Soilborne Plant Diseases Caused by *Pythium* spp.: Ecology, Epidemiology, and Prospects for Biological Control

Frank N. Martin* and Joyce E. Loper**

USDA-ARS, 1636 East Alisal St., Salinas, CA 93905; Horticulture Crops Lab, 3420 NW Orchard Ave., Corvallis, OR 97330

* Frank Martin: Phone: (831) 755–2873; Fax: (831) 755–2814; E-mail: fmartin@asrr.ars.usda.gov

** Joyce Loper: Phone: (541) 750–8771; Fax: (541) 750–8764; E-mail: loperj@bcc.orst.edu

**DEDICATION**

This paper is dedicated with gratitude and best wishes to Joe Hancock in honor of his exceptional contributions to knowledge of the genus *Pythium*, the field of plant pathology, and the many students and colleagues that he has encouraged with his words and example.

**TABLE OF CONTENTS**

I. Introduction ......................................................................................................................... 114

II. Ecology of *Pythium* spp. ................................................................................................. 115

   A. Survival in the Soil ............................................................................................................ 116

      1. Oospores ..................................................................................................................... 116

      2. Sporangia .................................................................................................................... 117

   B. Propagule Germination ..................................................................................................... 117

   C. Zoospore Formation ......................................................................................................... 118

   D. Saprophytic Phase of the Lifecycle ................................................................................... 118

   E. Infection of the Plant Host ................................................................................................. 119

      1. Host Sensitivity ............................................................................................................. 119

      2. Host Recognition by the Pathogen ............................................................................... 119

         a. Sporangial Germination ........................................................................................... 119

         b. Zoospore Development ......................................................................................... 120

         c. Timing of the Infection Process .............................................................................. 121

III. Epidemiology ................................................................................................................... 121

   A. Influence of Environment on Disease Severity and Ecology of *Pythium* spp. ................................................................................................................................. 121
1. Soil Moisture ................................................................................................................ 121
2. Soil Temperature ........................................................................................................... 123
3. Soil pH .......................................................................................................................... 124
4. Indirect Influence of Environment on *Pythium* spp. ................................................. 125
5. Influence of Environmental Factors on Host Susceptibility ........................................ 126

B. Inoculum-Disease Relationships .................................................................................. 126
1. Variations in Pathogen Virulence ................................................................................ 126
2. Temporal Variations in Inoculum Density .................................................................... 127
3. Spatial Variations in Inoculum Density ....................................................................... 127
4. Dose-Response Relationships .................................................................................... 128
5. Influence of the Host on Disease Progression .............................................................. 129

C. Conduciveness of Agricultural Soils to Disease ............................................................. 129

D. Management of Disease by Manipulating Organic Matter ............................................ 131

E. Suppressive Soils .......................................................................................................... 132

IV. Biological Control of Soilborne Plant Diseases caused by *Pythium* spp. ..................... 132

V. Description of Biocontrol Agents .................................................................................. 133
   A. Fungal Biocontrol Agents .......................................................................................... 133
      1. Saprophytic *Pythium* spp. .................................................................................... 133
      2. *Trichoderma* and *Gliocladium* spp. ................................................................ 136
   B. Bacterial Biocontrol Agents ...................................................................................... 137
      1. Fluorescent Pseudomonads .................................................................................... 137
      2. *Burkholderia cepacia* .......................................................................................... 138
      3. *Enterobacter cloacae* .......................................................................................... 139
      4. Gram-Positive Bacteria .......................................................................................... 139

VI. Mechanisms by which Biocontrol Agents Suppress Disease ....................................... 140
   A. Interference with Survival ......................................................................................... 141
   B. Interference with Saprophytic Colonization .............................................................. 141
   C. Interference with Zoospore Viability or Development ............................................... 143
   D. Interference with Sporangial or Oospore Germination ............................................... 143
      1. Long Chain Fatty Acids .......................................................................................... 143
      2. Ethanol and Acetylaldehyde .................................................................................... 144
   E. Interference with Germtube or Mycelial Growth in the Spermosphere or Rhizosphere .......................................................... 144
      1. Antibiosis ................................................................................................................ 144
      2. Temporal Patterns of Antifungal Metabolite Production ......................................... 145
      3. Factors Influencing Concentrations of Antifungal Metabolites *In Sito* .................... 146
   F. Parasitism ................................................................................................................... 147
   G. Competition for Nutrients ........................................................................................ 147
      1. Carbon and Nitrogen Compounds .......................................................................... 147
      2. Siderophore-Mediated Iron Competition ................................................................ 148
H. Induction of Host Response ................................................................. 149

VII. Enhancement of Biocontrol .............................................................. 150

A. Screening Programs to Identify Antagonists of *Pythium* spp. .......... 150

B. Improvement of Biocontrol Agents through

   Genetic Manipulation ........................................................................... 151

   1. Enhancement of *In Situ* Antifungal Metabolite
      Production by *Pseudomonas* spp. .................................................... 151

   2. Altered Metabolite Production of *Gliocladium virens* .................... 152

   3. Enhanced Establishment of Biocontrol Agents in
      Agricultural Environments .............................................................. 153

C. Deployment Tactics to Enhance Biological Control ........................... 153

   1. Fermentation .................................................................................. 153

   2. Formulation ................................................................................... 154

   3. Seed Priming ................................................................................ 154

   4. Mixtures of Biocontrol Agents ....................................................... 155

   5. Identification of Plant Genotypes Amenable to Biocontrol ............... 156

   6. Inoculation of Substrates that Can Support
      Biological Control Activities of Microbial Antagonists ................... 156

   7. Specific Applications ...................................................................... 157

Conclusions ............................................................................................. 157

A. Contributions of Research on *Pythium* spp. to the Field
   of Biological Control ........................................................................... 158

   1. Biological Control Agents Can Be Remarkably
      Flexible in the Mechanisms by Which They Inhibit Plant Pathogens .... 159

   2. Variability Is Directly Related to the Capacity of
      Biological Organisms to Respond to Their Environments:
      It Is An Intrinsic Characteristic of Biological Systems,
      Including Biological Control .......................................................... 159

   3. Biological Control Requires Effective Antagonists(s) and an
      Environment That Supports Their Biocontrol Activities .................... 159

   4. Timing Is Everything ..................................................................... 159

B. Practical Significance ......................................................................... 160

Acknowledgments .................................................................................. 160

References .............................................................................................. 160

ABSTRACT: Soilborne root diseases caused by plant pathogenic *Pythium* species cause serious losses in a number
of agricultural production systems, which has led to a considerable effort devoted to the development of biological
agents for disease control. In this article we review information on the ecology and biological control of these
pathogens with the premise that a clear understanding of the ecology of the pathogen will assist in the development
of efficacious biocontrol agents. The lifecycles of the pathogens and etiology of host infection also are reviewed,
as are epidemiological concepts of inoculum-disease relationships and the influence of environmental factors on
pathogen aggressiveness and host susceptibility. A number of fungal and bacterial biocontrol agents are discussed
and parallels between their ecology and that of the target pathogens highlighted. The mechanisms by which these
microbial agents suppress diseases caused by *Pythium* spp., such as interference with pathogen survival, disruption

Copyright © 1999, CRC Press LLC — Files may be downloaded for personal use only. Reproduction
of this material without the consent of the publisher is prohibited.  

113
of the process of plant infection, and induced host resistance, are evaluated. The possibilities for enhancement of efficacy of specific biological control agents by genetic manipulation or deployment tactics are discussed, as are conceptual suggestions for consideration when developing screening programs for antagonists.

**KEY WORDS:** soilborne plant diseases, *Pythium* spp., ecology, epidemiology, biological control, oospores, sporangia.

I. INTRODUCTION

Biological control of pests that plague agricultural plants has been an increasingly active area of research for the past several decades. A sizable fraction of the research effort devoted to development of biological control of plant disease has focused on soilborne diseases caused by *Pythium* spp., a large and diverse genus of oomycete fungi. The ecology of *Pythium* spp. and the etiology and epidemiology of diseases caused by this important group of plant pathogens also have been the subject of many classic studies in plant pathology. Research in these areas has accelerated since the 1970s when the fungicide metalaxyl, which is toxic specifically to *Pythium* spp. and other oomycetes, became available. Since that time, plant pathologists, armed with an invaluable new tool for assessing diseases caused by oomycetes, have gained an enhanced appreciation for costs associated with infection of agricultural plants by *Pythium* spp. This review was conceived from the recognition that this group of phytopathogenic fungi is the subject of both classic and current studies on pathogen ecology, disease etiology, soil microbiology, and biological control that represent some of the best research in these areas of plant pathology.

Biological control of plant disease is still a relatively new area of research, and one maturing only as fast as we can build solid concepts and principles gleaned from successful and failed attempts to suppress diseases through the use of biological agents. Biological control is thought to have the greatest chance for success when it is developed with a clear understanding of the biology of the target pathogen and the etiology of the diseases it causes. Fortunately, the literature provides examples of successful biological control based on careful consideration of the host-pathogen interaction as it occurs in agricultural fields or other production systems. The strong knowledge base of the ecology of *Pythium* spp. has illuminated multiple points in the lifecycle of these pathogens that are sensitive to antagonism by other microorganisms. The purpose of this review is to summarize information on the ecology of phytopathogenic *Pythium* spp. and the etiology and epidemiology of the soilborne diseases that they cause and to discuss selected examples of biological control within the context of this information. No attempt was made to present an exhaustive review of the biological control agents that have been evaluated for suppression of plant diseases caused by *Pythium* spp. Instead, we direct the reader to excellent reviews on this topic (Paulitz, 1992; Whipps, 1992; Whipps and Lumsden, 1991). Here, we focus on the pathogens’ biology and ecology, highlighting those stages of the pathogens’ lifecycle that is susceptible to disruption by microbial antagonists.

The genus *Pythium* consists of approximately 120 species that occupy diverse habitats ranging from terrestrial ecosystems to salt water estuaries. Many species are plant pathogens, whereas others are strict soil saprophytes (Van der Platts-Niterink, 1981), or are parasites of insects (Saunders et al., 1988), mammals (de Cock et al., 1987), algae, or fish (Van der Platts-Niterink, 1981). Certain nonphytopathogenic species show promise as biological control agents capable of protecting plants from attack by pathogenic species (Lifshitz et al., 1984a; Martin and Hancock, 1987; Martin and Semer, 1994; Paulitz and Baker, 1987a,b; Veselý, 1979). Some phytopathogenic species
have broad host ranges, whereas other species infect a narrow spectrum of plants. For example, *P. aphanidermatum* and *P. ultimum* infect a number of economic crops while *P. graminicola* and *P. spinosum* have more restricted host ranges. Likewise, different levels of virulence also may be observed among species capable of infecting a specific host (Abad et al., 1994; Chamswarng and Cook, 1985; Hancock, 1985; Ingram and Cook, 1990; Kilpatrick, 1968; Larkin et al., 1995a,b; Lee and Hoy, 1992; McCarter and Littrell, 1970; Moulin et al., 1994; Southern et al., 1976). Additional background material on the biology and ecology of the genus *Pythium* may be found in Hendrix and Campbell (1973, 1983) or the proceedings of the Symposium on the genus *Pythium* (1974), on pathogenesis and host specificity in Martin (1995), on zoospore development in Deacon and Donaldson (1993), and on population genetics in Francis and St. Clair (1997).

Soilborne diseases caused by phytopathogenic *Pythium* spp. afflict many plant species. *Pythium* spp. are the most important pathogens infecting seeds or seedlings before emergence from the soil, resulting in preemergence damping-off (Hendrix and Campbell, 1973). The fungus also infects roots and the hypocotyl of seedlings after emergence, which can kill the emerged seedlings (called post-emergence damping-off). Seedlings of many plant species can survive root infection after emergence (Chi and Hanson, 1962; Halpin and Hanson, 1958; Larkin et al., 1996; Leach, 1947; Mellano et al., 1970), although surviving seedlings typically exhibit symptoms of reduced vigor and growth (Hancock, 1991; Hodges and Coleman, 1985; Hoy and Schneider, 1988b). *Pythium* spp. also infect roots of mature plants, typically causing necrotic lesions on root tips or fine feeder roots and, less commonly, on tap roots. With some hosts, such as alfalfa and carrot, infection of root tips can stimulate excessive adventitious root branching. Mature plants infected by *Pythium* spp. typically exhibit poor growth; yield losses can be especially large for crops such as sugar beet or carrots, whose tap roots are the harvestable products. *Pythium* spp. can infect roots and significantly reduce plant growth and yield even when necrotic symptoms typically associated with infection are not produced (Larkin et al., 1995a; Lee and Hoy, 1992; Stanghellini and Kronland, 1986). Because fine feeder rootlets and root hairs are destroyed by *Pythium* spp., infected root systems utilize nitrogen and other nutrients inefficiently (Cook, 1992). Sites of infection by *Pythium* spp. are important points of entry for infection of roots by other phytopathogens, which then cause significant economic losses to growers. Furthermore, rhizosphere populations of beneficial microorganisms, such as fluorescent pseudomonads (Mazzola and Cook, 1991) or vesicular arbuscular mycorrhizae (Afek et al., 1990), can be reduced by *Pythium* spp. infection.

Control measures for reducing damage caused by infection by *Pythium* spp. usually consist of modifications of cultural practices and application of chemical pesticides. Resistant varieties of some plants are available, but efforts to breed plants for resistance have not been widely successful. Distinct approaches are employed to control diseases on plants at different stages of development. For example, seed treatment with a fungicide protects seeds or emerged seedlings from infection, whereas cultural practices such as planting bed preparation or irrigation are modified to reduce root infection as plants mature.

II. ECOLOGY OF *PYTHIUM* SPP.

When evaluating the discussion that follows, the reader should keep in mind the source of pathogen inoculum (i.e., natural substrates or culture medium) that was used in the different investigations, because virulence of *Pythium* spp. may vary with the nutritional status of the medium used to produce the inoculum (Abad et al., 1994; Johnson et al., 1981). Response to germination stimulants also differs between sporangia produced in natural substrates and defined culture media (Nelson and Craft, 1989). Hence, it is possible that experimenta-
A. Survival in the Soil

1. Oospores

Oospores are the primary survival structures for most *Pythium* species. These thick-walled sexual spores are resistant to desiccation and may survive in soil for long periods of time in the absence of suitable hosts or organic substrates that support saprophytic growth. For example, culture-produced oospores of *P. ultimum* survived air-drying and were still viable after 8 months of storage (Lumsden and Ayers, 1975), and those of *P. aphanidermatum* and *P. graminicola* were recovered from soil after 11 months (Peethambaran and Singh, 1977). *Pythium aphanidermatum* survived as oospores formed in oat roots that were buried in fallow field soil for 16 months (Stanghellini and Nigh, 1972). De Vay et al. (1982) also reported survival of *P. aphanidermatum* and *P. ultimum* for 5 months in naturally infested field soil. Oospores of *P. hydnosporum* formed in mushroom compost survived for at least 18 months (Al Hassan and Fergus, 1973). Hoppe (1966) reported that oospores of a *Pythium* sp. were still viable after 12 years in an air-dried muck soil.

Oospores of *Pythium* spp. exhibit constitutive dormancy; some of them do not germinate even under conditions conducive to germination of mature oospores. The molecular events involved in the conversion from dormant to germinable oospores have not been established in *Pythium* spp., but Jiang et al. (1989) postulated that karyogamy between haploid nuclei from the antheridial and oogonial gametangia must occur before oospores of *Phytophthora* spp. can germinate. For some species, such as *P. ultimum*, the maturation process is accompanied by a reduction in thickness of the oospore wall. For other species, such as *P. aphanidermatum*, conversion to germinability is not accompanied by an observable change in wall thickness (Lumsden and Ayers, 1975). Constitutive dormancy enhances ecological fitness of *Pythium* spp. because it prevents the germination of all propagules at the same time and therefore precludes the possibility that all propagules will germinate during a single period that is unfavorable for continued growth or survival.

A number of factors, including age of the spores, may influence the conversion of dormant to germinable oospores. Freshly harvested, culture-produced oospores of *P. aphanidermatum* germinated at a low frequency immediately after harvest (27%) but with increasing frequency after 1 to 2 weeks (Adams, 1971; Ayers and Lumsden, 1975). Similarly, culture-produced oospores of *P. ultimum* were initially dormant and unable to germinate, but 90% of the oospores germinated after 6 weeks incubation in a nonsterile soil extract (Ayers and Lumsden, 1975). Similar levels of conversion also were observed when oospores were dried on agar disks or coverslips and placed on soil, although some conversion was observed following several weeks of incubation. When added to field soil, significant levels of conversion to germinable propagules were observed following 3 weeks of incubation (Lumsden and Ayers, 1975). Likewise, Johnson and Arroyo (1983) observed 85% conversion of thick-walled to thin-walled oospores after 45 days in dried agar films on glass slides incubated in soil. Stasz and Harman (1980) observed a 40% conversion rate after 3 to 6 weeks incubation when culture-produced oospores of *P. ultimum* were added to a pasteurized soil mix. The rate of oospore conversion of *P. ultimum* appears to be influenced by CO$_2$ concentration (Johnson, 1988). Soil matrix potential and temperature had no apparent influence on conversion when kept constant, but alternating wetting and drying increased conversion (Lifshitz and Hancock, 1984). Continuous saturated soil conditions, however, have been found to reduce viability of *P. aphanidermatum* oospores; this may be due to depletion of endogenous carbon reserves in the oospore caused by respiratory activity of the oospore and exudation (Mondal et al., 1995a,b) or perhaps by parasitism of the oospores by soil bacteria as suggested for
The importance of light on stimulating germinability of oospores in culture has been observed for *P. ultimum* (Johnson, 1988), *P. aphanidermatum* (Schmitter, 1972), *P. splendens* (Guo and Ko, 1994), and *P. sylvaticum* (F. N. Martin, unpublished).

The above studies relate to the conversion of culture-produced oospores and may not accurately reflect the behavior of the fungus under natural conditions in the field. For example, only 10% of oospores of *P. aphanidermatum* produced in field soil were capable of germination (Trujello and Hine, 1965), whereas 90% of culture-produced oospores germinated following 6 weeks of incubation in non-sterile soil extract (Ayers and Lumsden, 1975). However, oospores of *P. ultimum* produced following saprophytic colonization of organic substrates or culture produced oospores added to soil required 60 to 90 days to convert to germinable propagules (Hancock, 1981), whereas oospores of another isolate produced in culture required only 40 days (Lumsden and Ayers, 1975).

Once the thick-walled oospore of *P. ultimum* has matured into a thin-walled structure, it is capable of germination, but it also is susceptible to desiccation or lysis induced by other soil microbes. Hancock (1981) observed that some thin-walled oospores maintained at a constant matric potential lysed in association with soil bacteria. Qian and Johnson (1987) also examined the lysis of culture-produced oospores in 11 natural field soils. Variations were observed among the different soils, but lysis generally increased with increases in soil pH, carbon: nitrogen ratio, % organic matter, and available P; lysis was negatively correlated with % clay.

### 2. Sporangia

Sporangia and hyphal swellings are asexual reproductive structures formed by *Pythium* spp. Their morphology varies from spherical hyphal swellings produced by *P. ultimum*, *P. sylvaticum*, and *P. heterothallicum* to inflated filamentous or lobate forms typified by sporangia of *P. aphanidermatum*. Spherical structure can survive long periods of time in dry soil and therefore function as important inocula for saprophytic colonization of substrates or attack of a susceptible plant. For example, the spherical hyphal of *P. ultimum* survived for 11 months in an air-dried soil (Stanghellini and Hancock, 1971b). In contrast, inflated filamentous or lobulate sporangia are believed to be short-lived in soil. Culture-produced sporangia of *P. aphanidermatum* did not survive in an air-dried soil for more than 2 days (Stanghellini and Burr, 1973a). Nevertheless, long-term survival of filamentous and lobulate sporangia in soil has been reported. Sporangia of *P. aphanidermatum* and *P. graminicola* survived in soil for 6 and 4 weeks, respectively (Peethambaran and Singh, 1977), and viable lobulate sporangial forms similar to *P. aphanidermatum* were detected in an air-dried soil recovered from the field (De Vay et al., 1982).

**B. Propagule Germination**

Oospores and sporangia of *Pythium* spp. are quiescent in the soil and do not germinate in the absence of chemical stimulants. A number of amino acids, carbohydrates, and volatile compounds (such as ethanol or aldehydes) present in root and seed exudates, plant debris, or organic matter can stimulate germination of sporangia or oospores and induce a chemotactic response of germ tube growth (Lifshitz et al., 1986; Nelson, 1987, 1990; Nelson and Craft, 1989; Paulitz, 1991; Stanghellini and Hancock, 1971a, 1971b; and references therein). When nutrient concentrations are great enough to
stimulate propagule germination but not prolonged growth of the hyphal germ tube, some species are capable of reforming a resting structure. For example, secondary sporangia formed from oospores or primary sporangia of *P. ultimum* are smaller than the primary sporangium but are fully capable of surviving in the soil (Paulitz and Baker, 1988b; Stanghellini and Hancock, 1971a). Secondary sporangia formation by some *Pythium* spp., such as *P. aphanidermatum*, has not been observed (Stanghellini and Burr, 1973a).

### C. Zoospore Formation

Sporangia and oospores of all *Pythium* spp. germinate directly by producing a germ tube, but sporangia of some species also produce zoospores. In the presence of certain nutrients or high moisture, sporangia of these species are stimulated to produce motile zoospores rather than to germinate directly. Once released into the soil solution, zoospores have only a limited time in which to infect a susceptible plant. If free water is present, they are attracted to germinating seeds or roots where they encyst and penetrate the host. If conditions are not suitable for infection, the zoospores may encyst in the soil, where they remain viable as long as soil moisture content and temperature are favorable. When the soil is kept moist, zoospores of *P. aphanidermatum* can persist for at least 7 days, but air drying the soil for as little as 2 days eliminates them (Stanghellini and Burr, 1973b). Temperature also influences survival; encysted zoospores of *P. aquatile* survived better in sand or partially sterilized soil at 10°C than at 20°C (Hardman et al., 1989). Persistence of encysted zoospores varies among *Pythium* species; for example, encysted zoospores of *P. aquatile* persisted in soil for longer periods than those of *P. intermedium* (Hardman et al., 1989), while fungicide tolerant strains of *P. oligandrum* could be recovered 80 days after addition of zoospores to moist soil (Madsen et al., 1995); however, in this study it is unclear if survival was due to cyst survival or saprophytic colonization of the soil.

### D. Saprophytic Phase of the Lifecycle

In general, *Pythium* spp. are considered primary colonizing sugar fungi (sensu Garrett, 1970) because they rapidly colonize fresh organic substrates in the soil. For example, propagules of *P. ultimum* germinate, hyphae grow saprophytically in the tissue, and new sporangia are produced within 44 h after organic substrates are added to soil (Watson, 1971); oospores are formed shortly thereafter. *Pythium aphanidermatum* colonizes organic substrates within a similar time frame (Trujillo and Hine, 1965). While it has not been examined extensively, mycelia of *Pythium* spp. appear to be relatively short-lived in soil (Agnihotri and Vaartaja, 1967; Peethambaran and Singh, 1977; Stanghellini and Burr, 1973b) and cannot survive desiccation. Mycelia also appear to be short-lived in colonized organic tissue in soil. When organic matter fragments colonized by *P. oligandrum* were recovered from field soil and plated on a medium that inhibited oospore germination, the fungus could be recovered readily for up to 4 days after substrate incorporation into the soil. After this time period, *P. oligandrum* could not be detected, presumably due to the ephemeral nature of its mycelia and globose sporangia (Martin and Hancock, 1986).

*Pythium* spp. can quickly colonize organic matter present in soil, but they also are sensitive to competition from other soil microorganisms. This was exemplified by the results of Barton (1961), who treated small wood blocks with a glucose solution and added them to soils infested with *P. mamillatum*. The pathogen colonized the blocks within 24 h, except when the blocks were incubated previously in soils that lacked the pathogen. Retreatment of the blocks with nutrient solution did not restore their susceptibility to colonization by the pathogen. Barton concluded that metabolites produced by saprophytic microorganisms inhibited *P. mamillatum*, even in the presence of additional nutrients. *Pythium ultimum* also is intolerant of saprophytic competition for substrate colonization in field soil; prior occupation by other saprophytes prevents colonization of or-
ganic matter by the pathogen (Hancock, 1977; Martin and Hancock, 1986; Rush et al., 1986). *Pythium nunn*, which colonizes substrates already occupied by *P. ultimum* (Paulitz and Baker, 1988a), appears to be an exception to the apparent intolerance of the genus to competition.

E. Infection of the Plant Host

1. Host Sensitivity

Germinating seeds are particularly vulnerable to infection by *Pythium* spp., because propagule germination and germtube growth are enhanced by the large quantities of exudates released by seeds imbibing water during the germination process. Factors that enhance seed exudation increase the size of the spermosphere, defined as the soil surrounding the seed that contains sufficient quantities of seed exudates to stimulate microbial growth and development. Such factors enhance seed infection because propagules of *Pythium* spp. distributed throughout a larger volume of soil are stimulated to germinate and grow. For example, a germinating bean seed in soil maintained at field capacity stimulates germination of *P. ultimum* sporangia within a distance of 10 mm (Stanghellini and Hancock, 1971a). Reducing the moisture content of the soil reduces the distance that nutrients diffuse into the soil from the seed (i.e., the size of the spermosphere) and therefore the number of fungal propagules that could potentially initiate an infection. Quantities and composition of seed exudates also vary with seed age (Harman et al., 1978; Woodstock and Taylorson, 1981) and genotype (Chen, 1988b), which correlates with susceptibility to preemergence damping-off. For example, both the quantity of root exudates and incidence of preemergence damping-off typically are less for younger seeds than for older seeds (Fukui et al., 1994a; Hering et al., 1987). The condition of the seed also influences the size of the spermosphere; for example, the soybean spermosphere extended 5 mm for intact and 7.5 mm for scarified seeds 5 h after planting when soil moisture was −0.03 MPa (Schlub and Schmitthenner, 1978). Agronomic practices that reduce the quantity or quality of seed exudates diffusing into the soil also reduce the incidence of seed infection by *Pythium* spp. Such strategies include osmopriming of the seed (Osburn and Schroth, 1989; Rush, 1991, 1992), soaking the seeds in water prior to planting (Fukui et al., 1994a), planting pre-germinated seed (Hall and Sumner, 1994; Hering et al., 1987), or treatment of the seed with biological control agents that are capable of rapidly utilizing or altering the composition of the exudates, thereby reducing the size of the spermosphere.

Sensitivity of a plant to post-emergence damping-off by *Pythium* spp. generally decreases with age. Halpin and Hanson (1958) reported that ladino and red clover were resistant to plant death caused by *P. debaryanum*, *P. ultimum*, *P. irregular*, *P. splendens*, and *P. paroecandrum* 2 days following seeding, whereas alfalfa and sweet clover were resistant after 3 days. Similarly, red clover and alfalfa resist damping-off caused by *P. debaryanum* when the seedlings are 5 days old, although infection of older plants was occasionally observed (Chi and Hanson, 1962). Mellano et al. (1970) observed that roots of *Anterrhinum majus* were susceptible to infection and plant death caused by *P. ultimum* when seedlings were 15 days old or younger; as the plants aged to 25 days old, the roots became less susceptible to infection.

2. Host Recognition by the Pathogen

a. Sporangial Germination

Sporangia produced by *P. ultimum* on most culture media germinate in response to a wide variety of organic substrates, but fastidious sporangia raised on metabolically active plant material (such as germinating seeds) do not respond to these stimulants, although they will germinate in response to seed exudates. Nelson and Hsu (1994) have suggested that responsive-
ness of sporangia to germination stimulants may be due at least in part to the nutritional status of the propagule, as the specificity of the sporangial germination response changes with age of the propagule. Components of seed exudates that stimulate germination of fastidious sporangia of *P. ultimum*, which were produced on seed exudates, were identified by GC mass spectroscopy as saturated, long-chain fatty acids (greater than 12 carbons in their fatty acid chain) (Ruttledge and Nelson, 1997). Based on these results, the authors suggested that long chain fatty acids also are important germination stimulants in nature. The capacity of *Pythium* spp. to respond to these exudates may be important in differentiating a susceptible seed from other organic material in soil.

**b. Zoospore Development**

In the presence of free water, motile zoospores respond chemotactically to exudates from roots or germinating seeds. Once the zoospore arrives at the root or seed surface, it attaches, encysts, germinates, and a germ tube penetrates susceptible tissue. Elegant biochemical studies of host-pathogen interactions occurring at each step in this process reveal a level of specificity that was not appreciated previously (reviewed in Deacon and Donaldson, 1993; Martin, 1995). The attraction of zoospores and subsequent germination of cysts of *Pythium* spp. on root surfaces appear to be moderated by specific compounds present in the exudates. Zoospores respond differentially to various components of root exudates; some compounds present in exudates induce a chemotactic response, whereas others induce cyst germination (Donaldson and Deacon, 1993b). For example, L-glutamine attracts zoospores of *P. aphanidermatum*, but it does not stimulate cyst germination; glucose has the converse influence on zoospores of this species. A differential effect of root polysaccharides on induction of zoospore encystment also was observed (Donaldson and Deacon, 1993c).

Some amino acids that did not induce chemotaxis were found to inhibit the attraction normally induced by other amino acids, leading Deacon and Donaldson (1993) to postulate that specific amino acids and carbohydrates in the root exudates, as well as root surface polysaccharides, may contribute to host range specificity. This concept was initially proposed by Tripanthi and Grover (1978) to explain host range specificity of *P. butleri*; root exudates of resistant plants contained arabinose and arginine, which reduce zoospore attraction and encystment. Arabinose and arginine are not present in root exudates of susceptible plants, and if these amino acids were added to the rhizosphere of susceptible plants disease severity was reduced.

In the early stages of zoospore encystment, glycoproteins stored in large peripheral vesicles of the zoospore are secreted to form an adhesive that attaches the cyst to the root (Estrada-Garcia et al., 1989, 1990a). Initial attachment is subsequently followed by deposition of a cell wall around the cyst (Grove and Bracker, 1978). For *P. aphanidermatum*, germination typically occurs 9 to 15 min after encystment (Jones et al., 1991), and the emerging germ tube exhibits a chemotropic response (Jones et al., 1991; Mitchell and Deacon, 1986a; Royle and Hickman, 1964). The appropriate concentrations of divalent cations, in particular Ca$^{2+}$, are necessary for cyst adhesion to the root and subsequent germination (Donaldson and Deacon, 1992, 1993a).

Specific carbohydrates on the root surface also moderate root recognition by zoospores and the subsequent process of infection. Mitchell and Deacon (1986b) observed that treatments altering carbohydrate residues on the root surface reduced or prevented zoospore encystment for several *Pythium* spp. Longman and Callow (1987) observed that root mucilage contains fucose-containing polysaccharides that induce *P. aphanidermatum* zoospores to bind to cress roots; treatment of roots with compounds that block or remove terminal fucose residues significantly reduced zoospore bind-
ing. Zoospores also encysted preferentially on regions of the root richest in terminal fucose residues (Longman and Callow, 1987), although results from similar studies by Jones et al. (1991) contrast with these findings. Other plant polysaccharides also induce zoospore encystment: the polyuronates alginate, polygalacturonic acid, and gum arabic induce zoospore encystment of three *Pythium* spp. (Donaldson and Deacon, 1993c; Jones et al., 1991). Some of these compounds may be involved in triggering a host recognition response in the zoospore. Treatment of zoospores with isolated root mucilage (Longman and Callow, 1987) or a fractionated portion of root mucilage containing low amounts of uronic acid and fucose as the major 6-deoxy sugar (EstradaGarcia et al., 1990b) prevented subsequent zoospore encystment on roots.

The above discussion on mechanisms involved in host infection by zoospore inoculum presents a plausible view of the initial stages of infection, but one gained from experimentation under artificial conditions in the laboratory. Under natural conditions, the plant may produce different combinations or concentrations of the various compounds found to influence zoospore behavior. In addition, the presence of other microbes in the rhizosphere will undoubtedly change the quantity and quality of exudates available to the pathogen, which may provide an avenue for disruption of the infection process by a biocontrol agent.

c. Timing of the Infection Process

Seeds or roots of host plants are infected by germ tubes arising from oospores, sporangia, or zoospore cysts. The most striking characteristic of the process is the remarkable rapidity at which infection occurs. In a scenario outlined by Osburn et al. (1989), sporangia of *P. ultimum* began to germinate in response to exudates of germinating bean seed in as little as 1.5 h (Stanghellini and Hancock, 1971b). With a germ tube growth rate of 300 µm/h, the bean seed was colonized within 24 h after planting (Stanghellini and Hancock, 1971a). *Pythium aphanidermatum* exhibited a similarly rapid germ tube growth rate in the soil (Tedla and Stanghellini, 1992). A rapid colonization of germinating seeds also has been observed by others evaluating cotton (Nelson, 1988, 1990), soybean (Schlub and Schmitthenner, 1978), chickpea (Kaiser and Hannan, 1983), sugar beet (Martin and Hancock, 1987), wheat (Hering et al., 1987), and pea (Lifshitz et al., 1986; Parke, 1990). While the timing of specific infection events of intact plant roots in the soil is not known, it would be expected to occur in a similar rapid manner.

III. EPIDEMIOLOGY

A. Influence of Environment on Disease Severity and Ecology of *Pythium* spp.

The behavior of *Pythium* spp. in the soil is moderated by environmental factors such as moisture, temperature, soil pH, and the presence of specific soil minerals. These environmental parameters can influence fungal growth or development directly or indirectly, through effects on competing or antagonistic soil microorganisms. The environment also can have profound effects on host susceptibility and symptom expression.

1. Soil Moisture

Plant diseases caused by *Pythium* spp. are more prevalent in fields with wet soils than in fields with low soil moisture, and experimental evidence substantiates the importance of soil moisture in disease development. Biesbrock and Hendrix (1970) observed that root rot of peach caused by two *Pythium* spp. was more
severe in wet soils than in drier soils. Severity of infection of barley by *P. irregulare*, *P. volutum*, and *P. graminicola* increased as the matric potential increased from 50 to 90% of field capacity (Bratoloveanu and Wallace, 1985). A positive correlation between disease severity with *P. ultimum* and soil moisture also has been reported for bean (Piezarka and Abawi, 1978b), poinsettia (Bateman, 1961), and soybean (Schlub and Lockwood, 1981). For soybean, increasing soil moisture from $-0.18$ to $-0.0018$ MPa reduced seedling emergence from 66 to 15%. Disease incidence in the field correlated positively with the number of days that soil water matric potential was greater than $-0.05$ MPa. A similar response between soil moisture and disease incidence was also observed in greenhouse evaluations for safflower infection by *P. ultimum* (Mundel et al., 1995). Likewise, Stanghellini and Burr (1973b) observed that oospore germination and host colonization by *P. aphanidermatum* occurred between 0 and $-0.1$ MPa. Soil moisture also was important for wheat seed infection by *P. irregulare* and *P. ultimum* var. *sporangiiferum*; maximum infection levels were obtained at $-0.01$ MPa, with little infection below $-0.04$ MPa (Hering et al., 1987). In a pasteurized field soil, Fukui et al. (1994a) observed that wheat embryo infection by *P. ultimum* var. *sporangiiferum* was maximized at $-0.01$ MPa and was negligible when the soil was dryer than $-0.2$ MPa. Soil moisture also influenced the relationship between inoculum density and embryo infection of wheat: 8.2, 21.2, and 36.0 propagules/g soil of *P. ultimum* var. *sporangiiferum* caused 50% infection when soil was maintained at $-0.011$, $-0.045$, and $-0.088$ MPa, respectively. In contrast, Hancock and Grimes (1993) observed that infection of alfalfa feeder roots by *P. ultimum* and *P. irregulare* in a well-drained sandy soil was minimally affected at moisture levels ranging from greater than $-0.02$ to 0.5 MPa in a field plot and from 0 to $-2.2$ MPa in greenhouse experimentation.

Fluctuations in soil moisture can differentially influence the severity of disease caused by *Pythium* spp. Periodic wetting and drying of the soil increased infection of peach roots by *P. vexans* but did not change the number of infections caused by *P. irregulare* (Biesbrock and Hendrix, 1970). The differential response of the two species was attributed to the capacity of *P. vexans* to produce and discharge zoospores under fluctuating moisture conditions, whereas *P. irregulare* does not produce zoospores.

The effects of soil moisture on disease are undoubtedly due to both direct and indirect effects on the pathogen and host, the latter of which can be mediated by other components of the soil microflora. Soil moisture influences the motility of zoospores, which requires free water, and the type of reproductive spores formed by *Pythium* spp. For example, formation of oospores by *P. ultimum* predominated in wet soils, whereas sporangia were more common in soil with a lower moisture content (Bainbridge, 1970). Soil matric potential also influences the saprophytic activity of the pathogen; *P. ultimum* has very low saprophytic activity in soils at $< -1$ MPa or in saturated soils (Lifshitz and Hancock, 1983). Soil moisture mediates the composition of microbial communities in organic matter; at high moisture levels, *Pythium* spp. are the primary saprophytes, but as the soil moisture decreases, other fungi predominate (Kouyeas, 1964). The size of the spermosphere is also influenced by soil moisture, with germination of pathogen propagules stimulated at greater distances from the germinating seed as soil moisture increases (Stanghellini and Hancock, 1971a).

As soil moisture increases, the concentration of CO$_2$ increases and O$_2$ decreases. Whereas the concentration of CO$_2$ in ambient air is 0.03%, it can range from 3.5 to 9.2% in soil at field capacity (Keeney et al., 1985; Papavizas and Davey, 1962; Tabak and Cooke, 1968). Because *Pythium* spp. are more tolerant of high CO$_2$ concentrations than many other soil microorganisms (Burges and Fenton, 1953; Gardner and Hendrix, 1973; Macauley and Griffin, 1969; Mitchell and Mitchell, 1973; Papavizas
and Davey, 1962), it enjoys an environment with reduced microbial competition at high soil moistures. For example, Gardner and Hendrix (1973) reported an increase in the inoculum densities of *P. vexans* and *P. irregulare* in infested field soils as CO₂ concentration increased from 0.03% to 15%; corresponding variations of O₂ concentrations from 5 to 21% had no effect on the pathogens. In contrast, others have reported that O₂ concentrations have a significant influence on linear growth and oospore production of *P. irregulare* (Mitchell and Mitchell, 1973), linear growth of *P. ultimum* (Brown and Kennedy, 1966), and conversion of thick-walled oospores of *P. ultimum* to thin-walled germinable propagules (Johnson, 1988).

### 2. Soil Temperature

Numerous examples of the influence of temperature on disease severity have been reported, only some of which are presented here (consult Hendrix and Campbell, 1973, 1983 for additional references). Generalizations can be made about the influence of temperature on diseases caused by particular *Pythium* spp., with the caveat that variations may be observed among isolates and plant hosts. Disease caused by *P. ultimum* and *P. irregulare* tend to be more severe at cooler soil temperatures. Leach (1947) observed less preemergence damping-off of sugar beet and watermelon caused by *P. ultimum* at 30 to 35°C than at 12 to 25°C. Likewise, this species caused less disease of bean at 27 to 28°C than at temperatures below 21°C (Piecarka and Abawi, 1978; Sippell and Hall, 1982), less disease of safflower below 15°C compared with greater than 15°C (Mundel et al., 1995) and was the most virulent on soybean at 15 to 20°C (Thomson et al., 1971). In contrast, Hershman et al. (1986) reported that *P. ultimum* and *P. irregulare* were equally virulent at temperatures ranging from 15 to 30°C. Ingram and Cook (1990) observed host-specific effects of temperature on disease incidence; preemergence damping-off caused by *P. ultimum* var. *sporangiferum* occurred at 15 to 25°C for wheat, 10 to 25°C for lentils, and 5 to 25°C for pea. *Pythium irregulare* caused damping-off on pea at 5°C only, and caused no detectable symptoms on the other two plant hosts. Hancock (1991) observed that root infection of alfalfa by this species was more severe at 16 to 21°C compared with higher temperatures. In contrast to *P. ultimum* and *P. irregulare*, disease caused by *P. aphanidermatum* and *P. myriotylum* is more severe at higher temperatures. *Pythium aphanidermatum* and *P. myriotylum* caused more damage to rye and tomato at temperatures above 27°C than at lower temperatures; no damage was observed at 15°C (Littrell and McCarter, 1970). Enhanced disease severity at higher temperatures does not necessarily reflect only the ability of the pathogen to infect the host. Mitchell (1975) observed similar levels of root infection of rye by *P. myriotylum* at 25 and 35°C, but root rot and mortality were observed only at 35°C. Undoubtedly, host susceptibility is influenced by temperature.

Generalizations about the influence of temperature on disease severity caused by a specific *Pythium* sp. should be approached with caution, as intraspecific variation in this characteristic has been reported. Nelson and Craft (1991) observed different levels of virulence on turfgrass for specific isolates of *P. graminicola* and *P. aphanidermatum* when experiments were conducted at 13 and 28°C in a potting soil mixture with colonized wheat seed as pathogen inoculum. Similar levels of intraspecific variation in temperature-moderated virulence were observed for *P. aristosporum*, *P. torulosum*, and *P. vanterpoolii* when trials were done on a smaller scale with agar inoculum. Using a similar technique, Abad et al. (1994) obtained similar results for several *Pythium* spp. infecting turfgrass. Hodges and Campbell (1994) also reported intraspecific variation in temperature moderated virulence of *P. torulosum*, *P. vanterpoolii*, and *P. graminicola* on turfgrass.
Soil temperature can influence the development of plant disease through effects on spore germination or germ tube growth of the pathogen. For example, zoospore discharge for *P. iwayamai* and *P. okanoganense*, the causal agents of snow mold of wheat, did not occur unless the temperature was below 15 or 10°C, respectively; maximum zoospore discharge was at 1°C (Lipps, 1980). Maximum germination frequency of culture-produced oospores of *P. aphanidermatum* in autoclaved soil occurred at 30°C; lower germination frequencies were observed at 10°C and 35°C (Adams, 1971). Rates of germ tube growth from germinating culture-produced oospores of this species in field soil also was greater at 27°C (636 µm/h) than at 20°C (146 µm/h; Tedla and Stanghellini, 1992).

3. Soil pH

Soil pH can have a significant influence on the expression of disease caused by *Pythium* spp. Buchholtz (1938) observed a positive correlation between soil pH and stand counts of sugar beets in soils naturally infested with *P. debaryanum*; soils above pH 6.6 had greater stands than fields with a lower pH. Soil pH also influences the incidence of cavity spot of carrot, which is more common in fields below pH 6.5 (Scaife et al., 1983), with little disease observed in soils above pH 8.0 (White, 1988). From assaying field soils for pH, inoculum densities of *Pythium* spp., and levels of wheat seed embryo infection, Fukui et al. (1994a) concluded that there was a negative correlation between levels of plant infection and soil pH ranging from pH 4.4 to pH 7.2. The influence of soil pH on infection levels was examined experimentally by manipulation of pH in two field soils and quantifying the frequency of embryo infection; maximum levels of infection were observed between pH 4.8 to 6.9, with a decrease at pH 7.6. In contrast, Bateman (1962) observed that infection of poinsettia by *P. ultimum* was reduced when plants were grown at pH 5.5 or below.

Commonly, *Pythium* spp. can be isolated only from field soils within a restricted range of pH. For example, recovery of *Pythium* spp. was reduced or eliminated in field soils below pH 5.5 (Barton, 1958), pH 4.5 (Remy, 1949), or much below pH 3.6 (Dick and Ali-Shtayeh, 1986). Warcup (1952) observed that *P. ultimum* was abundant in soils from pH 6.8 to 7.2 but rare in soils of pH 5.3 to 5.5. In contrast, Johnson and Doyle (1986) recovered *Pythium* spp. from field soil at pH 4.3.

Soil pH has a significant influence on saprophytic activity of *Pythium* spp., but these influences appear to vary within and among species. Lifshitz et al. (1984a) observed greater saprophytic activity of a hyphal-swelling isolate of *P. ultimum* at pH 5.0 than at pH 7.3. Likewise, Paulitz and Baker (1987a) observed a limited effect of soil pH ranging from 5.0 to 6.7 on the saprophytic activity of a hyphal swelling isolate of *P. ultimum* or *P. nunn* in pasteurized soil. In contrast to these findings, Martin and Semer (unpublished) observed that in autoclaved soil, *P. ultimum* had a maximum saprophytic activity at pH 6.5, approximately a 50% reduction in activity at pH 4.5 or 8.5, and no activity at pH 3.5. While *P. aphanidermatum* had no saprophytic activity at pH 3.5, a maximum was observed from pH 5.5 to pH 8.5. In field soil naturally infested with both pathogens, similar levels of saprophytic activity were observed between pH 4.5 and 7.5 for *P. aphanidermatum* and pH 5.5 to 7.5 for *P. ultimum*.

Soil pH also influences other aspects of the lifecycle of *Pythium* spp., including susceptibility to lysis and formation of resting structures. Barton (1958) attributed his inability to recover *P. mamillatum* from field soils below pH 5.0 to the lack of fungal saprophytic activity, lysis of fungal propagules, and inability of the fungus to form resting structures; increasing soil pH resulted in the recovery of the pathogen in these soils. *In vitro* investigations also identified a positive correlation between pH and linear growth rates (*P. aphanidermatum*, Adams, 1971; *P. ultimum*, Griffin, 1958), oospore con-
version (P. ultimum, Lumsden and Ayers, 1975; Quin and Johnson, 1987) and oospore germination (P. aphanidermatum, Adams, 1971; P. aphanidermatum, P. ultimum, Lumsden et al., 1975, 1976). The influence of soil pH on these phases of the pathogen life cycle have been suggested to be responsible for the poor saprophytic activity of P. aphanidermatum in field soils ranging from pH 5.1 to 6.1 (Lumsden et al., 1976) and to contribute to soil suppressiveness in traditional Mexican chinampa agroecosystems (Lumsden et al., 1987). In contrast, Kobayashi and Ko (1985) observed a moderate negative correlation (r = –0.47 and –0.65) in several tests between the pH of natural soils and germination of culture-produced sporangia of P. splendens.

Soil pH is the primary factor mediating the bioavailability of many soil minerals or compounds (Lindsay, 1979). Therefore, alterations in pH can influence Pythium spp. by changing the availability of nutrients or toxic compounds in the soil. For example, addition of CaO to soil suppresses damping-off of pea by P. ultimum by increasing the pH and converting inorganic ammonium salts to NH₄⁺ (Lewis and Lumsden, 1984), which inhibits many Pythium spp. (Chun and Lockwood, 1985; Lewis and Lumsden, 1984).


In addition to direct effects of the environment on Pythium spp., environmental conditions moderate the biological balance between the pathogen and other microorganisms in the soil. This was exemplified by the results of Lifshitz and Hancock (1983) when they examined the influence of temperature on the saprophytic activity of P. ultimum. Temperature had a parallel influence on saprophytic activity of the pathogen in sterile soil and on mycelial growth in culture, both of which are maximized between 27°C and 30°C. In contrast, the optimum temperature for maximum saprophytic activity in nonsterilized field soil was 19 to 21°C, presumably because the pathogen confronted competition from other microbes in nonsterilized soil at higher temperatures (Lifshitz and Hancock, 1983). A similar response also was observed for pathogenic attack of sugar beet by P. aphanidermatum (Tedla and Stanghellini, 1992). In natural field soil, the temperature optimum for infection was 27°C, but plant infection and oospore germination at 20°C dramatically increased following soil treatment with antibiotics to selectively reduce bacterial populations, suggesting that temperature moderated interactions between P. aphanidermatum and other components of the resident microflora. Likewise, Fukui et al. (1994a) observed that adjustment of soil pH from 5.3 to 7.4 reduced embryo infection of wheat seed by pathogenic Pythium spp. This effect was ameliorated by the addition of chloramphenicol to the soil, presumably by reducing populations of antagonistic bacteria. The addition of fungicides to the soil also can shift the ecological balance in favor of phytopathogenic Pythium spp. Katan and Lockwood (1970) observed that soil amendment with pentachloronitrobenzene (to which P. ultimum is insensitive) increased colonization of alfalfa residues by the pathogen due to selective inhibition of other soilborne competitors.

Physical and chemical characteristics of soil can influence the composition of Pythium spp. in the soil by placing one species at a competitive advantage over another. This was observed in suppressive soil from the San Joaquin Valley of California, in which CL⁻ concentrations appeared to favor the CL⁻ tolerant non-pathogenic species P. oligandrum over P. ultimum (Martin and Hancock, 1986). Because soil pH differentially influences various Pythium spp., it influences the community structure of the genus in the soil. Of the several physiochemical soil characteristics evaluated by Dick and Al-Shtayeh (1986), soil pH appeared to exert the greatest influence on relative population densities of various Pythium spp. Soil moisture also can determine which genera of fungal saprophytes predominate in organic substrates; in a study by Kouyeas (1964), Pythium spp. were the primary
saprophytes at the higher moisture levels, but as the moisture decreased other fungi predominated.

5. Influence of Environmental Factors on Host Susceptibility

Environmental conditions influence disease, in part by altering the growth or susceptibility of the plant host. For instance, tomato roots grown under oxygen stress are more sensitive to infection and exhibit enhanced lipoxygenase activity (Chérif et al., 1997). Altered cell membranes resulting from the enhanced lipoxygenase activity may be more sensitive to infection by the pathogen. Brown and Kennedy (1966) related the high incidence of soybean seed rot under conditions of low O₂ (conditions simulating flooded conditions) to the release of enhanced quantities of exudates, which stimulate germination of propagules of *P. ultimum* and seed infection by the pathogen. Enhanced seed exudation under conditions of high soil moisture is also thought to contribute to the greater infection of pea by *P. ultimum* as soil moisture is increased (Flentje, 1964; Kerr, 1964). Increases in preemergence damping-off at lower temperatures have been attributed to delayed seed germination and emergence, thereby keeping the seedling at a stage susceptible to infection for a longer period of time (Chi and Hanson, 1962; Leach, 1947). Similar conclusions also have been drawn for the influence of soil pH on infection of wheat by *P. graminicola* (Kauraw, 1979) and of three different hosts by *P. ultimum* (Griffin, 1958).

B. Inoculum-Disease Relationships

1. Variations in Pathogen Virulence

Significant differences in host range exist among the various phytopathogenic *Pythium* spp. Perhaps more importantly (and less well documented) are the levels of intraspecific variation in virulence that may exist among members of a pathogen population. Hampton and Buchholtz (1962) observed variation in virulence among isolates of *P. graminicola* when tested on six different crop varieties. Isolates recovered from fields that had been monocropped to corn, oat, or setaria appeared to be more virulent on these hosts than isolates recovered from wheat, barley, or rye in fields that had been subjected to crop rotation. The authors concluded that increased pathogen virulence was selected by monoculture of a particular host. Intraspecific variation in virulence also has been reported for *P. myriotylum* on bean (Gay, 1969), *P. aphanidermatum* and *P. myriotylum* on 12 different plant species (McCartter and Littrell, 1970), *P. aphanidermatum* on tomato (Grover and Dutt, 1973), *P. ultimum* on geranium (Chagnon and Bélanger, 1991), *P. ultimum* and *P. irregulare* on parsley (Hershman et al., 1986), *P. graminicola* on rice (Cother and Gilbert, 1993), *P. ultimum* on tomato fruit (Francis et al., 1994), and *P. arrhenomanes* on sugarcane (Deep and Lipps, 1996; Hoy and Schneider, 1988a). While these reports indicate variation in pathogen virulence, inoculum sources for the studies were colonized culture media, and the inoculum densities were not standardized among the different isolates in each study. Therefore, it is possible that differences in disease severity may be due to variation in inoculum levels (due to intraspecific variation in sporulation or germination frequency) rather than virulence.

Using carefully quantified levels of oospore inoculum, Mitchell (1975) examined the relationship between inoculum density of *P. myriotylum* and infection of rye seedlings. In the course of these investigations several isolates from the same field were identified that exhibited different levels of virulence (D. J. Mitchell, personal communication). Unexpectedly, some isolates were also found to lose their virulence following repeated subculturing, a trait that was not restored by growing the pathogen in association with the host. Using colonized wheat seed as an inoculum source, Nelson and Craft (1991) observed variation in
virulence among isolates of *P. graminicola* and *P. aphanidermatum* on turfgrass grown at two different temperatures. Abad et al. (1994) also observed intraspecific variation in virulence of a species infecting turfgrass in growth chamber tests in a sand/peat mixture; using colonized grass blades as an inoculum source, disease incidence on creeping bent grass ranged from 7 to 52% among 14 isolates of *P. vanterpoolii*. Seven other species were also identified that had isolates that were either low in virulence (1 to 15% disease incidence) or not pathogenic. Clearly, variation in pathogen virulence is an important consideration to keep in mind when selecting isolates for conducting screening trials and is an area of research that needs a more thorough evaluation using quantified natural inoculum.

2. Temporal Variations in Inoculum Density

Oospores and sporangia of some *Pythium* spp. are capable of long-term survival in the field, but their populations are not stable and fluctuations in inoculum density throughout the year are commonly observed. For example, Lumsden et al. (1976) reported that populations of *P. ultimum* and other low-temperature species increased in the fall months and decreased in the spring. Hancock (1977) observed a similar seasonal variation for inoculum densities of *P. ultimum* in the San Joaquin Valley of California, which were highest in the cooler winter months and lowest in the warmest midsummer months. In both studies, major increases in the population size of *P. ultimum* were attributed to saprophytic colonization of crop debris following its incorporation into the soil. Seasonal variation in populations of many *Pythium* spp. in cultivated and uncultivated soils was examined by Ali-Shtayeh et al. (1986), who detected maximum population sizes and species diversity in the winter months and the lowest values in the summer months. Similar observations on seasonal fluctuations of population densities and species composition have been reported in New York (Pieczarka and Abawi, 1978a), Australia (Bratoloveanu and Wallace, 1985), the Middle East (Ali-Shtayeh, 1986), and Nigeria (Aderungboye and Esuruoso, 1976). In the last example, soil moisture was thought to be responsible for population decreases. Seasonal fluctuations in populations of *P. aphanidermatum* were observed in golf course turf in Ohio (highest in November through January; Hall et al., 1980), but populations of this species were relatively stable in sugar beet fields in Arizona (Stanghellini et al., 1982). The presence of a susceptible host in the field can also contribute to increases in pathogen inoculum density (Soufi and Filonow, 1992), as well as to the diversity of species present in the soil (Ali, 1985). It has been suggested that root phenology may contribute to seasonal fluctuations in root infections, and hence pathogen population densities derived from root infections (Hancock, 1988).

In addition to seasonal fluctuations, short-term fluctuations in inoculum density have also been detected. Population sizes of *Pythium* spp. that produce hyphal swellings vary significantly among samples collected only 24 h apart (Hardman and Dick, 1987). Based on regression analysis of inoculum densities and environmental parameters, temperature exerted a more pronounced effect than rainfall on these short-term population fluctuations of *Pythium* spp. More detailed observations in sand or partially sterilized soil maintained at constant moisture confirmed the importance of temperature on recoverability of different spore forms (Hardman et al., 1989).

3. Spatial Variations in Inoculum Density

From surveys of natural field soils in Australia, Bratoloveanu and Wallace (1985) observed that *Pythium* spp. were more abundant in the surface 10 cm than at depths of 10 to 20 cm. Others have reported that maximum population densities are in the surface 10 cm for *P. irregulare* (Pankhurst et al., 1995), surface 15 cm for *P. aphanidermatum* (Burr, 1973; Stanghellini and Phillips, 1975), 15 to 20 cm
for *P. ultimum* (Allmaras et al., 1987; Kraft and Allmaras, 1985), and 15 to 30 cm for *P. splendens* (Aderungboye and Esuruoso, 1976). The distribution of inoculum in the soil strata corresponds to the distribution of roots and crop debris that are available for saprophytic colonization by *Pythium* spp. (Pankhurst et al., 1995). The concentration of inoculum high in the soil profile is likely to be responsible for the greater number of infection sites on shallow roots than on deeper roots (Hancock, 1985).

Propagules of *Pythium* spp. are not uniformly distributed but are clustered in the soil. Intrafield variation in inoculum densities has been reported for *P. ultimum* (Pieczarka and Abawi, 1978a). In a greenhouse nursery soil consisting mainly of sand, the inoculum density of *P. aphanidermatum* varied from 6 to 132 propagules/g of soil in samples collected as little as 7 cm apart (Stanghellini and Phillips, 1975). Inoculum of this species was also variable among fields and was clustered in soil sampled from within a single sugar beet field, with high densities in rhizosphere soil adjacent to the tap roots (Stanghellini et al., 1982). A highly clustered distribution was observed in the sugar beet rhizosphere as well, although some plants were identified that had a random or uniform distribution of the pathogen (Stanghellini et al., 1983). Necrotic lesions symptomatic of cavity spot of carrot, caused primarily by *P. violae* and to a lesser extent by *P. sulcatum*, also exhibited a clustered distribution on the surface of roots (Phelps et al., 1991). Unfortunately, it is difficult to quantify soil populations of these species, so the distribution of the pathogen in the soil, and how this distribution affects patterns of plant infection could not be determined.

### 4. Dose-Response Relationships

Development of models to correlate the amount of disease expected with a specific level of inoculum has been attempted by several labs. Most frequently this has been approached by addition of different amounts of culture-produced inoculum to sterilized or pasteurized soil and assaying for the extent of damping-off (Bhatti and Craft, 1992; Bratoloueanu and Wallace, 1985; Ferris, 1982; Hawthorne, 1988; Kerr, 1964; Pieczarka and Abawi, 1978; Schlub and Lockwood, 1981; Sippel and Hall, 1982; Stasz and Harman, 1980). Depending on the investigation (and the range of inoculum densities tested), authors have concluded either a linear relationship between inoculum density (ID) and disease severity or a linear relationship at the lower IDs with disease severity leveling off as the inoculum is increased. The results of Stasz and Harman (1980) are particularly interesting because graphs reflecting the relationship between inoculum density and disease severity in two different pasteurized soils were shaped the same, but the soils differed in the number of oospores required to incite a specific level of disease, suggesting that physiochemical or biological differences between the soils moderated disease expression. Ferris (1982) observed that IDs from 10 to 600 sporangia/g of soil of *P. ultimum* had similar levels of disease on soybean in pasteurized soil. Similar results were observed for *P. ultimum* on soybean by Schlub and Schmitthenner (1978) and pea by Flentje and Saksena (1964), leading Ferris (1982) to suggest that above a threshold level of ID, other factors such as temperature, soil moisture, or seed quality may be more important than ID for expression of disease.

A more detailed examination of the relationship between propagule density and root infection was pursued by Mitchell (1975, 1978), who added oospore inoculum to pasteurized soil and assayed root infection by the pathogen (not just damping-off). The inoculum level required to infect 50% of the plants (ID₅₀) was 20 oospores/g of soil for *P. myriotylum* on rye, with 30% infection at 10 oospores/g of soil (Mitchell, 1975). Surprisingly, similar results were observed in both autoclaved and untreated field soils. Similar levels of inoculum (ranging from 15 to 43 oospores/g of soil) were needed for 50% plant infection by *P. myriotylum, P. polymastum*, or *P. aphanidermatum* on several different host seedlings, while infection by...
zoospore inoculum required significantly higher levels (250 and 281 zoospores/g of soil for *P. aphanidermatum* and *P. ostracodes*, respectively; Mitchell, 1978). Using a similar approach with cucumber, Kusunoki and Ichitani (1984) reported that 30 oospores of *P. butleri* g of sand caused 100% infection of seedlings. Likewise, Smith et al. (1997) examined the relationship between the number of zoospores of *P. aphanidermatum, P. aristosporum,* and *P. torulosum* and seedling mortality for eight host species by adding zoospore suspensions to seedlings growing in autoclaved vermiculite; distinct differences in the amount of inoculum necessary to cause plant death were observed for the various host-pathogen combinations.

Using field soils naturally infested with the pathogens, Fukui et al. (1994a) examined the relationship between inoculum density of *Pythium* spp. (total isolates were quantified with no delineation of individual species, IDs were adjusted by diluting field soil with pasteurized soil) and incidence of wheat embryo infection. A curvilinear response was observed, with the level of infection related to inoculum density below 200 propagules/g of soil; above this level inoculum level increases had smaller effects on increasing infection. Using a modification of the Lineweaver-Burke double reciprocal plot technique, the relationship between inoculum density and disease incidence was calculated. Depending on the soil, the inoculum level causing 50% infection ranged from 58.7 to 107.6 propagules/g of soil. Knowledge of the individual species and their inoculum levels in the soil would provide an interesting correlation between ID and disease severity.

**5. Influence of the Host on Disease Progression**

The availability of susceptible plant tissue often limits plant disease epidemics. The relationship between susceptible tissue availability and severity of *Pythium* damping-off was examined by Neher et al. (1987, 1992) using two closely related plant species that had either a rapid synchronous germination and short period of susceptibility (*Glycine max*) or a longer asynchronous germination with a longer period of susceptibility (*Glycine soja*). The theory that damping-off epidemics could be enhanced by increasing the availability of susceptible tissue was tested by varying times of seeding and soil infestation with *P. aphanidermatum*. Because stands of *G. max* of mixed age had greater levels of disease than stands that were uniform in age, the authors concluded that synchronous germination of the host did not determine the progress of *Pythium* damping-off epidemics. For postemergence damping-off, the density of susceptible tissue can have an important influence on the expression of disease; planting fewer seeds or interplanting with resistant plants can reduce the extent of disease by limiting plant to plant spread (Augspurger and Kelly, 1984; Burdon and Childvers, 1975a,b, 1976a,b). A computer model of post-emergence damping-off of cress was developed to examine the spread of disease as influenced by plant density and the number of disease foci (Mao et al., 1988), although its applicability to portray field epidemics is unknown.

**C. Conduciveness of Agricultural Soils to Disease**

In contrast to the dose-response relationships observed in experimental systems, the relationship between natural population densities of pathogenic *Pythium* spp., and disease incidence or severity of plants grown in field soil is far less consistent. DeVay et al. (1982) observed that ID of *P. ultimum* in six soils was correlated positively (*r* = 0.94) with incidence of cotton damping-off, but others have reported contradictory observations. For example, Garber et al. (1979) observed that ID of *P. ultimum* in six soils was correlated positively (*r* = 0.94) with incidence of cotton damping-off, but others have reported contradictory observations. For example, Garber et al. (1979) observed that the inoculum density of *P. ultimum* in different field soils did not always correspond with the level of cotton damping-off in those soils. Likewise, Liddell et al. (1989) observed no correlation between
ID of *P. ultimum* or *P. irregulare* and disease severity on carrot in different field soils. In greenhouse tests, Kaiser and Hannan (1983) reported that less damping-off of chickpea was observed in some field soils that had a high ID of *P. ultimum* compared with others with a lower ID. Fukui et al. (1994a) made a similar observation when assessing the extent of embryo infection by pathogenic *Pythium* spp. of wheat seeds placed in various field soils.

The capacity of *Pythium* spp. to grow saprophytically is a key factor determining the relationship between ID and disease severity in the field. When a field soil is amended with organic matter, populations of *Pythium* spp. commonly increase, but the magnitude of the saprophytic increase in population size can vary dramatically among field soils. For example, population sizes attained by *P. ultimum* growing saprophytically on cotton leaves differed significantly among field soils, even when IDs of the fungus were very similar in the soils prior to addition of the organic amendment (Hancock, 1977; Lifshitz and Hancock, 1981, 1984; Martin and Hancock, 1986). Because the magnitude of population increase on organic matter (rather than the initial ID of the pathogen) correlated with subsequent disease incidence, saprophytic activity was considered to be a reliable indicator of seedling disease incidence on alfalfa or cotton (Lifshitz and Hancock, 1981). A similar relationship between saprophytic activity and disease expression was reported by Frank (1972), who used a baiting technique (sorghum seed soaked in pimaricin). A positive correlation ($r = 0.62$ at $P = 0.01$) was observed between recovery of seeds colonized by *Pythium* spp. and subsequent pod rot of peanut observed in the field. While this assay did not differentiate between species pathogenic on peanut and saprophytic species, such as *P. oligandrum*, the author concluded that the results reflect conditions that favor *Pythium* spp. in general, and hence are indicative of the activity of pathogenic species. Because radial growth of *P. aphanidermatum* in various field soils was correlated with the incidence of damping-off of tomato, Grunwald et al. (1997) proposed radial growth as a criterion useful in evaluating the conduciveness of soils.

The importance of saprophytic competition with indigenous microflora in inoculum-disease relationships of *Pythium* spp. was highlighted in a series of classic papers by Bouhot and colleagues. Various field soils containing natural inoculum of *Pythium* spp. were diluted with sterile soil, and the diluted soil was amended with organic material (oatmeal) and placed around the hypocotyl of cucumber seedlings (Bouhot, 1975a,b,c). Regression analysis of ID vs. disease incidence for the different soil dilutions was used to calculate an inoculum potential unit for the soil, which indicated the potential for a given amount of inoculum to cause disease. The inoculum potential units of soils differed: in some soils, a low ID would cause comparatively high levels of disease, whereas in other soils the converse was observed (Bouhot and Joannes, 1979). This approach was subsequently modified to provide a less disruptive and more realistic approach for quantification of inoculum potential (Bouhot, 1979). The unknown test soil, along with standardized soil samples with a high (sterile soil) and low inoculum potential, were amended with field soil naturally infested with the *Pythium* spp. The inoculum of these soils were tested as before with one exception; half the sample was tested immediately, while the other half was incubated for a specific period of time prior to testing. The ratio of the inoculum potential of the soils before and after incubation were compared relative to the standard soils to determine the relative conduciveness of each soil. The explanation presented to account for the different inoculum potential of soils before and after incubation were compared relative to the standard soils to determine the relative conduciveness of each soil. The explanation presented to account for the different inoculum potential of soils was saprophytic competition from other soil microflora (Bouhot and Joannes, 1979), in particular members of Mucorales that compete with *Pythium* spp. for organic substrates (Bouhot, 1981). Similarly, Ricci and Messcaen (1976) reported that soil fumigation reduced the ID of *Pythium* spp. that was required to reduce the emergence of bean by 50%, presumably by reducing populations...
of indigenous microorganisms that serve as natural antagonists of phytopathogenic *Pythium* spp.

**D. Management of Disease by Manipulating Organic Matter**

Fresh organic matter serves as a nutrient source for *Pythium* spp., which are adept primary colonists, as well as for indigenous microflora, including those microorganisms that antagonize phytopathogenic *Pythium* spp. Therefore, organic matter can enhance or suppress disease, depending on its relative influences on phytopathogenic *Pythium* spp. and their indigenous antagonists. For example, infection of wheat seed and roots by *Pythium* spp. (mostly *P. irregulare*) and the number of propagules of *Pythium* spp. in soil was higher in fields that had been under pasture the previous year, as opposed to those in wheat or lupine (Pankhurst et al., 1995). The build up in inoculum was attributed to a larger amount of organic matter in fields rotated to pasture, which supported saprophytic growth of the *Pythium* spp. Watson (1971) reported that, due to saprophytic increases in inoculum of *P. ultimum* on the crop debris, amendment of soil in commercial lettuce fields with lettuce crop debris initially enhanced the ID of *P. ultimum* and disease incidence. Subsequently, however, disease incidence decreased, presumably as the saprophytic activity of soil microorganisms that suppressed *P. ultimum* increased. In long-term experiments in Oregon with up to 40 years of consistent crop management, Pythium root rot of wheat was less severe when nitrogen was provided by incorporating manure or pea vine residue into the soil rather than by adding urea-ammonium nitrate (Smiley et al., 1996). In the same set of experiments, Pythium root rot was more severe when wheat was in rotation with fallow rather than pea, an effect attributed to the higher microbial activity and more diverse microbial species distribution in soils with the pea rotation. High organic matter content of soils, resulting from centuries of incorporation of large amounts of vegetable and animal manure, is also thought to be one factor contributing to suppression of *Pythium* spp. in Mexican chinampa agricultural systems (Lumsden et al., 1987). Organic amendments, including sewage sludge, sugar mill filter press cake, and cotton gin trash, consistently suppressed Pythium root rot of sugar cane in greenhouse trials (Dissanayake et al., 1995) and may provide an economically acceptable approach for management of the disease in production agriculture.

Some of the most compelling evidence for the importance of soil organic matter in Pythium diseases is provided from studies of diseases of horticultural crops in commercial planting media. Like field soil, planting mixes used in the horticultural industry can vary dramatically in conduciveness to seedling diseases caused by *Pythium* spp. Planting mixes amended with certain mature composts are suppressive against such diseases (Chen et al., 1988a,b; Hoitink and Fahy, 1986; Mandelbaum and Hadar, 1990; Schuler et al., 1989). Disease suppression is attributed to the activities of suppressive microorganisms present in the compost, based on the following evidence: (1) severity of disease generally correlates negatively with microbial activity (Chen et al., 1988a,b; Craft and Nelson, 1996); (2) heating suppressive compost to 60°C for 5 days destroys suppression (Chen et al., 1987), which is consistent with the hypothesis that a heat-sensitive component(s) of the compost microflora contributes significantly to disease suppression; (3) suppressiveness is reduced when nutrients are added to the planting mixture (Chen et al., 1988a,b; Mandelbaum and Hadar, 1990), which is consistent with the hypothesis that nutrient competition between the compost microflora and phytopathogenic *Pythium* spp. contributes to disease suppression. Disease suppression is attributed, at least in part, to a rapid depletion of nutrients that would otherwise be available to *Pythium* spp. by the microorganisms present in compost. As a result, compost decreases the time when nutrient
availability is sufficiently high to stimulate oospore and sporangial germination by *Pythium* spp. (Chen et al., 1988b; Mandelbaum and Hadar, 1990). It is likely, however, that specific components of the compost microflora contribute disproportionately to disease suppression, through the production of antifungal metabolites or by other mechanisms, which may explain exceptions to the general correlation between disease suppression and high microbial activity (Craft and Nelson, 1996).

Like compost, certain types of peat suppress *Pythium* damping-off diseases when incorporated into planting mixtures. In general, suppressiveness of peat is correlated to its level of decomposition (Boehm and Hoitink, 1992; Inbar et al., 1991). For example, *Pythium* root rot of poinsettia and populations of *P. ultimum* were suppressed by light peat, which is decomposed only slightly, but were not suppressed by dark peat, which is highly decomposed (Boehm and Hoitink, 1992). Organic matter decomposition level is correlated negatively with microbial activity (Boehm and Hoitink, 1992); the suppressive peat supports greater microbial activity than the nonsuppressive, highly decomposed peat. Although the diversity of microorganisms inhabiting the light and dark peats do not differ significantly, the composition of the microbial inhabitants differ between the planting mixes containing the two peat sources. *Pseudomonas* spp. and other taxa capable of inducing suppression of *Pythium* damping-off predominate in the suppressive mixes, whereas other genera of bacteria predominate in the conducive mix (Boehm et al., 1993). Effective bacterial biocontrol agents could be isolated from both suppressive and conducive mixes, but the majority of antagonists were associated with the less-decomposed, suppressive mixes. When bacterial agents were added to planting mixes, they were more effective in mixes containing the slightly decomposed, light peat rather than the highly decomposed, dark peat. If highly decomposed dark peat is amended with compost, however, it can become suppressive (Boehm et al., 1993). Therefore, both microbial activity and the composition of the microflora present in light peat contribute to suppression of *P. ultimum* (Boehm and Hoitink, 1992; Boehm et al., 1993).

### E. Suppressive Soils

A certain amount of suppressiveness exists in virtually all field soils, as indicated by comparisons of disease severity in natural and sterilized soils. In some soils, however, edaphic factors and components of the microflora may conspire to further suppress establishment of phytopathogenic *Pythium* spp. or disease caused by the pathogen. A review of the different soils reported to be suppressive to *Pythium* spp. may be found in Whipps and Lumsden (1991); an additional reference on suppressiveness of forest soils is Bouhot and Perrin (1980). Many suppressive soils have physical or chemical characteristics that suppress the pathogen directly or indirectly by altering a biological component(s) of the ecosystem to the detriment of the pathogen. For example, the poor saprophytic activity of *P. aphanidermatum* in field soils ranging from pH 5.1 to 6.1 (Lumsden et al., 1976) may be responsible for its suppression in traditional Mexican chinampa agroecosystems with soils in this pH range (Lumsden et al., 1987). In contrast, soils from Hawaii that are suppressive to *P. splendens* have neutral or slightly alkaline pHs (Kobayashi and Ko, 1985) and high calcium contents (Kao and Ko, 1986). Disease suppression in these soils has been attributed to a combination of high calcium levels and large microbial populations (Kao and Ko, 1986). Soils in the San Joaquin Valley of California that are suppressive to *P. ultimum* have high chloride concentrations that favor the Cl-tolerant, nonpathogenic species *P. oligandrum* over *P. ultimum* (Martin and Hancock, 1986).

### IV. BIOLOGICAL CONTROL OF SOILBORNE DISEASES CAUSED BY *Pythium* SPP.

The sensitivity of pathogenic *Pythium* spp. to competition and antagonism during its saprophytic phase of growth, even to the extent
witnessed in suppressive soils, is one characteristic of the pathogen that invites optimism among scientists attempting to manage disease through biological control. The perception of certain plant pathologists that seedling diseases caused by *Pythium* spp. are amenable to biological control has resulted in the focus of much biological control research on these diseases. The basis of this perception was summarized by Paulitz and Fernando (1995) as follows: “(1) in the case of damping-off diseases, the period of susceptibility of the host tissue is limited, so the biological control agent has to remain effective only from sowing until shortly after seedling emergence; (2) the infection court of the seed represents a small surface area and does not move or expand, encountering new inoculum; (3) a large population of the biocontrol agent can be applied easily to the seed to inundate and protect the infection court; (4) also, the dry seed coat does not normally contain a high population of resident microflora, making it easier to establish the biocontrol agent without competition and interference from preexisting microbes occupying that niche.”

Scientists that share this view of *Pythium* damping-off as an amenable target for biological control typically take the approach of applying large numbers of a biological control agent to seed or seedlings in an attempt to protect the plant during the time it is most susceptible to infection. Microorganisms applied to seeds must be active immediately after the seed is planted to successfully protect germinating seed from infection by the pathogen. Typically, biocontrol agents used successfully as seed treatments for suppression of damping-off are Gram-negative bacteria, which grow and produce metabolites on seed surfaces soon after seeds are planted, or other organisms applied in formulations or in concert with seed priming to enhance their activities on germinating seeds.

In contrast to the view summarized by Paulitz and Fernando (1995), other scientists perceive that *Pythium* damping-off diseases are difficult to suppress biologically because propagules of the pathogen germinate rapidly in response to seed or root exudates and quickly infect seeds or roots (Whipps and Lumsden, 1991). These scientists recognize that the activity of a biological control agent must coincide with a very short period of host susceptibility, and contrast this to the period of time needed for many biological control agents (such as bacteria and fungi inoculated as dormant spores) to become active. Clearly, the capacity of dormant propagules of *Pythium* spp. to infect susceptible plant tissues quickly is viewed as a key characteristic of the pathogen, even though scientists disagree about whether or not this characteristic presents an opportunity or an obstacle for biological control. Therefore, research on biocontrol of diseases caused by *Pythium* spp. is focused on the timing of interactions between biocontrol agents and target pathogens, a focus that is reflected in the following discussion.

**V. DESCRIPTION OF BIOCONTROL AGENTS**

Biocontrol agents that suppress plant diseases caused by *Pythium* spp. represent diverse species of bacteria and fungi (see Whipps and Lumsden, 1991 for a list of effective antagonists). Here, we describe some of the most promising biocontrol agents, and, where information is available, we discuss the influence of environmental parameters on the antagonist, pathogen, and disease severity.

**A. Fungal Biocontrol Agents**

1. **Saprophytic Pythium spp.**

   Like many other *Pythium* spp., *P. oligandrum* and *P. nunnii* are aggressive primary colonizers of organic matter, but they are not pathogenic on plants. Because of an overlapping ecological niche with phytopathogenic species of *Pythium*, association with pathogen suppressive soils, and antagonism in culture to patho-
genic species, these species should be ideally suited as biocontrol agents. In repeated field evaluations in Florida, certain isolates of *P. oligandrum* significantly reduced disease and were as efficacious as metalaxyl in controlling damping-off of tomato caused by *P. ultimum* and *P. aphanidermatum* when applied to seedlings prior to transplanting (Martin and Semer, 1992, and unpublished). These isolates are nonpathogenic on a number of crop plants (Martin and Hancock, 1987; F. N. Martin and C. R. Semer, unpublished) and, from investigations in culture, were found to colonize only external cortical cells of tomato roots (F. N. Martin, unpublished). This observation contrasts with those of Benhamou et al. (1997) (more extensive colonization of cortical tissue) and Al-Rawahi and Hancock (1997) (unable to colonize cortical tissue); these differences may be reflective of intraspecific variation among isolates of *P. oligandrum*.

A number of greenhouse trials also have evaluated the efficacy of *P. oligandrum* for control of damping-off. Veselý (1977, 1979) observed that application of oospores to sugar beet reduced damping-off incidence to a similar level as thiram treatment. Martin and Hancock (1987) observed a similar response when using isolates of *P. oligandrum* recovered from suppressive soils pelleted onto sugar beet seeds; preemergence damping-off due to *P. ultimum* was controlled to a level similar to that observed with fungicide treatments. Shortly after planting, the oospores of *P. oligandrum* germinated and mycelia colonized the seed surface and endosperm, preventing subsequent invasion by pathogenic *Pythium* spp. While the biocontrol agent colonized the emerging radicle and protected it from pathogen infection, this colonization was limited to association with the seed coat and suggested that this isolate of the fungus was not rhizosphere competent. Additional trials on cress (Al-Hamdani et al., 1983; McQuilken et al., 1990, 1992), sugar beet and carrot (Lutchmeah and Cooke, 1985), sugar beet (Walther and Gindrat, 1987), and cucumber (Thinggaard et al., 1988) have indicated that seed treatment with *P. oligandrum* can control other phytopathogenic *Pythium* spp., *Phoma betae*, and *Mycocentrospora acerina* in greenhouse evaluations.

Several field trials have also been conducted to evaluate efficacy of seed pelleting with *P. oligandrum* in protecting against damping-off. Using a powdered preparation of oospores (referred to as Polygandron, Table 1) pelleted onto the surface of sugar beet seeds, Veselý (1979, 1989) reported levels of protection from damping-off similar to that provided by thiram treatments. Treated seeds also had a higher germination rate and produced healthier and larger plants than untreated seeds. Zahradnicek et al. (1990) reported similar results, with better stand counts when 10 g of Polygandron/kg of seed was used compared with 50 g/kg. Oospore preparations of a *P. oligandrum* isolate recovered from the Pacific Northwest, U.S.A., protected chickpea seeds from seed rot and preemergence damping-off caused by phytopathogenic *Pythium* spp. in the field (Trapero-Casas et al., 1990).

Amendment of field soil with oospores of *P. oligandrum* can reduce the incidence of disease caused by indigenous populations of phytopathogenic *Pythium* spp. when cropped to susceptible hosts in the greenhouse. Amendment of the soil with as few as 7 to 37 propagules/g of soil of the biocontrol agent significantly reduced preemergence damping-off of sugar beet (59% for untreated check, 37 to 11% for oospore amendment, and 8% for fungicide check) (Martin and Hancock, 1987). These levels of control were enhanced when the soils also were amended with CaCl₂. Because treatment with the salt alone had no effect on disease, it appears that disease reductions may be due to a shift in the ecological balance of the spermosphere/rhizosphere that favors the biocontrol agent over the pathogen. This behavior parallels observations with soils in California, which have high chloride concentrations that place the chloride-tolerant *P. oligandrum* at a competitive advantage over the chloride-sensitive *P. ultimum* (Martin and Hancock, 1986).
McQuilken et al. (1992) also evaluated the biocontrol activity of *P. oligandrum* applied as a soil amendment, but they obtained mixed results.

In the initial description of soil suppressiveness induced by *P. numm*, Lifshitz et al. (1984) reported that amendment of raw field soil with 1 to 10% of soil containing high populations of *P. numm* reduced preemergence damping-off of cucumber in greenhouse trials. The ability of *P. numm* to control damping-off of cucumber in greenhouse and growth chamber trials was investigated further by Paulitz and Baker (1987a,b). The authors concluded that *P. numm* and *P. ultimum* occupied overlapping ecological niches and that disease was suppressed by
P. nunn in warm (26°C rather than 17 or 22°C) soils of neutral pH over a broad range of soil moistures. Infestation of soil with P. nunn and certain organic substrates suppressed disease more effectively than when P. nunn was added alone, leading Paulitz and Baker (1987a) to conclude that “rapid saprophytic increases of P. nunn, when the inoculum densities of P. ultimum are relatively low, are favorable for disease suppression.” When tested in greenhouse trials at 1000 propagules/g of soil, P. nunn suppressed root rot of sweet orange caused by Phytophthora parasitica; however, at this level P. nunn also reduced the growth of the host (Fang and Tsao, 1995). The ability of P. nunn to control disease in field trials has not been reported.

2. Trichoderma and Gliocladium spp.

Isolates of Trichoderma and Gliocladium spp. are effective antagonists of many soilborne diseases, including those caused by Pythium spp. We refer the reader to some excellent reviews describing the biological control activities and ecology of these fungi (Chet, 1987; Papavizas, 1985); this information will not be reiterated here. Certain strains of Trichoderma spp. and Gliocladium spp. are available commercially for biological control of plant diseases caused by Pythium spp. (Table 1). These fungi are secondary invaders of organic matter in soil, and the majority of naturally occurring isolates are not aggressive colonists of the rhizosphere. A rhizosphere-competent mutant of Trichoderma harzianum, strain 1295–22, which suppresses Pythium root rot and blight of turfgrass caused by P. graminicola (Lo et al., 1996), Pythium damping-off of a number of crop plants caused by P. ultimum (Harman et al., 1989) and other soilborne plant diseases (Harman et al., 1989) has been developed as a commercial product in the U.S. (Table 1).

Gliocladium virens GL-21 is an EPA-registered product (SoilGard, Table 1) for ornamental and food crop plants grown in greenhouses and nurseries (Lumsden et al., 1996). Applied in a granular formulation to potting mixes, GL-21 suppresses damping-off diseases of vegetable and ornamental seedlings caused by P. ultimum and R. solani. Other isolates of G. virens applied as seed treatments suppress damping-off of cotton (Howell and Stipanovic, 1983). Gliocladium virens is now classified as Trichoderma virens (Samuels, 1996). Due to the prevalence of the first name in the biological control literature, however, we refer to the fungus as G. virens throughout this review, while recognizing the similarities in the ecological characteristics of G. virens and Trichoderma spp.

Trichoderma spp. are favored by slightly acid soil conditions (Schupp and Frei, 1969; Warcup, 1951) and are more common in moist soil than in dry soil (reviewed in Lui and Baker, 1980; Widden and Abitol, 1980). Colonization of organic matter in soil is influenced by temperature. For example, T. viride is an active saprophyte at temperatures ranging from 5 to 15°C, whereas T. hammatum and T. koningii are more active saprophytes at higher soil temperatures (20 to 25°C) (Widden and Hsu, 1987). Population densities of T. harzianum are positively correlated with soil moisture and do not vary with soil pH between 6.2 and 7.9 (Eastburn and Butler, 1988a,b). In the central valley of California, population densities of T. harzianum in the field were found to be highest in the winter months with a peak in early summer, an observation that correlated with seasonal rainfall (Eastburn and Butler, 1988a,b). An experimental comparison of environmental influences on saprophytic activity revealed that T. harzianum was most active between –0.05 and –0.1 MPa and 15 to 21°C (Eastburn and Butler, 1991), conditions that also favor many phytopathogenic Pythium spp. Trichoderma harzianum suppresses Pythium damping-off at soil temperatures between 17°C and 34°C (Harman et al., 1981), but is more effective at 26°C than at, 19°C (Lifshitz et al., 1986).
B. Bacterial Biocontrol Agents

In five greenhouse studies in which bacteria were screened directly for suppression of Pythium damping-off, approximately 3 to 13% of bacteria isolated on a variety of culture media suppressed disease when applied as seed inoculants (Elad and Chet, 1987; Hagedorn et al., 1989; Loper, 1988; Sugimoto et al., 1990; Williams and Asher, 1996). Therefore, the capacity to suppress Pythium damping-off, at least to some degree, is not rare among isolates of bacteria that can be cultured from the rhizosphere or spermosphere. Fluorescent pseudomonads comprise a large proportion of the effective strains, ranging from 33 to 100%. Furthermore, approximately 30% of the strains of fluorescent pseudomonads that were isolated from roots of cotton or wheat were effective antagonists for suppression of Pythium damping-off in greenhouse experiments (Hagedorn et al., 1989; Weller and Cook, 1986). Far fewer were effective in the field trials (Weller and Cook, 1986). Therefore, although bacteria known to suppress Pythium damping-off are a heterogeneous group, representing many genera of Gram-negative and Gram-positive bacteria (Hagedorn et al., 1989), much research has focused on the fluorescent pseudomonads as effective antagonists of the disease.

1. Fluorescent Pseudomonads

Strains of Pseudomonas fluorescens (Hagedorn et al., 1989; Howell and Stipanovic, 1980), P. putida, P. aureofaciens, and P. aeruginosa (Buysens et al., 1996) are the most common species of fluorescent pseudomonads reported to suppress Pythium damping-off. One of these strains was commercialized in the late, 1980s as the product Dagger-G for suppression of seedling emergence diseases of cotton, but it was later removed from the market. This decision has been attributed to limitations in the shelf life of commercial formulations of Dagger-G, in which the bacterium lost viability (Powell and Jutsum, 1993). Although we are not aware of other fluorescent pseudomonads that have been commercialized for biocontrol of diseases caused by Pythium spp., there are many reports of successful biocontrol in the field (Callan et al., 1991, 1997; Hagedorn et al., 1993; Mathre et al., 1995; Miluse and Rothrock, 1997; Parke et al., 1991; Weller and Cook, 1986) or in commercial greenhouses (Rankin and Paulitz, 1994), and the bacteria have a number of characteristics that appear ideally suited for suppression of Pythium spp.

The population size of a fluorescent pseudomonad applied to seed surfaces typically increases in the spermosphere for 24 to 48 h after seeds are planted and then stabilizes or gradually declines with time (Fukui et al., 1994b,c,d; Loper, 1988; Osburn et al., 1989). The rapid increase in population size and metabolic activity of Pseudomonas spp. soon after seeds are planted corresponds well to the period in which seeds are infected by Pythium spp. Bacteria applied to seeds or seed pieces subsequently become established on roots of seedlings and mature plants, but their populations decline with distance from the seed. For example, P. fluorescens 2 to 79 established populations of 10^3 to 10^4 per cm on root segments located 6 to 7.5 cm below the seed; cultivable cells of the bacterium were not detected on deeper portions of roots of field-grown plants evaluated 35 to 68 days after planting (Weller, 1984). Similarly, populations of a fluorescent pseudomonad were not detected on potato roots deeper than 6 cm in a silty-clay-loam soil, or deeper than 12 cm in sandy loam soil (Bahme and Schrot, 1987). Irrigation water or rain that percolates through the soil enhances the downward distribution of bacterial cells to lower portions of the root surface (Bahme and Schrot, 1987; Liddell and Parke, 1989; Parke et al., 1986). Nevertheless, populations of fluorescent pseudomonads in the rhizosphere of field-grown plants are low, typically less than 10^3 cfu/cm on root segments located deeper than 5 cm from the soil surface, even in irrigated fields. Although inoculum of Pythium
spp. is commonly located within the top 10 to 20 cm of the soil, the rhizosphere population size of fluorescent pseudomonads may not be adequate to suppress the pathogen consistently on portions of the root located below 5 cm from the source of the inoculum (e.g., the seed coat).

The fluorescent pseudomonads are a diverse group of organisms, which are differentially influenced by environmental factors such as soil temperature, moisture, and pH. For example, the rhizosphere population size of *P. fluorescens* ANP15 on maize was greater than that of *P. aeruginosa* 7NSK2 at 18°C, whereas the converse was observed at 30°C (Seong et al., 1991). Populations established by three other strains of *P. fluorescens* were higher at temperatures below 20°C than at 24°C (Bowers and Parke, 1993b; Loper et al., 1985). Temperatures maximizing rhizosphere population size differ from those maximizing growth rate in culture (Loper et al., 1985; Seong et al., 1991), indicating that factors other than growth rate alone determine population size. Competition with soil microorganisms that predominate at temperatures greater than 20°C may contribute to the lower population size of fluorescent pseudomonads commonly observed in soils above 20°C. Soil temperatures favoring disease caused by the “cooler temperature” *Pythium* spp., such as *P. ultimum* and *P. irregulare*, and higher soil temperatures favoring disease caused by *P. aphanidermatum* and *P. myriotylum* appear to be conducive to rhizosphere colonization by fluorescent pseudomonads, although different strains may be the optimal antagonists of low- and high-temperature *Pythium* spp.

Rhizosphere populations are established by fluorescent pseudomonads in soils varying in soil matric potential from −0.001 to −0.4 MPa (Howie et al., 1987; Liddell and Parke, 1989; Loper et al., 1985), but matric potentials optimizing rhizosphere populations vary among strains of *Pseudomonas* spp. For example, certain strains colonize the surfaces of roots grown in wet soils (greater than −0.01 MPa) poorly (Howie et al., 1987; Loper et al., 1985; Parke et al., 1986), whereas the rhizosphere population size of another strain is maximized in wet soil (−0.001 MPa) (Liddell and Parke, 1989). Strains that can establish rhizosphere populations in wet soils are likely to be more reliable antagonists of diseases caused by *Pythium* spp., which can be severe even in water-saturated soils.

Soil pH varying from 5.4 to 8.0 has little influence on the rhizosphere population size established by *P. fluorescens* on bean roots (Loper and Henkels, 1997) or wheat roots (Howie et al., 1987). Therefore, fluorescent pseudomonads are likely to establish rhizosphere populations in soils with pHs spanning the range conducive to plant diseases caused by *Pythium* spp.

2. *Burkholderia cepacia*

*Burkholderia cepacia* is a common inhabitant of rhizosphere and bulk soil. Although certain strains of the species cause sour skin of onion, many strains are not phytopathogenic, and some of these can suppress plant disease. Strain AMMD of *B. cepacia* suppresses disease caused by *Pythium ultimum* and *P. sylvaticum* on pea in growth chamber experiments (Parke, 1990) and enhances stand and yield of pea in fields containing *Pythium* spp. and *Aphanomyces euteiches* (Bowers and Parke, 1993; King and Parke, 1993; Parke et al., 1991). Seed treatment with strain AMMD decreases the incidence of pea seed infection by *Pythium* spp. by 44 to 60% during first 48 hours after planting. Disease is suppressed at soil temperatures ranging from 16 to 28°C in controlled experiments done in growth chambers (Parke, 1990). Other strains of *B. cepacia* also suppress diseases caused by *Pythium* spp. in the greenhouse (Mao et al., 1997) or in the field (Milus and Rothrock, 1997). When applied to seed surfaces, *B. cepacia* can establish large spermosphere and rhizosphere populations. Populations of *B. cepacia* AMMD increased on pea seeds during the first 24 h after planting, for example, and represent-
ed an increasing proportion of bacterial populations that could be cultured from seed surfaces during this time period (Parke, 1990). Other antagonistic strains of *B. cepacia* represented 10 to 80% of the culturable bacterial populations in the rhizosphere of corn 2 weeks after seeds inoculated with these strains were planted (Hebbar et al., 1992b). These strains could establish rhizosphere populations on all portions of the root under varied field conditions (Hebbar et al., 1992a). One strain of *B. cepacia* has been registered as a seed treatment for suppression of diseases of vegetable crops caused by *Pythium* spp. (Table 1). At present, however, uncertainties regarding the taxonomic distinction between biocontrol strains and clinical isolates of *B. cepacia*, which cause serious infections of humans with cystic fibrosis, have raised cautions regarding the use of these biocontrol agents.

### 3. Enterobacter cloacae

*Enterobacter cloacae* was first identified as an antagonist of *Pythium* spp. by Hadar et al. (1983), who observed that pregerminated seeds were less sensitive than nonpregerminated seeds to rot by *Pythium* spp. Strains of *E. cloacae* isolated from germinating seed increase emergence of cucumbers, peas, and beets from soil containing *Pythium* spp. in growth chamber experiments (Hadar et al., 1983). Nevertheless, *E. cloacae* does not suppress *Pythium* damping-off on all plant hosts; it was ineffective on corn, snapbean, soybean, and lima bean. Disease suppression is inversely correlated to the amount of seed exudate (Nelson et al., 1986), an observation that has prompted investigations of the role of nutrient availability in biological control activity of *E. cloacae*. In growth chamber experiments done in naturally infested field soil, *E. cloacae* is as effective as metalaxyl in suppression of *Pythium* damping-off at 25°C but is not as effective as metalaxyl at 15°C, perhaps because seeds exude more carbon at 15°C than at 25°C (Nelson, 1988).

### 4. Gram-Positive Bacteria

In contrast to the Gram-negative bacteria described in the previous paragraphs, Gram-positive bacteria in the genus *Bacillus* form spores that are resistant to environmental stresses. This characteristic is viewed as a beneficial one for commercial development of biocontrol agents because formulations comprised of spores are relatively stable compared with those comprised of Gram-negative bacterial cells and exhibit shelf lives that are acceptable for agricultural use (Backman et al., 1997). Spores present in these formulations require time to germinate and grow, however, and the delay in their activities following application to agricultural soils is likely to limit their efficacy in suppressing preemergence damping-off caused by *Pythium* spp. The commercial biological control formulation Kodiak is comprised of spores of *B. subtilis* GB03. Twenty-four hours after Kodiak-treated cotton seeds are planted, a period of time during which seed is particularly vulnerable to infection by *Pythium* spp., 27 to 59% of the spores have not yet germinated on seed surfaces and probably are not actively suppressing the pathogen (Mahaffee and Backman, 1993). On soybean seed, 72% of spores of *B. cereus* UW85 germinate within the first 24 h after planting in the field (Halverson et al., 1993), indicating only that most of the spores had germinated by the end of this critical infection period. Although strains of *Bacillus* spp. have been known to enhance stands and seedling vigor of agricultural crops for decades (Broadbent et al., 1977), the positive effects on stand have rarely been attributed to suppression of *Pythium* spp. specifically. In some cases, however, *Bacillus* spp. have been shown to suppress diseases caused by *Pythium* spp. In field experiments, for example, *Bacillus* sp. L324-92 suppresses *Pythium* root rot of wheat caused primarily by *P. irregulare* and *P. ultimum*, as well as take-all disease and Rhizoctonia root rot (Kim et al., 1997a). *Bacillus cereus* UW85 suppresses damping-off of alfalfa and soybean caused by *Phytophthora* spp. (Handelsman
et al., 1990), and there are a few reports describing the promise of this bacterial antagonist for suppression of Pythium damping-off in the field (Jardine, 1992) or greenhouse (Smith et al., 1997).

Like *Pseudomonas* spp., the population size of *Bacillus* spp. applied to seed surfaces typically increases for a period after seeds are planted and then stabilizes (Liu and Sinclair, 1992) or gradually decreases with time (Holl and Chanway, 1992). In field trials, rhizosphere populations are commonly detectable many months after seed application (Halverson et al., 1993; Juhnke et al., 1987; Kim et al., 1997b; Liu and Sinclair, 1992). Rhizosphere population size typically decreases with distance from the seed; for example, *B. cereus* UW85 can persist in the rhizosphere of soybean throughout a growing season, but the bacterium remains primarily on portions of the root located only 2 to 3 cm from the seed (Halverson et al., 1993). Similar results were reported for *Bacillus* sp. L324–92R₁₂ in the rhizosphere of wheat (Kim et al., 1997b). In contrast, *B. mycoides* and *B. pumilus* have been detected in root segments located 20 to 30 cm below the inoculation site in the rhizosphere of wheat (Maplestone and Campbell, 1989) and *B. megaterium* was detected on root segments located 20 cm below the inoculation site in the rhizosphere of soybean (Liu and Sinclair, 1992). Soil texture could differentially influence dispersal of *Bacillus* spp. in the rhizosphere of soybean throughout a growing season, but the bacterium remains primarily on portions of the root located only 2 to 3 cm from the seed (Halverson et al., 1993). Similar results were reported for *Bacillus* sp. L324–92R₁₂ in the rhizosphere of wheat (Kim et al., 1997b). In contrast, *B. mycoides* and *B. pumilus* have been detected in root segments located 20 to 30 cm below the inoculation site in the rhizosphere of wheat (Maplestone and Campbell, 1989) and *B. megaterium* was detected on root segments located 20 cm below the inoculation site in the rhizosphere of soybean (Liu and Sinclair, 1992). Soil texture could differentially influence dispersal of *Bacillus* spp. in the rhizosphere, and movement of certain strains is enhanced in sandy soils over clay soils (Maplestone and Campbell, 1989). Therefore, dispersal of antagonistic strains of *Bacillus* spp. to depths of the rhizosphere soil where most propagules and most root infections by *Pythium* spp. occur is likely to depend on the planting depth and soil texture. Positive results from field trials testing strains for disease suppression indicate that the rhizosphere population size of *Bacillus* spp. appears adequate to suppress root rot caused by *Pythium* spp. in at least certain locations.

*Bacillus* spp. are facultative anaerobes and can persist under the low oxygen environments characterizing the wet soils where diseases caused by oomycetes are particularly severe (Handelsman et al., 1990). At least certain *Bacillus* spp. establish higher rhizosphere populations in moist soils of neutral pH than in drier soils at more acidic pHs (Reddy and Rahe, 1989). Like *Pseudomonas*, however, *Bacillus* is a diverse genus comprised of strains that are differentially influenced by environmental factors such as soil temperature. For example, the population size of *B. subtilis* B–2 in the onion rhizosphere was greater at soil temperatures of 22 to 25°C than at lower temperatures (17 to 19°C) (Reddy and Rahe, 1989). In contrast, *Bacillus* sp. L324–92R₁₂ persists on roots of winter wheat throughout a growing season, including months with frozen soil (Kim et al., 1997b). Therefore, it appears that strains of *Bacillus* spp. should persist under the full range of soil temperatures favoring disease caused by *Pythium* spp.

VI. MECHANISMS BY WHICH BIOCONTROL AGENTS SUPPRESS DISEASE

Effective biocontrol agents can disrupt various stages in the life cycle of a plant pathogen. Here, we present representative examples from the literature in which a biological control agent is known to interfere with certain processes fundamental to the ecology or pathogenesis of *Pythium* spp. We selected examples that illustrate the multiple stages in the life cycle of *Pythium* spp. that are susceptible to interference by biocontrol, while acknowledging that many of the potential effects of biocontrol agents may not be fully recognized. For example, antibiotics can inhibit the pathogen at many stages in its life cycle, but we discuss antibiosis in the context of suppressing germtube or hyphal growth by *Pythium* spp. in the spermosphere or rhizosphere because this stage has
been the focus of most studies evaluating antibiosis as a mechanism of biological control. Similarly, seed exudates can serve as stimulants of propagule germination and as substrates for growth of the pathogen, and biocontrol agents that utilize exudates can suppress disease by interfering with growth or development of the pathogen. While conceptually distinct, these processes are difficult to separate in studies evaluating interactions between biocontrol agents and target pathogens in situ. Indeed, catabolism of seed exudates by a biocontrol agent is likely to influence both growth and development of the target pathogen, albeit to different extents. Therefore, we encourage the reader to consider these examples as a starting point in conceptualizing the complexity of interactions that occur between phytopathogenic *Pythium* spp. and their antagonists in the soil, spermosphere, or rhizosphere.

A. Interference with Survival

A wide variety of soilborne fungi, including Plasmodiophoromycetes (Dylewski and Miller, 1983), Chytridomycetes (Karling 1942), Oomycetes, Deuteromycetes (Dreschler, 1938, 1943, 1952, 1961, 1963; Pemberton et al., 1990; Sneh et al., 1977), and *Fusarium merismoides* (Hoch and Abawi, 1979) can parasitize oospores of *Pythium* spp. in culture. Bacteria in the genus *Actinoplanes* also parasitize oospores of *Pythium* in culture (Khan et al., 1993; Sutherland and Lockwood 1984). Our current understanding of mycoparasitism has been developed almost exclusively from observations of interactions between *Pythium* spp. and its parasites that occur in culture. Parasitized oospores of *Pythium* spp. have been observed in field soil (Ayers and Lumsden, 1977; Dreschler, 1938; Hoch and Abawi, 1979; Pemberton et al., 1990; Sneh et al., 1977); indicating that parasitism contributes to reducing pathogen inoculum in nature. This was demonstrated experimentally by adding *H. catenoides* to pathogen-infested field soil and observing parasitized oospores of *Pythium* spp. (Ayers and Lumsden, 1977). Certain strains of *Actinoplanes* that parasitized oospores of *Pythium* spp. in culture were reported to suppress *Pythium* damping-off and root rot of table beets and beans in field experiments. However, this occurred when artificial inoculum of the pathogen and *Actinoplanes* were mixed together 10 days prior to planting (Khan et al., 1997); efficacy in naturally infested field soil has yet to be demonstrated. Nevertheless, we lack convincing evidence demonstrating that parasitism of oospores contributes significantly to the capacity of *Actinoplanes* spp. or other biocontrol agents to suppress *Pythium* damping-off or root rot under natural field conditions. Because parasitized propagules of *Pythium* spp. can be retrieved from field soil, however, it is likely that parasitism is a component of natural processes regulating the densities of viable propagules of the pathogen in the field.

B. Interference with Saprophytic Colonization

Saprophytic colonization of soil organic matter by phytopathogenic *Pythium* spp. is suppressed in the presence of certain strains of *P. oligandrum* and *P. nunn*. In soils with natural, enriched, or augmented populations of these fungal antagonists, populations of phytopathogenic *Pythium* spp. and the incidence of diseases they cause are low in comparison to soils lacking the antagonists. *Pythium nunn* was isolated from a field soil that became suppressive following periodic amendment in the lab with organic matter (Lifshitz et al., 1984a). Although *P. nunn* grows slowly relative to *P. ultimum*, it can displace *P. ultimum* from colonized organic matter in field soil incubated under laboratory conditions. In contrast, prior colonization of organic matter by *P. oligandrum* impedes subsequent increases in populations of *P. ultimum* (Martin and Hancock, 1986).
Both *P. oligandrum* (Al-Hamdani, 1982; Deacon and Henry, 1978; Foley and Deacon, 1986; Laing and Deacon, 1990; Lewis et al., 1989; Lutchmeah and Cooke, 1984; Ribeiro and Butler, 1995; Veselý, 1978; Whipps, 1987), and *P. nunn* (Lifshitz et al., 1984b) parasitize hyphae of a number of fungi in culture, including pathogenic *Pythium* spp. in the initial descriptions of the species, Drechsler (1943, 1946) observed hyphae of *P. oligandrum* coiling around the hyphae of other *Pythium* spp. when grown in dual culture. While *P. oligandrum* penetrates hyphae of many fungal hosts, conflicting results on its ability to penetrate hyphae of *Pythium* spp. have been reported. From evaluating fungal interactions in water agar, Lutchmeah and Cooke (1984) and Liang and Deacon (1991) did not observe hyphal penetration of *P. ultimum* or *P. graminicola* and *P. vexans*, respectively, while Lewis et al. (1989) and Berry et al. (1993) reported penetration of these same and two additional species. Because different isolates of *P. oligandrum* were used in these studies, it is possible that differences in penetration are due to intraspecific variation. One species that *P. oligandrum* had no effect on was *P. aphanidermatum*. In fact, this species was found to penetrate hyphae of *P. oligandrum* and eight other species (Berry et al., 1993; Jones and Deacon, 1995).

Another mechanism by which *P. oligandrum* may influence a number of fungal pathogens, including *Pythium* spp., is through hyphal interference, in which cellular processes, morphology, and finally the viability of fungal hyphae are disrupted without penetration by the antagonist. In dual culture, Lutchmeah and Cooke (1984) and Berry et al. (1993) observed that within 30 min or approximately 45 min, respectively, after *P. oligandrum* made contact, mycelia of *P. ultimum* lost opacity and become disorganized. Lewis et al. (1989) reported similar results for *P. ultimum* and two other species, but this process took between 1 to 8 h. In conflicting reports, Laing and Deacon (1991) observed that *P. oligandrum* had no effect on *P. graminicola* or *P. vexans*, while Berry et al. (1993) observed coagulation of cytoplasm in these two species within 50 to 59 min after initial contact between hyphae. It was suggested by Lutchmeah and Cooke (1984) that hyphal interference may be an early stage in the process of mycoparasitism.

The morphological changes in fungal mycelia characterizing hyphal interference can occur in response to antibiotics or enzymes produced by the perpetrating organism. *Pythium nunn* produces cellulase and beta 1,3-glucanase when grown in the presence of cell walls from *Pythium* spp. (Elad et al., 1985), and *P. oligandrum* produces several enzymes capable of degrading cell walls of pathogenic *Pythium* spp. (Lewis et al., 1989). Such enzymes may provide a mechanism by which the mycoparasites can penetrate hyphae of *Pythium* spp. or induce cellular alterations associated with hyphal interference. Alternatively, cell wall-degrading enzymes may promote nutrient transfer among fungi rather than facilitating internal colonization of a fungal host. The process of nutrient transfer from a phytopathogenic to a saprophytic species of *Pythium* can be thought of as a specialized form of nutrient competition (Baker, 1987).

The occurrence of parasitism or hyphal interference in culture media does not necessarily indicate that these processes occur in soil, and evidence for the latter has rarely been conclusive. For *P. oligandrum* and *P. nunn*, the results of ecological studies argue against mycoparasitism as a major determinant of its biocontrol activity. Individual pieces of organic matter retrieved from soils infested with *P. oligandrum* and *P. ultimum* typically contain one or the other species and rarely contain both species (Martin and Hancock, 1986). This spatial separation contrasts with the close association between a mycoparasite and its host fungus that characterizes mycoparasitism. Therefore, Martin and Hancock (1985, 1986) proposed that, in *Pythium*-suppressive soils in central California, *P. oligandrum* suppresses *P. ultimum* by utilizing nutrients in soil organic matter that would otherwise be available for saprophytic growth of the pathogen. Nutrient competition also appears to contribute to the suppression of phytopathogenic *Pythium* spp. by *P. nunn*. In organic
mater co-colonized by *P. ultimum* and *P. nun*, the pathogen exhibits the following classic signs of nutrient deprivation: (1) a greater proportion of germinating sporangia lyse, (2) greater numbers of secondary sporangia are produced, and (3) a greater proportion of secondary sporangia are aborted relative to *P. ultimum* on organic matter in the absence of *P. nun* (Baker, 1987; Lifshitz et al., 1984a). As a result, the inoculum density of *P. ultimum* in soil containing organic substrates is decreased by the presence of *P. nun* (Paulitz and Baker, 1987b). Another argument against mycoparasitism or hyphal interference as a primary mechanism of disease control is the observation that *P. oligandrum* can protect tomato transplants from damping-off caused by *P. aphanidermatum* (Martin and Semer, 1992; and unpublished), a species that is not susceptible to mycoparasitism or hyphal interference by *P. oligandrum* (in fact, *P. aphanidermatum* parasitizes hyphae of *P. oligandrum*; Berry et al., 1993; Jones and Deacon, 1995).

C. Interference with Zoospore Viability or Development

Zoospores of *P. aphanidermatum* are attracted chemotactically to cucumber roots, where the zoospores accumulate and encyst, cysts germinate, and germ tubes infect the plant tissue. Fluorescent pseudomonads (*P. corrugata* or *P. fluorescens*) applied to the roots can reduce the attraction, encystment, and germination of zoospores (Zhou and Paulitz, 1993). Zhou and Paulitz (1993) speculated that these effects may result from the utilization by *P. fluorescens* or *P. corrugata* of carbon and nitrogen compounds present in root exudates that are required for zoospore attraction and infection of roots.

Certain strains of *Pseudomonas* spp. produce rhamnolipid biosurfactants that are nonionic surfactants that destroy zoospores of *P. aphanidermatum* and other zoosporic fungi (Stanghellini and Miller, 1997). Nonionic surfactants disrupt the plasma membrane, resulting in lysis of fungal structures such as zoospores that lack a cell wall (Stanghellini and Tomlinson, 1987). Surfactants added to nutrient solutions reduce infection of cucumber by *P. aphanidermatum* and consequently enhance survival of cucumber grown hydroponically (Stanghellini et al., 1996). The intriguing possibility that biosurfactant production contributes to suppression of *P. aphanidermatum* by *Pseudomonas* spp. is a current subject of research by Stanghellini and colleagues (personal communication).

D. Interference with Sporangial or Oospore Germination

Because phytopathogenic *Pythium* spp. depend on seed exudates for propagule germination, they are vulnerable to suppression by other microorganisms that utilize stimulatory components of the exudates.

1. Long-Chain Fatty Acids

Sporangia produced by *P. ultimum* on plant material germinate in response to a specific component(s) of seed exudates rather than indiscriminately responding to any one of a large number of carbon and nitrogen compounds that are released from germinating seed (Nelson and Craft, 1989; Nelson and Hsu, 1994). Certain antagonistic strains of *E. cloacae* and *Pseudomonas* spp. can utilize long-chain fatty acids (LCFA) such as linoleic acid (van Dijk and Nelson, 1998), which constitute the principal germination stimulants present in cotton seed extract (Ruttledge and Nelson, 1997). The importance of LCFA catabolism in suppression of *Pythium* damping-off has been investigated primarily in strains of *E. cloacae* that can protect seed from colonization and infection by *P. ultimum* (Nelson, 1988; Nelson et al., 1986). A number of characteristics of *E. cloacae* have been evaluated as contributors to its biological control activity (Costa et al., 1994; Howell et al., 1988; Loper et al., 1993; Lorito et al., 1993a; Nelson et al., 1986). Recent evidence from Nelson and collaborators indicates that
the utilization of sporangial germination stimulants present in seed exudates by *E. cloacae* contributes significantly to its activity as a biological control agent of Pythium damping-off. The capacity of seeds or seed exudates to stimulate sporangial germination is reduced or eliminated if *E. cloacae* first grows on these substrates (Nelson, 1990). Therefore, Nelson and colleagues speculated that *E. cloacae* removes a stimulant(s) present in seed exudates by using it as a growth substrate(s). In support of this speculation, a mutant of *E. cloacae* with a single transposon insertion in fadAB is defective in both linoleic acid utilization and biological control activity. The mutant is restored for linoleic acid utilization and biocontrol activity if a plasmid containing wild-type fadAB is introduced into the mutant (van Dijk and Nelson, 1997). The differential biocontrol activities of the fadAB mutant and the parental strain cannot be attributed to differences in their population sizes on seed surfaces because *E. cloacae*’s capacity to suppress preemergence damping-off of cucumber is independent of its capacity to grow in the spermosphere (Roberts et al., 1996). A mutant of *E. cloacae* 501 that does not grow on seed surfaces suppresses preemergence damping off of cucumber as well as the parental strain. Therefore, it appears that the utilization of long-chain fatty acids specifically, rather than the more general capacity to grow on a range of compounds present in seed exudates, is required for biological control by *E. cloacae*. These results provide convincing evidence for the concept that a biocontrol agent can suppress disease by interfering with a signal from a plant host that triggers an essential step in the development of *P. ultimum*.

2. Ethanol and Acetaldehyde

Because ethanol and acetaldehyde, like LCFAs, are stimulants of sporangial germination, biological control agents that remove these compounds from seed exudates can suppress Pythium damping-off. Many strains of *P. fluorescens* and *P. putida* utilize ethanol (St-anier et al., 1966) and *E. cloacae* utilizes both ethanol and acetaldehyde as sole carbon sources (Gorecki et al., 1985). Strains of *P. fluorescens* (Harman et al., 1982), *P. putida* (Paulitz, 1991; Paulitz et al., 1992), and *E. cloacae* (Gorecki et al., 1985) also reduce stimulatory properties of seed exudates. Similarly, pea seeds treated with *T. harzianum* release lower levels of ethanol and acetaldehyde than do untreated seeds (Gorecki et al., 1985). While the capacities to utilize ethanol and acetaldehyde and to reduce the stimulatory properties of seed exudates are correlated in these studies, a causal effect has not been established.

E. Interference with Germtube or Mycelial Growth in the Spermosphere or Rhizosphere

1. Antibiosis

Of the many antifungal metabolites produced by bacteria or fungi that inhabit seed or root surfaces or soil, this review focuses on several that are known to contribute to suppression of soilborne diseases caused by *Pythium* spp. In addition to these, antibiotics produced by *Bacillus cereus* UW85 play a significant role in suppression of cottony leak of cucumber, a postharvest disease caused by *P. aphanidermatum* (Smith et al., 1993). *Bacillus cereus* UW85 produces zwittermycin A (He et al., 1994) and kanosamine (Mîlner et al., 1996), which contribute to suppression of damping-off and root rot of soybean caused by *Phytophthora megasperma* f. sp. medicaginis (Händelsman et al., 1990; Silo-Suh et al., 1994). Recently, a novel class of butyrolactones that suppress *Pythium* spp. have been identified in culture supernatants of *Pseudomonas aureofaciens* 63–28 (Gamard et al., 1997). Butyrolactone production appears to be one mechanism by which strain 63–28 suppresses infection of T-DNA-transformed pea roots (Benhamou et al., 1996), but its role in disease suppression in a soil environment has not yet been evaluated. Uncharacterized antifungal metabolites produced by other biocontrol agents, such as
Trichoderma spp. (Ghisalberti and Sivasithamparam, 1991; Lifshitz et al., 1986), *P. nunn* (Elad et al., 1985), *P. oligandrum* (Bradshaw-Smith et al., 1990; Whipps, 1987), Chaetomium globosum (Di Petro et al., 1992), and Bacillus subtilis (Berger et al., 1996), may also contribute to suppression of Pythium damping-off.

The role of antifungal metabolites in suppression of soilborne diseases caused by *Pythium* spp. has been established by one or more of several experimental approaches. A purified antifungal metabolite may be added to soil or seed, and the effect on disease examined. For example, treatment of cotton seed with pyoluteorin, a polyketide antibiotic produced by *P. fluorescens* strain Pf-5, mimics the disease suppression obtained by seed treatment with the producing strain (Howell and Stipanovic, 1980). These data provide evidence that the antifungal compound is active on the seed surface, but provide no evidence for the *in situ* production of the compound in the spermosphere in concentrations adequate for control of the pathogen. In a second approach, the biological control activity of one or more mutants, which are deficient in antibiotic production, are compared with that of a parental strain that produces the metabolite. This approach has demonstrated the involvement of several antifungal metabolites, including oomycin A (Howie and Suslow, 1991), pyoluteorin (Maurhofer et al., 1994b), and 2,4-diacetylphloroglucinol (Fenton et al., 1992) in suppression of Pythium damping-off by *P. fluorescens*. A similar approach has also been used to evaluate the contribution of gliovirin (Howell and Stipanovic, 1983) and gliotoxin (Wilhite et al., 1994) produced by *G. virens* in disease control. These antibiotics contribute to the biocontrol activities of producing strains, but typically they are not the sole determinant of biological control. For example, mutants of *G. virens* that are deficient in gliotoxin production exhibit approximately 40% of the biological control activities of the parental gliotoxin-producing strain; the residual biocontrol activity of gliotoxin-deficient mutants is attributed to nutrient competition between *G. virens* and *P. ultimum* (Lumsden et al., 1996). In some cases, the presence of certain antifungal metabolites in the spermosphere and rhizosphere, detected analytically or by specific antibodies, has been associated with the presence of a biocontrol agent that produces the metabolite. For example, inhibitory concentrations of gliotoxin and gliovirin were detected in soil and in a soilless potting mix containing *G. virens* (Lumsden et al., 1992a,b). Similarly, pyoluteorin and 2,4-diacetylphloroglucinol were detected in the rhizosphere of wheat inoculated with *P. fluorescens* CHAO (Maurhofer et al., 1995) that produces these metabolites.

Taken together, the evidence that antifungal compounds produced by some fungal and bacterial antagonists contribute to biocontrol of Pythium diseases is compelling. Therefore, much research is focused currently on identifying factors influencing the *in situ* production of antifungal compounds by biocontrol agents inhabiting seed or root surfaces.

2. Temporal Patterns of Antifungal Metabolite Production

Secondary metabolites, such as antifungal compounds inhibitory to *Pythium* spp., are not produced uniformly throughout the growth phase of producing organisms. Instead, antifungal metabolite production typically occurs during late exponential or early stationary phase within the growth cycle of Gram-negative bacteria (Chater and Bibb, 1997) or after the onset of sporulation of fungi (Bennett and Ciegler, 1983) and spore-forming bacteria (Champness and Chater, 1994). For an antifungal compound to be produced *in situ* in concentrations adequate to prevent infection by *Pythium* spp., the producing organism must have reached the stage in its life cycle where antifungal metabolite production occurs. Because *Pythium* spp. commonly infect seeds within hours after planting, quick onset of antifungal metabolite production by spermosphere inoculants is particularly critical for suppression of preemergence damping-off. Several groups evaluating...
biocontrol agents for suppression of *Pythium* spp. have appreciated the relationship between growth cycle and antifungal metabolite production. In a recent study evaluating biocontrol of *Pythium* damping-off with a strain of *B. subtilis* that produces an uncharacterized antifungal compound, for example, scientists estimated the number of vegetative cells and spores of the antagonist in the rhizosphere because “the ability to form spores and the number of sporulating cells in the rhizosphere, is likely to be a major factor affecting biocontrol activity” (Berger et al., 1996).

Scientists evaluating biocontrol agents that produce characterized antibiotics known to contribute significantly to biocontrol activity can assess temporal patterns of metabolite production directly. For example, gliotoxin concentrations in a peatmoss-vermiculite potting mix are at a maximum (approximately 0.65 mg gliotoxin per gram) within 48 h after the mix is amended with a sodium-alginate formulation of *G. virens* (Lumsden et al., 1992a,b). Gliotoxin concentrations subsequently declined and only low levels were detected in the potting mix after 14 days (Lumsden et al., 1992a,b). Cellular proteins associated with gliotoxin production were detected serologically 16 and 64 h after conidial germination, but these proteins were not detected in conidia (Ridout et al., 1993). In contrast to the typical case in which secondary metabolite production is associated with sporulation, gliotoxin appears to be produced early in the growth cycle of *G. virens* (Wilhite and Straney, 1996). Thus, both gliotoxin accumulation and disease suppression are transient in a planting mix amended with *G. virens*; both reach maximum levels within 4 days after inoculation and diminish to trace levels after 14 days. Temporal variations in gliotoxin levels also determine the mode of action of *G. virens*; although gliotoxin inhibits mycelial growth and sporangial germination of *P. ultimum* in culture (Roberts and Lumsden, 1990), the primary effect of *G. virens* on the pathogen in potting medium is to stunt and distort new hyphae arising from sporangia. Inhibition of sporangial germination was not observed in potting medium, presumably because sporangia of *P. ultimum* germinated before toxic levels of gliotoxin accumulated (Harris and Lumsden, 1997).

Some researchers have evaluated the expression of genes involved in antifungal metabolite biosynthesis by biocontrol agents in the soil or on seed surfaces as an alternative approach to the direct quantification of antifungal metabolites in these environments. Studies of in situ gene expression typically use a reporter gene (composed of a gene conferring an easily detected phenotype that lacks its endogenous promoter, such that it will be transcribed only if placed downstream from an exogenous promoter). The reporter gene is fused to a promoter of a biosynthetic gene. Consequently, the easily detected product of a reporter gene “reports” the transcriptional activity of the antifungal metabolite biosynthesis gene(s). Gene fusions to *lacZ* or *inaZ* reporters have enabled researchers to confirm that antibiotic biosynthesis genes are expressed by *Pseudomonas* spp. inhabiting the rhizosphere or spermosphere. The temporal patterns of antibiotic gene expression by *P. fluorescens* are especially important to suppression of preemergence damping-off by *Pythium* spp. For example, transcription of oomycin A biosynthesis genes by *P. fluorescens* HV37a on cotton seed was not detected until 10 to 12 h after the seeds were planted, which was slow relative to the infection of the seed coat by *P. ultimum* (many seeds were infected 6 h after planting) (Howie and Suslow, 1991). Kraus and Loper (1995) speculated that temporal differences in expression of the pyoluteorin biosynthetic genes on seeds could contribute to a differential role of pyoluteorin in suppression of *Pythium* damping-off on two host plants.

### 3. Factors Influencing Concentrations of Antifungal Metabolites In Situ

Antifungal metabolite production by biocontrol agents is affected profoundly by media composition and growth conditions in culture, and presumably by the chemical composition and physical environment that biocontrol...
agents encounter in soil. Temperature, pH, and the quantity and composition of available carbon substrates are particularly important factors influencing antifungal metabolite production by biocontrol agents. For example, the production of pyoluteorin, 2,4-di-acetylphloroglucinol, and oomycin A production by *Pseudomonas* spp. is influenced differentially by carbon sources found in seed or root exudates. Oomycin A production is enhanced by glucose and decreased by amino acids (Guterson, 1990). In contrast, pyoluteorin production by *P. fluorescens* Pf-5 is enhanced by glycerol and diminished by glucose (Kraus and Loper, 1995). Production of 2,4-di-acetylphloroglucinol appears to be influenced differently in different strains of *Pseudomonas* spp. Strain F113 produces optimized yields of the metabolite at 12°C in cultures with a high surface to volume ratio. Fructose, sucrose, and mannitol promote 2,4-diacetylphloroglucinol production by strain F113 (Shanahan et al., 1992), whereas glucose promotes production by strain Pf-5 (Nowak-Thompson et al., 1994). Given the various effects of environmental factors on antibiotic production by different strains, it is clear that the concentrations of metabolites produced by biocontrol agents *in situ* will vary with the environment and composition of seed or root exudates. Such variation is likely to influence the success of biological control. For example, soil pH, temperature, and texture influence the transcription of oomycin A biosynthesis genes by Hv37a in the cotton spermosphere and have parallel effects on suppression of Pythium damping-off (Howie and Suslow, 1987; Howie et al., 1988).

*Gliocladium virens* GL-21 also requires carbon substrates for antifungal metabolite production, but in Soilgard, an EPA-registered product for suppression of Pythium diseases, these substrates are provided in the formulation rather than exclusively by seed or root exudates, as was the case for the fluorescent pseudomonads discussed in the previous paragraph. Different nutrients in the formulation alter the capacity of GL-21 to suppress Pythium damping-off, presumably due to changes in gliotoxin production (Lewis and Papavizas, 1985). Organic matter content of potting mix and soil temperature (optima at 25 to 30°C) influence biocontrol activity and gliotoxin production by GL-21 in a similar manner (Lumsden et al., 1992a,b). These parallel effects highlight the importance of variations in antifungal metabolite production as important sources of variability in the efficacy of biological control.

In addition to their effects on metabolite production, environmental conditions influence the stability of antifungal compounds in soil and other natural substrates. For example, gliotoxin is very unstable above pH 7.0 (Brian and Hemming 1945; Weindling 1941; Weindling and Emerson, 1936), and pyoluteorin can be adsorbed and inactivated in agricultural soils (Howell and Stipanovic, 1980).

**F. Parasitism**

Theoretically, mycelia present on seed or root exudates prior to infection of a plant host, like those found in organic matter, are susceptible to parasitism. Parasitism is unlikely to be an important mechanism for suppression of preemergence damping-off because *Pythium* spp. infect seeds rapidly relative to the rate at which parasitism occurs. For a biocontrol agent to protect a germinating seed by parasitizing germ tubes of the plant pathogen, mycoparasitism would have to take place during the brief period (typically less than 12 h) between propagule germination and infection of the plant host. In contrast, mycoparasitism is not an immediate process; it takes time for cellular recognition to occur and parasitism to take place.

**G. Competition for Nutrients**

**1. Carbon and Nitrogen Compounds**

Bacteria that grow rapidly immediately after seeds are planted and become distributed over a large proportion of the seed surface can
reduce infection of seeds by *Pythium* spp., possibly because they utilize components of seed exudates that would otherwise be available for growth and development of *Pythium* spp. (Elad and Chet, 1987; Fukui et al., 1994d). For example, strains of *Pseudomonas* spp. that grow immediately after seeds are planted (with a lag phase of less than 4 h) are more effective in suppressing *Pythium* damping-off than strains that have a lag phase of 8 to 12 h on seed surfaces (Fukui et al., 1994d). Suppression of *Pythium* damping-off by *Burkholderia cepacia* or *Pseudomonas* spp. depends on the dose of the bacterium that is placed on the seed (Fukui et al., 1994d; Parke, 1990). Inoculation of seeds with high cell densities (10^7 cfu/seed) of *Pseudomonas* spp. results in a uniform distribution of bacteria on the entire seed surface, whereas inoculation of seeds with lower cell densities (10^4 cfu/seed) results in relatively few discrete microcolonies of bacterial cells localized on indented junctures between plant cells. Even when population sizes of 10^7 cfu/seed are ultimately achieved by *Pseudomonas* spp. inoculated at 10^4 cfu/seed, the bacteria form discrete microcolonies that cover only 40 to 50% of the seed surface (Fukui et al., 1994b). Because large portions of the seed are devoid of bacterial colonies, competition for carbon is likely to occur only on a small proportion of the seed surface. Indeed, the population size of one strain of *Pseudomonas* spp. on seed surfaces was not reduced in the presence of a second strain inoculated at 10^4 cfu/seed, but was reduced if the coinoculated strain was applied at 10^8 cfu/seed. The reduction in population size was attributed to competition for the limited supply of carbon available for microbial growth, because addition of a carbon source (salicylic acid) used by only one strain released that strain from inhibition by a second strain inoculated at a high cell density (Fukui et al., 1994c). These experiments provide evidence that carbon source is indeed limiting the population size attained by other microorganisms, including phytopathogenic *Pythium* spp., in the spermosphere.

### 2. Siderophore-Mediated Iron Competition

Siderophores are low-molecular-weight compounds produced by microorganisms under iron limiting conditions that chelate the ferric ion (Fe^{3+}) with a high specific activity and serve as vehicles for the transport of Fe^{3+} into a microbial cell (Guerinot, 1994). The pyoverdine siderophores (also called the fluorescent siderophores, pyoverdins, or pseudobactins) produced by *Pseudomonas* spp. inhibit mycelial growth of *P. ultimum* (Misaghi et al., 1982) on culture media in which iron availability is limited. In contrast, pyoverdines do not inhibit sporangial germination by the pathogen (Paulitz and Loper, 1991); effects of pyoverdines on oospore germination or zoospore release or encystment have not been reported.

The contribution of pyoverdine production to biocontrol activity of fluorescent pseudomonads has been assessed by the same approaches used to assess the role of antifungal metabolite production (reviewed in Loper and Buyers, 1991). Experiments comparing biocontrol activities of mutants deficient in pyoverdine production (Pvd−) to wild-type strains (Pvd+) have concluded that pyoverdine production contributes to suppression of *Pythium* damping-off of cotton (Loper, 1988) and *Pythium* root rot of wheat (Becker and Cook, 1988) but contributes little to suppression of preemergence damping-off of cucumber caused by *P. ultimum* (Kraus and Loper, 1992; Paulitz and Loper, 1991). Several possible explanations for the variable contribution of pyoverdines to the suppression of damping-off and root rot diseases caused by *Pythium* sp. have been proposed (Paulitz and Loper, 1991). For example, concentrations of pyoverdines that are required for suppression of *Pythium* spp. may not be produced on seed surfaces of certain hosts, such as cucumber, within the...
short time period after planting during which fungal infection occurs. In contrast, pyoverdines may be produced in adequate concentrations by *Pseudomonas* spp. inhabiting the rhizosphere or surfaces of seeds of certain plant hosts such as cotton, which produce more seed exudates and emerge more slowly than cucumber and thus are susceptible to infection by *Pythium* spp. for a longer period of time. A second reason for discrepancies in the literature is that many strains of *Pseudomonas* spp. produce siderophores, such as pyochelin (Cox et al., 1980) and salicylic acid (Meyer et al., 1992) in addition to a pyoverdine, and these siderophores can suppress *Pythium* damping-off (Buysens et al., 1996). For example, *Pseudomonas aeruginosa* 7NSK2, which suppresses damping-off caused by *P. splendens*, produces both pyoverdine and pyochelin. Mutants deficient in the production of only one siderophore suppress *Pythium* damping-off, but a double mutant that produces neither pyoverdine nor pyochelin does not suppress the disease (Buysens et al., 1996). Although siderophore production contributes to biological control, this contribution was not detected unless both siderophores produced by the strain were considered. Similarly, production of pyochelin or salicylic acid contributes to suppression of *Pythium* damping-off of wheat or maize by *P. fluorescens* CHA0 grown under low iron conditions (Schmidli-Sacherer et al., 1997). In contrast, antibiotic or HCN production is the major determinant of biocontrol activity of CHA0 against *Pythium* damping-off of the dicotyledenous hosts cress and cucumber. Therefore, the contribution of siderophore production to disease suppression varied with plant host, a relationship that was detected through comparison of biocontrol activities of near-isogenic derivatives of the biocontrol agent with specific mutations in regulatory or structural genes influencing metabolite production. Experiments comparing disease suppression by isogenic Pvd− and Pvd+ strains evaluated the contribution of pyoverdine production to biological control activity of a certain strain of *Pseudomonas* spp., but did not necessarily evaluate the importance of iron competition in biological control. Fluorescent pseudomonads commonly utilize ferric-pyoverdines produced by other strains (Raaaijmakers et al., 1994) and ferric-siderophores that are produced by various genera of rhizosphere bacteria and fungi (Jurkevitch et al., 1992). Pvd− and Pvd+ strains, which have the same capacity to utilize ferric-siderophores, are likely to place similar demands on the biologically available pool of iron in the rhizosphere. Given this possibility, the conceptual model proposing that Pvd+ strains deplete such microhabitats of available iron, whereas Pvd− strains do not impoverish such environments of iron, may require reassessment. There is a clear need for new approaches in which the effect of a siderophore-producing rhizosphere bacterium on the iron status of a target pathogen can be evaluated. A microbial iron sensor, comprised of a bacterial strain containing a fusion of an iron-regulated promoter to a reporter gene that can be monitored in natural habitats (Loper and Lindow, 1994), provides a method for assessing iron availability to microorganisms in the rhizosphere that should be useful in testing our current models of microbial iron competition. Other possible contributions of pyoverdines to disease suppression exist, however, including a proposed role in inducing systemic resistance in the plant.

**H. Induction of Host Response**

Certain strains of *Pseudomonas* spp. suppress stem rot initiated from infections by *P. aphanidermatum* on roots spatially separated from the bacterial antagonists (Zhou and Paulitz, 1994). Stem rot of cucumber was suppressed even when separated roots were inoculated simultaneously with *Pseudomonas* spp. and *P. aphanidermatum*. Therefore, Zhou and Paulitz (1994) speculated that the response induced by the bacterial antagonists influences later stages of disease (production and spread of secondary inoculum and movement of the...
pathogen in the root system) rather than initial stages of pathogenesis (zoospore encystment and penetration). Indeed, analysis of the ultrastructure of bacterized roots has demonstrated that certain strains of *P. fluorescens* can enhance the deposition of infection barriers in root cells and restrict growth of fungal pathogens to the epidermis and outer root cortex (Benhamou et al., 1996). The strains described by Zhou and Paulitz (1994) are members of a growing collection of rhizosphere bacteria that suppress disease through induced systemic resistance (ISR), which operates by induction of plant-resistance responses rather than through direct interactions with the target pathogen. It also has been suggested that one mechanism by which the biocontrol agent *P. oligandrum* induces reduced infection of tomato roots by *Fusarium oxysporum* f. sp. radicis-lycopersici is by inducing host defense reactions (Benhamou et al., 1997). Whether a similar mechanism is functioning in suppression of phytopathogenic *Pythium* spp. has yet to be determined. A recent report indicates that certain composts can suppress disease on parts of the plant that are not in direct contact with the compost, a phenomenon attributed to induction of resistance responses in the plant (Zhang et al., 1996). Like the more well-known systemic acquired resistance (SAR) induced by salicylic acid or a necrosis-inducing pathogen, ISR is manifested by reduced symptoms on a portion of a plant distal to placement of the inducing agent. Further similarities between ISR and SAR are obscure, however, and recent evidence suggests that salicylic acid accumulation in the plant, which is required for SAR, is not required for ISR (Pieterse et al., 1996).

Several characteristics of *Pseudomonas* spp. have a proposed role in ISR. *Pseudomonas fluorescens* CHA0 and a Pvd<sup>-</sup> mutant derived from this strain differ in their capacities to induce resistance of tobacco to tobacco necrosis virus, which implies that pyoverdine siderophores produced by certain strains can induce ISR (Maurohofer et al., 1994a). Salicylic acid production (rather than pyoverdine production) (De Meyer and Höfte, 1997; Leeman et al., 1996) and the O-side chain of the bacterial lipopolysaccharide (Leeman et al., 1995) appear to be involved in the ISR induced by other strains of *Pseudomonas* spp. Pyoverdines produced by different strains of *Pseudomonas* spp. differ in their capacities to induce resistance of radish to Fusarium wilt, a response that is maximized under conditions of low iron availability (Leeman et al., 1996). The proposed role of pyoverdines in the ISR provides an alternative to siderophore-mediated iron competition as sole function for this class of molecules in biological control of soilborne plant disease.

**VII. ENHANCEMENT OF BIOCONTROL**

Efforts to improve the efficacy or consistency of biocontrol of *Pythium* damping-off are focused on the identification of superior antagonists by screening naturally occurring microorganisms, genetic improvement of known antagonists, and enhanced deployment of existing biocontrol agents.

**A. Screening Programs to Identify Antagonists of *Pythium* spp.**

The biocontrol agents currently registered for management of diseases caused by *Pythium* spp. (Table 1) are naturally occurring isolates that have been obtained from agricultural systems. *Pythium oligandrum* is a component of the microflora indigenous to *Pythium*-suppressive soils, and its activities in concert with soil edaphic factors serve to suppress *Pythium* damping-off in central California. Most existing biocontrol agents have been obtained from soils without notable suppressive qualities, however, and such soils continue to be a rich source of bacterial and fungal antagonists. Indeed, recent reports of effective, previously undescribed antagonists (Kim et al., 1997a) indicate that the potential of naturally occurring
microflora for suppression of phytopathogenic *Pythium* spp. has not yet been explored fully. There is good reason to believe that well-designed and properly executed screening programs will be successful in identifying effective antagonists.

Of paramount importance in the goal of developing effective biological control of pathogenic *Pythium* spp. is the development of realistic screening programs for assessment of antagonists. While the question of host range specificity and intraspecific variation in virulence has not been addressed adequately for the genus as a whole, the data do suggest the importance of considering these factors when selecting isolates of the pathogen to be used in screening trials. Screening programs should consider the full diversity of *Pythium* spp. that cause disease in production agriculture, rather than focusing exclusively on a single species or using only a single isolate. Furthermore, potential antagonists should be evaluated for disease suppression under a range of environmental conditions favoring disease, so that selected biocontrol agents will be effective under the full spectrum of field conditions where disease constrains crop production. Because the source of pathogen inoculum can influence virulence and pathogen response to germination stimulants, inoculum used in screening programs should be similar to what the host encounters in nature. Depending on the species, such inoculum can be produced by amending moistened autoclaved soil with fresh organic substrates and a culture of the pathogen (Martin, 1992). Using this approach, inoculum of *P. ultimum* containing more than 10,000 propagules/g of soil has been routinely obtained (Martin, unpublished). In view of the common lack of continuity between greenhouse and field evaluations, it also is important to begin realistic field evaluations early on in the screening program. While it may be difficult to identify natural fields with high enough pathogen pressure to provide consistent disease expression, altering crop rotation practices to saprophytically increase pathogen inoculum can be helpful. Cover cropping with beans or peas and disking under chopped crop debris and disking under chopped crop debris prior to flowering significantly increased the inoculum density of pathogenic *Pythium* spp. and levels of disease expression in field trials. Greater increases were obtained when the crop debris was sprayed with homogenized tank-fermented cultures of the pathogens prior to disking (Martin and Semer, unpublished). It is clear from the literature that sources of variability in biological control should be considered as early as possible in screening programs, and efforts to test potential antagonists in field environments mimicking those occurring in commercial agriculture are essential in formulating realistic expectations of the efficacy of selected biocontrol agents.

**B. Improvement of Biocontrol Agents through Genetic Manipulation**

Efforts to enhance biocontrol agents have been directed primarily to (1) enhancing activity of biocontrol agents immediately after seeds are planted to improve suppression of preemergence damping-off, (2) enhancing rhizosphere competence of biocontrol agents to improve suppression of root rot, and (3) enhancing compatibility of biocontrol agents with other agricultural practices.

1. **Enhancement of In Situ Antifungal Metabolite Production by Pseudomonas spp.**

Expression of antifungal metabolite genes by *Pseudomonas* spp. in the spermosphere of at least some crop plants is slow relative to timing of seed infection by *Pythium* spp. (Howie and Suslow, 1991; Kraus and Loper, 1995). Even at the time following seed inoculation when gene expression by *P. fluorescens* is at a peak, *in situ* expression of oomycin A and pyoluteorin biosynthesis genes is low relative to levels expressed by bacteria grown under optimized conditions.
conditions in culture (Howie and Suslow, 1991; Kraus and Loper, 1995). Therefore, efforts to enhance in situ production of antibiotics by bacteria inhabiting the spermosphere and rhizosphere have been pursued actively with *P. fluorescens*.

Derivatives of *Pseudomonas* spp. that overproduce antifungal metabolites or produce them constitutively have been derived by manipulation of regulatory genes or by placing operons encoding structural genes for antibiotic biosynthesis under the control of an exogenous promoter. For example, a derivative of Hv37a in which oomycin A biosynthesis genes are transcribed from a *tac* promoter, which is very efficient in *Pseudomonas* spp., expresses the genes at elevated levels in the spermosphere of cotton and is superior to wild-type Hv37a in suppression of preemergence damping-off of cotton (Gutterson, 1990). This increased suppression has been attributed to an earlier expression of antibiotic biosynthesis genes in the spermosphere, which presumably results in adequate concentrations of oomycin A soon after the seed is planted and before seeds are infected by *P. ultimum*.

The production of antifungal metabolites by antagonistic strains of *Pseudomonas* spp. is under complex genetic control (see Thomasow and Weller, 1995 for a recent review) that responds to environmental signals, cell density (Pierson and Pierson, 1996), and physiological state of the producing strain (Sarniguet et al., 1995). The relative concentration of $\sigma^70$ (Schnider et al., 1995), the primary sigma factor of the bacterial cell, and $\sigma^5$ (Sarniguet et al., 1995), which controls the transcription of many genes expressed in response to starvation, osmotic stress, and during the transition to stationary phase (Hengge-Aronis, 1996; Kolter et al., 1993, Loewen and Hengge-Aronis, 1994), is one component of this complex regulatory system. An RpoS– mutant of Pf-5, which overproduces pyoluteorin and 2,4-diacyltelphloroglucinol, is superior to the wild type in suppression of postemergence damping-off of cucumber (Sarniguet et al., 1995). A derivative of CHA0 containing multiple copies of *rpoD*, which has a similar phenotype to an RpoS– mutant, is also more effective than the wild type in suppressing damping-off of cucumber (Maurhofer et al., 1992; Schnider et al., 1995). These data indicate that alterations in a single regulatory locus can enhance the production of antifungal metabolites and biocontrol activity of *P. fluorescens*.

Efforts to enhance biocontrol activity of *Pseudomonas* spp. by increasing the expression of antifungal metabolite biosynthesis genes have not been an unqualified success. High concentrations of antifungal metabolites can be phytotoxic, especially on more sensitive plant species. Therefore, the net effect of strains with enhanced production of pyoluteorin or 2,4-diacyltelphloroglucinol on the health of plants grown in soils infested with *Pythium* spp. is a function of the sensitivity of the plant to the antifungal compounds as well as to infection by *Pythium* spp. (Maurhofer et al., 1995).

### 2. Altered Metabolite Production of *Gliocladium virens*

Efforts to enhance the efficacy of *G. virens* by enhancing antifungal metabolite production have also been attempted. A mutant of *G. virens* that produces 25% more glioviren than the...
wild type was equivalent to the parent in suppression of Pythium damping-off of cotton in the greenhouse (Howell and Stipanovic, 1983). This mutant was impaired in mycoparasitism and growth rate, however, and an overproducing mutant that is not compromised ecologically may indeed be superior to the wild type in suppressing disease. Lumsden et al. (personal communication) have suggested that a strain of G. virens that does not convert gliotoxin to dimethylgliotoxin, a metabolite produced by the wild-type strain that has no antifungal activity, might be likely to accumulate greater concentrations of gliotoxin than does the wild type, and hence be more efficacious in pathogen control. Efforts for improvement of G. virens may also be directed at derivation of mutants deficient in production of viridin, an intermediate in the biosynthesis of the phytotoxin viridiol that is produced by virtually all strains of G. virens (Jones and Hancock, 1988). Treatment with low levels of sterol biosynthesis inhibitors reduced production of viridiol but not biomass production or biosynthesis of gliotoxin or glio-virin by G. virens grown in culture (Stipanovic and Howell, 1994).

C. Deployment Tactics to Enhance Biological Control

1. Fermentation

Commercialization of microbial inoculants for disease suppression requires the development of fermentation procedures tailored to optimize efficacy, survival of formulation processes and storage, and economic production of biological control agents. The physiological status or differentiation state of microbial antagonists harvested from fermentation can have profound effects on the survival and activities of these antagonists when they are applied to soil or seed surfaces. For example, Trichoderma spp. or G. virens grow to higher populations in soil when inoculum is comprised of chlamydomospores rather than conidia (Lewis and Papavizas, 1985). Although Gram-negative bacteria do not form spores,
recent evidence indicates that the stationary phase of the bacterial growth cycle approximates a differentiation state in which cells are more resistant to stresses, including exposure to desiccation (Potts, 1994), that may be encountered during formulation, in storage, and on plant surfaces. Indeed, stationary-phase cells of \textit{P. fluorescens} encapsulated on wheat seeds survive long-term storage better than cells in the exponential growth phase (Slininger et al., 1996).

The substrates on which microorganisms are grown have dramatic influences on metabolite production, and these influences can translate into altered efficacy of a biocontrol agent harvested from fermentation. For example, two isolates of \textit{G. virens} that produce glioviren suppress \textit{Pythium} damping-off only when inoculum of the biocontrol agents is grown on certain substrates (Howell, 1991). Howell (1991) speculated that the presence of gliovirin in a formulated product is needed to suppress infection of the seed by \textit{P. ultimum} until the biocontrol agent begins to produce glioviren \textit{in situ}. The importance of growing \textit{G. virens} on substrates that promote gliotoxin production while suppressing the production of viridin (and its phytotoxic derivative viridiol) was also recognized by Ridout and Lumsden (1993), who developed antisera to proteins associated with gliotoxin production to use in defining such substrates.

\section*{2. Formulation}

Formulation technology provides opportunities to enhance numerous characteristics of biological control agents, including shelf life, efficacy, growth and survival in the environment, and compatibility with agricultural practices and machinery. Several excellent reviews describe recent innovations in formulating microbial antagonists, thereby enhancing efficacy of biocontrol (Fravel et al., 1998; Lumsden et al., 1995), and these innovations are not reiterated here. Instead, we focus on factors limiting biological control of soilborne diseases caused by \textit{Pythium} spp. and specific ways in which formulation technology has enabled scientists to overcome those limitations.

As stated above, the short period of plant susceptibility to preemergence damping-off by \textit{Pythium} spp. has by necessity been a focus of programs developing biological controls of this disease. Successful suppression of preemergence damping-off requires that the agent responsible for biocontrol is delivered to the soil or seed surface prior to the infection period. Where the primary mechanism of disease suppression is direct inhibition of the pathogen by an antifungal metabolite(s), adequate concentrations of the metabolite must be present in the formulated product or produced by the biocontrol agent immediately after the seed is planted. The broad-spectrum antibiotic gliotoxin, which is responsible for approximately 60\% of the biocontrol activity of \textit{G. virens} GL-21, is produced after a granular formulation of the fungus, such as Soilgard (Table 1), is incorporated into the soil and remains active for only days thereafter (Lumsden et al., 1992a, 1996). The lack of gliotoxin in the formulation itself is viewed as an advantage, because it lessens risks to humans or animals that could inadvertently ingest the product (Lumsden et al., 1996). Nevertheless, \textit{G. virens} suppresses disease most reliably if applied to soilless mix several days before planting, presumably because gliotoxin is produced by \textit{G. virens} and accumulates to effective concentrations before infection by \textit{Pythium} spp. occurs (Lumsden and Locke, 1989). The presence of a food base in the formulation allows \textit{G. virens} to grow and produce gliotoxin soon after its introduction into soilless planting mix (Lumsden and Locke, 1989). The presence of specific carbon sources and microbial metabolites in formulations also influence survival of biocontrol agents and growth once they are introduced into the environment, and manipulation of such factors undoubtedly provides opportunities for enhancement of biocontrol activity.

\section*{3. Seed Priming}

The efficacy of certain biological control agents can be enhanced by incorporating them into seed priming processes, a variety of proce-
dures that enhance seed germination and vigor through the addition of moisture. Even in the absence of specific microbial inoculants, primed seeds, compared with seeds that are not primed prior to planting, emerge in greater frequencies from soils infested with *Pythium* spp. (Hadar et al., 1983). Seed priming reduces quantities of seed exudates and the numbers of surface wounds, both of which reduce seed infection by *Pythium* spp. (Osburn and Schroth, 1988, 1989). Bacteria such as *E. cloacae* that are normally associated with seed surfaces can multiply on seed during the hydration process, and the enhanced populations of antagonistic bacteria are also thought to reduce incidence of *Pythium* damping-off after seeds are planted in infested soils (Hadar et al., 1983; Taylor et al., 1985). Biopriming, in which specific biological control agents are incorporated into the seed priming process, can be very effective in suppressing disease. For example, *Trichoderma* spp. incorporated into a solid matrix priming process increased the rate of seedling emergence and suppressed pre- and postemergence damping-off caused by *Pythium* spp. (Harman and Taylor, 1988; Harman et al., 1989; Osburn and Schroth, 1988). In six field plantings of sh2 sweet corn, biopriming with *P. aureofaciens* AB254 was as effective as seed treatment with the fungicide metalaxyl in suppression of *Pythium* damping-off (Mathre et al., 1995). Biopriming has great promise for enhancing the efficacy, shelf life, and consistent performance of biological control agents (Callan et al., 1997).

4. Mixtures of Biocontrol Agents

For the last several decades, investigations into biological control of plant disease has focused on the use of single microbial inoculants to suppress disease, a focus reflected in this review. There is a growing view among plant pathologists, however, that the application of a single antagonist is not likely to be the best approach to provide sustainable disease management, especially under the full spectrum of environmental conditions conducive to disease. Biocontrol mixtures, comprised of compatible microbial antagonists that complement the activities of their coinoculants, offer a promising approach to enhance the consistent efficacy of biological control. For example, strains of *G. virens* fall into two groups: those that produce gliotoxin and those that produce gliovirin (Howell et al., 1993). Because the activity spectra of the two antibiotics differ, a mixture of gliotoxin- and gliovirin-producing strains of *G. virens* is expected to suppress a wider spectrum of plant diseases than would a single strain of the antagonist. Similarly, *P. nunn* was more effective in greenhouse trials than *T. harzianum* T–95 in suppressing *Pythium* damping-off of cucumber in a Colorado soil amended with organic matter (in the form of bean leaves), but the converse was observed in the soil that was not amended with organic matter. A mixture of the two biocontrol agents was as effective as the better individual inoculant in both amended and nonamended soils (Paulitz et al., 1990). Therefore, the mixture provided more consistent disease suppression in soils varying in organic matter content. The success of mixtures depends on complex interactions among microbial antagonists, the pathogen(s) and other components of the microflora, however, and mixtures do not always suppress diseases caused by *Pythium* spp. better than individual antagonists do. Certain antagonists are incompatible with one another and therefore compromise the biocontrol activity of a mixture. For example, *T. hamatum* suppresses rot of pea seed caused by *Pythium* spp., but its biocontrol activity is compromised by fluorescent pseudomonads in soils containing relatively low levels of iron (Hubbard et al., 1983). The antagonism of *T. hamatum* by fluorescent pseudomonads, attributed to pyoverdine-mediated iron competition (Hubbard et al., 1983), is likely to compromise the success of a mixture comprised of microbial antagonists in these taxa. Furthermore, even mixtures comprised of compatible microbial antagonists are not always more efficacious than their components...
applied individually. *Enterobacter cloacae* is compatible with both *G. virens* and *T. harzianum* in potting mix, but a mixture comprised of *E. cloacae* and the fungal biocontrol agents was not better than single antagonists in suppressing lettuce damping-off caused by *P. ultimum* (Lynch et al., 1991). More tests will be required to determine if these mixtures are more reliable than individual inoculants over a range of conditions, which would be beneficial even if mixtures are not superior to individual antagonists in every individual trial.

Biocontrol agents can also be coinoculated into soil or onto seeds with organisms such as mycorrhizal fungi or nitrogen-fixing organisms that promote plant health by enhancing nutrition and through other physiological effects. Although the literature includes contradictory reports about the biocontrol activities of mycorrhizal fungi (Linderman, 1994), certain isolates of vesicular-arbuscular mycorrhizal fungi suppress disease and root infection by *Pythium* spp. (Hwang et al., 1993; St. Arnaud et al., 1994), and disease suppression by these species is sometimes enhanced in the presence of other biocontrol agents (Calvet et al., 1993). The concept of the mycorhizosphere has been proposed to describe the rhizosphere of mycorrhizal plants, which differs from the rhizosphere of nonmycorrhizal plants in quantity and quality of root exudates and the presence of a hyphal network that physically expands the volume of soil accessible to plant roots (Linderman, 1994). Although little is known about microbial communities associated with the mycorhizosphere, investigators are testing the hypothesis that mycorrhizal fungi suppress disease by altering the size and composition of microbial populations associated with plant roots, resulting in a more suppressive community of microorganisms rather than through direct effects on plant pathogens. At this time, however, the literature contains contradictory reports as to the compatibility of mycorrhizal fungi with other taxa of biological control agents (Marscher and Crowley, 1997; Paulitz and Linderman, 1989 and references therein).

5. Identification of Plant Genotypes Amenable to Biocontrol

There have been many indications in the literature that cultivars of a given plant species can vary in their amenability to biological control. Rhizosphere populations of *P. oligandrum* are established differently on different cultivars of cantaloupe, cauliflower, and tomato (Al-Rawahi and Hancock, 1997). *Burkholderia cepacia* AMMD suppressed *Pythium* damping-off of four cultivars of pea, but the magnitude of disease suppression differed among the cultivars (King and Parke, 1993). A recent report demonstrates that tomato cultivars vary in amenability to biological control of *Pythium* damping-off by *B. cereus* UW85 (Smith et al., 1997). At this time, the plant genotypic characteristics that influence disease suppression by UW85 have not been identified, but one can envision that plant genotypes may differ in the composition or quantity of root exudates, which would affect both the population sizes and activities of biocontrol agents in the rhizosphere. The identification of tomato genotypes that vary in amenability to biological control lays the groundwork for future studies elucidating the plant host’s role in biological control, a question of great practical and conceptual significance.

6. Inoculation of Substrates that can Support Biological Control Activities of Microbial Antagonists

There is now good evidence that phenotypes critical to suppression of *Pythium* spp. are not expressed uniformly by microbial antagonists in all environments into which they are introduced (Kraus and Loper, 1995; Maurhofer et al., 1995). Although there has been great effort devoted to the testing of microbial antagonists inoculated into soil or onto seed planted in field soil, such inoculants have met with mixed success in their capacity to suppress disease, especially those caused by pathogens that infect
plants for extended periods throughout one or many growing seasons (Kloepper et al., 1989). Therefore, one approach for enhancing efficacy is to place effective antagonists in environments that will promote the timely expression of key phenotypes on plant surfaces.

The capacity of composts or peats to suppress disease requires two fundamental components: the presence of effective microbial antagonists and the energy to support the metabolic activities of these antagonists (Boehm et al., 1993, 1997). Disease suppression in planting mixtures containing compost or suppressive peats generally is attributed to the combined activities of the many components of microbial communities inhabiting these substrates, but, at least in certain cases, it can be enhanced by adding specific microbial antagonists. Similarly, the activities of effective biocontrol agents can sometimes be enhanced by placing them into substrates (such as composts) that support microbial activity. For example, bacterial antagonists were more effective in suppressing Pythium damping-off when introduced into planting mixtures containing suppressive peat rather than conducive peat (Boehm et al., 1993). During the last decade, there have been tremendous gains in our knowledge of the microbial ecology of disease-suppressive systems, such as composts, and in our understanding of the population biology and biocontrol activities of individual microbial antagonists. There are now enormous opportunities to couple our knowledge of these areas to enhance the consistent activities of both approaches.

7. Specific Applications

One of the factors impeding the commercial success of introduced biological control agents is their sensitivity to environmental factors that fluctuate in the field and vary from site to site. In contrast to agricultural fields, environmental conditions in greenhouses are more uniform and can be controlled to favor the establishment and activities of microbial antagonists. Some hydroponic and soilless planting media, such as rockwool, are essentially sterile when planting occurs, so microbial antagonists can become established without the pressure of competition from indigenous microorganisms that characterizes a soil environment. Furthermore, inoculum of biocontrol agents can be introduced into irrigation or fertilization systems at a frequency needed to optimize efficacy. Crops grown in hydroponics or in soilless culture generally have a high economic value, and can support costs associated with introduction of biocontrol agents. For these reasons, these systems appear to be ideally suited for biological control to succeed (Paulitz, 1997). Indeed, a number of microbial antagonists, including *P. fluorescens* (McCullagh et al., 1996; Moulin et al., 1994; Paulitz et al., 1992b; Rankin and Paulitz, 1994), *S. griseofulvis* (Mycostop; Postma et al., 1996), *P. oligandrum* (Thinggard et al., 1988), and *G. virens* (Soilgard; Lumsden et al., 1996), suppress diseases caused by *Pythium* spp. in these systems, many at levels considered to be acceptable economically.

CONCLUSIONS

In this review, we presented information on biological control of disease caused by *Pythium* spp. from the perspective of the ecology and life cycle of the pathogen, rather than from the perspective of the microbial antagonist. This presentation arose from our views that biological control agents can and must interfere with critical stages in the life cycle of the pathogen or in the development of disease if biocontrol is to be successful. Our intent was to draw parallels between knowledge gained from studies focused on the pathogen, those focused on the biological control agent, and those focused on the interactions between the pathogen and the biocontrol agent. Indeed, certain aspects of the life cycle of *Pythium* spp. have received a great deal of attention by investigators in search of
effective biological control agents. For example, saprophytic growth of *Pythium* spp. in a field soil commonly correlates directly with disease severity, and antagonists that compete with *Pythium* spp. for organic substrates in soil are associated with suppressive soils and, in some cases, can be used as inoculants to manage disease. For many biocontrol systems, however, extensive knowledge of how microbial antagonists and target pathogens interact in culture has not yet been translated into known effects on the life cycle of the pathogen in agricultural soils. For example, while we know a great deal about the genetics of antibiotic production by fluorescent pseudomonads and have compelling evidence for the role of these compounds in disease control, our knowledge of the influences of these compounds on specific aspects of the fungal life cycle are limited. The discussion herein focused on the influences of these compounds on mycelial growth because we could find little information on their influences on propagule germination or formation. Because information on biological control was organized in parallel with information on the ecology of the pathogen in this review, the unevenness of our knowledge of how biological control interferes with the life cycle of *Pythium* spp. was highlighted. Where the information was available, we drew parallels between the population sizes and distribution of phytopathogenic *Pythium* spp. and microbial antagonists as influenced by environment, but this information is available for only a subset of antagonists that have promise for suppression of disease caused by these pathogens.

Much of what is known about the mechanisms of suppression of plant diseases caused by *Pythium* spp. is focused on inhibition of mycelial growth of the pathogen. In this review, we highlighted a series of elegant studies elucidating processes of zoospore release, encystment and host recognition by *Pythium* spp. This information has elucidated several specific processes that could be targeted by biological control agents, and a few recent studies have focused on the capacities of antagonists to interfere with zoospore production or development. Similarly, recent evidence indicates that certain biocontrol agents can enhance resistance responses in the host. Future studies that collectively consider the whole life cycle of the pathogen as well as stages of disease development offer great hope for expanding biological control conceptually and designing novel strategies for biological management of disease.

Research evaluating disease suppression by indigenous or introduced microorganisms has contributed much to our current views of the biology of *Pythium* spp. For example, the disparity in relationships of inoculum to disease among different agricultural fields has been recognized for decades, but the identification of specific antagonists from disease suppressive soils, and an understanding of the ways in which such antagonists suppress the fungus highlights the importance of saprophytic competition in the life cycle of the pathogen. Similarly, many environmental factors that influence disease severity do so through indirect effects on components of the soil microflora that suppress *Pythium* spp. Recent information that certain catabolic mutants of *E. cloacae*, which are incapable of utilizing specific components of seed exudates, no longer suppress damping-off disease indicates that host recognition by the pathogen involves a level of specificity that was not previously appreciated. With the precedence of these examples and many others, reports in the literature have increasingly considered data on the ecology of the pathogen within the context of its microbial coinhabitants, a trend that enriched the understanding of the complex interactions that govern disease incidence and severity in agricultural systems.

**A. Contributions of Research on Pythium spp. to the Field of Biological Control**

Diseases caused by *Pythium* spp. have been the subject of a large proportion of studies eval-
Evaluating the biological control of plant disease. These studies have contributed to basic concepts of biological control that have broad applicability to other pathosystems. Here, we present a few principles that have been developed (at least in part) from information gained from studies of biological control of plant diseases caused by *Pythium* spp.

1. Biological Control Agents Can Be Remarkably Flexible in the Mechanisms by Which They Inhibit Target Plant Pathogens

A biological control agent can utilize distinct mechanisms of disease suppression on different plant hosts susceptible to the same disease. As described herein, antibiotic production by *P. fluorescens* contributes differentially to biocontrol of *Pythium* damping-off of different plant hosts, apparently due to differences in the quantities of antibiotic(s) produced in situ.

2. Variability Is Directly Related to the Capacity of Biological Organisms to Respond to Their Environments; It Is An Intrinsic Characteristic of Biological Systems, Including Biological Control

Multiple sources of variability in biological control have been elucidated in studies directed at suppression of plant diseases caused by *Pythium* spp. Studies described herein demonstrate intraspecific and interspecific variation in the ecology of the pathogen; variation in efficacy of biological control agents depending on their density on seed surfaces; variation in amenability of plant host to biological control, even at the cultivar level; and variation in expression of traits required for biocontrol activity in response to plant and microbial signals, environmental parameters, and physiological status of the antagonist. The literature on the ecology of *Pythium* spp. is impressive in its diversity and emphasizes the ecological heterogeneity of the genus. Despite the inter- and intraspecific heterogeneity of the genus, much biological control research is focused on *P. ultimum*, and interactions elucidated through a focus on that species may not translate directly to interactions with other species of the pathogen.

3. Biological Control Requires an Effective Antagonist(s) and an Environment That Supports Their Biocontrol Activities

Studies evaluating composts and soilless planting mixtures have demonstrated that antagonists are not equally effective in suppressing *Pythium* damping-off in all planting substrates; instead, their efficacy is enhanced when introduced into substrates that support their biocontrol activities. Using concepts and techniques from microbial ecology, scientists have identified characteristics of planting mixtures that appear to promote the population sizes and biocontrol activities of antagonists. These studies are particularly valuable to the field of biocontrol because they illustrate the power of ecological approaches and concepts for building our collective understanding of in situ activities of biocontrol agents.

4. Timing is Everything

Because *Pythium* spp. can infect seeds soon after they are planted, Pythium damping-off has become an important model system in which temporal aspects of biological control have been explored. Infection of seeds by *Pythium* spp., because of its discrete spatial and temporal nature, has been a subject of detailed studies of interactions between biocontrol agents and the target pathogen that have been difficult to achieve in other systems. The studies have demonstrated, for example, that subtle temporal changes in expression of genes
required for biocontrol activity can alter the efficacy of a biological control agent.

**B. Practical Significance**

Research into the biology and biological control of *Pythium* spp. has had important practical outcomes, altering the practices for management of diseases caused by the pathogen in numerous agricultural environments. For example, plant residue management is an important component of disease management practices and recommended planting dates are set with consideration of soil temperature and moisture, factors that influence the pathogen directly and indirectly through effects on the antagonistic microflora. Critics that consider commercially available microbial inoculants as the sole outcome of biological control research fail to recognize the full spectrum of practical information that can arise from these studies. Even when viewed by this criterion, biological control of *Pythium* has enjoyed success, including the products described herein (Table 1). To date, however, biological control agents applied as seed or soil inoculants have been successful only in suppression of seedling disease; such agents have not reliably suppressed root rot caused by *Pythium* spp., they have not consistently enhanced plant growth through reduction of nonlethal root lesions caused by the pathogen in agricultural soils. Biocontrol proponents are optimistic that these aspects of the disease will ultimately be suppressed once superior antagonistic strains and methods enhancing their establishment are developed. Constraints to management of infection of mature plants by *Pythium* spp. are nonetheless daunting due to differential distributions of biocontrol agents and phytopathogenic *Pythium* spp. in the soil and field variations in the establishment and activities of biological control agents, especially under the full spectrum of environmental conditions conducive to disease. Perhaps for these reasons, certain biocontrol programs focus on hydroponics (including rockwool) or container horticulture, where *Pythium* spp. pose a tremendous constraint to the industry and commercial levels of disease management can be attained. Studies evaluating suppression of diseases caused by *Pythium* spp. have been among the first to demonstrate the tremendous potential for biological control in these horticultural systems.

**ACKNOWLEDGMENTS**

The authors are indebted to Joe Hancock, Bob Lumsden, Dave Mitchell, Jennifer Parke, Tim Paulitz, and an anonymous reviewer for their review of this manuscript.

**REFERENCES**


Hengge-Aronis, R. 1996. Back to log phase: σ as a global regulator in the osmotic control of gene ex-


Copyright© 1999, CRC Press LLC — Files may be downloaded for personal use only. Reproduction of this material without the consent of the publisher is prohibited.


Marschner, P. and Crowley, D. E. 1996. Root colonization of mycorrhizal and nonmycorrhizal pepper...


Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to several hosts. *Phytopathology* 68: 1754–1759.


Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Phytophthora* and *Pythium* to several hosts. *Phytopathology* 68: 1754–1759.


Paulitz, T. C., Ahmad, J. S., and Baker, R. 1993. Integration of *Pythium nunn* and *Trichoderma har-


Stanghellini, M. E. and Tomlinson, J. A. 1987. Inhibitory and lytic effects of a nonionic surfactant on
various asexual stages in the life cycle of *Pythium* and *Phytophthora* species. *Phytopathology* **77**: 112–114.


学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，
提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。
图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：
图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具