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UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Study of *Pythium* root diseases of hydroponically grown crops, with emphasis on lettuce

by

Cornelia Gull

Submitted to the Faculty of Natural and Agricultural Sciences
Department of Microbiology and Plant Pathology
UNIVERSITY OF PRETORIA

In partial fulfillment of the requirements for
the degree of MSc(Agric)

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The Lord, without Whom nothing is possible.

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CHAPTER 1

GENERAL INTRODUCTION

The history of hydroponics dates back to the seventeenth century, with commercial use commencing in the early 1940's (Zinnen, 1988; Stanghellini & Rasmussen, 1994). Hydroponic systems are currently employed worldwide to grow high cash value crops such as vegetable, flower, foliage and bedding plants. According to Paulitz (1997), the proportion of vegetables produced in hydroponic systems has been increasing in Europe and Canada, particularly for tomato (*Lycopersicon esculentum* Mill.), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L.), peppers (*Capsicum* spp.) and spinach (*Spinacea oleracea* L.). Minor crops include watercress (*Nasturtium officinale* L.), various herbs and spices. In South Africa hydroponically grown crops mainly include tomato, cucumber, pepper, lettuce, brinjal (*Solanum melongena* L.) and strawberry (*Fragaria* sp.), covering approximately 800 hectares (P. Langenhoven - personal communication). Plants are grown using nutrient solutions with or without solid substrates for root support. Hydroponic systems without substrates include the nutrient film technique, deep flow technique, trough culture, and ebb-and-flow systems. Plants can also be cultured in sand, rockwool, or in bags containing peat or sawdust. The nutrient solution can either be recirculated (closed system) or drained after one use (open system) (Jenkins & Averre, 1983; Bates & Stanghellini, 1984; Stanghellini & Rasmussen, 1994).

Although initial capital investment is high, hydroponic systems have several advantages over conventional cultivation in soil. Firstly, inert media, mechanically supporting the plants, provide more consistent rooting conditions for the crop (Zinnen, 1988). Secondly, nutrient regimes and watering are tailored to fit the physiological age of the crop and prevailing environmental conditions. Plant nutrition and the physical environment can be tightly controlled by the grower, resulting in higher yields, better quality and control of crop scheduling. All the elements in the nutrient solution are readily available to the plant, so competition for nutrients can be reduced and greater plant densities can be used (Paulitz, 1997). The third advantage is the avoidance, theoretically at least, of certain root diseases (Bates & Stanghellini, 1984; Zinnen, 1988; Goldberg & Stanghellini, 1990b).

Cultivation in hydroponic systems results in a decrease in the diversity of root-infecting microorganisms compared to conventional culture in soil, but certain types of diseases have become more prominent and damaging in these systems (Stanghellini & Rasmussen, 1994; Paulitz, 1997). Infectious agents, once introduced into the system, are favoured as a result of the abundance of a genetically uniform host, a physical environment with a more constant temperature and moisture regime and a mechanism for the rapid and uniform dispersal of root-infecting agents throughout the cultural system (Favrin *et al.*, 1988; Zinnen, 1988; Stanghellini & Rasmussen, 1994). Hydroponic systems lack the microbial diversity and biological 'buffering' found in natural soils (Paulitz, 1997). Without competition from other microbes the pathogen may quickly become established in the substrate and cause severe disease.

The most important fungal pathogens in hydroponic systems are zoosporic species, being favoured by an aquatic environment (Price & Fox, 1986; Goldberg et al., 1992; Stanghellini & Rasmussen, 1994; Sanchez *et al.*, 2000). *Pythium* is one of the most common and destructive pathogens of crops in recirculating hydroponic systems (Goldberg & Stanghellini, 1990a; Cherif *et al.*, 1994; Stanghellini *et al.*, 1996, 2000). According to Hendrix & Campbell (1973), *Pythium* spp. have a poor competitive ability in soil relative to other root-colonising organisms but often act as primary colonisers of plant tissue. However, in hydroponic production systems, low populations of other microbes and the effective dissemination of zoospores through the nutrient solution increase the potential for disease development (Rankin & Paulitz, 1994).

Root and crown rot and yield reductions caused by *Pythium* spp. have been reported on various hydroponically grown vegetable crops (Stanghellini *et al.*, 1984) particularly cucumber, lettuce, spinach, peppers and tomato (Moulin *et al.*, 1994; Buysens *et al.*, 1995). In South Africa *Pythium* and *Phytophthora* are responsible for most of the root diseases in hydroponically grown crops and are particularly a problem in recirculating systems (A. H. Thompson - personal communication). *Pythium* is present in nearly all hydroponic systems and often infects plants through sites of damage, such as root injury caused during transplanting, or by mineral toxicities, nutrient stagnation or excessive temperatures (Morgan, 1999).

Whilst *Pythium aphanidermatum* (Edson) Fitzp. is probably the most widely reported (Jenkins & Averre, 1983; Rankin & Paulitz, 1994; McCullagh *et al.*, 1996; Wulff *et al.*, 1998) various *Pythium* species are capable of causing disease. Damage caused by *Pythium* ranges from very

severe (100 % loss) to light to moderate root or stem damage. In contrast to soil culture where older plants are not as susceptible to damage by *Pythium* spp., damage can be severe in older hydroponically grown plants, with extensive root rot and subsequent plant death (Jenkins & Averre, 1983). According to Stanghellini & Kronland (1986), Moulin *et al.* (1994) and Cherif *et al.* (1997), yield losses can also occur in the absence of any obvious root necrosis and *Pythium* is consistently isolated even from apparently healthy root systems. Factors influencing infection include inoculum density, soil moisture, soil temperature, pH, cation composition, light intensity, and presence and numbers of other microorganisms. Which factor is more important in a given instance often depends on the *Pythium* sp. involved (Hendrix & Campbell, 1973).

The reservoir water used in a hydroponic system as well as the root residues which remain in the hydroponic substrate after a crop has been harvested, could be possible sources of continuous infestation (Menzies & Belanger, 1996; Sanchez *et al.*, 2000). Gardiner *et al.* (1990) noted that during outbreaks of *Pythium* root rot, populations of fungus gnats (*Bradysia impatiens* Johannsen) were very high. It has therefore been suggested that fungus gnat as well as shore flies (*Scatella stagnalis* Fallen) could be potential vectors of *Pythium* (Goldberg & Stanghellini, 1990a; Rankin. & Paulitz, 1994; Stanghellini & Rasmussen, 1994).

To implement effective control procedures it is necessary to ascertain the source(s) responsible for introduction of the pathogen (Goldberg & Stanghellini, 1990b; Stanghellini & Rasmussen, 1994) and to identify the *Pythium* species responsible for yield reductions (Moulin *et al.*, 1994). In this study *Pythium* species. infecting the most important crops in selected hydroponic systems in South Africa were identified, their pathogenicity assessed and the disinfestation of gravel substrate investigated.

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CHAPTER 2

PYTHIUM SPECIES ASSOCIATED WITH WILT AND ROOT ROT OF HYDROPONICALLY GROWN CROPS IN SOUTH AFRICA

Abstract

Eight *Pythium* species, *P. acanthicum*, *P. aphanidermatum*, *P. coloratum*, *P. diclinum*, *P. irregulare*, *P. myriotylum*, *P. perplexum*, *P. spinosum*, and representatives of five heterothallic *Pythium* groups, F, G, HS, P and T, were isolated from roots and crowns of crops and from nutrient solutions, substrates, water sources and run-off water in 11 hydroponica in South Africa. *P.* group F was isolated most frequently and from the greatest variety of crops, followed by *P. irregulare*, *P. spinosum*, *P. aphanidermatum* and *P.* group HS. Various new *Pythium*/host associations for South Africa were recorded, e.g. *P. acanthicum* on strawberry, *P. aphanidermatum* on parsley, *P. coloratum* on lettuce, *P. irregulare* on Chinese cabbage and lettuce, *P. perplexum* on tomato, *P.* group G on lettuce, and *P.* group HS on cucumber and lettuce. In artificial inoculation studies, *P.* group F proved to be pathogenic to various lettuce cultivars and to the herbs endive, fennel and sorrel. *P. spinosum* was highly virulent on cucumber.

INTRODUCTION

Root infection by *Pythium* species poses a major constraint to the cultivation of crops in hydroponic systems (Stanghellini & Rasmussen, 1994). Of the various *Pythium* species associated with hydroponically-grown crops, *Pythium aphanidermatum* (Edson) Fitzp. has been reported the most frequently and from the greatest variety of crops (Bates & Stanghellini, 1984; Stanghellini, 1984; Zhou & Paulitz, 1993; Chérif *et al.*, 1994; Moulin *et al.*, 1994; Menzies & Belanger, 1996; Stanghellini *et al.*, 1996; Paulitz, 1997). Another important species is *Pythium ultimum* Trow. It has been recorded as a pathogen on hydroponically grown cucumber (*Cucumis sativus* L.), geranium (*Pelargonium hortorum* Baily), lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) (Zinnen, 1988; Hausbeck *et al.*, 1989). *Pythium dissotocum* Drechsler and *Pythium tracheiphilum* Matta are known to attack hydroponically grown lettuce, the latter particularly under

cool conditions (Tortolero & Sequeira, 1978; Stanghellini & Kronland, 1986; Moller & Hockenhull, 1997). *P. dissotocum* is mostly associated with subclinical infections, though infection can result in significant yield losses (Stanghellini & Kronland, 1986; Favrin *et al*, 1988). *Pythium myriotylum* Drechsler commonly occurs in irrigation water and causes root rot of cucumber, lettuce and tomato (Gill, 1970; Jenkins & Averre, 1983; Schuerger & Pategas, 1985). Other *Pythium* species and groups that have been associated with hydroponically grown crops include *Pythium spinosum* Sawada, *Pythium irregulare* Buisman, *Pythium coloratum* Vaartaja and *Pythium* groups F and G (Favrin *et al*, 1988; Chen *et al*., 1992; McCullagh *et al*., 1996; Chérif *et al*., 1997).

It is estimated that commercial hydroponica in South Africa cover an area of approximately 800 ha. Although local production manuals, e.g. Lewies (1998) and Thompson & Labuschagne (2001), refer to the presence of *Pythium* species such as *P. aphanidermatum*, *Pythium aristosporum* Vanterpool, *P. dissotocum*, *P. myriotylum* and *P.* groups F and G on crops in closed hydroponic systems, no survey of *Pythium* species occurring in hydroponica in the country has yet been conducted. To propose control measures based on accurate experiments it is necessary to determine which *Pythium* species are responsible for yield reductions in a particular hydroponicum (Moulin *et al*., 1994). The purpose of this study therefore was to identify the *Pythium* species and groups associated with root disease of crops in selected hydroponica in South Africa.

MATERIALS AND METHODS

Survey of *Pythium* species in hydroponic systems

Celery (*Apium graveolens* L. var. *dulce* (Mill.) Pers.), Chinese cabbage (*Brassica rapa* L. subsp. *Pekinensis* Laur.), cucumber, lettuce, parsley (*Petroselinum crispum* (Mill.) A.W. Hill), strawberry (*Fragaria* sp.) and tomato plants exhibiting stunted growth, wilt and root rot, as well as plants without visible disease symptoms, were collected between 1998 and 2001 from eight hydroponica in South Africa (Table 1). Samples were also taken of other potential sources of *Pythium* infestation, e.g. water sources, growth media and substrates (Goldberg & Stanghellini, 1990; Stanghellini & Rasmussen, 1994), in five of the above and three additional hydroponica.

Plants were collected at various stages of maturity and transported in plastic bags to the laboratory in Pretoria. Four to five water samples were taken at 5 to 10 minute intervals at four hydroponica by submerging a sterilised 1 l Schott bottle 2-3 cm deep into the water source until the bottle was filled with water. Run-off water was collected from three to five randomly selected hydroponic beds in four hydroponica. The contents of the bottles from each source at each hydroponicum were pooled and taken to the laboratory. Growth medium and substrate samples were randomly collected in plastic bags at five hydroponica.

In the laboratory, roots and crowns were rinsed in sterile distilled water (SDW) till clean. In accordance with Stanghellini & Kronland (1986), no further surface-disinfestation was applied. Five root tip segments, *ca.* 10 mm in length, and five internal crown tissue sections (*ca.* 2 mm^3) per plant were excised and plated on the selective medium of Masago *et al.* (1977), modified by Botha & Coetzer (1996): 1% water agar + 50 mg benomyl, 25 mg nystatin, 50 mg tolclofos-methyl, 20 mg rifampicin and 50 mg ampicillin l^{-1}. Plates were incubated at 25 °C.

Isolation of *Pythium* from water and substrate samples was accomplished by baiting with citrus leaf discs (Grimm & Alexander, 1973). About 200 ml of each water sample was transferred to a 250 ml plastic cup and five 5-mm-diameter leaf discs, sprayed with 70% ethanol and rinsed in SDW, were floated on the surface for 3-4 days. The discs were then plated on the above selective medium and incubated at 25 °C. With the growth media and substrates, approximately 80 ml of each sample was placed in a plastic cup and SDW added to a volume of 200 ml. Growth media containing perlite was covered with a mesh before adding water to prevent the perlite from levitating to the surface. Leaf discs were floated, plated and incubated as described previously.

After 3-6 days incubation, hyphal tips from *Pythium* colonies were transferred to 1% water agar supplemented with 30 µg l^{-1} ß-sitosterol to enhance development of sexual structures (Botha & Coetzer, 1996). Morphological observations were made directly on the culture plates after one week's incubation at 25 °C in the dark. Sporangia were produced by incubating 5-mm-diameter plugs from water agar cultures for 24 hours at 25 °C in non-sterile soil extract under near-UV light. Zoospores were released by exposing the cultures for 4 hours to 4 °C and then returning them to room temperature (*ca.* 25 °C). Sexual and asexual structures were observed under a

compound microscope at 400x and 1000x magnification. The key compiled by Dick (1990) based on oogonial criteria was used in conjunction with Van der Plaats-Niterink (1981) to identify the isolates, and the identifications were verified by W. J. Botha of the Agricultural Research Council - Plant Protection Research Institute (PPRI), Pretoria. Isolates were maintained on cornmeal or V8-juice agar plugs in SDW in McCarty bottles stored at 25 °C. Voucher cultures of representative isolates were deposited in the National Collection of Fungi, PPRI, Pretoria.

Pythium host range in gravel culture and ebb-and-flow systems

In a separate study, various crops in gravel culture hydroponicum #1 (Table 1) were screened for root infection by *Pythium* species. The crops included the lettuce cultivars Butter *Lutetia*, Lolla Rossa *Sesam*, Lolla Bionda *Bergamo*, Cos *Bambi*, Cos *Junior*, Cos *Wallop*, Cos *Pinnocio*, Batavia Red *Ascona*, Red Oak Leaf *Red Salad Bowl* and Green Oak Leaf *Krizet*, the herbs basil (*Ocimum basilicum* L.), chive (*Allium schoenoprasum* L.), fennel (*Foeniculum vulgare* L), mint (*Mentha* spp.), rocket (*Delphinium* spp.), sorrel (*Rumex* spp.) and watercress (*Nasturtium officinale* L.), the Oriental vegetables Chinese cabbage, pak choi (*Brassica rapa* L. subsp. *chinensis* L.) and tah tsai (*Brassica chinensis* L. var. *rosularis* L.), and other crops such as celery, endive (*Cichorium endiva* L.) cv. Oxalie, radicchio (*Cichorium intybus* L.) cv. Firebird, and viola (*Viola* spp.). A similar survey was conducted on cucumbers grown in the ebb-and-flow hydroponicum (#11 in Table 1). Diseased and symptomless plants collected at the two hydroponica were processed as described in the previous section.

Subcultures of selected *Pythium* isolates from each host were maintained on V8-juice agar at 25 °C for inoculation purposes. Pathogenicity of the isolates was determined by artificially inoculating 4-week-old cucumber, endive 'Oxalie', fennel, sorrel, and lettuce cultivar Batavia Red *Ascona*, Butter *Lutetia* and Lolla Bionde *Bergamo* seedlings in a hydro culture system in the greenhouse. The system consisted of 5 l plastic vessels, 23 cm in diameter, with four 3-cm-diameter holes spaced 9 cm apart in the lid of each vessel. Each vessel was filled with a nutrient solution consisting of 0.9 g Agrasol® 'O 3:2:8 (Fleuron, P.O. Box 31245, Braamfontein, 2017), 0.6 g calcium nitrate monohydrate and 0.3 g Micromix® (Fleuron) l^{-1} tap water. Aeration was provided by a compressor supplying air through a 5-mm-diameter tube inserted through a hole in the lid of each vessel (Bates & Stanghellini, 1984). The 4-week-old seedlings were

transplanted from steam-pasteurised Canadian peat moss growth medium into the holes in the lids of the vessels, one seeling per hole, their roots submerged in the nutrient solution and their shoots supported by strips of foam rubber. Three vessels were used for each crop / isolate.

Cucumber seedlings were inoculated with a zoospore suspension of an isolate of *P. spinosum*, whereas the other crops were each inoculated with a *P.* group F isolate from the respective crop. The isolates were cultured for 7-10 days on V8-juice agar. Zoospores were released as described above, immobilised by vortexing, and the zoospore cysts enumerated with the aid of a haemacytometer (Moulin *et al.*, 1994; Wulff *et al.*, 1998). Each vessel was inoculated by adding 15 ml of a 10^5 ml^{-1} zoospore cyst suspension to the nutrient solution one week after transplanting.

Symptoms (wilting and root rot) were recorded weekly. Plants were harvested 4 weeks after transplanting. Three root segments, *ca.* 10 mm long, were excised from each plant in each container, rinsed in SDW, and plated on the above selective medium. After incubation for 3-6 days at 25 °C, colonies were transferred to water agar supplemented with 30 µg l^{-1} ß-sitosterol for identification.

RESULTS

Survey of *Pythium* species in hydroponic systems

A total of 143 isolates, representing eight *Pythium* species and five heterothallic groups, were collected fom the 280 plant, 32 water source, 49 substrate and 30 run-off water samples (Table 1). *P.* group F was the most prevalent, representing 41% of all the isolates. Twenty per cent of the isolates were identified as *P. irregulare*, 6% as *P. spinosum*, and 5% as *P. aphanidermatum* and *Pythium* group HS, respectively. *Pythium acanthicum* Drechsler, *Pythium diclinum* Tokunaga, *P. myriotylum*, *Pythium perplexum* Kouyeas & Theohari, and *Pythium* groups G, P and T each contributed 2% or less to the total number of isolates.

Besides being encountered the most frequently, *P.* group F was also isolated from the greatest number of crop species (5/7) and hydroponica (5/11). It did, however, occur preferentially in recirculating gravel systems and, despite its prevalence in roots, borehole water and substrates,

could not be isolated from any of the run-off water samples. *P. irregulare* was isolated from three of the seven crop species in two recirculating gravel and two open dripper systems. It also frequently occurred in run-off water. *P. aphanidermatum* and *P.* groups HS were each isolated from two crop species, and the first two also from substrates and/or borehole water in three hydroponica. *P. acanthicum*, *P. coloratum*, *P. perplexum* and *P.* groups P and T were each isolated from one crop species only, whereas *P. diclinum* and *P. myriotylum* were not associated with a specific host. *P. coloratum*, although apparently infecting only lettuce, was isolated from water and/or substrate sources in three of the five recirculating gravel systems.

As almost 70% of the plant samples comprised lettuce, albeit from only two hydroponica, it was not surprising that this crop yielded the greatest diversity of *Pythium* species/groups (*P. coloratum*, *P. irregulare*, *P.* groups F, G, HS and T). Cucumber, which represented 15% of the plant samples, also yielded a variety of species/groups (*P. aphanidermatum*, *P. irregulare*, *P. spinosum*, *P.* groups F and HS). Of the crop species which contributed less than 10% to the total number of plant samples, celery, parsley and tomato each produced only one *Pythium* species/group, and Chinese cabbage two. A notable exception was strawberry, of which the three plants (1.1% of the total) from one hydroponicum yielded *P. acanthicum* and *P.* groups F and P. Strawberry was also the only crop from which a species/group (*P.* group F) could be isolated from crown tissue.

As indicated above, *P. coloratum* occurred only, and *P.* group F mostly, in recirculating gravel systems. *P. aphanidermatum* and *P. spinosum*, on the other hand, were isolated only from ebb-and-flow and open dripper systems. Due to the greater number of gravel system samples that were processed, a somewhat greater diversity of *Pythium* species/groups was retrieved from these systems, but the diversity was not consistently related to the number or variety of samples processed per hydroponicum.

Pythium host range in gravel culture and ebb-and-flow systems

Crops that were sampled in gravel system #1 showed stunting of the aboveground parts and slight to moderate browning of the root tips. Cucumber plants from which isolations were made in the ebb-and-flow system exhibited severe wilting of the foliage and were weakly anchored due to underdeveloped root systems. Symptomless infection without typical wilting and root rot

associated with *Pythium* disease also occurred in some crops, e.g. basil, celery, chive, mint, pak choi and tah tsai.

P. group F was isolated from all the crops evaluated in the gravel culture system, and *P. irregulare* from the lettuce cultivars Lolla Rossa *Sesam*, Cos green *Junior* and Batavia Red *Ascona* (Table 3). The incidence of *Pythium* in roots of the various lettuce cultivars ranged from 70 to 100% (mean 85%). Compared to the 7-83% (mean 38%) of the other crops, this was relatively high. Cucumber plants in the ebb-and flow system yielded only *P. spinosum.*

The different *Pythium* isolates varied in the time required for disease symptoms to appear after artificial inoculation, but all inoculated plants eventually developed some root rot and wilting or stunting of aboveground parts. Cucumber plants inoculated with *P. spinosum* showed symptoms of disease shortly after inoculation. The symptoms characteristically comprised wilting and stunting of aboveground parts which eventually resulted in the death of more than half of the plants. Watersoaked lesions appearing on the stems and root systems were severely necrotic. The herbs and lettuce cultivars inoculated with *P.* group F mainly showed stunting of aboveground parts and light to moderate root rot, but severe wilting was not observed. Roots of inoculated plants consistently yielded the same *Pythium* species/group the particular crop or cultivar was inoculated with. Uninoculated plants remained healthy. Symptoms observed on the crops are depicted in Figs 1-5.

DISCUSSION

The 11 hydroponica surveyed in this study comprised only a portion of the total number of hydroponic units in South Africa, and the *Pythium* species and groups that were isolated therefore do not represent the entire hydroponicum industry. Nevertheless, various new host / pathogen associations have been established for South Africa, e.g. *P. acanthicum* on strawberry, *P. aphanidermatum* on parsley, *P. coloratum* on lettuce, *P. irregulare* on Chinese cabbage and lettuce, *P. perplexum* on tomato, *P.* group G on lettuce, *P.* group HS on cucumber and lettuce, *P.* group P on strawberry, *P.* group T on lettuce and strawberry, and *P.* group F on all the crops it was isolated from. The study is also the first to report the isolation of *P. coloratum*, *P. diclinum*, *P. perplexum*, and *P.* groups HS and P from any crop or substrate in South Africa.

In accordance with the observation by Domsch *et al.* (1980) that nematosporangiate *Pythium* species preferentially occur in aquatic or semi-aquatic habitats, more than twice as many of the species/groups in this study (*P. coloratum*, *P. diclinum*, *P. myriotylum*, *P.* group F, *P.* group T) produce filamentous sporangia as those that produce strictly globose or subglobose sporangia (*P. acanthicum*, *P. irregulare*). Of all the species/groups, *P.* group F was isolated the most frequently and from the greatest variety of crops. Indeed, the only crops it could not be isolated from were parsley and tomato. This is in conflict with Chérif *et al.* (1997), who described *P.* group F as a common, albeit not very virulent, pathogen of hydroponically-grown tomatoes. It should, however, be kept in mind that tomato was sampled in only one hydroponicum, where only *P. perplexum* was the only *Pythium* sp. present at the time of sampling. *P.* group F is important in hydroponica (Rafin *et al.*, 1995) and is known to occur in hydroponica in South Africa (Lewies, 1998; Thompson & Labuschagne, 2001), and has also been associated with root rot of cabbage, wheat and pine cultivated conventionally (Linde *et al.*, 1994; Botha & Coetzer, 1996; Meyer & Wehner, 2000). Pathogenicity of *P.* group F to endive, fennel, sorrel and lettuce, as found in the present study, has not previously been reported anywhere in the world.

The second-most prevalent species, *P. irregulare*, is one of the most widespread and pathogenic *Pythium* species in temperate zones (Domsch *et al.*, 1980) and is commonly associated with hydroponically-grown crops elsewhere (Schuerger & Pategas, 1985; Favrin *et al.*, 1988), but not referred to by Lewies (1998) and Thompson & Labuschagne (2001) as a pathogen in hydroponica in South Africa. It has, however, been reported from 14 field-grown plant species in 10 families in the country (Crous *et al.*, 2000), including cucumber (Botha & Coetzer, 1996). *P. irregulare* produces a growth factor that stimulates the growth of pathogens such as *Rhizoctonia solani* J. G. Kühn, *Aphanomyces euteiches* Drechsler and several other *Pythium* species (Yang, 1969), and could therefore be particularly significant in disease complexes. *P. coloratum*, the third most prevalent species in this survey, is also not listed by Lewies (1998) and Thompson & Labuschagne (2001) as present in hydroponica in South Africa. Although it was isolated only from lettuce in gravel culture, it occurred commonly in substrates and run-off water in gravel systems, which is in accordance with Favrin *et al.* (1988) who could isolate the species only from potting mixes in British Colombian greenhouses. *P. coloratum* is a relatively rare species in soil (Van der Plaats-Niterink, 1981), but has been reported to cause root rot of cucumber and onion (*Allium cepa* L.) (Favrin *et al.*, 1988; Shishkoff, 1989).

The remaining species/groups contributed between <1 and 6% to the total number of isolates. Of these, *P. aphanidermatum*, *P. myriotylum* and *P.* group G have been described by Lewies (1998) and Thompson & Labuschagne (2001) as present in South African hydroponica. *P. myriotylum*, *P.* group G, and particularly *P. aphanidermatum* are also regarded as important in hydroponica elsewhere (Gill, 1970; Jenkins & Averre, 1983; Favrin *et al.*, 1988; McCullagh *et al.*, 1996; Stanghellini *et al.*, 1996). Indeed, *P. aphanidermatum* is considered to be the dominant *Pythium* in many types of hydroponic systems all over the world (Favrin *et al.*, 1988; Cherif *et al.*, 1994; Moulin *et al.*, 1994; Sanchez *et al.*, 1999). Being a thermotolerant species (Van der Plaats-Niterink, 1981) causing root damage mainly at higher temperatures (Hendrix & Campbell, 1973), it understandably is also very common in South African soils and has locally been reported as a root pathogen of 19 plant species in nine families (Crous *et al.*, 2000). The reason for its relative paucity in the present study is unclear, but could not have been due to temperatures in local hydroponica being too low as these temperatures are essentially the same as those in the environment. Particularly conspicuous in its absence was *P. ultimum*, another common *Pythium* species in hydroponica elsewhere (Mc Cullagh *et al.*, 1996; Cherif *et al.*, 1997) and the commonest *Pythium* species in South Africa, with 32 host species in 20 families recorded in Crous *et al.* (2000). The absence of *P. ultimum* in local hydroponica remains unresolved, but is nevertheless in accordance with Lewies (1998) and Thompson & Labuschagne (2001).

In conclusion, this study has shown that *Pythium* is omnipresent in South African hydroponica. The presence or absence of some, but not all, previously reported "hydroponic" species/groups was confirmed and new species and groups have been added to the list. Most of the species/groups have not previously been reported in South Africa from their present hosts in conventional production systems, the exceptions being *P. aphanidermatum*, *P. irregulare* and *P. spinosum* which are associated with root rot of greenhouse-cultivated cucumber (Botha & Coetzer, 1996). It is furthermore evident from the results that the *Pythium* species/group composition of hydroponica is not consistent. For instance, the first survey of gravel system #1 yielded *P. coloratum*, *P. irregulare* and *P.* groups F, G and HS from three crop species, whereas only *P.* group F and, to some extent, *P. irregulare* could be isolated from 16 crop species and 10 lettuce cultivars in the second survey. A similar shift occurred at the ebb-and-flow system with the apparent 'disappearance' of *P. aphanidermatum*. It is thus clear that hydroponica should be

monitored regularly to remain abreast of changes in *Pythium* populations.

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TABLE 1 Hydroponica in South Africa surveyed for *Pythium* species

Hydroponicum	System	Locality	Number of samples collected [a]
1	Recirculating gravel[b]	Pretoria	Le (121), Ce (15), GM (6), GC (23) BW (11), WW (19)
2	Recirculating gravel[b]	Vereeniging	Le (70), Ce (6), Ch (12), GC (6), BW (6), WW (5)
3	Recirculating gravel[b]	Port Elizabeth	BW (3)
4	Recirculating gravel[b]	Muldersdrift	WW (3)
5	Recirculating gravel[b]	Skeerpoort	St (3)
6	Open dripper[c]	Centurion	RS (3)
7	Open dripper[c]	Marikana	Cu (3), RW (2), DW (2), WW (3)
8	Open dripper[c]	Kemptonpark	Pa (5)
9	Open dripper[d]	Benoni	Cu (21), PE (7)
10	Open dripper[d]	Badplaas	To (6)
11	Ebb-and-flow[e]	Benoni	BW (8), Cu (18), QS (4)

[a]Ce = celery, Ch = Chinese cabbage, Cu = cucumber, Le = lettuce, Pa = parsley, St = strawberry, To = tomato, BW = borehole water, DW = dam water, RW = river water, WW = run-off water, GC = granite chips, GM = growth medium (Canadian peat moss), PE = perlite, QS = quarts sand, RS = river sand.

[b]Recirculating systems consisting of gullies with granite chips as substrate.

[c]Open dripper systems consisting of plastic bags with perlite as substrate.

[d]Open dripper systems consisting of plastic bags with pine wood shavings as substrate.

[e]Ebb-and-flow system consisting of gullies filled with coarse quartz sand.

TABLE 2 ***Pythium*** **species and groups isolated from commercial hydroponica in South Africa**

Pythium sp / group[a]	% Incidence[b]	Host	Number of isolates[c]		Hydroponic system[d]
P. acanthicum	1.5	Strawberry	2	Roots	5
P. aphanidermatum	5	Cucumber	1	Roots	11
		Parsley	1	Roots	8
			1	Sand	6
			4	Borehole water	11
P. coloratum	11	Lettuce	2	Roots	1, 2
			3	Growth media[e]	1
			6	Run-off water	1, 4
			5	Gravel substrate	2
P. diclinum	1.5		2	Run-off water	2
P. group F	41	Celery	5	Roots	2
		Chinese cabbage	4	Roots	2
		Cucumber	2	Roots	9
		Lettuce	39	Roots	1, 2
		Strawberry	1	Roots	5
			1	Gravel substrate	1, 2
			1	Growth media[e]	1
			5	Borehole water	1, 2, 3
P. group G	1.5	Lettuce	2	Roots	1
P. group HS	5.5	Cucumber	2	Roots	9
		Lettuce	4	Roots	1
			1	Gravel substrate	2
			1	Borehole water	1
P. group P	2	Strawberry	3	Stem	5
P. group T	2	Lettuce	3	Roots	2

TABLE 2 (continued)

P. irregulare	20	Cucumber	4	Roots	7, 9
		Lettuce	16	Roots	1, 2
		Chinese Cabbage	4	Roots	2
			2	Gravel substrate	2
			3	Run-off water	1, 2, 7
P. myriotylum	2		3	Gravel substrate	2
P. perplexum	1.5	Tomato	2	Roots	10
P. spinosum	5.5	Cucumber	8	Roots	9, 11

[a] Identified according to the keys compiled by Dick (1990) based on oogonial criteria and the revised key of Van der Plaats-Niterink (1981).

[b] Percentage frequency of species/group out of a total of 143 isolates.

[c] Number of isolates recovered from different hosts and other sources.

[d] Hydroponic systems according to Table 1.

[e] Canadian peat moss growth media for seedlings.

TABLE 3 ***Pythium*** **species and groups isolated from crops in gravel culture hydroponicum #1 and ebb-and-flow hydroponicum #11** [a]

Crop	% *Pythium* incidence in roots[b]	*Pythium* sp./group isolated[c]
GRAVEL CULTURE		
Lettuce cultivars		
Batavia Red *Ascona*	83	*P.* Group F, *P. irregulare*
Butter *Lutetia*	75	*P.* Group F
Cos green *Bambi*	93	*P.* Group F
Cos green *Junior*	97	*P.* Group F, *P. irregulare*
Cos green *Wallop*	73	*P.* Group F
Cos green *Pinnocio*	83	*P.* Group F
Lolla Rossa *Sesam*	70	*P.* Group F, *P. irregulare*
Lolla Bionda *Bergamo*	100	*P.* Group F
Green Oak Leaf *Krizet*	100	*P.* Group F
Red Oak Leaf *Red Salad Bowl*	80	*P.* Group F
Herbs		
Basil	80	*P.* Group F
Chives	17	*P.* Group F
Fennel	60	*P.* Group F
Mint	27	*P.* Group F
Rocket	43	*P.* Group F
Sorrel	7	*P.* Group F
Watercress	7	*P.* Group F
Oriental vegetables		
Chinese cabbage	20	*P.* Group F
Pak choi	30	*P.* Group F
Tah tsai	53	*P.* Group F

TABLE 3 (continued)

Other crops		
Celery *Victoria*	27	*P.* Group F
Endive *Oxalie*	40	*P.* Group F
Raddiccio *Firebird*	33	*P.* Group F
Violas	83	*P.* Group F
EBB-AND-FLOW CULTURE		
Cucumber	70	*P. spinosum*

[a] See Table 1.

[b] Percentage root segments out of 30 yielding the particular species/group.

[c] Identified according to the keys compiled by Dick (1990) based on oogonial criteria and the revised key of Van der Plaats-Niterink (1981).

FIGURE 1 a **Butterhead Lettuce uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 1 b **Butterhead Lettuce uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 2 a **Endive uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 2 b **Endive uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 3 a **Sorrel uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 3 b **Sorrel uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 4 a **Fennel uninfected (left) and naturally infected (right) with *P.* group F in a gravel culture system**

FIGURE 4 b **Fennel uninfected (left) and artificially infected (right) with *P.* group F in a water culture system**

FIGURE 5 a **Cucumber uninfected (left) and naturally infected (right) with *P. spinosum* in a ebb-and-flow system**

FIGURE 5 b **Cucumber uninfected (left) and artificially infected (right) with *P. spinosum* in a water culture system**

CHAPTER 3

PATHOGENICITY OF *PYTHIUM* SPECIES/GROUPS TO HYDROPONICALLY-GROWN BUTTER HEAD LETTUCE

Abstract

The pathogenicity of five *Pythium* species and representatives of three heterothallic groups to butter head lettuce was determined at 21°C and 28°C in three hydroponic systems, viz. static nutrient solution, hydroculture and a recirculating gravel system. Overall, *P. spinosum* was the most aggressive species at 21°C, followed by *P. irregulare* and *P.* group HS. *P. myriotylum* was the only species which consistently showed greater virulence at 28°C than at 21°C. No significant differences in virulence at the two temperatures were evident between *P. coloratum*, *P. diclinum*, and *P.* groups F and T. This is the first report describing the pathogenicity of the above species/groups to butter head lettuce in South Africa.

INTRODUCTION

Recirculating nutrient systems provide an ideal environment for spread of, and infection by, zoosporogenic plant pathogens (Chérif *et al.*, 1994; Stanghellini *et al.*, 2000). It is therefore not surprising that *Pythium* species are commonly associated with hydroponically-grown crops (Jenkins & Averre, 1983; Stanghellini *et al.*, 1984, 1996; Goldberg & Stanghellini, 1990; Moulin *et al.*, 1994; Buysens *et al.*, 1995; Wulff *et al.*, 1998) However, despite their prevalence, only a few *Pythium* species are highly virulent under hydroponic conditions, the most being considered as "minor pathogens" that reduce plant growth without causing obvious disease symptoms (Chérif *et al.*, 1997). Virulence of a species furthermore frequently depends on the strain involved (Jenkins & Averre, 1983) and on environmental conditions, particularly temperature (Hendrix & Campbell, 1973). Observations made during the present study showed that *Pythium* is associated with severe root rot and wilting of lettuce in commercial hydroponic

systems during the hot summer months.

The previous chapter (Chapter 2) indicated the presence of eight *Pythium* species and five heterothallic groups in hydroponica in South Africa. Isolates of some of the species/groups induced symptoms similar to those observed in commercial hydroponica when artificially inoculated into the hosts they have originally been isolated from. The purpose of the present study was to determine the pathogenicity and relative virulence of the most prevalent *Pythium* species/groups to butter head lettuce (*Lactuca sativa* L. var. *capitata* L.), the main target crop in this investigation.

MATERIALS AND METHODS

The *Pythium* species and groups listed in Table 1 were screened for pathogenicity in three separate experiments, viz. (i) static nutrient solution in controlled environment cabinets, (ii) aerated hydroculture system in a greenhouse, and (iii) recirculating gravel culture hydroponic system in a greenhouse. Limited space and facilities precluded inclusion of all the species/groups at the same time. The various experiments therefore had to be conducted twice, each time with a different group of isolates plus a control.

(i) Static nutrient solution

Three-week-old butter head lettuce *Lutetia* seedlings were transferred from steam-pasteurised growth medium (Canadian peat moss) to lidded 250 ml plastic cups, one seedling per cup. The cups contained a nutrient solution consisting of 0.45 g Agrasol® 'O 3:2:8 (Fleuron, P.O. Box 31245, Braamfontein, 2017), 0.3 g calcium nitrate monohydrate and 0.15 g Micromix® (Fleuron) l^{-1} tap water, with a pH of 7.0. Each seedling was supported by the lid of the cup, its roots submerged in the nutrient solution.

Twenty seedlings were inoculated with each of the *Pythium* isolates indicated in Table 1. Inoculum was prepared by blending a 5-day-old V8-juice agar Petri dish culture of each isolate for 15 seconds in 100 ml sterile distilled water in a Waring blender (Jenkins & Averre, 1983; Moulin *et al.*, 1994). Ten millilitres of inoculum suspension was added to each cup (Sanogo & Moorman, 1993), four days after transfer of the seedlings to the cups. Sterile blended V8-juice

agar served as control. Ten of the cups inoculated with each *Pythium* isolate were randomly arranged in a growth cabinet at 21°C and the other 10 in a growth cabinet at 28°C.

Seventeen days after inoculation the seedlings were removed from the cups, and their roots and shoots separated and weighed. Root rot was assessed according to a 0-4 scale (0 = healthy, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100% rottted). Five root segments, *ca.* 10 mm long, were excised from each seedling and plated on BNPRA selective medium (Roux & Botha, 1997). The identity of the *Pythium* species growing from each root segment after incubation for 3-6 days at 25°C was confirmed on water agar supplemented with 30 µg ml^{-1} ß-sitosterol (Botha & Coetzer, 1996).

Data were analysed statistically according to the GENSTAT 5 programme. Treatment differences were tested by means of one-way analysis of variance and Fisher's protected *t*-test was used to separate treatment means at 5% level of significance.

(ii) Hydroculture system

Plastic containers, 23 cm in diameter and with a capacity of 5 l, each fitted with a lid containing four 3cm-diameter holes spaced 9 cm apart, were filled with a nutrient solution consisting of 0.9 g Agrasol® 'O 3:2:8, 0.6 g calcium nitrate monohydrate and 0.3 g Micromix® l^{-1} tap water. Aeration was provided by a compressor supplying air through a 7-mm-diameter tube inserted through a hole in the lid of each container (Bates & Stanghellini, 1984). The nutrient solution was replaced on a weekly basis, and pH and electrical conductivity of the solution were maintained at 6.9 and 2.1 σ, respectively (Chérif et al., 1994).

A 4-week-old butter head lettuce seedling reared in steam-pasteurised Canadian peat moss was transplanted into each hole in the lid of each container, its roots submerged in the nutrient solution and the shoot supported by a strip of foam rubber. One week after transplanting, three containers with four plants in each were inoculated with one of the *Pythium* isolates indicated in Table 1. Inoculum was prepared from 5-day-old V8-juice agar Petri dish cultures. Two cultures per isolate were blended in 500 ml sterile distilled water. Each inoculum solution was divided in three and added to the nutrient solutions of three containers. Blended uncolonised V8-juice

agar served as control. Inoculation was repeated after two weeks. Plants were harvested three weeks after the first inoculation and processed as above.

(iii) Recirculating gravel system

The recirculating gravel system comprised four units, each with a 100 l reservoir feeding three troughs, 13 cm wide, 8 cm deep and 250 cm long, positioned at an incline of 1:13. The troughs were filled with previously unused, untreated 9.5mm-diameter granite chips. Nutrient solution with the same composition as in (ii) above was constantly circulated through the gravel in the troughs by means of an IDRA® 300 l h^{-1} submersible pump, returning to the reservoir by gravity flow. The nutrient solution was replaced once a week and the pH and electrical conductivity were maintained at 6.9 and 2.1 σ, respectively.

Sixteen four-leaf-stage butter head lettuce seedlings were transplanted from steam-pasteurised Canadian peat moss into each trough, with a 20 cm spacing between plants (Jenkins & Averre, 1983). One week after transplanting, each reservoir was inoculated with one of the *Pythium* isolates (Table 1). Inoculum was prepared from 5-day-old cultures on V8-juice agar in Petri dishes. Six cultures per isolate were blended in 500 ml sterile distilled water and the suspension added to a reservoir. Blended uncolonised V8-juice agar served as control. Plants were harvested three weeks after inoculation and processed as above.

RESULTS

All the *Pythium* species/groups caused significant root rot and reduced shoot and/or root development in at least one of the experiments (Tables 2-4). Wilting of aboveground parts was more evident at 28°C. Overall, the reduction in shoot growth resulting from infection with *Pythium* was 27.5% at 21°C and 18.8% at 28°C. The corresponding percentages for reduction in root growth was 25.5 and 27.9, whereas mean root rot rating at 21°C was 1.7 and 1.8 at 28°C. *P. spinosum* was the most aggressive species at 21°C, reducing shoot and root growth on average by 49.4% and 43.6%, respectively, compared to 11.9% and 22.9% at 28°C. Reduction in shoot growth due to inoculation with *P. spinosum* was significantly greater at 21°C than at 28°C in all three experiments, and reduction in root growth in two experiments. Root rot

ascribable to *P. spinosum* was almost similar at the two temperatures, the mean ratings being 2.1 at 21°C and 2.2 at 28°C.

P. irregulare and *P.* group HS also retarded plant growth to a greater extent at 21°C than at 28°C. At 21°C, inoculation with *P. irregulare* and *P.* group HS resulted in a mean reduction in shoot growth of 38.7% and 29.1%, respectively, and 34.0% and 29.5% in root growth. The corresponding percentages at 28 °C were 16.0, 13.5, 24,0 and 31.6. *P.* group HS reduced shoot growth significantly more at 21°C than at 28°C in two of the experiments, but no differences in root growth were evident between temperatures. With *P. irregulare*, shoot mass was significantly lower at 21 °C than at 28 °C in all three experiments, and root mass in two. As with *P. spinosum*, little or no differences in root rot were evident between temperatures (mean 1.6 for *P.* group HS at both temperatures, and 2.1 and 1.8 for *P. irregulare* at 21°C and 28°C, respectively). Although *P. spinosum*, *P. irregulare* and *P.* group HS generally were more virulent at 21°C, they also suppressed plant growth at 28°C in some of the experiments. Indeed, *P. irregulare* reduced root growth significantly more at 28°C than at 21°C in the recirculating gravel system.

The only species which consistently showed enhanced virulence at 28°C was *P. myriotylum*. On average, it reduced shoot and root growth at 28 °C by 29.4% and 38.6%, respectively, compared to 11.8% and 12.8% at 21°C. Mean root rot rating was 2.2 at 28°C and 1.5 at 21°C. The remaining species/groups did not differ much in aggressiveness between temperatures, although *P.* groups F and T tended to be somewhat more aggressive at 28°C, depending on the experiment.

In the absence of infection by *Pythium*, shoot mass of the lettuce seedlings at termination of the experiments was on average 42% higher at 21°C than at 28°C, and root mass 12%. However, plant vigour, particularly shoot growth, varied considerably between experiments. Mean shoot mass of control plants in static nutrient solution was 11.8 g at 21°C and 8.9 g (25% lower) at 28°C, compared to 159.7 g at 21 °C and 87.6 g (45% lower) at 28 °C in the recirculating gravel system. In the hydroculture system, shoot mass at 21 °C was 64.6 g at 21 °C and 70.3 g (9% higher) at 28°C. The overall reduction in shoot growth at 21°C as a result of infection with

Pythium was 27.6% in static nutrient solution, 30.4% in the hydroculture system and 24.6% in the recirculating gravel system, compared to 16.6%, 28.2% and 11.5%, respectively, at 28°C. The corresponding percentages reduction in root growth were 29.8, 29.1 and 17.6 at 21 °C, and 29.4, 29.3 and 25.1 at 28°C. Overall root rot rating at 21°C was 2.5 in static nutrient solution, 1.1 in the hydroculture system and 1.4 in the recirculating gravel system, and 3.1, 1.8 and 0.6, respectively, at 28°C.

The various *Pythium* species/groups could readily be isolated from the roots of plants with which they were inoculated. Some root necrosis, not due to infection by *Pythium*, was evident in control plants in all the experiments, particularly in static nutrient solution.

DISCUSSION

From the results presented above it is evident that all the *Pythium* species and groups evaluated in the study were pathogenic to butter head lettuce. This occurred regardless of whether the particular species or group had originally been isolated from lettuce or not. Indeed, the two most aggressive isolates, viz. *P. spinosum* at 21°C and *P. myriotylum* at 28°C, were both from other sources, though the *P. myriotylum* isolate originated from gravel substrate in a lettuce-growing hydroponicum. Two of the other non-lettuce root isolates, *P. coloratum* and *P. diclinum*, were also from hydroponica in which lettuce was grown.

As far as could be established, this is the first report of *P.* groups F, HS and T as pathogens of butter head lettuce, at least in South Africa. It is also the first time that Koch's postulates have been confirmed with *P. irregulare* on lettuce in South Africa, though the species is known as a pathogen of lettuce in other parts of the world (Ellis & Cox, 1951). Other species that have been reported to be pathogenic to lettuce elsewhere are *P. spinosum* (Ellis & Cox, 1951) and *P. myriotylum* (Jenkins & Averre, 1983). The latter is an interesting species, as far as the genus *Pythium* is concerned, in that it produces a toxin capable of causing leaf necrosis and stunting in tomato plants (Csinos & Hendrix, 1978). *P. myriotylum* also has an antagonistic relationship with *Rhizoctonia solani* J.G. Kühn (Garren, 1970), but acts synergistically with *Fusarium solani* (Mart.) Appel & Wollenw. in plant attack (Frank, 1972; García & Mitchell, 1975). These

attributes could contribute to *P. myriotylum* being one of the most important pathogens in hydroponica worldwide (Thompson & Labuschagne, 2001), particularly at high temperatures.

In general, temperature preferences of the various *Pythium* species/groups, as far as virulence is concerned, corresponded with what has been described in literature. Various reports refer to greater damage caused by *P. irregulare* and *P. spinosum* at lower temperatures (15-20°C), and by *P. myriotylum* at higher temperatures (Hendrix & Campbell, 1973; Hancock, 1991; Martin, 1995; Ben-Yephet & Nelson, 1999; Chellemi *et al.*, 2000). Extrapolated from growth rate (Van der Plaats-Niterink, 1981; Botha & Coetzer, 1996) and the results of the present study, *P. coloratum*, *P. diclinum* and *P.* group F can be classified as intermediate temperature pathogens, though the ability of *P.* group F to grow at >40 °C implies that it is also capable of causing damage at higher temperatures. With an optimal growth temperature of 30.5°C, and more damage caused to butter head lettuce at 28°C than at 21°C, *P.* group T should be regarded as a high-temperature pathogen. Results implicate *P.* group HS as a low-temperature pathogen of butter head lettuce, at least as far as its effect on shoot growth is concerned.

Temperatures in South African hydroponica vary considerably, depending on season and locality, but the minimum in winter and maximum in summer in the two lettuce-growing units surveyed in Chapter 2 are between 15-22°C and 30-36°C, respectively. Considering the diverse spectrum of *Pythium* species/groups with different temperature preferences present in these hydroponica, it is evident that the lettuce plants are at risk throughout the year. Nevertheless, the almost 1.5 times greater overall suppression of shoot growth at 21°C than at 28°C in the present study indicates that butter head lettuce is more susceptible to damage by the complex of *Pythium* species occurring in hydroponica at lower temperatures. This was not expected as butter head lettuce is a cool-weather crop (Morgan,, 1999), growing optimally at 12-21°C, and prone to heat stress that can aggravate injury by pathogens at temperatures above 25°C. It would thus appear if heat stress is subsidiary to the virulence of the *Pythium* species/groups present at a particular temperature in determining the extent of damage to the plant in commercial hydroponica. The issue nevertheless remains confounded. For instance, Stanghellini & Kronland (1986) reported yield reductions of 35-54% and 12-17% in lettuce infected with *Pythium dissotocum* Drechsler at 18°C and 28°C, respectively, and incidentally

also found uninfected lettuce to have a 29% higher shoot mass at 28°C than at 18°C. However, with spinach (*Spinacea oleracea* L.) which is also a cool-season crop, infection by *P. dissotocum* resulted in more stunting at 30°C than at 20°C (Bates & Stanghellini, 1984).

Besides the differences in temperature preferences, virulence of the various *Pythium* species/groups also varied considerably between systems. Overall, root rot rating and percentage reduction in plant mass were less in the recirculating gravel system than in static nutrient solution and the hydroculture system. It can be argued that the inoculum concentration in the gravel system was about seven and three times lower than in static nutrient solution and the hydroculture system, respectively, but more vigorous growth of the plants in the gravel system probably also contributed to their relative resistance. The poor growth of butter head lettuce in static nutrient solution was expected, considering the hypoxic environment and limited availability of nutrients. Nutrient supply, particularly the volume of nutrient solution available per plant, probably contributed to better growth in the gravel system than in the hydroculture system. Based on container capacity, each plant in the gravel system was exposed to a continuous supply of 2.08 l nutrient solution, 1.7 times more than the 1.25 l per plant in the hydroculture system. This ratio is reflected in the 1.8 times higher mass of control plants in the gravel system than in the hydroculture system, notwithstanding the fact that high levels of nutrients can predispose plants to infection by *Pythium* (Moorman, 1986; Gladstone & Moorman, 1989).

More effective pathogen dispersal in the hydroculture system could also have contributed to the increased severity of disease. In the gravel system, propagules have to be dispersed throughout the entire recycling process in order to infect all plants, whereas in the hydroculture system the entire root system of each plant is confined and constantly exposed to the pathogen (Jenkins & Averre, 1983). Nevertheless, the fact remains that growth conditions can have a marked effect on the virulence of a particular *Pythium* species (Zinnen, 1988).

P. group F, the species/group most commonly isolated from lettuce roots by far (Chapter 2), proved to be moderately virulent in static nutrient solution and the hydroculture system, but did not cause significant root rot or impediment of plant growth in the recirculating gravel system,

an environment which it seemed to prefer (Chapter 2). It is unlikely that this disparity could have been due to the different isolates of *P.* group F that were used. The isolate from wallop lettuce included in the recirculating gravel system was the same one evaluated in the hydroculture system, where it reduced shoot growth to approximately the same extent as the cos lettuce isolate in static nutrient solution, despite being introduced into the static nutrient solution at three times the concentration as in the hydroculture system. It is also unlikely that the lower initial inoculum concentration in the gravel system played a role, considering the 117% and 25% higher root rot rating in this system than in the hydroculture system at 21°C and 28°C, respectively.

Differences in virulence of *P.* group F in different substrates have been described before. Moulin *et al.* (1994) reported damping-off of cucumber (*Cucumis sativus* L.) in soil, inconsistent growth reduction in hydroponic culture, and no effect in rockwool culture, by *P.* group F or its homothallic counterpart, *Pythium flevoense* Van der Plaats-Niterink. Evidence indicates that oxygen concentration of the substrate could be a determining factor. Chérif *et al.* (1997) showed that tomato (*Lycopersicon esculentum* Mill.) plants inoculated with *P.* group F rapidly developed symptoms of infection and root decay in nutrient solution with moderate or low oxygen concentration, whereas highly oxygenated plants remained healthy and displayed significantly less root colonisation by the pathogen. A possible explanation for this phenomenon was that increases in lipoxygenase in tomato roots grown under oxygen stress and inoculated with *P.* group F could lead to degradation and disorganisation of membrane lipids, hence facilitating root colonisation by the pathogen and appearance of decay. Unlike *Pythium aphanidermatum* (Edson) Fitzp. for instance, *P.* group F also induces important defence reactions in cucumber plants, including formation of papillae and deposition of wall apposition and phenolic compounds (Rey *et al.*, 1996), but it is unclear if these defence mechanisms are still effective under poor aeration conditions (Chérif *et al.*, 1997). While the relatively hypoxic conditions in static nutrient solution in the present study could have aggravated disease, it is unlikely that hypoxia *per se* contributed to the differences in disease severity between the hydroculture and recirculating gravel systems. Results suggest that other stress factors may also have been involved.

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TABLE 1. ***Pythium*** **species/groups included in the present study**

Pythium sp./group	Hydroponicum[a]	Source[a]	Experiment[b]
P. coloratum		Growth medium[c]	(i)[¶], (ii)[¶], (iii)[¶]
P. diclinum		Run-off water	(i)[¶], (ii)[¶]
P. irregulare		Wallop lettuce	(i)[¶¶], (ii)[¶¶], (iii)[¶¶]
P. myriotylum		Gravel substrate	(i)[¶¶], (ii)[¶], (iii)[¶¶]
P. spinosum		Cucumber	(i)[¶], (ii)[¶¶], (iii)[¶]
P. group F		Cos lettuce	(i)[¶]
P. group F		Wallop lettuce	(ii)[¶¶], (iii)[¶]
P. group HS		Butter head lettuce	(i)[¶¶], (ii)[¶¶], (iii)[¶¶]
P. group T		Green oak lettuce	(i)[¶¶] , (ii)[¶]

[a] Refer to Chapter 2, Table 1.

[b] (i) Static nutrient solution, (ii) Hydroculture system, (iii) Recirculating gravel system.

[¶] Included in first run of experiment, [¶¶] Included in second run of experiment.

[c] Canadian peat moss growth medium for seedlings.

TABLE 2. **Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in static nutrient solution in growth cabinets.**

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21°C	28°C	21°C	28°C	21°C	28°C
P. coloratum[c]	**16.3**	13.1	**16.2**	3.4	**2.9**	**2.2**
P. diclinum[c]	**34.4**	**23.2**	**28.5**	**35.1**	1.8	**3.5**
P. irregulare[d]	**41.3***	8.0	**36.7***	**24.2**	**2.8**	**2.8**
P. myriotylum[d]	11.8	**24.1***	7.3	**20.8***	**2.8**	**3.3**
P. spinosum[c]	**58.4***	8.7	**76.4***	**38.0**	**3.7**	**2.7**
P. group F[c]	**26.7**	**21.8**	**35.3**	**24.9**	**2.7**	**2.9**
P. group HS[d]	**28.9***	5.4	**31.2**	**38.6**	**2.8**	**3.4**
P. group T[d]	2.9	**28.6***	6.9	**30.5***	0.3	**3.6**

[a] Each value is the mean of 10 replicate cups with one plant in each, evaluated 17 days after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$);
* indicates a significantly greater reduction at the particular temperature within parameters according to Fisher's protected t-test ($P \leq 0.05$).

[b] Each value is the mean of 10 replicate cups with one plant in each, evaluated 17 days after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75% , 4 = 100% rotted; values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$).

[c] Included in first experiment.

[d] Included in second experiment.

TALBLE 3. Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in a hydroculture system.

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21 °C	28 °C	21 °C	28 °C	21 °C	28 °C
P. coloratum[c]	28.6	**25.0**	23.9	**29.5**	**2.2**	1.3
P. diclinum[c]	36.3	**27.5**	23.5	**33.9**	**2.5**	1.8
P. irregulare[d]	**37.8***	**27.9**	**51.3***	17.2	**1.6**	**2.4**
P. myriotylum[c]	14.1	**39.7***	18.5	**29.9***	0.3	**2.4**
P. spinosum[d]	**49.9***	**23.7**	**25.5**	27.8	0.6	**3.7**
P. group F[d]	23.8	**34.9**	**30.1**	**49.3***	0.6	0.8
P. group HS[d]	**27.3**	**22.1**	**31.0**	25.0	**0.9**	0.8
P. group T[c]	25.5	**24.7**	28.7	21.9	0.4	0.8

[a]Each value is the mean of three replicate containers with four plants in each, evaluated three weeks after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$); * indicates a significantly greater reduction at the particular temperature within parameters. (according to Fisher's protected t-test ($P \leq 0.05$).

[b]Each value is the mean of three replicate containers with four plants in each, evaluated three weeks after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75%, 4 = 100% rotted; values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$).

[c] Included in first experiment.

[d] Included in second experiment.

TALBE 4. Effect of inoculation with *Pythium* species/groups on butter head lettuce seedlings grown at 21 and 28 °C in a recirculating gravel system.

Pythium sp./group	Reduction in shoot mass (%)[a]		Reduction in root mass (%)[a]		Root rot rating[b]	
	21 °C	28 °C	21 °C	28 °C	21 °C	28 °C
P. coloratum[c]	15.4	4.0	14.4	**15.9**	0.4	0.6
P. irregulare[d]	**36.9***	**12.2**	**14.1**	**30.7***	2.0	0.3
P. myriotylum[d]	9.4	**24.3***	**12.6**	**65.2***	1.3	0.9
P. spinosum[c]	**39.9***	3.3	28.9*	2.8	2.0	0.3
P. group F[c]	14.8	12.1	9.3	5.1	1.3	1.0
P. group HS[d]	**31.1***	**12.9**	**26.3**	**31.1**	1.1	0.6

[a] Each value is the mean of three troughs with 16 plants in each, evaluated three weeks after inoculation and representing percentage reduction compared to the control; in each column, values printed in bold differ significantly from the control according to LS Means ($P \leq 0.05$); * indicates a significantly greater reduction at the particular temperature within parameters. (according to Fisher's protected t-test ($P \leq 0.05$).

[b] Each value is the mean of three troughs with 16 plants in each, evaluated three weeks after inoculation; root rot was rated according to a scale 0 = healthy, 1 = 25%, 2 = 50%, 3 = 75% , 4 = 100% rotted.

[c] Included in first experiment.

[d] Included in second experiment.

CHAPTER 4

CHEMICAL DISINFESTATION OF GRAVEL SUBSTRATE USED IN RECIRCULATING HYDROPONIC SYSTEMS

Abstract

The chemical disinfectants calcium hypochlorite, chlorine dioxide + activator, dazomet, formaldehyde, glutaraldehyde, hydrogen peroxide, hydrogen peroxide + formic acid, metham-sodium, methyl bromide + chloropicrin, N-alkyl dimethyl benzyl ammonium chloride, polydimethyl ammonium chloride and sodium hypochlorite, were evaluated in one or more experiments for the eradication of *Pythium* and *Fusarium* populations in naturally-infested gravel utilised as substrate in a recirculating hydroponic system. Overall, the *Fusarium* population was more resistant to chemical treatment than the *Pythium* population. Total control of both populations could be obtained in at least one of the experiments with dazomet at 20 and 30 g m^{-2} and formaldehyde at 10 ml l^{-1}. Metham-sodium at 20, 10 and 5 ml l^{-1} reduced the *Pythium* population to zero and was also highly effective against *Fusarium*. Under semi-commercial conditions, significant, but not total control of *Pythium* was achieved with formaldehyde at 5 ml l^{-1}, hydrogen peroxide + formic acid at 25 ml l^{-1}, methyl bromide + chloropicrin at 100 g m^{-2}, polydimethyl ammonium chloride at 10 ml l^{-1} and sodium hypochlorite at 50 ml l^{-1}. Hydrogen peroxide + formic acid at 25 ml l^{-1} and polydimethyl ammonium chloride at 10 ml l^{-1} were correspondingly effective against *Fusarium*.

INTRODUCTION

Root diseases caused by *Pythium* spp. are particularly important in hydroponic systems (Jenkins & Averre, 1983; Zinnen, 1988; Cherif & Belanger, 1991; Cherif *et al.*, 1994, 1997; Stanghellini *et al.*, 1996; Sanchez *et al.*, 2000). Recirculating closed cultural systems that employ a common reservoir for distributing nutrient solution to and from various separate production units provide an ideal environment for disease spread. If the pathogen is accidentally introduced into such a

system at any site, rapid and uniform distribution is virtually guaranteed and control often difficult to achieve (Stanghellini *et al.*, 1984; Paulitz, 1997). Inoculum may be transmitted from diseased plants in run-off water into the reservoir and from there be further disseminated throughout the entire production facility (Mebals *et al.*, 1997b).

Attempts at controlling *Pythium* spp. in hydroponica have met with varying success. Disinfestation of nutrient solutions by heat, ozonation, UV-radiation, filtration, amendment with surfactants or incorporation of antagonists has proved to be effective in preventing the spread of *Pythium* in recirculating systems (Zhou & Paulitz, 1993; Runia, 1994, 1995; Wohanka, 1995; McCullagh *et al.*, 1996; Menzies & Belanger, 1996; Stanghellini *et al.*, 1996; Mebalds *et al.*, 1997b). However, the volume of water in circulation in such systems renders treatments like these rather costly. Furthermore, while zoospores are relatively sensitive, the treatments are not always effective against mycelial propagules retained in the substrate (Hendrix & Campbell, 1973).

Infected root residues that persist in the hydroponic substrate after the crop has been harvested constitute a major source of inoculum (Menzies & Belanger, 1996; Sanchez *et al*, 2000). Removal of the residues and disinfestation of the recycled substrate are essential for maintaining a pathogen-free system (Stanghellini & Rasmussen, 1994). Various compounds are available that can be used for this purpose, e.g. surfactants (Stanghellini & Tomlinson, 1987; Stanghellini *et al.*, 1996) fumigants (Garibaldi & Gullino, 1995; Mappes, 1995), and chemicals applied through irrigation or drenching (Handreck & Black, 1984; Fritsch & Huber, 1995). This paper reports on the efficacy of selected chemical disinfectants against *Pythium* and *Fusarium* spp. in naturally-infested hydroponic gravel substrate. *Fusarium* was included as reference (Runia, 1995) because it is also an important pathogenic genus in hydroponic systems and generally more resistant to chemicals than *Pythium* (Picket-Popoff & Parker, 1994; Minuto *et al.*, 1995).

MATERIALS AND METHODS

The study comprised three experiments, viz. (i) a pilot trial in which gravel collected from commercial beds was transferred into smaller containers and treated with chemicals, (ii) a large-scale screening where chemicals were applied *in situ* to the gravel in commercial hydroponic

beds, and (iii) a small-scale refinement, conducted similarly to the pilot trial, to optimise dosage rates of selected chemicals. The chemicals that were tested are listed in Table 1. Treatment with sterile water (SW) served as control, except in the large-scale screening where the water was not sterilised.

(i) Pilot trial

Approximately 60 l of gravel was obtained from a *Pythium*-infested commercial hydroponic system producing butter head lettuce (*Lactuca sativa* L. var. *capitata* L.). The gravel was collected from four hydroponic beds in the system, pooled, mixed and dispensed into thirty 2 l plastic containers, each with ten 5-mm-diameter holes in the bottom. The gravel in each of three containers was treated at 23°C with one of the chemicals indicated in Table 1. Basamid was sprinkled on the surface of the gravel and then drenched into the gravel with 2 l of SW. The other chemicals were either suspended or dissolved in SW and 2 l of the suspension/solution drenched through the gravel in each container, the excess liquid draining through the holes at the bottom. Containers receiving dazomet, hydrogen peroxide, hydrogen peroxide + formic acid, and metham-sodium were sealed with tight-fitting lids to enhance the fumigative action of the chemicals. Metham-sodium was applied to gravel in the containers with and without a saucer underneath to establish if enhanced retention of the compound could increase its efficacy.

After 48 hours, the gravel in each container was flushed twice with 2 l SW to remove chemical residues. The gravel was then transferred to a clean container without holes and the root residues that remained were extricated by adding 1 l of SW to the container and swirling it until the residues had risen to the surface. The supernatant was collected in a clean Erlenmeyer flask and decanted through filter paper to retrieve the root segments. Fifteen *ca.* 5-mm-long root segments from each replicate were plated, five segments per plate, on a *Pythium*-selective medium (Roux & Botha, 1997), and a further 15 segments on RBGU medium (Van Wyk *et al.*, 1986) selective for *Fusarium*. Plates were incubated for four days at 25 °C and the number of root segments yielding *Pythium* or *Fusarium* were recorded.

(ii) Large-scale screening

Three blocks, each comprising eleven 27 x 0.5 x 0.1 m hydroponic beds, were randomly designated in the commercial recirculating gravel system from which gravel was collected for the first experiment. Temperatures in the hydroponicum varied between 13 and 27 °C. One bed in each block was treated with one of the chemicals indicated in Table 1. Dazomet was sprinkled onto the gravel in the beds and then drenched into each bed with 300 l of water, whereas methyl bromide + chloropicrin was released from pressurised canisters into the beds. The other chemicals were applied in 300 l of water to the respective beds. Beds receiving dazomet and methyl bromide + chloropicrin were covered with plastic sheeting.

After 48 hours, each bed was rinsed with 300 l of water to remove chemical residues. Ten gravel samples of approximately 700 ml each were collected along the length of each bed. The samples from each bed were pooled and transferred to a clean container. Two litres of water was added to the gravel, the container was swirled until root segments floated in the water, and the segments were collected in an Erlenmeyer flask. The presence of *Pythium* and *Fusarium* in the root segments was determined as described above.

(iii) Small-scale refinement

Approximately 60 l of gravel was randomly collected from control beds in the previous large-scale screening. The gravel was pooled, mixed, and dispensed into thirty 2 l plastic containers, each with ten 5-mm-diameter holes in the bottom. The gravel in each of three containers was treated at 25 °C with one of the chemicals indicated in Table 1. Containers receiving formaldehyde, hydrogen peroxide + formic acid and metham-sodium were sealed as before during the exposure period. The same procedures for collection of root segments and enumeration of *Pythium* and *Fusarium* were followed as in the pilot trial.

RESULTS

The dominant *Fusarium* species in all three experiments was *Fusarium oxysporum* Schltdl. emend. W.C. Snyder & H.N. Hansen, with *Fusarium solani* (Mart.) Appel. & Wollenw. the

second most prevalent. Although the *Pythium* species were not identified, it can be assumed that the population comprised mainly *Pythium coloratum* Vaartaja, *Pythium irregulare* Buisman and *Pythium* groups F, G and HS, previously (Chapter 2) shown to be present in the hydroponicum concerned.

The *Fusarium* population was more resistant to chemical treatment than the *Pythium* population, with a mean overall reduction of 42% compared to 80% (Tables 2 & 3). In the pilot trial, all the chemicals that were evaluated, except chlorine dioxide + activator, significantly reduced the incidence of *Pythium*, whereas calcium hypochlorite, dazomet, glutaraldehyde, metham-sodium, PDAC and sodium hypochlorite were effective against *Fusarium*. Application of metham-sodium with a saucer underneath the container did not enhance the fungicidal action of the compound.

Efficacy was more pronounced in the large-scale screening, with the incidence of *Pythium* and *Fusarium* being significantly reduced by all the chemicals included in the experiment. Dazomet at 20 and 30 g m^{-2} and formaldehyde at 10 ml l^{-1} provided total (100%) control of both populations, whilst metham-sodium at 10 and 20 ml l^{-1} reduced the *Pythium* population to zero. The efficacy of metham-sodium against *Pythium* was verified in the small-scale refinement, where even the 5 ml l^{-1} application rate resulted in total control. Total control of *Pythium* in the refinement experiment was also achieved with PDAC at 5 and 10 ml l^{-1}, whereas significant control was evident with hydrogen peroxide + formic acid at 25 and 30 ml l^{-1} and formaldehyde at 10 ml l^{-1}, but not at 5 ml l^{-1}. None of the chemicals significantly reduced the incidence of *Fusarium*. Indeed, gravel treated with formaldehyde at 5 ml l^{-1} and hydrogen peroxide + formic acid at 30 ml l^{-1} yielded 54% and 60% higher *Fusarium* counts, respectively, than the control.

DISCUSSION

Pathogen colonisation of even a single plant, or plant residue for that matter, constitutes a serious threat to the entire plant population in a recirculating hydroponic system (Stanghellini *et al.*, 1990b). Spread of the pathogen from such an infection site can obviously be restricted by addition of fungicides to the nutrient solution. However, no fungicides are presently registered

for use in hydroponic systems in South Africa. It is also unlikely that any will be registered in the foreseeable future as the hydroponic industry, due to the limited area it occupies, is not a priority to chemical companies. Furthermore, the rapid succession and short duration of crop cycles do not allow sufficient time for the lag period between application and harvesting prescribed for most fungicides, hence precluding their usage.

Total eradication of the residual inoculum therefore remains the only way of rendering an infested hydroponic substrate suitable for replanting. Results of the present study indicate that relatively few of the chemical disinfectants that were tested are capable of achieving this. However, it should be kept in mind that the initial screening and eventual small-scale refinement were biased towards the pathogen, with limited contact time allowed for the chemicals. Of the compounds that did not make the grade in these experiments, hydrogen peroxide and chlorine have been evaluated before in hydroponic systems. Hydrogen peroxide has thus far been effective only under experimental conditions, in which tomato mosaic virus was inactivated at 400 ppm and conidia of *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen were killed at 100 ppm (Runia, 1995; Menzies & Belanger, 1996). Even at 12 500 ppm in the present study, hydrogen peroxide provided only moderate suppression of *Pythium* and had no significant effect on *Fusarium*, despite having sporicidal activity and not readily being inactivated by organic matter. The mixture of hydrogen peroxide + formic acid was much more effective against *Pythium* than hydrogen peroxide on its own. This mixture is being marketed commercially in the Netherlands under the trade name Reciclean®, the addition of formic acid being aimed at cleaning emitters of irrigation systems from calcium deposits (Anonymous).

Chlorine compounds have been tested frequently in hydroponica. Stanghellini *et al.* (1996) used sodium hypochlorite at a rate of 10% to successfully surface-sterilise an entire hydroponic system in which the efficacy of a non-ionic surfactant was evaluated. Previously, Bates & Stanghellini (1984) reported chlorine to be effective for controlling *Pythium aphanidermatum* (Edson) Fitz. root rot of cucumber (*Cucumis sativus* L.) and tomato (*Lycopersicon esculentum* Mill.), but found sodium hypochlorite at concentrations of 1 to 6 $\mu g\ ml^{-1}$ to be ineffective against *P. aphanidermatum* and *P. dissotocum* Drechsler, and phytotoxic to spinach (*Spinacea*

oleracea L.), when applied to the nutrient solution. Activity of chlorine dioxide towards *Pythium* and *Fusarium* has been reported by Mebalds *et al.* (1997b). Chlorine, regardless of being applied as chlorine gas, sodium hypochlorite or calcium hypochlorite (all of which yield hypochlorous acid [HClO] and then atomic oxygen), nevertheless remains one of the most effective and safest chemical disinfectans of water sources, and is also employed in the dairy and food industries. Chlorine has an oxidative action and destroys cellular material of vegetative bacteria and fungi, though not spores. Death of almost all microorganisms usually occurs within 30 minutes but, in the presence of organic material, an excess of chlorine has to be applied to ensure microbial destruction since the organic material interferes with the action of chlorine by reacting with it (Runia, 1994; Mebalds *et al.*, 1997b). It was interesting to note that the efficacy of calcium hypochlorite and sodium hypochlorite in the present study did not differ significantly, despite the almost 17 times lower application rate of the former. It must be noted however that disinfestation of the infected root residues that persist in the substrate after the crop has been harvested (as in the present study) is a much more difficult to achieve than disinfestation of irrigation water alone. Chlorine, incapable of penetrating plant tissue effectively, therefore proved to be ineffective in the present study.

Dazomet, formaldehyde and metham-sodium were the only chemicals which provided total control of *Pythium* in the large-scale screening, whereas application of PDAC resulted in a 93% reduction, as well as total control of *Pythium* in the pilot trial and small-scale refinement. Dazomet and formaldehyde also eradicated *Fusarium*, while significant reductions of 93% and 76% in *Fusarium* populations were evident with metham-sodium and PDAC, respectively. PDAC is a quaternary ammonium compound (QAC) commonly used for disinfecting purposes in the horticultural and flower industries. For a QAC, PDAC performed exceptionally well, particularly when considering that it was applied at a lower dosage than the commercially recommended rate. As a rule, QACs are effective only against fungal structures not containing a cell wall, e.g. zoospores, but in the present study PDAC obviously also killed walled propagules such as mycelium, conidia and chlamydospores. This wide spectrum of activity is in accordance with the results of a study conducted by the ARC-Plant Protection Research Institute (unpublished data) which showed PDAC to eradicate sclerotia of *Sclerotium rolfsii* Sacc. However, with *Rhizoctonia solani* J.G. Kühn, Muller & Wehner (1999) obtained a reduction in

sclerotial viability of between 30% and 60%, depending on the concentration of PDAC used.

Formaldehyde, dazomet and metham-sodium all have a fumigative action. Formaldehyde is commonly used for sterilising gravel, pipelines and reservoirs, but should not come in contact with living plants during media sterilisation. (Harris, 1992). Dazomet and metham-sodium are both rapidly converted to methyl isothiocyanate (MITC) when released into the environment (Tomlin, 1994). MITC is a general biocide with activity against many fungi, insects and nematodes, but unfortunately also plants. Treated substrates can therefore not be planted until free of MITC, usually some time after application (Handreck & Black, 1984; Tomlin, 1994; Fritsch & Huber, 1995). Dazomet had the advantage over metham-sodium in that it eradicated *Fusarium*, and furthermore is less expensive to apply at recommended rates. However, in terms of cost-effectiveness, formaldehyde should be the preferred choice. The compound is highly soluble in water, self-dispersing and relatively easy to use, though extremely irritating to mucous membranes and toxic to virtually all forms of life (Buckle, 1981). Based on the results of this study formaldehyde has nevertheless been used successfully and safely as sterilant in some commercial gravel recirculating hydroponic systems at a rate of 10 ml l^{-1}.

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TABLE 1. Chemicals screened in the various experiments

Chemical	Product name	Rate (product)	Experiment		
			(i)	(ii)	(iii)
Calcium hypochlorite	HTH	3 g l^{-1}	√		
Chlorine dioxide + activator	Purogene	2 ml l^{-1}	√		
Dazomet (98%)	Basamid	20 g m^{-2}		√	
		30 g m^{-2}	√	√	
Formaldehyde	Formalin	5 ml l^{-1}		√	√
		10 ml l^{-1}		√	√
Hydrogen peroxide + formic acid *	Reciclean	25 ml l^{-1}	√	√	√
		30 ml l^{-1}			√
Glutaraldehyde	EcoSanitizer	10 ml l^{-1}	√		
Hydrogen peroxide		12.5 ml l^{-1}	√		
Metham-sodium	Herbifume	5 ml l^{-1}			√
		10 ml l^{-1}		√	√
		20 ml l^{-1}	√	√	
Methyl bromide + chloropicrin	Methyl bromide	100 g m^{-2}		√	
N-alkyl dimethyl benzyl ammonium chloride 5% (NDBAC)	Desogerme	500 ppm	√		
Polydimethyl ammonium chloride 12% (PDAC)	Sporekill	5 ml l^{-1}			√
		10 ml l^{-1}	√	√	√
Sodium hypochlorite	Jik	50 ml l^{-1}	√	√	

* 35% hydrogen peroxide mixed with 15% formic acid in a 1:1 ratio.

TABLE 2. Effect of chemical treatment on the survival of *Pythium* species in root residues in gravel used as substrate in a recirculating hydroponic system

Chemical	Rate	Incidence (%) [a]		
		(i) [b]	(ii) [c]	(iii) [d]
Calcium hypochlorite	3 g l^{-1}	26.7 bc		
Chlorine dioxide + activator	2 ml l^{-1}	75.6 a		
Dazomet (98%)	20 g m^{-2}		0 b	
	30 g m^{-2}	4.4 c	0 b	
Formaldehyde	5 ml l^{-1}		17.8 b	71.1 a
	10 ml l^{-1}		0 b	37.8 b
Hydrogen peroxide + formic acid	25 ml l^{-1}	2.2 c	6.7 b	28.9 b
	30 ml l^{-1}			35.6 b
Glutaraldehyde	10 ml l^{-1}	4.4 c		
Hydrogen peroxide	12.5 ml l^{-1}	44.4 b		
Metham-sodium	5 ml l^{-1}			0 c
	10 ml l^{-1}		0 b	0 c
	20 ml l^{-1}	8.9 c	0 b	
Metham-sodium with saucer	20 ml l	6.7 c		
Methyl bromide + chloropicrin	100 g m^{-2}		17.8 b	
NDBAC [e]	500 ppm	26.7 bc		
PDAC [f]	5 ml l^{-1}			0 c
	10 ml l^{-1}	0 d	4.4 b	0 c
Sodium hypochlorite	50 ml l^{-1}	11.1 c	24.4 b	
Control		77.8 a	66.8 a	84.4 a

TABLE 2 (continued)

[a] Mean of three replicates of 15 root segments each; values in columns followed by the same letter do not differ significantly according to Fisher's protected t-test ($P \leq 0.05$).

[b] Pilot trial.

[c] Large-scale screening.

[d] Small-scale refinement.

[e] N-alkyl dimethyl benzyl ammonium chloride.

[f] Polydimethyl ammonium chloride.

TABLE 3. **Effect of chemical treatment on the survival of *Fusarium* species in root residues in gravel used as substrate in a recirculating hydroponic system**

Chemical	Rate	Incidence (%)[a]		
		(i)[b]	(ii)[c]	(iii)[d]
Calcium hypochlorite	3 g l^{-1}	33.3 bc		
Chlorine dioxide + activator	2 ml l^{-1}	93.3 a		
Dazomet (98%)	20 g m^{-2}		0 d	
	30 g m^{-2}	15.6 e	0 d	
Formaldehyde	5 ml l^{-1}		64.4 ab	51.1 a
	10 ml l^{-1}		0 d	26.7 a
Formic acid + hydrogen peroxide	25 ml l^{-1}	91.1 a	22.2 cd	24.4 a
	30 ml l^{-1}			53.3 a
Glutaraldehyde	10 ml l^{-1}	37.8 bc		
Hydrogen peroxide	12.5 ml l^{-1}	77.8 a		
Metham-sodium	5 ml l^{-1}			13.3 a
	10 ml l^{-1}		4.4 d	28.9 a
	20 ml l^{-1}	2.2 cd	4.4 d	
Metham-sodium with saucer	20 ml l	2.2 cd		
Methyl bromide + chloropicrin	100 g m^{-2}		80.0 a	
NDBAC[e]	500 ppm	73.3 ab		
PDAC[f]	5 ml l^{-1}			33.3 a
	10 ml l^{-1}	51.1 b	15.6 cd	31.1 a
Sodium hypochlorite	50 ml l^{-1}	37.8 bc	40.0 bc	
Control		95.6 a	64.4 ab	33.3 a

TABLE 3 (continued)

[a] Mean of three replicates of 15 root segments each; values in columns followed by the same letter do not differ significantly according to Fisher's protected t-test ($P \leq 0.05$).

[b] Pilot trial.

[c] Large-scale screening.

[d] Small-scale refinement.

[e] N-alkyl dimethyl benzyl ammonium chloride.

[f] Polydimethyl ammonium chloride.

CHAPTER 5

GENERAL DISCUSSION

The study presented in this report constitutes the first extensive survey of *Pythium* species associated with hydroponically-grown crops in South Africa. Thompson & Labuschagne (2001) previously referred to the presence of four *Pythium* species and two heterothallic groups on seven crop species in South African hydroponica, whereas Botha & Coetzer (1996) reported the isolation of eight species and two groups from six vegetable species, some of which were cultivated hydroponically. The present study included seven vegetable crops in the first survey and an additional 12 crop species in the second survey, which together yielded eight *Pythium* species and all five the existing heterothallic groups. This represents 35% of all the *Pythium* species/groups thus far reported in South Africa (Crous *et al.*, 2000; Thompson & Labuschagne, 2000/2001), and expands the list of entries by 22%. Only two of the species reported from hydroponica by Thompson & Labuschagne (2001), viz. *Pythium dissotocum* Drechsler and *Pythium aristosporum* Vanterpool, could not be isolated. The absence of *P. dissotocum* in the present study remains unclear as it is known to be associated with hydroponically grown lettuce (Stanghellini & Kronland, 1986). However, the identification by Thompson & Labuschagne (2001) of *P. aristosporum* from a hydroponic system growing non-graminaceous crops is queried as this species has only been isolated from members of the Poaceae, also in South Africa (Van der Plaats-Niterink, 1981; Meyer & van Dyk, 2002). *Pythium sylvaticum* W.A. Campb. & J.W. Hendrix, the only *Pythium* species previously reported from lettuce in South Africa, was also not found. Although *P. sylvaticum* appears to be common in aquatic environments (Van der Plaats-Niterink, 1975; Shokes & McCarter, 1976), no reference to its occurrence in a hydroponic system could be traced.

Some of the *Pythium* species/groups seemed to prefer specific types of hydroponic systems. For instance, *Pythium coloratum* Vaartaja and *Pythium* group F occurred mostly in recirculating gravel systems, whereas *Pythium aphanidermatum* (Edson) Fitz. and *Pythium spinosum* Sawada were isolated only from ebb-and-flow and open dripper systems. However, none of the hydroponica surveyed were free of *Pythium* and most contained a *Pythium* population that was neither host-specific nor temperature-restricted, and hence capable of causing losses to diverse

crops throughout the year. Mention should be made here of *P.* group F, which was the dominant species/group, in recirculating gravel systems at least. Although *P.* group F reduced the growth of various crops significantly, it did not kill the plants, and can thus be classified as a successful pathogen. This pathogenic competence undoubtedly contributed to the predominance of *P.* group F and should render it difficult to control.

From the above it is clear that management of pythiasis in a hydroponicum would depend on total and persistent suppression of the entire *Pythium* population. Results obtained in Chapter 4 indicated that a number of chemical disinfectants are capable of eradicating *Pythium* from infested gravel substrate. However, substrates are not the only source of pathogens as they can also be introduced through seed/seedlings, air, water and insects (Stanghellini & Rasmussen, 1994). To be successful, a disease management protocol should therefore include (a) use of pathogen-free seedlings reared in steam-pasteurised growth medium, (b) sterilisation of irrigation water by means of an effective treatment such as ozonation or chlorination, (iii) disinfestation of the substrate, and (iv) proper insect control. Regular monitoring of the irrigation water for the presence of *Pythium*, and other potentially pathogenic organisms, would be required to maintain quality control in such a system.

Fungicides have been excluded from the above strategy. As indicated in Chapter 4, no fungicides are registered for use in hydroponica in South Africa. Various compounds, particularly metalaxyl and fosetyl-Al (Morgan, 1999), have nevertheless been tested successfully for the control of *Pythium* in hydroponic systems. However, as most of them are active only against the *Oomycota* (besides *Pythium*, particularly *Phytophthora* and *Peronosporales* species), they would be of little value against other fungoid pathogens such as protozoa (e.g. *Spongospora subterranea* [Wallr.] Lagerh.) and fungi (mostly *Botrytis*, *Chalara*, *Colletotrichum*, *Fusarium*, *Microdochium*, *Olpidium*, *Rhizoctonia*, *Sclerotinia* and *Verticillium* species) known to infect crops in hydroponica, not to mention viruses (e.g. *cucumber green mottle mosaic*, *lettuce big vein*, *melon necrotic spot* and *tomato mosaic* viruses) and bacteria (e.g. *Clavibacter michiganense*, *Erwinia carotovora* and *Ralstonia solanacearum*) (Staunton & Cormican, 1978; Evans, 1979; Daughtrey & Schippers, 1980; Davies, 1980; Tomlinson & Faithfull, 1980; Jenkins & Averre, 1983; Vanachter *et al.*, 1983 Tomlinson & Thomas, 1986; Van Voorst *et al.*, 1987; Pategas *et al.*, 1989; Brammall & Lynch, 1990; Linde *et al.*, 1990;

Stanghellini *et al.*, 1990a, b; Stanghellini & Rasmussen, 1994; Morgan, 1999). Besides being ineffective against non-target organisms, persistent use of such fungicides can also lead to the development of iatrogenic diseases (Griffiths, 1981). Furthermore, it is commonly known that fungi rapidly develop resistance against selective systemic fungicides.

Another, and very important, reason for the exclusion of fungicides from a control strategy is the growing concern about their negative impact on consumers and the environment, and the global shift towards organic production. This concern is also applicable to other chemicals, including those that were evaluated in the present study, and will have to be addressed by the hydroponic industry. Although hydroponic systems, by nature of their reliance on synthetic fertilisers, are not amenable to organic farming, the intensive cropping practices inherent to hydroponic production render it eminently suited to alternative disease control. Alternative control strategies applicable to hydroponica obviously include resistant cultivars, which is the prerogative of the plant breeding/genetic engineering fraternity, and the use of introduced antagonists. In this regard it is interesting to take cognisance of the existence of a product named Polygandrum®, a formulation of *Pythium oligandrum* Drechsler marketed by Plant Production Institute, Slovakia, as seed or soil treatment for the control of *Pythium ultimum* Trow.

A novel approach to disease control worth mentioning here is the induction of systemic resistance to infection. This can be achieved by exposing plants to UV radiation (Runia, 1995), or to compounds such as salicylic acid (Schneider & Ulrich, 1994), oxalate (Doubrava *et al.*, 1988), phosphates (Gottstein & Kuc, 1989), unsaturated fatty acids (Cohen *et al.*, 1991), jasmonic acid (Cohen *et al.*, 1993), DL-3-amino-n-butanoic acid (Cohen, 1994), silicon (Chérif *et al.*, 1992) or chitosan (Walker-Simmons *et al.*, 1983). Besides inducing resistance in plants, chitosan is also known to initiate the formation of structural barriers in host tissue (El Ghaouth *et al.*, 1994) and to cause morphological and cytological alterations in the pathogen (Benhamou, 1992; El Ghaouth *et al.*, 1992). In addition to the above, antifungal compounds produced by plants have potential as natural fungicides, and some are known to induce systemic plant defence mechanisms, e.g. extracts from giant knotweed (*Reynoutria sacchalinensis* (Nakai) F. Schmidt (Daayf *et al.*, 1997), spinach (*Spinacea oleracea* L.) and rhubarb (*Rheum raponticum*

L.) (Doubrava *et al.*, 1988). Plants can also be "immunised" against disease by prior inoculation with the particular pathogen (Dalisay & Kuc, 1995), a different pathogen (Stroember & Brishammer, 1991), extracts of pathogenic organisms (Ricci *et al.*, 1989), or through the action of plant growth-promoting rhizobacteria (Wei *et al.*, 1991).

Lastly, the use of glucosinolate-containing brassicaceous crops seems to be an alternative disease control option tailor-made for hydroponic production. Rotation with brassicaceous crops and incorporation of brassica residues into soil or other growth media are known to suppress a variety of pests and disease organisms, including fungi, nematodes, insects, bacteria and weeds. The suppressive effect is due to the presence of ß-D-thioglucosidic compounds referred to as glucosinolates (GSLs) in the Brassicaceae and other families of the order Capparales (Brown & Morra, 1997). GSLs *per se* are not toxic but are hydrolysed in the presence of water to biologically active compounds such as organic cyanides, ionic cyanate, oxazolidinethiones and isothiocyanates (ITCs), by the enzyme myrosinase which occurs endogenously in brassica tissues. Of the various GSL hydrolysis products, ITCs are considered the most toxic. Indeed, methyl isothiocyanate, which proved to be highly effective as sterilant in Chapter 4, is a synthetic derivative of ITC. ITCs are general biocides that interact nonspecifically and irreversibly with proteins and amino acids (Fenwick *et al.*, 1983; Kawakishi *et al.*, 1983; Kawakishi & Kaneko, 1987). As ITCs are volatile, the utilisation of brassicaceous crops in the control of pests and diseases have been termed "biofumigation" (Kirkengaard *et al.*, 1993; Angus *et al.*, 1994). It certainly would be worthwhile to investigate biofumigation in hydroponic systems in South Africa, with brassicaceous plants as rotation crops.

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STUDY OF *PYTHIUM* ROOT DISEASE OF HYDROPONICALLY GROWN CROPS, WITH EMPHASIS ON LETTUCE

by

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RESUMé

Eight species of *Pythium*, *P. acanthicum*, *P. aphanidermatum*, *P. coloratum*, *P. diclinum*, *P. irregulare*, *P. myriotylum*, *P. perplexum*, *P. spinosum*, and representatives of five heterothallic groups, F, G, HS, P and T, were isolated from roots and crowns of crops and from nutrient solutions, substrates, water sources and run-off water in 11 commercial hydroponic systems in South Africa. *P.* group F was isolated most frequently and from the greatest variety of crops, followed by *P. irregulare*, *P. spinosum*, *P. aphanidermatum* and *P.* group HS. *P. acanthicum* was recorded for the first time on strawberry in South Africa, *P. aphanidermatum* on parsley, *P. coloratum* on lettuce, *P. irregulare* on Chinese cabbage and lettuce, *P. perplexum* on tomato, *P.* group G on lettuce, and *P.* group HS on cucumber and lettuce.

The pathogenicity of five of the above *Pythium* species and three heterothallic groups to butter head lettuce was determined at 21 °C and 28 °C in three hydroponic systems, viz. static nutrient solution, hydroculture and a recirculating gravel system. Overall, *P. spinosum* was the most aggressive species at 21 °C, followed by *P. irregulare* and *P.* group HS. *P. myriotylum* was the only species which consistently showed greater virulence at 28 °C than at 21 °C. No significant differences in virulence at the two temperatures were evident with *P. coloratum*, *P. diclinum* and *P.* groups F and T.

Various chemical disinfectants were evaluated for the suppression of *Pythium* and *Fusarium* populations in naturally-infested gravel utilised as substrate in recirculating gravel systems. Overall, the *Fusarium* population was more resistant to chemical treatment than the *Pythium* population. Total control of both *Pythium* and *Fusarium* was obtained with dazomet at 20 and 30 g m^{-2} and formaldehyde at 10 ml l^{-1}. Metham-sodium at concentrations as low as 5 ml l^{-1} reduced the *Pythium* population to zero and was also highly effective against *Fusarium*. Significant, albeit not total, control of *Pythium* was achieved with formaldehyde at 5 ml l^{-1}, hydrogen peroxide + formic acid at 25 ml l^{-1}, methyl bromide + chloropicrin at 100 g m^{-2}, polydimethyl ammonium chloride at 10 ml l^{-1} and sodium hypochlorite at 50 ml l^{-1}. Formic acid + hydrogen peroxide and polydimethyl ammonium chloride were also highly effective against *Fusarium*.

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STUDIE VAN *PYTHIUM* WORTELSIEKTE BY HIDROPONIESE GEWASSE, MET DIE KLEM OP BLAARSLAAI

deur

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SAMEVATTING

Agt spesies van *Pythium*, *P. acanthicum*, *P. aphanidermatum*, *P. coloratum*, *P. diclinum*, *P. irregulare*, *P. myriotylum*, *P. perplexum*, *P. spinosum*, en verteenwoordigers van vyf heterotalliese groepe, F, G, HS, P en T, is geïsoleer vanaf wortels en krone van gewasse in 11 kommersiële hidroponiese sisteme in Suid-Afrika, asook vanuit voedingsoplossings, substrate, waterbronne en afloopwater in die hidroponikums. *P.* groep F is mees dikwels geïsoleer en vanaf die grootste verskeidenheid van gewasse, gevolg deur *P. irregulare*, *P. spinosum*, *P. aphanidermatum* en *P.* groep HS. *P. acanthicum* is vir die eerste keer in Suid-Afrika geïsoleer vanaf aarbei, *P. aphanidermatum* vanaf pietersielie, *P. coloratum* vanaf blaarslaai, *P. irregulare* vanaf Chinese kool en blaarslaai, *P. perplexum* vanaf tamatie, *P.* groep G vanaf blaarslaai en *P.* groep HS vanaf komkommer en blaarslaai.

Die patogenisiteit van vyf van die bogenoemde *Pythium* spesies en drie heterotalliese groepe teenoor botterslaai is by 21 °C en 28 °C bepaal in drie hidroponiese sisteme, nl. statiese voedingsoplossing, hidrokultuur en 'n hersirkulerende gruissisteem. *P. spinosum* was die aggressiefste spesie by 21 °C, gevolg deur *P. irregulare* en *P.* groep HS. *P. myriotylum* was die enigste spesie wat konsekwent meer aggressief was by 28 °C as by 21 °C. Die aggressiwiteit van *P. coloratum*, *P. diclinum* en *P.* groepe F en T by die twee temperature het nie betekenisvol

verskil nie.

Verskeie chemiese ontsmettingsmiddels is geëvalueer vir die onderdrukking van *Pythium* en *Fusarium* populasies in natuurlik-besmette substraatgruis afkomstig van 'n hersirkulerende gruissisteem. Die *Fusarium* populasie was oor die algemeen meer bestand teen chemiese behandeling as die *Pythium* populasie. Volkome beheer van *Pythium* sowel as *Fusarium* is verkry met dasomet teen 20 en 30 g m^{-2} en formaldehied teen 10 ml l^{-1}. Metam-natrium teen 'n konsentrasie so laag as 5 ml l^{-1} het die *Pythium* populasie uitgewis en was ook baie doeltreffend teen *Fusarium*. Betekenisvolle, alhoewel nie totale, beheer van *Pythium* is behaal met formaldehied teen 5 ml l^{-1}, mieresuur + waterstofperoksied teen 25 ml l^{-1}, metielbromied + chloropikrien teen 100 g m^{-2}, polidimetiel-ammoniumchloried teen 10 ml l^{-1} en natriumhipochloriet teen 50 ml l^{-1}. Mieresuur + waterstofperoksied en polidimetiel-ammoniumchloried was ook besonder doeltreffend teen *Fusarium*.