ROLE OF *PYTHIUM* SPP. IN THE DEVELOPMENT OF CAVITY SPOT ON CARROT IN THE FRASER VALLEY OF BRITISH COLUMBIA

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Title of Thesis/Project/Extended Essay

The role of Pythium spp. in the development of cavity spot on carrots in the Fraser Valley of British Columbia.

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Abstract

Cavity spot of carrots is characterized by dark, elliptical, sunken lesions on the carrot root. Several species of fungi, including a total of 120 isolates of *Pythium* species, were isolated from cavity spot lesions on carrots grown in the Fraser Valley of British Columbia during 1990 to 1992. *In vitro* pathogenicity tests performed on seedlings, mature carrot roots, and carrots planted in soil showed that only isolates of *Pythium* spp. were capable of causing cavity spot symptoms. The 120 *Pythium* isolates were grouped on the basis of morphological similarities and identified using a combination of morphological and molecular techniques. The following species were found to be capable of causing cavity spot: *Pythium violae*, *P. sulcatum*, *P. sylvaticum*, *P. ultimum var. ultimum*, *P. irregulare*, *P. paroecandrum*, *P. acanthicum*, and *Pythium* group-G. The first four species have been reported to cause cavity spot in other carrot-growing areas of the world.

Greenhouse tests to determine the effects of soil moisture, temperature, and carrot age showed that high soil moisture (near field capacity) or flooding, and a temperature of 15 °C enhanced cavity spot development. Carrot age (1 to 3 months old), did not have any significant effect on cavity spot development.

An enzyme cup-plate assay using pectin as the substrate was developed to determine relative pectolytic enzyme production by selected *Pythium* isolates. The pathogenicity of these species on mature carrot roots *in vitro* was found to be correlated with pectolytic enzyme production. Pathogenicity was also found to be correlated with *Pythium* growth rate, with a higher

probability of slow-growing species being pathogenic than faster growing species.

The process of infection of root tissue was examined using histopathology. Lesion development was due to cell wall breakdown and cell death, and hyphae and oospores were found within the carrot tissue. Enzyme production was implicated in the development of cavity spot lesions.

Forty-one carrot cultivars were evaluated *in vitro* for their response (resistant or susceptible) to pathogenic isolates of *Pythium* (including *P. violae, P. sulcatum, P. irregulare,* and *P. acanthicum*). The most resistant carrot cultivars included Panther, E-0792, Six Pak, Caropride, Fannia, and Navajo; Paramount and Celloking were among the most susceptible.

Dedication

To the memory of my father, Joseph N. Benard,

who always made me feel that I could accomplish anything.

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Chapter I

An Introduction to *Pythium* species

The currently accepted concept of the genus *Pythium* was introduced by Pringsheim in 1858, based upon descriptions of *P. monospermum* and *P. entophytum*. Zopf (1890) transferred the latter species to *Lagenidium* in 1890, leaving the type species as *P. monospermum* Pringsh. The genus *Pythium* has been placed in the families Saprolegniaceae (Pringsheim, 1858; Cornu, 1872; Berlese and de Toni, 1888) and Peronosporaceae (de Bary, 1881; Fischer, 1892), but now belongs to the family Pythiaceae, order Peronosporales, and class Oomycetes (Schroter, 1897). Over 200 species have been described, although the most recent comprehensive keys recognize 87 species and 2 varieties (van der Plaats-Niterink, 1981), and 125 species and 4 varieties (Dick, 1990).

Host Range and Distribution

Most species of *Pythium* are phytopathogenic; however, some species in this genus exist as animal pathogens (Alfaro and Mendoza, 1990; Campbell, 1990; Cooper *et al.*, 1991; de Cock *et al.*, 1987); as fish pathogens (Scott and O'Brien, 1962); as insect parasites (Saunders *et al.*, 1988); as nematode parasites (Sadebeck, 1887); as mycoparasites (Drechsler, 1943; Laing and Deacon, 1991; Lifshitz *et al.*, 1984a); and as strict saprophytes (Martin, 1991d).

Plant Pathogens

Life cycle. Singh and Singh (1984) devised a general life cycle for plant pathogenic *Pythium* species within the soil: seed and/or root exudates activate the oospores, which germinate to form germ tubes, and may directly

attack the host plant tissues or form zoospores. Environmental conditions and species determine the form of germination (Ayers and Lumsden, 1975; Lumsden and Ayers, 1975). Germ tubes and zoospores follow the concentration gradient of root exudate, and upon contact with the host, zoospore aggregation around the feeder roots, followed by encystment (forming cystospores), is likely. Encystment on the root cap cells can occur within seconds of contact, with penetration and cell death occurring in as little as 15 minutes (Goldberg *et al.*, 1989). If host contact is not immediate, or if antagonistic microorganisms are abundant, the germ tube may lyse or form secondary oospores (Hendrix and Campbell, 1973).

Cystospores germinate and form appressoria. Infection pegs penetrate the host intercellularly, or via root hairs or epidermal cells. Pathogenic success may be dependent upon the production of cell wall degrading enzymes, such as pectinases (Winstead and McCombs, 1961; Dube and Prabakaran, 1989) and cellulases (Winstead and McCombs, 1961; Deacon, 1979; Dube and Prabakaran, 1989).

Mycelium enters the host and invades cortical tissues, which causes root cell necrosis and root hair plasmolysis and necrosis. Stele tissues may also be invaded. Ungerminated seeds and young seedlings are killed. More mature seedlings are stunted due to the destruction of feeder roots. Sporangia may be formed on necrotized tissue. Upon depletion of nutrients or in the presence of antagonistic microorganisms, thick-walled oospores are formed within host tissue. These oospores are released upon disintegration of the colonized host tissues, and may germinate in the presence of an uncolonized substrate and colonize it, forming more oospores. The oospores are capable of long term survival in the soil (Burr and Stanghellini, 1973).

In the absence of a suitable host, *Pythium* species survive predominantly via resistant resting structures (sporangia, oospores, and zoospores), which are more important than survival by saprophytic persistence (Hendrix and Campbell, 1973). Mycelium is probably not an important survival structure, and is probably transitory in the soil due to attack by antagonistic microorganisms (Hancock, 1981).

Diseases. *Pythium* species cause a wide variety of plant diseases, but they mostly attack succulent or juvenile tissues, such as seeds, seedlings, feeder roots, root tips, fruits, and stems. *Pythium* diseases occur on virtually all types of plants, ranging from ornamentals to trees and from vegetables to fruits. *Pythium* species can be found from the tropics to the temperate zones, in cultivated fields, in virgin soils, and in greenhouses and nurseries. They may cause disease on their own, or may be part of a complex involving other *Pythium* species or other pathogens.

Perennial plants have particular problems with *Pythium*-caused diseases, due to the subclinical nature of some of these diseases. One such example is decline, in which there is a slow to sudden deterioration of established plants, followed by reduction in growth and vigour of replants. These problems are the direct result of diseased root systems, in which the feeder roots are killed, leading to a decreased ability to obtain nutrients from the soil (Hendrix *et al.*, 1966). In the case of apple replant disease, a synergistic effect between *P. irregulare* and *Cylindrocarpon lucidum* is a suspected cause (Braun, 1991). *P. irregulare* has also been found to be associated with crown rot of apple trees in New York State (Jeffers *et al.*, 1982). Forest trees, such as slash pine, are also vulnerable to *Pythium*-caused diseases such as damping-off (Huang and Kuhlman, 1990). *Pythium* species are implicated in other

subclinical or cryptic diseases. For example, species such as *P. irregulare* cause preemergence and postemergence damping-off and root-forking in alfalfa (Hancock, 1983, 1985, 1991).

Sugarcane stubble decline is a complex disease in which *P. arrhenomanes* appears to play an important role (Hoy and Schneider, 1988b, Lee and Hoy, 1992). Hoy and Schneider (1988a,b) concluded that, due to its ability to cause extensive rotting of the primary root tips under conducive conditions and its widespread distribution, *P. arrhenomanes* should be considered an important pathogen. They concluded that it was part of a species complex, due to the lack of complete disease control with metalaxyl as compared with fumigation. However, in view of the fact that there appears to be a wide variation in sensitivity to metalaxyl (Cook and Haglund, 1982; Cook *et al.*, 1983; Cook and Zhang, 1985), this conclusion may require re-examination.

Pythium species also attack turfgrasses, causing root and crown rots, seed rots, damping-off, and foliar blights (Smiley, 1983). *P. aphanidermatum*, *P. myriotylum*, and *P. ultimum* cause Pythium blight, one of the major diseases of turfgrasses in the United States and Canada (Couch and Smith, 1991). The role of root-infecting species of *Pythium* is less clear, but there is evidence of disease involvement and pathogenicity. *P. aphanidermatum* (Kraft *et al.*, 1967) and *P. torulosum* are associated with root rots of some turfgrasses (Endo, 1961). *P. aristosporum* and *P. arrhenomanes* cause root disease under conditions of high humidity and temperature (Hodges and Coleman, 1985). Root rot of ryegrass is caused by *P. irregulare* and *P. violae* (Dewan and Sivasithamparam, 1988) and by *P. dissimile* and *P. splendens* (Vestberg, 1990). *P. vanterpoolii* and *P. graminicola* are pathogenic to

seedlings and to crowns of mature bent grass (Ichitana *et al.*, 1986). Nelson and Craft (1991) confirmed that isolates of *P. aphanidermatum, P. aristosporum, P. graminicola, P. torulosum,* and *P. vanterpoolii* were capable of causing crown and root rot on creeping bentgrass and perennial ryegrass.

There are many species of *Pythium* associated with wheat (van der Plaats-Niterink, 1981). Diseases on wheat include snow rot caused by *P. iwayamai* and *P. okanoganense* (Lipps and Bruehl, 1978) and root rot caused by *P. irregulare* (Chamswarng and Cook, 1985), *P. ultimum* (Cook and Haglund, 1982; Chamswarng and Cook, 1985), *P. volutum* and *P. sylvaticum* (Chamswarng and Cook, 1985), and *P. aristosporum* (Lipps and Bruehl, 1978; Chamswarng and Cook, 1985).

Soil-borne pathogenic species of *Pythium* do not necessarily require soil to cause infection. For example, *P. aphanidermatum* is one of the most common root pathogens of hydroponically grown vegetables (Bates and Stanghellini, 1984; Goldberg *et al.*, 1992; Stanghellini and Russell, 1971) and infection of feeder roots by *P. dissotocum* causes yield loss in hydroponically grown lettuce (Stanghellini and Kronland, 1986). Shore flies effectively vector *P. aphanidermatum,* inoculating water tanks in hydroponic systems (Goldberg *et al.*, 1992). *Pythium* species may also be transmitted by larvae of the fungus gnat, *Bradysia impatiens,* which ingest oospores and mycelium and then feed on cucumber roots (Gardiner *et al.*, 1990; Jarvis *et al.*, 1992).

Saprophytes

Pythium species are adapted for primary colonization of nutrient - containing virgin substrates. There are some strictly saprophytic *Pythium* species, and many of the plant pathogens are also facultative saprophytes

(Martin, 1991d). Most *Pythium* species manage to exist saprophytically largely because they are well adapted as primary colonizers and are tolerant to adverse conditions. For the most part, however, they are poor competitors, losing out to other organisms in highly populated substrates. Barton (1961) found that *P. mamillatum* was a poor saprophytic competitor, but could survive saprophytically in soil that had limited microbial competition. Hendrix and Campbell (1973) extrapolated these results to include many other *Pythium* species.

In the case of *P. ultimum*, however, the problem of direct competition is not a detriment to survival. Stanghellini and Hancock (1971a) found that *P. ultimum* was able to avoid competition and antagonism in the early stages of saprogenesis and pathogenesis: its persistent sporangia were able to respond to low exogenous nutrients with rapid germination and germ tube extension, and were able to produce secondary sporangia and retract and conserve protoplasm under starvation conditions. *P. ultimum* is thus considered to be in a strong competitive position as a saprophyte due to its capacity to respond quickly to nutrients from plant exudates or residues (Hancock, 1977). Nelson and Craft (1989) concluded that sporangia of *P. ultimum* germinated in response to molecules other than amino acids and sugars, contrary to the suggestions of previous researchers studying *P. ultimum* (Agnihotri and Vaartaja, 1967; Gindrat, 1976; Schlub and Schmitthenner, 1978), *P. irregulare* (Agnihotri and Vaartaja, 1970), and *P. aphanidermatum* (Kraft and Erwin, 1967; Chang-Ho, 1970). Regardless of the exact nature of the stimulatory plant exudates, the result is that dormant propagules germinate rapidly in response to appropriate stimuli, and infect

susceptible seeds and seedlings within hours of planting (Lifshitz *et al.*, 1986; Nelson, 1987; Stanghellini and Hancock, 1971a, b).

As well as being effective primary colonizers, *Pythium* species are adapted to conditions that are unfavourable for the growth of other organisms. The saprophytic activity of *Pythium* species is enhanced under conditions of poor soil gas exchange (high CO_2, low O_2) (Griffin, 1963; Gardner and Hendrix, 1973), and high soil moisture (Mircetich, 1971).

Mycoparasites

Mycoparasitism is described as one fungus existing in close association with, and receiving at least a portion of its nutrition from, another (negatively affected) fungus. There are two basic types of mycoparasitism: in biotrophic mycoparasitism, the parasite has a sustained relationship with the living host; in necrotrophic mycoparasitism, the parasite kills the host, sometimes before contact. Mycoparasitism is of particular interest due to its potential role in the biological control of plant pathogens, such as other *Pythium* species.

There are five known *Pythium* species that are mycoparasites. Three of these species, *P. oligandrum* (Laing and Deacon, 1991; Whipps *et al.*, 1988), *P. acanthicum* (Haskins, 1963), and *P. periplocum* (Foley and Deacon, 1986), produce ornamented oogonia; the other two, *P. nunn* (Laing and Deacon, 1991; Lifshitz *et al.*, 1984 a,c) and an unidentified species, *Pythium* SWO (Foley and Deacon, 1985; Laing and Deacon, 1991), have smooth-walled oogonia.

Lifshitz *et al.* (1984a) found that *P. nunn* was a necrotrophic mycoparasite with a limited host range and different modes of parasitism, depending upon the host species. For example, *P. nunn* coiled around the

hyphae of *P. ultimum* and *P. vexans* and lysed them. In contrast, *P. nunn* formed appressorium-like structures and then penetrated and parasitized the hyphae of *P. aphanidermatum*, *Rhizoctonia solani*, and two species of *Phytophthora*. For other fungal species tested by Lifshitz *et al.* (1984a), there was no mycoparasitism, and in a few cases (against two species of *Trichoderma*) *P. nunn* itself was destroyed.

Laing and Deacon (1991) found that *P. oligandrum* and *Pythium* SWO were more aggressive than *P. nunn*, both to individual fungal hosts and across different hosts. Hosts ranged from highly resistant (e.g. *Fusarium oxysporum*), to intermediate, to susceptible (e.g. *Botrytis cinerea* and *R. solani*). Laing and Deacon (1991) found that all the mycoparasites tested had an identical mode of parasitism: growth of susceptible host hyphae stopped soon after contact, followed by host lysis or coagulation/vacuolation of host contents; then (in about 50% of cases) penetration took place.

Identification of Pythium

Identification Using Morphological Techniques

Pythium species are identified morphologically based on presence or absence and characteristics of structures such as sporangia, oogonia, antheridia, zoospores, oospores, hyphal swellings and appressoria, as well as colony characteristics. These fungi have coenocytic, hyaline hyphae, although cross septa occur in old hyphae and at positions of spore delimitation. Hyphae are usually 5 - 7 (up to 10) µm wide, and protoplasmic streaming may be observed in young cultures. The mycelium is usually colourless (white), but may appear yellowish or greyish lilac. Colonies may exhibit distinct

growth patterns, such as radiate, chrysanthemum (acute triangles), rosette (obtuse lobes), or any combination. As well, colony growth rate at a given temperature and on a given medium is a useful characteristic for taxonomy (van der Plaats-Niterink, 1981).

Asexual reproduction in *Pythium* occurs via zoosporangia and zoospores. During sporangial germination, protoplasm flows from the sporangium through a discharge tube to an apical vesicle in which zoospores are formed. Under wet conditions, the vesicle wall disappears, and the zoospores swim away via two lateral cilia. They then encyst as spherical forms, and germinate via single germ tubes. Secondary spore formation may be observed as a repetition of the cycle of discharge, encystment, and germination by a single zoospore (Middleton, 1943).

The sporangia are filamentous or spherical, and are delimited from the rest of the mycelium by cross septa. In many *Pythium* species, the filamentous sporangia may be non-inflated and virtually indistinguishable from the vegetative hyphae, or they may be dendroid or inflated. The dense protoplasmic content may aid in differentiation of filamentous sporangia from vegetative hyphae. Spherical sporangia may be proliferating or non-proliferating. There are two methods of proliferation: the filament of a secondary sporangium grows up through the primary sporangium and its discharge tube to form a new sporangium outside and above the old one; or a new sporangium forms inside the old, empty sporangium.

Undifferentiated hyphal swellings, which are usually indistinguishable from young spherical sporangia, occur in many *Pythium* species, and can germinate to form a new thallus. They may be intercalary or terminal, and

occasionally catenulate (in chains). In a couple of species, thick-walled chlamydospores have also been observed.

Sexual reproduction occurs via oogonia and antheridia. The female oogonia are terminal or intercalary. Terminal oogonia tend to be more spherical, while intercalary oogonia tend to be more limoniform (lemon-shaped). The oogonial wall may be smooth or echinulate (spiny); the spines may be short or long, sharp or blunt, conical or curved; the spines may be papillate, digitate, or mammiform (Middleton, 1943).

The male antheridia have various shapes, including bell-shaped, lobed, clavate, trumpet-shaped, cylindrical, with or without waves or furrows, and borne terminally or intercalary. They may be stalked or sessile, and their origin may be monoclinous (from the same hypha), diclinous (from a distinct hypha), or hypogynous (the lower part of the oogonial stalk forms the antheridium). Contact with the oogonium may occur through apical contact or broad lengthwise contact, and penetration occurs via a small fertilization tube. The result of fertilization is the oospore, which is usually single and smooth-walled. Oospores may have thick or thin walls, and may be plerotic or aplerotic.

Whether an oospore is plerotic or aplerotic may be a source of confusion during identification, as there are differences of opinion in the literature as to the degree to which an oospore need fill an oogonium in order to be considered plerotic. The concept of an aplerotic index was recently introduced by Dick (1990), whereby an oospore is considered plerotic if it fills more than 60 - 65% of the oogonial volume. This seemingly arbitrary designation has led to confusion across taxonomic keys, and even within the key by Dick (1990). For example, oospores of *P. aristosporum*, *P. volutum*, and

P. myriotylum are considered aplerotic by van der Plaats-Niterink (1981), have an aplerotic index of <65%, but are listed as plerotic (aplerotic index >60%) by Dick (1990). Dick's introduction of the aplerotic index makes the decision of whether an oospore is plerotic or aplerotic a difficult one, and has created more taxonomic problems than it has solved. The concept should either be abandoned or reconsidered using some other criteria.

Most *Pythium* species are homothallic, although a few species are heterothallic (e.g. *P. sylvaticum*). The appearance of the contact zone between compatible strains of heterothallic species is a distinctive trait for species identification. There are a few species for which failure to develop sexual structures is a basis for identification (e.g. *Pythium* 'group G'); however, absence of a characteristic is an ambiguous basis for identification.

Other structures of taxonomic significance are appressoria, which are produced by many species of *Pythium*. They occur in various sizes, may be subglobose, or sickle-, sausage-, or club-shaped, and may occur singly, in clusters, or in chains (van der Plaats-Niterink, 1981).

A great many difficulties are encountered in using morphology for *Pythium* identification, both within and between existing keys. Dick's (1990) key to the genus *Pythium* emphasizes structural measurements, but Middleton (1943), after measuring 200 oogonia of each species included in his key, concluded that although each species had a certain range and mean size, the ranges and means were so similar, overlapping and often identical, that no taxonomic value could be assigned to them. Van der Plaats-Niterink (1981) includes such measurements in her key. As well, there are obvious technical difficulties with trying to determine measurements of

zoosporangial volumes, and Dick (1990), whose key often hinges on such values, fails to adequately explain the methods for determining them.

Identification Using Molecular Biology Techniques

Molecular biology techniques may help to redefine relationships among the various *Pythium* species, and to simplify potentially tedious and time-consuming morphological identification. DNA sequences selected from a genus-conserved but species-variable region of the mitochondrial or nuclear genome may facilitate the selection of DNA probes useful for identification of unknown *Pythium* species. Martin (1991b) constructed probes for two species of *Pythium*: for *P. oligandrum*, a small single-copy unique region of the mitochondrial DNA showed the greatest variability (Martin, 1991a), and clones sequenced from this region, along with some of the inverted repeat region, hybridized strongly to *P. oligandrum* and *P. acanthicum*. Clones sequenced from only the small single copy unique region were specific for only certain isolates of *P. oligandrum*. For *P. sylvaticum*, a reasonably isolate-specific probe was constructed from an insertion-deletion event in the inverted repeat region. Martin (1991c) found that a few isolates of *Pythium* contain circular mitochondrial plasmids, which may also be used to construct isolate-specific probes.

Chen (1992) and Chen *et al.* (1992a) examined restriction fragment length polymorphisms (RFLPs) in nuclear and mitochondrial ribosomal DNA (rDNA) amplified by the polymerase chain reaction (PCR). Differences between species, but not within species, were observed in the rDNA internal transcribed spacer (ITS) regions. Digestion with restriction enzymes showed species-specific banding patterns. The authors concluded that species-specific

variations in the ITS region of rDNA could be used to develop specific probes to identify *Pythium* species, at least in the three heterothallic species (Chen, 1992) and five homothallic species (Chen *et al.*, 1992a) that were examined. Lévesque *et al.* (1993) used PCR to amplify the ITS region of nuclear rDNA with universal primers. Dot blot hybridization using probes made from the various ITS fragments showed that this technique may be useful for identification of isolates of *Pythium* species.

Identification Using Cellular Proteins and Isozymes

Clare (1963) and Clare *et al.* (1968) reported that *Pythium* species could be differentiated on the basis of protein banding patterns. However, Glynn and Reid (1969) rejected this claim as they observed nearly identical intergeneric protein banding patterns between species of *Verticillium* and *Fusarium*. Separation of proteins via SDS-polyacrylamide gel electrophoresis and isozymes via starch gel analysis was found to have limited taxonomic usefulness as many *Pythium* species (particularly morphologically similar ones) could not be reliably distinguished by comparing soluble proteins and isozymes due to considerable intraspecific variation (Chen *et al.*, 1991; Chen *et al.*, 1992b).

The usefulness of isoelectric focusing techniques for differentiating proteins from morphologically closely related *Pythium* species was investigated by Adaskaveg *et al.* (1988). They found distinguishing protein bands for each of the six species they studied and little variation was found within species, even between isolates from widely disparate collections. Varieties of *P. ultimum* could not be distinguished, however, and it was

suggested that the validity of the varieties within this species may require reconsideration (Adaskaveg *et al.*, 1988).

Despite its shortcomings, morphological identification of *Pythium* species still remains the major taxonomic tool. However, molecular techniques will likely soon be available for the routine identification of many *Pythium* species. Although protein and isozyme analysis may provide some useful information, problems such as the strict requirements for method standardization (Adaskaveg *et al.*, 1988) make it doubtful that either will become important in *Pythium* taxonomy.

Management and Control of Phytopathogenic Pythium spp.

Chemical Control

Resting structures, such as oospores, may persist in the soil for many years, making elimination of *Pythium* virtually impossible. Acceptable levels of disease control may be achieved through control of pathogenic *Pythium* populations using various chemical fungicides. Utkhede and Smith (1991) tested several fungicides against *P. ultimum*, which is associated with crown rot of apple trees (Jeffers *et al.*, 1982), and against *P. sylvaticum*. Their results showed that there were major differences in the effectiveness of the various fungicides against these two species. This finding suggests that there may be differences in fungicidal efficacy among *Pythium* species, potentially making *Pythium*-caused diseases difficult to effectively control with these fungicides.

The introduction and use of metalaxyl, a fungicide selective against the Peronosporales, has caused selection for resistant strains in species such as *P. aphanidermatum* (Sanders, 1984) and *Phytophthora infestans* (Davidse *et al.*,

1981). Another problem with the use of metalaxyl has been varying results in its efficacy against *Pythium* species. Cook and Haglund (1982) found that yield response in wheat to application of metalaxyl varied from no response to up to 25% greater yield. The difference in responses to metalaxyl may be attributed to what appears to be a high variability in susceptibility of species and strains of *Pythium* to metalaxyl. Cook and Zhang (1985) found that species with relatively low sensitivity to metalaxyl comprise 25-75% of the total *Pythium* population in the U.S. Pacific Northwest.

Even when a chemical has proven to be effective against *Pythium* diseases, it may be unregistered for use on a specific crop or on a given growth medium. For example, metalaxyl appears to be effective in controlling *Pythium* species in hydroponic systems (Bates and Stanghellini, 1984), but this type of use is unregistered. Furthermore, metalaxyl is not registered for use in Canada on fruit-bearing apple trees, although it appears to be effective against the *Pythium* and *Phytophthora* species that incite crown rot of apple trees (Utkhede and Smith, 1991).

The use of fumigants, although recommended in the past (Wilhelm, 1965), has become less common due to the expense and the general trend towards less environment-damaging chemicals. The advances in fungicide development in the 1970's and early 1980's has reduced the need for soil fumigants to control *Pythium* species. In view of the fact that most *Pythium* species are excellent primary colonizers, the resultant biological vacuum created by the use of broad spectrum fumigants would likely lead to "the boomerang effect" (Kreutzer, 1978). Rapid recolonization by any surviving inoculum could lead to a worse pathogen problem than the original, due to the lack of microbial competition and antagonism.

The use of fungicide combinations may enhance levels of disease control while reducing the development of resistance, but caution must be exercised to ensure that the fungicide combination is an improvement over the components used individually. Couch and Smith (1991) tested several combinations of fungicides to determine their interactive effects against the species that cause Pythium blight on turfgrasses. They found that the commonly used mixture of chloroneb and mancozeb was antagonistic for control of *P. aphanidermatum*. This finding of an unexpected and negative interaction between two fungicides exemplifies why more studies are needed to determine the synergistic, antagonistic, or additive effects of various fungicide combinations in disease control.

Biological Control

The possible mechanisms through which biological control of *Pythium*-caused diseases may be achieved include mycoparasitism, competition, predation, antibiosis and enzymatic degradation.

Mycoparasitism. Some mycoparasites have been studied for their potential in controlling soilborne plant pathogenic fungi. Two of the mycoparasitic *Pythium* spp. with ornamented oogonia appear to have some pathogenicity to plants: *P. acanthicum* may cause blossom-end-rot and fruit-rot of watermelon (Drechsler, 1939) and is highly pathogenic to tomato seedlings (Robertson, 1973); *P. periplocum* may cause cotton wilt (van der Plaats-Niterink, 1981) and blossom-end-rot of watermelon (Drechsler, 1939). This pathogenicity may limit their use as biocontrol agents, at least for certain plants. *P.nunn* and *P.oligandrum*, however, appear to be relatively non-phytopathogenic (van der Plaats-Niterink, 1981; Lifshitz *et al.*, 1984c; Martin

and Hancock, 1987), and therefore, have more potential to be biocontrol agents of plant pathogens.

Paulitz and Baker (1988) demonstrated that *P. nunn* was capable of reducing the density of *P. ultimum* sporangia in soil, and was also able to displace *P. ultimum* from bean fragments via secondary colonization. Paulitz and Baker (1987a, b) showed that *P. nunn* was able to suppress Pythium damping-off of cucumbers in soil. In the *P. nunn* - *P. ultimum* interaction, biocontrol may have been achieved through either competition or mycoparasitism. Bradshaw-Smith *et al.* (1991) showed that *P. oligandrum* was an effective antagonist against the three major footrot pathogens of peas: *Fusarium solani*, *Phoma medicaginis*, and *Mycosphaerella pinodes*. *P. oligandrum* completely parasitized *P. medicaginis*, and partially parasitized and produced a volatile inhibitor against the other two pathogens; however, inadequate nutrients may decrease the antagonistic ability of *P. oligandrum*. *P. oligandrum* appears to be capable of controlling damping-off caused by *P. ultimum* on sugar beet (Veseley, 1977, 1979; Martin and Hancock, 1987) and on cress (Al-Hamdani *et al.*, 1983).

It seems clear that mycoparasites show promise as biological control agents of diseases caused by pathogenic *Pythium* species; however, information on modes of parasitism, aggressiveness, host range, and 'disease escape' (Paulitz and Baker, 1987a,b; Whipps *et al.*, 1988; Laing and Deacon, 1990), as well as information on ecophysiology, inoculum production, and oospore biology (Lewis *et al.*, 1989), are needed for a proper evaluation of biocontrol potential and practical use.

Competition. Since *Pythium* species are generally poor secondary colonizers that are unable to invade previously colonized niches (Barton,

1961; Hine and Trujillo, 1966), competition for nutrients should have potential in biological control. The mechanism of nutrient competition in biological control was exemplified when Pythium damping-off of cucumber was controlled by applying two species of antagonistic bacteria to the cucumber seeds (Elad and Chet, 1987). Tedla and Stanghellini (1992) concluded that nutrient competition was responsible for suppression of *P. aphanidermatum* by bacteria in a soil environment. Paulitz (1991) found that a strain of *Pseudomonas putida* reduced *P. ultimum*-induced damping-off of pea and soybean seedlings via competition for seed volatiles. When Parke (1990) applied *Ps. cepacia* to pea seeds to successfully control damping -off caused by *P. ultimum* and *P. sylvaticum*, the mechanism of disease control may have been nutrient competition.

It has been suggested that mycoparasitic fungi may use nutrient (substrate) competition as an alternate mechanism of antagonism (Foley and Deacon, 1986). *P. oligandrum* and other mycoparasitic *Pythium* species play a role in soil suppressiveness to *P. ultimum* and other plant pathogenic *Pythium* species (Lifshitz *et al.*, 1984b; Martin and Hancock, 1986); this may be explained as much by nutrient competition as by parasitism.

Predation. *Pythium* species are fed upon by soil dwelling organisms, such as springtails, protozoa, mites, and nematodes. For example, Williets *et al.* (1989) suggested that there is potential for using Collembola in an integrated pest management strategy against *P. ultimum*. They found that *Onychiurus auranticus* had a feeding preference for young, actively growing mycelium of *P. ultimum*. This mechanism of biocontrol requires much more research to define its potential.

Antibiosis and enzymatic degradation. When one organism secretes metabolites (e.g. enzymes, lytic substances, volatile or non-volatile toxins) that are toxic to another organism, it is termed antibiosis. Many antibiotics, produced by both bacteria and other fungi, are known to be effective against *Pythium* species. Nelson and Craft (1992) developed an effective miniaturized and rapid bioassay for the selection of soil bacteria that are suppressive to blight of turfgrasses caused by *P. aphanidermatum*. Gurusiddaiah *et al.* (1986) characterized an antibiotic produced by a strain of *Ps. fluorescens* that showed excellent activity against the wheat pathogen, *P. aristosporum*. Howell and Stipanovic (1980) demonstrated suppression of *P. ultimum* induced damping-off of cotton seedlings by another strain of *Ps. fluorescens*. Di Pietro *et al.* (1992) attributed the antagonistic behaviour of *Chaetomium globosum* to antibiosis. Although antibiotic production may be used to effectively suppress specific *Pythium* diseases, antibiotics may occasionally be phytotoxic at the concentrations required for optimum disease suppression (Maurhofer *et al.*, 1992).

Fungal species have also demonstrated antibiotic-attributed antagonism against *Pythium* species. Thompson and Burns (1989) concluded that antagonistic metabolites of *Penicillium claviforme* incorporated into sugar beet seed pellets had potential for control of *P. ultimum* - induced diseases. *Gliocladium virens* is known to produce an antibiotic that is also effective against *P. ultimum* (Howell and Stipanovic, 1983), and has been shown to control damping-off of zinnia, cotton, and cabbage (caused by *P. ultimum*) in non-sterile soilless mix (Lumsden and Locke, 1989). *Trichoderma harzianum*, which was used in combination with *G. virens* to effectively control post-emergence damping-off and root rot of cucumbers

caused by *P. ultimum*, is thought to work via antibiotic production (Wolffhechel and Funck Jensen, 1992). *T. harzianum* was shown to also be antagonistic to *P. aphanidermatum* (Fajola and Alasoadura, 1975; Mukhopadhyay *et al.*, 1986). Devaki *et al.* (1992) controlled damping-off of tobacco seedlings, caused by *P. aphanidermatum* and *P. myriotylum*, with *T. harzianum*, but only in sterile soil. There are other reports of the ineffectiveness of *T. harzianum* in natural soil (Papavizas, 1985; Adams, 1990). The difference in the effectiveness of *T. harzianum* in sterile vs. natural soil may be due to its ability to rapidly colonize the biological vacuum that is left after soil sterilization (Devaki *et al.*, 1992).

Various organisms are capable of secreting lytic enzymes that are effective against *Pythium* species. As mentioned previously, Parke (1990) applied *Ps. cepacia* to pea seeds and successfully controlled Pythium damping -off. Although the mechanism of protection remains undetermined, *Ps. cepacia* is known to inhibit mycelial growth and lyse zoospores of *P. aphanidermatum* (Parke, 1990). Mitchell and Hurwitz (1965) demonstrated that a lytic *Arthrobacter* strain protected young tomato seedlings from damping-off caused by *P. debaryanum*.

Cultural Control

In cases where there are no available biological or chemical controls, or where these exist but are not economically or aesthetically viable, cultural control measures may be the only available alternatives for disease management. Cultural controls may also be used effectively in conjunction with biological and/or chemical controls. The use of tolerant or resistant plant varieties is one important cultural control measure. Hendrix and

Campbell (1973) stated "control of *Pythium* diseases by resistant cultivars may be a promising and enduring approach to effective control." One of the first demonstrations of varietal resistance to a *Pythium* disease was against root rot of sugarcane (Rands and Dopp, 1938). Adegbola and Hagedorn (1970) discovered host resistance to Pythium blight of bean. Chagnon and Belanger (1991) found that certain cultivars of geranium exhibited a defense reaction and thus were able to recover from attack by *P. ultimum* and resume plant growth, while other cultivars were not. Howard and Williams (1976) developed two methods for screening carrot varieties for resistance to Pythium root dieback.

O'Brien *et al.* (1991) tested the effects of several cultural practices (planting depth, hilling, cultivation depth) on the severity of bean root rot, a disease in which several *Pythium* species play an important causal role. They found that shallow planting significantly reduced disease severity. They suggested that an integrated approach that combines cultural methods that enhance root system development through improved drainage and aeration with other approaches, such as tolerant varieties and/or fungicides, may lead to optimal control of root rot. This same idea can likely be applied to most, if not all, *Pythium*-caused plant diseases.

Barbetti and MacNish (1984) found that leaving a field fallow from August to March, or spring cultivation before sowing to oats, resulted in reduced severity of root rot of subterranean clover upon its subsequent replanting. Barbetti (1991) found that complete removal of subterranean clover from a pasture for at least one season resulted in significant reductions of lateral and tap root disease upon replanting, but only for the first season. There was no corresponding increase in total herbage production, however.

Ploetz *et al.* (1985) discovered that tillage to 15 cm, combined with multicropping to rye and soybeans, may affect population dynamics of soilborne fungi, including *Pythium* species.

Kulkarni *et al.* (1992) used a combination of soil solarization and host resistance to control *P. aphanidermatum*-caused dieback and collar and root rots of periwinkle. Plant mortality was reduced by 72-91%. Solarization alone increased leaf and root yields by 26-52% for both resistant and susceptible varieties tested.

Enhancement of competition among soil microorganisms by organic amendments that increase microbial activity can cause lysis of propagules of pathogenic fungi and increase plant growth (Cook and Baker, 1983). Huang and Kuhlman (1991) tested 17 chemical soil amendments for use against damping-off of slash pine seedlings, and found one that significantly reduced disease incidence and promoted seedling growth.

Microbial activity and biomass in container media, as determined by the rate of hydrolysis of fluorescein diacetate (FDA), were found to be important in suppression of Pythium damping-off (Chen *et al.*, 1988a, b; Inbar *et al.*, 1991; Boehm and Hoitink, 1992). Addition of various mature composts to peat increased microbial activity, which resulted in long term suppression of Pythium root rot and damping-off (Chen *et al.*, 1988a, b; Boehm and Hoitink, 1992). Chen *et al.* (1988a) and Inbar *et al.* (1991) developed methods for predicting the level of suppressiveness to damping-off caused by *Pythium* species by measuring FDA hydrolysis. There are limitations to these models, since there are other factors that may outweigh the suppressive effects, such as high moisture (Griffin, 1963); however, these models should prove

particularly useful under the controlled conditions of greenhouse and potting regimes.

In the case of hydroponics, filtration may be a viable method of removing *Pythium* zoospores, thereby reducing disease; however, the difficulties in completely excluding *Pythium* vectors can make recontamination likely (Goldberg *et al.*, 1992).

Chapter II

Role of *Pythium* spp. in Cavity Spot Development on Carrots in British Columbia

Cavity spot is an important and widespread disease of carrot (*Daucus carota* L.), and is a problem in many carrot growing areas worldwide (Long, 1985). The disease, first described in 1961 (Guba, et al., 1961), is characterized by dark, sunken, elliptical lesions that occur horizontally on the carrot root. It is now accepted that various species of *Pythium* cause cavity spot (Groom and Perry, 1985, Lyshol, et al., 1984, Punja, 1990, Vivoda, et al., 1991, White, 1986, White, 1988). The difficulty in isolating *Pythium* spp. from cavity spot lesions has resulted in the disease previously being attributed to a number of diverse causes, including: calcium deficiency (Guba, et al., 1961, Maynard, et al., 1961, Maynard, et al., 1963), *Rhizoctonia* (Mildenhall and Williams, 1970), pectolytic anaerobic bacteria (Perry and Harrison, 1979), high ammonium-N (De Cock, et al., 1981, Scaife, et al., 1983), fungus gnat larvae (Hafidh and Kelly, 1982), ammonium fertilizer injury (Goh and Ali, 1983), high (>6.5) soil pH (Scaife, et al., 1983), aliphatic acids (Perry, 1983), and stress from flooding and high soil temperature (Soroker, et al., 1984). The occurrence of cavity spot and its associated *Pythium* spp. is widespread, and has been reported from carrot production areas worldwide, including the United Kingdom (Green and Makin, 1985, Lyons and White, 1992, Perry and Harrison, 1979, White, 1986), France (Montfort and Rouxel, 1988), Israel (Jacobsohn, et al., 1984, White, et al., 1993), the United States (Guba, et al., 1961, Vivoda, et al., 1991), Canada (McDonald and Sutton, 1992, Ormrod, 1973), and Australia (Erceg, et al., 1993).

Among over 200 species of *Pythium* that have been described in the literature, seven species have been implicated as the cause(s) of cavity spot: *P. violae* (Groom and Perry, 1985, Vivoda, et al., 1991, White, 1986, White, et al., 1993, Lyons and White, 1992, Montfort and Rouxel, 1988, White and Wakeham, 1987); *P. sulcatum* (White, 1986, Lyons and White, 1992, Erceg, et al., 1993); *P. ultimum* Trow (Vivoda, et al., 1991, White, 1986); *P. sylvaticum* (White, 1986); *P. intermedium* (White, 1986); *P. irregulare* (White, 1986) and *P. aphanidermatum* (White, 1986). Four other species, *P. coloratum*, *P. dissotocum*, *P. tardicrescens*, and *P. rostratum* (White, 1988), have also been found associated with cavity spot and may have the ability to induce cavity spot-like lesions.

Although hyphae (Groom and Perry, 1985, Punja and Benard, 1992) and oospores (Punja and Benard, 1992) have been observed within the diseased carrot tissue after the collapse of the cells of the epidermis and secondary phloem (cavity formation), the actual method of penetration by the fungus into the host tissue is unknown. During the formation of cavity spot lesions, a dark discoloured area initially forms beneath the epidermal layer. The epidermis remains intact, then as the lesion develops, a cavity forms and the epidermis collapses. At this point, the lesion rarely enlarges further, and the likelihood of isolation of the pathogen past this point decreases with time.

The method by which the fungus spreads in the field is also unknown. However, cavity spot lesions may occur on any part of the surface of mature carrot roots, and in any number, and this may vary from one root to another. Lesions usually tend to occur more frequently on the upper third of the taproot (Vivoda, et al., 1991). There are no other symptoms of disease on the plant. In the absence of a host, *Pythium* spp. survive in the soil

saprophytically and by forming resistant resting structures (oospores, zoospores, and sporangia) (Hendrix and Campbell, 1973, Plaats-Niterink, 1981).

In the field, factors that have been found to enhance the incidence of cavity spot include: early field planting (Vivoda, et al., 1991), high soil moisture or waterlogged soils (Vivoda, et al., 1991, Perry and Harrison, 1979, Jacobsohn, et al., 1984), cool soil temperatures (Vivoda, et al., 1991, Montfort and Rouxel, 1988), soil pH (Scaife, et al., 1983), high ammonium N fertilizer (De Cock, et al., 1981, Goh and Ali, 1983), and carrot variety (Vivoda, et al., 1991, McDonald and Sutton, 1993).

The disease cycle for cavity spot-causing *Pythium* spp. is presently unknown. Based on what is known about cavity spot, carrots, and *Pythium* spp., a possible series of events could be as follows:

1. Oospores are stimulated to germinate by seed and/or root exudates. Resultant zoospores, if any, attack host tissues when conditions are wet. Mycelium is formed and also infects the host.

2. Pathogenesis occurs as a result of enzyme secretion by mycelium.

3. Mycelium survives in the host tissues and oospores are produced in the host, in response to antagonism by other microorganisms, or because of changing environmental conditions.

4. *Pythium* spp. overwinter in the soil in the form of oospores, zoospores, and sporangia.

Few methods for control of cavity spot of carrot are currently available. Fungicides and fumigants may be used to reduce *Pythium* population levels in the soil. Metalaxyl and mancozeb have shown effectiveness in reducing the incidence of cavity spot (Lyshol, et al., 1984, White, 1988, McDonald and

Sutton, 1993, McDonald and Sutton, 1992). Unfortunately, as *P. sulcatum* is relatively insensitive to metalaxyl (White, 1988), this treatment may be ineffective where this species is predominant. The use of resistant carrot varieties may prove to be an effective method of disease control. There are several carrot varieties that show some resistance to cavity spot, such as Six Pak (McDonald and Sutton, 1993, Benard and Punja, 1993), Panther (Benard and Punja, 1993), Nandor and Redca (Sweet, et al., 1989). White *et al.* found differences in susceptibility of different cultivars to different species of *Pythium* (White, et al., 1988). They found Nandor to be somewhat resistant to *P. violae* and *P. sulcatum* but susceptible to *P. intermedium*.

Cavity spot was first recognized as a problem on carrots grown in the Fraser Valley of British Columbia in 1973 (Ormrod, 1973). The incidence of cavity spot among commercial fields is now widespread; however, the severity is erratic and can vary from one year to the next. In general, the problem is known to be accentuated by cool temperatures (Montfort and Rouxel, 1988, Vivoda, et al., 1991) and high soil moisture (Perry and Harrison, 1979, Jacobsohn, et al., 1984, Vivoda, et al., 1991), by early plantings of susceptible cultivars (Vivoda, et al., 1991), and by continuous cropping of carrots in the same soil (Green and Makin, 1985).

Little is known about the etiology of cavity spot on carrots grown in soils in the Fraser Valley of B.C., and the role of *Pythium* spp. reported from other geographical areas (White, 1986, Vivoda, et al., 1991, White, et al., 1987) in cavity spot development has not been researched. In preliminary studies, the association of *Pythium* spp. with cavity spot lesions on carrots originating from B.C. (Punja, 1990) and Ontario (McDonald and Sutton, 1992) has been reported.

Based on this information, the objectives of the work described in this chapter were to: i) establish the pathogenicity of *Pythium* spp. and other soilborne fungi associated with cavity spot lesions; ii) determine the growth characteristics and identity of *Pythium* spp. isolated from cavity spot lesions on commercially grown carrots over a 4 year sampling period; and iii) investigate the influence of temperature, soil moisture, and carrot age on cavity spot development. Preliminary results from this study have been published (Punja, 1990, Benard and Punja, 1993, Punja and Benard, 1992).

Materials and Methods

Samples

Commercially grown carrot roots with cavity spot lesions representing several different cultivars were collected from a total of seven fields located in the Fraser Valley of B.C. In addition, root samples were obtained from the B.C. Coast Vegetable Co-operative in Richmond, B.C. and from various retail outlets. The samples were collected throughout the growing season (May-October) in each of 4 years (1989-1992). From two fields sampled in 1990 that yielded a high frequency of infected roots, soil samples were obtained and taken to the laboratory for use in further experiments.

Isolation Procedures

The presence of lesions resembling cavity spot (Fig. 1) was recorded from all collected samples, and isolations were usually made within 24-48 hr after the roots were obtained. Pieces of tissue (ca. 2 mm^2) were cut from the edges of lesions (in the case of small lesions, the entire lesion was cut out) and

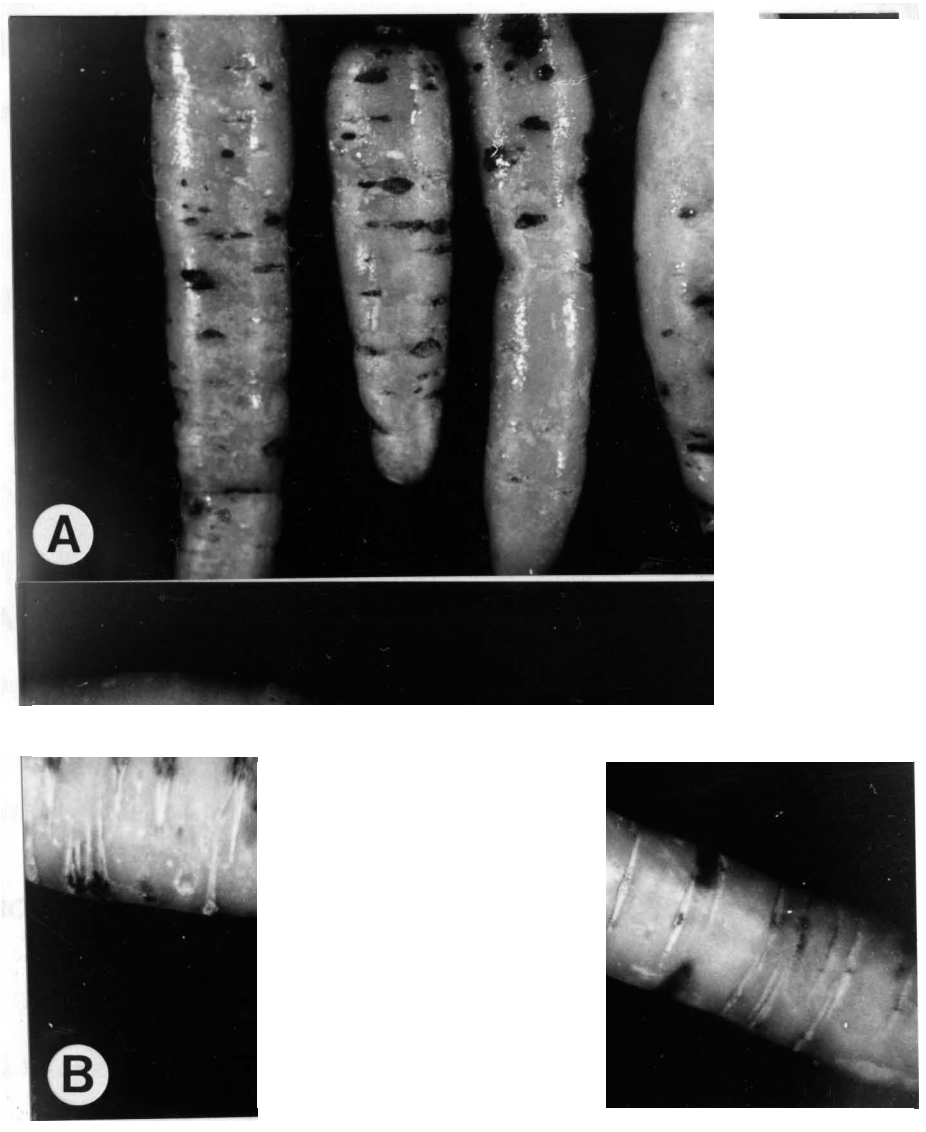

Figure 1. A) Cavity spot lesions on mature carrots from the Fraser Valley of British Columbia. B) Close-up of carrot root with lesions at various stages of development. Note dark areas under epidermis in young lesions.

one of three isolation techniques was used: a) surface sterilized in 0.3% NaOCl for 3 min; b) washed under running tap water for 3-5 hr, followed by surface sterilization; and c) washed in running tap water for 3 hr. In 1989 and 1990, all tissue segments were initially plated onto each of V-8 agar, 1.5% Bacto water agar (WA), corn meal agar (CMA), and *Pythium*-selective medium (PVPP = corn meal agar, pimaricin, vancomycin, penicillin, PCNB (Jeffers and Martin, 1986); with or without rose bengal). In studies conducted in 1991 and 1992, only PVPP medium (sometimes containing 400 mg/L ampicillin) was used. The petri dishes were incubated at 23-25 °C or at 15 °C and examined periodically for up to 3 weeks for fungal colony development. Subcultures were made onto either V-8 agar, potato dextrose agar (PDA), potato carrot agar (PCA) (Plaats-Niterink, 1981), WA, or CMA and maintained at room temperature (24-28 °C) or at 15 °C. All of the fungi recovered were identified to genus, and included predominantly *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phoma*.

Pathogenicity Tests

Three or four selected isolates from each of the four major genera of fungi recovered from cavity spot lesions were grown on V-8 agar or PDA for 10 days. To determine the pathogenicity of these isolates, three procedures were used:

a) Seedling assay — Carrot seeds cv. Danvers were surface sterilized in 0.5% NaOCl for 1 min, rinsed in water, and 10 seeds were arranged approximately 2 cm from the edges of 100 X 25 mm petri dishes containing 1.5% water agar. The dishes were sealed with parafilm and incubated under fluorescent lamps at 24-28 °C. After 12 days, a mycelial plug (0.5 cm diameter)

of the isolate to be tested was placed in the centre of each dish. The percentage of seedlings that were infected or killed was determined after 12-18 days and a pathogenicity rating was assigned to each isolate based on the following scale: 0 = 100% survival; 1 = 75-99% survival; 2 = 50-74% survival; 3 = 25-49% survival; 4 = 0-24% survival.

b) Root inoculation — Mature carrot roots were surface sterilized in 0.3% NaOCl for 3 min and rinsed twice in distilled water. The roots were placed in polystyrene containers lined with moist paper towels and inoculated with mycelial plugs (0.5 cm diameter) spaced at approximately 2 cm intervals along the length of the root. Four replicate roots were included for each isolate. The roots were kept at 20-24 °C for 7-10 days and the presence of lesions was recorded. Carrot cultivars tested included Paramount, Eagle, and several that were unknown.

c) Greenhouse tests — Three-month-old carrot plants cv. Danvers grown in pasteurized soil:sand mix in the greenhouse (temperature range of 16-24 °C) were inoculated with isolates of *Rhizoctonia, Fusarium, Phoma* and *Pythium* that were recovered from cavity spot lesions in 1989-1990. Inoculum of all fungi was produced on vermiculite:V-8 (500 cm^3 vermiculite, 50 mL V-8 juice, 0.5 g $CaCO_3$, 200 mL water) or potato dextrose broth (PDB). Cultures were grown for 12-15 days at 23-25 °C. Inoculum was added to each 15-cm-diameter pot, which contained three carrot plants, at the rate of 50 cm^3 of infested vermiculite or 50 g fresh weight of mycelium. The inoculum was mixed into the soil around each root, and the soil was moistened to saturation. Subsequently, the pots were watered as required. The presence of lesions resembling cavity spot on harvested, washed roots was rated 4 weeks

after inoculation. For each treatment, there were three replicate pots, each containing three plants. The experiment was repeated once.

Soil Treatment and Disease Development in Naturally Infested Field Soil

Muck soil obtained from a commercial field located in Cloverdale, B.C. that yielded a high frequency of carrots with cavity spot lesions in 1990 was used. The soil was either used without treatment, or was autoclaved (for 30 min at 121 °C and 103.4 kPa), microwaved (for 1 min at high power, 700 Watt microwave), drenched with metalaxyl (40 µg/L a.i.) or inoculated with one of three *Pythium* isolates recovered from infected roots from the same field. The isolates were grown in PDB for 10 days and 100 g of mycelium was mixed into 300 cm^3 of soil. Following treatment, aliquots of soil (150 cm^3) were placed in 200 cm^3 styrofoam cups and 2 month-old carrot seedlings cv. Danvers were transplanted into the cups (two seedlings/cup). For each treatment, there were six replicate cups. The soil was moistened to saturation and the cups were placed at constant 20 °C in a growth chamber (16 hr light:8 hr dark photoperiod, relative humidity 80%) for 4 weeks. The roots were subsequently harvested and washed and the number of lesions resembling cavity spot was recorded.

Influence of Soil Moisture, Temperature and Root Age on Disease Development on Soil-Grown Carrots

Natural field soil was autoclaved and artificially infested with an isolate of *P. violae* following the procedure described above. The initial soil moisture was adjusted to either 60% or 48% as determined by drying

representative subsamples at 80 °C for 24 hr and weighing, or was kept flooded. Aliquots of infested soil (150 cm^3) contained in polystyrene cups each received two carrot seedlings and the cups were placed at 15 °C, 20 °C, or 25 °C. At each temperature, four replicate cups were included. Water was added to the cups as needed to maintain the soil moisture content at 48% or 60% (± 5%). The roots were harvested after 3 weeks (flooded soil) or 6 weeks (48% and 60% moisture) and washed and the number of lesions resembling cavity spot was recorded. The experiment was repeated once.

To determine the influence of root age, artificially infested soil adjusted to a moisture content of 60% was planted with carrots that were obtained 1, 2, or 3 months after seeding into vermiculite. The roots were recovered after 4 weeks and rated for the number of cavity spot lesions. The experiment was repeated once.

Growth and Morphological Characteristics of Pythium *spp.*

From the isolations made from cavity spot lesions over the 1989-1992 growing seasons, 120 isolates of *Pythium* were recovered. To group these isolates based on growth rate, measurements of radial growth were made after subculturing each of the isolates onto V-8 agar, CMA and PCA and incubating at 15 °C (and sometimes at 25 °C) for 72 hr. Growth rate was expressed as colony diameter attained after 72 hr at 15 °C on V-8 or PCA. Although different growth rates were observed on the two media, the differences were not great enough to change growth rate classification (fast, slow, or intermediate). The frequency of isolation of slow-growers vs. fast-growers vs. isolates with an intermediate growth rate was analyzed using chi-square goodness of fit.

To further group the 120 isolates based on morphological characteristics, cultures that had been grown on CMA and PCA for 10-14 days were examined for the presence of oospores, hyphal swellings, sporangia, and other morphological characteristics that are used to identify *Pythium* spp. (Plaats-Niterink, 1981). Isolates were initially grouped according to their similarities in taxonomic characteristics. A total of 15 morphologically distinct groups was determined. Representative type cultures of several *Pythium* spp. (*P. violae, P. sulcatum, P. mamillatum, and P. sylvaticum)* were also examined to compare both growth rate and morphological characteristics.

Virulence of Pythium *spp.*

To establish the virulence of 36 representative isolates of *Pythium* spp. selected from each of the morphological groups identified above, the *in vitro* seedling assay described above was used. Cultures were selected, without knowledge of pathogenicity, from each of the 15 morphological groups, and grown on CMA or PCA for 8-12 days at 15 °C. Mycelial plugs (0.5 cm diameter) were transferred from the margins of colonies to Petri dishes containing carrot seedlings.

All 120 isolates were tested for pathogenicity to mature carrot roots. Mycelial plugs were placed on the surface of freshly harvested field-grown carrots of cultivars Paramount and Eagle and of several undetermined varieties. The roots were surface sterilized in 0.3% NaOCl for 3 min, rinsed thrice in distilled water, and placed in plastic containers lined with moistened paper towels. Each root was sprayed with distilled water and inoculated with 8-10 evenly spaced plugs, incubated at 15 °C for 7-10 days, and assessed for lesion development at 24-48 hr intervals. Lesion sizes and rate of lesion

development were recorded, and virulence was rated after 10 days using the following method: non-pathogenic = little or no lesion development; moderately virulent = < 75% of plugs producing lesions, < 50 % of lesions ≥ 5 mm in diameter ; highly virulent = ≥ 75% of plugs producing lesions, ≥ 50 % of lesions ≥ 5 mm in diameter. The relationship between growth rate and pathogenicity was analyzed using a contingency table and chi-square analysis. Several isolates of *Pythium*, including *P. violae*, *P. sulcatum*, and *P. ultimum* were also included for comparison in these tests.

In another experiment, carrot roots were wounded by scraping the epidermis with a scalpel. Wounded and non-wounded carrots were inoculated with 7 different isolates of *Pythium* spp. to determine if wounding influenced lesion development. Four slow-growing isolates (3 of which were pathogenic) and 3 fast-growing isolates (1 of which was pathogenic) were used.

Identification of Pythium *Isolates Using Species-Specific DNA Probes*

To further confirm the identity of the isolates of *Pythium* in each morphological group, the internal transcribed spacer (ITS) of the nuclear ribosomal DNA (rDNA) of 18 representative *Pythium* isolates from most of the morphological groups identified above was amplified by the polymerase chain reaction (PCR) and compared with species-specific probes for known *Pythium* spp. by non-isotopic dot blot hybridization (André Lévesque, personal communication). DNA extraction was conducted according to a modified Stratagene Protocol II-Extraction from whole tissue. The mycelium was homogenized in a Dounce homogenizer. Isolates were grown at 15 °C for 5-14 days in a broth that contained a 1:1 mixture of 1/4 strength potato-dextrose broth and the following medium: 0.5 g L-asparagine, 1.0 g KH_2PO_4,

250 mg $MgSO_4 \cdot 7H_2O$, 5 mg $CaCl_2 \cdot H_2O$, 1 mg $FeSO_4 \cdot 7H_2O$ per L of distilled water, adjusted to pH 6.0 with KOH.

Results

Isolation of Fungi

A number of fungal genera were isolated from cavity spot lesions during 1989-1990, especially when nutrient-rich agar media (e.g. V-8 agar) were used. Most of these fungi were identified as species of *Fusarium* (31 isolates), *Phoma* (18 isolates), *Rhizoctonia* (24 isolates), and *Pythium* (15 isolates). During 1991 and 1992, a total of 105 additional isolates of *Pythium* spp. were obtained when PVPP medium was used. The most effective technique for isolating these *Pythium* spp. was washing the tissues in running tap water for 3 hr, plating onto PVPP agar containing 22 mg/L rose bengal, and incubating at 15 °C. PVPP medium was essential for isolation of slow-growing species. Subcultures were best maintained on PCA, or on WA amended with penicillin and streptomycin if there was evidence of bacterial contamination.

Pathogenicity Tests

a) Seedling assay — Among the 24 isolates of *Fusarium* spp. that were tested for pathogenicity to carrot seedlings, only one was pathogenic and killed <25% of the seedlings (Table 1). Among the 19 isolates of *Rhizoctonia* tested, two were moderately pathogenic and killed >50% of the seedlings,

Table 1. Pathogenicity of fungal isolates recovered from cavity spot lesions during 1989-1990 to carrot seedlings using an *in vitro* method

Genus	Number of isolates tested	Pathogenicity[a]
Fusarium	23	0
	1	1
Rhizoctonia	17	0
	2	3
Phoma	12	0
Pythium	5	0
	3	1
	4	2
	9	3
	15	4

[a]Rated 12-18 days after inoculation according to the following scale: 0 = 100% survival; 1 = 75=99% survival; 2 = 50-74% survival; 3 = 25-49% survival; 4 = 0-24% survival.

while the remainder were non-pathogenic. None of the 12 *Phoma* isolates tested was found to be pathogenic. Among 36 isolates of *Pythium* tested, the range was from 5 non-virulent isolates (killed none of the seedlings) to 15 highly virulent isolates (killed >75% of the seedlings) (Table 1).

b) Root inoculation — None of the isolates of *Fusarium, Rhizoctonia,* or *Phoma* tested on mature roots produced any lesions. Among the *Pythium* isolates tested, several were found to produce small lesions or pits on the carrot surface.

c) Greenhouse tests: — No symptoms developed when 3-month-old carrots were inoculated with isolates of *Fusarium* or *Phoma*. Inoculation with *Rhizoctonia* isolates resulted in the development of large, dark, sunken lesions. Inoculation with *Pythium* isolates gave rise to cavity spot-like lesions on the roots.

Influence of Soil Treatment, Soil Moisture, Temperature, and Plant Age

In natural field soil, the development of cavity spot-like lesions was found to depend on the treatment the soil received (Table 2). No lesions developed when the soil was sterilized by autoclaving or microwaving prior to use, or when the fungicide metalaxyl was added. Lesions developed in all of the pots that received *Pythium* spp., and also in the control. Plants died when the soil was sterilized by microwaving and *Pythium* was added (Table 2).

The incidence of cavity spot was greatest when artificially infested soil was kept flooded or at 60% moisture content (about -1/3 bar) and at 15 °C (Table 3). At 60% moisture content, lesion numbers were significantly higher

Table 2. Influence of treatments of field soil on development of cavity spot on carrots subsequently planted into the soil

Treatment[a]	Average number of lesions per root[b]
None	2.4
Autoclaved	0
Microwaved	0
Metalaxyl[c]	0
Pythium -1	4.0
Pythium -2	4.0
Pythium- 3	3.0
Microwaved + *Pythium*-1	N/A[d]

[a] Treatments of natural field soil were made prior to transplanting carrots into the soil.

[b] Represents an average of two seedlings per pot, six replicate pots per treatment, and one repetition of the experiment.

[c] Applied as a drench containing 40 µg/mL a.i.

[d] Plants dead.

Table 3. Influence of moisture and temperature on development of cavity spot on carrots planted in soil artificially infested with *Pythium* species

Soil moisture	Temperature (°C)	Average number of lesions per root
Flooded[a]	15	2.3
	15	4.0
60 %[b]	15	5.2
	20	4.1
	25	1.9
48 %[b]	15	2.3
	20	0
	25	0
LSD= 1.3 (P=0.05)		

[a]Rated after 3 weeks.

[b]Rated after 6 weeks.

(P=0.05) than at 48% at all of the temperatures tested. Greatest disease incidence at both soil moisture levels was observed at 15 °C, with significantly fewer lesions developing at 20 and 25 °C (Table 3). When carrots of three different ages were planted into infested soil, there was no significant difference in the number of lesions that developed after 4 weeks (data not shown).

Growth and Virulence of Pythium *spp.*

The 120 isolates of *Pythium* were tentatively placed into 15 groups based on morphological criteria. Measurements of growth on V-8 agar and PCA at 15 and 25 °C indicated that 41% were fast-growers (growing more than 8.0 cm in 72 hr), 51.5% were slow-growers (growing less than 4.0 cm in 72 hr), and 7.5% grew at an intermediate rate (Table 4). The difference in the number of isolations of slow-growers vs. fast-growers was not significant ($0.1<P<0.25$) if one assumes a similar opportunity for isolating a slow-grower or a fast-grower. However, upon inclusion of the isolates with intermediate growth rate and assumption of an equal probability of isolating a species with any growth rate, the result is very highly statistically significant ($P<0.001$).

When a comparison of growth rate and pathogenicity of 36 selected isolates of *Pythium* was made using the *in vitro* seedling assay, the fast-growing isolates ranged from non-virulent (22%) to highly virulent (22%), while most slow-growing isolates (89%) were moderately to highly virulent (Table 4).

When all 120 isolates were tested for pathogenicity using mature carrot roots, the results indicated that 92% of the slow-growing isolates were pathogenic, while only 51% of the fast-growing isolates and none of the

Table 4. Comparison of selected fast- and slow-growing *Pythium* isolates for pathogenicity using an *in vitro* carrot seedling method

Growth rate[a]	Virulence[b]	No. of isolates
Fast	0	4
(>8.0 cm in	1	3
72 h)	2	3
	3	4
	4	4
Slow	0	1
(<4.0 cm in	1	0
72 h)	2	1
	3	5
	4	11

[a]Average growth rate on V-8 agar or PCA at 15 °C.

[b]0 = 100% survival; 1 = 75=99% survival; 2 = 50-74% survival; 3 = 25-49% survival; 4 = 0-24% survival.

intermediate growers were pathogenic (Table 5). These results were highly significant ($P<0.001$), and indicate that pathogenicity is correlated with growth rate.

When the effects of wounding were examined, it was found that in the case of each pathogenic isolate, virulence appeared to be enhanced, i.e. lesions were larger on the wounded carrots as compared with the non-wounded roots. In the case of each non-pathogenic isolate, wounding did not result in lesion formation, i.e. wounding did not cause a non-pathogenic isolate to become pathogenic.

Identification of Pythium *Isolates Using Species-Specific DNA Probes*

The dot blot hybridization technique showed that the isolates pathogenic to carrot included: *P. violae, P. sylvaticum, P. irregulare, P. sulcatum, P. ultimum* var. *ultimum, P. paroecandrum, P. acanthicum,* and *Pythium* Group-G. *P. mamillatum* was identified as a non-pathogenic species that was occasionally isolated from cavity spot lesions. Following this identification, the numbers of isolates within each species was determined (Table 6). The identification of three of the isolates as *P. ultimum* Trow, *P. irregulare* Buisman, and *P. violae* Chesters & Hickman was subsequently confirmed by Dr. Donald J. S. Barr of Agriculture Canada's National Identification Service, Economic Fungi, Ottawa, Canada. The results obtained from the molecular analysis were comparable to what was determined from the morphological identification, except that one pathogenic, slow-growing group that morphologically appeared to resemble *P. rostratum* was not confirmed using the DNA probes.

Table 5. Comparison of *Pythium* isolates for growth rate and pathogenicity on mature carrot roots

Growth rate / 72 hr[a]	Number of isolates		No. of isolates/ total isolates
	Pathogenic	Non-pathogenic[b]	
>8.0 cm	25	24	49/120
4.0-8.0 cm	0	9	9/120
<4.0 cm	57	5	62/120

[a] Average growth rate on V-8 agar or PCA at 15 °C.

[b] Pathogenic = cavity spot-like lesions produced; non-pathogenic = no lesions present.

Table 6. Identification of species of *Pythium* isolated from cavity spot lesions during 1990-1992 using morphological and molecular techniques

Species	Total no. of isolates	Pathogenic isolates	Non-pathogenic isolates
P. violae	41	38	3
P. sulcatum	20	18	2
P. ultimum	17	7	10
P. irregulare	13	8	5
P. sylvaticum	11	5	6
P. acanthicum	9	4	5
P. paroecandrum	2	1	1
Pythium Group-G	1	0	1
P. mamillatum	2	1	1
Unidentified	4	1	3
Total	120	83	37

Discussion

The pathogenicity tests performed with the various fungi isolated from cavity spot lesions over 3 years of study indicated that only *Pythium* spp. were able to cause cavity spot-like lesions. In addition, the soil treatment experiments showed that the presence of *Pythium* spp. was necessary for the development of cavity spot, since lesions developed only when *Pythium* inoculum was present. These findings substantiate the work of other researchers who have reported *Pythium* spp. to be the causal agent(s) of cavity spot in other carrot growing areas of the world (White, 1986, Montfort and Rouxel, 1988, Vivoda, et al., 1991, Erceg, et al., 1993, White, et al., 1987, Groom and Perry, 1985). Although numerous biotic (Perry and Harrison, 1979, Guba, et al., 1961, Hafidh and Kelly, 1982, Mildenhall and Williams, 1970) and abiotic (De Cock, et al., 1981, Goh and Ali, 1983, Maynard, et al., 1961, Maynard, et al., 1963, Scaife, et al., 1983, Soroker, et al., 1984) factors previously have been suggested as possible causes of cavity spot, the role of *Pythium* spp. may have been overlooked due to the difficulty in isolating the various slow-growing pathogenic *Pythium* spp., which can be rapidly overgrown by other fungi and bacteria on conventional isolation media. White (1988) noted that successful isolation of *Pythium* spp. was more frequent from younger carrot plants, with isolation frequency decreasing as the crop matured. He concluded that this supported the general view that *Pythium* spp. are colonizers of juvenile tissue. Our research, however, indicated that lesion age and not plant age, affected the success of isolation of *Pythium* spp. In older lesions that were healed over, *Pythium* spp. were often absent or were very difficult to isolate, while with young lesions, the probability of isolation was greater. Similar

results were obtained by Groom and Perry (1985) who reported they could reisolate *P. violae* from lesions on 2, 4, and 11 day old inoculated tissues, but not after 21 days. Our results also indicated that there was no difference in the extent of cavity spot development on carrots from 1-3 months of age. Vivoda *et al.* (1991), however, reported that cavity spot development was affected by carrot age, with 5 month old roots being more susceptible than 3-4 month old roots. A possible explanation for this discrepancy may be that while young carrots are equally susceptible, older roots develop more cavity spot lesions over time due to extended exposure to pathogen inoculum in soil. In addition, carrot cultivar and pathogenicity of isolate or species present can also influence the extent of cavity spot development on mature roots (Chapter 3). McDonald and Sutton (1993) found that plant age was positively correlated with disease incidence in the field, but concluded that plant age was not a major determinant of final levels of cavity spot.

Among the isolation techniques used, the best method to recover *Pythium* spp. was washing the tissues in running tap water for 3 hr, plating onto PVPP agar containing 22 mg/L rose bengal, and incubating at 15 °C. Washing tissues in water without use of bleach ensured that bleach-sensitive and surface-growing *Pythium* spp. could still be isolated, while decreasing the level of contamination. Rose bengal was useful for eliminating bacterial contaminants; however, rose bengal was not always used as it appeared to inhibit the growth of some isolates. The relatively low temperature of 15 °C was used because it reduced the growth of other organisms, particularly bacteria, and allowed some *Pythium* spp. that do better at lower temperatures to grow.

The proportion of isolations of *Pythium* spp. in each growth rate category was very highly statistically significant ($P<0.001$), based on an assumption of equal proportions. The proportions of isolates that should be expected, however, should be based on the average growth rates of all the species that could possibly be isolated. An extrapolation of the growth rates of 73 *Pythium* spp. on CMA over 24 hr (Middleton, 1943), shows that 56/73 of the species listed would be considered to have a slow growth rate (<4.0 cm/72 hr) if measured at 72 hr, while 17/73 would be considered to have an intermediate growth rate (4.0-8.0 cm/72 hr), and 0/73 would be fast-growers (>8.0 cm/72 hr). Compared with Middleton's growth rate information, the result of our research is very highly statistically significant ($P<0.001$). This extrapolation is not entirely accurate, however, as colonies tend to have a slower initial growth rate due to the effects of transferring to a new medium. The fact that different media were used (CMA vs. PCA) may not significantly affect the 24 hr growth rates: a comparison of growth rates given for *Pythium* spp. at 25 °C on PCA (Plaats-Niterink, 1981) vs. the same on CMA at 25 °C (Middleton, 1943) shows them to be very similar. Sampling methods may be responsible for the unexpected pattern of isolations.

Among the three techniques used in this study to evaluate the pathogenicity of *Pythium* isolates, the mature root pathogenicity test proved to be the best. The seedling assay was useful to separate the non-pathogenic fungi from those that were pathogenic, but did not provide any indication of the ability of the latter to cause cavity spot. All the isolates tested that were found to be capable of causing cavity spot on mature carrot roots were also pathogenic to seedlings; however, the degree of pathogenicity of an isolate to seedlings was not necessarily correlated to its pathogenicity to mature carrot

roots. Furthermore, some of the isolates found to be pathogenic to seedlings were not capable of producing cavity spot lesions on mature carrot roots. Of the isolates tested, those capable of producing lesions in greenhouse inoculations were also able to cause cavity spot on mature carrot roots, and vice versa; however, greenhouse inoculations required a great deal of time and space, and inoculum levels of *Pythium* spp. in the soil were difficult to standardize, so this technique was less satisfactory.

The isolates of *Pythium* collected in this study were initially grouped according to their growth rate and morphology (Plaats-Niterink, 1981), and molecular biology techniques were used to confirm the species identification. The close relationships between some isolates were indicated by cross hybridization using the dot blot hybridization technique (results not shown). A close, almost indistinguishable, relationship was found between many, if not all, isolates of *Pythium* Group-G and *P. ultimum* var. *ultimum*. Cross hybridization also occurred among isolates of *P. irregulare, P. sylvaticum,* and *P. paroecandrum,* indicating a close phylogenetic relationship probably exists among these species.

Many of the *Pythium* spp. isolated in this study have been reported by other researchers to be causal agents of cavity spot, including: *P. violae* (Lyons and White, 1992, White, 1986, Montfort and Rouxel, 1988, White, et al., 1993, Vivoda, et al., 1991, White, et al., 1987, Groom and Perry, 1985); *P. sulcatum* (Lyons and White, 1992, Erceg, et al., 1993); *P. ultimum* Trow (White, 1986, Vivoda, et al., 1991); and *P. sylvaticum* (White, 1986). Some previously unreported species were identified in this study as being able to cause cavity spot, such as *P. acanthicum, P. paroecandrum,* and *Pythium* Group-G. These species may be unique to the organic soils found in the lower Fraser Valley of

British Columbia, or they may have been excluded during isolation by other researchers. Our results implicating several different species as causal agents of cavity spot are similar to those reported by White *et al.* (White, 1986, White, et al., 1987, White, 1988), who implicated 11 different species as causal agents of cavity spot in the U.K.

Among the species recovered in our study, *P. violae* and *P. sulcatum* appear to be the most important based on their repeated isolation from carrots from different sources over several years, and their consistently high virulence *in vitro*. *P. irregulare* and *P. ultimum* Trow may also be important causes of cavity spot in British Columbia, given their pathogenicity and frequency of isolation. Vivoda *et al.* (1991) tested isolates of *P. irregulare*, but found them to be non-pathogenic, although White (1986) found that isolates of *P. irregulare* isolated in the U.K. were capable of producing some lesions on carrots.

Some apparently non-pathogenic species were also found associated with cavity spot, such as *P. mamillatum*. White (1988) reported isolating *P. rostratum*, *P. dissotocum*, and *P. intermedium* from cavity spot lesions, and *P. tardicrescens*, *P. oligandrum*, and *P. coloratum* from the asymptomatic periderm of carrots with cavity spot, but no pathogenicity tests were performed. The role, if any, of these non-pathogenic *Pythium* spp. remains unknown. It is possible that at least some of them are, in fact, pathogenic and/or they may play a secondary role in lesion development, accelerating or increasing progress of the disease after invasion by pathogenic species.

In many of the cavity spot-infested fields cropped to carrots in the Fraser Valley, symptoms such as forked root and discoloured lateral roots were also observed. Carrot diseases with symptoms such as discoloured

lateral roots and forked, stunted, or necrotic taproots have been described as rusty root (Barr and Kemp, 1955, Kalu, et al., 1976, Sutton, 1975), brown root (Mildenhall, et al., 1971), Pythium root dieback (Stanghellini and Hancock, 1971b, Wisbey, et al., 1977), lateral root dieback (Wisbey, et al., 1977), or root dieback (White, 1986, Kalu, et al., 1976, Howard, et al., 1978, Liddell, et al., 1989, Chang-Ho, 1970). These diseases are thought to be caused by many of the *Pythium* spp. known to cause or be associated with cavity spot, such as *P. sulcatum* (Kalu, et al., 1976, Howard, et al., 1978), *P. irregulare* (Howard, et al., 1978), *P. ultimum* (Liddell, et al., 1989), and *P. violae* (White, 1986). The ability of the cavity spot-forming *Pythium* isolates recovered in this study to induce symptoms of these diseases was not determined, but they could be involved as part of a disease syndrome.

The possible role of other microorganisms (such as the fungi and bacteria that are frequently isolated from cavity spot lesions) in the development of cavity spot symptoms is unknown. Bacteria may be important, since bacterial contamination of *Pythium* isolates was frequent and difficult to eliminate. When present, these contaminants were sometimes observed to reduce isolate vigour, or conversely, lesion development *in vitro* was sometimes enhanced. Anaerobic bacteria have been previously implicated to be involved in cavity spot development (Perry and Harrison, 1979); their role may be to increase lesion size following initial infection through the epidermis by *Pythium*. On naturally infected carrots, cavity spot lesions are generally <1 cm in width, while roots inoculated *in vitro* develop much larger lesions. The limitation of lesion size in the field may be due to various host defense mechanisms that are known to occur in

carrot (Punja, et al., 1992) or to non-conducive environmental conditions following infection.

The occurrence of cavity spot in the field is enhanced by environmental factors such as high soil moisture (Jacobsohn, et al., 1984, Guba, et al., 1961, Perry, 1972, McDonald and Sutton, 1993) and cool temperatures (Vivoda, et al., 1991, McDonald and Sutton, 1993). We found that conditions of high moisture (soil saturation or flooding), especially at a temperature of 15 °C, significantly increased cavity spot incidence. Similar results have been reported from California (Vivoda, et al., 1991). Disease incidence in the field has also been positively correlated with cumulative rainfall and negatively correlated with soil temperature (McDonald and Sutton, 1993). The Fraser Valley of British Columbia has cool, wet springs, relatively mild winters, and rich organic soils, all of which are apparently conducive to cavity spot development. In addition, carrots are sometimes repeatedly cropped in the same field, allowing for the buildup of higher populations of *Pythium*. In the absence of a suitable host, *Pythium* spp. can survive by forming resistant resting structures, such as sporangia, zoospores, and oospores (Burr and Stanghellini, 1973), or possibly can infect alternate hosts. For example, *P. violae*, which causes cavity spot on carrot in California, was shown to reside on roots of cowpea, broccoli, celery, cucumber, sugarbeet, and watermelon without causing symptoms (Schrandt, et al., 1994). The mycelium of *Pythium* is probably transitory in the soil due to attack by antagonistic microorganisms (Hancock, 1981). Many of the *Pythium* spp. identified as causal agents of cavity spot are capable of survival and growth at low temperatures (Plaats-Niterink, 1981, Middleton, 1943).

With *P. ultimum*, which is important in cavity spot development in California (Vivoda, et al., 1991) and the U.K. (White, 1988), direct competition is not a detriment to survival, since it is able to avoid competition and antagonism in the early stages of saprogenesis and pathogenesis (Stanghellini and Hancock, 1971a). *P. ultimum* can respond rapidly to nutrients in plant exudates or residues (Hancock, 1977), and to various other carbon and nitrogen sources (Nelson and Craft, 1989). It is not known whether the other *Pythium* spp. that cause cavity spot, such as *P. violae* and *P. sulcatum*, respond similarly, since little information is available on their ecology. Their slow growth in culture (and presumably in nature) does not appear to reduce their ability to be important pathogens of carrot.

The relative importance of the various *Pythium* spp. that have been implicated as causal agents of cavity spot should be examined, in addition to the role, if any, of the non-pathogenic species frequently isolated from cavity spot lesions. Investigations into the possible role of other microorganisms in the development of cavity spot, as well as the possibility of additive or even synergistic effects of abiotic factors, are required to obtain a better understanding of this disease.

Chapter III

Cavity Spot on Carrots in British Columbia: Pathogenesis by *Pythium* spp. and Identification of Cultivar Resistance

Cavity spot of carrots is a disease that is characterized by elliptical, sunken lesions on the carrot root (Chapter 2). A dark discoloured area initially forms beneath the epidermal layer, and as the lesion develops, a cavity forms and the epidermis collapses (Guba, et al., 1961). While cavity spot has been attributed to a number of causes, there is now considerable evidence to implicate various *Pythium* spp. as the causal agents (Groom and Perry, 1985, Lyshol, et al., 1984, Punja, 1990, Vivoda, et al., 1991, White, 1986, White, et al., 1987). In particular, *P. violae* (Groom and Perry, 1985, Vivoda, et al., 1991, White, 1986), *P. ultimum* (Vivoda, et al., 1991), and *P. sulcatum* (Erceg, et al., 1993, White, 1989) have been identified as the most important species responsible.

In many *Pythium* diseases, hydrolytic enzymes (Endo, 1961, Nemec, 1972) and toxins (Desilets and Belanger, 1991, Ichihara, et al., 1985, Mojdehi, et al., 1990) play an important role during pathogenesis. Extracellular pectinase production by *P. ultimum* on cucumber has been clearly demonstrated (Cherif, et al., 1991). Secretion of cell wall degrading enzymes and toxic metabolites has been demonstrated for *P. ultimum* (Ichihara, et al., 1985, Mellano, et al., 1970), *P. aphanidermatum* (Winstead and McCombs, 1961), and *P. butleri* (Janardhanan and Akhtar, 1974), resulting in host cell wall degradation and tissue maceration (Napi-Acedo and Exconde, 1965). Several techniques have been developed to examine pectic enzyme activity and include use of: colour indicators (Winstead and McCombs, 1961, Smith, 1958),

diffusion assays (Chang, et al., 1992, Hankin and Anagnostakis, 1975), viscometry (Mellano, et al., 1970, Winstead and McCombs, 1961), and histopathology utilizing labelled probes (Cherif, et al., 1991). The involvement of extracellular pectinases during cavity spot development on carrot and during the infection process has not been previously investigated. Furthermore, it is not known whether differences in virulence between *Pythium* isolates that cause cavity spot (Chapter 2) could be related to their ability to secrete pectinases.

Carrot cultivars have been shown to differ in susceptibility to cavity spot development under field conditions (McDonald and Sutton, 1992, Odermatt and Snow, 1989, Sweet, et al., 1989); however, it has proven difficult to correlate results from *in vitro* root inoculations to field results for the purpose of evaluating cultivars for resistance (White, et al., 1987, White, et al., 1988), L. Thijssen, personal communication). White *et al.* (White, et al., 1988) reported that differences in varietal susceptibility to different *Pythium* species existed, but they were unable to correlate any of the laboratory findings to results from field evaluations. The availability of an *in vitro* screening procedure for evaluating cultivars for tolerance to cavity spot could expedite breeding for resistance to this disease.

The objectives of this study were to: i) determine the extent of pectolytic enzyme production by *Pythium* species isolated from cavity spot lesions and correlate this to virulence; ii) develop an *in vitro* procedure to evaluate the resistance or susceptibility of carrot cultivars to cavity spot; and iii) investigate the mechanism of infection and symptom development by *Pythium* spp. on carrot using histopathology.

Materials and Methods

Mycelial Growth and Pectolytic Enzyme Production

The isolates of *Pythium* used in this study are described elsewhere (Chapter 2). A total of 38 isolates were grown on potato-carrot agar (PCA) for 96 hr, at which time five plugs (0.5 cm diameter) were placed in 10 mL vials containing 1±0.01 g of sliced autoclaved carrots and 7.5 mL broth. The broth was comprised of 0.5 g asparagine-L, 1.0 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.005 g $CaCl_2 \cdot H_2O$, 0.001g $FeSO_4 \cdot 7H_2O$ per L of distilled water, adjusted to pH 6.0 with KOH. The vials were incubated at 15 °C for 96 hr, and the mycelium was carefully removed from each vial with forceps and dried for 48 hr at 60 °C to obtain dry weight (±0.05 mg). Seventy-five µl of the remaining broth was pipetted into each of four wells (0.5 cm diameter) spaced evenly 2 cm from the edge of a 100x15 mm petri plate containing 16 mL of agar. The agar consisted of (per L): 20 g Anachemia brand agar, 0.1 g pectin (Sigma, from citrus fruits), 0.045 g Apron® (35% metalaxyl) to prevent *Pythium* growth, pH 7.0. The plates were incubated at 37 °C for 96 hr, then flooded with a 1% hexadecyltrimethylammonium bromide (C-TAB) solution for 30 min, after which the C-TAB was poured off and plates were allowed to dry at room temperature for approximately 24 hr. The plates were examined for clear circular zones around the wells. Each clearing zone was measured across two diameters, using at least two replicate plates for each isolate. The experiment was repeated three times, and the results were averaged. The data were expressed as zone size (cm) per mycelial dry weight (mg) for each isolate, the value for which was termed 'enzyme activity'. The data were analyzed to

determine if there were significant differences between isolates. The Kruskal-Wallis and Mann-Whitney Tests were used due to a large difference in standard deviations of the enzyme activity values between non-pathogenic isolates (S.D.=0.167) and moderately (S.D.=5.17) and highly (S.D.=5.69) pathogenic isolates.

Pectinase (from *Aspergillus niger*) from Sigma (one unit releases 1.0 µmole of galacturonic acid from polygalacturonic acid per min at pH 4.0 and 25 °C) was used to estimate the enzyme activity in the assay described above. Several concentrations of pectinase (from 0.1 to 8 units) were tested and an activity curve was developed. Simple nonlinear regression analysis showed that there was a significant relationship between units of pectinase and zone size (Fig. 2). Using this curve, the relative enzyme activity of the *Pythium* isolates was determined.

Histopathology

Cavity spot lesions on roots inoculated *in vitro* and from natural infections were prepared for light microscopy. For artificial inoculations, several inoculum plugs of two highly virulent isolates of *P. violae* were placed on the roots, which were incubated in a sealed plastic container at 15 °C for 2-10 days. Lesioned areas (0.25 cm^2) from both artificially and naturally infected carrot roots, at different stages of lesion development, were cut and fixed in formalin acetic acid for ≥24 hr. The tissues were then dehydrated through a 10%-100% ethanol series (100% twice), followed by 30 min in 1:1 100% xylene:100% ethanol, then three 1 hr treatments in 100% xylene, 30 min in xylene/wax mixture, and 0.75-1.0 hr in hot wax (shaken every 10 min). The tissues were then embedded in wax, sectioned using a rotary

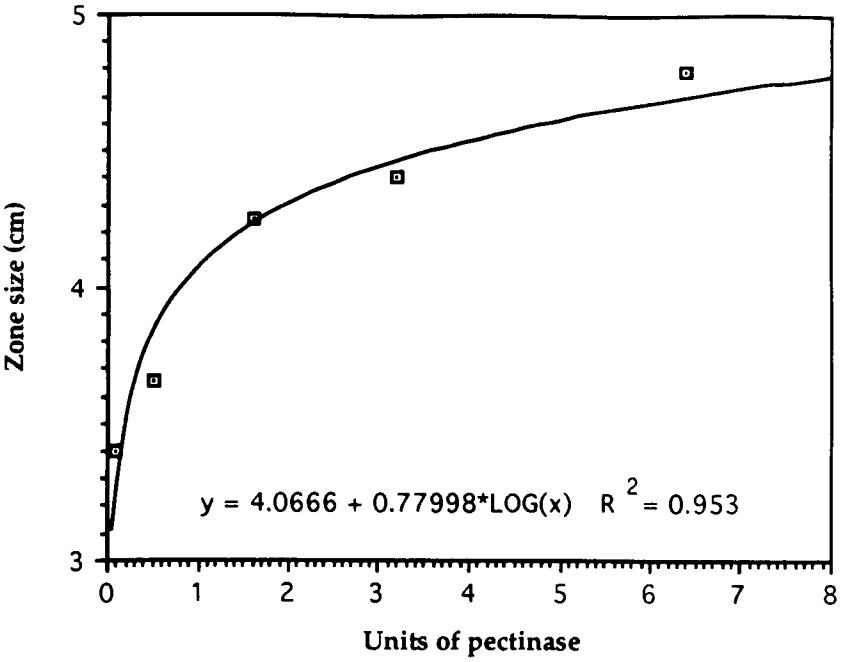

Figure 2. Pectinase enzyme activity curve using pectinase from *Aspergillus niger* in a cup-plate assay.

microtome (10-12 μm), and stained with fast green or aniline blue and examined under the light microscope.

To examine possible differences between resistant and susceptible carrot varieties in the pathogen-host interaction, carrots of varieties Celloking, Eagle, Six Pak, and Gold Pak were inoculated with *P. violae*. Lesioned and healthy areas, as well as lesions at different stages of development, were sectioned and stained as described above.

Evaluation of Carrot Cultivars

Selected field-grown cultivars that were included in the British Columbia Ministry of Agriculture, Fisheries, and Food carrot varietal demonstrations in Cloverdale, B.C. were evaluated in 1991 and 1992. In 1993, selected cultivars from commercial and experimental field plots were evaluated. See Appendix 1 for seed sources. Three carrot roots from each of 36 cultivars were tested in 1991. Three sets of three carrot roots from 4 of the cultivars tested in 1991 and four sets of three carrot roots from 14 of the cultivars tested in 1991 were retested during the 1992 growing season; one previously untested variety (Dakota) was also included. Three carrots from 9 varieties were tested twice in 1993. The mature root pathogenicity test (Chapter 2) was used. Ten inoculum plugs of highly pathogenic isolates of *P. violae, P. sulcatum, P. irregulare,* and *P. acanthicum* were placed on the upper 10-15 cm of surface sterilized carrot roots and incubated on moist paper towels in a sealed container at 15 °C, and examined for cavity spot lesion development every 1-2 days, for up to 14 days. To categorize the carrot varieties into resistant, intermediate, or susceptible classes, average final lesion diameter for each set of carrots was calculated using two methods.

First, the inoculum plugs that resulted in successful infections were considered, and second, all of the plugs were considered (using "0" as the value where no lesions developed). In situations where more than one lesion developed from a single plug, only the largest lesion was included. The data from the two methods were averaged, and the resulting lesion diameter was used to determine category (resistant, intermediate, or susceptible). The range of values for each category was also determined for each set of data by observation of the carrots and examination of the generated data. All data sets from a given season were combined. The data from each of the three years were kept separate.

Results and Discussion

Mycelial Growth and Pectolytic Enzyme Production

The cup-plate assay for pectinase activity used in this study showed development of clear zones after 24-96 hr (Fig. 3). Enzyme activity of the 38 isolates tested was plotted against the virulence of the isolates on mature carrot roots as described previously (Chapter 2). The highly virulent isolates generally had relatively high levels of enzyme activity, while the non-pathogenic isolates had low enzyme activity. Enzyme activity was significantly higher ($P<0.005$) in the highly virulent isolates as compared with the non-pathogenic isolates. The moderately virulent isolates had both high and low enzyme activity (Fig. 4), but enzyme activity was significantly different in these isolates as compared with non-pathogenic isolates ($P<0.03$) and as compared with highly pathogenic isolates ($P<0.001$). The enzymes

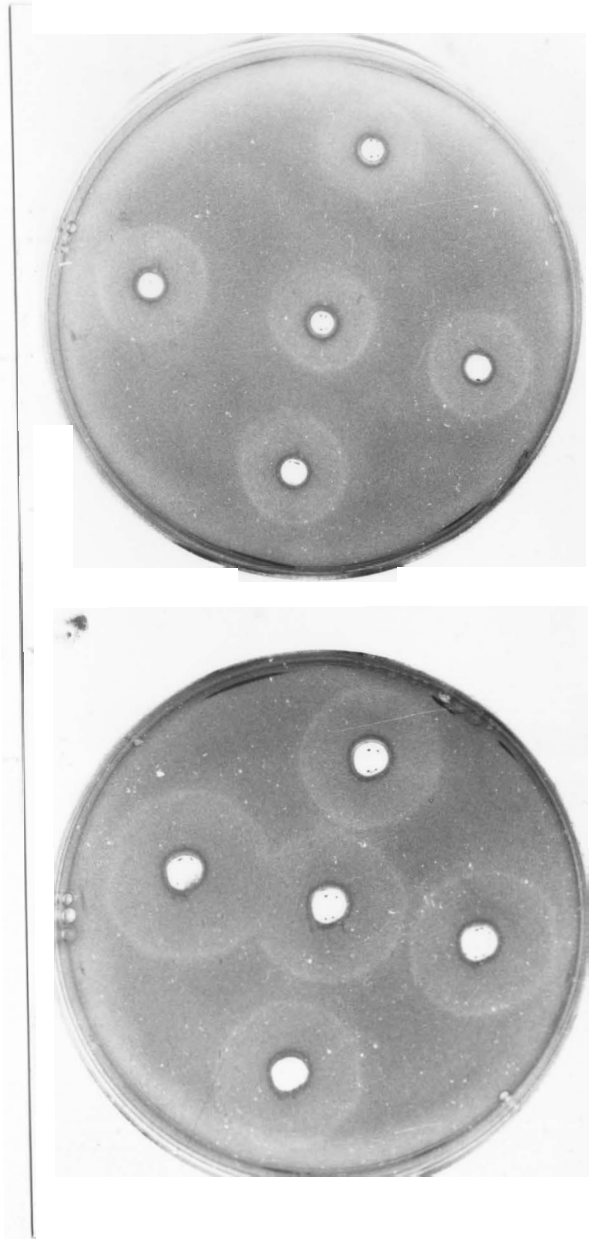

Figure 3. Enzyme assay plates showing zones of clearing due to pectolysis after 96 hr. Top plate contains broth from weakly virulent isolate and bottom plate contains broth from virulent isolate. Both isolates had comparable mycelial dry weights.

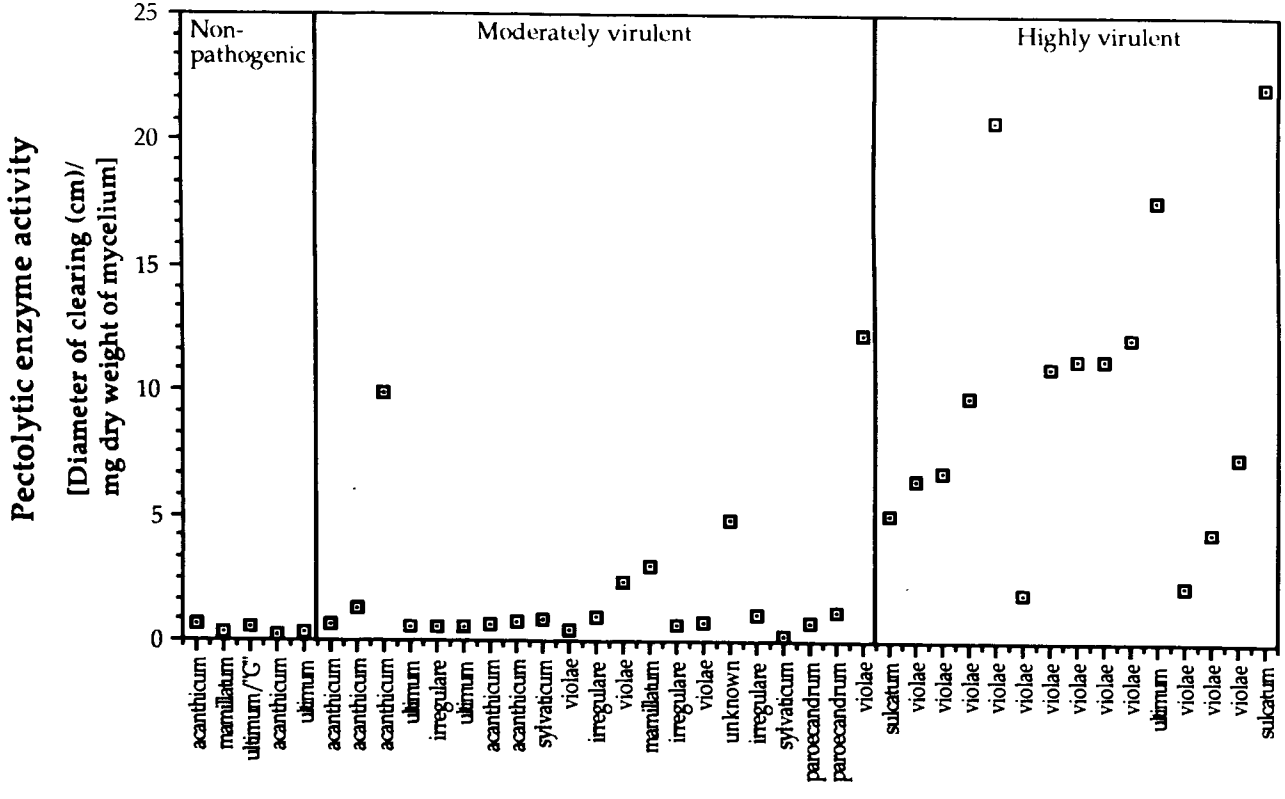

Figure 4. Relationship between pectolytic enzyme activity of culture broth and pathogenicity of selected *Pythium* isolates.

involved may have included polygalacturonase, secreted at concentrations of up to 11.6 units/mg mycelial dry weight for highly virulent isolates and up to 10 units/mg mycelial dry weight for moderately virulent isolates.

In many of the diseases caused by *Pythium* spp., infection is dependent upon the production of cell wall degrading enzymes, such as pectinases (Winstead and McCombs, 1961, Dube and Prabakaran, 1989) and cellulases (Winstead and McCombs, 1961, Dube and Prabakaran, 1989, Deacon, 1979). Winstead and McCombs (1961) correlated the symptoms of cottony leak disease on cucumber with the activities of pectinolytic and cellulolytic enzymes produced by *P. aphanidermatum*. Cherif *et al.* (1991) confirmed the involvement of pectinases produced by *P.ultimum* in development of disease symptoms on cucumber. The isolates in our study that exhibited high enzyme activity were usually also highly pathogenic, and included *P. violae* and *P. sulcatum*; therefore, it is probable that pectolytic enzymes have an important role in cavity spot development, although the exact nature of these enzymes was not determined in this study.

Histopathology.

During cavity spot lesion development, the cells below the epidermis darkened and collapsed, and a cavity developed (Fig. 5A). The epidermis eventually collapsed into the expanding cavity. Lesion size was restricted, presumably by host reaction to the pathogen (Fig. 5B). Fungal structures (e.g. oospores, hyphae) were observed within the diseased tissue. Groom and Perry (1985) showed hyphae ramifying through the periderm and secondary phloem tissues of carrot two days after inoculation with *P. violae*.

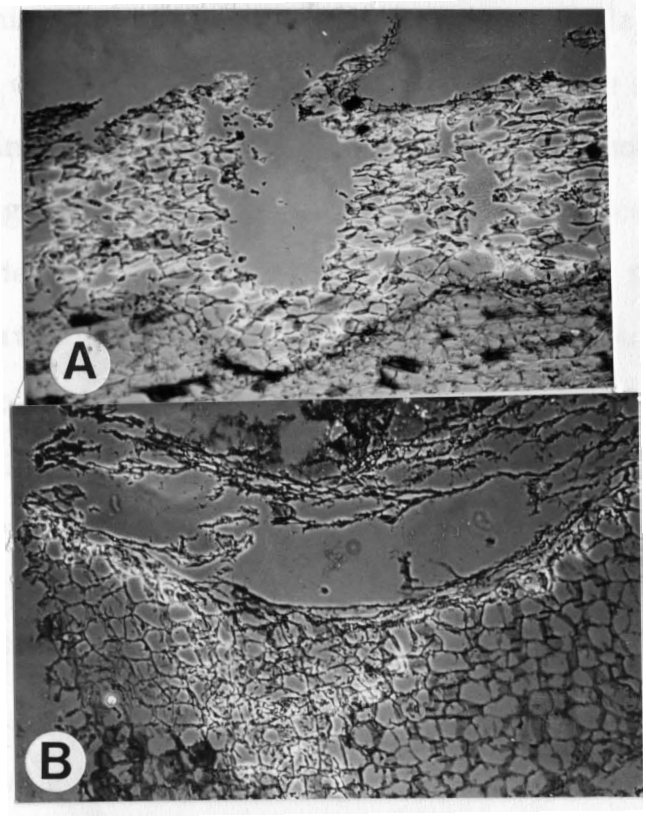

Figure 5. Histopathology of cavity spot lesions on carrot. A) Section through a developing lesion, showing collapse of cells beneath epidermis, and zone of infection. Note oospore and hyphae within carrot tissue. B) Section through mature lesion, showing cavity development.

Evaluation of carrot cultivars.

The *in vitro* root inoculation procedure yielded results within 72 hr of inoculation. Large expanding lesions were visible (Fig. 6A). It was also possible to clearly distinguish a susceptible cultivar from a resistant one (Fig. 6B) using this method. Carrot cultivars were placed into susceptible, intermediate, or resistant classes based on the average size of lesions (Fig. 7). Response to infection was determined using two methods (all plugs and only plugs causing infection) to include the percent successful infection as a criterion for determining carrot susceptibility to cavity spot, and to give more emphasis to average lesion diameter, which was the most consistent criterion for susceptibility. Average lesion diameter was considered the most important criterion for determining whether a cultivar was resistant or susceptible because percent successful infection was variable, and could be influenced by factors other than cultivar (e.g. level of moisture on the carrot). Furthermore, lesion size is a greater factor in determining cullage than the total number of lesions; i.e. a carrot is more likely to be rejected during grading if it has a few large lesions that if it has a greater number of very small lesions.

The results from the three years of study showed that of the cultivars tested in multiple years, Panther, E0792, Caropride, Fannia, and Navajo were among the most resistant (Fig. 7). Six Pak, Imperator, and XPH 3507 were tested in only one year, but appeared very resistant. There were a few inconsistencies among the 19 varieties tested in more than one year: Eagle was found to be resistant in 1991, susceptible in 1992, and intermediate in 1993; Carochoice was found to be resistant in 1991, intermediate in 1992, and

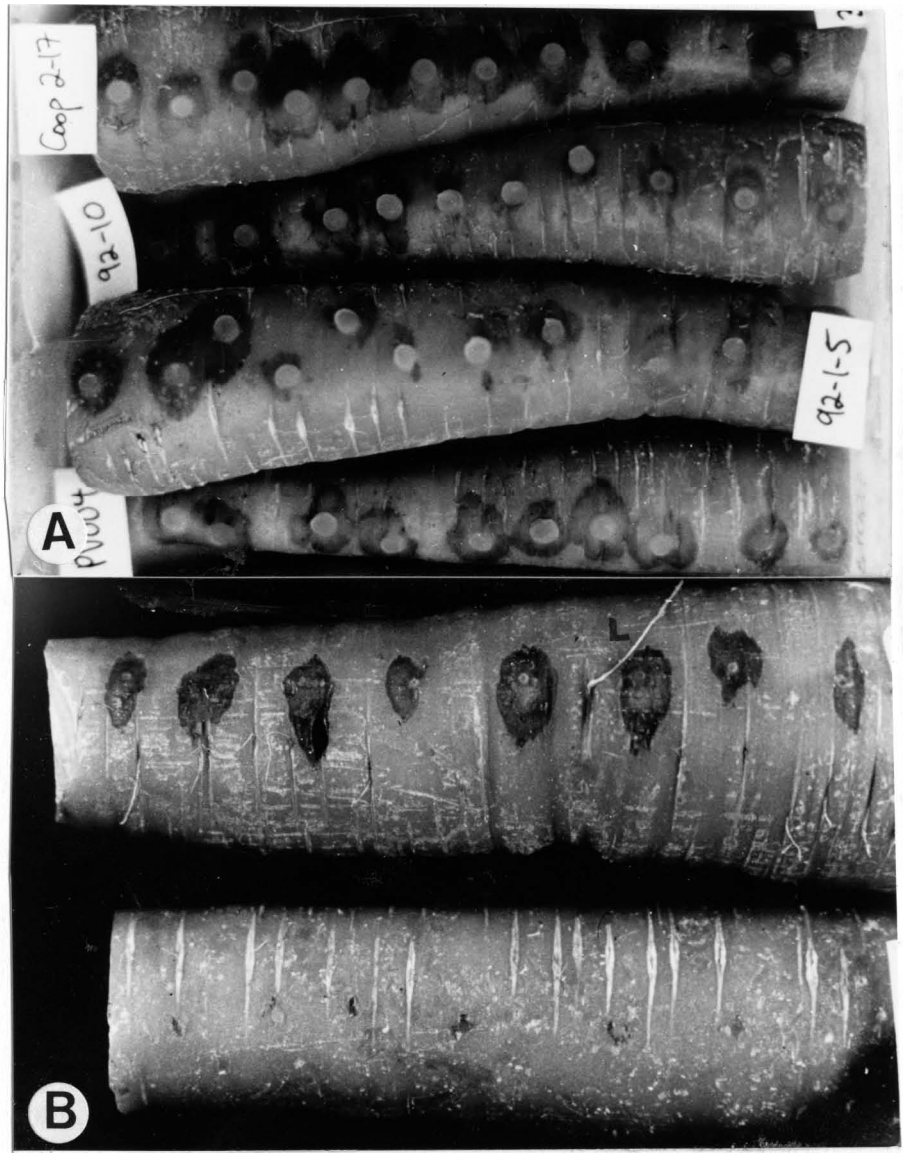

Figure 6. A) Mature carrot root pathogenicity test showing lesion development 72 hr after inoculation. B) Response of susceptible carrot cultivar (top) and resistant (bottom) to *in vitro* inoculation with *Pythium* spp.

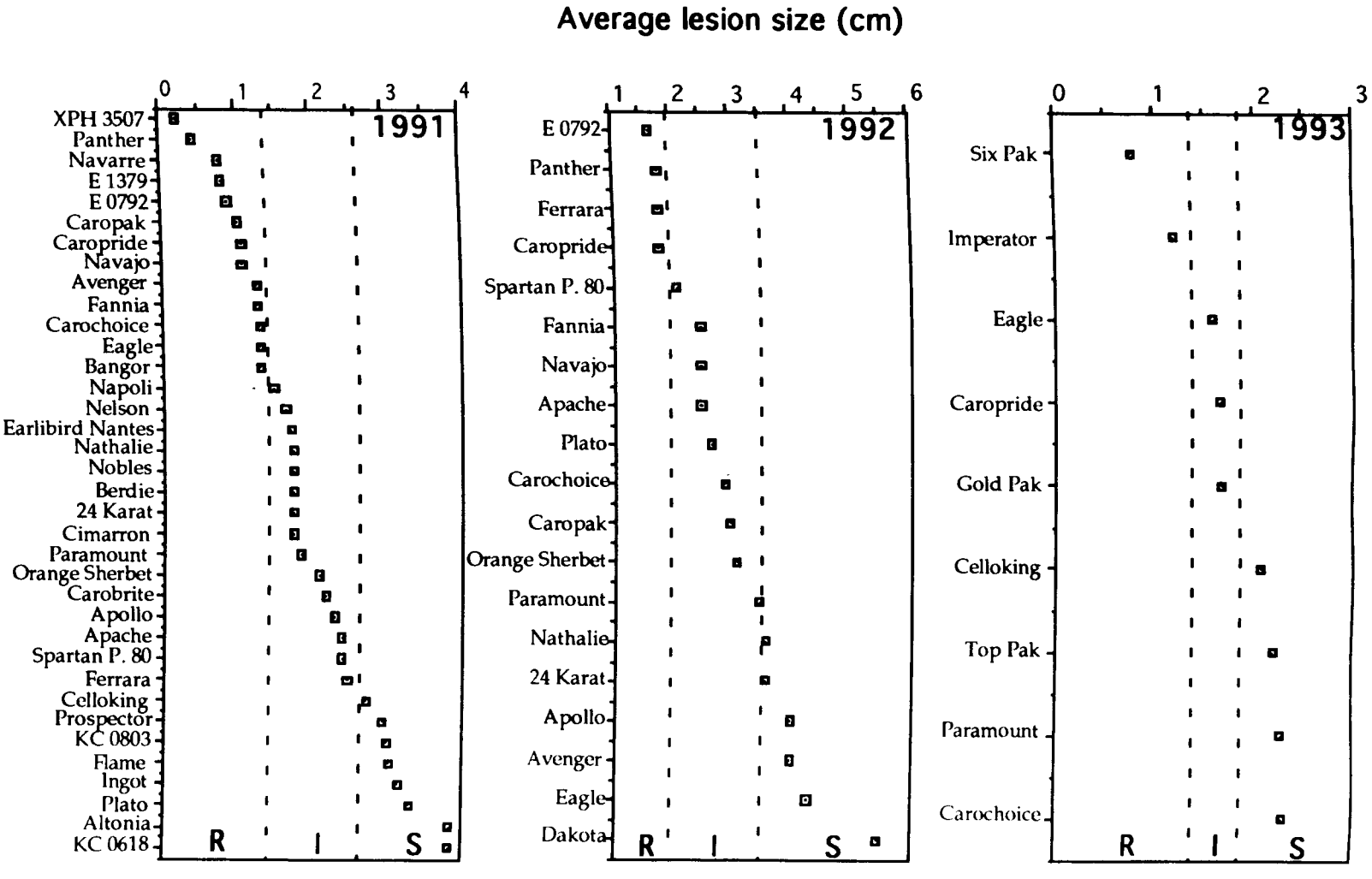

Figure 7. Results of *in vitro* carrot variety evaluations (1991-1993), showing response of cultivars to *Pythium* infection - resistant (R), intermediate (I), and susceptible (S).

susceptible in 1993; and Avenger was found to be resistant in 1991, but susceptible in 1992. Lesion sizes that defined the class boundaries differed between years because of differences in carrot size; i.e. with increasing root size, the maximum lesion size for a cultivar to be considered resistant also increased. The ages of the roots may also have differed between years and could have influenced the reaction of these carrot varieties. Although previously no differences with regard to carrot age and cavity spot incidence were observed *in vitro* (Chapter 2), other researchers have noted increased susceptibility of older carrots (Vivoda, et al., 1991). Since many of the carrots we tested were more than 3 months old, and the isolate of the*Pythium* spp. used in the different years to inoculate the carrots sometimes varied, this may also account for some of the discrepancies. Differences in soil fertility could also have affected cultivar performance between years. The relative ranking of most of the varieties tested in multiple years (16/19) remained fairly constant with regard to susceptibility or resistance to cavity spot. Comparison of our 1991 data with the results from the British Columbia Ministry of Agriculture, Fisheries, and Food carrot varietal demonstrations in Cloverdale, B.C. showed relatively similar results (Harvie Snow, pers. comm.). The only significant difference occurred with Eagle, which was ranked as more resistant than Caropak, Caropride, Navajo, Avenger, Fannia, and Carochoice in field trials. The variations in results between years with Eagle in our study suggest that growing conditions may affect its susceptibility to cavity spot. For most cultivars, the *in vitro* results were comparable to field results. In 1992, such comparisons could not be made because weather conditions (warm and dry) were not conducive to cavity spot development and disease incidence among

all the field-tested varieties was low. This further points to the advantages of *in vitro* screening vs. field evaluation for cultivar resistance.

Sweet *et al.* (1989) reported differences in cultivar resistance to cavity spot in field trials, and reported that cultivars Nandor and Redca show partial resistance. Six Pak has also been shown to have partial resistance to cavity spot in the field, based on lesion size and the number of carrots that developed lesions (McDonald and Sutton, 1992, McDonald and Sutton, 1993). White et al. (1987) found no useful genetic resistance among 19 carrot cultivars following *in vitro* inoculation with *P. violae, P. sulcatum,* and *P. intermedium.* In a further study, significant differences in susceptibility to cavity spot were found among seven carrot cultivars tested *in vitro,* but these differences did not correlate with field observations (White, et al., 1988).

McDonald and Sutton (1993) concluded that cultivar resistance is a major factor that determines final levels of cavity spot in the field. The basis for resistance to cavity spot that exists among some carrot cultivars may be horizontal resistance (McDonald and Sutton, 1992), a level of unspecific resistance to pathogens that all plants possess to varying degrees. Resistance of carrot roots to *P. aphanidermatum* has been attributed to the production of host phenolics that are toxic to the pathogen (Dube and Prabakaran, 1989). The mechanism of resistance to cavity spot is not known and is worthy of further investigation.

Chapter 4

General Summary and Recommendations for Future Research

Several species of *Pythium* were shown to be the causal agents of cavity spot on carrots in the Fraser Valley of British Columbia. These included *P. violae, P. sulcatum, P. sylvaticum, P. ultimum, P. irregulare, P. acanthicum, P. paroecandrum,* and *P. mamillatum.* Other species of *Pythium,* such as *Pythium* Group-G, were isolated from cavity spot lesions, but were not pathogenic. Isolation of *Pythium* spp. from carrot tissues was optimal following washing in running tap water for 3-5 hr, and plating onto PVPP medium. The growth rates of most of the species isolated were either slow or fast, and most (92%) of the slow growers were found to be pathogenic to mature carrot roots, while about half (51%) of the fast growers were similarly pathogenic, and none of the isolates with intermediate growth rate was pathogenic.

A temperature of 15 °C and conditions of high moisture enhanced cavity spot development, while carrot ages from 1-3 months had no significant effect.

Most of the isolates of *Pythium* tested were capable of producing pectolytic enzymes, but the most virulent isolates tended to have the greatest enzyme production per mycelial dry weight, while moderately virulent isolates varied in their enzyme activity.

During screening of carrot varieties for resistance using an *in vitro* technique, several cultivars with resistance were identified, including: Panther, E 0792, Six Pak, and Fannia. The most susceptible cultivars included

several that are frequently grown in the Fraser Valley of B.C, including Celloking, Paramount, and Carochoice.

There are several potential methods for control of cavity spot that require further investigation. Changes in cultural practices may help reduce the incidence of this disease. Based on information already known, the effects of temperature, moisture, planting time and location, and soil pH should be considered by the grower when making decisions as to when and where to plant carrots. As well, this information could be used to create a forecasting system to predict disease occurrence. However, past predictions of disease incidence have occasionally been incorrect (White, 1988), so further investigation into the effects of environmental factors on cavity spot development and the ecology of *Pythium* spp. is required to make disease prediction feasible.

Disease control may be aided through sanitation and crop rotation. Inoculum (expressed as colony forming units/unit of soil) must be present in the soil for disease to occur. As such, any method that decreases *Pythium* spp. population levels in the soil, such as removal of carrot debris that may be harbouring overwintering structures, and crop rotation with a non-host crop, such as onion (Schrandt *et al.*, 1993), should help to decrease disease severity.

The use of resistant carrot cultivars should reduce disease incidence in areas where environmental conditions favour disease development. There is also the potential for introducing resistance into susceptible, but otherwise desirable, varieties via breeding.

Another possibility for control lies in the area of biological control. There are several microorganisms found in soil that are antagonistic to *Pythium* spp. (Singh and Singh, 1984). Mechanisms of antagonism include

competition, antibiosis, lysis, parasitism, and predation. Control of diseases caused by other *Pythium* spp. has been achieved using these methods. Examples include: coating of cucumber seeds with antagonistic bacteria, which prevented damping-off (Elad and Chet, 1987); use of a fluorescent pseudomonad, which produced a chemical effective against damping-off in cotton (Howell and Stipanovic, 1980); use of *Arthrobacter*, which protected tomato plants against damping-off (Mitchell and Hurwitz, 1965); use of non-plant pathogenic *P. nunn*, which parasitized the hyphae of *P. ultimum*, displacing it from a previously occupied substrate (Paulitz and Baker, 1988). In addition, *Pythium* spp. are preyed upon by various nematodes, protozoans, mites, and springtails. Thus, there is much potential in the area of biological control for control of plant diseases caused by *Pythium* spp., and research is warranted.

Finally, the pathogen-host interaction should be elucidated. There may be a defense response by the host that explains why lesions are usually quite small. The basis for the differences in varietal susceptibility needs to be elucidated. It is not clear why only some species (and particularly mostly slow growing species) cause cavity spot, especially since both pathogenic and non-pathogenic species are isolated from cavity spot lesions.

The possibility that cavity spot in the field is influenced by interactions with other microorganisms in the soil should not be overlooked. As well, the role of secondary invaders should be studied to determine their effects on lesion development, and on the effects to the disease.

Appendix I. Seed sources for carrot variety trials

- Asgrow Canada, Bradford, Ontario, L3Z 2B2
- Bejo Seeds U.S.A., 1323 Fir St., Mount Vernon, Washington, 98273
- Breeder's Seeds Ltd., 17 Summerwood Lane, Halsall, Ormskirk, Lancashire, L39 8RQ
- Enza Zaden, Postbox 7, 1600 AA Enkhuizen, Holland
- Ferry-Morse Seed Co., P.O. Box 4983, Modesto, California, 95352
- Harris Moran Seed Co., P.O. Box 3091, Modesto, California, 95352
- Northrup King Co., P.O. Box 1827, Gilroy, California, 95021
- W. Osborne International Seed Co., 1679 Hwy. 99 South, Mount Vernon, Washington, 98273
- PetoSeed, P.O. Box 4206, Saticoy, California, 93004
- Stokes Seed Ltd., P.O. Box10, St. Catharines, Ontario, L2R 6R6
- Sunseeds Genetics, P.O. Box 1438, Technology Parkway, Building 11, Suite A, Hollister, California, 95024-1438

References

Adams, P. B. 1990. The potential of mycoparasites for biological control of plant diseases. Annu. Rev. Phytopathol. 28:59-72.

Adaskaveg, J. E., M. E. Staghellini, R. L. Gilbertson and N. B. Egen. 1988. Comparative protein studies of several *Pythium* species using isoelectric focusing. Mycologia 80:665-672.

Agnihorti, V. P. and O. Vaartaja. 1967. Root exudates from redpine seedlings and their effects on *Pythium ultimum*. Can. J. Bot. 45:1031-1040.

Agnihotri, V. P. and O. Vaartaja. 1970. Effects of seed exudates of *Pinus resinosa* on the germination of sporangia and on the population of *Pythium irregulare* in the soil. Plant Soil 32:246-249.

Al-Hamdani, A. M., R. S. Lutchmeah and R. C. Cooke. 1983. Biological control of *Pythium ultimum*-induced damping-off by treating cress seed with the mycoparasite *Pythium oligandrum*. Plant Pathol. 32:449-454.

Alfaro, A. A. and L. Mendoza. 1990. Four cases of equine bone lesions caused by *Pythium insidiosum*. Equine Vet. J. 22:227-228.

Barbetti, M. J. and G. C. MacNish. 1984. Effects of cultivation and cultural practices on subterranean clover root rot. Aust. J. Exp. Agric. Anim. Husb. 24:550-554.

Barbetti, M. J. 1991. Effects of clover-free rotations upon the severity of root rot and yield in regenerating subterranean clover pastures. Aust. J. Agric. Res. 42:1195-1204.

Barr, D. J. S. and W. C. Kemp. 1955. *Olpidium brassicae*, tobacco necrosis virus, and *Pythium* spp. in relation to rusty root of carrots in Ontario and Quebec. Can. Plant Dis. Surv. 55:77-82.

Barton, R. 1961. Saprophytic activity of *Pythium mamillatum* in soils II. Factors restricting *P. mamillatum* to pioneer colonizations of substrates. Trans. Br. Mycol. Soc. 44:105-118.

Bates, M. L. and M. E. Stanghellini. 1984. Root rot of hydroponically grown spinach caused by *Pythium aphanidermatum* and *P. dissotocum*. Plant Dis. 68:989-991.

Benard, D. and Z. K. Punja. 1993. Role of *Pythium* spp. in the development of cavity spot on carrots in British Columbia (Abstr.). Phytopathology 82:1091.

Berlese, A. N. and J. B. de Toni. 1888. Phycomycetes. Saccardo, syll. Fung 7:181-322.

Boehm, M. J. and H. A. J. Hoitink. 1992. Sustenance of microbial activity in potting mixes and its impact on severity of Pythium root rot of Poinsettia. Phytopathology 82:259-264.

Boehm, M. J. and H. A. Hoitink. 1992. Sustenance of microbial activity in potting mixes and its impact on severity of Pythium root rot of Poinsettia. Phytopathology 82:259-264.

Bradshaw-Smith, R. P., W. M. Whalley and G. D. Craig. 1991. Interactions between *Pythium oligandrum* and the fungal footrot pathogens of peas. Mycol. Res. 95:861-865.

Braun, P. G. 1991. The combination of *Cylindrocarpon lucidum* and *Pythium irregulare* as a possible cause of apple replant disease in Nova Scotia. Can. J. Plant Pathol. 13:281-297.

Burr, T. J. and M. E. Stanghellini. 1973. Propagule nature and density of *Pythium aphanidermatum* in field soil. Phytopathology 63:1499-1501.

Campbell, C. K. 1990. Pythiosis. Equine Vet. J. 22:227-228.

Chagnon, M. C. and R. R. Belanger. 1991. Tolerance in Greenhouse geraniums to *Pythium ultimum*. Plant Dis. 75:820-823.

Chamswarng, C. and R. J. Cook. 1985. Identification and comparative pathogenicity of *Pythium* species from wheat roots and wheat-field soils in the Pacific Northwest. Phytopathology 75:821-827.

Chang, T. T., X. Y. Yang and W. H. Ko. 1992. A sensitive method for detecting production of extracellular enzymes by fungi on solid media. Mycologia 84:923-926.

Chang-Ho, Y. 1970. The effects of pea root exudate on the germination of *Pythium aphanidermatum* zoospore cysts. Can. J. Bot. 48:1401-1514.

Chen, W., H. A. J. Hoitink and L. V. Madden. 1988a. Microbial activity and biomass in container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Phytopathology 78:1447-1450.

Chen, W., H. A. J. Hoitink and A. F. Schmitthenner. 1988b. The role of microbial activity in suppression of damping-off caused by *Pythium ultimum*. Phytopathology 78:314-322.

Chen, W., J. W. Hoy and R. W. Schneider. 1991. Comparisons of soluble proteins and isozymes for seven *Pythium* species and applications of the biochemical data to *Pythium* systematics. Mycol. Res. 95:548-555.

Chen, W. 1992. Restriction fragment length polymorphisms in enzymatically amplified ribosomal DNAs of three heterothallic *Pythium* species. Phytopathology 82:1467-1472.

Chen, W., J. W. Hoy and R. W. Schneider. 1992. Species-specific polymorphism in transcribed ribosomal DNA of five *Pythium* species. Exp. Mycol. 16:22-34.

Chen, W., R. W. Schneider and J. W. Hoy. 1992b. Taxonomic and phylogenetic analysis of ten *Pythium* species based on isozyme polymorphisms. Phytopathology 82:1234-1244.

Cherif, M., N. Benhamou and R. Belanger. 1991. Ultrastructural and cytochemical studies of fungal development and host reactions in cucumber plants infected by *Pythium ultimum*. Physiol. Molec. Plant Pathol. 39:353-375.

Clare, B. G. 1963. Starch gel electrophoresis of proteins as an aid in identifying fungi. Nature 200:803-804.

Clare, B. G., N. T. Flentje and M. R. Atkinson. 1968. Electrophoretic patterns of oxidoreductases and other proteins as criteria in fungal taxonomy. Aust. J. of Biol. Sci. 21:275-295.

Cook, R. J. and W. A. Haglund. 1982. Pythium root rot: A barrier to yield of Pacific Northwest wheat. Wash. State Coll. Agric. Rec. Bull. XB0913.

Cook, R. J. and K. F. Baker. 1983. The nature and practice of biological control of plant pathogens. Amer. Phytopath. Soc., St. Paul, MN.

Cook, R. J., B.-X. Zhang and A. Doerr. 1983. Failure of metalaxyl to control all of the Pythium population pathogenic on roots of Pacific Northwest wheat. Phytopathology 73:957.

Cook, R. J. and B. X. Zhang. 1985. Degrees of sensitivity to metalaxyl within the *Pythium* spp. pathogenic to wheat in the Pacific Northwest. Plant Dis. 69:686-688.

Cooper, R. C., Jr., N. Allison and J. G. Boring. 1991. Apparent successful surgical treatment of intestinal Pythiosis with vascular invasion in a dog. Canine Pract. 16:9-12.

Cornu, M. 1872. Monographie des saprolegniees. Ann. Sci. Nat. 15:5-198.

Couch, H. B. and B. D. Smith. 1991. Synergistic and antagonistic interactions of fungicides against *Pythium aphanidermatum* on perennial ryegrass. Crop Prot. 10:386-390.

Davidse, L. C., D. Looijen, L. J. Turkensteen and D. van der Wal. 1981. Occurrences of metalaxyl-resistant strains of *Phytophthora infestans* in dutch potato fields. Neth. J. Plant. Pathol. 87:65-68.

de Bary, A. 1881. Untersuchungen über die Peronosporeen und Saprolegnieen und die Grundlagen eines naturlichen Systems der Pilze. Abhandl. Senckenb. Naturf. Ges. 12:225-370.

de Cock, P. C., A. Hall and R. H. E. Inkson. 1981. Cavity spot of carrots. Anal. de Edafologia Agrobiologia XL:307-316.

de Cock, A. W. A., L. Mendoza, A. A. Padhye, L. Ajello and L. Kaufmen. 1987. *Pythium insidiosum* sp. nov. the etiologic agent of Pythiosis. J. Clin. Microbiol. 25:344-349.

Deacon, J. W. 1979. Cellulose decomposition by *Pythium* and its relevance to substrate groups of fungi. Trans. Br. Mycol. Soc. 72:469-477.

Desilets, H. and R. R. Belanger. 1991. An *in vitro* system for studying the effects of *Pythium ultimum* metabolites on *Pelargonium xhortorum*. Phytopathology 81:202-206.

Devaki, N. S., S. S. Bhat, S. G. Bhat and K. R. Manjunatha. 1992. Antagonistic activities of *Trichoderma harzianum* against *Pythium aphanidermatum* and *Pythium myriotylum* on tobacco. J. Phytopathol. 136:82-87.

Dewan, M. M. and K. Sivasithamparam. 1988. *Pythium* spp. in roots of wheat and ryegrass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis* var. *tritici*. Soil Biol. Biochem. 20:801-808.

Di Pietro, A., M. Gut-Rella, J. P. Pachlatko and F. J. Schwinn. 1992. Role of antibiotics produced by *Chaetomium globosum* in biocontrol of *Pythium ultimum*, a causal agent of damping-off. Phytopathology 82:131-135.

Dick, M. W. 1990. Keys to Pythium. College of Estate Management, Whiteknights, Reading, U.K. 64 pp.

Drechsler, C. 1939. Several species of *Pythium* causing blossom-end rot of watermelon. Phytopathology 29:391-422.

Drechsler, C. 1943. Two species of *Pythium* occuring in southern states. Phytopathology 33:285-299.

Dube, H. C. and K. Prabakaran. 1989. Cell wall degrading enzymes of *Pythium*. Persp. Plant Pathol. 1989:181-188.

Elad, Y. and I. Chet. 1987. Possible role for competition for nutrients in biocontrol of Pythium damping-off by bacteria. Phytopathology 77:190-195.

Endo, R. M. 1961. Turfgrass diseases in southern California. Plant Dis. Rep. 45:869-873.

Erceg, D., K. Sivasithamparam and P. O'Brien. 1993. Identification of *Pythium* isolates associated with cavity spot disease of carrots in Western Australia (Abstr.). 6th International Congress of Plant Pathology, Montreal, Canada, July 28-August 6 1993:149.

Fajola, A. O. and S. O. Alasoadura. 1975. Antagonistic effects of *Trichoderma harzianum* on *Pythium aphanidermatum* causing the damping-off disease of tobacco in Nigeria. Mycopathologia 57:47-52.

Fischer, A. 1892. Phycomycetes. Rabenhorst, Kryptogamenflora 4:505.

Foley, M. F. and J. W. Deacon. 1985. Isolations of *Pythium oligandrum* and other necrotrophic mycoparasites from soil. Trans. Br. Mycol. Soc. 85:631-639.

Foley, M. F. and J. W. Deacon. 1986. Physiological differences between mycoparasitic and plant-pathogenic *Pythium* spp. Trans. Br. Mycol. Soc. 86:225-231.

Gardner, D. E. and F. F. Hendrix Jr. 1973. Carbon dioxide and oxygen concentrations in relation to survival and saprophytic growth of *Pythium irregulare* and *Pythium vexans* in soil. Can. J. Bot.

Gindrat, D. 1976. Components of unbleached commercial chitin stimulate *Pythium ultimum* in sugarbeet spermosphere. Phytopathology 66:312-316.

Glynn, A. N. and J. Reid. 1969. Electrophoretic patterns of soluble fungal proteins and their possible use as taxonomic criteria in the genus *Fusarium*. Can. J. Bot. 47:1823-1831.

Goh, K. and M. S. Ali. 1983. Effects of nitrogen fertilizers, calcium and water regime on the incidence of cavity spot in carrot. Fert. Res. 4:223-230.

Goldberg, N. P., M. C. Hawes and M. E. Stanghellini. 1989. Specific attraction to and infection of cotton root cap cells by zoospores of *Pythium dissotocum*. Can. J. Bot. 67:1760-1767.

Goldberg, N. P., M. E. Stanghellini and S. L. Rasmussen. 1992. Filtration as a method for controlling Pythium root rot of hydroponically grown cucumbers. Plant Dis. 76:777-779.

Green, C. D. and T. Makin. 1985. Soil-borne transmission of cavity spot of carrots, grown in north Lincolnshire for processing. Crop Prot. 4:351-358.

Griffin, D. M. 1963. Soil physical factors and the ecology of fungi. II. Behaviour of *Pythium ultimum* at small soil water suctions. Trans. Br. Mycol. Soc. 46:368-372.

Groom, M. R. and D. A. Perry. 1985. Induction of 'cavity spot-like' lesions on roots of *Daucus carota* by *Pythium violae*. Trans. Br. Mycol. Soc. 84:755-757.

Guba, E. F., R. E. Young and T. Ui. 1961. Cavity spot disease of carrot and parsnip roots. Plant Dis. Rep. 45:102-105.

Gurusiddaiah, S., D. M. Weller, A. Sarkar and R. J. Cook. 1986. Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. Antimicrob. Agents Chemother. 29:488-495.

Hafidh, F. T. and W. C. Kelly. 1982. Cavity spot of carrots caused by feeding of the fungus gnat larvae. J. Amer. Soc. Hort. Sci. 107:1177-1181.

Hancock, J. G. 1977. Factors affecting soil population of *Pythium ultimum* in the San Joaquin Valley of California. Hilgardia 45:107-121.

Hancock, J. G. 1981. Longevity of *Pythium ultimum* in moist soil. Phytopathology 71:1033-1037.

Hancock, J. G. 1983. Seedling diseases of alfalfa in California. Plant Dis. 67:1203-1208.

Hancock, J. G. 1985. Fungal infection of feeder rootlets of alfalfa. Phytopathology 75:1112-1120.

Hancock, J. G. 1991. Seedling and rootlet diseases of forage alfalfa caused by *Pythium irregulare*. Plant Dis. 75:691-694.

Hankin, L. and S. L. Anagnostakis. 1975. The use of solid media for detection of enzyme production by fungi. Mycologia 67:597-607.

Haskins, R. H. 1963. Morphology, nutrition and host range of a species of *Pythium*. Can. J. Microbiol. 9:451-457.

Hendrix, F. F. J., W. M. Powell and J. H. Owen. 1966. Relation of root necrosis caused by *Pythium* species to peach tree decline. Phytopathology 56:1229-1232.

Hendrix, F. F. and W. A. Campbell. 1973. *Pythiums* as plant pathogens. Annu. Rev. Phytopathol. 11:77-98.

Hine, R. B. and E. E. Trujillo. 1966. Manometric studies on residue colonization in soil by *Pythium aphanidermatum* and *Phytophthora parasitica*. Phytopathology 56:334-336.

Hodges, C. F. and L. W. Coleman. 1985. *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. Plant Dis. Rep. 69:336-340.

Howard, R. J. and P. H. Williams. 1976. Methods for detecting resistance to *Pythium* and *Rhizoctonia* root diseases in seedling carrots. Plant Dis. Reptr. 60:151-156.

Howard, R. J., R. G. Pratt and P. H. Williams. 1978. Pathogenicity to carrots of *Pythium* species from organic soils of North America. Phytopathology 68:1293-1296.

Howell, C. R. and R. D. Stipanovic. 1980. Suppression of *Pythium ultimum*-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotc pyoluteorin. Phytopathology 70:712-715.

Howell, C. R. and R. D. Stipanovic. 1983. Gliovirin, a new antibiotic from *Gliocladium virens* and its role in the biological control of *Pythium ultimum*. Can. J. Microbiol. 29:321-324.

Hoy, J. W. and R. W. Schneider. 1988. Role of *Pythium* in sugarcane stubble decline: effects on plant growth in field soil. Phytopathology 78:1692-1696.

Huang, J. W. and E. G. Kuhlman. 1990. Fungi associated with damping-off of slash pine seedlings in Georgia. Plant Dis. 74:

Huang, J. W. and E. G. Kuhlman. 1991. Formulation of a soil amendment to control damping-off of slash pine seedlings. Phytopathology 81:163-170.

Ichihara, A., M. Hashimoto and K. Sakamura. 1985. (3S, 5Z) - (-) -3-Hydroxy-5-dodecenoic acid, a phytotoxic metabolite of *Pythium ultimum*. Agric. Biol. Chem. 49:2207-2209.

Ichitana, T., T. Tani and T. Umakoshi. 1986. Identification of *Pythium* spp. pathogenic on Manila grass (*Zoysia matrella* Merr.). Trans. Mycol. Soc. Jpn. 27:41-50.

Inbar, Y., M. J. Boehm and H. A. J. Hoitink. 1991. Hydrolysis of fluorescein diacetate in sphagnum peat container media for predicting suppressiveness to damping-off caused by *Pythium ultimum*. Soil Biol. Biochem. 23:479-483.

Jacobsohn, R., D. Zutra, H. Dan and Y. Kelman. 1984. Studies of carrot cavity spot. J. Hort. Sci. 59:529-535.

Janardhanan, K. K. and H. Akhtar. 1974. Production of a toxic metabolite and pectolytic enzyme by *Pythium butleri*. Mycopathologia and Mycologia Applicata 52:325-330.

Jeffers, S. N., H. S. Aldwinckle, T. J. Burr and P. A. Arneson. 1982. *Phytophthora* and *Pythium* species associated with crown rot in New York apple orchards. Phytopathology 72:533-538.

Jeffers, S. N. and S. B. Martin. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Dis. 70:1038-1043.

Johnson, L. F., P. Qian and R. S. Ferris. 1990. Soil matric potential effects on changes in wall morphology, germination, and lysis of oospores of *Pythium ultimum*. Phytopathology 80:1357-1361.

Kalu, N. N., J. C. Sutton and O. Vaartaja. 1976. *Pythium* spp. associated with root dieback of carrot in Ontario. Can. J. Plant Sci. 56:555-561.

Kraft, J. M. and D. C. Erwin. 1967. Stimulation of *Pythium aphanidermatum* by exudates from mung bean seeds. Phytopathology 57:866-868.

Kraft, J. M., R. M. Endo and D. C. Erwin. 1967. Infection of primary roots of bentgrass by zoospores of *Pythium aphanidermatum*. Phytopathology 57:86-90.

Kreutzer, W. A. 1978. Soil Treatment. In: Plant Pathology: An Advanced Treatise, Vol.3. J.G. Horsfall and A.E. Dimond (eds.). Academic Press, New York 431-476.

Kulkarni, R. N., A. Kalra and N. S. Ravindra. 1992. Integration of soil solarization with host resistance in the control of dieback and collar and root rot diseases of periwinkle. Trop. Agric. 69:217-222.

Laing, S. A. K. and J. W. Deacon. 1990. Aggressiveness and fungal host ranges of mycoparasitic *Pythium* species. Soil Biol. Biochem. 22:905-911.

Laing, S. A. K. and J. W. Deacon. 1991. Video microscopical comparison of mycoparasitism by *Pythium oligandrum, P. nunn* and an unnamed *Pythium* species. Mycol. Res. 95:469-479.

Larsson, M. and B. Gerhardson. 1992. Disease progression and yield from root diseases caused by soilborne pathogens of spinach. Phytopathology 82:403-406.

Lee, Y. S. and J. W. Hoy. 1992. Interactions among *Pythium* species affecting root rot of sugarcane. Plant Dis. 76:735-739.

Levesque, C. A., T. C. Vrain and S. H. DeBoer. 1993. Production of species specific probes for different *Pythium* species using amplified ribosomal DNA. Phytopathology (In press):in press.

Lewis, K., J. M. Whipps and R. C. Cooke. 1989. Mechanisms of biological disease control with special reference to the case study of *Pythium oligandrum* as an antagonist. In *Biotechnology of Fungi for Improving Plant Growth*, ed. J.M. Whipps and R.D. Lumsden. Cambridge, U.K. : Cambridge University Press. pp. 191-217.

Liddell, C. M., R. M. Davis, J. J. Nunez and J. P. Guerard. 1989. Association of *Pythium* spp. with carrot root dieback in the San Joaquin Valley of California. Plant Dis. 73:246-249.

Lifshitz, R., M. Dupler, Y. Elad and R. Baker. 1984a. Hyphal interactions between a mycoparasite, *Pythium nunn*, and several soil fungi. Can. J. Microbiol. 30:1482-1487.

Lifshitz, R., B. Sneh and R. Baker. 1984b. Soil suppressiveness to a plant pathogenic *Pythium* species. Phytopathology 74:1054-1061.

Lifshitz, R., M. E. Stanghellini and R. Baker. 1984c. A new species of *Pythium* isolated from soil in Colorado. Mycotaxon 20:373-379.

Lifshitz, R. and M. T. Windham. 1986. Mechanism of biological control of preemergence damping-off of pea by seed treatment with *Trichoderma* spp. Phytopathology 76:720-725.

Lipps, P. E. and G. W. Bruehl. 1978. Snow rot of winter wheat in Washington. Phytopathology

Long, E. 1985. Cavity spot - the culprits are *Pythium* fungi. Grower, March 14:15-16.

Lumsden, R. D. and W. A. Ayers. 1975. Influence of soil environment on the germinability of constitutively dormant oospores of *Pythium ultimum*. Phytopathology 65:1101-1107.

Lumsden, R. D. and J. C. Locke. 1989. Biological control of damping-off caused by *Pythium ultimum* and *Rhizoctonia solani* with *Gliocladium virens* in soilless mix. Phytopathology 79:361-366.

Lyons, N. F. and J. G. White. 1992. Detection of *Pythium violae* and *Pythium sulcatum* in carrots with cavity spot using competition ELISA. Ann. Appl. Biol. 120:235-244.

Lyshol, A. J., L. Semb and G. Taksdal. 1984. Reduction of cavity spot and root dieback in carrots by fungicide applications. Plant Pathol. 33:193-198.

Martin, F. N. and J. G. Hancock. 1986. Association of chemical and biological factors in soils suppressive to *Pythium ultimum*. Phytopathology 76:1221-1231.

Martin, F. N. and J. G. Hancock. 1987. The use of *Pythium oligandrum* for biological control of pre-emergence damping-off caused by *P. ultimum*. Phytopathology 77:1013-1020.

Martin, F. N. 1991a. Linear mitochondrial molecules and intraspecific mitochondrial genome stability in a species of *Pythium*. Genome 34:156-162.

Martin, F. N. 1991b. Selection of DNA probes specific for isolates or species in the genus *Pythium*. Phytopathology 81:742-746.

Martin, F. N. 1991c. Characterization of circular mitochondrial plasmids in three *Pythium* spp. Curr. Genet. 20:91-97.

Martin, F. N. 1991d. Pythium. QK 1:39-49.

Maurhofer, M., C. Keel, U. Schnider, C. Voisard, D. Hass and G. Defago. 1992. Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHA0 on its disease suppressive capacity. Phytopathology 82:190-195.

Maynard, D. N., B. Gersten, E. F. Vlach and H. F. Vernell. 1961. The effects of micronutrient concentration and calcium levels on the occurrence of carrot cavity spot. J. Amer. Soc. Hort. Sci. 78:339-342.

Maynard, D. N., B. Gersten, R. E. Young and H. F. Vernell. 1963. The influence of plant maturity and calcium level on the occurrence of carrot cavity spot. J. Amer. Soc. Hort. Sci. 83:506-510.

McDonald, M. R. and J. C. Sutton. 1992. Horizontal resistance to cavity spot in commercial carrot cultivars (Abstr.). Can. J. Plant Pathol. 14:245.

McDonald, M. R. and J. C. Sutton. 1993. Influence of cultivar, plant age, rainfall, and soil temperature on the development of cavity spot of carrot (Abstr.). 6th International Congress of Plant Pathology, Montreal, Canada, July 28-August 6 1993:

Mellano, H. M., D. E. Munnecke and J. J. Sims. 1970. Relationship of pectic enzyme activity and presence of sterols to pathogenicity of *Pythium ultimum* on roots of *Antirrhinum majus*. Phytopathology 60:943-950.

Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of the genus *pythium*. Memoirs of the Torrey Botanical Club 20:1-171.

Mildenhall, J. P. and P. H. Williams. 1970. *Rhizoctonia* crown rot and cavity spot of muck-grown carrots. Phytopathology 60:887-889.

Mildenhall, J. P., R. G. Pratt, P. H. Williams and J. E. Mitchell. 1971. Pythium brown root and forking of muck-grown carrots. Plant Dis. Rep. 55:536-540.

Mircetich, S. M. 1971. The role of *Pythium* in feeder roots of diseased and symptomless peach trees and in orchard soils in peach tree decline. Phytopathology 61:357-360.

Mitchell, R. and R. Hurwitz. 1965. Suppression of *Pythium debaryanum* by lytic rhizosphere bacteria. Phytopathology 55:156-158.

Mojdehi, L. L., H. A. Singleton, Meloux and G. R. Waller. 1990. Reproduction of symptoms of a root disease of wheat by toxic metabolites produced by two *Pythium* species and their partial characterization. J. Phytopathol. 128:246-256.

Montfort, F. and F. Rouxel. 1988. La maladie de la "tache" de la carrote due a *Pythium violae* Chester et Hickman: donnees symptomatologiques et etiologiques. Agronomie Paris 8:701-706.

Mukhopadhyay, A. N., A. Brahmbhatt and G. J. Patel. 1986. *Trichoderma harzianum* a potential biocontrol agent for tobacco damping-off. Tob. Res. 12:26-35.

Napi-Acedo, G. and O. R. Exconde. 1965. Penetration and infection of corn roots by *Pythium arrhenomanes*. Philippine Agricultura 49:279-293.

Nelson, E. B. 1987. Rapid germination of sporangia of *Pythium* species in response to volatiles from germinating seeds. Phytopathology 77:1108-1112.

Nelson, E. B. and C. M. Craft. 1989. Comparative germination of culture-produced and plant-produced sporangia of *Pythium ultimum* in response to soluble seed exudates and exudate components. Phytopathology 79:1009-1013.

Nelson, E. B. and C. M. Craft. 1991. Identification and comparative pathogenicity of *Pythium* spp. from roots and crown of turfgrasses exhibiting symtoms of root rot. Phytopathology 81:1529-1536.

Nelson, E. B. and C. M. Craft. 1992. A miniaturized and rapid bioassay for the selection of soil bacteria suppressive to Pythium blight of turfgrasses. Phytopathology 82:206-210.

Nemec, S. 1972. Histopathology of *Pythium* infected strawberry roots. Can. J. Bot. 50:1091-1096.

O'Brien, R. G. O., P. J. O'Hare and R. J. Glass. 1991. Cultural practices in the control of bean root rot. Aust. J. Exp. Agric. 31:551-555.

Odermatt, W. and H. Snow. 1989. 1989 carrot varietal demonstrations. British Columbia Ministry of Agriculture and Fisheries, Cloverdale, B.C.

Ormrod, D. J. 1973. Newsletter: Cavity spot. British Columbia Ministry of Agriculture and Fisheries, Cloverdale B.C.

Papavizas, G. C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu. Rev. Phytopathol. 23:23-54.

Parke, J. L. 1990. Population dynamics of *Pseudomonas cepacia* in the pea spermosphere in relation to biocontrol of *Pythium*. Phytopathology 80:1307-1311.

Paulitz, T. C. and R. Baker. 1987a. Biological control of Pythium damping-off of cucumbers with *Pythium nunn*: population dynamics and disease suppression. Phytopathology 77:335-340.

Paulitz, T. C. and R. Baker. 1987b. Biological control of Pythium damping-off of cucumbers with *Pythium nunn*: influence of soil environment and organic amendments. Phytopathology 77:341-346.

Paulitz, T. C. and R. Baker. 1988. Interactions between *Pythium nunn* and *Pythium ultimum* on bean leaves. Can. J. Microbiol. 34:947-951.

Paulitz, T. C. 1991. Effects of *Pseudomonas putida* on the stimulation of *Pythium ultimum* by seed volatiles of pea and soybean. Phytopathology 81:1282-1287.

Perry, D. A. 1972. Excess potash link to cavity spot. Grower, April:19.

Perry, D. A. and J. G. Harrison. 1979. Cavity spot of carrots. II. The effects of soil conditions and the role of pectolytic anaerobic bacteria. Ann. Appl. Biol. 93:109-115.

Perry, D. A. 1983. Effect of soil cultivation and anaerobiosis on cavity spot of carrots. Ann. Appl. Biol. 103:541-547.

Plaats-Niterink, A. J., Van der. 1981. Monograph of the genus *Pythium*. Studies in Mycology, Centraalbureau voor Schimmelcultures, Baarn 21:1-242.

Ploetz, R. C., D. J. Mitchell and R. N. Gallaher. 1985. Population dynamics of soilborne fungi in a field multicropped to rye and soybeans under reduced tillage in Florida. Phytopathology 75:1447-1451.

Pringsheim, N. 1858. Beitrage zur morphologie und systematik der algen. 2. die saprolegnieen. Jb. wiss. Bot. 1:284-306.

Punja, Z. K. 1990. Association of *Pythium* spp. and other soil borne fungi with cavity spot lesions on fresh market carrots grown in muck soils (Abstr.). Can. J. Plant Pathol. 12:338.

Punja, Z. K. and D. Benard. 1992. Incidence, pathogenicity, and pathogen-host interactions of *Pythium* spp. causing cavity spot on carrots in the Fraser Valley of British Columbia (Abstr.). Can. J. Plant Pathol. 13:284.

Punja, Z. K., S. Chittaranjan and M. M. Gaye. 1992. Development of black root rot caused by *Chalara elegans* on fresh market carrots. Can. J. Plant Pathol. 14:299-309.

Robertson, G. I. 1973. Pathogenicity of *Pythium* spp. to seeds and seedling roots. N. Z. J. Agric. Res. 16:367-373.

Sadebeck, R. 1887. Original berichte gelehrter gesellschaften. Botanisches Centralblatt 29:318-319.

Sanders, P. L. 1984. Failure of metalaxyl to control pythium blight on turfgrass in pennsylvania. Plant Dis. 68:776-777.

Saunders, G. A., J. O. Washburn, D. E. Egerter and J. A. Anderson. 1988. Pathogenicity of fungi isolated from field-collected larvae of the western treehole mosquito, *Aedes sierrensis* (Diptera: Culicidae). J. Invert. Path. 52:360-363.

Scaife, M. A., M. K. Turner, A. Barnes and J. Junt. 1983. Cavity spot of carrots - observations on a commercial crop. Ann. Appl. Biol. 102:567-575.

Schlub, R. L. and A. F. Schmitthenner. 1978. Effects of soybean seed coat cracks on seed exudation and seedlings quality in soil infested with *Pythium ultimum*. Phytopathology 68:1186-1191.

Schrandt, J. K., R. M. Davis and J. J. Nunez. 1993. Host range and influence of nutrition, temperature, and pH on growth of *Pythium violae* from carrot. Plant Dis. (In press).

Schroter, J. 1897. Pythiaceae. Engler & Prantl, nat PflFam. 1:104-105.

Scott, W. W. and A. H. O'Brien. 1962. Aquatic fungi associated with diseased fish and fish eggs. Progve. Fish Cult. 24:3-14.

Smiley, R. W. 1983. Compendium of turfgrass diseases. Amer. Phytopathol. Soc., St. Paul, MN

Smith, W. K. 1958. A survey of the production of pectic enzymes by plant pathogenic and other bacteria. J. Gen. Microbiol. 18:33-41.

Soroker, E., Y. Bashan and Y. Okon. 1984. Reproducible induction of cavity spot in carrots and physiological and microbial changes occurring during cavity spot formation. Soil Biol. Biochem. 16:541-548.

Stanghellini, M. E. and J. D. Russel. 1971. Damping-off of tomato seedlings in commercial hydroponic culture. Prog. Agric. Ariz. 23:15-16.

Stanghellini, M. E. and J. G. Hancock. 1971a. The sporangium of *Pythium ultimum* as a survival structure in soil. Phytopathology 61:157-154.

Stanghellini, M. E. and J. G. Hancock. 1971b. Radial extent of the bean spermosphere and its relation to the behavior of *Pythium ultimum*. Phytopathology 61:165-168.

Stanghellini, M. E. and W. L. Kronland. 1986. Yield loss in hydroponically grown lettuce attributed to subclinical infection of feeder roots by *Pythium dissotocum*. Plant Dis. 70:1053-1056.

Sutton, J. C. 1975. *Pythium* spp. produce rusty root of carrots in Ontario. Can. J. Plant Sci. 55:139-143.

Sweet, J. B., R. E. Beale and I. R. Wright. 1989. Cavity spot disease in six carrot cultivars treated with a metalaxyl and thiram fungicide. Tests of Agrochemicals and Cultivars No. 10 (Ann. Appl. Biol. Supplement) 114:38-39.

Tedla, T. and M. E. Stanghellini. 1992. Bacterial population dynamics and interactions with *Pythium aphanidermatum* in intact rhizosphere soil. Phytopathology 82:652-656.

Thompson, R. J. and R. G. Burns. 1989. Control of *Pythium ultimum* with antagonistic fungal metabolites incorporated into sugar beet seed pellets. Soil Biol. Biochem. 21:745-748.

Utkhede, R. S. 1987. Chemical and biological control of crown and root rot of apple caused by *Phytophthora cactorum*. Can. J. Plant Pathol. 9:295-300.

Utkhede, R. S. and E. M. Smith. 1991. *Phytophthora* and *Pythium* species associated with root rot of young apple trees and their control. Soil Biol. Biochem. 23:1059-1063.

Veseley, D. 1977. Potential biological control of damping-off pathogens in emerging sugar beet by *Pythium oligandrum*. Phytopathol. Z. 90:113-115.

Veseley, D. 1979. Use of *Pythium oligandrum* to protect emerging sugar-beet. In *Soil-Borne Plant Pathogens*, ed. B. Schippers & W. Gams. Academic Press: London. pp. 593-595.

Vestberg, M. 1990. Occurrence and pathogenicity of *Pythium* spp. in seedling roots of winter rye. J. Agric. Sci. Finl. 62:275-284.

Vivoda, E., R. M. Davis, J. J. Nunez and J. P. Guerard. 1991. Factors affecting the development of cavity spot of carrot. Plant Dis. 75:519-522.

Wagenvoort, W. A., I. Babik and G. R. Findenegg. 1985. The effect of oxygen supply and calcium levels in hydroponic culture on the occurrence of carrot cavity spot. Soilless Culture 1:67-72.

Whipps, J. M., K. Lewis and R. C. Cooke. 1988. Mycoparasitism and plant disease control. Fungi and Biological Control Systems 161-187.

White, J. G. 1986. The association of *Pythium* spp. with cavity spot and root dieback of carrots. Ann. Appl. Biol. 108:265-273.

White, J. G., B. D. Dowker and T. C. Crowther. 1987. Screening carrot cultivars against *Pythium* spp. associated with cavity spot. Tests of Agrochemicals and Cultivars No. 8 (Ann. Appl. Biol. Supplement) 110:138-139.

White, J. G. and A. J. Wakeham. 1987. Responses of *Pythium* spp. associated with cavity spot of carrots to metalaxyl and related fungicides. Tests of Agrochemicals and Cultivars No. 8 (Ann. Appl. Biol. Supplement) 110:52-53.

White, J. G. 1988. Studies on the biology and control of cavity spot of carrots. Ann. Appl. Biol. 113:259-268.

White, J. G., B. D. Dowker, T. C. Crowther and A. J. Wakeham. 1988. Laboratory screening of carrot cultivars with reported differential field performance for cavity spot to three *Pythium* spp. Tests of Agrochemicals and Cultivars No. 9 (Ann. Appl. Biol. Supplement) 112:76-77.

White, J. G. 1989. Cavity spot of carrots indicates widespread occurrence of little known slow-growing *Pythium* spp. Proceedings of the International Symposium on *Phytophthora;* Dublin (Abstr.) 36.

White, G. 1991. Curing spotty carrots. Grower, June:9-10.

Wilhelm, S. 1965. *Pythium ultimum* and the soil fumigation growth response. Phytopathology 55:1016-1020.

Williets, H. M., A. R. Thompson and G. E. L. Morris. 1989. Laboratory studies on the selection of *Pythium ultimum*, a plant-pathogenic fungus, by *Onychiurus auranticus* (Collembola). Aspects Appl. Biol. 22:373-378.

Winstead, N. N. and C. L. McCombs. 1961. Pectinolytic and cellulolytic enzyme production by *Pythium aphanidermatum*. Phytopathology 51:209-268.

Wisbey, B. D., R. J. Copeman and T. A. Black. 1977. Other incitants associated with Pythium root dieback of muck-grown carrots in British Columbia. Can. J. Plant Sci. 57:235-241.

Wolffhechel, H. and D. F. Jensen. 1992. Use of *Trichoderma harzianum* and *Gliocladium virens* for the biological control of post-emergence damping-off and root rot of cucumbers caused by *Pythium ultimum*. J. Phytopathol. 136:221-230.

Zopf, W. 1890. Die pilze in morphologischer, physiologischer, biologischer und systematischer beziehung. A. Schenk, Handb. Bot. 4:271-755.