The Mycota

Edited by
K. Esser

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The Mycota

A Comprehensive Treatise
on Fungi as Experimental Systems
for Basic and Applied Research

Edited by K. Esser

V *Plant Relationships*
2nd Edition

Volume Editor:
H.B. Deising

Springer

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Karl Esser
(born 1924) is retired Professor of General Botany and Director of the Botanical Garden at the Ruhr-Universität Bochum (Germany). His scientific work focused on basic research in classical and molecular genetics in relation to practical application. His studies were carried out mostly on fungi. Together with his collaborators he was the first to detect plasmids in higher fungi. This has led to the integration of fungal genetics in biotechnology. His scientific work was distinguished by many national and international honors, especially three honorary doctoral degrees.

Holger B. Deising
(born 1956) studied agricultural sciences and botany at the University of Kiel, Germany. His PhD thesis focused on nitrate reduction by *Sphagnum* species. After graduating in 1987 he worked on the infection structures of plant-pathogenic rust fungi and he qualified as a lecturer at the University of Konstanz in 1996. In 1997 he became a Full Professor for Phytopathology and Plant Protection at Martin-Luther-University Halle-Wittenberg. His research stays include McMaster University (Hamilton, ON, Canada), the University of Georgia (Athens, GA, USA), and Purdue University (West Lafayetta, IN, USA). His scientific interest is directed to various aspects of fungus–plant interactions, with special focus on the differentiation and function of fungal infection structures and pathogenicity factors in the causal agent of maize anthracnose and stalk rot, *Colletotrichum graminicola*. Another area of research includes molecular mechanisms conferring fungicide resistance in several plant-pathogenic fungi.

Series Preface

Mycology, the study of fungi, originated as a subdiscipline of botany and was a descriptive discipline, largely neglected as an experimental science until the early years of this century. A seminal paper by Blakeslee in 1904 provided evidence for selfincompatibility, termed "heterothallism", and stimulated interest in studies related to the control of sexual reproduction in fungi by mating-type specificities. Soon to follow was the demonstration that sexually reproducing fungi exhibit Mendelian inheritance and that it was possible to conduct formal genetic analysis with fungi. The names Burgeff, Kniep and Lindegren are all associated with this early period of fungal genetics research.

These studies and the discovery of penicillin by Fleming, who shared a Nobel Prize in 1945, provided further impetus for experimental research with fungi. Thus began a period of interest in mutation induction and analysis of mutants for biochemical traits. Such fundamental research, conducted largely with Neurospora crassa, led to the one gene: one enzyme hypothesis and to a secondNobel Prize for fungal research awarded to Beadle and Tatum in 1958. Fundamental research in biochemical genetics was extended to other fungi, especially to Saccharomyces cerevisiae, and by the mid-1960s fungal systems were much favored for studies in eukaryotic molecular biology and were soon able to compete with bacterial systems in the molecular arena.

The experimental achievements in research on the genetics andmolecular biology of fungi have benefited more generally studies in the related fields of fungal biochemistry, plant pathology,medicalmycology, and systematics. Today, there ismuch interest in the geneticmanipulation of fungi for applied research. This current interest in biotechnical genetics has been augmented by the development of DNA-mediated transformation systems in fungi and by an understanding of gene expression and regulation at the molecular level. Applied research initiatives involving fungi extend broadly to areas of interest not only to industry but to agricultural and environmental sciences as well.

It is this burgeoning interest in fungi as experimental systems for applied as well as basic research that has prompted publication of this series of books under the title The Mycota. This title knowingly relegates fungi into a separate realm, distinct from that of either plants, animals, or protozoa. For consistency throughout this Series of Volumes the names adopted for major groups of fungi (representative genera in parentheses) are as follows:

Pseudomycota

Division:	Oomycota (Achlya, Phytophthora, Pythium)
Division:	Hyphochytriomycota

Eumycota

Division:	Chytridiomycota (Allomyces)
Division:	Zygomycota (Mucor, Phycomyces, Blakeslea)
Division:	Dikaryomycota

Subdivision:	Ascomycotina
Class:	Saccharomycetes (Saccharomyces, Schizosaccharomyces)
Class:	Ascomycetes (Neurospora, Podospora, Aspergillus)
Subdivision:	Basidiomycotina
Class:	Heterobasidiomycetes (Ustilago, Tremella)
Class:	Homobasidiomycetes (Schizophyllum, Coprinus)

We have made the decision to exclude from The Mycota the slime molds which, although they have traditional and strong ties to mycology, truly represent nonfungal forms insofar as they ingest nutrients by phagocytosis, lack a cell wall during the assimilative phase, and clearly show affinities with certain protozoan taxa.
The Series throughoutwill address three basic questions:what are the fungi,what do they do, andwhat is their relevance to human affairs? Such a focused and comprehensive treatment of the fungi is long overdue in the opinion of the editors.

A volume devoted to systematics would ordinarily have been the first to appear in this Series. However, the scope of such a volume, coupled with the need to give serious and sustained consideration to any reclassification of major fungal groups, has delayed early publication. We wish, however, to provide a preamble on the nature of fungi, to acquaint readers who are unfamiliar with fungi with certain characteristics that are representative of these organisms and which make them attractive subjects for experimentation.

The fungi represent a heterogeneous assemblage of eukaryotic microorganisms. Fungal metabolism is characteristically heterotrophic or assimilative for organic carbon and some nonelemental source of nitrogen. Fungal cells characteristically imbibe or absorb, rather thaningest,nutrients andtheyhave rigid cellwalls.The vastmajorityof fungi are haploid organisms reproducing either sexually or asexually through spores. The spore forms and details on theirmethod of production have been used to delineate most fungal taxa.Although there is amultitude of spore forms, fungal spores are basically only of two types: (i) asexual spores are formed followingmitosis (mitospores) and culminate vegetative growth, and (ii) sexual spores are formed following meiosis (meiospores) and are borne in or upon specialized generative structures, the latter frequently clustered in a fruit body. The vegetative forms of fungi are either unicellular, yeasts are an example, or hyphal; the latter may be branched to form an extensive mycelium.

Regardless of these details, it is the accessibility of spores, especially the direct recovery of meiospores coupled with extended vegetative haploidy, that have made fungi especially attractive as objects for experimental research.

The ability of fungi, especially the saprobic fungi, to absorb and grow on rather simple and defined substrates and to convert these substances, not only into essential metabolites but into important secondarymetabolites, is also noteworthy.Themetabolic capacities of fungi have attracted much interest in natural products chemistry and in the production of antibiotics and other bioactive compounds. Fungi, especially yeasts, are important in fermentation processes. Other fungi are important in the production of enzymes, citric acid and other organic compounds as well as in the fermentation of foods.

Fungi have invaded every conceivable ecological niche. Saprobic forms abound, especially in the decay of organic debris. Pathogenic forms exist with both plant and animal hosts. Fungi even grow on other fungi. They are found in aquatic as well as soil environments, and their spores may pollute the air. Some are edible; others are poisonous. Many are variously associated with plants as copartners in the formation of lichens and mycorrhizae, as symbiotic endophytes or as overt pathogens. Association with animal systems varies; examples include the predaceous fungi that trap nematodes, the microfungi that grow in the anaerobic environment of the rumen, the many insectas-

sociated fungi and themedically important pathogens afflicting humans. Yes, fungi are ubiquitous and important.

There are many fungi, conservative estimates are in the order of 100,000 species, and there are many ways to study them, from descriptive accounts of organisms found in nature to laboratory experimentation at the cellular and molecular level. All such studies expand our knowledge of fungi and of fungal processes and improve our ability to utilize and to control fungi for the benefit of humankind.

We have invited leading research specialists in the field of mycology to contribute to this Series. We are especially indebted and grateful for the initiative and leadership shown by theVolumeEditors in selecting topics and assembling the experts.We have all been a bit ambitious in producing these Volumes on a timely basis and therein lies the possibility of mistakes and oversights in this first edition.We encourage the readership to draw our attention to any error, omission or inconsistency in this Series in order that improvements can be made in any subsequent edition.

Finally, we wish to acknowledge the willingness of Springer-Verlag to host this project, which is envisioned to require more than 5 years of effort and the publication of at least nine Volumes.

Bochum, Germany
Auburn, AL, USA
April 1994

Karl Esser
Paul A. Lemke
Series Editors

Addendum to the Series Preface

In early 1989, encouraged by Dieter Czeschlik, Springer-Verlag, Paul A. Lemke and I began to plan The Mycota. The first volume was released in 1994, 12 volumes followed in the subsequent years, and two more volumes (Volumes XIV and XV) will be published within the next few years. Unfortunately, after a long and serious illness, Paul A. Lemke died in November 1995. Thus, it was my responsibility to proceed with the continuation of this series, which was supported by JoanW. Bennett for Volumes X–XII.

The series was evidently accepted by the scientific community, because several volumes are out of print. Therefore, Springer-Verlag has decided to publish completely revised and updated new editions of Volumes I, II, III, IV, V, VI, VIII, and X. I am glad that most of the volume editors and authors have agreed to join our project again.I would like to take this opportunity to thank Dieter Czeschlik, his colleague, Andrea Schlitzberger, and Springer-Verlag for their help in realizing this enterprise and for their excellent cooperation for many years

Bochum, Germany
May 2008

Karl Esser

Volume Preface to the Second Edition

Joseph G.H. Wessels, in a review on fungal growth and morphogenesis, described fungi as the natural complement to plant life. He speculated that plants could probably have arisen without animals evolving, but raised doubts whether plants could have ever have evolved without the advent of fungi. The intimacy of trans-kingdom relationships between fungi and plants could not have been circumscribed any clearer.

The first edition of volume V *Plant Relationships* was been published in *The Mycota* series more than ten years ago. In their preface George Carroll and Paul Tudzynski ,the editors, commented on the large number of fungal and vascular plant species (respectively estimated to be in the order of 10^6 and $300–350\times10^3$) and emphasized the enormous number of interactions displayed by fungi and plants. The large number of fungi and plants reflects the complexity of the mechanisms of the interactions between them. Therefore, only examples can be given of fungal lifestyles and their interactions with plants, either mutualistic or pathogenic.

Significant methodological progress has been made in almost all areas of mycological research (e.g. transcriptomics, proteomics, metabolomics) since the first edition was published. For example, molecular genetics has experienced strong support from various genome sequencing efforts. To date, more than 50 fungal and several oomycete genomes have been sequenced and genome-wide gene expression profiling, functional screens for genes in yeasts or bacteria, the labelling of gene products, and the efficiency of targeted inactivation of genes have improved. These advances were accompanied by improvements in light and electron microscopy which, together with the utilization of molecular tools, allowed a proportional development in our knowledge of the cell biology of fungal interactions. Last but not least, increased sensitivies in the detection of biomolecules through analytical chemistry enabled our understanding of the chemical basis of both mutualistic and pathogenic fungus–plant interactions.

The second edition of *The Mycota*, volume V, reflects the substantial progress made in various areas of fungus–plant interactions. Organized in three parts, i.e. profiles in pathogenesis and mutualism, mechanisms of pathogenic and mutualistic interactions, and plant response to pathogen ingress, this book provides an overview of fundamental aspects of fungal lifestyles.

Chapters 1–5 focus on different fungal systems, characterize their profiles, and give examples both for pathogenic *Phytophthora* species belonging to the Oomycota and for fungi with biotrophic, necrotrophic, or mutualistic lifestyles.

Chapters 6–16 focus on mechanisms of the interactions, with detailed discussions on specific aspects, such as spore release and distribution (which are of critical importance to the success of pathogens), the basis of the specificity of fungus–plant interactions, and signal transduction. Protein secretion, the role played by cell wall-degrading enzymes and toxins, and the induction of programmed cell death represent further areas of research that significantly helped our understanding fungal pathogenicity.

The last four chapters of this section, 13–16, describe in detail mechanisms of mutualism, i.e. mycorrhizal and endophytic interactions, as well as interactions between fungi and algae in lichens. Finally, chapters 17 and 18 describe the response of plants

towards attacking pathogenic fungi and focus on signal perception and transduction and the mechanisms of defense.

Forty-six internationally acknowledged scientists specialized in different areas of eukaryotic microbiology (mycology), microbial genetics and genomics, plant pathology, plant molecular biology, and plant genetics contributed to this volume. I would like to express my gratitude to all authors for their tremendous efforts, to the series editor, Karl Esser, who provided many helpful comments, and to Springer-Verlag for continuous assistance and patience.

I hope that the second edition of *Plant Relationships*, volume V in *The Mycota* series, will be appreciated by a wide variety of professional biologists, as it allows one to keep pace with the rapidly developing field of fungal research. In addition, the text and high-quality illustrations may provide a source for teaching at graduate level; and the different chapters can be used by young researchers as a helpful introduction to the relevant literature on fungus–plant interactions.

Halle (Saale), Germany
October 2008

Holger B. Deising
Volume Editor

Volume Preface to the First Edition

The number of fungal species has been loosely estimated to be on the order of 1 million, while the number of vascular plants is known with considerably greater certainty to lie between 300000 and 350000. Clearly, any volume which purports to deal with interactions between these two vast assemblages of organisms must do so concisely and selectively. In the chapters to foIlow, we have made no attempt to be allinclusive, but rather have chosen examples from which general conclusions about fungus/plant interactions might be drawn. The materials presented here come from the core literature on plant pathology and from research on fungal mutualisms and on evolutionary biology. A variety of approaches are evident: biochemistry, molecular biology, cellular fine structure, genetics, epidemiology, population biology, ecology, and computer modeling. The frequent overlap of such approach es within single reviews has resulted in a rich array of insights into the factors which regulate fungus/plant interactions. In these chapters, such interactions have also been considered on a variety of scales, both geographic and temporal, from single plant cells to ecosystems, from interactions which occur within minutes of contact to mechanisms which have presumably evolved during the course of several hundred million years.

Volume V consists of two parts: Volume V, Part A, and Volume V, Part B. While section headings provide signposts, we wish to make the rationale for the organization of these volumes absolutely dear. Part A begins with a brief introduction to both volumes. A series of reviews follows (Chaps. 1-6) which deal with the temporal sequence of events from the time fungal spores make contact with a host plant until the point where fungal hyphae are either firmly ensconced within a host or the attempted infections have been repulsed. Chapters 7-12 deal with metabolic interactions between host and fungus within the host plant after infection and particularly with the roles played by low molecular weight fungal metabolites such as toxins and phytohormones in pathogenic as weIl as mutualistic associations.

Chapters 1-8 of Part B are grouped in a section labeled, “Profiles in Pathogenesis and Mutualism”; here, interactions between fungi and host plants are explored in a variety of important model systems. These reviews focus less on processes per se and more on the specific fungi or groups of fungi as examples of pathogens or mutualists on plants. Chapters 9-12 of Part B move from discussions of physiological interactions between individuals to considerations of interactions at an expanded geographic scale, within populations of plants. Here, Chapter 9 provides a treatment of dassical plant epidemiology, while Chapter 11 provides the same focus for mutualistic mycorrhizal associations. Chapter 10 covers the fuzzy area between population biology and microevolution in a genus of ubiquitous and pleurivorous pathogens; Chapter 12 ofters much the same approach for mutualistic endophytes of grasses.

Chapters 13-16 of Part B ofter a view of an expanded temporal scale and consider the evolution of plant/fungus interactions. Chapter 13 considers the flexibility of the fungal genome, the ultimate substrate on which evolutionary forces must act. Chapter 14 discusses the evolutionary relationships between pathogenic and mutualistic fungi

in one situation which has been particularly weIl worked out, the clavicipitaceous endophytes of grasses. Chapter 15 considers the evolutionary interplay between fungi and plants as illuminated through the use of mathematical and computer-driven models. The final chapter in the volume (Chap. 16) deals with overall evolution of fungal parasitism and plant resistance and provides an appropriate coda for this series of essays.

Who is the audience for these volumes? Who might and will read them with profit? Basic literacy in mycology, in particular, and in modern biology, in general, has been assumed as a background for these chapters, and they clearly are not intended for the biological novice. However, we do expect that these volumes will be appreciated by a wide variety of professional biologists including, for example: teachers of upper division courses in general mycology engaged in the valiant (but often futile) attempt to keep their lectures up-to-date; graduate students contemplating literature reviews in connection with a thesis project; nonmycologists who wish to know wh at the fungi might have to ofter in the way of model systems for the study of some fundamental aspect of host/parasite interactions; evolutionary biologists who have just become aware that fungi offer advantages in studying the evolutionary consequences of asexual reproduction. These, and many others, will read these chapters with pleasure. On the whole we are very pleased with the contributions presented here and believe they will prove informative and useful as entrees into the literature on fungus/plant interactions for some years to come.

Eugene, Oregon, USA — George Carroll
Münster, Germany — Paul Tudzynski
March 1997 — *Volume Editors*

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Profiles in Pathogenesis and Mutualism

1 Cellular and Molecular Biology of *Phytophthora*–Plant Interactions

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CONTENTS

I. Introduction

There are over 60 species of *Phytophthora* and many are aggressive plant pathogens that cause extensive losses in agricultural crops, horticulture and natural ecosystems (Erwin and Ribeiro 1996). Some species, such as *P. infestans* the causal agent of late blight of potato and *P. sojae* the cause of soybean root rot, have a limited host range. Others, such as *P. cinnamomi* and *P. nicotianae*, have extremely broad host ranges, with both of these pathogens infecting over 1000 different plant species (Erwin and Ribeiro 1996; Hardham 2005). The genus *Phytophthora* belongs to the class Oomycetes and is now grouped with a variety of other protists within the Stramenopile cluster (Adl et al. 2005; Harper et al. 2005; Yoon et al. 2002). The Stramenopiles also include the coloured algae, the diatoms and the apicomplexans (i.e. malarial parasites). One of the distinguishing structural characteristics of organisms classified within the Stramenopiles is possession of flagella adorned with tubular tripartite hairs called mastigonemes (Barr 1992; Patterson and Sogin 1992). Modern molecular analyses of gene sequences have strengthened evidence of the close phylogenetic relationships between these different groups of Stramenopile organisms (Gunderson et al. 1987; Van de Peer et al. 1996) and have provided a basis for informative comparative studies of infection strategies.

Species of *Phytophthora* produce biflagellate, asexual spores called zoospores and, in most cases, these motile zoospores are instrumental in initiating plant infection. The zoospores are formed within a multinucleate cell called a sporangium that subsequently cleaves to form and release the uninucleate zoospores (Hardham and Hyde 1997). *Phytophthora* sporangium superficially resemble fungal conidia and during vegetative growth *Phytophthora* species form hyphae whose appearance and life style are also similar to those of fungi. These similarities in morphology and mode of nutrient acquisition between *Phytophthora* and fungi are accompanied by similarities in aspects of their infection strategies (Hardham 2007). In both cases, the development of disease requires the pathogen to establish initial contact with a

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potential host, to attach onto and then to penetrate the host surface and to obtain nutrients from the plant in order to grow and reproduce. Most *Phytophthora* species are necrotrophs that obtain the nutrients they need from dead or dying plant cells. However, while none are true biotrophs, a number of species are hemibiotrophs that initially establish a biotrophic relationship with their host plant before turning to a necrotrophic life style.

The establishment of infection through the activity of a motile *Phytophthora* spore contrasts with the situation in most fungi, however, there are clear parallels in the mechanisms employed by *Phytophthora* and fungi, in plant penetration and colonization. Both groups of organisms secrete effector molecules required for pathogenicity, including cell wall degrading enzymes and proteins that are transported into the plant cell cytoplasm (see Chap. 9). In susceptible hosts, these effectors successfully orchestrate colonization of the plant. In resistant hosts, on the other hand, these effectors or other elicitors trigger plant defence and there are strong similarities in the plant's response to attempted invasion by *Phytophthora* or fungi. In this chapter, we explore current understanding of the cellular and molecular basis of the interactions between plants and their *Phytophthora* pathogens, focusing on key aspects of *Phytophthora* pathogenicity, plant recognition of *Phytophthora* invasion and plant defence responses.

II. Establishing Plant Infection

A. Targeting Preferred Infection Sites

Species of *Phytophthora* may arrive at and initiate infection of potential host plants as hyphae, sporangia or zoospores (Fig. 1.1). These three cell types differ in the distances they may travel before reaching a host. Dissemination through hyphal growth restricts the spread of disease to the vicinity of a pre-existing infection site. In contrast, production of caducous sporangia that detach from the mycelial mass may facilitate pathogen dispersal over large distances if the sporangia are blown in the wind, as is believed to have occurred for *P. infestans* during the spread of the late blight disease through Europe in the 1840s (Aylor 2003; Erwin and Ribeiro 1996). Not all species of *Phytophthora*, however, produce caducous sporangia. Sporangia may germinate either directly through production of hyphae or indirectly though cleavage of their multinucleate cytoplasm and subsequent release of uninucleate motile zoospores. Zoospores swim at speeds of up to about 200 μm/s and can cover distances of several centimetres. The movement of zoospores may allow infection at nearby sites as occurs, for example, when zoospores of *P. infestans* are released from sporangia that have landed on the surface of a leaf. Zoospores may also allow the pathogen to spread over much greater distances if they get into water that is moving through the environment. The zoospores of soil-borne *Phytophthora* species, for example, may be carried downhill in streams or water-logged soils.

Phytophthora zoospores are able to swim through the action of two flagella that emerge from the centre of a groove along the ventral surface of the spore (Fig. 1.1A). The zoospore flagella are typical eukaryotic flagella based on a microtubular axoneme that consists of nine microtubule doublets surrounding a central pair of microtubules (Hardham 1987a). The axonemal microtubules are connected by protein complexes that form radial spokes and a variety of other linkages; flagellar function is achieved by the sliding of adjacent microtubule doublets relative to their neighbours, a process powered by the mechanochemical protein, dynein (Silflow and Lefebvre 2001).

Being able to swim enhances the chance that the zoospores initiate disease because they are chemotaxis and electrotactically attracted to potential infection sites on the surface of host plants (Gow 2004; Tyler 2002). In general, these tactic responses appear to be non-specific in that the zoospores move towards both host and non-host plants, being attracted by gradients of compounds such as sugars and amino acids diffusing from the plant surface (Carlile 1983). Specific recognition of a chemoattractant produced by a host plant is, however, known to occur (Tyler et al. 1996). Two isoflavones secreted by soybean roots attract zoospores of the soybean pathogen, *P. sojae*, but not zoospores of several *Phytophthora* species that do not cause disease on soybean (Morris and Ward 1992). Specificity of attraction in terms of targeting zoospore movement to particular locations on the plant is also known. For instance, zoospores of a number of soil-borne species swim to the root elongation zone rather than the root cap or root hair regions (Carlile 1983; Van West et al. 2002). Zoospores also swim towards wounds and may show auto-aggregation phenomena. There is evidence that targeting to different regions of a root surface may involve electrotaxis, a process in which the cells are able to detect and swim towards anodic or cathodic regions of the

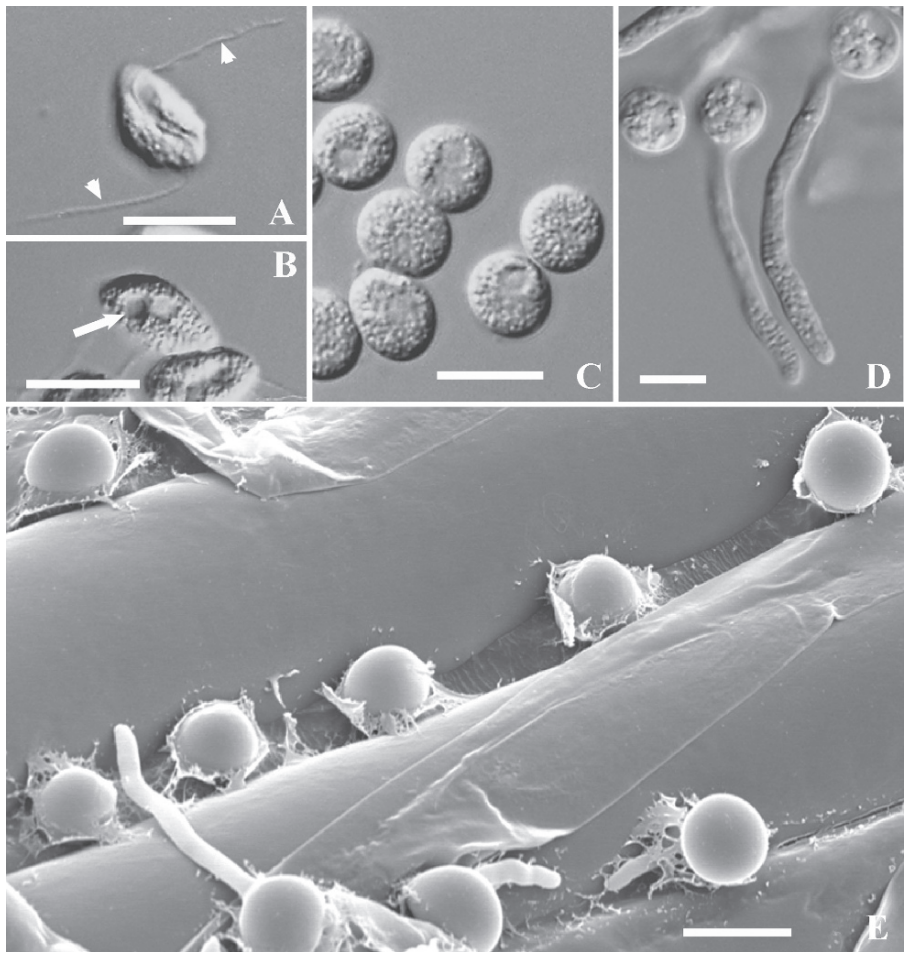


Fig. 1.1. *Phytophthora* zoospores and cysts. **A** *P. nicotianae* zoospore showing emergence of the two flagella (*arrowheads*) from the centre of the ventral groove. **B** *P. nicotianae* zoospore showing the water expulsion vacuole (*arrow*) at the anterior end of the cell. **C** *P. nicotianae* cysts. **D** *P. nicotianae* cysts 2 h after germination. **E** Scanning electron micrograph of *P. nicotianae* cysts that have targeted and settled in the grooves between the epidermal cells of a tobacco (*Nicotiana tobacum*) root. Material secreted by the spores coats the cyst and nearby plant surface. *Bars* 10 µm

root (Van West et al. 2002). At an even finer spatial scale, zoospores of foliar pathogens may be preferentially attracted to stomata and zoospores of root pathogens may target the grooves between adjacent epidermal cells (Fig. 1.1E; Gees and Hohl 1988; Hardham 2001, 2005; Judelson and Blanco 2005).

Like the zoospores of other Stramenopiles, *Phytophthora* zoospores are said to have heterokont flagella because the two flagella have different morphologies. The anteriorly directed flagellum is shorter than the posterior flagellum and possesses two rows of tubular hairs called mastigonemes about 1 µm in length (Hardham 1987a). Observations of zoospore motility suggest that the anterior flagellum pulls the cell forward while the posterior flagellum acts like a rudder, occasionally bending to change the swimming direction. Both flagella form quasi-sinusoidal waves that emanate from the base of the flagella and propagate to their tip. This form of beating of the anterior flagellum would normally propel the cell backwards but the two rows of rigid mastigonemes reverse the thrust of flagellar beat, causing the zoospore to be pulled forwards (Cahill et al. 1996; Jahn et al. 1964). Until recently, there has been little information on the nature of the components that make up the mastigonemes of *Phytophthora* or other stramenopile species. The first advances arose from immunocytochemical studies using monoclonal antibodies directed towards zoospore surface molecules that revealed that the shaft of *P. nicotianae* mastigonemes is made of a 40-kDa glycoprotein (Robold and Hardham 1998). Amino acid sequence data have now been obtained following immunoprecipitation purification of the mastigoneme protein and these data used to clone the corresponding gene (M. Arikawa, T. Suzaki, L.M. Blackman and A.R. Hardham, unpublished data). The results indicate that the *Phytophthora*

mastigoneme protein Pn14B7 is related to the Sig1 and Ocm1 proteins recently cloned from two algal Stramenopile, *Scytosiphon lomentaria* and *Ochromonas danica*, respectively (Honda et al. 2007; Yamagishi et al. 2007).

As yet we have little information on the identity of zoospore proteins involved in the reception of chemotaxis or electrotaxis signals, however, recent studies of *P. infestans* genes encoding the α-subunit of a trimeric G-protein (Dong et al. 2004; Latijnhouwers et al. 2004) and a bZIP transcription factor (Blanco and Judelson 2005) indicate that both these proteins play a role in zoospore motility. Silencing of these two genes inhibits zoospore motility by causing the cells to turn more frequently or spin in tight circles. Unfortunately it has not been possible to use these mutants to assess the contribution of zoospore motility and taxis to pathogen virulence because silencing the genes also produced aberrations during infection structure development. Regulation of flagellar activity is known to involve controls of cytoplasmic Ca^{2+} concentration and two calcium-binding proteins, calmodulin and centrin, have been localized within the flagella apparatus of *P. cinnamomi* zoospores (Gubler et al. 1990; Harper et al. 1995). Genes encoding centrin, a dynein light chain protein and a radial spoke protein were recently cloned from *P. cinnamomi* and are currently being further characterized (R. Narayan, L.M. Blackman and A. R. Hardham, unpublished data).

Phytophthora zoospores are not surrounded by a cell wall and their outer surface is that of the plasma membrane (Hardham 1987b). Water from their surroundings enters the zoospores down its chemo-osmotic gradient and, in order to maintain cell volume and homeostasis, must be pumped out of the cell. Zoospores achieve this through the operation of a contractile vacuole (often called a water expulsion vacuole; Fig. 1.1B) that consists of a reticulate spongiome surrounding a central bladder (Mitchell and Hardham 1999; Patterson 1980). It is not known exactly how contractile vacuoles function in any protist but it is believed that H^+-pumping ATPases power the accumulation of solutes within the spongiome, accompanied by the passive influx of water (Stevens and Forgac 1997). Localization of vacuolar H^+-ATPase in the spongiome of *P. nicotianae* zoospores is consistent with this hypothesis (Mitchell and Hardham 1999). Water is believed then to be transferred from the spongiome to the bladder which periodically fuses with the plasma membrane and contracts to expel the accumulated water.

Phytophthora zoospores are able to swim for many hours utilizing endogenous energy stores, thought to be predominantly polysaccharides (such as mycolaminarins) and lipids (Bimpong 1975; Wang and Bartnicki-Garcia 1974). They inherit many, if not the majority, of their proteins from the sporangium and early inhibitor studies suggested that mRNA and protein synthesis were not required for zoospore function (Penington et al. 1989). However, more recently labelling studies have shown that new proteins are synthesized in *P. infestans* zoospores (Krämer et al. 1997) and proteomic analyses have identified polypeptides that are more abundant in zoospores than in any other stage of the life cycle of *P. palmivora* (Shepherd et al. 2003). In addition, transcriptome and other studies have identified genes that are preferentially expressed in *Phytophthora* zoospores (Ambikapathy et al. 2002; Connolly et al. 2005; Judelson and Blanco 2005; Škalamera et al. 2004). Proteins synthesized in zoospores may function during this motile phase or they may be required to function in the cysts that are formed by zoospore encystment. For example, one gene that is highly expressed in *P. nicotianae* zoospores is that encoding Δ^1-pyrroline-5-carboxylate reductase, an enzyme involved in proline biosynthesis (Ambikapathy et al. 2002). High levels of proline may be required for osmoregulation in the wall-less *Phytophthora* zoospores as they are in some other protists (Steck et al. 1997). In contrast, cell wall degrading enzymes (e.g. cellulase) encoded by genes identified in the transcriptome study (Škalamera et al. 2004) may be synthesized in readiness for secretion by germinated cysts during plant invasion.

B. Attaching to the Plant Surface

Having reached the surface of a potential host plant, *Phytophthora* zoospores adjust their swimming pattern so that the ventral surface faces the plant (Hardham and Gubler 1990). While they maintain this orientation, the zoospores encyst (Fig. 1.1C, E). This is a rapid process during which the two flagella are detached, rendering the spores non-motile, and material is secreted from three different categories of spherical vesicles in the zoospore peripheral cytoplasm (Fig. 1.1E). A network of cortical cisternae also fragments, apparently fusing with the plasma membrane, possibly thereby bringing about a rapid and wholesale change in the composition of the spore plasma membrane (Hardham 1989). The material that is secreted during zoospore encystment includes adhesion proteins that firmly attach the spores to the plant surface (Hardham and Gubler 1990). Attachment of pathogen spores or other cells to the surface of their hosts is an important aspect of the infection process (Epstein and Nicholson 1997;

Tucker and Talbot 2001). Not only does it prevent the pathogen being dislodged before it penetrates the plant, but the close contact also aids the reception of signals that guide pathogen growth and that trigger the development of specialized infection structures. Strong adhesion also facilitates host penetration by hyphae or appressoria.

The secretion of adhesive and other proteins from encysting zoospores is complete within about 2 min and a cellulosic cell wall capable of withstanding cell turgor is formed within 5–10 min (Hardham and Gubler 1990). As the cell wall forms, the pulsing of the contractile vacuole slows down and become undetectable (Mitchell and Hardham 1999). Zoospore encystment is triggered by a range of physical and chemical factors and there is evidence for a role of cell surface receptors and of the phospholipase D signal transduction pathway in induction of this process (Bishop-Hurley et al. 2002; Hardham and Suzaki 1986; Latijnhouwers et al. 2002).

The regulated secretion triggered during zoospore encystment involves exocytosis of the contents of the so-called large peripheral, dorsal and ventral vesicles (Fig. 1.2; Hardham 1995, 2005; Hardham and Hyde 1997; Škalamera and Hardham 2006). Material released from the dorsal vesicles includes a high molecular weight glycoprotein that forms a mucilage-like covering that coats the cysts and the nearby plant surface (Figs. 1.1E, 1.2A, B; Gubler and Hardham 1988). This material may protect the young cysts from physical or chemical damage but evidence to support this hypothesis has not yet been obtained. Material released from the ventral vesicles includes a 220-kDa adhesive protein, named Vsv1, that attaches the cyst to the plant (Fig. 1.2C, D). Cloning of the gene encoding Vsv1 in *P. cinnamomi* has revealed that, apart from short N- and C-terminal sequences, the bulk of the PcVsv1 protein is composed of 47 copies of a domain approximately 50 amino acids in length that shows homology to thrombospondin type 1 repeats found in a number of adhesive extracellular matrix proteins in animals and secreted adhesins in apicomplexan malarial parasites (Adams and Tucker 2000; Robold and Hardham 2005; Tomley and Soldati 2001). Homologues of the PcVsv1 adhesive occur in other *Phytophthora* species and in species of *Pythium*, *Plasmopara* and *Albugo*, suggesting that the Vsv1 protein may be a spore adhesive used throughout the plant pathogenic Oomycetes.

Until recently, studies of the large peripheral vesicles in the zoospore cortex indicated that their contents were not secreted during encystment but that the vesicles moved away from the plasma membrane and became randomly distributed within the cyst cytoplasm (Gubler and Hardham 1990). However, in zoospore transcriptome studies in *P. nicotianae*, cloning of a gene encoding a complement control protein has given rise to evidence that some of the contents of large peripheral vesicles are secreted during encystment (Škalamera and Hardham 2006). Evidence for the selective secretion of PnCcp proteins from the large peripheral vesicles comes from double immunolabelling of PnCcp and Lpv proteins at both the light and electron microscope levels (Fig. 1.2E–I). In motile zoospores both proteins are localized to the large peripheral vesicles but in young cysts, PnCcp proteins are absent from the vesicles and instead coat the cyst surface. In mammals, proteins containing complement control protein modules play a number of roles in signalling and adhesion (King et al. 2003). Their role in the infection of plants by *Phytophthora* zoospores remains to be elucidated.

In addition to the adhesives secreted by zoospores during their encystment, other *Phytophthora* genes encoding putative adhesives that may function in hyphae or germinated cysts have been cloned and characterized. Hyphae and cysts of *P. nicotianae* (formerly *P. parasitica*) have been shown to synthesize and secrete a 34-kDa glycoprotein, termed CBEL, that contains two cellulose-binding domains (Séjalon-Delmas et al. 1997; Villalba Mateos et al. 1997). Silencing of the expression of the CBEL gene interferes with adhesion of the hyphae to cellophane membranes and with morphogenetic changes normally induced by contact with cellulose in vitro (Gaulin et al. 2002). Silencing of CBEL expression does not have a great effect on pathogenicity on host tobacco plants. Another family of secreted proteins that may play a role in adhesion of germinated cysts is the Car proteins (*c*yst-germination-specific *a*cid *r*epeat) from *P. infestans* (Görnhardt et al. 2000). The Car proteins contain multiple copies of an octapeptide repeat, a motif found in mammalian mucin proteins (Guyonnet Duperat et al. 1995). *Car* genes are expressed during cyst germination and appressorium differentiation and the Car proteins are secreted onto the germling surface. By analogy with the functions of mammalian mucins, the Car proteins have been hypothesized as playing roles in protecting the germlings from desiccation or physical damage and in germling adhesion (Görnhardt et al. 2000).

C. Penetration of the Host Surface

Zoospores may carry mRNA transcripts and proteins that function in the cysts that are formed

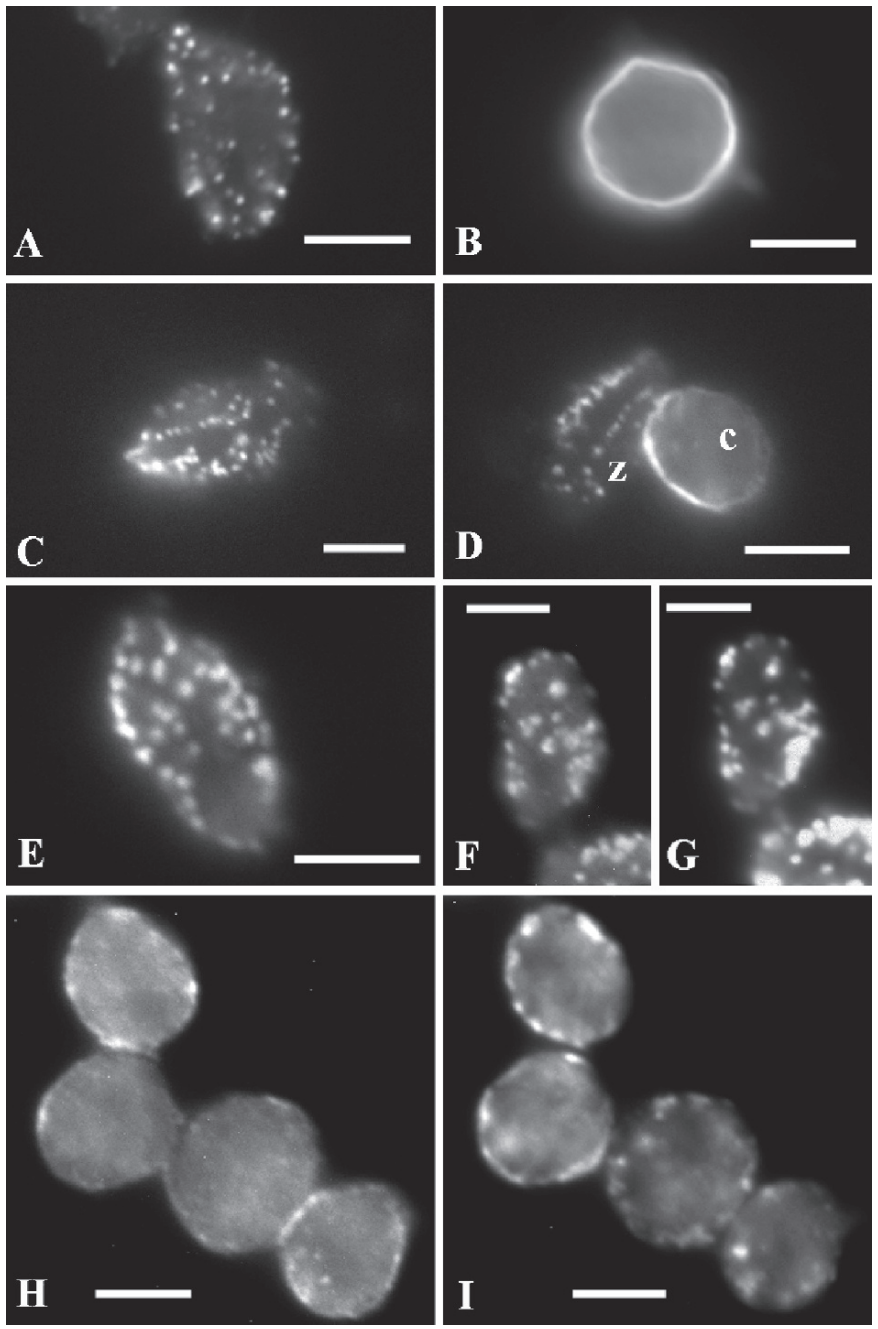


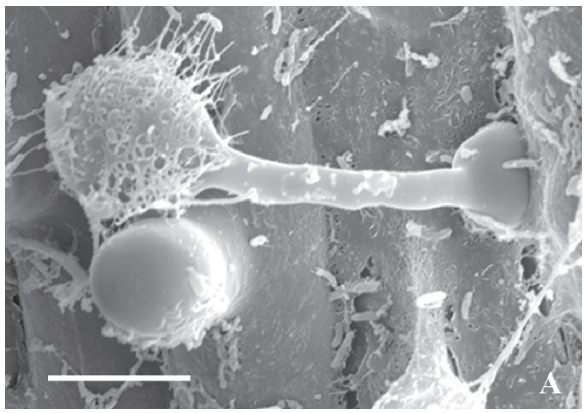
Fig. 1.2. Regulated secretion of cortical vesicles by *P. nicotianae* zoospores. **A** Dorsal vesicles in a zoospore labelled with monoclonal antibody 8E6 (Gautam et al. 1999). **B** Cyst showing 8E6 labelling of the contents of the dorsal vesicles that have been secreted onto the cyst surface. **C** Labelling of the ventral vesicles in a zoospore with monoclonal antibody Vsv1. The ventral vesicles preferentially line the ridges of the ventral groove. **D** Vsv1 labelling of zoospore (*z*) ventral vesicles and secreted material along one surface of a young cyst (*c*). **E** Large peripheral vesicles labelled with monoclonal antibody Lpv1 in a zoospore. **F, G** Double-labelling of large peripheral vesicles in zoospores with PnCcp polyclonal antibody (**F**) and Lpv1 (**G**). Both antibodies label the same cortical vesicles in the zoospores. **H, I** Double-labelling of cysts with PnCcp polyclonal antibody (**H**) and Lpv1 (**I**). PnCcp antigens appear on the surface of young cysts but Lpv1 proteins remain in vesicles in the cell cortex. *Bars* 5 μm

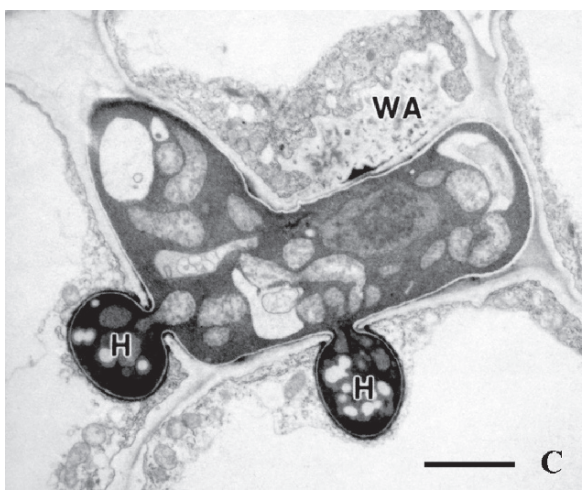
during zoospore encystment; nevertheless, the process of encystment also triggers a new pattern of gene expression and protein synthesis, producing proteins required for cyst germination and germling growth and development (Avrova et al. 2003; Ebstrup et al. 2005; Grenville-Briggs et al. 2005; Krämer et al. 1997; Shan et al. 2004b; Shepherd et al. 2003). Proteins encoded by the genes that are up-regulated perform a range of functions, include DNA, RNA and protein synthesis, signalling, cell structure and growth. A number of studies have highlighted the increased abundance of heat shock and other proteins involved in scavenging reactive oxygen species and in protecting the pathogen against stress, functions that would be important for the pathogen's survival of the plant defence response (Avrova et al. 2003; Ebstrup et al. 2005; Shan and Hardham 2004). Future studies promise to elucidate the role of other cyst proteins identified in the gene discovery projects with exciting results for our understanding of molecular changes occurring during early infection events.

Spatial and temporal aspects of spore germination in many organisms are typically influenced by environmental factors, however, in *Phytophthora* the site of cyst germination is pre-determined and the polarity of germ tube emergence with respect to the adjacent plant is set up by the motile zoospore before it encysts (Hardham and Gubler 1990). In soil-borne *Phytophthora* species, such as *P. cinnamomi* and *P. nicotianae*, the zoospores approach the root and alter their mode of swimming so that they swim parallel to the root surface, frequently turning by 180 degrees so that they swim backwards and forwards over the same section of root surface, all the while maintaining an orientation such that their ventral surface faces the root. Just before encystment, motility decreases and, often quite suddenly, the flagella detach and the cell adopts a more spherical shape (Fig. 1.1C–E). The cysts typically germinate 20–30 min later and the germ tube emerges from the centre of what had been the ventral surface of the zoospore (Figs. 1.1D, E, 1.3A, B). Because most zoospores orient their ventral surface towards the root before encystment, the germ tube consequently forms directly opposite the plant surface and grows chemotropically towards a suitable penetration site (Hardham and Gubler 1990; Miller and Maxwell 1984). In root pathogenic species, the preferential targeting of the zoospores to grooves between epidermal cells is accompanied by subsequent preferential penetration along the anticlinal wall between the cells (Figs. 1.1E, 1.3A; Enkerli et al. 1997; Hardham 2001). In foliar pathogenic species whose zoospores target stomatal complexes, subsequent penetration of the leaf occurs via stomatal apertures (Gees and Hohl 1988; Judelson and Blanco 2005).

Phytophthora hyphae may penetrate the plant surface either along anticlinal walls or directly through the outer periclinal wall without any detectable modification of hyphal morphology (Hardham 2001). In some cases, however, penetration of the plant surface is preceded by the development of an appressorium or appressorium-like swelling of the hyphal apex (Fig. 1.3A, B; Bircher and Hohl 1997; Grenville-Briggs et al. 2005; Judelson and Blanco 2005). The appressoria of some species, such as *P. infestans*, appear to be differentiated cells that are separated from the subtending hyphae by a cross wall (Fig. 1.3B; Gees and Hohl 1988). In other cases, this degree of differentiation is not evident. In *P. infestans*, appressoria formation is induced by factors similar to those that trigger fungal appressorial differentiation, including surface topography and hydrophobicity (Bircher and Hohl 1997) and is accompanied by changes in patterns of gene expression and protein synthesis (Grenville-Briggs et al. 2005). In *P. nicotianae* and *P. cinnamomi*, the hyphal swellings that develop over an anticlinal wall tend to be disk-shaped structures oriented along the groove (Fig. 1.3A). Those that form over the periclinal wall generally assume a more globular shape (Hardham 2001). The fact that the appressorium-like swellings are flat discs rather than spherical expansion of the tip when formed over anticlinal walls suggests that swelling of the hyphal apex does not result simply from the inhibition of hyphal growth by the unyielding plant surface. Instead, their formation is indicative of apical differentiation to produces a structure better able to penetrate the underlying cell wall. Although it has been shown that Oomycete hyphae can exert a force at their apex similar to that generated by fungal hyphae (Money et al. 2004), as yet there are no data on turgor pressures within *Phytophthora* appressoria or on the mechanical pressures they exert during penetration of the plant surface.

Like fungal phytopathogens, *Phytophthora* species also use cell wall degrading enzymes to penetrate and colonize the plant (see Chap. 10). Although there is still only limited in planta evidence of the activity of wall digestion during penetration, loss of pectin from the host cell walls was recently demonstrated during infection by a species of the closely-related genus, *Pythium* (Boudjeko et al. 2006). *Phytophthora* hyphae secrete a range of enzymes that break down the polymers found in plant cell walls and genome and EST sequencing projects have catalogued the genes that encode them. In three *Phytophthora* species for which genome sequence data are available,





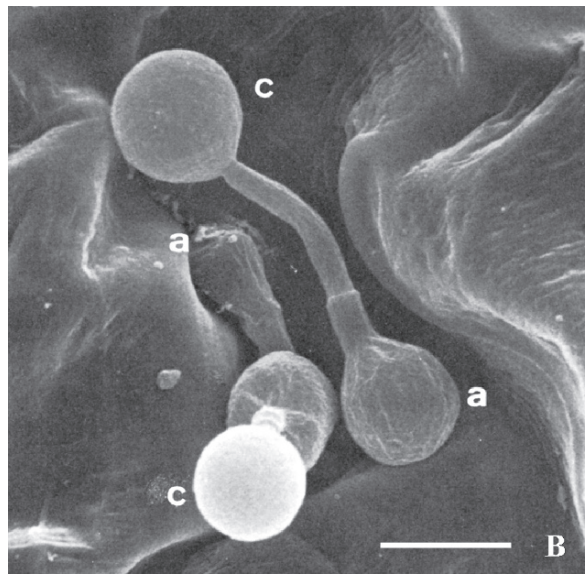


Fig. 1.3. Plant penetration and nutrient acquisition. **A** Germinated cyst of *P. cinnamomi* that has formed a disc-shaped appressorium-like structure as it grows between two epidermal cells of an alfalfa root. *Bar* 10 µm. **B** Appressoria (*a*) that have differentiated at the hyphal apices of germinated cysts (*c*) of *P. infestans*. *Bar* 20 µm. Micrograph reproduced with permission from Gees and Hohl (1988). **C** Two haustoria (*H*) formed by an intercellular hypha of *P. sojae* growing in a soybean (*Glycine max*) root. One root cell has deposited a cell wall apposition (*WA*) adjacent to the hypha. *Bar* 2.5 µm. Micrograph reproduced with permission from Enkerli et al. (1997)

namely *P. ramorum*, *P. sojae* and *P. infestans*, genes encoding glucanases, polygalacturonases, pectin esterases, pectin lyases and xylanases have been identified (http://www.genome.jgi-psf.org; http://www.broad.mit.edu/tools/data/seq.html). To date, only a small number of these genes have been characterized in any detail (Brunner et al. 2002b; Götesson et al. 2002; McLeod et al. 2003; Torto et al. 2002; Yan and Liou 2005).

The gene family that has been studied in most detail is that encoding endopolygalacturonase, an enzyme that degrades the polygalacturonan backbone of pectin molecules (Götesson et al. 2002; McLeod et al. 2003; Torto et al. 2002; Yan and Liou 2005). Polygalacturonases and other pectin degrading enzymes are secreted early in infection. Their activity exposes other wall polymers to attack and causes tissue maceration by disrupting the middle lamella that normally glues adjacent plant cells together. The gene family encoding endopolygalacturonase in *Phytophthora* contains over 20 members (Götesson et al. 2002). The reasons for such a large gene family have not been fully elucidated but are likely to include a need for enzymes specialized to digest the diverse range of pectin molecules. During fungal infection of host plants, a cascade of expression of different members of the polygalacturonase gene family has been demonstrated. In *Botrytis cinerea*, one gene is constitutively expressed and it is thought that the products that are released by digestion of wall pectin by the enzyme encoded by this gene trigger the expression of other members of the multigene family (Ten Have et al. 2001). In *P. cinnamomi*, of the 20 or so polygalacturonase genes, only a small number have been found to be expressed during in vitro culture in both defined and undefined media or during plant infection; one of these genes is under glucose catabolic repression (E. Landgren, A. Götesson, L.M. Blackman and A.R. Hardham, unpublished data).

Cell wall degrading enzymes are just one category of molecules that are secreted by *Phytophthora* hyphae as they grow. As in fungi, mate-

rial to be secreted into the external environment is thought to be transported to and released from the hyphal apex in small apical vesicles.

Although not visible in the light microscope, *Phytophthora* hyphae contain a cluster of such vesicles in the apical cytoplasm, similar to the Spitzenkörper of fungal hyphae. In addition to degradative enzymes, these apical vesicles are likely to contain adhesins, enzymes involved in cell wall synthesis and modification, hyphal wall components and molecules involved in counterdefence (Gaulin et al. 2002; Rose et al. 2002; Shapiro and Mullins 2002; Tian et al. 2005). *Phytophthora* spores and hyphae contain recognizable dictyosomes (Hardham 1987b) and proteins, glycoproteins and polysaccharides destined for secretion are synthesized and packaged in the endoplasmic reticulum and Golgi apparatus (Dearnaley and Hardham 1994). Fusion of the apical vesicles at the hyphal apex also contributes membrane to the expanding plasma membrane, including proteins that function as receptors, channels and enzymes involved in cell wall synthesis (Loprete and Hill 2002).

As in fungal hyphae, transport and distribution of apical vesicles and other cell components in the hyphal apex is dependent on the function of cytoskeletal elements, namely microtubules and actin microfilaments, which form longitudinal arrays along the hyphae (Heath 1995; Temperli et al. 1990). Near the hyphal apex, organelles are typically stratified along the hypha. Behind the cluster of apical vesicles in the very tip, the cytoplasm contains mitochondria and nuclei before becoming increasingly filled with spherical and tubular vacuoles (Ashford and Allaway 2007; Heath and Kaminskyj 1989). Experimental studies using cytoskeletal inhibitors indicate that microtubules regulate long-distance movement of hyphal organelles while actin microfilaments facilitate movement of the apical vesicles to their site of fusion at the tip (Heath 1995; Temperli et al. 1991). A cap of actin microfilaments is also sometimes seen at the apex of *Phytophthora* hyphae (Walker et al. 2006). A function in stabilizing the expanding apical dome has been suggested from studies of other Oomycetes (Jackson and Heath 1990; Kaminskyj and Heath 1995) and the lack of the actin cap has been correlated with active invasion of the surrounding medium (Walker et al. 2006). Mathematical modelling has been used to show that the rate of extension and morphology of *Phytophthora* hyphae can be predicted using parameters reflecting the rate of movement of the cluster of apical vesicles, the so-called vesicle supply centre, and the rate of vesicle fusion with the plasma membrane at the hyphal tip (Dieguez-Uribeondo et al. 2004). A decrease in the number of apical vesicles emanating from the apical cluster leads to slower hyphal growth; inhibition of movement of the vesicle cluster leads to isotropic expansion of the hyphal apex.

D. Nutrient Acquisition to Support Pathogen Growth and Reproduction

Having penetrated the plant surface, the mode of subsequent growth within the plant depends on the life style of the pathogen, that is, whether it is a necrotroph or a hemibiotroph. During necrotrophic growth, hyphae may grow intercellularly or intracellularly, acquiring the nutrients they need from dead and dying cells. However, during the initial biotrophic phase of hemibiotrophic species (*P. capsici*, *P. infestans*, *P. nicotianae*, *P. palmivora*, *P. sojae*), hyphal growth is restricted to the apoplast and disruption of host cells is minimized. During biotrophic growth, nutrients are acquired through the development of specialized haustoria that form predominantly in mesophyll cells for foliar pathogens or in cortical cells for root pathogens (Fig. 1.3C). In contrast to the situation in many biotrophic fungi, distinct haustorial mother cells do not differentiate and instead haustoria develop directly from the intercellular hyphae (Enkerli et al. 1997; Jeun and Buchenauer 2001). Formation of haustoria involves localized dissolution of the plant cell wall and invagination of the plant plasma membrane by the invading pathogen cell (Fig. 1.3C; Enkerli et al. 1997). *Phytophthora* haustoria may be globose or finger-like projections that contain the normal complement of organelles apart from nuclei (Coffey and Gees 1991). Throughout their operational lifetime, *Phytophthora* haustoria remain surrounded by the invaginated host plasma membrane, commonly termed the extrahaustorial membrane. By analogy with the situation in fungal–plant interactions, it is likely that this domain of the plant plasma membrane becomes specialized such that its properties support nutrient uptake by the haustorium. The extrahaustorial membrane is separated from the haustorial wall by an electron-dense, extrahaustorial matrix. Again, by analogy with fungal haustorial complexes, it is likely that components within the extrahaustorial matrix are of both plant and *Phytophthora* origin.

Research in recent years on biotrophic fungi has uncovered molecular evidence of specializations of both the haustorial membrane and the extrahaustorial membrane that facilitate nutrient uptake by

the haustoria (Chap. 4). These features include the localization of amino acid and sugar transporters within the haustorial membrane and a concentration of H^+-ATPase to power nutrient transport (Hahn et al. 1997; Struck et al. 1996; Voegele and Mendgen 2003). Evidence for similar specializations in association with *Phytophthora* haustoria has yet to be uncovered. However, studies of *Phytophthora* proteins that are secreted from haustoria have made a major contribution to our understanding of the translocation of pathogen effector (avirulence) proteins into the host cell cytoplasm (Birch et al. 2006; Whisson et al. 2007; Chap. 9). As discussed below, an RXLR motif has been shown to direct the translocation of proteins from the apoplast into the plant cell cytoplasm from where they may orchestrate changes in host cell organization and metabolism or may be recognized by the host cell and trigger a defence response.

III. *Phytophthora* Effectors and Elicitors of the Plant Defence Response

Plants resist attack from most micro-organisms through the activation of defence reactions elicited either directly or indirectly by molecules produced by the invading microbe. Microbial elicitors may be molecules that are common to a wide group of micro-organisms. For instance, many of the elicitors that trigger basal defence responses are essential pathogen components that contain highly conserved domains, or microbe-associated molecular patterns (MAMPs), and are present across a range of organisms (Bent and Mackey 2007; Jones and Takemoto 2004). Examples of MAMP-containing elicitors include bacterial flagellin (Zipfel et al. 2004), fungal chitin (Kaku et al. 2006) and *Phytophthora* cell wall heptaglucans (Cheong et al. 1993). In many cases, the function of elicitors in this category is not specifically related to microbial pathogenicity. In contrast, other elicitors that are recognized by the plant have functions that are directly involved in the infection process. Such elicitors include not only structural components of the infection apparatus but also proteins that enable the pathogen to evade, suppress or manipulate host defences (see Chaps. 9, 18).

Because many elicitors were first isolated by genetic mapping and complementation on the basis of their induction of plant resistance, i.e. their avirulence activity, until recently, they were referred to as avirulence proteins (Martin et al. 2003). This focus on avirulence functions tended to be somewhat confusing. Why would a pathogen produce a molecule that was recognized by potential hosts and that triggered defences that subsequently thwarted infection? The explanation, as indicated above, is that these molecules are important components of the pathogen's infection machinery. The recent introduction and rapid acceptance of the term "effectors" to encompass these molecules in both their virulent and avirulent forms is a helpful development that makes their role in pathogenicity more apparent. During evolution and the on-going arms race between plants and their pathogens, the sequence of effector genes has changed in order to help pathogens avoid detection by their plant hosts. Application of modern genomic and bioinformatic approaches is greatly facilitating the identification of elicitors and effectors. A variety of such molecules has been isolated and characterized from *Phytophthora* pathogens, including pathogenicity effectors that function extracellularly or within the host cell cytoplasm as well as conserved cell wall constituents that contain MAMPs. As oomycete effectors are discussed in detail in Chap. 9, with an emphasis on the molecular level, the role(s) of these molecules is discussed here only briefly.

A. Extracellular *Phytophthora* Effectors

1. Inhibitors of Plant Enzymes

In response to pathogen attack, plants produce a variety of hydrolytic enzymes including glucanases, chitinases and proteases in defence against pathogen infection (Stintzi et al. 1993). In the case of the first two groups of enzymes, their activity also generates elicitor-active oligosaccharides that trigger further defence responses (Stintzi et al. 1993). Fungal, bacterial and oomycete pathogens have evolved mechanisms to protect themselves against these degradative enzymes by secreting effector proteins that inhibit enzymatic activity and, in the case of glucanase and chitinase inhibitors, arrest the production of potent elicitors (Abramovitch and Martin 2004).

Phytophthora cell walls are rich in β-1,3-glucans, including those that constitute the