almost half of the 368 species cited by Abbott and Hollemberg (1976) for the coast of California were found living on or in other algae or invertebrates (Goff 1983). The analysis of 11 floristic studies from around the world, focussing on rhodophycean taxa, indicated that 23% to 79% of the species were reported growing attached to, or boring into, other living organisms (Correa and McLachlan, unpubl.)

Traditionally, these associations have been classified as epiphytic or endophytic when the host is a plant (algae, seagrasses, *etc.*). Thus, an epiphyte is an organism that uses a plant as substratum for attachment. Although in some cases such as algal partners, there might be penetration of the host by rhizoids (Rawlence and Taylor 1970, Hallam *et al.* 1975, Gonzalez and Goff 1989), much of the thallus develops outside the host. An endophyte, on the other hand, is an organism that lives its vegetative life embedded within the tissues of a host plant. These definitions must be considered as indicators of only the spatial relationship between an organism and its algal host, and no other connotations should be ascribed to them.

In general, little more than the spatial relationship is presently known for most epiphytic and endophytic algal associations. Some epiphytic associations have received special attention as a result of their high degree of host specificity (Ducker and Knox 1984). However, the possibility that host-specificity results from physiological dependence of the epiphytes upon their hosts, involving translocation of substances, remains equivocal and precludes generalizations. While the rhodophycean *Polysiphonia lanosa* (L.) Tandy lives almost exclusively associated with the phaeophycean *Ascophyllum nodosum* (L.) Le Jolis throughout most of the

host range (Lobban and Baxter 1983), translocation of photosynthates from *A. nodosum* to *P. lanosa* is negligible, and likely to have no impact as metabolic substrates of biological importance (Harlin and Craigie 1975, Turner and Evans 1977). Although *P. lanosa* is certainly carbon independent (Bidwell 1958), exchange of inorganic ions (Perrot 1974, Perrot and Perrot 1981) and translocation of glutamic acid (Citharel 1972a, b) have been demonstrated in the *P. lanosa-A. nodosum* association. Exchange of substances has also been demonstrated to occur between the foliose bangiophycean *Smithora naiadum* (Anders.) Hollenb. and its hosts, the seagrasses *Phyllospadix* and *Zostera* (Harlin 1973b). The once believed obligate nature of the association for the epiphyte, based upon translocation of substances became unsustainable, after Harlin (1973a) demonstrated that artificial seagrass was equally colonized by the epiphyte, indicating that ecological rather than host-related factors were responsible for the habit of *S. naiadum*.

Epiphytic associations have also received some attention from an ecological perspective, where it has been suggested that algal epiphytes, in general, may have a detrimental effect on their hosts (Chapter 3 for references).

A high degree of intimacy, and dependence upon their host has been achieved by rhodophycean parasitic species. Among algal associations, parasites have received most attention (Evans *et al.* 1978 and Goff 1982a). Goff (1982a) listed more than 100 species meeting the morphological criteria of reduced size and pigmentation and the establishment of cellular connections with their hosts, always other red algae, for them to be considered parasites. As Goff (1982a) indicated, most algal parasites are closely related to their hosts (adelphoparasites). Experiments using radioactive photosynthate-precursors indicate that, indeed, a physiological relationship exists, and that in general, carbon translocated from the host is inversely correlated with the degree of pigmentation displayed by the parasite (Evans *et al.* 1973, Goff 1979a, Callow *et al.* 1979, Court 1980, Kremer 1983).

Other aspects of parasitic associations which have been investigated include cellular interactions (Kugrens and West 1973, Goff 1976, 1979b, Wetherbee 1979, Goff and Coleman 1984, 1985, 1987, Zuccarello and Goff 1988, Wetherbee and Quirk 1982a, b), host-specificity (Nonomura

and West 1981, Goff 1982a), and effects of parasitic infections on host performance (Nonomura 1979, Apt 1984).

Endophytism by pigmented algae has, on the other hand, been the most neglected type of organismic association involving algal partners, as studies have concentrated mainly on taxonomic and life history aspects of the endophytic taxa. Rhodophycean endophytes belong to the family Acrochaetiaceae, a taxonomically complex group which includes filamentous species with relatively simple vegetative and reproductive structures. The taxonomic aspects of this group are beyond the scope of my study, and extensive discussions can be found in the works by Garbary (1979a), Dixon and Irvine (1977), Woelkerling (1973), and Lee and Lee (1988). Acrochaetioid endophytes are widely distributed, and under field conditions they seem to be obligately associated with their hosts. The improvement of culture techniques to establish laboratory cultures developed during the late 60's has resulted in clear demonstration that many of these species develop and reproduce well in the absence of their hosts (Swale and Belcher 1963, West 1968, 1970, 1972, 1979; Boney and White 1968, White and Boney 1969, 1970; Boney 1970, 1972, 1975, 1978, 1980; Woelkerling 1971, Garbary 1979b, c; Garbary and Rueness 1980, Küiper 1983, Magne and Abdel-Rhaman 1983, Abdel-Rhaman 1984, 1985a, b; Tam *et al.* 1987). Furthermore, some of these studies have shown that an assumed high degree of host specificity, a feature used in the past to erect new species, was artificial (White and Boney 1969, Boney 1980, Garbary 1979b, Tam *et al.* 1987).

Brown endophytes have been generally ascribed to the genus *Streblonema* (Setchell and Gardner 1922, 1925, Dangeard 1931, Andrews 1977, Yoshida and Akiyama 1979, Apt 1988a). However, as indicated by Pedersen (1984) there is a confusion regarding the taxonomy of *Streblonema* and related groups. This led Apt (1988a) to indicate that his identification of the gall-causing brown endophyte isolated from some species of Laminariales should be considered tentative. The presence of algal galls associated with brown endophytes is common, but in only one study the etiological nature of the endophyte, as gall-inducer, has been experimentally demonstrated (Apt 1988a).

Green endophytes comprise, among others, species of *Phaeophila, Acrochaete, Blastophysa, Entocladia* and *Endophyton.* As with rhodo-

phycean endophytes, a number of culture studies have been undertaken to reassess their taxonomic status (South 1968, Yarish 1975, Nielsen 1979, 1980, O'Kelly 1980, 1982, 1983, O'Kelly and Yarish 1980, 1981, Iima and Tatewaki 1987). Only two of those studies dealt with host-specificity (O'Kelly 1980, Iima and Tatewaki 1987).

In eastern Canada, the knowledge of endophytic associations was until recently limited to the check list provided by South (1984), where earlier morphological and some culture studies were referenced. More recently, Nielsen and McLachlan (1986) studied small green algae in the region, some of which were endophytic in various algal hosts. In that study, the rhodophycean *C. crispus* was reported, for the first time, to host a green filamentous endophyte, identified as *Endophyton ramosum* Gardner based on field collected material only. Concurrently, Correa (1986) had undertaken field and culture studies of pigmented endophytes associated with *C. crispus*. It soon became apparent that wild *C. crispus* hosted several species of green and brown endophytes, which were particularly abundant in the central portion of fully developed fronds (Correa *et al.* 1987). Furthermore, it was evident that infections by pigmented algal endophytes were common in *C. crispus* and affected intertidal and subtidal populations in Maritime Canada and subtidal populations off the coasts of Denmark and Sweden (Correa *et al.* 1987). Culture studies on some of these green endophytes demonstrated that the two most common species were *Acrochaete operculata* Correa & Nielsen and *A. heteroclada* Correa & Nielsen, and that the former species had been misidentified by Nielsen and McLachlan as *E. ramosum* (Correa *et al.* 1988).

A number of aspects remained to be investigated following the studies by Correa (1986) and Correa *et al.* (1987, 1988). For example, the explanation regarding the absence of endophytes detected in the holdfast of wild *C. crispus* remained equivocal. Correa *et al.* (1987), suggested that, based on the available information (Chen and Taylor 1976, Tveter-Gallagher and Mathieson 1980), the absence of endophytes in the holdfast of the host could be explained by the presence of a mechanical barrier, the cuticle, which permanently covered the holdfast of *C. crispus*. Accordingly, Correa *et al.* (1987) suggested an infection pattern involving zoospores settling and penetrating the fronds only at the apices, where the

cuticle was constantly peeled off during the normal growth of the plant, exposing "unprotected" tissue. Subsequent *in vitro* infection of free living fronds of *C. crispus* with both *A. operculata* and *A. heteroclada* indicated, however, that infection could take place at any point along the frond, the role of the cuticle during the infection process was then questionable (Correa *et al.* 1988). Unfortunately, no holdfasts or discoid, young plants, were used in the latter study. The most intriguing observation during the *in vitro* infections of *C. crispus* was the different development of *A. operculata* in the two life-history phases of the host. While infecting germlings could rapidly invade the cortex, reach the medulla, and colonize adjacent tissue of sporophytic fronds, they were unable to do so in the gametophytic fronds, where they remained localized (Correa *et al.* 1988). The use of only one isolate of each endophyte and one of the host raised concerns regarding whether the infection pattern by *A. operculata* was isolate-specific or it represented a more general response of the endophyte to a particular feature of *C. crispus*. Finally, Correa *et al.* (1988) did not consider fully developed infections, nor the nature of the cellular interactions at the host-endophyte interface as infection progressed.

Taking the above information into consideration, the present study was undertaken to gain additional insight into various aspects of the association between *C. crispus* and its chlorophycean endophytes, *A. operculata* and *A. heteroclada*.

My efforts were concentrated in three general areas: 1) host-specificity, 2) fine structural characterization of the different stages of infection and 3) the effect of endophytic infections on the performance of the host and on the perception of *C. crispus* as food by crustacean grazers. By integrating the results obtained in the three areas investigated, an assessment of the potential role of *A. operculata* and *A. heteroclada* as disease agents of *C. crispus* becomes possible.

Chondrus crispus is probably one of the most studied seaweeds, owing to its ecological importance as a major user of primary space and to its value as a source of carrageenan, a hydrocolloid of multiple uses in the food, pharmaceutical, cosmetic and coating industries (Glicksman 1987, McLachlan 1985, Levring *et al.* 1969, Waaland 1981). Numerous studies have been done on its structure (McLachlan *et al.* 1989, Tveter and Mathieson 1976), harvesting (Pringle and Semple 1988, Pringle *et al.*

1990), ecology (Craigie and Pringle 1978, Prince and Kingsbury 1973, Lazo *et al.* 1989, Dudgeon *et al.* 1989, Bhattacharya 1985), life history (Chen and McLachlan 1972, Taylor and Chen 1973), and cell wall chemistry (McCandless *et al.* 1973, Gordon-Mills and McCandless 1975, 1977; Gordon-Mills *et al.* 1977, Chopin *et al.* 1987). These and other topics regarding the biology of *C. crispus* have been periodically and thoroughly reviewed (Harvey and McLachlan 1973, Chopin 1986, Pringle and Mathieson 1987, Taylor and Chen 1990). More recently, two reviews have concentrated on the farming of *C. crispus* in eastern Canada, a venture that appears successful (Craigie and Shacklock 1989, Craigie 1990) after just 20 years of effort on outdoor cultivation (Simpson *et al.* 1978, Simpson and Shacklock 1979, Bidwell *et al.* 1985). Consequently, the present study will point to potential sources of frond mortality in natural populations of the host, and also lead to better understanding the origin of certain diseases already reported (Craigie and Shacklock 1989, Craigie 1990) in farmed *C. crispus*.

CHAPTER 1

HOST SPECIFICITY

INTRODUCTION

Host specificity is an intrinsic feature of spatially intimate organismic associations. However, in those associations where algae take part as obligate epi- or endophytes, or epi- or endozoans, the study of host specificity has been neglected, resulting in a poor understanding of the mechanisms involved.

Host specificity may be determined by factors external to the host, *i.e.* ecologically-determined, where the host is irrelevant for the successful growth and reproduction of the symbiont, although the particular niche it provides is unique. The red alga *Smithora naiadum* (Anders.) Hollenb. was considered an obligate epiphyte, restricted to the sea grasses *Phyllospadix scoulery* Hook. and *Zostera marina* L., until Harlin (1973a) demonstrated that spores of *S. naiadum* colonized and developed on polypropylene strips introduced in the natural habitat of the epiphyte. Another example is *Porphyra nereocystis* Anders. This red alga occurs almost exclusively on stipes of *Nereocystis luetkeana* (Mert.) Post. & Rupr., while in laboratory cultures the epiphyte develops normally in absence of the host. This behavior suggests that the host is required only for attachment, although indirectly it may provide a particular set of ecological conditions permitting development of the epiphyte (Dickson and Waaland 1985).

Alternatively, host specificity may be regulated by characters of the host, either structural or metabolic. This is suggested for the association between the obligate brown epiphyte, *Notheia anomala* Harvey & Bailey and its brown host, *Hormosira banksii* (Tur.) Decaisne (Hallam *et al.* 1980). In spite of having a pigmented thallus, *N. anomala* survives in unialgal culture only if filtered extract of the receptacles of *H. banksii* is added to the medium (Hallam *et al.* 1980). In red-algal parasites, experimental evidence also seems to indicate that host specificity is associated with metabolites produced by the host (Evans *et al.* 1978, Nonomura and West 1981, Goff 1982a). Recently, it has been indicated that the structure and dynamics of the thallus surface in the basiphyte is important in determining host specificity in the closely related, obligate epiphytes, *Microcladia coulteri* Harv. and *M. californica* Farl. (Gonzalez and Goff 1989).

It is among endophytic, non-parasitic algae that the phenomenon of specificity acquires particular relevance as the resulting intimate physical contact creates ideal conditions for cellular interactions and recognition processes (see Ducker and Knox 1984). There are a number of genera with endophytic species among Chlorophyta (*Endophyton* Gardn., *Acrochaete* Pringsh., *Entocladia* Reinke), Phaeophyta (*Streblonema* Derb. & Sol.) and Rhodophyta (*Audouinella-Acrochaetium-Rhodochorton* complex). Species of *Audouinella* (*sensu* Woelkerling 1983) were once considered highly host specific, and new species were described based on occurrences in specific hosts (Rosenvinge 1909, Børgesen 1916, 1919). After more refined culturing techniques became available, it was realized that this was incorrect. Some audouinelloid species were not only able to grow isolated from their hosts, but also to infect a wide variety of algae (White and Boney 1969, Garbary 1979b, Boney 1980).

Host specificity of green and brown endophytes has received little attention, even though they have been found to induce tissue damage, galls, and deformations in some ecologically and economically important hosts (Andrews 1977, Yoshida and Akiyama 1979, Apt 1988a, Correa *et al.* 1988). Among these endophytes, only the chlorophycean *Endophyton ramosum* Gardn. (O'Kelly 1980) and *Blastophysa rhizopus* Reinke (Iima and Tatewaki 1987), have been experimentally assayed to test host specificity.

In all studies where host specificity by a pigmented endophyte has been assessed under laboratory conditions, the elucidation of taxonomic and life-history aspects has been the main focus, with little consideration of the role played by the host in the association. Furthermore, the presence of photosynthetic pigments and the relative ease of isolation of the endophytes from their hosts, suggest that they are nutrient-independent. This has led to the erroneous impression that endophytism is an "ecological accident", and that chemical and structural features of the host have minor, if any, relevance in determining whether or not infection will occur. It must be appreciated, however, that regardless of the driving force in establishing an endophytic habit, *i.e.* a particular niche, nutrients, *etc.*, the symbiont must enter into the host by breaching, using mechanical or enzymatic means, or both, structurally and chemically diverse components found in its way. Host specificity will then be determined by the interaction between the

composition of the host(s) (chemical and structural) and the mechanisms of infection used by the symbiont. This view, mostly overlooked in research on algal associations, has been one of the main paradigms in the study of pathology of higher plants. It is now generally accepted that, in spite of the enormous number of bacteria and fungi which could potentially establish a functional association (parasitic, pathogenic, *etc.*) with a given species of higher plant, only few in fact do so in a highly specific manner (Heath 1981, 1984). This is believed to result from mechanisms evolved in bacterial and fungal symbionts to overcome preformed chemical and structural barriers, such as cuticle, cell walls, secondary metabolites, *etc.*, as well as active responses of the host triggered by the symbionts themselves, such as papilla formation, toxins, *etc.* (reviewed by Heath 1986). In pathogenic associations, where specificity has received most attention, basic compatibility is achieved when a pathogenic species establishes a functional association with one host species (Heath 1981) by *specific* accommodation to the particular host that renders preformed and induced barriers ineffective. Once one of these already highly specific associations is established, an even narrower specificity may evolve when strains or cultivars within the initially susceptible species become resistant to the pathogen (Heath 1981). In this context, the polysaccharide fraction of the cell walls in higher plants, and their pathogens, have been indicated to play an active role during the establishment of fungal and bacterial infections. Currently, at least two mechanisms are known by which oligosaccharides, released by either host plants or from infecting organisms, trigger the production of various antibiotic substances by the host. In the first case, the sequence of events begins with the cleavage, by an enzyme released from the host, of 7-glucose fragments from the cell wall of the fungus. Alternatively, the oligosaccharide fragments come from the host cell wall, released by the action of enzymes produced by bacteria or fungi. Regardless of the mechanism by which the oligosaccharides are produced, they combine with a receptor in the host to form an "activated signal molecule" recognized by the genome of the host cells, which in turn results in the synthesis of antibiotics that can inhibit growth of the microorganism (reviewed by Darvill and Albersheim 1984, Albersheim *et al.* 1986). Consequently, a microorganism will succeed in establishing an intimate, eventually functional, association with a potential

host if mechanisms have evolved in it to (a) prevent formation of the "activated signal molecule" or (b) inactivate the antibiotics produced by the host (Heath 1986). Furthermore, the release of cell wall fragments certainly represents the chemical basis for recognition and establishment of highly specific associations between higher plants and their fungal and bacterial symbionts.

In algal associations, host specificity has been studied only at the species level, and the host species in question treated as a chemically and structurally homogeneous entity, even though it is widely recognized that some algal species, particularly in the Rhodophyta, show alternation of structurally different generations, such as crusts and erect fronds (reviewed by West and Hommersand 1981). Structural differences are apparent even between parts of a single plant, *e.g.* frond, stipe, and holdfast in *Chondrus crispus* Stackh. (Gordon and McCandless 1973, Tveter-Gallagher and Mathieson 1980, Correa and McLachlan 1988, Chapter 2). On the other hand, different phases in species of the Gigartinaceae with alternation of iso- and heteromorphic generations as *C. crispus* and *Iridaea cordata*, although structurally similar, are known to have different chemical composition in their cell walls (McCandless *et al.* 1973, 1975).

Whether or not in algae these factors represent selective barriers to infection by foreign organisms is not known. The first suggestion that intraspecific differences could be involved in determining the host range for an algal endophyte came to light during attempts to reproduce experimentally the infection of *C. crispus* by the chlorophycean endophyte *Acrochaete operculata* Correa and Nielsen (Correa *et al.* 1988). It took a longer time to infect gametophytic than sporophytic fronds, and when infection did occur, it developed following a different pattern, consisting of highly localized growths of the endophyte within the host (Correa *et al.* 1988). Some features of *C. crispus* could be visualized as potentially responsible for the apparent differential susceptibility to infection by *A. operculata* displayed by the two phases of the host. Fine structural information on the outermost cell wall (cuticle *sensu* Hanic and Craigie 1969) seems to indicate that sporophytic fronds have a thinner cuticle, with fewer lamellae (Correa and McLachlan 1988). The outermost cell wall may represent not only a structural barrier, as potentially toxic substances could accumulate in it to be discarded by periodic shedding of the cuticle.

The toxicity of brominated compounds has been suggested (Fenical 1975) and experimentally demonstrated for bacteria (Paul and Fenical 1987), microalgae (McLachlan and Craigie 1964, 1966) and invertebrates (Thompson 1985, Walker *et al.* 1985, reviews by Bakus *et al.* 1986 and Davis *et al.* 1989). Bromine has been reported from several species of red algae (Fenical 1975, Fenical and Norris 1975, Pedérsen *et al.* 1979, 1981), including *C. crispus*, where it appears to accumulate in the cuticle (Pedérsen *et al.* 1980). Unfortunately, Pedérsen *et al.* (1980) did not indicate the life-history phase of the plants used in their analysis, leaving the role of bromine, as a potentially selective and localized (perhaps cuticle-bound) deterrent of algal infection, an open question. More important perhaps, the polysaccharide fraction of the cell wall, which may make up to 70% of the alcohol-extracted dry weight of the frond of *C. crispus* (Craigie and Leigh 1978), consists of lambda-family carrageenan in the sporophyte, which is chemically different from the kappa-family carrageenan found in the gametophyte (McCandless *et al.* 1973).

As indicated above, successful colonization by a given endophytic species will also be determined by the mechanism of gaining access into the host(s). This became apparent when another chlorophycean endophyte, *A. heteroclada* Correa and Nielsen, was shown to invade equally sporophytic and gametophytic fronds of *C. crispus* (Correa *et al.* 1988), indicating that intraspecific differences in the host, important for *A. operculata*, seem irrelevant for *A. heteroclada*. A major difference, however, was detected between the two endophytic species in the sequence of events leading to the final penetration of the host, and that might be influencing the lack of selectivity displayed by *A. heteroclada*. While penetration of *C. crispus* by *A. operculata* occurred immediately, during germination of the zoospores, penetration by *A. heteroclada* was preceded by establishment of an early epiphytic stage (Correa *et al.* 1988). This infection pattern shown by *A. heteroclada* may represent a more generalist strategy, allowing infection of a wider diversity of hosts by a rapid colonization of the surface, followed by a later penetration of the host after some "weakening" of the thallus has been induced by the epiphytic stage.

In spite of these suggestive results, little could be concluded by Correa *et al.* (1988) regarding the factors determining the successful establishment of each endophyte, as only a single isolate of *C. crispus* was

used as potential host. Consequently, the present study was designed to evaluate experimental evidence of host specificity in *A. operculata* and *A. heteroclada,* to illustrate at which stage during infection specificity is expressed, and to suggest possible determinants involved in the establishment of fully compatible associations between host and symbionts. To accomplish this, potential hosts were selected to provide a wide diversity of cell-wall composition, considered of primary importance as *A. operculata* and *A. heteroclada* grow mostly within the intercellular matrix (Correa *et al.* 1988).

MATERIALS AND METHODS

Seven isolates of *A. operculata*, and three of *A. heteroclada*, were involved in this study (Table 1). The procedure followed to establish unialgal cultures of the endophytes consisted initially in selecting infected fronds of *C. crispus*, which were brushed and rinsed in running tap water. Smaller pieces of infected tissue were then excised and immersed in a fresh 0.6-1.2 % solution of sodium hypochlorite for up to 1 min, followed by four rinses, 2 min each, in sterile seawater. Sonication with five to ten 2-min bursts of 50 $kc \cdot s^{-1}$ was subsequently applied to 25 mm^2 pieces of infected tissue contained in a sterile flask with 50 mL of autoclaved seawater. The seawater was changed after each burst. Diatoms and other epiphytes were successfully eliminated by this intensive cleaning treatment.

Crude cultures were initiated by inoculating 2 x 0.5 mm fragments of infected, cleaned tissue, into 60 x 20 mm plastic Petri dishes containing SWM-3 medium (McLachlan 1977), or enriched seawater (SFC medium) modified after McLachlan (1982) by using 2 mM $NaNO_3$ and 100 µM Na H_2PO_4. In the latter case, Tris, $Na_2 SiO_3$, and Mo were omitted, and the concentration of Co decreased to 0.02 µM. The SFC medium was filtered through 0.22 µm pore-size membrane filters, and the SWM-3 medium was autoclaved. Crude cultures were maintained at 15 °C, light regime of 16 $h \cdot d^{-1}$, and photon flux density (PFD) of 20-40 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Germlings initiated from swarmers or outgrowths of the endophytes from the pieces of tissue used as inocula, were segregated into unialgal, non clonal cultures. These were allowed to grow actively for at least a month, before being transferred to slow-growth conditions, 10 °C, a light regime of 12 $h \cdot d^{-1}$, at less than 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PFD. Material was obtained, as needed, from these stock cultures. All procedures were performed in a laminar flow hood.

Observations on the infection of sporophytic fronds of *C. crispus* by *A. operculata* (isolate PF1a 161085) and *A. heteroclada* (isolate PCV 300585), were carried out at 5, 10, 15 and 20 °C. For each temperature, light regimes of 8 or 16 $h \cdot d^{-1}$ and 40-55 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were used. Results indicated that infection by both endophytes occurred regardless of the temperature-light regime combination, but developed more slowly at 5 and

10 °C. At 20 °C, bacteria became active and in some cases accelerated degradation of the fronds. Consequently, 15 °C and 16 h light·d^{-1} were the selected conditions to carry out all the experiments in the present study, and they are referred to as standard conditions of culture.

Thirty nine unialgal isolates from 26 rhodophycean species were tested as potential hosts for infection by *A. operculata* and *A. heteroclada*. The hosts included 16 carrageenophytes and 7 agarophytes (Table 2). The sporophytic and gametophytic phases of species of *Chondrus* and *Iridaea* were treated as different hosts. When thalli were brought into unialgal cultures from vegetative tips of field collected material, only very small fragments from the tips were used as inocula, after being cleaned following the procedure described above. These plants were used in the cross-inoculation trials only after being in culture for at least 2 mo, sufficient time for development of potential algal contaminants not eliminated during the cleaning treatment. Microscopic observation were performed to further ensure the unialgal condition of the fronds. *C. crispus* from several localities (Table 2) within Maritime Canada was tested to assess local variation as a possible factor related to host specificity. Two *C. crispus* isolates, the gametophytic JC009BH and the sporophytic JC010BH, were obtained from stock cultures maintained (as C. *crispus* BH) at the National Research Council of Canada (Halifax), originally established by J. McLachlan from spores released by mature fronds collected at Basin Head, Prince Edward Island.

Each infection trial was performed by inoculating a 22 x 60 mm plastic Petri dish containing 30 mL of SFC medium with 2 mL of actively reproducing suspension of one or the other endophyte. A freshly cut fragment of potential host (experimental plant), and a piece of sporophytic *C. crispus* (positive control), each about 10 mm in length, excised from apical portions of the fronds, were added to a Petri dish prepared as indicated above. A positive control was necessary to ensure that a negative infection in the experimental plants was not caused by a loss of infectiveness of the endophytic inoculum. Triplicates (at least) were established for each experimental plant, and trials were carried out under standard conditions of culture. Culture medium was changed every 2 wk, and the observation period was for at least 2 mo. Similar procedures and conditions were used in additional cross-inoculation experiments of *C.*

crispus from various localities (Table 2), with all isolates of the two endophytes listed in Table 1. Light microscopy observations of infected and non-infected fronds were done using free-hand sections of alive material mounted in culture medium.

To quantify infection, 30 gametophytic and 30 sporophytic frond fragments of *C. crispus* BH, each about 10 mm in length, were individually inoculated with *A. operculata* as indicated above and cultured under standard conditions. Culture medium was not changed during the 20-d experiment period. At the end of the experiment, fragments were gently brushed to remove germlings that did not invade the cell wall of the host, and counts were performed in 3 haphazardly-selected positions at the apex (distal) and base (proximal) of each frond. The area covered in each count was 0.02 mm^2, and the counts were averaged for each position.

To assess infection susceptibility in immature *C. crispus*, unialgal cultures of the two phases of this species were established from tetra- and carpospores released by plants collected at Peggy's Cove, Nova Scotia. After germination on glass coverslips, plants were cultured for 3 wk under standard conditions, with weekly changes of medium. Coverslips were then transferred to new Petri dishes containing 30 mL of fresh medium and 2 mL of inoculum from one or the other endophyte. Following a period of 30 d under standard conditions, all discoid plants were observed under a dissecting microscope, and the degree of infection was recorded using arbitrary categories based on the proportion of the host surface occupied by the endophytes. "High" represented plants having at least three-fourths of their surface infected, "Light" included hosts with only few endophytic cells scattered over the surface, and the remaining host plants were classified as "Regular", or "Non Infected".

Scanning electron microscopy (SEM) was used to assess penetration of the cuticle during early infection of *C. crispus*. Sporophytic and gametophytic apices of the host (JC010BH) were incubated with *A. operculata* for 5 d under standard conditions. Fragments were fixed for 1 h at room temperature in culture medium to which was added 2% glutaraldehyde. This was followed by rinses in culture medium with gradually decreasing salinity, several short rinses in distilled water and finally freeze-dried and gold-coated.

The elemental composition at the surface of gametophytic and sporophytic fronds of *C. crispus* (JC010BH) was performed on bulk samples, processed as for standard SEM, while coated with carbon rather than gold. Five frond fragments from each phase were analysed, and several readings from each fragment were taken. The analysis was done in a JEOL JXA-35 scanning electron microscope equiped with a electron probe X-ray micro analyser connected to a Kevex 8000 energy dispersive X-ray analyser having a quantum detector. The electron microscope was operated at 25 kV, and samples were analysed using a take-off angle of 35°.

RESULTS

The process of infection by *Acrochaete operculata* and *A. heteroclada* consists of at least three stages: settling of the zoospores, germination with subsequent penetration of the host and establishment of the endophytic habit followed by reproduction. A susceptible host is considered one in which the sequence of all stages is completed. A non-susceptible host is, on the other hand, one in which the development of the infection is arrested at any of the stages.

Zoospores of *A. operculata* settled and germinated on controls and experimental plants, as well as on the surfaces of the plastic culture dishes. In no case, however, were the germlings able to attach firmly to the plastic substratum. The events that follow settlement proceed regardless of the nature of the substratum. These consist of unipolar germination with an elongating germination tube that receives the evacuated cytoplasm from the zoospore. A septum developed and separated the empty zoospore cell wall from the first cell. This cell elongates, and divides to form a two-celled germling. Usually at this point, the germling branches irregularly, with more branches produced by each sub-apical cell.

The orientation of the filament, immediately after germination, follows two patterns. On plastic substratum the germlings developed parallel to the surface. No apparent adhesive material was produced by these germlings, which became free-living shortly after germination. Where some germlings remained associated with the plastic, minimal agitation freed them. A similar development occurred on all agarophytes, *Devaleraea, Palmaria, Polyides,* and some carrageenophytes, where zoospore of *A. operculata* attached, but did not penetrate, the thallus (Table 3). In all those species, zoospores of *A. operculata* attached to the surface, germinated and developed into multicelled germlings. These were, however, easily removed either by gentle brushing or by periodic peeling of the cuticle. It is emphasized that a truly epiphytic habit, that is vegetative filaments firmly attached to experimental plants never developed, and when cuticle-peeling occurred, no vegetative fragments of the endophyte were found attached to the newly exposed surface.

In the second pattern of development, the filamentous germlings of *A. operculata* penetrated the surface of experimental plants, passing through the cuticle, and reaching the cortex. Penetration was observed in species of *Chondrus, Gigartina, Gymnogongrus, Phyllophora, Iridaea, Rhodoglossum,* and *Furcellaria* (Table 3). Although the penetration pattern was consistent among replicates for a particular experimental plant, differences in the occurrence of penetration were sometimes observed between species of the same genus (*G. devoniensis* and *G. furcellatus*), or even strains of the same species (*F. lumbricalis*) (Table 3). With the exception of the sporophytic phases of *I. cordata* and *C. crispus*, penetration was highly patchy, localized in few sites along the thallus of the experimental fronds. The positive control used for each experimental frond, on the other hand, had developed extensive areas of heavy infection, characterized by large endophytic cells of irregular profile embedded in the cortex of the host (Fig. 1). Cross section of these controls revealed a network of endophytic filaments that had colonized the medullary tissue (Fig. 2). In contrast to the extensive colonization of the positive controls by *A. operculata,* however, most experimental plants showed limited, if any, development of the endophyte (Figs 3-12). The lowest density of penetrating germlings was observed in *G. chiton* (Fig. 3), where the usually single-celled endophytes appeared compacted by the surrounding cortical cells of the host. In *C. canaliculatus,* endophytic cells displayed a circular profile on a surface view, and the parietal chloroplast and pyrenoids were distinct (Fig. 4). In spite of the apparent absence of compression of endophytic cells by the cortical cells of the host, cross sections through the infection sites showed that *A. operculata* had not developed beyond the cortex of *C. canaliculatus* (Fig. 5). On one occasion, a single, unbranched endophytic filament was found in the outer medulla of *G. acicularis* (Fig. 6). This stage of development, however, did not compare with the infection level in the positive control for *G. acicularis* (Figs 1, 2). Relatively large number of endophytic cells were sometimes observed in few sites along the thallus of experimental isolates, as in *P. pseudoceranoides* (Fig. 7). These endophytic cells, however, remained undivided and restricted to the upper cortex of the host (Fig. 8). Very few *A. operculata* cells penetrated *G. devoniensis,* and they developed into 2- or 3-celled germlings, always restricted to the cortex (Fig. 9). As in *G.*

chiton (Fig. 5), compression of *A. operculata* by cortical cells of *F. lumbricalis* resulted in deformation of the endophytic cells, which displayed a highly condensed cytoplasm that obscured cellular details (Fig. 10). Similarly, most infecting cells did not penetrate beyond the first layer of cortical cells in *F. lumbricalis* (Fig. 11). The few invasive cells observed to reach deeper zones of the cortex appeared compressed and remained unbranched (Fig. 12).

Penetration of host cells was not observed, although minor cortical disorganization and cell deformation occurred in *C. canaliculatus* (Fig. 5) and *F. lumbricalis* (Fig. 11). Furthermore, germlings that remained within the cortex usually did not become reproductive.

The third stage of infection following penetration by *A. operculata* was successfully completed only in the sporophytic phase of *C. crispus* (Figs 13-15), including all controls (Table 3, Figs 1, 2, 13, 14), and in the sporophytic phase of *Iridaea cordata* (Figs 16-18). There were no observable differences, regarding post-penetration events, between controls and sporophytic *C. crispus* from various localities. Penetration first became apparent at the bases of the fronds where the invasive germlings soon developed into a filamentous network throughout the cortex. By the end of the experimental period, most of the surface of the hosts had been entirely replaced by cells of *A. operculata* (Fig. 13). At this time, endophytic cells located immediately beneath the cuticle had enlarged, displaying a highly variable profile (Fig. 14). From the cortical-borne network, invasive vegetative filaments could be found deeply embedded within the medullary tissue of *C. crispus* (Fig. 15). There was no evidence of penetration of host cells by the endophytic filaments.

The sporophytic phase of *I. cordata* was the only experimental plant in which an infection developed similar to that in the comparable phase of *C. crispus*. Again, the infection occurred at high densities at the base of the fronds (Fig. 16). In a surface view (Fig. 17), infection corresponded to the pattern described for the sporophytic phase of *C. crispus* (Fig. 14), and maturity of the endophytic, cortex-borne cells was evident as numerous brown spots representing emptied sporangia (Fig. 17). At this stage, vegetative filaments of *A. operculata* were deeply embedded in the medulla of the host (Fig. 18) with no evidence of cell penetration.

Perhaps the most noticeable difference between the sporophytic phases of *C. crispus* and *I. cordata* was the distribution of the infection. This decreased gradually and smoothly from the proximal ends of the thalli, toward the apices in *C. crispus*, while in *I. cordata* it showed a generally abrupt transition from heavy infection at the basal half of the thallus to minor infection at the apices. Also, infection in *I. cordata* developed more slowly than in *C. crispus*, advancing as a front, perpendicular to the longitudinal axis of the fronds (Fig. 16).

The development of *A. operculata* in gametophytic fronds of *C. crispus* from all localities was different from that in the controls. At low magnification, infection appeared as discrete, dark spots scattered on the surface of the fronds (Fig. 19). Each spot corresponded to a single invasive event, endophytic filaments displaying little, if any, lateral colonization (Fig. 20). Observations in cross section confirm the highly localized development of *A. operculata* which rarely grew into the medulla (Fig. 21). Sometimes, small areas of cortical cells of gametophytic *C. crispus*, especially the JC009BH isolate, became highly pigmented as noted in surface view (Fig. 20). This seemed to be a response to penetration, leading to early arrest of the infection. When these areas were sectioned, local cortical disorganization and increased thickness were evident, together with disruption of the surface and elimination of the endophytic filaments (Fig. 21). Regardless of the presence of these pigmented areas, the infection never spread over the rest of the gametophytic fronds, even in those experimental plants maintained for over a year. An additional difference was the length of time (about 4 mo) required for the infection to develop to the stage displayed in Figures 19-21.

Differences in development of the infection by *A. operculata* in the two phases of *C. crispus* persisted when other isolates of the endophyte (Table 1) were tested.

The frequency and density of infection by *A. operculata* was different in the two phases of *C. crispus*. Fewer gametophytic fronds were infected, and these were penetrated by a relatively smaller number of endophytic germlings. In both phases, however, the proximal portions of the fronds displayed the greatest degree of infection (Table 4).

Experiments with 3-wk old gametophytic and sporophytic plants of *C. crispus* revealed a similar differential susceptibility as observed in adult

fronds of the two phases. Fewer (76.3%) gametophytic plants were infected (2.9% heavily), well below the 99.5% infection (69% heavily) of the sporophytic plants (Table 5).

One experimental isolate (JW026, Table 2) displayed a somewhat inconsistent pattern of infection. In the first trial, one out of three male fronds of *R. californicum* was heavily penetrated by *A. operculata* germlings, which eventually invaded the medulla. A second trial was performed with seven replicates, and again one of the fronds developed the same pattern of infection. However, there were important differences with the infection in sporophytic *C. crispus* used as a control. Endophytic filaments were less numerous, abnormally thick and contorted. The reproductive structures of *A. operculata*, normally embedded within the cortex of controls and sporophytic *I. cordata*, developed outside the fronds of *R. californicum* as terminal cells of vegetative filaments protruding from the host. Moreover, this infection took place only at the base of the fronds, the center and the apical portions remaining uninfected for up to 4 mo.

The SEM study of early infection of *C. crispus* indicated that zoospores of *A. operculata* settled, uniformly distributed, on the surface of the host, and the cuticle of sporophytic (Fig. 22) and gametophytic (Figs 23-25) fronds was equally penetrated. Penetration sites did not show morphological evidence of mechanical disruption (Figs 24, 25), although changes in the arrangement of the cuticular fibrils were often noted in areas surrounding the penetrating germlings (Fig. 24). The presence of a non-fibrilar zone, located between the modified cuticle and the wall of the infecting cell (Figs 24, 25), was commonly found as if the cuticle had been hydrolyzed (Fig. 25).

Energy dispersive x-ray microanalysis indicated similar elemental composition for the surface of the two phases of *C. crispus* (Figs 26, 27), with sulfur and potassium being the most abundant elements. Bromine, on the other hand, was not detected under the conditions of the analysis.

Acrochaete heteroclada was a generalist endophyte, with swarmers and germlings attaching firmly either to plastic or algal substrata. The infection pattern displayed by this endophyte was consistent throughout the range of experimental plants tested as potential hosts. The zoospores, after settling over the surface of the experimental plants, germinated unipolarly

and developed into more or less radial, superficial, monostromatic plants. These soon became several layers thick, coalesced with adjacent plants, and vegetative filaments grew downwards which penetrated throughout the cortex of all offered hosts, including control *Chondrus crispus* (Fig. 28) and experimental fronds of *C. canaliculatus* (Fig. 29), *Gymnogongrus furcellatus* (Fig. 30), *G. linearis* (Fig. 31), *Gracilaria mammillaris* (Fig. 32), and *G. chilensis* (Fig. 33). Penetration was achieved by individual filaments (Figs 28, 30, 33), or by groups of irregularly shaped clusters of cells (Figs 31, 32). The endophytic filaments consisted of relatively short cells with generally compacted cytoplasm, and appeared homogeneous in pigmentation. Most of the cortex was uniformly replaced in some cases (Figs 28, 33), whereas patchy replacement occurred in others (Figs 30-32). No evidence of cell penetration was found, in spite of deformation of some host cells in contact with the endophytic filaments. Generally, however, vegetative filaments followed the contour of the host cells (Fig. 33).

The pattern of infection was consistent between different isolates of *A. heteroclada*, which responded in the same fashion when infecting *C. crispus* (from different localities), *Gelidium vagum* and *Gymnogongrus furcellatus*. Also, there were no differences in susceptibility of young gametophytic and sporophytic plants of *C. crispus* to infection by *A. heteroclada* (Table 4).

DISCUSSION

It is apparent that *A. operculata* is a highly host specific endophyte which establishes a fully compatible association only with the sporophytic phases of *C. crispus*, and a similar behavior was noted in the closely related sporophytic *I. cordata*. The compatibility between host and endophyte is characterized by a quick and generally massive penetration of the host thallus during germination of the infective zoospores, followed by a rapid spreading of the endophyte via vegetative filaments, into the cortex first, and the medulla later. At the same time, vegetative cells of the endophyte embedded in the cortex of the hosts differentiate into sporangia, and the released zoospores reinitiate the cycle, accelerating the process of colonization. This infective cycle occurred in all sporophytic *C. crispus* - *A. operculata* pairing, regardless of combinations of isolates of either host or endophyte. Thus the phase of the host itself, and not the origin of the isolates, regulates susceptibility. The development of infection by *A. operculata* in all sporophytic and gametophytic isolates of *C. crispus* and *I. cordata* agrees with the original description of the infection reported by Correa *et al.* (1988), and strongly supports preliminary information pointing to intraspecific differences as potential determinant of the host range for this pigmented endophyte.

Considering the structure of the thallus and cell-wall composition of the experimental plants used in this study, and the development of *A. operculata* during infection (Correa *et al.* 1988), host specificity could come about as the result of the interaction between germinating zoospores and the outermost cell wall of the hosts. Alternatively, host specificity could become expressed later on, during the interaction of growing vegetative filaments of the endophytes with the intercellular matrix and cell walls of the host.

For *A. operculata*, the evidence strongly suggests that factor(s) other than superficial chemical composition, or texture, limit the range of hosts susceptible to infection. Topography of the host surface seems irrelevant as zoospores settle and germinate on both plastic and algal substrata, indicating a lack of host specificity at these early events of infection. Furthermore, in the particular case of *C. crispus*, the alleged accumulation

of bromine in its outermost cell wall (Pedérsen *et al.* 1980), which might have represented a toxic threat for epi- and endophytic organisms (McLachlan and Craigie 1964, 1966), could not be substantiated. In the present study, bromine was not detected in either phase of *C. crispus*, suggesting that if present it was below the 0.1% detection limit determined by the conditions in which the analysis was performed (Vaughan 1983).

The lack of specificity at early stages of infection has also been reported for endophytic species of *Audouinella* (White and Boney 1969, Boney 1972, Tam *et al.* 1987) and for parasitic red algae (Nonomura 1979, Goff 1982a). This should be expected, however, as rhodophycean spores are non motile, and contact with a substratum is likely a random event. Furthermore, in the few reported instances where the host is required for germination, diffusible compounds, not topography or chemical composition of the surface, have been suggested to stimulate the spores (Goff 1976, 1982a, Stegenga and Borsje 1976). Poor host specificity is also common in fungal-higher plant associations, where settlement and germination seem to be events usually independent from the host (Goodman *et al.* 1986).

The first evidence of host specificity in *A. operculata* became apparent after germination. Only some experimental fronds, all carrageenophytes, were successfully penetrated by the endophyte. The frequency of penetration was, however, relatively low with the exception of the natural host and sporophytic plants of *I. cordata,* where abundant penetration occurred. This observation suggests that development of the infection by *A. operculata* is first affected by potential hosts during penetration. It is not clear how this occurs, because the mechanisms of infection by *A. operculata*, whether mechanical, enzymatic or both, are not fully understood. However, evidence from SEM observations of the penetration sites in *C. crispus* shows that the inner-side of the cuticle at the host-endophyte interface always appears clearly defined, with sharp borders, and no indication of inward bending characteristic of mechanical penetration of higher plants by fungi (Cooper 1981, Kolattukudy 1985). This, together with additional SEM and TEM information (Chapter 2), seems to indicate that enzymes, probably proteases, may be involved in facilitating access of the endophyte into the host. Algal cuticles are reportedly proteinaceous (Hanic and Craigie 1969, Gerwick and Lang

1977), including that of *C. crispus* (J.S. Craigie pers. comm.). If this is indeed the case, the failure of *A. operculata* to penetrate non carrageenophytes may be owing to lack of appropriate enzymes.

It is also during penetration and early colonization of the cortex of *C. crispus*, that intraspecific differences between the two phases of the host seem to translate into differential susceptibilities to infection by *A. operculata* as indicated by higher infection densities reached in the sporophytic fronds in the short-term infection experiments. Initially, it was not clear whether this difference originated during the passage through the cuticle as a result of a selective obstruction of penetration at this level by the gametophytic fronds, or later during consolidation of the invasive germlings in the subjacent cell wall. Although some differences in the fine structure of the cuticle of the two phases of *C. crispus* have been reported (Correa and McLachlan 1988), SEM observations of early infections showed similar cuticle penetration of sporophytes and gametophytes. The passage of large numbers of germlings through the cuticle of both phases, with no evidence of mechanical disruption, indicates that at least for the association between *C. crispus* and *A. operculata*, this external structure does not represent a qualitative barrier to invasion, as seems to be the case for the hosts of the chlorophycean endophytes *Blastophysa rhizopus* (Iima and Tatewaki 1987) and *Endophyton ramosum* (O'Kelly 1982). When growing rapidly, cuticle shedding in *C. crispus* is almost continuous. This, together with a larger number of lamellae in the gametophytic phase (Correa and McLachlan 1988, Chapter 2), may have some effect on the number of *A. operculata* germlings reaching the internal cell wall. However, as intensive cuticle peeling is localized at the apices of the fronds, it is unlikely that this mechanism has an important overall impact on penetration success. The phenomenon of cuticle shedding, generally considered as an effective cleaning mechanism common to many macroalgae (McArthur and Moss 1977, Filion-Myklebust and Norton 1981, Sieburth and Tootle 1981, Moss 1982, Russell and Veltkamp 1984, Johnson and Mann 1986, Gonzalez and Goff 1989), is inoperative in hosts susceptible to infection by *A. operculata*. This may represent a species-specific adaptation of the endophyte to overcome the dynamic first barrier of the host, as indicated to occur during the establishment of the basic

compatibility between fungi and bacteria, and higher plant hosts (Heath 1981).

The absence of penetration at sites of artificial wounding of gametophytic fronds reported previously (Correa *et al.* 1988) and through the cut surfaces of experimental fronds in the present study, add further support to the suggestion that cuticle is not a significant factor in determining host specificity in *A. operculata.* Although absence of penetration owing to wound-healing responses cannot be absolutely excluded, it seems unlikely considering that healing responses take about three weeks to be completed (Correa pers. obs., Azanze-Corrales and Dawes 1989), which is sufficient time for zoospores of *A. operculata* to settle, germinate and gain access into *C. crispus.*

The full expression of host specificity did not became apparent until development of *A. operculata* within the host plants began. Several lines of evidence indicate that chemical composition of the cell wall, and not internal tissue structure (spatial configuration of cells and cell walls within the thallus) is responsible for the highly specific pattern of infection displayed by *A. operculata* in the sporophytic phases of *C. crispus* and *I. cordata.* Although life-history generations in both *C. crispus* and I. *cordata* are anatomically indistinguishable, gametophytic fronds were not infected for *I. cordata.* For *C. crispus* infection was "light", incomplete, and followed a different pattern of development compared to the sporophytic fronds. Additionally, susceptibility to infection by *A. operculata* of the discoid germlings from the two phases of *C. crispus* was similar to that of fully developed fronds. Crustose germlings and fronds in *C. crispus* differ in a number of anatomical features, including organization of the cuticle, cell size, and shape, and volume of intercellular matrix (Chapter 2, Tveter-Gallagher and Mathieson 1980), while having the same cell-wall composition (Tveter-Gallagher and Mathieson 1980, Chen and Craigie 1981). The type of carrageenan characteristic for each generation begins to be produced at the spore stage (Gordon-Mills and McCandless 1975). Thus, hosts anatomically similar, but with different cell-wall composition are infected differently by *A. operculata,* whereas hosts structurally different but with comparable cell wall composition display similar susceptibility to infection. Consequently, it is suggested that diversity in the carrageenan fraction plays a major role in determining the degree of

host specificity shown by *A. operculata*. Only experimental plants known to produce carrageenans of the lambda family (McCandless *et al.* 1973, 1975) were susceptible to the endophyte. Whether specificity is mediated by enzymes or results from particular physical properties of the cell walls conferred by the different families of carrageenans, or both, remains equivocal. The fine structure of the host-endophyte interface (Chapter 2) provides circumstantial evidence for the involvement of enzymes in hydrolyzing the intercellular polysaccharide matrices during growth of the endophyte. An enzyme-substrate system operating at the host-endophyte interface could account for the observed degree of host specificity in *A. operculata*. This has been also suggested for the association between the parasitic rhodophycean *Harveyella mirabilis* and its host *Odonthalia floccosa* (Goff and Cole 1976). Highly specific enzymes able to degrade various types of cell-wall polysaccharides, including kappa (Weigl and Yaphe 1966, McLean and Williamson1979) and lambda (Weigl and Yaphe 1966, Johnston and McCandless 1973) carrageeenans, have been isolated from bacteria. However, occurrences of these enzymes in algae have not been documented in spite of repeated suggestions of enzymatic digestion of cell wall in a number of algal symbiotic associations where invasion of the host occurs (Rawlence 1972, Rawlence and Taylor 1972, Nonomura 1979, Goff and Cole 1976, Goff 1982a, Gonzalez and Goff 1989). In this context, it is interesting to note that in symbiotic associations involving red algal endophytes, the colonization of the host has been considered mainly a mechanical event. Germlings of *Acrochaetium endophyticum* Batt. grow along the surface of *Heterosiphonia plumosa* (Ellis) Batt. until the area of contact between two host cells is found, and only then penetration takes place, always following an apparently weaker interface between adjacent cells (White and Boney 1969). Furthermore, Garbary (1979b) reported swelling and deformation of the host cells at the site of penetration by some audouinelloid endophytes, interpreted as the result of physical disrupture and pressure exerted by the endophytic germlings during infection.

Acrochaete heteroclada showed no host specificity in the laboratory experiments. Two developmental features are suggested to account for this non selectivity of *A. heteroclada*. One is the immediate development of a prostrate system after germination (Correa *et al.* 1988) that firmly attaches this alga to any substratum. Secondly, when germlings of *A. heteroclada*

attach to algae, filaments produced by the prostrate system penetrate through the cuticle into the cortex of the hosts, anchoring the symbiont to a more stable substratum. Fine structural evidence (Chapter 2) strongly suggests that penetration is mechanical and, consequently, chemical composition of the hosts may not affect the development of the infection, as if it were a process mediated by enzymes. Rapid penetration may account for the high diversity of hosts susceptible to infection by *A. heteroclada,* and explains the failure of the "self-cleaning" mechanism of cuticle peeling in this association. A rapid penetration through the cuticle seems effective in some epiphytic associations, and in *Microcladia coulteri* Harv. this feature has been suggested to account for the broad range of hosts susceptible to infection by this species (Gonzalez and Goff 1989).

The range of host specificity displayed by *A. operculata* and *A. heteroclada* in laboratory cultures agrees with field observations. *A. operculata* has been found only in *C. crispus* (Correa pers. obs., R. Nielsen in litt.), although there is no information yet available on its distribution in the two generations of the host. This information may prove to be extremely difficult to obtain, as wild fronds of *C. crispus* generally co-host several intermingled brown and green endophytes (Correa *et al.* 1987), making identification almost impossible unless parallel culture studies are conducted. In spite of an intensive search, Nielsen and McLachlan (1986) did not find *A. operculata* in *M. stellatus*, a species with heteromorphic alternation of generations whose foliose gametophytic phase coexists with, and is structurally similar to, *C. crispus*, but produces carrageenan of the kappa family (McCandless *et al.* 1983). European species of Gigartinaceae were not screened for the presence of *A. operculata,* but it would be expected that sporophytic fronds of any other species producing carrageenan of the lambda family also hosts this endophyte.

Interestingly, Correa *et al.* (1987) examined numerous holdfasts of *C. crispus* from several sites in Maritime Canada and found no evidence of infection by either green or brown endophytes. They suggested that the presence of a "non disrupted" cuticle was responsible for the lack of endophytic infection. It seems unlikely, however, that this structure plays a significant role in preventing infection by algal endophytes, as the fine structure of the "non disrupted" cuticle (Correa and McLachlan 1988) in well developed holdfasts is similar to the cuticle present in the young

discoid plants of *C. crispus* (Chapter 2), and young holdfasts (Tveter-Gallagher and Mathieson 1980), regardless of life history phase. Furthermore, as established in this study, those discoid plants are indeed infectable, and their susceptibility follows the pattern of susceptibility observed in adult fronds. Consequently, the absence of endophytes in field-collected holdfasts may result from ecological factors, rather than from structural resistance, localized at the cuticle level, in the adhesive discs of *C. crispus*. Interestingly, field-collected *Petrocelis* plants did not appear to host *A. operculata* (C. Maggs in verb.), even though this crustose life-history phase is known to produce carrageenan of the lambda family (Craigie 1990b). This supports the hypothesis that under certain conditions, as the crustose habit, ecological factors may be superimposed on chemical compatibility between host and endophyte.

Although *A. heteroclada* was originally described based on a single isolate from *C. crispus* (Correa *et al.* 1988), subsequently it has been also found associated with *M. stellatus* (pers. obs.) and other red and brown algae (Nielsen in litt), in concurrence with the broad host range displayed in laboratory experiments.

Host-specificity studies of algal associations are extremely important as they provide insight into underlying factors determining susceptibility of a particular host. A decade ago Andrews *et al.* (1979) suggested, for the first time, that haploid and diploid stages of a single algal species could be differentially susceptible to infection. However, previous observations from field-collected material (Saito *et al.* 1977, Goff and Coleman 1984), and from laboratory experiments (Nonomura 1979, Goff and Coleman 1984, Gonzalez and Goff 1989) indicated no correlation between infection and reproductive phases in the various algal symbiotic associations considered. Mine is the first experimental account of differential susceptibility of algal life history-generations to infection by a foreign organism, and also for the first time the polysaccharide fraction of the cell wall in algae is indicated as a main determinant of host specificity.

Finally, in the specific case of the association between *C. crispus* and *A. operculata*, the difference in susceptibility to infection might reduce, at least partially, the competiveness of the sporophytic phase, and may help to explain the gametophytic dominance detected in a number of populations of the host in eastern Canada (McLachlan in press.). Similarly, increased

susceptibility of the sporophytic phase of *C. crispus* could be detrimental by facilitating bacterial diseases secondary to infection by *A. operculata.* A bacterial disease has been detected in sporophytic fronds growing in large-scale cultures (J. S. Craigie in verb.).

FIGURES

Figs 1-25. Host specificity in *Acrochaete operculata.*

Fig. 1. Surface view of sporophytic *Chondrus crispus* (JC010BH) used as positive control for *Gigartina acicularis,* and selected as representative, infected positive control. Irregularly shaped endophytic cells occupy most of the surface. Scale= 20 μm

Fig. 2. Transverse section through the same individual frond displayed in Fig.1, showing numerous endophytic filaments embedded in the medulla of the host. Scale= 20 μm

Fig. 3. Surface view of *Gymnogongrus chiton,* showing two infecting cells with no distinct cellular details. Scale= 50 μm

Fig. 4. Surface view of *Chondrus canaliculatus* showing a group of eight endophytic cells. Parietal chloroplast and pyrenoids are distinct in the larger cells. Scale= 50 μm

Fig. 5. Transverse section through the same frond displayed in Fig. 4. Infecting cells are restricted to the cortex, which appears slightly disorganized. Scale= 50 μm

Fig. 6. Transverse section through *Gigartina acicularis* showing a single, unbranched filament reaching the outer medulla. Note the absence of other endophytic cells in the vicinity of this invading filament. Scale= 50 μm

Fig. 7. Surface view of a site with high density of infection, in a *Phyllophora pseudoceranoides* frond. Scale= 50 μm

Fig. 8. Transverse section through the same site displayed in Fig. 7, to show the endophytic cells restricted to the outer cortex of the host. Scale= 50 μm

Fig. 9. Transverse section through *Gymnogongrus devoniensis* showing a two-celled endophytic filament. Scale= 20 μm

Fig. 10. Surface view of *Furcellaria lumbricalis* (CB028) showing deformed endophytic cells with highly condensed cytoplasm. Scale= 50 µm

Fig. 11. Transverse section through *Furcellaria lumbricalis* (CB028) showing a one-cell germling penetrating the cuticle and deforming a subjacent host cell. Empty cell wall of the zoospore still visible (arrowhead). Scale= 20 µm

Fig. 12. A single, unbranched, and compressed endophytic filament reaching the inner cortex of *Furcellaria lumbricalis* (CB028). Scale= 50 µm

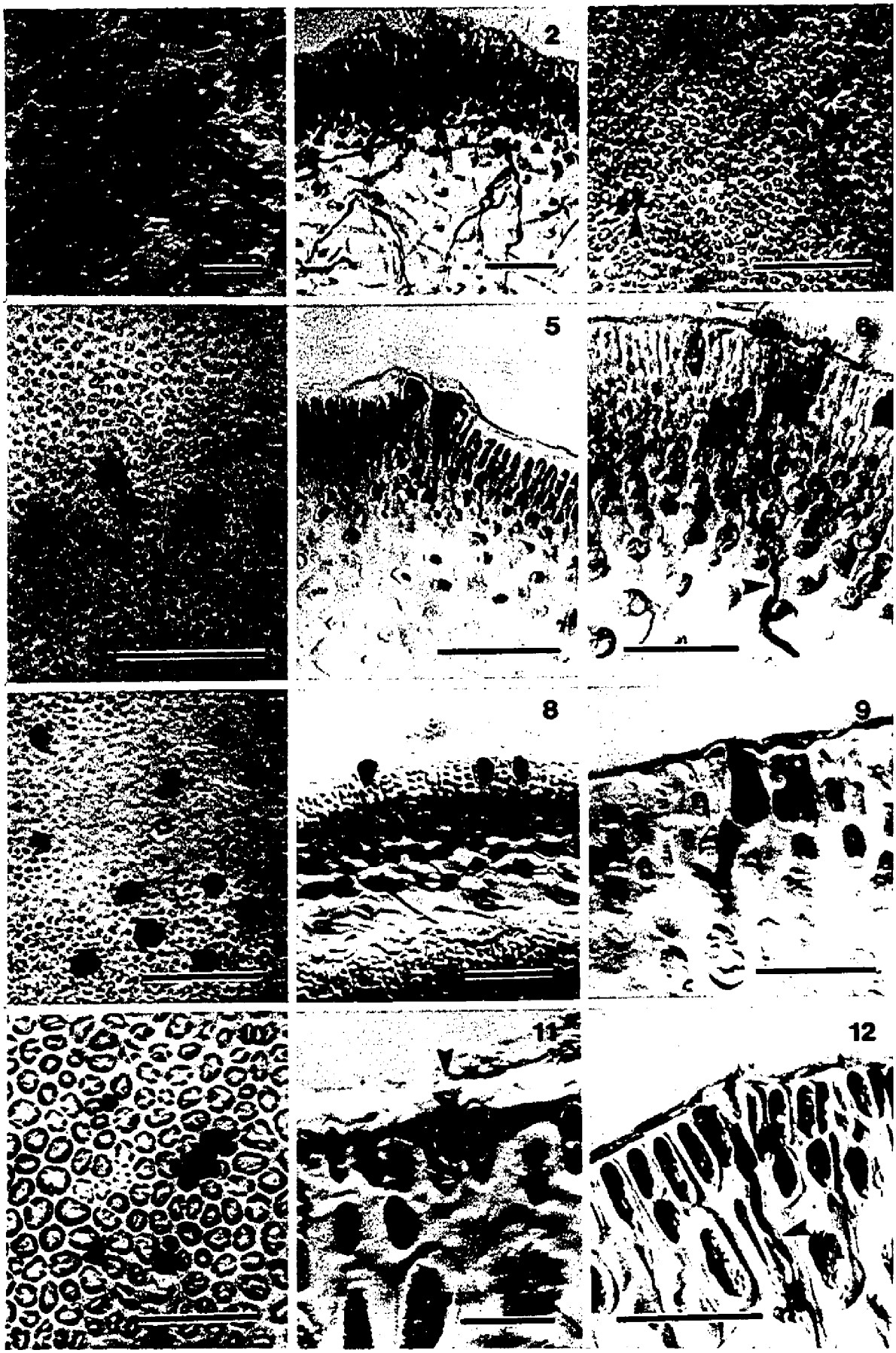

2
5
8
9
11
12

Fig. 13. Heavily infected basal portion of an experimental sporophytic frond of *Chondrus crispus* (JC010BH). Scale= 0.5 μm

Fig. 14. Surface view of an area of intermediate density of infection, at the transitional zone between newly formed host tissue and heavily infected base (same frond shown in Fig. 13). Endophytic cells irregular in shape, with parietal plastids and pyrenoids are easily recognized. Scale= 25 μm

Fig. 15. Transverse section through a heavily infected portion of a sporophytic frond (*c.f.* Fig. 13), showing invasive filaments in the medullary tissue of the host. Scale= 20 μm

Fig. 16. Frond of *Iridaea cordata* showing the characteristic infection pattern, in which greatest development occurred at the base of the frond. Scale= 5 mm

Fig. 17. Surface view of a heavily infected site in *Iridaea cordata* (base of frond displayed in Fig. 16). Most of the host cortical cells have been replaced by *Acrochaete operculata* cells. Brownish tips of emptied sporangia (arrowheads) are apparent. Scale= 50 μm

Fig. 18. Transverse section through a heavily infected site in *Iridaea cordata* (base of frond displayed in Fig. 16). Greenish color of the cortex indicates the replacement of host cells by the endophyte. An invasive endophytic filament (arrowhead) is seen in the medulla of the host. Scale= 20 μm

Fig. 19. Surface view of a gametophytic *Chondrus crispus* frond (JC009BH). Scattered dark spots over the surface of the frond represent the highly localized infection sites. Scale= 0.5 μm

Fig. 20. Close up of two infection sites in a gametophytic frond (same shown in Fig. 19). Filaments resulting from each invasive event have not colonized the surrounding host tissue. Subtle localized increase in host pigmentation is suggested. Scale= 50 μm

Fig. 21. Transverse section through two infection sites, similar to those in Fig. 20. Infections remain localized in the cortex, surrounded by apparently normal host tissue. Cortical disorganization (arrowhead) without endophytic filaments, seems to correspond to zones of increased pigmentation in Fig. 20. Scale= 50 μm

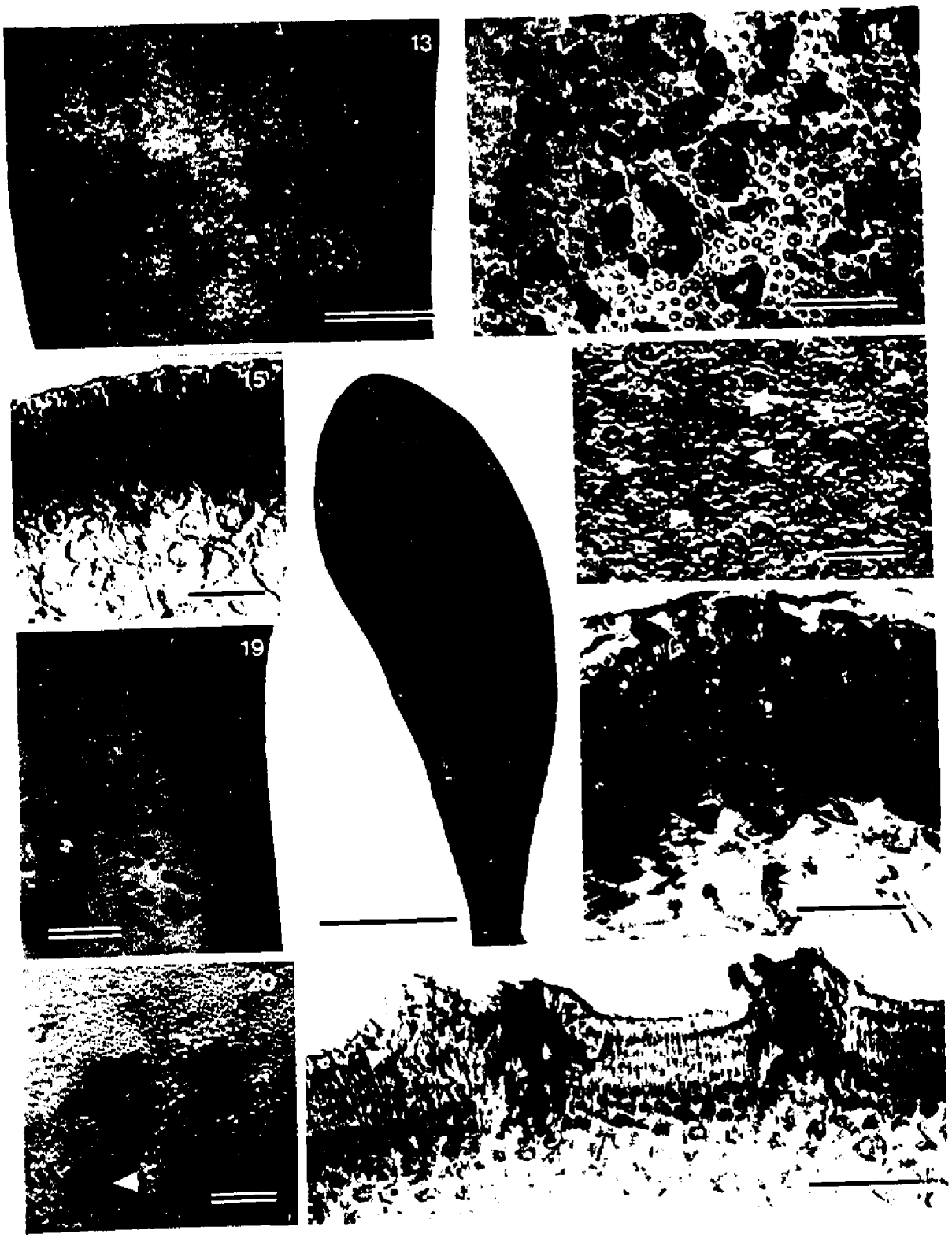
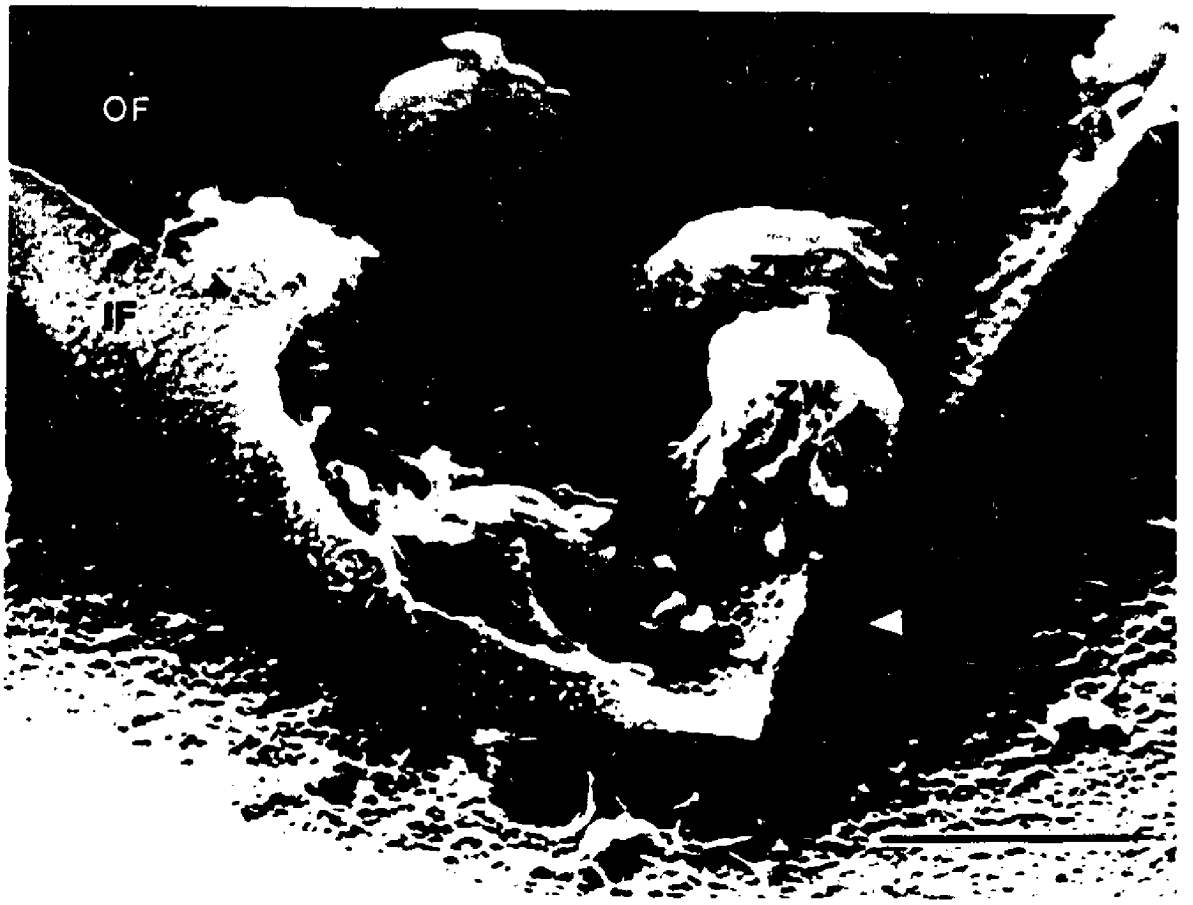

13
14
15
19

Fig. 22-25. Scanning electron microscopy of early penetration of the cuticle of *Chondrus crispus* by *Acrochaete operculata.*

Fig. 22. Penetrating germlings (arrowheads) passing through a lamella, at the cuticle of a sporophytic frond. The collapsed zoospore walls (ZW) remain attached to the outer face (OF) of the lamella. IF: inner face of the lamella. Scale= 5 μm

Figs 23-25. Gametophytic *Chondrus crispus.* Inner face of the most superficial lamella of the cuticle.

Fig. 23. Multiple penetration sites (arrows point some of them) showing the more or less homogeneous distribution of the infecting germlings. Scale= 10 μm


OF
IF

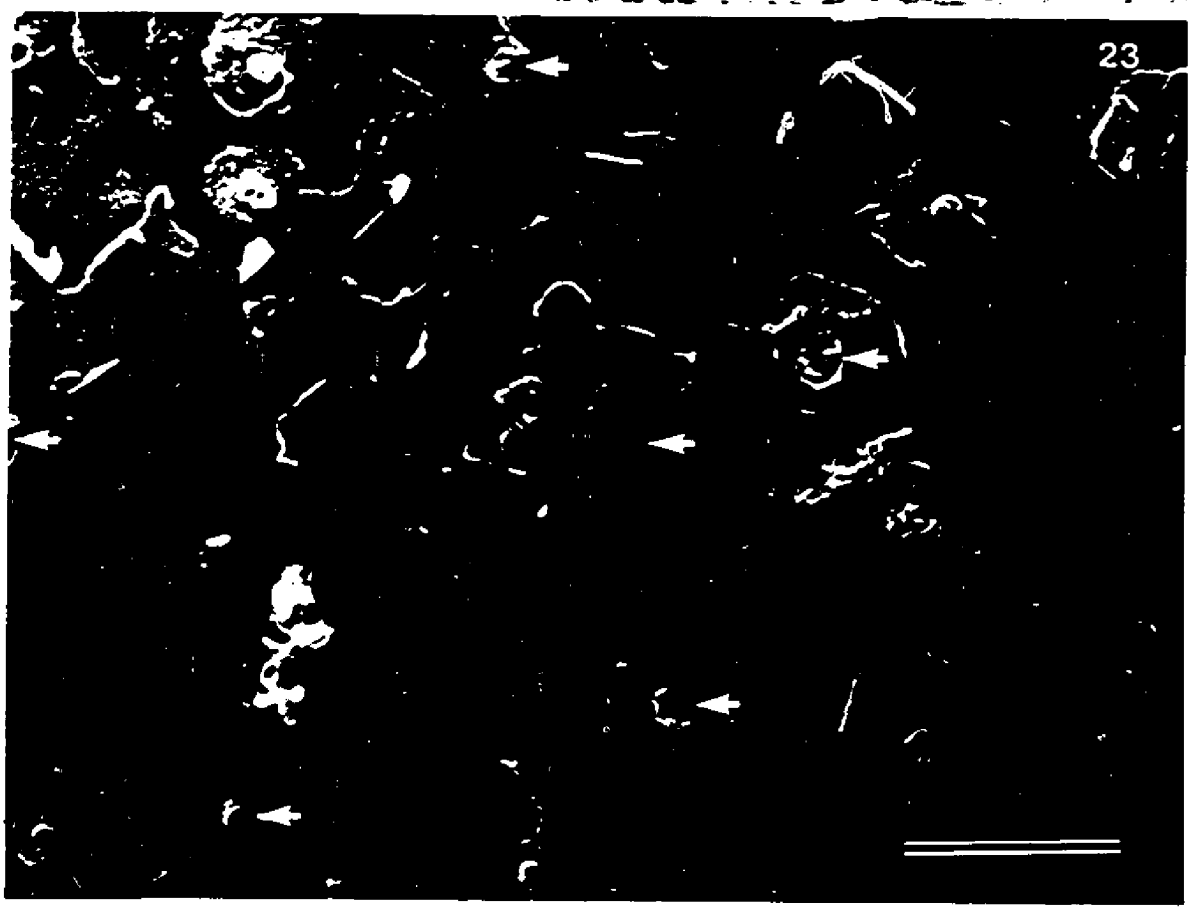
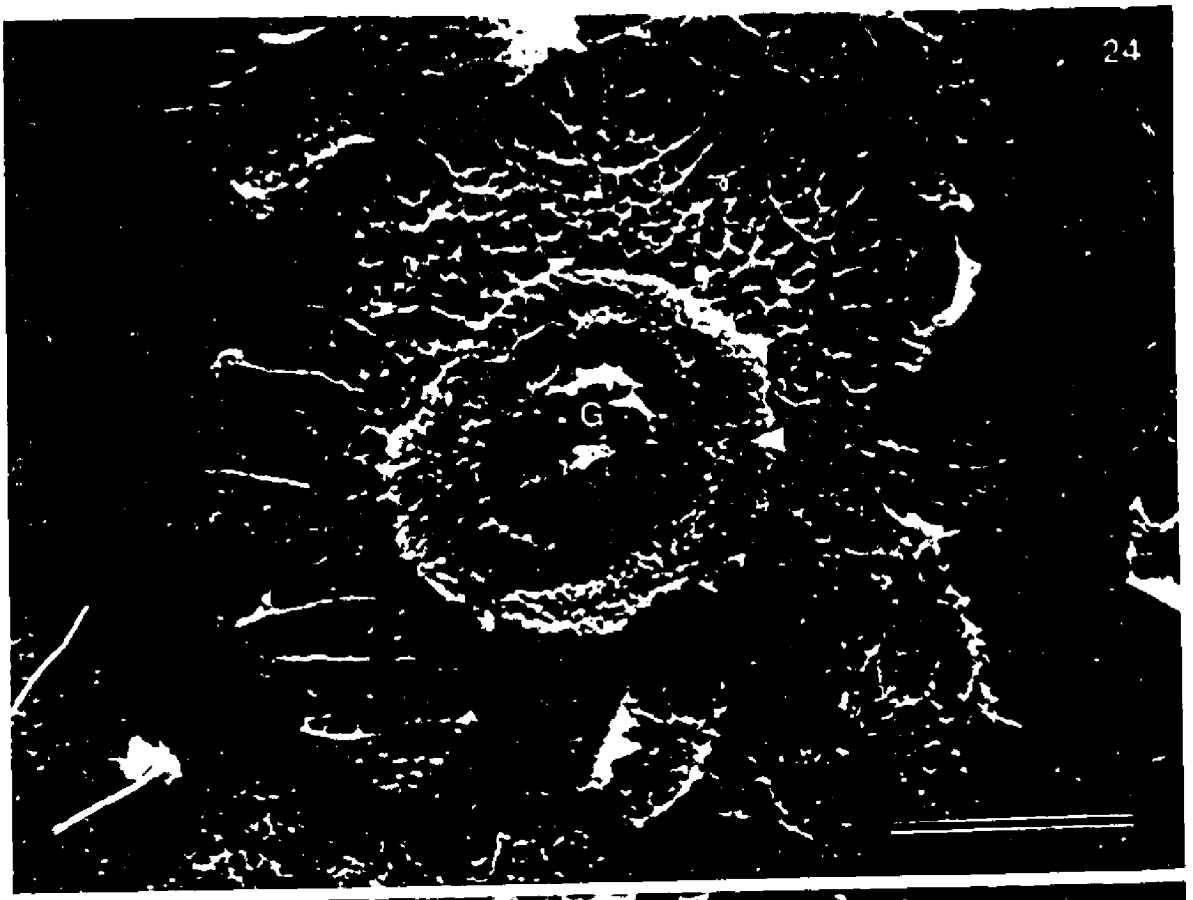
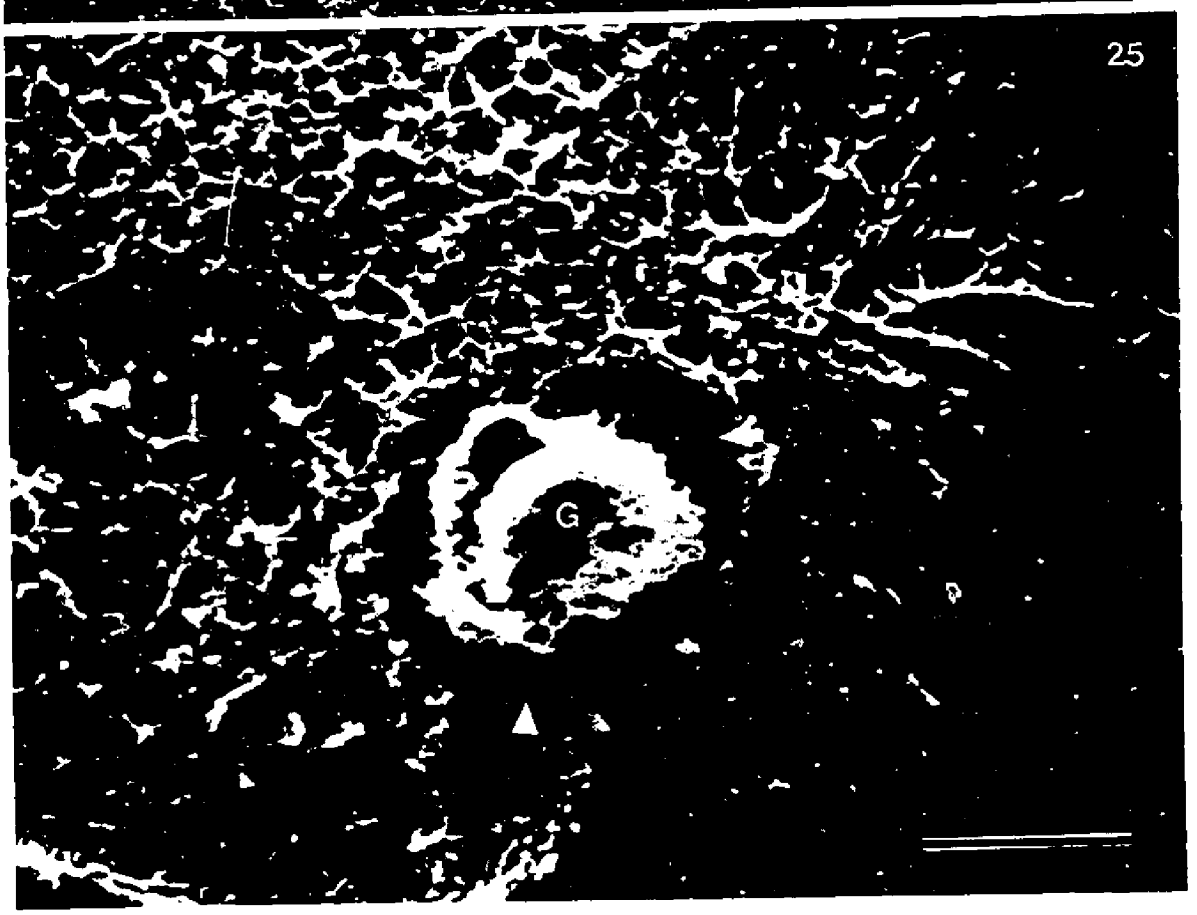

23

Fig. 24. Microfibrilar rearrangement at the site of penetration. A ring of modified lamella (arrowhead) surrounds the penetrating germling (G). Scale= 5 µm

Fig. 25. Alternatively, the ring seen in Fig. 24 is not apparent. The hole in the lamella (arrows) is larger than the penetrating filament (G). Scale= 2 µm

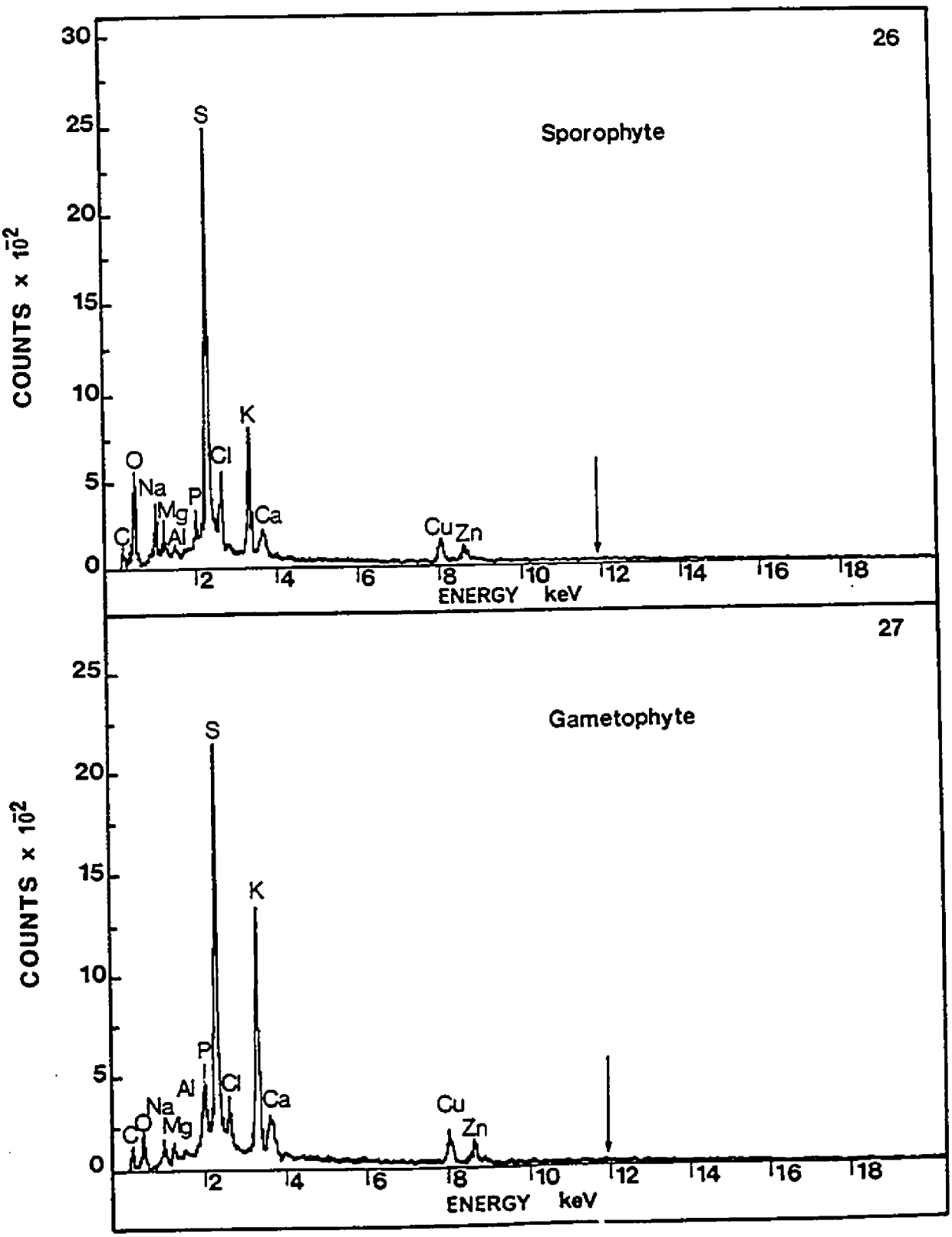

24
G


25
G

Figs 26-27. X-ray microanalysis spectra. Arrows indicate the expected (11.92 keV) site for Br.

Fig. 26. Characteristic spectrum for sporophytic, wild fronds.

Fig. 27. Characteristic spectrum for gametophytic, wild fronds.


26
Sporophyte
COUNTS x 10⁻²
30
25
20
15
10
5
0
S
K
Cl
O
Na
P
Mg
Al
Ca
Cu
Zn
2
4
6
8
10
12
14
16
18
ENERGY keV
27
Gametophyte
COUNTS x 10⁻²
25
20
15
10
5
0
S
K
P
Al
Cl
Na
Mg
Ca
Cu
Zn
2
4
6
8
10
12
14
16
18
ENERGY keV

Figs 28-33. Host specificity in *Acrochaete heteroclada.* Transverse sections through the fronds of some representative isolates are shown. H= host tissue, Ah= *Acrochaete heteroclada.*

Fig. 28. Sporophytic *Chondrus crispus* (JC010BH) with cortex replaced by endophytic filaments. Scale= 25 μm

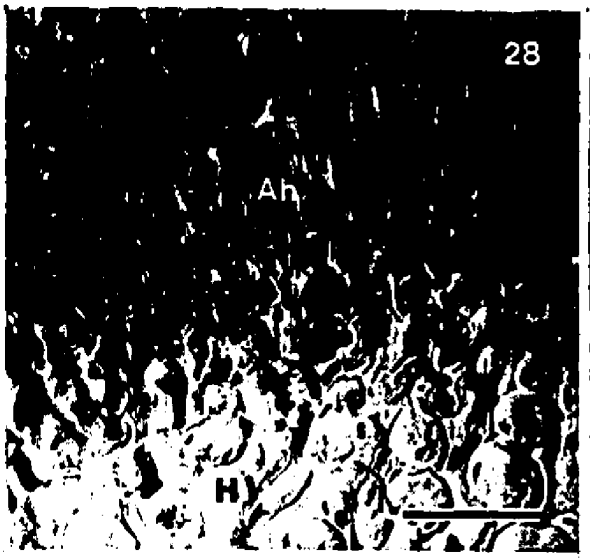
Fig. 29. *Chondrus canaliculatus.* Light penetration; most of the cortex remains intact. Scale= 25 μm

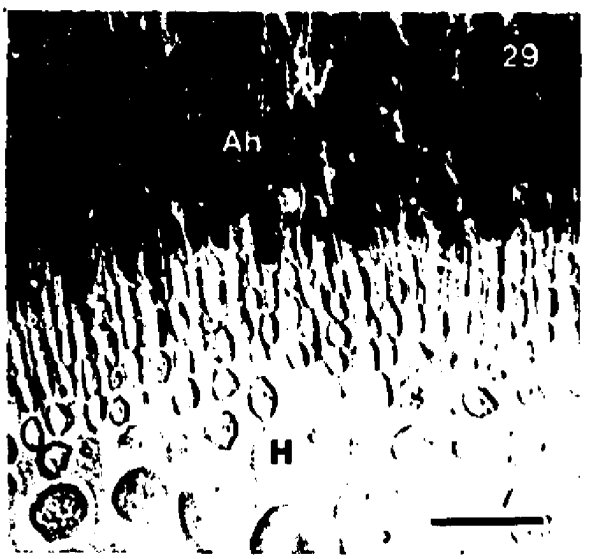
Fig. 30. *Gymnogongrus furcellatus.* Most of the cortex has been replaced, with no penetration of the medulla. Scale= 25 μm

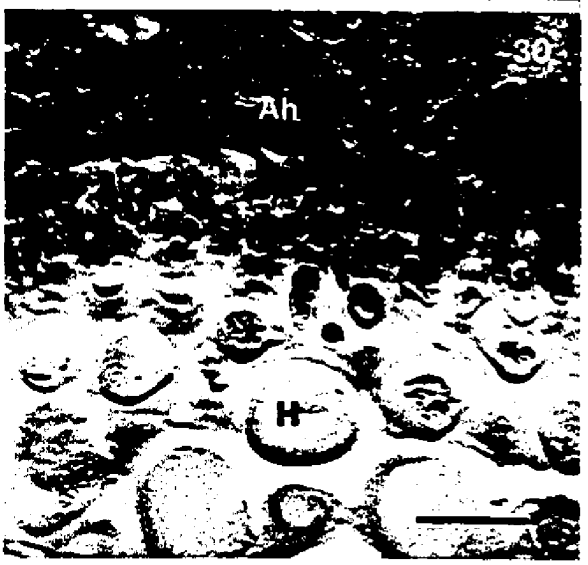
Fig. 31. *Gymnogongrus linearis.* Heavy penetration by clusters of endophytic cells which reach the outer medulla. Scale= 50 μm

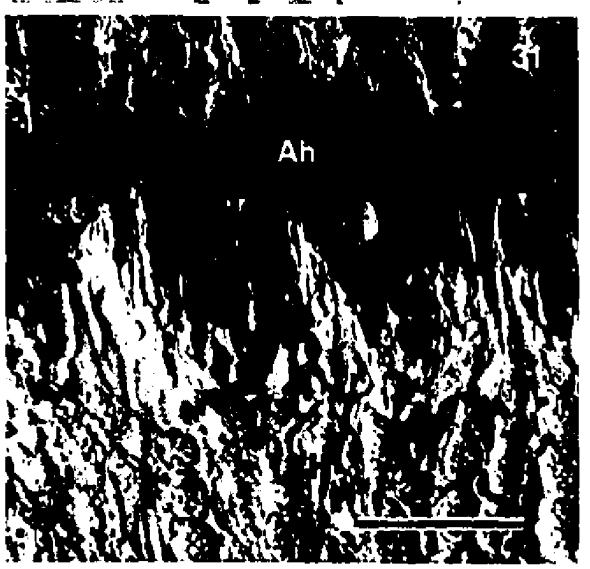
Fig. 32. *Gracilaria mammillaris.* Localized penetration, with fragments of the cuticle in place (arrowhead). Scale= 50 μm

Fig. 33. *Gracilaria chilensis.* The cortex has been entirely replaced, and some filaments have reached the medulla. Scale= 50 μm


28
Ah
H


29
Ah
H


30
Ah
H


31
Ah


32
Ah
H


Ah
H

TABLES

Table 1. Isolates of *Acrochaete operculata* and *A. heteroclada*

ISOLATE	COLLECTION DATE	LOCALITY(1)
Acrochaete operculata		
PF1 161085	October, 1985	Pubnico, N.S.
PF1a 161085	October, 1985	Pubnico
Pub 161085-2	October, 1985	Pubnico
Pub 161085-2-1	October, 1985	Pubnico
KH 040687-1-1	June, 1987	Ketch Harbour, N.S.
KH 040687-1-6	June, 1987	Ketch Harbour
Ru 260687-1	June, 1987	Rustico, P.E.I.
Acrochaete heteroclada		
PC2V 300585	May, 1985	Peggy's Cove, N.S.
IH 070587-5-1	May, 1987	Indian Harbour, N.S.
IH 070587-7-2	May, 1987	Indian Harbour

(1) Coordinates: Pubnico 43°36' N, 65°48' W, Ketch Harbour 44°29' N, 63°33' W, Rustico 46°25' N, 63°14' W, Peggy's Cove 44°29' N, 63°55' W, Indian Harbour 44°31' N, 63°56' W.

Table 2. Isolates of red algae used in cross-infection experiments.

ISOLATE	SPECIES	ORIGIN	PHASE	LOCALITY
CARRAGEENOPHYTES (1)				
JC001PC	Chondrus crispus Stackh.	T	G^{K}	Peggy's Cove, N.S.
JC002PC	C. crispus	C	S^{λ}	Peggy's Cove
JC003KH	C. crispus	T	G^{K}	Ketch Harbour, N.S.
JC004KH	C. crispus	C	S^{λ}	Ketch Harbour
JC005TB	C. crispus	T	G^{K}	Tor Bay, N. S.
JC006TB	C. crispus	C	S^{λ}	Tor Bay
JC007PP	C. crispus	T	G^{K}	Point Prim, P.E.I.
JC008PP	C. crispus	C	S^{λ}	Point Prim
JC009BH	C. crispus	T	G^{K}	Basin Head, P.E.I.
JC010BH	C. crispus	C	S^{λ}	Basin Head
JC011	C. canaliculatus (C. Ag.) Grev.	T	G^{K}	Chile
LC012	C. giganteus Yendo	T	G^{K}	Japan
JSC013	Gigartina skottsbergii Setch. & Gardn.	VT	G^{K}	Chile
MG014	G. acicularis (Roth) Lamour.	T	G^{K}	England
CM015	Gymnogongrus chiton (Howe) Silva & De Cew	VT	G^{K}	British Columbia, Can
CM016	G. devoniensis (Grev.) Schott.	C	G^{K}	Nova Scotia, Can
JC017	G. furcellatus (C. Ag.) J. Ag.	T	G^{K}	Chile

CM018	G. linearis (C. Ag.) J. Ag.	VT	G^{K}	British Columbia Can.
LC019	Gymnogongrus sp.	T	G^{K}	Japan
JC020	Iridaea cordata (Turn.) Bory	T	G^{K}	Washington State, USA
JC021	I. cordata	C	S^{λ}	Washington State, USA
JC022	Mastocarpus stellatus (Stackh.) Guiry	VT	G^{K}	Nova Scotia, Can
CM023	M. stellatus	C	G^{K}	Wales
CM024	Phyllophora traillii Holmes	?	G^{K}	North Ireland
JC025	P. pseudoceranoides (Gmel.) Newroth & Taylor	VT	G^{K}	Prince Edward Island, Can
JW026	Rhodoglossum californicum (J. Ag.) Abbott	T	$G^{K}(2)$	California, USA
JW027	R. californicum	T	$G^{K}(3)$	California, USA
JM028	Furcellaria lumbricalis (Huds.) Lamour.	T	$G^{K,\beta}$	Nova Scotia, Can
JM029	F. lumbricalis	?	$G^{K,\beta}$	Nova Scotia, Can
AGAROPHYTES				
CM030	Ahnfeltia fastigiata (Post. & Rupr.) Makienko	?	G	British Columbia, Can
JC031	A. plicata (Huds.) Fries	VT	?	Nova Scotia, Can
JC032	Gelidium crinale (Tur.) Lamour.	VT	?	Spain
JC033	G. vagum Okamura	VT	?	British Columbia, Can
JC034	Gracilaria chilensis Bird, McLachlan & Oliveira	C	S	Chile
JC035	G. mammillaris	VT	?	Tampa, USA

	(Montag.) Howe			
JC036	G. tikvahiae McLachlan	VT	S	Nova Scotia, Can
OTHERS				
IN037	Devaleraea ramentacea (L.) J. Ag.	VT	G	Nova Scotia, Can
CB038	Palmaria palmata (L.) O. Kunt.	VT	G	Nova Scotia, Can
IN039	Polyides rotundus (Grev.) Papenf.	VT	?	Nova Scotia, Can

(1) Carrageenan composition from McCandless et al. (1982, 1983) and Craigie (1990b). Three families of carrageenan are recognized (Craigie 1990b): Kappa (κ), includes κ, ι, μ, ν, and o types; Beta (β), includes β, ϖ, γ, and φ types; Lambda (λ) includes α, θ, π, δ, λ, and ξ types.

(2) Male

(3) Female

Abbreviations: T tetraspores, C carpospores, VT vegetative tips, S sporophyte, G gametophyte, ? information not available.

Table 3. Summary of cross-infection experiments using *Acrochaete operculata* (PF1a 161085). Control, *Chondrus crispus* (BH). + success, - failure.

ISOLATE	PH	EP	PE	EN	EPc	PEc	ENc
CARRAGEENOPHYTES							
JC001PC	G	-	+	-	-	+	+
JC002PC	S	-	+	+	-	+	+
JC003KH	G	-	+	-	-	+	+
JC004KH	S	-	+	+	-	+	+
JC005TB	G	-	+	-	-	+	+
JC006TB	S	-	+	+	-	+	+
JC007PP	G	-	+	-	-	+	+
JC008PP	S	-	+	+	-	+	+
JC009BH	G	-	+	-	-	+	+
JC010BH	S	-	+	+	-	+	+
JC011	G	-	+	-	-	+	+
LC012	G	-	+	-	-	+	+
JSC013	G	-	-	-	-	+	+
MG014	G	-	+	-	-	+	+
CM015	G	-	+	-	-	+	+
CM016	G	-	+	-	-	+	+
JC017	G	-	-	-	-	+	+
CM018	G	-	-	-	-	+	+
LC019	G	-	+	-	-	+	+
JC020	G	-	+	-	-	+	+
JC021	S	-	+	+	-	+	+
JC022	G	-	-	-	-	+	+
CM023	G	-	-	-	-	+	+
CM024	G	-	-	-	-	+	+
JC025	G	-	+	-	-	+	+
JW026	G	-	+	-	-	+	+
JW027	G	-	+	-	-	+	+
JM028	G	-	+	-	-	+	+

JM029	G	-	-	-	-	+	+
AGAROPHYTES							
CM030	G	-	-	-	-	+	+
JC031	?	-	-	-	-	+	+
JC032	?	-	-	-	-	+	+
JC033	?	-	-	-	-	+	+
JC034	S	-	-	-	-	+	+
JC035	?	-	-	-	-	+	+
JC036	S	-	-	-	-	+	+
OTHERS							
IN037	G	-	-	-	-	+	+
CB038	G	-	-	-	-	+	+
IN039	?	-	-	-	-	+	+

Abreviations: PH life history phase of the experimental plant, G gametophyte, S sporophyte, EP epiphytic on experimental plants, PE penetration of experimental plants, EN endophytic in experimental plants, EPc epiphytic on control, PEc penetration of control, ENc endophytic in control.

Table 4. Comparison of infection density by *Acrochaete operculata* (PF1a 161085) in *Chondrus crispus*. Values are means (± 1SD) of endophytic cells per 0.02 mm^2 of host surface.

	Sporophytes (n= 30, infected= 30)	Gametophytes (n= 30, infected= 23)
Distal (1)	1.2 (± 1.1)	0.1 (± 0.3)
Proximal(2)	27.7 (± 7.8)	5.7 (± 4.1)

(1) tip (ca 3 mm) of the experimental fragments
(2) base (ca 5 mm) of the experimental fragments

Table 5. Number of 3-wk old *Chondrus crispus* plants(1) (Peggy's Cove) infected by *Acrochaete operculata* (PF1a 161085) and *A. heteroclada* (PC 2V 300585).

	Acrochaete operculata		Acrochaete heteroclada	
INFECTION	Sporophyte	Gametophyte	Sporophyte	Gametophyte
Heavy	1034 (69.0%)	26 (2.9%)	1003 (98.5%)	357 (87.5%)
Regular	262 (17.5%)	42 (4.6%)	15 (1.5%)	51 (12.5%)
Light	194 (13.0%)	622 (68.8%)	- -	- -
Not infected	8 (0.5%)	214 (23.7%)	- -	- -
Total	1498	904	1018	408

(1) At this stage of development, plants were discoid with frond initials ≤1 mm in height.

CHAPTER 2

FINE STRUCTURAL ASPECTS OF THE INFECTIONS

INTRODUCTION

Fine structural studies have been an integral part of research leading to understandings of symbiotic associations between higher plant hosts, and their bacteria or fungal symbionts, particularly in parasitic or pathogenic interactions (reviewed by Akai 1973, Cooper 1981, Asada *et al.* 1982, Ingram 1982, Bailey 1986, Goodman *et al.* 1986, Nishimura *et al.* 1987, Ralton *et al.* 1987). This has resulted in a continually-growing body of knowledge of various aspects of host-pathogen associations, including, (a) surface signalling and thigmotropisms (Wynn 1976, Staples and Hoch 1987, Hoch *et al.* 1987), (b) mechanism of host penetration (Kolattukudy 1985, Kunoh 1984, Staples and Hoch 1984, Kolattukudy and Crawford 1987), and (c) cellular changes in both partners taking place during establishments of either compatible or incompatible interactions.

Although macroalgae host a number of pathogenic organisms, including bacteria, fungi, and other algae (Tsekos 1982, Apt 1984, 1988a, b, c, Pueschel and van der Meer 1985, van der Meer and Pueschel 1985, Molina 1986, Porter 1986, Raghukumar 1986, 1987a, b; Apt and Gibor 1989, Craigie and Shacklock 1989, Craigie 1990a, earlier reviews by Andrews 1976, Andrews *et al.* 1979, Kohlmeyer and Kohlmeyer 1979, Goff and Glasgow 1980, Tseng 1981, Goff 1983), our understanding of algal pathologies caused by biological agents is still poor. Areas that deserve special consideration are the host-pathogen interface, mechanisms of infection and subsequent spreading of the pathogen within the host, and the characterization of damage and the timing and extent to which it takes place in infections where host-cell damage occurs. Intracellular organization and relative abundance of membrane-bound organelles, including changes in the fine structure of host-cell walls, may provide useful additional information regarding whether or not algal hosts react against invasion by pathogens.

Only a few studies have reported fine structural features of lesions induced by pathogenic microorganism in their algal hosts. These have included bacterial-associated galls on *Prionitis lanceolata* (Harv.) Harv. (McBride *et al.* 1974, Apt and Gibor 1989), *Gigartina teedii* (Roth) Lamour. (Tsekos 1982) and *Polyneuropsis stolonifera* Wynne, McBride

& West (McBride *et al.* 1974), and the associations between *Pythium marinum* Sparrow and *Porphyra perforata* J. Ag. (Kazama and Fuller 1970), *Petersenia palmariae* van der Meer & Pueschel and *Palmaria mollis* Setchell & Gardner (Pueschel and van der Meer 1985), and *Petersenia pollagaster* (Petersen) Sparrow and *Chondrus crispus* Stackh. (Molina 1986). No such data exist, however, for algal pathologies caused by other algae, as in *Streblonema*-induced hyperplasia on species of Laminariales (Apt 1988a, and earlier studies cited there in), or in the "brown-spot" disease of cultivated *Undaria* sp. caused by *Streblonema aecidioides* (Rosenvinge) Foslie (Yoshida and Akiyama 1979). Instead, most fine-structural studies of associations involving algal partners have concentrated on non-pathogenic, parasitic relationships established between rhodophycean symbionts and their usually closely related rhodophycean host (reviews by Evans *et al.* 1978, Goff 1982a). This research has provided valuable information regarding interspecific cellular interactions taking place during infection of the host, particularly the passage of parasite nuclei into host cells via secondary pit-connections, which has been newly indicated as a regulatory mechanism of parasitism (Goff and Coleman 1984, 1985, 1987, Lewin 1984). Morphological evidence for possible transport of nutrients has also been reported (Wetherbee and Quirk 1982a, b). Pueschel (1980), however, has manifested his reservations about pit-plugs as a route for nutrient transport.

The poorly understood association between the highly specific, obligate rhodophycean epiphyte *Polysiphonia lanosa* (L.) Tandy and its phaeophycean host *Ascophyllum nodosum* (L.) Le Jolis has also received attention at the ultrastructural level. Attempts were made to elucidate the sequence of events taking place during the infection process, with emphasis on cellular changes of both the penetrating portion of the epiphyte and the surrounding host tissue (Rawlence 1972, Rawlence and Taylor 1972).

At present, it is difficult to summarize the phenomena, at cellular and tissue levels, taking place during invasion of healthy algal thalli by a foreign organism. In general, variable degrees of deformation in the external morphology of the host, sometimes accompanied by hypertrophy and hyperplasy, may occur during infection by either red-algal parasites (Kugrens and West 1973a, Goff 1976, 1982a, Fredericq *et al.* 1989), filamentous *Streblonema*-like brown endophytes (Andrews 1977, Apt

1988a, Yoshida and Akiyama 1979), fungi (Kohlmeyer and Demoulin 1981, Apt 1988b), or bacteria (Tsekos 1982, Apt and Gibor 1989). Penetration of host cells does not normally occur in infections by red-algal parasites nor in infections by streblonemoid endophytes, although in red-algal parasitism, nuclear transfer from some adelphoparasites has been documented (Goff and Coleman 1984, 1987, Zuccarello and Goff 1988). Furthermore, the establishment of secondary pit connections between partners has been clearly demonstrated (Kugrens and West 1973a, Wetherbee and Quirk 1982a, b, Goff 1982, Goff and Coleman 1984, 1985, 1987). Haustorial-like structures penetrating host cells have been described for two parasitic Corallinaceae, *Kvaleya epilaeve* Adey & Sperapani and *Ezo epyessoense* Adey, Masaki & Akioka, although their function, comparable to similar structures in fungi, has not been established (Adey and Sperapani 1971, Adey *et al.* 1974). During fungal infections, host-cell penetration seems to be common (Kazama and Fuller 1970, Kohlmeyer 1973, Walker *et al.* 1979, Pueschel and van der Meer 1985, Molina 1986, Raghukumar 1986, 1987, Apt 1988b), although there are reports of fungi which remain in the intercellular matrix, as *Blodgettia bornetii* Wright in *Cladophora catenata* (L.) Kütz. emend. v. d. Hoek (= *C. fuliginosa* Kütz.) (Testrake and Aldrich 1984), *Phycomelaina laminariae* (Rostrup) Kohlmeyer in *Laminaria saccharina* (L.) Lamour. (Schatz 1980), and *Mycosphaerella* spp. in various hosts (Kohlmeyer and Kohlmeyer 1972, Kohlmeyer and Hawkes 1983). Bacteria, on the other hand, although apparently restricted to the intercellular matrix, as established for *P. lanceolata* (Mc Bride *et al.* 1974, Apt and Gibor 1989), *P. stolonifera* (Mc Bride *et al.* 1974), and *G. teedii* (Tsekos 1982), have also been reported intracellularly in *Bryopsis hypnoides* Lamour. (Burr and West 1970), *Caulerpa prolifera* (Forsskål) Lamour. (Dawes and Lohr 1978), and in *Halimeda tuna* (Ell. *et* Soll.) Lamour. and *Udotea petiolata* (Tuna) Börgesen (Mariani Colombo 1978).

The impact of infection by an alien organism on the fine structure of algal host cells ranges from minor changes detected in red algal parasitism, including decrease in floridean starch, dispersed cytoplasmic matrix, and contortion of chloroplasts (Kugrens and West 1973a), to disintegration of host cells at the culmination of the infection process in *P. perforata* infected by *P. marinum* (Kazama and Fuller 1970) and in *P. mollis*

infected by *P. palmariae* (Pueschel and van der Meer 1985). Furthermore, host cell destruction may occur secondarily to cell wall degradation by extracellular fungi (Schatz 1980) or bacteria (Mc Bride *et al.* 1974). Thus, it appears that cellular damage of the host caused by algal infections is relatively unimportant and highly localized, whereas fungal and bacterial infections may induce severe cell and cell-wall disruption leading to the development of macroscopic lesions representing decaying host tissue (Fuller *et al.* 1966, Andrews 1977, Goff 1983, van der Meer and Pueschel 1985, Molina 1986, Craigie and Shacklock 1989, Craigie 1990a). There are other cases, however, as in the *Spathulospora-Ballia* association, where the fungus penetrates the host cell but has little effect on it or on contiguous hosts cells (Kohlmeyer and Kohlmeyer 1975, Walker *et al.* 1979). Similarly, there was no cell damage reported for extra- and intracellular bacterial infections in chlorophycean hosts (Burr and West 1970, Dawes and Lohr 1978, Mariani-Colombo 1978)

In nature, the rhodophycean species *C. crispus* is known to host an epiphytic bacterial flora which does not appear to represent a threat to the welfare of the fronds (Sieburth and Tootle 1981). This flora is removed periodically by peeling of the outer layers of the cell wall, a self-cleaning phenomenon that, together with epidermis shedding, seems to occur in a number of macroalgae (McArthur and Moss 1977, Filion-Myklebust and Norton 1981, Sieburth and Tootle 1981, Moss 1982, Russell and Veltkamp 1984). Some pathogens, however, are apparently not purged by this mechanism, as documentation exists for *Didymosphaeria danica* infecting field-collected fronds of *C. crispus* in England (S. Stanley in verb.) and in Rhode Island (Goff 1983), and *Petersenia* sp. infecting wild population of *C. crispus* in southern Nova Scotia and Prince Edward Island (J. S. Craigie in verb.).

Filamentous brown and green (but not red) algal endophytes are commonly found associated with *C. crispus* in Nova Scotia, Prince Edward Island and northern Denmark (Correa *et al.* 1987). The central portion of the fronds is the area more frequently infected, and endophytes were not found in the holdfast. Fronds at various stages of infection can be found in the wild, particularly in localities protected from strong wave action. These fronds range from morphologically unaltered, with pigmented endophytes visible only under microscopic examination, to heavily infected

ones, with no macroscopic evidence of damage other than greenish (or simply darker) pigmentation of the central areas. Broken fronds, sometimes necrotic and heavily grazed at the fracture points are also commonly found, and seem to represent the step that follows heavy infection by algal and bacterial endophytes. Although suggestive, these field observations do not unequivocally establish whether algal endophytes or bacteria are the cause of necrosis, nor which organism was the primary invader. It seems likely, though, that bacteria are secondary invaders. Bacteria commonly found in seawater and on healthy seaweeds are generally benign to algae, although under certain conditions, such as frond disruption, they can cause degradation of cell-wall components of numerous algae, including *C. crispus* (Yaphe and Baxter 1955, Bellion *et al.* 1982, Sarwar *et al.* 1983). In fact, bacterial isolates from necrotic lesions of farmed *C. crispus* failed to reproduce the infection under laboratory conditions, unless a wound was made in the cortex of the host (L. Staples and J.S. Craigie in verb.).

The two most common green algal endophytes of *C. crispus* are *Acrochaete operculata* and *A. heteroclada.* These species were isolated into unialgal culture, and general morphologies and life histories, in absence of the host, as well as preliminary information on patterns of infection, have been reported (Correa *et al.* 1988).

A number of algal species are being successfully cultivated on a commercial scale, and diseases have become a concern, as sooner or later they affect crops (Tseng 1981). Attempts to establish large-scale cultivation of *C. crispus* have been ongoing in Canada and France since the early 1970's (Chopin 1986, Deveau 1989), and by 1987 the first 100 metric tonnes of farmed dry material was shipped for extraction (Craigie and Shacklock 1989). As summarized by Craigie and Shacklock (1989) and Craigie (1990 a), two diseases have seriously hampered the development of *C. crispus* aquaculture. One was caused by the fungus *Petersenia pollagaster* which affected mainly the apical zone of the fronds. The second, of bacterial origin, affects mainly the central portion of the fronds, inducing softening, decay and erosion of areas up to *ca.* 3 mm in diameter. When these holes coalesce, the fronds weaken and eventually break. An interesting correlation seems to exist between the development of these bacterial lesions and the presence of green algal filaments, identified as *A.*

operculata (pers. obs.), colonizing extensive areas of the cortex of *C. crispus*.

In this chapter I attempt to characterize, at cellular and tissue levels, sequences of events taking place during infection of *C. crispus* by *A. operculata* and *A. heteroclada*, from spore settlement to the onset of reproduction and host decay. Special consideration is given to the *A. operculata-C. crispus* association, as the life-history phases of the host are affected differentially by this endophyte (Correa *et al.* 1988, Chapter 2).

As outlined in Chapter 2, the outcome of any interorganismal association, including those where a pathogen is involved, will depend both on the mechanism evolved in the pathogen to complete the different stages of the infection process, and on the presence and type of preformed and induced "barriers" developed by the host (Keen 1986), which tend to attenuate or eliminate the effectiveness of the pathogen (Heath 1981). In this context, there have been several studies reporting ultrastructural information on *C. crispus* (Cottler 1971, Gordon and McCandless 1973, Tveter-Gallagher *et al.* 1980). The plant, including holdfast, stipe and frond, has been considered a structurally homogeneous entity, particularly regarding the layer of material making up the interface between the outside and the cellular component of the thallus cortex. Preliminary information has indicated, however, that the fine structure of the host outer cell wall, of primary importance in the so called self-cleaning process (Sieburth and Tootle 1981), is indeed highly variable not only within a plant, but also between phases of the host (Correa and McLachlan 1988). Therefore, a characterization of cellular and extracellular components of different portions of the thallus of *C. crispus*, with emphasis in the outer cell wall, and including sporophytic and gametophytic fronds, was done. An effort is made to relate this information to the differential localization of algal endophytes along the thallus of field-collected material as reported by Correa *et al.* (1987).

MATERIALS AND METHODS

Transmission electron microscopy

A) Preliminary observations

Early in the fine structural study of the interaction between *C. crispus* and the endophytes *A. operculata* and *A. heteroclada*, it was clear that fixation in glutaraldehyde followed by postfixation in osmium tetroxide would not result in cellular details necessary to describe and interpret different aspects of these symbiotic associations. Membranous structures of host cells were the most affected, showing very low reactivity (poor contrast), where, initially, host cells were interpreted as altered after endophytic action. This interpretation was soon discarded, however, as samples of uninfected *C. crispus* showed cells displaying the same poor definition of membranes, similar to previous ultrastructural studies of this alga (Cottler 1970, McCandless *et al.* 1977, Tveter-Gallagher *et al.* 1980).

By contrast, the combined use of potassium ferrocyanide (K4) and osmium (see below) resulted in a consistent general contrast enhancement in all membranous structures in *C. crispus* and in the endophytic cells. The use of iron compounds as potential "fixatives" for cell membranes was introduced by Elbers *et al.* (1965), who studied the reaction of potassium ferricyanide (K3) and lead nitrate with a phospholipid fraction isolated from bovine brain. Subsequently, K4 and K3 combined with osmium tetroxide have been used in a variety of plant and animal tissues for selective staining of different cellular components, particularly membrane systems (Hepler 1980, 1981; McDonald 1984). This technique has been apparently overlooked in phycological studies, with few exceptions. Pueschel (1987) used it with several red algae (*C. crispus* not included) to describe the fine structure of pit plugs. Unfortunately, because of the scope of Pueschel's paper, no information was presented regarding the results of the technique on the general morphology and contrast of cell organelles.

My preliminary results also indicated that the Golgi complex was selectively reactive to the treatment, although this occurred only when *in block* staining with uranyl acetate and post-staining with lead citrate was included. Similar responses by the Golgi complex had been noted by

Hepler (1981) in lettuce root cells, but he considered this to be an example of non-specific reaction of the OsFeCN reagent, which in his study enhanced dramatically the contrast of the nuclear envelope and endoplasmic reticulum. I have failed to find such a reaction in any cell of *C. crispus*, *A. operculata* or *A. heteroclada.*

Visualization of microtubular components in zoospores of mature *A. operculata,* not achieved with the standard procedure, was comparable to microtubular contrast enhancement by the OsFeCN mixture in various types of animal cells (McDonald 1984), and reveals the versatility of this procedure as a general contrast enhancer.

Variations in the OsFeCN treatment (see below), including longer times of fixation, addition of acrolein, use of K3 instead of K4, and omission of calcium chloride, did not induce any noticeable change in the preservation nor in the contrast of organelles. Although postfixation at room temperature produced results similar to on-ice postfixation, the sharpness of the images decreased owing to coarse granularity throughout the cell, which is particularly undesirable at high magnifications.

The use of K3 and K4, in consequence, was adopted as the routine additive to the OsO_4 solutions during postfixation throughout most of the present study. Although the chemical mechanism for tissue staining by the OsFeCN mixtures has been described in detail by White *et al.* (1979), the so-called "specificity" of the reaction (Hepler 1981) with some membranes is a phenomenon poorly understood and in need of further research.

B) Material

To study the outer cell wall (OCW), sporophytic and gametophytic plants of *C. crispus,* including holdfasts, separated by the presence of tetrasporangia or cystocarps, were collected in the intertidal zone, Peggy's Cove, Nova Scotia. The plants were transported to the laboratory in plastic bags maintained on ice in a cooler. The material was sorted and processed within 2 h after being collected. Samples were taken from the discoid holdfasts, stipes, central portion of the fronds above the first dichotomy but before the last undivided branches and apices (above the last dichotomy). Discoid germlings and young plants (PC isolates) were obtained by culturing either tetraspores or carpospores from mature *C. crispus* fronds collected in the same locality. Spores were settled on glass cover slips to

facilitate subsequent manipulations. Non-infected fronds of *C. crispus* (isolate BH) were obtained, as needed, from unialgal stock cultures maintained in the laboratory.

Infected *C. crispus* was obtained by inoculating unialgal tetrasporophytic and gametophytic fronds (BH isolate) or young plants (PC isolate) with actively-reproducing *A. operculata* (isolate PF1a 161085) and *A. heteroclada* (isolate PC 2V 300585). After a period of about 24 h, inoculum and enriched seawater medium were discarded, and infected material was transferred to fresh medium and cultured at 10-15 °C, 25-50 μmol m^{-2} s^{-1} PFD, and 18-h light (24 h period). Samples were collected at various intervals of time, from 24 h to 4 mo after inoculation, with the exception of young plants, which were collected once, 30 d after inoculation with the endophytes.

Glutaraldehyde-osmium procedure

With some changes, I followed the method described by Pueschel (1979). Large pieces of infected gametophytic and tetrasporophytic fronds of the host were immersed in cold 5% glutaraldehyde in 0.1M sodium cacodylate buffer, pH 7-7.2 (hereafter standard buffer), with added 0.2M sucrose. Smaller slices (about 0.3 mm thick) were cut in fixative and transferred to fresh 5% glutaraldehyde containing 3% acrolein at 4 °C for at least 24 h. Samples were then rinsed in four changes of cold standard buffer with decreasing concentrations of sucrose, followed by two rinses in pure standard buffer. Postfixation with 2% osmium tetroxide in standard buffer was for 8 h at 4 °C, followed by two rinses in cold standard buffer, three rinses in cold distilled water, dehydration through an ethanol series (on ice up to 95%), two changes in propylene oxide, infiltration over four days and embedding in Spurr's (hard type) resin (Spurr 1969). Infiltration was performed under constant rotation of the vials containing the samples, and the final step, using pure resin, was in vacuum (7.8×10^2 mbar). Embedding in fresh resin was accomplished by polymerization of the plastic at 70 °C for at least 12 h. Sections (60-90 nm) were collected on formvar-coated grids, stained with 1% aqueous uranyl acetate for 15 min followed by 10 min in lead citrate (Reynolds 1963). Variations in the standard procedure included longer postfixation times (up to 19 h), use of acetone instead of ethanol during dehydration, longer staining times, *in*

block staining with 0.5% aqueous uranyl acetate and the use of saturated ethanolic uranyl acetate during staining.

Osmium tetroxide-potassium ferrocyanide procedure (OsFeCN)

Samples were fixed in 5% glutaraldehyde in standard buffer plus 0.2M sucrose and 10 mM calcium chloride (acrolein was omitted) for 3 h and 1 h, respectively. Four rinses in standard buffer with added 10 mM calcium chloride and gradually decreased concentrations of sucrose were followed by two rinses in pure standard buffer plus 10 mM calcium chloride. Postfixation was in 2% osmium tetroxide in standard buffer plus 1.1% potassium ferrocyanide (K4) for 1 h. This was followed by four rinses in standard buffer and three rinses in distilled water. Dehydration was in an ethanol series. All solutions were kept, and all steps performed, on ice up to 95% ethanol. Infiltration and subsequent processing was as for the standard procedure. Variation in the OsFeCN treatment included different times of fixation in glutaraldehyde (to 24 h at 4 °C), addition of acrolein (3%) to the glutaraldehyde mixture, omission of calcium chloride, various concentrations of potassium ferrocyanide (0.8% to 3%), substitution of K4 by potassium ferricyanide (K3), postfixation at room temperature (23-24 °C), and *in block* staining with 0.5% aqueous uranyl acetate before the dehydration series.

Five to ten fronds were sampled each time, with six to fifteen blocks per frond. Thin sections for the final observations were collected from the central region of each block.

Scanning Electron Microscopy

Infected and non-infected specimens for scanning electron microscopy were obtained from the above sources. These materials were fixed for 1-24 h in 2% glutaraldehyde in culture medium or filtered seawater, rinsed in pure seawater to which distilled water was gradually added to decrease salinity. Once in pure distilled water, samples were frozen in Freon 22 at liquid-nitrogen temperature, after which they were transferred to liquid nitrogen and dried in a Virtis (Mod. 10-010) freeze-dryer for at least 24 h. Samples were gold-coated, and observed in a JEOL (Mod. 35) scanning electron microscope.

Zoospores were obtained by adding glutaraldehyde to give a 2% final concentration in Petri dishes with sporulating *A. operculata* or *A. heteroclada*. The zoospore solutions were maintained for 1 h at 4 °C, filtered through 1 µm pore-size nucleopore filters, and rinsed with seawater of gradually-decreasing salinities. Once in pure distilled water, the filters were processed and viewed in the same way as the samples described above.

RESULTS

1. Characterization of non-infected *C. crispus*.

a) Holdfast and discoid germlings.

The fine structure of holdfasts and discoid germlings was similar (Figs 1-6). Cells were arranged in filaments perpendicular to the surface (Figs 1, 3). The apical cell of each filament, 10-15 µm diam., located close to the surface of the holdfast, was characteristically lens-shaped (Figs 1, 5), with the flat side towards the substratum and the convex side facing the outer cell wall (OCW). The OCW is defined here as the acellular layer of material deposited between the immediate cell wall of the outermost cortical cells and the surface of the disc. In discoid germlings and holdfasts, the OCW was 4-9.5 µm thick and characterized by a more or less homogeneous appearance, resulting from a network of delicate microfibrils embedded in a finely granular matrix (Fig. 1). Sometimes, at the interface with the medium, the OCW displayed a layer of granular material, 250-400 nm thick, quite dense at the inner side (Figs 1, 2), but becoming diffuse towards the outside (Fig. 2).

Cells of the holdfast or in the discoid germlings ranged in shape from discoid at the surface, underneath the apical cell (Fig. 1), and similar in size to the apical cell, through more or less isodiametric, 7-13 µm in diameter, at the center (Fig. 3), to cylindrical or irregular at the base of the holdfast (Fig. 6). Chloroplasts, in general not prominent, were more developed in the superficial cells (Figs 1, 5), with the exception of discoid germlings growing under multidirectional illumination, where chloroplasts developed profusely also in the basal cells (Fig. 6). Most of the intracellular space of the superficial cells was occupied by vacuoles (Figs 1, 5), resulting in organelles restricted to the periphery of the cells (Fig. 5), or sometimes in a "cytoplasmic septum" (Fig. 1), where the nucleus was generally located. Plastids were much less apparent in the isodiametric, central cells, where granules of floridean starch and osmiophilic inclusions prevailed and a large vacuole was not apparent (Fig. 3). Pit-plugs were commonly observed between cells of the same filament (Figs 3, 5), and between cells of adjacent filaments (Fig. 6).

Each cell, regardless of the position within the tissue, was surrounded by an immediate cell wall, clearly delimited from the intercellular matrix (Figs 1, 3, 6) and from the OCW (Fig. 5). This was true for holdfasts, discoid germlings and for other parts of the thallus. Although the ultrastructure of the microfibrils appeared similar in both immediate cell wall and intercellular matrix, the surface of contact between the two usually appeared as a compacted band of microfibrils belonging to the immediate cell wall (Fig. 4). Perhaps the most striking feature in both holfasts and discoid germlings was the reduced intercellular matrix, which was virtually nonexistent between cells of the same filament (Figs 1, 3, 5).

No structural differences were found between sporophytic and gametophytic holdfasts, nor between sporophytic and gametophytic discoid germlings.

b) Fronds

Cortical cells of *C. crispus* showed variable morphologies (Figs 7, 8), although the most common was ovoid, 3.5-4.5 x 5.5-6.5 μm in transverse sections (Fig. 7). All cortical cells, however, displayed a circular profile in sections parallel to the surface of the frond (Fig. 45). Cells from the cortex of rapidly growing apical portions of fronds contained inconspicuous plastids, a highly developed endoplasmic reticulum (ER), Golgi complex, and mitochondria (Fig. 8). The typical cortical cell, on the other hand, displayed a fully developed, parietal chloroplast, a centrally located nucleus with a prominent nucleolus and a small basal vacuole (Fig. 7). Mitochondria were seen throughout the cell. Golgi complexes were usually located in the upper portion of the cells (Fig. 7), and sometimes the *cis* saccules were selectively filled with a highly electron-opaque substance (Fig. 9). The ER was restricted mainly to the periphery of the cell (Fig. 7) and to the perinuclear zone (Fig. 8), where ribosomes attached to the flat vesicles (Fig. 10). The plasmalemma, irregular in shape, was usually apparent, although at some points it appeared discontinuous while emitting short projections into the inner immediate cell wall (Fig. 7).

Subcortical cells (Fig. 11), larger (4-6 x 7-10 μm) than cortical cells, also displayed variable morphologies. The cytoplasm usually contained abundant, large membranous structures (convoluted vacuole

perhaps), which made the process of embedding particularly difficult . The lobed chloroplast, together with other organelles, were located at the periphery of the cell where ER developed profusely. More than one nucleus, up to 5, were relatively common in these cells. The plasmalemma was convoluted, with finger-like projections protruding into the immediate cell wall (Fig. 11).

Medullary cells (Fig. 12) showed a convoluted profile with a large central vacuole and highly reduced chloroplast. Plasmalemmal projections into the immediate cell wall occurred throughout the cell.

Each cell was surrounded by an immediate cell wall (Figs 7, 8, 11, 12), which gradually increased in thickness from cortical (0.3-0.6 μm) to medullary (1-2 μm) cells. The intercellular matrix, poorly represented in the cortex, occupied most of the medullary space. This resulted in medullary cells separated by distances several times their diameter from adjacent cells, and which appeared embedded in the finely fibrillar network of intercellular matrix.

No differences in the cells or in the walls, including intercellular matrix, were detected between sporophytic and gametophytic fronds. The OCW, however, displayed a highly variable, fine-structural organization, determined not only by the location within the thallus, stipe, central portion, or apex, but also by the life-history phase of *C. crispus*.

In the stipe of gametophytic fronds, the OCW appeared similar to the OCW of holdfasts and discoid germlings, with a thick, finely fibrillar matrix, evenly distributed between the cortical cells and the outside (Fig. 13). No modifications of the homogeneous appearance were found at the interface between OCW and the medium, with the exception of occasional development of highly localized, electron dense deposits (Fig. 13). However, when the interface between OCW and the medium was examined at the central portion of gametophytic fronds, a lamellar organization (cuticle *sensu* Hanic and Craigie 1969) became apparent (Fig. 14). The number of lamellae in this region varied from 1 to 6, and sometimes they were seen becoming separated from the frond. Usually, however, at least one lamella remained in close contact with the thallus. The zone of the OCW apparently involved in the assembly of the lamellae was characteristically more electron dense than deeper portions (Fig. 14). At the apices, the multilamellar organization of the OCW was more

conspicuous (Fig. 15), up to 17 lamellae noted. Peeling of the external lamellae was common and it occurred in flakes of up to 5 layers, although 2- or 3-lamellar flakes were more frequent (Fig. 15). The basal region of the multilamellar OCW (Fig. 16) consisted of regularly-spaced dark bands (about 50 nm thick), separated from each other by a granular to fibrillar, lighter layer (about 100 nm thick). Towards the surface, splitting occurred between the darkened layers (Fig. 17), resulting in individual lamellae consisting of an electron dense compacted core, sandwiched between two lighter, fibrillar layers (Fig. 17).

In sporophytic fronds, the interface between OCW and the medium at the stipe was similar to gametophytic fronds, although in the former the homogeneous, non-lamellar appearance extended to most of the central portion of the fronds. At the apices, a lamellar appearance did develop (Figs 18-20), however, the structure and the spatial organization of the lamellae were different from their gametophytic counterpart. The banding was sometimes difficult to determine, as if lamellae were embedded in the homogeneous region of the OCW, with the dark layers closely packed, leaving almost no space between them (Fig. 18). When lamellae were clearly outside the homogeneous region of the OCW, they usually lacked the spatial regularity of those in the gametophytic fronds (but see Figs 110-112), and instead appeared undulating and anastomosing (Fig. 19), with a granular appearance (Fig. 20) and with various degrees of compaction. A feature frequently found at the apical regions of sporophytic fronds, especially those growing rapidly, was the presence of an amorphous layer of finely granular material with almost no electron opacity (Fig. 21). Sometimes this material replaced the OCW, appeared split into two or three layers of similar electron opacity, and were shed as the lamellae (see Fig. 34).

2. Infection of sporophytic *C. crispus* by *A. operculata*

a) Fronds

The infection cycle begins when quadriflagellated zoospores (Figs 22, 23) from mature sporangia of *A. operculata* are released into the medium and establish contact with the flaky surface of the host (Fig. 24). Motile spores are the only infective cells, as in no instance were vegetative

filaments used as inocula found to penetrate directly into the host. Zoospores were pyriform (Fig. 22), 3-4 x 3.5-5 μm, with flagella 3-4 times the length of the cell, connected to a papilla at the anterior end of the cell (Fig. 23).

After a relatively short period of swimming, the zoospores attached to the surface of the host by the anterior end, as they did to the bottom of the plastic dishes used in the inoculations. By the end of the second hour after inoculation, settled zoospores still retained their shape (Fig. 25), but flagellar remains were no longer seen. During the following 72 h, the attachment point of the settled zoospores enlarged (Fig. 26), and adhesive material, apparently produced by the spore during the ongoing process of germination, surrounded the germlings and extended onto the surface of the host as a thin pad of radially arranged material (Fig. 27). At this stage of development, germlings of *A. operculata* had initiated the penetration of the host (Fig. 28), by means of a germination tube, generally narrower (*ca.* 1 5 μm) than the diameter of the original zoospore. Observations with the SEM indicated that zoospores settled regularly over the surface of the fronds, in spite of peeling of the cuticle at the surface of the host, a phenomenon that was actively occurring during early infection, particularly at the apices. When a lamella (or several) became separated from the host while being penetrated by the endophytic germlings, the tensile force applied to the germling while being pulled could sometimes be visualized by the rupture of the germling cell wall (Fig. 29). Depending on how deeply the germling had advanced into the host at the time of cuticle peeling, two outcomes were observed. If penetration was superficial, the germling was pulled out of the host by the lamella (Fig. 30), whereas if penetration was deep, fracture of the germling into two parts usually occurred. The emptied zoospore cell wall remained attached to the outer face of the lamella, and both were discarded together. A short remnant of the germling cell wall could sometimes be seen protruding from the inner face of the shed lamella (Fig. 31). The fibrillar organization of this face tended to disappear at the zone of contact with the invading germling (Fig. 31). The rest of the germling remained embedded in the host, and only the upper portion of the penetrating filament, where the fracture took place, was seen protruding from the newly exposed host surface (Fig. 32). Penetration of the host during germination was the

norm, and seldom were zoospores seen germinating and developing outside the host. In some cases, secondary penetrations were indicated by marks, left by germlings removed during the sample processing, on the surface of the OCW which appeared digested (Fig. 33).

After the zoospores settled on the surface of the host, germination proceeded with the formation of an elongated, cylindrical tube that carried the cytoplasm evacuated from the spore. When evacuation of cytoplasm was completed, a septum formed separating the now emptied zoospore cell wall, from the first cell of the filamentous endophyte (Fig. 34). At this stage, some degree of organelle polarization was evident within the cytoplasm, especially in germlings penetrating the host diagonally. Numerous membranous vesicles, of various sizes, were localized mainly at the tip of the cell and along the "host face" of the germlings, while the chloroplast occupied the opposite side, at the periphery of the cell (Fig. 34), where a double membrane enclosed the thylakoids and pyrenoid. Only one pyrenoid was present, located at the anterior end (Figs 34, 37). The relatively large nucleus occupied the center of the cell, which had a fully developed cell wall continuous with that of the zoospore (Fig. 34). The cell wall of the germling had at least two layers. An external, darker layer, clearly continuous with the cell wall of the zoospore, that appeared to fade towards the tip of the cell (Fig. 34). There was also an internal layer which surrounded the first vegetative cell only and which was well developed at the septum (Fig. 34).

Penetration appeared to occur smoothly, with no evidence of inward bending or compression of any component of the host OCW (Fig. 34). Even in slightly more developed infection stages, within a post-inoculation period of 5 d, and when hosts had a more compacted OCW, the outermost lamella and subjacent OCW ended abruptly at the contact with the cell wall of the penetrating germling (Fig. 35). Again, no deformation of the host surface was observed, a phenomenon better appreciated when the cell wall of the germlings became detached from the host (Fig. 36). Properly oriented sections indicated that evacuation of the cytoplasm from the zoospore into the first cell occurred through a narrow passage (Fig. 35). The effectiveness of cuticle shedding in removing infecting endophytes was evident mainly on germlings of the one-cell stage (Fig. 37). In a number of early infections, particularly those taking place in hosts with non-

lamellar OCW, alteration of the matrix surrounding the infecting germling was apparent, as indicated by areas of low electron opacity and changed texture, where caverns of digested OCW were commonly observed (Fig. 38). It is worthwhile to mention that bacteria, although frequent on the surface of the fronds, never preceded the infecting germlings. Sometimes bacteria remained associated with the external layer of the OCW, surrounding the penetration sites. Furthermore, when digestion of the OCW was observed (Fig. 38), bacteria were not found in or near the areas being digested, even though serial sections were performed through these infection sites.

Further penetration of the frond occurred by elongation of the first cell of the endophyte, followed by mitosis, resulting in a two-cell germling (Fig. 39). Growth soon slowed in the superficial cell, and the chloroplast developed rapidly, occupying most of the intracellular space with thylakoids irregularly arranged throughout the cell. The apical cell, on the other hand, continued elongating and dividing, penetrating deeper into the host mainly through the intercellular matrix (Figs 39, 40). These usually uniseriate *A. operculata* germlings constituted the primary invasive filaments. Very little damage of host cells was observed at this stage of infection, and it consisted mainly of changes in cell shape induced by the advancing apical cell (Fig. 40). Organelles, pit plugs, plasmalemma, and immediate cell wall remained unaltered (Fig. 40).

Sometimes, the most superficial cell of the primary filament enlarged to several times its original diameter (Fig. 41), inducing localized damage to the host which affected mainly the cortical cells adjacent to the endophyte. Regardless of the stage of infection at which host-cell damage occurred, during the development of the primary filament of the endophyte or later during maturation, it consisted of an initial disorganization of the cytoplasm, which appeared highly granular, with a swollen ER (Fig. 42). Also the chloroplast displayed uneven reactivity to stains, and its outer double membrane was altered or absent (Fig. 42). The nucleus, still visible, showed accumulation of dark material inside the nuclear membrane, which appeared abnormally blurred, with irregular distribution of nuclear pores (Fig. 42). Although the cells remained surrounded by the immediate cell wall, the plasmalemma was not apparent (Fig. 42). It was common to find osmiophilic deposits in the cytoplasm of cortical cells of

the host facing the endophytic filaments (Fig. 43), together with changes in staining properties of the nuclear membrane, which appeared disrupted. Eventually, the initial changes led to the loss of most internal cellular organization, such that there remained only fragments of disorganized chloroplast and degenerated mitochondria embedded in an amorphous matrix, granular in aspect, and irregularly stained (Fig. 44). No evidence of plasmalemma was observed at this stage (Fig. 44).

If any cellular damage of the host occurred during early infection, it involved only the cells in immediate contact with the endophyte, the other cortical cells remaining structurally unchanged (Fig. 45).

As the primary filament advanced into the host, no compression of the fibrillar intercellular matrix was observed. However, at the interface between the endophyte and the host, a pale area, which appeared to correspond to digested intercellular matrix, was commonly found surrounding the tip of the apical cell in the primary filament (Fig. 46). The presence of this halo at the tip of the apical cell generally coincided with small pocket-like invaginations in the plasmalemma of *A. operculata* filled with electron dense vesicles (Fig. 47). At some distance from the tip of the apical cell, a pale area was not evident at the host-endophyte interface, and the cell wall of the endophyte was in close contact with the intercellular matrix of the host (Fig. 48). A conspicuous cell wall surrounding the endophytic filaments, including apical cells, was present throughout the infection process, so that direct contact between the protoplast of the endophyte and the host cell wall was lacking (see Figs 46, 48).

When primary infective filaments reached the inner cortex, vegetative cells of *A. operculata* enlarged (Fig. 49), became irregularly shaped, and usually branched. These branches ran initially parallel to the surface, through the intercellular matrix perpendicular to the cortical filaments of the host (Fig. 50), and secondary filaments developed from them, some of which grew into medullary tissue of *C. crispus*, while the others grew into the cortex, this time towards the surface of the host. The large, irregular cells remained uninucleated, with numerous mitochondria in the vicinity of the centrally located nucleus, which usually displayed a prominent nucleolus. Abundant starch granules appeared embedded between the thylakoids, which were irregularly arranged in the chloroplast

(Fig. 49). Numerous Golgi complexes were also frequently present in these cells, grouped near the nucleus (Fig. 51). One, generally two, and sometimes three, bilenticular pyrenoids, each surrounded by a pair of hemispherical starch plates were commonly observed (Fig. 52). A single thylakoid ran through the center of the homogeneously granular matrix, and it appeared continuous with the irregularly arranged thylakoids forming the bulk of the chloroplast (Fig. 51).

The secondary filaments colonizing the inner cortex and medulla of *C. crispus* did so through intercellular matrix. As during early penetration of the cortex, endophytic cells contacting subcortical (Figs 53, 54) and medullary cells of *C. crispus* did not induce apparent structural changes in the organelles located in the cytoplasm adjacent to the contact area (Fig. 53) nor in the immediate cell wall (Fig. 54) of the host cells. At this stage, as in the cortex, the host-endophyte interface comprised cell wall components of the two partners (Fig. 54). Endophytic filaments reaching the medulla of the host showed a condensed chloroplast and a slightly irregular profile (Fig. 55), which later developed into the appearance of many small, radial projections (Fig. 56) resulting from transverse sectioning of cell-wall ridges. These ridges were accompanied by cytoplasmic and chloroplast protrusions, and extended along the longitudinal axis of the endophytic filaments. Occasionally, some ridges enlarged which induced mechanical deformation, compression, of the primary cell wall in host medullary cells (Fig. 57).

At the same time the medullary tissue was being infected, secondary filaments of the endophyte began rapid expansion into the cortex of the host. During this stage, numerous host cells suffered severe structural damage (Figs 58-60), which included mechanical disruption of the immediate cell wall (Fig. 58) resulting, in many cases, in endophytic cells penetrating it (Fig. 59). This caused gradual disorganization of the host cell protoplasts, to a point where abnormal chloroplasts were the only structures that remained recognizable (Fig. 60). It is important to consider that direct damage of host cells was accompanied by disruption of the spatial organization of the cortex of *C. crispus*, resulting from the pressure exerted by the endophyte against the cortical filaments and the destruction of pit-connections among cells of the same filament.

In the meantime, cells of *A. operculata* located beneath the OCW enlarged, apparently as an initial expansion of the central vacuole, and protruded from the surface of the host (Fig. 61), resulting in the rupture of the OCW at multiple points in the infected areas (Fig. 60). The apical end of these endophytic cells generally contained a highly condensed chloroplast, with up to three pyrenoids, and their profile became irregular (Fig. 62). Some of these large cells differentiated into sporangia. Initially, a large nucleus adopted a central position, starch granules were displaced to the periphery and the plasmalemma became separated from the thick, bilayered cell wall (Figs 63, 64). No membrane was seen to overlie the cell wall, and only fine cytoplasmic threads remained in contact with it (Fig. 64). The separation between protoplast and cell wall appeared to occur not by condensation of the former, but by active production and extracellular accumulation of an amorphous, electron transparent material. In more advanced stages of maturation, accumulation of the extracellular substance resulted in expansion of the sporangium perimeter leaving the protoplast of the sporangium mother cell suspended (Fig. 65). Eventually, cell division occurred (Fig. 66), producing between 8 and more than 20 cells. Immature zoospores were restricted to the proximal portions of the sporangium (Fig. 66), apparently after the development of a coarsely fibrillar plug at the apex (Fig. 67). The coarse fibrils, embedded in the same amorphous substance as the zoospores, did not extend into the zoospore region (Fig. 67). The development of the zoospores continued, as Golgi complexes produced numerous vesicles that accumulated at the anterior end (Fig. 68). Starch granules were still present, the nucleus was located at the back of the cell, and the anterior end appeared enlarged to form the papilla (Fig 68), which served as insertion point for the flagella (Fig. 69). A uniseriate, immature eyespot could be seen beneath the double membrane of the incompletely developed chloroplast (Fig. 70). At this stage, the zoospores appeared naked (Fig. 70). After zoospores matured, they were released into the medium, leaving large emptied spaces open to the exterior, surrounded by the sporangium cell wall (Fig. 71). These large openings were not repaired, as the surrounding cortical cells of *C. crispus* were severely damaged (Fig. 71) and probably non-functional, as indicated by positive staining with Evans blue of host cells at equivalent infection stages. Gradually, more and more endophytic cells became

sporangia and released zoospores, so creating more unhealed openings. Eventually, the entire cortex of the host collapsed, as it was replaced by endophytic cells of *A. operculata* undergoing rapid differentiation into sporangia. This process left some fragments of OCW intact and a few disintegrated host cell as the only evidence that this was originally cortical tissue of *C. crispus* (Fig. 72).

When the massive production of sporangia by *A. operculata* began, the surface of *C. crispus* did not appear extensively damaged, as only few sporangia had released the zoospores, and most of the OCW was intact (Fig. 73). At this stage, the presence of hairs (up to 200 µm long) characteristic of the genus *Acrochaete* was noted (Fig. 73-77). They usually appeared supported by a lenticular basal cell underneath the OCW (Fig. 74). Organelle and plasmalemma remains could be observed in this degenerating supportive cell (Fig. 75), which rested on a more or less isodiametric, globose cell containing a highly developed chloroplast and a variable number of pyrenoids (Fig. 74). A second type of hair was found, which lacked of a lenticular basal cell, and issued directly from an ordinary vegetative cell (Fig. 76). No cellular remains were observed at the base of these hairs (Fig. 76). Immature hairs, on the other hand, were shorter and showed a swollen, fusiform tip (Fig. 77).

As zoospore release progressed, disruption of the OCW became more extensive, facilitating the entry of bacteria (Fig. 78). Bacteria were common inhabitants of the surface of *C. crispus,* while they were never observed invading healthy tissue. The cortex of *C. crispus* was severely damaged at this point by bacterial digestion of cell wall and cellular remains (Fig. 79). The fibrillar structure of immediate cell walls and intercellular matrix disappeared, and bacteria were seen penetrating decaying host cells (Fig. 79). Cells of *A. operculata,* on the other hand, seemed unaffected by the bacterial activity taking place in their vicinity (Figs 78, 79).

As the cortex collapsed, cellular interactions between *A. operculata* and medullary cells of *C. crispus* became more conspicuous. The most noticeable change was the presence, in host cells, of a plasmalemma interrupted by gradually increasing invaginations (Fig. 80), which eventually filled the otherwise empty cytoplasm (Fig. 81). Alternatively, host cells swelled greatly, leaving only a thin layer of immediate cell wall

between the cell wall of the endophytic filaments and the protoplast of *C. crispus*, which appeared depleted (Figs 82, 84). Occasionally, fragments of disrupted organelles (perhaps mitochondria) were observed at this stage of the infection in the affected cells (Fig. 83). Concurrent with the swelling of host cells, endophytic filaments enlarged (Fig. 82), leading first to ruptures of the host-cell plasmalemma at various points (Fig. 83), and finally to lysis of the medullary cells of *C. crispus*. The cell wall of *A. operculata* at this stage was thick, with at least two layers of different electron opacity (Fig. 84). During the occurrence of these events, bacteria digested their way from the collapsed cortex into the medulla of the host, completing breakdown of the tissue initiated by *A. operculata* (Fig. 85). In spite of the almost complete absence of normal *C. crispus* tissue in the infection site, there was no macroscopic evidence regarding the magnitude of the damage, with the exception of a gradual increase in the softness of the frond.

When *A. operculata* developed outside the host, germination gave rise to a cylindrical filament, of more or less uniform diameter (Fig. 86). As during penetration of the host, a septum separated the emptied zoospore cell wall from the first vegetative cell, which displayed a parietal chloroplast, with a large central space apparently filled by vacuoles (Fig. 87). Structures such as nucleus, Golgi, excretory vesicles, and pyrenoid appeared concentrated at the apical end of the cell (Fig. 88). At this region, the plasmalemma showed numerous pocket-like invaginations (not followed by the cell wall), probably resulting from the fusion with Golgi vesicles that appeared to be migrating towards the periphery of the cell (Fig. 89). The parietal arrangement of the chloroplast, surrounded by a conspicuous darker membrane and containing the typical bilenticular pyrenoid, was easily recognized (Fig. 89). Proceeding towards the posterior end, the pocket-like invagination of the plasmalemma disappeared, and Golgi complexes and secretory vesicles occupied most of the space left by the parietal chloroplast (Fig. 90). Some ER could be seen scattered, surrounding the Golgi complexes (Fig. 90). At the nuclear region, the nucleus was located at the center of the cell, the chloroplast became slightly reduced in comparison to the apical region, and mitochondria and Golgi were present in variable number (Fig. 91). In sections posterior to the nucleus, membranous vacuoles seemed to push the

chloroplast and other organelles against the cell wall (Fig. 92). At the vacuolar and nuclear regions of *A. operculata,* the cell wall was electron dense, usually with only one distinguishable layer (figs 91, 92), whereas towards the tip of the cell, the cell wall was electron translucent, with loose fibrils (Figs 89, 90).

b) Germlings

Penetration of sporophytic germlings of *C. crispus* by *A. operculata* proceeded as infection of the fronds, and was performed only by germinating zoospores attached to the surface of the host (Fig. 93), with vegetative filaments not observed to penetrate. The protoplast of the zoospore migrated into the germination tube through a narrow passage, close to the border of the slightly flattened zoospore cell wall (Fig. 93). As in the infection of fronds, no inward deformations of the OCW were observed during penetration, and when this process finished, the emptied cell wall of the zoospore remained adhering to the surface (Fig. 93). Owing to the reduced intercellular matrix, endophytic filaments soon became contorted, spreading throughout the tissue, and exerted pressure against the immediate cell walls of adjacent host cells (Fig. 94). No cellular damage or structural changes other than light compression of the primary cell wall and minor cell deformations were observed at this stage (Fig. 94). Subsequent stages in the development of the infection of sporophytic germlings were not studied.

3. Infection of gametophytic *C. crispus* by *A. operculata*

a) Fronds

The early stages of penetration, as passage through the multilamellar OCW, was similar to the penetration of sporophytic fronds, with no evidence of mechanical disruption of the cuticle (see SEM of early penetration of gametophytic fronds in Chapter 2). Nevertheless, only a few *A. operculata* germlings gained access into the cortex of *C. crispus* where they developed slowly. During their establishment in the cortex, I did not observe any evidence of digestion of the intercellular matrix of the host at the tips of the advancing apical cells of the endophyte (Fig. 95). Most endophytic filaments were restricted to the cortex, and the few that

gained access into the inner cortex or outer medulla, did so in a limited fashion and always via the intercellular matrix (Fig. 96). At this stage of infection, cell division in the vegetative filaments of *A. operculata* was still active. Owing to the inability of the endophyte to spread further into the host, those filaments soon became contorted, with the surface of the cells, particularly those in the inner cortex, consisting of numerous projections of the cell wall and cytoplasm (Fig. 97). No structural modification of the intercellular matrix surrounding these cells was observed (Fig. 97). In the upper cortex, failure of the infecting germlings to invade nearby host tissues through the intercellular matrix led to the apical cells of endophytic filaments to break through the immediate cell wall of cortical cells (Figs 98, 99). This occurred mainly at the periphery of the infection site, which at this stage had developed into a nodule of green filaments circumscribed by morphologically normal host tissue (see Chapter 2). In some instances the affected host cells appeared to be cut in half by the endophyte (Fig. 98), whereas in others, they became compressed against the remaining immediate cell wall and adjacent cells (Fig. 99). Typically, however, host-cell damage was not extensive, as mainly those cells directly in contact with the endophytic apical cells suffered lysis (Figs 98, 99). As growth of the endophyte continued, the superficial cells of *A. operculata* enlarged, resulting in additional damage to host cells caused by compression (Fig. 100), and eventually, in disruption of the OCW (Fig. 101). At this stage, most of those enlarged cells underwent degenerative changes, lysis, opening of the apical end and evacuation of cytoplasmic remains (Fig. 101). As subjacent cells of *A. operculata* pushed their way towards the disrupted OCW, the infection nodule protruded from the surface of the frond to form a papule (Fig. 102). Although most of the papule surface retained a continuous OCW (Figs 101, 102), the uppermost region appeared open (Fig. 103), as a result of the cytoplasmic evacuation of abortive cells of *A. operculata*..

At the center of the infection nodule, below the superficial cells undergoing degenerative changes, the enlarged endophytic cells came in close contact with each other, and the cytoplasm in most of them became vacuolated, or replete with starch granules, or both (Fig. 104). Structurally normal (Fig. 104) and Evans blue negative host cells surrounded the lesion. Only few isolated relicts of *C. crispus* tissue,

showing signs of severe cellular damage, were observed within the lesion (Fig. 105). Cells of *A. operculata*, also in advanced stages of deterioration and recognizable by the pyrenoid and remains of starch granules, were not uncommon (Fig. 106). Finally, cell destruction and probably the concurrent release of lytic enzymes, appeared to produce areas of digested tissue (Fig. 107). In addition to the highly localized cellular damage caused in *C. crispus*, it took *A. operculata* at least four months to reach this stage of development in the gametophytic fronds of the host.

Sometimes, superficially located cells of *A. operculata*, rather than becoming abortive, underwent differentiation into sporangia, and these followed the same sequence of stages described for sporangia formation in sporophytic *C. crispus*. The release of zoospores did not appear to facilitate subsequent bacterial infection. All tissue and cellular degenerative processes described above developed in the absence of secondary bacterial infection.

b) Germlings

Penetration of gametophytic germlings followed the pattern described for sporophytic germlings, although the density of infecting *A. operculata* successfully developing into the host was low. As in the sporophytic phase, the evolution of the infection into later stages was not monitored.

4. Infection of gametophytic and sporophytic *C. crispus* by *A. heteroclada*.

a) Fronds

No differences were noticed between the development of the infection in gametophytic and sporophytic *C. crispus* fronds. Therefore the description of the different stages is applicable to both life-history phases of the host. Infection began, as in *A. operculata*, by settlement of zoospores on the surface of the fronds. Swarmers were pyriform, 4-5.5 x 3.5-4.5 µm, with an anterior papilla and usually 4 (Fig. 108), but sometimes 2 or 6 (Fig. 109) flagella, 2.5-3.5 times the length of the cell. The terminal end of these flagella appeared to decrease gradually in diameter (Fig. 109). After zoospores attached to the OCW, unipolar germination occurred, resulting in an elongated germling. If the cytoplasm

distributed homogeneously during elongation, this was followed by the development of a medial transverse septum, which resulted in a two-cell germling. Alternatively, the cytoplasm migrated along with the newly produced cell wall, resulting in a one-cell germling after a septum developed between the emptied cell wall of the zoospore and the evacuated cytoplasm. At this stage, the elongated germlings rested on the outermost lamella of the OCW, with no evidence of penetration (Fig. 110). Cells were about 3-4 μm in diameter and 2 to 4 times as long, with a parietal chloroplast, discontinuous at the host-side of the filament, which contained regularly arranged thylakoids, one of them passing through a bilenticular pyrenoid partially surrounded by two hemispherical starch plates (Fig. 110). The chloroplast was delimited by a continuous double membrane (Fig. 110). The nucleus was located in the host-side of the cell, and its double membrane appeared periodically interrupted by pores (Fig. 110). At the border of the pores, the nuclear envelope became continuous with ER, which appeared closely associated to mitochondria (Fig. 110). The rest of the cytoplasm presented a densely granular texture owing to the large number of ribosomes present (Fig. 110). There was a clearly distinguishable cell wall surrounding *A. heteroclada* germlings, including the area of contact with the lamellar OCW of the host (Fig. 110). The inner cell wall of the endophyte consisted of an irregularly distributed electron dense, more or less granular material, which was replaced outside by a homogeneous, finely fibrillar layer, that was not apparent at the host-endophyte interface (Fig. 110).

Penetration of the host began shortly after germination had concluded, when the tip of an apical cell of *A. heteroclada* changed direction of its elongation axis from parallel to the surface to more or less perpendicular, pushing the lamella inwards (Fig. 111). It appeared that elasticity of the lamella was poor, as minor deformations caused by the infecting germlings resulted in initial fractures (Fig. 111) that facilitated subsequent access to more basal lamellae, and eventually, to the homogeneous portion of the OCW (Fig. 112) which provided a more stable substratum. After the passage through the multilamellar OCW, the filamentous germlings of *A. heteroclada* usually were depleted of chloroplasts and other organelles, which seemed to remain localized at the terminal end of the advancing cells (Fig. 112). The lumen of these

apparently emptied filaments was, however, delimited by plasmalemma (Fig. 113).

Once the penetrating germlings reached the homogeneous OCW, usually within a week after inoculation, endophytic filaments began rapidly branching, although they remained restricted to the superficial region of the host thallus. This stage was characterized by a rapid colonization of extensive areas of the host, always through the amorphous OCW. While embedded in the OCW, *A. heteroclada* did not induce fine structural changes in the subjacent cortical cells of *C. crispus*, nor in their primary cell wall (Fig. 114). Concurrent with the expansion of the infected area, the endophyte became polystromatic in the older portion of the infection, and by the end of the third week of incubation, penetration into the cortex of *C. crispus* by cells which originated at the basal stratum of *A. heteroclada* began (Fig. 115). Most of the organelles in the invasive cells were displaced to the proximal end, leaving a vacuolated apical region (Fig. 115), before cell division began. The first row of host cells in the vicinity of the invasive filaments underwent rapid, fine-structural changes, similar to those already described during infection of *C. crispus* by *A. operculata*.. This resulted in cytoplasmic disorganization and disruption of cell organelles, with no intracellular components observed (Fig. 115).

As penetration of the cortex of *C. crispus* proceeded, more filaments invaded the host, mainly through the intercellular matrix (Fig. 116). It should be noted that branching of these filaments was rare. Penetration then resulted in massive damage of cortical cells of the host throughout the infected areas (Fig. 116). At this stage two types of *A. heteroclada* cells were observed in the cortex of the host. The subapical cells were cylindrical, 1.5-6 x 15-20 μm, and displayed poorly recognizable organelles, particularly the chloroplast (Fig. 116). The tip-rounded apical cells of *A. heteroclada*, on the other hand, were relatively short, with parietal, cup-shaped chloroplast and nucleus localized at the proximal end (Fig. 116), leaving the distal half of the cell occupied by membranous vesicles of various shapes and sizes (Fig. 117). Some of these vesicles were filled with electron dense granules (Figs 117, 118), and appeared to fuse with the plasmalemma at the tip (Fig. 117). A continuous cell wall surrounded the endophytic filaments throughout the infection process, and no apparent differences were noticed in this respect at the tip of the apical,

penetrating cells (Figs 117-119). The granules seemed to migrate towards the cell wall of the apical cell, with which they fused (Fig. 118). During penetration of the cortex, no evidence of deformation of the fine fibrillar structure of the intercellular matrix, indicating mechanical force applied against it, was observed. To the contrary, it was common to find, at the tip of the apical cells, a discrete, narrow zone of intercellular matrix where the microfibrils appeared to disintegrate (Fig. 118). Furthermore, dissolution of the microfibrils was associated with highly electron dense granules (sometimes rod-like structures), located at the surface of the apical cell wall of the endophytic filaments, and at some distance from the endophyte, embedded in the host intercellular matrix (Fig. 118). In the latter situation, a halo or a path of digested intercellular matrix indicated that these granules were moving away (but in the same direction as) from the apical cell of the endophyte (Fig. 118). Artifactual separation of the plasmalemma from the cell wall in the endophytic cells was sometimes observed, probably due to an uneven action of the fixative solutions (Figs 117, 123).

As apical cells of *A. heteroclada* penetrated deeper into the cortex, more host cells underwent lysis following disruption of the plasmalemma (Fig. 119). These cells, however, still remained surrounded by their immediate cell walls. Finally, endophytic filaments of *A. heteroclada* took over the cortex, replacing the decaying host cells. At this stage the "cortex", which was mainly composed of *A. heteroclada* cells, became separated from the medullary tissue. The latter was soon colonized and degraded by bacteria.

Concurrent with the changes taking place in the cortex of *C. crispus*, the OCW of the host had disappeared, replaced by the actively growing superficial filaments of *A. heteroclada*, which at this stage had developed into more or less cushion-like plants. Later, the terminal, slightly tapered cells of these filaments became sporangial mother cells (Figs 120-122). The apical chloroplast was cup-shaped with numerous starch granules (Fig. 120), and up to five pyrenoids. The remaining space was occupied by a basal nucleus, a membranous center and Golgi complexes located above the nucleus (Fig. 121). At the center of the area delimited by the Golgi complexes, ER developed profusely (Fig. 122). Mitochondria were also abundant throughout the cell. Cells located below the future sporangia, at

the center of the cushion-like plants, did not differ markedly from the apical, invasive cells, except for their isodiametric to short-cylindrical shape. The chloroplast was apical, as were most organelles, including the nucleus, whereas the basal portion of the cell was mainly occupied by membranous vesicles (Fig. 123). Thick cell walls coalesced with those of adjacent cells, preventing the passage of bacteria into the cortex of *C. crispus*. In addition to the characteristic position of the nucleus (basal), the sporangium-mother cell differed from the subjacent cells by lacking a noticeable vacuole (Fig. 120).

DISCUSSION

The plant of *C. crispus* is a chemically and morphologically complex entity, at both the cellular and tissue levels. At least two parts of the thallus can be visualized as potentially important elements involved in the colonization of the host by either *A. operculata* or *A. heteroclada,* the two endophytes considered in the present study.

The first is the outer cell wall (OCW), which was found to vary from a single, homogeneous layer of fibrillar material at the holdfast and stipe, to a composite structure at the apices, with a multilamellar stratum superimposed on the homogeneous layer present in the basal region of the plant.

There have been numerous, fine structural studies where the OCW of various algae has been illustrated (Bisalputra *et al.* 1967, Hanic and Craigie 1969, Scott and Dixon 1973, Borowitzka and Larkum 1977, McArthur and Moss 1977, Borowitzka and Vesk 1978, Pedersén *et al.* 1979, 1981, Pueschel 1979, Hawkes 1980, Moss 1982, Cole *et al.* 1985, Mariani *et al.* 1985, among others), including *C. crispus* (Cottler 1971, Gordon and McCandless 1973, Pedersén *et al.* 1980, Tveter-Gallagher and Mathieson 1980) and its close relatives *Iridaea flaccida* (S & G) Silva, *I. cordata* var. *cordata* (Turn.) Bory, and *I. cordata* var. *splendens* (S & G) Abbott (Gerwick and Lang 1977), and *Gigartina teedii* (Roth) Lamour. (Tsekos and Schnepf 1983). Only two of these studies, however, focussed specifically on both the chemical and morphological structure of the OCW of red algae (Hanic and Craigie 1969, Gerwick and Lang 1977), and where the term "cuticle" was applied to the lamellar portion of the OCW. Although morphologically similar to that of higher plants (Martin and Juniper 1970), there are important biochemical differences between the cuticle in higher plants (reviewed by Kolattukudy 1984) and algae (Hanic and Craigie 1969, Gerwick and Lang 1977). The cuticle in higher plants consists mainly of the complex, structural fatty acid cutin which is embedded in a matrix of waxes (Kolattukudy 1984), whereas a proteinaceous (possibly conjugated with carbohydrates) composition has been found in algal cuticles, either by *in situ* histo- and cytochemical analysis (Pueschel 1979, Diannelides and Kristen 1988, Cole *et al.* 1985,

Tsekos and Schnepf 1983), or by characterization of the lamellar composition following chemical or mechanical isolation (Hanic and Craigie 1969, Gerwick and Lang 1977). Green fluorescence of the cuticle of *C. crispus* suggests that, as in other algae, the lamellar portion of the OCW in this species is proteinaceous (Gordon and McCandless 1973). This agrees with information reporting 16.3% N in H_2SO_4-extracted *C. crispus* cuticle, with proline as the principal aminoacid (Buggeln and Craigie 1973). Recently, the proteinaceous nature of this structure was confirmed by infra-red analysis (J.S. Craigie in verb.) using highly purified preparations of *C. crispus* cuticle isolated after its normal release from the thallus.

The homogeneous portion of the OCW in *C. crispus*, exposed to the environment in the holdfasts, stipes and part of the central portion of the fronds, displays a relatively uniform arrangement of microfibrils. There is little direct information regarding the chemical composition of this layer, although it gives a positive stain with Toluidine blue and the periodic acid-Schiff's reaction (PAS) (Figs 18 and 25 in Gordon and McCandless 1973). These reactions indicate the presence of polysaccharides, which is also supported by the birefringence pattern under polarizing light (Gordon-Mills and McCandless 1977).

The second component of the *C. crispus* thallus that is likely to play a role during the process of infection by either *A. heteroclada* or *A. operculata* is the intercellular matrix. The two endophytes clearly develop into the host mainly via intercellular space (Correa *et al.* 1988, this Chapter). The microfibrillar fine structure of the intercellular matrix remains more or less consistent throughout the plant, and in many cases there are no major structural differences with the microfibrils from the immediate cell wall. These observations are in general agreement with the fine structural information available for *C. crispus* (Gordon and McCandless 1973, McCandless *et al.* 1977). In the holdfasts and discoid germlings, however, the microfibrils appeared more compacted, resulting in a highly electron dense intercellular matrix. Similar observations were made on *C. crispus* germlings studied by Tveter-Gallagher and Mathieson (1980). Perhaps the most noticeable feature regarding the intercellular matrix was its relative poor development in holdfasts, discoid germlings, and cortex of the frond. This contrasts with the medulla, where the cells

are widely separated by large amounts of intercellular matrix. These features remain regardless of the life history phase of *C. crispus*. However, in spite of fine structural similarities in the intercellular matrix of sporophytes and gametophytes, there are important chemical differences. It is now accepted that the gametophytic generation produces carrageenan of the kappa family while the sporophytic generation produces carrageenan of the lambda family (McCandless *et al.* 1973) following the pattern of generation-determined carrageenan type in species of the Gigartinaceae (McCandless *et al.* 1983) and Phyllophoraceae (McCandless *et al.* 1982). Efforts to localize the carrageenan *in situ* using histochemistry and immunohistochemistry indicate that both kappa and lambda carrageenan, and their precursors, occur in the intercellular matrix and immediate cell wall (Gordon-Mills and McCandless 1975, 1977). Further support comes from fine structural observations of both intercellular matrix and primary cell wall before and after incubation in hot $NaHCO_3$ (McCandless *et al.* 1977), a standard procedure used for carrageenan extraction (Craigie and Leigh 1978). It was shown that after extraction, the microfibrillar structure of the intercellular matrix and immediate cell walls became highly disorganized, and microfibrils were reduced in density. In the same study (McCandless *et al.* 1977), birefringence was lessened in the immediate cell walls and eliminated from the intercellular matrix after extraction with hot aqueous bicarbonate. This process also eliminated the signal for sulfur by removing the sulfated polysaccharide carrageenan, as detected by energy-dispersive x-ray analysis (EDX) in non treated samples.

The question of whether the microfibrils, as observed under the TEM, are the morphological representation of the different types of carrageenans remains equivocal, although there are no other apparent alternatives (reviewed by McCandless 1981, Craigie 1990b). Cellulose, for example, represents less than 1% of the dry weight of *C. crispus* (Young 1966), whereas the carrageenan fraction may comprise up to 70 % of the alcohol-extracted dry weight of the fronds (Craigie and Leigh 1978). Using EDX, McCandless *et al.* (1977) found a gradient of sulfur from the innermost area of the immediate cell wall to the intercellular matrix, correlating with a similar gradient of density and concentration of microfibrils as observed at the TEM. This, according to McCandless *et al.*

(1977), was further support for the hypothesis that carrageenans and microfibrils are closely associated .

It is apparent that the OCW, intercellular matrix and eventually the immediate cell wall represent a highly diverse array of barriers for any invasive, alien organism. In the coming discussion, I address the possible mechanisms developed in *A. operculata* and *A. heteroclada* to cross these barriers. This results in successful development and reproduction of *A. operculata* in the susceptible host, the sporophytic phase of *C. crispus,* but generally in a failure of the process in the gametophytic phase of the same host. Cellular interactions in the highly host-specific *C. crispus-A. operculata* association are then compared with those taking place between *C. crispus* and the host-generalist *A. heteroclada.*

The first contact between *A. operculata* and *C. crispus* takes place at the surface of the OCW of the host. Zoospores distributed regularly on the surface of *C. crispus* fragments, whether from the stipe, central frond or apical frond, indicate that no selection of settling site is involved. Attachment to the host probably occurred by the release of adhesive material stored in vesicles concentrated at the flagellar end of the zoospores before release. A similar mechanism has been documented for a number of green and brown motile algal spores (Evans and Christie 1970, Bråten 1975, Henry and Cole 1982).

In general, infecting organisms can germinate on the surface of the host, and "search" for an aperture or a weak point to achieve penetration. Such a mechanism has been described for some rhodophycean endophytes (White and Boney 1969), and is characteristic of fungal pathogens that penetrate their host through natural openings, such as stomata (Wynn 1976, Staples and Macko 1980, Wynn 1981, Wynn and Staples 1981, Staples *et al.* 1985, Hoch and Staples 1987, Hoch *et. al.* 1987, Staples and Hoch 1987). Alternatively, spores may germinate directly through the cuticle, which is indeed the route followed by *A. operculata.* As revealed by SEM observations during penetration of the OCW, most zoospores penetrate the host directly, during germination. Furthermore, it appears that a successful infection *requires* penetration during germination and that penetration has to proceed *through* the OCW. In fact, germinated zoospores and large plants of *A. operculata* are unable to infect the host (Correa *et al.* 1988), even after being mechanically inserted into *C. crispus*

fronds, where they remain until the host heals the opening, killing the filaments (unpubl. inf.). The reason for this is not known, but there may be some physiological (*e.g.* enzyme production) induction in *A. operculata* zoospores which might be triggered *only* after recognition of the appropriate substratum. On the other hand, some rhodophycean parasites, like *Harveyella mirabilis,* appear to require the disruption of the OCW by grazers to gain access into their host (Goff 1976, 1982a).

The process of penetration of the OCW by *A. operculata* could be mechanical or enzymatic. The morphological evidence gathered in this study suggests that enzymes might be involved. This was indicated by marked disorganization of the internal, homogeneous layer of the OCW surrounding penetrating *A. operculata* germlings, which sometimes culminated with formation of digested "caverns". When the lamellar portion of the OCW is involved, there is no indication of inward bending or tearing apart of this structure, as expected if it were a mechanical penetration (Politis and Wheeler 1973, Jordan *et. al.* 1988). On the contrary, penetration seemed to induce no deformation, which is clearly perceived at the contact point between lamellae and endophyte, where the former structure ends abruptly, and by the sharp clean holes observed at the SEM, similar to those of fungal pathogens known to penetrate their hosts enzymatically (reviewed by Cooper 1981).

The OCW, particularly the multilamellar cuticle, seems to be the only structure that may have some effect on diminishing infection by *A. operculata* germlings. The continuous peeling of the lamellae will eliminate individual endophytes that have not reached the more stable cortex (see below). Once the cortex has been reached, however, infection proceeds, and no mechanism seems to have evolved in the sporophytic phase of *C. crispus* to prevent further colonization of the tissues. Quite differently, early stages of fungal pathogen invasion in higher plants are characterized by various structural changes which take place in the tissues of non susceptible hosts. Perhaps the most common is the formation of a papilla beneath the fungal appressorium (Aist 1976, 1983; Aist and Israel 1977, Cooper 1981, Sherwood and Vance 1982, Hargreaves and Keon 1986, Aist and Gold 1987). In some cases, rapid, massive but highly localized cell death occurs at the site of infection and is known as hypersensitive response (Goodman *et al.* 1986). The phenomenon of

lignification is also reportedly associated with infecting fungal germlings (Sherwood and Vance 1981). The apparent absence of any structure, produced by sporophytic *C. crispus* in response to infecting *A. operculata* during penetration or subsequent colonization of adjacent tissues, supports the contention that this phase of the host is highly susceptible to *A. operculata.* Furthermore, this susceptibility is not restricted to certain areas of the plant, as was first apparent in field-collected plants of *C. crispus* (Correa *et al.* 1987). This has two important implications. First, we should be looking for features other than those which are host-constitutive to explain the absence of infection in holdfasts from wild *C. crispus.* Second, although for other potential infecting organisms the multilamellar cuticle may represent a non-specific barrier, it does not interfere noticeably with the process of infection by *A. operculata.* The differential distribution of the cuticle along the frond, or fine structure variations between phases (see below), was not correlated with any particular susceptibility pattern in the host.

The sequence of events leading to the completion of the infection cycle by *A. operculata,* characterized by reproduction of the endophyte, begins with a rapid penetration of the cortex by the primary infective filaments. When the endophyte reaches the inner cortex, it grows mainly parallel to the surface, that is perpendicular to the cortical filaments of the host. It is at this stage that the actual spreading of the infection into adjacent tissues starts. What determines this infection pattern is not known. It certainly could represent a prefixed route determined either by areas of the host wall offering less resistance to the passage of the invasive cells, as might occur during infection by some rhodophycean endophytes and penetrating rhizoids of algal epiphytes (White and Boney 1969, Rawlence and Taylor 1972, Garbary 1979b, Gonzalez and Goff 1989). Alternatively, it could represent a more subtle and precise matching between enzymes produced by *A. operculata,* and wall components of the host. So far there is no evidence indicating that the intercellular matrix of *C. crispus* is weaker than the immediate cell walls. There is, however, morphological evidence showing disorganization or absence of microfibrils from localized areas of the intercellular matrix, at the tip of the advancing endophytic filaments. This, together with the absence of any indication of microfibril compression at those same points, strongly suggests that

enzymatic degradation of this fraction of the host cell wall is taking place. In spite of repeated suggestions that enzymatic digestion of host cell walls occurs in a number of algal associations (Rawlence 1972, Rawlence and Taylor 1972, Nonomura 1979, Goff and Cole 1976, Goff 1982a, Gonzalez and Goff 1989), no direct documentation is available. The presence of cell-wall degrading enzymes by fungal pathogens and their potential role in pathogenesis is well documented (Cooper 1983, Collmer and Keen 1986, Goodman *et al.* 1986, Keon *et al.* 1987). It appears that some correspondence exists between the images produced by certain biotrophic fungi and *A. operculata* during the penetration of the intercellular matrix of their respective hosts (Cooper 1981). In fungi, enzymes involved in breaking down the host cell wall seem to be localized in vesicles at the tip of the advancing hypha, as indicated by cytochemical studies (Hislop *et al.* 1974). Although no such vesicles were visualized at the tip of advancing apical cells of *A. operculata,* the pocket-like invaginations of the plasmalemma, consistently found at those areas, may represent points where exocytosis of material, including enzymes, could take place. Interestingly, immediate cell walls of the cortical cells were not breached at this stage of infection (uninfected intercellular matrix still being abundant). Later, disruption occurs after clear deformation and compression of the host microfibrils at the tip of the endophytic filament, with no indication of fibrillar digestion. A similar phenomenon was observed in the medulla of *C. crispus*. Although no deformation of the fibrillar component of the intercellular matrix surrounding the endophytic filaments was noted, obvious deformation and compression of fibrils in the immediate cell walls was apparent. Thus, if the hypothesis of enzymatic digestion of the host cell wall is correct, this may result from a heterogeneous distribution of lambda carrageenan and its precursors within the cell wall of the host. Immunologic and histochemical studies in *C. crispus* have indicated that this is indeed the case, lambda carrageenan being mainly restricted to the immediate cell walls throughout the frond, and to the intercellular matrix in the cortex (Gordon-Mills and McCandless 1975). The persistence of some immediate cell wall material after the tissue has been exposed to hot aqueous bicarbonate (McCandless *et al.* 1977), a treatment that extracts carrageenans (Craigie and Leigh 1978), indicates the presence of a highly resistant component, perhaps other than carrageenan. This also may

explain the relatively late and apparently non enzymatic disruption of the immediate cell wall of *C. crispus* by *A. operculata.*

Up to the lateral subcortical spreading of infection, cellular damage of the host was minor and affected mainly those cells in direct contact with the endophyte. The OCW, including the lamellar structure, was still in place. As soon as new filaments issued by the subcortical endophytic cells of *A. operculata* began to grow towards the surface, host cell damage became more extensive. The cause of cell death is still unclear, although it is probably the result of various factors, such as compression, reduced nutrient availability by competition with endophytic cells, and in later stages, lytic enzymes released from adjacent, lysed cells. It is at this stage that penetration of the immediate cell wall occurs more frequently, resulting in closer contact between the cell wall of the endophyte and plasmalemma of the cortical cells of *C. crispus.* These cellular interactions, however, involved highly deteriorated, dead host cells, clearly resulting from a rapidly-growing endophyte in surroundings where "suitable space" in the intercellular matrix to grow, had become limited. Consequently, although some degree of heterotrophy can not be completely ruled out (Linskens 1963, Ducker and Knox 1984), it seems unlikely that penetration of host cells is its morphological expression. In biotrophic associations between fungi and higher plants, penetration of host cells and the development of haustoria to obtain nutrients occur early during the infection, and the host cells involved remain structurally intact and functional (Goodman *et al.* 1986). To the contrary, penetration of the immediate cell wall of host cells by *A. operculata*, and the establishment of a closer contact with the host plasmalemma, occurs very late in the infection cycle, just prior to reproduction of the endophyte. Furthermore, host cells appear to be already undergoing degradative changes *before* penetration by the endophytes, suggesting that little, if any, metabolic gain is obtained by the endophyte once penetration is achieved. In this regard, it has been demonstrated by Correa (1986) and Correa *et al.* (1988) that *A. operculata* and various other species of pigmented algal endophytes are able to grow and reproduce outside *C. crispus.* Therefore, it is unlikely that the host represents an essential nutrient source for them.

At the onset of the endophyte reproduction, host damage caused directly by *A. operculata* reached its maximum. Most of the outer cortical

cells became severely distorted, in advanced stages of degeneration, and nonfunctional (see Chapter 3). This resulted in a cessation of cuticle formation, which in turn led to accumulation of bacteria on the surface, as a prelude to the release of zoospores. After the zoospores are released, emptied sporangia walls in the cortex tend to collapse, together with surrounding decaying host cortical tissue. As no healing of this wounds takes place, bacteria invade the host. Bacteria appear to be much more destructive than *A. operculata*, and they rapidly digest cells wall and cell remains. However, they were unable to gain access into the fronds until disruption of the surface was accompanied by severe damage of the cortex, which indicates that bacteria not only need an opening, but also decaying, non functional host cells, in order to colonize the inner tissues of *C. crispus*. This agrees with observations indicating that bacterial infection of unialgal *C. crispus* does not normally occur in culture, even though bacteria are normal contaminants, and in spite of the large areas of both cortex and medulla exposed during cutting to propagate these fronds. Further support of the hypothesis that bacteria may be mainly restricted to secondary infections, following primary infection by algal endophytes, comes from field-collected fronds of *C. crispus*. In this material I have seldom seen evidence of bacterial damage without the concurrence of algal endophytes, although heavy endophytic infections with no evidence of bacterial degradation of the fronds are common.

The developmental pattern of *A. operculata* in the sporophytic fronds of *C. crispus* is highly conservative regardless of conditions of culture. The consistency of this pattern extends to wild fronds. There may be various advantages to the endophyte, the most evident perhaps occurring during the critical stage of reproduction. *A. operculata* sporangia, both in nature and culture, develop embedded in the cortex of the host. The endophytic development of sporangia ensures complete maturation of the zoospores before release. This prevents exposure to the environment of this extremely fragile structure, particularly in the intertidal range of *A. operculata*. Desiccation, salinity, abrasion and grazing, among other factors, are likely to operate with higher intensity at the surface of the fronds. The fragility of the sporangia and the potential role of the host thallus in lessening the impact of external stimuli are apparent when sonication is used. Sporangia of plants growing in unialgal culture, or

protruding from the surface of the host are rapidly lysed by even very short exposures (30-sec bursts of 50 kc/s), whereas sporangia inside the thallus resist 4 or more min with no apparent damage (unpubl. inf.).

The development of the infection in gametophytic fronds of *C. crispus* differed greatly from that in sporophytic fronds, and was in agreement with the preliminary account provided by Correa *et al.* (1988). However, as in sporophytic fronds, the infection was initiated by zoospores, which after making contact with and settling on the OCW penetrated in a similar manner to penetration of the sporophytic counterpart.

No evidence of tearing or inward bending of the lamellar cuticle was observed during penetration of the gametophytes. This points to enzymatic rather than mechanical penetration, and suggests that the chemical composition of this structure is similar in the two generations of *C. crispus*. Owing to the larger number of lamellae found in the gametophytic cuticle, it seems likely that fewer *A. operculata* germlings will reach the cortex of the host before being discarded together with older lamellae. In spite of this potential resistance of the cuticle to penetration, no impact on the final outcome of the infection appears related to it. This was evident as those germlings reaching the cortex of the host developed following a pattern leading to a highly localized lesion, rather than spreading throughout the frond, as in the sporophytic hosts.

Colonization of the gametophytic fronds by *A. operculata* is initially via the intercellular matrix, with minor mechanical damage by compression of the immediately adjacent host cells. However, unlike the rapid invasion of the cortex followed by extensive spreading into neighboring tissues observed in sporophytic fronds, the advance of endophytic apical cells into deeper areas of the cortex in gametophytic fronds soon slows down greatly. Continuous cell division, on the other hand, together with abnormally early branching in the endophytic filaments, results in an accumulation of *A. operculata* cells in a nodule made of atypical contorted and enlarged vegetative cells, which compressed otherwise normal host tissue. This impasse in the development of the infection may last years, with no noticeable changes in either partner, as occurred when fronds infected at this stage were transferred to slow-growing conditions. If growth is stimulated, the endophytic cells in the nodule push their way

towards the surface, eventually disrupting the OCW, rather than spreading throughout the host.

The formation of a papule in gametophytic fronds infected by *A. operculata* resembles the early stages of gall formation induced by either bacteria (Tsekos 1982, Apt and Gibor 1989) or *Streblonema* (Apt 1988a). However, unlike to gall development, no hyperplasia takes place in the cortex of *C. crispus,* the bulk of the papule consisting of *A. operculata* cells. It is then clear that the inability of *A. operculata* to spread in gametophytic fronds is not mediated by host cellular responses. In this respect, the extrusion of the endophyte during the late stages of papule development is similar to the disruption of the surface of *Undaria* caused by *S. aecidioides* (Yoshida and Akiyama 1979).

The pattern of development of *A. operculata* in gametophytic fronds of *C. crispus* strongly indicates incompatibility between the two partners. Although it is not clear at what level this incompatibility is operating, one can speculate that a set of enzymes may have evolved in *A. operculata,* allowing breaking down of cell walls of the sporophytic, but not those of the gametophytic phase of *C. crispus.* In addition to the observation that early arrest of the infection process occurs at the intercellular matrix level (but not OCW level), at least two other lines of evidence support this hypothesis. First, I found no clear indication of digestion of the intercellular matrix at the advancing tip of endophytic filaments when growing in gametophytic fronds, although morphological evidence suggesting cell wall degradation exists for infected sporophytes. Second, there are no fine structural differences between the two phases, either preformed or induced, to which the observed differences in behavior of *A. operculata* germlings can be attributed.

A passive resistance, resulting from the inability of *A. operculata* to digest the cell walls of gametophytic *C. crispus,* seems to be the most supportable hypothesis. However, an active response of the host leading to resistance can not be absolutely ruled out. This alternative hypothesis comes from fine structural observations on a considerable number of *A. operculata* cells which become abortive, and eventually disintegrate, while in the papule, perhaps as the result of host products specifically released. This would agree with the present understanding of some incompatible associations between fungal pathogens and their hosts plants, where the

latter respond by producing, upon recognition, enzymes that breakdown fungal cell walls, a process that stops fungal colonization (Benhamou *et al.* 1989, 1990; review by Boller 1987)

In spite of differences in development of infection by *A. operculata* in sporophytic and gametophytic fronds of *C. crispus*, cellular changes were similar in the two phases of the host. Comparison at the cellular level with other similar associations is not possible. To my knowledge, this is the first fine structural account of the infection of an algal host by a pigmented algal endophyte. There have, however, been reports describing cellular changes in algal hosts during the establishment of parasitic red algae. Cellular changes in those parasitic associations usually consist of disorganization in the structure of the organelles, and they are rarely accompanied by disruption and plasmolysis (Kugrens and West 1973, Goff 1976, 1982a). Some cellular changes described in those parasitic associations were also present in *C. crispus* cells. The accumulation of osmiophilic material at the endophyte side of host cortical cells was similar to that observed in *O. floccosa* and identified as phospholipids (Goff 1976). Vesiculation of medullary cells of infected *C. crispus* also appeared as in medullary cells of infected *O. floccosa* (Goff 1976). The presence of vesicles in the cytoplasm of various types of cells seems to be a common response to stress situations. This phenomenon has been reported during cell fusion of incompatible isolates of *Griffithsia pacifica* Kylin (Koslowsky and Waaland 1987), and in incompatibility reactions in fungi (Lane and Carlile 1979) and higher plants (Moore and Walker 1983). However, in spite of the morphological similarities, the origin of the vesicles differs, as the outcome for those cells involved. In medullary cells of sporophytic *C. crispus*, vesicles seem to be associated with changes in the plasmalemma, as in *O. floccosa* (Goff 1976), and appear shortly before cell collapse. In the latter species, however, vesiculation does not seem to precede cell lysis (Goff 1976). In *G. pacifica*, Koslowsky and Waaland (1987) suggested that the vesicles may have resulted from chloroplast disruption, and although vesiculation was extensive, subsequent cell death did not occur. In heterografts of *Solanum* and *Sedum*, on the other hand, vesicles were associated with the dilation of ER in *Sedum* cells, which eventually died (Moore and Walker 1983).

There are some similarities in host cell changes between the *A. operculata-C. crispus* and *P. lanosa-A. nodosum* associations. Although most of *P. lanosa* thallus develops as an epiphyte, penetrating rhizoids are highly destructive during invasion of *A. nodusum*. Host cells became severely deformed by compression, which lead first to ER disruption and changes in contrast of mitochondria and chloroplast membranes and finally to the complete digestion and lysis of *A. nodosum* cells surrounding the rhizoid (Rawlence 1972). The main difference between these two systems is that, while rhizoidal penetration of *A. nodosum* is localized, resulting in an overall minor destruction of host tissue, endophytic filaments of *A. operculata* spread throughout the frond, inducing decay in large areas of the host.

The nutritional dependence of algal parasites upon their hosts has probably led to the evolution of mechanisms, in the parasite, that prevent or minimize host cell damage (Goff 1976, 1982a). Features like small size, endophytic portion of the thallus restricted to the intercellular matrix of the host and reproductive thallus developed outside the host in gall-like structures, may be interpreted as parasitic adaptations to minimize physical damage of the host at both tissue and cellular level. Furthermore, transfer of material from the host to the parasite seems negligible in terms of the host carbon budget (Kremer 1983). On the other hand, as indicated above, there seems to be no dependence of *A. operculata* upon nutrients from *C. crispus*, and there is certainly no morphological nor developmental evidence that suggests adaptations of the endophyte to minimize host damage. On the contrary, gradual deterioration of host cells induced by *A. operculata* resembles damage to algal hosts caused by pathogenic bacteria (McBride *et al.* 1974) or fungi (Kazama and Fuller 1970). The degenerative changes occurring in cells of fronds infected by *A. operculata*, which lead to cellular death and host dysfunction as demonstrated by poor growth and regeneration performance (Chapter 3), together with large areas of the host being affected, strongly indicate that this endophyte is a potential pathogen of the sporophytic phase of *C. crispus*. Whether or not the pathogenicity of this endophytic species will manifest in nature is still equivocal, most of the evidence being circumstantial. Some developmental features of *C. crispus* (McLachlan *et al.* 1989) may diminish the effect of the infection, as heavily infected

fronds, if destroyed, will be replaced by new ones issued by the holdfast. Although experimental evidence indicates that holdfasts are susceptible to infection by *A. operculata,* this structure has not been found infected in wild plants (Correa *et al.* 1987). Natural frond replacement, however, does not operate in farmed *C. crispus,* where individual fronds are maintained in suspension. All fronds are susceptible, and if lost there is no holdfast equivalent to replace them. Consequently, infection by *A. operculata* may represent a much more serious threat to farmed than to wild sporophytic *C. crispus,* not only as primary cause of tissue decay, but also as a facilitating factor of secondary bacterial and possibly fungal infections.

Fine structural observations of *A. operculata* germlings outside the host indicate that the process of germination is highly conservative, following the same pattern observed during infection of *C. crispus.* Concentration of nucleus, Golgi system, and secretory vesicles seems to reflect cellular elongation at the tip of the germling. Pocket-like invaginations at the surface of the cell, occurring by fusion of Golgi-originated secretive vesicles with the plasmalemma, probably represent the discharge site for vesicle material (possibly wall material), and occurs only at the tip of the germlings. Similar observations were made at the tip of apical cells of *A. operculata* during penetration of the host, suggesting that wall material produced in the Golgi system was being deposited in the expanding apical cell wall of the endophyte. This pattern of cell wall deposition is common in a number of other organisms with apical growth, including algae (Mishra 1969, Rawlence and Taylor 1972, Toth 1976, Pueschel and Cole 1985), fungi (Grove *et al.* 1970, Farkas 1979, Wessels and Sietsma 1981, Boller 1987) and higher plants (reviewed by Schnepf 1986). In addition, fine structural observations of germlings outside *C. crispus* support the contention that *A. operculata* is an obligate endophyte, as no adhesive sheath providing attachment to the substratum developed surrounding these germlings. This sheath of adhesive material, ultrastructurally represented by a layer of microfibrillar material outside the cell wall, is a conspicuous feature in a number of algae using various living organisms or rocks as substrata (Baker and Evans 1973, Bråten 1975, Chen and Taylor 1976, Toth 1976, Hardy and Moss 1978, Fletcher 1981).

The taxonomic value of the so-called hairs has been a matter of controversy, and even the use of the term "hair" has been criticized (O'Kelly and Yarish 1981). Nielsen (1979) defined the *Acrochaete*-type hair as consisting "of a narrow terminal part and a swollen base which is separated from the underlying cell by a wall...", but O'Kelly and Yarish (1981) have suggested that the term "hair" should be applied only if a nucleus is formed within the hyaline structure at maturity, or a nuclear division is associated with its initiation. My findings, although preliminary, are in agreement with the general description of the *Acrochaete*-type hairs (Nielsen 1979) and with hairs reported to develop in unialgal plants of *A. operculata* (Correa *et al.* 1988). This indicates that within a single species, different hair morphologies may develop. It is still uncertain whether the two types of hairs found in *A. operculata,* usually borne by a single plant, represent successive developmental stages. More important perhaps is the failure to detect any remnant of plasmalemma or other organelle inside the elongated portion of the hairs, regardless of the presence or absence of a swollen base. Consequently, if the definition by Huber (1892, in Yarish and O'Kelly 1981) is to be maintained, these hyaline structures in *A. operculata* should be referred to as setae.

Although facing the same type of obstacles in *C. crispus,* that is OCW, intercellular matrix, and immediate cell wall, *A. heteroclada* follows a different pattern of development, and also apparently has additional mechanisms to circumvent those barriers, as compared to *A. operculata.* The initiation of the association between *A. heteroclada* and *C. crispus* begins by the contact of a zoospore with the surface of the frond. Germination generally occurs outside, on the surface of the host, and it is followed by penetration of the OCW. The main difference between *A. heteroclada* and *A. operculata* at this stage is that while germlings of the latter do not produce deformation of the OCW, *A. heteroclada* certainly does. The inward bending of the lamellar cuticle suggests a primary mechanical, rather than enzymatic mechanism of penetration. When the homogeneous layer of the OCW is reached, deformation of the host ceases, and a new important difference for *A. operculata* emerges. Instead of rapidly penetrating the cortex of the host, as *A. operculata* does, *A. heteroclada* begins an extremely rapid colonization of the homogeneous OCW, initially as a monolayer of radiating filaments, which eventually

develops into a multilayered, discoid, cushion-like plant. While the multilamellar cuticle is very effective in removing *A. heteroclada* germlings during the first period, owing to its external germination, this effectiveness declines drastically once the endophyte reaches the homogeneous OCW. As the branches expand laterally very rapidly, it appears that much more force is required to remove this structure than a non-branched cylindrical cell oriented perpendicular to the surface, as *A. operculata* germlings. More importantly, as *A. heteroclada* germlings rapidly advance within the homogeneous OCW, they coalesce with adjacent germlings, and shortly after inoculation, large areas of the frond are infected. Although little cellular damage takes place at this stage of infection, it seems likely that cortical cells of *C. crispus* are under severe physiological stress. *Acrochaete heteroclada* plants became polystromatic over extensive areas, were actively growing and reproducing, and probably acting as a nutrient and light filter, similarly to what has been indicated to occur in a number of host-epiphyte associations (Sand-Jensen 1977, Bulthius and Woelkerling 1983, Sand-Jensen and Revsbech 1987). Other potential sources of stress for the cortical cells of *C. crispus* subjacent to *A. heteroclada* may be changes in pH and redox conditions at the host-endophyte interface (Terry and Edyvean 1981). Although the points indicated above may not result in immediate death of host cells, the frond as a whole reduces its performance (see Chapter 3).

Penetration of the cortex of *C. crispus* proceeds after a period of superficial colonization, and it is still unclear what triggers this process. Cortical cells of the host, probably weakened during the pre-invasive period, undergo rapid organelle disorganization, disruption and lysis, in a sequence similar to that observed during infection by *A. operculata.* Similarly, penetration is always via intercellular matrix, and in contrast to *A. operculata,* no penetration of host cells appears to take place. As in infection by *A. operculata,* fine structural changes in the microfibrillar organization of the intercellular matrix, leading to an absence of microfibrils at the tip of apical, penetrating cells of *A. heteroclada,* suggest degradation of the intercellular matrix. Other differences between infection by *A. operculata* and *A. heteroclada* were observed when the invasive filaments of the latter reached the medulla of the host. While at this stage *A. operculata* began colonization of adjacent tissues, including

penetration into the medulla, and reinvasion of the cortex; penetrating filaments of *A. heteroclada* do not proceed into the medulla. Whether this arrest in host penetration is a response to changes in chemical composition of the intercellular matrix, or a shortage in nutrients or light (or both), is not known. My experiments on host specificity, however, indicate that *A. heteroclada* is a generalistic endophyte, able to infect a number of hosts that, considered as a whole, certainly represent a higher diversity in cell wall composition than one could find within a generation of *C. crispus* alone. This makes it unlikely that potential chemical differences between intercellular cell wall at the cortex and medulla are responsible for the observed infection pattern of *A. heteroclada.* Furthermore, this endophytic species develops similarly in both sporophytic and gametophytic *C. crispus,* whose cortical intercellular matrix, as discussed above, is chemically distinct.

As invasion of the cortex of the frond by *A. heteroclada* is generally synchronous, a large number of filaments penetrating at the same time results in extensive areas of the cortex with severe cell destruction. As with *A. operculata,* cellular changes in the host are caused directly by *A. heteroclada,* with no participation of concurrent bacterial infections.

Breakdown of the fronds infected by *A. heteroclada* results from a total replacement of the cortex of *C. crispus* by the endophyte, after which it becomes separated from the medulla. Concurrent bacterial infection occurs and is responsible for the final digestion of the remains of *C. crispus.* Unlike infection by *A. operculata,* where the onset of reproduction initiates the massive collapse of the cortex of the host, and facilitates secondary infection by bacteria, reproduction of *A. heteroclada* occurred at the surface of the cushion-like plants, outside the cortex, and had no direct impact on the development of the infection, other than production of new inoculum.

The pattern of development displayed by *A. heteroclada* results in plants which are certainly more exposed to the environment. In the field it is likely that both abiotic and biotic factors play an important role in determining the fate of the infection. However, this apparent disadvantage in comparison with the well protected habit of *A. operculata* may be compensated for by a larger diversity of hosts potentially susceptible to colonization by *A. heteroclada.*

FIGURES

Figs 1-6. Non infected sporophytic *C. crispus*. Holdfasts and discoid germlings.

Fig. 1. Cross section of the upper surface of a discoid germling to show spatial arrangement and shape of vegetative cells, including the apical cell (AC). Most of the few intracellular structures are at the periphery, or in the "cytoplasmic septum" (arrow-heads) left by the large vacuole (V). Note the reduced intercellular matrix and the thick outer cell wall (OCW), with the layer of granular material (arrows) at the outer interface. Scale= 5 μm

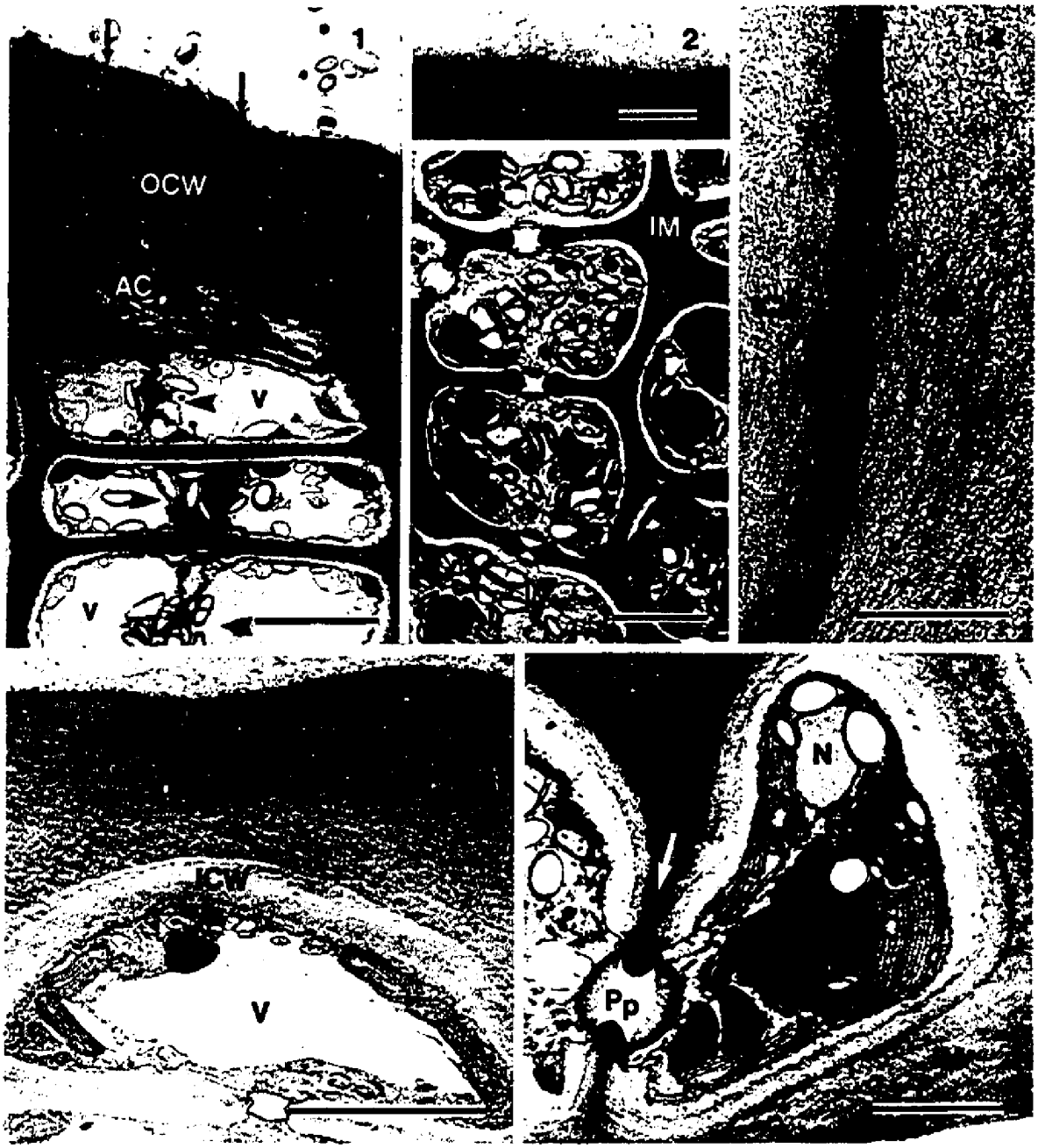
Fig. 2. Enlargement of Fig. 1 showing material deposited at the interface between the outer cell wall and the medium. Scale= 1 μm

Fig. 3. Cross section through the central portion of a discoid holdfast, showing more or less isodiametric vegetative cells, arranged in filaments, with pit-plugs distinguishable. Large osmiophilic inclusions are abundant. Little intercellular matrix (IM) evident, but distinctive from the immediate cell wall. Scale= 5 μm

Fig. 4. Interface between intercellular matrix (IM) and immediate cell wall (ICW). Scale= 1 μm

Fig. 5. Cross section of field-collected holdfast showing the lens-shaped apical cell with a large vacuole (V) and parietal chloroplast, separated by a distinct, immediate cell wall (ICW) from the outer cell wall (OCW). Scale= 5 μm

Fig. 6. Cross section at the base of a discoid germling grown under multidirectional illumination. Cell shows a highly developed chloroplast, with an apically located nucleus (N) and characteristic pit-plug (Pp) which interrupts the continuity of the immediate cell wall (arrow). The intercellular matrix (IM) appears slightly more developed than in upper portions of the disc. Scale= 2 μm


1
OCW
AC
V
V
2
IM
3
ICW
V
N
Pp

Figs 7-21. Non infected fronds of sporophytic and gametophytic *C. crispus*.

Fig. 7. Sporophytic *C. crispus*. Cross section of typical cortical cell, showing a parietal chloroplast (Cl), central nucleus (N) with prominent nucleolus (Nu), endoplasmic reticulum (ER), mitochondria (m) profiles, apical Golgi bodies (G) and basal vacuole (V). Note the continuous plasmalemma (arrows). The immediate cell wall (ICW) surrounds the entire cell and is distinct from the intercellular matrix (IM). Scale= 1 μm


IM
ICM
V

Fig. 8. Sporophytic *C. crispus*. Cross section through two cortical cells, at the apex of a frond growing rapidly. Note the highly developed endoplasmic reticulum (ER) and the poor development of the chloroplast (Cl). Nucleus (N) with a prominent nucleolus (Nu) occupies most of the cell. Scale= 2 μm

Fig. 9. Sporophytic *C. crispus*. Golgi body with *cis* saccules (arrowhead) selectively stained. *In block* staining with uranyl acetate, post-stained with lead citrate. Scale= 0.5 μm

Fig. 10. Sporophytic *C. crispus*. Enlargement of Fig. 8 showing the endoplasmic reticulum with associated ribosomes at the periphery of the nucleus (N). Scale= 0.5 μm

Fig. 11. Sporophytic *C. crispus*. Cross section through a subcortical cell, with cytoplasm full of membranous structures, which appear to displace the nucleus (N) and other organelles. Plasmalemmal finger-like projections, protruding into the immediate cell wall (ICW), are well developed at the vesicle pole of the cell (arrowheads). Scale= 1 μm

Fig. 12. Sporophytic *C. crispus*. Cross section through a medullary cell with a convoluted profile resulting from plasmalemmal intrusions of the thick, immediate cell wall (ICW), which do not reach the intercellular matrix (IM). Scale= 2 μm

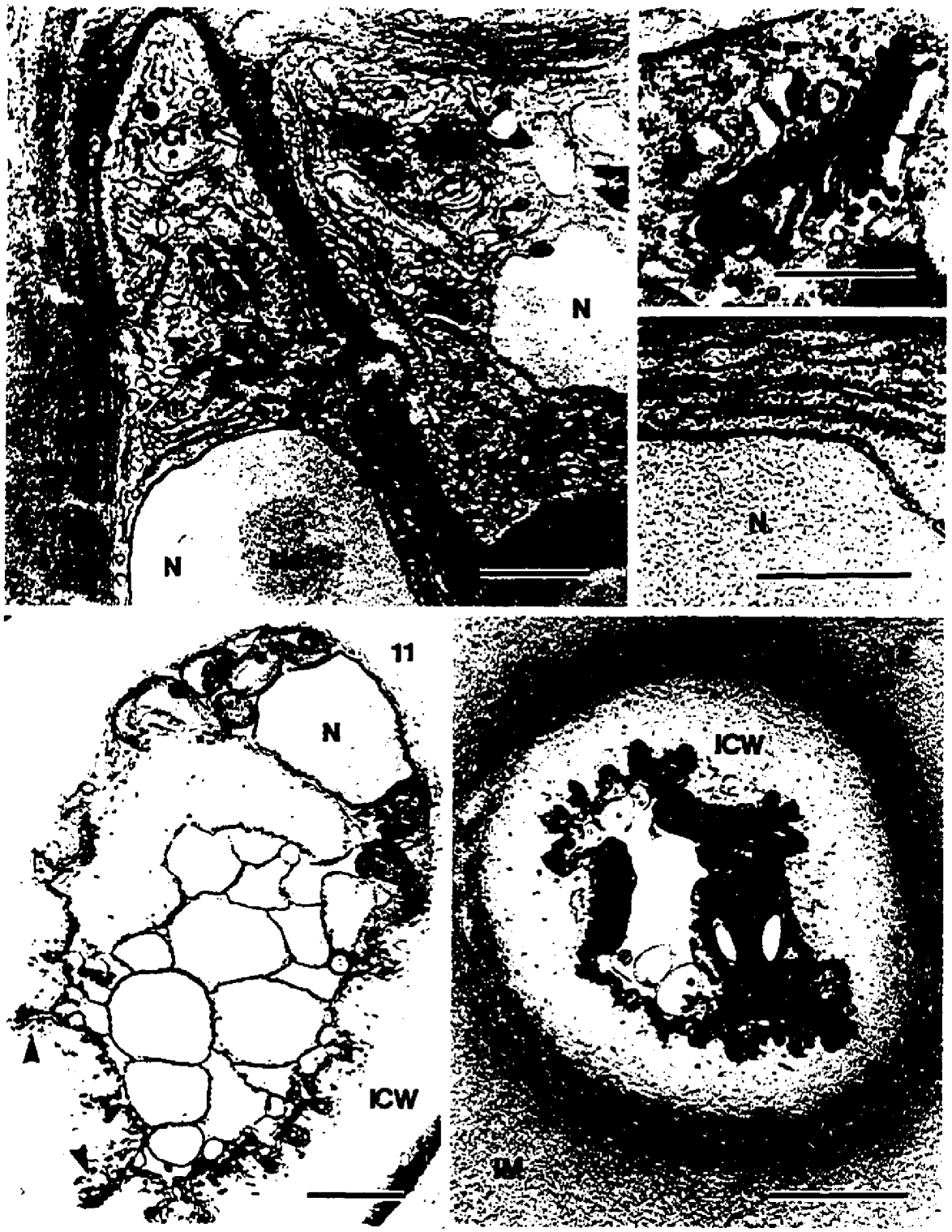

N
N
N
11
N
ICW
ICW

Fig. 13. Gametophytic *C. crispus*. Cross section through the stipe showing the homogeneous outer cell wall (OCW) that extends between the cortical cells (arrowheads) to the outside (*). Note an electron dense, small flake-like deposit (arrow) at the surface of the outer cell wall. Scale= 2 μm

Fig. 14. Gametophytic *C. crispus*. Cross section through the outer cell wall (OCW) at the central portion of the fronds , showing the lamellar portion of the outer cell wall, the cuticle (Cu), lying on the homogeneous stratum. A narrow portion of the outer cell wall at the base of the cuticle appears electron dense. Scale= 1 μm

Fig. 15. Gametophytic *C. crispus*. Cross section through the outer cell wall at the apex, showing a twelve-layer cuticle. The uppermost lamellae are being peeled away. Scale= 1 μm

Fig. 16. Gametophytic *C. crispus*. Cross section through the inner part of the cuticle, showing details of the alternate electron dense and electron translucent bands. Scale= 0.25 μm

Fig. 17. Gametophytic *C. crispus*. Individual lamella with an electron dense core between 2 electron translucent layers. Scale= 0.25 μm

Fig. 18. Sporophytic *C. crispus*. Cross section through the outer cell wall, at the apex of the frond, showing an electron dense, coarsely granular zone (arrows), between the homogeneous region and the exterior. Scale= 1 μm

Fig. 19. Sporophytic *C. crispus*. Cross section through the outer cell wall, apical region of the frond, showing the cuticle only, with irregularly arranged lamellae. Scale= 1 μm

Fig. 20. Enlargement of Fig. 19, showing the coarsely granular structure of the anastomosing lamellae. Scale= 0.5 μm

Fig. 21. Sporophytic *C. crispus*. Cross section through the outer cell wall, apical region, showing electron translucent, homogeneous layer (between arrowheads) replacing the multilamellar structure. Scale= 0.25 μm

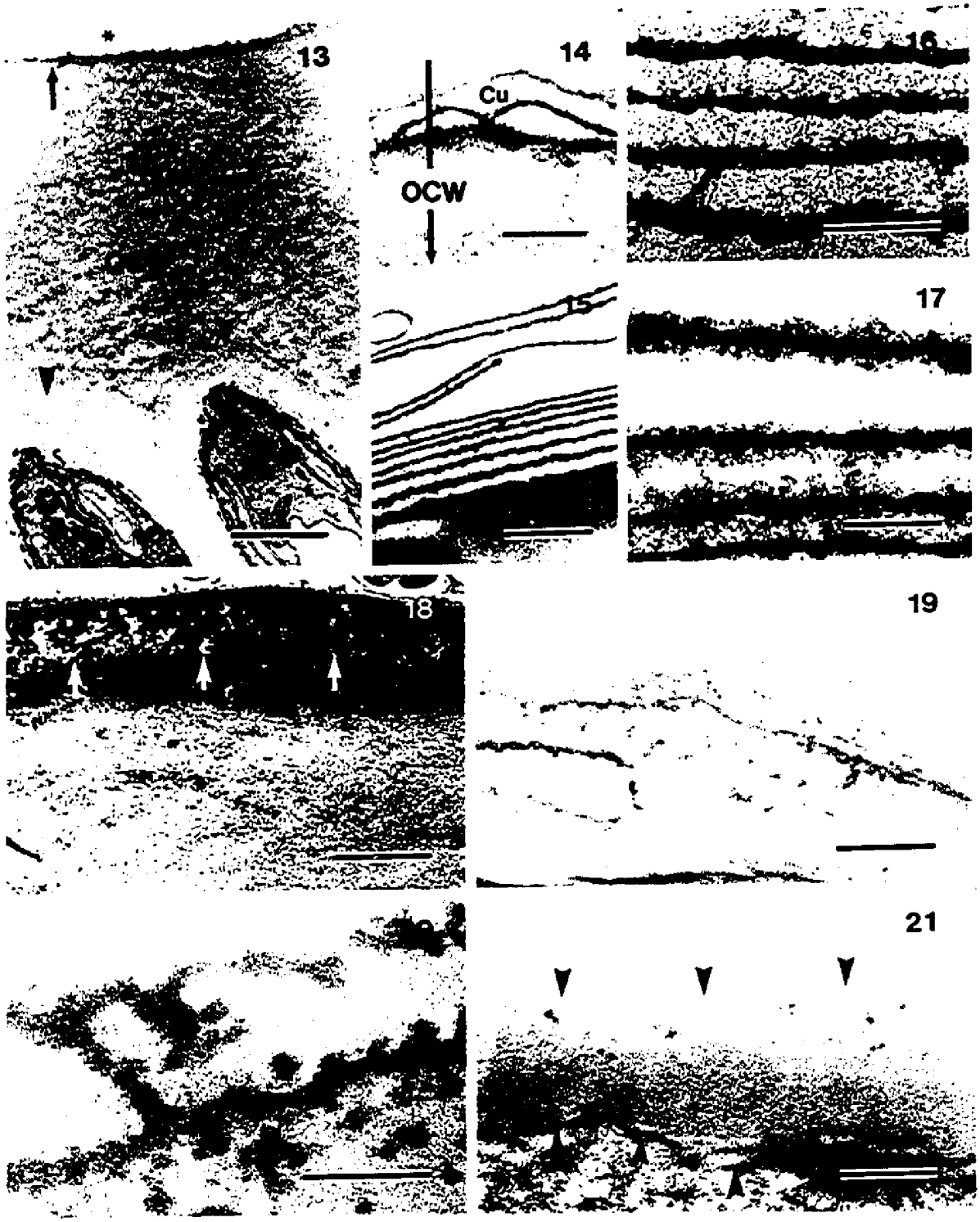

13
14
Cu
OCW
16
15
17
18
19
21

Fig. 22. Quadriflagellate zoospore of *Acrochaete operculata.* Scale= 2 μm

Fig. 23. Zoospore of *A. operculata,* with 4 flagella connected to the anterior papilla (arrowhead). Scale= 2 μm

Fig. 24. Sporophytic *C. crispus.* Surface view of a non-infected frond showing a flaky appearance from the peeling cuticular lamella. Scale= 10 μm

Figs 25-33. Early events (within 5 d after inoculation) at the surface of sporophytic *C. crispus* fronds during the infection by *A. operculata.*

Fig. 25. Zoospore, shortly after settling. Pyriform shape retained, with the flagellar end attached to the surface of the host. Scale= 2 μm

Fig. 26. *A. operculata* germling showing an enlarged attaching point and a loss of pyriform shape. Scale= 2 μm

Fig. 27. As in Fig. 26, but from above, showing adhesive pad (arrows head) extending over surface of the host immediate to the infecting germling. Scale= 2 μm

Fig. 28. A partially detached germling showing a narrow germination tube (Gt), a clean, sharp penetration hole (arrowhead), and the adhesive pad (arrow). Scale= 2 μm

Fig. 29. Beginning of the fracture (arrows) of a penetrating germling while pulled out by the lamella (La). Scale= 2 μm

Fig. 30. Germling successfully pulled out by the peeling lamella (La). The germ tube (Gt) appears intact and clean, and sharp edges can be seen in the penetration hole (arrowheads). Scale= 5 μm

Fig. 31. Inner face of a shed lamella showing a ring of zoospore cell-wall remains (arrowhead) after fracture occurred. The hole connects with the emptied zoospore wall, still attached to the outer side of the lamella. Note

the absence of fibrils in the lamellar area around the penetration site (arrow). Scale= 2 μm

Fig. 32. Fractured germling after unsuccessful removal by a peeling lamella. The fracture apparently occurred above the first cell (arrowhead), *i.e.* at the zoospore cell wall, as the fracture point does not seem to affect the subjacent cell, which looks turgid and closely associated with the host outer cell wall. Scale= 2 μm

Fig. 33. Marks of the lamellar digestion (arrowheads) left by germlings growing on the surface. Scale= 5 μm

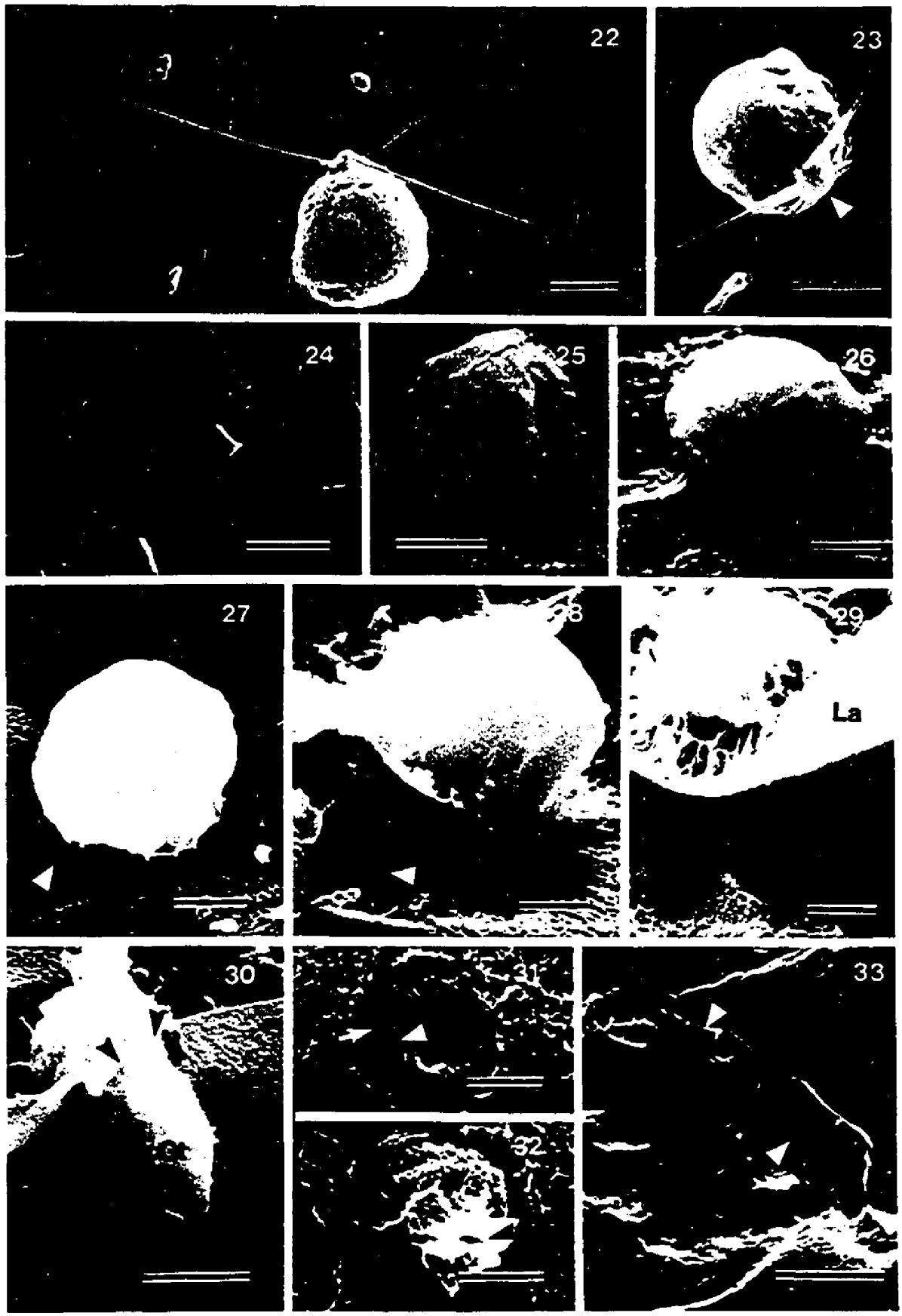

22
23
24
25
26
27
28
29
La
30
31
32
33

Fig. 34-92. Penetration and post penetration events during the infection of sporophytic *C. crispus* by *A. operculata*. Unless stated otherwise, photographs correspond to cross sections.

Fig. 34. Medial section of a one-cell old germling passing through the outer cell wall (OCW) of the host, which does not show evidence of deformation. The emptied cell wall of the zoospore (ZW) remains outside, and a septum (Se) separates it from the first vegetative cell (VC). Note the different cell wall structure at the emptied zoospore and at the septum. The parietal chloroplast (Cl) locates towards outside, while a large number of vesicles (Ve) concentrate at the host-side of the cell. An immature pyrenoid (Py) is embedded in the anterior end of the chloroplast. A double membrane is clearly distinguishable in the centrally-positioned nucleus (N). The tip of the invading cell is reaching the outer cortex (OC), where the immediate cell wall (ICW) of outermost cortical cells can be seen. Scale= 2 μm


34
ZW
Se
CI
N
RV

Fig. 35. Penetration of an area of the frond with a more compacted host outer cell wall, which ends abruptly at the penetration site (arrow). The emptied zoospore cell wall (ZW) remains outside. Scale= 2 μm

Fig. 36. Sharp edge of the outer cell wall of the host (arrow), visualized after slight separation from the penetrating germling (Gt). Scale= 2 μm

Fig. 37. A one-cell old germling that has been removed by a peeling lamella (La). Scale= 2 μm

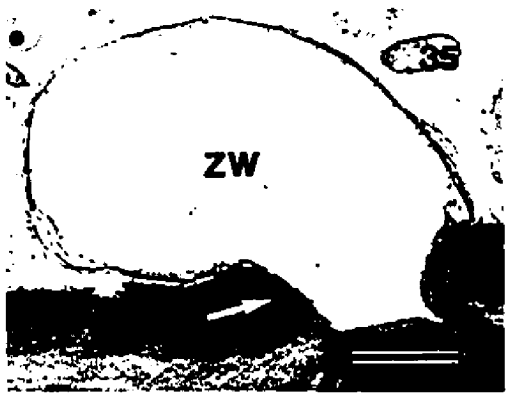
Fig. 38. Structural changes in the organization of the host outer cell wall (OCW) indicating digestion of the area (arrowheads) surrounding the penetrating germling (Gt). Compare with the adjacent normal outer cell wall. Loss of the matrix is evidenced by the formation of "caverns" (arrow) in the digested areas. Scale= 1 μm

Fig. 39. A two-cell primary invasive filament (E) penetrating through the intercellular matrix, at the upper cortex. Host cells (H) appear unaffected. Scale= 5 μm

Fig. 40. Tip of an invasive filament (E) inducing some deformation of host cells (H), which otherwise seem unaffected. Scale= 5 μm

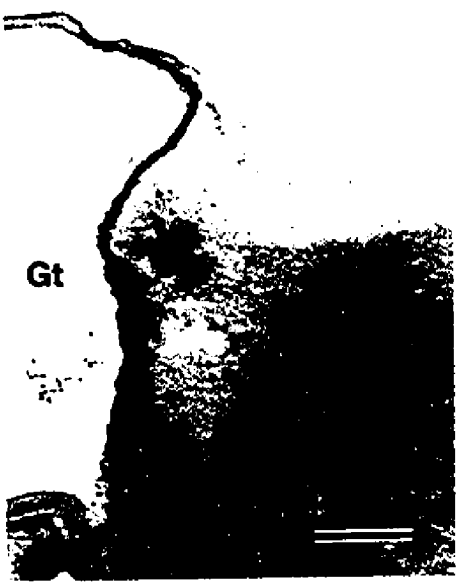
Fig. 41. Enlarged superficial cell of *A. operculata*. Host cells (arrows) surrounding the endophyte have been damaged by compression. Zoospore cell wall remains attached to the surface of the host. Scale= 5 μm

Fig. 42. Early degenerative changes in a host cortical cell during infection, characterized by chloroplast (Cl) disorganization, swelling of endoplasmic reticulum (ER) and highly granular cytoplasm. The nucleus (N) is still identifiable, but the nuclear envelope shows electron dense deposits, and the nucleolus (Nu) is abnormally granular. Plasmalemma is not apparent. Scale= 1 μm


35
ZW


Gt
36


37
La


38
Gt


39
E


40
H


41

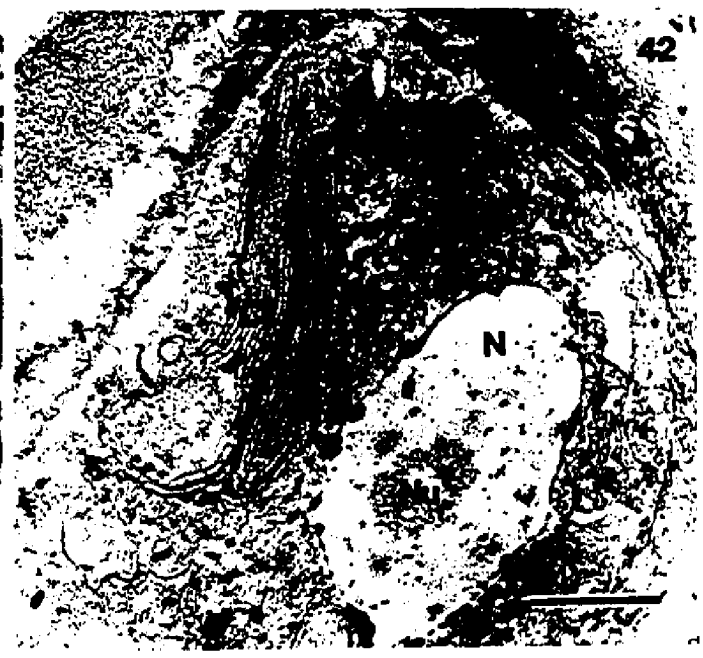

42
N

Fig. 43. Cortical cell of the host (H) with osmiophilic deposits (arrowheads) at the cytoplasm facing an endophytic cell (E). Note some compression of the immediate cell wall at this point. Scale= 0.5 μm

Fig. 44. Advanced stage of degeneration of a host cortical cell (H) closely associated with an endophytic cell (E). Chloroplast (Cl) remains are still recognizable, as well as degenerated mitochondria (arrowheads). There is no indication of the plasmalemma. Scale= 0.5 μm

Fig. 45. Section parallel to the surface of the frond showing four host cells around the endophyte (E) with various degrees of damage, and structurally normal adjacent cortical cells (arrowheads). Scale= 2 μm

Fig. 46. Tip of an apical endophytic cell (E) advancing through the intercellular matrix (IM) of the host. An electron translucent area (arrowheads) at the interface between the endophyte cell wall and the intercellular matrix is apparent. Scale= 2 μm

Fig. 47. Pocket-like invaginations of the endophyte plasmalemma, filled with small electron dense granules, usually associated with the advancing tip. Scale= 0.25 μm

Fig. 48. Host-endophyte interface at some distance from the advancing tip of an apical cell (E) of *A. operculata* in the vicinity of a host cell (H). No evidence of digestion of the intercellular matrix (IM) is apparent where in contact with the cell wall of the endophyte (arrowhead). Scale= 1 μm

Fig. 49. Enlarged cell of *A. operculata* at the inner cortex of the host, embedded in the intercellular matrix (IM). The single nucleus (N), centrally located, displays a prominent nucleolus (Nu). The parietal chloroplast shows abundant starch granules (St), scattered among irregularly-arranged thylakoids. Scale= 5 μm

Fig. 50. Section through an endophytic filament (E) originated from the enlarged *A. operculata* cells (*i.e.* Fig. 49), running through the

intercellular matrix (IM), perpendicular to the filaments of host cells (H). Scale= 2 μm

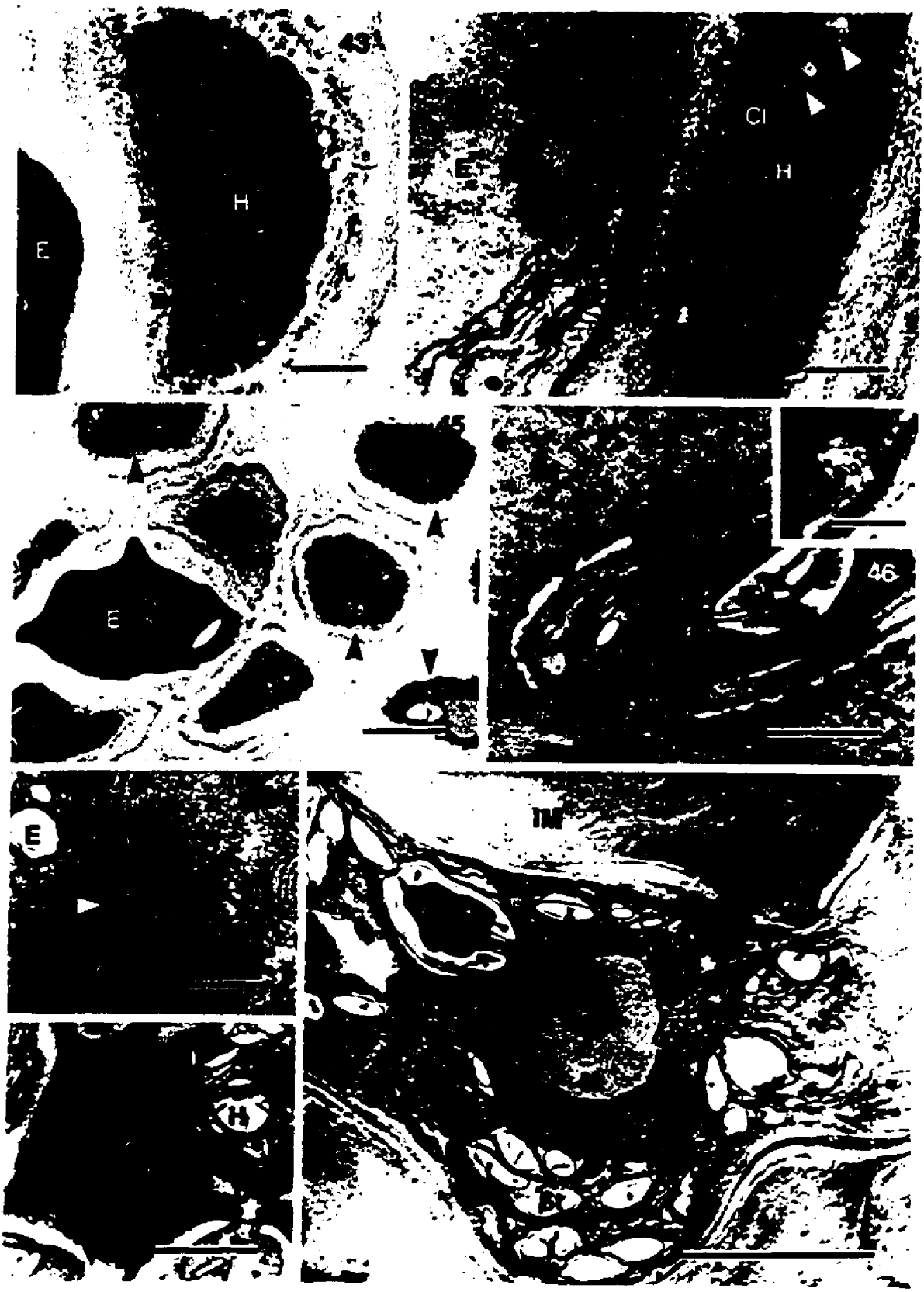

E
H
Cl
H
E
46
E
H
TM

Fig. 51. Enlargement of the perinuclear cytoplasm of Fig. 49, showing the numerous Golgi systems (G), mitochondria (m), and endoplasmic reticulum, developed in association with the nucleus (N). Starch granules (St) embedded in the chloroplast are also shown. Scale= 1 μm

Fig. 52. Bilenticular pyrenoid (Py) characteristic of *A. operculata*, surrounded by two bilenticular starch plates (SP), and traversed by a single thylakoid (arrow). Portion of the endophyte cell wall (EW) is also observed. Scale= 1 μm

Fig. 53. Endophytic cell (E) embedded in the intercellular matrix (IM), and contacting the immediate cell wall (ICW) of a subcortical host cell (H). There is no morphological evidence of host-cell damage. All membrane-bound organelles, including nucleus, chloroplast mitochondria, and endoplasmic reticulum are intact. No distortion of the immediate cell wall is evident. Scale= 2 μm

Fig. 54. An enlargement of Fig. 53, to show the host-endophyte interface, comprising cell wall of *A. operculata* (EW) in contact with the immediate cell wall (ICW) of the subcortical cell. Note the host plasmalemma (arrows) clearly recognizable outside the highly developed endoplasmic reticulum. Scale= 1 μm

Fig 55. Section through an endophytic filament (E) embedded in the intercellular matrix (IM) at the medulla of the host, showing an irregular profile. Immediate cell walls (ICW) of host cells are clearly distinct from the intercellular matrix. Scale= 2 μm

Fig. 56. Section through an endophytic filament (medullary region) with cell wall and cytoplasmic projections into the intercellular matrix. No contact with the immediate cell wall (ICW) occurs at this point. The highly condensed chloroplast surrounds a central pyrenoid (Py). Scale= 1 μm

Fig. 57. Mechanical deformation of the immediate cell wall (arrowheads) of a medullary host cell (H) caused by the endophyte (E). Scale= 1 μm

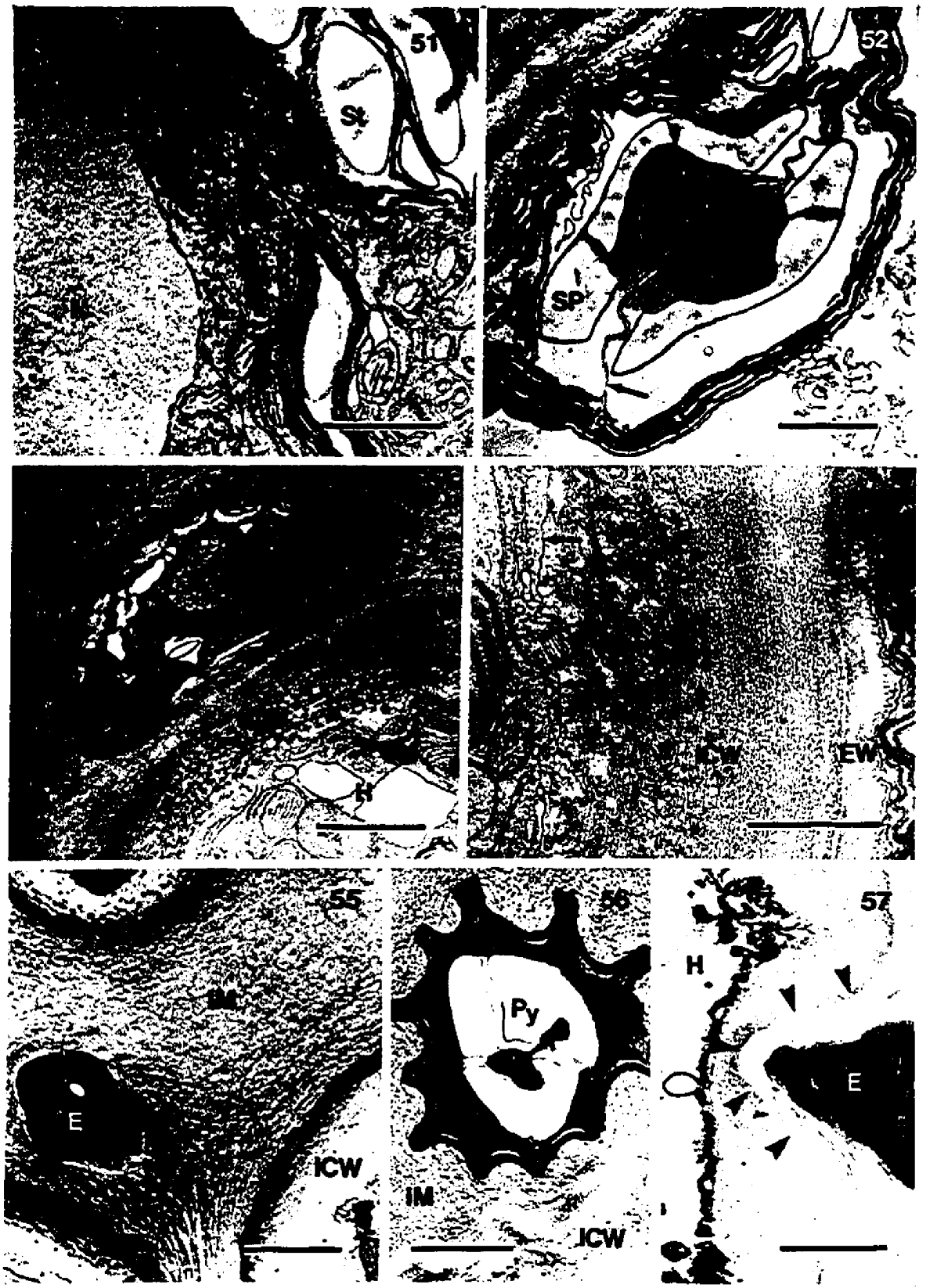

51
St
52
SP
H
EW
55
E
ICW
56
Py
IM
ICW
57
H
E

Fig. 58. Section parallel to the surface to show disruption of the immediate cell wall (arrowheads) of a host cortical cell (H) by a thick-walled endophyte (E), which is compressing the host protoplast. Evidence of host cell degeneration begins to show in the nucleus and chloroplast. Scale= 1 μm

Fig. 59. Host-endophyte interface from Fig. 59 showing the absence of immediate cell wall between the cell wall of the endophyte (arrows) and the host cell (H). Note the highly disorganized organelles, particularly the chloroplast (Cl). Plasmalemma of the host cell is not apparent. Scale= 1 μm

Fig. 60. Enlarged endophytic cell (arrowhead), sharing a common immediate cell wall (arrows) with a host cell (H) in advanced stage of degeneration. Scale= 1 μm

Fig. 61. Outer cortex of the host showing enlarged endophytic cells (E), one of which has disrupted the host outer cell wall (arrowheads). Note the almost complete absence of host cells. Scale= 10 μm

Fig. 62. Surface view of a superficial, cortex-borne endophytic cell, with two pyrenoids (Py) embedded in a highly developed chloroplast occupying most of the intracellular space. The profile is irregular, and several host cells (arrowheads) appear severely damaged. Scale= 5 μm

Fig. 63. Transverse section through a sporangium mother cell. The protoplast, with a prominent nucleus and large amount of starch granules, has become detached from the thick cell wall. Scale= 5 μm

Fig. 64. An enlargement of Fig. 63, to show the bilayered sporangium cell wall, and cytoplasmic threads (arrowheads) still in contact with it. Scale= 1 μm

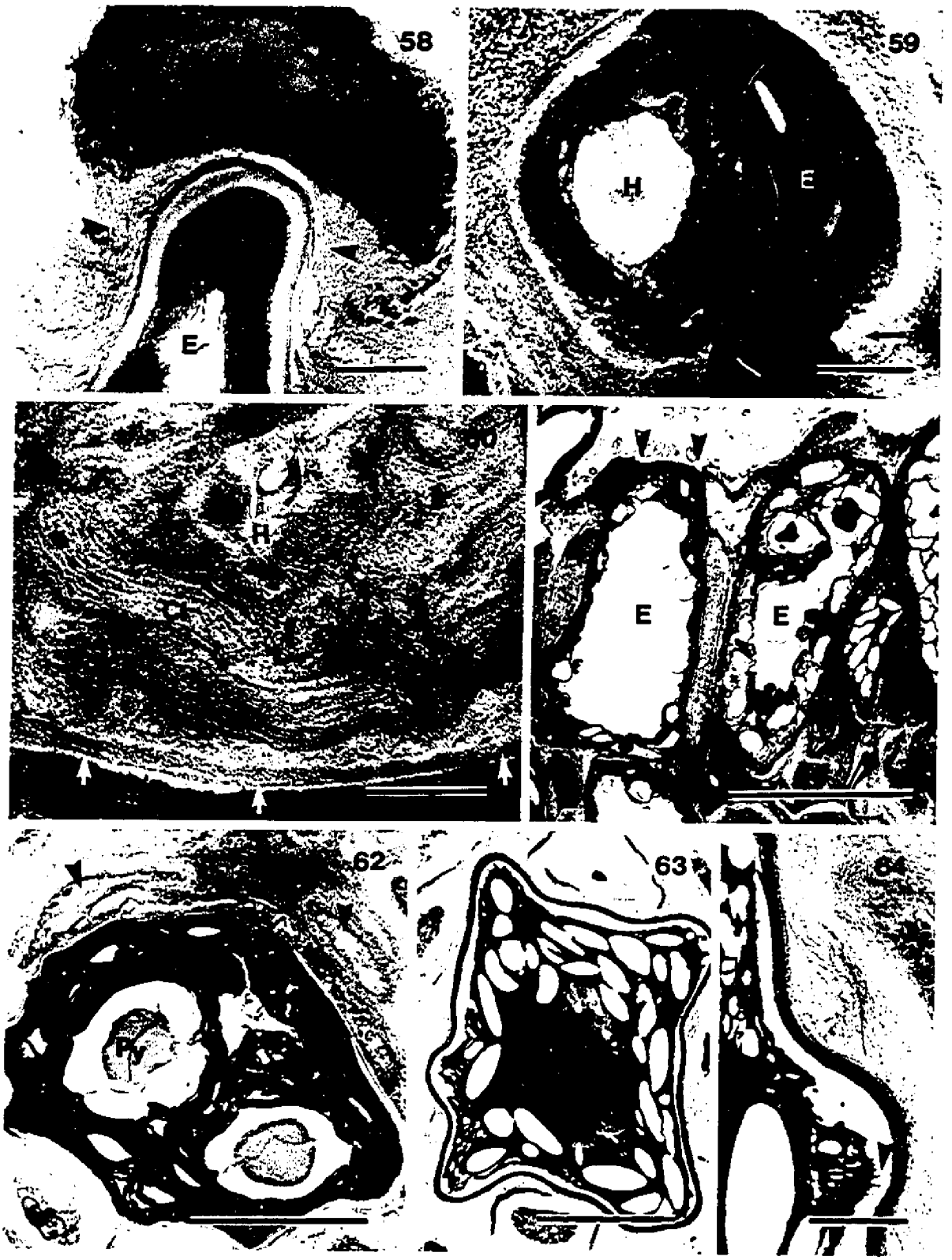

58
E
59
H
E
H
E
E
62
Pv
63
64

Fig. 65. Transverse section through a swollen sporangium, with distended cell wall (arrowheads), and protoplast embedded in an electron translucent material. Scale= 5 μm

Fig. 66. Maturing sporangium containing several zoospores, embedded in the cortex of the host. Note adjacent host cells in advanced stages of degeneration. Scale= 5 μm

Fig 67. Apex of a sporangium near maturity. Coarse fibrillar network, restricted to the tip, appears embedded in an amorphous material with almost no electron opacity. Scale= 2 μm

Figs 68-70. Zoospores in the sporangium.

Fig. 68. The anterior end contains large number of vesicles (Ve), apparently secreted by the Golgi bodies near the basally-located nucleus (N). The anterior papilla (arrowhead) conects with the rest of the cell by a narrow neck. Starch granules (St) are present at the posterior region. Scale= 2μm

Fig. 69. Tangential section through the papilla. Three of the flagella are in the plane of the section and their microtubular components evident (arrows). Scale= 0.5 μm

Fig. 70. The surface of the zoospore appears naked, and in some areas microtubules (arrow) are seen between the plasmalemma and the chloroplast (Cl). The immature eyespot (arrowhead) lies immediately beneath the double membrane surrounding the thylakoids. Scale= 0.5 μm

Fig. 71. Section parallel to the surface of the host, showing two recently evacuated sporangia. No cell or amorphous substance remains can be seen, and the lumen of each sporangium, outlined by the sporangium cell wall, remains open. Host cells, still surrounded by immediate cell walls, show no internal structure. Scale= 2 μm

Fig. 72. Advanced stage of infection of cortical tissue. There are multiple disruptions of the outer cell wall (OCW), which connect the outside with the interior of the frond. Host cells are barely recognizable, and most of the tissue is composed of endophytic cells (E) and emptied sporangia (S). Scale= 10 μm

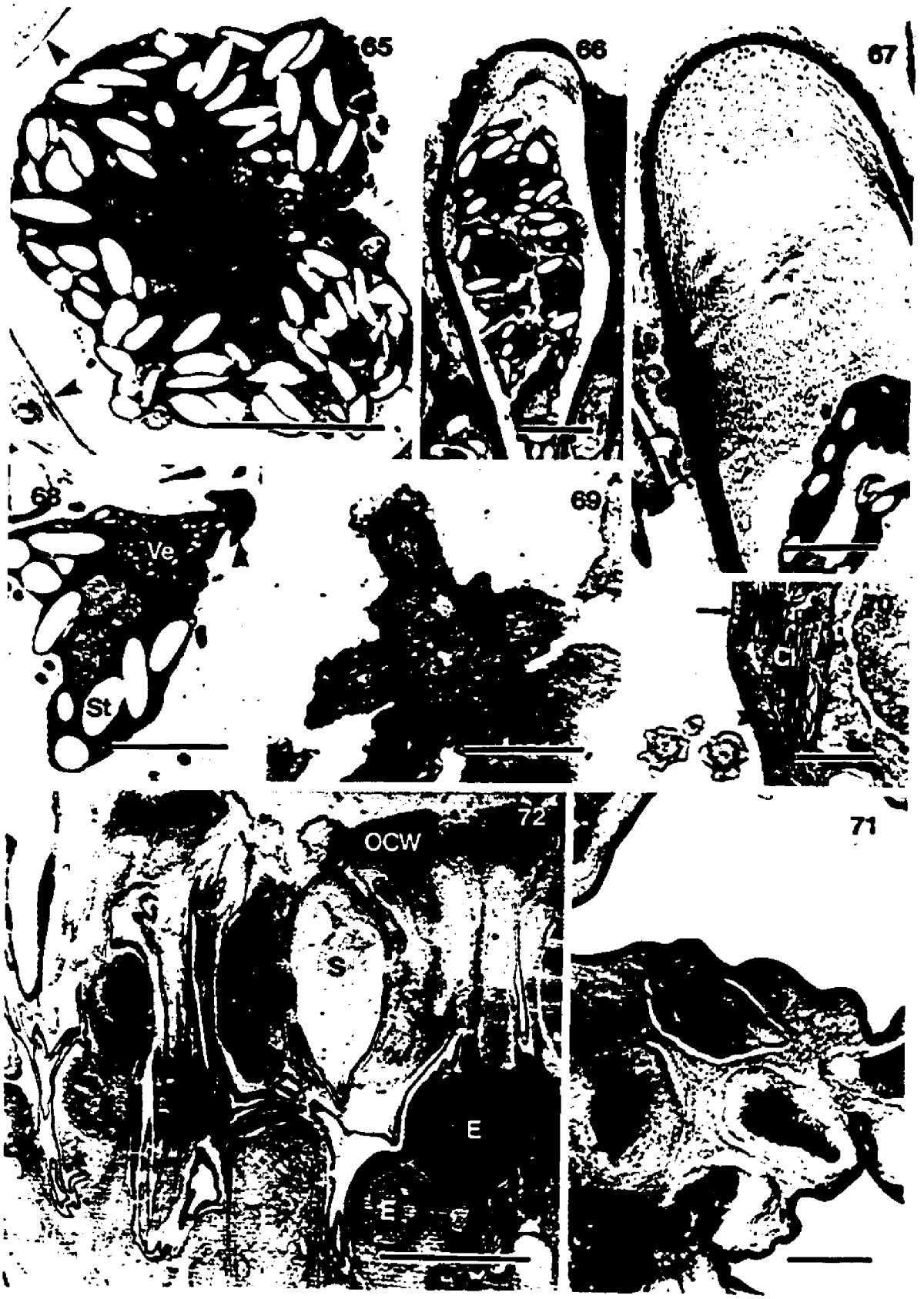

65
66
67
68
Ve
St
69
Cl
71
72
OCW
S
E
E

Fig. 73. Surface view of a frond at the time of sporangial maturation. Many sporangial apices are seen protruding from the outer cell wall. Setae of the endophyte are also present. Note the coalescence of two or more emptied sporangia (arrowhead). Scale= 20 μm

Fig. 74. *Acrochaete*-type setae, with a lenticular, bulbose and septated base (arrow) resting on a globose vegetative cell, both underneath the outer cell wall. Scale= 10 μm

Fig. 75. Enlargement of Fig. 74 showing membranous remains, apparently mitochondria (m) and plasmalemma. Scale= 1 μm

Fig. 76. Setae attached directly to a globose basal cell. Scale= 2 μm

Fig. 77. Swollen, fusiform tip of a short, apparently immature setae. Scale= 2 μm

Fig. 78. Terminal stages of the infection, characterized by bacterial colonization of the cortex. Note that although the outer cell wall (OCW) is still recognizable, bacteria (B) have digested most intercellular matrix and the immediate cell wall of host cells (H). Endophytic cell (E) appears unaffected. Scale= 5 μm

Fig. 79. Endophytic cell (E) at the cortex of the host, surrounded by bacteria. Note the absence of fibrillar organization of the intercellular space. A bacterium (arrow) appears to be invading a decaying host cell (H). Scale= 5 μm

Fig. 80. Medullary host cell (H) with its immediate cell wall (ICW) compressed by an endophytic filament (E). Early inward plasmalemmal invaginations (arrow). Scale= 1 μm

Fig. 81. Advanced stage of cytoplasmic vesiculation in a medullary host cell (H). These membranous vesicles appear to originate at the plasmalemma (arrow). Note the adjacent endophytic cell (E). Scale= 1 μm

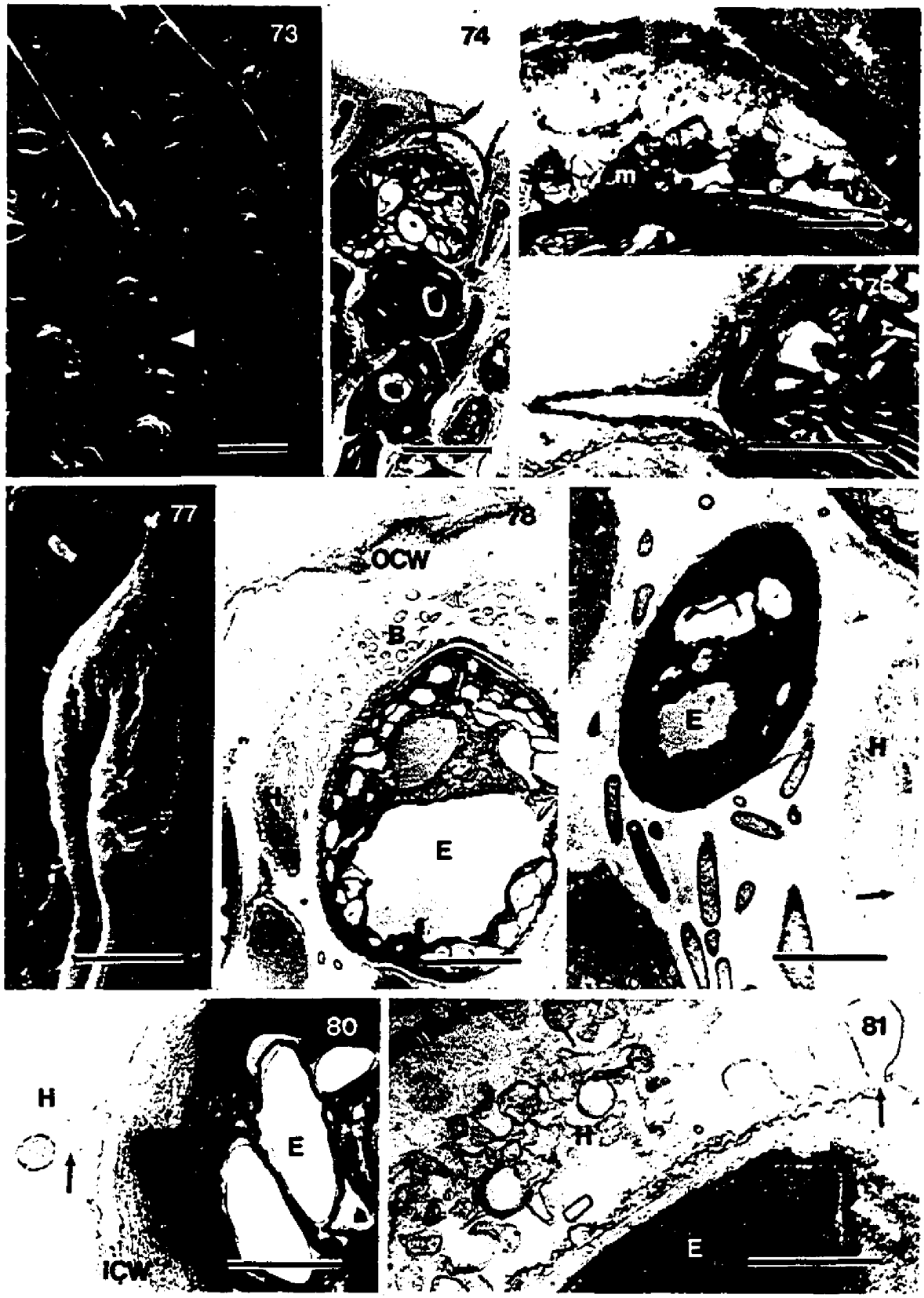

73
74
m
76
77
78
OCW
B
E
E
H
80
H
E
ICW
81
H
E

Fig. 82. Enlarged endophytic filament (E) inducing deformation of two swollen host medullary cells (H). Note thylakoids scattered throughout the endophytic cell. Plasmalemma of the host cells appears detached (arrow) from the thin remains of the immediate cell wall. Only a small reduced area with intercellular matrix (IM) is observed. Scale= 2 μm

Fig. 83. Host medullary cell (H) in close contact with an endophytic cell (E), at a late degenerative stage, with discontinuous plasmalemma (arrow), and double membrane-bound structures (degenerative mitochondria?), some of them disrupted (arrowhead). Scale= 2 μm

Fig. 84. Enlargement of Fig. 82 to show the bilayered cell wall (EW) of the endophyte (E) in close contact with a thin host immediate cell wall (arrowheads). Note the absence of plasmalemma in the host cell(H), whereas the endophytic counterpart is clearly visible and highly convoluted. Scale= 0.25 μm

Fig. 85. Bacterial secondary infection of the medulla. Rod-like bacteria (B) appear digesting intercellular matrix (IM) of the host, producing large caverns. The immediate cell wall (ICW) of two host cells (H) appears unaffected. Scale= 1 μm

Figs 86-92 *A. operculata* germlings growing outside the host.

Fig. 86. Scanning electron micrograph of a 5-d old germling, with the zoospore cell wall (ZW) and germination tube (Gt). Scale= 5 μm

Fig. 87. Longitudinal section of a one-celled, 5-d old germling showing the emptied zoospore cell wall (ZW) and the germination tube consisting of a vegetative cell, with a large central vacuole (V). Scale= 5 μm

Fig. 88. A section through the same germling as in Fig. 87, to show the apical concentration of organelles, and the large posterior vacuole (V). Scale= 5 μm

Fig. 89. Cross section through the tip of a one-celled, 5-d old germling. Parietal chloroplast (Cl) consist of parallely oriented thylakoids, one of which passes through the bilenticular pyrenoid (Py), typically surrounded by two hemispheric starch plates (SP). Golgi (G) and endoplasmic reticulum are well developed, and vesicles originated in the Golgi appear to migrate towards the plasmalemma. Note the numerous pocket-like invaginations of the plasmalemma (arrowheads). Scale= 1 μm

Fig. 90. Cross-section posterior to Fig. 89. Parietal chloroplast (Cl) appears displaced by several Golgi bodies (G) and secretive vesicles. Endoplasmic reticulum and mitochondria (m) are also present. The pocket-like invaginations of the plasmalemma are uncommon (arrowhead). Scale= 1 μm

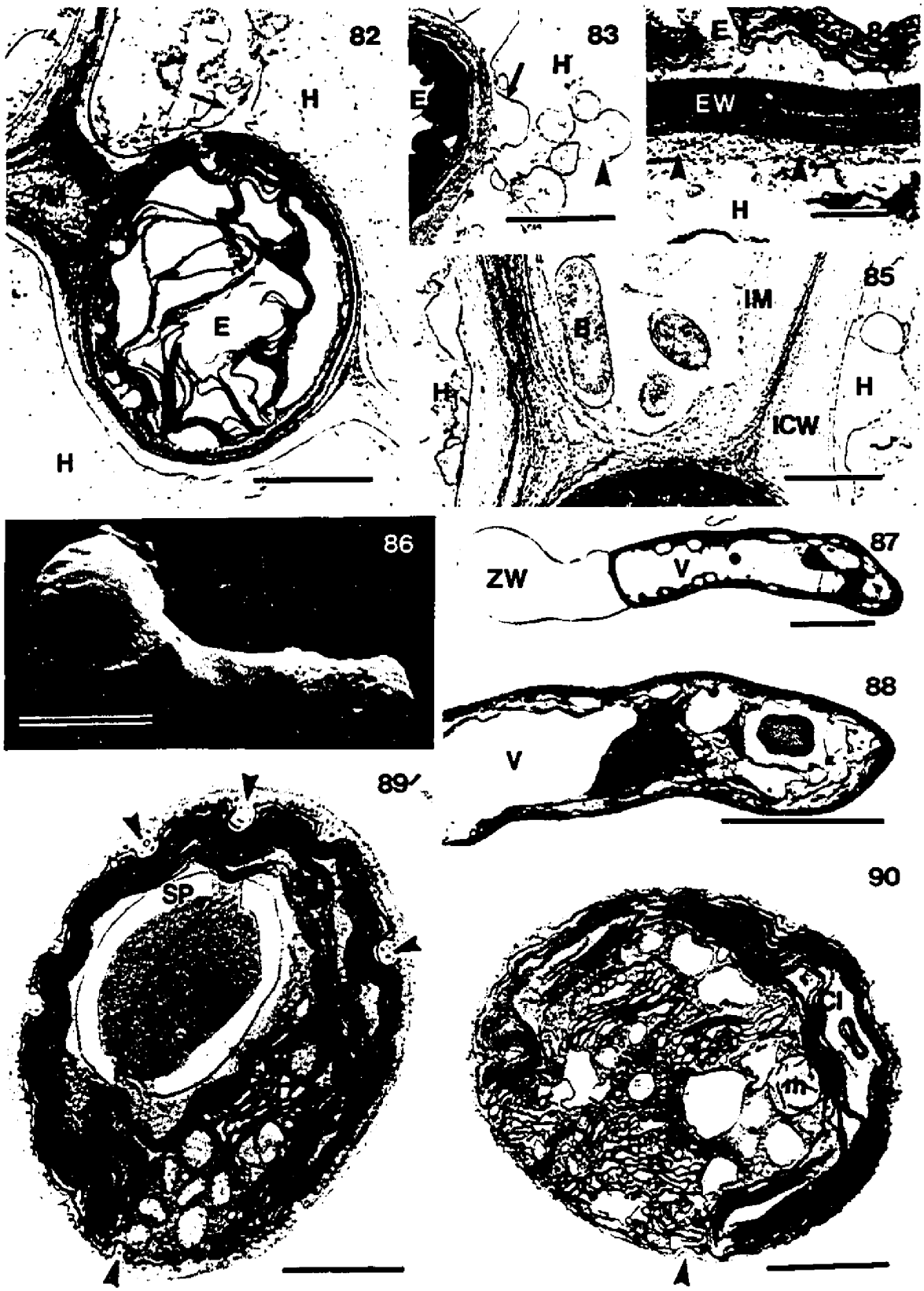

82
H
E
H
83
H
E
EW
E
H
85
IM
E
H
H
ICW
86
87
ZW
V
88
V
89
SP
90

Fig. 91. Cross-section at the level of the nucleus (N), which occupies most of the space. Golgi (G) decreases in abundance, and mitochondria and endoplasmic reticulum are scattered throughout the section. The chloroplast remains parietal and, as in more apical regions, is partially open. Scale= 1 μm

Fig. 92. Cross-section at the vacuole (V) region. All organelles, including chloroplast (Cl) with a pyrenoid (Py), are displaced towards the periphery of the cell. Membranes (arrows) appear to compartmentalize the space. Scale= 1 μm

Fig. 93. Penetration of a discoid *C. crispus* germling by *A. operculata*. The zoospore cell wall (ZW) appears empty and flattened against the host outer cell wall (OCW), which does not show evidence of inwards deformation or compression. Note the narrow passage (arrowheads) through which the cellular content of the endophyte (E) migrated, and the thick septum (Se) separating the zoospore cell wall from the endophytic cell. Scale= 5 μm

Fig. 94. Vegetative endophytic filament (E) growing through the reduced intercellular matrix of a discoid host germling. The host immediate cell walls (ICW) are not disrupted, but deformed. No damage or degenerative changes can be seen in the organelles of the host cells (H). Scale= 2 μm

Fig. 95-107. Infection of gametophytic *C. crispus* by *A. operculata*.

Fig. 95. Tip of an endophytic apical cell (E) growing in the cortical intercellular matrix (IM) of the host. No evidence of host cell wall digestion is apparent. Scale= 1 μm

Fig. 96. Endophytic filament (E) invading the subcortical portions of the frond through the intercellular matrix (IM). No changes are apparent in the immediate cell wall nor in the organelles of the adjacent host cell (H). Scale= 2 μm

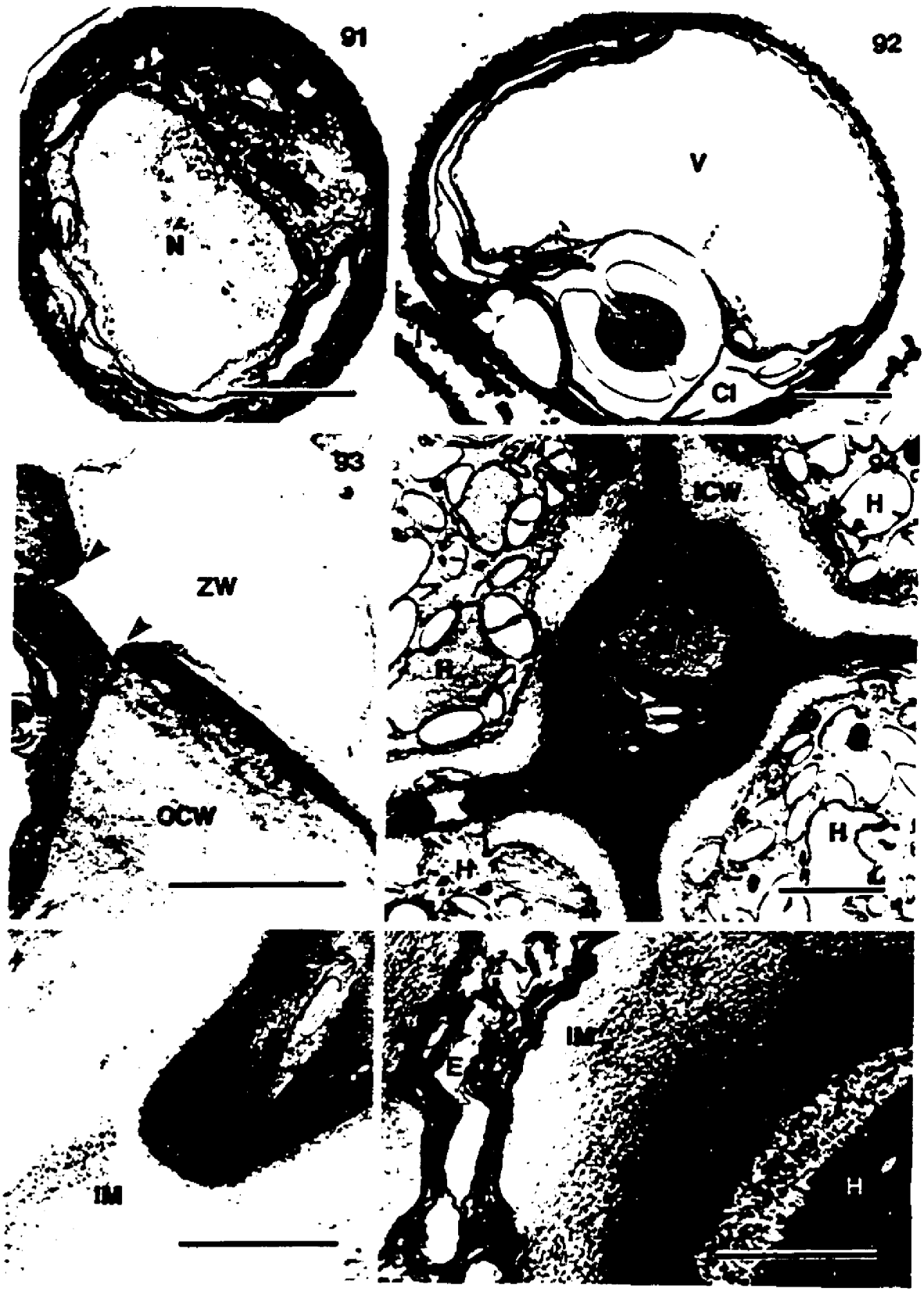

91
N
92
V
Cl
93
ZW
OCW
ICW
H
H
H
IM
IM
H

Fig. 97. Cross-section of an endophytic filament (E) embedded in the intercellular matrix (IM) at the inner cortex, with a highly contorted profile. Projections involve both cell wall and cytoplasm of the endophyte. Scale= 1 μm

Fig. 98. Endophytic filament (E) splitting a cortical host cell (H). Scale= 2 μm

Fig. 99. Endophytic filament (E) breaching the immediate cell wall (arrowheads), and the affected host cell (H) appears severely damaged, with no organelles clearly recognizable. The host cell immediately above, however, shows no indication of organelle degeneration. Scale= 2 μm

Fig. 100. Section parallel to the surface, showing the effect of compression on two host cortical cells (H) by an enlarged endophytic cell (E). Scale= 2 μm

Fig. 101. Disruption of the outer cell wall (arrowhead) of the host by the localized growth of the endophyte. A superficial endophytic cell (E) has degenerated and lysed and most of its content evacuated. Host cells surrounding the infection are structurally unaffected. Scale= 5 μm

Fig. 102. Scanning electron micrograph of a papule similar to the one in Fig. 101. Scale= 50 μm

Fig. 103. As Fig. 102, but viewed from above to show the cellular disruption (arrowheads) at the center of the papule. Scale= 50 μm

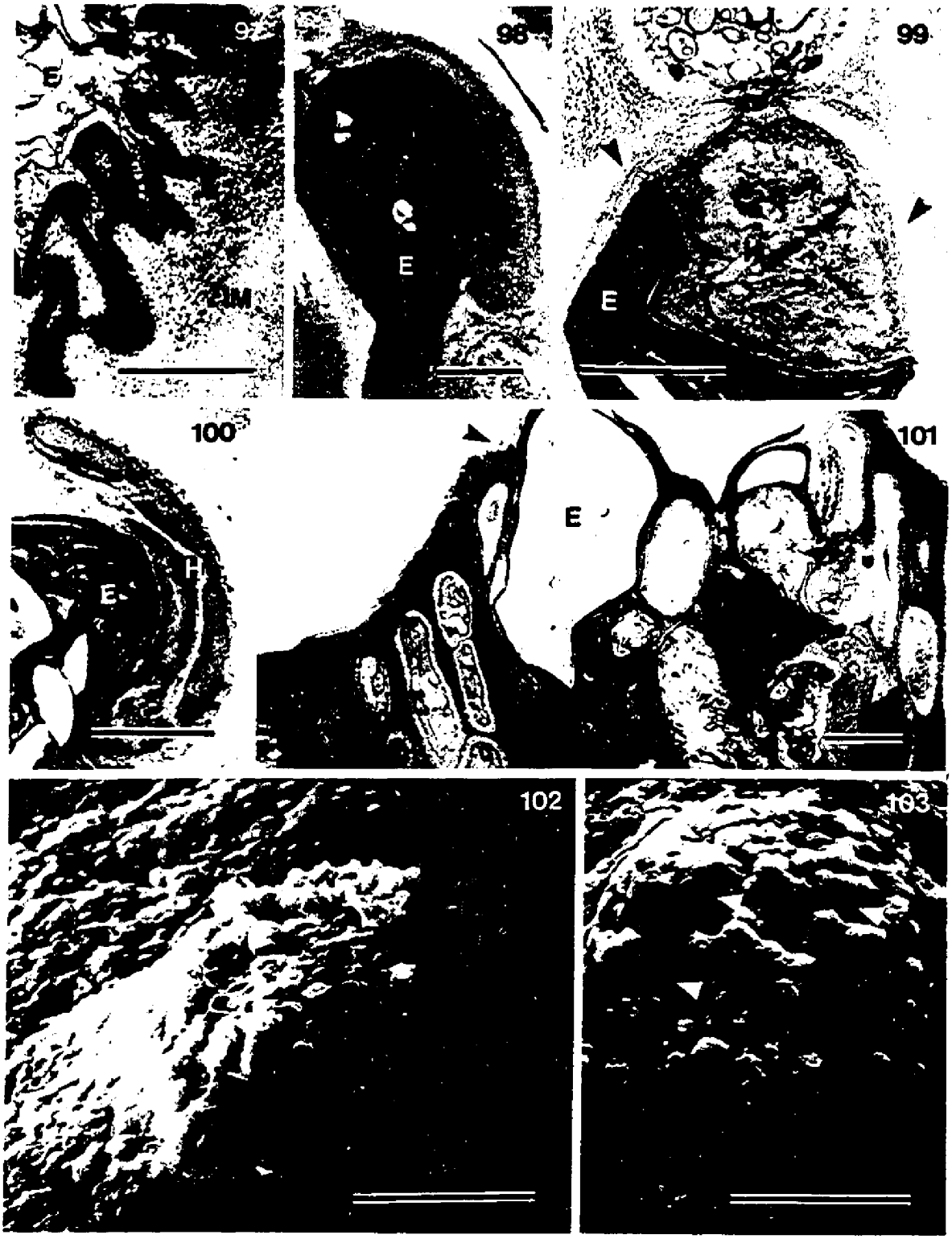
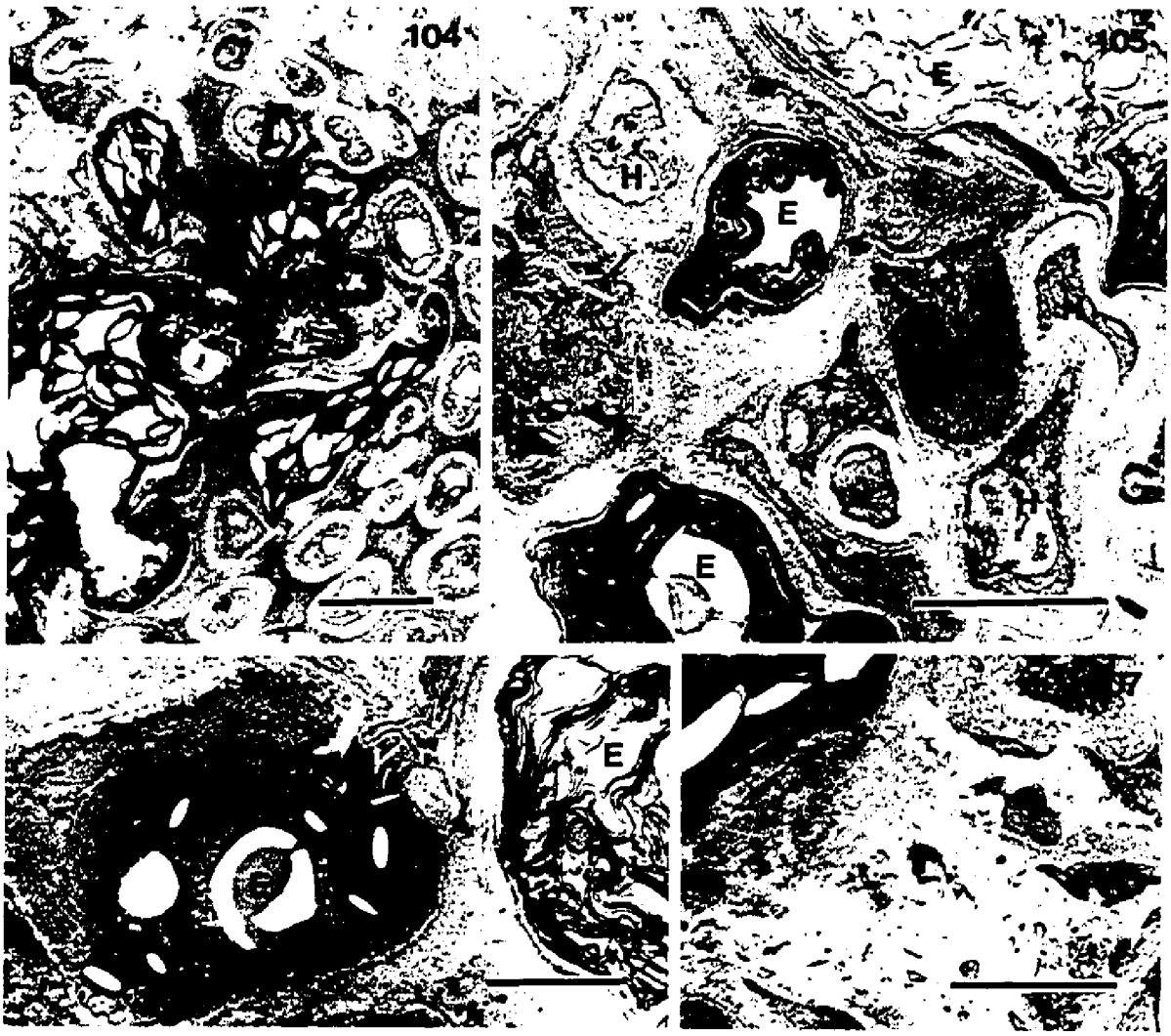

97
E
M
98
E
99
E
100
E
H
101
E
102
103

Fig. 104. Section parallel to the surface and below the lysed cells, showing the large, irregularly shaped endophytic cells surrounded by non-infected cortical tissue. Scale= 5 μm

Fig. 105. The center of the lesion, showing severely deteriorated host cells (H) surrounded by mainly structurally normal endophytic filaments (E). Scale= 5 μm

Fig. 106. Endophytic (E) and host (H) cells in advanced stage of deterioration in the center of the lesion. The starch plates and pyrenoid are the only recognizable structures of the endophytic cell. Compare with the normal endophytic cell to the right. Scale= 2 μm

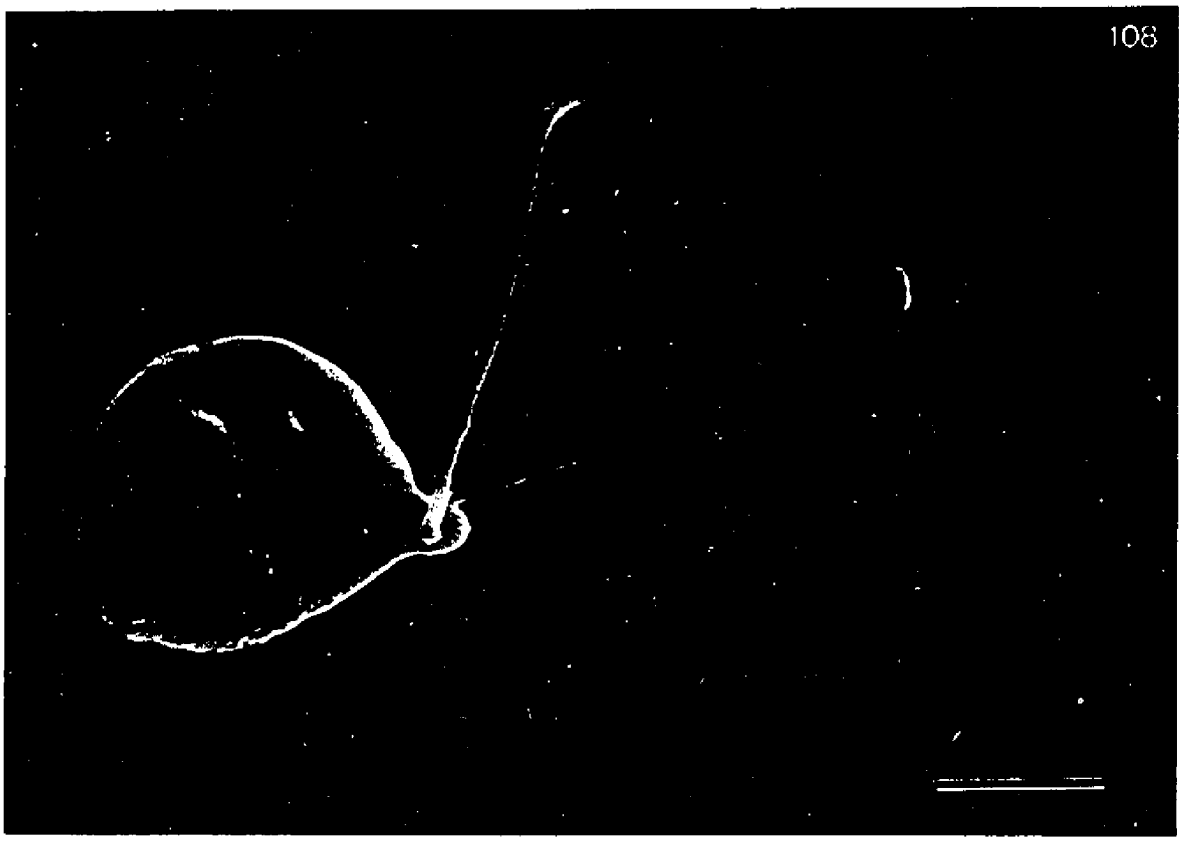
Fig. 107. Area of digested tissue, at the center of the lesion, after host cell fragmentation. Scale= 2 μm


104
105
E
H
E
E
H
E

Figs 108-123. Infection of sporophytic *C. crispus* by *A. heteroclada.*

Fig. 108. Zoospore with four flagella attached to the anterior papilla. Scale= 2 μm

Fig. 109. Zoospore with six flagella which appear to decrease in diameter towards the tip. Scale= 2 μm


108


109

Fig. 110. Section through a germling, resting on the multilamellar outer cell wall (OCW), prior to penetration of the host. A parietal chloroplast (Cl), with an external double membrane (arrowhead) occupies most of the space. The pyrenoid (Py) is divided by a single thylakoid, and it is surrounded by two hemispheric starch plates (SP). Mitochondria (m) and endoplasmic reticulum are present throughout the cell, and the latter organelle can be seen continuous with the double membrane (arrow) of the nucleus (N). A continuous cell wall, bilayered in some areas, surrounds the entire germling. Scale= 1 μm

110

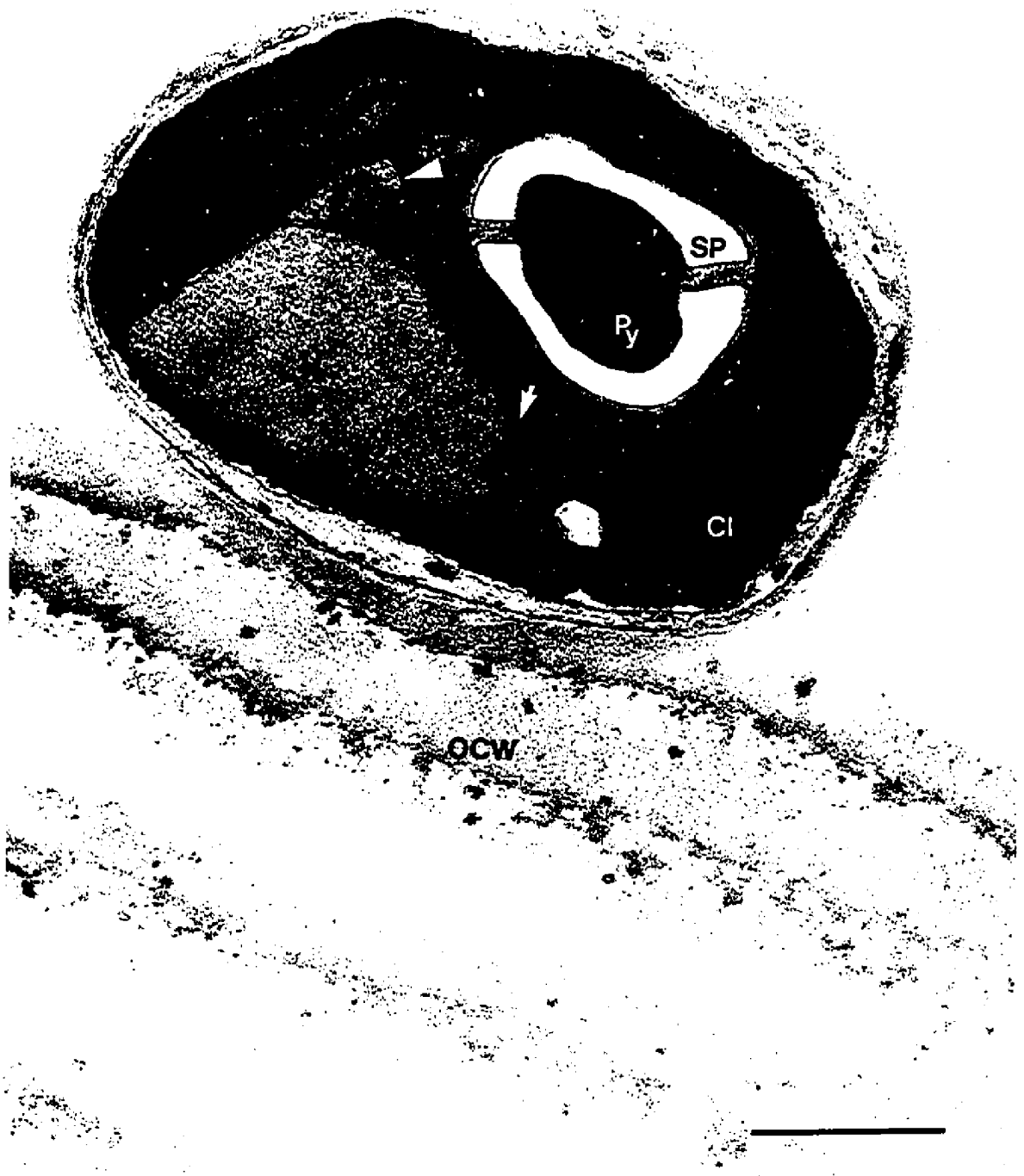


Fig. 111. Penetration of the cuticle with clear inwards bending of the lamellae (arrows). Nucleus (N) begins to migrate along the germination tube. Scale= 1 μm

111

Fig. 112. A filamentous germling (E) has completed penetration of the cuticle and the advancing cells have reached and penetrated the homogeneous zone of the outer cell wall (OCW). The cell located in the multilamellar zone appears mostly depleted of organelles. Scale= 2 μm

Fig. 113. Enlargement of Fig. 112 to show plasmalemma (arrowhead) outlining the lumen of the otherwise empty endophytic cell (E). Scale= 0.5 μm

Fig. 114. Host-endophyte interface during the stage of outer cell wall (OCW) colonization by the endophyte (E). The three host cells are separated from the endophytic filament by a thin layer of outer cell wall and their individual immediate cell wall (arrowheads). No alteration of the organelles in the host cell, including nucleus (N), mitochondria (m) and chloroplasts (Cl) has yet occurred. Scale= 2 μm

Fig. 115. Beginning of the cortical penetration, through the intercellular matrix (IM). Penetrating endophytic cell (E) contains most organelles including nucleus (N) and chloroplast (Cl) at the proximal end, leaving a vacuolated apical end. A nearby host cell (H) shows advanced degenerative changes, and although the immediate cell wall (ICW) remains distinctive, no membrane-bound organelles can be recognized. Scale= 2 μm

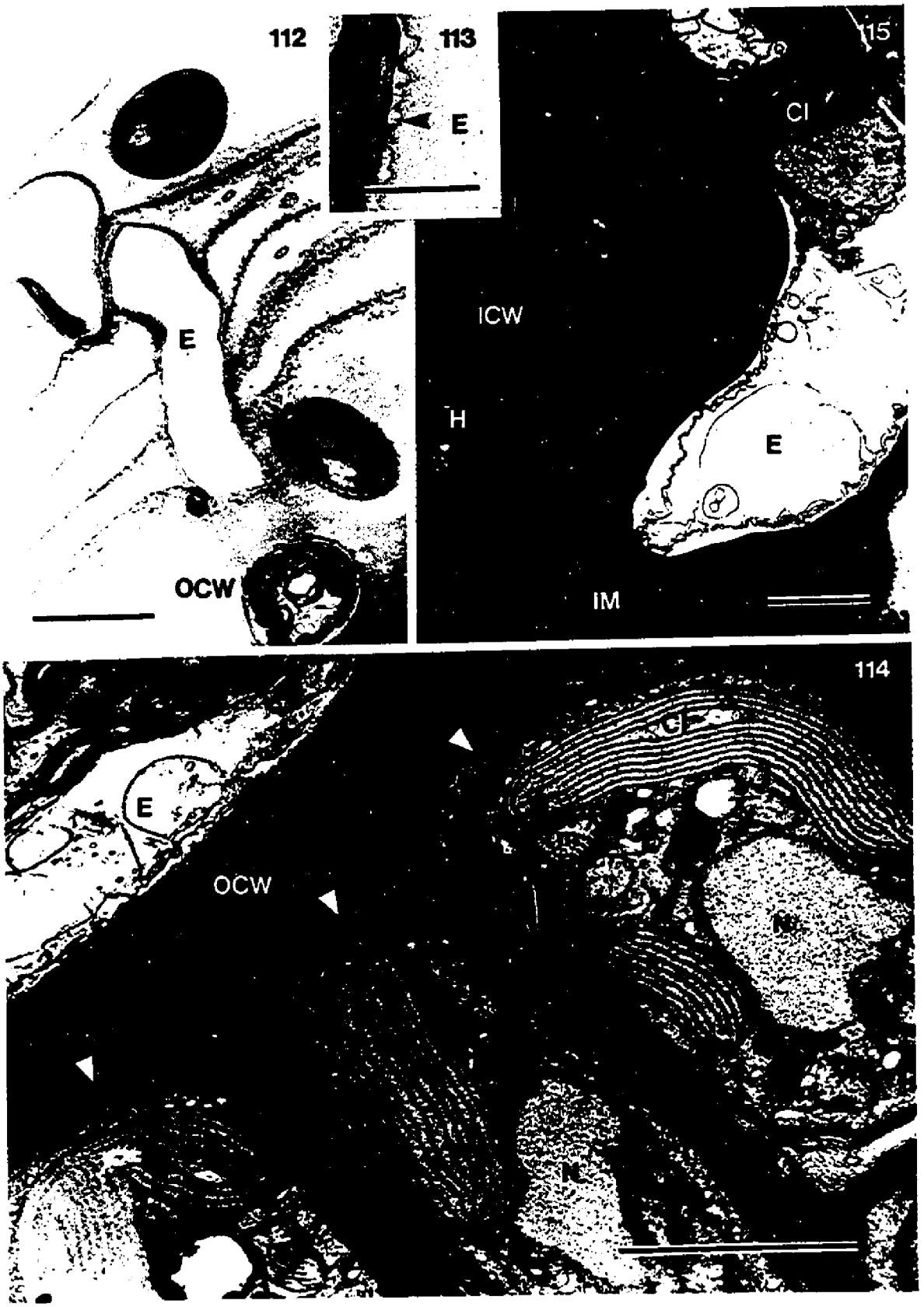

112
E
OCW
113
E
115
Cl
ICW
H
E
IM
114
E
OCW
C
N
N

Fig. 116. Massive penetration of cortical tissue. Host cell alteration is evident, as they appear elongated and lacking of internal structure. Subapical (arrowheads) and apical (arrows) endophytic cells are recognizable. Scale= 5 μm

Fig. 117. Detail of the tip of an apical endophytic cell, embedded in the host cortical tissue, showing cytoplasm with abundant membranous structures. Some of these membranes appear continuous with the plasmalemma and filled with electron dense granules (arrow). Scale= 2 μm

Fig. 118. Host-endophyte interface at the tip of an apical endophytic cell (*i.e.* Fig. 117). Electron dense granules appear to migrate from the lumen of the vesicle (Ve) to the innerside of the endophyte cell wall to which they fuse. A narrow area of the intercellular matrix has lost its microfibrillar structure (arrowheads), and no evidence of deformation of the remaining intercellular matrix can be seen. Loss of the microfibrillar organization appears associated with electron dense deposits, some of them located at the outer side cell wall of the endophyte, others at some distance from the tip (arrows), where a "trail" of digested intercellular matrix is apparent. Scale= 0.5 μm

Fig. 119. Two host cortical cells (H) in the vicinity of an infecting filament (E). Compression has greatly reduced the cell diameter to barely larger than the hardly recognizable nucleus (N). Breaking down of the organelles has resulted in an homogeneously stained cytoplasm. The beginning of host cell fragmentation is seen (arrow). Scale= 2 μm

Fig. 120. Sporangium mother cell of *A. heteroclada*, located at the surface of a cushion-like plant, displaying a cup-shaped chloroplast with numerous starch granules, and a basal nucleus. Scale= 5 μm

Fig. 121. Enlargement of Fig. 120 to show the spatial arrangement of organelles in the cytoplasm of the sporangium mother cell. Golgi bodies (G) adopt a circular arrangement between the apical chloroplast (Cl) and

the basal nucleus (N). Mitochondrial profiles appear scattered throughout the cytoplasm. Scale= 2 μm

Fig. 122. The Golgi area of the sporangial mother cell showing the highly developed endoplasmic reticulum. Scale= 0.5 μm

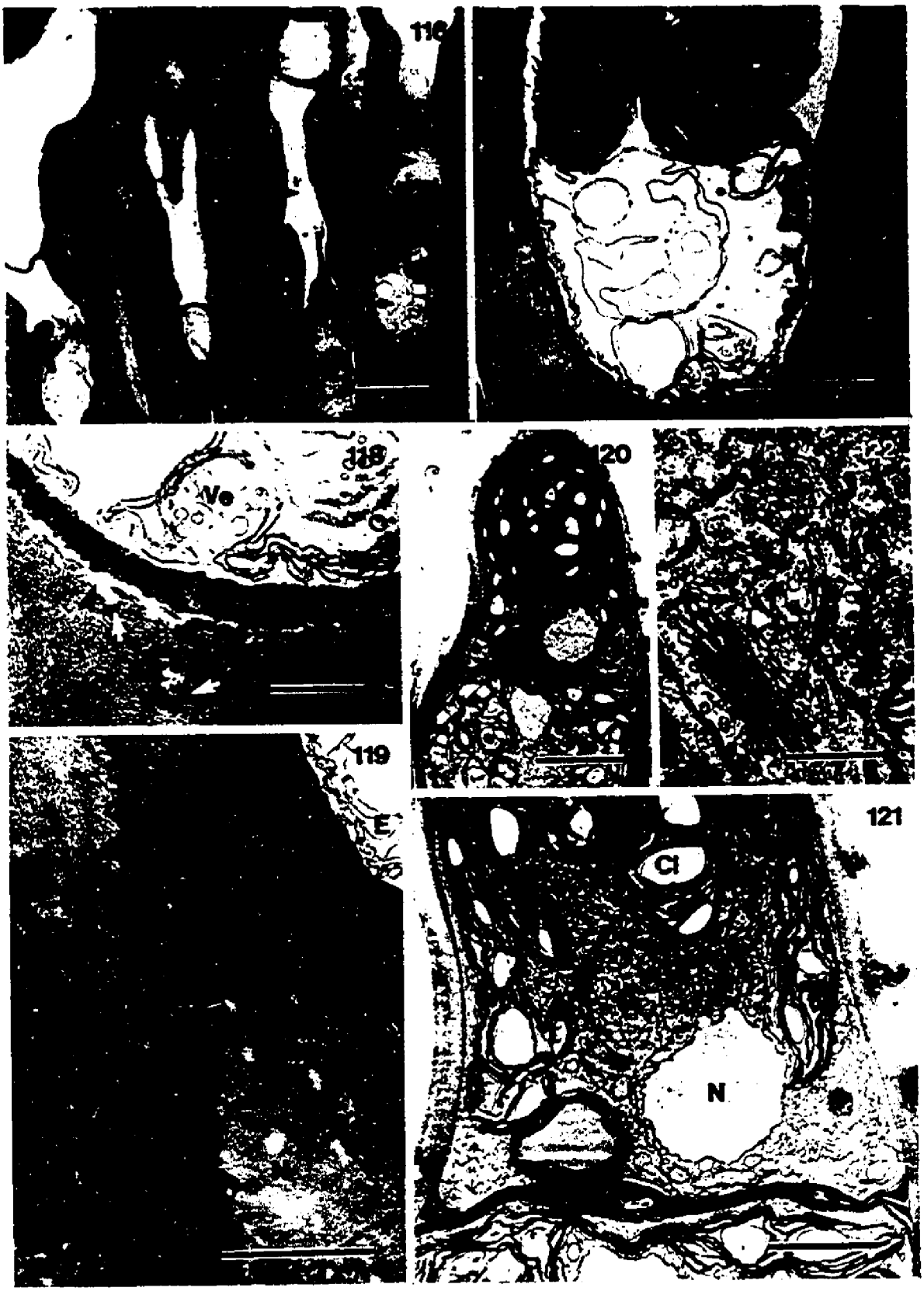
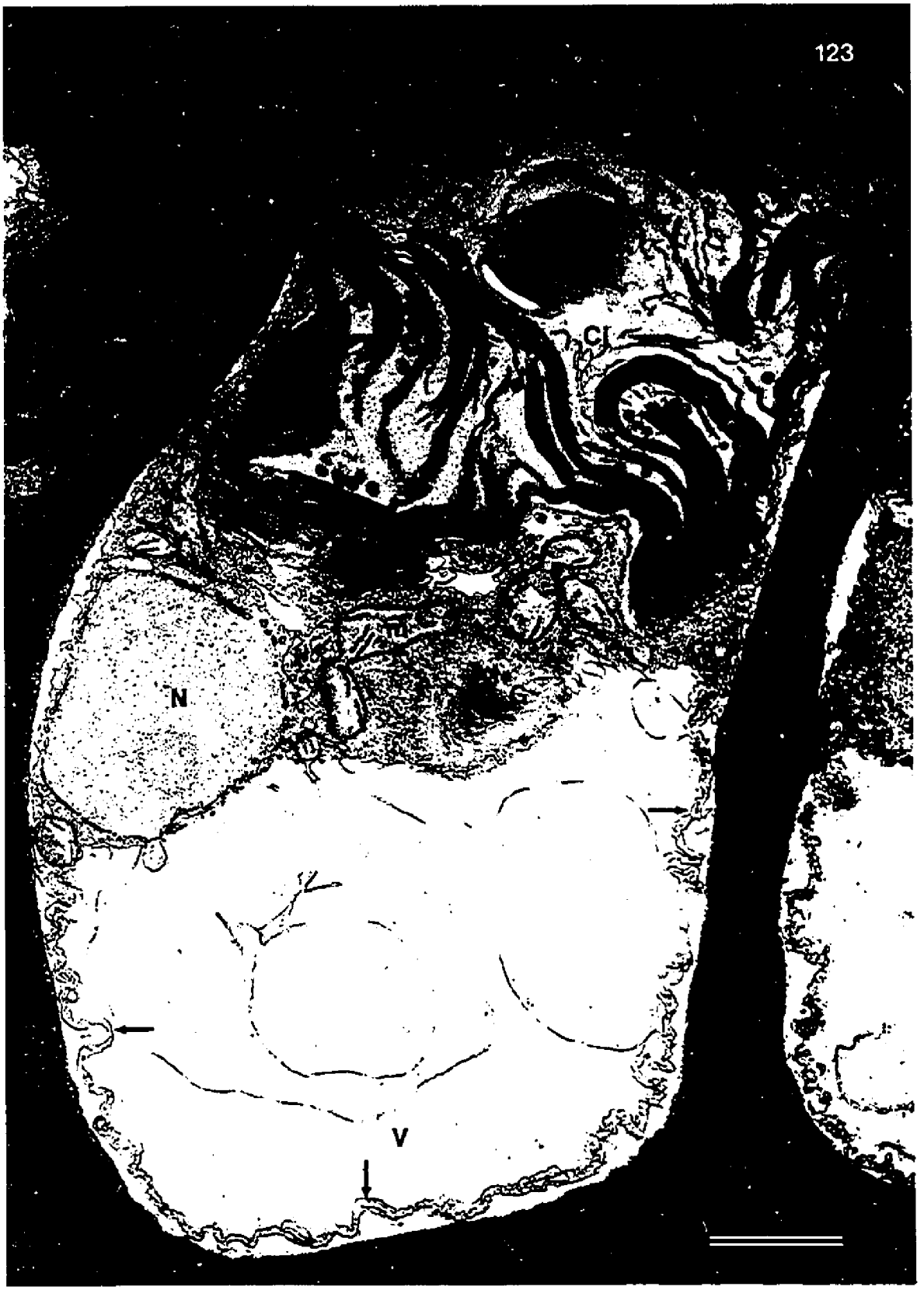

116
118
Ve
120
122
119
E
121
Cl
N

Fig. 123. Vegetative cell of *A. heteroclada,* at the center of the cushion. Most organelles, including chloroplast (Cl), nucleus (N), mitochondria (m) and Golgi (G), are displaced to the upper portion of the cell by a large, convoluted vacuole (V). The latter structure is intimately associated with the plasmalemma, giving the impression of a double membrane (arrows). Scale= 1 μm


123
N
V

CHAPTER 3

EFFECTS OF INFECTIONS ON PERFORMANCE OF THE HOST

INTRODUCTION

Algae serving as hosts for a large diversity of organism are common in marine habitats. By far, the majority of these organisms colonize the surface of the hosts (Ducker and Knox 1984, Wahl 1989). The effect upon their hosts will certainly depend on a number of aspects, including the nature of the epibionts, whether animal, other algae, bacteria, *etc.*, the densities at which they occur and parts of the host being affected. It appears, however, that algal individuals supporting epiphytes are disadvantaged compared to those that do not (Wahl 1989).

It has been suggested that hosts, including algae and sea grasses, might benefit from their epiphytes by deriving nutrients, either directly or indirectly (Linskens 1963, Harlin 1973b, Ducker and Knox 1984). Water retained by epiphytes could retard desiccation rates of their intertidal hosts (Penhale and Smith 1977). The list of potential disadvantages, on the other hand, is much larger and includes weight added to the host (Oswald and Seed 1986), alteration of mechanical properties (*sensu* Denny 1988) of the thallus (Dixon *et al.* 1981) including surface friction and drag (Lilly 1968, Lubchenco 1983, D'Antonio 1985), chemical changes at the host-epiphyte interface (Terry and Edyvean 1981), shading (Sand-Jensen 1977, Bulthuis and Woelkerling 1983, Sand-Jensen and Revsbech 1987), and competition for nutrients (Libes 1986, Sand-Jensen 1977, Sand-Jensen and Revsbech 1987). As Wahl (1989) indicated, if host and epibiont have the same trophic requirements, the epibionts will act as a filter, reducing the amount of resource, whatever it is, that reaches the host. Some of these aspects, especially the role that shading by epiphytes may have on growth of the hosts, has been discussed in a recent review on the physiological basis for competition among marine macroalgae (Carpenter 1990). Additional negative effects on the host may be caused by "accidental" grazing by herbivores eating epiphytes (Shacklock and Doyle 1983, Bronmark 1985, D'Antonio 1985, Brawley and Fei 1987).

Macroalgae may also host a variety of algal endophytes, some of which are known to obtain nutrients from their hosts and therefore considered parasites (Evans *et al.* 1978, Goff 1982a). Others, with well developed pigmentation and including species of Chlorophyta, Phaeophyta

and Rhodophyta, appear to be carbon independent. Regardless of the nature of the nutrition of the endophyte, little is known about how these symbionts affect the hosts at an organismic level. As outlined in the previous chapters, research on parasitic associations has concentrated on assessing changes of the host at the cellular level (Kugrens and West 1973a, Goff 1976), or in detecting translocation of substances between the partners (Evans *et al* . 1973, Callow *et al* . 1979, Goff 1979a, Court 1980, Kremer 1983). This lack of information has resulted in the general impression that parasitic red algae are not detrimental to the host (Martin and Pocock 1953, Goff 1982, 1983), and is supported by data from the only carbon-budget study on an algal parasitic system (Kremer 1983). It was suggested that infection by even several individuals of the parasitic *H. mirabilis* does not affect the vitality of the mature host, *R. confervoides* by reducing its energetic resources. There is, however, information indicating that parasitized hosts are less vigorous, have fewer growing tips and are more susceptible to removal from the population (Richards 1891, in Goff 1976). Furthermore, Goff (1976) noted that local tissue destruction after infection of *O. floccosa* by *H. mirabilis* resulted in hollow branches which likely caused debilitation of the frond. The only two studies where experiments have been specifically designed to evaluate the effect of parasitic infections on the host showed that infections reduced growth rates and elongation of the hosts (Nonomura 1979, Apt 1984).

Our knowledge regarding effects of endophytic infections on algal hosts, when the endophytes are pigmented algae is even more incomplete than for parasitic associations. Most if not all studies have focussed on life history and morphology, attempting to elucidate the usually complex taxonomic status of these cryptic green (South 1968, Yarish 1975, Nielsen 1979, O'Kelly and Yarish 1981, O'Kelly 1982, 1983, Nielsen and McLachlan 1986, Iima and Tatewaki 1987, Kogame and Yoshida 1988), red (White and Boney 1969, 1970; Garbary 1979b, Tam *et al.* 1987), and brown (Searles and Leister 1980, Pedersen 1984) species. Casual comments in those papers seem to indicate that at last some acrochaetioid endophytes do not interfere with the host (Tam *et al.* 1987). Brown ectocarpoid endophytes, however, were able to emerge from inner tissues and overgrew pieces of *Laminaria digitata* (Hudson) Lamouroux in culture (Russell 1983), suggesting that under some condition, pigmented

endophytes are detrimental to the host. Brown streblonemoid endophytes have been found associated with (Andrews 1977), and proven to be the cause of (Apt 1988a), algal galls. The commercially valuable brown alga *Undaria* sp has been reported to host *Streblonema*-like endophytes, which turn the infected areas thicker and coarser than the rest of the frond (Yoshida and Akiyama 1979). In heavy infections the quality of *Undaria* as food is lowered, and the endophyte is considered a pathogen.

The rhodophycean *C. crispus* hosts a number of brown and green endophytes, although no red species have yet been reported (Nielsen and McLachlan 1986, Correa *et al.* 1987). Field observations indicate that the frequency of endophytic infection in *C. crispus* is high (Correa *et al.* 1987), and that numerous broken fronds are also heavily infected. Furthermore, laboratory-infected fronds of *C. crispus* undergo significant cellular damage during infection by either *A. operculata* or *A. heteroclada*, the two most common green endophytes recorded so far. Taking this into consideration, it seems reasonable to expect a negative effect of the infection by endophytes upon *C. crispus*. In this chapter, I address the following questions in an attempt to quantify such effects:

1. Does the observed cellular damage translate into a poor performance (biomass accumulation, carrageenan yield, and regeneration capacity) of the host when grown under conditions of favorable growth?
2. Is "toughness" of the frond affected?
3. Are feeding responses of crustacean grazers on *C. crispus* modified by endophytic infections?

MATERIALS AND METHODS

All material used in this study, except *Ulva* sp., came from stock cultures of either non infected sporophytic or gametophytic *C. crispus* (isolate BH, Chapter 1), or material of the same host isolate infected by *A. operculata* (isolate PF1a 161085) or *A. heteroclada* (isolate PC2V 300585). These stock cultures were being mantained at the National Research Council (NRC) in Halifax, and in the case of *Ulva* sp., the material was provided by L. Staples (NRC Aquaculture Research Station, Finck Cove).

Effect of infection on biomass accumulation and carrageenan yield

Culture experiments were done under controlled laboratory conditions to assess effects of infections by *A. operculata* and *A. heteroclada* on biomass accumulation in sporophytic and gametophytic fronds of *C. crispus*. A total of four combinations, each consisting of one species of endophyte infecting one phase of the host, were done at different times. Infected material was obtained from stock cultures that had been inoculated at least 6 wk prior to the experiment, but maintained under conditions of slow growth: 8-10 ^{o}C, less than 10 µmol m^{-2} s^{-1} photon flux density (PFD), and a light period of 12 h d^{-1}. Apical fragments of 5-10 mm in length were excised from the tip of the fronds. These fragments had estimated endophytic densities of less than 500 cells/mm^2 in the *A. operculata*-sporophyte combination. A different procedure had to be followed with the *A. operculata*-gametophyte combination, as the endophyte remained localized near the penetration site (see Chapter 2). Areas with the heaviest infection, usually more 50% of the host surface occupied by coalescing lesions, were selected from the stock cultures. As these heavy infections were always located at the base of large fronds, excised fragments did not have an apical meristem. These fragments were incubated at 45-50 µmol m^{-2} s^{-1}, 15 ^{o}C, and 12 h d^{-1} to allow regeneration of the apical meristem. No quantification of the density of infection was attempted in the *A. heteroclada* combinations, owing to the development of polystromatic endophytic plants (Correa *et al.* 1988, Chapter 2). In these cases, the excised apical fragments had infections concentrated in at basal region (~3 mm above the cutting edge). The

density of infection was estimated at less than 20% of the surface of the host occupied by *A. heteroclada*. The apical areas, including the meristem, were free from endophytic filaments.

When infected material was ready, each apex was blotted, weighed, and transferred into a numbered 250 ml flask containing 175 mL of SFC culture medium. Thirty infected apices were used in each host-endophyte combination. Controls consisted of apices from non-infected fronds, or for the *A. operculata*-gametophyte combination, apices regenerated from basal portions of non infected gametophytic fronds. Each infected apex was paired with a control piece, having similar shape and wet weight and also growing in an individual flask. The flasks, 60 for each host-endophyte combination, were incubated on a single shelf, in a growth chamber programmed at 15 °C, 40-50 μmol m^{-2} s^{-1} PFD, and a light period of 18 h d^{-1}. Culture medium was changed every 10 d and increases in blotted wet weight were recorded every 20 d. The position of the paired flasks on the shelf was rotated at 10-d intervals to minimize unevenness in illumination. At the end of the experiments, the fragments were individually dried at 70 °C, to constant weight and stored.

The biomass accumulated at each time interval (t), 20, 40, and 60 d, was calculated for individual apices using the expression $(W_t-W_0) / W_0$, where W_0 is the wet weight of a given apex at the beginning of the experiment and W_t is the wet weight of the same apex at time (t). This was done to normalize differences in initial weights between replicates. The means were positively correlated with the variance, and the latter were not homogeneous as indicated by the F_s test (Sokal and Rohlf 1981). Independence of the variances from the means and homocedasticity of variances were achieved by logarithmic transformation, after which t-test for paired observations was applied (Sokal and Rohlf 1981).

For the extraction of carrageenan, apices were dried overnight at 60 °C under vacuum, and pooled in groups to at least 300 mg dry weight. This is the minimum weight recommended by Craigie and Leigh (1978) for carrageenan analysis. Samples were then ground in a ball-mill until a homogeneous powder was produced (30 s), reweighed, and processed according to Craigie and Leigh (1978). The white fibrillar product obtained at the end of the procedure represented the total carrageenan. This material was dried overnight at 60 °C under vacuum, and weighed.

Carrageenan yield was calculated by dividing the total carrageenan by the weight of the sample before extraction and expressed as a percentage.

Effect of infection on regeneration

Fragments of about 16 mm^2 were excised from three areas along large sporophytic fronds infected with *A. operculata*. Fragments from the base were heavily infected (>2000 endophytic cells/ mm^2 of host), whereas those from the central portions were moderately infected (500-1500 endophytic cells/mm^2); fragments from the apical regions were less densely infected (<100 endophytic cell/mm^2). It should be noted that in fragments considered heavily infected, precautions were taken to avoid using fragments where most of the cortex of the host had been replaced. Although more than 2000 endophytic cells/mm^2 could be counted in those fragments, there was still recognizable, fully pigmented host cortical tissue. All procedures were done under sterile conditions. Fragments were transferred to 250 mL aeration flasks containing 200 mL of SFC medium, and cultured in triplicate at 15 °C, 40-50 µmol m^{-2} s^{-1} PFD, and a light period of 16 h d^{-1}. The experiments lasted 30 d, with the culture medium changed weekly. Fragments of approximately the same size, and excised from equivalent areas, were obtained from non-infected sporophytic fronds and used as controls. Control fragments were cultured in parallel with the infected fragments. At the end of the experiment, each fragment was observed under a dissection microscope and scored as positive regeneration if at least one upright emerged at any position on the fragment.

The pattern of development of *A. operculata* in the gametophytic phase of *C. crispus* (see Chapter 2) did not permit a reasonably accurate estimate of the number of endophytic cells per unit area of host surface. Consequently, infected fragments were subjectively classified as heavily infected (> 50% of the surface occupied by coalescing lesions), or lightly infected (< 10% of the surface occupied by lesions). Those fragments with more than 10% but less than 50 % of their surface affected, were classified as moderately infected. Controls were obtained from non-infected gametophytic fronds. Control and infected fragments were subjected to the same experimental procedure outlined for the *A. operculata*-sporophyte association.

As described in Chapter 2, *A. heteroclada* develops initially within the outer cell wall of the host, with little penetration into the cortex during this early stage. Furthermore, soon after spreading begins, the endophyte becomes polystromatic (Correa *et al.* 1988). Consequently, I considered it more meaningful to classify the level of infection subjectively, according to microscopic observations of the surface of the fragments. Fifty percent or more of the surface occupied by *A. heteroclada* was considered heavy infection, less than 10% was light infection, and intermediate values were classified as moderate infection. As there are no differences between sporophytic and gametophytic fronds regarding the pattern of infection by *A. heteroclada,* the same criterion was used in both cases. Non-infected control fragments of the two phases were obtained from unialgal stock cultures as outlined above. The rest of the experimental procedure followed that described for the *A. operculata*-sporophyte association.

Effect of infection on toughness

For purposes of my study, toughness is defined as the degree of resistance of tissue to a penetrating device. To assess whether endophytic infections could alter the normal toughness of *C. crispus* by softening the tissues, an apparatus was used following the principles outlined in Littler and Littler (1980). It consisted of a gelometer, modified by J.S. Craigie (NRC, Halifax), based on an earlier model described by Goring (1956), to which a special sample holder (Fig. 1) was adapted. The sample holder consisted of a cylindrical base of solid acrylic with a cylindrical hole, 1.5x10 mm, drilled in the center. A lid was made of the same material and also had a 1.5 mm perforation in the center. Samples were held between the base and the lid. The latter was secured by two bronze screws providing an even and gentle pressure on the tissue. The gelometer had a cylindrical plunger, 1 mm in diameter, that descended at a constant speed until it perforated the sample. The movement of the plunger was electronically controlled at 0.36 mm s^{-1}, and the pressure exerted upon the sample was detected by a load cell, calibrated to either 100 or 500 g, hooked to a chart recorder.

Measurements were not made near the borders of the samples, and when more than one measurement was obtained from a single sample, these were at least 3 mm apart. In those instances, samples were

transferred into a Petri dish with fresh SFC medium between readings, to prevent desiccation.

During preliminary trials, it became apparent that variability in the values mainly resulted from variability in the thickness of the fronds, making the reading units (g/mm^2) meaningless. To normalize the values, perforated samples were immediately sectioned by hand (10-20 µm thick) in the vicinity of each perforation. Sections were then mounted in a drop of SFC medium and their thickness measured under the microscope. During the passage through the sample, the plunger did not tear it apart, but it pushed the tissue in such a way that a disc of ~1 mm in diameter was removed from the frond. Knowing the diameter of the disc and the thickness at the perforation point the values are expressed as g/mm^3.

The effect of *A. operculata* on toughness of sporophytic tissue was assessed by testing fragments of fronds with different densities of infection. Light and heavily infected fragments were selected using the same criterion as for experiments on regeneration. Terminal infection was also tested by including fragments whose cortex had been replaced by endophytic cells, discernable by observation with a dissection microscope (dark field, 50X). For comparison with toughness measurements in infected tissue, values of toughness were obtained from non-infected fronds. All fronds used as source of infected and non-infected tissue had a preconditioning period of at least 3 wk at 15 °C, 40-50 µmol m^{-2} s^{-1} PFD, and a light period of 16 h d^{-1}. SFC medium was changed every 5 d.

The effect of *A. heteroclada* infection was assessed for toughness on both sporophytic and gametophytic fronds of *C. crispus*. Both infected and non-infected material used in these observations was preconditioned for at least 3 wk, as were fronds infected by *A. operculata*. Infected fronds of the two phases had been incubated for at least a month with the endophyte under conditions of slow growth (see above). Shortly before performing the measurements, infected material was monitored using hand sections and light microscopy to assess the stage of the infection. Microscopic observations revealed that infections were at the point where heavy penetration of the cortex had begun. This material did not show signs of abnormal softness characteristic of more advanced infections associated with secondary infections by bacteria.

In an attempt to compare information obtained from laboratory grown *C. crispus* with wild fronds, a single collection was made in October 1989 at Finck Cove, Nova Scotia. Fronds broken at some point above the first dichotomy were collected from the intertidal population of *C. crispus* and transported to the laboratory on ice. Intact fronds were also collected in the vicinity of the broken fronds. Measurements on these fronds were done within 2 h of collection. Holes in the broken fronds were punched only in a narrow zone, 3-5 mm below the broken margins. Measurements on the intact fronds were performed in the central portion, above the first dichotomy.

Statistical analysis consisted of t-test of the differences between two means from large samples, or by a single classification ANOVA (model I) with unequal sample size followed by a Tukey-Kramer test for multiple comparisons among pairs of means (Sokal and Rohlf 1981). Prior to these analyses, homocedasticity using either the Fs ratio for testing the significance of differences between two variances, or the F max-test for more than two variances, were performed. No transformations of data were required as graphic tests indicated normality (Sokal and Rohlf 1981).

Effect of infection on feeding responses

In this part of my study I attempted to evaluate how infection by *A. operculata* and *A. heteroclada* modified the feeding responses of two common crustacean herbivores, *Gammarus oceanicus* Segerstråle and *Idotea baltica* Pallas. The animals were collected at the NRC Aquaculture Research Station (Finck Cove) from tanks of *C. crispus*. These animals are always present in the tanks, introduced into the culture system with incoming water. Alternatively, they were provided by P.S. Shacklock from his own experimental stocks of animals.

Two aspects of the feeding responses received particular attention.

1. Food preferences: These consisted of offering potential foods simultaneously to individual grazers so they could express a choice without interference from other individuals (Peterson and Renaud 1989). The general procedure consisted in pretreating the animals with an abundant diet of the same combination of plants to be used in the experiments, at least 24 h in advance, to provide a similar short-term feeding history. Consequently, animals were not starved before the experiments. Although

generally used in feeding experiments, starvation produces undesirable trends where preferences for items generally consumed poorly or not at all is increased. Furthermore, *I. baltica* was particularly sensitive to the absence of food, eating each other shortly after all food had been removed. After the pretreatment, each animal (8-12 mm long) was offered a pair of fronds of similar blotted wet weights (difference less than 2%), each representing a different food item (excepting the 3-choice experiment). The experiments were done in 250 mL flasks with aeration and containing 175 mL of 0.45 µm filtered seawater. Replicate flasks containing pairs of algae, similar to the experimental combination of food items but without herbivores (control), were incorporated into the design to quantify autogenic changes in the algae. Experimental and control replicates, covered by black plastic bags, were left for 24 h in a growth chamber programmed at 20 °C (optimum feeding temperature, J.S. Craigie in verb.).

The following algal combinations were tested for preference by *I. baltica:*

a) Non-infected sporophytic *C. crispus* vs. non-infected gametophytic *C. crispus.*

b) Non-infected sporophytic *C. crispus* vs. *Ulva* sp.

c) Non-infected gametophytic *C. crispus* vs. *Ulva* sp.

d) Non-infected sporophytic *C. crispus* vs. sporophytic *C. crispus* infected by *A. operculata.*

e) Sporophytic *C. crispus* infected by *A. operculata* vs. *Ulva* sp.

f) Sporophytic *C. crispus* infected by *A. operculata* vs. non-infected sporophytic and gametophytic *C. crispus* (3-choice experiment).

g) Non-infected gametophytic *C. crispus* vs. gametophytic *C. crispus* infected by *A. heteroclada.*

h) Non-infected sporophytic *C. crispus* vs. sporophytic *C. crispus* infected by *A. heteroclada.*

Fragments remaining from the 3-choice experiment were washed in several rinses of SFC medium, and incubated for one month in individual 250 mL flasks containing 175 mL of medium changed weekly. Conditions of culture were 15 °C, 16 h light d^{-1} and 40-50 µmol m^{-2} s^{-1} PFD. The progress of regeneration or decay in each fragment was monitored and recorded.

The combinations d and g were also tested with *G. oceanicus*, following the same procedure described for *I. baltica* trials.

At the end of each trial, experimental and control fronds were blotted and their weight recorded. The difference in weight, whether gained or lost, was expressed as a percentage of the original weight (algal mass removed or gained). Statistical analysis was performed by t-tests for each food combination, comparing the differences between mean values of biomass changes in flasks with herbivores and mean values due to autogenic changes, according to the procedure developed by Peterson and Renaud (1989). For the 3-choice experiment, t-tests were performed on all possible combinations.

2. Palatability: this was the second aspect studied in relation to the feeding behavior of *I. baltica* and *G. oceanicus,* which consisted of allowing individual grazers to feed for a constant time on individually offered foods. Thus, feeding rates of a grazer on alternative foods items can compared as they are independent (Peterson and Renaud 1989). The animals went through the same pretreatment described for the experiments on preference, after which they were randomly assigned to flasks containing either infected or non-infected fronds. Controls without grazers were included to assess autogenic changes of the tested algae. Two combinations of food were used:

a) Gametophytic *C. crispus* infected by *A. heteroclada* vs. non-infected gametophytic *C. crispus.*

b) Sporophytic *C. crispus* infected by *A. operculata* vs. non-infected sporophytic *C. crispus.*

Experiments were done under the same conditions as the preference experiments and each trial lasted 24 h. The pieces were blotted, weighted, and the values corrected for autogenic changes in the controls. Palatability was expressed as mg of alga consumed per mg of grazer per day. Comparisons for the differences between means in each combination of food were performed by t-tests.

Finally, preliminary attempts were done to assess the presence of chemical attractants produced by either infected or non-infected sporophytic *C. crispus.* Infected fronds (500 mg wet wt) were macerated in a tissue homogenizer, and the resulting supernatant and fragments of

tissue were added to 50 mL of a 2% agar solution at a temperature just above gelification. After mixing the algal macerate with the agar, the resulting solution was poured onto a sterile plastic Petri dish and left to solidify. Similar procedure was followed with non infected fronds. An agar plate without algal material was prepared as control. The agar (Difco) used in these preparations was prewashed several times (once overnight) with excess of 0.45 μm filtered seawater, followed by several rinses in distilled water, ethanol, and ether.

Blocks of solidified agar and agar plus algal macerate were cut and transferred to plastic cylinders, 60 mm long and 10 mm wide, used as sample holders. These cylinders were made of plastic net, 0.25 mm mesh, which allowed free exchange of water, but prevented the agar fragments from protruding through the holes. Six of these sample holders were suspended by a fishing line at about 30 mm from the bottom of a plastic container filled with 15 L of 0.45 μm filtered seawater. The container was cylindrical, so sample holders hung attached to the lid in a circular fashion, about 30 mm from the walls.

Two trials were performed using *I. baltica*. In the first, three sample holders were filled with agar plus macerate of sporophytic *C. crispus* infected by *A. operculata*, and three with agar only. Twenty five individuals of *I. baltica* were added and the container was covered with a black plastic bag. The container was mantained at room temperature (~25 ^{o}C), with constant but gentle aeration. The number of grazers clasping each cylinder was recorded periodically during a 27-h period. The second experiment was run to test whether or not attractants from sporophytic *C. crispus* infected by *A. operculata* exerted a stronger effect on *I. baltica* than attractants from non-infected *C. crispus* alone. In this experiments, macerate of sporophytic *C. crispus*, infected or not, were used to prepare the agar blocks. Conditions were similar to the first experiment, and the trial lasted 21 h.

RESULTS

Effect of infection on biomass accumulation and carrageenan yield

The effect of *A. operculata* on biomass accumulation was influenced by the life-history phase of *C. crispus*. Although there was an initial period of similar accumulation of biomass in infected and control sporophytic apices, by the 40th day of the experiment infected fronds had increased, on average, only 11 times their original weight, whereas the control, non-infected apices had increased, on average, 19 times their original weight (Fig. 2). At the end of the experiment, the differences between infected and control apices became even greater, with twice the biomass accumulated in the control group (Fig. 2). Infected fronds appeared shorter and narrower than controls, although no deformations or fragmentation was observed. The differences between infected and control fronds throughout the experiment were highly significant ($p<0.001$).

In the *A. operculata*-gametophyte combination (Fig. 3), infected fronds had accumulated significantly ($p<0.001$) more biomass than controls by the 20th day of the experiment. These differences were less at the end of the experiment with average values for biomass accumulation of 67 and 74 times their original weight in control and infected thalli respectively and were not significantly different ($p>0.5$).

Chondrus crispus infected by *A. heteroclada* showed similar responses, regardless of the life-history phase (Figs 4, 5). Control apices had accumulated, by the 20th day of incubation, significantly ($p<0.001$) greater mean biomass than the infected apices in the two phases of the host, and those differences were greater by the end of the experiments (Figs 4, 5). At this stage, most fronds appeared overgrown by the endophyte, and some of them showed softening at their basal portions.

Carrageenan yield from the gametophytic fragments was not affected by infection with *A. operculata* (Fig. 6), whereas in the sporophytic phase it was 46.0% compared to 52% in the controls. Infections of both phases of the host by *A. heteroclada*, on the other hand, resulted in values of about 20% for total carrageenan yield, less than half of the amount obtained from controls (Fig. 6).

Effect of infection on regeneration

The effect of *A. operculata* on the regeneration capacity of *C. crispus* was affected by the density of the endophyte at the beginning of the experiment and was also influenced by the phase of the host (Figs 7, 8). In the sporophytic phase (Fig. 7), there was no evidence of regeneration in any of the 99 heavily infected fragments, and in most of them a characteristic deformation of the cut edges, accompanied by softening following bacterial infection, was evident. With moderate densities of infection, almost 50% of the fragments regenerated (Fig. 7), producing one or two uprights, and less than 10% showed signs of bacterial-induced softening. Interestingly, even light infection appeared to have a negative effect on regeneration, although no sign of bacterial softening was observed in these fragments. At the end of the experiment, all fragments used as control, whether from the apical, central or basal regions of the fronds, and regardless the phase of the host, had developed at least one, generally several (up to 32) upright initials. These uprights occurred along the cut edges of the fragments, from the cortical tissue, as on many occasions two rows of uprights developed from one edge, with one row issued by each exposed cortical side. In no case did bacterial infection developed to produce softening or discoloration of control fragments.

Gametophytic fragments infected by *A. operculata* at moderate and low densities showed normal regeneration, as in the controls (Fig. 8). Even in the heavy-density fronds, over 75% of the fragments regenerated (Fig. 8), with usually two or more uprights developing from the edges. While regeneration along the cut edge was usual in fragments with moderate and light infections, some of the heavily infected fragments formed uprights from any area of the cortex which did not appear affected by the endophyte. No bacterial infection developed in experimental or control fragments.

The effect of *A. heteroclada* on regeneration of *C. crispus* was also influenced by the density of the infection at the beginning of the experiment. However, in this case there were no marked differences between the two phases of the host (Figs 9, 10). Sporophytic fragments infected by *A. heteroclada* (Fig. 9) showed a regeneration pattern similar to those infected by *A. operculata.* No regeneration occurred at heavy densities of the endophyte, and most fragments were soft, indicating severe

bacterial infection, which was further evident from cortical tissue separated from the medulla. With moderate infection, more than 90% of the fragments regenerated (Fig. 9), usually with one or two (up to 12) uprights developing from the edges. With light infection, regeneration frequency was 100% and developed as in controls (Fig. 9). There were no indications of bacterial damage in fragments with moderate or light densities of *A. heteroclada.*

In gametophytic fragments infected by *A. heteroclada,* heavy infection resulted in only 5% regeneration (Fig.10). In these cases, the one or two small uprights that developed were overgrown by *A. heteroclada* at the time of observation. Softening affected less than 10% of the fragments.

Effect of infection on toughness

Infection by *A. operculata* resulted in a significant ($p<0.001$) decrease in the force required to perforate sporophytic fronds of *C. crispus* (Fig. 11, Tables 1, 2). This force was inversely related to the density of the infection, ranging from 411 g/mm^3 for fronds with light infection, a value only slightly less than non infected fronds ($0.025<p<0.05$, Table 1, Fig. 11), to less than 50 g/mm^3 in fronds with terminal infection.

The force required to perforate fronds, infected by *A. heteroclada,* of both gametophytes ($p<0.001$) and sporophytes ($p<0.001$) was significantly less compared to non-infected controls (Fig. 12). The mean values for the sporophytic phase was 431 g/mm^3 in non-infected and 214 in infected fronds. Equivalents values in gametophytes were 657 and 298 for non-infected and infected fronds respectively (Fig. 12).

Although the force required to perforate wild fronds of *C. crispus* was higher (Fig. 13) than for laboratory-grown fronds, a similar relationship was observed. Intact fronds were perforated by applying an average force of 840 g/mm^3 while less than 600 g/mm^3 ($p<0.05$) was required to perforate zones near the fracture point in broken fronds. In microscopic examination of the sections to assess thickness of the fronds, it became apparent that the testing areas in those broken fronds were heavily infected with various brown and green endophytes. Furthermore, infection was more intense in the cortex of the host and towards the fracture point. Infection of intact fronds was minor or absent.

Effect of infection on feeding responses

The buccal appendages of *I. baltica* and *G. oceanicus* (Figs 14-19) appear well adapted to tear apart and grind algal material. In *I. baltica* (Figs 14-16) the mandibles are strong and have 4-6 sharp tips (Fig. 16) which meet at the ventro-medial line (Fig. 14). At the base of the mandible, and only visible when removed from the animal, a discoid, ornamented structure (Fig. 16) serves to grind the pieces torn by the sharp external part. Adjacent appendages, like maxillae and maxillipeds have sharp claw-like chitinous projections (Fig. 15). *Gammarus oceanicus* has buccal appendages similar in general structure to those in *I. baltica* (Figs 17-19). Mandibles are prominent, with a massive base and a double row of 4-6 sharp, pointed projections (Fig. 17). Also as in *I. baltica,* an ornamented discoid structure, in this case equipped with concentrically arranged, sharp-edged rings (Fig. 18), is located at the base of each mandible. Auxiliary appendages associated with the mandibles terminate in sharp, elaborate needle-like projections (Fig. 19).

Individuals of *I. baltica* clearly preferred *Ulva* sp. over non-infected sporophytic (Fig. 20) and gametophytic (Fig. 21) *C. crispus,* and when the two life-history phases were offered, the gametophytic fronds were by far the preferred item (Fig. 22). When the alternative items were sporophytic fronds infected by *A. operculata* and non infected sporophytic fronds, the isopods preferred infected over non-infected fronds (Fig. 23). Furthermore, *I. baltica* removed more algal biomass from *A. operculata*-infected sporophytic fronds than from *Ulva* sp. (Fig. 24), although the differences were not significant ($p>0.05$). In the 3-choice experiment, a clear ranking was established from the highly preferred sporophytic fronds infected by *A. operculata* to the least preferred item, non-infected sporophytic fronds (Fig. 25). A similar response appeared to be triggered by infection of gametophytic and sporophytic fronds of *C. crispus* by *A. heteroclada* (Figs 26, 27). In both sporophytic (Fig. 26) and gametophytic (Fig. 27) trials, fronds infected by *A. heteroclada* were preferred.

The amphipod *G. oceanicus* responded similarly to *I. baltica* when facing infected versus non-infected fronds as alternative foods. This was demonstrated with gametophytic fronds infected by *A. heteroclada* (Fig. 28) and in sporophytic fronds infected by *A. operculata* (Fig. 29). The infected fronds were highly preferred over their non infected counterparts.

Complementary information obtained in the experiments on palatability supports that for food preferences displayed by *I. baltica* and *G. oceanicus*. Fronds infected by either species of endophyte were eaten significantly more rapidly than non-infected counterparts. Feeding rates averaged more than half the wet weight of the herbivores per day when *I. baltica* was offered to *A. heteroclada*-infected gametophytic *C. crispus* (Fig. 30), and 40% of the herbivore's wet biomass per day in the *Idotea/C.crispus-A. operculata* combination (Fig. 31). Consumption rates were consistently lower in *G. oceanicus,* with an average of 15% and 8.2% of the biomass of the individual grazers per day when feeding upon *C. crispus* infected by *A. heteroclada* (Fig. 32) and *A. operculata* (Fig. 33) respectively. Those values, however, were significantly higher than consumption rates for control, non-infected fronds.

In addition to the quantitative information, it was clear that the way in which animals grazed varied according to whether or not the fronds were infected. This was particularly evident in the preference experiments done with *I. baltica*. The animals consistently bit the edges of the non-infected fronds, leaving no marks on the surface. When feeding on infected fronds, however, they grazed mainly on the surface, removing extensive areas of cortical tissue. This behavior had major effects on the regeneration of those fronds. As demonstrated by the incubation of fragments left after the 3-choice experiment, discoloration, softening and decay of the infected fronds occurred rapidly, and in some cases after heavy grazing, a few hours were sufficient for complete break down to occur. In comparison, all non-infected fronds, regardless of how extensively they had been consumed, regenerated many uprights along the damaged borders.

Although these must be considered as preliminary results, the chemoreception experiments suggest that *I. baltica* was able to detect substances produced by destruction of *C. crispus* tissue, regardless of whether or not it was infected by *A. operculata*. The number of animals clasping the cylinders with algal macerate was consistently greater than those clasping cylinders containing agar only (Fig. 34). Also, more animals were recorded on cylinders with macerate of sporophytic *C. crispus* infected with *A. operculata* than sample holders containing macerate of non-infected *C. crispus* (Fig. 35).

DISCUSSION

The results of my experiments indicate that *Chondrus crispus* was adversely affected when infected by either *Acrochaete operculata* or *A. heteroclada*. The effects are influenced by the species of endophyte involved, and the life-history phase of the host. Furthermore, the density of infection plays an important role in determining the intensity at which a particular feature (regeneration, toughness, *etc.*) is affected.

Most of the effect on sporophytic fronds by infections of *A. operculata* was related to the combination of the infection pattern of the endophyte, which develops exclusively inside the host and the growth pattern of *C. crispus*. Fronds of *C. crispus* gain biomass by producing new tissue at the apices and along the borders (Chopin and Floc'h 1987). As infection spreads through the frond, host-cell death gradually increases, follows the advancing front of the infection and, therefore, interferes with the two processes of biomass acquisition in the frond. This was evident at the end of the biomass accumulation trial, where infected fronds appeared shorter and narrower than non-infected controls. However, apart from their reduced size, these fronds developed normally. A normal development of the branching pattern in infected fronds was expected. This development is determined by the apical meristem, an area that owing to the fast-growing experimental conditions was never overgrown by *A. operculata*. However, subapical infections consolidated rapidly, damaging the marginal meristem and causing the cessation of frond widening. As indicated by observations on fine-structural aspects of the infection (Chapter 2), the consolidation of *A. operculata* in the cortex of sporophytic fronds results in massive cell disruption. This interrupts the normal process of growth, and therefore becomes the direct cause of diminution in biomass accumulation. The only studies where reduced growth has been reported involved hosts infected by parasitic red algae (Nonomura 1979, Apt 1984). In contrast to my findings, however, the reduced growth of infected plants was not found associated with extensive cellular damage. In one of those studies, it was indicated that the integrity of the tissues had not been affected as similar carrageenan yields were obtained from infected and non-infected fronds of *Hypnea musciformis* (Wulfen) Lamouroux (Apt

1984). In *C. crispus*, on the other hand, carrageenan yields were low in fronds infected by *A. heteroclada* and *A. operculata*. In infections with the latter endophyte, the low yield of carrageenan was noted only in sporophytic fronds, probably resulting from extensive degradation of the cell walls that occurs in this phase (but not in the gametophytic phase) of the host. Competition for nutrients between *A. operculata* and *C. crispus* cells might occur during early infection when host cells are not severely damaged. Such competition has been shown in various host-epiphyte associations (Enright 1979, Libes 1986, Sand-Jensen 1977, Sand-Jensen and Revsbach 1987). It seems unlikely, however, that this competition could have an impact on growth after endophytic infections become consolidated, owing to the resulting severe and extensive degradative changes in host cells.

Disruption of the host cells by *A. operculata* was related to the observed poor regeneration in sporophytic fragments. Regeneration of new uprights in non-infected *C. crispus* occurred at the cut edges, even though some fragments had areas with remnants of lateral meristem. This indicates that prior to regeneration, healing of the cut edges must take place. Wound healing in non-coenocytic algae has been studied in only few species, including *Udotea petiolata* (Mariani-Colombo and de Carli 1980), *Sargassum filipendula* C. Agardh (Fagerberg and Dawes 1976), *Fucus vesiculosus* L. (Fulcher and McCully 1969) and *Eucheuma alvarezii* var. *tambalang* Doty (Azanza-Corrales and Dawes 1989). It is apparent from these studies that wound healing involves dedifferentiation of cortical and medullary cells beneath the site of wounding, accompanied by active cell division. In the process, cells at the wound site become meristematic, and in species with apical growth, as *C. crispus*, the initiation of new fronds from the repaired cut edges occurs. Fine structural observations (Chapter 2) showed that as endophytic filaments of *A. operculata* become more numerous in the cortex and sporangium mother cells begin to enlarge, more host cells underwent degenerative changes. Consequently, I interpret the gradual loss of regenerability as caused by a parallel and gradual increase in the number of disrupted host cells. It is unlikely that with disrupted, non-functional host cells the dedifferentation process at the edges of infected tissue will occur. This, therefore, precludes wound healing and regeneration.

Gametophytic fronds of *C. crispus* infected by *A. operculata* were much less affected than sporophytic fronds. The failure of *A. operculata* to colonize newly formed host tissue produced by the fast growing apices allowed normal elongation and expansion of the fronds along the margins as in non-infected hosts (Chopin and Floc'h 1987). In the regeneration trials, relatively large areas of host cortical tissue were not affected by *A. operculata* in the "light" and "moderate" categories at the beginning of the experiments, and only few pockets remained in the "heavy" category. Recovery was, however, total in the first two groups and very high in the third, which indicates that areas of the cortex not directly affected by the endophyte were functional, able to heal wounds and generate new uprights. It is important to stress that even those heavily infected fragments which did not produce uprights, showed wound healing. This was not the case in sporophytic fragments, where the absence of regeneration was associated with overgrowth of the cut areas by *A. operculata,* in many instances showing evidence of bacterial decay.

The effect of *A. heteroclada* on *C. crispus* was not modified by the life history of the fronds. Prior to penetration of *C. crispus* by the endophyte, disruption of host cells is negligible (Chapter 2). Therefore, the poor biomass accumulation in infected fronds is interpreted as infecting plants acting as filters of nutrients, light or both. A similar phenomenon has repeatedly been reported to affect a number of physiological activities in plants hosting photosynthetic epiphytes (Sand-Jensen 1977, Bulthuis 1983, Bulthuis and Woelkerling 1983, Cambridge *et al.* 1986, Libes 1986, Silberstein *et al.* 1986, Sand-Jensen and Revsbech 1987). With further development, *A. heteroclada* gradually increases the surface of the host colonized and reaches the apices of the enlarging fronds. At this stage, enlargement of the host is seriously arrested and fronds become deformed. Furthermore if branching occurs, it is anomalous which suggests a dysfunction of the apical meristem. Concurrent invasion of the cortex, by the endophyte, in areas below the apical meristem results in final decay of the infected fronds.

Acrochaete heteroclada also interferes with some of the normal functions of the host tissues by disruption of the cortical cells. This observation is supported by the regeneration experiments where poor (gametophytes) or no (sporophytes) regeneration in the heavily infected

fragments coincided with the cortex being penetrated by the endophyte. As documented in Chapter 2, invasion of the host by *A. heteroclada* causes massive cortical cell damage and, therefore, should be considered responsible for the poor regenerative performance. On the other hand, in fragments with light or moderate infections, and where the cortex had not been invaded, regeneration was very high, indicating that although *A. heteroclada* was present on the surface, there was still functional host cortical tissue able to heal the wounds and issue new uprights before being overgrown by the endophyte.

The leathery texture of *C. crispus*, as in similar seaweeds, likely results from spatial arrangement of the cells combined with the physico-chemical properties of the cell wall including the immediate cell wall and intercellular matrix (Kloareg and Quatrano 1988). Consequently, any factor that alters the integrity of either the cellular or intercellular component or both will indirectly affect biomechanical properties. This is clearly the effect of *A. operculata* on sporophytic fronds and *A. heteroclada* on both sporophytic and gametophytic fronds of *C. crispus*. The destruction of the cortex of *C. crispus* and its replacement by endophytic cells, and disruption of the intercellular matrix during spreading of the infection, certainly contribute to weakening of the fronds. Lytic enzymes leaking from damaged host cells may also become part of the process of softening the tissues of *C. crispus*, aggravating the disruptive action of the endophytes. The relationship between tissue toughness and the integrity of cellular and cell-wall components of the thallus of *C. crispus* is substantiated by the comparatively small force required to perforate fronds at terminal stages of infection by *A. operculata*. In those fronds, secondary bacterial infection results in digestion of extensive areas of cortical and medullary intercellular matrix of the host (Chapter 2).

Field-collected *C. crispus* confirmed results of laboratory-infected material. The fronds became softer after infection by algal endophytes, which suggests that softening may result in frond fractures. Indeed, areas below the fracture points in broken fronds were heavily infected by brown and green endophytes, one of which corresponded to *A. operculata*, and these areas were consistently softer than intact, lightly or non-infected fronds. The extensive development of infections indicates that this likely preceded the fractures and were not secondary to them. It is unclear why

the perforation values for wild *C. crispus* were higher than the laboratory-grown fronds. Growth conditions may be involved, as suggested by increased carrageenan content caused by depletion of nitrogen and phosphorus (Neish *et al.* 1977, McCandless and Craigie 1979). Wild plants were collected in the fall, when the levels of nitrate and phosphate in the water are relatively low (Mathieson and Burns 1975), and the total carrageenan content maximum (Chopin 1986). Laboratory-grown fronds, on the other hand, were subjected to frequent changes of seawater enriched with nitrate and phosphate, which probably resulted in limited accumulation of carrageenan in the cell walls. Furthermore, high pH, which is likely to occur in laboratory cultures, has been shown to produce soft, floppy fronds (Simpson *et al.* 1978).

The involvement of carrageenan in the observed changes in toughness caused by endophytic infections is unclear. Kloareg and Quatrano (1988) suggested that one of the functions of carrageenan is as a structural material in a number of red algae, including *C. crispus,* and its diminution should therefore result in weakened fronds. In the present study, total carrageenan was lowered by endophytic infections and this phenomenon may be responsible for softer fronds.

It has been suggested that *C. crispus* is a poorly preferred food item because of its leathery texture (Lubchenco 1978, Lubchenco and Menge 1978). My experiments also indicate that, at least for *I. baltica,* non-infected tissue of either phase was not preferred when offered together with the highly palatable chlorophycean *Ulva* sp. Similarly, preferential grazing of *Enteromorpha* sp., a close relative of *Ulva,* by *I. baltica* in the presence of *C. crispus* has been demonstrated by Shacklock and Doyle (1983). Endophytic infection by *A. operculata* increases the attractiveness of sporophytic fronds to *I. baltica.* These infected fronds become preferred over non-infected sporophytic and gametophytic fronds, and are equivalent to *Ulva* in their attractiveness to *I. baltica.* Preferential feeding upon infected fronds by *I. baltica* extends to infections by *A. heteroclada,* regardless of the phase of the host. Similarly, *G. oceanicus* also responded by choosing infected over non-infected fronds of *C. crispus,* suggesting that this may be a common response of grazers. My study is, however, the first reported experimental evidence of changes in feeding responses by marine herbivores resulting from algal endophytic infections. My results also

stress the necessity of rigorous examination for algal endophytes before food preference experiments are undertaken with field-collected algal material.

Higher consumption rates of infected fronds confirm the food preference data, indicating that endophytic infections increase edibility (term according to Nicotri 1980, Watson and Norton 1985, 1987) of *C. crispus*. It is not clear, however, why the consumption rates of *G. oceanicus* were much lower than those of *I. baltica*. As the buccal parts seem to be equally well designed to tear apart and grind algal tissue, differences in consumption rates may represent physiological dissimilarities between the two grazers.

There have been numerous reports where crustacean mesoherbivores have been indicated to prefer the epiphytic component of host-epiphyte associations (Nicotri 1977, Brawley and Adey 1981, Norton and Benson 1983, Shacklock and Doyle 1983, D'Antonio 1985, Brawley and Fei 1987). In these cases, though, as the infecting algae are outside the host, minor damage of the basiphyte is to be expected, although some overgrazing may result in damage to the host (Shacklock and Doyle 1983, Craigie and Shacklock 1989). Contrarily, grazing activity stimulated following infections of a host by algal endophytes will always result in biomass losses of the host. This effect is aggravated by the poor capacity of wound healing at the infection sites which prevents tissue regeneration and accelerates secondary infection by bacteria. This was demonstrated with regeneration of fronds previously grazed by *I. baltica*. Non-infected fronds regenerated normally and infected fronds did not, with most of them undergoing complete bleaching and breakdown only a few hours after the grazers were removed.

The determinants of preferential feeding by the two crustacean grazers considered in the present study remain unknown, but at least two factors can be visualized as being potentially involved. One is the softening of the infected areas, with a decreased toughness, caused directly by host cell and tissue disruptions during endophytic infections and subsequently aggravated by secondary bacterial infections. Consumption rates support this view, as infected fronds were eaten faster than non infected *C. crispus* by both *I. baltica* and *G. oceanicus*. Additional support comes from observations of individual sporophytic fronds infected by *A. operculata*

during the preference trials, where a gradient in infection density occurred from heavily infected basal portions (softer areas), to light infections at the apices (tougher areas). In these cases, regardless of the grazer, the tissue removed was mainly from the basal, heavily infected areas, and grazing marks were rarely observed at the apical portions. A similar pattern of grazing marks was commonly found on heavily infected, field-collected fronds of *C. crispus*. Switching the mode of feeding by *I. baltica* and *G. oceanicus* from biting the edges of non infected fronds to deep browsing on the surface of heavily infected areas, where most of the cortex was removed, also suggests a response mediated by softening of the tissues. The second factor potentially involved in preferential feeding is the release of substances from the normal metabolism of the endophytes or from the disrupted host cells or both, which may serve as phagostimulants. This has been demonstrated for a number of algal products which induce feeding responses in various fish, gastropods and crustacean herbivores (Ache 1982, Carr and Derby 1986, Rittshof and Bonaventura 1986, Sakata *et al.* 1988). Certainly the two factors, tissue softening and release of phagostimulants, may influence the behavior of the grazers at the same time, although it is difficult to separate experimentally one from the other during the process of food selection by the herbivores. In fact it may prove to be impossible, as recent studies indicate that the senses of taste, touch and smell, anatomically distinct in terrestrial vertebrates, form a continuum in marine sensory systems (Rittschof and Bonaventura 1986). That *I. baltica* is able to detect substances released by non infected, disrupted *C. crispus* tissue embedded in agar blocks was suggested by the consistently higher number of grazer clasping the meshed cylinders containing those blocks compared to the number of individuals associated with the cylinders containing agar alone. Furthermore, there were consistently higher numbers of *I. baltica* on cylinders having agar plus sporophytic tissue infected by *A. operculata* than on those having agar plus non infected sporophytic tissue. This suggests that *I. baltica* is stimulated in a synergistic fashion by mixtures of phagostimulatory substances from both host and endophyte. Similar reaction has been reported for other crustaceans, where mixtures of substances trigger stronger feeding responses than when administered singly (Carr and Derby 1986).

Chondrus crispus is a perennial species and a main user of primary space. Its persistence is determined by interactions with its physical environment and other algae or invertebrates that compete with *C. crispus* for space (Mathieson and Prince 1973, Menge 1976, Lubchenco and Menge 1978, Lubchenco 1978, 1980, Menge and Lubchenco 1981, Chapman 1986, Dudgeon *et al.* 1989), and also with other organisms, that use it as substratum or food (Lilly 1968, Prince and Kinsburg 1973, Mathieson and Burns 1975, Pybus 1977, Shacklock and Croft 1981, Shacklock and Doyle 1983). The extent to which the laboratory-tested, negative effects of infections by the two most common green algal endophytes expresses in wild populations of *C. crispus* and how the phenomenon of endophytism fits into the broad and complex network of interactions between *C. crispus* and its physical and biotic environment are matters of conjecture. It seems reasonable to expect, however, that factors like those discussed in the present study may have some impact on the longevity and recovery (regeneration) of the fronds.

For example, wave action is recognized as a major disturbance agent which causes mortality in sessile invertebrates and algae (Koehl 1982, 1984, 1986, Denny *et al.* 1985, Biedka *et al.* 1987, Denny 1987, 1988, Denny *et al.* 1989). It seems reasonable that softening of the fronds of *C. crispus* by endophytic infections could facilitate their fracture by wave forces. Furthermore, if infected fronds are selectively grazed upon by herbivores, the removed biomass will accelerate the fracture process by producing flaws on the surface where the wave forces concentrate and amplify (Biedka *et al.* 1987, Denny *et al.* 1989). Interestingly, the architecture of *C. crispus,* with an extremely narrow stipe attached to the holdfast suggest that if an intact frond is going to break, the fracture will occur at the stipe-holdfast interface (McLachlan *et al.* 1989). Although this seems to be commonly the case, up to 30% of randomly collected fronds from intertidal populations of *C. crispus* in N.S. appeared broken above the first dichotomy (McLachlan pers. comm.). This indicates that the fracture occurred at a point weaker than the holdfast-stipe interface. The force required to break an intact frond above the first dichotomy is several times the force needed to separate the frond from the holdfast (White pers. comm.), reported to be 4-5 N mm^{-2} (McLachlan *et al.* 1989). This indicates that for a frond to break above the first dichotomy, softening of

the tissue must occur. This conclusion is supported by observations of endophytic infections and grazing marks commonly found at the fracture points in field-collected fronds (unpubl.). Finally, if endophytic infections at the fracture site are heavy enough, regeneration will be suppressed, and the frond lost from the population.

Another interesting aspect of the presently studied host-endophyte associations is the apparent differential susceptibility of the two phases of the host to *A. operculata*. It may be possible to integrate this information into explanation of gametophytic dominance in some populations of *C. crispus* (Bhattacharya 1985).

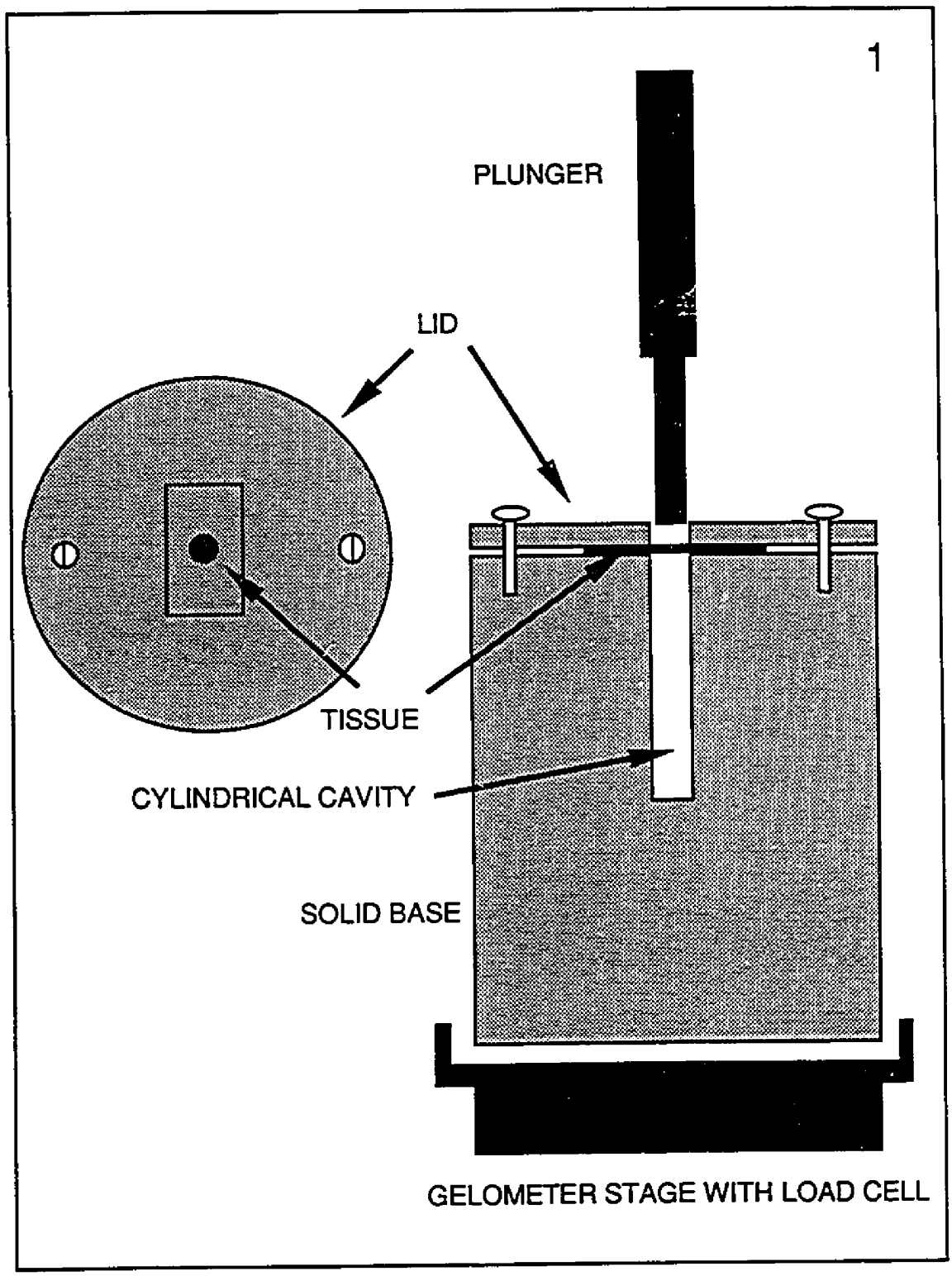
As previously indicated, *C. crispus* may be considered a "domesticated" species, and as such, susceptible to an array of pathogenic agents, some of which might be of little consequence under natural conditions. *A. operculata* and *A. heteroclada* certainly represent a threat to large-scale cultivation of *C. crispus*, by directly reducing growth rates, and reducing and contaminating carrageenan production. These endophytes could also facilitate secondary infections, softening tissues and stimulating selective grazing of infected areas by crustacean herbivores, always associated with cultivated *C. crispus*. The last three factors may accelerate the process of frond fragmentation, which could result in significant biomass losses (Craigie and Shacklock 1989, Craigie 1990 a). The use of crustacean grazers has been suggested as a biological control for epiphytes in a number of small and large scale algal culture systems (Shacklock and Doyle 1983, Brawley and Fei 1987), although these may prove to cause more damage than resulting benefits if endophytes or epi/endophytes are involved.

Contamination of the carrageenan extracts is an aspect that needs further attention. Preliminary assays (unpubl.) have demonstrated that if dried material of *A. heteroclada* or *A. operculata* from unialgal cultures is subjected to the same protocol followed for carrageenan extraction, a whitish, finely granular material (~9% of the initial dry weight) is obtained. The chemical nature of this extract has not been determined, but the presence of this endophyte-derived material in carrageenan preparations might interfere with the properties of the latter.

Finally, the negative effect of endophytic infections on regeneration performance may represent an additional problem for farmed *C. crispus*, whose propagation is based on vegetative propagation of clonal material.

FIGURES

Fig. 1. Diagram showing the device used to assess toughness of *Chondrus crispus*. The plunger was attached a the gelometer (see text). The solid base rested on the stage of the gelometer where the load cell was located.

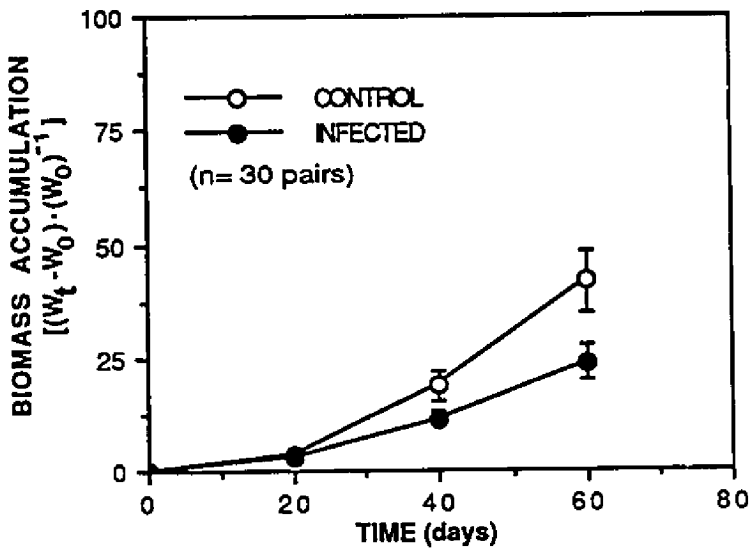

1
PLUNGER
LID
TISSUE
CYLINDRICAL CAVITY
SOLID BASE
GELOMETER STAGE WITH LOAD CELL

Figs 2-5. Effect of endophytic infection on biomass accumulation by *Chondrus crispus* apices. In all cases the control treatment consisted of uninfected apices of the corresponding life history phase of the host. Each point represents a mean value for a given treatment, and the vertical bars indicate ± 1 standard error.

Fig. 2. Sporophytic apices infected by *Acrochaete operculata.*

Fig. 3. Gametophytic apices infected by *Acrochaete operculata.*

2


100
75
50
25
0
0
20
40
60
80
CONTROL
INFECTED
(n= 30 pairs)
BIOMASS ACCUMULATION
[(Wt-W0)·(W0)-1]
TIME (days)

3

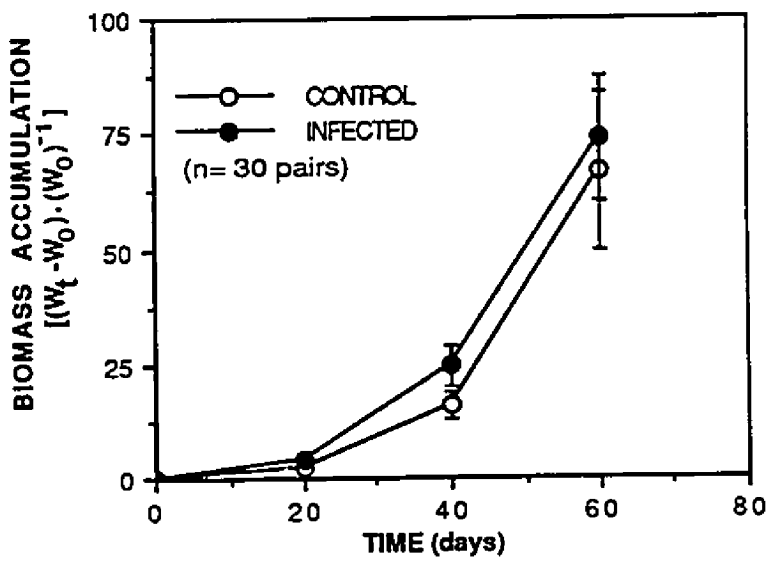
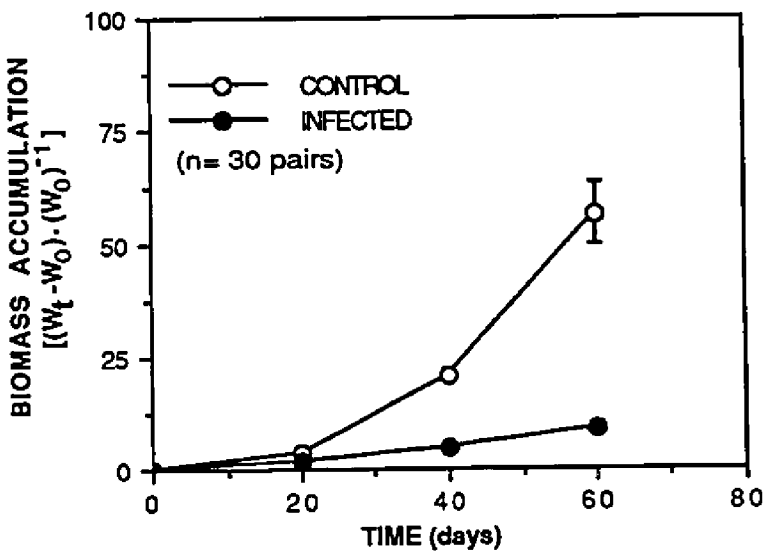

100
75
50
25
0
0
20
40
60
80
CONTROL
INFECTED
(n= 30 pairs)
BIOMASS ACCUMULATION
[(Wt-W0)·(W0)-1]
TIME (days)

Fig. 4. Sporophytic apices infected by *Acrochaete heteroclada.*

Fig. 5. Gametophytic apices infected by *Acrochaete heteroclada.*

4


100
75
50
25
0
0
20
40
60
80
CONTROL
INFECTED
(n= 30 pairs)
BIOMASS ACCUMULATION
[(Wt -W0)·(W0)-1]
TIME (days)

5

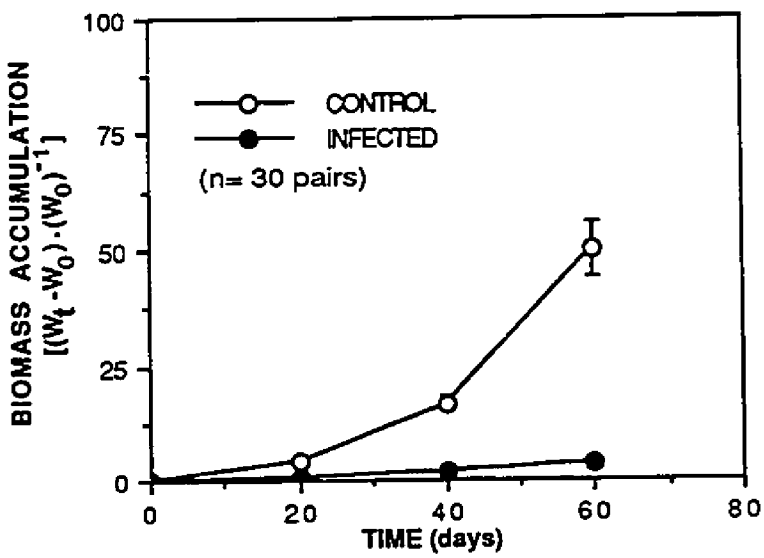
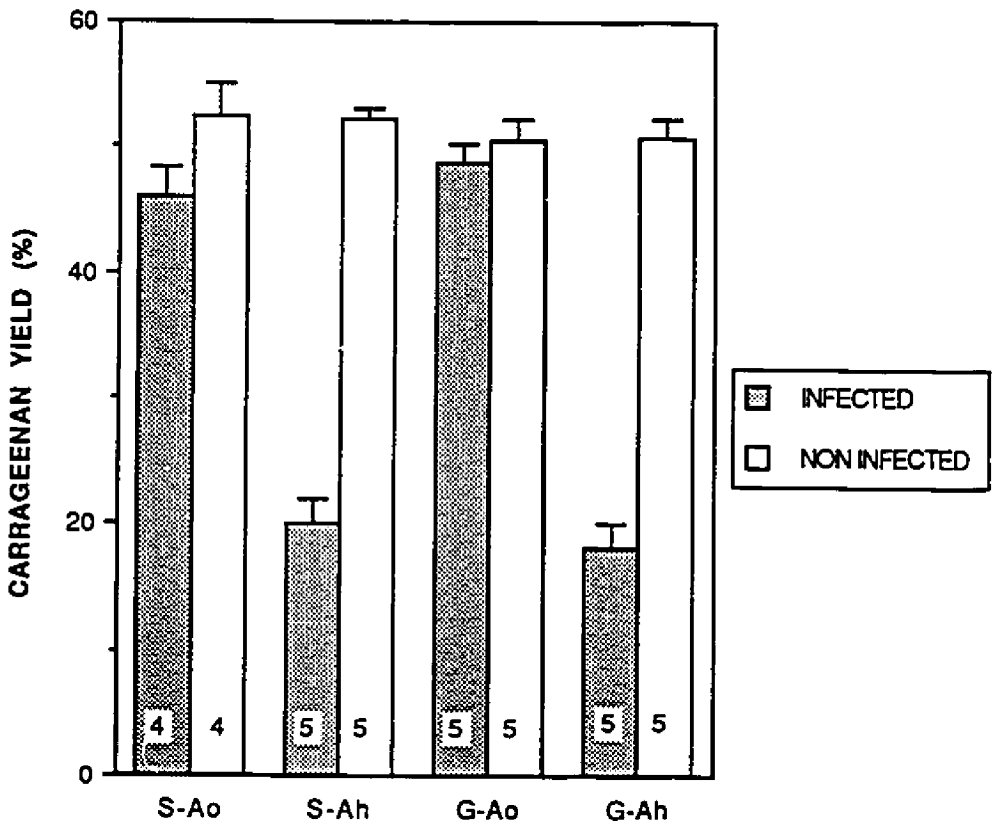

100
75
50
25
0
0
20
40
60
80
CONTROL
INFECTED
(n= 30 pairs)
BIOMASS ACCUMULATION
[(Wt -W0)·(W0)-1]
TIME (days)

Fig. 6. Carrageenan yield of fronds from the experiment on biomass accumulation. Initials indicate the host-endophyte combination, where S= sporophyte, G= gametophyte, Ao= *Acrochaete operculata,* Ah= *Acrochaete heteroclada.* Each bar represents a mean value (± 1 standard error). The number of replicates are shown at the base of each bar.

6

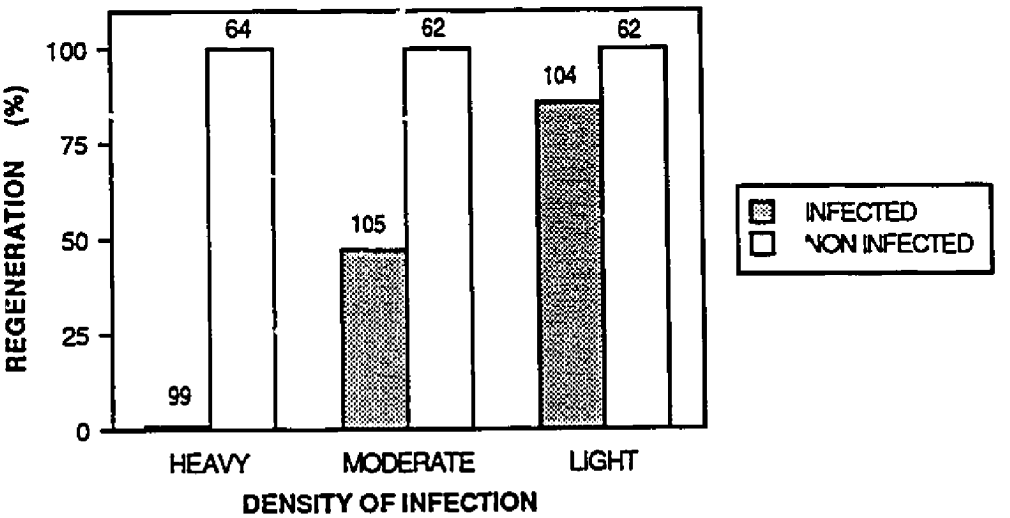

60
40
20
0
CARRAGEENAN YIELD (%)
4
4
5
5
5
5
5
5
S-Ao
S-Ah
G-Ao
G-Ah
INFECTED
NON INFECTED

Figs 7-10. Effect of endophytic infection on regeneration of *Chondrus crispus*. The number of fragments used in each treatment is indicated at the top of each bar.

Fig. 7 Sporophytic fragments infected by *Acrochaete operculata.*

Fig. 8. Gametophytic fragments infected by *Acrochaete operculata.*

7


REGENERATION (%)
100
75
50
25
0
64
62
62
104
105
99
HEAVY
MODERATE
LIGHT
DENSITY OF INFECTION
INFECTED
NON INFECTED

8

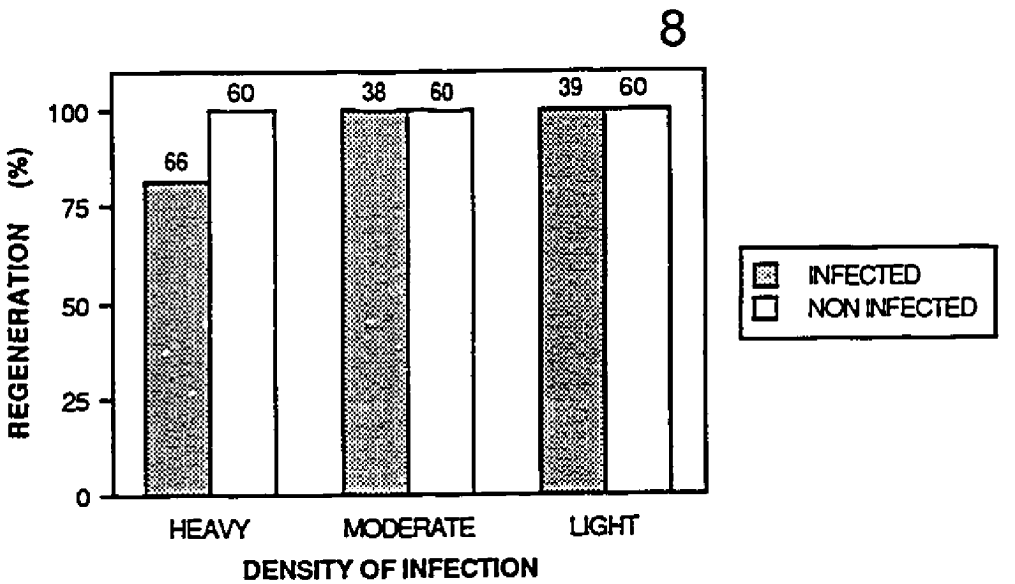
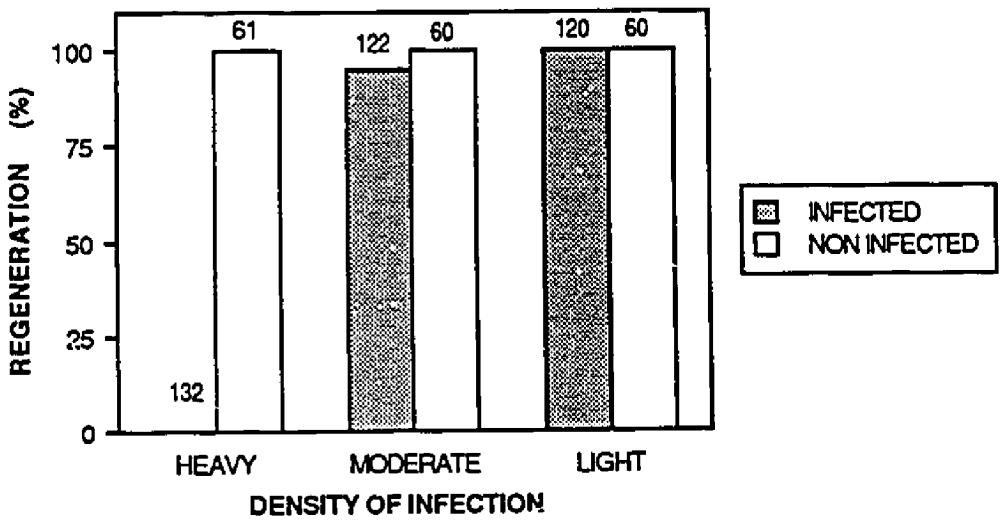

REGENERATION (%)
100
75
50
25
0
60
38
60
39
60
66
HEAVY
MODERATE
LIGHT
DENSITY OF INFECTION
INFECTED
NON INFECTED

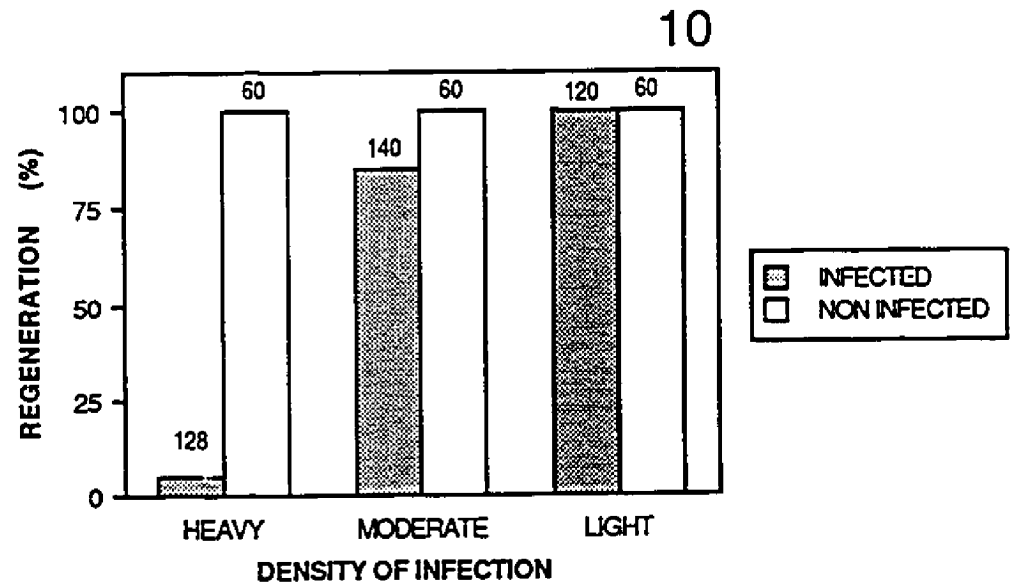
Fig. 9. Sporophytic fragments infected by *Acrochaete heteroclada*.

Fig. 10. Gametophytic fragments infected by *Acrochaete heteroclada*.

9


REGENERATION (%)
100
75
50
25
0
61
122
60
120
60
132
HEAVY
MODERATE
LIGHT
DENSITY OF INFECTION
INFECTED
NON INFECTED

10

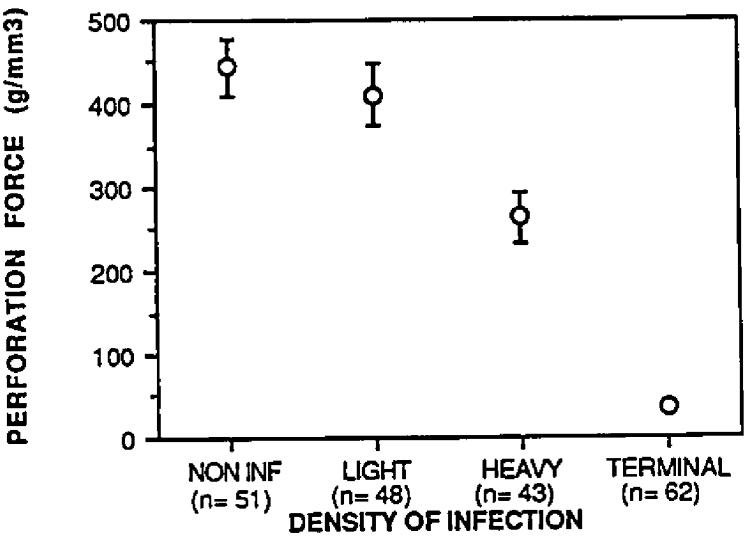

REGENERATION (%)
100
75
50
25
0
60
60
120
60
140
128
HEAVY
MODERATE
LIGHT
DENSITY OF INFECTION
INFECTED
NON INFECTED

Figs 11-13. Effect of endophytic infection on toughness of *Chondrus crispus*. Each point represents the mean (99% confidence limits) force required to perforate the tissue.

Fig. 11. Sporophytic *Chondrus crispus* infected by *Acrochaete operculata*. Light, heavy and terminal are categories indicating an increase in the density of infection (see text). Normal toughness was estimated in uninfected sporophytic fronds (NON INF). Laboratory-grown fronds.

Fig. 12. Gametophytic and sporophytic *Chondrus crispus* infected by *Acrochaete heteroclada*. Infected (INF) fronds are compared with uninfected (NON INF) controls in each phase of the host. Laboratory-grown material.

11


PERFORATION FORCE (g/mm3)
500
400
300
200
100
0
NON INF (n= 51)
LIGHT (n= 48)
HEAVY (n= 43)
TERMINAL (n= 62)
DENSITY OF INFECTION

12

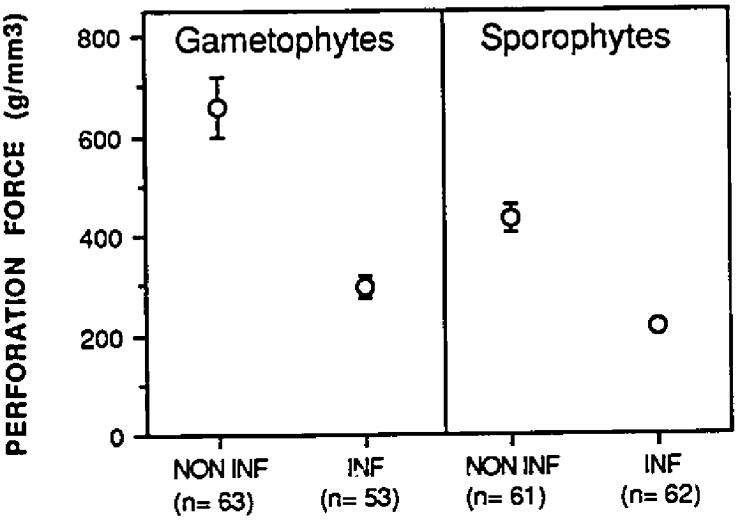
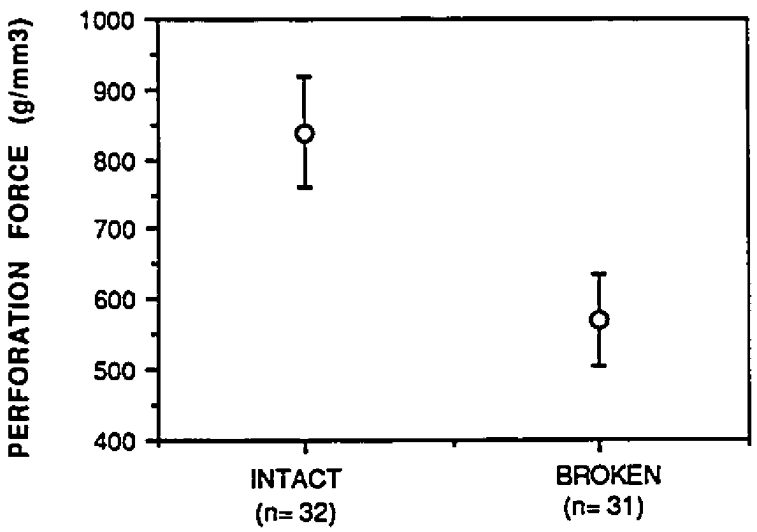

PERFORATION FORCE (g/mm3)
800
600
400
200
0
Gametophytes
Sporophytes
NON INF (n= 63)
INF (n= 53)
NON INF (n= 61)
INF (n= 62)

Fig. 13. Perforation values for intact wild fronds of *Chondrus crispus,* compared to values from areas immediately subjacent to fracture points in broken fronds. The broken fronds were heavily infected by green and brown algal endophytes, while infection in intact fronds, if any, was minor.

13


PERFORATION FORCE (g/mm3)
1000
900
800
700
600
500
400
INTACT
(n= 32)
BROKEN
(n= 31)

Figs 14-16. Buccal appendages of *Idotea baltica.*

Fig. 14. Ventral view of *Idotea baltica* showing the mouth region and the general arrangement of the buccal appendages. Note the mandibles (M) which meet at medial position. Scale= 1 μm

Fig. 15. Claw-like terminal portion of a maxilla.


14


15

Fig. 16. Mandible with 4 pointed, sharp projections at terminal end, and a discoid, ornamented structure, located at the proximal end (arrowhead).

Figs 17-19. Buccal appendages of *Gammarus oceanicus.*

Fig. 17. Mandible, showing a double row of teeth-like projections.

Fig. 18. Detail of the discoid, ornamented structure at the base of each mandible.

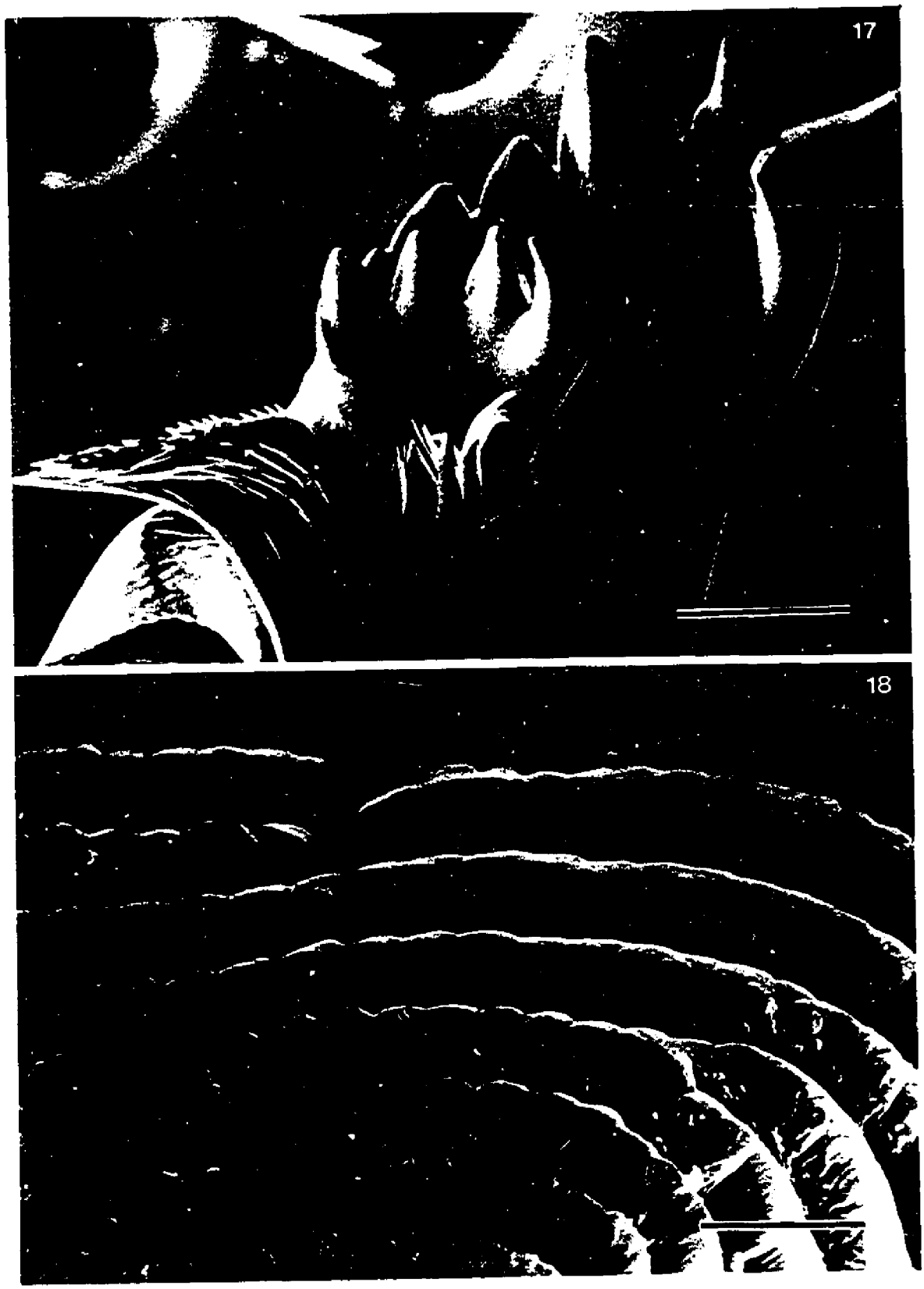

17
18

Fig. 19. Claw-like terminal portion of maxilla.


19

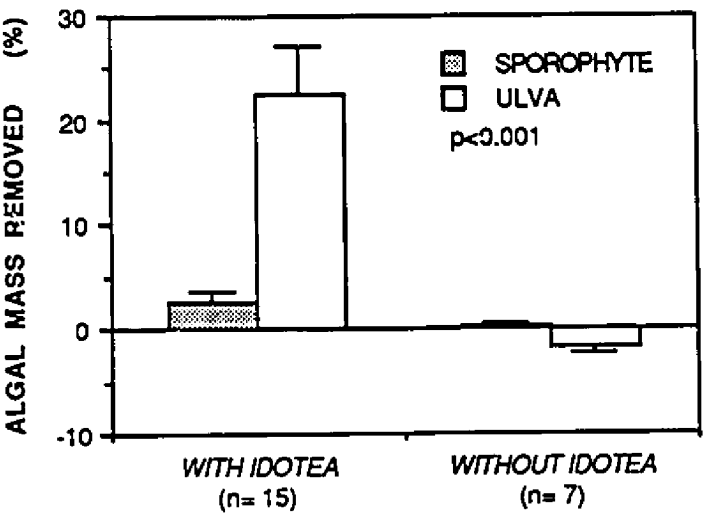
Figs. 20-29. Food preferences by *Idotea baltica* and *Gammarus oceanicus*. In all cases, values represent mean (± 1 standard error) changes in algal biomass in each treatment. Negative values occur when the tested specimens gained weight. The p value indicates the probability level used to test the significance of the differences between alternative foods in the treatments with grazers.

Figs 20-27. *Idotea baltica.*

Fig. 20. Non infected sporophytic fronds versus *Ulva* sp.

Fig. 21. Non infected gametophytic fronds versus *Ulva* sp.

20


ALGAL MASS REMOVED (%)
30
20
10
0
-10
SPOROPHYTE
ULVA
p<0.001
WITH IDOTEA
(n= 15)
WITHOUT IDOTEA
(n= 7)

21

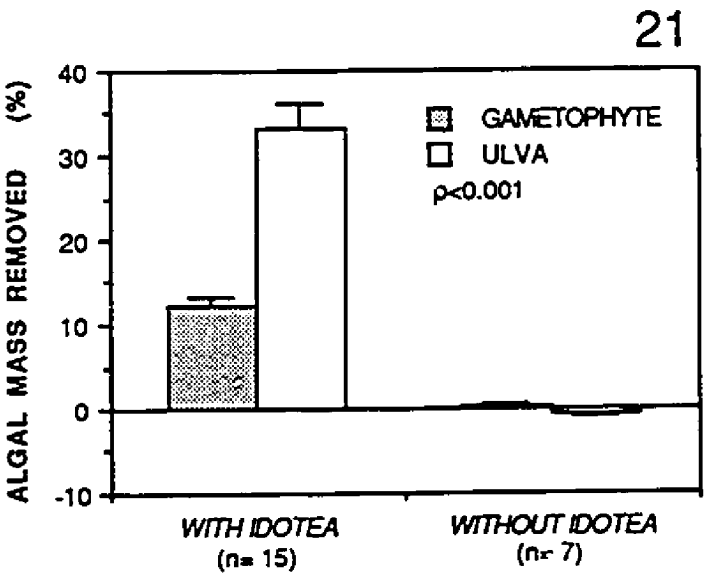
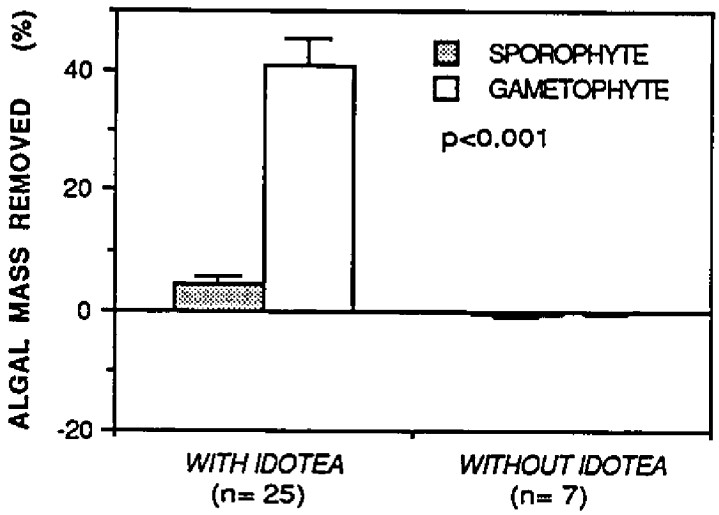

ALGAL MASS REMOVED (%)
40
30
20
10
0
-10
GAMETOPHYTE
ULVA
p<0.001
WITH IDOTEA
(n= 15)
WITHOUT IDOTEA
(n= 7)

Fig. 22. Sporophytic versus gametophytic fronds, both uninfected.

Fig. 23. Sporophytic fronds infected by *Acrochaete operculata* versus non infected sporophytic fronds.

22


ALGAL MASS REMOVED (%)
40
20
0
-20
SPOROPHYTE
GAMETOPHYTE
p<0.001
WITH IDOTEA
(n= 25)
WITHOUT IDOTEA
(n= 7)

23

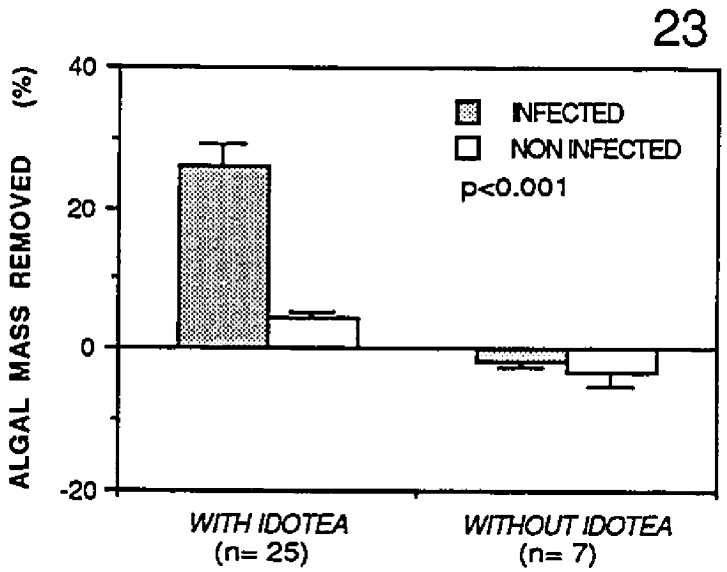
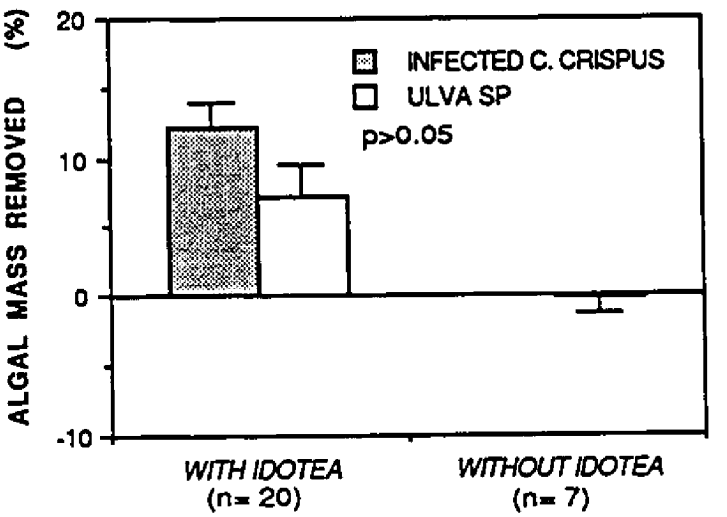

ALGAL MASS REMOVED (%)
40
20
0
-20
INFECTED
NON INFECTED
p<0.001
WITH IDOTEA
(n= 25)
WITHOUT IDOTEA
(n= 7)

Fig. 24. Sporophytic fronds infected by *Acrochaete operculata* versus *Ulva* sp.

Fig. 25. Ranking of preferences in the 3-choice experiment where sporophytic fronds infected by *Acrochaete operculata,* and non infected sporophytic and gametophytic fronds, were tested at simultaneously. The p value indicates the probability level used to test the differences on all possible pairs

24


ALGAL MASS REMOVED (%)
20
10
0
-10
INFECTED C. CRISPUS
ULVA SP
p>0.05
WITH IDOTEA
(n= 20)
WITHOUT IDOTEA
(n= 7)

25

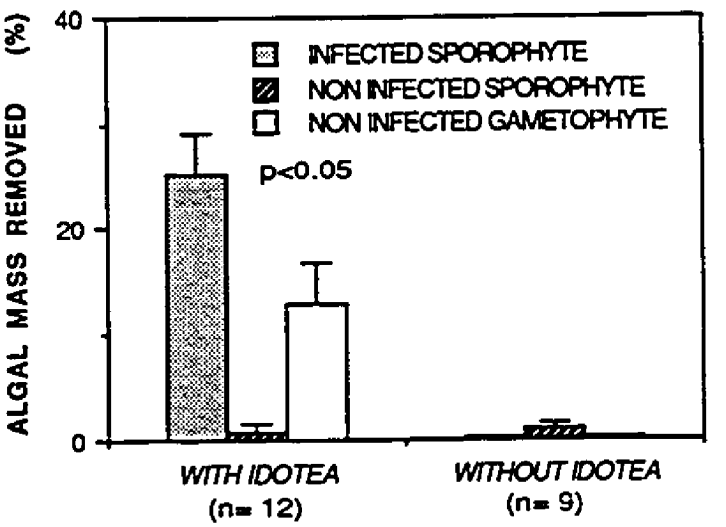
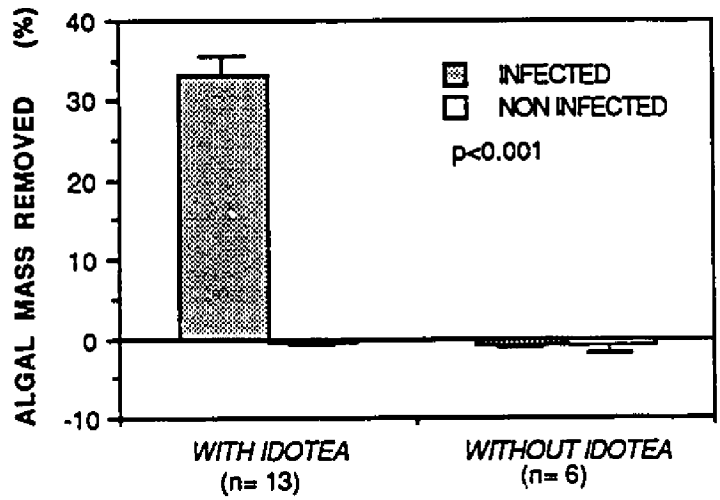

ALGAL MASS REMOVED (%)
40
20
0
INFECTED SPOROPHYTE
NON INFECTED SPOROPHYTE
NON INFECTED GAMETOPHYTE
p<0.05
WITH IDOTEA
(n= 12)
WITHOUT IDOTEA
(n= 9)

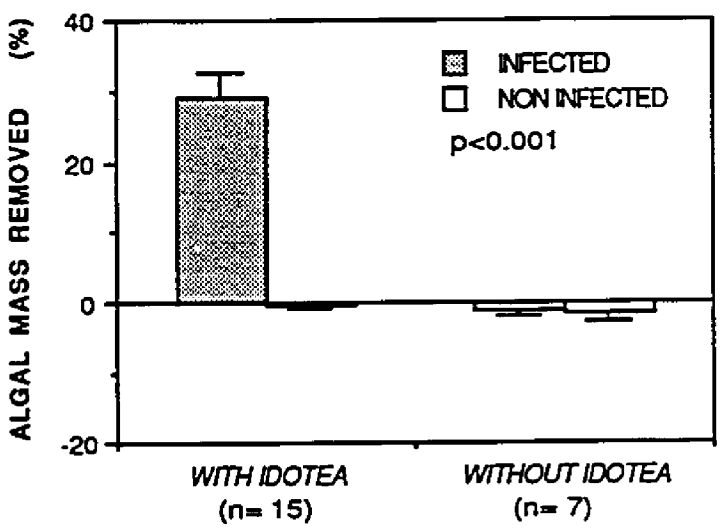
Fig. 26. Sporophytic fronds infected by *Acrochaete heteroclada* versus non infected sporophytic fronds.

Fig. 27. Gametophytic fronds infected by *Acrochaete heteroclada* versus non infected gametophytic fronds.

26


ALGAL MASS REMOVED (%)
40
30
20
10
0
-10
INFECTED
NON INFECTED
p<0.001
WITH IDOTEA
(n= 13)
WITHOUT IDOTEA
(n= 6)

27

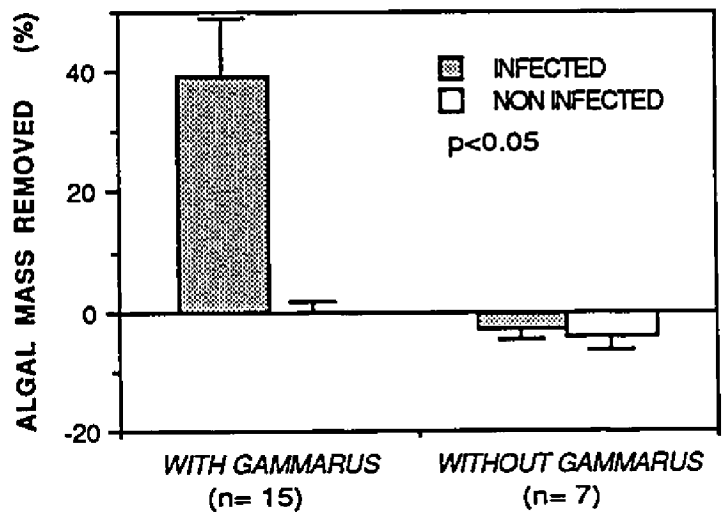

ALGAL MASS REMOVED (%)
40
20
0
-20
INFECTED
NON INFECTED
p<0.001
WITH IDOTEA
(n= 15)
WITHOUT IDOTEA
(n= 7)

Figs 28, 29. *Gammarus oceanicus.*

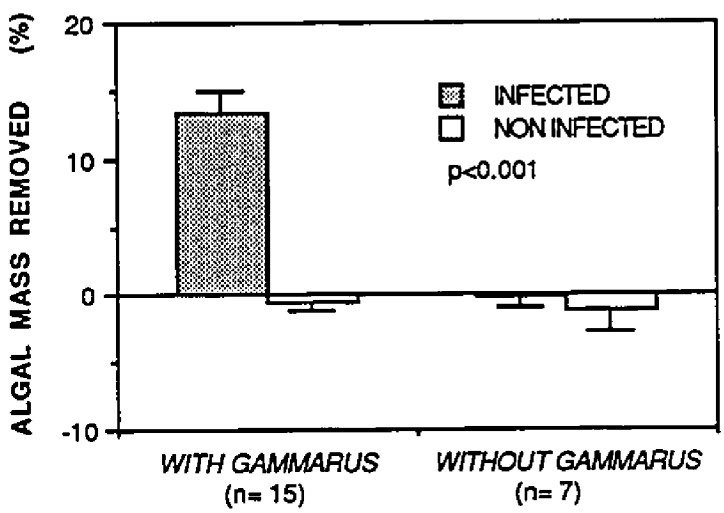
Fig. 28. Gametophytic fronds infected by *Acrochaete heteroclada* versus non infected gametophytic fronds.

Fig. 29. Sporophytic fronds infected by *Acrochaete operculata* versus non infected sporophytic fronds.

28


ALGAL MASS REMOVED (%)
40
20
0
-20
INFECTED
NON INFECTED
p<0.05
WITH GAMMARUS
(n= 15)
WITHOUT GAMMARUS
(n= 7)

29

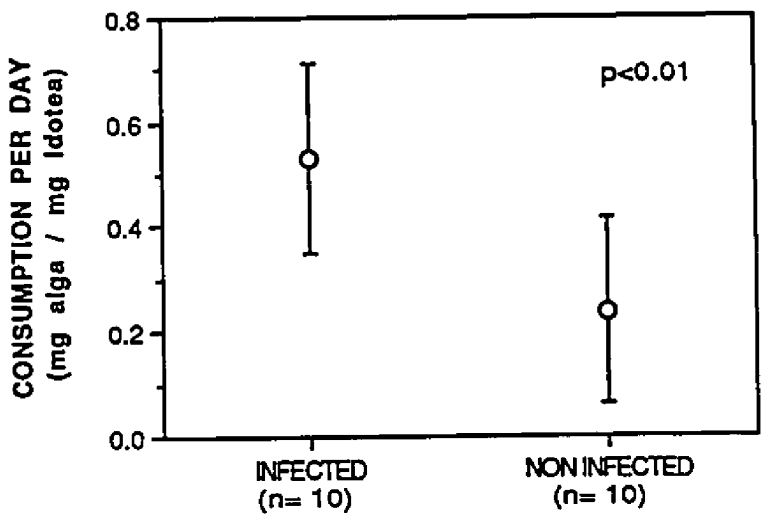

ALGAL MASS REMOVED (%)
20
10
0
-10
INFECTED
NON INFECTED
p<0.001
WITH GAMMARUS
(n= 15)
WITHOUT GAMMARUS
(n= 7)

Figs 30-33. Effect of endophytic infection on palatability of *Chondrus crispus* by *Idotea baltica* and *Gammarus oceanicus*. Points represent mean consumption (with 99% confidence limit) in each treatment. The p value is the probability level at which the differences in consumption rates between infected and non infected fronds were tested.

Fig. 30. Gametophytic fronds infected by *Acrochaete heteroclada*, and non infected fronds, consumed by *Idotea baltica*.

Fig. 31. Sporophytic fronds infected by *Acrochaete operculata*, and non infected sporophytic fronds, consumed by *Idotea baltica*.

30


CONSUMPTION PER DAY
(mg alga / mg Idotea)
0.8
0.6
0.4
0.2
0.0
p<0.01
INFECTED
(n= 10)
NON INFECTED
(n= 10)

31

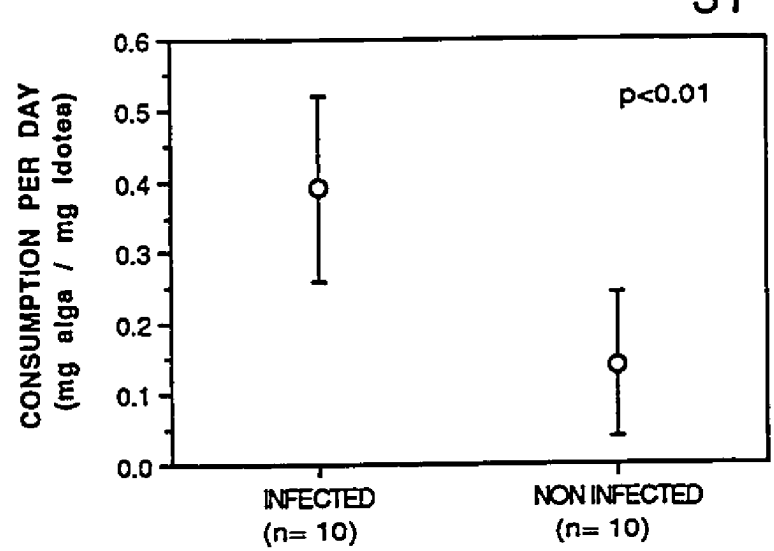
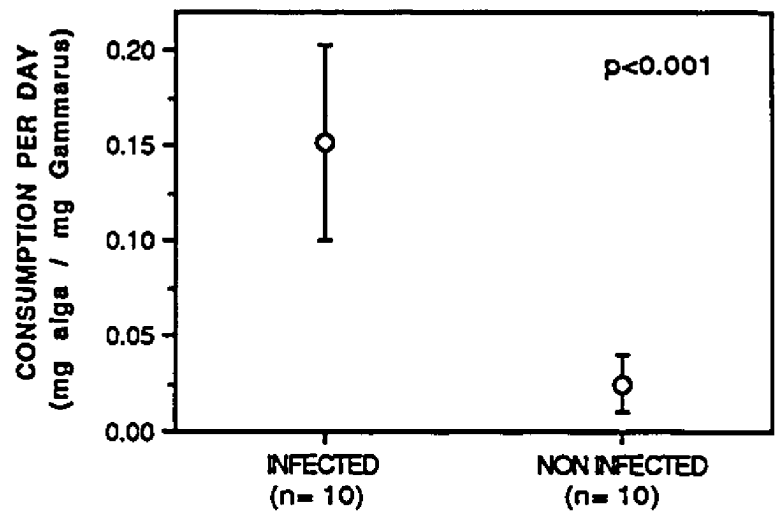

CONSUMPTION PER DAY
(mg alga / mg Idotea)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
p<0.01
INFECTED
(n= 10)
NON INFECTED
(n= 10)

Fig. 32. Algal material as in Fig. 30, but consumed by *Gammarus oceanicus*.

Fig. 33. Algal material as in Fig. 31, but consumed by *Gammarus oceanicus*.

32


CONSUMPTION PER DAY
(mg alga / mg Gammarus)
0.20
0.15
0.10
0.05
0.00
p<0.001
INFECTED
(n= 10)
NON INFECTED
(n= 10)

33

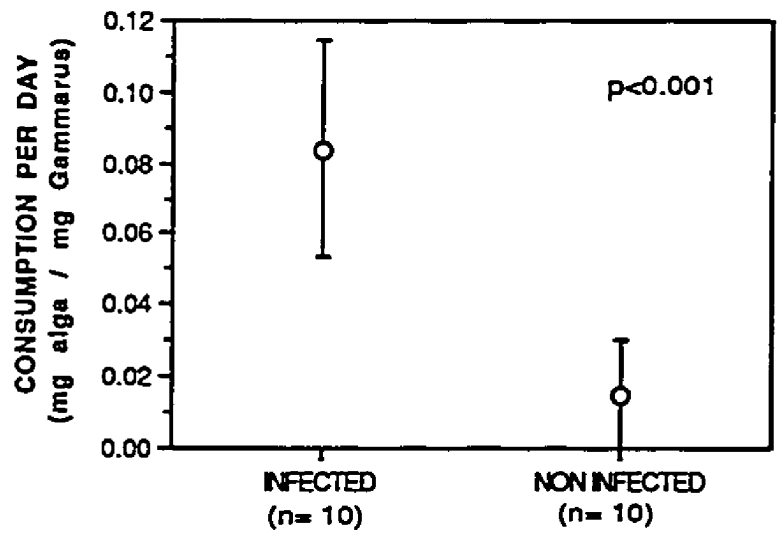
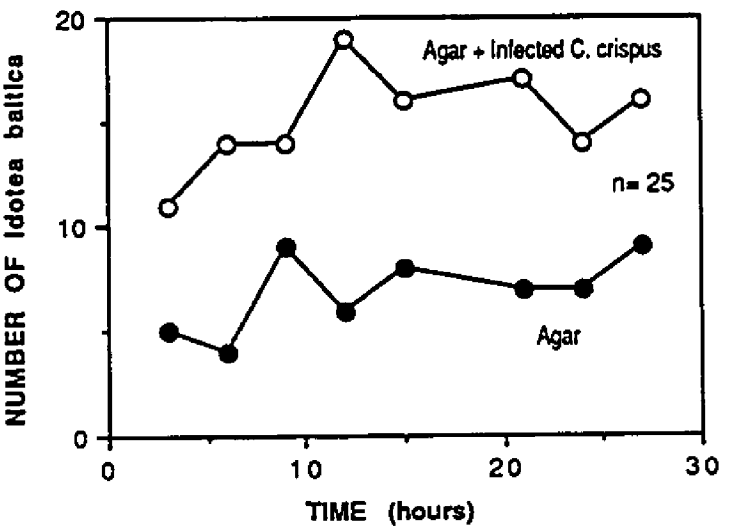

CONSUMPTION PER DAY
(mg alga / mg Gammarus)
0.12
0.10
0.08
0.06
0.04
0.02
0.00
p<0.001
INFECTED
(n= 10)
NON INFECTED
(n= 10)

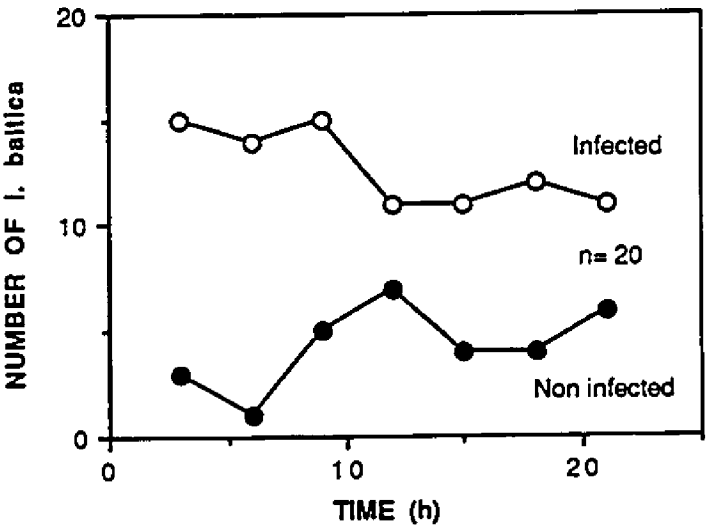
Fig. 34. Number of *Idotea baltica* gripping cylinders containing blocks of agar with macerate of *Chondrus crispus* infected by *Acrochaete operculata,* or blocks of agar alone.

Fig. 35. Number of *Idotea baltica* gripping cylinders containing blocks of macerate of agar with either sporophytic *Chondrus crispus* infected by *Acrochaete operculata,* or non infected sporophytic fronds.

34


20
10
0
0
10
20
30
NUMBER OF Idotea baltica
TIME (hours)
Agar + Infected C. crispus
n= 25
Agar

35


20
10
0
0
10
20
NUMBER OF I. baltica
TIME (h)
Infected
n= 20
Non infected

TABLES

TABLE 1. ANOVA table indicating effects of density of infection by *A. operculata* on toughness of sporophytic *C. crispus*.

Source of variation	df	SS	MS	Fs
Among densities	3	18801280	6267093	1043*
Error	200	1201476	6007	
Total	203	20002756		

* $p<0.005$

Table 2. Summary of Tukey-Kramer test for comparisons among pairs of means from samples of unequal size. The moduli of the difference between each pair of means are given below the diagonal, and the corresponding critical difference values are given above the diagonal. Experimentwise error $\alpha = 0.05$

	DENSITY OF INFECTION			
	Control	Light	Heavy	Terminal
Control	-	40.0	41.2	37.6
Light	33.6	-	41.8	38.3
Heavy	180.8*	147.2*	-	39.5
Terminal	407.9*	374.3*	227 1*	-

* Significative differences

FINAL REMARKS

FINAL REMARKS

I began this thesis indicating that the terms epiphyte and endophyte were used as the most direct way to classify associations involving algal partners, owing the lack of information on aspects other than the spatial relationship of the associates. The present study provides clear evidence that the association between *C. crispus* and its two commonest green algal endophytes goes beyond a solely intimate spatial relationship and results in effects on the host that can be visualized and quantified at the cellular and individual frond levels. This information allows us, for the first time in an algal endophytic association involving two fully pigmented partners, to assess the nature of the association in the context of symbiosis. Can the associations between *C. crispus* and its pigmented endophytes be considered symbioses? De Bary (1879) created the term symbiosis to describe the living together of dissimilarly named organisms, but with time the meaning became narrower and equated with associations leading to mutual benefit (Lewis 1973, Lewin 1982), or mutualism according to the broad interpretation of de Bary's definition (see Hall 1974). The controversy regarding the meaning of the term symbiosis has been lengthy. However recent authors seem to support the broad definition originally given by de Bary (Lewis 1973, Starr 1975, Goff 1982b, Ahmadjian and Paracer 1986, Smith and Douglas 1987, Douglas and Smith 1989). The use of the term symbiosis in its narrow connotation has been criticized as an "historical accident" (Ahmadjian and Paracer 1986), and in the view of Lewis (1985), its repetition in textbooks does not render it correct. Several features have been used in the description of symbiosis (Starr 1975, Whitfield 1979, Lewis 1985, Smith and Douglas 1987, Ahmadjian and Paracer 1986), which support the contention that both *A. operculata* and *A. heteroclada* establish a symbiotic association with their host *C. crispus*. For example, symbiotic associations should persist for an appreciable time relative to the life-spans of the partners (Ahmadjian and Paracer 1986, Whitfield 1979, Smith and Douglas 1987). There are some extreme views regarding the required time of contact between partners to consider a particular association as a symbiosis. Starr (1975) and Lewis (1985) have considered even transient associations as symbiosis, if there is evidence that at least one

partner is obviously affected (positively or negatively). It is clear that when *C. crispus* is infected, no mechanism in the host seems to prevent the completion of the life history or reproduction of either endophyte. Consequently, in nature it seems likely that several generations of a given endophyte infect the same frond before it is purged from the plant, and associations are long lasting in laboratory-maintained infected fronds.

Another feature that requires consideration during the assessment of an association as symbiotic is the necessity of the relationship between partners. In theory, a symbiosis is obligate for an organism that cannot survive, reproduce or both in the absence of its partner. Alternatively, the relationship is facultative when survivorship and reproduction of one partner are not dependent upon the presence of the other (Starr 1975, Smith and Douglas 1987). A complication in the above dichotomy arises in cases where a given organism grows isolated in laboratory culture, but in nature is found only associated with a specific partner. This led Brian (1966) to differentiate physiological obligacy, an absolute dependence on a partner, from ecological obligacy, the inability of one partner to survive in the natural environment separated from the other. Most parasitic red algae, for example, are physiologically obligated symbionts and cannot develop isolated from their hosts (Goff 1982 a). There are several features indicating that *A. operculata* is an ecologically obligate endophyte. The plant lacks a pseudoparenchymatous habit throughout its development (Correa *et al.* 1988), and there is no evidence of extracellular material surrounding the free living filaments that could be interpreted as involved in attachment. These data strongly suggest that this species cannot attach to any surface, and in nature the endophytic habit may be the only possibility for *A. operculata* to establish, develop and reproduce. In fact, this species is unable to establish an epiphytic habit even on the susceptible, sporophytic fronds of *C. crispus*. Furthermore, because of its fragility it is unlikely that the free-living habit could persist in nature where the plants would be easily disintegrated. The obligacy of *A. operculata* upon its host in nature is, however, certainly non physiological, as demonstrated by the ability of several isolates to grow and reproduce under diverse conditions and in absence of the host (also Correa *et al.* 1988). On the other hand, the consistent development of a pseudoparenchymatous habit by *A. heteroclada*

that permits attachment of the plants to diverse hosts and surfaces suggests a facultative endophytic relationship between this species and *C. crispus*.

Within symbiotic associations, the effect of one symbiont upon the other may be beneficial, innocuous or harmful. The present study indicates that infection by the two chlorophycean endophytes can be detrimental to *C. crispus*. Consequently, I suggest that *A. operculata* and *A. heteroclada* should be considered pathogens. There has been considerable debate in attempting to clarify the nomenclature used for symbionts inducing negative effects on their hosts (Hall 1974, Ahmadjian and Paracer 1986). Parasitism has been used extensively to classify detrimental associations. This probably has resulted from misunderstanding de Bary's definition of symbiosis in which all associations believed to be of mutual benefit for the two partners were erroneously grouped together. However, as clearly concluded by Hall in his review (1974), the term parasite should be restricted to organisms that develop on or in a living partner and from which they derive at least part of their nutrition, with no further implications regarding whether or not such unilateral "feeding" is detrimental. Furthermore, as indicated by the various examples given by Hall (1974) and Ahmadjian and Paracer (1986), nutrient withdrawal (parasitism) and the capacity to produce disease (pathogenicity) can be distinguished in theory and practice. This is true not only in fungal-higher plant associations, where most of the attention has concentrated, but also in algal interactions. Red algal parasites are characterized by obtaining at least part of their metabolic requirements from the host (Goff 1982 a), and have been rarely reported to cause damage or dysfunction to their partners. For at least two of these parasitic associations, however, experimental evidence has indicated a reduction or cessation of growth of infected plants (Nonomura 1979, Apt 1984). In the association between *Hypneocolax stellaris* and its host *Hypnea musciformis*, Apt (1984) considered the poor growth caused by the parasite to be a disease, and accordingly classified the parasite as a pathogen. This view is correct, as disease can be considered as a complex set of symptoms and signs induced by a pathogen whose presence causes disruption or impairment of vital functions in its host, or in part of it (Hall 1974, Ahmadjian and Paracer 1986, Mish 1983). I believe that growth, regenerative capacity and structural integrity are vital elements determining the performance of a frond of *C. crispus* as a whole.

The alteration of any one (or more than one) of these elements in a negative manner should be considered a disease, and the causing agents, *A. operculata* and *A. heteroclada* in this case, pathogens.

The question that now remains to be answered is whether diseases caused by these pigmented algal endophytes have an impact at a population level. The difficulties in doing experimental manipulations in the *C. crispus*-dominated zone of the intertidal area, together with the impossibility of detecting by eye endophytic infections *in situ* make it impractical to address this question.

To further assess the role of endophytism in the well being of *C. crispus,* it should also be recalled that, in addition to the chlorophycean species involved in the present study, there are brown filamentous endophytes that coexist with *A. operculata* and *A. heteroclada* (Correa *et al.* 1987). Preliminary results (unpubl.) with those taxa in culture indicate that they behave similarly to the chlorophycean species. Some of them penetrate the host immediately during germination (*A. operculata*-type infection), while others penetrate the host only after a period of growth embedded in the outer cell wall (*A. heteroclada*-type infection). Some of these isolates, as *A. operculata,* appear to develop preferentially in the sporophytic phase of the host. Furthermore, the similarity between infections by brown and green endophytes extends to cellular damage induced in the host by the penetrating filaments, and to the change in perception as food by crustacean herbivores (unpubl.). All this indicates that, in spite of the apparently large species diversity of pigmented algal endophytes associated with *C. crispus,* they can be grouped into two functional groups, *A. operculata*-type or *A. heteroclada*-type endophytes. Such an approach may be useful in gaining an understanding of the interactions between the two types of endophytes, which in fact may be acting synergistically upon *C. crispus.*

BIBLIOGRAPHY

Abbott, I. A. and Hollemberg, G. J. (1976). *Marine Algae of California.* Stanford University Press, Palo Alto, CA, 827 p.

Abdel-Rhaman, M. H. (1984). Le cycle de devéloppement de L'*Acrochaetium parvulum* (Rhodophycées, Acrochaetiales). *Cryptogamie Algologie* 5, 1-13.

Abdel-Rhaman, M. H. (1985a). Le cycle de devéloppement de L'*Acrochaetium gynandrum* (Rhodophycées, Acrochaetiales). *Cryptogamie Algologie* 6, 1-12.

Abdel-Rhaman, M. H. (1985b). Le cycle de devéloppement de L'*Audouinella rosulata* (Rhodophycées, Acrochaetiales). *Cryptogamie Algologie* 6, 171-184.

Ache, B. W. (1982). Chemoreception and thermoreception. In: *The Biology of Crustacea.* 3. Atwood, H. L. and Sandeman, D. C., eds. Academic Press, NY. pp. 369-398.

Adey, W. H. and Sperapani, C. P. (1971). The biology of *Kvaleya epilaeve,* a new parasitic genus and species of Corallinaceae. *Phycologia* 10, 29-42.

Adey, W. H., Masaki, T. and Akioka, H. (1974). *Ezo epiyessoense,* a new parasitic genus and species of Corallinaceae (Rhodophyta, Cryptonemiales). *Phycologia* 13, 329-344.

Ahmadjian, V. and Paracer, S. (1986). *Symbiosis. An Introduction to Biological Associations.* University Press of New England, Hanover, 212 pp.

Aist, J. R. (1976). Cytology of penetration and infection - Fungi. In: *Physiological Plant Pathology.* Heitfuss, R. and Williams, P. H., eds. Encyclopedia of Plant Physiology, New Series, Volume 4. Springer-Verlag, Berlin. pp. 197-221.

Aist, J. R. and Israel, H. W. (1977). Papilla formation: timing and significance during penetration of barley coleoptiles by *Erysiphe graminis* var. *hordei*. *Phytopathology* 67, 455-461.

Aist, J. R. (1983). Structural responses as resistance mechanisms. In: *The Dynamics of Host Defense*. Bailey, J. A. and Deverall, B. J., eds. Academic Press, NY. pp. 33-70.

Aist, J. R. and Gold, R. E. (1987). Prevention of fungal ingress: the role of papillae and calcium. In: *Molecular Determinants of Plant Diseases*. Nishimura, S., Vance, C. P. and Doke, N., eds. Japan Scientific Societies (Tokyo) and Springer-Verlag, Berlin. pp. 47-58.

Akai, S. (1973). Recent advance in studying the mechanism of fungal infection in plants. *Shokubutsu Byogai Kenkyu* 8, 1-60.

Albersheim, P., Darvil, A. G., Sharp, J. K., Davis, K. R. and Doares, S. H. (1986). Studies on the role of carbohydrates in host-microbe interactions. In: *Recognition in Micro-Plant Symbiotic and Pathogenic Interactions*. Lugtenberg, B., ed. Springer-Verlag, Berlin. pp. 297-309.

Andrews, J. H. (1976). The pathology of marine algae. *Biological Reviews* 51, 211-253.

Andrews, J. H. (1977). Observations on the pathology of seaweeds in the Pacific Northwest. *Canadian Journal of Botany* 55, 1019-1027.

Andrews, J. H., Vadas, R. L., Wheeler, W. N., Neushul, M., Woessner, J., Kohlmeyer, J., Dodds, J. A., Kazama, F. Y., and North, W. J. (1979). Pathology of seaweeds: current status and future prospects. *Experientia* 35, 429-450.

Apt, K. E. (1984). Effects of the symbiotic red alga *Hypneocolax steliaris* on its host *Hypnea musciformis* (Hypneaceae, Gigartinales). *Journal of Phycology* 20, 148-150.

Apt, K. E. (1988a). Ethiology and development of hyperplasia induced by *Streblonema* sp. (Phaeophyta) on members of the Laminariales (Phaeophyta). *Journal of Phycology* 24, 28-34.

Apt, K. E. (1988b). Morphology and development of hyperplasia on *Cystoseira osmundacea* (Phaeophyta) associated with *Haloguignardia irritans* (Ascomycotina). *American Journal of Botany* 75, 979-984.

Apt, K. E. (1988c). Galls and tumor-like growths on marine macroalgae. *Diseases of Aquatic Organisms* 4, 211-217.

Apt, K. and Gibor, A. (1989). Development and induction of bacteria-associated galls on *Prionitis lanceolata* (Rhodophyta). *Diseases of Aquatic Organisms* 6, 151-156.

Asada, Y., Bushnell, W. R., Ouchi, S. and Vance, C. P. (1982). *Plant Infection. The Physiological and Biochemical Basis.* Japan Scientific Societies Press (Tokyo) and Springer-Verlag (Berlin), 362 pp.

Azanza-Corrales, R. and Dawes, C. J. (1989). Wound healing in cultured *Eucheuma alvarezii* var. *tambalang* Doty. *Botanica Marina* 32, 229-234.

Bailey, J. A. (1986). *Biology and Molecular Biology of Plant-Pathogen Interactions.* Springer-Verlag, Berlin, 415 pp.

Baker, J. R. J. and Evans, L. V. (1973). The ship fouling alga *Ectocarpus*. I. Ultrastructure and cytochemistry of plurilocular reproductive stages. *Protoplasma* 77, 1-13.

Bakus, G. J., Targett, N. M. and Schulte, B. (1986). Chemical ecology of marine organisms: an overview. *Journal of Chemical Ecology* 12, 951-987.

Bellion, C., Hamer, G. K. and Yaphe, W. (1982). The degradation of *Eucheuma spinosum* and *Eucheuma cottonii* carrageenans by i-carrageenases and k-carrageenases from marine bacteria. *Canadian Journal of Microbiology* 28, 874-880.

Benhamou, N., Grenier, J., Asselin, A. and Legrand, M. (1989). Inmunogold localization of ß-1,3-glucanases in two plants infected by vascular wilt fungi. *The Plant Cell* 1, 1209-1221.

Benhamou, N, Joosten, M. and De Wit, P. (1990). Subcellular localization of chitinase and of its potential substrate in tomato root tissues infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici. Plant Physiology* 92, 1108-1120.

Bhattacharya, D. (1985). The demography of fronds of *Chondrus crispus* Stackhouse. *Journal of Experimental Marine Biology and Ecology* 91, 217-231.

Bidwell, R. G. S. (1958). Photosynthesis and marine algae. II. A survey of rates and products of photosynthesis in $C^{14}O_2$. *Canadian Journal of Botany* 36, 337-349.

Bidwell, R. G. S., McLachlan, J. and Lloyd, N. D. H. (1985). Tank cultivation of Irish moss, *Chondrus crispus* Stack. *Botanica Marina* 28, 87-97.

Biedka, R. F., Gosline, J. M. and De Wreede, R. E. (1987). Biomechanical analysis of wave-induced mortality in the marine alga *Pterygophora californica. Marine Ecology Progress Series* 36, 136-170.

Bisalputra, T., Rusanowski, P. C. and Walker, W. S. (1967). Surface activity, cell wall, and fine structure of pit connection in the red alga *Laurencia spectabilis. Journal of Ultrastructural Research* 20, 277-289.

Boller, T. (1987). Hydrolytic enzymes in plant disease defense. In: *Plant-Microbe Interactions. Molecular and Genetic Perspectives*. Kosuge, T. and Nester, E. W., eds. Macmillan Publishing Company, NY. pp. 385-413.

Boney, A. D. (1970). On the occurrence of structures resembling certain reproductive organs in cultures of *Acrochaetium infestans* Howe et Hoyt. *Nova Hedwigia* 19, 125-128.

Boney, A. D. (1972). *In vitro* growth of the endophyte *Acrochaetium bonnemaisoniae* (Batt.) J. et G. Feld. *Nova Hedwigia* 23, 173-186.

Boney, A. D. (1975). A filamentous red alga endozoic in a calcareous polyzoan. *Nova Hedwigia* 26, 477-482.

Boney, A. D. (1978). *Audouinella bonnemaisoniae* (Batt.) Dixon in *Bonnemaisonia nootkana* (Esper) Silva; *in situ* and *in vitro* studies. *Syesis* 11, 65-71.

Boney, A. D. (1980). Post-attachment responses of monospores of some endophytic *Audouinella* spp. (Nemaliales: Florideophyceae). *Nova Hedwigia* 33, 499-507.

Børgesen, F. (1916). The marine algae of the Danish West Indies. Part III. Rhodophyceae (1). *Dansk Botanisk Arkiv* 3, 3-80.

Børgesen, F. (1919). The marine algae of the Danish West Indies. Part IV. Rhodophyceae (5). *Dansk Botanisk Arkiv* 3, 305-368.

Borowitzka, M. A. and Larkum, A. W. D. (1977). Calcification in the green alga *Halimeda*. I. An ultrastructure study of the thallus development. *Journal of Phycology* 13, 6-16.

Borowitzka, M. A. and Vesk, M. (1978). Ultrastructure of the Corallinaceae. I. The vegetative cells of *Corallina officinalis* and *C. cuvierii*. *Marine Biology* 46, 295-304.

Bråten, T. (1975). Observations on mechanisms of attachment in the green alga *Ulva mutabilis* Føyn. An ultrastructural and light microscopical study of zygotes and rhizoids. *Protoplasma* 84, 161-173.

Brawley, S. H. and Adey, W. H. (1981). The effect of micrograzers on algal community structure in a coral reef microcosm. *Marine Biology* 61, 167-177.

Brawley, S. H. and Fei, X. G. (1987). Studies of mesoherbivory in aquaria and in an unbarricaded mariculture farm on the Chinese coast. *Journal of Phycology* 23, 614-623.

Brian, P. W. (1966). Obligate parasitism in fungi. *Proceedings of the Royal Society of London,* Series B, 168, 101-118.

Bronmark, C. (1985). Interactions between macrophytes, epiphytes and herbivores. *Oikos* 45, 26-30.

Buggeln, R. G. and Craigie, J. S. (1973). The physiology and biochemistry of *Chondrus crispus* Stackhouse. In: *Chondrus crispus*. Harvey, M. J. and McLachlan, J., eds. Nova Scotian Institute of Science, Volume 27 (Suppl.). pp. 81-102.

Bulthuis, D. A. (1983). Effects of *in situ* light reduction on density and growth of the seagrass *Heterozostera tasmanica* (Martens ex Aschers.) den Hartog in Western Port, Victoria, Australia. *Journal of Experimental Marine Biology and Ecology* 67, 91-103.

Bulthuis, D. A. and Woelkerling, Wm. J. (1983). Biomass accumulation and shading effects of epiphytes on leaves of the seagrass, *Heterozostera tasmanica,* in Victoria, Australia. *Aquatic Botany* 16, 137-148.

Burr, F. A. and West, J. A. (1970). Light and electron microscope observations on the vegetative and reproductive structure of *Bryopsis hypnoides*. *Phycologia* 9, 17-37.

Callow, J. A., Callow, M. E. and Evans, L. V. (1979). Nutritional studies on the parasitic red alga *Choreocolax polysiphoniae*. *New Phytologist* 83, 451-462.

Cambridge, M. L., Chiffing, A. W., Brittan, C., Moore, L. and McComb, A. J. (1986). The loss of seagrass in Cockburn Sound, Western Australia. II. Possible causes of seagrass decline. *Aquatic Botany* 24, 269-285.

Carpenter, R. C. (1990). Competition among marine macroalgae: a physiological perspective. *Journal of Phycology* 26, 6-12.

Carr, W. E. S and Derby, C. D. (1986). Chemically stimulated feeding behavior in marine animals. Importance of chemical mixtures and involvement of mixture interactions. *Journal of Chemical Ecology* 12, 989-1011.

Chapman, A. R. O. (1986). Population and community ecology of seaweeds. *Advances in Marine Biology* 23, 1-161.

Chen, L. C.-M. and McLachlan, J. (1972). The life history of *Chondrus crispus* in culture. *Canadian Journal of Botany* 50, 1055-1060.

Chen, L. C.-M. and Taylor, A. R. A. (1976). Scanning electron microscopy of early sporeling ontogeny of *Chondrus crispus*. *Canadian Journal of Botany* 54, 672-678.

Chen, L. C.-M and Craigie, J. S. (1981). Carrageenan analysis in apomictic *Gigartina stellata* - new puzzles. *Proceedings of the Interanational Seaweed Symposium* 10, 391-396.

Chopin, T. (1986). The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans-a review. *Canadian Technical Report of Fisheries and Aquatic Sciences* No. 1514, 68 pp.

Chopin, T. and Floc'h, J.-Y. (1987). Seasonal variations of growth in the red alga *Chondrus crispus* on the Atlantic French coast. I. A new approach by fluorescence labelling. *Canadian Journal of Botany* 65, 1014-1018.

Chopin, T., Bodeau-Bellion, C., Floc'h, J.-Y., Guittet, E. and Lallemand, J.-Y. (1987). Seasonal study of carrageenan structure from female gametophytes of *Chondrus crispus* Stackhouse (Rhodophyta). *Hydrobiologia* 151/152, 535-539.

Citharel, M. J. (1972 a). *Polysiphonia lanosa* (L.) Tandy est-il un simple épiphyte? *Comptes Rendus des Academie des Séances de la Société de Biologie Paris*, Série D 274, 1904-1906.

Citharel, M. J. (1972 b). Contribution à l'étude du metabolisme azoté des algues marines. Utilisation métabolique d'acide glutamique-^{14}C par *Ascophyllum nodosum* (Linne) Le Jolis et *Polysiphonia lanosa* (Linne) Tandy. *Botanica Marina* 15, 157-161.

Cole, K. M., Carol, C. M., Reid, P. E. and Sheath, R. G. (1985). Comparative studies on the cell walls of sexual and asexual *Bangia atropurpurea* (Rhodophyta). I. Histochemistry of polysaccharides. *Journal of Phycology* 21, 585-592.

Collmer, A. and Keen, N. T. (1986). The role of pectic enzymes in plant pathogenesis. *Annual Review of Phytopathology* 24, 383-409.

Cooper, R. M. (1981). Pathogen-induced changes in host ultrastructure. In: *Plant Disease Control. Resistance and Susceptibility.* Staples, R. C. and Toenniessen, G. H., eds. John Wiley & Sons, NY. pp. 105-142.

Cooper, R. M. (1983). The mechanisms and significance of enzymic degradation of host cell walls by parasites. In: *Biochemical Plant Pathology*. Callow, J. A., ed. Wiley, NY. pp. 101-135.

Correa, J. A. (1986). Endophytic Green Algae Associated with *C. crispus* Stackhouse. MSc Thesis, Acadia University. 117 pp

Correa, J. A., Nielsen, R., Grund, D. W. and McLachlan, J. (1987). Endophytic algae of Irish moss (*Chondrus crispus* Stackh.). *Hydrobiologia* 151/152, 223-228.

Correa, J. A. and McLachlan, J. (1988). Variation in the fine structure of the cuticle of *Chondrus crispus* Stackhouse (Rhodophyta). *British Phycological Journal* 23, 285-286.

Correa, J. A., Nielsen, R. and Grund, D. W. (1988). Endophytic algae of *Chondrus crispus* (Rhodophyta). II. *Acrochaete heteroclada* sp. nov., *A. operculata* sp. nov., and *Phaeophila dendroides* (Chlorophyta). *Journal of Phycology* 24, 528-539.

Cottler, M. H. (1971). Plasmalemmal extensions in *Chondrus crispus* (L.) Stackh. *Journal of Ultrastructure Research* 37, 31-36.

Court, G. J. (1980). Photosynthesis and translocation studies of *Laurencia spectabilis* and its symbiont *Janczewskia gardneri* (Rhodophyceae). *Journal of Phycology* 16, 270-279.

Craigie, J. S. (1990a). Irish moss cultivation: some reflections. In: *Economically Important Marine Plants of the Atlantic. Their Biology and Cultivation.* Yarish, C., Penniman, C. A. and Van Patten, P., eds. Connecticut Sea Grant College Program, Marine Sciences Institute, University of Connecticut, Groton, Connecticut. pp. 37-52.

Craigie, J. S. (1990b). Cell walls. In: *The Biology of Red Algae*. Cole, K. M. and Sheath, R., eds. Cambridge University Press, NY, (in press).

Craigie, J. S. and Leigh, C. (1978). Carrageenans and agars. In: *Handbook of Phycological Methods. Physiological and Biochemical Methods*. Hellebust, J. A. and Craigie, J. S., eds. Cambridge University Press, Cambridge. pp. 109-131.

Craigie, J. S. and Shacklock, P. F. (1989). Culture of Irish moss. In: *Cold Water Aquaculture in Atlantic Canada*. Boghen, A. D., ed. The Canadian Institute for Research on Regional Development, University of Moncton, Moncton, N.B. pp. 243-270.

Dangeard, P. (1931). Sur un *Ectocarpus* parasite provoquant des tumeurs chez le *Laminaria flexicaulis* (*Ectocarpus deformans* sp. nov.). *Comptes Rendus des Academie des Séances de la Société de Biologie Paris*, Série D 192, 57-60.

D'Antonio, C. (1985). Epiphytes on the rocky intertidal red alga *Rhodomela larix* (Turner) C. Agardh: negative effects on the host and food for herbivores?. *Journal of Experimental Marine Biology and Ecology* 86, 197-218.

Darvill, A. G. and Albersheim, P. (1984). Phytoalexins and their elicitors-A defence against microbial infection in plants. *Annual Review of Plant Physiology* 35, 243-275.

Davis, A. R., Targett, N. M., McConnell, O. J. and Young, C. M. (1989). Epibiosis of marine algae and benthic invertebrates: Natural products chemistry and other mechanisms inhibiting settlement and overgrowth. In: *Bioorganic Marine Chemistry* 3. Scheuer, P. J., ed. Springer-Verlag, Berlin. pp. 85-114.

Dawes, C. J., and Lohr, C. A. (1978). Cytoplasmic organization and endosymbiotic bacteria in the growing points of *Caulerpa prolifera*. *Revue de Algologie* 13, 309-314.

De Bary, A. (1879). *Die Erscheinung der Symbiose.* Verlag von Karl J. Trübner.

Denny, M. W. (1987). Lift as a mechanism of patch initiation in mussel beds. *Journal of Experimental Marine Biology and Ecology* 113, 231-245.

Denny, M. W. (1988). *Biology and the Mechanics of the Wave-Swept Environment.* Princeton University Press, Princeton. 329 pp.

Denny, M. W., Daniel, T. L. and Koehl, M. A. R. (1985). Mechanical limits to size in wave-swept organisms. *Ecological Monographs* 55, 69-102.

Denny, M. W., Brown, V., Carrington, E., Kraemer, G. and Miller, A. (1989). Fracture mechanics and the survival of wave-swept macroalgae. *Journal of Experimental Marine Biology and Ecology* 127, 211-228.

Deveau, L. E. (1989). Cultivation of *Chondrus crispus*. Programme and Abstracts of the 13th International Seaweed Symposium, Vancouver, Canada, August 13-18, 1989.

Diannelides, B. E. and Kristen, U. (1988). Comparative histochemical studies of reproductive and gametophytic tissue of marine red algae by means of fluorescent and light microscopy. *Botanica Marina* 31, 163-170.

Dickson, L. G. and Waaland, J. R. (1985). *Porphyra nereocystis:* a dual-daylength seaweed. *Planta* 165, 548-553.

Dixon, J., Schroeter, S.C. and Kastendiek, J. (1981). Effects of the encrusting bryozoan, *Membranipora membranacea,* on the loss of blades and fronds by the giant kelp, *Macrocystis pyrifera* (Laminariales). *Journal of Phycology* 17, 341-345.

Dixon, P. S. and Irvine, L. M. (1977). *Seaweeds of the British Isles. 1. Rhodophyta.* Part 1. *Introduction, Nemaliales, Gigartinales.* British Museum (Natural History), London, 252 p.

Douglas, A. E. and Smith, D. C. (1989). Are endosymbiosis mutualistic?. *Trends in Ecology and Evolution* 4, 350-352.

Ducker, S. C. and Knox, R. B. (1984). Epiphytism at the cellular level with special reference to algal epiphytes. In: *Cellular Interactions.* Linskens, H. F. and Heslop-Harrison, J. eds. Encyclopedia of Plant Physiology, New Series, Volume 17, Springer-Verlag, Berlin. pp. 113-123.

Dudgeon, S. R., Davison, I. R. and Vadas, R. L. (1989). Effect of freezing on photosynthesis of intertidal macroalgae: relative tolerance of *Chondrus crispus* and *Mastocarpus stellatus* (Rhodophyta). *Marine Biology* 101, 107-114.

Elbers, P. F., Ververgaert, P. H. J. T. and Demel, R. (1965). Tricomplex fixation of phospholipids. *Journal of Cell Biology* 24: 23-30.

Enright, C. T. (1979). Competitive interaction between *Chondrus crispus* (Florideophyceae) and *Ulva lactuca* in *Chondrus* aquaculture. *Proceedings of the International Seaweed Symposium* 9, 209-218.

Evans, L. V. and Christie, A. O. (1970). Studies on the ship-fouling alga *Enteromorpha.* I. Aspects of the fine-structure and biochemistry of swimming and newly settled zoospores. *Annals of Botany* 34, 451-466.

Evans, L. V., Callow, J. A. and Callow, M. A. (1973). Structural and physiological studies on the parasitic red alga *Holmsella*. *New Phytologist* 72, 393-402.

Evans, L. V., Callow, J. A. and Callow, M. A. (1978). Parasitic red algae: an appraisal. In: *Modern Approaches to the Taxonomy of Red and Brown Algae*. Irvine, D. E. G. and Price, J. H., eds. Systematics Association Special Volume No. 10. Academic Press, London. pp. 87-109.

Farkas, V. (1979). Biosynthesis of cell walls of fungi. *Microbiology Review* 43, 117-144.

Fagerberg, W. R. and Dawes, C. J. (1976). Studies on *Sargassum*. I. A light microscopic examination of the wound regeneration process in mature stipes of *S. filipendula*. *American Journal of Botany* 63, 110-119.

Fenical, W. (1975). Halogenation in Rhodophyta. *Journal of Phycology* 11, 245-259.

Fenical, W. and Norris, J. N. (1975). Chemotaxonomy in marine algae: chemical separation of some *Laurencia* species (Rhodophyta) from the Gulf of California. *Journal of Phycology* 11, 104-108.

Filion-Myklebust, C. and Norton, T. A. (1981). Epidermis shedding in the brown seaweed *Ascophyllum nodosum* (L.) Le Jolis, and its ecological significance. *Marine Biology Letters* 2, 45-51.

Fletcher, R. L. (1981). Studies on the marine fouling brown alga *Giffordia granulosa* (Sm.) Hamel in the Solent (South Coast of England). *Botanica Marina* 24, 211-221.

Fredericq, S., Hommersand, M. H. and Norris, J. N. (1989). Morphological observations on the adelphoparasite *Gracilariophila*

oryzoides (Gracilariales, Rhodophyta). *Japanese Journal of Phycology* 37, 167-179.

Fulcher, R. G. and McCully, M. E. (1969). Histological studies on the genus *Fucus*. IV. Regeneration and adventive embryony. *Canadian Journal of Botany* 47, 1643-1649.

Fuller, M. S., Lewis, B. and Cook, P. (1966). Occurrence of *Pythium* sp. on the marine alga *Porphyra*. *Mycologia* 58, 303-318.

Garbary, D. (1979a). Numerical taxonomy and generic circumscription in the Acrochaetiaceae (Rhodophyta). *Botanica Marina* 22, 477-492.

Garbary, D. (1979b). A revised species concept for endophytic and endozoic members of the Acrochaetiaceae (Rhodophyta). *Botaniska Notiser* 132, 451-455.

Garbary, D. (1979c). The effects of temperature on the growth and morphology of some *Audouinella* spp. (Acrochaetiaceae, Rhodophyta). *Botanica Marina* 22, 493-498.

Garbary, D. and Rueness, J. (1980). *Audouinella tetraspora*, a new member of the Acrochaetiaceae (Rhodophyta) from Norway. *Norwegian Journal of Botany* 27, 17-22.

Gerwick, W. and Lang, N. (1977). Structural, chemical and ecological studies on iridescence in *Iridaea* (Rhodophyta). *Journal of Phycology* 13, 121-127.

Glicksman, M. (1987). Utilization of seaweed hydrocolloids in the food industry. *Hydrobiologia* 151/152, 31-47.

Goff, L. J. (1976). The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae). V. Host responses to parasite infection. *Journal of Phycology* 12, 313-328.

Goff, L. J. (1979a). The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae). VI. Translocation of photoassimilated ^{14}C. *Journal of Phycology* 15, 82-87.

Goff, L. J. (1979b). The biology of *Harveyella mirabilis* (Cryptonemiales, Rhodophyceae). VII. Structure and proposed function of host-penetrating cells. *Journal of Phycology* 15, 87-100.

Goff, L. J. (1982a). The biology of parasitic red algae. In: *Progress in Phycological Research,* Volume 1. Round, F. E. and Chapman D. J., eds. Elsevier, NY. pp. 289-369.

Goff, L. J. (1982b). Symbiosis and parasitism: another viewpoint. *BioScience* 32, 255-256.

Goff, L. J. (1983). *Marine algal interactions: epibiosis, endobiosis, parasitism and disease.* In: Proceedings of the Joint China-U.S. Phycology Symposium. Tseng, C. K., ed. Science Press, Beijing. pp. 221-274.

Goff, L. J. and Cole, K. (1976). The biology of *Harveyella mirabilis* (Criptonemiales, Rhodophyceae). III. Spore germination and subsequent development witin the host *Odonthalia floccosa* (Ceramiales, Rhodophyceae). *Canadian Journal of Botany* 54, 268-280.

Goff, L. J. and Coleman, A. W. (1984). Transfer of nuclei from a parasite to its host. *Proceedings of the National Academy of Sciences U.S.A.* 81, 5420-5424.

Goff, L. J. and Coleman, A. W. (1985). The role of secondary pit connections in red algal parasitism. *Journal of Phycology* 21, 483-508.

Goff, L. J. and Coleman, A. W. (1987). Nuclear transfer from parasite to host. A new regulatory mechanism of parasitism. In:

Endocytobiology III. Annals of the New York Academy of Sciences, Volume 503. Lee, J. J. and Frederick, J. F. eds. pp. 402-423.

Goff, L. J. and Glasgow, J. C. (1980). *Pathogens of Marine Plants*. Special Report 7, California Sea Grant College Program, La Jolla. 236 pp.

Gonzalez, M. A. and Goff, L. J. (1989). The red algal epiphytes *Microcladia coulteri* and *M. californica* (Rhodophyceae, Ceramiaceae). II. Basiphyte specificity. *Journal of Phycology* 25, 558-567.

Goodman, R. N., Király, Z. and Wood, K. R. (1986). *The Biochemistry and Physiology of Plant Disease*. University of Missouri Press, 433 pp.

Gordon, E. and McCandless, E. L. (1973). Ultrastructure and histochemistry of *Chondrus crispus* Stackhouse. In: *Chondrus crispus*. Harvey, M. J. and McLachlan, J., eds. Nova Scotian Institute of Science, Volume 27 (Suppl.). pp. 111-133.

Gordon-Mills, E. and McCandless, E. L. (1975). Carrageenan in the cell walls of *Chondrus crispus* Stackh. (Rhodophyceae, Gigartinales). I. Localization with fluorescent antibody. *Phycologia* 14, 275-281.

Gordon-Mills, E. and McCandless, E. L. (1977). Carrageenan in the cell walls of *Chondrus crispus* Stackh. (Rhodophyceae, Gigartinales). II. Birefringence. Phycologia 16, 169-176.

Gordon-Mills, E. and McCandless, E. L. (1978). Carrageenan in the cell walls of *Chondrus crispus* Stackh. (Rhodophyceae, Gigartinales). III. Metachromasia and the topooptical reaction. Phycologia 17, 95-104.

Goring, D. A. I. (1956). A semimicro gelometer. *Canadian Journal of Technology* 34, 53-59.

Grove, S. N., Bracker, C. E. and Morré, D. J. (1970). An ultrastructural basis for hyphal tip growth in *Pythium ultimum*. *American Journal of Botany* 57, 245-266.

Hall, R. (1974). Pathogenism and parasitism as concepts of symbiotic relationships. *Phytopathology* 64, 576-577.

Hallam, N. D., Clayton, M. N. and Parish, D. (1980). Studies on the association between *Notheia anomala* and *Hormosira banksii* (Phaeophyta). *Australian Journal of Botany* 28, 239-248.

Hanic, L. A. and Craigie, J. S. (1969). Studies on the algal cuticle. *Journal of Phycology* 5, 89-102.

Hardy, F. G. and Moss, B. L. (1978). The attachment of zygotes and germlings of *Halidrys siliquosa* (L.) Lyngb. (Phaeophyceae, Fucales). *Phycologia* 17, 69-78.

Hargreaves, J. A. and Keon, J. P. R. (1986). Cell wall modifications associated with the resistance of cereals to fungal pathogens. In: *Biology and Molecular Biology of Plant-Pathogen Interactions*. Bailey, J. A., ed. Springer-Verlag, Berlin. pp. 133-140.

Harlin, M. M. (1973 a). "Obligate" algal epiphyte: *Smithora naiadum* grows on a synthetic substrate. *Journal of Phycology* 9, 230-232.

Harlin, M. M. (1973 b). Transfer of products between epiphytic marine algae and host plants. *Journal of Phycology* 9, 243-248.

Harlin, M. M. and Craigie, J. S. (1975). The distribution of photosynthate in *Ascophyllum nodosum* as it relates to epiphytic *Polysiphonia lanosa*. *Journal of Phycology* 11, 109-113.

Harvey, M. J. and McLachlan, J. (1973). *Chondrus crispus*. *Nova Scotian Institute of Science* 27 (Suppl.), 1-155.

Hawkes, M. W. (1980). Ultrastructure characteristics of monospore formation in *Porphyra gardneri* (Rhodophyta). *Journal of Phycology* 16, 192-196.

Heath, M. C. (1981). A generalized concept of host-parasite specificity. *Phytopathology* 71, 1121-1123.

Heath, M. C. (1984). Defense mechanisms of plants against fungal pathogens. In: *Infection Processes of Fungi*. Roberts, D. W. and Aist, J. R. eds. The Rockefeller Foundation. pp. 153-159.

Heath, M. C. (1986). Fundamental questions related to plant-fungal interactions: Can recombinant DNA technology provided the answers? pp. In: *Biology and Molecular Biology of Plant-Pathogen Interactions*. Bailey, J. A. ed. Springer-Verlag, Berlin. 15-27.

Henry, S. and Cole, K. M. (1982). Ultrastructure of swarmers in the Laminariales (Phaephyceae). I. Zoospores. *Journal of Phycology* 18, 550-569.

Hepler, P. K. (1980). Membranes in the mitotic apparatus of barley cells. *Journal of Cell Biology* 86, 490-499.

Hepler, P. K. (1981). The structure of the endoplasmic reticulum revealed by osmium tetroxide-potassium ferricyanide staining. *European Journal of Cell Biology* 26, 102-110.

Hislop, E. C., Barnaby, V. M., Shellis, C. and Laborda, F. (1974). Localization of a-L-arabinofuranosidase and acid phosphatase in mycelium of *Sclerotinia fructigena*. *Journal of General Microbiology* 81, 79-99.

Hoch, H. C. and Staples, R. C. (1987). Structural and chemical changes among the rust fungi during appressorium development. *Annual Review of Phytopathology* 25, 231-247.

Hoch, H. C., Staples, R. C., Whitehead, B., Comeau, J. and Wolf, E. D. (1987). Signaling for growth orientation and cell differentiation by surface topography in *Uromyces*. *Science* 235, 1659-1662.

Iima, M. and Tatewaki, M. (1987). On the life history and host-specificity of *Blastophysa rhizopus* (Codiales, Chaetosiphonaceae), an endophytic green alga from Muroran in laboratory cultures. *Japaneese Journal of Phycology* (Sôrui) 35, 241-250.

Ingram, D. S. (1982). A structural view of active defence. In: *Active Defence Mechanisms in Plants*. Wood, R. K. S., ed. Plenum Press, NY. pp. 19-38.

Johnson, C. R. and Mann, K. H. (1986). The crustose coralline alga, *Phymatolithon* Foslie, inhibits the overgrowth of seaweeds without relying on herbivores. *Journal of Experimental Marine Biology and Ecology* 96, 127-146.

Johnston, K. H. and McCandless, E. L. (1973). Enzymic hydrolysis of potassium chloride soluble fractions of carrageenan: properties of "λ–carrageenase" from *Pseudomonas carrageenovora*. *Canadian Journal of Microbiology* 19, 779-788.

Jordan, C. M., Endo, R. M. and Jordan, L. S. (1988). Penetration and colonization of resistant and susceptible *Apium graveolens* by *Fusarium oxysporum* f. sp. *apii* race: callose as a structural response. *Canadian Journal of Botany* 66, 2385-2391.

Kazama, F. and Fuller, M. S. (1970). Ultrastructure of *Porphyra perforata* infected with *Pythium marinum,* a marine fungus. *Canadian Journal of Botany* 48, 2103-2107.

Keon, J. P. R., Byrde, R. J. W. and Cooper, R. M. (1987). Some aspects of fungal enzymes that degrade cell walls. In: *Fungal Infection of Plants.* Pegg, G. F. and Ayres, P. G., eds. Cambridge University Press, Cambridge. pp. 133-192.

Kloareg, B. and Quatrano, R. S. (1988). Structure of the cell walls of marine algae and ecophysiological functions of the matrix polysaccharides. *Oceanography and Marine Biology Annual Review* 26, 259-315.

Koehl, M. A. R. (1982). The interaction of moving water and sessile organisms. *Scientific American* 247, 124-134.

Koehl, M. A. R. (1984). How do benthic organisms withstand moving water?. *American Zoologist* 24, 57-70.

Koehl, M. A. R. (1986). Seaweeds in moving water: form and mechanical function. In: *On the Economy of Plant Form and Function.* Givnish, T. J., ed. Cambridge University Press, Cambridge. pp. 603-634.

Kogame, K. and Yoshida, T. (1988). Observations on *Bolbocoleon piliferum* Pringsheim (Chaetophoraceae, Chlorophyta) newly found in Japan. *Japanese Journal of Phycology* 36, 52-54.

Kohlmeyer, J. (1973). Fungi from marine algae. *Botanica Marina* 16, 201-215.

Kohlmeyer, J. and Kohlmeyer, E. (1972). Is *Ascophyllum nodosum* lichenized?. *Botanica Marina* 15, 109-112.

Kohlmeyer, J. and Kohlmeyer, E. (1975). Biology and geographical distribution of *Spathulospora* species. *Mycologia* 67, 629-637.

Kohlmeyer, J. and Kohlmeyer, E. (1979). *Marine Mycology.* Academic Press, New York.

Kohlmeyer, J. and Demoulin, V. (1981). Parasitic and symbiotic fungi on marine algae. *Botanica Marina* 24, 9-18.

Kohlmeyer, J. and Hawkes, M. W. (1983). A suspected case of Mycophycobiosis between *Mycosphaerella apophlaeae* (Ascomycetes) and *Apophlaea* spp. (Rhodophyta). *Journal of Phycology* 19, 257-260.

Kolattukudy, P. E. (1984). Biochemistry and function of cutin and suberin. *Canadian Journal of Botany* 62, 2918-2933.

Kolattukudy, P. E. (1985). Enzymatic penetration of the plant cuticle by fungal pathogens. *Annual Review of Phytopathology* 23, 223-250.

Kolattukudy, P. E. and Crawford, M. S. (1987). The role of polymer degrading enzymes in fungal pathogenesis. In: *Molecular Determinants of Plant Diseases.* Nishimura, S., Vance, C. P. and Doke, N., eds. Japan Scientific Societies Press (Tokyo) and Springer-Verlag (Berlin). pp. 75-93.

Koslowsky, D. J. and Waaland, S. D. (1987). Ultrastructure of selective chloroplast destruction after somatic cell fusion in *Griffithsia pacifica* Kylin (Rhodophyta). *Journal of Phycology* 23, 638-648.

Kremer, B. P. (1983). Carbon economy and nutrition of the alloparasitic red alga *Harveyella mirabilis. Marine Biology* 76, 231-239.

Kugrens, P. and West, J. A. (1973 a). The ultrastructure of the alloparasitic red alga *Choreocolax polysiphoniae*. *Phycologia* 12, 175-186.

Küiper, J. (1983). The life history of *Chromastrum alariae* (Jónsson) Papenfuss (Rhodophyta, Acrochaetiaceae). *Acta Botanica Neerlandica* 32, 129-151.

Kunoh, H. (1984). Cytological aspects of penetration of plant epidermis by fungi. In: *Infections Processes of Fungi*. Roberst, D. W. and Aist, J. R., eds. The Rockefeller Foundation. pp. 137-146.

Lane, E. B. and Carlile, M. J. (1979). Post fusion somatic incompatibility in plasmodia of *Physarum polycephalum*. *Journal of Cell Science* 35, 339-354.

Lazo, M. L., Greenwell, M. and McLachlan, J. (1989). Population structure of *Chondrus crispus* Stackhouse (Gigartinaceae, Rhodophyta) along the coast of Prince Edward Island, Canada: distribution of gametophytic and sporophytic fronds. *Journal of Experimental Marine Biology and Ecology* 126, 45-58.

Lee, Y. P. and Lee, I. K. (1988). Contribution to the generic classification of the Rhodochortaceae (Rhodophyta, Nemaliales). *Botanica Marina* 31, 119-131.

Levring, T., Hoppe, H. A. and Schmid, O. J. (1969). *Marine Algae: A Survey of Research and Utilization*. Cram, De Gruyter & Co., Hamburg. 421 pp.

Lewin, R. (1982). Symbiosis and parasitism - Definitions and evaluations. *BioScience* 32, 254-260.

Lewin, R. (1984). New regulatory mechanism of parasitism. *Science* 226, 427.

Lewis, D. H. (1973). Concepts in fungal nutrition and the origin of biotrophy. *Biological Reviews* 48, 261-278.

Lewis, D. H. (1985). Symbiosis and mutualism: crisp concepts and soggy semantics. In: *The Biology of Mutualism. Ecology and Evolution*. Boucher, D. H., ed. Croom Helm, Kent, Australia. pp. 29-39.

Libes, M. (1986). Productivity-irradiants relationship of *Posidonia oceanica* and its epiphytes. *Aquatic Botany* 26, 285-306.

Lilly, G. R. (1968). Some aspect of the ecology of Irish moss, *Chondrus crispus* (L.) Stackh., in Newfoundland waters. *Fisheries Research Board of Canada, Technical Report* 43, 44 pp.

Linskens, H. (1963). Beitrag zur Frage der Beziehungen zwischen Epiphyt und Basiphyt bei marinen Algen. *Pubblicazioni della Stazione Zoologica di Napoli* 33, 274-293.

Littler, M. M. and Littler, D. S. (1980). The evolution of thallus form and survival strategies in benthic marine macroalgae: field and laboratory tests of a functional form model. *The American Naturalist* 116, 25-44.

Lobban, C. S. and Baxter, D. M. (1983). Distribution of the red algal epiphyte *Polysiphonia lanosa* on its brown algal host *Ascophyllum nodosum* in the Bay of Fundy, Canada. *Botanica Marina* 26, 533-538.

Lubchenco, J. (1978). Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. *The American Naturalist* 112, 23-39.

Lubchenco, J. (1980). Algal zonation in a New England rocky intertidal community: an experimental analysis. *Ecology* 61, 333-344

Lubchenco, J. (1983). *Littorina* and *Fucus:* effects of herbivores, substratum heterogeneity, and plant escapes during succession. *Ecology* 64, 1116-1123.

Lubchenco, J. and Menge, B. A. (1978). Community development and persistence in a low rocky intertidal zone. *Ecological Monographs* 59, 67-94.

Magne, F. and Abdel-Rhaman, M. H. (1983). La nature exacte de L'*Acrochaetium polyidis* (Rhodophycées, Acrochaetiales). *Cryptogamie Algologie* 4, 21-35.

Mariani-Colombo, P. (1978). Occurrence of endophytic bacteria in siphonous algae. *Phycologia* 17, 148-151.

Mariani-Colombo, P. and de Carli, M. E. (1980). Morphological and ultrastructural aspects of regeneration following wounding in the *Udotea petiolata* thallus. *Cytobios* 27, 147-155.

Mariani, P., Tolomio, C. and Braghetta, P. (1985). An ultrastructural approach to the adaptive role of the cell wall in the intertidal alga *Fucus virsoides*. *Protoplasma* 128, 208-217.

Martin, J. T. and Juniper, B. E. (1970). *The Cuticles of Plants*. St Martins Press, New York. 347 pp

Mathieson, A. C. and Prince, J. S. (1973). Ecology of *Chondrus crispus*. In: *Chondrus crispus*. Harvey, M. J. and McLachlan, J., eds. Nova Scotian Institute of Science 27 (Suppl.). pp. 53-79.

Mathieson, A. C. and Burns, R. L. (1975). Ecological studies of economic red algae. 5. Growth and reproduction of natural and harvested populations of *Chondrus crispus* Stackhouse in New Hampshire. *Journal of Experimental Marine Biology and Ecology* 17, 137-156.

McArthur, D. M. and Moss, B. L. (1977). The ultrastructure of cell walls in *Enteromorpha intestinalis* (L.) Link. *British Phycological Journal* 12, 359-368.

McBride, D. L., Kugrens, P. and West, J. A. (1974). Light and electron microscopic observations on red algal galls. *Protoplasma* 79, 249-264.

McCandless, E. L. (1981). Biological control of carrageenan structure: effects conferred by the phase of life cycle of the carrageenophyte. *Proceedings of the International Seaweed Symposium* 8, 1-18.

McCandless, E. L. and Craigie, J. S. (1979). Sulfated polysaccharides in red and brown algae. *Annual Review of Plant Physiology* 30, 41-53.

McCandless, E. L., Craigie, J. S. and Walter, J. A. (1973). Carrageenan in the gametophytic and sporophytic stages of *Chondrus crispus*. *Planta* 112, 201-211.

McCandless, E. L., Craigie, J. S. and Hansen, J. E. (1975). Carrageenans of gametangial and tetrasporangial stages of *Iridaea cordata* (Gigartinaceae). *Canadian Journal of Botany* 53, 2315-2318.

McCandless, E. L., Okada, W. T., Lott, J. N. A., Vollmer, C. M. and Gordon-Mills, E. M. (1977). Structural studies of *Chondrus crispus:* the effect of extraction of carrageenan. *Canadian Journal of Botany* 55, 2053-2064.

McCandless, E. L., West, J. A. and Guiry, M. D. (1982). Carrageenan patterns in the Phyllophoraceae. *Biochemical Systematics and Ecology* 10, 275-284.

McCandless, E. L., West, J. A. and Guiry, M. D. (1983). Carrageenan patterns in the Gigartinaceae. *Biochemical Systematics and Ecology* 11, 175-182.

McDonald, K. (1984). Osmium ferricyanide fixation improves microfilament preservation and membrane visualization in a variety of animal cell types. *Journal of Ultrastructure Research* 86, 107-118.

McLachlan, J. (1977). Effects of nutrients on growth and development of embryos of *Fucus edentatus* Pyl. (Phaeophyceae, Fucales). *Phycologia* 16, 329-338.

McLachlan, J. (1982). Inorganic nutrition of marine macroalgae in culture. In: *Synthetic and Degradative Processes in Marine Macrophytes*. Srivastava, L. M., ed. Walter de Gruyter, Berlin. pp. 71-98.

McLachlan, J. (1985). Macroalgae (seaweeds): industrial resources and their utilization. *Plant & Soil* 89, 137-157.

McLachlan, J. and Craigie, J. S. (1964). Algal inhibition by yellow ultraviolet-absorbing substances from *Fucus vesiculosus*. *Canadian Journal of Botany* 42, 287-292.

McLachlan, J. and Craigie, J. S. (1966). Antialgal activity of some simple phenols. *Journal of Phycology* 2, 133-135.

McLachlan, J., Quinn, J. and MacDougall, C. (1989). The structure of the plant of *Chondrus crispus* Stackhouse (Irish moss). *Journal of Applied Phycology* 1, 311-317.

McLean, M. W. and Williamson, F. B (1979). k-carrageenase from *Pseudomonas carrageenovora*. *European Journal of Biochemistry* 93, 553-558.

Menge, B. A. (1976). Organization of the New England rocky intertidal community: role of predation, competition, and environmental heterogeneity. *Ecological Monographs* 46, 355-393.

Menge, B. A. and Lubchenco, J. (1981). Community organization in temperate and tropical rocky intertidal habitats; prey refuges in relation to consumer pressure gradients. *Ecological Monographs* 51, 429-450.

Mish, F. (1983). *Webster's Ninth New Collegiate Dictionary.* Thomas Allen & Son Limited, Ontario. 1563 pp.

Mishra, A. K. (1969). Fine structure of the growing point of the coenocytic alga, *Caulerpa sertularioides. Canadian Journal of Botany* 47, 1599-1603.

Molina, F. I. (1986). *Petersenia pollagaster* (Oomycetes): an invasive fungal pathogen of *Chondrus crispus* (Rhodophyceae). In: *The Biology of Marine Fungi.* Moss, S. T., ed. Cambridge University Press. Cambridge. pp. 165-175.

Moore, R. and Walker, D. B. (1983). Studies of vegetative compatibility-incompatibility in higher plants. VI. Grafting of *Sedum* and *Solanum* callus tissue *in vitro. Protoplasma* 115, 114-121.

Moss, B. L. (1982). The control of epiphytes by *Halidrys siliquosa* (L.) Lyngb. (Phaeophyta, Cystoceiraceae). *Phycologia* 21, 185-191.

Neish, A. C., Shacklock, P. F., Fox, C. H. and Simpson, F. J. (1977). The cultivation of *Chondrus crispus.* Factors affecting growth under greenhouse conditions. *Canadian Journal of Botany* 55, 2263-2271.

Nicotri, M. E. (1977). The impact of crustacean herbivores on cultured seaweed populations. *Aquaculture* 12, 127-136.

Nicotri, M. E. (1980). Factors involved in herbivore food preference. *Journal of Experimental Marine Biology and Ecology* 42, 13-26.

Nielsen, R. (1979). Culture studies on the type species of *Acrochaete, Bolbocoleon* and *Entocladia* (Chaetophoraceae, Chlorophyceae). *Botaniska Notiser* 132, 441-449.

Nielsen, R. (1980). A comparative study of five marine Chaetophoraceae. *British Phycological Journal* 15, 131-138.

Nielsen, R. and McLachlan, J. (1986). Investigations of the marine algae of Nova Scotia. XVI. The occurrence of small green algae. *Canadian Journal of Botany* 64, 808-814.

Nishimura, S., Vance, C. P. and Doke, N. (1987). *Molecular Determinants of Plants Diseases.* Japan Scientific Societies Press (Tokyo) and Springer-Verlag (Berlin), 293 pp.

Nonomura, A. M. (1979). Development of *Janczewskia morimotoi* (Ceramiales) on its host *Laurencia nipponica* (Ceramiales, Rhodophyceae). *Journal of Phycology* 15, 154-162.

Nonomura, A. M. and West, J. A. (1981). Host-specificity of *Janczewskia* (Ceramiales, Rhodophyta). *Phycologia* 20, 251-258.

Norton, T. A. and Benson, M. R. (1983). Ecological interactions between the brown seaweed *Sargassum muticum* and its associated fauna. *Marine Biology* 75, 169-177

O'Kelly, C. J. (1980). Host-specificity and the mechanisms of speciation and dispersal in the endophytic algal genus *Endophyton* Gardner (Chlorophyta). *Journal of Phycology* 16 (Suppl.), 31.

O'Kelly, C. J. (1982). Observations on marine Chaetophoraceae (Chlorophyta). III. The structure, reproduction and life history of *Endophyton ramosum*. *Phycologia* 21, 247-257.

O'Kelly, C. J. (1983). Observations on marine Chaetophoraceae (Chlorophyta). IV. The structure, reproduction and life history of *Acrochaete geniculata* (Gardner) comb. nov. *Phycologia* 22, 13-21.

O'Kelly, C. J. and Yarish, C. (1980). Observations on marine Chaetophoraceae (Chlorophyta). I. Sporangial ontogeny in the type species of *Entocladia* and *Phaeophila*. *Journal of Phycology* 16, 543-554.

O'Kelly, C. J. and Yarish, C. (1981). Observations on marine Chaetophoraceae (Chlorophyta). II. On the circumscription of the genus *Entocladia* Reinke. *Phycologia* 20, 32-45.

Oswald, R. C. and Seed, R. (1986). Organization and seasonal progression within the epifaunal communities of coastal macroalgae. *Cahieres des Biologie Marine* 27, 29-40.

Paul. V. J. and Fenical, W. (1987). Natural products chemistry and chemical defence in tropical marine algae of the Phylum Chlorophyta. In: *Bioorganic Marine Chemistry I.* Scheuer, P. J., ed. Springer-Verlag, Berlin. pp. 1-29.

Pedersen, P. M. (1984). Studies on primitive brown algae (Fucophyceae). *Opera Botanica* 74, 1-76.

Pedérsen, M., Saenger, P., Rowan, K. S. and Hofsten, A. (1979). Bromine, bromophenols and floridorubin in the red alga *Lenormandia prolifera*. *Physiologia Plantarum* 46, 121-126.

Pedérsen, M., Roomans, G. M. and Hofsten, A. (1980). Blue iridescence and bromine in the cuticle of the red alga *Chondrus crispus* Stackh. *Botanica Marina* 23, 193-196.

Pedérsen, M., Roomans, G. M. and Hofsten, A. (1981). Bromine in the cuticle of *Polysiphonia nigrescens:* localization and content. *Journal of Phycology* 17, 105-108.

Penhale, P. A. and Smith, W. O. (1977). Excretion of dissolved organic carbon by eelgrass (*Zostera marina*) and its epiphytes. *Limnology and Oceanography* 22, 400-407.

Penot, M. (1974). Ion exchange between tissues of *Ascophyllum nodosum* (L.) Le Jolis and *Polysiphonia lanosa* (L.) Tandy. *Zeitschrift für Pflanzenphysiologie* 73, 125-131.

Penot, M. and Penot, M. (1981). Ions transport and exchange between Ascophyllum nodosum (L.) Le Jolis and some epiphytes. Proceedings of the International Seaweed Symposium

Peterson, C. H. and Renaud, P. E. (1989). Analysis of feeding preference experiments. *Oecologia* 80, 82-86.

Politis, D. J. and Wheeler, H. (1973). Ultrastructural study of penetration of maize leaves by *Colletotrichum graminicola. Physiological Plant Pathology* 3, 465-471.

Porter, D. (1986). Mycoses of marine organisms: an overview of pathogenic fungi. In: The *Biology of Marine Fungi.* Moss, S. T., ed. Cambridge University Press, Cambridge. pp. 141-153.

Prince, J. S. and Kingsbury, J. M. (1973). The ecology of *Chondrus crispus* at Plymouth, Massachusetts. 2. Field studies. *American Journal of Botany* 60, 964-975.

Pringle, J. D. and Mathieson, A. C. (1987). *Chondrus crispus* Stackhouse. In: Case Studies of Seven Commercial Seaweed Resources (Doty, M. S., Caddy, J. F. and Santelices, B., eds.). *FAO Fisheries Technical Paper* No. 281, pp. 49-122.

Pringle, J. D. and Semple, R. E. (1988). Impact of harvesting on Irish moss (*Chondrus crispus* Stackhouse) frond size class structure. *Canadian Journal of Fisheries and Aquatic Sciences* 45, 767-773.

Pringle, J. D., Ugarte, R. and Semple, R. E. (1990). Annual net primary production calculated from eastern Canadian Irish moss fishery data. *Hydrobiologia*(in press).

Pueschel, C. M. (1979). Ultrastructure of tetrasporogenesis in *Palmaria palmata* (Rhodophyta). *Journal of Phycology* 15, 409-424.

Pueschel, C. M. (1980). Pit connections and translocation in red algae. *Science* 209, 422-423.

Pueschel, C. M. (1987). Absence of cap membranes as a characteristic of pit plugs of some red algal orders. *Journal of Phycology* 23: 150-156

Pueschel, C. M. and Cole, K. M. (1985). Ultrastructure of germinating carpospores of *Porphyra variegata* (Kjellm.) Hus (Bangiales, Rhodophyta). *Journal of Phycology* 21, 146-154.

Pueschel, C. M. and van der Meer, J. P. (1985). Ultrastructure of the fungus *Petersenia palmariae* (Oomycetes) parasitic on the alga *Palmaria mollis* (Rhodophyceae). *Canadian Journal of Botany* 63, 409-418.

Pybus, C. (1977). The ecology of *Chondrus crispus* and *Gigartina stellata* (Rhodophyta) in Galway Bay. *Journal of the Marine Biological Association of the United Kingdom* 57, 609-628.

Raghukumar, C. (1986). Fungal parasites of the marine green algae, *Cladophora* and *Rhizoclonium*. *Botanica Marina* 29, 289-297.

Raghukumar, C. (1987 a). Fungal parasites of marine algae from Mandapam (South India). *Diseases of Aquatic Organisms* 3, 137-145.

Raghukumar, C. (1987 b). Fungal parasites of the green alga *Chaetomorpha media. Diseases of Aquatic Organisms* 3, 147-150.

Ralton, J. E., Smart, M. G. and Clarke, A. E. (1987). Recognition and infection processes in plant pathogen interactions. In: *Plant Microbe Interactions. Molecular and Genetic Perspectives.* Kosuge, T. and Nester, E. W., eds. Macmillan Publishing Company, NY. pp. 217-252.

Rawlence, D. J. and Taylor, A. R. A. (1970). The rhizoids of *Polysiphonia lanosa. Canadian Journal of Botany* 48, 607-611.

Rawlence, D. J. (1972). An ultrastructural study of the relationship between rhizoids of *Polysiphonia lanosa* (L.) Tandy (Rhodophyceae) and the tissue of *Ascophyllum nodosum* (L.) Le Jolis (Phaeophyceae). *Phycologia* 11, 279-290.

Rawlence, D. J and Taylor, A. R. A. (1972). A light and electron microscopy study of rhizoid development in *Polysiphonia lanosa* (L.) Tandy. *Journal of Phycology* 8, 15-24.

Reynolds, E. S. (1963). The use of lead citrate at high pH as a electron-opaque stain in electron microscopy. *Journal of Cell Biology* 17, 208-212.

Rittshof, D. and Bonaventura, J. (1986). Macromolecular cues in marine systems. *Journal of Chemical Ecology* 12, 1013-1023.

Rosenvinge, L. K. (1909). The marine algae of Denmark. Contributions to their natural history. Part I. Introduction. Rhodophyceae I. (Bangiales and Nemaliales). *Mémoires de l'Académie Royale des Sciences et des Lettres de Danemark* 7, 1-151.

Russell, G. (1983). Formation of an ectocarpoid epiflora on blades of *Laminaria digitata*. *Marine Ecology Progress Series* 11, 181-187.

Russell, G. and Veltkamp, C. J. (1984). Epiphyte survival on skin-shedding macrophytes. *Marine Ecology Progress Series* 18, 149-153.

Saito, Y., Yoneta, T. and Yoshikawa, M. (1977). The relationships of parasite and host in the red algae *Janczewskia tokidae* and *Laurencia nipponica*. *Bulletin of the Japanese Society of Phycology* 25, 311-317.

Sakata, K., Sakura, T. and Ina, K. (1988). Algal phagostimulants for marine herbivorous gastropods. *Journal of Chemical Ecology* 14, 1405-1416.

Sand-Jensen, K. (1977). Effect of epiphytes on eelgrass photosynthesis. *Aquatic Botany* 3, 55-63.

Sand-Jensen, K. and Revsbech, N. P. (1987). Photosynthesis and light adaptation in epiphyte-macrophyte associations measured by oxygen microelectrodes. *Limnology and Oceanography* 32, 452-457.

Sarwar, G., Sakata, T. and Kakimoto, D. (1983). Isolation and characterization of carrageenan - decomposing bacteria from marine environments. *Journal of General and Applied Microbiology* 29,145-155.

Schatz, S. (1980). Degradation of *Laminaria saccharina* by higher fungi: a preliminary report. *Botanica Marina* 23, 617-622.

Schnepf, E. (1986). Cellular polarity. *Annal Review of Plant Physiology* 37, 23-47.

Scott, J. L. and Dixon, P. S. (1973). Ultrastructure of tetrasporogenesis in the marine red alga *Ptilota hypnoides*. *Journal of Phycology* 9, 29-46.

Searles, R. B. and Leister, G. L. (1980). North Carolina marine algae. IX. *Onslowia endophytica* gen. et sp. nov. (Phaeophyta, Sphacelariales) and notes on other new records for North Carolina. *Journal of Phycology* 16, 35-40.

Setchell, W. A. and Gardner, N. L. (1922). Phycological contributions II to VI. II. New species of *Myrionema*. III. New species of *Compsonema*. IV. New species of *Hecatonema*. V. New species of *Pylaiella* and *Streblonema*. VI. New species of *Ectocarpus*. *University of California Publications in Botany* 7, 333-427.

Setchell, W. A. and Gardner, N. L. (1925). The marine algae of North America. Part III. Melonophyceae. *University of California Publications in Botany* 8, 381-898.

Shacklock, P. F. and Doyle, R. W. (1983). Control of epiphytes in seaweed cultures using grazers. *Aquaculture* 31, 141-151.

Shacklock, P. F. and Croft, G. B. (1981). Effects of grazers on *Chondrus crispus* in culture. *Aquaculture* 22, 331-342.

Sherwood, R. T. and Vance, C. P. (1982). Initial events in the epidermal layer during penetration. In: *Plant Infection. The Physiological and Biochemical Basis*. Asada, Y., Bushnell, W. R., Ouchi, S. and Vance, C. P., eds. Japan Scientific Societes Press (Tokyo) and Springer-Verlag (Berlin). pp. 27-44.

Sieburth, J. M. and Tootle, J. L. (1981). Seasonality of microbial fouling on *Ascophyllum nodosum* (L.) Le Jol., *Fucus vesiculosus* L., *Polysiphonia lanosa* (L.) Tandy and *Chondrus crispus* Stackh. *Journal of Phycology* 17, 57-64.

Silberstein, K., Chiffings, A. W. and McComb, A. J. (1986). The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook. *Aquatic Botany* 24, 355-371.

Simpson, F. J. and Shachlock, P. F. (1979). The cultivation of *Chondrus crispus*. Effect of temperature on growth and carrageenan production. *Botanica Marina* 22, 295-298.

Simpson, F. J., Neish, P. F., Shacklock and Robson, D. R. (1978). The cultivation of *Chondrus crispus:* effect of pH on growth and production of carrageenan. *Botanica Marina* 21, 229-235.

Smith, D. C. and Douglas, A. E. (1987). *The Biology of Symbiosis*. Edward Arnold (Publishers), Baltimore, 302 pp.

Sokal, R. R. and Rohlf, F. J. (1981). *Biometry*. W.H. Freeman and Company, NY, 859 pp.

South, G. R. (1968). Aspects of the development and reproduction of *Acrochaete repens* and *Bolbocoleon piliferum*. *Canadian Journal of Botany* 26, 101-113.

South, G. R. (1984). A checklist of marine algae of eastern Canada, second revision. *Canadian Journal of Botany* 62, 680-704.

Spurr, A. R. (1969). A low-viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructure Research* 26, 31-43.

Staples, R. C. and Macko, V. (1980). Formation of infection structures as a recognition response in fungi. *Experimental Mycology* 4, 2-16.

Staples, R. C. and Hoch, H. C. (1984). A sensing mechanism in rust uredospore germlings responsive to host morphology starts the cell

cycle. In: *Infections Processes of Fungi.* Roberst, D. W. and Aist, J. R., eds. The Rockefeller Foundation. pp. 126-136.

Staples, R. C., Hoch, H. C., Epstein, L., Laccetti, L. and Hassouna, S. (1985). Recognition of host morphology by rust fungi: responses and mechanisms. *Canadian Journal of Plant Pathology* 7, 314-322.

Staples, R. C. and Hoch, H. C. (1987). Infection structures - Form and function. *Experimental Mycology* 11, 163-169.

Starr, M. P. (1975). A generalized scheme for classifying organismic associations. In: *Symbiosis.* Jennings, D. H. and Lee, D. L., eds. Cambridge University Press, Cambridge. pp. 1-20.

Stegenga, H. and Borsje, W. J. (1976). The morphology and life history of *Acrochaetium dasyae* Collins (Rhodophyta, Nemaliales). *Acta Botanica Neerlandica* 25, 15-29.

Swale, E. M. F. and Belcher, J. H. (1963). Morphological observations on wild and cultured material of *Rhodochorton investiens* (Lenormand) nov. comb. (*Balbiania investiens* (Lenorm.) Sirodot). *Annals of Botany* 27, 281-290.

Tam, C. E., Cole, K. M. and Garbary, D. (1987). *In situ* and *in vitro* studies on the endophytic red algae *Audouinella porphyrae* and *A. vaga* (Acrochaetiales). *Canadian Journal of Botany* 65, 532-538.

Taylor, A. R. A. and Chen, L. C.-M. (1973). The biology of *Chondrus crispus* Stackhouse: Systematics, morphology and life history. In: *Chondrus crispus.* Harvey, M. J. and McLachlan, J., eds. Nova Scotian Institute of Science, Volume 27 (Suppl.). pp. 1-21.

Taylor, A. R. A. and Chen, L. C.-M. (1990). Chondrus Stackhouse. In: *Biology of Economic Seaweeds.* Akatsuka, I., ed. Dr. W. Junk Publishers, The Netherlans (In press).

Terry, L. A. and Edyvean, R. G. J. (1981). Microalgae and corrosion. *Botanica Marina* 24, 177-183.

Testrake, D. and Aldrich, H. C. (1984). Ultrastructure of two associations involving marine fungi and green algae. *Botanica Marina* 27, 515-519.

Thompson, J. E. (1985). Exudation of biologically-active metabolites in a sponge (*Aplysina fistularis*). I. Biological evidence. *Marine Biology* 88, 23-26.

Toth, R. (1976). The release, settlement and germination of zoospores in *Chorda tomentosa* (Phaeophyceae, Laminariales). *Journal of Phycology* 12, 222-233.

Tsekos, I. (1982). Tumour-like growths induced by bacteria in the thallus of a red alga , *Gigartina teedii* (Roth) Lamour. *Annals of Botany* 49, 123-126.

Tsekos, I. and Schnepf, E. (1983). The ultrastructure of carposporogenesis in *Gigartina teedii* (Roth) Lamour. (Gigartinales, Rhodophyceae): auxiliary cell, cystocarpic plant. *Flora* 173, 81-86.

Tseng, C. K. (1981). Commercial cultivation. In: *The Biology of Seaweeds.* Lobban, C. S. and Wynne, M. J., eds. Botanical Monographs, Volume 17. University of California Press, Berkeley. pp. 680-725.

Turner, C. H. C. and Evans, L. V. (1977). Physiological studies on the relationship between *Ascophyllum nodosum* and *Polysiphonia lanosa. New Phytologist* 79, 363-371.

Tveter, E. and Mathieson, A. C. (1976). Sporeling coalescence in *Chondrus crispus* (Rhodophyta). *Journal of Phycology* 12, 110-118.

Tveter-Gallagher, E. and Mathieson, A. C. (1980). An electron microscopic study of sporeling coalescence in the red alga *Chondrus crispus. Scanning Electron Microscopy* 3, 570-580.

Vaughan D. (1983). *Energy Dispersive X-Ray Microanalysis. An Introduction.* Kevex Corporation, CA. 52 pp.

van der Meer, J. P. and Pueschel, C. M. (1985). *Petersenia palmariae* n. sp. (Oomycetes): a pathogenic parasite of the red alga *Palmaria mollis* (Rhodophyceae). *Canadian Journal of Botany* 63, 404-408.

Waaland, J. R. (1981). Commercial utilization. In: *The Biology of Seaweeds.* Lobban, C. S. and Wynne, M. J., eds. Botanical Monographs, Volume 17. University of California Press, Berkeley. pp. 726-741.

Wahl, M. (1989). Marine epibiosis. I. Fouling and antifouling: some basic aspects. *Marine Ecology Progress Series* 58, 175-189.

Walker, D. C., Hughes, G. C. and Bisalputra, T. (1979). A new interpretation of the interfacial zone between *Spathulospora* (Ascomyctes) and *Ballia* (Florideophyceae). *Transactions of the British Mycological Society* 73, 193-206.

Walker, R. P., Thompson, J. E. and Faulkner, D. J. (1985). Exudation of biologically-active metabolites in a sponge (*Aplysina fistularis*). II. Chemical evidence. *Marine Biology* 88, 27-32.

Watson, D. C. and Norton, T. A. (1985). Dietary preferences of the common periwinkle, *Littorina littorea* (L.). *Journal of Experimental Marine Biology and Ecology* 88, 193-211.

Watson, D. C. and Norton, T. A. (1987). The habitat and feeding preferences of *Littorina obtusata* (L.) and *L. mariae* Sacchi et Rastelli. *Journal of Experimental Marine Biology and Ecology* 112, 61-72.

Weigl, J. and Yaphe, W. (1966). The enzymatic hydrolysis of carrageenan by *Pseudomonas carrageenovora:* purification of a k-carrageenase. *Canadian Journal of Microbiology* 12, 939-947.

Wessels, J. G. H. and Sietsma, J. H. (1981). Fungal cell walls: a survey. In: *Plant Carbohydrates II.* Tanner, W. and Loewus, F. A., eds. Encyclopedia of Plant Physiology, New Series, Volume 13 B. Springer-Verlag, Berlin. pp. 352-394.

West, J. A. (1968). Morphology and reproduction of the red alga *Acrochaetium pectinatum* in culture. *Journal of Phycology* 4, 89-99.

West, J. A. (1970). The life history of *Rhodochorton concrescens* in culture. *British Phycological Journal* 5, 179-186.

West, J. A. (1972). Environmental control of hair and sporangial formation in the marine red alga *Acrochaetium proskaneri* sp. nov. *Proceedings of the International Seaweed Symposium* 7, 377-384.

West, J. A. (1979). The life history of *Rhodochorton membranaceum,* an endozoic red alga. *Botanica Marina* 22, 111-115.

West, J. A. and Hommersand, M. H. (1981). Rhodophyta: Life Histories. In: *The Biology of Seaweeds.* Lobban, C. S. and Wynne, M. J., eds. Botanical Monographs, Volume 17. University of California Press, Berkeley. pp. 133-193.

Wetherbee, R. (1979). "Transfer connections": specialized pathways for nutrient translocation in a red alga? *Science* 204, 858-859.

Wetherbee, R. and Quirk, H. M. (1982 a). The fine structure and cytology of the association between the parasitic red alga *Holmsella australis* and its red algal host *Gracilaria furcellata. Protoplasma* 110, 153-165.

Wetherbee, R. and Quirk, H. M. (1982 b). The fine structure of secondary pit connection formation between the red algal alloparasite *Holmsella australis* and its red algal host *Gracilaria furcellata. Protoplasma* 110, 166-176.

White, E. B. and Boney, A. D. (1969). Experiments with some endophytic and endozoic *Acrochaetium* species. *Journal of Experimental Marine Biology and Ecology* 3, 246-274.

White, E. B. and Boney, A. D. (1970). *In situ* and *in vitro* studies on some endophytic and endozoic *Acrochaetium* species. *Nova Hedwigia* 19, 841-881.

White, D. L., Mazurkiewicz, J. E. and Barnett, R. J. (1979). A chemical mechanism for tissue staining by osmium tetroxide-ferrocyanide mixtures. *Journal of Histochemistry and Cytochemistry* 27, 1084-1091.

Whitfield, P. S. (1979). *The Biology of Parasitism: An Introduction to the Study of Associating Organisms.* Edward Arnold, London, 271 pp.

Woelkerling, W. J. (1973). The morphology and systematics of the *Audouinella* complex (Acrochaetiaceae, Rhodophyta) in northeastern United States. *Rhodora* 75, 529-621.

Woelkerling, W. J. (1983). The *Audouinella* (*Acrochaetium-Rhodochorton*) complex (Rhodophyta): present prospectives. *Phycologia* 22, 59-92.

Wynn, W. K. (1976). Appressorium formation over stomates by the bean rust fungus: response to a surface contact stimulus. *Phytopathology* 66, 136-146.

Wynn, W. K. (1981). Tropic and taxic responses of pathogens to plants. *Annual Review of Phytopathology* 19, 237-255.

Wynn, W. K. and Staples, R. C. (1981). Tropisms of fungi in host recognition. In: *Plant Disease Control, Resistance and Susceptibility*. Staples, R. C. and Toenniessen, G. H., eds. John Wiley & Sons, New York. pp. 45-69.

Yarish, C. (1975). A cultural assessment of the taxonomic criteria of selected marine Chaetophoraceae (Chlorophyta). *Nova Hedwigia* 26, 385-430.

Yaphe, W. and Baxter, B. (1955). The enzymic hydrolysis of carrageenin. *Applied Microbiology* 3, 380-383.

Yoshida, T. and Akiyama, K. (1979). *Streblonema* (Phaeophyta) infection in the frond of cultivated *Undaria* (Phaeophyceae). *Proceedings of the International Seaweed Symposium* 9, 219-223.

Young, E. G. (1966). Periodate oxidation of the insoluble residue after severe extraction of some marine algae. *Proceedings of the International Seaweed Symposium* 5, 337-346.

Zuccarello, G. and Goff, L. J. (1988). Host infection and the development of the adelphoparasite *Gracilariophila oryzoides*. *Journal of Phycology* 24 (Suppl.), 10.