COMPARATIVE STUDIES ON MYCOPARASITIC PYTHIUM SPECIES

by

Steven Alexander Kirk Laing

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To my mother, Janette, my father, Alexander, my sister, Tracey, and my brothers, John and Andrew

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DECLARATION

I declare that the work in this thesis is my own, that my thesis has been composed by myself and that none of the material contained therein has been submitted for any other degree or profession qualification.

S. A. K. Laing

CONTENTS

ABSTRACT

Pythium mycoparasiticum is described as a new species. Its mycoparasitic behaviour was compared with those of *P. oligandrum* and *P. nunn* against nine species of potential host fungi - *P. graminicola*, *P. vexans*, *Rhizoctonia solani* (two isolates), *Fusarium culmorum*, *F. oxysporum* f sp *lycopersici*, *Botrytis cinerea*, *Botryotrichum piluliferum*, *Trichoderma aureoviride* and *Phialophora* sp.

In interactions on filter paper or cellulose film, the degree of aggressiveness of each mycoparasite and the degree of resistance of host fungi were assessed by the difference in cellulose breakdown caused by the hosts alone or in dual inoculations with mycoparasites. *P. oligandrum* was the most aggressive mycoparasite, active against most hosts; *P. nunn* was least aggressive, with a narrow host range, and *P. mycoparasiticum* was intermediate in these respects. Of the hosts, *Pythium* spp and *R. solani* were least susceptible to parasitism and *T. aureoviride* and *Phialophora* were most susceptible. *B. cinerea*, and some other hosts to a lesser degree, was susceptible to *P. oligandrum* but not to *P. nunn*. Some of these findings were confirmed by the abilities of mycoparasites to grow on potato-dextrose agar precolonized by the host fungi; this assay method was not as sensitive as others, but it could be used to compare potential host ranges of mycoparasitic *Pythium* spp and the cellulolytic mycoparasites, *Trichoderma harzianum* and *Gliocladium roseum*.

Interactions between individual hyphae were studied by videomicroscopy on films of water agar. The incidence and timing of different events were assessed from replicate interactions on videotape and statistically analysed. The results generally confirmed those of interactions on cellulosic substrates, but several new details of inter-

actions were observed. All three mycoparasitic Pythium spp had similar modes of parasitism, different from those of T. harzianum and G. roseum, used in a small comparative study. They did not affect host hyphae before contact, and none showed tropism to host hyphae. Soon after contacts involving parasite or host tips, the susceptible host hyphae stopped growing and generally either lysed at the point of contact or exhibited vacuolation/coagulation of their cytoplasm, starting at this point; this was often followed by penetration by a mycoparasite. In some cases the parasites penetrated directly, sometimes after proliferating on the host surface. The parasites branched at points of contact and penetrated from these branches. Lysis and cytoplasmic coagulation typically preceded penetration or even the origin of a penetrating branch from the mycoparasites. Based on the timing of these events and the number of hosts affected, P. nunn was significantly the least aggressive mycoparasite, whereas P. oligandrum and P. mycoparasiticum were equally aggressive. The hosts differed in resistance as on cellulosic substrates.

Mycoparasitism by P. oligandrum was investigated by inactivating the parasite or host hyphae with fine beams of intense light prior to contact on water agar. The results demonstrated an essential role of mycoparasitic activity in host stoppage, lysis and cytoplasmic coagulation. However, lysis also required activity by the host. The findings are discussed in relation to postulated surface recognition events and the respective roles of host- and mycoparasite-derived wall-lytic enzymes.

In limited tests, P. nunn was found to be unique among mycoparasitic Pythium spp in its ability to utilize inorganic (nitrate) nitrogen. Germination of oospores of P. oligandrum was found to depend on length of storage in culture and on conditions of aeration and illumination.

Germination was stimulated by peptone and, to a lesser degree, malt extract, and also by acetaldehyde among the volatile metabolites that were tested.

The results of all these studies are discussed in relation to the roles or potential roles of the mycoparasites in biocontrol of plant pathogens.

SECTION 1

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Introduction

There is increasing interest in the interactions between fungi, particularly in relation to the potential for use of antagonistic fungi as inoculants for control of plant pathogens or other deleterious organisms. Recent texts in this area include those of Burge (1988) and Whipps and Lumsden (1989). Of special interest in this respect are mycoparasitic fungi, and this thesis is concerned with three such fungi - P. oligandrum Drechsler, P. nunn Lifshitz, Stanghellini & Baker and a species newly described here, P. mycoparasiticum. Published work on the activities of these fungi is described below and placed in the broader context of mycoparasitism and other antagonistic interactions involving fungi.

1.1 Fungal Interactions

Park (1960) divided the possible interactions between a pair of species into two categories, these being antagonism and symbiosis. Antagonism involves one of the species being harmed, whereas in a symbiotic relationship neither is harmed. Antagonism was then further divided into (1) antibiosis, in which one species prevents growth of the other, usually by production of a chemical, but gains no direct benefit; (2) competition, which is the direct rivalry for a limited environmental resource; and (3) exploitation, whereby one species inflicts harm by the direct use of the other species (Park, 1960). According to Culver (1981), however, this division of possible interactions is not quite complete, so he proposed an all-encompassing classification of inter-

actions, as shown in Table 1.1 below.

Table 1.1 Classification of possible interactions between two species

		Effect of species A on B		
		0	-	+
Effect of	0	-	Amensalism	Commensalism
species B	-	Amensalism	Competition	Predation
on A	+	Commensalism	Predation	Mutualism

Although this classification has much in common with that given by Park (1960), amensalism is also included. In the table, commensalism may be synonymous with antibiosis, mutualism with symbiosis, and predation with exploitation. Predation can be further subdivided into direct parasitism and indirect parasitism (predation), with the former being more closely associated with the soil fungi. These titles are, however, descriptions of interactions between two species, and to use them as a description of a single species may be misleading, particularly since many species, and indeed single organisms, do interact in more than one way. Other discussions of interactions between fungi or between microorganisms in general are those of Cooke & Rayner (1984), Culver (1981) and Park (1960). To a large degree they present variations on the themes and terms outlined above. This thesis is concerned with one method of antagonism, or interference competition (Lockwood, 1981), namely mycoparasitism. This is discussed in detail below, but two other methods of antagonism, competition and antibiosis will be referred to in less detail further on, the latter with particular reference to antagonism by Trichoderma and Gliocladium species.

1.1.1 Mycoparasitism

The term mycoparasite is used to define fungi that parasitise other

fungi (Boosalis, 1956; Barnett & Binder, 1973). Mycoparasites were previously termed hyperparasites (Boosalis, 1964) due to the fact that early studies were on parasites that preyed upon parasites of higher plants with the goal of biological control of plant diseases. This label does not accurately describe parasites of non-parasites (Cooke, 1977) so it will be avoided to prevent confusion.

Whipps, Lewis & Cooke (1988) recently defined a mycoparasite as "A fungus existing in intimate association with another from which it derives some or all of its nutrients while confering no benefit in return". The origins of this definition may be traced to a detailed analysis of the behaviour of P. oligandrum by Deacon (1976) which, in turn, was based on the definition of parasitism in Federation of British Plant Pathologists (1973). Examples of mycoparasitism can be found among all groups of fungi from the chytrids to the higher basidiomycetes (De Vay, 1956). The host ranges of these mycoparasites range from broad, as in the case of Trichoderma spp, to narrow such as a single host genus, as is exhibited by many of the mycoparasitic chytrid species. The range of host species may give an indication to the ecology of the fungal species in question, as to how much it relies on parasitism for its main source of nutrition. Parasites can be separated into two groups, as follows, based on the nature of the host-parasite interaction. (1) Biotrophic parasites - characterized by their feeding on tissues of living hosts and by the fact that, at least initially, they do not kill host cells; they set up a physiological balance with the host cells and depend on the functioning of these cells for their nutrition. (2) Necrotrophic parasites - these characteristically kill the host cells at an early stage in the parasitic process, by means of toxins or enzymes or simply by penetrating and disrupting cellular membranes, but they can continue to feed on the dead host cells. These

groupings were proposed initially for plant parasitic fungi (Gaumann, 1946), but have been adopted for fungal parasites (Barnett & Binder, 1973).

1.1.2 Biotrophic Mycoparasites (Barnett & Binder, 1973)

Biotrophic, or "balanced", mycoparasites include the "obligate" parasites as well as those that can be cultured on non-living media. The term obligate, however, is inappropriate, for basically it indicates only that we do not know the nutritional or other conditions required for the growth of the parasite in the absence of the living host. Biotrophic mycoparasites also tend to have, at most, a few potential hosts or are confined to parasitism of well-defined taxonomic groups of hosts, such as the Mucorales (Jeffries, 1985). To some degree, this may be because, over many years of association with the host, some of these parasites have lost the ability to synthesize one or more components required for growth, and have come to depend on the host for its supply. However, it is equally likely that they have particular features that are involved in escaping recognition by their hosts, thus enabling them to grow in association with living host cells. Thus it seems that the parasite's survival depends on the closeness of adaptation of its life cycle to that of its hosts. There are three distinct types of biotrophic mycoparasitism, termed internal, contact and haustorial (Barnett & Binder, 1973).

1) Internal mycoparasites

This group is tentatively termed biotrophic as although they appear to cause little or no harm to the host, particularly during early development, they may destroy the host protoplasm prior to sporulation. The group is comprised of several chytrids, but little is known about the nutritional relationship between parasite and host.

2) Biotrophic contact mycoparasites

All members of this small group are imperfect fungi and their hosts are ascomycetes or imperfect fungi. They show host specificity from the time of spore germination, as they require the presence of a host exudate for germination. The actual mode of parasitism is by means of specialised branches, which contact the host hypha - then may partially or completely surround it, or touch end-to-end to a short branch of the host. Evidence suggests that a required nutrient is held within the cells of most hosts and very little escapes into the substrate before autolysis. The contact cells must, therefore, function in some way to increase the permeability of the host cell membrane to this nutrient. This mode of parasitism appears to cause little direct harm to the host but does result in growth reduction.

3) Biotrophic haustorial mycoparasites

These parasites are classified in the Mucorales and are characterised by haustoria - that is, specialised parasitic structures formed within the host cell with a large surface area apparently for nutrient transfer from host to parasite. These haustoria resemble those of specialised fungal parasites of higher plants even in fine-structure (Manocha & Lee, 1974). The host ranges are generally restricted to other members of the Mucorales, but some haustorial biotrophs may attack members of the Ascomycotina and Hyphomycetes (Deuteromycotina). As in the contact mycoparasites, there appears to be at least one neccesary host-produced nutrient or stimulant required before spore germination. The mode of parasitism involves parasitic hyphae making contact with the host cell, then usually forming a conspicious appressorium-like swelling, followed by penetration of the host wall by a slender infection peg, and formation of a branched haustorium.

1.1.3 Necrotrophic mycoparasites

The necrotrophic, or destructive mycoparasites, are a diverse group of fungi with generally a very wide host range in their parasitic phase. There is a problem with necrotrophs, however, in that they generally exhibit saprophytic properties also, allowing them to be easily cultured on laboratory media. As biotrophic mycoparasites are often obligate, so necrotrophic mycoparasites are facultative or opportunistic parasites, with most having attributes enabling them to grow successfully in competition with other fungi on dead host mycelia or other organic matter. Barnett & Binder (1973) stated that necrotrophic mycoparasites kill their hosts by excretion of a toxic substance which kills the host cells. This statement is endorsed by some workers (eg Pachenari & Dix, 1980) but not by others, including Ayers & Adams (1981) and Foley & Deacon (1986b) who report necrotrophic mycoparasitism without toxin production by the parasite. Ayers & Adams (1981) also report destructive mycoparasites which are "biotrophic in that their development is favoured by living rather than dead host structure". Many plant pathogens termed biotrophic are in fact destructive of their hosts, generally during or after spore formation. Biotrophy in plant pathology does, correctly, not infer non-destructive parasitism but simply parasitism of living (including dying) but not dead tissue, and conversely necrotrophy describes parasitism of only dead tissue. Therefore the term destructive mycoparasitism will be used here to describe mycoparasitism leading to death of the host cell, and necrotrophic mycoparasitism to describe parasitism of fungal tissue killed by the parasite prior to invasion. Infection by necrotrophic fungi is usually characterized by direct penetration of the host hypha by the hypha of the mycoparasite, or by coiling of the parasitic hyphae around the hyphae of the host (Lumsden, 1981). There is often a directional

stimulus toward the host hyphae before contact, mediated by diffusible substances (Baker, 1987) and lectins are reported to be involved in recognition (Baker, 1987); this will perhaps partly affect the host range of a mycoparasite. Penetration is preceeded by internal disruption of the host cell following initial contact, such that the cell is effectively dead prior to penetration. At the site of penetration, the mycoparasite may produce hook-like structures, presumed to be appressoria (Lifshitz et al., 1984a), which probably aid penetration of the host fungal cell wall, as in fungal-plant interactions. However there are examples in which the hyphae involved in penetrating and internally colonising the host are morphologically similar to vegetative hyphae (Hoch & Fuller, 1977). Ingrowths of the host cell wall (papillae) at the site of contact of the parasitic hyphae have also been observed, but these do not appear to prevent penetration by the parasite, again a mirror of certain fungal-plant parasitic interactions (Manners, 1982). The role of hyphal coiling with regard to mycoparasitism is less clear. It is often associated with mycoparasitism involving penetration, but has been shown to occur without host penetration, in interactions between a single parasite and different hosts (Hoch & Fuller, 1977). Although hyphal coiling was once considered indicative of a parasitic relationship (Drechsler, 1943), Deacon (1976) reports that extensive hyphal coiling is more commonly associated with host resistance. The view of the ecological importance of necrotrophic mycoparasitism in the soil ranges from "insignificance" (Griffin, 1972) to "may cause a substantial impact" (Baker, 1987). This reflects the difficulty in assessing the in vivo interactions of many necrotrophic fungal associations observed in vitro (Lumsden, 1981). This difficulty may be due to the range of factors, inherent and environmental, which determine successful necrotrophic mycoparasitic relationships, and these will be

discussed in the next section. Biotrophic mycoparasitic relationships, on the other hand, are less likely to fluctuate in this manner due to the close association and evolution of the parasite with the host.

1.2 Factors affecting mycoparasitism

A number of factors can influence a mycoparasitic interaction. These factors may be inherent or extrinsic. Inherent factors are those determined by the genetic make-up and variability of the host and parasite; extrinsic factors are the influences of the environment on their interaction. These aspects have been reviewed by Barnett & Binder (1973) and Lumsden (1981) respectively.

1.2.1 Inherent factors

The genetics of the host and parasite are probably the most fundamental factors in determining if a mycoparasitic interaction is possible. With regard to the expression of these genes it seems that the host cell wall, the stage of the host life-cycle and the presence or absence of growth factors required by the mycoparasite are of most importance in determining the extent of a given interaction. Baker (1987) also regards tropic substances, host-parasite recognition chemicals and the range of wall-degrading enzymes produced by the parasite as possible features determining the host ranges of mycoparasites. Once a parasite has made contact with the host, the cell wall of the host is the first major obstacle the mycoparasite must encounter in a mycoparasitic interaction. Fungal cell walls differ between groups in their chemical composition but there are often differences - at least in degree - between old and young hyphae of the host, and this has been shown to affect the resistance against attack. Barnett & Lilly (1962) found that Gliocladium roseum (Link) Bain develops more profusely around

younger hyphae and immature structures of numerous fungi than around some mature parts, which may become totally resistant. Deacon (1976) and Foley & Deacon (1986b) showed that only hyphal tips of Rhizoctonia solani Kuhn were susceptible to invasion by hyphae of Pythium oligandrum. This may be due to a change in wall thickness or perhaps in wall structure with hyphal age. It may also be due to the degree of nutrient leakage or host enzyme activity around the host hyphae, younger hyphae perhaps being more involved in external breakdown of large macromolecules by host enzymes, creating a microclimate more amenable to successful parasitism by mycoparasitic fungi. Deacon (1976) also observed that melanized hyphae of several fungi were unaffected by the presence of P. oligandrum in conditions in which non-melanized hyphae were parasitized. Since the degree of melanization can be markedly affected by environmental factors, particularly aeration (Smith & Griffin, 1971), this is an example of a genetic/environmental interaction. The stage of development of the host also affects the host-parasite interaction. Certain mycoparasitic fungi characteristically parasitise reproductive structures of their hosts, and in some cases they do not grow on the normal vegetative hyphae of the host (Barnett & Binder, 1973). Several sclerotial mycoparasites seem largely to be of this type (Ayers & Adams, 1981; Turner & Tribe, 1976), as are the mycoparasites such as Verticillium lecanii (Zimm) Viégas (Spencer, 1980; Spencer & Atkey, 1981) and Darluca filum (Bivona ex Fr.) Cast (Svensrud & Calpouzos, 1972) that characteristically grow on sporulating pustules of rusts and powdery mildews. The presence of host-produced growth factors required by the parasite will also determine if a mycoparasitic interaction is successful. Pythium species, for example, require sterols or sterol precursors for oogonium production (Child, Defago & Haskins, 1969; Hendrix & Campbell, 1973). Mycoparasitic Pythium species are therefore unlikely

to be able to persist or proliferate when parasitising other Pythium species except, perhaps, when the Pythium host has access to a sterol source or the underlying substrate is sterol-rich.

1.2.2 Environmental factors

Environment affects mycoparasitism not only at the "interaction level", but also at the "population level". Although Barnett & Binder (1973) stated that, in general, the favourable environment for host and parasite is the same, it is obvious, particularly with necrotrophic mycoparasites, that this is not the case. Environmental factors that have been recognised to influence the host-parasite interaction of soil mycoparasites are nutrition of the host and parasite, temperature, pH, moisture, gas exchange, and soil type. Host nutrition is one of the most important factors affecting mycoparasitism (Boosalis, 1964), particularly the ratio of carbon to nitrogen in the medium. Barnett and Lilly (1958) reported that high available nitrogen in the medium rendered the normally resistant Physalospora ilicis (Shleich.) Sacc. susceptible to parasitism by Calcariosporum parasiticum (Barnett). It appears, however, that the nutrition of the parasite, as well as that of the host, may be equally important, particularly in necrotrophic interactions. Rhizoctonia solani did not infect either Mucor recurvus Butler or Rhizopus nigricans Ehrenb when the host and parasite were grown together on water agar, whereas the presence of some sugars induced heavy parasitism (Butler, 1957). Increases in the inoculum densities of mycoparasites in soils have been achieved by the addition of supplements, as has been shown by Boosalis (1956) and Lifshitz, Sneh & Baker (1984). Boosalis (1956) showed enhanced incidence of mycoparasitism by Trichoderma spp and Penicillium vermiculatum Dangeard on R. solani when the soil was amended with dried, ground soybean leaves and stems, these

apparently providing nutrients that enhanced parasitism of R. solani hyphae. Lifshitz, Sneh & Baker (1984) showed very high levels of mycoparasitism of Pythium spp, Phytophthora spp and R. solani by the mycoparasite Pythium nunn after five weekly additions of bean leaf meal to the soil, and also reported an increase in population density of P. nunn, apparently at the expense of phytopathogenic Pythium spp. In all studies concerned with mycoparasitism in the soil, it was found that temperature affected the intensity of mycoparasitism (Lumsden, 1981), and indeed it may be critical (Baker, 1987). The optimum temperature for infection and decay of sclerotia of Sclerotinia sclerotiorum (Lib) de Bary by Sporidesmium sclerotivorum was 20-25°C (Adams & Ayres, 1980); mycoparasitic Trichoderma spp did not protect seeds from attack by phytopathogenic Pythium spp or R. solani at temperatures below 17°C or above 30°C (Harman et al., 1981), and soil suppressiveness to Pythium ultimum Trow was induced by P. nunn at 26°C but not at 19°C (Lifshitz, Sneh & Baker, 1984).

The effect of pH on mycoparasitism in soil is less well documented but researchers have shown that mycoparasites seem to prefer certain pH ranges (Lumsden, 1981; Foley & Deacon, 1985), and this may affect their distributions. Similarly, water content of the soil is bound to affect the microenvironment of host and parasite and may influence any possible interaction. Additionally, the degree of soil saturation will determine the dominant soil fungal flora at any given time. Sneh et al. (1977) showed that the type of mycoparasite associated with oospores of Phytophthora spp was affected by water content. In flooded soil, parasitism by chytrids was predominant over parasitism by hyphomycetes. Conversely, at low soil moisture levels there was no infection by chytrids, but many oospores were infected by hyphomycetes. This is due to the effect of different soil saturation levels on the production and motility of the chytrid zoospores.

Levels of oxygen and carbon dioxide in the soil can influence mycoparasitic activities, as may soil type, but little work has been carried out to assess their relative importance.

The increasing interest in mycoparasitism is undoubtedly due to its potential role in controlling diseases caused by phytopathogenic fungi. A greater understanding of the factors affecting the mycoparasitic activity in natural soils may lead to the more rapid development of biological control methods in agriculture, in ways that are better adapted to the varying demands of modern farming. One fungal species which has already been considered, and indeed tested, for its biological control of damping-off is Pythium oligandrum. P. oligandrum is one of a small group of mycoparasitic Pythium species, and it is this group that will be discussed next.

1.3 The mycoparasitic Pythium spp

The genus Pythium is best known for its plant pathogenic species, but it also contains a small group of mycoparasitic species. Four such species have been well characterised, and a fifth has been reported but is as yet unnamed.

Mycoparasitism within the genus was first recognized by Drechsler (1938, 1946) in three fungi, P. oligandrum, P. acanthicum Drechsler and P. periplocum Drechsler which he had described earlier (1930). Recently, a fourth species, P. nunn, which also displays mycoparasitic properties was isolated in the United States (Lifshitz, Stanghellini & Baker, 1984). Another mycoparasitic species was tentatively reported in 1978 by Deacon & Henry and was later reisolated by Foley & Deacon (1985). It has been provisionally termed Pythium SWO to denote its smooth walled oogonia, in contrast to the echinulate oogonia of P. acanthicum, P. oligandrum and P. periplocum. The recently described P.

nunn also has smooth-walled oogonia and has, until recently, been unavailable for taxonomic comparison with Pythium SWO, pending a patent application on its use as a biocontrol agent. Although the emphasis below will be on the three species P. acanthicum, P. oligandrum and P. periplocum, due to the greater amount of available information on them, an account of P. nunn and Pythium SWO will also be given.

P. oligandrum, P. acanthicum and P. periplocum have been isolated periodically from diseased plant tissues, but there is little evidence to suggest that they are the causal agents of plant disease, despite frequent reports to the contrary (eg Middleton, 1941; Waterhouse & Waterson, 1966). Even when P. oligandrum was isolated from rotting pea plants by Dreschler (1946), it was reported to occur together with Pythium species that were well known phytopathogens and that could, therefore, have caused the symptoms. Observations that the hyphae of P. oligandrum, P. acanthicum and P. periplocum coiled profusely around the hyphae of phytopathogenic Pythium spp in culture led Drechsler to suggest that the three species occurred less as primary parasites of plants than as secondary invaders deriving their nutrition partly from mycelia of the primary invaders and partly from plant tissues freshly killed by the primary invaders. It is, however, impossible to discount plant parasitism by these predominantly mycoparasitic species, as phytopathogenic isolates of P. acanthicum and P. periplocum have been obtained from diseased watermelon fruits (Dreschler, 1946).

An association of P. oligandrum with cellulolytic fungi was reported by Tribe (1966). Earlier, Tribe (1961) had found P. oligandrum on cellulose film buried in soil, which was surprising as P. oligandrum was not thought to be cellulolytic. However, when mycelia of another fungus, identified as Botryotrichum piluliferum Sacc.& March., were found growing on the film near to P. oligandrum, Tribe suggested that P.

oligandrum was deriving its nutrition from small molecules, perhaps sugars and organic acids, which were released by B. piluliferum. The ability of a non-cellulolytic fungus to utilise cellulose breakdown products released by the actions of extra-hyphal enzymes of a cellulolytic fungus has been termed secondary sugar saprophytism (Garrett, 1970; Hedger & Hudson, 1974). Although Tribe (1966) found no evidence to suggest that P. oligandrum was an aggressive mycoparasite, Deacon (1976) and Deacon & Henry (1978), in similar studies, concluded that P. oligandrum, P. acanthicum and P. periplocum are aggressive mycoparasites.

Deacon (1976) found that P. oligandrum could grow on cellulosic substrata in the presence of several cellulolytic fungi but not alone, and that when it grew well it markedly inhibited the growth of the other fungi. There were, however, differences in the degree of apparent susceptibility of cellulolytic fungi to mycoparasitism by P. oligandrum.

Although, P. oligandrum, P. acanthicum and P. periplocum, as well as P. nunn and Pythium SWO, are described as mycoparasites, this may not be their only means of nutrition because there is evidence of saprophytism and perhaps slight plant pathogenicity by these species. Indeed their mode of mycoparasitism is unspecialised, so the relative importance of mycoparasitism as compared to saprophytism in vivo is difficult to ascertain. However, the term mycoparasite does separate them from other members of the genus which are also unspecialised parasites of plants and show some saprophytic ability. Moreover the mycoparasitic Pythium spp show markedly different nutritional characteristics from the phytopathogenic Pythium spp, as will be discussed next.

1.3.1 Morphology and physiology of the mycoparasitic Pythium spp

In agar culture, the mycoparasitic Pythium spp produce numerous

fine branches from the main radiating hyphae, and their colonies usually lack the abundant coarse aerial mycelia typical of many phytopathogenic Pythium spp (Drechsler, 1946; Lifshitz, Stanghellini & Baker, 1984). With regard to physiological characteristics, Foley & Deacon (1986a) summarized the differences between the mycoparasitic species (P. oligandrum, P. acanthicum, P. periplocum and Pythium SWO) and non-mycoparasitic Pythium species. The main nutritional differences concern nitrogen and vitamin requirements and the utilisation of carbon sources in pure culture, and also differences in the responses to nutrient dilution and in growth on gallic acid agar.

The mycoparasitic Pythium spp cannot utilize inorganic nitrogen sources as can many of the phytopathogenic Pythium spp; instead they require an organic nitrogen source, such as amino acids. Similarly, they require exogenous thiamine or at least the pyrimidine moiety of thiamine for growth, unlike most phytopathogenic Pythium spp (Ridings et al., 1969; Leonian & Lilly, 1938; Foley & Deacon, 1986a).

Although all of the Pythium species tested by Foley & Deacon (1986a) were able to grow well on glucose and cellobiose, and relatively well on trehalose, only the mycoparasites were able to utilize mannitol to at least some degree as sole sugar source, confirming the earlier results of Child, Defago & Haskins (1969) for one isolate of P. acanthicum. Conversely, none of the mycoparasites was able to degrade cellulose, whereas several, but not all, of the non-mycoparasites were able to do so (Deacon, 1979).

Foley & Deacon (1986a) also reported that, in contrast to that of non-mycoparasitic species, the radial growth rate of mycoparasitic Pythium spp was reduced by a component of commercial potato extract and, where tested (not Pythium SWO), their growth was severely disrupted on agar containing rose bengal and gallic acid. On the latter medium, the

hyphae of P. acanthicum, P. oligandrum and P. periplocum were pink and the medium was unchanged from its original plum-purple colour, whereas almost all other Pythium spp had white hyphae and changed the colour of the medium to green. The physiological basis of this difference is unknown.

1.3.2 Fungal hosts of the mycoparasitic Pythium spp

There is much difficulty in accurately defining host ranges for the mycoparasitic Pythium spp due to the number of possible modes of interaction with fungi. Haskins (1963) tested a single isolate of P. acanthicum against a wide range of fungi on potato-dextrose agar plates, the criterion of parasitism being the ability of P. acanthicum to overgrow colonies of the other fungi and to coil round their hyphae. The use of such a loose definition of parasitism was reflected in the results which showed that of 94 fungal hosts tested, 67 were parasitized by P. acanthicum with the production of oogonia and 10 were parasitized without oogonium production. Only seven fungi were not parasitized and 10 were antagonistic to P. acanthicum. The range of potential host fungi tested included members of the Oomycetes, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina; members of each group were parasitised, but other members of each group showed antagonism to P. acanthicum.

P. oligandrum also has a wide host range, and, again, fungi differ markedly in their susceptibility to it (Deacon, 1976). The criteria of parasitism used in this study were far more rigorous than those used by Haskins (1963) in that P. oligandrum was expected to grow in association with the fungal 'hosts' and simultaneously to reduce the growth and activities of the 'host' in conditions in which P. oligandrum could not grow alone. This was done by using a carbon source such as cellulose, nitrate as sole nitrogen source and no vitamins in the medium. More-

over, oogonium production by Pythium spp requires an exogenous source of sterols or sterol precursors (Haskins, et al. (1964); Hendrix & Campbell, 1973), which were not supplied in the medium. So oogonium production by the mycoparasite must have been supported by host-derived sterols. A comparison of the results of these tests with empirical results gained from experiments with microscopic observation led Deacon (1976) to conclude that the coiling of hyphae of the mycoparasite around the hyphae of the 'host' was more indicative of resistance to mycoparasitism than susceptibility as previously thought by Drechsler (1946) and Haskins (1963).

Deacon & Henry (1978) compared P. oligandrum with P. acanthicum, including the isolate used by Haskins (1963), and found that not only were the host ranges similar, but also the degrees to which the mycoparasites affected the different hosts were similar.

A series of experiments carried out by Whipps (1987b) using P. oligandrum amongst other mycoparasites, showed that under different cultural conditions interactions between fungi may be quite different thereby stressing that an interaction observed under laboratory conditions may not necessarily occur in a natural environment. However, he also noted that P. oligandrum was consistently mycoparasitic on each of the three different media that he used and thus inferred that it was more likely to behave in vivo as it does in vitro.

Perhaps due to its infrequent isolation, little work has been undertaken to ascertain the host range of P. periplocum. Like P. acanthicum and P. oligandrum, it does show a high degree of antagonism to Botryotrichum piluliferum (Deacon & Henry, 1978; Foley, 1983) and may in fact show a similar host range to these other mycoparasites. Foley (1983) also compared Pythium SWO with P. oligandrum and found that it too had a comparable host range.

Although a comparative study of the host range of P. nunn has not been made in similar tests, it is reported to show varying degrees of mycoparasitism toward a range of fungi (Lifshitz et al., 1984a; Elad et al., 1985).

1.3.3 Mechanisms of antagonism

The mycoparasitic Pythium species, like most unspecialised parasites of fungi or plants, are necrotrophic and thus able to utilise nutrients from dead organic matter. So proof of parasitism is difficult to obtain, particularly as they can grow in close proximity to other fungi without apparently affecting growth of the other fungi. There is also a tendency for fungal hyphae to lyse, or at least release nutrients from older parts of their hyphae, even in the absence of other organisms, if subjected to nutrient stress (Ko & Lockwood, 1970).

The first definitive evidence of mycoparasitism by Pythium spp was provided by Hoch & Fuller (1977) who, by a combination of light and electron microscopy, showed two probable patterns and one possible pattern of mycoparasitism by P. acanthicum (isolate PRL 2142). The first of these three patterns of antagonism involved slow penetration of host, the second involved fast penetration, and the third no penetration but extensive hyphal coiling by the mycoparasite. The first pattern was typified by the interaction with Phycomyces blakesleeanus Burgeff wherein young, actively growing hyphae of the host were penetrated by hyphae of P. acanthicum, with subsequent degeneration of the host hyphal contents and internal growth by the mycoparasite. Initial contact by the mycoparasite was followed by the development of localized wall ingrowths (papillae) by the host hyphae, similar to those seen in some plant cells during attempted penetration by fungi (Manners, 1982). As in the plant response, these structures often do not seem to prevent penetration.

Penetration appeared to be by a combination of enzymic activity and mechanical forces by P. acanthicum, but the mycoparasite did not form any specialised structures, such as appressoria, and instead invaded by apparently unmodified vegetative hyphae. There seemed to be a greater susceptibility to invasion in the younger hyphal tips of P. blakesleeanus than in more mature regions, and the mycoparasite displayed tropism towards them, even from 100 µm distance.

The second type of behaviour seen by Hoch & Fuller (1977) was between P. acanthicum and Corticium sp, a member of the Basidiomycotina, or Rhizoctonia solani. Here the response to contact by the mycoparasite was extremely rapid, with papilla formation occurring almost immediately following contact, and the host cytoplasm soon becoming moribund. Penetration of the host cell generally occurred within 8-16 minutes of initial contact by the mycoparasite. As the mycoparasite colonized the host, it promoted similar host cell responses as it reached septa of adjacent, hitherto unaffected host cells. Rapid responses were also seen by Lutchmeah & Cooke (1984) when hyphae of P. oligandrum contacted cells of R. solani, Mycocentrospora acerina (Hartig) Deighton and P. ultimum on agar plates. P. oligandrum grew past the affected cells, however, and only hyphal branches that arose from branches of the main hyphae of the parasite invaded R. solani or M. acerina. P. ultimum, as above, was not penetrated. The host behaviour observed by Lutchmeah & Cooke (1984) was likened to hyphal interference, first described by Ikediugwu & Webster (1970) as a contact inhibition elicited by hyphae of some members of the Basidiomycotina.

The third type of interaction recognised by Hoch & Fuller (1977) was typified by that between P. acanthicum and Pythium aphanidermatum (Edson) Fitzp. and was quite different from those mentioned above. Although Hoch & Fuller (1977) observed the hyphae of P. acanthicum

coiling extensively around the hyphae of P. aphanidermatum, further detailed examination under the scanning electron microscope revealed that P. aphanidermatum was never successfully penetrated by the mycoparasite. As P. aphanidermatum in culture seems to be little affected by P. acanthicum, hyphal coiling is not as strongly indicative of mycoparasitism as was once believed, an observation that agrees with the reports of Deacon (1976). However, Lutchmeah & Cooke (1984) observed that hyphal tips of P. ultimum lost opacity within 5-30 minutes after being contacted by P. oligandrum, but penetration did not occur. Penetration of hyphae of certain phytopathogenic Pythium spp has been reported recently by Lewis et al. (1989).

Lifshitz et al. (1984a) carried out similar studies to those above for P. nunn. They reported conspicuous hyphal coiling by P. nunn around hyphae of P. ultimum and Pythium vexans de Bary, generally followed by lysis of host hyphae. In contrast, P. nunn penetrated and eventually parasitized hyphae of P. aphanidermatum, Phytophthora parasitica Dastur, Phytophthora cinnamomi Rands and hyphal tips of R. solani, on which it formed appressorium-like structures.

More recently, studies of this nature have been carried out with P. oligandrum (Whipps, Lewis & Cooke, 1988; Lewis, Whipps & Cooke, 1989). Unlike the observational studies of Lifshitz et al. (1984a), interactions were observed prior to contact, and could thus be divided into four stages, namely "target location", "recognition", "contact and penetration", and "nutrient acquisition" (Whipps, Lewis & Cooke, 1988). They observed that Pythium oligandrum was able to detect host hypha over ranges of up to 100 µm, in that P. oligandrum formed lateral branches which grew towards the host (Lewis, Whipps & Cooke, 1989). Unfortunately, since all the hosts examined were susceptible to parasitism by P. oligandrum it is not clear whether such tropic responses were to all, or

just susceptible, host hyphae. It was believed that such location involved the detection of metabolite gradients by the parasite and that post-contact recognition is lectin-mediated (Elad, Barak & Chet, 1983; Barak et al., 1985) though no further investigation of this had been undertaken.

With regard to mechanisms of interaction, Lewis et al. (1989) were able to divide interactions involving susceptible hosts into three categories. The first category was that of fast lysis, whereby the host hypha lysed, evacuating its hyphal contents soon after the cell was penetrated. Subsequent cessation of growth of the host hypha and a loss of opacity was then observed. The mycoparasite, meanwhile, continued to grow, and branched profusely in the area of interaction. Ten of the fourteen hosts examined fell into this category.

The second category was fast granulation, in which the host hyphal contents became granulated and disorganised leading to loss of opacity and cessation of hyphal growth soon after contact, but rarely involving lysis. Only R. solani fell into this category.

The third category was "slow, no lysis" and involved only pythiaceous hosts. These interactions were typically non-lytic but between 1 and 8 h following contact with P. oligandrum, the host hyphal contents became disorganised and lost opacity.

In all interactions the mycoparasite ramified through the host mycelium, often exiting the host hyphae as fine branches only to cause further lysis and penetration of other hyphae, thereby parasitising the entire colony.

A different approach was tried by Elad et al. (1985) following the scanning electron microscopy of Lifshitz et al. (1984a) which had revealed that host hyphal cell walls showed signs of enzymic degradation in areas of parasitism by P. nunn. Elad et al. (1985) examined the

possible roles of wall-degrading enzymes in mycoparasitism. The mycoparasite was shown to be able to produce the enzymes β-1,3-glucanase, cellulase, and chitinase in different amounts in response to the presence of the different fungal species of differing wall composition. Despite showing that P. nunn produces a range of such enzymes, it is important to note that all hyphae have the ability to degrade their own walls, an essential feature of hyphal growth (Burnett & Trinci, 1979).

Furthermore, in conditions of nutrient stress, hyphae may lyse as a result of autolysis (Ko & Lockwood, 1970). Elad et al. (1985) showed that the amount of each enzyme produced was significantly greater than that produced by a range of phytopathogenic Pythium spp. P. oligandrum showed levels of production of wall-degrading enzymes comparable with those of the phytopathogens, so a high level of enzyme production may be peculiar to P. nunn and should not be attributed to the mycoparasitic Pythium spp as a group. Work by Lewis, Whipps & Cooke (1989) confirmed the production of β-1,3-D-glucanase by P. oligandrum (Elad et al., 1985) but these workers were apprently unable to detect cellulase or chitinase production by this fungus as Elad et al. (1985) had been able to for P. nunn.

Elad et al. (1985) also showed that P. nunn produces non-volatile substances that inhibit mycelial growth of R. solani and Pythium spp in culture and in soil. This type of activity was not observed by Foley & Deacon (1986b) in culture filtrates of P. oligandrum, and has not been reported for the other mycoparasitic Pythium species. Whipps (1987b) and Lewis, Whipps & Cooke (1989) did find, however, that P. oligandrum grown on cellophane overlying agar plates was able to reduce the subsequent growth by host species inoculated onto the plates. Although in other experiments no production of volatile growth inhibiting compounds had been found, the production of non-volatile inhibitory compounds was

suspected by these workers.

1.3.4 Use of mycoparasitic Pythium spp as biological control agents

Two factors have made mycoparasitic Pythium spp attractive as potential biocontrol agents of soil-borne plant pathogens. Firstly, they are antagonistic to a range of soil-borne fungi and, secondly, they show negligible pathogenicity to plants (Klemmer & Nakano, 1964; Kilpatrick, 1968; Plaats-Niterink, 1975; Deacon & Henry, 1978; Pieczarka & Abawi, 1978; Martin & Hancock, 1987). However, there have been some reports of phytopathogenicity by isolates of P. acanthicum, P. periplocum and P. oligandrum (Dreschler, 1930, 1946; Haskins, 1963; Robertson, 1973).

P. oligandrum has been studied as a potential biocontrol agent and has been patented for use on sugar beet in several countries (Vesely, 1977, 1978, 1981, UK Patent GB2027448B; US Patent 4,259,317). It is effective against damping-off of sugar beet caused by pathogens such as P. ultimum (Vesely, 1977, 1978, 1981; Martin & Hancock, 1987; Walther & Gindrat, 1987b) and Phoma betae Frank (Walther & Gindrat, 1987b). P. oligandrum is also effective against damping-off of cress by P. ultimum, and against seedling disease of carrots caused by Mycocentrospora acerina (Al-Hamdani, Lutchmeah & Cooke, 1983; Lutchmeah & Cooke, 1985). All the above work has involved application of oospores of P. oligandrum to the seeds prior to sowing (Vesely, 1981; Lutchmeah & Cooke, 1985).

A different approach has been used with P. nunn to try to reduce the populations of plant-pathogenic fungi in soil (Lifshitz, Sneh & Baker, 1984). The addition of dried bean leaves to soil containing P. nunn resulted in an increase in the soil population of P. nunn and a corresponding decrease in the levels of phytopathogen. Suppression of

P. ultimum was enhanced if rolled oats were used rather than bean leaves as an organic substrate for the added P. nunn (Paulitz & Baker, 1987a), but other substrates such as cotton leaves, alfalfa, or wheat straw did not significantly influence disease incidence (Paulitz & Baker, 1987b). Furthermore, temperature, pH and soil matric potential also influence disease suppression by P. nunn (Paulitz & Baker, 1987a). The routine addition of P. nunn to the soil may prove difficult, however, because this fungus does not readily produce oospores (Lifshitz, Stranghelli & Baker, 1984). Work by Martin & Hancock (1986) demonstrated a potential natural role of P. oligandrum in control of other Pythium spp, and the workers further demonstrated that the relative population levels of mycoparasite and its hosts were influenced by Cl^- contents of soil. This evidence suggests that P. oligandrum might be used, like P. nunn above, to suppress pathogen populations in plant residues in addition to its use as a seed inoculant.

1.4 Antagonism by Trichoderma and Gliocladium spp

Even more attention has been devoted to the potential of antagonistic members of the genera Trichoderma and Gliocladium in biocontrol programmes than for mycoparasitic Pythium spp in this respect. The mechanisms of antagonism by Trichoderma and Gliocladium species in vivo are rather more difficult to ascertain than in the case of mycoparasitic Pythium spp, due to the known ability of several Trichoderma and Gliocladium spp to produce antibiotics as well as to parasitise their hosts (Dennis & Webster, 1971,a,b,c,; Chet, 1987). As a result, mycoparasitic Trichoderma and Gliocladium spp more closely fit Cook & Baker's (1983) outline of the "ideal" antagonist. Indeed Trichoderma harzianum Rifai or related species have reduced seedling diseases caused by Rhizoctonia solani (eg Hadar, Chet & Henis, 1979), cucumber root rot

caused by Rhizoctonia solani (Lewis & Papavizas, 1980), white rot of garlic caused by Sclerotium cepivorum Berk (de Oliveira et al., 1984) and pre-emergence damping-off of pea caused by Pythium spp (Lifshitz et al., 1986) - and these are but a few examples. Gliocladium spp have reduced damping-off of cotton seedlings by Pythium ultimum and Rhizoctonia solani (Howell, 1982) and damping-off and blight of snapbean caused by Sclerotium rolfsii Sacc (Papavizas & Lewis, 1989) amongst other reports.

Despite these successful demonstrations, it remains unclear whether the primary mode of antagonism is mycoparasitism, antibiosis, competition or a combination of all these. Chet (1987) forwards mycoparasitism as being the principal mechanism of antagonism against soil-borne pathogens and describes it as a process involving chemotropic growth before contact, followed by recognition of the host by the mycoparasite, and excretion of extracellular enzymes by the mycoparasite to enable penetration or elicit host lysis. Directed growth of hyphae in response to a gradient of exudates produced by the host mycelium has been observed (Dennis & Webster, 1971c) with Trichoderma hamatum (Bon) Bain. The role of lectins and agglutinins has been implicated in the host-recognition by Trichoderma (Chet, 1987). Lectins present on the hyphae of pathogenic fungi, such as R. solani, bind to agglutinins on Trichoderma cell walls and by doing so are thought to trigger a host-recognition response (Barak et al., 1985).

The stimulation of excretion of extracellular enzymes, particularly β-1,3-glucanase and chitinase, was shown by Elad et al. (1982) when T. harzianum was grown on different media or on cell walls of the pathogen Sclerotium rolfsii. Protease and lipase activity were also detected in the medium when the antagonist attacked mycelium of S. rolfsii (Elad et al., 1982). Differences in the levels of production of hydrolytic enzymes between isolates of T. harzianum were noted when mycelium of S.

rolfsii, Rhizoctonia solani and Pythium aphanidermatum were attacked in soil and this phenomenon was correlated with the ability of each of the Trichoderma isolates to control the respective soil-borne pathogen.

There is plentiful evidence of host hyphal invasion by mycoparasitic Trichoderma and Gliocladium spp. Lewis & Papavizas (1980) observed hyphae of R. solani invaded by hyphae of an antagonistic Trichoderma isolate. A more detailed investigation by Elad et al. (1983b) using scanning electron microscopy and fluorescence microscopy revealed that Trichoderma harzianum or T. hamatum attached to either S. rolfsii or R. solani by hyphal coils, hooks or appressoria. Sites of lysis and penetration were found in the host hyphae following removal of the parasitic hyphae. Tu (1980) observed penetration of Sclerotinia sclerotiorum by hyphae of Gliocladium virens Miller et al. and Howell (1982) presented evidence of penetration of R. solani, and coagulation and disintegration of cytoplasm of Pythium ultimum, by G. virens.

Although there is direct evidence for mycoparasitism, the production of antibiotic substances may be the primary mode of antagonism by Trichoderma and Gliocladium spp. Dennis & Webster (1971a, b) reported that species of Trichoderma were capable of producing non-volatile and volatile antibiotics, and Howell & Stipanovic (1983) reported the production of a new antibiotic, gliovirin, by G. virens which is highly toxic to P. ultimum. An ultraviolet light-induced mutant of G. virens deficient for gliovirin production was overgrown by P. ultimum in culture and did not protect cotton seedlings from damping-off by this pathogen. A mutant with enhanced gliovirin production was more inhibitory to P. ultimum in culture than the parent isolate, and despite having a reduced growth rate showed similar seedling disease suppression as the parent. The obvious importance of antibiosis as a means of antagonism in this example was highlighted in later work by Howell

(1987) when he produced mutants of G. virens with no mycoparasitic activity. He found that these had similar efficacy as biocontrol agents of cotton seedling disease caused by R. solani, as mycoparasitic strains, indicating that mycoparasitism did not appear to be a major mechanism for antagonism by G. virens in this instance. However, production of antibiotics depends on availability of nutrients (Cook & Baker, 1983), so changes in nutrient availability can effect the degree and means of antagonism between parasite and host (Whipps, 1987b). Thus it would appear that means of antagonism can change in their importance according to circumstance, particularly for the mycoparasitic Trichoderma and Gliocladium spp.

Fuller appraisals of Trichoderma spp, and Trichoderma and Gliocladium spp are given in reviews by Chet (1987) and Papavizas (1985) respectively.

1.5 Oospores of Pythium spp and their germination

Members of the genus Pythium commonly produce two types of resting structure. Sporangia, generally producing zoospores, are of greatest importance in the short term (Stanghellini, 1974), whereas the thick-walled oospores are commonly considered as the primary long-term resting structure of Pythium spp in soil. Only the latter will be reviewed here due to their current and potential future use as inocula for biological control by mycoparasitic Pythium spp (Vesely, 1981; Lutchmeah & Cooke, 1985; Martin & Hancock, 1987; Walther & Gindrat, 1987b). Their study has, until relatively recently been concerned more with their taxonomic significance (eg Drechsler, 1930) than with their behaviour. In the last twenty years, however, there has been a marked increase in the amount of information about the behaviour of these propagules. This has mainly concerned two phytopathogenic species, P. aphanidermatum and P.

ultimum, in attempts to determine the role of oospores in initiating infection. However, the increased interest in the use of microorganisms as biocontrol agents has led to attempts to try to use oospores of P. oligandrum as biocontrol inocula, and the related physiological studies carried out, therefore, have more in common with work on fungicide formulation and efficacy than with the natural role of oospores in natural environments.

1.5.1 Germination of oospores of P. aphanidermatum and P. ultimum

In one of the earlier attempts to investigate factors influencing oospore germination by Pythium spp, Adams (1971) studied the effects of temperature, soil pH and incubation period. He found that the optimum temperature range for germination was equivalent to the optimum range for mycelial growth of this fungus, and observed that germination occurred as early as 2 h and was maximal after 10 h. However, the optimal pH for germination, at 7.5, seemed unusually high since this fungus can cause extensive damage to plants over a broad range of pH, reaching as low as pH 3.5. Maximum germination levels that he obtained were around 50-60%. By introduction of nutrients (casein and gallic acid) to the germination medium, it was possible to increase the germination level to 95% (Flowers & Litrell, 1972) at an optimal pH lower than 6.0. However, germination levels were not so high as this when Stanghellini & Russell (1973) used other carbon nutrient sources.

The importance of light in germination was found by Schmitthener (1972), in that exposure to light increased germination of P. aphanidermatum oospores. The amount of light required appeared to be related to the amount and form of calcium supplied.

Endogenous dormancy was considered by Stanghellini & Russell (1973) to affect the amount of germination in different conditions. They

described two stages in the germination process. The first stage, pre-germination, involved the adsorption of the endospore - that is, the conversion of the oospore from being thick- to thin-walled and a reduction in the size of the central reserve globule. This stage, they found, required calcium. The second stage, germination, required an exogenous carbon source. Conditions for germination were thus thought to be improved in soils with high moisture contents due to the increased nutrient availability (Stanghellini & Burr, 1973). This requirement for nutrients for germination led Burr & Stanghellini (1973) to conclude that the oospores were not constitutively but exogenously dormant - that is, dormancy was not an innate property of the oospore itself, but a condition whereby development is delayed due to unfavourable environmental conditions (Sussman & Halvorson, 1966).

Ayers & Lumsden (1975) found a major difference between the levels of germination of oospores of P. aphanidermatum, P. ultimum and P. myriotylum, and they presumed that marked differences must exist with regards to optimal conditions for oospore germination in different Pythium spp. The difference was in the "pre-germination" stage, ie change from being thick- to thin-walled. Oospores of P. aphanidermatum were able to change fairly rapidly (1-2 days in favourable conditions) whereas oospores of P. ultimum required much longer for this (2-6 weeks). This led Lumsden & Ayers (1975) to conclude, after studies on the greater resistance to environmental stress by thick- as opposed to the thin-walled oospores, that the thick-walled oospores were constitutively dormant. This, however, was refuted by Johnson (1988) who was able to convert oospores of P. ultimum from being thick- to thin-walled very rapidly (69% in 2 days) when subjected to appropriate conditions, notably full aeration, and optimum pH and light levels. These followed a study (Johnson & Arroyo, 1983) where 30% thick-walled

oospores were converted to a thin-walled form within 48 h in both cotton rhizosphere and non-rhizosphere soil. It may thus be presumed that oospores of most Pythium species are exogenously dormant, and only require the discovery of optimal conditions for optimal germination levels.

1.5.2 Germination of oospores of P. oligandrum

The first observations made on oospore germination by P. oligandrum were by Drechsler (1946) who noted that though a small proportion of oospores germinated after resting for 40 to 50 days when immersed in a shallow layer of water, practically all oospores germinated after 150-200 days of aging. He described the process of germination whereby the reserve globule changed from a spherical to an irregular shape, refringement bodies became less conspicuous and the dark, inner lining making up about two-thirds of the oospore wall showed radial markings and eventually broke down, leaving a thin-walled spherical oospore. However, Drechsler (1946) described the outcome of germination to be the production of a vesicle yielding laterally bicilliate zoospores in most instances. Only in some cases did oospores germinate to form mycelia.

Although there have been many studies in which oospores of P. oligandrum were used for biocontrol of soil-borne fungal pathogens (Vesely, 1979; Al-Hamdani et al., 1983; Martin & Hancock, 1987; Lutchmeah & Cooke, 1985), the degree of oospore germination in such work was seldom examined. Foley & Deacon (1985) used a most probable number analysis to estimate the population of P. oligandrum that could be retrieved from soil supplemented with oospores of P. oligandrum, and this indicated that between 48 and 60% of oospores were capable of establishing colonies on agar plates pre-colonised by a susceptible host fungus. In the same study, maximum germination was found to be 68%

after 5 days when oospore preparations were plated onto malt extract agar. Walther & Gindrat (1987b) studied differences between isolates and the effects of method of culture and subsequent storage on germination of the oospores of P. oligandrum. They found that different isolates exhibited different germination levels, and that this varied depending on the time and method of culture, and the time and method of storage of oospores. Maximum germination was achieved when oospores were aged for 7 days in sterile distilled water after culture for 10 days in a carrot- based medium, and when storage was not longer than c 10 days in myo-inositol to protect against desiccation. Unlike Drechsler (1946), Walther & Gindrat (1987b) reported no thinning of the oospore wall in germinating oospores. Furthermore, they suggested that young oospores may have constitutive dormancy, differing from the conclusion of Ayers & Lumsden (1975) for P. myriotylum.

1.6 Aims and objectives of the work in this thesis

Although there had been several studies of the mode of parasitism by P. oligandrum and, to a lesser degree, by P. nunn, there was no substantial comparative study of the behaviour of these mycoparasites against a range of potential host fungi at the "level" of interactions between individual hyphae. A principal aim of this study was to make such a comparison, for three mycoparasites that had different growth rates and other physiological attributes, namely P. oligandrum, P. mycoparasiticum (formerly Pythium SWO) and P. nunn. Thereby it was hoped to compare their aggressiveness across a range of hosts, and the degrees of resistance of the individual hosts across the range of mycoparasites. A video-microscopical technique was developed for this so that, for the first time, aspects of the interactions could be quantified and statistically analysed.

A second objective was to conduct a similar study of interactions at the level of "whole colonies", so that evidence on aggressiveness of the mycoparasites and host susceptibility based on the responses of individual hyphae could be related to the ability of the mycoparasites to control growth by the various hosts.

The final objective was to examine the taxonomy of isolates previously referred to as Pythium SWO, and to conduct comparative physiological studies on this and other fungi with which it might be confused. During this, and as a supplement to it, an attempt was made to investigate some of the factors associated with production and germination of the oospores of the mycoparasites, in recognition of the potential role of oospores in biocontrol programmes involving mycoparasitic Pythium spp.

SECTION 2

SECTION 2

Materials and Methods

2.1 Culture media

Distilled water agar (WA)	Agar (Oxoid No 3), 20 g; distilled water, 1 l
Potato-dextrose agar (PDA)	Potato extract (Oxoid), 4 g; dextrose, 20 g; agar 20 g; distilled water, 1 l
Cornmeal agar (CMA)	Cornmeal extract (Difco), 20 g; glucose, 20 g; agar, 20 g; distilled water, 1 l
Sunflower seed extract	Sunflower seeds (60 g) boiled in distilled water for 1 h, then homogenized, filtered through muslin and diluted to 4% w/v in distilled water
Sunflower seed agar (SSA)	Sunflower seed extract, 50 ml; distilled water, 950 ml; agar, 20 g
Carrot extract	Carrots (60 g) homogenized in 400 ml distilled water, sieved through 5 mm mesh and diluted to 7.5% w/v in distilled water
Carrot extract agar (CA)	Carrot extract (7.5%) as above, 1 l; agar 20 g
Mineral nutrient solution (MNS)	KH_2PO_4, 1.23 g; KCl, 0.5 g; $MgSO_4.7H_2O$, 0.5 g; $FeCl_3.6H_2O$, 1.0 mg; $ZnSO_4.7H_2O$, 0.9 mg; $MnSO_4.4H_2O$, 0.4 mg; distilled water, 1 l

All culture media were sterilised by autoclaving at 121°C for 15

minutes.

2.2 Fungal cultures

The fungi used in this work, together with their origins and relevant accession numbers, are shown in Table 2.1.

Cultures were maintained by fortnightly subculturing onto plates of PDA which were incubated in darkness at 25°C. Inoculum discs of the isolates were removed from the youngest part of the colony and were placed at the plate margins to enable juvenile mycelia to grow for longer than if the inoculum was centrally placed. In addition, the fungi were stored on slopes of sunflower-seed enriched CMA under sterilised mineral oil at 4°C and inoculum discs from plates of CMA were stored under sterilised water in Universal bottles at 4°C.

Isolate CGH of P. oligandrum, and isolates AR5A and AR7A of P. mycoparasiticum were isolated from natural environments during this study using the pre-colonised plate technique of Deacon & Henry (1978). The former was identified using the keys of Waterhouse (1968).

2.3 Experimental methods

2.3.1 Liquid culture

The method of Foley & Deacon (1986a) was used in these studies. Cultures were grown in medical flats of 100 ml capacity (120 mm in length) containing 10 ml of culture medium. Glucose was added to the basal medium (MNS, Section 2.1) as a carbon source (20 g l^{-1}), along with nitrogen in the form of either sodium nitrate ($NaNO_3$, 2 g l^{-1}) or D,L-asparagine (1.51 g l^{-1}). The media were used with or without the addition of thiamine hydrochloride (100 µg l^{-1}). Medical flats containing these media were sterilised by autoclaving at 121°C for 15 min.

Table 2.1 Table of fungal cultures

	Culture collection accession, or culture number	Origin
Pythium spp		
P. aphanidermatum	CBS 634.70	From *Solanum* sp
P. graminicola Subram.	IMI 91329	From sugar cane
P. mycoparasiticum	AR7A	Arable field, Lasswade, Lothian
	AR5A	Arable field, Straiton, Lothian
P. nunn	ATCC 20692	Ex sandy loam soil, Colorado, USA
	ATCC 20693	Ex sandy loam soil, Colorado, USA
P. oligandrum	CGH	Wasteland soil, Edinburgh
	4410b	Ex loam soil, Whiteknights, England
P. scleroteichum Drechsler	CBS 294.37	From sweet potato
P. vexans	CBS 270.38	From alfalfa
Other species		
Botryotrichum piluliferum	145 A	See Deacon (1976)
Botrytis cinerea Sardina	ESCA SCC*	From grapes
Gliocladium roseum	Gr53	From glasshouse soil, Penicuik
Fusarium culmorum (W G Sm.) Sacc.	CD 9	From wheat
F. oxysporum Schlect fsp *lycopersici*	ESCA SCC	From tomatoes

Table 2.1 (Cont'd) Table of fungal cultures

	Culture collection accession, or culture number	Origin
Phialophora sp (lobed hyphopodia)	IMI 187786	From wheat
Rhizoctonia solani	GM1 (AG5) T125	Supplied by R. T. Sherwood. From wheat (Deacon & Scott, 1985)
Trichoderma aureoviride Rifai	ESCA SCC	Origin unknown
Trichoderma harzianum	T95	See Chang *et al.* (1986)

* East of Scotland College of Agriculture Stock Culture Collection

Inocula of fungi consisted of plugs (5 mm diameter) cut from the margins of colonies on plates of potato-dextrose agar (PDA). The inocula were placed centrally on one of the broad sides of each flat, which was then placed horizontally in the final position for incubation, to partly immerse the inoculum. The experiments were incubated in a lit growth room at 25°C for 14 days.

Assessments of growth in liquid media were made by visual inspection and measurement of colony extension along the length of the flats. Attempts were made to assess growth as mycelial dry weights, but the weights were too small to be satisfactorily compared.

2.3.2 Production and germination of oospores

Oospores were produced using a similar method to that of Walther & Gindrat (1987b), whereby Roux bottles (1 litre capacity) containing 100 ml of carrot extract (Section 2.1) were autoclaved for 15 min at 121°C, then inoculated on the broad side with a 11 mm diameter disc of P. oligandrum (isolate 4410b) and incubated at 20°C in darkness, for 21 days. The cultures were then removed, rinsed twice in sterile distilled water (SDW), then left for 7 days in SDW. For experiments comparing the effect of length of the culture period on germination, medical flats (100 ml capacity) were used containing 10 ml of carrot extract. Also, in these experiments some of the cultures were left in SDW for 7 days, and others were not.

After the appropriate length of culture and treatment as above, the colonies from three replicate flasks were harvested onto cellulose acetate filters, rinsed twice with SDW under vacuum in a Buchner funnel, suspended in a volume of SDW equivalent to half of the initial culture volume and then homogenized at 13,500 rpm for 120 sec using an Ultra-Turrax T25 blender. The resulting suspension was centrifuged for 15 sec at the top speed (36,000 rpm) of a bench centrifuge, the supernatant was discar-

ded and the pellet was resuspended in SDW to achieve an oospore concentration of 10^5 spores ml^{-1}.

To assess oospore germination, aliquots of oospore suspensions were mixed with equal volumes of various test solutions, SDW being used as a control, the mixing being done by holding tubes for 15 sec on a vortex mixer. One hundred µl of a sample was placed on each of several clean microscope slides. These were then placed in a moist chamber and incubated for 18 h at 25°C. Assessment was made by counting the number of germinated oospores out of the total number of oospores. Five replicates of each treatment were prepared and five counts of between 130 and 160 oospores were made for each replicate.

2.3.3 Plant pathogenicity tests

Strips of PDA 7 x 0.75 cm were cut from near the margins of fungal colonies on PDA and were placed on a layer of vermiculite in trays, 20 x 15 cm, such that five parallel strips ran breadthwise in each tray. Fifty seeds of wheat (cv Avalon), pea (cv Waverley), Brussels sprout (cv Roodnerf) and spring onion (unknown cultivar) were surface sterilised by immersion in methylated spirits for 10 sec before immersion in a 0.05% solution of mercuric chloride for 2 min. The seeds were then washed thoroughly for c. 30 min to remove any traces of the sterilising solution. After drying the seeds on sterilised filter paper, they were pressed into the agar strips, 10 seeds per strip for Brussels sprout and spring onion, five seeds per strip for wheat and pea. The strips were then covered with an appropriate layer of vermiculite for each type of seed and the trays were then watered and placed in a glasshouse at 20°C in natural day/night regimes. Emergence of the seedlings was monitored daily and the trays incubated until no further emergence was expected (2 wk). Then the strips of agar and seeds were excavated, and the healthy

seedlings were counted. The experiment involved a comparison of P. oligandrum (isolate CGH), P. nunn (isolate 20693), P. mycoparasiticum (isolates AR7A and AR5A), and P. aphanidermatum (CBS 634.70), with uncolonised strips of agar as controls.

2.3.4 Interactions on cellulose

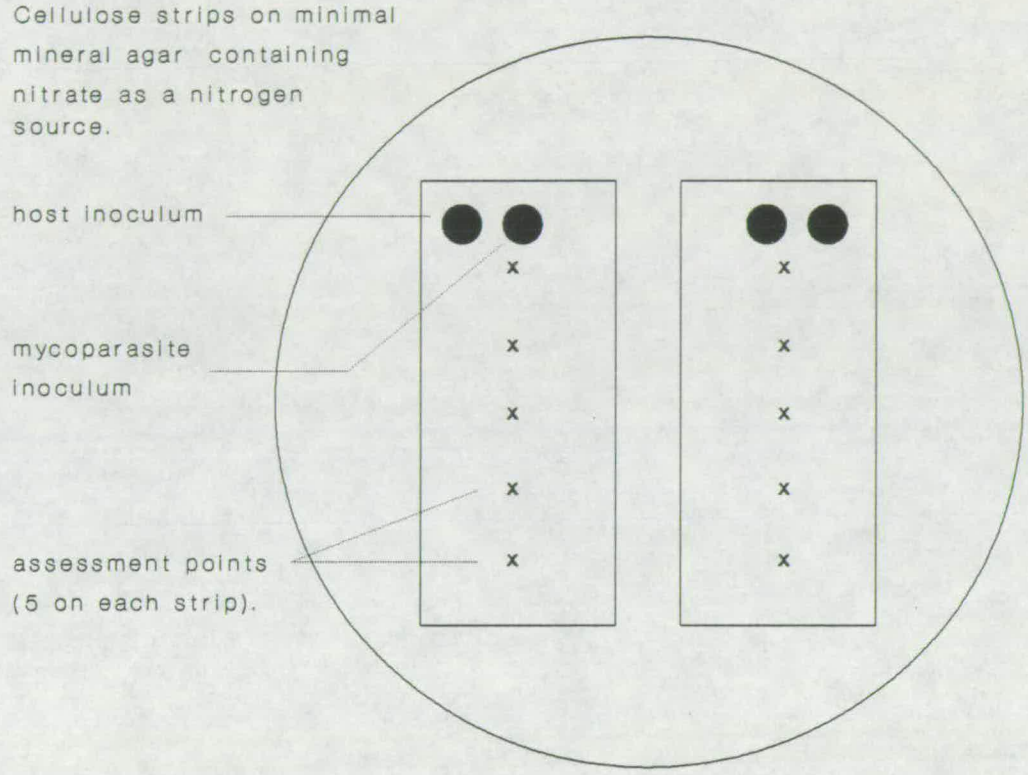
2.3.4.1 Growth on filter paper

A modification of the method of Deacon (1976) was used to study the effects of the mycoparasites on growth of host fungi on filter paper. Wads of five oven-dry filter paper circles (Whatman No 3, 7 cm diameter) were accurately weighed into 250 ml flasks and saturated with 15 ml mineral nutrient solution (Section 2.1) supplemented with $NaNO_3$ (2 g l^{-1}), thiamine (100 µg l^{-1}) and biotin (10 µg l^{-1}). After autoclaving for 30 min at 121°C, the wads were inoculated at their margins with 10 mm discs of fungi, cut from the margins of colonies on PDA. The three cellulolytic fungi used were F. culmorum, B. piluliferum and B. cinerea. The flasks were incubated in darkness at 25°C. Some received no further treatment. Others, after 2 days, received an inoculum disc of a mycoparasitic Pythium species, placed alongside the original inoculum disc in a juxtaposed position. All flasks were incubated for a total of 6 weeks at 25°C. Then their contents were oven-dried to constant weight at 80°C and weighed. After allowance for the weight of nutrients added (using uninoculated controls), the loss in dry weight of the wads, approximating to the weight of material respired, was calculated.

2.3.4.2 Juxtaposed inocula on cellulose film

Unlaquered cellulose film (Rayophane PU 525, supplied by British Sidac Ltd, Merseyside, UK) was cut into strips 6 x 2 cm, and autoclaved

Figure 2.1. Diagrammatic representation of interactions of colonies juxtaposed on cellulose strips.



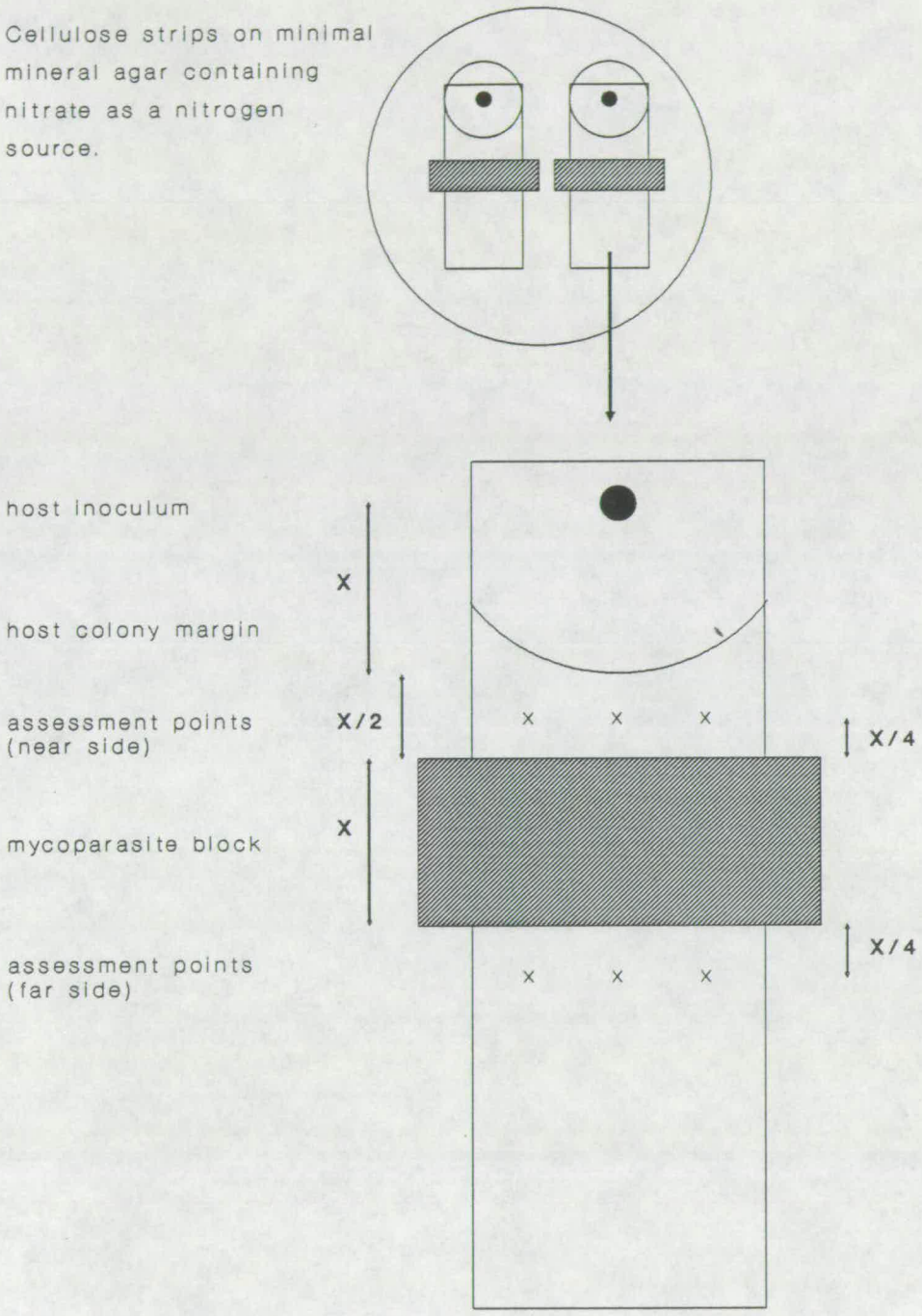
in distilled water for 15 minutes at 121°C. Two strips were placed in parallel on agar plates, the agar containing 2 g $NaNO_3$ and 20 g agar (Oxoid No 3) in 1 litre mineral nutrient solution (Section 2.1). The strips were inoculated at one end with 5 mm diameter discs of test fungi, either singly or in paired combinations (Fig 2.1). In the latter case a cellulolytic partner was allowed to grow for 24 h before a mycoparasitic Pythium sp was added. In paired inoculations, the inoculum discs were placed side by side and 5 mm apart. The plates (four replicates) were incubated at 25°C for 6 days in total.

Cellulolysis was assessed by means of a simple penetrometer which comprised a dissecting needle with a small petri dish attached to the top. The shaft of the needle was supported in a length of glass tubing held in a clamp stand. The point was lowered momentarily onto the cellulose film and raised again if it did not puncture the film, and weights were added progressively to the dish until the film was punctured (Deacon & Henry, 1978). If any given weight was insufficient to cause puncturing, the penetrometer was raised, further weights were added and the penetrometer lowered to an adjacent point on the film. In this way it was hoped to reduce inadvertent weakening of the film either by leaving the penetrometer in position while adding more weights, or by repeatedly probing the same position. The weight required to cause puncturing was recorded initially for five points spaced 10 mm apart along the length of each strip, the first point being 5 mm from the inoculated end of the strip (Fig 2.1); 55g was required to puncture uninoculated film.

2.3.4.3 Opposed inocula on cellulose film

Agar plates with strips of cellulose film were prepared as above, inoculated with host (cellulolytic) fungi and incubated for 24 h to enable the host fungi to grow. The mycoparasite inocula were added as

Figure 2.2. Diagrammatic representation of interactions of colonies opposed on cellulose strips.



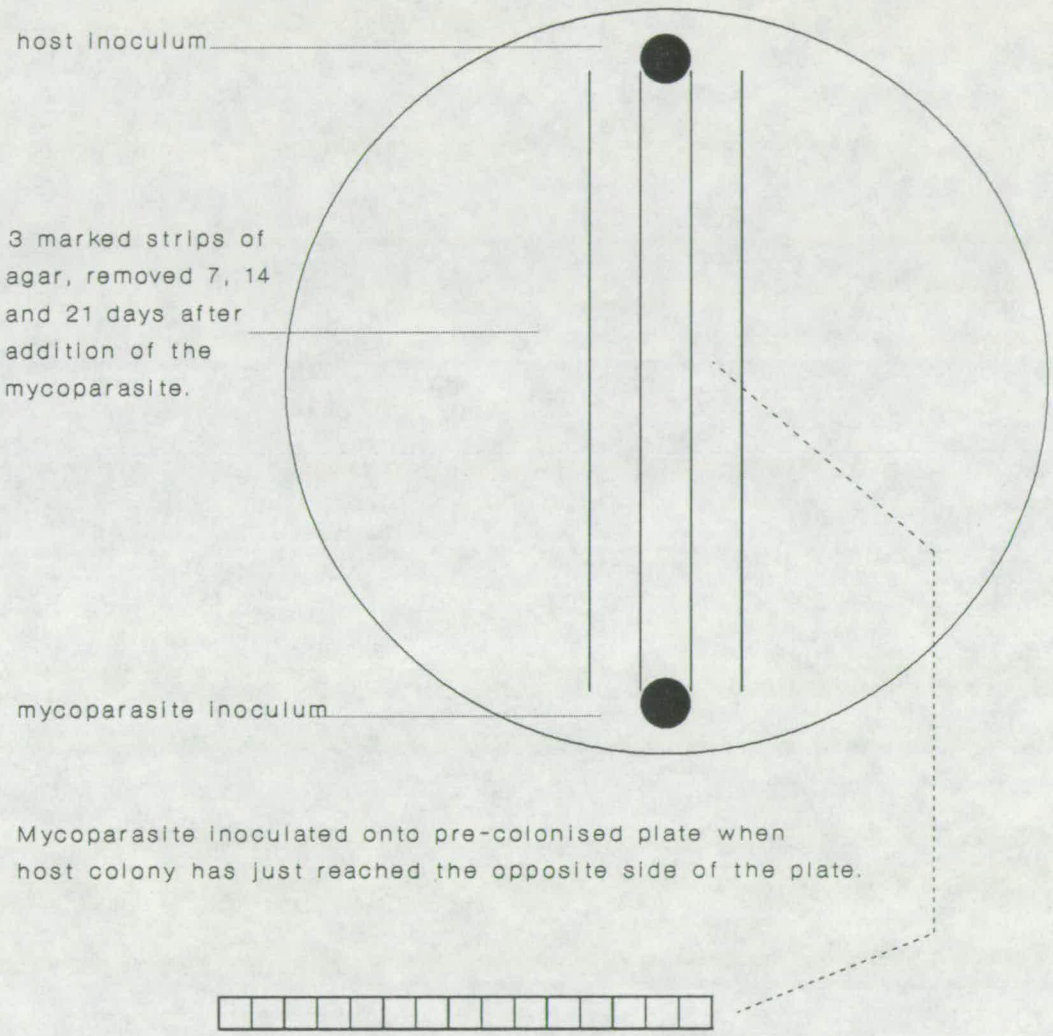
blocks of agar cut from the margins of colonies on PDA. These blocks were 25 mm long and were placed across the strip of cellulose film ahead of the advancing margins of the host colonies as shown in Fig 2.2. The width of each block was selected to represent the equivalent of 24 hours' extension of the host colony margin and the blocks were placed at such a distance that the host colony margin could reach them in 12 h.

Cellulose breakdown was assessed by penetrometer, as above, except that test points were on either side of the mycoparasite block at distance equivalent to 6 h host growth on each side (Fig 2.2). Measurements of the strength of the film were made with a penetrometer at these points on each side of the block after 7, 14 and 21 days, a different assessment point (but at the same distance from the parasite block) being used each time. There were four replicates per treatment.

2.3.5 Growth of mycoparasites on pre-colonised agar plates

Plates of PDA were inoculated at the margin with host fungi and incubated at 25°C until the colony margin just reached the opposite side of the plate. Then a 5 mm diameter inoculum disc of a mycoparasite was placed at the youngest margin of each pre-colonised plate and the plates were reincubated at 25°C. Growth by a mycoparasite across the plate was measured by marking four parallel lines 5 mm apart on the base of the plate such that three strips were marked, the centre strip joining the host and mycoparasite inoculum discs (Fig 2.3). After 7 days' incubation, the central strip of agar was removed with a sharp scalpel, cutting from the host inoculum block to avoid the possibility that spores of the mycoparasite would be inadvertently carried forward. The strip was then cut into successive 5 mm pieces (15 in all) which were incubated on PDA plates to detect outgrowth by the mycoparasite. This was usually evident by visual observation because of the characteristic

Figure 2.3. Diagrammatic representation of interactions on plates of agar pre-colonised by host fungi.



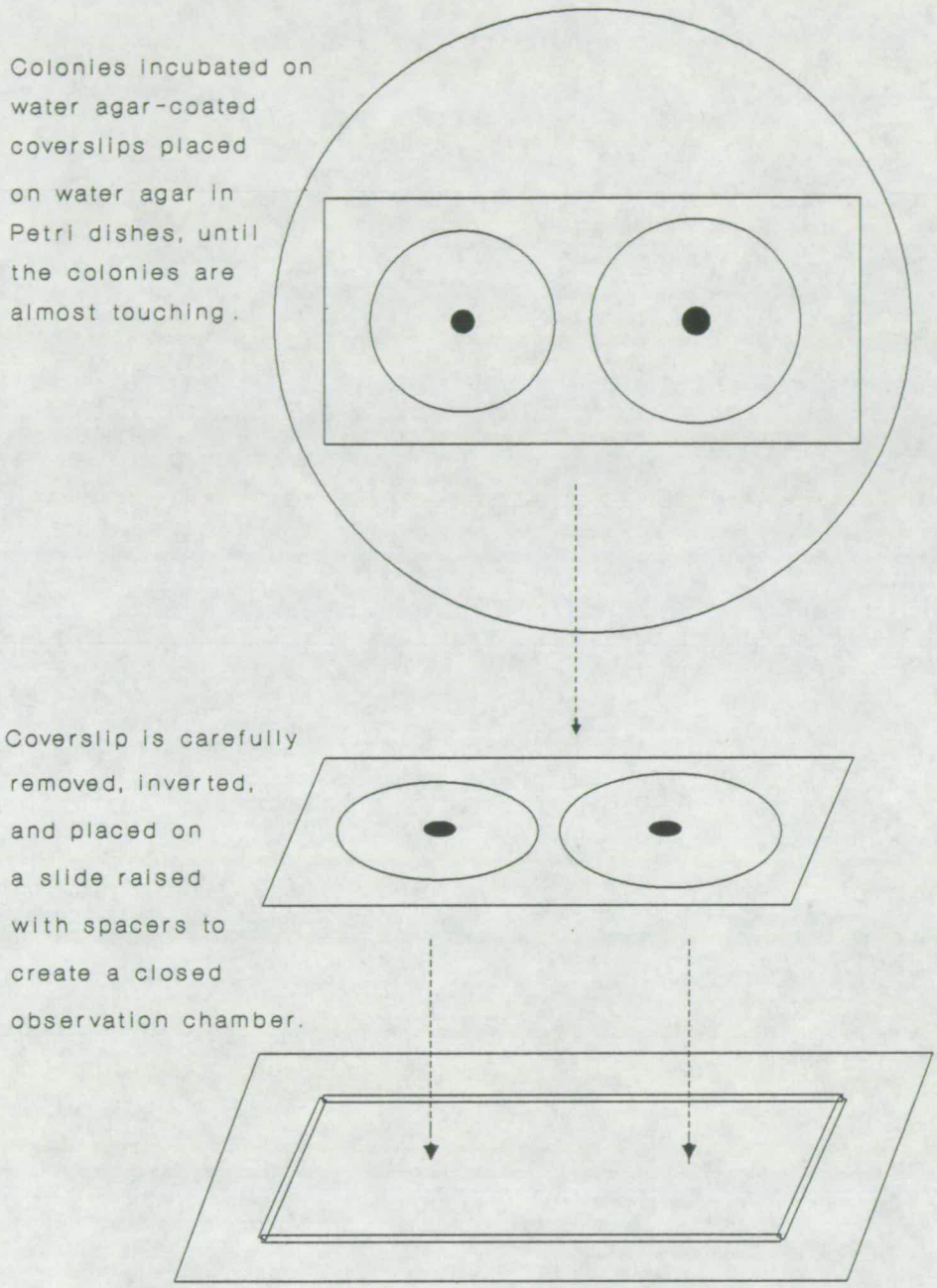
colony morphology of each fungus used, but in cases of doubt the inocula were transferred to carrot agar which enabled the production of the characteristic oogonia of the mycoparasites. After 14 days and 21 days this process was repeated for the two remaining marked strips, so that progress in growth of the mycoparasite could be followed.

2.3.6 Interactions on agar-coated coverslips

Glass coverslips (35 mm by 64 mm) were sterilised by autoclaving at 121°C for 15 min and dipped in sterile molten water agar at normally 92°C for 1 or 2 seconds. Excess agar was allowed to drip off so that only a thin adhering film remained. The coverslips were then laid on solidified water agar in Petri dishes. When the agar on the coverslips had set, a small ($5 mm^2$) inoculum block from the margin of a colony of a host fungus on PDA was placed near one end. The plate was incubated for a variable time (normally 1 to 2 days) until the host had grown about 10 mm from its inoculum, and an inoculum block of the parasite was then placed beyond the colony margin. In all cases the distance and relative timings of inoculation were designed to ensure that the contact between the host and mycoparasite would occur about 24 h later when the plates were incubated at 20°C. In practice, however, a series of plates was prepared with an increasing range of distance between the inoculum blocks to ensure that colony interactions were available for viewing the following day.

When colonies of the host and parasite had almost touched, the coverslip was removed from the agar plate and the inoculum blocks were carefully removed to avoid disruption of the mycelia. The coverslip was then inverted onto an observation chamber (Fig 2.4) consisting of a large microscope slide with a rectangle of glass spacers 2 mm high. The inverted coverslip was sealed to the chamber with vaseline to prevent

Figure 2.4. Diagrammatic representation of method of producing interaction plates.



drying. The upper surface of the coverslip was cleaned of excess agar and the hyphal interactions were observed microscopically.

A Leitz Orthoplan microscope was fitted with a Ploempak incident fluorescence unit between the objectives and the eyepiece assembly. The fluorescence unit was fitted with a clear filter block (TK400) containing a dichroic mirror to enable the passage of all wavelengths of light. A colour video camera (Panasonic WVP 100E) with the front lens removed was attached to a photographic extension tube in the eyepiece housing. A beam-splitting prism directed 80% of light through the camera and 20% through the eyepieces, enabling simultaneous observation through the eyepieces or on a video screen. The camera was attached, through a Panasonic WV-PS01AE/B power supply unit, to a video recorder (Toshiba DV 80-B) which in turn was connected to a Sony Trinitron 14 inch (625 line) colour television. All observations were made with conventional transmitted light supplied from a 120 W tungsten lamp. Exposure of hyphae to intense light was achieved by a 200 W mercury vapour lamp. The output from this was focused and narrowed to a fine beam, c. 10 µm diam, using the focusing and diaphragm of the Ploempak attachment, and this beam was directed, as incident light, down through the objective and onto the specimen, from which it was reflected back through the objective and into the camera. During normal observations the best image on the video screen was found to be obtained by using x 70 phase contrast objectives. Interactions were recorded on Scotch videotape cassettes, and times in 0.1 sec intervals were superimposed on the recordings, using an integral date-time facility in the camera. The effective magnification of the system (x 70 objective and x 1.25 magnification in the Ploempak unit) was such that objects of 10 µm dimension were measurable as 45 mm on the video screen.

For analysing the interactions of hyphae, videotapes were replayed

using the digital frame freeze and frame shift facilities of the video recorder. This enabled 100 individual frames to be analysed per second of "real time", although only 7 in every 10 frames were usable because others showed interference lines. Events that occurred over periods ranging from tenths of seconds to several minutes were analysed either by comparison of "polaroid photographs" using a Mitsubishi P61-B video copy processor attached to the video recorder, or by tracing onto an acetate overlay, calibrated with the image of a slide micrometer on the video screen.

SECTION 3

SECTION 3

The taxonomy, physiology and plant pathogenicity of mycoparasitic *Pythium* species

3.1 Introduction

As described in the Introduction, there are four named *Pythium* species with mycoparasitic activities and a fifth organism, provisionally termed *Pythium* SWO, which is also a mycoparasite. This fifth organism was studied intensively (see later sections of this thesis) and will be described as a new species, *P. mycoparasiticum* sp nov Laing and Deacon (publication in preparation). The evidence on which this will be based, and the form in which the publication will be submitted, is detailed in this section. In the rest of this thesis, the name *P. mycoparasiticum* will be used, pending formal publication.

Also included in this section are some studies on the physiology of *P. mycoparasiticum*, in comparison with other mycoparasitic *Pythium* spp, and an attempt to optimise the production and germination of oospores of some of these fungi.

3.2 *Pythium mycoparasiticum*: background

The existence of a mycoparasitic *Pythium* species with smooth-walled oogonia was first reported by Deacon & Henry (1978), who isolated it using a pre-colonised plate technique whereby plates of PDA are colonised by *Phialophora* sp (lobed hyphopodia) and inoculated with soil organic matter at the colony margin. It was subsequently isolated using the same method from 17% of 164 samples of soil, sediment and similar natural materials in Britain (Foley, 1983; Foley & Deacon, 1985) and was termed *Pythium* SWO to denote its smooth-walled oogonia. The fungus

originally isolated by Deacon & Henry was provisionally identified as P. sclerotеichum Drechsler by Dr J. Stamps, Commonwealth Mycological Institute, and as P. vexans de Bary by Dr A. J. van der Plaats-Niterink, Centraalbureau voor Schimmelcultures, Baarn, Netherlands. A culture was deposited at the Commonwealth Mycological Institute (as accession no. IMI 211458, representing isolate CH7 of Deacon & Henry, 1978). Attempts have been made to obtain a sub-culture of this, but there is, apparently, some difficulty in reviving it from storage, so the culture may no longer be available. None of the isolates obtained by Deacon & Henry (1978) or Foley & Deacon (1985) was retained at Edinburgh. J. W. Deacon (personal communication) experienced difficulty in maintaining viability of the fungus during prolonged storage, and after a two-year absence from Edinburgh, he was unable to revive any of the stored cultures. Any comparison of the current cultures isolated during my work with the fungi previously studied by Deacon & Henry (1978) and Foley & Deacon (1985, 1986a) must, therefore, be based on information that was recorded by these workers in various publications. However, in taxonomic practice (Hawksworth, 1974), which is governed by the International Code of Botanical Nomenclature (Stafleu et al., 1972), this does not pose a barrier to the description of P. mycoparasiticum as a new species. Such a description is based on a nominated holotype, for which one of my cultures (AR7A) will serve. The problems, such as they are, relate only to correlating the characteristics of this holotype with those of previously studied fungi for which the terms Pythium SWO (Foley & Deacon, 1985, 1986a) or "possibly P. sclerotеichum" (Deacon & Henry, 1978) were used.

The description of another new mycoparasitic Pythium with smooth-walled oogonia - P. nunn (Lifshitz, Stanghellini & Baker, 1984) - made it desirable to delay taxonomic work on "Pythium SWO" until cultures

were available for comparison. Cultures of P. nunn were initially unavailable, pending patent applications in the USA for their potential use as biocontrol agents of plant pathogens. Only in 1986 were isolates ATCC 20692 and ATCC 20693 of P. nunn made available for general distribution. Isolate ATCC 20693 is a subculture of the holotype culture (CUP 61143) of P. nunn, held by the Colorado State University (Lifshitz, Stanghellini & Baker, 1984; Baker, 1984). Both of these cultures have been used in work in this thesis (see this and later sections), although most attention has been given to isolate ATCC 20693, as the subculture of the holotype.

3.3 Physiological studies on mycoparasitic Pythium spp

3.3.1 Colony growth and temperature requirements

Colony growth rates of three of the five mycoparasitic Pythium spp were recorded on four different media, and on dilutions of one of these media. Two isolates of P. mycoparasiticum, two of P. nunn and two of P. oligandrum were assessed on water agar (WA), cornmeal agar (CMA) and sunflower seed agar (SSA), as well as PDA at full, one-quarter and one-tenth dilutions. The colony extension rates are recorded in Table 3.1.

From the dilution of PDA plates it appears that P. oligandrum and P. mycoparasiticum grow better on ¼ strength rather than full strength formulations of this medium, unlike P. nunn which grew best at the full concentration. When few or no nutrients were available (WA), the isolates of P. oligandrum and P. nunn showed reduced growth rates whereas colonies of P. mycoparasiticum extended at rates comparable to those on diluted PDA and higher (though not significantly so) than on full-strength PDA. All three species showed lower than maximum extension rates on CMA, the reduction being especially pronounced for P.

Table 3.1 Colony growth rates (mm radial extension 24 h^{-1}) of three mycoparasitic *Pythium* spp on different agar media at 25°C ; means ± s.e. for 4 replicates.

	P. oligandrum Isolate:		*P. mycoparasiticum*		*P. nunn*	
	CGH	4410b	AR7A	AR5A	20692	20693
PDA	28.2 ± 0.5	26.0 ± 0.5	7.2 ± 0.1	5.2 ± 0.2	12.5 ± 0.7	12.2 ± 0.4
One-quarter PDA	30.7 ± 0.4	27.4 ± 0.5	8.3 ± 0.1	8.1 ± 0.1	10.3 ± 0.6	10.1 ± 0.4
One-tenth PDA	26.3 ± 0.4	24.0 ± 0.4	7.4 ± 0.1	5.3 ± 0.1	10.0 ± 0.5	9.7 ± 0.4
WA	25.7 ± 0.3	22.2 ± 0.4	8.6 ± 0.5	6.3 ± 0.7	7.4 ± 0.5	7.6 ± 0.5
SSA	27.6 ± 0.6	24.8 ± 0.4	13.5 ± 0.2	13.6 ± 0.1	12.6 ± 0.4	13.1 ± 0.4
CMA	20.5 ± 0.2	16.2 ± 0.7	5.2 ± 0.2	3.7 ± 0.2	10.7 ± 0.3	10.8 ± 0.2

Table 3.2 Colony extension rates (mm radial extension 24 h^{-1}) of *P. mycoparasiticum* (isolate AR7A), *P. scleroteichum* and *P. vexans* on PDA and SSA at 25°C ; means ± s.e. for 4 replicates.

	PDA	SSA
P. mycoparasiticum	7.2 ± 0.1	13.5 ± 0.2
P. scleroteichum	18.9 ± 0.4	18.1 ± 0.5
P. vexans	12.0 ± 0.3	12.9 ± 0.1

oligandrum and P. mycoparasiticum. However, colony extension growth by P. nunn was ≡ on SSA and on PDA, the rates of c 12-13 mm 24 h^{-1} seeming to be near-maximum for the temperature used. P. oligandrum behaved similarly in this respect, with near maximum extension rates of c 25-30 mm 24 h^{-1} on SSA or PDA, but P. mycoparasiticum showed a pronounced and significantly greater extension rate on SSA than on any other medium, the near-maximum rate being c 13.5 mm 24 h^{-1}.

For comparative purposes, colony extension by P. mycoparasiticum (isolate AR7A) was compared with that by the two species to which Pythium SWO had been assigned - P. scleroteichum (CBS 294.37) and P. vexans (CBS 270.68) on both PDA and SSA (Table 3.2).

As seen from these results, the growth of neither P. scleroteichum nor P. vexans was significantly affected by the medium used, whereas P. mycoparasiticum grew markedly better on SSA than on PDA. This experiment was done as part of that described in Table 3.1, accounting for the identical value for P. mycoparasiticum.

The cardinal temperatures for growth of P. mycoparasiticum were investigated on plates of PDA incubated at 2°C intervals, the minimum temperature for growth being 6°C and the maximum 30°C. The maximum growth rate was recorded at 22°C. These figures compare with 7°C minimum, 37°C maximum, 30°C optimum for P. oligandrum (Van der Plaats-Nitirink, 1981) and 7°C minimum, 42°C maximum and 34°C optimum for P. nunn (Lifshitz, Stanghellini & Baker, 1984).

3.3.2 Nitrogen and vitamin requirements

Tests on the nitrogen and vitamin requirements of P. oligandrum, P. nunn and P. mycoparasiticum were made by the method of Foley & Deacon (1986a). The fungi were inoculated into medical flats (100 ml capacity) containing 10 ml nitrogen-free mineral nutrient solution (Section 2.1)

supplemented with either $NaNO_3$ (2.0 g l^{-1}) or D,L-asparagine (1.51 g l^{-1}) with or without thiamine hydrochloride (100 µg l^{-1}). The phytopathogen P. aphanidermatum, was used for comparison. It was intended that growth would be assessed as dry mycelial weights, but in repeated tests the weights were very low and variable despite the observed extensive growth by some species in some conditions. This problem had previously been found by Foley (1983) and could not be overcome. Instead, the diameters of the colonies along the lengths of the flats, and estimates of density were used as the criteria of growth (Table 3.3).

P. aphanidermatum grew well with either nitrogen source, and to a similar degree in the presence or absence of thiamine, confirming an earlier report (Foley & Deacon, 1986a) of its ability to utilise nitrate as sole nitrogen source and its self-sufficiency for thiamine. P. oligandrum and P. mycoparasiticum did not grow in the presence of nitrate and grew only poorly in the presence of organic nitrogen, whether or not this was supplemented with thiamine. There was, however, an indication of better growth by these fungi in the presence than in the absence of thiamine, consistent with the report that P. oligandrum and P. mycoparasiticum require both organic nitrogen and thiamine for growth (Foley & Deacon, 1986a). P. nunn was able to grow on both nitrogen sources with or without the addition of thiamine, although the colonies were denser in the presence of thiamine. P. nunn grew equally well in all media but again the colonies were denser in the presence than in the absence of thiamine. All these findings were confirmed in repeated tests. They were always complicated by the poor growth of mycoparasites in defined liquid media and by the possibility of nutrient carry-over from the PDA inoculum discs, which had to be used in order to ensure even the small degree of growth that was observed. Nevertheless,

Table 3.3 Colony lengths and densities (in parentheses) of four Pythium species after 14 days in liquid medium containing different nitrogen sources in the presence or absence of thiamine; means (± se) of 4 replicates

	Nitrate		Asparagine	
	No thiamine	+ Thiamine	No thiamine	+ Thiamine
P. aphanidermatum	7.4 ± 0.7 (+++)*	7.0 ± 0.1 (+++)	6.6 ± 0.3 (+++)	7.8 ± 0.6 (+++)
P. oligandrum (CGH)	0.0 (+)	0.0 (+)	3.6 ± 0.3 (+)	4.1 ± 0.2 (+)
P. mycoparasiticum (AR7A)	0.0 (+)	0.0 (+)	1.3 ± 0.2 (+)	1.9 ± 0.4 (+)
P. nunn 20692	3.4 ± 0.2 (++)	3.8 ± 0.3 (+++)	3.5 ± 0.3 (++)	3.8 ± 0.2 (+++)
P. nunn 20693	3.2 ± 0.3 (++)	3.6 ± 0.4 (+++)	3.0 ± 0.4 (++)	3.6 ± 0.2 (+++)

* +, diffuse colony; ++, intermediate colony; +++, dense colony

the results indicated clearly that P. nunn was unique among the mycoparasitic Pythium spp in being the only one to utilise nitrate as sole nitrogen source; the other four species - P. oligandrum, P. acanthicum, P. periplocum and P. mycoparasiticum - cannot do so (Table 3.3; Foley & Deacon, 1986a).

3.3.3 Growth and oospore production in non-defined liquid media

For comparison with the work on defined liquid media, and in an attempt to produce oospores for further work, the mycoparasites were grown in Roux bottles (1 l capacity) containing 100 ml of either carrot extract (Section 2.1) or 1% molasses. The use of carrot extract followed the report by Walther & Gindrat (1987b) of its ability to support the production of oospores by P. oligandrum. The fungi were inoculated, as discs from the margins of colonies on PDA, on one of the broad sides of the flats, which were then laid on this side and incubated for 21 d at 20°C. Mycelial dry weights of the colonies were determined by filtering the flask contents under vacuum through weighed filter papers, and washing the mycelial mats retained on the filters. These were then dried to constant weight in an oven at 80°C.

As shown in Table 3.4, the mycelial dry weights for all species were approximately twice as large on carrot extract compared with on 1% molasses, but all of the fungi grew substantially better compared with on the defined media used earlier. Scrapings from the dried filter papers also revealed that abundant oogonia had been produced by P. oligandrum and P. mycoparasiticum - more so on carrot extract than molasses - but P. nunn failed to produce oospores on either medium. Lifshitz, Stangellini & Baker (1984) had earlier reported that P. nunn does not readily form oospores on a range of media that support their production by other Pythium spp, although it did so on rolled oat agar

Table 3.4 Mycelial oven-dry weights (mg) of mycoparasitic *Pythium* spp after 21 days in liquid media prepared from molasses or carrot extract; means (± se) of 3 replicates

	Carrot extract	Molasses (1%)
P. oligandrum (CGH)	116 ± 21	51 ± 11
P. mycoparasiticum (AR7A)	101 ± 18	41 ± 12
P. nunn (20693)	132 ± 11	63 ± 8

supplemented with dry bean leaves.

3.3.4 Germination of oospores

As described above and in Section 2.3.2, oospore-rich colonies of P. oligandrum and P. mycoparasiticum were prepared on carrot extract medium. After 21 days' growth the mycelia were removed from the medium by filtration through membrane filters, rinsed twice in sterile distilled water (SDW), then left for 7 days at room temperature in flasks containing 100 ml SDW. The colonies were then harvested onto cellulose acetate filters, rinsed twice with 50 ml SDW under vacuum, resuspended in 50 ml SDW and homogenized at 13,500 rpm for 120 sec on an Ultra-turrax T25 blender. The resulting suspension was bench centrifuged at 36,000 rpm for 15 sec, the supernatant was discarded and the pellet was resuspended in SDW to achieve an oospore concentration of 10^5 spores ml^{-1} (Section 2.3.2). Although all these procedures were done in clean conditions it was not feasible to maintain sterility. Subsequently, the final preparations contained few mycelial fragments.

In initial tests, several isolates of P. oligandrum and isolate AR7A of P. mycoparasiticum were used. To assess the germination of their oospores, aliquots of oospore suspension were mixed with an equal volume of SDW and 100 µl of the resulting suspension was placed on each of several clean microscope slides which were then placed in a moist chamber and incubated for 18 h at 25°C (Section 2.3.2). Isolate 4410b of P. oligandrum achieved up to 8% germination in these conditions, compared with less than 0.5% for all other isolates, so it was used in preference to the other isolates in the following experiments. It is notable that a small number of zoospores were observed on some slides incubated as above, but only with oospores of isolate 4410b of P. oligandrum and not with any other isolate of this species, nor with P.

mycoparasiticum (AR7A). The zoospores are assumed to have been formed following germination of the oospores, as reported by Drechsler (1946) after oospores of the fungus had been aged by storage.

3.3.4.1 Effects of environment on oospore germination by P. oligandrum

The effects of aeration and light on oospore germination were studied, using spore suspensions in water or supplemented with 0.5% (final concentration) bacteriological peptone. The oospore suspensions, as above, were incubated on microscope slides at 25°C. Differences in aeration were achieved by placing a coverslip on some of the slides prior to incubation, while leaving others exposed to air in the moist chambers. Some of these chambers were incubated in light, others in the dark. Assessments of germination were made 18 h later by examining at least 130 oospores on each of 5 replicate slides. As shown in Table 3.5, for oospores kept in darkness, germination was suppressed in the presence of a coverslip, whereas at least some oospores germinated in uncovered preparations. Furthermore, the presence of bacteriological peptone significantly enhanced the amount of germination over that in SDW, although it did not induce germination on the slides covered by cover-slips.

In similar experimental conditions, in a further experiment, exposure to light completely suppressed germination by "aerated" oospores in the presence of water or peptone (Table 3.6).

3.3.4.2 Effects of nutrients on oospore germination by P. oligandrum

Following the results of earlier experiments, all further work was done with oospores incubated uncovered and in darkness. The effects of two nutrient sources on germination were studied in these conditions by supplementing oospore suspensions with either bacteriological peptone or

Table 3.5 Effect of aeration and the presence of nutrients on germination of oospores of P. oligandrum in darkness

	% Germination*
Suspension covered	0.0
Suspension covered, bacteriological peptone 0.5%	0.0
Suspension exposed	8.6 ± 0.3
Suspension exposed, bacteriological peptone 0.5%	37.4 ± 1.7

* Means ± se for 5 replicate slides after 18 h at 25°C

Table 3.6 Effect of light and the presence of nutrients on germination of oospores of P. oligandrum

	% Germination*
In light	0.0
In light, 0.5% peptone	0.0
In dark	8.8 ± 0.3
In dark, 0.5% peptone	30.4 ± 0.9

* Means ± se for 5 replicate slides after 18 h at 25°C

malt extract. These were selected to represent, essentially, mixtures of amino acids and sugars respectively. The nutrients were added at a series of concentrations up to 1% (final concentration), the final oospore concentration in all cases being 5×10^4 oospores ml^{-1}.

As shown in Table 3.7, both nutrient sources stimulated germination relative to that in the distilled water control but at any given concentration bacteriological peptone was superior to malt extract. The highest recorded germination was c 39% but examination of the oospores showed no obvious difference, such as in thickness of the oospore wall, between spores that had, and those that had not germinated. For both nutrient sources the highest percentage germination occurred at the high nutrient concentrations, although bacteriological peptone caused a significant ($P = 0.001$) stimulation at even 0.01% concentration whereas malt extract caused no significant increase in germination, relative to that in distilled water, at 0.1% concentration. In this and all other experiments a few zoospores were occasionally seen in preparations containing distilled water, but never in suspensions supplemented with nutrients.

3.3.4.3 Effects of volatile compounds on oospore germination by *P. oligandrum*

The effects of two volatile compounds on oospore germination were investigated using spore suspension mixed with either acetaldehyde or ethanol. These compounds were selected for study because they are commonly released from germinating seeds (Bewley & Black, 1983) and have previously been investigated for their effects on germination of sporangia of *Pythium* spp (Nelson, 1987). A series of final concentrations from 25 mM to 0.0025 mM were used for acetaldehyde and from 50% to 0.005% for ethanol.

As shown in Tables 3.8 and 3.9, both compounds inhibited germin-

Table 3.7 Effect of nutrients on the germination of oospores of *P. oligandrum*

Concentration (%)	% Germination	
	Bacteriological peptone	Malt extract
0 (distilled water)	8.6 ± 0.3	7.9 ± 0.5
1	37.4 ± 0.6	16.6 ± 1.0
0.5	39.1 ± 1.3	18.9 ± 0.5
0.1	30.4 ± 0.9	9.2 ± 0.3
0.01	22.5 ± 1.6	-*
0.001	9.9 ± 0.3	-

* Not tested

(Means ± se for 5 replicate slides after 18 h at 25°C)

Table 3.8 Effect of acetaldehyde on the germination of oospores of *P. oligandrum*

Concentration (mM)	% Germination
0 (distilled water)	11.1 ± 0.6
25	1.1 ± 0.2
12.5	4.4 ± 0.5
2.5	9.4 ± 0.5
0.5	9.3 ± 0.4
0.25	27.0 ± 1.1
0.025	9.2 ± 0.6
0.0025	9.4 ± 0.5

* Means ± se for 5 replicate slides after 18 h at 25°C

Table 3.9 Effect of ethanol on the germination of oospores of *P. oligandrum*

Concentration (%)	% Germination
0 (distilled water)	8.7 ± 0.2
50	0.0
5	2.4 ± 0.3
0.5	11.3 ± 0.4
0.05	8.4 ± 0.2

* Means ± se for 5 replicate slides after 18 h at 25°C

ation, relative to that in distilled water, at high concentrations. At lower concentrations, however, they either had no influence on germination or, for acetaldehyde at 0.25 mM, caused a significant stimulation of germination. In this last respect it is notable that acetaldehyde was markedly stimulatory ($P = 0.001$) at 0.25 mM but had no stimulatory effect at 0.5 mM, which was below the inhibitory concentration. There was an indication that ethanol was stimulatory at 0.5% concentration, and it is possible that at a slightly higher or lower concentration (but below the inhibitory 5.0% level) it might have had a larger effect. However, further experiments were confined to an investigation of acetaldehyde.

3.3.4.4 Comparison of the effects of nutrients and acetaldehyde on oospore germination by P. oligandrum

The effect of acetaldehyde at the most active concentration found previously was comparable with that of both malt extract and bacteriological peptone at their maximum stimulatory concentrations. These soluble nutrient sources were then used in combination with acetaldehyde, in an attempt to see if the total percentage of oospore germination could be enhanced.

The results (Table 3.10) showed that all these treatments significantly increased the amount of germination relative to that in distilled water controls, but the effects of the treatments did not differ markedly from one another. From these results it seems that only some 30-40% of oospores could be induced to germinate by any or all of these compounds, and the evidence strongly suggests that the same component of the oospore population is inducible by any of these treatments.

Table 3.10 Effects of nutrients, acetaldehyde and their combination on the germination of oospores of P. oligandrum

	% Germination
Control (SDW)	10.3 ± 0.5
0.5% bacteriological peptone (BP)	30.5 ± 0.8
0.5% malt extract (ME)	25.9 ± 0.8
0.25 mM acetaldehyde	32.5 ± 0.4
0.5% BP, 0.25 mM acetaldehyde	34.3 ± 1.0
0.5% ME, 0.25 mM acetaldehyde	25.7 ± 0.9

* Means ± se for 5 replicate slides after 18 h at 25°C

Table 3.11 Effect of culture duration on the germination of oospores of P. oligandrum

	% Germination in 0.5% bacteriological peptone	
Culture duration (days)	Direct harvest	Harvest after 7 days in SDW
7	18.0 ± 0.6	40.8 ± 0.7
14	40.5 ± 1.9	46.2 ± 1.4
21	48.1 ± 1.2	41.5 ± 1.8
28	49.1 ± 0.9	46.0 ± 1.6
35	23.6 ± 0.6	27.1 ± 1.7
42	12.9 ± 1.2	13.0 ± 0.6

* Means ± se for 5 replicate slides after 18 h at 25°C

3.3.4.5 Effect of culturing and harvesting conditions on oospore germination

A number of cultures of P. oligandrum isolate 4410b were prepared in 10 ml carrot extract in medical flats (Section 2.3.2). They were harvested, two at a time, at weekly intervals. One culture of the pair was used immediately to prepare an oospore suspension; the other mycelial mat was rinsed and reincubated in SDW for a further 7 days before being used to prepare an oospore suspension. In all cases the oospores were resuspended at a lower concentration (5×10^4 spores ml^{-1}) than in the previous experiments. Counts with a haemacytometer (not presented) showed that the number of oospores produced did not differ significantly between the shortest and longest culture durations, suggesting that most, if not all, oospore production occurred within the first 7 days.

The final oospore suspensions were supplemented with 1% bacteriological peptone (final concentration 0.5%) to stimulate germination, and incubated for 18 h on slides as in previous experiments.

As shown in Table 3.11, the percentage germination of oospores from 7 day cultures was significantly ($P=0.001$) lower if the spores had been "directly" harvested rather than reincubated in water for a further 7 d. However, there was no such difference between the "direct" and "reincubated" populations at the subsequent harvest times. Further, there was a clear trend towards a reduction in oospore germination as the period of culture in carrot extract was extended beyond 28 days, and a marked reduction in oospore germination from the oldest (42 day) cultures.

It was notable in further tests that oospores from 7 day "directly harvested" cultures did not increase their germinability if they were stored as oospore suspensions in distilled water. In other words, the beneficial effect of 7 days' incubation in distilled water shown for 7-

day mycelia in Table 3.11 applied only if the mycelia were intact and not if the mycelia had been macerated. The implication of this is that the transfer of mycelia to distilled water after 7 d enables oospores to mature or to gain further nutrients from the nutrient starved mycelium and thereby increases the ability of the oospores to germinate when induced to do so by appropriate compounds.

3.4 Plant pathogenicity tests

In order to determine the phytopathogenicity of P. oligandrum (isolate CGH), P. mycoparasiticum (isolates AR5A and AR7A) and P. nunn (isolate 20693) in comparison with a phytopathogen, P. aphanidermatum (CBS 634.70), strips from the edges of colonies of these fungi on PDA were placed on a layer of vermiculite in trays (see Section 2.3.3). Fifty seeds of wheat (Triticum aestivum, cv Avalon), pea (Pisum sativum, cv Waverley), Brussels sprout (Brassica campestris, cv Roodnerf) and spring onion (Allium cepa, cultivar unknown) surface sterilised in mercuric chloride (see Section 2.3.3), were pressed in the agar strips which were then covered with an appropriate layer of vermiculite for each type of seed and the trays were then watered and placed in a glasshouse at 20°C in natural day/night regimes. Seedling emergence was monitored daily until no further emergence was expected (2 wk). The seeds in the agar strips were excavated and the healthy, emerged seedlings were counted.

As shown in Table 3.12, P. aphanidermatum caused a severe reduction in emergence of all four plant species, whereas high rates of germination were found in the uncolonized agar controls. All of the mycoparasites had no significant effect on seedling emergence. The resulting seedlings were as healthy as those in the controls when their roots were excavated and examined microscopically.

Table 3.12 Number of seedlings that emerged (max 50) when sown over uncolonized agar or agar colonized by different Pythium spp in trays of vermiculite

Inoculum	Wheat	Pea	Brussels sprout	Spring onion
None (agar control)	50	47	44	47
P. oligandrum	45	46	44	47
P. nunn	48	45	41	43
P. mycoparasiticum AR7A	48	47	46	47
P. mycoparasiticum AR5A	46	47	42	47
P. aphanidermatum	12	0	0	28

3.5 Taxonomy of P. mycoparasiticum

When grown on rich media such as CMA, SSA or CA, colonies of P. mycoparasiticum are thin, submerged or grow on the surface with no aerial mycelia. On PDA, however, the colonies are flat producing no aerial hyphae, and have a mealy appearance. The diameters of the hyphae vary, main hyphae being up to 5 µm wide with many short branches that are sometimes convoluted, of irregular width and branch repeatedly forming fascicles. Sporangia were not observed on any medium and were not produced when colonized grass blades were immersed in buffered pond water (Mitchell & Deacon, 1985) or distilled water. Also, in limited tests, P. mycoparasiticum was not found to release zoospores from germinated oospores in conditions in which P. oligandrum occasionally did so (Section 3.3.4). Oogonia and antheridia were produced only on media containing sterols or on PDA plates precolonized by the susceptible host fungus, Phialophora sp. Their features and many other aspects described below were best observed by projecting microscope images onto a video screen. Drawings were also made by tracing from the screen. The oogonia were smooth-walled, generally globose but occasionally subglobose and produced terminally on usually short side branches of the main hyphae but occasionally on longer hyphae. The diameter of oogonia varied from 16 to 28 µm but usually was between 18 and 25 µm. The oogonia were heavily invested by antheridia and antheridial branches (Fig 3.1, Plates 3.1 to 3.13), the origins of which were difficult to discern when the oospores were mature, but in younger material the antheridia were clearly seen to arise from hyphae of different origins, not closely related to the oogonial hyphae in most cases. On this basis the antheridia are characterized as diclinous, but were occasionally monoclinous. Each oogonium bore between one and six antheridia but generally from two to four. The antheridia were clavate or distinctly

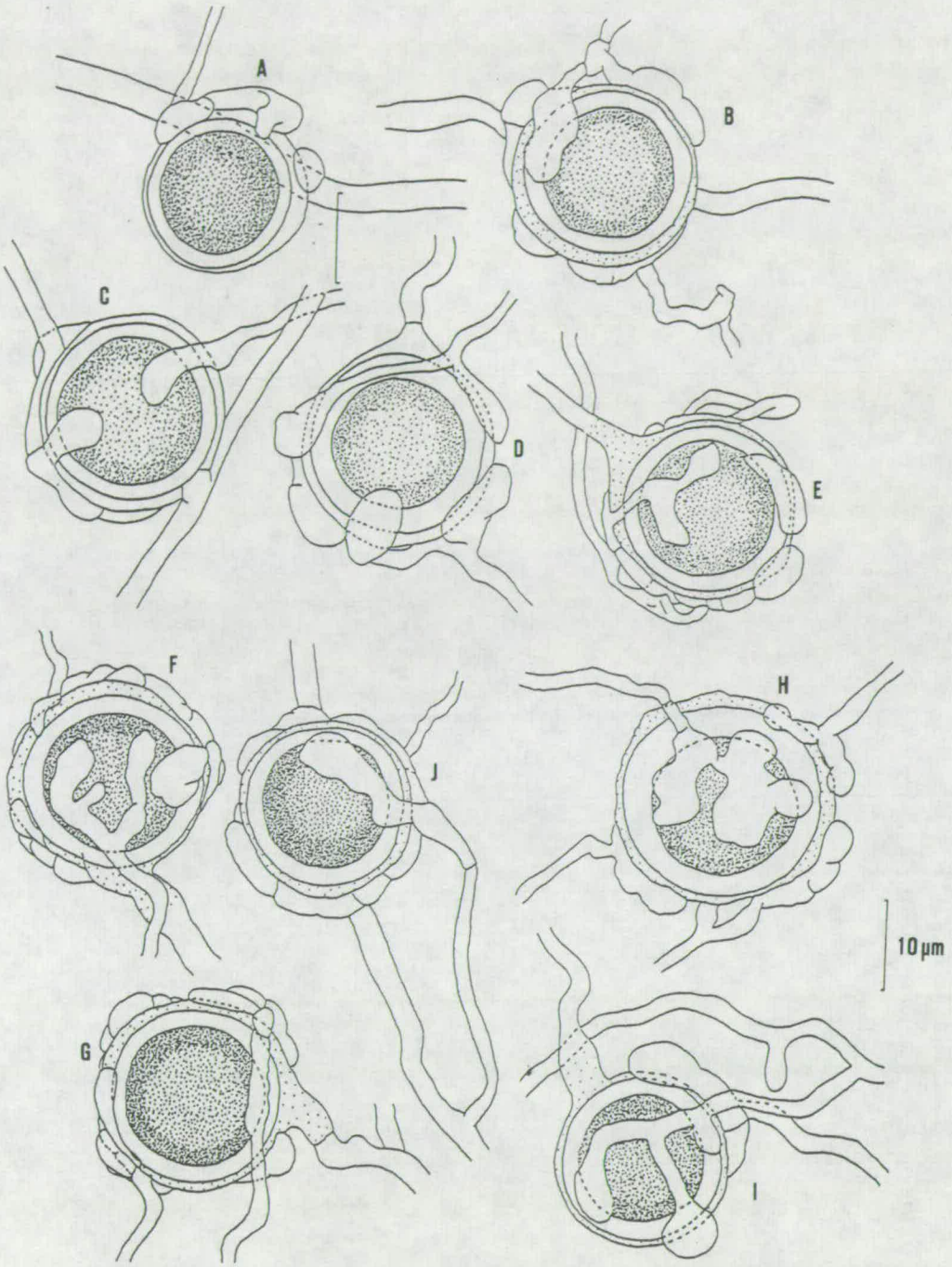


Fig 3.1 Sexual apparatus of Pythium mycoparasiticum developed in plate cultures of carrot agar.

A-I Mature oogonia: all diclinous

J Mature oogonium: monoclinous

Plates 3.1 -3.13 Sexual apparatus of Pythium mycoparasiticum developed in plate cultures of carrot agar; photographed with a Leitz Orthomat fully automatic microscope camera at a uniform magnification. Bar represents 10 µm.

Plates 3.1 -3.4 Photographs of oogonial structure in different planes of focus

Plate 3.1 Focus to show lobed antheridium (arrowed).

Plate 3.2 Focus to show antheridial hypha encompassing oogonium.

Plate 3.3 Focus to show antheridial structures adpressed to the oogonial wall.

Plate 3.4 Focus to show antheridial structures and thick oospore wall.

Plates 3.5 -3.11 Photographs of different oogonia showing antheridial hyphae around the oogonium. Note distinct reserve globule in Plate 3.9 (arrowed).

Plates 3.12 -3.13 Preparations stained with cotton blue in lactophenol.

Plate 3.12 Photograph showing stained antheridial structures surrounding an oogonium.

Plate 3.13 Photograph showing oogonial hypha and attachment (arrowed) and antheridial hypha tightly adpressed to about a third of the circumference of the oogonium. Note also thick oospore wall and reserve globule.

Plate 3.1

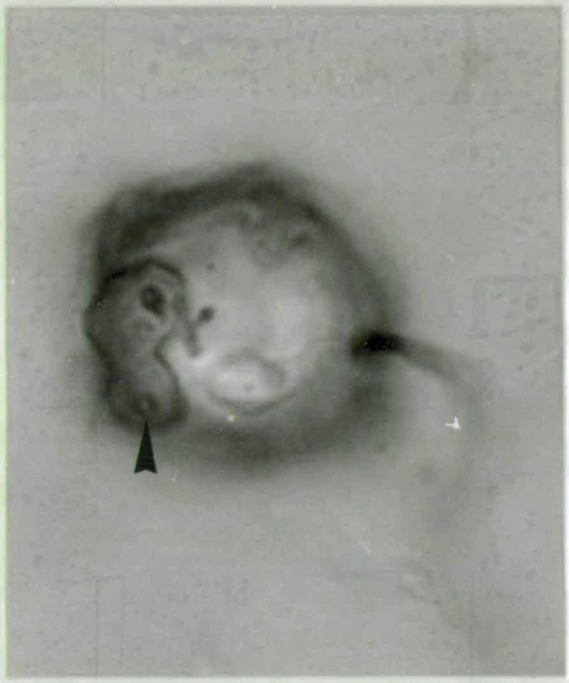


Plate 3.2

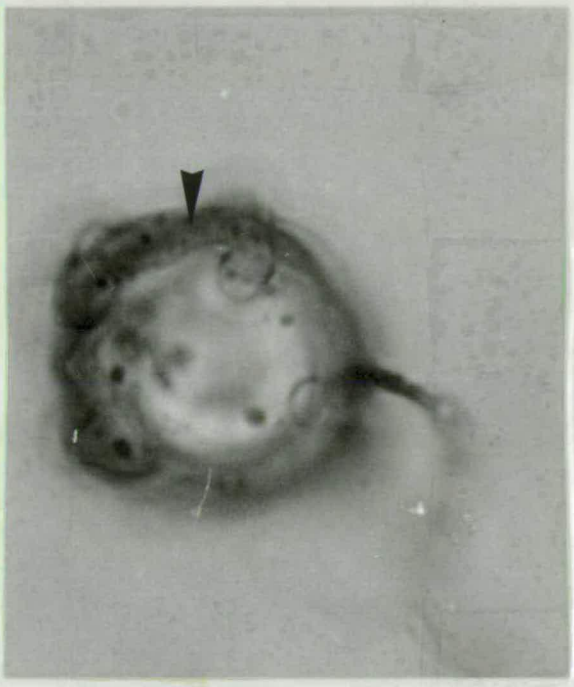


Plate 3.3

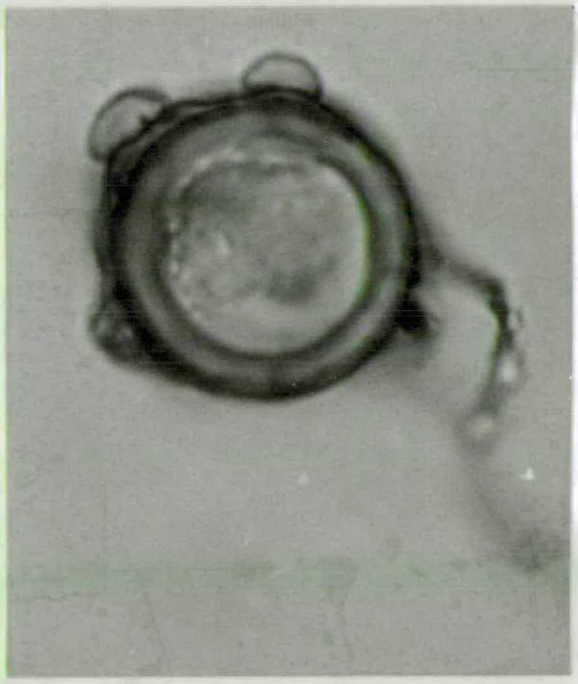


Plate 3.4

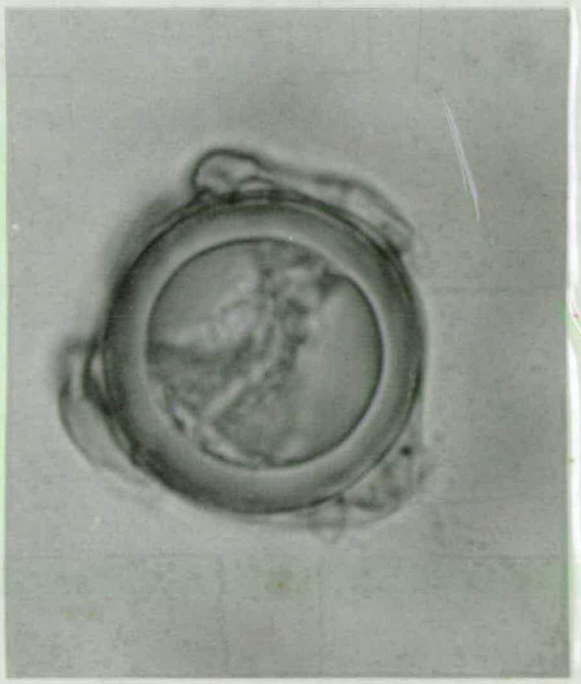


Plate 3.5

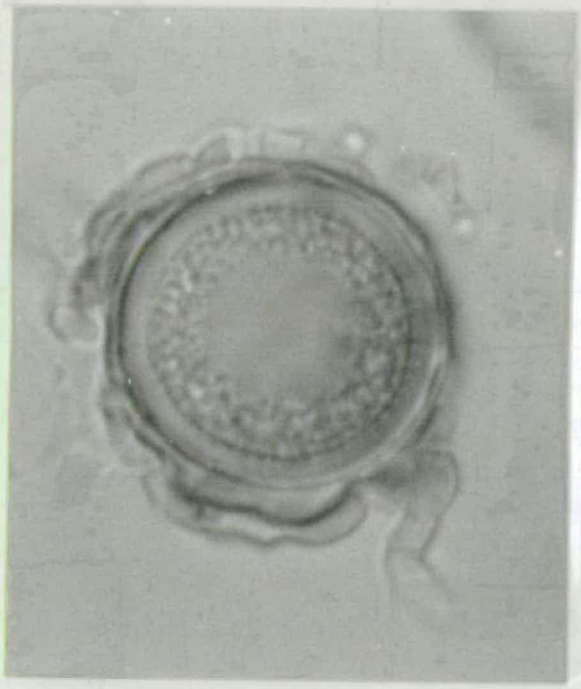


Plate 3.6

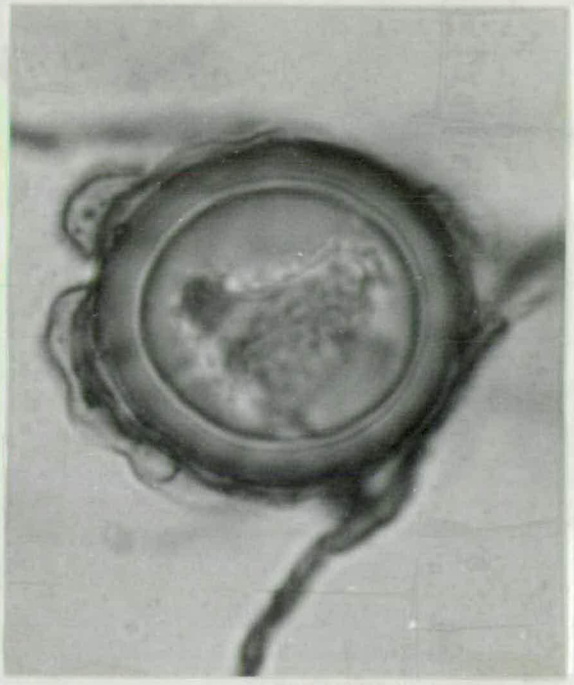


Plate 3.7

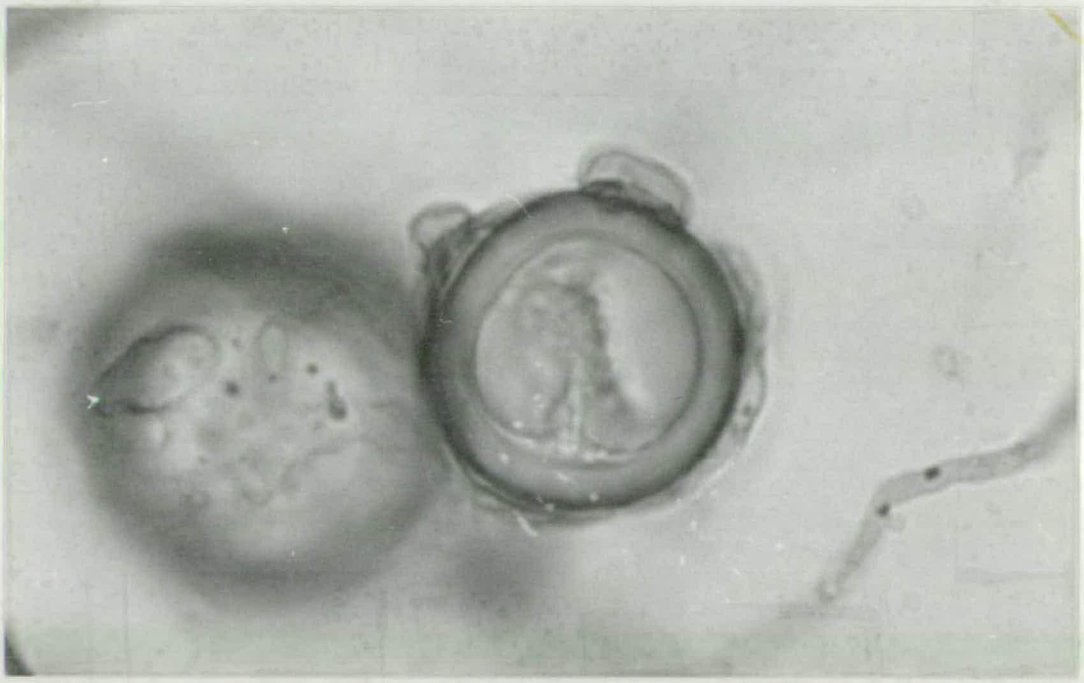


Plate 3.8

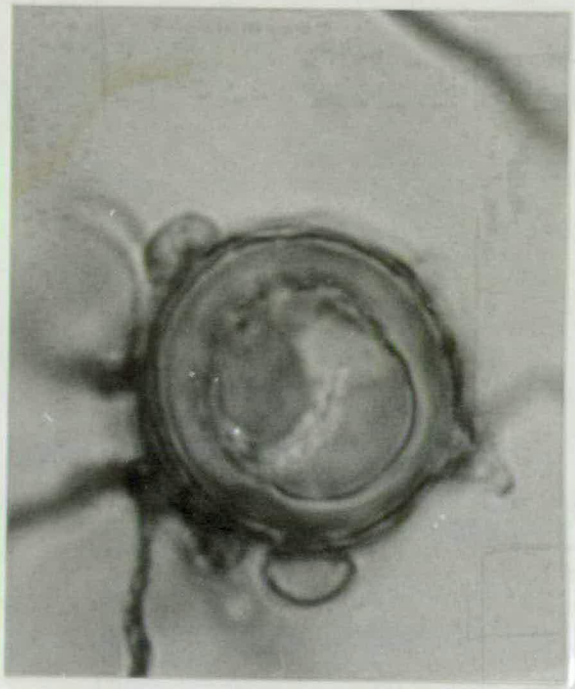


Plate 3.9

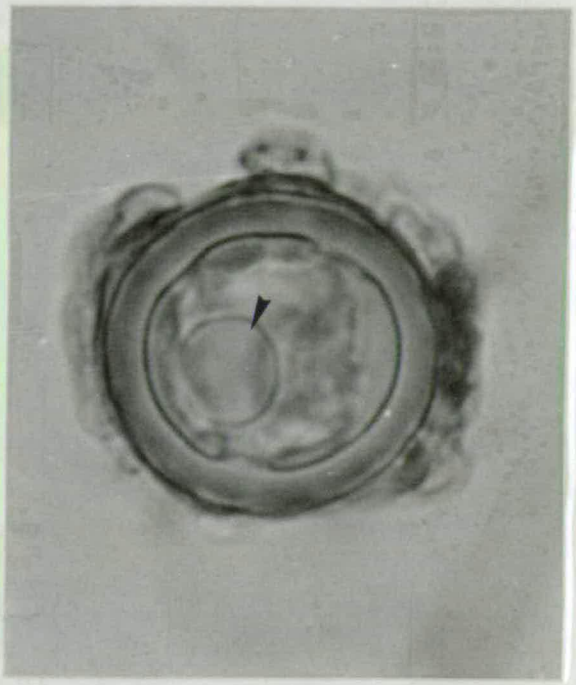


Plate 3.10

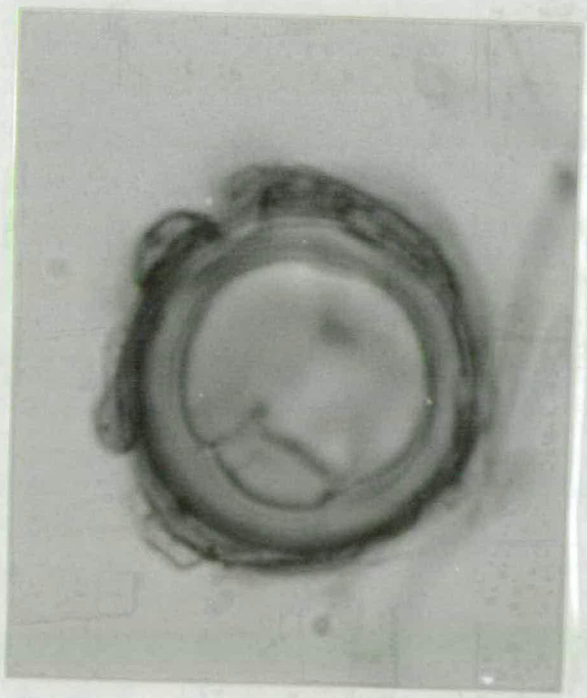


Plate 3.11

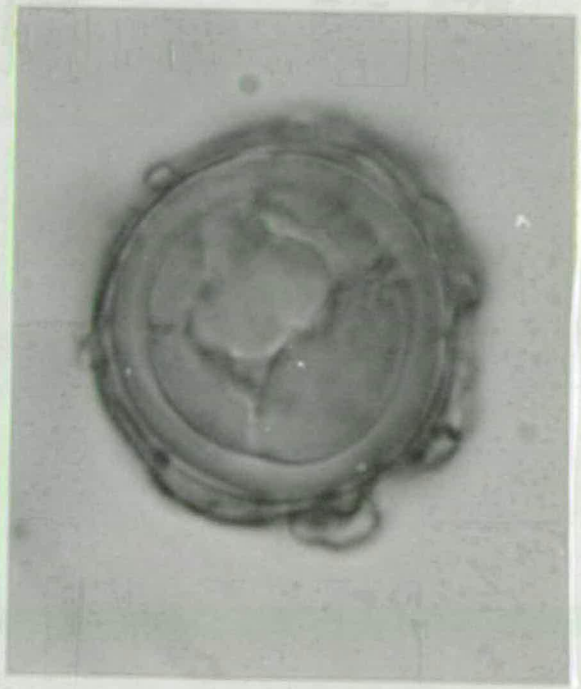


Plate 3.12

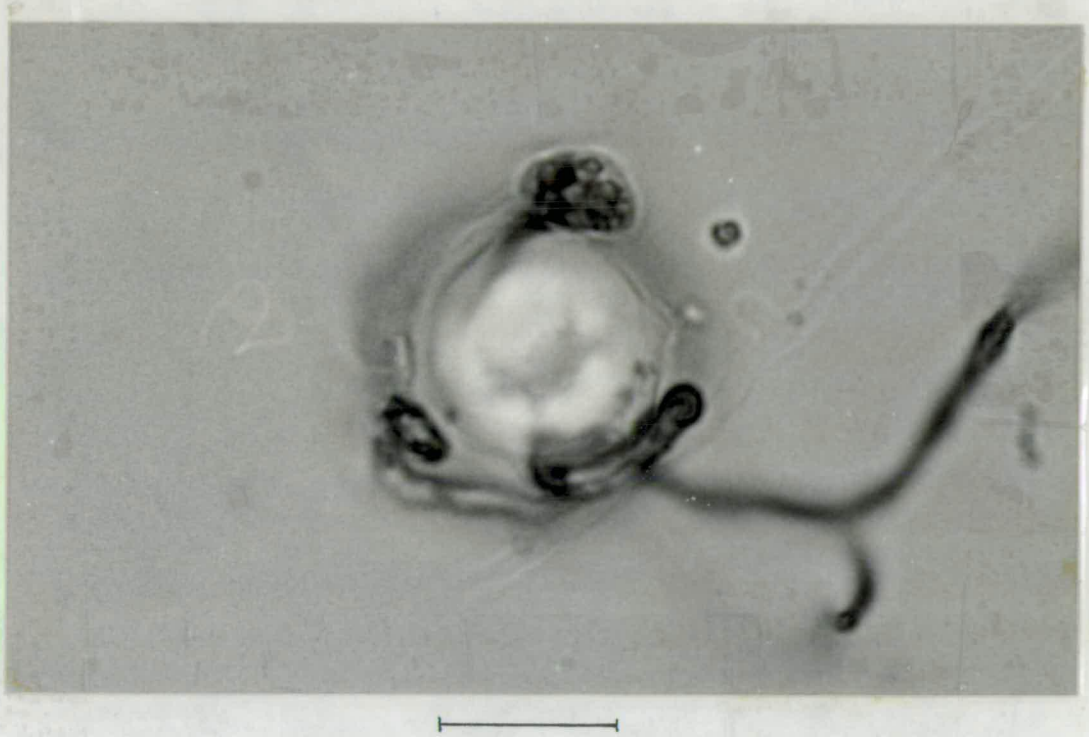
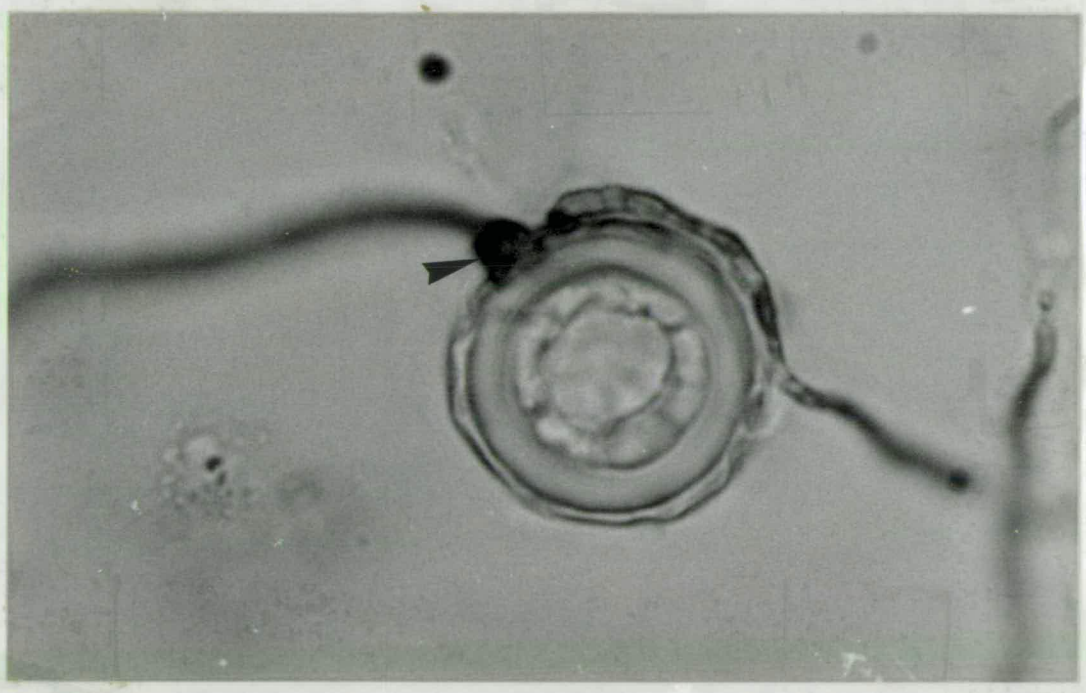


Plate 3.13



lobed and not clearly delimited from the supporting hyphae; they were broadly adpressed to the oogonium (Fig 3.1, Plates 3.1 to 3.13). The antheridia arose singly or in multiples from branched hyphae that extensively and intimately invested the oogonia and that had irregular, furrowed contours. The antheridial cells were between 6 and 12.5 µm long, though were generally between 7 and 10 µm, and they varied in width between 4 and 6 µm at the broadest point. Oospores were spherical to sub-spherical in shape and yellowish in colour as the cultures aged. They had a smooth appearance and were aplerotic, though they often nearly filled the oogonium. The oospores varied between 15 and 25 µm diameter, though generally they were between 18 and 23 µm. They had characteristically thick walls between 1.1 and 3.5 µm thick, though usually 1.7 to 2.7 µm thick (Fig 3.1, Plates 3.1 to 3.13).

As this combination of features is not shared by any previously described species of Pythium, the fungus merits description as a new species, as follows.

3.6 Formal description of Pythium mycoparasiticum

Hyphae principales 3-5 µm latae. Sporangia ignota. Oogonia diametro (16-)-18-25-(-28) µm, laevia, globosa vel subglobosa, ramos breves hypharum interdum hyphas longiores terminantia. Parietes oogoniorum tenues, 0.5-1 µm in crassitiei. Antheridia in quoque oogonio (1-)-2-4(-6); (6-)-7-10-(12.5) µm longa, 4-6 µm lata; clavulata vel distincte lobata, ad oogonia insigniter et late adpressa. Hyphae antheridiferae longae, saepe ramificantes, irregulariter constrictae, oogonia involvens, plerumque exorientes uno vel plus quam uno filo myceliali, omnis filum separatem stipite oogonii (antheridia sic diclina), aliquando monoclina. Oosporae diametro (15-)-18-23-(25) µm, sphaericae, aperoticae, laeves, parietes notabiliter crassi, (1-)1.7-

2.7(-3.5) µm in crassitiei.

Ex solo agrario, Lasswade, Scotia, 1986.

Colonies on cornmeal, carrot or sunflower agar thin, submerged, or grow on the surface with no aerial mycelia. On potato dextrose agar, colonies are flat producing no aerial hypha and have a mealy appearance. Main hyphae up to 5 µm wide. Sporangia and zoospores unknown. Oogonia smooth, globose or sub-globose, terminal on short side branches or occasionally on long hyphae, rarely intercalary (16-) 18-25 (-28) µm diam. Antheridia 1-6 (mostly 2 to 4) per oogonium, clavate or distinctly lobed and not clearly delimited from supporting hyphae, broadly adpressed to the oogonium, arising singly or in multiples from branched hyphae that entangle the oogonium and have irregular, furrowed contours; antheridial cells (6-) 7-10 (-12.5) µm long and 4-6 µm at the broadest point, arising from one, two or more hyphae unconnected to the oogonial stalk, but occasionally from the oogonial stalk. Oospores spherical to sub-spherical, yellowish, smooth, aplerotic but nearly filling the oogonium (15-) 18-23 (-25) µm diam, with a wall (1.1-) 1.7-2.7 (-3.5) µm thick.

Cardinal temperatures: minimum 6°C, optimum 22°C, maximum 30°C. Daily radial growth on potato-dextrose agar at 25°C, 7.2 mm (16 mm on carrot agar).

Holotype: isolate AR7A, from soil near Lasswade, Scotland 17.xi. 1986.

P. mycoparasiticum (as Pythium SWO) is common in agricultural lands of moderate pH (5 to 7), having been isolated from 19 out of 51 such soils from sites ranging from the north of Scotland (Ross-shire and Angus) to the south of England (Herefordshire, Hampshire) (Foley & Deacon, 1985). It was not as common as P. oligandrum, isolated by the same technique (PDA plates pre-colonised by Phialophora sp (Deacon &

Henry, 1978)), but was probably out-competed by this faster growing species on the isolation plates. Both species have, however, been found in the same soil samples.

Deacon & Henry (1978) recorded that Pythium SWO did not rot cucumber fruits on wound inoculation. The holotype and another culture isolated in this study did rot reduce the emergence of wheat (Triticum aestivum), pea (Pisum sativum), Brussels sprout (Brassica campestris) or spring onion (Allium cepa) seedlings when seeds were sown over agar discs pre-colonised by the fungus (Section 3.4).

3.6.1 Comments on classification

Despite repeated attempts, the production of sporangia by this fungus has never been observed (this study; Foley & Deacon, 1986a). Although this could potentially raise objections for its inclusion in the genus Pythium which is, in part, characterized by the mode of zoospore release from sporangia, the fungus is in other respects typical of the genus Pythium, and there are precedents for including non-sporangial species in this genus. Such species include P. sclerotiechum, P. buismaniae van der Plaats-Niterink, P. artotorgus (Mont) de Bary, P. echinocarpon Ho & Tokunga and P. acanthophoron Sideris, all of which are accepted species of the genus (Middleton, 1943; Waterhouse, 1968; Van der Plaats-Niterink, 1981). The mycoparasite P. nunn produces hyphal swellings that resemble sporangia but have not been induced to release zoospores (Lifshitz, Stanghellini & Baker, 1984), placing this species in a similar category.

The distinctive morphological feature of P. mycoparasiticum is the conspicuous envelopment of the oogonium by antheridia and antheridial hyphae. Except in young material, the precise arrangements of these are difficult to discern. In this respect, P. mycoparasiticum closely

resembles P. scleroteichum, which also has a relatively slow growth rate, lacks sporangia and has non-echinulate oogonia. P. mycoparasiticum has, however, a thick oospore wall, which distinguishes it from P. scleroteichum on a morphological basis. The mycoparasitic habit of P. mycoparasiticum also is distinctive, as is its dependence on organic nitrogen and thiamine (or the pyrimidine moiety of thiamine) (this study; Foley & Deacon, 1986a, for Pythium SWO).

Thickness of the oospore wall is an accepted feature distinguishing species of Pythium (Middleton, 1943; Van der Plaats-Niterink, 1981). However, as it was the only clear morphological criterion by which P. mycoparasiticum could be distinguished from P. scleroteichum, special attention was paid to the differences between these species. P. scleroteichum is a little-known species isolated only from sweet potato (Ipomoea batatas) in the USA. A holotype was not cited in its original description (Drechsler, 1934) but it is evident that several isolates from different parts of the USA were examined. The oospore wall was recorded as being 0.8-1.4 (mostly 0.9-1.3) µm in thickness. The culture held at the Centraalbureau voor Schimmelcultures, cited as "Pythium scleroteichum Drechsler (CBS 294.37) fr root Ipomoea batatas, USA, comm C. Drechsler, 1937" was examined by Van der Plaats-Niterink (1981). She recorded the oospore wall thickness as 0.8-1.5 µm, and this has subsequently been confirmed in this laboratory. Therefore, the normal range of thickness of the oospore wall of P. mycoparasiticum is clearly different from that of P.scleroteichum.

There are, however, other non-morphological characteristics which separate P. mycoparasiticum and P. scleroteichum. Drechsler made no mention of mycoparasitism by P. scleroteichum in his original (1934) or subsequent (1940, 1946) papers in which he described and discussed mycoparasitic Pythium spp. Work by Foley (1983) confirmed that P.

scleroteichum (CBS 294.37) is not mycoparasitic. Isolate CBS 294.37 of P. scleroteichum also utilizes nitrate-nitrogen and is self-sufficient for thiamine (Foley & Deacon, 1986) and it is one of the most highly cellulolytic Pythium spp (Deacon, 1979). None of these features is showed by P. mycoparasiticum (Foley & Deacon, 1986a; Sections 4 and 5 of this thesis). Thus differentiation exists between P. mycoparasiticum and P. scleroteichum on behavioural and physiological as well as on morphological criteria. There seems no reasonable grounds for considering them as con-specific.

Although similar to P. nunn in mycoparasitic behaviour (Section 5) and in the production of non-echinulate oogonia, these species differ in morphology in that P. nunn lacks the conspicuous envelopment of the oogonium by antheridia or antheridial hyphae. Also, P. nunn can use nitrate as sole nitrogen source - the only mycoparasitic Pythium species that does (Section 3.3.2).

As described in Section 3.3.1, the colony growth rates of P. mycoparasiticum are of interest because this fungus grows at distinctly different rates on different media. Growth is stimulated on sunflower seed agar (SSA) and carrot agar (CA), compared with on potato-dextrose agar (PDA), cornmeal agar (CMA), water agar (WA) or various dilutions of PDA. In comparative tests the growth of two other slow growing species, P. scleroteichum (CBS 294.37) and P. vexans (CBS 270.38), was not stimulated by SSA (Section 3.3.1).

3.7 Discussion

Many of the experiments carried out in this section were designed to supplement the work of Deacon (1976), Deacon & Henry (1978) and Foley & Deacon (1986a) to ascertain if P. nunn showed the same physiological behaviour as reported for the other four mycoparasitic Pythium spp.

Also, it was relevant to compare the behaviour of isolates AR7A and AR5A (*P. mycoparasiticum*) with that previously reported for the isolates termed "possibly *P. scleroteichum*" (Deacon & Henry, 1978) and *Pythium* SWO (Foley & Deacon, 1986a), because all these were suspected to represent the same species. Finally, and in an unrelated context, a limited study was made of oospore production and germination by *P. oligandrum*, but time did not permit a more detailed study of this, nor a comparison of the mycoparasites in this respect.

P. nunn and *P. mycoparasiticum* are superficially similar, being relatively slow-growing and having smooth-walled oogonia. In both these features they differ from *P. oligandrum*, *P. acanthicum* and *P. periplocum*. However, *P. nunn* and *P. mycoparasiticum* differed from one another in several respects, quite apart from the conventional morphological criteria used to separate species of the genus. The most notable difference was that *P. nunn* could utilize nitrate as sole nitrogen source, whereas *P. mycoparasiticum* required organic nitrogen. Foley & Deacon (1986a) had previously found that *P. oligandrum*, *P. acanthicum*, *P. periplocum* and "*Pythium* SWO" require organic nitrogen. So *P. nunn* is unique among the mycoparasitic *Pythium* spp in utilising inorganic nitrogen. Thiamine requirements were more difficult to establish due to the poor growth of these fungi in defined liquid media, as previously found by Foley (1983), and the possibility that small amounts of thiamine might have been carried over in the inoculum discs. However *P. nunn* grew relatively better when thiamine was added to the medium, as did the other mycoparasites that were tested, indicating that it, too, might require thiamine or one of the moieties of thiamine. It was notable that all three mycoparasites, *P. oligandrum*, *P. mycoparasiticum* and *P. nunn*, produced much denser and larger colonies on carrot extract or 1% molasses medium than in defined liquid medium based on

glucose and mineral salts. In the non-defined media P. nunn produced the largest biomass but did not produce oospores, unlike P. oligandrum and P. mycoparasiticum. These points may be related, because oospore production presumably is associated with autolysis of the mycelium and conversion of its resources into oospore reserves, as found in a different context by Christias & Lockwood (1973) for conversion of mycelial biomass into sclerotia of several fungi. But direct comparisons of mycelial dry weights by different Pythium spp should be made with caution, because Foley (1983) found a considerable fall in mycelial biomass of P. ultimum on prolonged culture in liquid media. The failure of P. nunn to produce oospores in the liquid media here is consistent with the report by Lifshitz, Stanghellini & Baker (1984) that P. nunn forms sexual stages only in a few conditions, most notably on Lima bean-based media.

Another notable difference between P. mycoparasiticum and P. nunn was in their linear growth rates on different agar media. The colony extension rate of P. mycoparasiticum was greatly increased relative to that on PDA, on sunflower seed agar or carrot agar, whereas P. nunn grew similarly on any medium used. Foley & Deacon (1986a) previously reported that a constituent of commercial potato extract is partly inhibitory to the growth of P. oligandrum and Pythium SWO. P. nunn does not seem to be sensitive to this inhibitor. However, in the experiments here P. mycoparasiticum did not respond markedly to dilution of PDA. Instead, it seemed to require a constituent of sunflower seed agar and carrot agar for maximum extension growth. Some care is needed in interpreting such effects, because all of the fungi produced thin colonies, without aerial mycelia, on such media (unlike PDA), so the linear extension rates of their colonies were not necessarily a reflection of enhanced growth (ie biomass production) per se. Nevertheless, it is possible

that sterols in these media promoted growth (and thus enabled oospore production). In this respect Child et al. (1969) reported that some sterols stimulate the growth of another mycoparasite, P. acanthicum, and also extend the range of carbon sources that this fungus can use in their presence.

The availability of cultures of P. nunn for comparative work revealed that P. mycoparasiticum is a taxonomically distinct species and it will be described as such (paper in preparation). In morphological characteristics it is most similar to P. scleroteichum but differs from this in thickness of the oospore wall. Supporting its separation as a distinct species is its range of physiological characteristics described and discussed earlier in this section. That there are now five distinct mycoparasitic species of Pythium is interesting and lends weight to the comment by Hendrix & Campbell (1973) that the "traditional" view of Pythium spp as seedling pathogens is much too restrictive. Three of these mycoparasitic species were compared by Deacon & Henry (1978), but these three species - P. acanthicum, P. oligandrum and P. periplocum - are rather similar to one another and Hendrix & Campbell (1973) considered that, for most purposes, they could be regarded as a species complex, or species grouping. By this, they perhaps meant that it serves little purpose to distinguish them taxonomically. The more recent discovery of two further mycoparasitic species (P. nunn and P. mycoparasiticum) that are conspicuously different from the others in physiology and/or morphology raises interesting issues about the origin of mycoparasitism in the genus and justifies the work described later, where the mycoparasitic behaviour of these fungi is compared.

The initial results for oospore germination by P. oligandrum confirmed the findings of Walther & Gindrat (1987b) that different isolates show different inherent germination rates under identical

culture conditions. Only one isolate (4410b) of P. oligandrum displayed "base" germination rates approaching 10% and so was selected for further study. Germination by its oospores was completely suppressed in the presence of light or when spore suspensions were kept beneath a coverslip, presumably because oxygen was limiting, although this assumption was not tested experimentally. Even the presence of a nutrient source that could stimulate germination was ineffective in covered suspensions. Germination was enhanced more by the presence of a mixture of amino acids than by a mixture of sugars, but the amount of germination never exceeded 40% of the oospore population. The volatile compounds that were tested were highly suppressive at high concentrations, and exerted no effect at low concentrations. However, within a critical concentration range acetaldehyde was found to be as stimulatory as peptone, and it evidently triggered germination by the same component of the oospore population because the same percentage germination occurred in the presence of both acetaldehyde and peptone as in the presence of either stimulant alone.

Unless this maximum observed percentage of germination was governed by other factors such as self-inhibitors, it seems that the non-responding proportion of the oospore population was constitutively dormant. As noted in the Introduction (Section 1.5), the concept of constitutive dormancy in Pythium has been challenged. But indirect evidence for it was found in the experiments where the length of culture or of post-culture storage in distilled water was varied. The percentage germination of oospore populations was reduced if cultures were maintained on carrot extract medium for extended times, over 28 days. On this basis it can be postulated that the oospores that germinate when harvested from 28 day cultures subsequently enter a dormant phase if the culture time is prolonged. Also of interest was the confirmation of the finding

by Walther & Gindrat (1987b) that oospore germinability is increased (for oospores from short culture periods) if the mycelia are washed and incubated in distilled water. This did not occur if the mycelia were macerated and the resulting oospores were stored in water, indicating that the enhanced germinability might be in response to the increase in nutrient-status of spores that accumulate autolytic products from starved mycelia. Lastly, it is notable that the germinability of oospores harvested from starved cultures (ie after transfer of mycelia to water) did not fall appreciably even on prolonged storage of oospores in water at room temperature - past the time at which their germinability falls if they are kept on mycelia in carrot extract. This again implicates mycelial connections in the changes in oospore germinability. The mechanisms involved merit detailed study because they are of interest both for fungal physiology and for the potential commercial production of oospore inocula for biocontrol programmes (Lutchmeah & Cooke, 1985; Martin & Hancock, 1987; Walther & Gindrat, 1987b).

SECTION 4

SECTION 4

Colony Interactions

4.1 Introduction

The work in this section was carried out to extend previous studies such as those of Drechsler (1943), Tribe (1966), Deacon (1976) and Deacon & Henry (1978) on the host ranges of mycoparasitic *Pythium* spp. These workers had identified apparent differences in resistance of 'host' fungi to the mycoparasites, *P. oligandrum*, *P. acanthicum* and *P. periplocum*, based on the degree to which these parasites could grow across, or sporulate on, colonies of hosts, and also based on the degree to which the host activities were reduced in the presence of mycoparasites (see Introduction).

The major difference in this work was that it involved a comparison of *P. oligandrum*, *P. mycoparasiticum* and *P. nunn* across a range of host fungi, because no such comparison had previously been made. It was thus expected that the experiments would reveal potential differences in host susceptibility *per se* and potential differences in the inherent parasitic ability (equivalent to pathogenicity) of the mycoparasites themselves.

As in the work of Deacon (1976), Deacon & Henry (1978) and Foley & Deacon (1986a), use was made of the fact that none of the mycoparasitic *Pythium* spp can degrade cellulose, whereas most of the selected host fungi could do so. So the degree of susceptibility of a host fungus to antagonism by a parasite could be assessed by the degree to which cellulolysis was reduced in dual cultures compared with in monoculture of the host. But this approach is impractical for some other mycoparasites such as *Trichoderma* or *Gliocladium* spp, so in more limited tests in

which these fungi were included the criterion of parasitism (or, conversely, of host susceptibility) was taken to be the rate at which a mycoparasite could grow across agar plates precolonised by various hosts.

The experimental methods were described in Sections 2.3.4 and 2.3.5.

4.2 Materials and methods

4.2.1 Interactions on filter paper

Wads of five Whatman No 3 filter paper circles (7cm diam, 3.5 g air-dry weight) were accurately weighed into plugged 250 ml conical flasks and saturated with 15 ml mineral solution. This solution consisted of minimal mineral solution (Section 2.1) with $NaNO_3$ (2 g l^{-1}), thiamine hydrochloride (100 µg l^{-1}) and biotin (10 µg l^{-1}). After autoclaving for 30 min at 121°C, the wads were inoculated at the margin with a 10 mm diameter inoculum disc of a host fungus (on PDA) and incubated at 25°C (Section 2.3.4.1). Some flasks were left undisturbed for six weeks, others received a 10 mm diameter inoculum disc of a mycoparasite, placed beside the original inoculum disc two days later, and were then reincubated for a total of six weeks. The contents of all flasks were then oven dried (80°C) to determine the weight loss from breakdown of the filter paper. Control (uninoculated) flasks were used to provide a correction factor for converting the initial air-dry weights of the filter paper into oven-dried weights. The experiment comprised the three fungal hosts that grew well in these conditions (F. culmorum, B. piluliferum and B. cinerea), tested alone or in combination with each mycoparasite, and the three mycoparasites alone; there were three replicates for each treatment.

4.2.2 Interactions on cellulose film: juxtaposed inocula

As described in Section 2.3.4.2 and shown in Fig 2.1, two strips of autoclaved cellulose film, 6 x 2 cm, were placed in parallel on plates of mineral nutrient agar containing nitrate as sole nitrogen source. Each strip was inoculated at one end with an agar inoculum disc (5 mm diameter) of a host fungus and the plates were incubated for 24 h at 25°C. Then, for some strips, an inoculum disc of a mycoparasite was placed beside the original inoculum disc but 5 mm distant from it and the plates were reincubated. "Host control" plates did not receive an inoculum of a mycoparasite. "Mycoparasitic control" plates received only an inoculum of the mycoparasite, and uninoculated controls were also included in the experiments.

After six days incubation, the strength of the cellulose film was assessed with a needle penetrometer (Section 2.3.4.2) at five points along each strip, the first assessment point being 5 mm from the edge of the original inoculum and other points being spaced at 10 mm intervals from this first point (Fig 2.1).

The mean weight supported by the penetrometer at the five points along each strip of cellulose film constituted one replicate result. Two replicate plates (each with two cellulose strips) were used to obtain the presented results, calculated as means with standard errors for the replicates. The experiments involved ten host fungi (Table 2.1), each of which was assessed in the presence or absence of three mycoparasites, P. oligandrum (isolate CGH), P. mycoparasiticum (isolate AR7A) and P. nunn (isolate 20693).

4.2.3 Interactions on cellulose film: opposed inocula

These experiments were performed as above, the host fungi being allowed to grow for 24 h before a mycoparasite was introduced. But the

inocula of the mycoparasites consisted of blocks of agar cut from the margins of colonies on PDA, the blocks being 25 mm long and placed across the strip of cellulose film ahead of the advancing margins of the host colonies (Fig 2.2). The width of each block was selected to represent the equivalent of 24 hours extension of the host colony margin (eg 10 mm, if the host colony advanced 10 mm in 24 h) and the blocks were placed at such a distance that the host colony would reach them in 12 h (Section 2.3.4.3).

Cellulose breakdown was assessed by penetrometer on both sides of the mycoparasite block. Four strips were assessed, at three separate points on each side. Assessment was carried out after 7, 14 and 21 days.

4.2.4 Growth of mycoparasites on precolonised agar plates

Petri dishes (9 cm diameter) containing 12 ml of PDA were inoculated at the margin with a single inoculum disc of a host fungus and incubated for varying times at 25°C until the colony had just reached the furthest edge of the agar plate. Then a 5 mm diameter inoculum disc of a mycoparasite was placed on the colony margin of the host and the plates were marked on the base with four parallel lines, each 5 mm apart (Fig 2.3) such that three strips were marked, the central strip joining the host and mycoparasite inoculum discs. After seven days incubation, the central strip of agar was removed with a sharp scalpel, cutting from the host inoculum block to avoid the possibility that spores of the mycoparasite would be inadvertently carried forward. The strip was then cut into successive 5 mm pieces (15 in all) which were incubated on PDA plates to detect outgrowth by the mycoparasite (Section 2.3.5). This was usually evident by visual observation because of the characteristic colony morphology of each fungus used, but in cases of doubt the inocula were transferred to carrot agar which enabled the production of the

characteristic oogonia of the mycoparasites.

4.2 Results

4.2.1 Interactions on filter paper

As described in Section 4.2.1, wads of filter paper were inoculated with three host fungi in the presence or absence of inocula of mycoparasites; controls consisted of mycoparasites or hosts alone. After 6 weeks at 25°C the weight loss of the filter paper wads was assessed.

The results (Table 4.1) show that the three mycoparasites alone caused no weight loss, consistent with earlier findings that P. oligandrum and P. mycoparasiticum are non-cellulolytic and cannot utilize nitrate - the form in which nitrogen was supplied (Foley & Deacon, 1986a). P. nunn also was non-cellulolytic, because all other components of the medium, including the nitrogen source, were suitable for its growth (see Section 3.3.2).

The three host species - F. culmorum, B. piluliferum and B. cinerea - all caused substantial breakdown of the filter paper (Table 4.1). In the presence of the mycoparasites, the breakdown caused by these hosts was reduced, though to different degrees depending on the host/parasite pairing. In the presence of P. oligandrum, all three hosts were severely impaired in their cellulolytic behaviour; indeed B. cinerea caused almost no breakdown in these circumstances. When each host was paired with P. mycoparasiticum, the reduction in cellulolysis was less than that caused by P. oligandrum (25-34% as opposed to 64-98% reduction). P. nunn had an even lesser effect on the hosts than did P. mycoparasiticum, and no effect at all on F. culmorum. On the basis of these findings, P. oligandrum was considered to be a more aggressive parasite than was P. mycoparasiticum, and this in turn was more

Table 4.1 Weight loss (mg) of filter paper wads caused by cellulolytic fungi in the presence or absence of mycoparasitic *Pythium* spp

Values are means, with standard errors of the means, for three replicates after 6 weeks at 25°C. Figures in parentheses are percentage reductions of weight loss by the cellulolytic fungi alone compared with

Cellulolytic fungus	Mycoparasite: None	*P. oligandrum*	*P. mycoparasiticum*	*P. nunn*
None	0.0	0.0	0.0	0.0
F. culmorum	517 ± 3.9	184 ± 8.2 (64.4)	390 ± 2.9 (24.6)	544 ± 17.3 (+ 5.2)
B. piluliferum	400 ± 28.3	70 ± 1.8 (82.5)	266 ± 12.1 (33.5)	330 ± 85.3 (17.5)
B. cinerea	564 ± 45.1	11 ± 1.1 (98.0)	373 ± 44.3 (33.8)	472 ± 47.1 (16.3)

aggressive than P. nunn. This pattern with regard to the parasites was seen in the case of each host. Considering the host fungi per se, it is difficult to assess their relative degree of susceptibility to parasitism because they caused different amounts of breakdown of filter paper when used alone. However, the values for percentage reduction in cellulolytic activity caused by the presence of the mycoparasites suggest that B. cinerea was, overall, the most susceptible host to antagonism by the mycoparasites, and F. culmorum was the least susceptible, with B. pulilliferum occupying an intermediate position.

4.3.2 Interactions on cellulose film: juxtaposed inocula

As described in Section 2.3.4.2, strips of cellulose film were inoculated at one end with cellulolytic host fungi, with or without the addition (after 24 h) of inocula of the mycoparasites. Strength of the cellulose film was then assessed after 6 days at five points of 10 mm intervals along its length. For each strip the weights supported by the film at the five points were averaged, and these averages for four replicate strips were used to obtain means (with standard errors) of the weight supported in each treatment.

Uninoculated control strips of cellulose film were punctured by a weight of 55 g applied to the needle penetrometer. Any reduction from this weight was considered to represent cellulolysis by the fungi (Deacon, 1979).

As shown in Tables 4.2, 4.3 and 4.4, the ten 'host' fungi differed considerably in cellulolytic ability - a difference visually confirmed by their density of growth on the plates (not presented) where the cellulose film was the sole carbon source. P. graminicola, F. culmorum, B. cinerea, F. oxysporum, R. solani (GM1) and T. aureoviride were the most highly cellulolytic; B. piluliferum, R. solani (T125) and Phialo-

Table 4.2 Weight supported (g) after 6 days, when cellulose film was inoculated with cellulolytic fungi in the presence or absence of *P. oligandrum* (juxtaposed inocula)*

Cellulolytic fungus	*P. oligandrum*:			[Oospore production]
	Absent	Present	% Decrease	
P. graminicola	3.8 ± 0.6†	4.5 ± 0.3	1.3	[0]
R. solani GM1	8.8 ± 1.4	14.0 ± 0.7	11.4	[2]
R. solani T125	29.5 ± 1.6	38.8 ± 1.0	35.3	[2]
F. culmorum	4.5 ± 0.3	26.2 ± 1.7	43.1	[0]
B. piluliferum	25.5 ± 1.8	40.0 ± 1.1	48.7	[5]
F. oxysporum	7.2 ± 0.9	37.5 ± 1.5	61.3	[5]
B. cinerea	5.2 ± 0.2	38.2 ± 1.6	66.4	[4]
T. aureoviride	13.8 ± 0.5	37.5 ± 1.5	76.4	[4]
Phialophora sp	31.2 ± 1.8	50.0 ± 0.4	78.9	[5]
P. vexans	52.8 ± 0.9	53.5 ± 0.3	N/A*'	[0]
Control	55.0 ± 0.0	54.8 ± 0.2	N/A	[0]

* Values are mean weights supported by penetrometer on four replicate strips of film, each assessed at five points along its length

'* N/A not applicable

† mean ± s.e.

Table 4.3 Weight supported (g) after 6 days, when cellulose film was inoculated with cellulolytic fungi in the presence or absence of *P. mycoparasiticum* (juxtaposed inocula)*

Cellulolytic fungus	*P. mycoparasiticum*:			[Oospore production]
	Absent	Present	% Decrease	
P. graminicola	3.8 ± 0.6†	4.2 ± 0.7	0.7	[0]
B. cinerea	5.2 ± 0.2	9.0 ± 0.8	4.5	[1]
R. solani GM1	8.8 ± 1.4	8.8 ± 1.4	5.4	[0]
R. solani T125	29.5 ± 1.6	34.5 ± 2.5	19.6	[0]
F. culmorum	4.5 ± 0.3	14.8 ± 2.5	20.3	[1]
B. piluliferum	25.5 ± 1.8	35.5 ± 0.9	33.6	[3]
F. oxysporum	7.2 ± 0.9	25.8 ± 0.6	35.4	[1]
T. aureoviride	13.8 ± 0.5	42.0 ± 2.4	68.5	[3]
Phialophora sp	31.2 ± 1.8	50.5 ± 0.3	78.9	[5]
P. vexans	52.8 ± 0.9	53.2 ± 0.2	N/A*'	[0]
Control	55.0 ± 0.0	55.0 ± 0.0	N/A	[0]

* Values are mean weights supported by penetrometer on four replicate strips of film, each assessed at five points along its length

*' N/A not applicable

† mean ± s.e.

Table 4.4 Weight supported (g) after 6 days, when cellulose film was inoculated with cellulolytic fungi in the presence or absence of *P. nunn* (juxtaposed inocula)*

Cellulolytic fungus	*P. nunn*: Absent	Present	% Decrease	[Oospore production]
R. solani GM1	8.8 ± 1.4†	7.8 ± 1.0	-3.2	[0]
P. graminicola	3.8 ± 0.6	4.0 ± 0.7	0.3	[0]
B. cinerea	5.2 ± 0.2	8.0 ± 1.2	4.5	[0]
R. solani T125	29.5 ± 1.6	31.0 ± 3.2	5.9	[0]
F. culmorum	4.5 ± 0.3	10.0 ± 1.3	10.9	[0]
B. piluliferum	25.5 ± 1.8	28.8 ± 0.8	10.9	[0]
F. oxysporum	7.2 ± 0.9	22.8 ± 1.3	28.7	[0]
T. aureoviride	13.8 ± 0.5	29.5 ± 4.5	38.8	[0]
Phialophora sp	31.2 ± 1.8	43.8 ± 2.5	52.6	[0]
P. vexans	52.8 ± 0.9	53.5 ± 0.3	N/A*'	[0]
Control	55.0 ± 0.0	48.8 ± 1.2	N/A	[0]

* Values are mean weights supported by penetrometer on four replicate strips of film, each assessed at five points along its length

*' N/A not applicable

† mean ± s.e.

phora sp were apparently less highly cellulolytic, and P. vexans was essentially non-cellulolytic. The only qualification that needs to be made is that B. piluliferum grows much more slowly than do the other fungi on sugar-containing media (eg PDA), as well as on cellulose film and thus cellulose breakdown appeared to be reduced overall because of the non-breakdown of cellulose beyond its much slower extending colony margins. In all cases there was no evidence of cellulose breakdown (assessed by the penetrometer) ahead of the colony margins of the host fungi.

The mycoparasites P. oligandrum and P. mycoparasiticum when used alone did not cause weakening of the cellulose film; P. nunn caused a very slight weakening (mean 48.8 g supported) but this did not vary along the length of the cellulose strips as might be expected if it were the result of cellulolysis. So P. nunn can also be considered as essentially non-cellulolytic in these conditions.

Table 4.2 shows the effect of co-inoculation with P. oligandrum on cellulolytic activity of the various host fungi, ranked in order of increasing apparent sensitivity to the mycoparasite. P. graminicola was apparently the most resistant host, because there was no significant effect of P. oligandrum in reducing the weakening of the cellulose caused by P. graminicola. R. solani (GM1) was similarly little affected by the presence of P. oligandrum, and the other host fungi showed varying degrees of inhibition of cellulolysis, the most marked inhibition being seen for T. aureoviride and Phialophora sp. Although the results for P. vexans are included in Table 4.2, they can be discounted because the method was clearly unsuitable for detecting the activity of this host fungus on cellulose film.

Table 4.3 shows equivalent results for the hosts in the presence of P. mycoparasiticum, as does Table 4.4 in the presence of P. nunn.

P. graminicola was again unaffected by the presence of these mycoparasites. Conversely, T. aureoviride and Phialophora sp were the most markedly affected by the presence of P. mycoparasiticum or P. nunn, as was found in the presence of P. oligandrum. These findings suggest that the hosts that are most resistant or susceptible to the activities of any one mycoparasite are also most resistant or susceptible to the activities of the other mycoparasitic Pythium spp. Further support for this view is seen in the fact that R. solani (GM1) was seemingly resistant to the effects of all three mycoparasites, whereas F. oxysporum, B. piluliferum and F. culmorum showed intermediate degrees of susceptibility to all three mycoparasites. Only two results were at variance with these general findings. First R. solani (isolate T125) was less markedly affected by the presence of P. nunn than in the presence of P. oligandrum or P. mycoparasiticum, although its order of susceptibility among the hosts was unchanged. Second, and more importantly, B. cinerea was markedly affected by P. oligandrum but was among the most resistant host fungi to the influences of P. mycoparasiticum and P. nunn.

When the cellulose strips were assessed microscopically, P. oligandrum and P. mycoparasiticum were seen to have formed oogonia in the presence of some of the host fungi. P. nunn did not produce oogonia on any of the test strips. The production of oogonia of P. oligandrum and P. mycoparasiticum was scored on a 0-to-5 basis, as follows:

0 No oogonia observed

1 A few oogonia near the mycoparasite inoculum block

2 Oogonia abundant but only near the mycoparasite inoculum

3 Oogonia present along part of the length of the film

4 Oogonia present along whole length of film

5 Oogonia present and abundant along whole length of film

As shown in the final columns of Tables 4.2, 4.3 and 4.4 oogonia were not produced by the mycoparasites in the presence of P. graminicola or P. vexans, but this can be explained by the inability of these host fungi to produce sterols, which were not supplied in the agar medium but are necessary for reproduction by the mycoparasites (Haskins, et al., 1964). Oogonia were formed abundantly by P. oligandrum and P. mycoparasiticum in the presence of many of the host fungi that were most susceptible to antagonism (as evidenced by a reduction in cellulolysis) but were formed sparsely or not at all in the presence of the more resistant host fungi. In general, therefore, the degree of reduction in activity of a host fungus was inversely related to the degree of growth of these mycoparasites, oogonium production being the only easily assessable criterion of growth of the mycoparasites in the conditions of these experiments.

The results in Tables 4.2, 4.3 and 4.4 also suggest that there were marked differences in the aggressiveness of the different mycoparasites across the range of hosts. In order to assess this, the results for percent reduction in cellulolysis caused by the presence of the mycoparasites were subjected to analysis of variance, the data for eight hosts being used for this purpose, as shown in Table 4.5. Results for P. vexans were excluded from the analysis for reasons given earlier, and those for B. cinerea were excluded because this host was affected quite differently in the presence of the different mycoparasites. In almost every instance for each of the eight host fungi, the degree of inhibition of cellulolysis caused by the presence of P. oligandrum was greater than that caused by the presence of P. mycoparasiticum, and this in turn was greater than in the presence of P. nunn.

Table 4.5. Mean percent reduction in cellulolysis caused by the presence of mycoparasitic *Pythium* spp

Cellulolytic fungus	Mycoparasite: *P. oligandrum*	*P. mycoparasiticum*	*P. nunn*	Mean* 5% LSD = 13.14)
P. graminicola	1.3	0.7	0.3	0.8
R. solani GM1	11.4	5.4	-3.2	4.5
R. solani T125	35.3	19.6	5.9	20.3
F. culmorum	43.1	20.3	10.9	24.8
B. piluliferum	48.7	33.6	10.9	31.1
F. oxysporum	61.3	35.4	28.7	41.8
T. aureoviride	76.4	68.5	38.8	61.2
Phialophora sp	78.9	78.9	52.6	70.1
Mean % reduction* (5% LSD = 8.05)	46.6	32.8	18.5	

* *B. cinerea* and *P. vexans* were excluded from the analysis

4.3.3 Interactions on cellulose film: opposed inocula.

The results of the previous experiments suggested that the host fungi differed in susceptibility to antagonism to the mycoparasitic Pythium spp and that the mycoparasites differed in aggressiveness to the host fungi. In all but one instance (involving B. cinerea as a host) these differences seemed to be valid in general, irrespective of the particular host-mycoparasite combination; in other words they reflected inherent differences in host susceptibility across the range of mycoparasites or inherent differences in aggressiveness of the mycoparasites across the range of host fungi. But interpretation of the results could be challenged on the basis that the hosts differ in linear extension rate, providing opportunities for some of the hosts to escape the influences of the mycoparasites by outgrowing them. Similarly, the mycoparasites differ in linear extension rates (see Section 3.3.1) and thus might have different abilities to antagonise the host fungi at the host colony margins.

The experiments below were designed to remove these potential sources of variability. Essentially as described in Section 2.3.4.3, a strip of agar colonised by a mycoparasite was placed across each strip of cellulose film ahead of the advancing colony margin of the host fungus. The breadth of this strip was varied for particular hosts, such that it represented 24 hours' extension of the host colony margin, and in all cases the strip was positioned such that the host margin would contact it initially after 12 h (the hosts having been inoculated on the strips 24 h previously so that their colonies were well-established before the mycoparasites were introduced). Controls were prepared with strips of uncolonised PDA in place of strips of PDA colonised by the mycoparasites (see Fig 2.2).

At 7, 14 and 21 days after the mycoparasites had been added, the

strength of the cellulose strips was assessed with a needle penetrometer in three positions, at distances equivalent to 6 h host growth on either side of the mycoparasite strip (Fig 2.2). Four replicate cellulose strips were used for each host-mycoparasite combination and for the hosts and mycoparasites alone.

As shown in Table 4.6, there was no weakening of the cellulose film in the presence of the mycoparasites alone (weight 55 g supported on the penetrometer) at any time up to three weeks of incubation. In the presence of the hosts alone the assessments in the 'fore' and 'aft' positions (ahead and behind the control blocks of PDA) showed extensive cellulolysis after only one week; and in most cases the cellulose film was punctured by the weight of the penetrometer alone (6.62 g). Only R. solani (T125) failed to cause "complete" weakening of the film after 2 or 3 weeks, but this was because the fungus had grown poorly on one replicate strip of film. Visual observation confirmed that the host fungi had grown on the cellulose film under the PDA "barriers", and tests at various points along the cellulose strips showed that there was no detectable cellulolysis ahead of the colony margins of the host fungi.

Table 4.7 shows that the presence of a PDA block bearing P. oligandrum caused substantial reductions in cellulolysis by some of the host fungi in the vicinity of the mycoparasite. The reduction in cellulolysis by R. solani (GM1), R. solani (T125) and F. culmorum was only temporary, being evident at only 7 days, especially in the region of the film distant from the host inoculum; in these cases the effect was no longer evident after 14 days. The effect of P. oligandrum on P. graminicola was initially quite marked and persisted to at least some degree even after 21 days. This was true also for B. piluliferum and F. oxysporum, which after 7 days had caused no significant weakening of the

Table 4.6 Weight (g, max 55) applied to a penetrometer that punctured cellulose film inoculated with cellulolytic fungi in the presence of an inoculum block of PDA

Assessments (means ± s.e. of 4 replicates) were made on the near side and far side of an uninoculated block of PDA positioned as shown in Fig 2.2

	Weight supported (g)					
	7 days		14 days		21 days	
	H/S*	F/S*	H/S*	F/S*	H/S*	F/S*
P. graminicola	5.4 ± 0.9	5.5 ± 1.0	0.0	0.0	0.0	0.0
R. solani GM1	0.0	0.0	0.0	0.0	0.0	0.0
R. solani T125	8.8 ± 1.2	11.2 ± 1.2	3.8 ± 3.8	3.8 ± 3.8	3.8 ± 3.8	3.8 ± 3.8
F. culmorum	0.0	0.0	0.0	0.0	0.0	0.0
B. piluliferum	0.0	0.0	0.0	0.0	0.0	0.0
B. cinerea	0.0	0.0	0.0	0.0	0.0	0.0
F. oxysporum	0.0	5.0 ± 0.2	0.0	0.0	0.0	0.0
T. aureoviride	0.0	0.0	0.0	0.0	0.0	0.0
Phialophora sp	0.0	0.0	0.0	0.0	0.0	0.0
P. oligandrum	55.0	55.0	55.0	55.0	55.0	55.0
P. mycoparasiticum	55.0	55.0	55.0	55.0	55.0	55.0
P. nunn	55.0	55.0	55.0	55.0	55.0	55.0

* H/S Host side ("fore" position)

* F/S Far side ("aft" position)

Table 4.7 Weight (g, max 55) applied to a penetrometer that punctured cellulose film inoculated with cellulolytic fungi in the presence of an inoculum block of *P. oligandrum*

Assessments (means of 4 replicates) were made on the near side and far side of an inoculum block of *P. oligandrum*, position as shown in Fig 2.2

	Weight supported (g)					
	7 days		14 days		21 days	
	H/S*	F/S*	H/S*	F/S*	H/S*	F/S*
P. graminicola	11.2 ± 1.2	27.9 ± 1.6	8.8 ± 0.7	11.2 ± 0.7	6.2 ± 0.7	10.0 ± 1.2
R. solani GM1	0.0	2.5 ± 0.8	0.0	0.0	0.0	0.0
R. solani T125	2.5 ± 0.8	13.8 ± 2.1	0.0	0.0	0.0	0.0
F. culmorum	0.0	6.2 ± 0.7	0.0	0.0	0.0	0.0
B. piluliferum	12.5 ± 1.0	50.4 ± 1.4	7.5 ± 1.9	45.0 ± 1.7	0.0	37.5 ± 0.8
B. cinerea	47.5 ± 1.9	53.8 ± 0.7	46.2 ± 1.4	53.8 ± 0.7	33.8 ± 3.2	53.8 ± 0.7
F. oxysporum	7.5 ± 0.8	48.8 ± 1.4	1.2 ± 0.7	16.2 ± 1.4	0.0	11.2 ± 0.7
T. aureoviride	20.0 ± 1.2	51.2 ± 1.4	20.0 ± 1.2	47.5 ± 2.5	17.5 ± 1.4	48.8 ± 1.4
Phialophora sp	18.8 ± 0.7	53.8 ± 0.7	17.5 ± 0.8	53.8 ± 0.7	16.2 ± 0.7	52.5 ± 0.8

* H/S Host side ("fore" position)

* F/S Far side ("aft" position)

cellulose film on the "far side" of the block containing P. oligandrum but had caused a significant weakening of the film in this position after 3 weeks. In contrast, the inoculum of P. oligandrum represented an apparently insurmountable barrier to the advance of B. cinerea, T. aureoviride and Phialophora sp along the strips of cellulose film, because little or no weakening of the film beyond the position of the mycoparasite was seen even after 3 weeks. Also, in these cases, there was little or no further weakening of the film between 7 and 21 days in a position close to the original colony margins of these host fungi.

On the basis of these results, it seems that P. graminicola, R. solani (GM1 and T125) and F. culmorum were among the more resistant hosts to antagonism by P. oligandrum; B. piluliferum and F. oxysporum were intermediate in susceptibility to antagonism, and B. cinerea, T. aureoviride and Phialophora sp were highly susceptible to antagonism by P. oligandrum.

Table 4.8 presents equivalent results to those above except P. mycoparasiticum was used as the 'barrier' inoculum. In this case it was found that all of the host fungi continued to degrade the cellulose to at least some degree during the 3 weeks of the experiment, both in the positions 'fore' and 'aft' of the block containing the mycoparasite. P. graminicola, R. solani (GM1) and F. culmorum were least susceptible; R. solani (T125), B. piluliferum, B. cinerea and F. oxysporum were intermediate in susceptibility, as evidenced by their failure to cause almost complete weakening of the film beyond the mycoparasite inoculum at 7 days; T. aureoviride and Phialophora sp were most susceptible, as evidenced by the retention of some strength by the film beyond the mycoparasite block even after 3 weeks. The only qualification that need be made in these respects is that R. solani (T125) might more correctly be placed in the resistant category rather than the intermediate category

Table 4.8 Weight (g, max 55) applied to a penetrometer that punctured cellulose film inoculated with cellulolytic fungi in the presence of an inoculum block of *P. mycoparasiticum*

Assessments (means of 4 replicates) were made on the near side and far side of an inoculum block of *P. mycoparasiticum*, position as shown in Fig 2.2

	Weight supported (g)					
	7 days		14 days		21 days	
	H/S*	F/S*	H/S*	F/S*	H/S*	F/S*
P. graminicola	5.0 ± 0.9	5.9 ± 0.9	0.0	0.0	0.0	0.0
R. solani GM1	0.0	0.0	0.0	0.0	0.0	0.0
R. solani T125	0.0	17.5 ± 2.8	0.0	0.0	0.0	0.0
F. culmorum	0.0	0:0	0.0	0.0	0.0	0.0
B. piluliferum	0.0	18.8 ± 0.9	0.0	0.0	0.0	0.0
B. cinerea	0.0	55.0 ± 0.0	0.0	0.0	0.0	0.0
F. oxysporum	0.0	12.5 ± 0.8	0.0	1.2 ± 0.7	0.0	0.0
T. aureoviride	0.0	31.2 ± 1.4	0.0	27.5 ± 1.9	0.0	18.8 ± 2.5
Phialophora sp	3.8 ± 0.7	47.5 ± 1.9	1.2 ± 0.7	33.8 ± 0.7	0.0	21.2 ± 0.7

* H/S Host side ("fore" position)

* F/S Far side ("aft" position)

Table 4.9 Weight (g, max 55) applied to a penetrometer that punctured cellulose film inoculated with cellulolytic fungi in the presence of an inoculum block of *P. nunn*

Assessments (means of 4 replicates) were made on the near side and far side of an inoculum block of *P. nunn*, position as shown in Fig 2.2

	Weight supported (g)					
	7 days		14 days		21 days	
	H/S*	F/S*	H/S*	F/S*	H/S*	F/S*
P. graminicola	5.0 ± 0.7	7.5 ± 0.9	0.0	0.0	0.0	0.0
R. solani GM1	0.0	0.0	0.0	0.0	0.0	0.0
R. solani T125	0.0	8.8 ± 1.4	0.0	0.0	0.0	0.0
F. culmorum	0.0	0.0	0.0	0.0	0.0	0.0
B. piluliferum	0.0	6.2 ± 1.4	0.0	0.0	0.0	0.0
B. cinerea	0.0	53.8 ± 0.7	0.0	0.0	0.0	0.0
F. oxysporum	0.0	26.2 ± 1.4	0.0	0.0	0.0	0.0
T. aureoviride	0.0	40.0 ± 1.2	0.0	36.8 ± 1.4	0.0	23.8 ± 2.1
Phialophora sp	0.0	23.8 ± 1.3	0.0	13.8 ± 0.7	0.0	11.2 ± 0.7

* H/S Host side ("fore" position)

* F/S Far side ("aft" position)

of hosts, because its relatively poor ability to weaken the film after one week (Table 4.8) was also found in control strips of film in the absence of a mycoparasite (Table 4.6).

An essentially similar pattern of results to those above was seen in the presence of inoculum blocks colonised by P. nunn (Table 4.9). Again, P. graminicola, R. solani (GM1 and T125), and F. culmorum were resistant to the effects of the mycoparasite; B. piluliferum, F. oxysporum and B. cinerea showed intermediate susceptibility, reflected in less weakening of the film after 7 days behind the parasitic inoculum, and T. aureoviride and Phialophora sp were the most susceptible, the effects of the mycoparasite on their cellulolytic activities being evident even after 3 weeks on the far side of the parasitic inoculum.

As in the previous experiments, a major difference was seen in the effects of the different mycoparasites on B. cinerea. This fungus was highly susceptible to antagonism by P. oligandrum, but only weakly susceptible to the effects of P. mycoparasiticum and P. nunn. Irrespective of this, a comparison of Tables 4.7, 4.8 and 4.9 suggests that P. nunn was similar to P. mycoparasiticum in its aggressiveness across the range of host fungi, although both of these mycoparasites were much less aggressive in general than was P. oligandrum.

4.3.4 Growth of mycoparasites on pre-colonised agar plates

In order to confirm and extend the findings of previous experiments in this section, plates of PDA were inoculated at the margin with discs of host fungi and incubated at 25°C until the host colony margins had just reached the opposite edge of the agar plates. Then an inoculum disc of a mycoparasite was placed on the host colony margin and the rate of growth of the mycoparasite across the precolonised plate was assessed as explained in Section 2.3.5. Assessments were made on three replicate

plates of each host (for each mycoparasite) after 7, 14 and 21 days at 25°C.

The advance of P. oligandrum across the colonies of various host fungi is shown in Table 4.10. This mycoparasite was found to make no detectable growth across colonies of P. graminicola, P. vexans, R. solani (T125) or T. aureoviride at any time up to 3 weeks, and it made only poor growth across colonies of R. solani (GM1). In contrast, it had completely covered the colonies of F. culmorum, B. piluliferum, F. oxysporum and Phialophora sp after 1 or 2 weeks' incubation, and it made progressive growth across colonies of B. cinerea, covering these completely after 3 weeks (Table 4.10).

In identical conditions, P. mycoparasiticum (Table 4.11) grew only across the colonies of Phialophora sp - poorly after 7 days but completely after 14 days' incubation. P. nunn (Table 4.12) grew only across colonies of Phialophora sp and B. piluliferum, and in both cases it had not fully colonised the agar plates even after 3 weeks (Table 4.12).

As an extension of this experiment, further colonies of the host fungi were inoculated with discs of T. harzianum and G. roseum in conditions identical to those used for the Pythium mycoparasites (Tables 4.13 and 4.14). The results were quite different from those of the mycoparasitic Pythium spp. T. harzianum made no growth across colonies of R. solani (T125) or T. aureoviride, and very little growth across F. culmorum or F. oxysporum. It advanced rapidly across colonies of P. graminicola, P. vexans, R. solani (GM1), Phialophora sp and B. cinerea, having completely covered these by 2 weeks if not earlier. T. harzianum made much poorer (but progressive) growth across colonies of B. piluliferum. G. roseum had an inherently slower rate of colony extension than did T. harzianum, but it progressively grew across colonies of all of the host fungi except R. solani (T125) which did not support any growth

Table 4.10 Growth of *P. oligandrum* after 7, 14 and 21 days across plates of potato-dextrose agar previously colonised by host fungi

Host	Distance covered (mm)*		
	7 days	14 days	21 days
P. graminicola	0.0	0.0	0.0
R. solani GM1	6.7 ± 1.7	18.3 ± 3.3	20.0 ± 2.8
R. solani T125	0.0	0.0	0.0
F. culmorum	75.0	75.0	75.0
B. piluliferum	61.7 ± 13.3	75.0	75.0
B. cinerea	3.3 ± 1.7	36.7 ± 4.4	75.0
F. oxysporum	75.0	75.0	75.0
T. aureoviride	0.0	0.0	0.0
Phialophora sp	75.0	75.0	75.0
P. vexans	0.0	0.0	0.0

* Means ± standard error for three replicate plates

Table 4.11 Growth of *P. mycoparasiticum* after 7, 14 and 21 days across plates of potato-dextrose agar previously colonised by host fungi

Host	Distance covered (mm)*		
	7 days	14 days	21 days
P. graminicola	0.0	0.0	0.0
R. solani GM1	0.0	0.0	0.0
R. solani T125	0.0	0.0	0.0
F. culmorum	0.0	0.0	0.0
B. piluliferum	0.0	0.0	0.0
B. cinerea	0.0	0.0	0.0
F. oxysporum	0.0	0.0	0.0
T. aureoviride	0.0	0.0	0.0
Phialophora sp	28.3 ± 3.3	75.0	75.0
P. vexans	0.0	0.0	0.0

* Means ± standard error for three replicate plates

Table 4.12 Growth of *P. nunn* after 7, 14 and 21 days across plates of potato-dextrose agar previously colonised by host fungi

Host	Distance covered (mm)*		
	7 days	14 days	21 days
P. graminicola	0.0	0.0	0.0
R. solani GM1	0.0	0.0	0.0
R. solani T125	0.0	0.0	0.0
F. culmorum	0.0	0.0	0.0
B. piluliferum	21.7 ± 3.3	38.3 ± 3.3	66.7 ± 4.4
B. cinerea	0.0	0.0	0.0
F. oxysporum	0.0	0.0	0.0
T. aureoviride	0.0	0.0	0.0
Phialophora sp	16.7 ± 4.4	28.3 ± 3.3	58.3 ± 8.8
P. vexans	0.0	0.0	0.0

* Means ± standard error for three replicate plates

Table 4.13 Growth of *T. harzianum* after 7, 14 and 21 days across plates of potato-dextrose agar previously colonised by host fungi

Host	Distance covered (mm)*		
	7 days	14 days	21 days
P. graminicola	75.0	75.0	75.0
R. solani GM1	58.3 ± 3.3	75.0	75.0
R. solani T125	0.0	0.0	0.0
F. culmorum	1.7 ± 1.7	11.7 ± 3.3	11.7 ± 3.3
B. piluliferum	3.3 ± 1.7	18.3 ± 3.3	28.3 ± 6.7
B. cinerea	75.0	75.0	75.0
F. oxysporum	0.0	0.0	5.0 ± 1.6
T. aureoviride	0.0	0.0	0.0
Phialophora sp	20.0 ± 13.2	75.0	75.0
P. vexans	65.0 ± 5.8	75.0	75.0

* Means ± standard error for three replicate plates

Table 4.14 Growth of G. roseum after 7, 14 and 21 days across plates of potato-dextrose agar previously colonised by host fungi

Host	Distance covered (mm)*		
	7 days	14 days	21 days
P. graminicola	15.0 ± 5.7	36.7 ± 4.4	48.3 ± 3.3
R. solani GM1	16.7 ± 1.7	31.7 ± 3.3	75.0
R. solani T125	0.0	0.0	0.0
F. culmorum	11.7 ± 3.3	35.0 ± 5.8	41.7 ± 3.3
B. piluliferum	20.0 ± 2.9	75.0	75.0
B. cinerea	6.7 ± 1.7	31.7 ± 3.3	75.0
F. oxysporum	16.7 ± 4.4	38.3 ± 3.3	75.0
T. aureoviride	13.3 ± 1.7	33.3 ± 1.7	48.3 ± 3.3
Phialophora sp	13.3 ± 1.6	75.0	75.0
P. vexans	21.7 ± 3.3	38.3 ± 4.4	75.0

* Means ± standard error for three replicate plates

by G. roseum (Table 4.14). Its rate of advance was greatest across colonies of B. piluliferum and Phialophora sp, which were completely covered by G. roseum after 2 weeks incubation.

4.4 Discussion.

The experiments in this section were similar to those of Deacon (1976), Deacon & Henry (1978) and Foley & Deacon (1986b) in that different mycoparasites were tested against a range of host fungi in conditions in which the colony interactions could be quantified, giving comparative data on host susceptibility and aggressiveness of the mycoparasites. Previous experiments of this type involved comparisons of P. oligandrum, P. acanthicum and P. periplocum, all of which are similar in gross morphology, growth rate and other physiological features (Deacon & Henry, 1978; Foley & Deacon, 1986a). However, the present work involved a comparison of three mycoparasites - P. oligandrum, P. mycoparasiticum and P. nunn - quite different from one another. For example, they differ from one another in growth rate, production of echinulate or smooth-walled oogonia and, according to previous reports, in aspects of their mycoparasitic behaviour (Deacon, 1976; Lifshitz et al., 1984a).

The main criterion used to assess host susceptibility was the degree to which cellulolysis by a host fungus was reduced in the presence of a mycoparasite. In this respect, weight loss of cellulosic substrates has often been used as a criterion of cellulolysis, but penetrometry has been used only more recently, following the development of the method by Deacon & Henry (1978). It has the advantage that it can be used to assess strength (of cellulose film) at selected points. Its use has been validated to the demonstration that cellulose degradation assessed by this means is strongly correlated to degradation

determined by cellulose weight loss (Deacon, 1979).

The cellulolysis assays could be used in studies with mycoparasitic Pythium spp, because none of these can degrade cellulose (Deacon, 1976, 1979; Deacon & Henry, 1978; Foley & Deacon, 1986a; this study, section 4.3.2). Unfortunately, such assays could not be used for T. harzianum or G. roseum, both of which are cellulolytic. A secondary criterion of host susceptibility, first suggested by Deacon (1976) in this respect, was the ability of a host colony to support the growth of a mycoparasite on agar plates pre-colonised by the host. This had previously been used as a criterion of host susceptibility (or of antagonism by a parasite) by Haskins (1963) but in a qualitative rather than quantitative way. In interpreting the results of both types of study mentioned above, it is recognised that many interacting factors are potentially involved, only the net effect of all these being recorded. For example, if a host fungus 'escapes' from the zone of influence of a mycoparasite on a strip of cellulose film, by virtue of its rapid growth rate, then it might cause extensive degradation of the cellulose even if its individual hyphae are highly susceptible to parasitism. This was demonstrated by Foley & Deacon (1986b) by inoculating filter paper wads with susceptible host fungi - B. piluliferum and Phialophora sp - at different times before addition of P. oligandrum. An attempt was made to overcome this potential difficulty by using two types of cellulolysis test - one in which the host colony was allowed to establish before the mycoparasite was introduced behind the colony margin, and one in which the mycoparasite was placed as a potential barrier beyond the margins of established colonies on cellulose film. But this latter test raised further interpretational problems because it assessed mainly the degree of susceptibility of the host hyphal tips, which are known to be more susceptible than are older regions of host

hyphae to parasitism by P. oligandrum (Deacon, 1976).

The main interpretational difficulty in assessing the ability of a mycoparasite to grow across a pre-colonised agar plate is to decide whether the mycoparasite is utilising host-derived nutrients or nutrients that remain in the agar plate after the host has grown. No attempt was made to determine this, but in previous work with liquid cultures Foley & Deacon (1986b) found that P. oligandrum could not grow in the presence of even some moderately resistant hosts (eg F. culmorum) whereas the culture filtrates of these hosts would support abundant growth by P. oligandrum, suggesting that appropriate nutrients were available outside of the host hyphae but could not be utilised when the host itself was active. On this basis the ability to grow across pre-colonised plates may be a reasonable criterion of host resistance (or parasitic activity) even if some nutrients remain unutilised in the agar.

If these potential difficulties are accepted, then the results of these experiments seem to provide clear evidence of differences in host susceptibility to the different mycoparasites, as summarised in Table 4.15.

P. graminicola was resistant to parasitism by P. oligandrum, P. mycoparasiticum and P. nunn by any of the criteria used: its colonies on agar did not support growth by the mycoparasites, its ability to degrade cellulose in juxtaposed interactions was unaffected by them, and it was able to grow past a substantial barrier (an agar block pre-colonised by any of these mycoparasites), and degrade cellulose film on the distal side of this barrier. Foley & Deacon (1986b) had previously reported that P. graminicola is highly resistant to parasitism by P. oligandrum - a finding now extended to include parasitism by P. mycoparasiticum and P. nunn. P. vexans may also be resistant to parasitism

Table 4.15 Categorisation of susceptibility of host fungi, based on degree of reduction of host cellulolytic activity in the presence of various mycoparasites

	Mycoparasite:		
	P.oligandrum	P.mycoparasiticum	P.nunn
Category			
Highly resistant host	P. graminicola	P. graminicola	P. graminicola R. solani GM1 R. solani T125 B. cinerea
Resistant host	R. solani GM1	R. solani GM1 B. cinerea R. solani T125 F. culmorum	F. culmorum B. piluliferum
Moderately susceptible host	R. solani T125 F. culmorum B. piluliferum	B. piluliferum F. oxysporum	F. oxysporum T. aureoviride
Highly susceptible host	F. oxysporum B. cinerea T. aureoviride Phialophora sp	T. aureoviride Phialophora sp	Phialophora sp

by all these fungi, but it was non-cellulolytic so the only criterion on which its resistance could be based was its inability to support the growth of the mycoparasites across pre-colonised plates.

The two isolates of R. solani (GM1 and T125) were found to be among the most resistant hosts to all three mycoparasites: their colonies supported little or no growth across pre-colonised plates, and they were able to degrade cellulose film in the presence of the mycoparasites, even growing past inocula of the mycoparasites positioned ahead of them on cellulose film. In the cellulolysis experiments T125 seemed to be less resistant than was isolate GM1 to any of the mycoparasites. But this was perhaps an artefact of the assessment method because GM1 was conspicuously the more highly cellulolytic, and it is possible that the penetrometer was less sensitive in detecting differences in cellulose strength when the cellulose was highly degraded than when only moderately degraded. Other mechanically-based assessments of cellulose degradation, such as viscometry or shear-strength, are known to be subject to similar constraints (Gascoigne & Gascoigne, 1960; Zeronian, 1977). In fact, the tests for growth on pre-colonised plates indicated the opposite of the results for cellulolysis, namely that isolate T125 was slightly the more resistant isolate of R. solani to parasitism. Nevertheless, on balance, all the results suggested that R. solani is highly resistant to parasitism by the three Pythium spp. This finding is interesting because it confirms those of Deacon (1976) and Foley & Deacon (1986b) for parasitism by P. oligandrum, where a different isolate of R. solani was used to those used here. But the results contrast with those of Al-Hamdani, Lutchmeah & Cooke (1983) who reported that R. solani is susceptible to this mycoparasite. Also, Haskins (1963) reported it to be susceptible to P. acanthicum. Further discussion of this is deferred to Section 5.4, where a likely

explanation of the discrepancies is proposed.

Based on cellulolysis data, F. culmorum was found to be quite highly resistant to parasitism by the three Pythium spp, although agar plates precolonised by F. culmorum supported good growth by P. oligandrum (but not by the other two mycoparasites) - a discrepancy that is not easy to explain. Foley & Deacon (1986b) had previously found that F. culmorum did not enable good growth by P. oligandrum in dual-inoculated liquid cultures, even though the mycoparasite could grow in the cultures (using sources of nitrogen and vitamins derived from F. culmorum) if the mycelia of F. culmorum were removed by filtration. Deacon (1976) had earlier categorised F. culmorum as moderately susceptible to parasitism by P. oligandrum based on reduction of its cellulolysis in the presence of the mycoparasite - a finding similar to that discussed here.

F. oxysporum and B. piluliferum were also found to be moderately susceptible to parasitism by P. oligandrum and P. mycoparasiticum (but somewhat less affected by P. nunn) based on results from cellulolysis. Both hosts supported extensive growth by P. oligandrum across their colonies on agar, yet neither of them supported growth by P. mycoparasiticum, and only B. piluliferum supported good growth by P. nunn across its colonies on agar. These results for the different tests (and the different mycoparasites) are difficult to interpret. They suggest that the correspondence between the different assessment methods breaks down in intermediate cases, ie when a host is moderately susceptible to parasitism. In previous work, B. piluliferum was suggested to be highly susceptible to parasitism by P. oligandrum, based on a reduction in cellulolysis (Deacon, 1976), but in the same study Deacon found that cellulolysis by B. piluliferum was unaffected if introduction of the mycoparasite was delayed until the host was well established on filter

paper wads. In this and some other cases, therefore, susceptibility to parasitism may depend on the age of the host colony or of individual hyphae that are contacted by a mycoparasite. This in turn may depend on the relative growth rates of colonies of the host and its parasites and thus on the ability (or not) of hyphae of the host to escape the influences of a mycoparasite.

Two hosts - T. aureoviride and Phialophora sp- were the most susceptible to the influences of all three mycoparasites on cellulose film - a finding compatible with earlier results of Deacon (1976) and Deacon & Henry (1978) who found that Phialophora sp is highly susceptible to antagonism by P. oligandrum, P. acanthicum and P. periplocum. Phialophora sp was also the only fungus of those tested (R. solani, P. ultimum, F. oxysporum and Phialophora sp) that enabled P. oligandrum to grow in its presence in liquid cultures (Foley & Deacon, 1986b). Of interest, Phialophora sp was the only fungus that enabled all three mycoparasites - P. oligandrum, P. mycoparasiticum and P. nunn - to grow across agar plates that it had already colonised. So, by any criterion, it seems to be highly susceptible to parasitism by the Pythium spp. T. aureoviride showed equivalent susceptibility to that of Phialophora sp on cellulose film, yet it did not enable any of the mycoparasites to grow across its colonies on PDA. A possible explanation of this is that T. aureoviride may produce fungitoxic compounds on nutrient-rich media such as PDA, but this was not investigated.

The only host fungus that showed a clear difference in response to the different mycoparasites - and a response that was different from that of the other hosts - was B. cinerea. It was one of the most susceptible hosts to antagonism by P. oligandrum on cellulose film but was apparently resistant to parasitism by P. mycoparasiticum and P. nunn in equivalent conditions. It enabled P. oligandrum to grow well across

agar plates that it had pre-colonised, whereas it enabled no growth by P. mycoparasiticum or P. nunn (although in this respect it was little different from most of the other host fungi used). Further discussion of these differences is deferred to Chapter 5, where evidence was presented that hyphae of B. cinerea release a diffusible fungistatic compound that prevents growth by P. mycoparasiticum and P. nunn but has no effect on P. oligandrum.

For comparison with all these findings it is interesting to note that T. harzianum and G. roseum grew across plates pre-colonised by P. graminicola, P. vexans and R. solani (GM1) which did not support growth by the mycoparasitic Pythium spp. Only G. roseum, and not T. harzianum, could grow across plates pre-colonised by T. aureoviride, which did not support growth by the Pythium spp. There were differences in degree of growth across plates of most other fungi. One notable exception was that R. solani (T125) did not support growth by any mycoparasite, for reasons unknown. Another notable exception was that T. harzianum did not grow across colonies of F. oxysporum, whereas G. roseum and P. oligandrum did so. On the other hand, all of the fungi - T. harzianum, G. roseum and the three mycoparasitic Pythium spp - grew well across agar plates pre-colonised by Phialophora sp. This is fortuitous because Phialophora sp was chosen almost arbitrarily, for the production of pre-colonised plates for selective isolation of mycoparasitic Pythium spp from soil (Deacon & Henry, 1978), and has also proved effective in isolation of both Trichoderma spp and G. roseum (Foley & Deacon, 1985). On the other hand, colonies of R. solani have been used for selective isolation of Verticillium bigattattum (W. Gams) and Gliocladium (van den Boogert & Jager, 1983) from soil, and in this report there was no reference to the isolation of mycoparasitic Pythium spp, which accords with the results of the experiments here.

The discussion so far has focused mainly on differences in resistance of host fungi to the mycoparasitic Pythium spp. But there was clear evidence also of differences in aggressiveness of the mycoparasites, irrespective of the host fungi used. On most or all host fungi, P. oligandrum was found to be the most aggressive of the three mycoparasites in terms of ability to reduce host cellulolytic ability. P. mycoparasiticum was less aggressive and P. nunn was least aggressive. These differences were most conspicuous with juxtaposed inocula. With 'opposed' inocula the greater aggressiveness of P. oligandrum compared with P. mycoparasiticum and P. nunn was also obvious, but differences between P. mycoparasiticum and P. nunn were then not apparent. The difference between P. oligandrum and the other mycoparasites was also seen on pre-colonised agar plates, insofar as P. oligandrum grew to at least some degree across agar pre-colonised by six host fungi, whereas P. nunn grew across only two and P. mycoparasiticum across only one host (Phialophora sp). For comparison, T. harzianum and G. roseum grew to at least some degree across colonies of eight host fungi, of the ten that were tested.

SECTION 5

SECTION 5

Hyphal interactions

5.1 Introduction

The results in Section 4 demonstrated significant differences in the effects of any one mycoparasitic Pythium species on a range of cellulolytic fungi and, similarly, differences in the ability of a mycoparasitic species to grow across agar precolonised by different fungi. Coupled with this, there was evidence that three mycoparasitic Pythium species differed one from another in ability to grow on, or reduce the growth of, individual fungal hosts. In other words, the mycoparasites appeared to differ in aggressiveness of their parasitism, and hosts differed in their susceptibility.

The experiments in this section were designed to relate such findings to observable hyphal interactions and to quantify or categorise these interactions. Previous work of this type was done by Hoch & Fuller (1977) for P. acanthicum on a range of fungal hosts, using light and electron microscopy, and by Lifshitz et al. (1984a) and Elad et al. (1985) for P. nunn using electron microscopy and enzyme assays. More recently, Lewis et al. (1989) carried out studies on interactions involving P. oligandrum against a range of host fungi as well as carrying out enzyme assays with this fungus. Other relevant work discussed in the Introduction was by Haskins (1963) for P. acanthicum, and Deacon (1976) for P. oligandrum. The major innovation in the present study was to adapt and employ video-microscopy for recording of interactions so that sequential and concomitant events could be analysed from video frames.

The experiments were essentially of chequer-board design, with each

of the three mycoparasites being tested against each of five 'hosts' selected on the basis of results in Section 4 for their contrasting responses or sensitivities to mycoparasitism. Additionally, P.oligandrum alone was tested against a further five host fungi, and more limited tests were included to study the behaviour of Trichoderma harzianum and Gliocladium roseum as mycoparasites.

5.2 Materials and Methods

The five host fungi used in all comparisons were: Pythium vexans, Botrytis cinerea, Fusarium oxysporum, Phialophora sp with lobed hyphopodia, and Trichoderma aureoviride. Isolates CGH (P. oligandrum), ATCC 20693 (P. nunn) and AR7A (P. mycoparasiticum) were used as mycoparasites.

Glass coverslips (35 mm by 64 mm) were sterilized by autoclaving at 121°C for 15 min and dipped in sterile molten 2% distilled water agar at normally 92°C for 1 or 2 seconds. Excess agar was allowed to drip off so that only a thin adhering film remained. The coverslips were then laid, agar face upwards, on solidified water agar in Petri dishes. When the agar on the coverslips had set, a small inoculum block from the margin of a colony of a host fungus on PDA was placed near one end. The plate was incubated for a variable time (normally 1 to 2 days) until the host had grown about 10 mm from its inoculum, and an inoculum block of the parasite was then placed beyond the colony margin (Fig 2.4). In all cases the distances and relative timings of inoculation were designed to ensure that contact between the host and mycoparasite would occur about 24 h later when the plates were incubated at 20°C (Section 2.3.6). In practice, however, a series of plates was prepared with an increasing range of distance between the inoculum blocks to ensure that material was available for viewing the following day.

When colonies of the host and parasite had almost touched, the coverslip was removed from the agar plate and the inoculum blocks were removed carefully to avoid disruption of the mycelia. The coverslip was then inverted on an observation chamber (Fig 2.4) consisting of a large microscope slide with a rectangle of glass spacers 2 mm high. The inverted coverslip was sealed with vaseline to prevent drying. The upper surface of the coverslip was cleaned and the hyphal interactions were observed microscopically.

With this technique, interactions between undisturbed colonies could be examined through the coverslip and agar film with oil-immersion objectives (x 70 and x 90 magnification), x 10 eyepieces and a 1.25 magnification factor from a Leitz Ploempak incident fluorescence attachment containing a blank (TK 400) filter block. Video recordings were made of some hyphal interactions to enable quantification of results but they were supported by many further observations by eye.

5.3 Results

The results are presented in three forms. First, a detailed account is given of five interactions that illustrate many of the important phases of the interactions. These accounts are supplemented with descriptions of other events from other interactions. On this basis, a catalogue of events was constructed to aid comparisons across the range of mycoparasites and hosts. Secondly, the results of individual parasite/host combinations are presented according to these categories of events. Thirdly, the results are analysed by statistical comparisons of quantitative data, using either chi-squared tests or t-tests.

5.3.1.1 Pythium oligandrum on Trichoderma aureoviride

The interaction described below involved a hyphal tip of P. oligandrum (diameter 5 μm) that approached a sub-apical region of a hypha of T. aureoviride (diameter 4 μm), the point of contact being 5 μm behind the host tip. The hypha of P. oligandrum was extending at 9 μm min^{-1} up to the time of contact, and its growth rate was uniform over the period of observation until contact occurred. The course of events is summarised in Fig 5.1. The tip of T. aureoviride was not extending, possibly due to trauma when the preparation was mounted, but the hypha showed no cytoplasmic abnormality, with normal cyclosis occurring. The parasite hypha made contact with the host hypha at an angle of 55° (Fig 5.2). The two hyphae were in the same focal plane, on the surface of the agar film. The tip of P. oligandrum grew over the host hypha, slightly deflecting this in the process (Fig 5.3) and its subsequent rate of growth slowed to 4 μm min^{-1}, averaged over the 3 min after contact, but had returned to the previous rate (9 μm min^{-1}) by 7 min after contact, its diameter decreasing to 4 μm in the process. At the time that the drop in parasite hyphal growth was observed, protoplasmic surging was seen in the hypha of T. aureoviride. All of the protoplasm was seen to move towards the host hyphal tip from older regions of the hypha. The surging began 170 sec after contact and lasted for 12 sec; its rate was measured at 1.2 μm sec^{-1} over this period by measuring the rate of displacement of identifiable protoplasmic contents. There was no visible discharge of material from the hypha during this period or subsequently, suggesting that there was a marked release of water from the hypha at the point of contact, or that there was a marked contraction of the cytoplasm. Soon after this protoplasmic surge had ceased, a branch was seen to have developed from the parasite hypha (Fig 5.4). It emerged at the point of contact from the side of the hypha in initial

Fig 5.1 Summary of an interaction between *P. oligandrum* and *T. aureoviride*

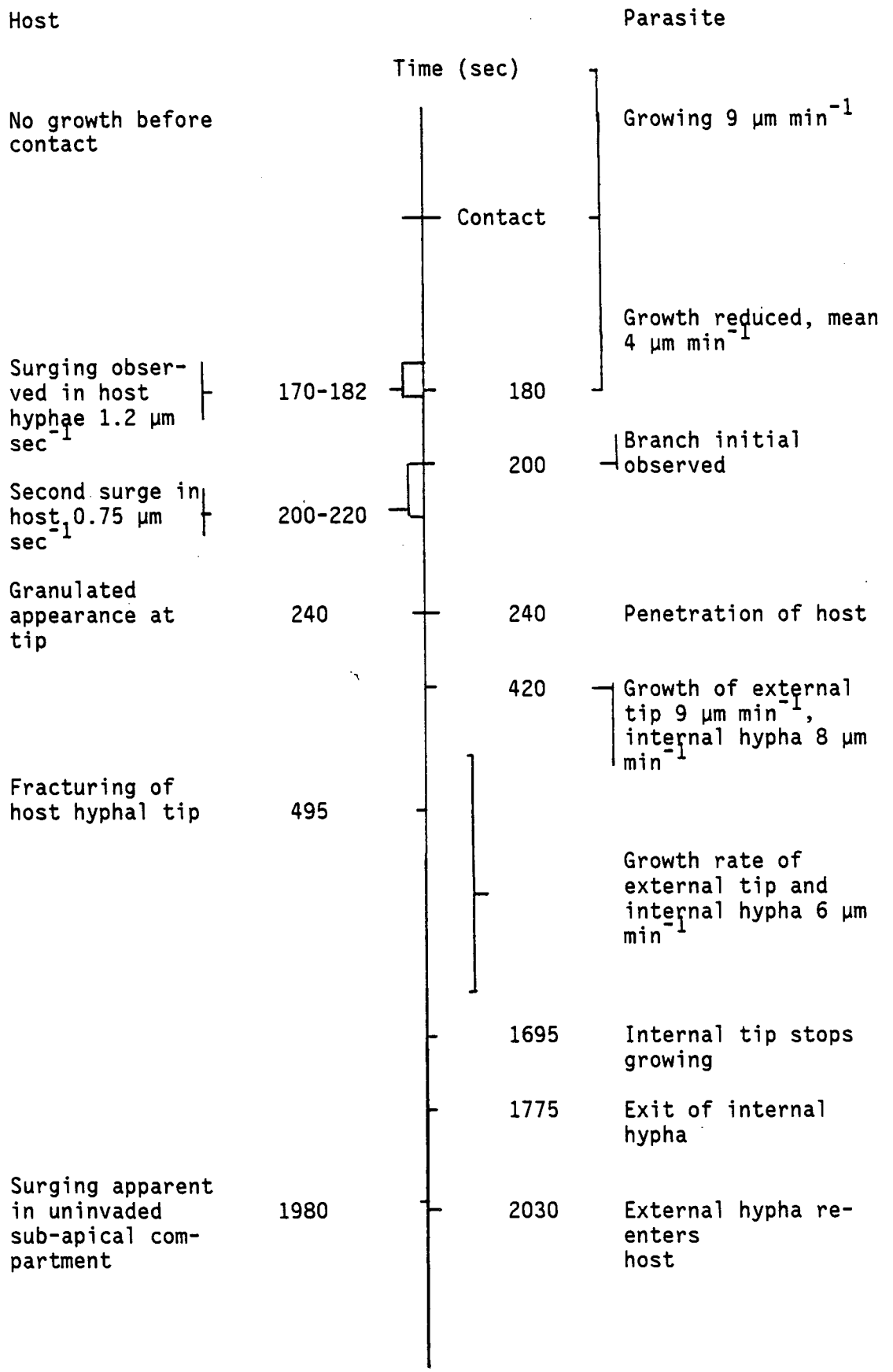
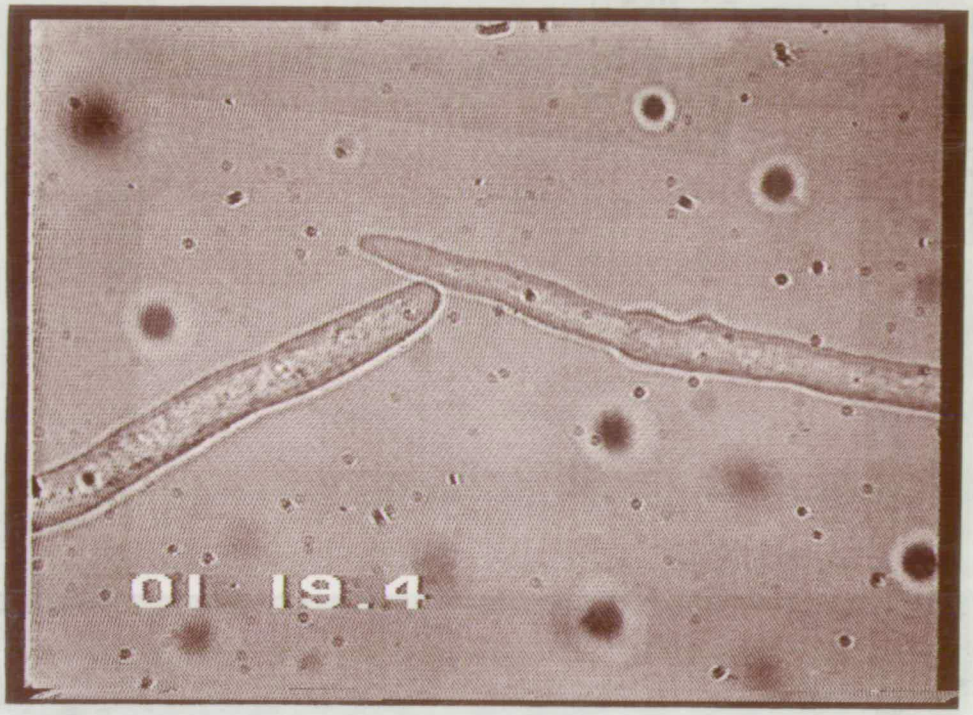


Fig 5.2 Contact of hyphae of P. oligandrum (left) and T. aureoviride. Note - subtract 80 s from time on pictures for times after contact. Bar represents 10 µm. ⟵⟶

Fig 5.3 60 sec post-contact. P. oligandrum hypha causing bending of T. aureoviride hypha as it grows over it.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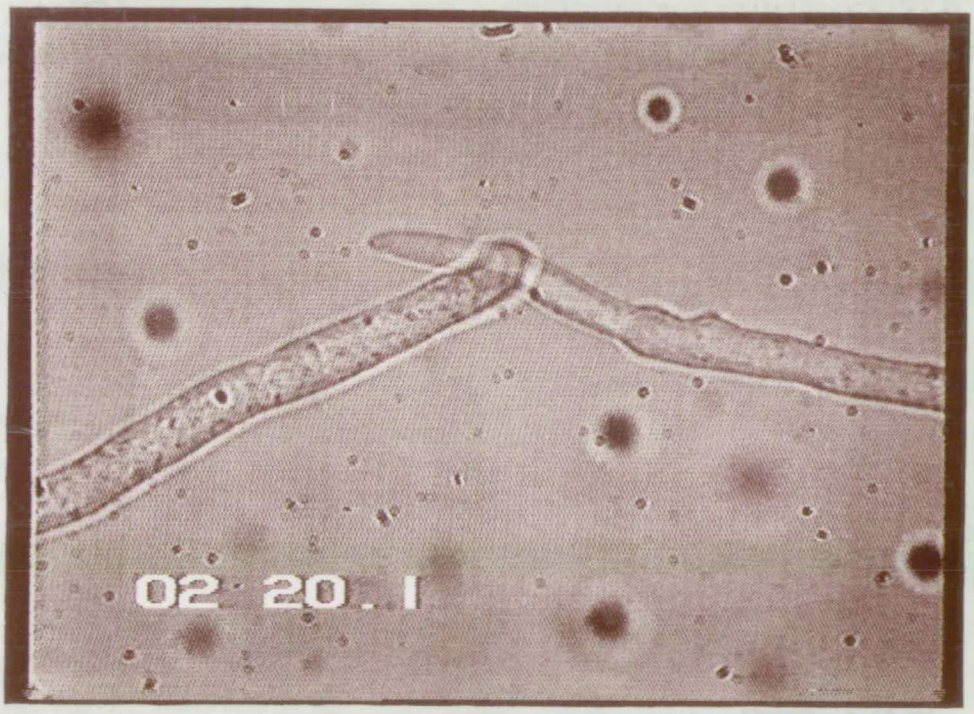
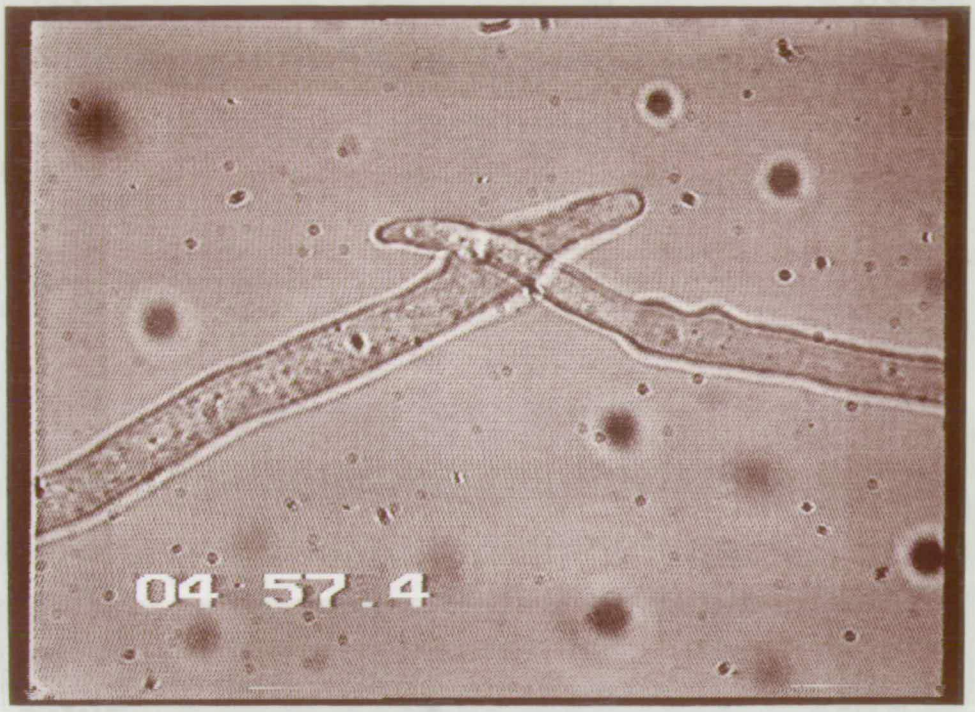

02 20.1

Fig 5.4 217 sec post-contact. Branching by *P. oligandrum* at the point of contact. Note bent appearance of host hypha.

Fig 5.5 270 sec post-contact. Penetration of host hypha by *P. oligandrum* branch. Host hypha has straightened and has a coagulated /vacuolated appearance.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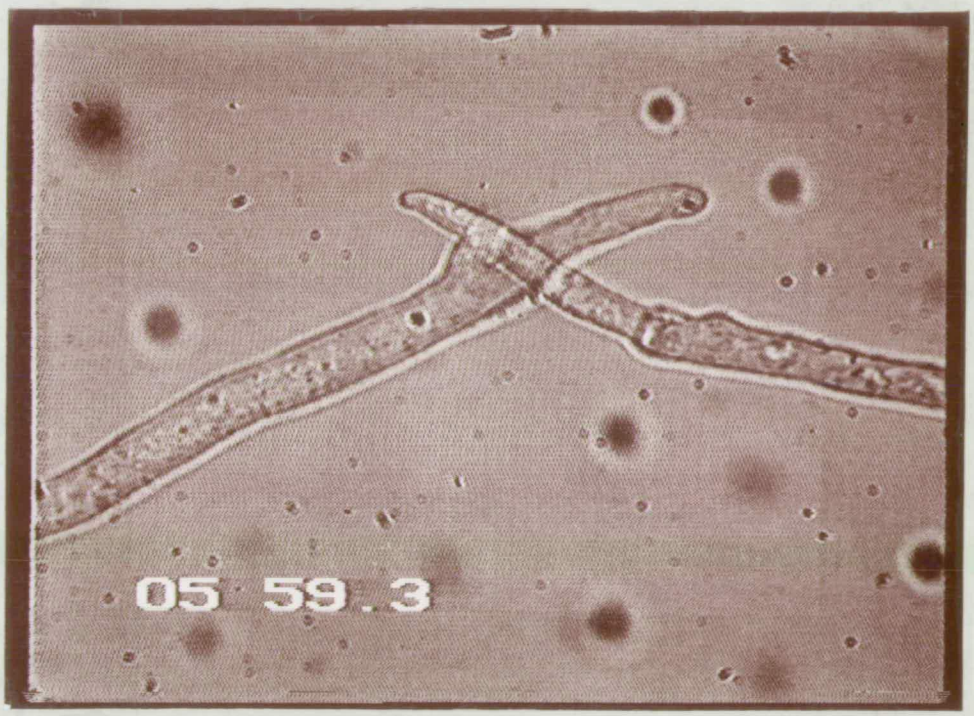
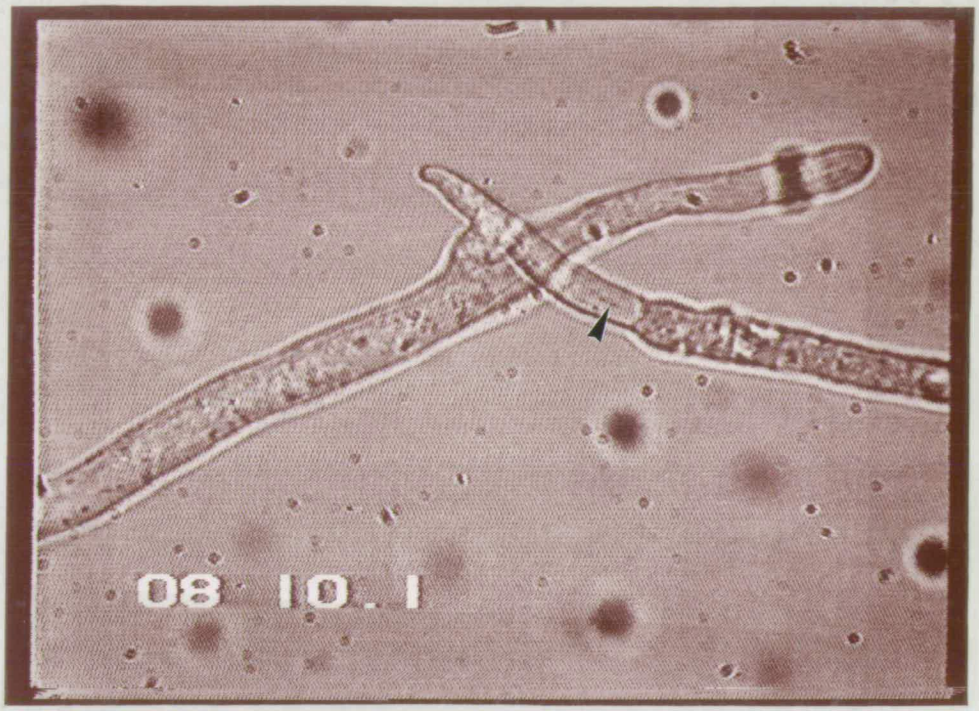

05 59.3

Fig 5.6 410 sec post-contact. Hyphal invasion, 350 sec after contact. An internal hypha (arrowed) is growing bisipetally, filling the host hypha.

Fig 5.7 452 sec post-contact. Growth of internal hypha toward host tip, which is beginning to bend.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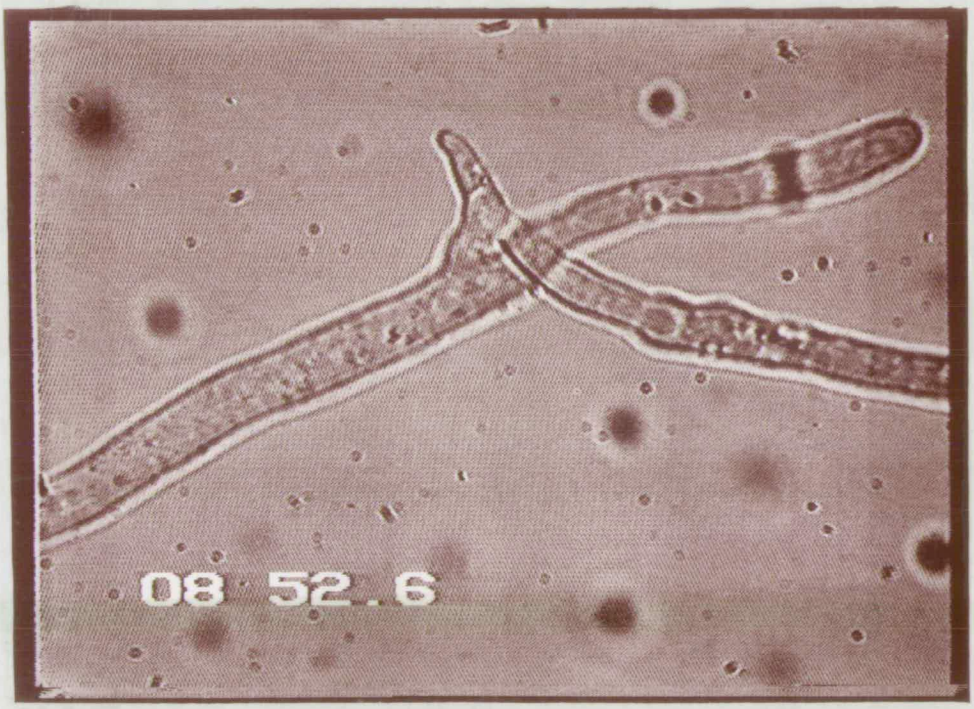
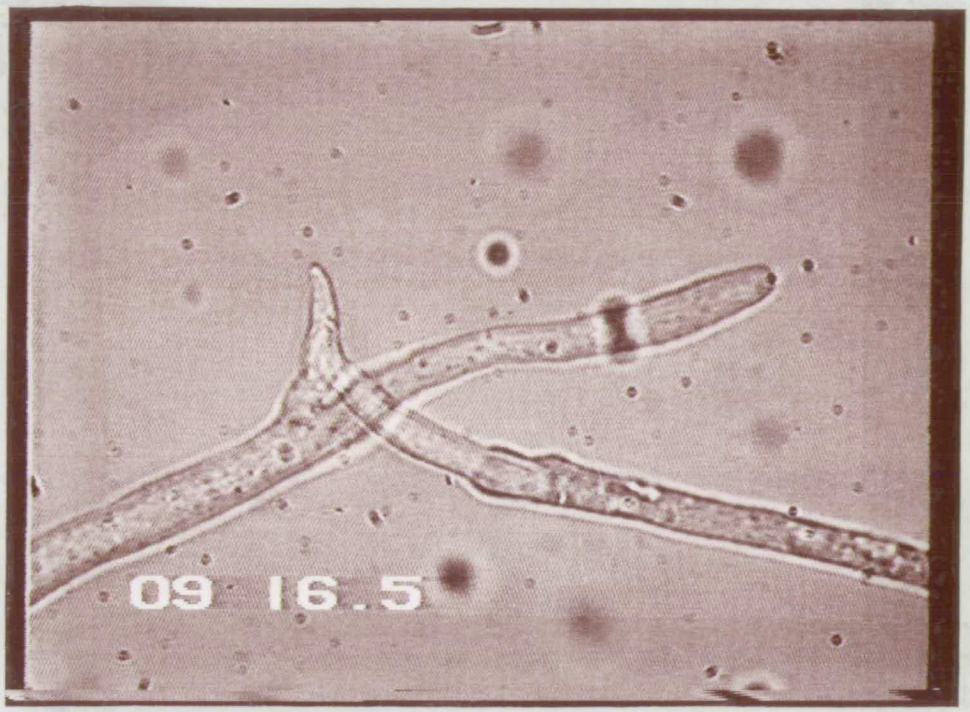

08 52.6

Fig 5.8 476 sec post-contact. The host tip has bent through 45° from its original position, due to growth of an internal hypha.

Fig 5.9 490 sec post-contact. The host tip ruptures leaving a "ghost" which soon becomes invisible (see later figures).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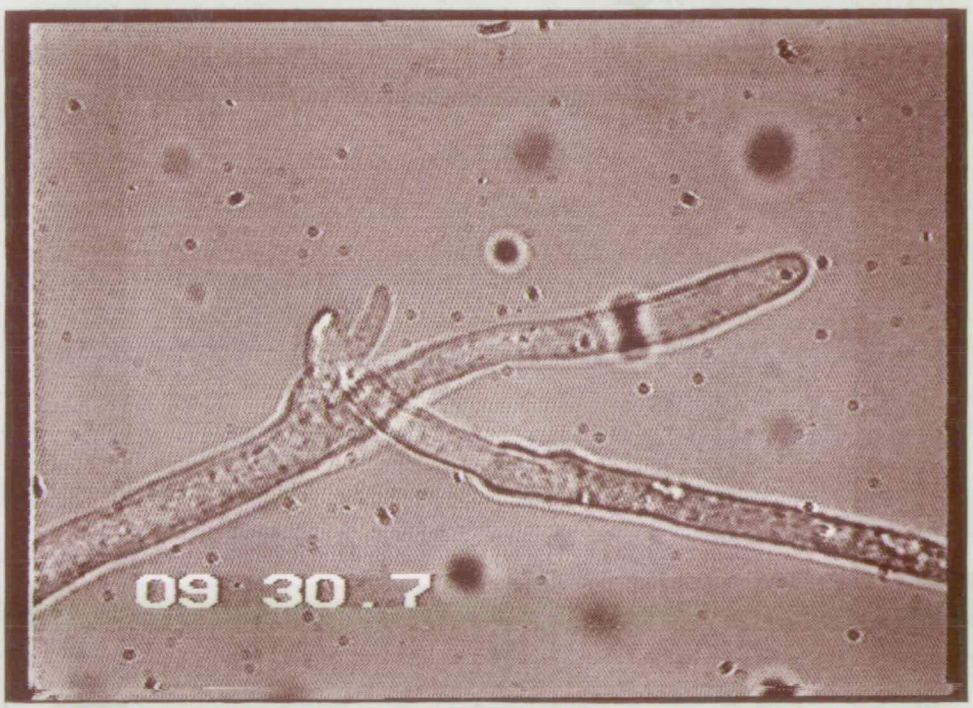
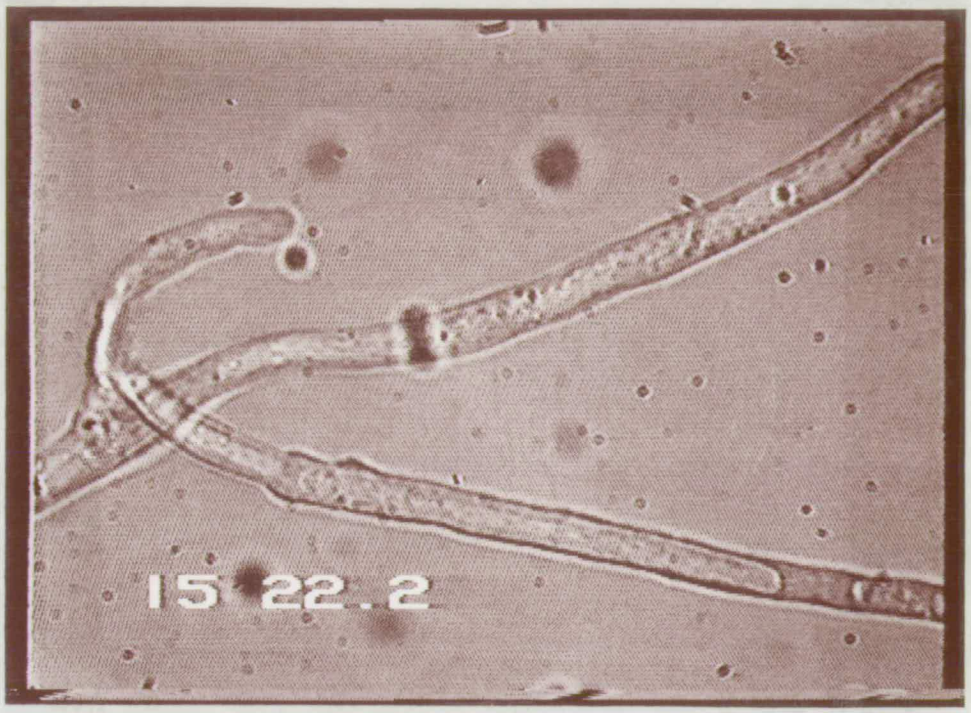

09 30.7

Fig 5.10 14 min post-contact. Initial (contacting) hypha has grown on; a branch has emerged from the host tip; bisipetally-directed parasite hypha has grown further.

Fig 5.11 c 22 min post-contact. On approach to first septum (arrowed), internal hypha exits at what appears to be a mass of coagulated host cytoplasm.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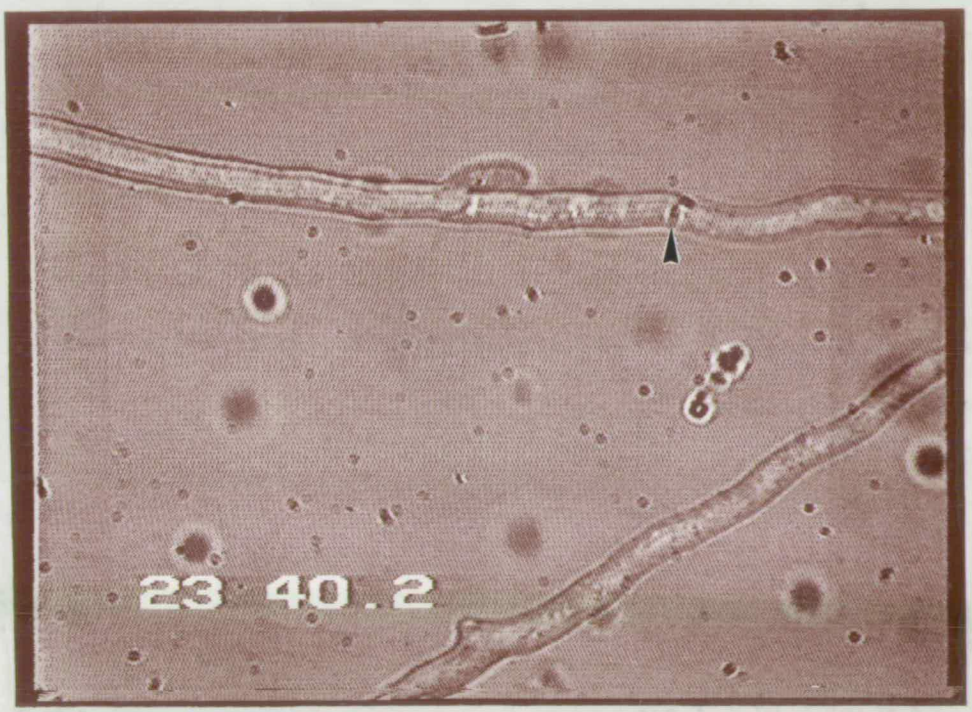
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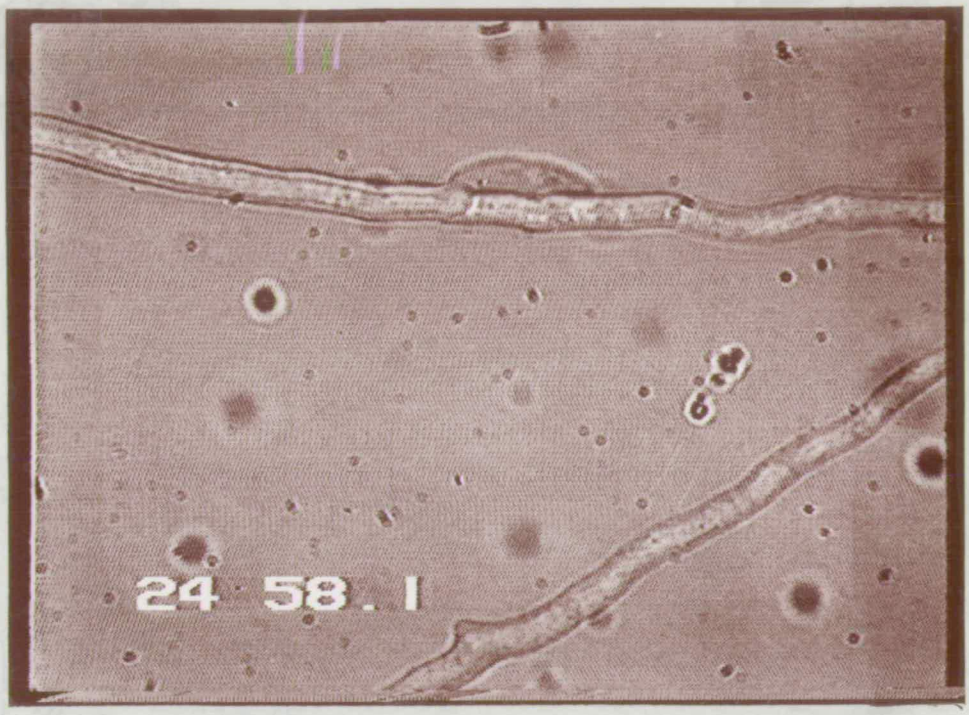
Fig 5.12 c 23 min post-contact. Exited hypha maintains contact with the host hypha.

Fig 5.13 c 29 min post-contact. Exited hypha passes first septum still remaining in contact.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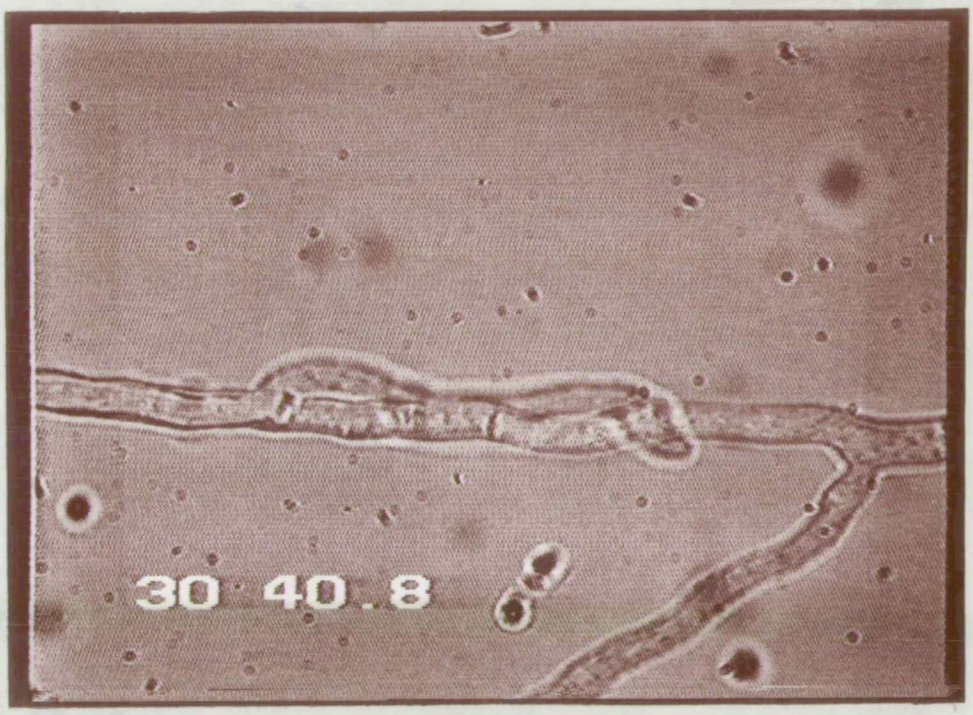
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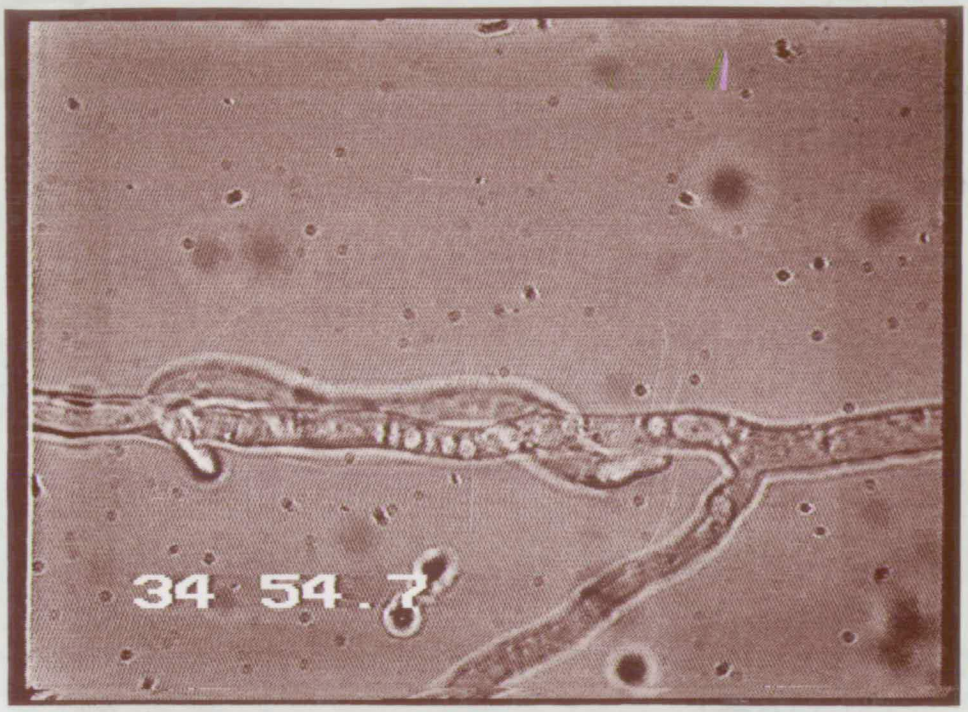
Fig 5.14 c 33 min post-contact. A branch from the internal hypha has exited opposite the first point of exit. The cytoplasm in the sub-apical host compartment begins to coagulate and vacuolate.

Fig 5.15 35 min post-contact. Re-penetration of host hypha by branch of mycoparasite (arrow).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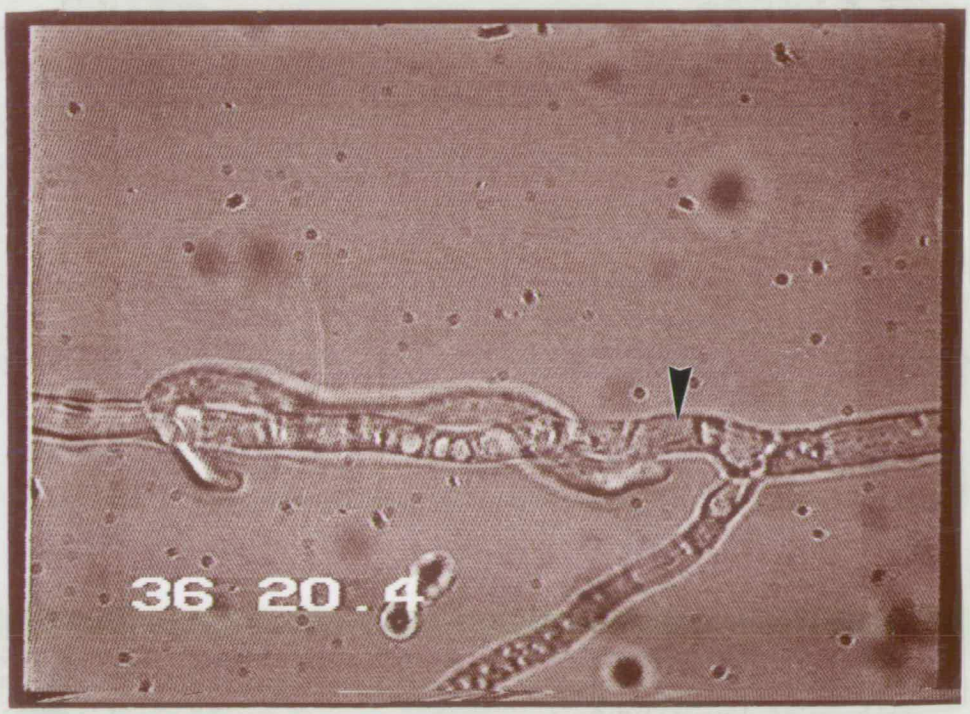

36 20.4

contact with the host. This was followed by a more prolonged surge of protoplasm toward the host tip at 210 sec post-contact, when penetration by the parasite appeared to begin. This second surge lasted for nearly 20 sec with a rate of 0.75 μm sec^{-1}. The surging ceased first at the tip and then successively further back in the host hypha. Once it had ceased, there was no further evidence of regulated protoplasmic movement in the host tip and sub-apical regions. Instead the tip became slightly granulated at 240 sec post-contact, and at this stage the branch of the mycoparasite appeared to have penetrated $\leq$ 3 μm into the host hypha. Forty sec later, the host tip became re-orientated with respect to the rest of the host hypha and the host hypha began to take on a more granular appearance further back (Fig 5.5). Concurrently, as the invading hypha reached the furthest side of the host wall it appeared to branch in both directions within the host. The branch growing tipwards was initially narrow ($\leq$ 1 μm in diameter); it grew to about 2 μm in length and then stopped temporarily. The branch that grew into the older part of the host hypha was wide enough to fill this (without causing distension of the host wall) and it grew rapidly (8 μm min^{-1} based on measurements over the period 9 to 15 min post-contact) (Fig 5.6). The branch of the invading hypha directed towards the host tip subsequently re-grew and filled the host tip, causing the tip of the host hypha to bend upwards from the point of invasion. The host tip was re-orientated through 90° in a period of 60 sec and finally appeared to fracture 495 sec post-contact, releasing a small amount of cytoplasm and leaving the cell wall as a "ghost" (Figs 5.2 to 5.9). The small hypha of the parasite retained the new orientation, and then grew from the point of fracture at 4 μm min^{-1}. Meanwhile both the main (external) hypha that had initially made contact with the host tip and the basipetally-directed internal hypha continued to grow, but at the

reduced rate of 6 μm min^{-1} (Fig 5.10). The internal hypha continued to grow within the host hypha for a further 20 min and then stopped, due probably to a dense coagulation of the host protoplasm as the invading hypha neared the first host septum. After stoppage for 70 sec its tip became re-orientated towards the lateral wall of the host hypha and it grew out of the host hypha, emerging at an angle of 30° to this (Fig 5.11). The hypha rapidly changed its direction of growth such that it grew alongside the host hypha at a rate of 5 μm min^{-1}, the hyphal tip remaining in contact with the host hypha (Fig 5.12). Over a period of 12 min it grew along, physically in contact with the host hypha, coursing over the top of this (Figs 5.13 and 5.14). At 29 to 30 min post-contact (8 min after it had emerged from the host), a branch emerged downward from the parasite at the point of hyphal exit and grew out of the host, although this played no further part in the interaction. After 33 min post-contact, surging became apparent in the uninvaded part of the sub-apical compartment of the host hypha toward the host septum which at this time lay half-way between the parasite hyphal tip and the point of exit (Fig 5.14), resulting in host granulation. At the same time a branch emerged from the parasite 2 μm from the tip at a point next to the host hypha; this tip re-invaded the host at the time that the surging was occurring. The internal hypha reached the opposite wall of the host hypha, then re-orientated to grow, basipetally-directed, inside the host hypha at a rate of 7 μm min^{-1} (Fig 5.15).

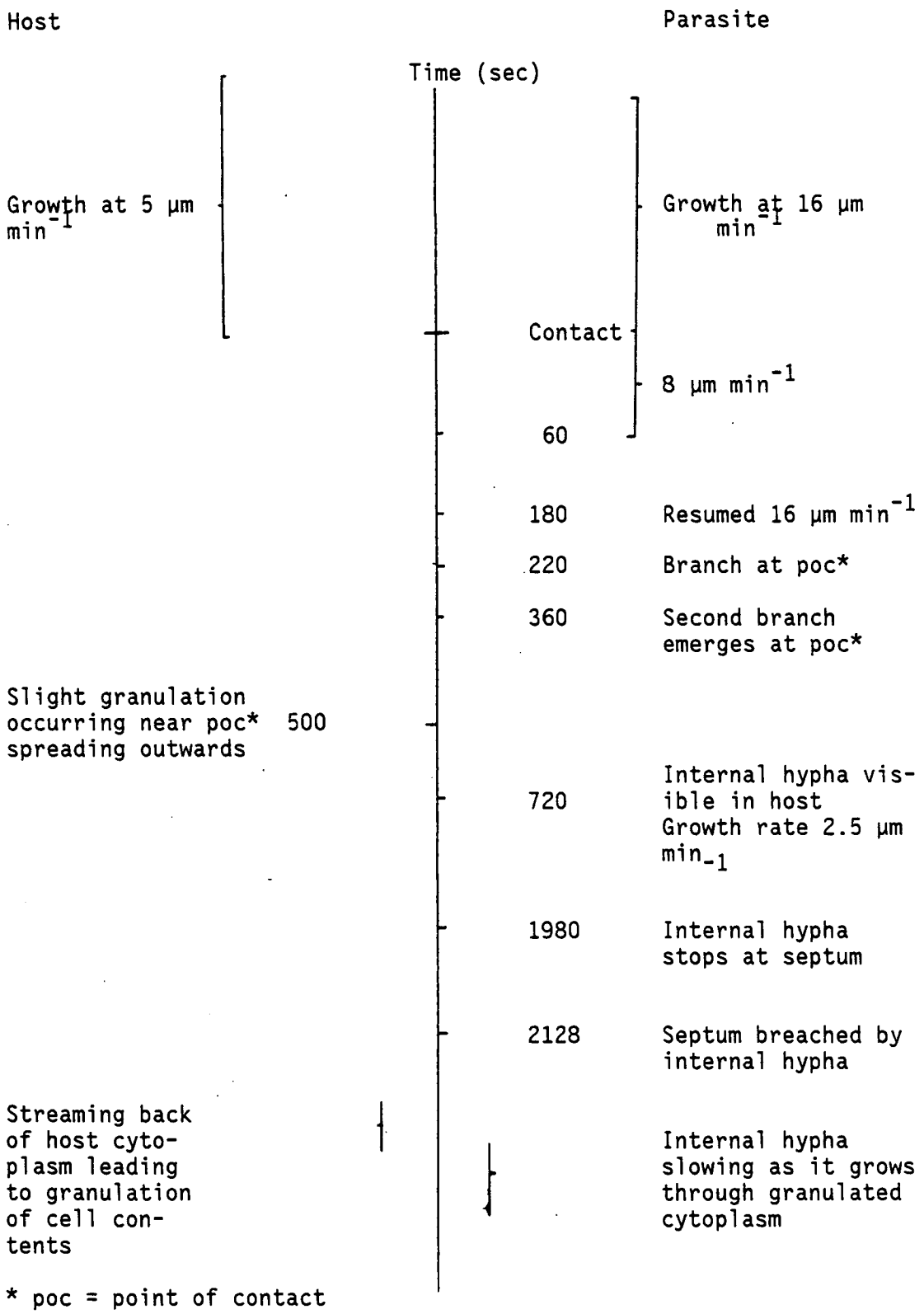
The whole interaction described above was characterised as a <u>direct penetrative interaction</u> involving an aggressive mycoparasite and a susceptible host.

5.3.1.2 Pythium oligandrum on Rhizoctonia solani (isolate GM1)

Unlike the previous interaction, the following one that involved P. oligandrum on R. solani (GM1) was characterised as a penetrative interaction with coiling, involving an aggressive mycoparasite and a partly resistant host The course of events is summarised in Fig 5.16.

A hyphal tip of P. oligandrum (5.5 µm diameter) approached the side of a hypha of R. solani (8 µm diameter) at a point 425 µm from the host tip. Before contact the parasite was growing at a steady rate of 16 µm min^{-1}, and the host at 5 µm min^{-1} at its tip. The point of initial contact was in the apical compartment of the host (Fig 5.17). The parasite made contact at an angle of 85° to the host hypha and caused a slight 'bending' displacement of the host hypha as it grew over and past it. A reduction in growth rate of the parasite hypha was observed in the first minute after contact, falling to 8 µm min^{-1}, but after 3 min the rate was again 16 µm min^{-1}. There was no obvious change in behaviour of the host until 480 sec post-contact. Meanwhile, however, a branch was seen to emerge from the mycoparasite at the point of initial contact, after 220 sec, and this branch began to grow under the host hypha but soon appeared to slow down. A second branch started to emerge from the same side of the parasite hypha at 360 sec and grew over the host hypha. At 480 sec post-contact there was a momentary protoplasmic movement in the host hypha towards the point of contact, and this probably represented the beginning of penetration by the mycoparasite. Slight granulation began to occur in the host hypha near the point of contact after 560 sec and this granulation progressed in both directions from the point of contact (Fig 5.18). The host cytoplasm was very dense, obscuring internal events involved in penetration and early growth of the penetrating hypha, but by 13 min post-contact an internal hypha of P. oligandrum was seen to have grown 15 µm from the point of

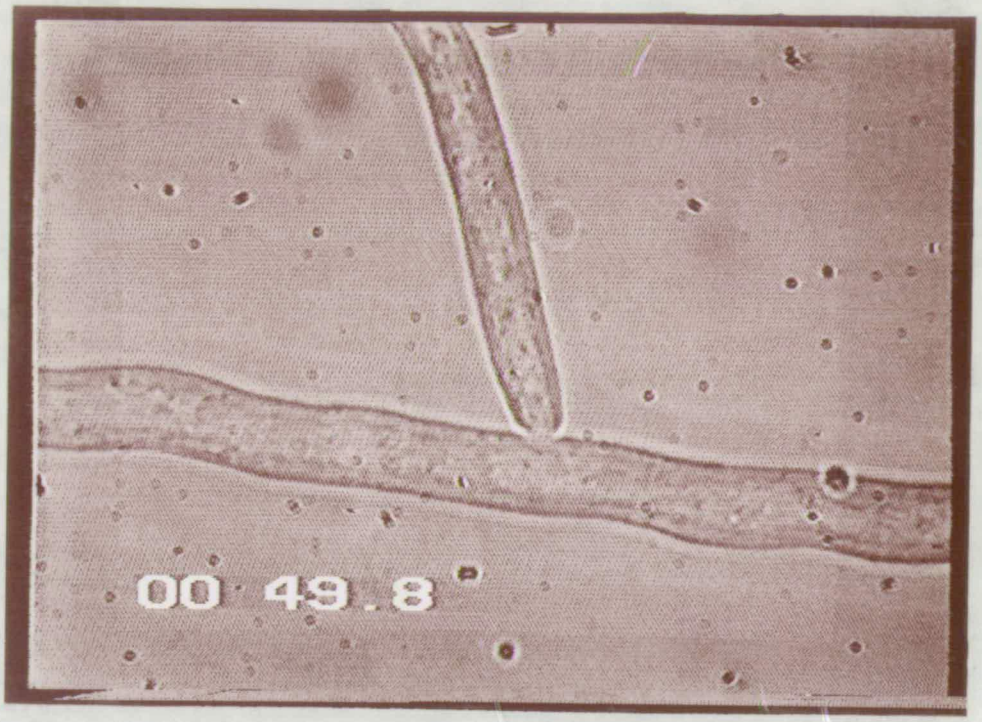
Fig 5.16 Summery of an interaction between P. oligandrum and R. solani GM1



* poc = point of contact

Fig 5.17 Hypha of *P. oligandrum* makes contact with side of hypha of *R. solani* (GM1). Bar represents 10 µm. ⟵⟶

Fig 5.18 540 sec post-contact. First parasite branch has stopped growing. A second branch (arrowed), starts to grow into the host hypha, causing granulation/vacuolation around point of contact.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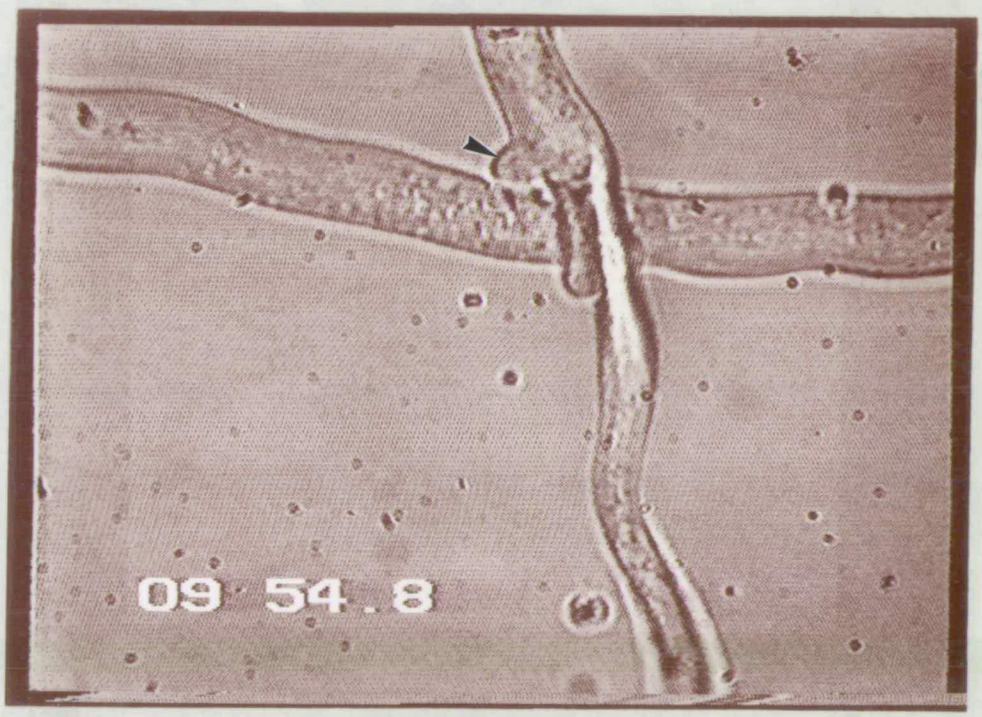
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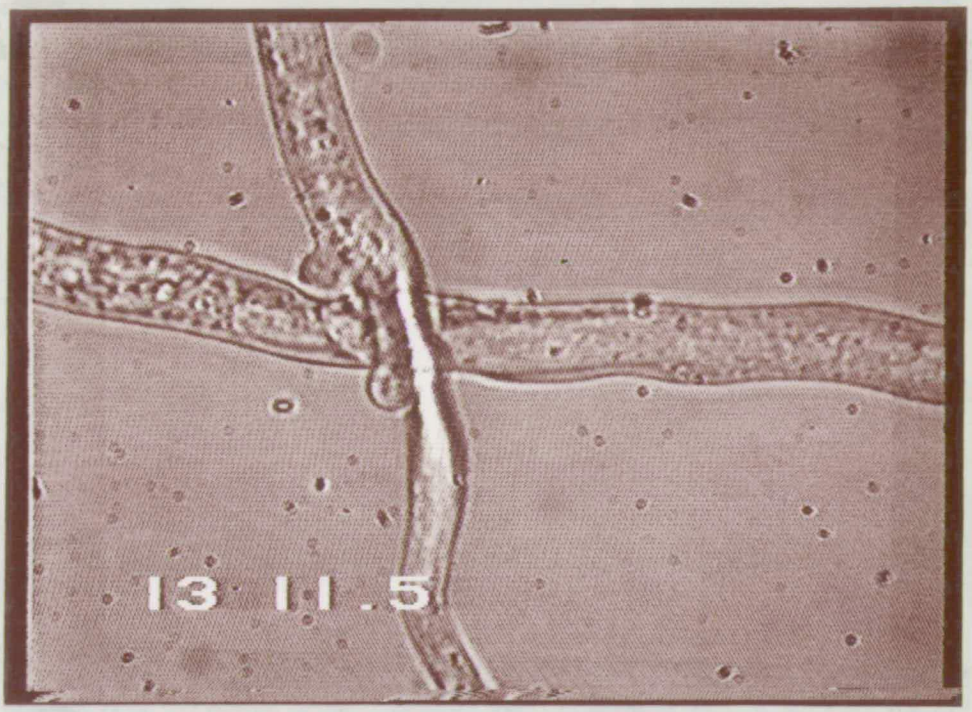
Fig 5.19 740 sec post-contact. Internal hyphae are growing in both directions in the host.

Fig 5.20 c 33 min post-contact. Internal hypha (arrow) approaches first septum which has bulged into the apical compartment.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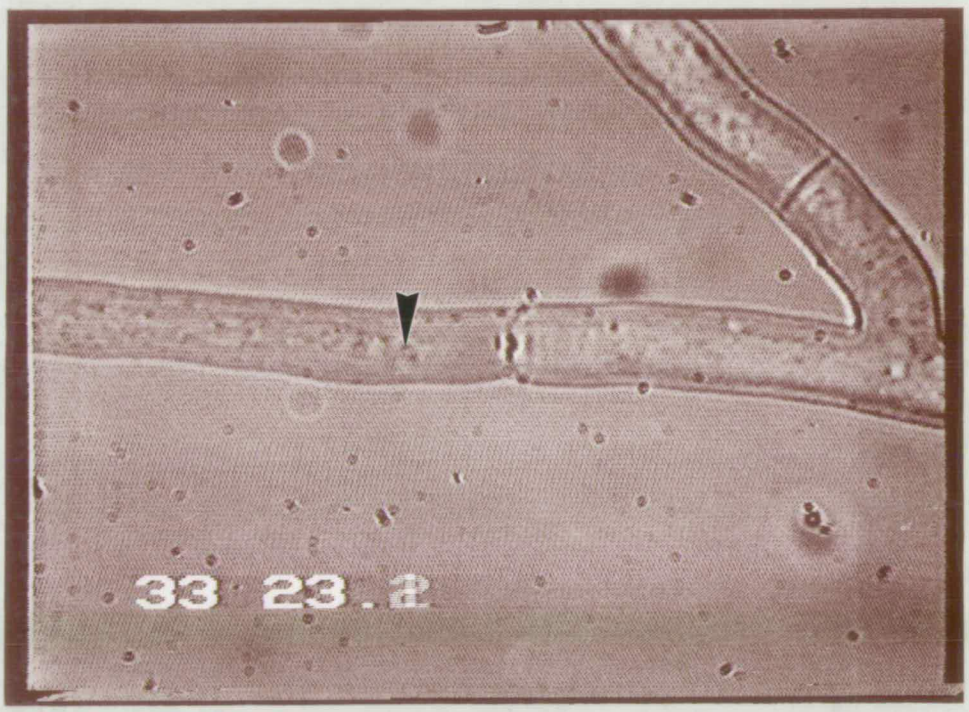
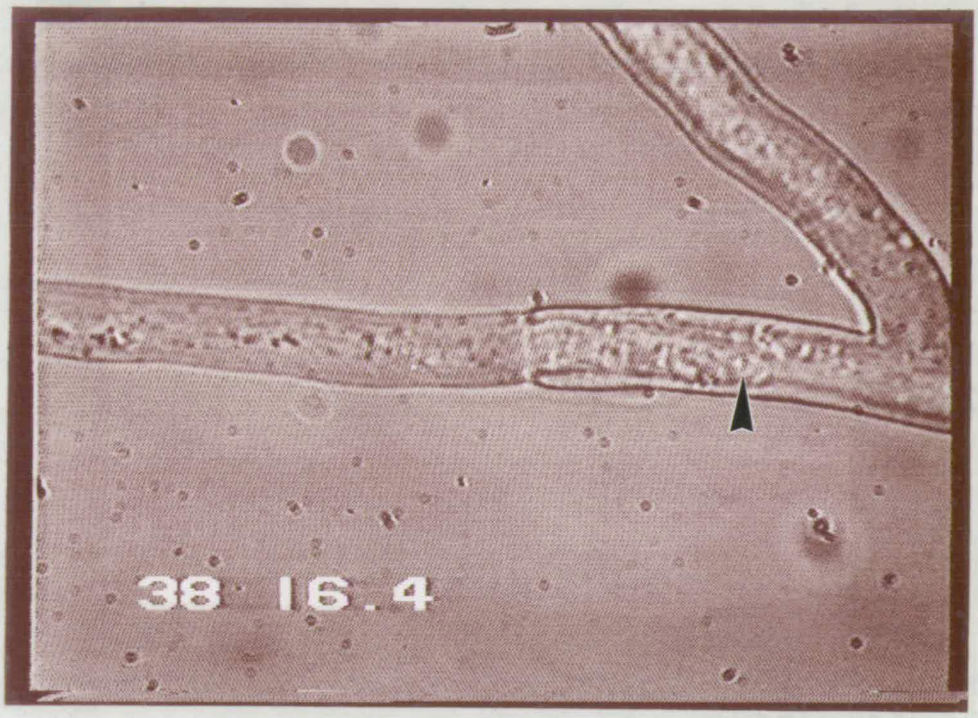

33 23.2

Fig 5.21 c 37 min post-contact. Internal hypha (tip arrowed) has grown through septum and caused coagulation of host contents (compare with Fig 5.20).

Fig 5.22 6 sec after above figure. A massive backward surge of cytoplasm in the R. solani hypha six seconds later more clearly reveals the internal hypha.


38 16.4

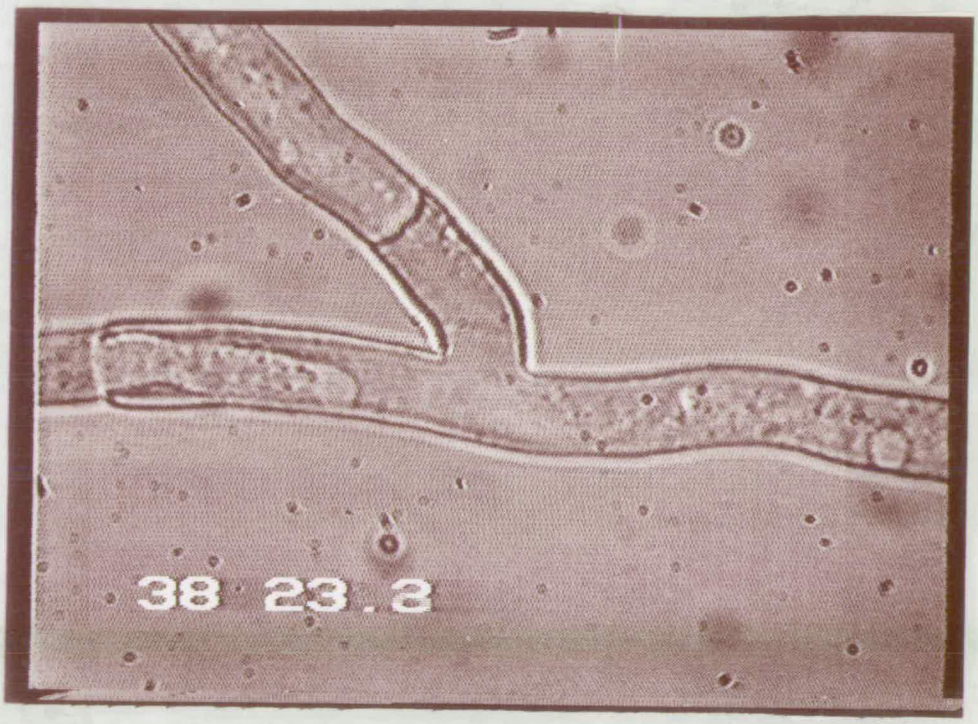
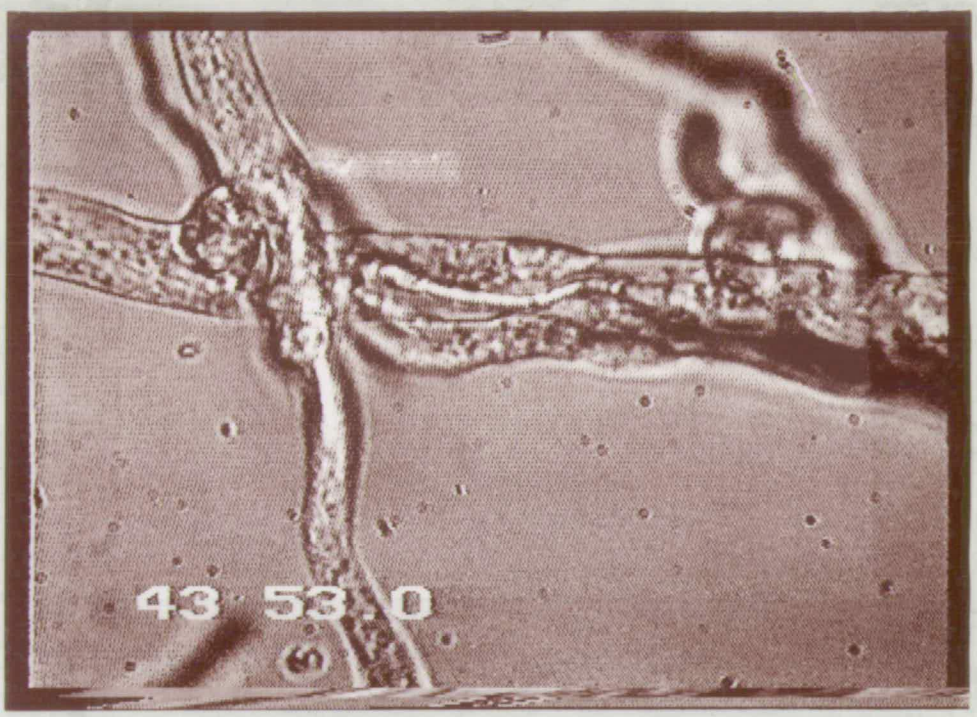

38 23.2

Fig 5.23 Area around initial contact area, 43 mins after contact. Note the proliferation of coiling branches maintaining close contact with the host hypha.

Fig 5.24 c 59 min post-contact. Internal hypha in sub-apical compartment. Note thinness of hypha compared to the much thicker diameter of the same hypha in Fig 5.22.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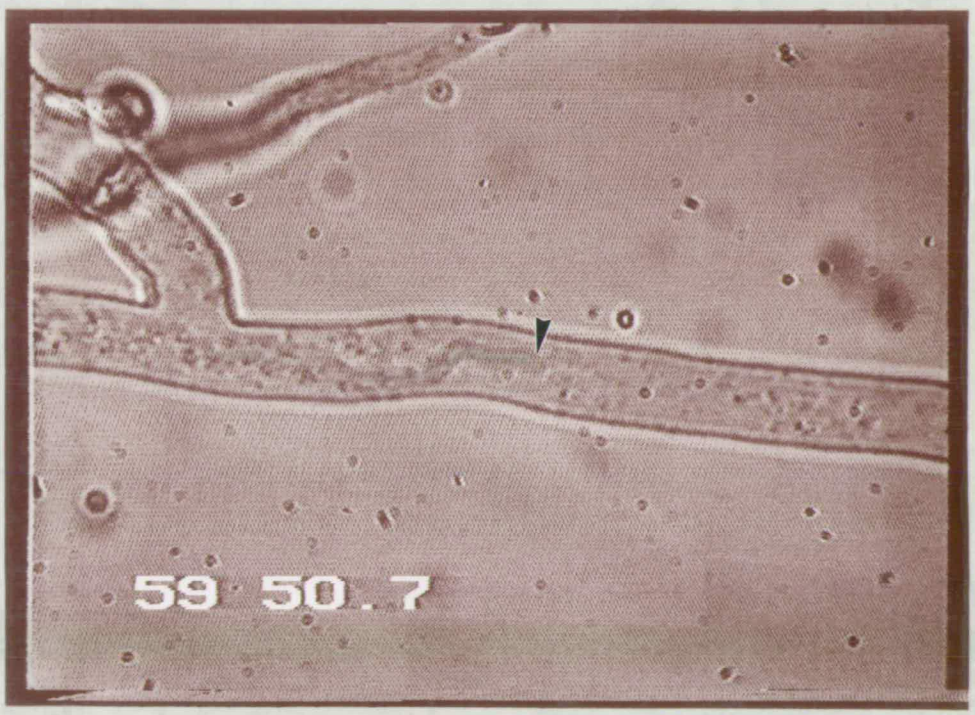
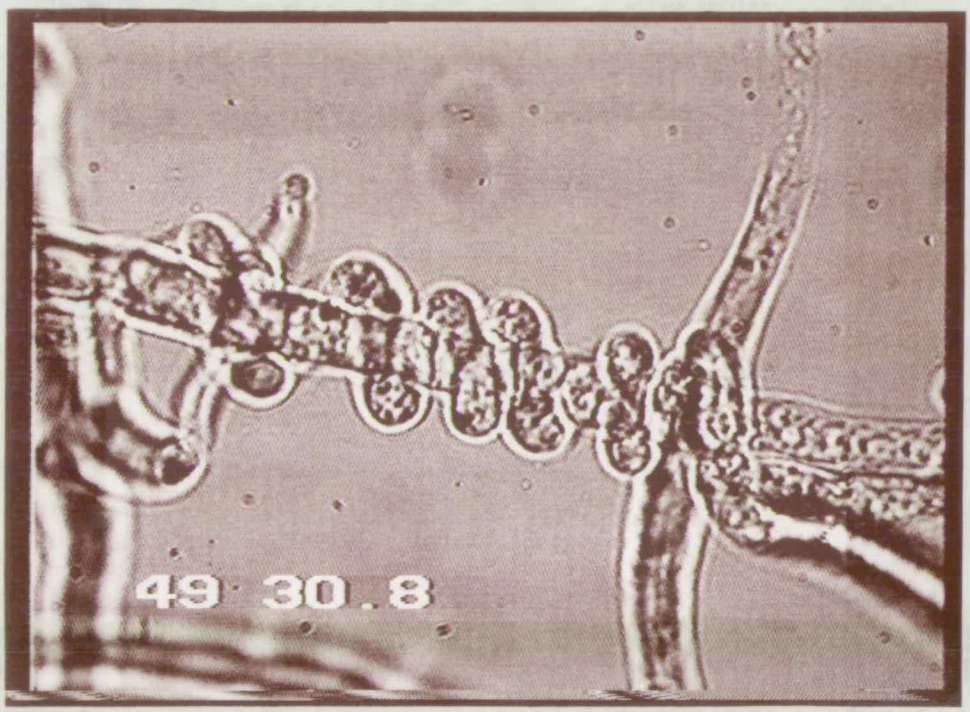

59 50.7

Fig 5.25 c 100 min post-contact. Tip of the invaded R. solani hypha, containing an apparently narrow and twisted parasite hypha. Host cell outline is irregular. Tip is 450 µm from initial point of contact, having grown for 5 mins after contact before stopping.

Fig 5.26 Coiling of P. oligandrum around a hypha of R. solani T125 48 mins after contact.


99 00.0


49 30.8

contact (Fig 5.19). The tip of the internal hypha was difficult to discern as it grew within the host, although the more mature regions of the internal hypha could be discerned more easily owing to a more granular appearance of the hyphal contents. The internal growth rate was slow (2.5 μm min^{-1}), and the diameter of the internal hypha seemed to vary between 1 and 5 μm. The course of the internal hypha was not straight but convoluted, as it apparently followed a path of least resistance. At 33 min post-contact (Fig 5.20), the internal hypha (growing away from the host tip) encountered a septum, where it temporarily stopped, its diameter at this time being rather narrow (2 μm diameter). The septum bulged toward the internal hypha, clearly delineating healthy and invaded host compartments. One hundred and forty-eight sec after contact was made with the septum, a slight jarring of the protoplasm was observed in the healthy compartment, denoting that the septum had been breached by the internal hypha. However, the density of the host cytoplasm partly prevented observation of the internal hypha (Fig 5.21) until 110 sec after the septum was penetrated, when the host cytoplasm suddenly appeared to stream back from the newly invaded compartment. The internal hypha appeared to have thickened in diameter as it entered this compartment (Fig 5.22). The protoplasm of the invaded compartment rapidly assumed a granulated appearance, which appeared to slow the internal hyphal growth rate and again narrow the internal hyphal diameter to 2 μm (Fig 5.24). This pattern was observed in almost identical fashion when the internal hypha reached another septum 115 min post-contact.

Meanwhile, towards the host tip, there was a proliferation of external branching and coiling near the point of initial contact (Fig 5.23). Also, by 50 min post-contact, the host tip was almost completely colonised by a thin, twisting internal hypha. After 100 min the host

tip cell was no longer smooth in outline but irregularly "bumpy" and its contents were granulated around the internal hypha, which by this stage had apparently reached the tip but had not exited from it (Fig 5.25).

A more typical example of coiling is shown in Fig 5.26 where P. oligandrum hyphae are seen coiled tightly around a hypha of R. solani T125.

5.3.1.3 Pythium mycoparasiticum on Fusarium oxysporum

The interaction below was characterised as a direct penetrative interaction with exit pegs. The course of events is summarised in Fig 5.27. A hyphal tip of P. mycoparasiticum (3 µm diameter) approached the side of a hypha of F. oxysporum (4.5 µm diameter) at a point 60 µm from the tip. Prior to contact the parasite was extending at a steady rate of 10 µm min^{-1} for the previous 6 min, the host at 4 µm min^{-1}. The parasite hypha made contact at an angle of 40° to the host hypha (Fig 5.28), but changed direction and grew alongside the host hypha for 180 sec before growing over the host hypha at an angle of 35° and a growth rate of 10 µm min^{-1}. Contact was maintained with the host hypha throughout this time and there was no evidence of host cytoplasmic dysfunction as the parasite contacted or grew alongside. At 220 sec after contact, cytoplasmic surging was visible in the host hypha as a slow and unsteady movement of protoplasm towards the host tip. At 420 sec a branch appeared on the mycoparasite hypha at a point adjacent to the host hypha but 20 µm further away from the host tip than the initial point of contact. Penetration appeared to be direct, as the branch grew down into the host hypha which started to show coagulation of the cytoplasm, particularly near the point of entry (Fig 5.29). The internal hypha then grew internally away from the tip, but a small branch soon emerged from it and began to grow tipwards. A second invasion

Fig 5.27 Summary of an interaction between Pythium mycoparasiticum and Fusarium oxysporum

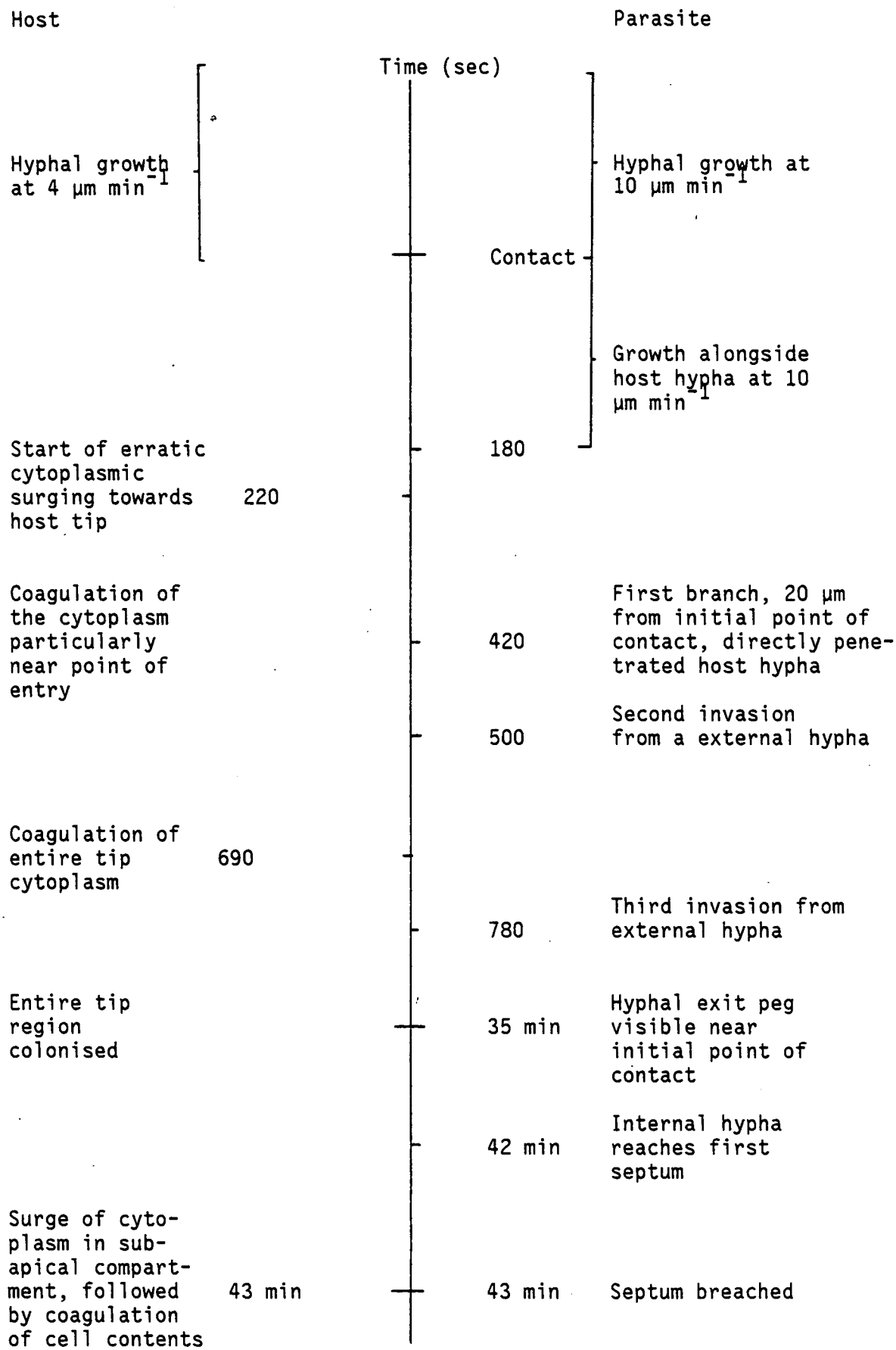
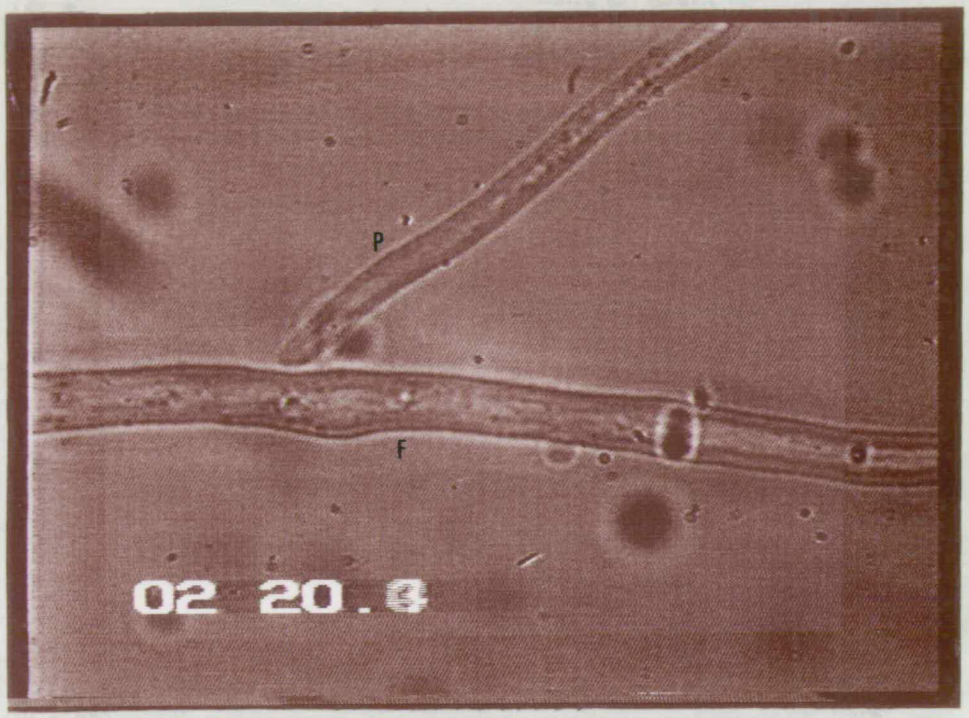


Fig 5.28 Hypha of *P. mycoparasiticum* (P) makes contact with hypha of *F. oxysporum* (F). Bar represents 10 µm. ⊢——⊣

Fig 5.29 525 sec post-contact. After growing along and over host hypha, the mycoparasite branched and penetrated (arrow). The host hyphal contents became coagulated/vacuolated in this region.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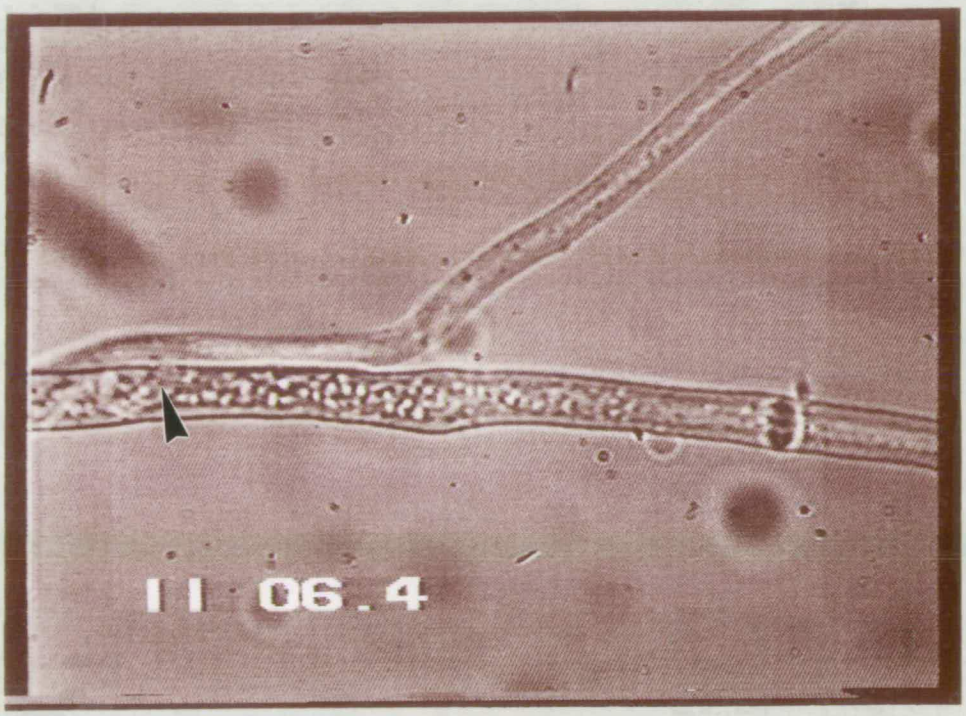
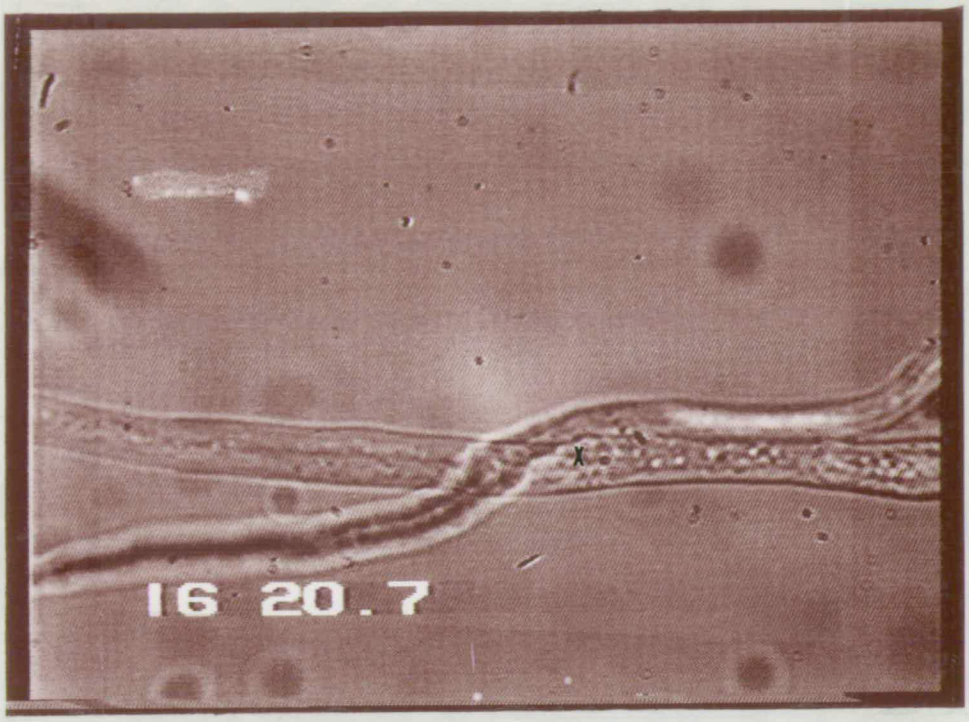

11 06.4

Fig 5.30 840 sec post-contact. A second penetration has occurred (at point marked x, but not clearly visible) and the invading hypha is growing alongside the first.

Fig 5.31 1120 sec post-contact. The three penetrating branches are clearly visible within the host hypha.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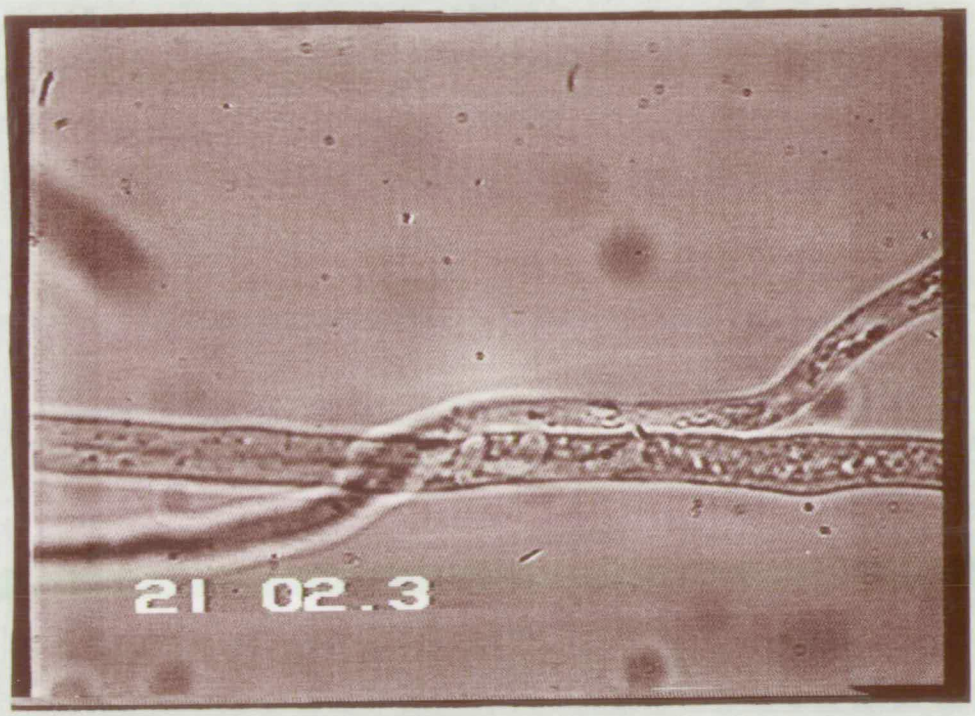
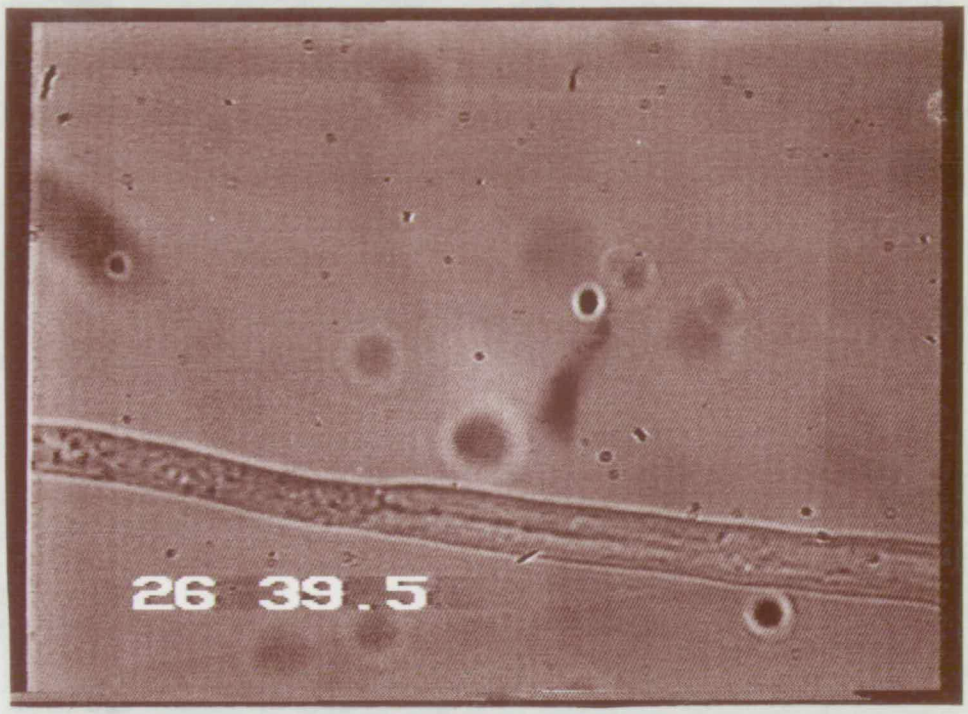

21 02.3

Fig 5.32 c 24 min post-contact. Internal hyphae growing side by side within host hypha. Note distension of the invaded area of the host hypha.

Fig 5.33 c 39 min post-contact. Internal hyphae approaching first septum, which has bulged into the parasitized apical cell. Note difference in host cytoplasm on each side.


26 39.5

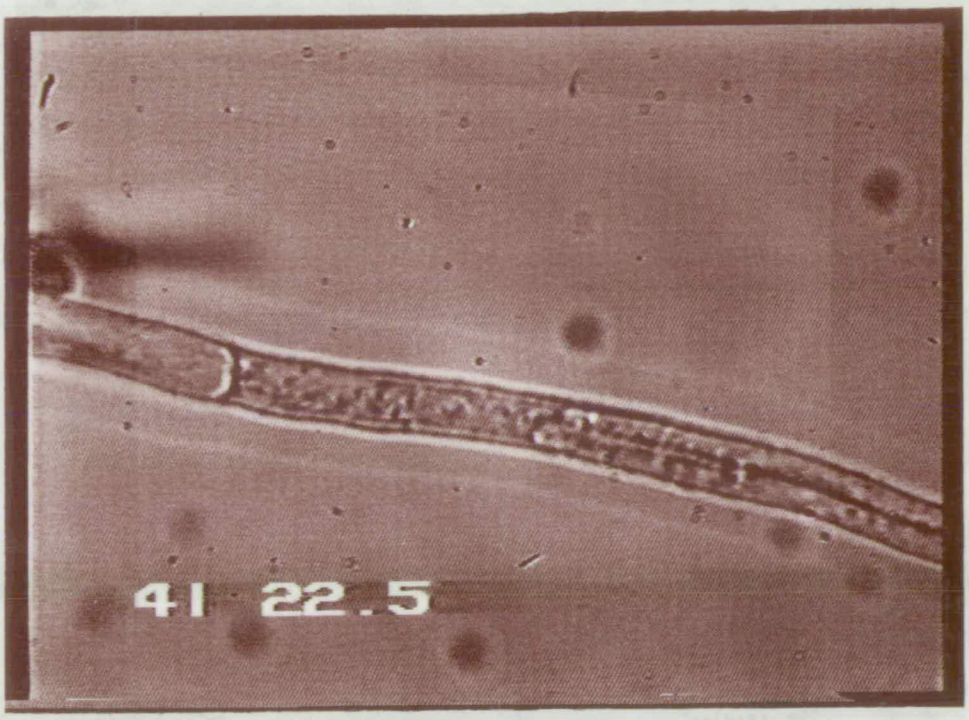
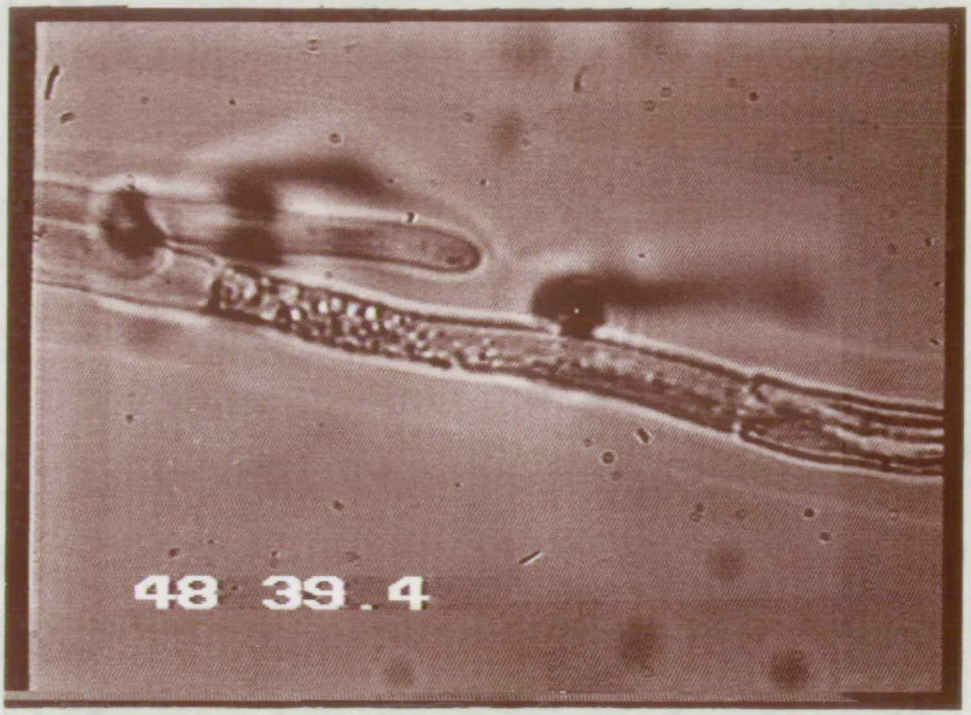

41 22.5

Fig 5.34 c 46 min post-contact. Internal hypha approaches second septum. Note one internal hypha has overtaken the other. Again note cytoplasmic differences on each side of septum.

Fig 5.35 c 47 min post-contact. Exit pegs (arrowed) of *P. mycoparasiticum* have emerged from a parasited hypha.


48 39.4

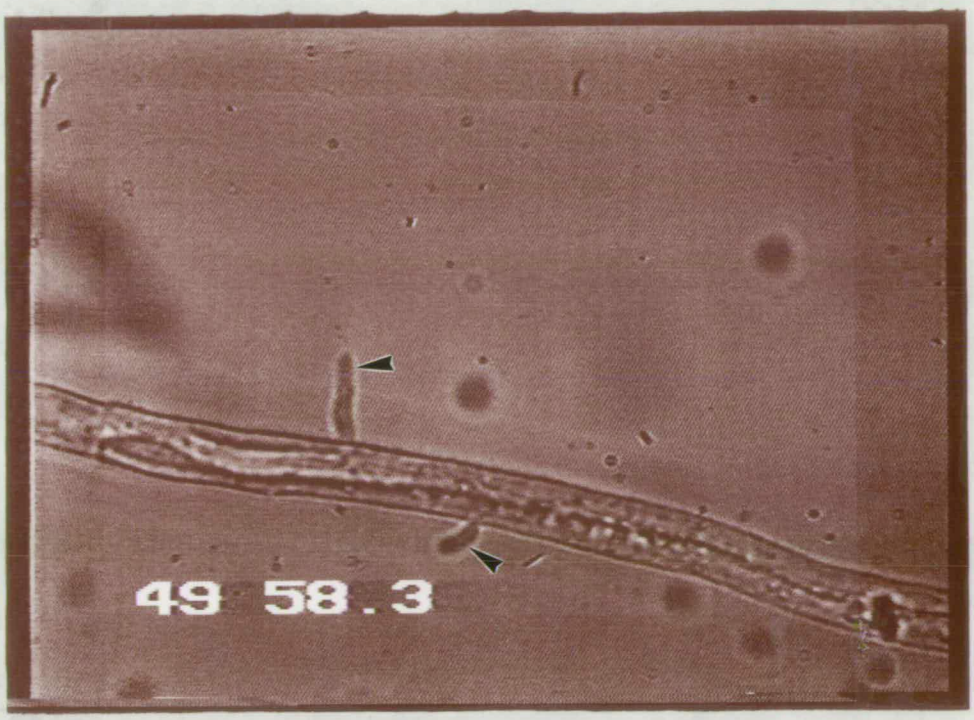
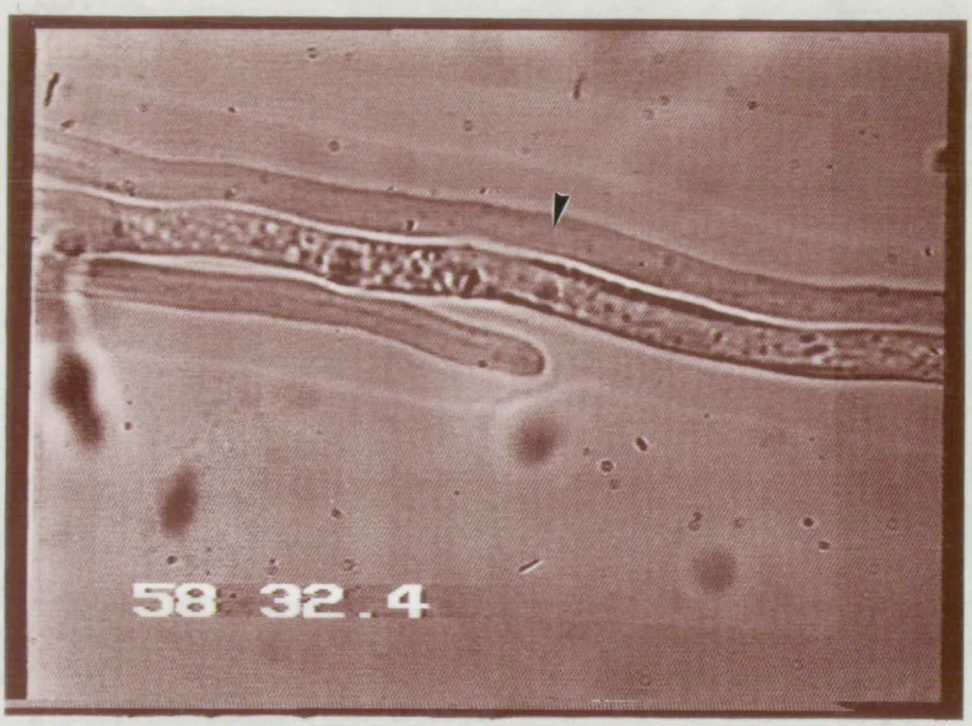

49 58.3

Fig 5.36 c 56 min post-contact. Host hypha (arrowed) adjacent to parasitised hypha is seemingly unaffected by it.

Fig 5.37 c 60 min post-contact. Exit peg of mycoparasite (arrowed) grows out from invaded hypha, up into the unaffected host hypha.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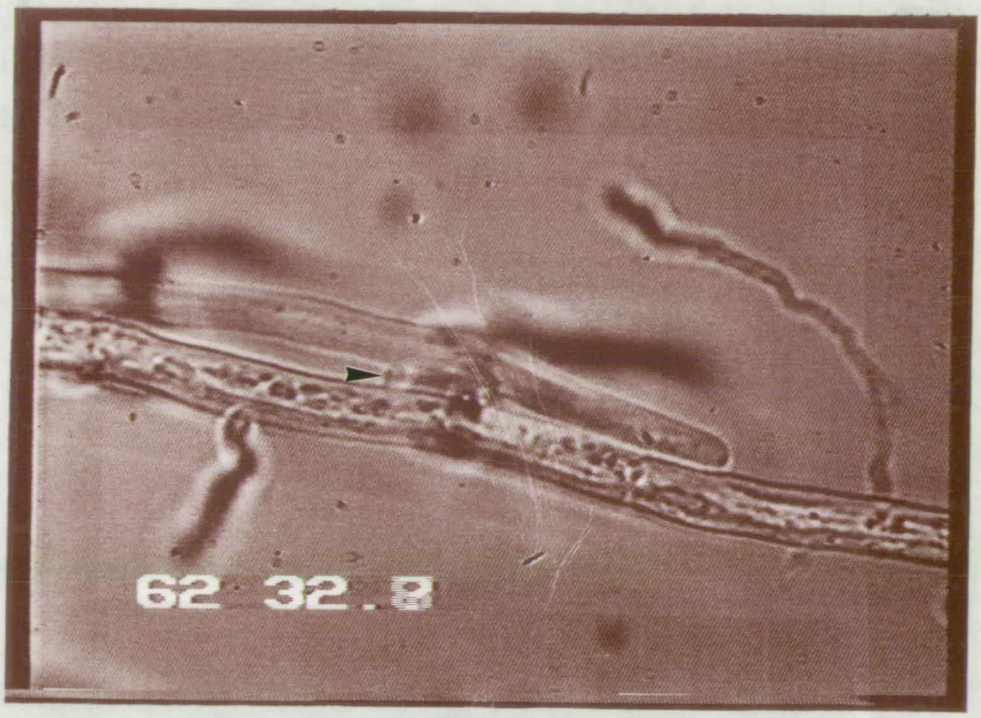
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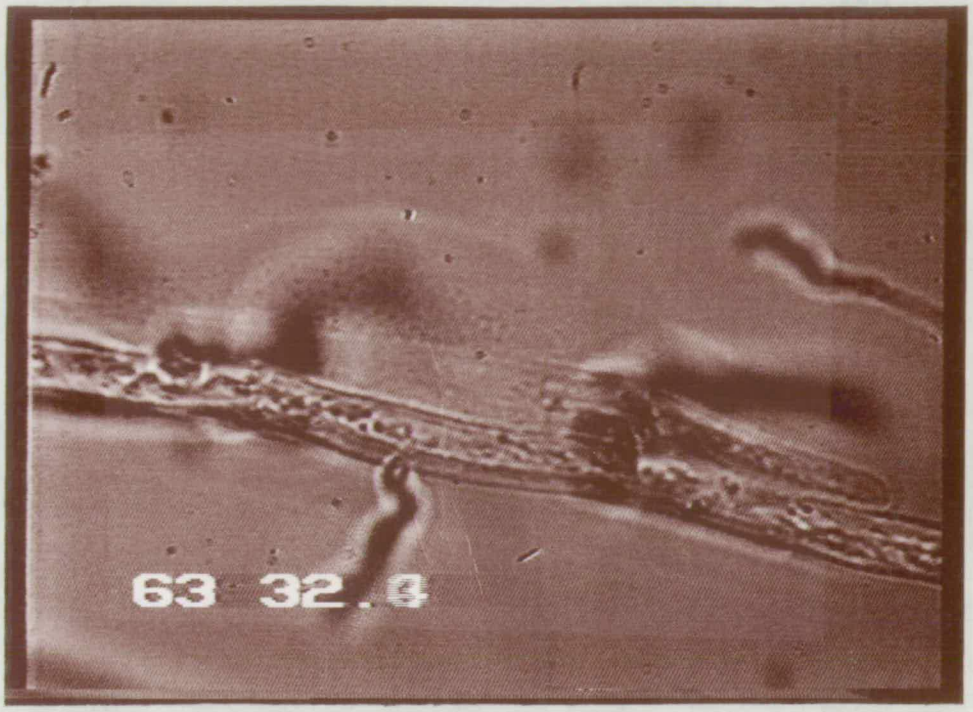
Fig 5.38 60 sec later; the exit peg has caused lysis of the adjacent host hypha.


63 32.4

occurred at 500 sec, from a point 5 µm nearer the initial point of contact than was the first penetration. The second invading branch also grew internally, away from the host tip and alongside the first invading hypha. The tips of these two hyphae grew side-by-side at a rate of 10 µm min^{-1} (Fig 5.30).

After 13 min post-contact, the entire tip area of the host appeared coagulated, and the external hypha of P. mycoparasiticum that initiated the infection had temporarily stopped growing. By 16 min post-contact, however, it had resumed growth and a third invasion had occurred, from a branch 5 µm from the point of initial contact. This invading branch again grew away from the host tip, but formed a branch that grew towards the tip. Only the branch that grew tipwards kept growing; the two other internal hyphae apparently blocked the progress of the third basipetally directed invading hypha (Fig 5.31). As these two (earlier) invading hyphae grew through the host hypha they appeared to cause its distension to accommodate their width (Fig 5.32).

At 26 min post-contact the microscope was scanned along the host hypha, which showed coagulation up to the first septum. The septum bulged and showed a clear delineation between the healthy and the invaded compartment. Although the internal hyphae were by this time 110 µm long, cytoplasmic contents were still seen to be entering them from the parent (external) hypha.

After 35 min the entire tip of the host hypha had been fully colonised by P. mycoparasiticum and the internal hypha had exited the host at the tip. At this time a small hyphal peg, approximately 3 µm long and 0.75 µm wide, was also seen to emerge from an internal hypha through the host wall at a point opposite the third invasion point.

As the two parallel, basipetally-directed internal hyphae approached the first septum (39 min after contact), the sub-apical

compartment was seen to be wider than the apical compartment and the septum bulged out towards the tip (Fig 5.33). One of the internal hyphae reached the septum at 42 min post-contact; 60 sec later, a surge of cytoplasm toward the septum was observed in the sub-apical compartment of the host. The surge appeared like a bursting, with a small amount of the cytoplasm of the sub-apical compartment rushing into the apical compartment through the septum. This was followed by the coagulation of the host cell contents as the internal hypha grew into the compartment (Fig 5.34). A second host hypha growing alongside the invaded hypha was seemingly unaffected by its parasitised neighbour (Fig 5.36). By this time a number of hyphal pegs of various lengths, but all of the same width (0.75 μm), were observed to have emerged from along the length of the invaded hypha of F. oxysporum (Fig 5.35). A third hypha of F. oxysporum was also growing alongside the invaded hypha, and when one of these emerging hyphal pegs made contact with it (15 μm from the host apex) this third host hypha lysed after 90 sec at the point of contact (Figs 5.37 and 5.38). The hyphal peg immediately invaded and began to grow internally along the third hypha.

5.3.1.4 Pythium oligandrum on Botrytis cinerea

The interaction below was characterised as penetrative, with host lysis. The course of events is summarised in Fig 5.39. A hyphal tip of B. cinerea (12 μm diameter), approached the side of a hypha of P. oligandrum (4 μm diameter), at a point 230 μm behind the tip of the mycoparasite. Prior to contact, the hypha of the host appeared to be slowing as it produced a sub-apical branch, and this was confirmed by measurement as it slowed from 10 μm min^{-1} to 6 μm min^{-1} in 3 min. The tip of the P. oligandrum hypha, which played no part in the interaction, was extending at a rate of 11 μm min^{-1}. On this basis the mycoparasitic

Fig 5.39 Summary of an interaction between *P. oligandrum* and *Botrytis cinerea*

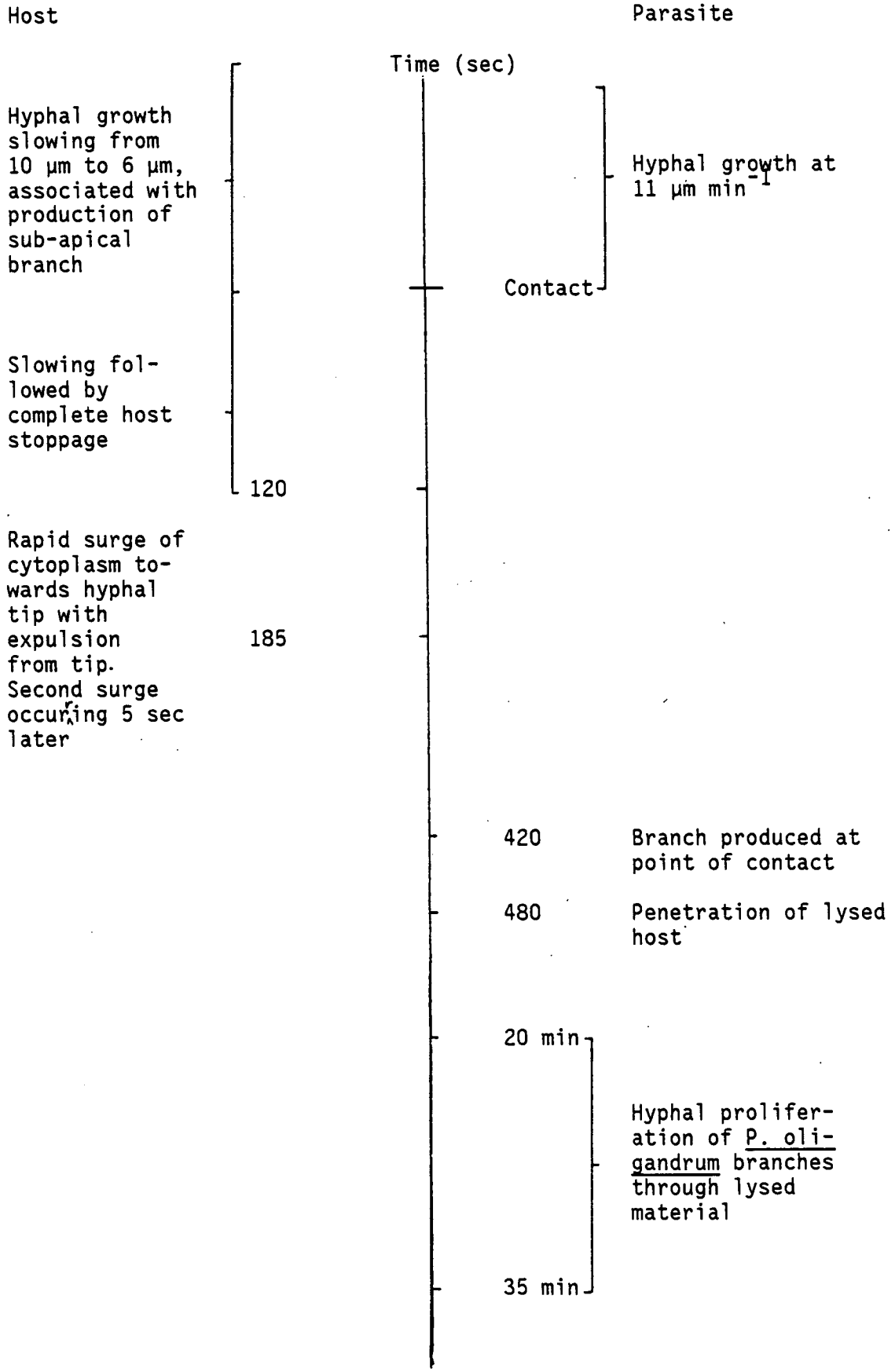
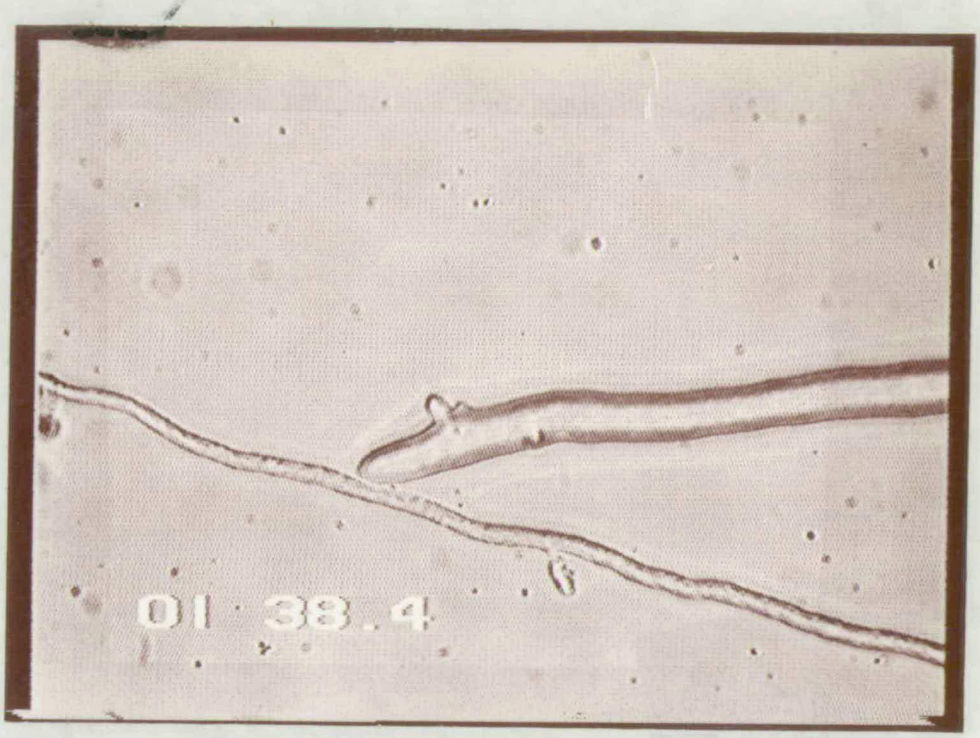


Fig 5.40 Hypha of B. cinerea making contact with side of P. oligandrum hypha. Bar represents 10 µm. ⊢——⊣

Fig 5.41 52 sec post contact. The tip of B. cinerea has grown a short distance along the parasite hypha.


01 38.4

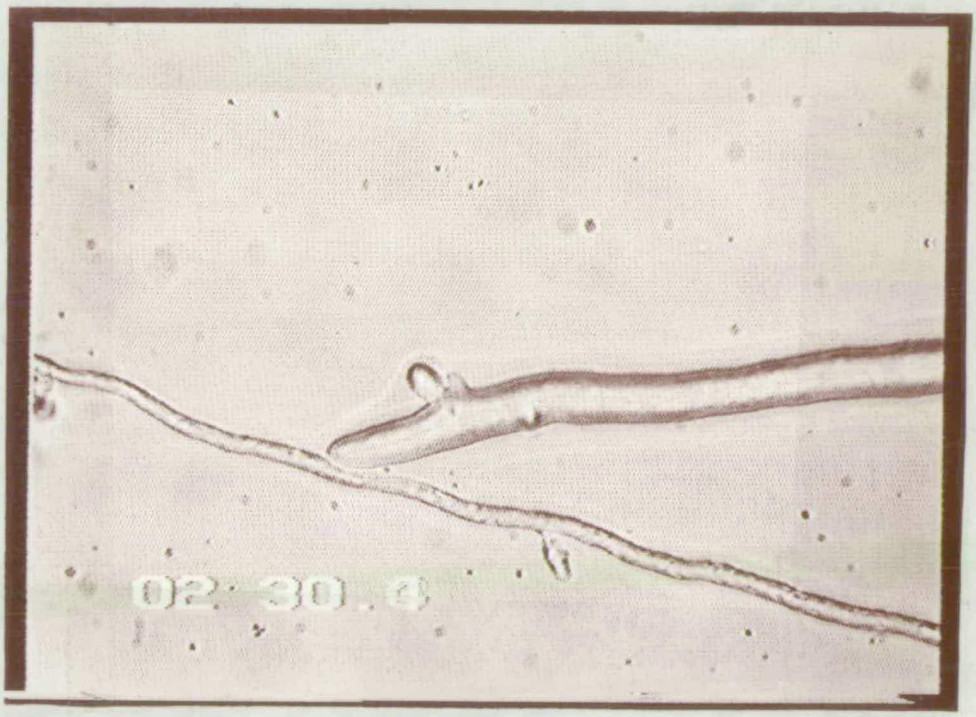
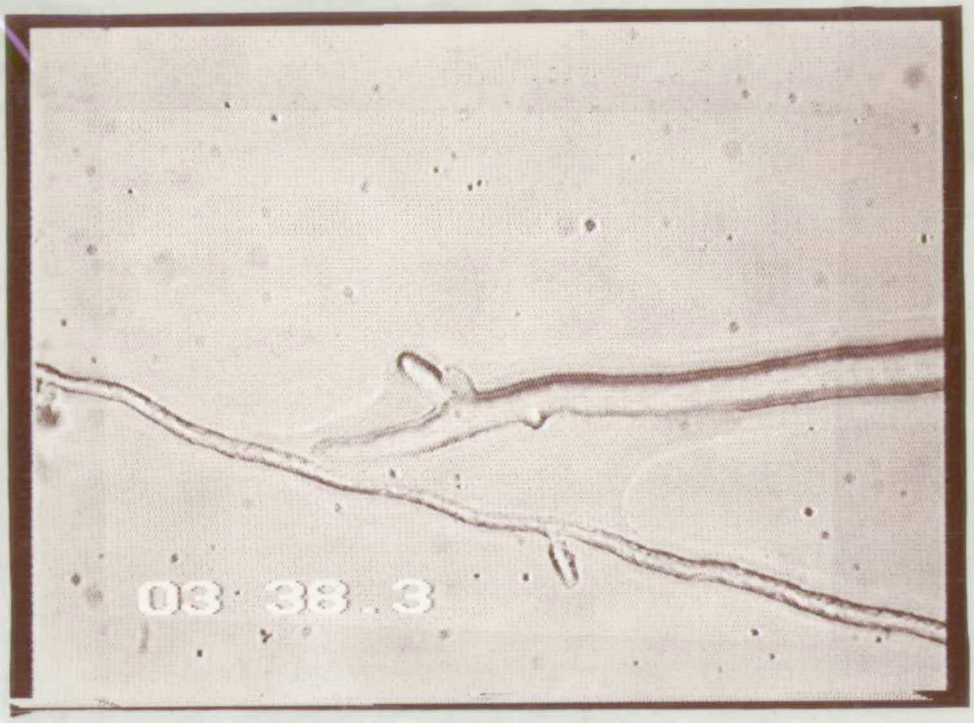

02 30.4

Fig 5.42 120 sec after contact, lysis has occurred at the point of contact. Lysed material can be seen between the hyphae.

Fig 5.43 5 sec later, following a second surge, the area of lysed material is $\underline{c}$ 520 μm^2.


03' 38.3

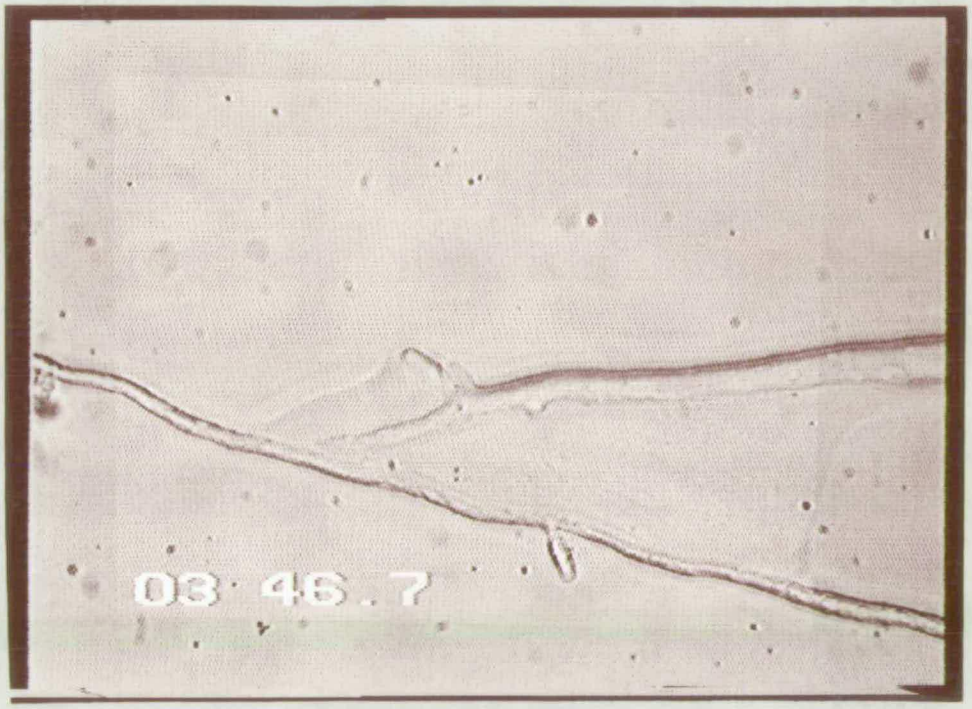
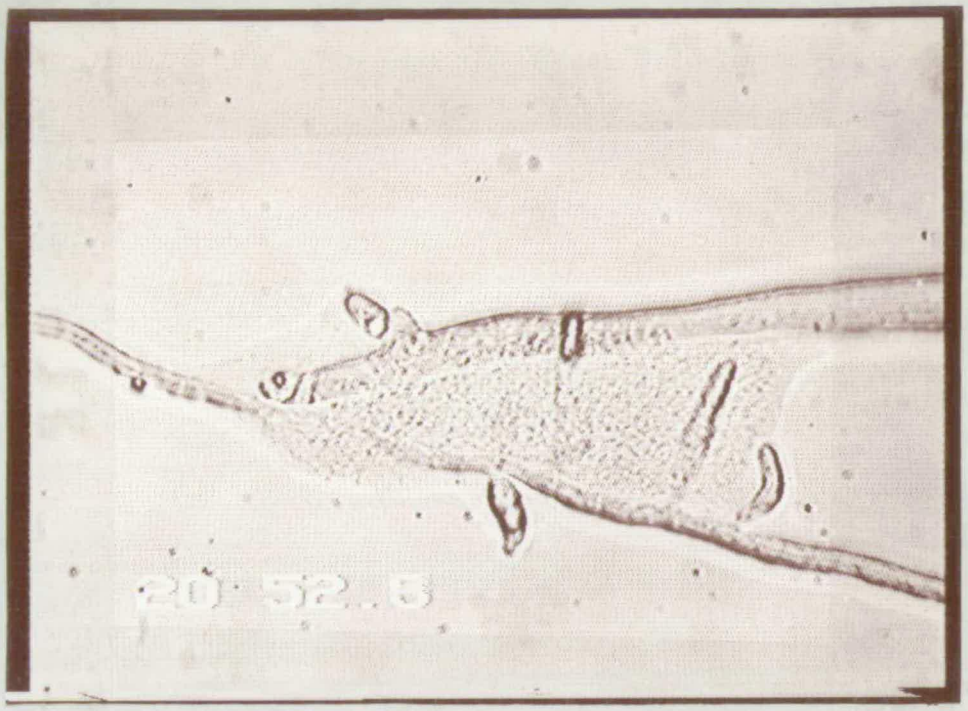
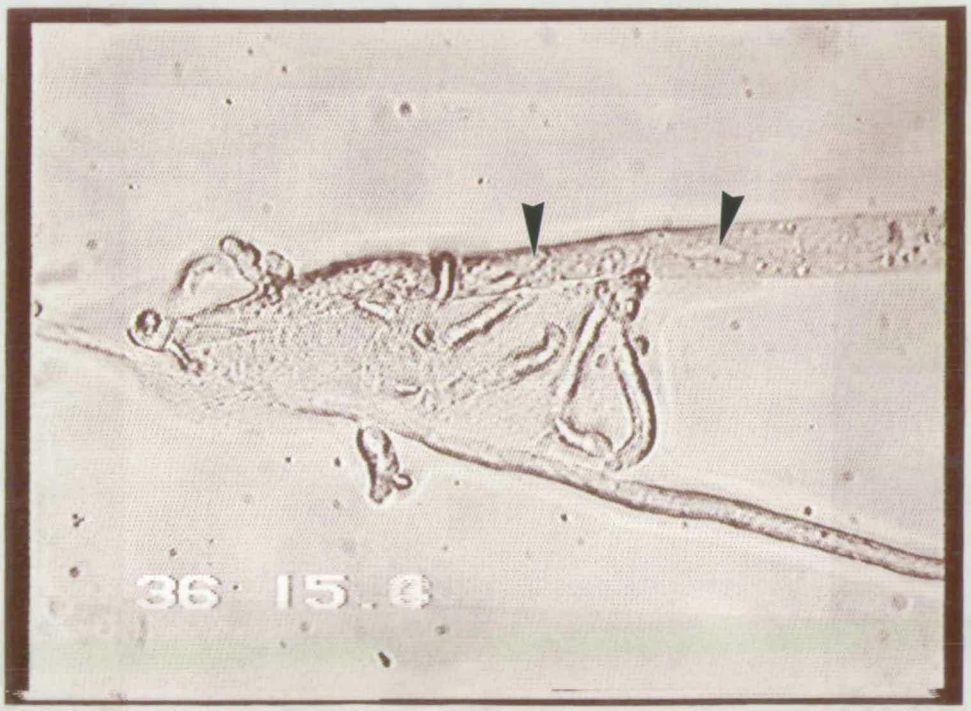

03' 46.7

Fig 5.44 c 19 min after contact, the host hypha has been invaded. Proliferation of hyphae by P. oligandrum occurs in the spilled host cytoplasm.

Fig 5.45 c 16 min later. Internal hyphae are visible (arrowed); parasite branches have proliferated in spilled host contents.


20· 52.5


36· 15.6

hypha was approximately 20 min old at the point of contact. The host tip made contact at an angle of 30° to the parasite hypha (Fig 5.40). On contact, the host slowed and ceased extension within 2 min, but it had evidently made some growth after contact because it had locally displaced the hypha of P. oligandrum and grown alongside it for a distance of 5 µm (Fig 5.41). At 185 sec post-contact, a rapid surge of the host cytoplasm was seen towards the tip, and the hyphal contents were expelled at the point of contact (Fig 5.42). This expulsion occurred with considerable force for 5 sec, the host protoplasm filling an area of c 150 μm^2 on the agar surface. Some component of the host protoplasm evidently blocked the region of wall lysis temporarily, but 5 sec later a second, more powerful, surge occurred, forcing out hyphal contents that occupied an area of c 520μm^2 (Fig 5.43). The second surge lasted 3 sec and was followed by smaller surges for the following 30 sec. Despite the force of the lysis, the host hypha remained attached to the parasite where they had been in contact prior to the lysis. The lysed hyphal contents obstructed the view of subsequent events, but a branch of P. oligandrum was seen growing into the host at the point of contact which was also the point from which lysis had occurred. However, this branching did not occur until 7 min after lysis, and penetration of the host was not seen until 60 sec later. The invading hypha was c 4 µm diameter and grew at an overall rate of 4 µm min^{-1}, but its growth rate was variable, sometimes dropping to as little as 1 µm min^{-1}, for reasons unknown. After 20 min, the main hypha of P. oligandrum was seen to have formed branches at a number of points within the area of lysed host contents (Fig 5.44). By 35 min these branches had grown through most of this material and some had penetrated the host hyphal ghost, but some also were seen to grow out of the lysed material (Fig 5.45). A scan along the host hypha at this time showed that both the apical and sub-

apical compartments were empty and that only behind the second septum, which bulged tipwards, did the host cytoplasm appear healthy.

5.3.1.5 Pythium nunn on Fusarium oxysporum.

A summary of the events in this interaction is shown in Fig 5.46. A hyphal tip of P. nunn (4 µm diameter) approached the side of a hypha of F. oxysporum (5 µm diameter) and made contact with it at an angle of almost 90°, 45 µm from the host tip (Fig 5.47). The hypha of the mycoparasite maintained a steady growth rate of 4.5 µm min^{-1}, and that of F. oxysporum was 3 µm min^{-1} prior to the time of contact. The mycoparasite grew over the host hypha, with a slightly reduced growth rate (3 µm min^{-1}) for the first 3 min after contact, but it then resumed its former growth rate of 4.5 µm min^{-1}. The host hyphal tip continued to grow at 3 µm min^{-1} during this time. At 20 min post-contact, the hypha of P. nunn formed a branch at the point of contact (Fig 5.48). The branch grew slowly over the host hypha, parallel to the parent hypha of P. nunn, but it stopped growing when it had done so (Fig 5.59). After a further 19 min, the host hyphal contents began to coagulate, especially near the point of contact, and the host tip stopped extending (Figs 5.50 to 5.53). No further interaction was observed during the period of recording (85 min). This interaction was characterised as involving vacuolation/coagulation without penetration.

5.3.1.6 General observations.

The five interactions described above were selected to illustrate all the possible events observed in interactions between mycoparasites and hosts, although these events occur in various combinations as described later. They enabled a "check list" to be constructed as a basis for comparing different host-parasite interactions at the level of

Fig 5.46 Summary of an interaction between P. nunn and F. oxysporum

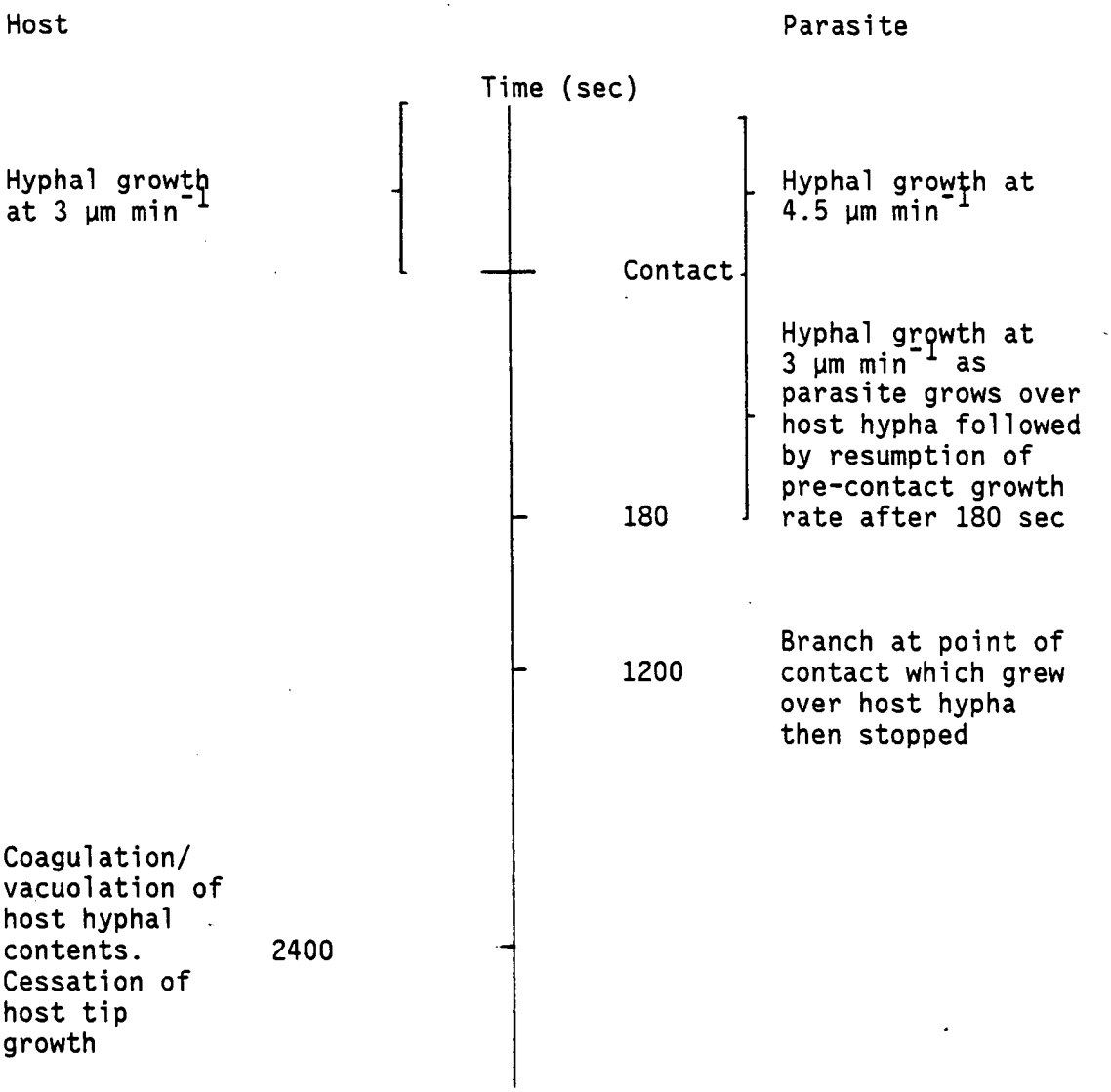
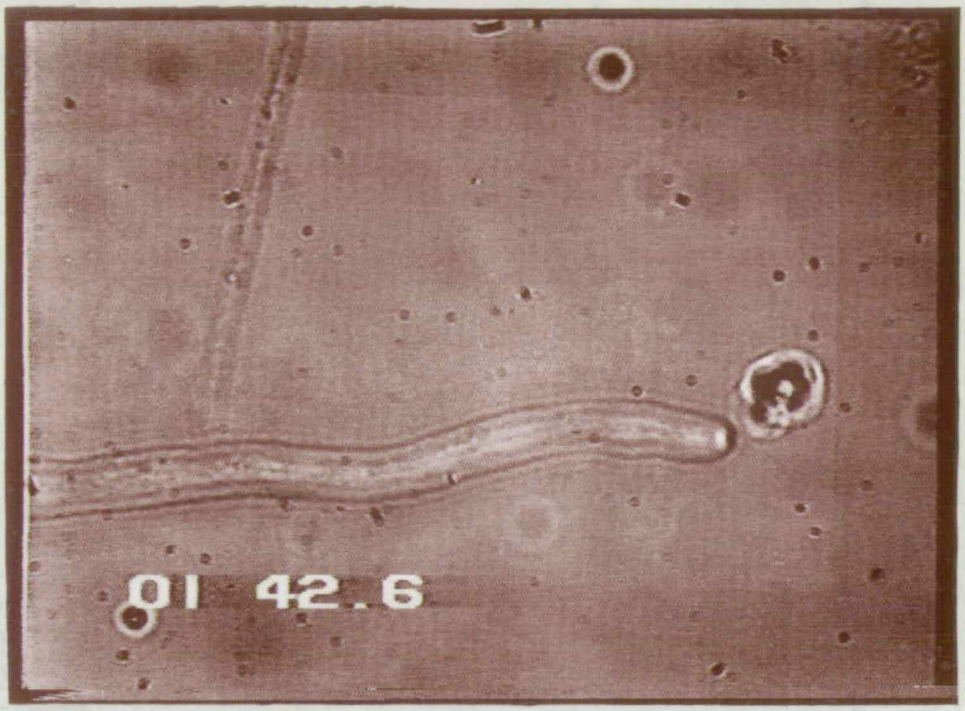


Fig 5.47 Apex of *P. nunn* (growing downwards) makes contact with *F. oxysporum* hypha. Bar represents 10 µm. ⊢——⊣

Fig 5.48 20 min post-contact. *P. nunn* branches at point of contact (arrow).


01 42.6

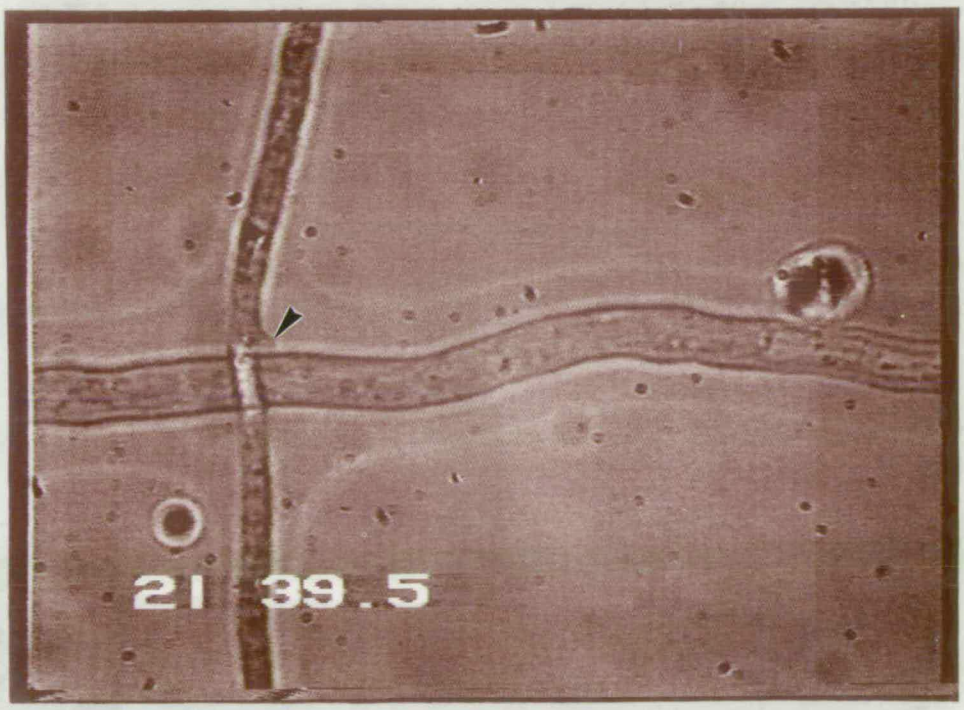
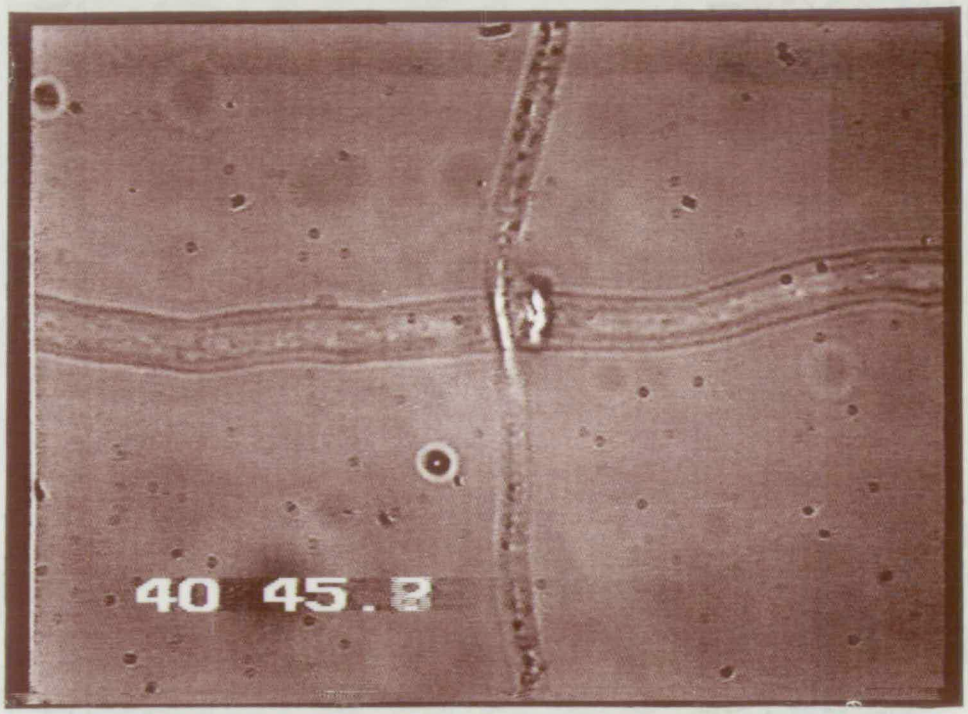

21 39.5

Fig 5.49 39 min post-contact. Branch of P. nunn has grown across the host hypha, then stopped growing.

Figs 5.50-52 2367-2410 sec post-contact, showing progressive stages of cytoplasmic disorganisation of host hypha.

Fig 5.50


40 45.8

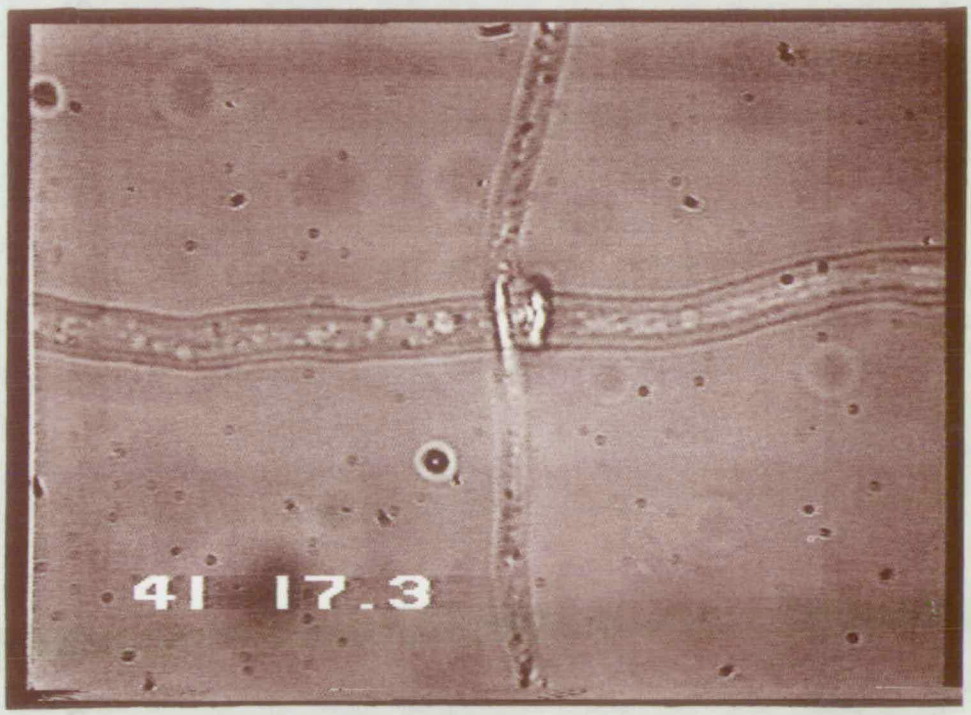
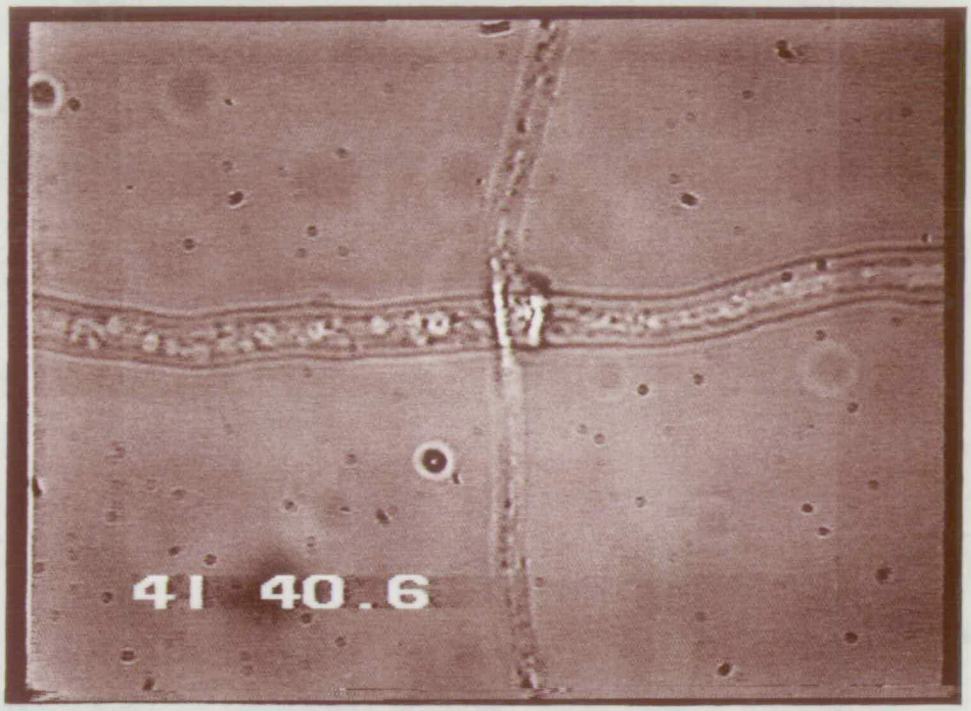

41 17.3

Fig 5.51

Fig 5.52


41 40.6

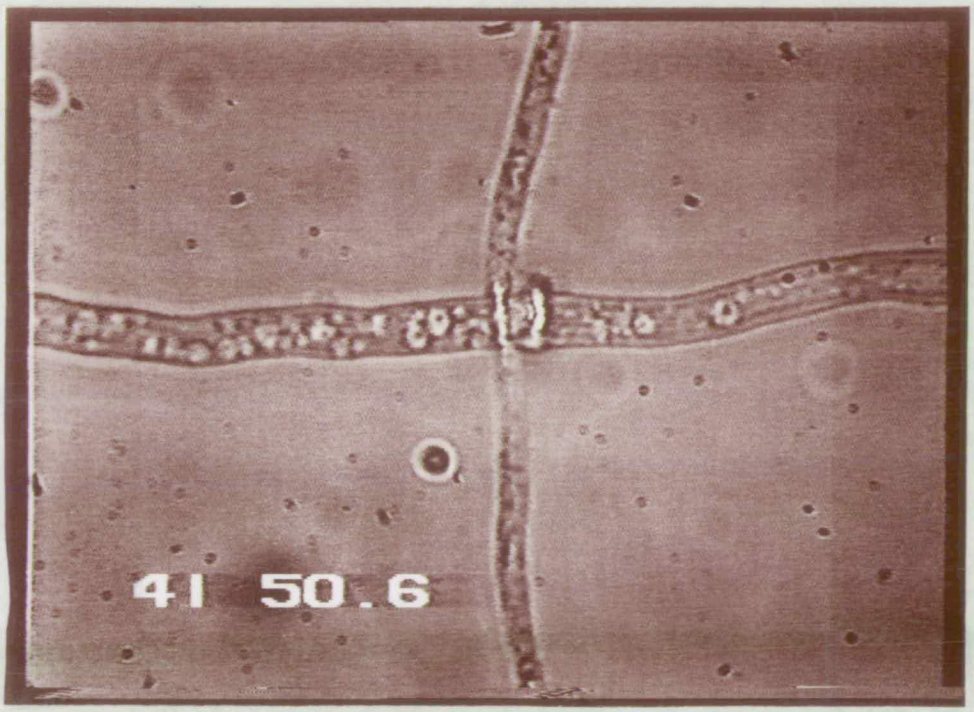
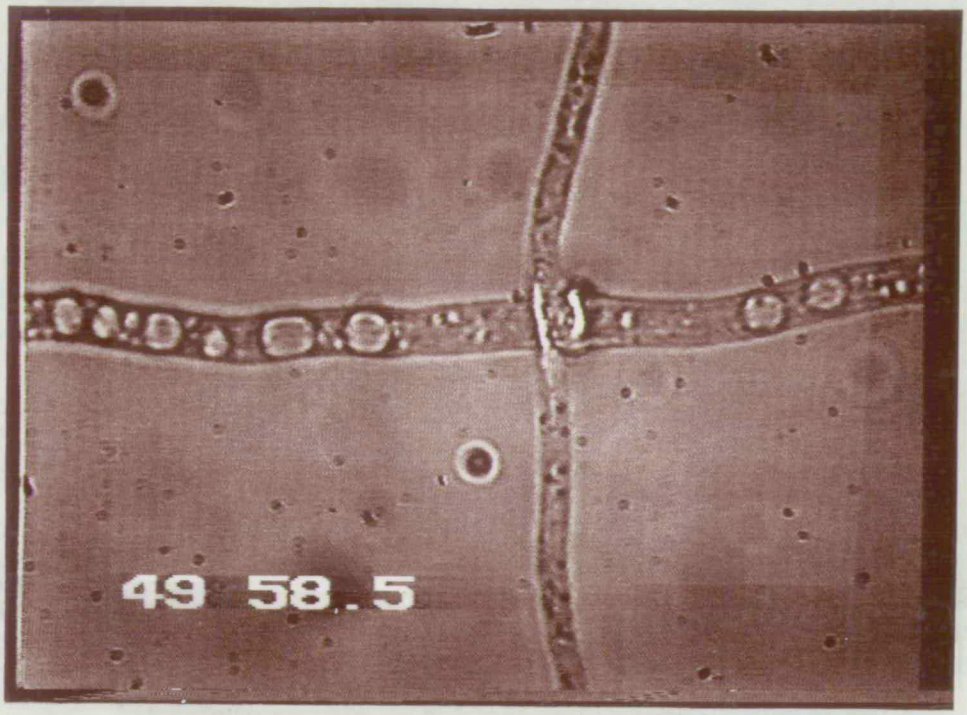

41 50.6

Fig 5.53 c 48 min post-contact. 8 min later and still no penetration by the parasite was observed.


49 58.5

individual hyphae. The form in which these events were recorded is shown in Fig 5.54.

Some types of information were found from experience to be of little value in comparative work because the events occurred apparently at random. This was the case, for example, with the direction of growth of the initial penetrating hypha - whether towards or away from the host tip. A summary of recordings of this is given in Table 5.1, where it is seen that a similar number of internal hyphae grew towards and away from the host tip.

It was also recognized that the mode of exit of an internal hypha from a host hypha was not directly relevant to a comparative study of mycoparasites and hosts. Such egress of an internal mycoparasitic hypha usually occurred at the extreme tip of a host hypha when the internal hypha was orientated in this direction. It was also seen frequently to occur in basipetally directed hyphae when these approached or made contact with an invading hypha from a different infection event. Lastly, the internal hypha sometimes exited through the lateral wall of the host when it reached or approached a septum delineating a damaged and a healthy host compartment. In several instances the internal hypha penetrated several such septa but finally exited when it encountered an accumulation of apparently dense protoplasm near a septum. Such a sequence was described in Section 5.3.1.1 and shown in Figs 5.11 to 5.15. Analysis of video frames preceding such an event revealed that the host hyphal contents had accumulated progressively closer to a septum as the internal hypha grew along the host compartment, and especially when the diameter of the mycoparasite hypha was similar to that of the host. In other words, the mycoparasite hypha had acted like a piston in a cylinder. The production of hyphal pegs by P. mycoparasiticum (see Fig 5.35) was, however, apparently different from all of

Fig 5.54 Interaction check list

1. Categorization

a) Parasite

b) Host

c) Type of interaction [(a) with respect to (b)]

d) Distance from tip

2. Size

a) Parasite hyphal diameter

b) "Host" hyphal diameter

c) Internal hyphal diameter

3. Growth rates

a) Pre-contact parasite growth rate

b) Pre-contact "host" growth rate

c) Changes in parasite growth rate after contact

d) Changes in host growth after contact

e) Host cytoplasmic streaming changes

4. Times (* after contact)

a) Time of first branching*

b) Time of lysis of host hyphae*

c) Time of vacuolation/coagulation of host cell contents*

d) Time of penetration of host*

5. Observations

a) Summary description of interaction

b) Full description

c) Special notes

Table 5.1 Numbers of initial penetrating hyphae that grew internally towards or away from the tip of the host hypha: summary of recordings from videotaped interactions where penetration occurred in the side of a host hypha

	Growth towards tip	Growth away from tip	Total
P. oligandrum	12	14	26
P. mycoparasiticum	4	6	10
P. nunn	1	1	2

these involving exit of a "main" internal hypha, because it involved the egress of a number of narrow branches of the internal hypha, each of these pegs having only a limited capacity for growth unless they made contact with a nearby host hypha. Indeed, branching of internal hyphae was seldom seen for the other mycoparasites, except where a branch developed near the initial penetration point and colonised the host hypha in the opposite direction to the main internal hypha (see Fig 5.7).

5.3.2 Summary descriptions of individual host-parasite interactions

The following descriptions record the main features of individual host-parasite interactions. They are based, wherever possible, on six interactions between an individual mycoparasite and an individual host fungus. At least three of these are tip-to-host side interactions (ie where the tip of the mycoparasite made contact with the lateral wall of a host hypha), and a minimum of three are side-to-host tip interactions (where a host tip contacted the side of a mycoparasite). This delineation was found to be necessary because the behaviour of fungi was often quite different when it involved a hyphal tip rather than a lateral wall of a mycoparasite or host. A third potential type of interaction (tip-to-tip) is theoretically possible, but it occurred very infrequently, and then apparently solely by chance.

The body of data described in this section was assembled over more than a year of study. It is based on videotapes of interactions but the main findings were supported by further observations that were not recorded on videotape. The conditions for all interactions were broadly similar, within the limitations set by the extended period of study, and for every host-parasite combination the replicate recordings (usually three) were on different plates, usually prepared at different times over

the course of the study period.

In a number of interactions it was difficult to measure the distance from the tip to the point of interaction if this was more than 500 µm from the tip. In such cases a value of > 500 µm is recorded (see tables below).

5.3.2.1 Interactions involving Pythium oligandrum

5.3.2.1.1 P. oligandrum versus Pythium graminicola

The main features of these interactions are shown in Table 5.2. In all these interactions the growth rate of the parasite was unaffected as its tip approached the host hypha, and vice versa. In interactions 1-3, P. oligandrum grew up to and over the hypha of P. graminicola with only a slight reduction in its growth rate for the first few minutes after contact, followed by a resumption of the pre-contact growth rate, a feature typical of many interactions. The mycoparasite hypha branched at the point of contact, the branch being seen after a mean of 250 ± 53 (s.e.) sec. However, the branch grew on without penetrating the host hypha, and no effect of the mycoparasite on the host hypha was observed. In side-to-host tip interactions (4-6) there was no change of behaviour of the mycoparasite or host following contact of their hyphae.

Categorisation of interaction: non-parasitic (resistant host).

5.3.2.1.2 P. oligandrum versus Pythium vexans

The main features of these interactions are shown in Table 5.3. There was no change in growth rate of mycoparasite or host hyphae as they approached one another. In tip-to-host side interactions, the mycoparasite grew over (interactions 1 and 2) or along (3 and 4) the host hypha. Branching of the mycoparasite occurred sooner when it grew

Table 5.2 Summary of observations from videotapes of interactions of hyphae of P. oligandrum and P. graminicola

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	40	270	N/A*	N/A	N/A	N/A	N/A	
2	70	330	N/A	N/A	N/A	N/A	N/A	
3	400	150	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
4	50	N/A	N/A	N/A	N/A	N/A	N/A	
5	200	N/A	N/A	N/A	N/A	N/A	N/A	
6	>500	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.3 Summary of observations from videotapes of interactions of hyphae of P. oligandrum and P. vexans

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	10	720	N/A*	N/A	N/A	N/A	N/A	Coiling
2	550	600	N/A	N/A	N/A	N/A	N/A	Coiling
3	>500	270	N/A	N/A	N/A	N/A	N/A	
4	>500	270	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
5	15	N/A	N/A	N/A	N/A	N/A	N/A	
6	35	360	N/A	N/A	N/A	N/A	N/A	Coiling
7	>500	N/A	120	600	N/A	N/A	N/A	

*1 Mycoparasitic tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

alongside the host, but this branching always occurred at the point of contact. The mycoparasite coiled around the host hypha in interactions 1 and 2.

In side-to-host tip interactions branching occurred in two of the three cases, at the point of contact, leading to coiling by the mycoparasite in interaction 6. No other change in behaviour of the mycoparasite or host was seen in any of these interactions during 60 minutes of observation. In interaction 7, the host quickly stopped growing after contact and the protoplasm coagulated after 10 min. However, P. oligandrum also stopped growing after about 15 min and it is suspected that the agar had dried, leading to these anomalous events. Categorisation of interaction: non-parasitic (resistant host) but with coiling in some instances.

5.3.2.1.3 P. oligandrum versus Rhizoctonia solani (isolate GM1)

The main features of these interactions are shown in Table 5.4. Growth rates of the mycoparasite and host hyphae were unaffected on approach to the opposing hyphae. In tip-to-host side interactions the mycoparasite grew over the host hypha and branched soon after contact (188 ± 27 sec) and at the point of contact. The branch gave rise to coiling hyphae in interactions 2 and 3 but there was only slight evidence of coiling in interaction 1. The host tip stopped growing after a variable time (from 300 sec to more than 17 min) following branching by the mycoparasite. Penetration of the host hypha (accompanied by cytoplasmic surging) was found always to follow stoppage of the host tip.

In side-to-host tip interactions (4-6) the host tip stopped growing soon after contact with the mycoparasite, and this preceded by 1-2 min the formation of a visible branch by the mycoparasite at the point of contact. Penetration by the mycoparasite (interactions 5 and 6) fol-

Table 5.4 Summary of observations from videotapes of interactions of hyphae of P. oligandrum and R. solani GM1

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	140	210	480	N/A*	N/A	840	1.0	Slight coiling
2	150	135	1200	N/A	N/A	1500	4.0	Coiling
3	425	220	300	N/A	N/A	350	2.5	Coiling
Side-to-tip*2								
4	10	150	60	60	N/A	N/A	N/A	Coiling
5	45	120	60	N/A	140	180	4.0	
6	>500	200	120	N/A	N/A	250	8.0	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

lowed soon after branch emergence and was preceded by host lysis in interaction 5. However, the mycoparasite did not penetrate the host in interaction 4; instead, the mycoparasite coiled around the host hypha, the contents of which coagulated soon after contact with the mycoparasitic hypha.

Categorisation of interaction: variable; host tips are susceptible to vacuolation/coagulation, penetration or lysis, but more mature regions of the host hyphae may remain unaffected for some time and elicit coiling by the mycoparasite.

5.3.2.1.4 *P. oligandrum* versus *R. solani* (isolate T125)

The main features of these interactions are shown in Table 5.5. Prior to contact, the growth rates of the mycoparasite and host hyphae were unaffected as they approached the opposing hyphae. In interactions 1 to 4, the mycoparasitic hypha grew over the host hypha, branching at the point of contact in each case, after a mean of 398 ± 66 sec. Although the host stopped growing in all four interactions, penetration was observed in only cases 1, 2 and 4 (Table 5.5) and this followed vacuolation/coagulation of the host hyphal contents. In interactions 1 and 2 coiling of branches of the parasite around the host hypha was observed, and this began prior to penetration.

In the side-to-host tip interactions, the host hyphae stopped growing soon after contact, and between 30 and 60 sec after branching the host hypha was penetrated. In interaction 5 host stoppage was followed by lysis very soon after contact; in interactions 6 and 7 the host contents became granulated 60 sec after host stoppage, which in both cases occurred 120 sec after contact.

Categorisation of interaction: variable; host tips are susceptible to vacuolation/coagulation of contents, lysis or penetration, but even

Table 5.5 Summary of observations from videotapes of interaction of hyphae of *P. oligandrum* and *R. solani* T125

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	25	270	300	540	N/A*	600	5.0	Coiling
2	50	480	480	480	N/A	1200	3.0	Coiling
3	55	300	1320	N/A	N/A	N/A	N/A	
4	90	540	1200	1200	N/A	1200	2.0	
Side-to-tip*2								
5	70	240	50	N/A	55	270	2.0	
6	100	480	120	180	N/A	540	6.0	
7	>500	180	120	180	N/A	220	2.0	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

slightly more mature regions may remain unaffected for some time and may elicit coiling by the mycoparasite.

5.3.2.1.5 P. oligandrum versus Fusarium culmorum

The main features of these interactions are shown in Table 5.6. In all these interactions the growth rate of the parasite was unaffected as its tip approached the host hypha and vice versa. In the tip-to-host side interactions, P. oligandrum grew up to and over the hypha of F. culmorum in interactions 1 to 4, and alongside the host for 20 µm before growing over it in interaction 5. In interactions 2 to 5 the main hypha of the mycoparasite continued to grow after passing the host hypha; in interaction 1, however, the mycoparasite hypha stopped 120 sec after contact and did not resume growth, although no reason for this could be discerned. Interactions 2 to 5 resulted in penetration, following lysis in interaction 2 and host protoplasmic vacuolation/coagulation in interactions 3 and 4. Interaction 1 resulted in host lysis 150 sec after contact.

In the side-to-host tip interactions, the host hyphae stopped growing after a mean of 177 ± 34 sec, with host hyphal lysis occurring in each case. In only one interaction (7) did the mycoparasite branch (after 420 sec) and this led to host penetration 60 sec later.

Categorisation of interaction: parasitic; host hyphae stopped growing relatively soon after contact, and such stoppage was normally followed by host lysis or vacuolation/coagulation. Branch formation by the parasite resulted in host penetration.

5.3.2.1.6 P. oligandrum versus Botryotrichum piluliferum

The main features of these interactions are shown in Table 5.7. Before contact, both the mycoparasitic hypha and the host hypha main-

Table 5.6 Summary of observations from videotapes of interactions of hyphae of *P. oligandrum* and *F. culmorum*

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	6	N/A*	120	N/A	150	N/A	N/A	Parasite stopped
2	150	500	420	N/A	540	600	6.0	
3	>500	180	160	180	N/A	210	8.0	
4	>500	360	400	420	N/A	400	8.5	
5	>500	210	240	N/A	N/A	240	5.0	
Side-to-tip*2								
6	60	N/A	240	N/A	420	N/A	N/A	
7	1000	420	120	N/A	310	480	6.0	
8	>500	N/A	170	N/A	170	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.7 Summary of observations from videotapes of interactions of hyphae of P. oligandrum and B. piluliferum

Replicate observation	Distance from tip to point of contact (μm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (μm min^{-1})	Comments
Tip-to-side*1								
1	15	80	120	N/A*	N/A	190	7.0	
2	20	120	120	N/A	N/A	120	4.0	
3	30	300	120	N/A	170	330	7.0	
4	90	200	420	N/A	N/A	540	2.0	
Side-to-tip*2								
5	3	220	90	N/A	160	260	3.0	
6	100	480	240	N/A	240	720	1.0	
7	>500	360	240	N/A	260	N/A	N/A	
8	>500	N/A	30	N/A	170	N/A	N/A	
9	>500	220	120	N/A	155	220	5.5	

*1 Mycoparasite tip contacted the side of the host hypha
*2 host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

tained a steady growth rate as they approached each other. In interaction 3 the mycoparasite grew over the host hypha; in interaction 4 the parasite grew alongside the host hypha for 15 µm before growing over and away. In interactions 1 and 2 the mycoparasite hyphal tip stopped growing after contact and the tip became swollen. In both instances, however, as with interactions 3 and 4, a branch emerged at the point of contact, after a mean time (overall) of 175 ± 56 sec, and these branches went on to penetrate the host hyphae. In interaction 3 branching and penetration was preceded by violent host lysis 170 sec after contact.

The side-to-host tip interactions consistently exhibited host hyphal lysis at a mean time of 197 ± 28 sec after contact. Branching by the mycoparasite occurred at the point of contact in all but interaction 8, leading to host hyphal penetration in interactions 5, 6 and 9.

Categorisation of interaction: parasitic; host hyphae stop growing relatively soon after contact. Host stoppage is often followed by lysis, particularly in side-to-host tip interactions. Host hyphal penetration occurs soon after branching in most cases.

5.3.2.1.7 *P. oligandrum* versus *Botrytis cinerea*

The main features of these interactions are shown in Table 5.8. Prior to contact, the growth rates of the mycoparasite and host hyphae were unaffected as they approached the opposing hyphae except in interaction 5 (see Section 5.3.1.4) where the host hypha slowed as it produced a sub-apical branch in the three minutes prior to contact. In the tip-to-host side interactions the parasite grew over and away from the host hypha in interactions 1 and 3, but stopped on contact in interaction 2, when its hyphal tip swelled. Branching at the point of contact occurred in all three cases after a mean time of 240 ± 17 sec, this first branch penetrating the host hypha in the first and second

Table 5.8 Summary of observations from videotapes of interactions of hyphae of *P. oligandrum* and *B. cinerea*

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	30	280	390	390	N/A*	410	8.0	
2	>500	210	180	N/A	180	240	4.0	
3	>500	220	960	960	N/A	1230	5.0	
Side-to-tip*2								
4	100	420	300	480	N/A	N/A	N/A	
5	230	540	120	N/A	120	600	4.0	Hyphal proliferation
6	>500	N/A	180	360	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

interactions. In interaction 3 the first branch did not invade the host; however a second branch did so. Host stoppage was characterised by vacuolation/ coagulation of the cell contents in interactions 1 and 3, and by host lysis in interaction 2.

In interactions 4 to 6 (side-to-host tip) host stoppage preceded vacuolation/coagulation of the host protoplasm in interactions 4 and 6 and host lysis in interaction 5. Although the parasite branched at the point of contact in interactions 4 and 5, only in interaction 5 did it go on to penetrate the host hypha.

Categorisation of interaction: parasitic; host vacuolation/coagulation is more prevalent than host lysis following stoppage of the host, the former occurring later after contact than the latter. Penetration was quite common but often slow.

5.3.2.1.8 *P. oligandrum* versus *Fusarium oxysporum*

The main features of these interactions are shown in Table 5.9. Growth rates of the mycoparasite and host hyphae were unaffected on approach to the opposing hyphae. In interactions 1, 3 and 4, the mycoparasitic hypha grew over the host hypha and grew away; in interaction 2 the narrow mycoparasite hypha (diameter 1 μm) contacted the host hypha and stopped growing. In this interaction the mycoparasite did not resume growth or form a branch. All four interactions exhibited host lysis following host stoppage. Branching at the point of contact after a mean 485 ± 33 sec in interactions 1, 3 and 4 resulted in host penetration between 15 and 60 sec later.

The side-to-host tip interactions also displayed host lysis in each case, this occurring at a mean time of 243 ± 14 sec after contact. Branching at the point of contact occurred in interactions 5 and 7; in the latter case it occurred 15 min after contact and this was the only

Table 5.9 Summary of observations from videotapes of interactions of hyphae of *P. oligandrum* and *F. oxysporum*

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	50	510	180	N/A*	220	540	7.0	
2	90	N/A	80	N/A	100	N/A	N/A	Parasite stopped
3	120	420	180	N/A	220	480	7.5	
4	150	525	360	N/A	480	540	10.0	
Side-to-tip*2								
5	10	160	180	N/A	220	N/A	N/A	
6	160	N/A	60	N/A	240	N/A	N/A	
7	>500	900	240	N/A	270	930	5.0	Hyphal proliferation

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

instance in which the host was penetrated.

Categorisation of interaction: parasitic; host hyphae stop growing and lyse soon after contact. Host penetration is relatively common, with rapid internal growth of the mycoparasite.

5.3.2.1.9 P. oligandrum versus Trichoderma aureoviride

The main features of these interactions are shown in Table 5.10. Before contact, both the mycoparasitic hypha and the host hypha maintained a steady growth rate as they approached each other, except in interaction 1 where prior to contact the host was observed to have stopped growing. In the tip-to-host side interactions the mycoparasite grew over the host hypha in all but interaction 4 where the mycoparasite grew alongside the host hypha for more than 50 µm before crossing it and growing away. In all examples branching occurred at the point of contact, after a mean time of 200 ± 75 sec. This led to penetration in four of the five interactions, interaction 2 being the exception, after a mean of 202 ± 47 sec. Host hyphal lysis was observed in three interactions, including the non-penetrative interaction and in interactions 3 and 5 where branching and penetration occurred more rapidly.

In side-to-host tip interactions, penetration did not occur, despite branching at the point of contact (in interactions 6 and 7). However, host lysis occurred in each at a mean 170 ± 20 sec after contact, and in each case lysis was violent, releasing a large amount of host hyphal contents.

Categorisation of interaction: parasitic; the large number of lytic events suggests that the host is sensitive to this parasite, particularly at host tips. Penetration, though only observed in tip-to-host side interactions, occurred quickly and internal growth rates were high.

Table 5.10 Summary of observations from videotapes of interactions of hyphae of P. oligandrum and T. aureoviride

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	5	205	N/A*3	N/A*	N/A	240	8.0	
2	30	480	120	N/A	325	N/A	N/A	
3	30	30	40	N/A	70	90	8.0	
4	160	145	240	N/A	N/A	310	8.0	
5	>500	140	150	N/A	170	170	10.0	
Side-to-tip*2								
6	40	390	180	N/A	210	N/A	N/A	
7	80	210	120	N/A	150	N/A	N/A	
8	160	N/A	120	N/A	150	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

*3 Host already stopped

* Not applicable

5.3.2.1.10 P. oligandrum versus Phialophora sp

The main features in these interactions are shown in Table 5.11. In all interactions, the growth rate of the parasite and the host was unaffected as the hyphae converged. Of the tip-to-host side interactions, the mycoparasitic hypha grew over the host hypha in interactions 1, 3, 4 and 5, and alongside before crossing over in interaction 2. The mycoparasite always branched at the point of contact, in a mean time of 214 ± 57 sec, this branch penetrating the host hypha in interactions 1 and 4. In interactions 3 and 5 penetration occurred from a branch of this first branch. Penetration followed host vacuolation/coagulation in these two latter interactions, host lysis occurring in the other three although penetration also occurred in interactions 1 and 4 as mentioned previously.

In interactions 6 to 8, penetration occurred from branches arising from the initial mycoparasite hypha at the point of contact. In interaction 7 this followed host lysis, and in interaction 8 it followed host vacuolation/coagulation. Branching occurred more slowly from the more mature parts of the mycoparasite hypha, with penetration in interaction 8 not occurring until after 40 min.

Categorisation of interaction: parasitic; penetration occurred in most cases following host lysis or vacuolation/coagulation. Internal mycoparasitic growth rates were generally high.

5.3.2.2 Interactions with Pythium mycoparasiticum

5.3.2.2.1 P. mycoparasiticum versus Pythium vexans

The main features of these interactions are shown in Table 5.12. Before contact, both the mycoparasitic hypha and the host hypha showed no change in growth rate as they approached the opposing hyphae. In

Table 5.11 Summary of observations from videotapes of interactions of hyphae of *P. oligandrum* and *Phialophora* sp

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	5	240	120	N/A*	200	360	6.0	
2	70	180	240	N/A	300	N/A	N/A	
3	160	110	420	480	N/A	600	9.0	
4	200	120	120	N/A	240	320	11.0	
5	>500	420	540	540	N/A	570	9.0	
Side-to-tip*2								
6	5	120	60	N/A	N/A	330	11.0	
7	>500	360	30	N/A	210	420	6.0	
8	>500	2400	420	420	N/A	2400	3.0	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.12 Summary of observations from videotapes of interactions of hyphae of P. mycoparasiticum and P. vexans

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	8	N/A*	N/A	N/A	N/A	N/A	N/A	
2	40	N/A	N/A	N/A	N/A	N/A	N/A	
3	180	1100	N/A	N/A	N/A	N/A	N/A	
4	>500	N/A	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
5	10	N/A	N/A	N/A	N/A	N/A	N/A	
6	20	N/A	N/A	N/A	N/A	N/A	N/A	
7	40	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

tip-to-host side interactions, the parasitic hypha grew up to and over the host hypha in interactions 2 and 3 and alongside the host hypha in interactions 1 and 4. Branching occurred only in interaction 3, and even then only 18 min after contact. There was no effect on the host hypha.

Branching by the mycoparasite was not observed in any of the side-to-host tip interactions and, as with the tip-to-host side interactions, there was no effect on the host hyphae.

Categorisation of interaction: non-parasitic (resistant host).

5.3.2.2.2 P. mycoparasiticum versus Botrytis cinerea

The main features of these interactions are shown in Table 5.13. Prior to contact, the growth rate of the host hyphae were unaffected as they approached the mycoparasitic hypha. The mycoparasitic hyphae, on the other hand, began to slow and eventually stopped between 5 and 20 µm from the host hypha, indicative of the production of a diffusible fungistatic compound by the host to which P. mycoparasiticum is sensitive. The term fungistatic rather than fungitoxic is used, as the mycoparasite did not appear to be affected internally after stoppage. Usually, this effect prevented tip-to-host side interactions, and only one such example was seen, when the parasitic tip contacted the host hypha near the host hyphal tip. In this single example, branching was observed at the point of contact 520 sec after initial contact, where the mycoparasitic hypha had grown up to, then along and eventually over the host hyphal tip. The host tip stopped growing after 480 sec and its contents coagulated after 720 sec. At 60 sec later the parasite penetrated the host hyphal tip. At the same time, the main mycoparasite hyphal tip stopped growing and began to swell, in a manner identical to the other observed pre-contact inhibitions. This effect was noticed soon after-

Table 5.13 Summary of observations from videotapes of interactions of hyphae of P. mycoparasiticum and B. cinerea

Replicate observation	Distance from tip to point of contact (μm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (μm min^{-1})	Comments
Tip-to-side*1								
1	10	520	480	720	N/A*	780	2.0	
Side-to-tip*2								
2	40	N/A	360	720	N/A	N/A	N/A	
3	200	N/A	180	600	N/A	N/A	N/A	
4	>500	N/A	120	600	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

wards in the internal hypha.

In interactions 2 to 4, the host hypha approached and grew over the mycoparasitic hypha, but then slowed and soon stopped. After between 10 and 12 min vacuolation/coagulation of the host cell contents was observed. Branching by the mycoparasite did not occur in any of the examples, possibly because of the production of a fungistatic compound by B. cinerea.

Categorisation of interaction: potentially parasitic, but the host inhibits pre-contact growth by the mycoparasite, and also hyphal branching, apparently by production of a diffusible compound. The host hyphae are, however, susceptible to stoppage and internal vacuolation/coagulation on contact with P. mycoparasiticum.

5.3.2.2.3 P. mycoparasiticum versus Fusarium oxysporum

The main features of these interactions are shown in Table 5.14. Prior to contact, the growth rates of the mycoparasite and host hyphae remained constant as they approached the opposing hyphae. In the tip-to-host side interactions, 1 to 3, the parasite grew along and over the host hypha in interaction 1, and over in interactions 2 and 3. In all three interactions the mycoparasite branched at the point of contact, after a mean time of 273 ± 82 sec, and in each example the branching was followed by penetration. This occurred from the first branch in interactions 1 and 2, but from a branch off the first branch in interaction 3. In interactions 2 and 3 vacuolation/coagulation of the host protoplasm was observed soon after host stoppage.

Of the five side-to-host tip interactions, all four that showed branching by the mycoparasite at the point of contact exhibited host hyphal penetration by this first branch. The host stopped growing after a mean of 165 ± 57 sec when contact occurred perpendicularly (inter-

Table 5.14 Summary of observations from videotapes of interactions of hyphae of P. mycoparasiticum and F. oxysporum

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	20	240	180	N/A*	N/A	270	8.0	Exit pegs
2	60	430	420	430	N/A	430	10.0	Exit pegs
3	65	150	180	210	N/A	280	2.0	
Side-to-tip*2								
4	35	N/A	240	N/A	390	N/A	N/A	
5	40	240	120	210	N/A	480	1.5	
6	40	540	420	420	N/A	540	2.0	
7	60	270	120	N/A	N/A	300	5.0	
8	65	420	180	N/A	290	420	2.5	Exit pegs

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

action 4, 5, 7 and 8), but continued to grow on for a longer period when the angle of contact was acute (interaction 6). Prior to penetration, vacuolation/ coagulation of the host protoplasm was observed in two cases (interactions 5 and 6) and lysis in one (interaction 8). In interaction 4, where no branching occurred, host lysis occurred after 390 sec.

Exit pegs were observed in three interactions (1, 2 and 8), and in two cases subsequently affected adjacent host hyphae.

Categorisation of interaction: parasitic; host hyphal penetration occurred in all cases where branching occurred at the point of contact, although internal growth rates varied greatly. Host vacuolation/coagulation and host lysis were also observed.

5.3.2.2.4 P. mycoparasiticum versus Trichoderma aureoviride

The main features of these interactions are shown in Table 5.15. Before contact, both the mycoparasitic hyphae and the host hyphae maintained a steady growth rate as they approached each other. In interactions 1 to 4 host penetration followed branching at the point of contact in each case. In interaction 3, however, this branching took place after 40 min despite the fact that the host hypha had stopped growing and its contents coagulated after 15 min. There was no obvious reason for this anomaly. In interactions 1 and 4 the host hypha lysed before it was penetrated, although in interaction 1 this lysis was slight. Exit pegs were also observed in interaction 1, one hour after contact.

Of the three side-to-host tip interactions, penetration was observed in interactions 5 and 6, where the mycoparasite branched at the point of contact. In all three interactions host hyphal lysis occurred between 30 and 120 sec after the host hypha had stopped growing.

Table 5.15 Summary of observations from videotapes of interactions of hyphae of P. mycoparasiticum and T. aureoviride

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	10	210	480	N/A*	540	600	6.0	Exit pegs
2	15	100	60	N/A	N/A	120	5.0	
3	40	2400	900	900	N/A	2400	5.0	
4	45	320	70	N/A	210	390	7.0	
Side-to-tip*2								
5	100	300	90	N/A	120	300	8.0	
6	130	720	120	N/A	140	720	6.0	
7	180	N/A	240	N/A	360	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Categorisation of interaction: parasitic; host penetration occurred in every interaction where the mycoparasite branched at the point of contact. In a number of interactions host lysis occurred. Internal growth rates were moderately high and consistent.

5.3.2.2.5 P. mycoparasiticum versus Phialophora sp

The main features of these interactions are shown in Table 5.16. In all interactions the growth rate of the mycoparasitic hypha was unaffected as its tip approached the host hypha, and vice versa. In interactions 1 to 3, the parasite grew up to and over the host hyphae. The host ceased growing, in each case exhibiting vacuolation/coagulation of the contents before the parasite branched at the point of contact. These branches invaded the host hypha after a mean 390 ± 52 sec.

In interactions 4 to 6, the host tip stopped at between 6 and 10 min after contact. In cases 4 and 6, after the parasite had branched at the point of contact, the host hypha underwent a massive lysis, and in each case the branch failed to penetrate. In interaction 5 the parasite did not branch, and after the host hypha had stopped it showed no change in appearance.

Categorisation of interaction: parasitic; host penetration, host lysis and the vacuolation/coagulation of cell contents are all observed. Internal growth rates were moderate.

5.3.2.2.6 P.mycoparasiticum versus miscellaneous fungi

Limited observations were made of interactions between P. mycoparasiticum and some other fungi (Table 5.17), but study of a fuller range of hosts (as with P. oligandrum) was precluded by the slow and sparse growth of P. mycoparasiticum which made replicated observations unacceptably time consuming.

Table 5.16 Summary of observations from videotapes of interactions of hyphae of *P. mycoparasiticum* and *Phialophora* sp

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	40	300	170	165	N/A*	300	4.0	
2	350	300	240	250	N/A	390	4.0	
3	>500	480	320	360	N/A	480	6.0	
Side-to-tip*2								
4	20	150	360	N/A	555	N/A	N/A	
5	25	N/A	360	N/A	N/A	N/A	N/A	
6	130	540	600	N/A	1140	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.17 Summary of observations from videotapes of interactions of hyphae of P. mycoparasiticum and miscellaneous fungi

Replicate observation	Distance from tip to point of contact (μm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (μm min^{-1})	Comments
P. graminicola								
Tip-to-side*1								
1	260	660	N/A*	N/A	N/A	N/A	N/A	
Side-to-tip*2								
2	80	N/A	N/A	N/A	N/A	N/A	N/A	
R. solani T125								
Side-to-tip*2								
3	60	260	240	N/A	250	300	7.0	
F. culmorum								
Tip-to-side*1								
4	40	420	460	460	N/A	490	4.0	
5	90	300	240	240	N/A	N/A	N/A	
B. piluliferum								
Tip-to-side*1								
6	6	300	220	300	N/A	480	3.0	
7	40	330	420	420	N/A	440	4.0	
Side-to-tip*2								
8	60	870	360	380	N/A	870	2.0	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Prior to contact, neither the growth rates of the mycoparasite hyphae nor the growth rates of any of the host hyphae were affected as they approached the opposing hyphae. With P. graminicola, P. mycoparasiticum branched at the point of contact 660 sec after meeting, but showed no further behavioural response and had no effect on the host. P. graminicola was similarly unaffected when its hyphal tip made contact with a hypha of P. mycoparasiticum (interaction 2 in Table 5.17).

In a single instance when a hyphal tip of R. solani (T125) approached the side of a hypha of P. mycoparasiticum (interaction 3), the host stopped and lysed and this was quickly followed by branching of the mycoparasite at the point of contact and by subsequent penetration.

In two cases where the tip of P. mycoparasiticum approached the side of a hypha of F. culmorum (interactions 4 and 5 in Table 5.17), the host hypha stopped growing and its contents coagulated. The mycoparasite branched at the point of contact, but penetration occurred in only one of the two cases. In the three interactions involving B. piluliferum the host stopped and its contents coagulated in each case, and this was accompanied by branching by the mycoparasite at the point of contact and by penetration. These miscellaneous interactions were not included in the quantitative comparison of results.

Categorisations of interactions (provisional, based on few observations):

P. graminicola: non-parasitic (resistant host).

R. solani (T125): hyphal tips susceptible to lysis and penetration.

F. culmorum: parasitic; penetration and vacuolation/coagulation observed.

B. piluliferum: parasitic; penetration and vacuolation/coagulation observed.

5.3.2.3 Interactions involving Pythium nunn

5.3.2.3.1 P. nunn versus Pythium vexans

The main features of these interactions are shown in Table 5.18. Prior to contact, the growth rates of neither the mycoparasite nor the host hyphae were affected. In the tip-to-host side interactions, the parasite grew up to and over the hyphae of P. vexans in interactions 2, 3 and 4, and along the host hypha in interaction 1. The contact did not elicit branching, and the host and mycoparasite were unaffected in any way.

Similarly for the side-to-host tip interactions, there was no branching by the parasite at the point of contact, and no detrimental effect of the mycoparasite on the host.

Categorisation of interaction: non-parasitic (resistant host).

5.3.2.3.2 P. nunn versus Botrytis cinerea

The main features of these interactions are shown in Table 5.19. It was not possible to study tip-to-host side interactions because the hyphal tips of P. nunn always stopped growing as they neared the colony of B. cinerea. This phenomenon occurred between 5 and 20 µm distance, as in the case of P. mycoparasiticum described earlier, and indicated the production of a fungistatic factor by B. cinerea.

In side-to-host tip interactions, the growth rate of the host was not affected as its tips approached hyphae of P. nunn. The host did not elicit branching by the mycoparasite at the point of contact. The host hyphae were unaffected in any apparent way.

Categorisation of interaction: non-parasitic (resistant host).

Table 5.18 Summary of observations from videotapes of interactions of hyphae of P. nunn and P. vexans

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate ($\mu m\ min^{-1}$)	Comments
Tip-to-side*1								
1	60	N/A*	N/A	N/A	N/A	N/A	N/A	
2	125	N/A	N/A	N/A	N/A	N/A	N/A	
3	150	N/A	N/A	N/A	N/A	N/A	N/A	
4	>500	N/A	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
5	220	N/A	N/A	N/A	N/A	N/A	N/A	
6	250	N/A	N/A	N/A	N/A	N/A	N/A	
7	>500	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.19 Summary of observations from videotapes of interactions of hyphae of P. nunn and B. cinerea

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Side-to-tip*1								
1	60	N/A	N/A	N/A	N/A	N/A	N/A	
2	80	N/A	N/A	N/A	N/A	N/A	N/A	
3	150	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

5.3.2.3.3 P. nunn versus Fusarium oxysporum

The main features of these interactions are shown in Table 5.20. Before contact, both the mycoparasitic hyphae and the host maintained a steady growth rate as they approached each other. In tip-to-host side interactions, the parasite branched at the point of contact in two of the three cases. In the first such interaction the host stopped and lysed before the parasite branched, but in the second case host stoppage and internal vacuolation/coagulation occurred only some time after branching. In the third interaction the host hypha kept growing and was seemingly unaffected by the parasite.

A similar pattern was seen in side-to-host tip interactions (4-6 in Table 5.20). In the two cases where branching occurred at the point of contact, the host was affected in some way. In interaction 4 the host tip stopped, its contents coagulated, and in interaction 5, the host tip stopped prior to branching by the mycoparasite and host lysis occurred just after the branch appeared. In this case the branch subsequently penetrated the host hypha. There was no obvious response by the mycoparasite or the host in interaction 6, in which the host tip made contact with an older region of the mycoparasitic hypha.

Categorisation of interaction: variable; the host was always affected when the parasite branched at the point of contact. But all events occurred relatively slowly, and there were no responses when the interaction involved older regions of either host or mycoparasitic hyphae.

5.3.2.3.4 P. nunn versus Trichoderma aureoviride

The main features of these interactions are shown in Table 5.21. The growth rates of the mycoparasite and the host were unaffected as they approached one another. The mycoparasite branched at the point of contact in three of four tip-to-host side interactions. In all three

Table 5.20 Summary of observations from videotapes of interactions of hyphae of *P. nunn* and *F. oxysporum*

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	15	1080	900	N/A*	960	N/A	N/A	
2	48	1200	2400	2400	N/A	N/A	N/A	
3	260	N/A	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
4	60	660	1440	1440	N/A	N/A	N/A	
5	100	900	840	N/A	930	990	2.5	
6	>500	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

Table 5.21 Summary of observations from videotapes of interactions of hyphae of P. nunn and T. aureoviride

Replicate observation	Distance from tip to point of contact (µm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (µm min^{-1})	Comments
Tip-to-side*1								
1	3	330	240	N/A*	290	N/A	N/A	
2	70	680	600	600	N/A	N/A	N/A	
3	260	450	380	380	N/A	N/A	N/A	
4	>500	N/A	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
5	110	N/A	300	N/A	315	N/A	N/A	
6	230	735	540	N/A	600	1020	1.0	
7	450	1200	60	1200	N/A	1500	2.0	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

cases branching was preceded by either hyphal stoppage and lysis (interaction 1) or internal vacuolation/coagulation (interactions 2 and 3) by the host. But there was no penetration by the mycoparasite in any of these interactions. In a fourth case (interaction 4), the mycoparasite did not branch at the point of contact and did not affect the host.

In the side-to-host tip interactions, P. nunn branched at the point of contact in two (interactions 6 and 7) of three cases. It subsequently penetrated the host, this being preceded by host lysis in interaction 6 and coinciding with host vacuolation/coagulation in interaction 7. Although there was no response by the mycoparasite in interaction 5, nevertheless the host tip lysed at the point of contact after 315 sec, almost immediately following stoppage of its growth.

Categorisation of interaction: parasitic, involving lysis and internal vacuolation/coagulation by the host, and in some instances, penetration by the mycoparasite.

5.3.2.3.5 P. nunn versus Phialophora sp.

The main features of these interactions are shown in Table 5.22. Growth rates of the mycoparasite and host hyphae were unaffected on approach to the opposing hyphae. In interactions 1 to 3, the mycoparasite hypha grew up to and over the host hypha, branching at the point of contact in interactions 1 and 3. In interaction 1 the first branch did not penetrate, but the host stopped and its contents became coagulated, and a second branch then penetrated the host hypha. In interaction 3, a branch emerged rapidly and the host stopped and lysed within 9 min of contact. In interaction 2, the mycoparasite did not branch at the point of contact and the host was unaffected.

In side-to-host tip interactions, the mycoparasite branched at the point of contact in cases 4 and 6. This was followed by host stoppage

Table 5.22 Summary of observations from videotapes of interactions of hyphae of P. nunn and Phialophora sp

Replicate observation	Distance from tip to point of contact (μm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (μm min^{-1})	Comments
Tip-to-side*1								
1	5	720	2160	2160	N/A*	2400	2.0	
2	30	N/A	N/A	N/A	N/A	N/A	N/A	
3	100	60	480	N/A	530	N/A	N/A	
Side-to-tip*2								
4	40	885	960	N/A	1020	1060	3.5	
5	80	N/A	N/A	N/A	N/A	N/A	N/A	
6	100	540	480	N/A	N/A	N/A	N/A	
7	>500	N/A	N/A	N/A	N/A	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

and lysis in interaction 4, where the branch subsequently penetrated the host. In interaction 6, branching occurred at 60 sec after the host hypha had stopped, but no other detrimental effect on the host hypha was observed. In two other interactions (5 and 7), the parasite did not branch at the point of contact and the host was not affected in any way. Categorisation of interaction: variable; branching by the mycoparasite occurred in only four of the seven interactions and this always resulted in detrimental effects on the host. But there was no effect on the host, and no response by the mycoparasite in the other three cases.

5.3.2.3.6 P. nunn versus miscellaneous fungi

As with P. mycoparasiticum, P. nunn does not grow as quickly or profusely as P. oligandrum and it also tends to grow at a different depth in the agar than do many host hyphae. So a full range of interactions could not be observed, but a few recorded cases are shown in Table 5.23.

In all these cases the growth rate of the mycoparasite and host hyphae were unaffected as they approached the opposing hyphae. In the only recorded interaction with P. graminicola, the tip of a hypha of P. nunn branched at the point of contact after 16 min, but had no effect on P. graminicola. P. nunn also branched at the point of contact with a hypha of R. solani (T125) but had no effect on the host (interaction 3 in Table 5.23). In an interaction with R. solani (GM1) and two interactions with F. culmorum (interactions 2, 4 and 5 in Table 5.23) there was no branching at the point of contact, nor was there any effect on the hosts, which continued growing after contact. In the only side-to-host tip interaction that was observed (interaction 6), a hyphal tip of F. culmorum stopped growing and lysed a few minutes after it made contact with a hypha of P. nunn, but the parasite did not branch at the

Table 5.23 Summary of observations from videotapes of interactions of hyphae of P. nunn and miscellaneous fungi

Replicate observation	Distance from tip to point of contact (μm)	Time (sec) after contact to: Branching	Host-stoppage	Coagulation	Lysis	Penetration	Internal growth rate (μm min^{-1})	Comments
P. graminicola								
Tip-to-side*1								
1	180	960	N/A*	N/A	N/A	N/A	N/A	
R. solani GM1								
Tip-to-side*1								
2	5	N/A	N/A	N/A	N/A	N/A	N/A	
R. solani T125								
Tip-to-side*1								
3	120	260	N/A	N/A	N/A	N/A	N/A	
F. culmorum								
Tip-to-side*1								
4	160	N/A	N/A	N/A	N/A	N/A	N/A	
5	220	N/A	N/A	N/A	N/A	N/A	N/A	
Side-to-tip*2								
6	50	N/A	240	N/A	550	N/A	N/A	

*1 Mycoparasite tip contacted the side of the host hypha

*2 Host hyphal tip contacted the side of mycoparasitic hypha

* Not applicable

point of contact. These miscellaneous interactions were not included in the quantitative comparison of results.

Categorisations of interactions (provisional, based on few observations):

P. graminicola:	non-parasitic (resistant host), but a branch was elicited at the point of contact.
R. solani (GM1):	non-parasitic (resistant host).
R. solani (T125):	non-parasitic (resistant host), but a branch was elicited at the point of contact.
F. culmorum:	variable; the parasite did not branch, but one host tip lysed on contact with P. nunn.

5.3.3 Quantitative comparison of the behaviour of mycoparasitic and host fungi

In much of the following account, attention is focused on specific events in mycoparasitic interactions and quantitative data are assembled (and combined) for particular parasites or particular hosts. In doing so, however, it is recognised that this can only be an approximation, to reveal major behavioural differences, because the behaviour of a particular parasite may vary with different hosts, and vice-versa. Only if many more replicate interactions had been studied (which was not feasible) would it have been possible to analyse quantitative data for individual host-parasite interactions.

Table 5.24 summarises the outcome of all interactions between the three mycoparasites and all hosts (excluding P. graminicola and P. vexans), while Table 5.25 presents similar summary data for interactions in which the mycoparasites branched at the point of contact.

Penetration only occurred when a branch emerged from the mycoparasite at or near the point of contact, never directly by the original

Table 5.24 Outcome of interactions against non-Pythiaceous hosts

	Number of interactions								
	Total	Lysis only	Lysis and penetration	Vacuolation/coagulation		Penetration only	Total lysed	Total vacuolated	Total penetrated
				Only	+ Penetration				
P. oligandrum	59	13	19	3	12	11	32	15	42
P. mycoparasiticum	24	4	5	3	8	3	9	11	16
P. nunn	20	4	3	4	2	0	7	6	5
Total	103	21	27	10	22	14	48	32	63

Table 5.25 Outcome of interactions against non-Pythiaceous hosts where branching of the mycoparasite occurred at the point of contact

	Number of interactions								
	Total	Lysis only	Lysis and penetration	Vacuolation/coagulation		Penetration only	Total lysed	Total vacuolated	Total penetrated
				Only	+ Penetration				
P. oligandrum	52	8	19	2	12	11	27	14	42
P. mycoparasiticum	18	2	5	0	8	3	7	8	16
P. nunn	13	3	3	4	2	0	6	6	5
Total	83	13	27	6	22	14	40	28	63

hypha of P. oligandrum involved in an interaction. It always followed host hyphal lysis or vacuolation/coagulation, never preceding these events. Penetration occurred overall in 63 of the 103 interactions (61.2%), and in 14 of these it was not preceded by lysis or vacuolation/coagulation. But perhaps of more interest is the finding that lysis or vacuolation/coagulation of the host could occur without penetration by a mycoparasite (in a total of 31 out of 103 instances) and also in some cases (12 out of 20 instances) when the mycoparasite did not branch at the point of contact. The implication of these findings is that the hosts could show adverse effects after contact with the mycoparasites, even when the mycoparasites themselves exhibited no obvious behavioural change.

These results will be analysed firstly in terms of the mycoparasites, and then in terms of the hosts.

5.3.3.1 Behaviour of mycoparasites

The results in Section 4 demonstrated marked differences in antagonism by Pythium spp at the level of colony interaction. The purpose of this section is to examine the evidence from interactions at the level of individual hyphae, described earlier in this section, with particular emphasis on quantitative analysis of the effects. Before considering the results, however, it is recorded that both the mean hyphal diameters and the mean extension rates of hyphae of the mycoparasites differed significantly ($P = 0.05$) on the water agar films (Table 5.26).

5.3.3.1.1 Pre-contact events

Prior to contact in each interaction, the growth rates of hyphae of the mycoparasites were recorded (Table 5.27). The most striking feature was that the growth of both P. mycoparasiticum and P. nunn was inhibited

Table 5.26 Mean hyphal diameters and growth rates of the three mycoparasites on water agar films

	Mean hyphal diameter (μm)	Mean growth rate (μm min^{-1})	No of observations
P. oligandrum	4.1 ± 0.1	11.7 ± 0.6	72
P. mycoparasiticum	2.8 ± 0.1	5.9 ± 0.5	32
P. nunn	3.5 ± 0.2	4.6 ± 0.4	30

Table 5.27 Mean growth rates of parasites as they approached host hyphae on water agar films

	Parasite growth rate (μm min^{-1}) [no of interactions]		
	P. oligandrum	*P. mycoparasiticum*	*P. nunn*
P. graminicola	12.3 ± 1.8 (3)		
P. vexans	18.2 ± 3.4 (4)	5.5 ± 0.3 (4)	2.5 ± 0.3 (4)
R. solani (GM1)	16.0 ± 0.6 (3)		
R.solani (T125)	12.0 ± 1.8 (4)		
F. culmorum	10.5 ± 0.9 (5)		
B. piluliferum	7.5 ± 1.3 (4)		
B. cinerea	10.0 ± 2.1 (3)	0.0*	0.0*
F. oxysporum	9.2 ± 2.8 (4)	7.3 ± 1.8 (3)	4.8 ± 0.2 (3)
T. aureoviride	10.8 ± 1.2 (5)	5.5 ± 1.2 (4)	4.1 ± 0.5 (4)
Phialophora sp	15.2 ± 0.7 (5)	5.3 ± 0.2 (3)	6.2 ± 0.4 (3)
All hosts	11.7 ± 0.6 (40)	5.9 ± 0.5 (14)	4.6 ± 0.4 (14)

* Based on at least 3 observations, not included in figures for all hosts

as they approached hyphae of B. cinerea, whereas the growth rate of hyphal tips of P. oligandrum was completely unaffected by B. cinerea. This difference was repeatedly confirmed. The other data in Table 5.27 suggest that there were variations in the growth rates of the mycoparasites in the presence of different hosts, but it must be recorded that direct comparisons are difficult to make because of the long period over which the study was done, and the low degree of replication in each case is reflected in some of the large standard errors. Also, recordings of individual hyphae in successive intervals as they approached the host hyphae revealed no evidence of either a slowing or increase in growth rate before contact, except in the presence of B. cinerea as noted above. Such recordings were made over at least 5 minutes before contact, and over a distance of usually at least 50 µm.

Of particular interest was the absence of any convincing evidence of tropic responses by the mycoparasites prior to contact. There was no evidence of a change in directional growth of the hyphal tips, nor of differential growth or orientation of branching in any of the 134 interactions studied. Instead, hyphal contacts were found to occur at random, and many instances of "near-misses" were observed and recorded - a feature that greatly extended the time required for this study.

5.3.3.1.2 Post-contact events

In almost all instances, hyphal tips of the mycoparasites continued to extend after contact with hyphae of other fungi. Often the direction of growth was unchanged, but if contact was made at an angle then the hypha of the mycoparasite was often deflected and grew alongside the other hypha. Also, in almost all cases the growth rate of the tip of the mycoparasite remained unchanged by contact with another hypha. Sometimes there was a temporary slowing of growth after contact, but the

rate reverted to the pre-contact rate soon afterwards. This slowing was in some instances perhaps more apparent than real, because measurements could only be recorded in a given plane of focus, and the mycoparasites typically grew over or under the hypha of the hosts.

The mycoparasites frequently branched at the point of contact with a host hypha, but the branch first appeared in a sub-apical position as the main hypha had grown on meantime. As shown in Table 5.28, this branching occurred in almost all cases with P. oligandrum, particularly when the hyphal tip contacted the lateral wall of a host hypha, and in most cases when a host hyphal tip contacted the lateral wall of P. oligandrum. This branching response was less frequent in interactions between P. oligandrum and the other Pythium spp (P. graminicola and P. vexans) which were resistant to parasitism (Table 5.29). Branching at the point of contact occurred somewhat less frequently from hyphae of P. mycoparasiticum, and even less frequently from P. nunn than from P. oligandrum (Table 5.28). It seldom occurred when these mycoparasites made contact with, or were contacted by other Pythium spp (Tables 5.30 and 5.31)

P. oligandrum also branched at points of contact with B. cinerea, whereas P. mycoparasiticum and P. nunn seldom did so (the phenomenon being observed only once, with P. mycoparasiticum). But this difference was caused by the inhibitory effect of B. cinerea on these fungi, mentioned earlier. If these 'exceptional' cases (involving B. cinerea and Pythium spp as hosts) are excluded, then there were still differences between the mycoparasites in their frequency of branching on contact with other fungi, as shown in Table 5.28.

The times after contact at which a discernible branch was seen are recorded in Tables 5.29, 5.30 and 5.31. For P. oligandrum, the mean times ranged from c 3 to 9 min, with an overall mean of 302 ± 23 sec;

Table 5.28 Mycoparasite branching at the point of contact with host hyphae

	Number of instances of branching at the point of contact	
	Tip-to-host side	Side-to-host tip
P. oligandrum	38/40 (31/33)*1 (28/30)*2	23/33 (19/27)*1 (17/24)*2
P. mycoparasiticum	12/15 (11/11)*1 (10/10)*2	8/17 (8/14)*1 (8/11)*2
P.nunn	7/14 (7/10)*1 (7/10)*2	6/16 (6/13)*1 (6/10)*2

*1 excluding *Pythium* spp as hosts

*2 excluding *Pythium* spp and *B. cinerea* as hosts

Table 5.29 Mean time between contact and onset of branch emergence by *P. oligandrum* (based on number of branching events in parentheses)

	Time to branch emergence (sec)	
	Tip-to-host side	Side-to-host tip
P. graminicola	250 ± 53 (3)	- (0)
P. vexans	465 ± 115 (4)	330 ± 30 (2)
R. solani GM1	188 ± 27 (3)	157 ± 23 (3)
R. solani T125	398 ± 66 (4)	313 ± 85 (3)
F. culmorum	312 ± 74 (4)	420 (1)
B. piluliferum	197 ± 64 (4)	320 ± 63 (4)
B. cinerea	250 ± 42 (3)	480 ± 60 (2)
F. oxysporum	485 ± 33 (3)	530 ± 370 (2)
T. aureoviride	200 ± 75 (5)	300 ± 90 (2)
Phialophora sp	214 ± 57 (5)	173 ± 96 (3)
All hosts	288 ± 26	320 ± 38

Table 5.30 Mean time between contact and onset of branching by *P. mycoparasiticum* (based on number of branching events in parentheses)

	Time to branch emergence (sec)	
	Tip-to-host side	Side-to-host tip
P. vexans	1100 (1)	- (0)
F.oxysporum	273 ± 83 (3)	368 ± 140 (4)
T. aureoviride	310 ± 63 (3)	510 ± 210 (2)
Phialophora sp	360 ± 60 (3)	345 ± 195 (2)
All hosts	542 ± 197 (10)	398 ± 68 (8)

N/A = no branching

Table 5.31 Mean time between contact and onset of branching by *P. nunn* (based on number of branching events in parentheses)

	Time to branch emergence (sec)	
	Tip-to-host side	Side-to-host tip
P. vexans	- (0)	- (0)
F. oxysporum	1140 ± 60 (2)	780 ± 120 (2)
T. aureoviride	487 ± 103 (3)	968 ± 232 (2)
Phialophora sp	390 ± 330 (2)	712 ± 172 (2)
All hosts	645 ± 153 (7)	820 ± 94 (6)

there was no difference overall between tip-to-host side (mean 288 sec) and side-to-host tip (mean 320 sec) interactions. Evidently P. oligandrum was as responsive to branching in the more mature parts of its hyphae as at the hyphal tips. Also, for P. oligandrum there was no discernible difference in response time to hyphae of resistant (P. graminicola, P. vexans), intermediate (R. solani isolates GM1 and T125), or susceptible (eg B. piluliferum, Phialophora sp) hosts as categorised in Section 5.3, although the number of branching events differed in interactions with these hosts.

The times after which branching was seen in P. mycoparasiticum (Table 5.30) did not differ substantially from those of P. oligandrum on the same range of hosts; the overall times were 542 sec for tip-to-host side, and 398 sec for side-to-host tip interactions, again suggesting that the mycoparasite P. mycoparasiticum was equally responsive in mature regions and at the hyphal tips. The most valid basis of comparison between P. mycoparasiticum and P. oligandrum is for the interactions with the three hosts Phialophora sp, T. aureoviride and F. oxysporum, which were common to both. The overall mean times for branching (tip-to-host side and side-to-host tip) were then: P. oligandrum, 299 ± 48 sec, P. mycoparasiticum, 335 ± 40sec. But there was evidence that the behaviour of the mycoparasites differed with different hosts. For example, with F. oxysporum as the host, P. mycoparasiticum formed a branch somewhat sooner than did P. oligandrum (respective means; 327 ± 52 sec and 503 ± 119 sec), whereas the converse was true with Phialophora sp as the host fungus (mean for P. oligandrum, 221 ± 47 sec, compared with 354 ± 70 sec for P. mycoparasiticum).

Compared with both of these mycoparasites, P. nunn was very slow to branch at the point of contact, irrespective of the host against which it was grown (Table 5.31). The difference in this respect, overall, was

at P=0·01
significant (Table 5.32).

In cases where any of these mycoparasites penetrated the host hyphae, such penetration usually occurred from branches that formed at, or near, the point of contact. But sometimes the first-formed branch did not penetrate, and instead invasion occurred from a branch of the mycoparasite that arose a short distance away from the point of initial contact. This occurred most often when the hypha of the mycoparasite grew alongside the host hypha, and in such cases it was common to see multiple entry points.

The numbers of interactions that resulted in penetration of a host are shown in Table 5.33 in relation to the total number of interactions recorded and those that resulted in branching by the mycoparasite at the point of contact. The table excludes Pythium spp as hosts. Table 5.34 shows a similar analysis but only for the hosts, Phialophora sp, F. oxysporum and T. aureoviride, which were tested against all the mycoparasites.

The interesting feature of these results is that P. nunn penetrated host hyphae much less often than did either P. mycoparasiticum or P. oligandrum. Such a difference cannot be accounted for by the failure of P. nunn to form a branch upon contact, although it did so less frequently than did the other mycoparasites. Instead, it penetrated from only one-third of the branching events, whereas P. oligandrum and P. mycoparasiticum penetrated from more than 80 percent of the branching events. The times between contact or branch initiation and penetration are summarised for the different mycoparasites in Tables 5.35, 5.36 and 5.37. Considering, first, the data for P. oligandrum which involved the most extensive host range, there is evidence of faster penetration into some hosts than into others, once a branch had formed. For example, penetration occurred more rapidly (after branching) when F. culmorum, F.

Table 5.32 Mean time (after contact) of branching by mycoparasites, averaged for all branching events with the hosts *F. oxysporum*, *T. aureoviride* and *Phialophora* sp (no. in parentheses)

	Time to onset of branching (sec)
P. oligandrum	299 ± 48 (15)
P. mycoparasiticum	335 ± 40 (16)
P. nunn	787 ± 89 (5)

Table 5.33 Numbers of interactions involving penetration and numbers involving branching by mycoparasites in all interactions with non-Pythiaceous hosts

	Number of interactions								
	Tip-to-host side			Side-to-host tip			Combined		
	Total observations	Branching	Penetration	Total observations	Branching	Penetration	Total observation	Branching	Penetration
P. oligandrum	33	31	28	26	20	14	59	51	42
P. mycoparasiticum	15	15	14	16	10	8	31	25	22
P. nunn	14	8	1	14	7	4	28	15	5

Table 5.34 Number of interactions involving penetration and numbers involving branching by mycoparasites in all interactions with F. oxysporum, Trichoderma aureoviride and Phialophora sp

	Number of interactions								
	Tip-to-host side			Side-to-host tip			Combined		
	Total observations	Branching	Penetration	Total observations	Branching	Penetration	Total observation	Branching	Penetration
P. oligandrum	14	13	11	9	7	4	23	20	15
P. mycoparasiticum	10	10	10	11	8	6	21	18	16
P. nunn	10	7	1	10	6	4	20	13	5

Table 5.35 Mean times (sec) between contact and penetration and between branching and penetration for *P. oligandrum* against non-Pythiaceous hosts (number of penetration events in parentheses)

	Time between contact and penetration			Time between branching and penetration		
	Tip-to-host side	Side-to-host tip	Overall	Tip-to-host side	Side-to-host tip	Overall
R. solani GM1	713 ± 184 (3)	215 ± 35 (2)	648 ± 242 (5)	708 ± 359 (3)	55 ± 5 (2)	483 ± 299 (5)
R. solani T125	1000 ± 200 (3)	343 ± 99 (3)	672 ± 178 (6)	570 ± 121 (3)	43 ± 15 (3)	360 ± 145 (6)
F. culmorum	362 ± 89 (4)	480 (1)	386 ± 73 (5)	50 ± 34 (4)	60 (1)	52 ± 13 (5)
B. piluliferum	295 ± 92 (4)	400 ± 160 (3)	340 ± 81 (7)	120 ± 77 (4)	93 ± 74 (3)	109 ± 50 (7)
B. cinerea	627 ± 306 (3)	600 (1)	620 ± 216 (4)	397 ± 318 (3)	60 (1)	300 ± 238 (4)
F. oxysporum	520 ± 20 (3)	930 (1)	622 ± 103 (4)	35 ± 13 (3)	30 (1)	34 ± 9 (4)
T. aureoviride	202 ± 47 (4)	- (0)	202 ± 47 (4)	72 ± 31 (4)	- (0)	72 ± 31 (4)
Phialophora sp	462 ± 71 (4)	375 ± 45 (3)	433 ± 46 (7)	250 ± 95 (4)	135 ± 75 (3)	195 ± 39 (7)
All hosts	515 ± 69 (28)	416 ± 63 (13)	484 ± 51 (41)	251 ± 64 (28)	72 ± 19 (13)	198 ± 46 (41)

Table 5.36 Mean times (sec) between contact and penetration and between branching and penetration for *P. mycoparasiticum* against non-Pythiaceous hosts or *B. cinerea* (number of penetration events in parentheses)

	Time between contact and penetration			Time between branching and penetration		
	Tip-to-host side	Side-to-host tip	Overall	Tip-to-host side	Side-to-host tip	Overall
F. oxysporum	327 ± 52 (3)	435 ± 51 (4)	388 ± 40 (7)	53 ± 48 (3)	68 ± 58 (4)	61 ± 34 (7)
T. aureoviride	370 ± 139 (3)	510 ± 210 (2)	426 ± 107 (5)	160 ± 116 (3)	0 ± 0 (2)	96 ± 75 (5)
Phialophora sp	390 ± 52 (3)	- (0)	390 ± 52 (3)	30 ± 30 (3)	- (0)	30 ± 30 (3)
All hosts	302 ± 46 (9)	460 + 65 (6)	401 ± 39 (15)	81 ± 41 (9)	45 ± 39 (6)	67 ± 29 (15)

Table 5.37 Mean times (sec) between contact and penetration and between branching and penetration for *P. nunn* against non-Pythiaceous hosts (number of penetration events in parentheses)

	Time between contact and penetration			Time between branching and penetration		
	Tip-to-host side	Side-to-host tip	Overall	Tip-to-host side	Side-to-host tip	Overall
F. oxysporum	- (0)	990 (1)	990 (1)	- (0)	90 (1)	90 (1)
T. aureoviride	- (0)	1260 ± 240 (2)	1260 ± 240 (2)	- (0)	292 ± 8 (2)	292 ± 8 (2)
Phialophora sp	2400 (1)	1040 (2)	1720 ± 680 (2)	1680 (1)	175 (1)	918 ± 762 (2)
All hosts	2400 (1)	1142 ± 120 (4)	1394 ± 268 (5)	1680 (1)	212 ± 49 (4)	506 ± 296 (5)

oxysporum, T. aureoviride and B. piluliferum were the hosts than when other fungi were the hosts (see final column of Table 5.35), and penetration (after branching) was usually faster in side-to-host tip than tip-to-host side interactions. Such differences were not always reflected in the times taken for penetration following the initial contact (Tables 5.35, 5.36 and 5.37). This point is considered again later, because it probably is more a reflection of the susceptibility of different hosts than of the responses of the mycoparasites per se.

Comparisons between the different mycoparasites are difficult in these respects, because there were relatively few penetrative interactions. But it is notable that P. mycoparasiticum, like P. oligandrum, tended to penetrate rapidly after branching whereas penetration by P. nunn sometimes took considerably longer.

Tables 5.38, 5.39 and 5.40 record the internal growth rates of hyphae of the mycoparasites following penetration. Among the notable features of the results is the finding (Table 5.38) that the growth rate of P. oligandrum differed markedly in the different hosts, being higher in T. aureoviride, Phialophora sp, F. oxysporum and F. culmorum than in the other hosts, particularly in hyphae of R. solani. Another interesting feature is that the rates for P. oligandrum were uniformly lower than those of the hyphae that initially made contact with the host (see Table 5.27 earlier). But this might in part be explained by the fact that the internal hyphae were branches of the initial contacting hyphae, which continued to grow, and hyphal branches often grow more slowly, at least initially, than the parent hyphae (Trinci, 1974). The internal hyphae of P. mycoparasiticum grew more slowly than those of P. oligandrum in the same hosts (cf Tables 5.39 and 5.38), and the internal hyphae of P. nunn grew significantly ($P=0.01$) more slowly than both (Table 5.40) although the data for P. nunn are based on few penetrative events. When

Table 5.38 Mean internal growth rate of *P. oligandrum* in non-Pythiaceous hosts (based on the numbers of observations in parentheses)

	Mean growth rate ($\mu m\ min^{-1}$)		
	Tip-to-host side	Side-to-host tip	Overall
R. solani GM1	2.5 ± 0.9 (3)	6.0 ± 2.0 (2)	3.9 ± 1.2 (5)
R. solani T125	3.3 ± 0.9 (3)	3.3 ± 1.3 (2)	3.3 ± 0.7 (6)
F. culmorum	6.9 ± 0.8 (4)	6.0 (1)	6.7 ± 0.7 (5)
B. piluliferum	5.1 ± 1.2 (4)	3.2 ± 1.3 (3)	4.1 ± 0.8 (7)
B. cinerea	5.7 ± 1.2 (3)	4.0 (1)	5.2 ± 0.9 (4)
F. oxysporum	8.2 ± 0.9 (3)	5.0 (1)	7.4 ± 1.0 (4)
T. aureoviride	8.5 ± 0.5 (4)	- (0)	8.5 ± 0.5 (4)
Phialophora sp	8.8 ± 1.0 (4)	6.7 ± 2.3 (3)	7.9 ± 1.1 (7)
All hosts	6.3 ± 0.5 (28)	4.8 ± 0.7 (14)	5.8 ± 0.4 (42)

Table 5.39 Mean internal growth rate of *P. mycoparasiticum* on non-Pythiaceous hosts (based on the numbers of observations in parentheses)

	Mean growth rate ($\mu m\ min^{-1}$)		
	Tip-to-host side	Side-to-host tip	Overall
F. oxysporum	6.7 ± 2.4 (3)	2.8 ± 0.8 (4)	4.4 ± 1.3 (7)
T. aureoviride	5.8 ± 0.5 (4)	7.0 ± 1.0 (2)	6.2 ± 0.5 (6)
Phialophora sp	4.7 ± 0.7 (3)	- (0)	4.7 ± 0.7 (3)
All hosts	5.7 ± 0.7 (10)	4.2 ± 1.0 (6)	5.1 ± 0.6 (16)

Table 5.40 Mean internal growth rate of *P. nunn* on non-Pythiaceous hosts (based on the numbers of observations in parentheses)

	Mean growth rate ($\mu m\ min^{-1}$)		
	Tip-to-host side	Side-to-host tip	Overall
F. oxysporum	- (0)	2.5 (1)	2.5 (1)
T. aureoviride	- (0)	1.5 ± 0.5 (2)	1.5 ± 0.5 (2)
Phialophora sp	2.0 (1)	3.5 (1)	2.8 ± 0·8 (2)
All hosts	2.0 (1)	2.2 ± 0.5 (4)	2.2 ± 0.4 (5)

these internal growth rates are compared with the growth rates before penetration, P. oligandrum achieved, overall, 49% of the 'external' rate, compared with 86% for P. mycoparasiticum but only 48% for P. nunn. This again suggests the poorer performance of P. nunn as a penetrative mycoparasite, supporting the earlier results for numbers of penetrations (Tables 5.33 and 5.34).

5.3.3.2 Behaviour of hosts

The comments above relate to the behaviour of the parasites; here the data from the interactions are analysed in relation to differences between the host fungi, the growth rates (before contact) and hyphal diameters of which are recorded in Table 5.41.

5.3.3.2.1 Pre-contact events

The extension rates of tips of the various hosts as they approached hyphae of the mycoparasites are recorded in Table 5.42, based on usually three replicates. With such a low degree of replication and considering that these hyphal tips might have varied in their positions in the colonies, it is difficult to draw any meaningful comparisons. In some instances the growth rates are higher than those recorded in Table 5.41, for tips that were not approaching hyphae of the mycoparasites, and in some cases they were lower. Indeed, the only notable feature is that the extension rates for P. vexans, B. cinerea and T. aureoviride were considerably lower as their tips approached hyphae of P. nunn than as they approached P. oligandrum and P. mycoparasiticum. This might indicate the production by P. nunn of growth inhibitory compounds to which these host spp are sensitive. But if this was the case, then it was not reflected in a slowing of individual tips as they approached the hyphae of P. nunn, because no reduction (nor enhancement) of growth was

Table 5.41 Mean host hyphal diameters and growth rates on water agar films

	Hyphal diameter (μm)	Hyphal growth rate (μm min^{-1})
P. graminicola	4.5 ± 0.6	6.8 ± 0.9
P. vexans	5.1 ± 0.3	5.9 ± 0.6
R. solani GM1	7.2 ± 0.3	4.7 ± 0.6
R. solani T125	4.4 ± 0.3	4.5 ± 0.5
F. culmorum	5.2 ± 0.5	3.8 ± 0.2
B. piluliferum	4.1 ± 0.1	2.1 ± 0.3
F. oxysporum	4.6 ± 0.2	2.8 ± 0.1
B. cinerea	6.9 ± 0.8	5.2 ± 0.9
T. aureoviride	3.5 ± 0.2	3.8 ± 0.4
Phialophora sp	3.7 ± 0.1	3.2 ± 0.3

Table 5.42 Mean growth rates of hosts as they approached mycoparasitic hyphae

	Host growth rate ($\mu m\ min^{-1}$) [no of interactions]		
	P. oligandrum	P. mycoparasiticum	P. nunn
P. vexans	7.3 ± 1.3 (3)	7.0 ± 1.0 (3)	4.0 ± 1.3 (3)
B. cinerea	6.7 ± 2.4 (3)	5.3 ± 0.9 (3)	2.7 ± 1.1 (3)
F. oxysporum	2.3 ± 0.3 (3)	2.8 ± 0.4 (5)	2.8 ± 0.2 (3)
T. aureoviride	3.8 ± 1.2 (3)	5.3 ± 2.4 (3)	2.5 ± 0.3 (3)
Phialophora sp	2.7 ± 0.3 (3)	4.0 ± 0.6 (3)	4.0 ± 0.8 (4)

Table 5.43 Times at which hyphal tips of host fungi ceased extension after contact with P. oligandrum

	Mean stopping time (sec) [no of interactions]	
	Tip-to-host side	Side-to-host tip
R. solani GM1	660 ± 275 (3)	80 ± 20 (3)
R. solani T125	825 ± 294 (4)	97 ± 23 (3)
F. culmorum	268 ± 61 (5)	177 ± 35 (3)
B. piluliferum	195 ± 75 (4)	144 ± 42 (5)
B. cinerea	510 ± 233 (3)	200 ± 53 (3)
F. oxysporum	200 ± 58 (4)	160 ± 53 (3)
T. aureoviride	138 ± 41 (5)	140 ± 20 (3)
Phialophora sp	288 ± 84 (5)	170 ± 25 (3)

noted by analysing the rates over the final few minutes before contact occurred. It is concluded, therefore, that the mycoparasites do not markedly influence any of these host fungi before contact - at least on water agar. Similarly, there was no evidence in any case of a change in hyphal orientation of the hosts before contact.

5.3.3.2.2 Post-contact events

When the tips of host hyphae made contact with the lateral walls of hyphae of the mycoparasites (side-to-host tip interactions) they showed a range of responses. The hyphae of pythiaceous hosts (P. vexans in the case of all three mycoparasites, and P. graminicola in the case of P. oligandrum) were unaffected; their tips grew past the mycoparasites, with no decrease in growth rate. The only exception was in one instance involving P. oligandrum and P. vexans, but this was probably an artefact caused by drying of the agar. In contrast, the tips of all other (non-pythiaceous) hosts stopped growing on contact with a hypha of P. oligandrum or P. mycoparasiticum, and sometimes (but not always) with P. nunn.

The occurrence of this host stoppage and the times after which it occurred are shown in Tables 5.43, 5.44 and 5.45. A similar pattern occurred in "tip-to-host side" interactions, ie when tips of mycoparasites made contact with sub-terminal regions of the host hyphae. In these cases, the hosts P. graminicola and P. vexans again continued growing after they were contacted by any of the three mycoparasites; all non-pythiaceous hosts stopped growing some time after being contacted by P. oligandrum or P. mycoparasiticum, and some, but not all, hyphae of each host continued to grow after a hyphal tip of P. nunn had made contact with them.

It is notable that in many instances the host tip stopped more

Table 5.44 Times at which hyphal tips of host fungi ceased extension after contact with *P. mycoparasiticum*

	Mean stopping time (sec) [no of interactions]	
	Tip-to-host side	Side-to-host tip
B. cinerea	N/A*	220 ± 72 (3)
F. oxysporum	260 ± 80 (3)	216 ± 57 (5)
T. aureoviride	378 ± 200 (4)	150+ ±46 (3)
Phialophora sp	257 ± 55 (3)	440 ± 80 (3)

* Not applicable

Table 5.45 Times at which hyphal tips of host fungi ceased extension after contact with *P. nunn*

	Mean stopping time (sec) [no of interactions]	
	Tip-to-host side	Side-to-host tip
F. oxysporum	1650 ± 750 (2/3)	1140 ± 300 (2/3)
T. aureoviride	407 ± 105 (3/4)	300 ± 139 (3/3)
Phialophora sp	1320 ± 840 (2/3)	720 ± 240 (2/4)

quickly in side-to-host tip than tip-to-host side interactions (Tables 5.43, 5.44 and 5.45), ie when the tip of a host made contact with the lateral wall of a mycoparasite. This difference was particularly marked in interactions involving R. solani and P. oligandrum, but there was less of a difference, if any, in interactions involving some of the other hosts (eg T. aureoviride, B. piluliferum and F. oxysporum) with P. oligandrum. It indicates a substantial difference in susceptibility between the tips and older regions of the hyphae of R. solani (compared with some other hosts) to the effects of the mycoparasite, consistent with the results for colony interactions on cellulose (Section 4).

For the few hosts that can be compared across the range of mycoparasites, there was no clear difference in the times at which stoppage occurred in response to P. oligandrum and P. mycoparasiticum respectively (Tables 5.43 and 5.44). But in every case the hosts stopped later after contact with P. nunn (Table 5.45) than with P. oligandrum or P. mycoparasiticum. The smallest difference in this respect was for T. aureoviride, suggesting that this host had, overall, the greatest susceptibility to the effects of the three mycoparasites.

Further information on some of these points is seen by comparing the times at which host tips stopped in the replicate observations on individual host-mycoparasite interactions (data presented earlier in Tables 5.2 to 5.23).

All events that followed stoppage involved some cytoplasmic disruption in the host. Often, the hyphal contents surged to the point of contact, but despite considerable relocation of the hyphal contents, there was no apparent leakage (as distinguishable from lysis) of materials from the hyphae in any case. This surging usually preceded, by a short time, the lysis or vacuolation/coagulation of the host hyphal contents.

The most conspicuous event that followed contact was localised lysis of the host. Its incidence and timing in different host-mycoparasite combinations are shown in Tables 5.46, 5.47 and 5.48. Lysis occurred rarely with the isolates of R. solani (tested against P. oligandrum only) - in a total of only two of 13 interactions, and both involved contact of a host tip with the lateral wall of P. oligandrum. In these two cases, however, it occurred quite rapidly after contact (55 and 140 sec). In contrast, lysis occurred commonly in interactions of F. culmorum, B. piluliferum, F. oxysporum and T. aureoviride with P. oligandrum, and in every instance when the tips of these host fungi made contact with hyphae of the mycoparasite. It was no faster in these cases than when R. solani contacted P. oligandrum and no faster in side-to-host tip interactions than in tip-to-host side. Pooling the results for all such interactions in Table 5.46, the mean time after contact when lysis occurred was 240 ± 36 sec for tip-to-host side interactions, and 203 ± 81 for side-to-host tip interactions.

This finding is interesting because it indicates that different regions of the apical compartments of the hosts - the extreme tips and sub-apical regions - were equally susceptible to localised wall lysis after contact with P. oligandrum. Inspection of Tables 5.2 to 5.11 (see earlier) shows that the mean distance of these sub-terminal contact points was 137 ± 44 µm from the tips of the hosts. Further examination of those tables reveals that, for any single host, there was no relationship between distance behind the apex and susceptibility to hyphal lysis on the part of host hyphae. In addition to these points, it may be noted that P. graminicola and P. vexans were insensitive to the effect of P. oligandrum or the other mycoparasites; they were not lysed and are excluded from Tables 5.46, 5.47 and 5.48.

The three host fungi for which comparisons can be made (F. oxy-

Table 5.46 Number and mean time after contact of lytic interactions involving *P. oligandrum*

	Time of lysis (sec) [no of interactions]				
	Tip-to-host side		Side-to-host tip		Total
R. solani GM1	-	[0/3]	140	[1/3]	[1/6]
R. solani T125	-	[0/4]	55	[1/3]	[1/7]
F. culmorum	345 ± 195	[2/5]	317 ± 133	[3/3]	[5/8]
B. piluliferum	170	[1/4]	197 ± 22	[5/5]	[6/9]
B. cinerea	180	[1/3]	130	[1/3]	[2/6]
F. oxysporum	255 ± 80	[4/4]	243 ± 25	[3/3]	[7/7]
T. aureoviride	188 ± 74	[3/5]	170 ± 20	[3/3]	[6/8]
Phialophora sp	247 ± 29	[3/5]	210	[1/3]	[4/8]
All hosts	240 ± 36	[14/33]	203 ± 51	[18/26]	

Table 5.47 Number and mean time after contact of lytic interactions involving *P. mycoparasiticum*

	Time of lysis (sec) [no of interactions]				
	Tip-to-side		Side-to-tip		Total
B. cinerea	N/A*		-	[0/3]	[0/3]
F. oxysporum	-	[0/3]	340 ± 50	[2/5]	[2/8]
T. aureoviride	375 ± 165	[2/4]	207 ± 77	[3/3]	[5/7]
Phialophora sp	N/A	[0/3]	848 ± 292	[2/3]	[2/6]
All hosts	375 ± 165	[2/10]	428 ± 131	[7/14]	

* Not applicable

Table 5.48 Number and mean time after contact of lytic interactions involving P. nunn

	Time of lysis (sec) [no of interactions]				
	Tip-to-host side		Side-to-host tip		Total
B. cinerea	N/A*		-	[0/3]	[0/3]
F. oxysporum	960	[1/3]	1440	[1/3]	[2/6]
T. aureoviride	290	[1/4]	458 ± 142	[2/3]	[3/7]
Phialophora sp	530	[1/3]	1020	[1/4]	[2/7]
All hosts	593 ± 196	[3/10]	844 ± 246	[4/13]	

* Not applicable

Table 5.49 Number and mean time after contact of vacuolation/coagulation of host cell contents with P. oligandrum

	Time of coagulation (sec) [no of interactions]				
	Tip-to-host side		Side-to-host tip		Total
R. solani GM1	-	[0/3]	60	[1/3]	[1/6]
R. solani T125	740 ± 231	[3/4]	180 ± 00	[2/3]	[5/7]
F. culmorum	300 ± 120	[2/5]	-	[0/3]	[2/8]
B. piluliferum	-	[0/4]	-	[0/5]	[0/9]
B. cinerea	675 ± 285	[2/3]	420 ± 60	[2/3]	[4/6]
F. oxysporum	-	[0/4]	-	[0/3]	[0/7]
T. aureoviride	-	[0/5]	-	[0/3]	[0/8]
Phialophora sp	510 ± 30	[2/5]	420	[1/3]	[3/8]
All hosts	577 ± 103	[9/33]	280 ± 67	[6/26]	

sporum, T. aureoviride and Phialophora sp) showed, overall, a lower incidence of lysis in the presence of P. mycoparasiticum and P. nunn than of P. oligandrum. They lysed in 17 of 23 interactions with P. oligandrum, compared with 9 of 22 for P. mycoparasiticum and 7 of 20 for P. nunn, but the difference was not significant by χ^2 analysis. There was, however, a significant difference in the time at which lysis occurred: overall, this time was 222 ± 22 for interactions of the three hosts with P. oligandrum, compared with 416 ± 104 for P. mycoparasiticum and 664 ± 116 for P. nunn.

The three hosts, F. oxysporum, T. aureoviride and Phialophora sp, showed some evidence of differential susceptibility to lysis by the range of mycoparasites: the number of lytic events in T. aureoviride for all mycoparasites was 14 (of 22 interactions), compared with 11 (of 21) for F. oxysporum and 8 (of 21) for Phialophora sp, although this difference was not significant by χ^2 analysis. A similar analysis for times at which lysis occurred was precluded by the few available data, which were not uniformly distributed for the different mycoparasites. But it is notable that the hyphae of T. aureoviride lysed, on average, sooner in the presence of each mycoparasite than did hyphae of F. oxysporum and Phialophora sp.

An alternative to host lysis involved vacuolation/coagulation of the host hyphal contents, the incidence and timing of which are shown in Tables 5.49, 5.50 and 5.51. There are no relevant data for the hosts P. graminicola and P. vexans, because these were unaffected by the mycoparasites. Also, there are no, or few, data for F. oxysporum and T. aureoviride in the presence of P. oligandrum or for T. aureoviride in the presence of P. mycoparasiticum, because in most instances the result of these interactions was host cell lysis.

Considering the results for P. oligandrum, an interesting differ-

Table 5.50 Number and mean time after contact of vacuolation/coagulation of host cell contents with *P. mycoparasiticum*

	Time of coagulation (sec) [no of interactions]		
	Tip-to-host side	Side-to-host tip	Total
B. cinerea	N/A*	640 ± 40 [3/3]	[3/3]
F. oxysporum	320 ± 110 [2/3]	315 ± 105 [2/5]	[4/8]
T. aureoviride	900 [1/4]	- [0/3]	[1/7]
Phialophora sp	258 ± 56 [3/3]	- [0/3]	[0/6]
All hosts	386 ± 110 [6/10]	510 ± 89 [5/14]	

* Not applicable

Table 5.51 Number and mean time after contact of vacuolation/coagulation of host cell contents with *P. nunn*

	Time of coagulation (sec) [no of interactions]		
	Tip-to-host side	Side-to-host tip	Total
B. cinerea	N/A*	- [0/3]	[0/3]
F. oxysporum	2400 [1/3]	1440 [1/3]	[2/6]
T. aureoviride	490 ± 110 [2/4]	1200 [1/3]	[2/7]
Phialophora sp	2160 [1/3]	- [0/4]	[1/7]
All hosts	1385 ± 520 [4/10]	1320 ± 120 [2/10]	

* Not applicable

ence was found between the behaviour of the two isolates of R. solani. Whereas neither isolate showed much evidence of lysis after contact (Table 5.46), isolate T125 showed a high incidence of vacuolation/coagulation of its hyphal contents, but isolate GM1 did so only in one instance (Table 5.49). In fact only 2 of the 6 interactions of GM1 with P. oligandrum showed any evidence of adverse effects prior to penetration of the host, and in both cases this occurred when the host tip contacted the mycoparasite. On this basis, R. solani GM1 was among the most resistant host fungi to mycoparasitism by P. oligandrum. But isolate T125 (6 out of 7 interactions involved some adverse effect) was apparently more sensitive to the activities of P. oligandrum.

Sensitivity was also shown by B. cinerea and F. oxysporum, affected in every instance, and F. culmorum, Phialophora sp and T. aureoviride, affected in most instances (Tables 5.46, 5.49).

Although there were differences between the hosts in that some exhibited a high degree of lysis and others exhibited mainly vacuolation/coagulation, nevertheless, pooling of the results for all hosts against all three mycoparasites revealed that the incidence of lysis (in a total 48 out of 103 interactions) was not markedly different from that of vacuolation/coagulation (in 32 of 103 interactions). The times after contact in which vacuolation/coagulation was observed were generally much longer in interactions with P. nunn than in those with P. oligandrum and P. mycoparasiticum. But there was no clear difference between the individual hosts in the timing of this event.

5.3.4 Interactions of Trichoderma harzianum and Gliocladium roseum with host fungi

In order to compare the mode of antagonism of the mycoparasitic Pythium spp with that of other reported mycoparasites, one isolate each

of Trichoderma harzianum (T95) and Gliocladium roseum (Gr53) were grown against two of the previously used hosts, Pythium graminicola and Phialophora sp, which were, respectively, resistant and susceptible to the mycoparasitic Pythium spp.

5.3.4.1 Interactions with Trichoderma harzianum (T95)

Both of the host fungi were inhibited by a diffusate of T. harzianum and their tips stopped growing long before they made contact with the T. harzianum hyphae. So only interactions in which tips of the mycoparasite made contact with the lateral walls of the host could be studied. In three videotaped interactions (Table 5.52), hyphae of T. harzianum showed no change of growth rate and no obvious change of hyphal orientation as their tips approached hyphae of P. graminicola. After contact, T. harzianum grew over and past the host in interactions 1 and 3, and alongside in interaction 2 (Table 5.52). The mycoparasite branched at the point of contact after 8 min in interaction 2 and this was followed 22 min later by vacuolation/coagulation of the host cytoplasm. Slight vacuolation/coagulation was also observed around the point of contact in the host hypha in interaction 3, after 40 min. Penetration of the host by T. harzianum was not observed in any instance.

T. harzianum showed no change in growth rate or hyphal orientation as its tips approached the lateral walls of Phialophora sp. In all three recorded interactions (Table 5.53) its tips grew over the host hyphae with no change in growth rate. In one of the three instances it formed a branch at the point of contact after 25 min and slight vacuolation/coagulation of the cytoplasm was discernible in the host hypha after 30 min.

Table 5.52 Summary of observations from videotapes of interactions of hyphae of T. harzianum and P. graminicola

Replicate observation	Type	Distance from tip (µm)	Time until branching (sec)	Host stoppage (sec)	Host vacuol-ation (sec)	Comments
1	Tip-to-host side	5	N/A	N/A*	N/A	
2	Tip-to-host side	40	480	N/A*	1800	
3	Tip-to-host side	130	N/A	N/A*	2400	

Table 5.53 Summary of observations from videotapes of interactions of hyphae of T. harzianum and Phialophora sp

Replicate observation	Type	Distance from tip (µm)	Time until branching (sec)	Host stoppage (sec)	Host vacuol-ation (sec)	Comments
1	Tip-to-host side	25	N/A	N/A*	N/A	
2	Tip-to-host side	35	1500	N/A*	1800	
3	Tip-to-host side	80	N/A	N/A*	N/A	

* Host had stopped prior to contact

5.3.4.2 Interactions with Gliocladium roseum (Gr53)

As in interactions with T. harzianum, the host hyphae stopped growing at some distance from colonies of G. roseum, so only tip-to-host side interactions could be recorded (Tables 5.54 and 5.55). The tips of G. roseum maintained a constant growth rate as they approached hyphae of either P. graminicola or Phialophora sp. They grew over the host hyphae and continued their growth away from the host. G. roseum did not branch at the point of contact with P. graminicola, and only slight internal vacuolation/coagulation was observed in one of three cases, after 960 sec. Similarly, no branching was seen at the point of contact with Phialophora sp, and there was no adverse effect on the host in three recorded interactions.

5.3.5 Interactions between mycoparasites

In this final series of interactions the mycoparasitic Pythium species were grown against each other in conditions identical to those used earlier. The findings are summarised briefly below and in Tables 5.56, 5.57 and 5.58. In no case was there a significant antagonistic effect in these interactions.

5.3.5.1 P. oligandrum versus P. mycoparasiticum

In both tip-to-host side and side-to-host tip interactions the growth rates of both mycoparasites were unaffected before or after contact with each other. Hyphal tips of the mycoparasites - P. oligandrum in interactions 1-3 and P. mycoparasiticum in interactions 4-6 - grew over the hypha of the other fungus and continued to grow in the same direction. Branching at the point of contact occurred in only one instance, by P. oligandrum in interaction 1 (Table 5.56). Otherwise, neither mycoparasite was adversely affected by, nor responded to, the

Table 5.54 Summary of observations from videotapes of interactions of hyphae of G. roseum and P. graminicola

Replicate observation	Type	Distance from tip (µm)	Time until branching (sec)	Host stoppage (sec)	Host vacuol-ation (sec)	Comments
1	Tip-to-host side	5	N/A	N/A*	960	
2	Tip-to-host side	40	N/A	N/A*	N/A	
3	Tip-to-host side	65	N/A	N/A*	N/A	

Table 5.55 Summary of observations from videotapes of interactions of hyphae of G. roseum versus Phialophora sp

Replicate observation	Type	Distance from tip (µm)	Time until branching (sec)	Host stoppage (sec)	Host vacuol-ation (sec)	Comments
1	Tip-to-host side	20	N/A	N/A*	N/A	
2	Tip-to-host side	35	N/A	N/A*	N/A	
3	Tip-to-host side	40	N/A	N/A*	N/A	

* Host hypha stopped prior to contact

Table 5.56 Summary of observations from vidoetapes of interactions of hyphae of P. oligandrum and P. mycoparasiticum

Replicate observation	Distance from tip to point of contact (μm)	Time until branching of P. oligandrum	Time until branching of P. mycoparasiticum
Tip-to-side			
1	80	480	N/A*
2	160	N/A	N/A
3	380	N/A	N/A
Side-to-tip			
4	45	N/A	N/A
5	220	N/A	N/A
6	300	N/A	N/A

*, no branching

Table 5.57 Summary of observations from videotaps of interactions of hyphae of P. oligandrum and P. nunn

Replicate observation	Distance from tip to point of contact (μm)	Time until branching of P. oligandrum	Time until branching of P. nunn
Tip-to-side			
1	5	N/A*	N/A
2	30	540	1380
3	260	540	N/A
Side-to-tip			
4	310	N/A	390
5	420	N/A	N/A
6	>500	N/A	480

*, no branching

Table 5.58 Summary of observations from videotapes of interactions of hyphae of *P. mycoparasiticum* and *P. nunn*

Replicate observation	Distance from tip to point of contact (μm)	Time until branching of *P. mycoparasiticum*	Time until branching of *P. nunn*
Tip-to-side			
1	12	600	N/A*
2	70	N/A	N/A
3	110	N/A	N/A
Side-to-tip			
4	30	N/A	N/A
5	120	N/A	N/A
6	>500	N/A	N/A

*, no branching

other during the time of observation.

5.3.5.2 P. oligandrum versus P. nunn

The hyphal tips of both P. oligandrum and P. nunn maintained a steady growth rate as they approached the opposing hyphae. These growth rates were maintained in each interaction as one hypha grew over and away from the other. Branching at the point of contact was more common than in the previous pairing; P. oligandrum branched at the point of contact with lateral walls of P. nunn in interactions 2 & 3, as did P. nunn on contact with lateral walls of P. oligandrum in interactions 4 & 5. P. nunn also formed a branch where the tip of P. oligandrum grew over it in interaction 2. Neither parasite was adversely affected by the other during the time of observation (Table 5.57)

5.3.5.3 P. mycoparasiticum versus P. nunn

In all cases the growth rates of both fungi were unaffected as a hyphal tip approached the side of the other hypha. In interactions 1, 2, 4, 5 and 6 the approaching hypha grew up to and over the other hypha without a change in growth rate; in interaction 3 the hypha of P. mycoparasiticum grew along the hypha of P. nunn for 35 µm before growing over and away. Branching occurred at the point of contact only in interaction 1 - by P. mycoparasiticum. Neither parasite was adversely affected by the other during the time of observation (Table 5.58).

5.3.6 Attempted disruption of mycoparasitic responses

Some of the observed effects of mycoparasites on host hyphae described earlier - for example, the rapid coagulation/vacuolation of cytoplasm - are reminiscent of hypersensitive responses of plant cells to invasion by parasitic fungi (Bailey, 1982) or of hyphal interference

as described in fungal pairings by Ikediugwu & Webster (1970). It seems clear that where contact occurs, recognition events (Keen, 1982) leading rapidly to loss of function or cytoplasmic integrity by the affected cell, must be involved. The rapid hyphal lysis observed when some host fungi made contact with, or were contacted by, mycoparasites is perhaps explicable in a different way - as the result of actions of enzymes of the mycoparasite on walls of their hosts. Lewis et al. (1989) and Elad et al. (1985) have reported that mycoparasitic Pythium spp produce enzymes capable of the lysis of host hyphal walls and, furthermore, that these enzymes are inducible by host wall components rather than being produced constitutively. Elad, Chet & Henis (1982) have reported similarly for Trichoderma spp that parasitize other fungi. However, the production of certain enzymes such as chitinase, by P. oligandrum has not, as yet, been substantiated (Lewis et al., 1989).

As a possible approach to investigating such mechanisms, an attempt was made initially to use dyes that might block potential receptors on host hyphae, as was done successfully by Mitchell & Deacon (1986) to demonstrate a role of receptors on root surface mucilage in inducing encystment by zoospores of Pythium spp. However, this approach was abandoned because dyes (at what were thought to be sufficient concentrations) either obscured observations or adversely affected the growth of the mycoparasites or hosts. Another possible approach that was considered involved supplementing the basal medium with sugars that might block potential receptors on the hyphae of hosts or mycoparasites, on the basis that Elad, Barak & Chet (1983) and Barak et al. (1985) have implicated lectins in host-mycoparasite interactions involving Trichoderma spp. However, this possible approach was not pursued because sugars would increase the density of colonies, again possibly obscuring

observation of interactions.

The approach finally adopted to investigate the possible roles of both recognition events and mycoparasite-derived enzymes was to inactivate hyphae of hosts or mycoparasites as they approached one another. The method involved the use of very fine beams (approximately 10 μm diameter) of intense light which could be accurately targeted onto specific areas of the fungal hyphae. The beams were produced from a 200 W mercury vapour lamp and were directed through the optics of the microscope (Leitz "Orthoplan") through a Ploempak incident fluorescence attachment. In place of a normal filter block, a blank (TK400) filter block was used so that all wavelengths of light generated by the lamp were directed onto the hyphae. The beam of light was focused using the focusing attachment of the Ploempak and it was restricted to 10 μm diameter by closing the field diaphragm.

All other conditions of the experiments were similar to those above: hosts and parasites were opposed on thin films of water agar and their interactions were recorded with a video-camera using transmitted light microscopy. However, when hyphae were about to make contact with one another a beam of intense light was focused on a selected hypha, or part of a hypha, by means of the Ploempak epifluorescence attachment. This beam was directed, by means of prisms in the Ploempak, down through the objective and onto the hyphae. The position of the beam was always in the centre of a microscope field of view, so the part of a hypha to be irradiated was moved into this position, using the moveable stage of the microscope. The duration of exposure was controlled by moving a barrier into the light path from the mercury vapour lamp.

The intensity of the light beam was not determined. This was not considered necessary because the light had to pass through a variable thickness of agar before reaching the target hypha, due to variation in

thickness of the agar film on different coverslips. Instead, interactions and their outcomes were categorized according to the observed effects of the light beam on the target hyphae - in some instances hyphal growth was severely disrupted and in other cases it was unaffected. The video-camera again proved useful in this respect, because it would have been unsafe to view the effect of the light beam (with a UV component) through the microscope eyepieces, but this could be observed on the video monitor.

All observations were made on interactions between P. oligandrum and the hosts F. oxysporum and T. aureoviride. These combinations were selected because it was found previously that the hosts normally showed lysis (always for F. oxysporum and in all side-to-host tip interactions with T. aureoviride) when they contacted hyphae of P. oligandrum (Tables 5.9 and 5.10). So a clear normal pattern of behaviour was available for comparison with the effects of irradiation.

5.3.6.1 Results

The irradiation treatments had variable and generally unpredictable effects on the individual treated hyphae (Tables 5.58, 5.59 and 5.60). Some treatments caused growth cessation and surging of protoplasm to the tip during the exposure; the cytoplasm then coagulated/vacuolated and lacked coordinated movement and the hypha did not resume growth. In other cases some protoplasmic surging occurred in the treated region during exposure but the hypha grew normally after irradiation. In yet other cases surging occurred during exposure and the hypha exhibited slight or erratic protoplasmic streaming after treatment. In two instances the treated hypha stopped and lysed. These variable responses, and the random nature of contact events, made it impossible to relate

Table 5.58 Examples of irradiation treatments that had no effect on the outcome of a mycoparasitic interaction

Host*	Irradiation site, duration and start time (sec) before contact	Contact site	Effect on exposed hypha		Outcome of interaction (times post-contact, sec)
			During exposure	After exposure	
F. oxy	Parasite tip, 60, 620 pre-contact	400 µm from parasite tip	Coagulation 0-300 µm from parasite tip	Some cytoplasmic movement	Host stopped (60), lysis at contact point (150)
F. oxy	Parasite tip, 60, 170 after contact	140 µm from host tip	Stopped 30 sec, erratic surging then no cytoplasmic movement	No cytoplasmic movement	Host stopped (120), surging in host (150), host lysis (240)
T. aureo	30 µm from parasite tip, 60, 30 post-contact	40 µm from parasite tip	Cytoplasmic surging	Normal cytoplasmic movement	Host stopped (140), lysis at contact point (260)
T. aureo	80 µm from parasite tip, 60, 10 post-contact	70 µm from parasite tip	Cytoplasmic surging	Normal cytoplasmic movement	Host stopped (60), lysis at contact point (100)
T. aureo	30 µm from host tip, 60, 250 pre-contact	30 µm from host tip	Surging then loss of cytoplasmic movement	No cytoplasmic movement	Surging in host (200), penetration
T. aureo	27 µm from parasite tip, 60 at time of contact	22 µm from parasite tip	Slight surging	No effect	Host stoped (90), penetrated (150)
T. aureo	70 µm from host tip, 60, 90 pre-contact	70 µm from host tip	No effect	No effect	Host lysis (150)

* Fusarium oxysporum or Trichoderma aureoviride

Table 5.59 Examples of irradiation treatment that caused partial disruption of normal parasitism

Host *	Irradiation site, duration and start time (sec) before contact	Contact site	Effect on exposed hypha		Outcome of interaction (times post-contact, sec)
			During exposure	After exposure	
F. oxy	Parasite tip, 40, 300 pre-contact	60 µm from parasite tip	Stopped at 20 sec, slight cytoplasmic streaming	Slight cytoplasmic streaming	Host stopped (90), vacuolated (150), penetration
F. oxy	125 and 135 µm from parasite tip (60 sec at each (120 pre-contact)	120 µm from parasite tip	Cytoplasmic surging, erratic cytoplasmic streaming	Erratic cytoplasmic streaming	Host overgrew parasite. Stopped (960), vacuolated (1800)
F. oxy	90 µm from host tip, 100, 220 pre-contact	90 µm from host tip	Vacuolation/ coagulation	Vacuolation/ coagulation	Parasite branched (270), host lysed at contact point (412)
F. oxy	Host tip, 50, 60 pre-contact	35 µm from host tip	Growth stopped (35 sec)	No cytoplasmic movement	Parasite branched (100) penetration (120)
T. aureo	130 µm from parasite tip, 60, at contact time	140 µm from parasite tip	Cytoplasmic surging, erratic streaming	Erratic cytoplasmic streaming	Host overgrew parasite, stopped (480), vacuolated (960)
T. aureo	40 µm from parasite tip, 60, 30 post-contact	40 µm from parasite tip	Surging then slight cytoplasmic streaming	Slight cytoplasmic streaming	Host stopped (80), vacuolated (600)
T. aureo	65 µm from parasite tip, 60, 480 pre-contact and 60, 30 pre-contact	60 µm from parasite tip	Surging, erratic streaming	Erratic streaming	Host overgrew parasite, stopped (240), lysed (290)

* See Table 5.58

Table 5.59 (Cont'd) Examples of irradiation treatment that caused partial disruption of normal parasitism

Host	Irradiation site, duration and start time (sec) before contact	Contact site	Effect on exposed hypha		Outcome of interaction (times post-contact, sec)
			During exposure	After exposure	
T. aureo	85 µm from parasite tip, 80, 240, pre-contact	75 µm from parasite tip	Surging	Normal at time of contact	Host overgrew parasite, stopped (150), vacuolated

Table 5.60 Examples of irradiation treatments that caused complete disruption of normal parasitism

Host *	Irradiation site, duration and start time (sec) before contact	Contact site	Effect on exposed hypha		Outcome of interaction (times post-contact, sec)
			During exposure	After exposure	
F. oxy	Parasite tip, 60, 480 pre-contact	10 µm from parasite tip	Stopped at 20 sec, vacuolation 140 sec, lysis 190 sec	Lysed with coagulated contents	Host grew at pre-contact rate, unaffected
F. oxy	Parasite tip, 60, 1080 pre-contact	50 µm from parasite tip	Stopped at 20 sec, lysed	Lysed hypha	As above
F. oxy	Parasite tip, 80, 240 pre-contact	250 µm from parasite tip	Stopped 25 sec, vacuolated (0-500 µm from tip)	Vacuolation/ coagulation	As above
F. oxy	Parasite tip, 60, 70 pre-contact	80 µm from parasite tip	Stopped 30 sec, erratic surging then no cytoplasmic movement	No cytoplasmic movement	As above
T. aureo	130 µm from parasite tip, 180, 200 pre-contact	130 µm from parasite tip	Strong, erratic surging	Strong erratic surging	As above
T. aureo	Parasite tip, 120, 300 pre-contact	10 µm from parasite tip	Stopped 32 sec	Coagulation 240 sec	As above
T. aureo	50 µm from parasite tip, 80, 30 pre-contact	40 µm from parasite tip	None	Stopped 200 vacuolated 240 sec	As above

* See Table 5.58

treatment per se to subsequent mycoparasitic events. So a correlative approach was used in which the outcome of an interaction was related to the observed effect of irradiation on a treated hypha. This revealed three categories of behaviour, termed "no disruption", "partial disruption" and "complete disruption" of the normal parasitic process (Tables 5.58, 5.59 and 5.60).

In cases of "no disruption" (Table 5.58) the hosts lysed or exhibited cytoplasmic vacuolation/coagulation within the range of times found for normal (unirradiated) interactions. These cases were common when host tips contacted parasite hyphae that had been irradiated but had recovered fully from the treatment, or one example when irradiation of the parasite was some time after contact and the host hypha had already stopped growing and exhibited cytoplasmic surging. They were also seen when parasite tips contacted host hyphae that had been irradiated but not fully inactivated (the interaction then leading to host lysis), or inactivated (when the interaction resulted in rapid penetration though without lysis).

In cases of "partial disruption" (Table 5.59) the host was affected by the parasite later than would be expected in normal interactions, or it showed cytoplasmic coagulation/vacuolation rather than normal lysis, or it grew over the treated hypha before being affected. Two cases of "partial disruption" of parasitism followed the complete inactivation of host hyphae by irradiation; others involved treatment of parasite hyphae that did not fully recover their normal functions and exhibited only slight or erratic cytoplasmic streaming.

Cases of "complete disruption" (Table 5.60) were those in which the host showed no adverse effect after its tip contacted a hypha of the parasite. Instead, the host tip grew across the parasite hypha and onwards at the pre-contact rate. In all such cases the parasite had

been permanently affected by exposure to irradiation. Its tip did not resume growth and the cytoplasm did not exhibit cyclosis at the point where it was contacted by the host.

To give a more detailed account of these studies two examples are detailed below.

5.3.6.1.1 P. oligandrum on F. oxysporum

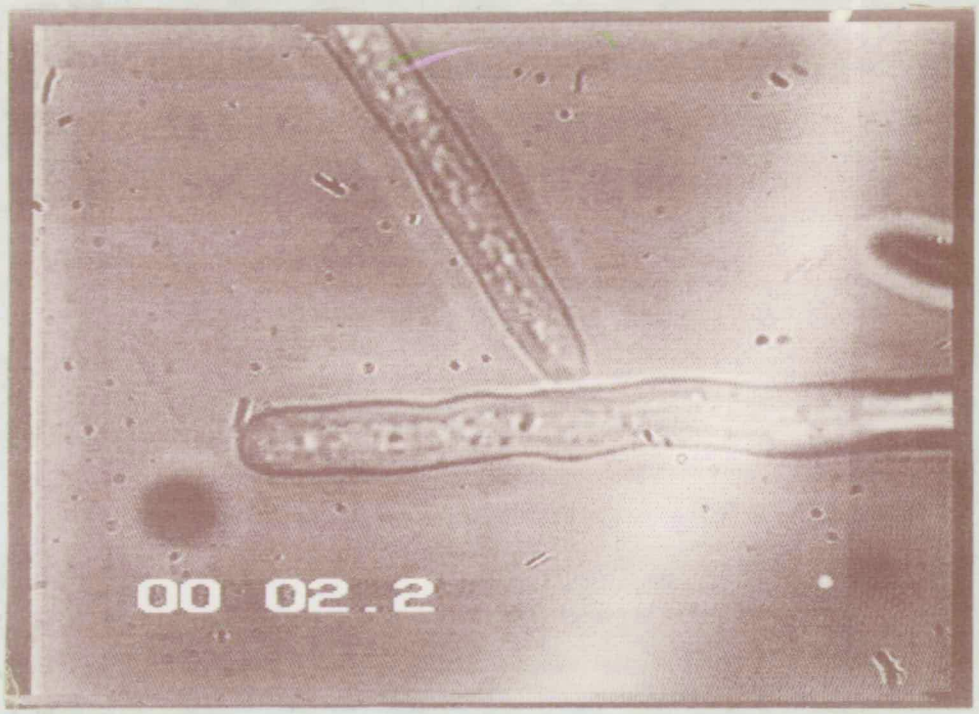
The sequence of this interaction is shown in Figs 5.55 to 5.62. Initially, single hyphae of F. oxysporum (diameter 5 μm) and P. oligandrum (5 μm diameter) were extending at rates of 4 μm min^{-1} and 12 μm min^{-1} respectively. Their paths were such that, with unchanged growth rates, the tip of P. oligandrum would contact the hypha of F. oxysporum at a point 37 μm behind its tip. The tip of F. oxysporum was irradiated for 60 sec, resulting in cessation of extension of the tip 40 sec after irradiation was begun. No protoplasmic movement could be observed in the host hypha following the exposure of its tip. The approaching hyphal tip of P. oligandrum (unirradiated) contacted the hypha of F. oxysporum at a point 30 μm behind the host tip 90 sec after irradiation ceased (Fig 5.55). After contact the tip of P. oligandrum continued to extend at 12 μm min^{-1}. Vacuolation/coagulation of the host cell began to occur 230 sec after contact by the mycoparasite. The first evidence of this was at the point of contact, after which coagulation was seen to spread from this area. The mycoparasite was seen to have branched at the point of contact after 450 sec, and this branch penetrated the host, being clearly observed within the host hypha after 520 sec (Fig 5.57).

A second hypha of F. oxysporum grew towards the hypha of P. oligandrum and contacted the lateral wall of the mycoparasite hypha, 60 μm behind the point of contact with the first hypha of F. oxysporum (Fig

Fig 5.55 *P. oligandrum* hyphal tip makes contact with irradiated *F. oxysporum* hypha (F_1) Note rounded-up appearance of *F. oxysporum* hyphal tip due to stoppage caused by irradiation. Bar represents 10 µm. ⟵⟶

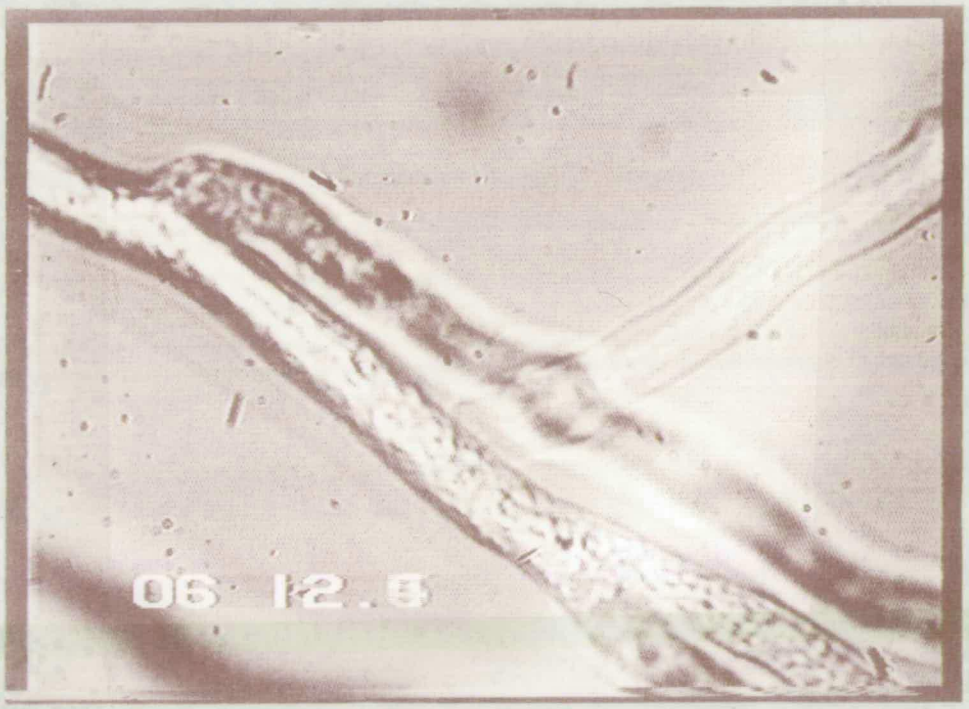
Fig 5.56 370 sec after first contact. *F. oxysporum* hypha (F_2) contacts *P. oligandrum* hypha 60 µm back from *P. oligandrum* tip.

P



F_1

P

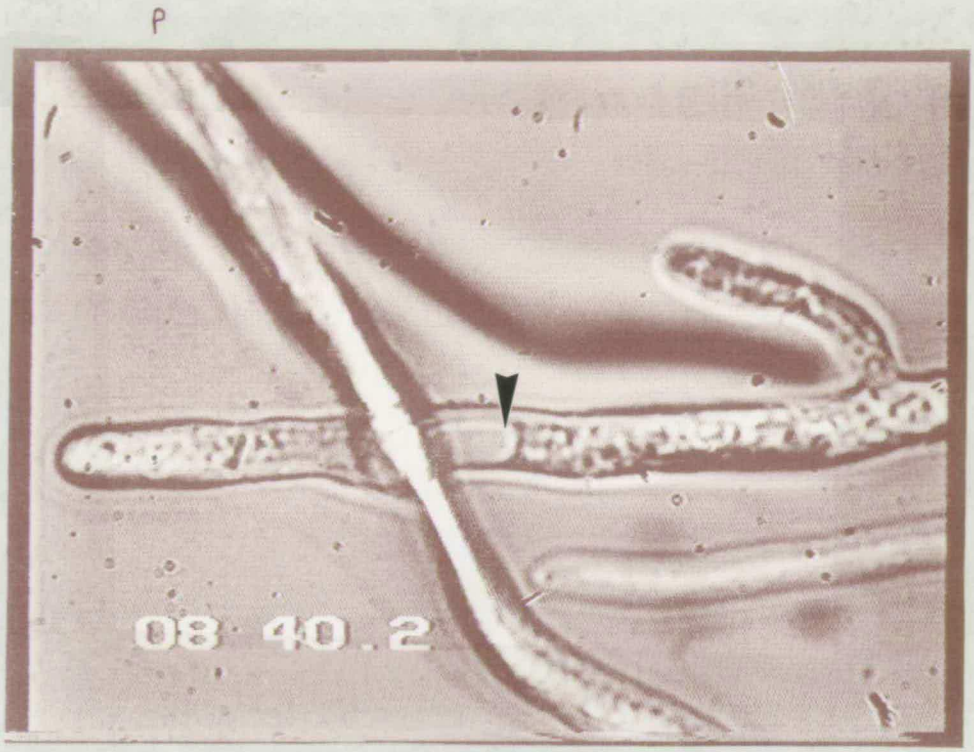


F_2

(F1, arrow)

Fig 5.57 520 sec after contact. F. oxysporum hypha clearly penetrated by P. oligandrum. Note approach of third hypha.

Fig 5.58 550 sec after first contact. Tip of P. oligandrum exposed to intense light. Note diamter of fine beam.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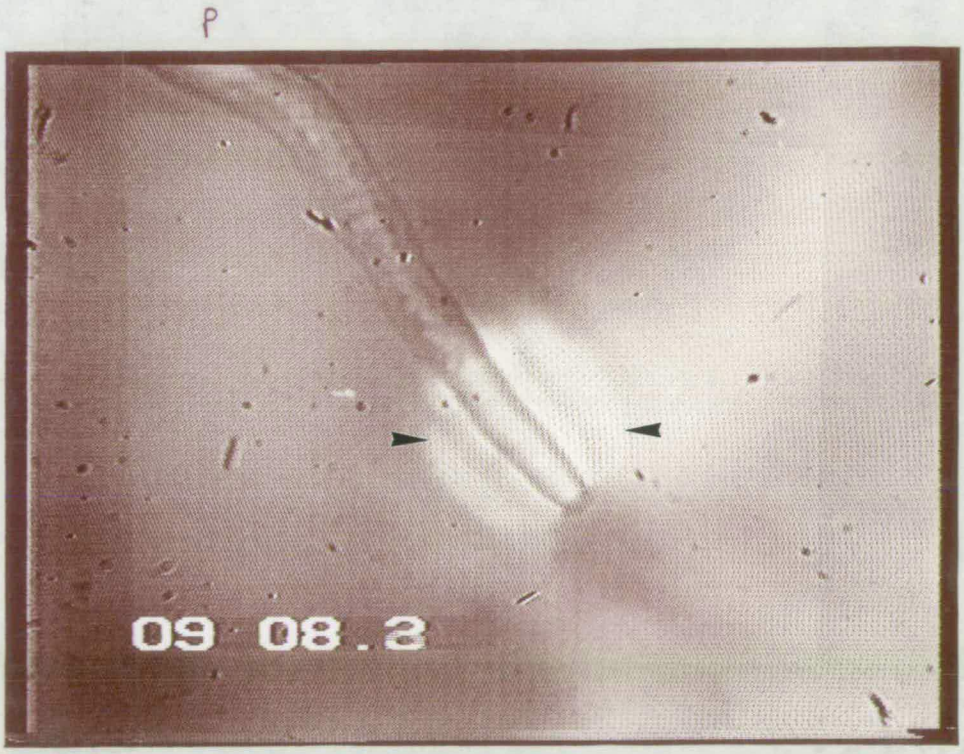
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Fig 5.59 615 sec after first contact. Third F. oxysporum hypha (F_3) makes contact with now irradiated P. oligandrum hypha (P).

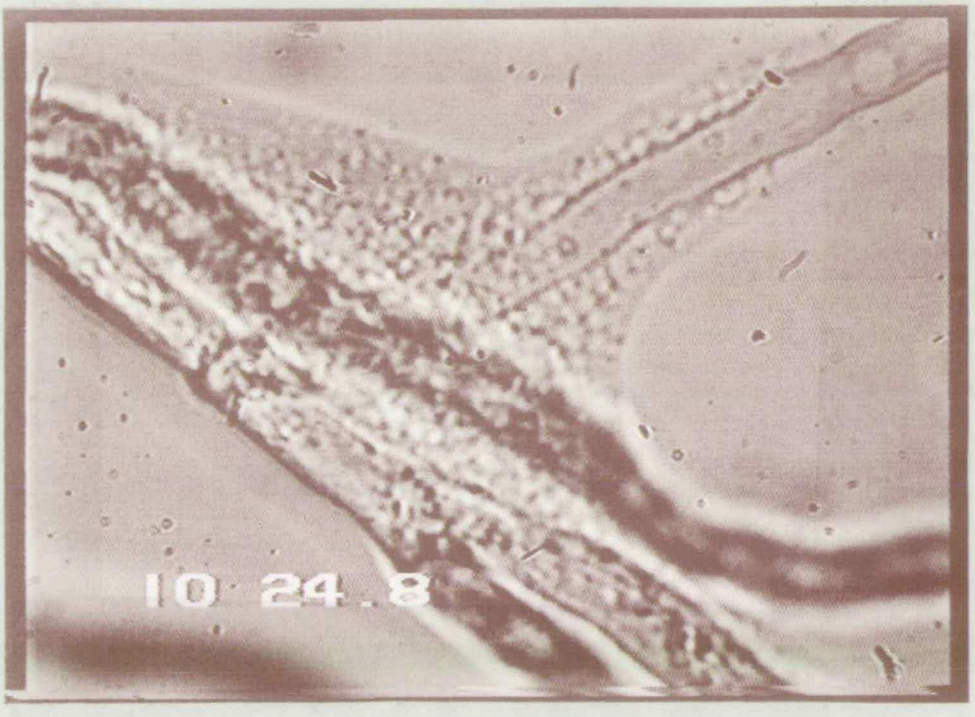
Fig 5.60 625 sec after first contact. Second F. oxysporum hypha (F_2) lyses 240 sec after it made contact with P. oligandrum hypha.

P

F_1

F_3

P

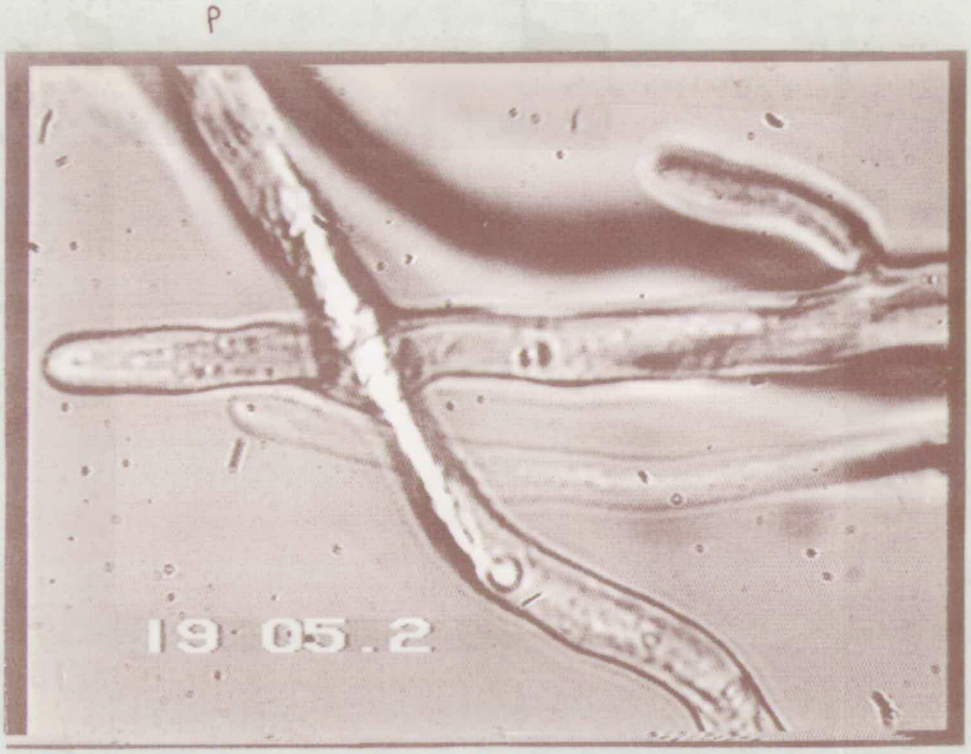


F_2

Figs 5.61 and 5.62 Third *F. oxysporum* hypha (F_3) continues to grow past irradiated *P. oligandrum* hypha without being affected. Photographs taken 9 and 29 min after third contact.

Fig 5.61

Fig 5.62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F3
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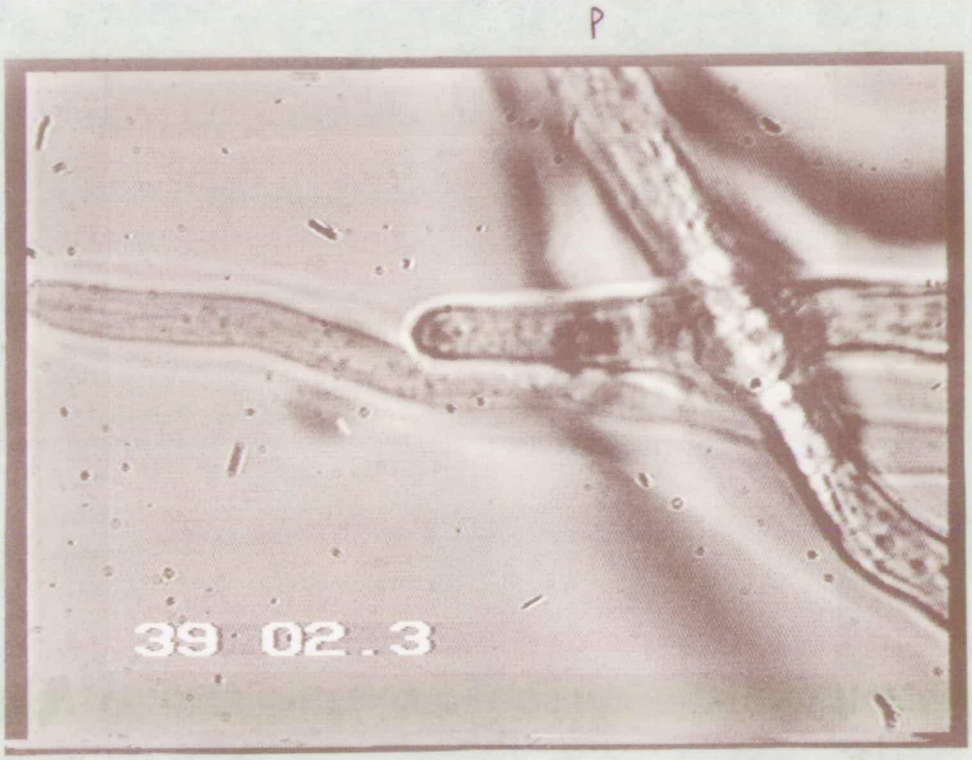
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F1
F3
39 02.3

5.56). Pre-contact growth rate of the host hypha (diameter 4.5 µm) was 3 µm min^{-1}, and contact occurred at 540 sec from the start of observations (320 sec after the initial contact of F. oxysporum and P. oligandrum). This second tip stopped within 120 sec of contact, followed by surging of the host protoplasm towards the tip which began at 150 sec post-contact. At 170 s after the second contact the tip of P. oligandrum was irradiated for 60 sec (Fig 5.58) resulting in the cessation of its growth. This had no effect on the second F. oxysporum hypha which lysed at the point of contact 240 sec after contact and 10 sec after the irradiation of the host was completed (Fig 5.60).

A third hyphal tip of F. oxysporum approached the hypha of P. oligandrum at a point about 5 µm closer to the parasite tip than the first host hypha had been contacted (Fig 5.57). Contact between the third host hypha and the now irradiated parasite hypha occurred 10 sec after irradiation was stopped (Fig 5.59). The tip of the F. oxysporum hypha (diameter 4 µm) was growing at 3 µm min^{-1} pre-contact and continued at this rate as it began to grow over the parasite hypha. During this time conspicuous surging of protoplasm toward the tip was observed in the P. oligandrum hypha culminating in a complete lack of protoplasmic movement in the mycoparasite by 140 sec after the end of irradiation. Meanwhile the third F. oxysporum continued to grow across and away from the parasite hypha, apparently unaffected (Figs 5.61 and 5.62).

In summary, this interaction involved three separate contact events between P. oligandrum and F. oxysporum. When a tip of P. oligandrum made contact with an irradiated hypha of F. oxysporum the response was like that in 3 of the 4 tip-to-host side interactions described in Section 5.3.2.1.8, in that the mycoparasite penetrated the host, although without causing lysis as was seen in all four of these inter-

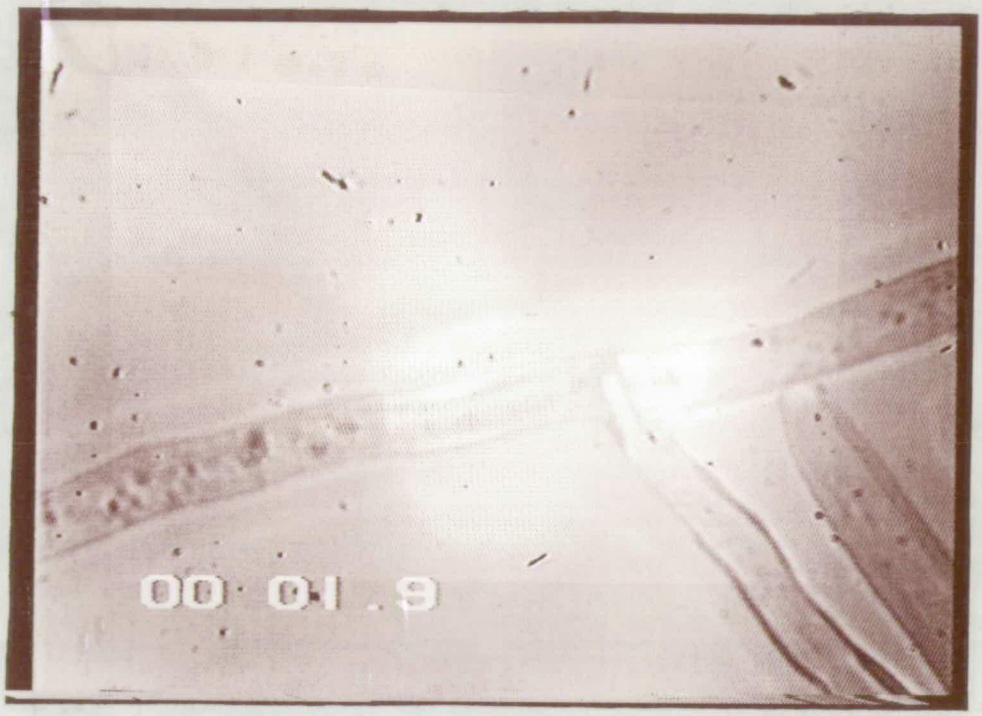
actions. When an unirradiated tip of F. oxysporum contacted a lateral wall of P. oligandrum, the host lysed, as expected. But in a subsequent identical contact event that followed irradiation of P. oligandrum the host hyphal tip was wholly unaffected. These three contact events were categorized as exhibiting, respectively, "partial disruption" of the normal mycoparasitic interaction (because of the absence of lysis), "no disruption" and "complete disruption" of the mycoparasitic interaction. In relation to the later observations in this section it is relevant to note that irradiation of the mycoparasite caused its tip to stop and the protoplasm to surge and then cease motility.

5.3.6.1.2 P. oligandrum on T. aureoviride

The sequence of this interaction is shown in Figs 5.63 to 5.66. A hypha of T. aureoviride (diameter 4.5 µm) and a hypha of P. oligandrum (diameter 5 µm) approached one another at respective extension rates of 5.5 and 11 µm min^{-1}. The mycoparasite was irradiated at a point centred 27 µm behind its tip for a total period of 60 sec. This treatment was begun immediately (< 10 sec) before the hyphae made contact, the contact point being 22 µm behind the extending tip of the mycoparasite. During irradiation the mycoparasite tip advanced 11 µm, the host tip made contact and began to grow across the mycoparasite hypha (Fig 5.63). The only observed effect of irradiation on P. oligandrum was that it caused a slight surging of protoplasm towards the tip, but this surging ceased when irradiation was completed. By 88 sec after contact the host had almost grown over the parasite hypha but had slowed almost to a halt, and a branch initial of the mycoparasite was clearly visible at the point of contact. After 113 sec the host protoplasm began to surge toward its tip, which now protruded just past the parasite hypha. The growth rate of the parasite remained at 11 µm min^{-1}. Between 150 and

Fig 5.63 T. aureoviride hyphal tip contacts side of P. oligandrum hypha under irradiation. Bar represents 10 µm. ⊢——⊣

Fig 5.64 236 sec after contact. T. aureoviride hypha penetrated by P. oligandrum branch (arrowed).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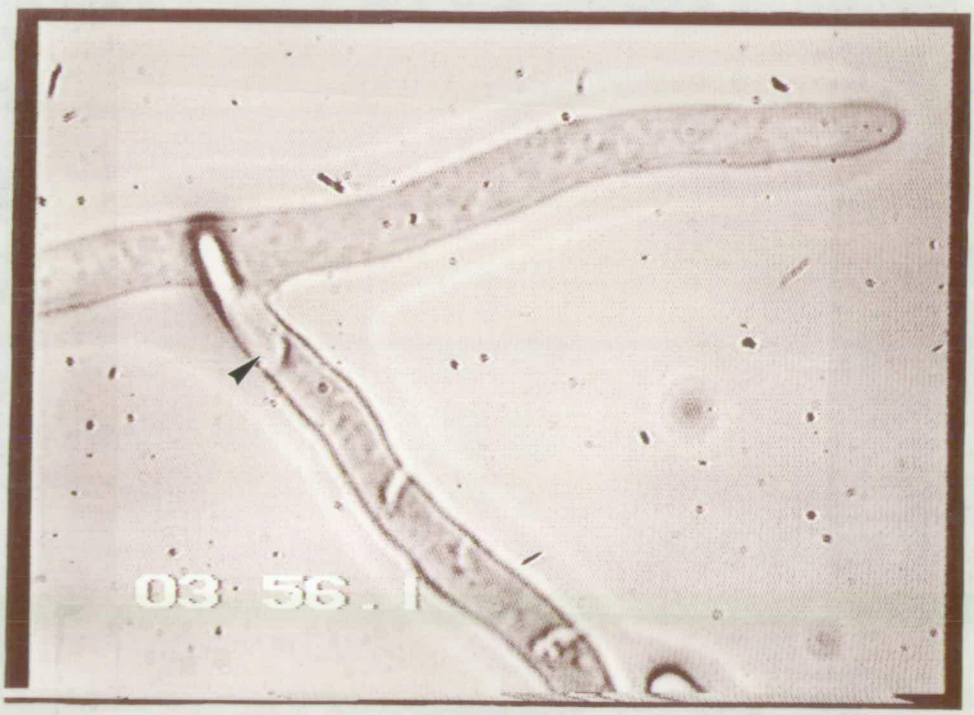
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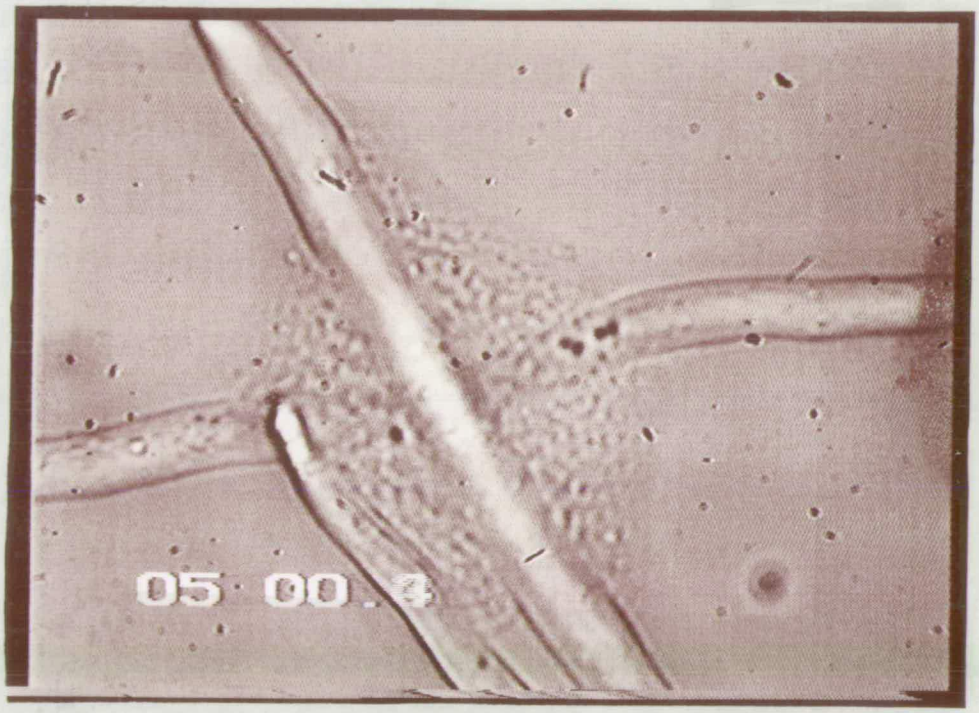
Fig 5.65 Contact of second T. aureoviride hypha with side of P. oligandrum. Mycoparasite hypha undergoing a second exposure to intense light

Fig 5.66 300 sec after second contact. Lysis of second T. aureoviride hypha at point of contact. Notice distance which T. aureoviride hypha has grown over P. oligandrum before lysing (tip just out of frame).


00 53.7


05 00.4

160 sec after contact cytoplasm was still flowing to the host tip, but this stopped when the parasite branch grew into the host hypha (Fig 5.64).

A second hypha of T. aureoviride (diameter 5 µm), growing alongside the first, neared the parasite hypha 8 min after the first contact event. Approximately 30 sec before the estimated time of contact, the hypha of P. oligandrum was irradiated for 60 sec at the same position as before. Surging in the mycoparasite was much more obvious than during the previous period of treatment; the protoplasmic movement continued after the exposure but was more erratic than during this. The second hypha of T. aureoviride made contact with the parasite (now 60 µm behind the parasite tip) 35 sec after irradiation began (Fig 5.65), growing at a rate of 5.5 µm min^{-1}. This rate was maintained for 4 min during which the host hypha grew over and away from the parasite. After 4 min, however, the host tip stopped growing, and after 290 sec the host hypha lysed at the point of contact (Fig 5.66).

In summary, both these contact events resulted in a pattern of response similar to that for untreated hyphae (Section 5.3.2.1.9) in that the host eventually lysed or was penetrated. However, the responses were, in the second contact, somewhat unusual because the host initially overgrew the mycoparasite hypha and lysis was correspondingly delayed. The responses are thus categorised as "no disruption" for the first and "partial disruption" for the second case.

It is notable that irradiation of the mycoparasite hypha did not result in cessation of its growth or protoplasmic movement but did cause a temporary disturbance of its behaviour in the first contact and a more pronounced disturbance of cytoplasmic movement during the second contact.

The findings presented in these examples and in Tables 5.58, 5.59

and 5.60 suggest a clear relationship between parasite activity (at least at the point of contact) and normal mycoparasitic events. Inactivation of the parasite previous to contact enabled a contacting host tip to grow unhindered after contact; partial recovery of the parasite after treatment led to a delay or perturbation in the effect on the host; complete recovery of the parasite after treatment was associated with the normal, rapid effect on the host; whereas parasite irradiation following contact, host stoppage and surging does not affect the normal outcome of events. Less dramatic disturbance of mycoparasitism was observed twice when the host hyphae were inactivated. In one case lysis (which was normal for F. oxysporum) was replaced by a halt in cytoplasmic movement or protoplasmic vacuolation/coagulation at the point of contact. In the other, branching preceded lysis, when normally lysis precedes branching or followed shortly afterwards. In all these respects, Tables 5.58, 5.59 and 5.60 show that the point of irradiation was not necessarily the point where contact occurred so possibly direct effects of the irradiation treatment can be discounted. These findings are also reinforced by the fact that different types of behaviour (among differently-treated hyphae) were sometimes seen in a single field of view (see examples 1 and 2), thus overcoming the potential objection that the results were based on independent events in space and time.

5.4 Discussion

The development of methods that enable observation and recording of the mycoparasitic interactions at high magnification was essential for these studies. In the conditions used, the hyphae were essentially undisturbed during observation, and their behaviour can be analysed in depth by repeated playback of videotapes and the use of "frame freeze" and other facilities. A time/date facility on the camera allowed

quantification of the events that occurred during interactions, some of which are reported for the first time.

Interactions were studied prior to contact in order to investigate possible pre-contact tropisms as reported and illustrated for P. oligandrum (Lutchmeah & Cooke, 1984; Whipps et al., 1988; Lewis et al., 1989), P. nunn (Lifshitz et al., 1984a), P. acanthicum (Hoch & Fuller, 1977) and mycoparasitic Trichoderma spp (Chet, Harman & Baker, 1981; Chet, 1987). The apparent randomness of contact between hyphae in all the interactions studied here strongly suggests a lack of tropic response before contact for any of the three mycoparasitic Pythium spp or for T. harzianum or G. roseum in the conditions of this study.

These findings are particularly interesting, as two of the host species used here - B. cinerea and F. oxysporum - were previously reported to elicit tropism by P. oligandrum (Lewis et al., 1989). A possible explanation for these contradictory findings is that the observations here were made on water agar, whereas other workers have used cellulose film or nutrient-based media. The mycoparasite might be expected to be most responsive to tropic factors on nutrient-poor media, but the hosts might not release them in these conditions. Alternatively, in previous studies on cellulose film these mycoparasites might not have responded to host-derived materials per se, but rather to the breakdown products of cellulose caused by the release of cellulase by the hosts. Ecologically, a tropic response towards a host involved in the breakdown of a "nutrient base" would be more beneficial than attraction simply to a host hypha which may be nutrient-depleted in the absence of utilisable underlying substrate.

The other pre-contact effect reported for mycoparasitic species is antibiosis or some other form of inhibition of host growth. The absence of any such pre-contact effect by P. oligandrum, P. mycoparasiticum or

P. nunn in this study is compatible with the report by Foley & Deacon (1986b) that P. oligandrum does not produce diffusible antibiotics. This seems also to be true for P. mycoparasiticum and P. nunn, although the latter was reported by Lifshitz, Sneh & Baker (1984) to produce a metabolite that partly inhibited growth and propagule germination by its hosts. "Near contact" effects such as those in hyphal interference by basidiomycetes (Ikediugwu & Webster, 1970) can also be excluded, because host hyphal tips were unaffected even in the final few seconds before they contacted the mycoparasite in the present study. Whipps (1987b) and Lewis et al. (1989) reported that agar plates which had previously supported colonies of P. oligandrum inhibited the growth of several plant pathogens compared to untreated controls. Although these results were attributed to antibiotic production, they could equally have resulted from depletion of nutrients.

In contrast to the mycoparasitic Pythium spp, both T. harzianum and G. roseum had obvious antagonistic effects on hyphal tips of two hosts (P. graminicola and Phialophora sp) before contact of the hyphae. The host tips stopped before making contact with these parasites such that no side-to-host tip interactions were observed with either parasite. The effect of antibiosis by either mycoparasite on Phialophora sp was somewhat different from that on P. graminicola, in that the hyphae of Phialophora sp narrowed progressively before they stopped growing.

The only other antibiotic events observed in the course of all these experiments were those caused by B. cinerea. It stopped the approaching tips of P. mycoparasiticum and P. nunn in a similar way to that in which Trichoderma and Gliocladium stopped the approaching tips of P. graminicola, although at a lesser distance. Although the metabolite from B. cinerea stopped hyphal extension by these two mycoparasites, it did not appear to affect the internal cytoplasmic streaming. Also of

interest is the fact that B. cinerea had no effect on P. oligandrum, though the reason why this differed from the other two mycoparasitic Pythium spp is unknown.

Despite the lack of effect of the mycoparasitic Pythium spp prior to contact with host hyphae, the meeting of hyphae often led to a rapid host response. The nature and timing of such responses seemed to depend on whether or not the host was susceptible to the mycoparasite, and also on the configuration of the interacting hyphae. The most obvious and rapid events were usually seen in side-to-host tip interactions, where the host tip stopped growing and this was usually preceded by a conspicuous surge of protoplasm towards the contact point. However, the tips of resistant "hosts", P. graminicola and P. vexans, were seldom if ever affected by contact; they grew over and away from the parasite hypha, although P. oligandrum was sometimes observed to coil around hyphae of P. vexans.

The first notable change in behaviour of the parasites after contact was a temporary slowing of the rate of hyphal tip extension, but the parasite tip always grew on and past (or along) the host hypha. Sometimes the parasite tip resumed its normal growth rate within 2-3 min. In other instances it slowed or nearly stopped while a branch emerged at the point of contact, but it sooner or later resumed normal growth. Slowing was most pronounced when the contact between hyphae occurred in the same plane and in a perpendicular orientation. In contrast, when the angle of contact was acute or the hyphae converged on slightly different planes (though still making contact), this slowing of the parasite was much reduced or unapparent. Branching at the point of contact was a common occurrence for all the mycoparasitic Pythium species. It occurred more often in tip-to-host side than in side-to-host tip interactions, but there was no evidence that the "responsive-

ness" of parasite hyphae (evidenced by their ability to branch) was influenced by the distance behind the parasite tip at which the contact point was located. Branching in tip-to-host side interactions generally preceded disruption of the host, whereas the opposite was almost always true in side-to-host tip interactions. Although apical branching is a common response of fungi to any trauma that causes a temporary arrest or slowing of apical growth (Robertson, 1958, 1965), the branch was almost always formed sub-apically, in the region where the tip had first made contact, and not from other regions of the (former) apex that would have made contact only seconds later. Since the resistant hosts did not branch after contacting parasite hyphae, and in most instances the parasite hyphae did not branch when making contact with other parasite hyphae, this branching cannot be explained simply by trauma. Furthermore, there is some evidence of specificity, as P. oligandrum never branched from older regions of its hyphae that were contacted by tips of P. graminicola, nor did P. mycoparasiticum or P. nunn branch when coming into contact with P. vexans (these hosts showing complete resistance to these parasites). Thus a physical (thigmotropic) response to the presence of an obstacle seems to be excluded. Branching could possibly reflect a recognition event mediated by lectins (Elad, Barak & Chet, 1983; Barak et al., 1985; Lewis et al., 1989) or other surface characteristics. Alternatively, the parasite branched in response to localized leakage of materials from traumatized host hyphae. Consistent with this, parasite branching often emerged after the host had stopped growing, and P. oligandrum, in particular, sometimes branched profusely in the spilled contents of lysed hosts.

Further evidence of tropism after contact was seen in the coiling of P. oligandrum around hyphae of some hosts, as first described by Drechsler (1943) when P. oligandrum grew on other Pythium spp. In this

work this was seen only round hyphae of P. vexans and the two isolates of R. solani but not P. graminicola. The mycoparasite also spiralled loosely on sub-apical compartments of T. aureoviride (Figs 5.11 to 5.14) after it had emerged from a parasitised apical compartment. These observations support the view (Deacon, 1976) that coiling indicates at least temporary host resistance, because sub-apical compartments are likely to be less susceptible than are apices, and R. solani and Pythium spp are among the more resistant hosts of P. oligandrum (Foley & Deacon, 1986a,b). Whipps (1987b) however observed coiling by P. oligandrum on hyphae of B. cinerea and F. oxysporum on water agar, and these host species were recorded as susceptible by Lewis et al. (1989) as well as in this study. It is possible (though not stated) that Whipps (1987b) observed coiling by the mycoparasite on older hyphal regions of these hosts, where their resistance to parasitism was perhaps greater than near the tips. If so, then the presence or absence of coiling by mycoparasites may only be indicative of host susceptibility or resistance in defined areas of the host and during a certain time after contact. Its function, at least in Trichoderma spp, was suggested to be that it enabled close contact with hyphae so that antibiotics produced by the mycoparasite could exert an intense localised effect (Dennis & Webster, 1971c).

For reasons that are unclear coiling was not observed in interactions involving P. mycoparasiticum or P. nunn, although P. nunn has been reported to coil round the hyphae of several fungi (Lifshitz et al., 1984a). It seems possible that all mycoparasites have an essentially similar mode of behaviour although they may differ in the degree to which they express particular features in different conditions. In support of this view, most mycoparasites are reported, in one study or another, to coil round host hyphae, and in the present study T. harzi-

anum was found to branch at the points of contact with its hosts in two of six videotaped interactions.

For P. oligandrum and P. mycoparasiticum, and to a much lesser extent for P. nunn, branching led sooner or later to penetration of the host. Once inside the host hypha, the internal hyphae of the parasites appeared to be able to grow easily through the host septa into healthy adjacent compartments. In some examples, however, the internal hypha evidently met some form of internal resistance, generally at or near a septum, and the internal hypha would then exit through a lateral wall but then repenetrate the host hypha at a point beyond the septum. P. mycoparasiticum also produced thin and often short hyphal pegs from an internal hypha and these emerged through the lateral walls of parasitized hyphae (eg Fig 5.35). In general, such "pegs" grew to only a limited extent but they were able to lyse adjacent host hyphae and then penetrate these. Lewis et al. (1989) also observed the production of fine exiting branches, but by P. oligandrum (not observed in this study); they were reported to arise in the later stages of interactions and led to parasitism of the whole host mycelium.

The behaviour of host hyphae after contact with (or by) the mycoparasites took one of several courses. In all instances, as noted earlier, the first sign of a deleterious effect was stoppage of the host tip, even if the tip was not directly involved in the interaction, although the hyphae of resistant hosts to any particular mycoparasite continued to grow after contact. The surging of host protoplasm that followed growth stoppage seemed to be a universal occurrence. The condensation of protoplasm in the apex caused by this surging might have been accompanied by expulsion of fluids through the hyphal walls, but there was no visible loss of cytoplasmic material. It seems likely that the surging reflected a change in the cytoskeletal components in the

hyphae. Microtubules and microfilaments have been implicated in apical growth (reviewed by Gooday, 1983), and microtubules are sensitive to depolymerisation by various treatments (Hoch & Staples, 1985). The association between trauma, tip stoppage and protoplasmic surging could thus be explained.

The most rapid event following stoppage and surging was lysis of host hyphae. Three important aspects of hyphal lysis were recorded. Firstly, lysis always occurred from a small zone at the initial point of contact rather than being generalised. Secondly, it preceded penetration by the parasite, and usually preceded the origin of a penetrating branch. Thirdly, the host and parasite were never seen to separate from one another at the point of initial contact, which was also the point of lysis, despite the force of expulsion of the cytoplasm, suggesting a tight adhesion of the hyphae at this point. Lectins might possibly be involved in this adhesion, as in the early stages of binding of nematophagous fungi to their nematode hosts (Nordbring-Hertz, 1988). The other frequent mode of host disorganisation was coagulation and vacuolation at the cytoplasm. This appeared first and then spread from the initial point of contact, suggesting a "cascade effect" that again might have resulted from disruption of microtubules and microfilaments comprising the cytoskeleton. These observations are not compatible with the view of Whipps et al. (1988) who stated that "In the majority of encounters penetration would seem to be an essential preliminary to, and probable cause of, mortality in parasitised hyphae". On the contrary, in the present study host lysis often preceded the development of a penetrating branch by the mycoparasite at the point of contact, and always preceded penetration per se. The vacuolation and coagulation of host cytoplasm, although generally occurring later after contact than did lysis, again typically preceded penetration and sometimes occurred

before the parasite produced a branch and even in several cases when no branch was produced

Instead of exhibiting lysis or cytoplasmic vacuolation/coagulation, some host hyphae were penetrated "directly" by the parasites without showing previous evidence of dysfunction, except for growth stoppage. Some of these "direct" penetrative events were as rapid as the lytic or coagulative events, but others were considerably delayed and were preceded by coiling or other forms of proliferation of the parasite on the host surface.

All of the features mentioned above (with the exception of coiling) were observed in interactions with all three mycoparasitic Pythium species, indicating that these fungi have an essentially similar mode of parasitism in the conditions used here. They caused lysis, or cytoplasmic coagulation, and they penetrated susceptible host hyphae but had no effect before contact. Moreover, the relationships between these events and host stoppage or parasite branching were similar for all three mycoparasites. Indeed, from previous comparative studies on P. oligandrum, P. acanthicum and P. periplocum (Deacon & Henry, 1978) it would seem that all five mycoparasitic Pythium spp share the same mode of mycoparasitism. Nevertheless, from the present study it was clear that they differ in their degree of aggressiveness as mycoparasites, these differences being reflected in both their host ranges (or, strictly, the range of hosts affected by them), and their effects on any single host.

P. nunn was least aggressive as a parasite. It antagonised fewest hosts, and it was the least consistent and the slowest in affecting any single host. P. mycoparasiticum grew as slowly as P. nunn on the water agar films, and yet it was much more aggressive than P. nunn, causing stoppage of a higher proportion of the host hyphae in the contact events and having a broader host range, insofar as it affected B. cinerea in

conditions in which P. nunn did not do so. P. oligandrum was the most aggressive, although in many respects it was similar to P. mycoparasiticum in its effects on the range of hosts and in its speed of effect. It is possible that only its higher growth rate compared to that of P. mycoparasiticum was responsible for its slightly higher degree of aggressiveness overall because it could rapidly overgrow and antagonize the hyphae in host colonies. Indeed, P. oligandrum was recorded as being significantly more aggressive than P. mycoparasiticum in the experiments in Section 4, involving whole colonies of host fungi, whereas in the inter-hyphal interactions in this chapter there was a much smaller difference in the recorded behaviour of these two fungi.

It is possible that P. nunn is more aggressive in other conditions or against other fungal hosts than those used here. However, it is notable that Lifshitz et al. (1984a) also recorded P. nunn as having a limited host range. These workers distinguished between a "slow reaction" and a "quick reaction" elicited by P. nunn, but even the latter involved massive coiling around host hyphae, and the hosts were said to lyse only after some hours. The definition of a fast reaction in the present study would be one in which a host compartment is irreversibly disrupted in 3-5 min after contact, and several adjacent compartments are penetrated and destroyed within one hour by internally growing hyphae that arise from the initial penetrating branch of a mycoparasite. An interesting supplementary form of evidence on the aggressiveness of the mycoparasites was obtained by comparing their internal growth rates within host hyphae. Considering the data combined for all host-parasite interactions with each mycoparasite (Tables 5.38, 5.39 and 5.40), it was found that the internal growth rate of P. oligandrum (5.8 ± 0.4 μm min^{-1}) was not significantly greater than that by P. mycoparasiticum, but both were significantly faster than P. nunn. When the

internal growth rates were expressed as a percentage of the "external" rates of hyphal growth by these fungi on the agar films, both P. oligandrum and P. nunn grew internally at 48-49% of the "normal" rate but P. mycoparasiticum grew at 86% of this value. This remarkable difference may indicate a high degree of specialisation for parasitism on the part of P. mycoparasiticum despite its normally slow growth.

Differences in host resistance to the mycoparasites were far more difficult to determine and quantify than were differences in aggressiveness of the mycoparasites. In part, this is because every interaction was an individual event, likely to be influenced by the physiological states and ages of the partners at the point and time of contact. What may be taken as a sign of resistance may vary also with the degree of aggressiveness of the parasite. Such distinctions at the "cellular" level pose similar problems in studies of necrotrophic parasitism of plants (Heath, 1976; Kiraly, Barna & Ersek, 1972). However, the evidence overall indicated that both P. graminicola and P. vexans were highly resistant to all mycoparasitic Pythium spp, confirming earlier reports that Pythium spp in general are resistant to parasitism by P. oligandrum (Deacon, 1976; Foley & Deacon, 1986b). The massive coiling by P. nunn that Lifshitz et al. (1984a) observed on hyphae of P. ultimum and P. vexans is consistent with this view (Deacon, 1976), although it differs from the view of the authors who considered coiling to be indicative of "pronounced mycoparasitism". Many of the other host fungi were susceptible to all or some of the parasites, especially P. oligandrum and P. mycoparasiticum. Hyphae of even F. oxysporum were affected by P. nunn in some instances, though never sooner than 15 min post-contact. This host was considered by Lifshitz et al. (1984a) to be resistant to parasitism, and Elad et al. (1985) suggested that the basis of this resistance was an outer layer of mucilaginous

material on the hyphal walls, which precluded their dissolution by parasite-derived wall-degrading enzymes. If hyphal coiling was used by these workers as an indication of parasitism, as seems to have been the case, then it is not surprising that they consider F. oxysporum to be resistant, because the most susceptible hosts observed in the present studies did not support coiling by the parasite but, rather, lysed or were penetrated directly.

Of the host species used in these studies B. cinerea showed a clear difference in susceptibility to the different mycoparasites, being resistant to P. nunn, as reported by Lifshitz et al. (1984a), but susceptible to P. oligandrum, as reported by Lewis et al. (1989) and also to P. mycoparasiticum. The basis of this difference in resistance to the mycoparasites is unclear (recognizing that it may be a reflection of the difference in aggressiveness of the mycoparasites rather than a difference in host resistance - the two are interlinked). It was noted earlier that B. cinerea produced a diffusate that inhibited approaching hyphae of P. mycoparasiticum and P. nunn, but this did not seem to influence the behaviour of the parasites in side-to-host tip interactions. In any case the diffusate was equally inhibitory to P. mycoparasiticum and P. nunn, and yet only P. nunn was unable to affect B. cinerea. The two isolates of R. solani were also notable in that their hyphal tips were highly susceptible to parasitism by P. oligandrum (and by P. mycoparasiticum in one interaction) but the sub-apical regions showed considerable resistance. Massive coiling by P. nunn on this host (Lifshitz et al., 1984a) is compatible with resistance (Deacon, 1976). These findings may explain the conflicting reports that R. solani is resistant (Deacon, 1976; Deacon & Henry, 1978; Foley & Deacon, 1986b) and susceptible (Al-Hamdani & Cooke, 1983) to parasitism by P. oligandrum. Al-Hamdani & Cooke (1983) proposed that this was because

different isolates of R. solani had been used but Deacon (personal communication) found that over 30 different isolates representing all anastomosis groups of R. solani were not substantially different in susceptibility. The likely explanation for the conflicting reports is that old and young (tip) hyphal regions were exposed to the parasite in the respective studies above, because the parasite was introduced at different times after the host was inoculated onto filter paper wads. The relative timing of inoculation is known to affect the results of such studies (Deacon, 1976).

There was no evidence from the present work to suggest that R. solani caused vacuolation, disappearance of cytoplasm, and apparent lysis of hyphae of P. oligandrum as reported by Walther & Gindrat (1987a). Possibly, the findings of Walther & Gindrat (1987a) were due to the rich medium on which the interacting hyphae were grown. In any case, it seems that the status of R. solani as a host for mycoparasites can vary substantially in different conditions and at different points along its hyphae. R. solani is even reported to be a mycoparasite in its own right (Butler, 1957). At least some of this variability may be due to the fact that "R. solani" is a name applied to a mycelial state of a range of fungi that belong to different anastomosis groups (Parmeter, 1970). Even in the present study there was evidence of a difference in susceptibility between two isolates of this "species", although this difference was not large enough to warrant separation of these isolates into different groupings in terms of their responses to the parasites.

There appeared to be little difference in the relative susceptibility of the other five hosts, F. culmorum, B. piluliferum, F. oxysporum, T. aureoviride and Phialophora sp to mycoparasitism by P. oligandrum and P. mycoparasiticum. However, T. aureoviride did seem to be more sus-

ceptible to the presence of P. nunn than did the other hosts, so, overall, it could be ranked as one of the most (if not the most) susceptible hosts.

Unlike the mycoparasitic Pythium spp, T. harzianum and G. roseum appeared to exhibit a very different mode of antagonism to the "direct" mycoparasitism discussed above. Antibiotics obviously play a far more important role in the antagonistic properties of these species, perhaps to the extent that mycoparasitism is of only secondary or even minor importance. The results obtained in these studies thus tend to support the findings of Howell (1987) that non-mycoparasitic mutants of Gliocladium virens were as antagonistic as the wild-type cultures that exhibited features of mycoparasitic behaviour. Trichoderma harzianum did, however, show some similarity to the behaviour of the mycoparasitic Pythium spp, in that it branched at the point of contact with some hyphae and caused, in some instances, cytoplasmic coagulation/vacuolation of the host hyphae after contact. Even though the production of antibiotics by Trichoderma spp and G. roseum is greater on rich than on nutrient-poor media, Whipps (1987b) found that these fungi were significantly inhibitory to several plant pathogens on water agar or soil extract agar - and more antagonistic in general than was P. oligandrum on any of three media tested. The present results support these findings and indicate, as mentioned earlier, that Trichoderma spp and G. roseum may exert more significant effects by antibiosis than they do by penetrative mycoparasitism per se.

The final point for discussion is the issue of the relative contributions of hosts and mycoparasites to the results of the interactions recorded in this chapter. Evidence relating to this was obtained by attempting to disrupt either parasite or host hyphae with intense light before contacts were established between the hyphae. Such treatments

strongly implicated mycoparasite activity as being essential for host stoppage, lysis, cytoplasmic coagulation/vacuolation and, of course, penetration. Total inactivation of the parasite, even by irradiation applied to regions that were not directly involved in the contact events, led to negation of mycoparasitism, while a partial debilitation led to a weakened effect on the host. In contrast, inactivation of the host caused little or no change in the normal course of parasitism, except that lysis did not occur where otherwise it would have been expected, or lysis was delayed, and followed parasite branching by more than 2 mins.

The possibility that irradiation caused changes in surface receptors or surface-located enzymes on the treated hyphae cannot be excluded, although it is noteworthy that lysis in delayed interactions did not force the hyphae apart, so if lectins were involved in this tight adhesion then they were also present on irradiated hyphae. Also, it is not possible to exclude a potential role for highly labile compounds in the normal course of parasitism. However, the simplest interpretation of the findings from the irradiation treatments is as follows. Because the mycoparasite had to be active at the time and place of contact in order to affect host hyphae, then host stoppage, lysis and cytoplasmic vacuolation/coagulation seemingly resulted from the induced release (after contact) or continuing release of substances from the parasite hypha (the localisation of lysis particularly implicating the former). The converse possibility, that the effects result from recognition of (or sensitivity to) an existing component of the parasite surface by the host, seems to be excluded. The nature and origin of the enzymes required for wall lysis is more problematical. On the one hand, the evidence above indicated a major role of the mycoparasite in such events. On the other hand, inactivated host hyphae occasionally showed

delayed lysis where contacted by a parasite tip, but did not always show lysis even though they appeared turgid. This seems to indicate at least some role of host activity in the lytic process. Inactivated host hyphae were unlikely to have been able to mobilize lytic enzymes to the points of contact, but Rosenberger (1979) found considerable amounts of nascent lysins in sub-apical hyphal walls, and these lysins could be released and show activity when walls were treated with surfactants. So the mycoparasite might locally "activate" the host's nascent wall lysins by releasing a substance that causes this effect. This possibility merits serious consideration because P. oligandrum has not been shown to produce chitinase (Lewis et al. 1989) and, anyway, might not be induced to produce it before penetration because chitin, the inducer, is overlaid by other components in fungal walls (Hunsley & Burnett, 1970). Whatever the mechanism involved, lysis preceded the emergence of a penetrating branch in most interactions, so it was not caused by penetration or even attempted penetration. But this does not preclude the release of lysins or other substances by the parasite at the site of future branch emergence, because vesicles and wall lysins must be transported to, and active at, such sites before a branch emerges (Trinci, 1979). Thus lysis and the rapid nature of its occurrence, often accompanying or even preceding branch emergence by the mycoparasite in normal interactions, could be explained, irrespective of the origin of the enzymes involved.

SECTION 6

SECTION 6

Concluding discussion

Different aspects of the work in this thesis have been discussed in Sections 3.6, 4.4 and 5.4. In this Concluding Discussion it is intended initially to compare the results of Sections 4 and 5 with reference to Section 3 and finally to review the work in the context of biological control.

There was a high degree of consistency between the results for interactions studied at the levels of "whole colony" (Section 4) and individual hypha (Section 5). The phytopathogenic Pythium spp, especially P. graminicola, were found to have a high degree of resistance to the mycoparasitic Pythium spp in cellulolysis assays and also in interactions between hyphae, and it is notable that none of the mycoparasites could grow across agar plates pre-colonised by P. graminicola or P. vexans. At the other extreme, the host species that proved highly susceptible to antagonism on cellulose were also seen to be parasitised very rapidly in interhyphal interactions. Phialophora sp in particular was highly susceptible in all experiments, and was the only host whose colonies were overgrown by all the mycoparasitic Pythium spp and by T. harzianum and G. roseum on agar plates. It is also overgrown by P. acanthicum and P. periplocum in similar conditions (Deacon & Henry, 1978; Foley & Deacon, 1985). T. aureoviride was also extremely susceptible to the mycoparasitic Pythium spp in all tests, but it was not overgrown by any of the three on plates of PDA. Possibly, on this richer medium T. aureoviride could produce antibiotics or other growth inhibitors. However, Dennis and Webster (1971a) found that three isolates of this species had little or no ability to produce water-

diffusible antibiotics, so this seems unlikely. Intermediate degrees of susceptibility were exhibited by most other fungi in these experiments on cellulose or in interhyphal interactions. Yet there were still clear differences between the hosts. The two isolates of R. solani were among the most resistant hosts on cellulose film and also at the level of individual hyphae. However, they were notably susceptible to parasitism by P. oligandrum and P. mycoparasiticum at their extreme tips, gaining substantial resistance at very short distances behind their tips. In this respect it is interesting that R. solani was delayed for only a relatively short time by a 'barrier' of mycoparasitic hyphae placed across cellulose film.

The results for B. cinerea show a degree of inconsistency between the experiments. This host was susceptible to P. oligandrum at both colony and cellular levels. However, on cellulose film with juxtaposed inocula, B. cinerea was almost completely unaffected by either P. mycoparasiticum or P. nunn, and on cellulose film with opposed inocula the growth of B. cinerea was only temporarily delayed by both of these mycoparasites. Yet in studies on interactions at the cellular level, P. mycoparasiticum was seen to be aggressively parasitic on B. cinerea (in side-to-host tip interactions) but was unaffected by P. nunn. It would appear, therefore, that the fungistatic factor produced by B. cinerea allows it to "escape" parasitism by P. mycoparasiticum, and this merits further study.

A similar comparison across the hosts and across the experimental methods reveals differences in aggressiveness of the three mycoparasitic Pythium spp. Again, there was a large degree of consistency, in that P. oligandrum was always more aggressive than P. mycoparasiticum or P. nunn. This may have been due partly to its higher growth rate, resulting in a greater number of contacts with host hyphae at the 'colony' level

of interactions, but this explanation cannot apply at the cellular level because many of the comparisons of aggressiveness were based on events that followed contacts between hosts and parasites. So it seems that P. oligandrum is inherently more aggressive to any single host and also has a wider host range of hosts that are susceptible to it than is the case with P. mycoparasiticum and P.nunn.

Comparing P. mycoparasiticum, P. nunn and P. oligandrum, the most noticeable trait is the similarity of the orders of susceptibility of the host fungi to the three parasites. Only B. cinerea did not fit neatly into this pattern, for reasons previously explained. However, the interactions at the cellular level revealed that P. mycoparasiticum is an aggressive mycoparasite, equivalent in many respects to P. oligandrum, and yet this was not clearly shown in studies on cellulosic substrates, perhaps because of the slower growth of P. mycoparasiticum and a correspondingly greater chance of the host hyphae being able to 'escape' infection by outgrowing the parasite. In nature it seems likely that these mycoparasites would have different ecological requirements or host ranges, because too great a similarity would lead to P. oligandrum out-competing P. mycoparasiticum, ultimately to its extinction. In this respect it is notable that Foley & Deacon (1985) isolated P. oligandrum more frequently than P. mycoparasiticum from soils and yet both fungi were sometimes isolated from the same sites (although always on different agar plates). There was an indication from the work of Foley & Deacon (1985) that dilution of a soil sample with sand led to an increased frequency of isolation of P. mycoparasiticum, coinciding with a decreased frequency of isolation of P. oligandrum. The explanation offered for this was that P. oligandrum was the more competitive, because of its faster growth rate, on the isolation medium and thus its presence tended to obscure the presence of P. mycoparasiticum. Urgent

attention should now be given to the respective ecologies of these two mycoparasites.

A final point of interest in comparisons of the methods used in this study concerns the growth of mycoparasites on pre-colonised agar plates. With the exception of T. aureoviride, already discussed, the hosts supported growth by P. oligandrum across their colonies in a manner that was broadly compatible with their susceptibilities as determined by other methods. However, very few host fungi supported growth by P. mycoparasiticum or P. nunn across pre-colonised agar plates, despite the fact that several of the host fungi were susceptible to one or other of these mycoparasites in other tests. Of interest also was the finding that T. harzianum and G. roseum grew across colonies of a large proportion of the host fungi, even those that did not support growth by P. oligandrum. The spectra of behaviour of the mycoparasites were thus different in this respect, perhaps indicating that pre-colonised agar plates could be used for a preliminary screen of antagonists to particular fungi. If the aim were to select antagonists with biocontrol potential against pathogens, then this technique would be seen to be too restrictive, because in the present study, for example, it would have eliminated P. mycoparasiticum and P. nunn, which were found by other methods to be antagonistic to some of the hosts. On the other hand, the ability of all five mycoparasites to grow across colonies of Phialophora sp suggests that plates pre-colonised by this fungus could provide a useful preliminary screen for antagonists. A rather similar approach has been used previously for selection (or selective enrichment) of antagonists by burial of host mycelia in soil. In this way Hadar, Chet & Henis (1979) were able to enhance populations of Trichoderma spp antagonistic to R. solani, and van den Boogert & Jager (1983) were able to isolate Verticillium biguttatum as an antagonist of

this host fungus.

P. nunn was found to be unique among the mycoparasitic Pythium spp in its ability to utilise inorganic nitrogen and was the only species that did not produce oospores in association with host fungi on cellulose. These points, coupled with its lower degree of aggressiveness in hyphal interactions than was shown by P. oligandrum or P. mycoparasiticum, indicate that it may antagonise fungi in a different way to them in soil. For example, it would seem to be better fitted for growth on organic substrates that do not have large amounts of organic nitrogen, and less fitted as a directly mycoparasitic organism. It was reported by Lifshitz, Sneh & Baker (1984) to produce substances that inhibit growth or propagule germination by other fungi, and Elad et al. (1985) showed that P. nunn can produce a greater range of wall lytic enzymes than P. oligandrum has so far been shown to produce. In all these respects its behaviour may be closer to that of Trichoderma and Gliocladium spp than to the seemingly specialised mycoparasites P. oligandrum and P. mycoparasiticum. Indeed, Paulitz and Baker (19887b) have shown that P. nunn is a particularly effective antagonist of Pythium spp in soils that are amended with organic residues, as is true also for Trichoderma and Gliocladium spp (Papavizas, 1985). However, direct comparative studies are needed in this regard, because Martin & Hancock (1986) have also suggested that P. oligandrum is effective in controlling populations of P. ultimum in such circumstances.

Compared to the mycoparasitic Pythium spp, antagonism by T. harzianum and G. roseum appeared to be of an essentially different nature, although only limited studies were made on these fungi. The importance of antibiosis seems to be more fundamental to their mode of antagonism as previously suggested by many workers (eg Dennis & Webster, 1971a,b,c; Pachenari & Dix, 1980). In a series of direct comparative studies,

Whipps (1987b) confirmed that antibiotic production by Trichoderma spp and G. roseum was generally greater than that by P. oligandrum and certainly on nutrient-rich media. Indeed, that study illustrated one of the unavoidable limitations in the present work, that comparisons of mycoparasites should ideally be made on a range of substrates. There is, however, always a limitation to the number of permutations of substrate, host and mycoparasite that can be included in detailed comparative work. A particularly fruitful line of future study for the mycoparasitic Pythium spp would be to select, if possible, non-mycoparasitic mutants for studies in antagonism, as Howell (1987) did for G. virens. This species is known to produce antibiotics (Dennis & Webster, 1971a) as perhaps a major means of antagonism, and the non-mycoparasitic mutants were as effective antagonists as were wild-type strains. The evidence in the present work suggests that direct parasitism of host fungi is the more important mode of antagonism by at least P. oligandrum and P. mycoparasiticum, but this cannot be substantiated until non-mycoparasitic mutants are tested in comparative studies.

As indicated by some of the comments above, much of the interest concerning mycoparasites is in their potential roles as biocontrol agents of plant pathogens. Many workers (see Introduction) have shown that mycoparasites can be used experimentally to reduce the populations of pathogens in the soil or to decrease the incidence of disease. As yet, however, their usage has not been fully implemented in commercial agriculture and only a few mycoparasite formulations have been marketed. For example, P. oligandrum is marketed as a product "Polygandron", for use in control of damping-off diseases, and Trichoderma viride is marketed as "Binab T" for use against Armillaria mellea (Vahl ex Fr), Kummer, Chondrostereum purpureum (Pers ex Fr) and a few other pathogens (Lynch, 1987). The limited exploitation of such fungi is probably

because many questions still remain unanswered as to the most effective usage of mycoparasites and the conditions in which they are likely to be active. The mycoparasitic Pythium spp appear essentially non-phytopathogenic (Section 3) and their spores seem unlikely to cause allergy (unlike the much smaller spores of Trichoderma spp). However, to be effective they would probably have to be introduced as oospores (as in "Polygandron"), and the preliminary studies in Section 3 suggest that only a relatively small proportion of oospores are likely to germinate readily, which makes their use problematical. The variability in germination levels with age of culture and time of storage as found in this study suggests that much work is still required before the use of seed coatings incorporating oospores of mycoparasitic Pythium spp could be relied on to give ensured levels of crop protection in differing circumstances. In future work P. mycoparasiticum could usefully be studied in parallel with P. oligandrum as a possible biocontrol agent. The characterisation of this fungus will, hopefully, add another potential weapon to the armoury of biocontrol agents that might find commercial application.

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