Pythium and *Phytophthora* associated with root disease of hydroponic lettuce

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DECLARATION

The work presented in this thesis is original and contains no material formerly published or written by another person, except where due acknowledgement has been specified. I hereby declare that I have not submitted this material to any institution for a degree or diploma.

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SUMMARY

Root rot disease of lettuce grown hydroponically has become a serious problem in Australia and worldwide. Farmers in Australia claim that they have suffered heavy yield losses of hydroponic lettuce in summer in recent years. The research reported in this thesis focused on root disease of lettuce grown in hydroponic systems in the Sydney area and included determination of the causes of this disease, isolation of pathogenic organisms and identification of pathogens by using morphological and physiological studies, as well as molecular techniques. The technique involving Inter Simple Sequence Repeats (ISSR) was also used to study the relationships among the populations of these pathogens. Moreover, the effect of the temperature of the nutrient solution on disease development was also investigated. The research reported here represents the first comprehensive survey of hydroponic lettuce farms in and near Sydney, New South Wales (NSW) in relation to root disease.

Two surveys investigated root disease severity on lettuce grown in hydroponic systems in the Sydney and Central Coast areas of NSW. Three different lettuce cultivars (Baby Cos, Red Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2003 to March 2004) in one farm (Leppington 1) in the first survey. In the second survey, four different lettuce cultivars (Baby Cos, Red Oak, Green Oak and Brown Mignonette) were surveyed five times over an 11 month period (May 2004 to March 2005) in four different farms (Leppington 1 and 2 and Central Coast 1 and 2). From these two surveys, it appears that root disease of hydroponic lettuce occurred at farms only in the warmer times of the year, when the nutrient solution temperature was 20-30.5°C, and not in the cooler times of the year, when the nutrient solution temperature was 13.5-18°C.

In order to isolate pathogenic organisms, lettuce plants were sampled during the two farm-based surveys. Isolations were carried out from the roots of the cultivars surveyed from the same farms and at the same times as the surveys. Two genera of oomycetes, *Pythium* (81 isolates) and *Phytophthora* (68 isolates), were the main microorganisms isolated from lettuce roots grown in hydroponic systems. *Pythium* was isolated all year round (from 60-100% of samples), but a disease problem at the farms only occurred in

the months with higher temperatures (November, January and March). *Phytophthora* was isolated nearly all year around (from 19-80% of samples).

Isolates of *Pythium* and *Phytophthora* were generally found to be pathogenic to lettuce plants at 25°C and 35°C, but not at 15°C, when lettuce were grown in potting mix. *Pythium coloratum* was found to be pathogenic to lettuce plants grown in an experimental hydroponic system when the nutrient solution temperature was between 22°C and 26°C. Other fungi, such as *Fusarium* spp. and *Rhizoctonia* spp., were also isolated but only infrequently and they were not associated with root disease in the farm at the time of isolation. Furthermore, they were not pathogenic to lettuce grown in potting mix at 15, 25 or 35°C.

The effects of the temperature of the nutrient solution on root disease of lettuce caused by *Pythium* and *Phytophthora* were examined in an experimental hydroponic system. Root rot disease occurred following inoculation with an isolate belonging to *Pythium* group F, or a combination of this isolate and *Phytophthora drechsleri*, under a temperature regime of 24-27°C but not at 16-17°C. Yield reduction was found in plants inoculated with an isolate belonging to *Pythium* group F, *Phytophthora drechsleri* and a combination of the two, at a nutrient solution temperature regime which involved exposure to 34°C for 10 hours, followed by 18-20°C for the remainder of the experiment.

Morphological features and physiological characteristics were used to identify 81 isolates of *Pythium* and 68 isolates of *Phytophthora* obtained from roots of hydroponic lettuce. Molecular techniques were also used for identification including polymerase chain reaction-random fragment length polymorphisms (PCR-RFLP) and sequencing of the internal transcribed spacer (ITS) region of rDNA. For population studies, the ISSR technique was used. The 81 isolates of *Pythium* could be divided into three groups on the basis of colony characteristics. Eighty *Pythium* isolates were identified as belonging to *Pythium* group F and one isolate as *Pythium coloratum*. All 68 *Phytophthora* isolates were identified as *Phytophthora drechsleri*. The optimum growth temperature of the isolates belonging to *Pythium* group F and the isolate of *Pythium coloratum* was 30°C. They also grew well at temperatures of 25°C and 35°C and could still grow at 40°C and 5°C. The optimum growth temperature for the isolates of *Phytophthora drechsleri* was

25°C but they were still able to grow at temperatures of 10°C and 35°C. An assessment of mating type was used as a biological marker for all *Phytophthora drechsleri* isolates. All isolates were found to be heterothallic and of the A_1 mating type. They produced oogonia with amphigynous antheridia when paired with the A_2 mating type of *Phytophthora cryptogea.*

When 81 *Pythium* isolates were examined using four primers with the ISSR technique, 11 groups were established. A slight correlation was found between the groups and the sampling times at which isolates in the groups were obtained. However, no correlations were found between the groups and either the farm or the geographic area from which isolates were obtained. Furthermore, there was no correlation between these groups and the lettuce cultivars yielding the isolates. Moreover, no correlations were found between the groups established by the ISSR technique and the three groups identified on the basis of colony characteristics.

The ISSR technique applied to *Phytophthora* isolates yielded six groups. A correlation was found between these groups and the sample times at which isolates were obtained, on the basis of cooler season samples (May and August together) compared with warmer season samples (November, January and March together). No correlation was found between the groups and either the farms or the geographic areas from which the isolates were obtained. Furthermore, there was no correlation between the groups and the lettuce cultivars yielding the isolates.

Based on the findings of this research, root rot disease management in hydroponic lettuce could be achieved by reducing the temperature of the nutrient solution in summer to 20°C or less, whilst maintaining it within a range favourable to lettuce growth. Moreover, methods to reduce the inoculum level of *Pythium* (and possibly *Phytophthora* as well) are worth investigating, as are methods of disease management based on biological control.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BGT	Botanic Gardens Trust
bp	base pair
BSA	Bovine serum albumin
CLA	Carnation Leaf Agar
cm	centimetre
CMA	Corn Meal Agar
dATP	deoxyadenosine triphosphate
dCTP	deoxycytosine triphosphate
DI	Disease Index
DNA	deoxyribonucleic acid
dTTP	deoxythymidine triphosphate
EDTA	ethylenediaminetetraacetic acid
g	gram
GLM	General Linear Model
h	hour
ha	hectare
ISSR	Inter Simple Sequence Repeats
ITS	Internal Transcribed Spacer
min	minute
mL	millilitre
mm	millimetre
NFT	Nutrient Film Technique
ng	nanogram
PCA	Potato Carrot Agar
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PPA	Peptone PCNB Agar
PSA	*Phytophthora* Selective Agar
PYSA	*Pythium* Selective Agar
rDNA	ribosomal DNA
RFLP	Restriction Fragment Length Polymorphisms
rpm	revolutions per minute
s	second
SNA	Spezieller Nährstoffarmer Agar
U	Unit
UTS	University of Technology Sydney
UV	Ultraviolet
WA	Water Agar
µL	microlitre
µm	micrometre

1 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Lettuce (*Lactuca sativa* L.) is one of the most important crops grown in hydroponic systems around the world (Hassall & Associates 2001). Unfortunately, root rot diseases of lettuce grown hydroponically have become a serious problem both in Australia (Tesoriero & Cresswell 1995) and worldwide (Stanghellini & Rasmussen 1994). Heavy yield losses have occurred in crops grown in hydroponic systems when pathogens have been introduced and conditions have been favourable for their development.

Hydroponic systems were developed to allow better quality crops, higher levels of production with shorter production times, reduced labour requirements and lower chemical use (Hassall & Associates 2001). However, nutrient film technique (NFT), the main hydroponic system for lettuce production, provides favourable conditions for pathogens that produce motile infective structures. Once such pathogens are introduced to the system, they spread rapidly between plants and can cause damage and yield losses under certain conditions. Unfortunately, no pesticides or fungicides are registered for controlling root diseases in hydroponic systems in New South Wales (NSW), Australia (Australian Pesticides and Veterinary Medicines Authority (APVMA) 2006). Disinfection products, however, have been used to try to reduce the pathogen density in order to reduce disease severity.

Pythium spp. and *Phytophthora* spp. are the main pathogens isolated from root rot diseases of plants grown in hydroponic systems (Stanghellini & Kronland 1986; Jamart 1999). Farmers in Australia claim that they have suffered increasingly heavy yield losses of hydroponic lettuce in summer over the period from 1995 to the present, over which time the crop has grown in importance (Tesoriero & Cresswell 1995; Tesoriero, pers. comm.). *Pythium* spp. and *Phytophthora* sp. have also been recorded as causing root rot diseases on lettuce grown in hydroponic systems in New South Wales (NSW) (Tesoriero *et al.* 1991). There is, however, a scarcity of studies, both locally and internationally, on root rot disease in hydroponic lettuce.

Morphological methods have traditionally been used to identify fungi and oomycetes. New molecular techniques, however, are now being used to confirm these identifications and to study the relationships between and within species (Levesque & De Cock 2004; Drenth *et al.* 2006). The following review provides a summary of the most important literature that is relevant to research undertaken in this study.

1.2 Hydroponics as a plant production system

1.2.1 Overview of hydroponics

Hydroponics is the growth of plants in a medium other than soil, using a mix of elements dissolved in water. It is considered to be a modern method of growing plants, but different types of hydroponic systems have been used to grow plants for hundreds of years. In 1699, Woodward grew plants in water to which he added small amounts of different garden soils (Harris *et al.* 1974; Sutherland 1986). In France, De Saussure in 1804 and Boussingault in 1851-1856 used only water and added chemicals to grow plants. In 1860, von Sachs published the first formulae for nutrient solutions. His work, and that of Wilhelm Knop in 1865, are the real beginnings of the science of hydroponics (Harris *et al.* 1974).

In the early 1930s, Gericke started the first commercial hydroponic system in California (Jones 1997; Donnan 1998). During the Second World War, the United States armed forces established hydroponic operations in several islands in the Western Pacific to supply fresh vegetables to troops stationed on the islands (Donnan 1998). In the 1970s, a significant advance in hydroponic systems occurred. This was the development of the Nutrient Film Technique (NFT) (Cooper 1975 & 1979), a method which recirculates the plant nutrient solution in a flat-bottomed channel or gully through the bare roots of plants (Seymour 1993). From the 1980s until the present time there have been rapid developments in hydroponic production systems (Hassall & Associates 2001). These developments include conveyor belt methods of planting, harvesting and packaging and total environment control using glasshouse production.

In Australia, commercial hydroponic production started in the early 1970s with open air lettuce production on the Gold Coast of Queensland and carnation production in Victoria (Hassall & Associates 2001). In the 1980s, Rockwool®, a supporting inorganic

medium which can hold the plant and the nutrient solution, was launched and rapidly gained favour for hydroponic production when combined with an European designed management system. Rockwool® was marketed under the Australian brand name, Growool® (Hanger 1993).

In a recent report on hydroponic production published by Hassall and Associates (2001), Holland is ranked first in terms of total hydroponic production area with 10,000 ha (Table 1-1) and Australia is ranked tenth with 500 ha. For hydroponic lettuce production, however, Australia is ranked first in the world with 240 ha. There are, however, no current statistical data for Australia regarding total hydroponic production area (Australian Bureau of Statistics, pers. comm.).

Table 1-1 The top ten hydroponic producing countries in the world. (Data from Hassall & Associates 2001, pp.10-11.)

Country	Date	Area (ha)
Holland	2001	10000
Spain	2001	4000
Canada	2001	2000
France	1996	1000
Japan	1999	1000
Israel	1996	650
Belgium	1996	600
Germany	1996	560
New Zealand	2001	550
Australia	1996	500

1.2.2 Advantages and disadvantages of hydroponic systems

Hydroponic systems have been created to grow better quality plants. These systems have many advantages and only a few disadvantages (Hanger 1993; Adams 1990; Morgan 1999; Hassall & Associates 2001). The advantages are listed below.

1. Growth is faster in a hydroponic system and the crop yield is greater than that in soil.
2. The amount of water needed in a hydroponic system is less than in soil; it is estimated to be 1/30 of that needed in soil-based production.

3. Hydroponic systems allow for the possibility of growth of a crop outside its natural climatic range, including continual growth throughout the year if appropriate temperature control is provided.
4. The amount of labour for tilling, cultivating, watering, weeding, fumigating and other traditional practices is less in a hydroponic system than in soil-based production.
5. The incidence of diseases in hydroponic systems is generally less than in soil, and there is no risk of weed growth.

However, there are some disadvantages of hydroponic systems as listed below.

1. They are expensive to establish and require a high level of maintenance and knowledge.
2. When the system is contaminated by disease, the disease can spread quickly.
3. A hydroponic system is a more complicated and involved process than a soil-based system.

1.2.3 Types of hydroponic systems

There are many different types of hydroponic systems with the differences based primarily on two aspects - whether nutrient usage involves recirculation or not and the nature of the plant support during growth. The two main alternatives for each aspect are described below.

1. Nutrient usage
   - Open or run-to-waste systems: nutrient solution is applied to the plants and then drained off as waste.
   - Closed or recirculating systems: nutrient solution is applied to the plants and, after the nutrient solution runs off, it is collected and recirculated.

2. Plant support during growth
   - Water-based systems: these are closed systems and three main types have been used (NFT, water culture and aeroponics).
   - Media systems: plants are supported by organic or inorganic media, such as Rockwool®, perlite, scoria and sand.

1.2.4 Nutrient Film Technique

This technique was developed in the 1970's by Cooper in England (Cooper 1975 & 1979) (Figure 1-1) and is the main hydroponic system used commercially for lettuce production. The nutrient solution moves slowly past the roots as a film or stream of dissolved minerals in a flat bottomed channel (Mason 1996).

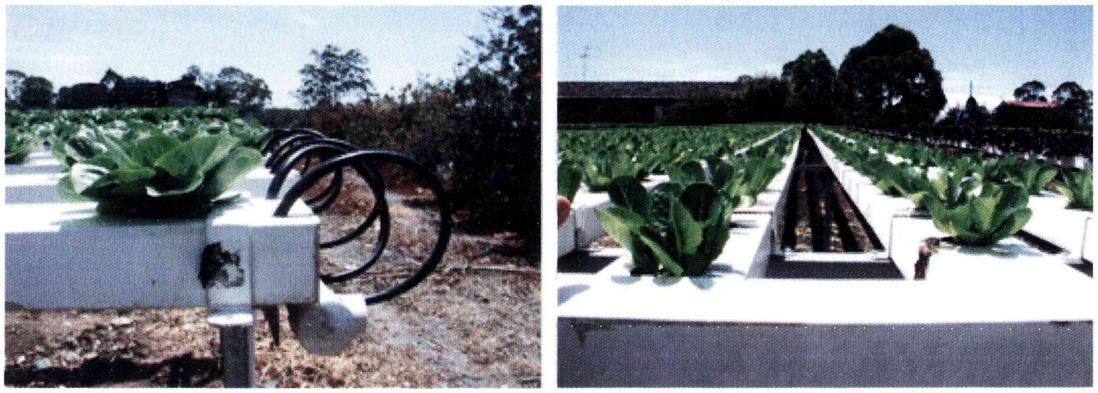

Figure 1-1 Nutrient Film Technique (NFT) for lettuce production. (Manicaro's Lettuce Farm, Leppington, NSW.)

This system has been used widely in commercial production especially for short term crops like lettuce and herbs. However, some problems may occur when NFT systems are used. Firstly, the roots will quickly dry out and become stressed if the flow of nutrient solution is interrupted, even for a short time. Secondly, excessive heating of the channels may occur in newly planted systems since channels are exposed to sunlight. Thirdly, the NFT channels can become blocked by the roots of vigorously growing plants (Mason 1996).

Furthermore, it is important to ensure that sufficient oxygen is available to the plants in NFT systems (Seymour 1993). This may require either agitating the nutrient within the tank or allowing air to reach the nutrient solution within the channels. Production advantages are achievable under NFT systems but vigilant management is required, along with high quality equipment and good system design (Seymour 1993).

1.3 Lettuce

1.3.1 General aspects

Lettuce is considered to be the most important salad vegetable grown today around the world. Lettuce probably originated in or near the Mediterranean basin and knowledge of it stretches back over 6,000 years to the time of the Sumerians, the first known civilisation who lived in Southern Iraq (Davis *et al.* 1997). Evidence of its long history is also found in Egyptian tomb paintings, dating from the Middle Kingdom about 4,500 years ago (Morgan 1999).

It is known that the Romans introduced lettuce to Britain. Later the Dutch and English brought lettuce to the northern part of America, and the French brought it to Canada. The British and other immigrants brought lettuce seeds when they came to Australia. Lettuce production has increased dramatically over the last 10 years in most countries around the world, especially in the United States, Great Britain, Australia, Japan and Spain (Hassall & Associates 2001).

Lettuce has been grown in soil and in hydroponic systems worldwide. In Australia, particularly NSW, approximately 90% of lettuce consumed is grown in hydroponic systems (Hassall & Associates 2001).

1.3.2 Types of lettuce

Lettuce belongs to the Asteraceae family. It is diploid with $2n = 18$ chromosomes (Davis *et al.* 1997). There are many types and varieties of lettuce around the world and they can be divided into many groups. The main lettuce types are described below.

1.3.2.1 Crisp Head

This type is also known as 'Iceberg' and it is the lettuce most widely available as a fresh market type grown in soil (Davis *et al.* 1997). Crisp Head lettuce are grown hydroponically, but are not as popular for hydroponic production as the more favoured gourmet varieties (Morgan 1999).

1.3.2.2 Butter Head

This type of lettuce is a high quality winter green salad grown in greenhouses in England, Holland and other European countries (Davis *et al.* 1997). It is light to dark green outside and creamy yellow inside. The inner leaves have an oily or buttery feel, giving rise to the name 'Butter Head'. Some cultivars have red pigmentation. Green Butter Head varieties include Esmeralda, Atlanta, Blondy, Big Boston, Conny and Dolly. Red Butter Head varieties include Red and Brown Mignonette (Figure 1-2 a), Manto, Bibb and Caddo (Morgan 1999).

1.3.2.3 Leaf Lettuce or Loose Leaf

There are numerous leaf lettuce varieties placed under the Leaf Lettuce or Loose Leaf types (*Lactuca sativa* var. *crispa*), because they do not produce hearted heads or fall into the Oak Leaf or Frilled categories (Morgan 1999). These varieties come in red and green leaves with a variety of leaf sizes, shapes and a range of colour intensities. There are many cultivars of this type grown in soil or hydroponic systems.

1.3.2.4 Oak Leaf

'Oak leaved' varieties were listed by Vilmorin in 1771 for the first time under the name '*Laitue epinard*' (Morgan 1999). This type is divided into three main groups - Pale Green, Brown and Dark Green. It is a Loose Leaf type with thin tender light green or brown/red leaves shaped like oak leaves. Oak Leaf cultivars are often listed in seed catalogues as simply Red or Green Oak Leaf (Figure 1-2 b and c).

1.3.2.5 Cos or Romaine

This is the oldest type of lettuce known (*Lactuca sativa* var. *longifolia*). It has been found as paintings on some Egyptian tomb walls and is thought to have been cultivated since approximately 4,500 years ago (Morgan 1999). This type develops an elongated head of stiff upright leaves, which are sometimes closed at the top or relatively open. Baby Cos (Figure 1-2 d) varieties are grown in soil and in hydroponic systems.

Figure 1-2 Different lettuce cultivars grown in the NFT system in the Sydney area.
(a) Brown Mignonette **(b) Red Oak**
(c) Green Oak **(d) Baby Cos**

1.4 Lettuce diseases

More than 75 diseases have been recorded on lettuce plants (Davis *et al.* 1997) and are caused by bacteria, nematodes, viruses, fungi and oomycetes. Abiotic or non-infectious diseases can be caused by adverse environmental conditions, saline soil, nutrient deficiencies or an excess of water.

1.4.1 Bacteria

Bacteria are very important organisms because of their beneficial effects but they can also be pathogenic to plants. Five bacterial genera cause the majority of plant diseases: *Pseudomonas*, *Xanthomonas*, *Erwinia*, *Clavibacter* and *Agrobacterium*. Bacteria cause many economically important diseases of lettuce crops, such as leaf spot (*Xanthomonas campestris* pv. *vitians*), corky root (*Rhizomonas suberifaciens*), soft rot (*Erwinia carotovora* subsp. *carotovora*), marginal leaf blight (*Pseudomonas marginalis* pv. *marginalis*) and varnish spot (*Pseudomonas cichorii*).

On the other hand, some bacterial species can be used as biological control agents for different types of plant pathogens (Kusakari & Tanaka 1986; Rankin & Paulitz 1994; Nakayama *et al.* 1999; Ongena *et al.* 1999; Bardin *et al.* 2004). Amer and Utkhede (2000) used *Bacillus subtilis* and *Pseudomonas putida* to control root rot disease of glasshouse-grown lettuce and cucumbers caused by *Pythium aphanidermatum* and *Fusarium oxysporum* f.sp. *cucurbitacearum*. *Pseudomonas chlororaphis* was used to control root rot disease of hydroponic peppers caused by *Pythium aphanidermatum* and *Pythium dissotocum* (Chatterton *et al.* 2004).

1.4.2 Nematodes

Nematodes have a dual role in causing plant diseases. Approximately 2,500 nematodes species are directly parasitic on plants and nematodes may also be vectors for some plant pathogenic viruses. Many nematode species cause diseases on lettuce, but very few have been studied in depth (Davis *et al.* 1997). The most important nematodes causing disease and yield losses in lettuce are needle nematode (*Longidorus africanus*), the lesion nematode (*Pratylenchus penetrans*), root-knot nematodes (*Meloidogyne* spp.) and the spiral nematode (*Rotylenchus robustus*).

1.4.3 Viruses

Six hundred plant diseases caused by viruses have been reported and new ones continue to be reported (Davis *et al.* 1997). Disease symptoms on lettuce plants caused by viruses vary from yellowing leaves to stunting of whole plants and small or no heads in head cultivars to dead plants. The main methods of virus transmission are insects, mites, fungi, nematodes, mechanical damage, grafting and sometimes seeds. In lettuce plants, insects such as aphids, thrips and whiteflies are the main vectors of viruses. Viruses cause many economically important diseases of lettuce crops, such as lettuce big-vein (lettuce big-vein virus (LBV)), lettuce mosaic (LMV), lettuce mottle (LMoV), lettuce chlorosis (LCV) and alfalfa mosaic (AMV).

1.4.4 Fungi

Generally fungi are microscopic, eukaryotic organisms and have vegetative structures that are usually filamentous and branched. They are spore bearing organisms that lack chlorophyll and have cell walls that contain chitin and cellulose. They are classified based on their spore production and morphology (Davis *et al.* 1997; Agrios 2005).

Fungi cause diseases on lettuce plants that range from root diseases to wilts or leaf spots (Table 1-2).

Table 1-2 Fungal diseases on lettuce plants and their symptoms (Davis *et al.* 1997).

Disease	Causal organism	Symptoms
Anthracnose	*Microdochium panattonianum*	Tan, water-soaked spots on outer leaves 2-3 mm in diameter. The centres fall out causing the main symptoms of this disease: shot-hole.
Bottom rot	*Rhizoctonia solani*	Lesions and small spots on the lower leaves where they touch the soil. Leaf blades eventually rot.
Drop	*Sclerotinia minor* or *S. sclerotiorum*	Wilt of outermost leaves which later lie flat on the soil surface and plants become yellowish. Black sclerotia are produced on the lower surface of the leaves touching the soil.
Fusarium wilt	*Fusarium oxysporum*	Plants wilt initially. Red-brown streaks run from the upper taproot into the cortex of the crown. Older affected heads exhibit a tipburn that is often limited to one side of the plant. Plants appear stunted and some fail to produce heads.
Powdery mildew	*Erysiphe cichoracearum*	Older plants affected on outer leaves. The fungus develops on both sides of leaf as white and powdery spores. Infected heads remain small and poor in quality.
Verticillium wilt	*Verticillium dahliae*	Wilt appears on the lower leaves at the rosette stage. Green or black vertical striations form in the tap root and at the crown. Outer leaves become yellow, die and remain closely appressed to the head.

1.4.5 Oomycetes

Prior to 1990, the Oomycetes were considered to be true fungi, belonging to the Kingdom Fungi (Agrios 2005; Deacon 2006). At this time, molecular and biochemical studies revealed that the Oomycetes were more closely related to plants than true fungi. As a result, the Oomycetes were transferred to the Kingdom Chromista and organisms belonging to the Oomycetes became known as fungal-like organisms (discussed in detail in section 1.6).

Oomycetes include some of the most economically important plant pathogens. *Pythium* spp., *Phytophthora* spp. and *Bremia* spp. are the main pathogens in this group and they can cause significant damage to and yield losses of lettuce plants when environmental conditions are favourable. Most of the diseases caused by these pathogens are in the root system and on the stem, which results in wilted leaves (Table 1-3). Seedlings and mature lettuces are affected by these diseases and if the seeds are affected they fail to germinate (Agrios 2005).

Pythium spp. are the main pathogens affecting lettuce plants grown in hydroponic systems. Diseases caused by these pathogens affect seedlings and root systems leading to collapse and damage of the plants with yield losses (Table 1-3) (Funck-Jensen & Hockenhull 1983; Stanghellini & Kim 1998; Labuschange *et al.* 2002). Sometimes *Pythium* spp. can cause yield losses of lettuces grown in hydroponic systems without visible root rot symptoms (Stanghellini & Kronland 1986). In Australia, *Pythium aphanidermatum* was recorded on cucumber and capsicum crops grown in soil-less systems in New South Wales (NSW) (Gillings & Letham 1989; Tesoriero *et al.* 1991). These authors also reported that *Pythium afertile*, *P. coloratum, P. myriotylum, P. torulosum* and *P. vexans* affected lettuces grown in soil-less systems.

Table 1-3 Oomycete diseases on lettuce plants and their symptoms (Davis *et al.* 1997).

Disease	Causal organism	Symptoms
Damping-off	*Pythium* spp.	Seeds soften and fail to germinate. Seedling infection starts at the roots which become water soaked and discoloured, and the cells soon collapse. The stem at the attachment point with the roots becomes soft and collapses. In older lettuces, the roots rot, stem lesions appear and the whole plant wilts.
Root rot	*Pythium* spp.	Infected roots on young and mature lettuce become brown and then black and the whole lettuce wilts. Some *Pythium* spp. affect lettuce plants without showing symptoms, but lettuce size and yield are reduced.
Root rot	*Phytophthora* spp.	Infected seedlings show root rot which leads to collapse in a few days or weeks. Older lettuces show root rot and wilting.
Downy mildew	*Bremia lactucae*	Light green lesions which are slightly chlorotic at early stages becoming yellow or necrotic later. They become translucent after invasion of infected tissue by secondary saprophytes.

Phytophthora spp. also cause root rot diseases on most vegetables grown in hydroponic systems, including lettuce (Table 1-3) (Forster *et al.* 1998; Jamart 1999). Tomato plants grown in hydroponic systems suffer from root rot disease caused by *Phytophthora parasitica* (Snapp & Shennan 1992). Peppers affected by *Phytophthora capsici* develop root rot disease (Stanghellini *et al.* 1996a). *Phytophthora* sp. has been isolated from lettuce roots and stems grown in hydroponic systems in Australia (Hutton & Forsberg 1991).

1.4.6 Abiotic disorder or non-infectious diseases

Abiotic disorders are caused by changes to some factors that affect the accessibility of water and nutrient to the plant. These factors are soil moisture, temperature, humidity, nutrient status, soil salinity and pH (Davis *et al.* 1997). The symptoms caused by these factors are difficult to describe; however, they are often similar to those caused by viruses. Moreover, these disorders are not transmitted between plants and can happen at any stage of plant growth. The most important effect of these disorders is that stressed plants may succumb to more aggressive diseases when pathogens are present (Davis *et al.* 1997).

1.5 Environmental conditions and disease development

Environmental conditions are very important for both plant growth and pathogen activities. Temperature and moisture are the most important conditions that affect lettuce plants, especially those grown in hydroponic systems (Stanghellini & Rasmussen 1994; Agrios 2005). Temperatures between 20 and 25°C are optimal for germination and growth of lettuce plants; higher temperatures, however, will inhibit germination and stress the plant (Davis *et al.* 1997). On the other hand, the optimum growth temperature of different pathogens varies amongst pathogens and even between isolates of a single pathogen species (Deacon 2006).

Pythium spp. and *Phytophthora* spp. are the main pathogens isolated from lettuce plants grown in hydroponic systems (Zinnen 1988; Hutton & Forsberg 1991; Stanghellini & Rasmussen 1994). Optimum growth temperatures for the majority of *Pythium* spp. are usually between 25 and 30°C (van der Plaats-Niterink 1981) and for the majority of *Phytophthora* spp. are from 20 to 25°C (Stamps *et al.* 1990; Erwin & Ribeiro 1996).

Hydroponic lettuce farmers in NSW have found, from experience, that one or two hot days (higher than 35°C) in early summer are enough to cause the outbreak of significant root rot disease in their lettuces (several farmers, pers. comm.). The relationship between nutrient solution temperature and root rot disease caused by *Pythium* spp. and *Phytophthora* spp. has been investigated in several studies using different plants (Funck-Jensen & Hockenhull 1983; Bates & Stanghellini 1984; Gold & Stanghellini

1985; Stanghellini & Kronland 1986; Pegg & Jordan 1991; Tesoriero & Cresswell 1995).

Tesoriero and Cresswell (1995) examined the relationship between disease severity and temperature in hydroponic lettuces and found that yield losses and damage were significant when roots were exposed to temperatures of 30-40°C for two hours in a water bath then returned to the hydroponic system and inoculated with *Pythium* or *Phytophthora*. *Phytophthora cryptogea* has been shown to cause damage to stems and roots of tomato plants grown in hydroponic systems at temperatures of 15°C but little or no damage was reported when the temperature was 25°C (Kennedy & Pegg 1990; Pegg & Jordan 1991; Kennedy *et al.* 1993). Jamart (1999) found that *Phytophthora cryptogea* was pathogenic to lettuce grown in hydroponic systems in a greenhouse at 20°C. *Phytophthora porri* was reported to cause stem rot on lettuce grown in soil at a temperature of 15°C in South Australia (Sitepu & Bumbieris 1981).

For *Pythium* spp., one study found that no significant disease symptoms occurred after lettuce roots were dipped in a water bath for 6 minutes at 42°C and then returned to a hydroponic system which had been inoculated with *Pythium* sp. (Funck-Jensen & Hockenhull 1983). Stanghellini and Kronland (1986) studied two temperature regimes with two *Pythium* spp. and found that *Pythium aphanidermatum* damaged lettuce when the temperature of the nutrient solution was above 25°C. However, *Pythium dissotocum* caused damage to and yield losses of lettuce when the temperature of the nutrient solution was below 20°C. Spinach plants grown in a hydroponic system died within 3-4 days after inoculation with *Pythium aphanidermatum* at nutrient solution temperatures between 21°C and 27°C; however, it was more aggressive at 27°C (Gold & Stanghellini 1985). Other researchers have also found that root rot disease caused by *Pythium aphanidermatum* is most destructive at nutrient solution temperatures above 25°C (Bates & Stanghellini 1984; Stanghellini & Rasmussen 1994). Stanghellini and Kronland (1986) reported that *Pythium dissotocum* was responsible for yield reduction in the absence of any visible root rot symptoms when they inoculated lettuce plants at glasshouse temperatures of 23 to 36°C (mean 28°C).

The relationship between nutrient solution temperature and root rot disease of hydroponic lettuces caused by *Pythium* spp. and *Phytophthora* spp. needs more study

especially at temperatures below 20°C and above 25-30°C. Moreover, the effects of brief exposure to higher temperatures need to be investigated.

Moisture is another important environmental factor, especially for soil-borne pathogens such as *Pythium* spp. and *Phytophthora* spp. These pathogens are favoured by high soil moisture (Agrios 2005) since they produce motile zoospores which require free water in which to move. In hydroponic systems, especially NFT, water is freely available, thereby favouring these pathogens. The zoospores of *Pythium* spp. and *Phytophthora* spp. can swim in the nutrient solution until they find new root systems where they can germinate and infect if the right conditions are available (Stanghellini & Rasmussen 1994).

The interaction between moisture and temperature in relation to root rot disease is very important. When the nutrient solution temperature is favourable to zoospores swimming in the nutrient solution, there is more chance that they will germinate and infect new roots. The other factor involved in disease initiation is the plant itself. If the nutrient solution temperature is in the range for optimal plant growth, the plant may be able to resist infection. However, higher or lower temperatures could stress the plant and active pathogens may enter their roots more easily (Stanghellini & Rasmussen 1994).

Diseases caused by *Pythium* spp. start when seeds or seedlings come into contact with zoospores or mycelium of *Pythium* spp. (Moulin *et al.* 1994; Agrios 2005). The seeds may have been placed in soil or seedling beds contaminated with *Pythium* spp., while the seedlings may have been placed in soil or hydroponic systems similarly contaminated. The pathogen produces pectinolytic enzymes that dissolve the pectins that hold the cells together, resulting in maceration of the tissues. *Pythium* species continue growing between and through the cells. Proteolytic enzymes break down the protoplasts and celluolytic enzymes cause collapse and disintegration of the cell wall. This infection will kill seeds and seedlings and turn them into a rotten mass consisting primarily of the pathogen and substances like suberin and lignin, which the pathogen cannot break down. When the seedling is well developed, however, and has well thickened and lignified cells, the pathogen cannot grow through it to the same extent and only small lesions develop. Rootlets can be attacked at any stage of growth. This

type of disease caused by *Pythium* becomes more severe when there is free water around for a long time (Agrios 2005).

The oospores and chlamydospores of *Phytophthora* species germinate when the environmental conditions are suitable, and the mycelium will grow further and produce zoosporangia that release zoospores (Toppe & Thinggaard 1998; Agrios 2005). The zoospores then swim around in the soil water or in nutrient solution and infect roots of susceptible hosts with which they come in contact. In wet conditions, *Phytophthora* spp. produce more mycelium and zoospores and the disease spreads to more plants, particularly in cooler weather. However, in dry, hot or extremely cold weather, the pathogen survives as oospores, chlamydospores or mycelium that can initiate infection when the conditions become favourable.

1.6 The phylum Oomycota (*Pythium* spp. and *Phytophthora* spp.)

The oomycetes have the following features which differentiate them from the true fungi (Erwin & Ribeiro 1996; Money 1998; West *et al.* 2003; Agrios 2005; Deacon 2006):

1. their cell walls contain glucans but not chitin;
2. they have diploid nuclei whereas the majority of true fungi have haploid nuclei;
3. they have ultrastructural features closer to plants than fungi, such as stacked, plate-like golgi cisternae and tubular mitochondrial cisternae; and
4. they react differently to a range of antifungal agents.

Some scientists argue that oomycetes should remain with the true fungi (Money 1998). This is because oomycetes have mycelia and they infect plants in the same way that true fungi do (Agrios 2005; Deacon 2006). However, the oomycetes have mycelium which lacks septa and molecular and biochemical studies suggest that oomycetes are more closely related to the heterokont algae (Abdelghani *et al.* 2004). Other scientists suggest that the term "fungus" should be expanded to permit a new definition which includes oomycetes (Money 1998). Nevertheless, it is obvious that oomycetes are different from true fungi and the way they infect plants is not the basis upon which these organisms are classified. For these reasons, organisms belonging to the phylum Oomycota will not be referred to as fungi in this study.

1.6.1 *Pythium* species

The genus *Pythium* was created by Pringsheim in 1858 with *Pythium monospermum* Pringsh. as the type species (Martin 1992). Van der Plaats-Niterink (1981) described and listed 87 species of *Pythium*, while Dick (1990) described over 120 species of *Pythium*. *Pythium* is classified in the family Pythiaceae, order Peronosporales and phylum Oomycetes (Martin 1992). Common characteristics of *Pythium* spp. include the following (Martin 1992):

1. the hyphae are hyaline, 5-7 μm in diameter (Figure 1-3 a) and lack septa, except when cultures are old or at points of spore differentiation;
2. the sporangia are asexual spores and are produced in a variety of shapes (filamentous, inflated filamentous, globose and spherical) and sizes, depending on the species;
3. sporangia germinate indirectly by the formation of motile zoospores or directly by the formation of a germ tube;
4. the asexual structures are often referred to as conidia or hyphal swellings, while those which release zoospores are referred to as zoosporangia;
5. oospores, which are sexual structures, differ among species in the following aspects: diameter; wall thickness and type (i.e. smooth or echinate); plerotic or aplerotic condition; number, origin and morphology of attachment of antheridia (Figure 1-3 b); and
6. growth is dependant on temperature and this may be used as a taxonomic aid.

Pythium spp. cause many diseases on a wide variety of plants. Most of these diseases occur on seeds (seed rot), seedlings (seedling damping off) or root systems, as well as soft rots of fleshy fruits in contact with the soil (Agrios 2005).

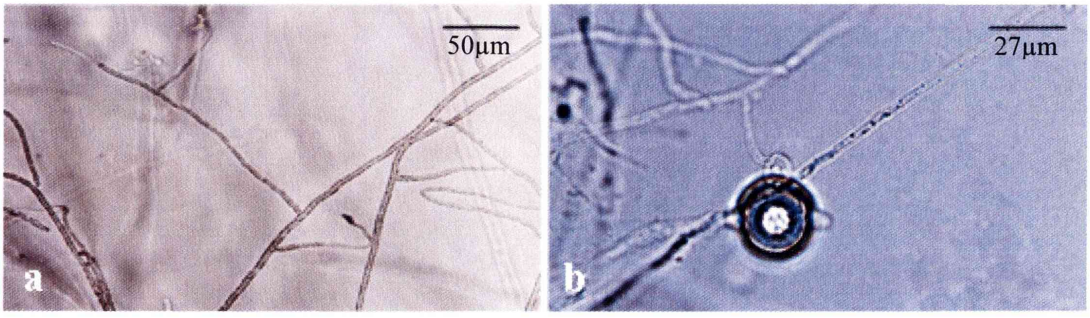

Figure 1-3 *Pythium* sp. a: hyphae and b: diclinous antheridia.

1.6.2 *Phytophthora* species

Phytophthora spp. belong to the family Pythiaceae, order Peronosporales and phylum Oomycetes. *Phytophthora* spp. have many common characteristics (Stamps *et al.* 1990; Mitchell & Kannwischer-Mitchell 1992; Erwin & Ribeiro 1996) which include the following:

1. non-septate hyphae, although in old cultures septa can be seen, with slight constrictions at the bases of right angled branches (Figure 1-4 a);
2. sporangia are ovoid or obpyriform to limoniform (Figure 1-4 b) and are produced in succession on typically sympodially branched sporangiophores of indeterminate growth;
3. differentiation of mature, laterally biflagellate zoospores within the sporangium;
4. globose oogonia with single, spherical oospores and thin or no periplasm (Figure 1-4 c); and
5. amphigynous or paragynous antheridial configurations.

Phytophthora spp. cause many diseases on different types of plants including root rot of seedlings of annual vegetables, rots of fully-developed fruits and root rots of fruit, ornamental and forest trees (Averre & Reynolds 1964; Hutton & Forsberg 1991; Agrios 2005). More than 80 species of *Phytophthora* have been described as plant pathogens (Agrios 2005).

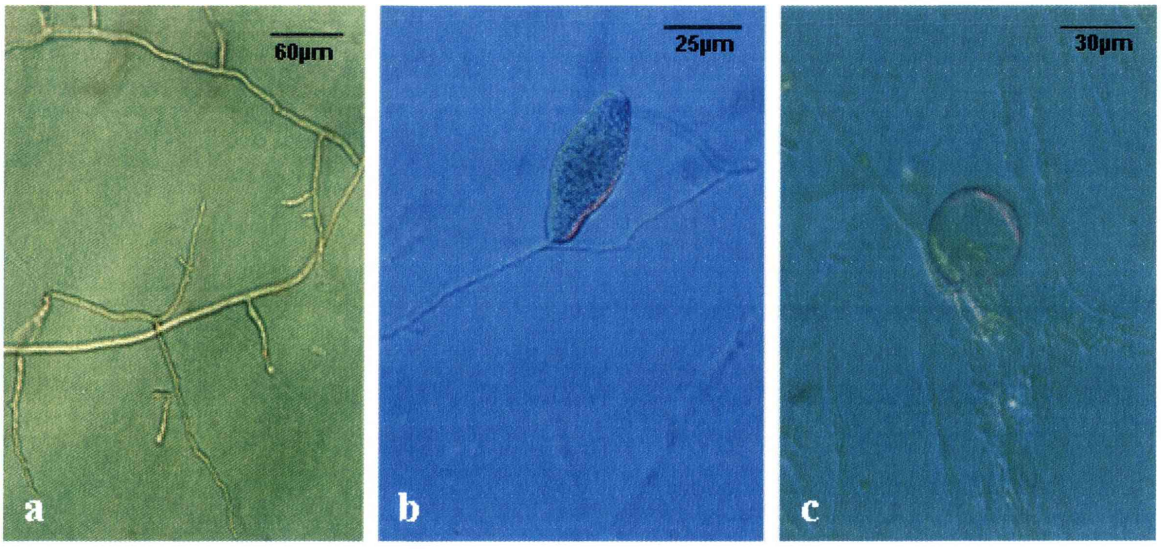

Figure 1-4 *Phytophthora* sp. a: hyphae; b: sporangium; c: globose oogonium with amphigynous antheridium.

1.7 Identification of *Pythium* spp. and *Phytophthora* spp.

Identification of plant pathogens is very important in helping to find effective disease control or management methods. Incorrect identification could lead to control strategies and control methods that are ineffective. Fungi, in general, and oomycetes in particular, have traditionally been identified using morphological characteristics, but this is difficult since many species produce overlapping characteristics which are often hard to differentiate. Many morphological features are similar among different groups of species and intraspecific morphological variation is frequently observed in different field isolates (van Os 2003). Moreover, this difficulty may be exacerbated by environmental factors (such as temperature) and physiological factors (such as nutrition) (Bates *et al.* 1993). Morphological characteristics, however, are still useful and often provide the basis for species identification.

More recently, molecular techniques based on several DNA methods have been developed to identify species and understand the relationships between them (Levesque & De Cock 2004; Drenth *et al.* 2006), with the aim of re-examing identifications and relationships determined by morphological methods. Moreover, several investigations have used these molecular techniques to study the variation between populations of one species obtained from different areas (Harvey *et al.* 2000; Pongpisutta 2005).

1.7.1 *Pythium* species

The main features used for morphological identification of *Pythium* spp. are sporangial size and shape, production of zoospores, formation and morphological features of oospores, and the number and shape of antheridia and their method of attachment. The most widely used keys for identification of *Pythium* spp. are those by Middleton (1943), Waterhouse (1968), van der Plaats-Niterink (1981) (who described 87 species) and Dick (1990) (who described 120 species). Some *Pythium* isolates do not produce sexual structures or produce oospores only which leads to difficulties in identification.

More recently, molecular techniques based on several DNA methods have been developed to identify *Pythium* spp. and also to understand the relationships between species (Bruns *et al.* 1991; Levesque *et al.* 1998). Most of these methods focus on the polymerase chain reaction (PCR) of the internal transcribed spacer (ITS) region of

ribosomal DNA (rDNA) (Figure 1-5). This region is known to be moderately variable and can often be used to distinguish different species by using PCR with universal primers that bind to the 5' end of each DNA strand and progress toward the 3' end (Deacon 2006). These variations have been used to identify different organisms, including oomycetes, to species level (Rafin *et al.* 1995; Matsumoto *et al.* 1999; Paul & Masih 2000; Paul 2001; Mathew *et al.* 2003).

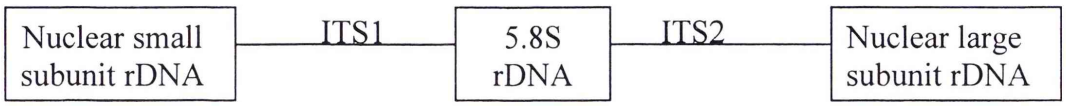

Figure 1-5 The Internal Transcribed Spacer (ITS) region of ribosomal DNA (rDNA) (Sambrook & Russell 2001)

Different methods have been used to identify *Pythium* species and to understand the relationships within *Pythium*. The most powerful and widely used technique for identification of *Pythium* spp. is restriction fragment length polymorphism (RFLP) of amplified ribosomal DNA (Levesque *et al.* 1994; Powell *et al.* 1996; Matsumoto *et al.* 2000; Harvey *et al.* 2000 & 2001; Wang *et al.* 2002). In this method, restriction enzymes are added to the PCR product to digest the ITS region, with the digestion products then used to distinguish between different *Pythium* species. Wang and White (1997) successfully used four restriction enzymes (*Cfo*I, *Hinf*I, *Mbo*I and *Taq*I) to distinguish between 36 *Pythium* species. RFLP was also used to identify *Pythium aphanidermatum* and *Pythium ultimum* isolated from potato plants in Tunisia (Triki *et al.* 2001).

Sequence analysis of the ITS region of rDNA has also been used to identify and study the relationships among isolates of *Pythium* species (Bailey *et al.* 2002; Mathew *et al.* 2003; Schurko *et al.* 2003; Levesque & De Cock 2004; Paul 2004). In this method, the ITS region is sequenced to distinguish *Pythium* spp. by comparing the nucleotides using a BLAST search (basic local alignment search tool) in the GenBank databases. Levesque and De Cock (2004) compared the sequences of 116 *Pythium* isolates in order to identify and study the relationships between these isolates. Kageyama *et al.* (1997 & 1998) studied the relationships between *Pythium ultimum* and *Pythium* HS group using their sequences and found it likely that the *Pythium* HS group represents asexual strains of *Pythium ultimum*. Scott *et al.* (2005) identified 130 *Pythium* isolates obtained from table beet in the Lockyer Valley in Queensland by using RFLP and sequencing the ITS

region. The majority of these isolates (91%) could be allocated to three main groups - Lockyer Valley *Pythium* A (LVP A), LVP B and LVP C. Sequences for isolates from these groups were compared by using a BLAST search in the GenBank databases and it was found that LPV A were 99% identical to *P. aphanidermatum*, LVP B were 96% identical to *P. ultimum* and LVB C were 98% identical to *Pythium dissotocum*. Sequences of isolates from the three main groups and three minor groups (LVP D, E and F) were placed in a phylogenetic tree, based on comparisons with sequences in GenBank databases (Figure 1-6).

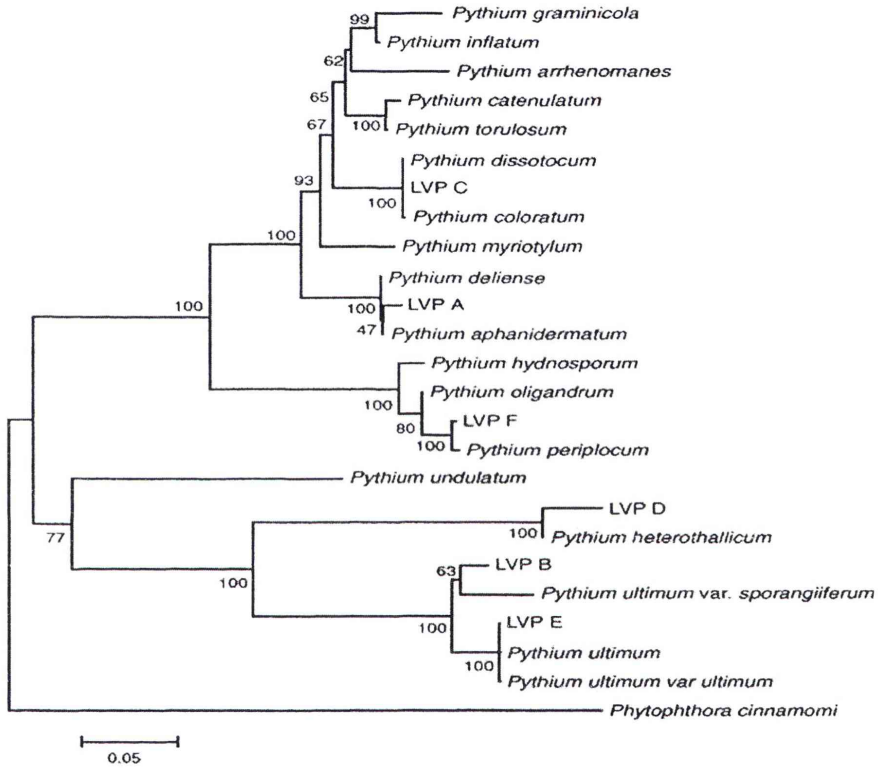

Figure 1-6 Phylogenetic tree of *Pythium* isolates from this study [Scott *et al.* 2005] and previously characterised *Pythium* spp. based on DNA sequence analysis of the region encompassing ITS I, 5.8S rDNA and ITS II. The numbers at each branch point are bootstrap values following resampling of the data 1000 times. [This study was based on 130 isolates of *Pythium* from the Lockyer Valley, Queensland (LVP), which were grouped into three main groups (A, B and C) and three minor groups (D, E and F).]

Other studies have used specific universal primers to amplify the ITS region within ribosomal DNA to differentiate between *Pythium* spp. (Wang *et al.* 2003; Mathew *et al.* 2003). Reverse dot blot hybridization with the immobilized entire ITS I region was used to identify species of oomycetes (*Pythium* and *Phytophthora*) (Levesque *et al.* 1998). Random amplified polymorphic DNA (RAPD) was used to identify *Pythium aphanidermatum* isolated from cucumber (*Cucumis sativus*) or from water from a cucumber greenhouse (Herrero & Klemsdal 1998). This method, however, used PCR of

genomic DNA at a low annealing temperature and this lead to a lack of reproducibility (Duncan *et al.* 1998). Amplified fragment length polymorphism (AFLP) has been used successfully to identify *Pythium* species and analyse population variation within a species. In this method genomic DNA is digested, both with regular and rare restriction enzymes, and adaptors are ligated to the resultant fragments (Vos *et al.* 1995; Duncan *et al.* 1998). According to Duncan *et al.* (1998), this technique produces more bands and provides more information on variation than other molecular methods. Garzon *et al.* (2005) used the AFLP fingerprinting method to identify and characterise populations of *Pythium aphanidermatum*, *P. irregulare* and *P. ultimum*.

The inter simple sequence repeats (ISSR) technique is a variant of PCR that uses simple sequence repeat primers (e.g. AC, AG) to amplify random regions between their target sequences (Zietkiewicz *et al.* 1994; Kahl 2001). The ISSR specifically targets the di- and trinucleotide repeat types of microsatellite which are tandemly repeated (Cregan & Quigley 1997). They are present in both coding and noncoding regions of the genome and are usually characterised by a high degree of length polymorphism. Moreover, ISSR uses primers that are anchored at the 5' or 3' end of a repeat region and extend into the flanking region.

The ISSR method has been used to study variation in populations of plant species (Archak *et al.* 2003) and some fungi (Grunig *et al.* 2001; Meng & Chen 2001). This method was found to be more effective and cheaper than the AFLP technique in detecting genetic variation in *Phialophora gregata* and also more effective than the RAPD method (Tymon & Pell 2005). Recently, the ISSR technique was used to study the variation among populations of *Phytophthora cinnamomi* isolated from soil in different geographic areas of New South Wales (NSW), Australia (Pongpisutta 2005). No studies have yet reported using the ISSR method to study the populations of *Pythium* species.

1.7.2 *Phytophthora* species

Morphological characteristics have traditionally been used to identify species within the genus *Phytophthora*. *Phytophthora* species were divided into six groups on the basis of their sporangial structure (non-papillate, semipapillate or papillate) and the form of the antheridium (amphigynous or paragynous) (Waterhouse 1970; Newhook *et al.* 1978;

Stamps *et al.* 1990). Also, the production of oogonia in a homothallic culture, or by using heterothallic mating types A_1 or A_2, was used to group *Phytophthora* spp. (Stamps *et al.* 1990; Erwin & Ribeiro 1996). Identifying *Phytophthora* species, using morphological characteristics alone, is difficult because many species produce overlapping characteristics which are often hard to differentiate.

Molecular techniques have provided the potential to confirm the identification of species (Erwin & Ribeiro 1996), particularly closely related species with few morphological differences (Klich & Mullaney 2004). Ribosomal DNA is regularly used for PCR-based diagnostics (Drenth *et al.* 1999 & 2006).

The ITS region (Figure 1-5) has been shown to be sufficient to distinguish different species in *Phytophthora* (Cooke & Duncan 1997; Cooke *et al.* 2000). This is because sequence changes in the non-coding ITS region of the nuclear rRNA occur at a rate that often allows species to be distinguished within a genus (White *et al.* 1990). The PCR-RFLP technique developed by Drenth *et al.* (1999 & 2006) and Cooke *et al.* (2000) has shown great applicability to the identification of a wide range of *Phytophthora* species and is likely to be used in a range of studies on this genus in the future. In addition, the RFLP method, based on the ITS region, has been used successfully to identify and study the relationships of *Phytophthora megasperma* (Forster & Coffey 1993).

The modern study of the population genetics of *Phytophthora* species started in the mid-1980s when the first studies on isozyme variation in *Phytophthora cinnamomi* (Old *et al.* 1984) and *Phytophthora infestans* (Tooley *et al.* 1985) were published. Since that time, reports outlining the use of molecular tools to determine genetic variation or diversity of *Phytophthora* species have described a number of diverse techniques.

There are several techniques for estimating variation using DNA based markers in *Phytophthora* species. Two of the most commonly used techniques for assessment of variation of this nature are analysis of the ITS region and microsatellite (SSR) techniques. Drenth *et al.* (1993), using DNA fingerprinting patterns developed using Southern blot techniques, were able to estimate the genotypic diversity of *Phytophthora infestans* isolated from potato and tomato from different locations in the Netherlands. DNA fingerprinting of isolates with probe RG57 revealed significantly more diversity

in the *Phytophthora infestans* population than had been detected previously and genotypic diversity varied among regions. Isolates collected from community gardens in west and central regions were different from each other as well as from isolates collected from commercial potato fields in other regions (Drenth *et al.* 1993). Recently, the ISSR technique was used to study the variation among populations of *P. cinnamomi* isolated from soil in different geographic areas of New South Wales (NSW), Australia (Pongpisutta 2005).

1.8 Aims of the research

The main aim of this study was to increase our understanding of root rot diseases of lettuce grown in hydroponic systems in the Sydney area by identifying the causes of these diseases and understanding their development. Farmers in Australia claim that they have suffered heavy yield losses of hydroponic lettuce in summer in recent years. The oomycetes *Pythium* spp. and *Phytophthora* spp. have been recorded on hydroponic lettuce in NSW and identified by the Plant Health Diagnostic Service of NSW Department of Primary Industries from hydroponic lettuce samples sent in by farmers (Tesoriero, pers. comm.). However, there has been no previous, comprehensive survey of hydroponic lettuce farms in the Sydney area of NSW in relation to root disease.

The incidence and severity of root rot diseases on several lettuce cultivars were assessed with a view to establishing whether root rot disease in hydroponic lettuce in NSW is a summer-specific problem. A survey was carried out at one farm in the Sydney area with a history of root rot disease in previous years. This farm was surveyed five times over 11 months, to cover all seasons. Fungi and oomycetes isolated from lettuce roots were identified to genus and pathogenicity tests were carried out to confirm that they caused root rot diseases. Furthermore, the optimum, minimum and maximum temperatures for growth in culture were investigated for the fungi and oomycetes isolated (Chapter 2).

To confirm the findings of the first survey, an expanded survey was carried out, involving other hydroponic lettuce farms as well as the previous farm. Two hydroponic farms in Sydney and two on the Central Coast of NSW were surveyed five times over 11 months to assess the incidence and severity of root rot disease on several lettuce cultivars and to confirm that root rot disease in hydroponic lettuce in NSW is a summer-

specific problem. *Pythium* spp. and *Phytophthora* spp. were isolated using selective media and were identified. Pathogenicity tests were carried out for any oomycetes that were different from those isolated in the first survey to confirm that they were the cause of the root rot diseases (Chapter 3).

The relationship between nutrient solution temperature and the severity of root rot diseases caused by *Pythium* spp. and *Phytophthora* spp. was investigated in an experimental hydroponic system. This relationship was studied by testing four temperature regimes of the nutrient solution (16-17°C, 24-27°C, 34°C continuously heated and 34°C for 10 hours followed by 18-20°C) with four different treatments (*Pythium* sp., *Phytophthora* sp., a combination of *Pythium* sp. and *Phytophthora* sp., and non-inoculated plants as control) (Chapter 4).

Isolates of *Pythium* spp. obtained from lettuce roots in the two surveys (Chapters 2 and 3) (81 isolates) were identified to species level by morphological methods. The main features used to identify *Pythium* were colony type, growth under nine different temperatures (5 to 45°C, with 5°C intervals), sporangial size and shape, production of zoospores, formation and morphological features of oospores, and the number and shape of antheridia and the method of attachment. Molecular techniques (PCR-RFLP and sequencing of ITS region) were also used to identify *Pythium* spp. to species level. ISSR markers were used to investigate the variation amongst these 81 *Pythium* isolates (Chapter 5).

Sixty-eight *Phytophthora* isolates were obtained from lettuce roots in the two surveys (Chapters 2 and 3). Molecular methods of PCR-RFLP and sequencing of the ITS region were used to identify these isolates to species level. Representative isolates were examined for their colony type, growth at nine different temperatures (5 to 45°C, with 5°C intervals), sporangial structure and production of oogonia in homothallic culture or in heterothallic culture using mating type A_1 or A_2. Variation amongst the 68 isolates was examined by using the ISSR method (Chapter 6).

Information from the different components of this study, reported in Chapters 2 to 6, are integrated in a general discussion of root rot disease in hydroponic lettuce (Chapter 7).

2 SURVEY OF ROOT ROT DISEASE IN HYDROPONIC LETTUCE IN THE SYDNEY AREA, ISOLATION OF FUNGI AND OOMYCETES, AND THEIR TEMPERATURE RESPONSES IN CULTURE

2.1 Introduction

Lettuce (*Lactuca sativa* L.) is considered to be the most important salad vegetable grown today around the world. Recently, lettuce production has occurred more often in hydroponic systems than in soil. Growth in hydroponic systems has many advantages over soil, such as higher yields, better quality, less time required for production, less water usage and potential reduction of soil-borne pathogens (Carpenter 1979).

Growing plants commercially in hydroponic systems started in the mid-1930s, with Western Europe the centre of hydroponic production (Zinnen 1988). In the 1970's, the Nutrient Film Technique (NFT) was developed as a new type of hydroponic system suitable for growing vegetables, especially lettuce (Cooper 1975 & 1979). With the NFT, the nutrient solution recirculates in a flat-bottomed channel or gully through the bare roots of plants (Seymour 1993). However, this system has an important disadvantage in that any pathogens that enter the nutrient solution can spread quickly throughout the entire system.

Hydroponic systems are now a popular method for growing vegetables and flowers in many countries around the world. Australia is recognised as the largest grower of hydroponic lettuce in the world with over 240 ha of production area (Hassall & Associates 2001). New South Wales (NSW) is the largest lettuce-producing state in Australia with almost 190 ha in production.

Lettuce can be affected by many types of pathogens, such as bacteria, fungi, oomycetes, nematodes and viruses. Lettuce can also be affected by abiotic conditions, such as nutrient deficiencies when grown in soil, waterlogging or salinity. The main diseases affecting lettuces and causing economic losses are damping off, root rot, big vein, mosaic, downy mildew and sclerotinia drop (Davis *et al.* 1997).

Root rot diseases are the main problem in hydroponic systems and, once they appear, farmers have few options to control the pathogens believed to be responsible for these diseases (Zinnen 1988). In this situation, farmers tend to destroy the diseased lettuce and shut down the system. Therefore these diseases have become a serious problem in hydroponic lettuce production in Australia and worldwide. Farmers in Australia claim that they have suffered heavy yield losses of hydroponic lettuce, particularly in summer, in recent years (Tesoriero & Cresswell 1995; Tesoriero, pers. comm.). Root rot diseases have been reported on lettuce grown in the majority of hydroponic systems studied (Stanghellini & Rasmussen 1994). There are no fungicides registered for use in hydroponic systems in NSW nor, indeed, worldwide (Tesoriero *et al.* 1991; Stanghellini & Rasmussen 1994; Australian Pesticides and Veterinary Medicines Authority (APVMA) 2006). Several researchers are investigating biological control and other control methods but the efficacy of these methods has not yet been demonstrated in commercial hydroponic production systems (Tesoriero *et al.* 1991; APVMA 2006; Tesoriero, pers. comm.).

Various studies have investigated the causes of root rot diseases in hydroponically grown vegetables including lettuce and, in these studies, *Pythium* spp. and *Phytophthora* spp. were the main pathogens isolated (Stanghellini & Kronland 1986; Jamart 1999). The pathogens *Fusarium* spp. and *Rhizoctonia* spp. were isolated infrequently from crop roots grown in hydroponic systems (Vanachter *et al.* 1983; Herr 1992). In Australia and, more specifically, in NSW, no comprehensive survey has been carried out to assess root rot disease on lettuce grown in hydroponic systems. Root pathogens have been isolated from a limited number of lettuce plants submitted to the Plant Health Diagnostic Service of NSW Department of Primary Industries (Tesoriero, pers. comm.).

Pythium spp. are the most important pathogens causing damping-off and root rot disease in plants, especially vegetables. Generally, *Pythium* spp. prefer warm temperatures and wet conditions in the soil environment for growth and reproduction (Agrios 2005). In hydroponic systems, *Pythium* spp. are known as common pathogens and they can be found in most hydroponic systems around the world (Vanachter *et al.* 1983; Stanghellini *et al.* 1984 & 1988; Zinnen 1988; Cherif & Belanger 1992). On lettuce grown in hydroponic systems, *Pythium* spp. are the main pathogens causing root rot disease and

yield losses. Stanghellini and Kim (1998) reported for the first time that *Pythium myriotylum* caused root rot disease in lettuce grown in hydroponic systems. Others have also reported *Pythium* spp. in hydroponic lettuce (Funck-Jensen & Hockenhull 1983; Stanghellini & Kronland 1986; Amer & Utkhede 2000; Labuschagne *et al.* 2002).

Phytophthora spp. also cause root rot diseases and yield losses in most vegetables grown in hydroponic systems (Snapp & Shennan 1992; Stanghellini *et al.* 1996a; Forster *et al.* 1998; Jamart 1999). *Phytophthora* spp. prefer temperatures of 15-23°C and wet conditions in the soil environment for growth and reproduction (Agrios 2005). Many *Phytophthora* spp. have been isolated from the roots and stems of lettuce, tomato, pepper and other plants grown in hydroponic systems (Hutton & Forsberg 1991; Stanghellini *et al.* 1996a).

Other pathogens have also been reported on vegetables grown in hydroponic systems. *Fusarium* spp. have been isolated from lettuce, spinach, pepper, tomato and cucumber grown in hydroponic systems where they caused root rot and wilt (Staunton & Cormican 1978 & 1980; Price 1980; Vanachter *et al.* 1983; Mihuta-Grimm *et al.* 1990; Stanghellini & Rasmussen 1994; Duffy & Defago 1997). *Rhizoctonia solani* caused bottom rot complex in glasshouse lettuce resulting in yield losses (Kooistra 1983).

The aims of this study were:

- to carry out a disease survey over a year on a hydroponic lettuce farm in the Sydney area, to assess the incidence and severity of root rot disease in several lettuce cultivars at young and mature stages, with a view to establishing whether root rot disease is a summer-specific problem;

- to isolate and identify the fungi and oomycetes associated with root rot diseases at the hydroponic lettuce farm surveyed in the Sydney area;

- to carry out pathogenicity testing for these fungi and oomycetes to confirm that they are the cause(s) of root rot diseases; and

- to establish the optimum temperatures for the main fungi and oomycetes isolated, as well as their minimum and maximum temperatures, using colony growth on agar media.

2.2 Materials and methods

2.2.1 Root rot disease survey

A disease survey was carried out over an 11 month period at a hydroponic lettuce farm in the Sydney area (Manicaro's Farm, 216 Rickard Road, Leppington, NSW) (Figure 2-1) approximately 40 km from the main vegetable market at Flemington (Figure 2-2). This farm had a history of root rot disease in previous years. Lettuce are grown outdoors in NFT systems using small diameter white cylindrical channels for young lettuce development (Figure 2-1 a) and large rectangular white channels for maturing lettuce (Figure 2-1 b). Nutrient solution is prepared using town water and lettuce seedlings are purchased from various suppliers.

Three lettuce cultivars (Brown Mignonette, Baby Cos and Red Oak) (Figure 2-3) were surveyed five times during 2003-2004 to cover a range of seasons. They were surveyed in May 2003, August 2003, November 2003, January 2004 and March 2004. The temperature of the nutrient solution in the growing channels was measured on each survey day. Additional information on air temperatures (daily maxima and mean monthly maxima) were obtained from the Bureau of Meteorology, NSW (pers. comm.). Both young (lettuces at the 6-8 leaf stage) and mature lettuces (18-27 leaves) were examined. Disease severity ratings on roots were assessed in 20 plants randomly selected from each cultivar at each stage of growth. A scale of 1 to 4 was used to assess disease severity (Figure 2-4) as follows:

1. healthy white roots;
2. generally healthy white roots, but with some brown colouration;
3. unhealthy roots, with most roots brown in colour; and
4. dead roots and/or black roots.

Root systems rated as 1 or 2 are considered to be healthy, whilst those rated as 3 or 4 are unhealthy or diseased.

Figure 2-1 Manicaro's Hydroponic Lettuce Farm (216 Rickard Road, Leppington, NSW), surveyed from May 2003 to March 2004. View downhill, towards Rickard Road (line of trees).
(a) young lettuce in small diameter cylindrical channels in foreground;
(b) maturing lettuce in large rectangular channels.

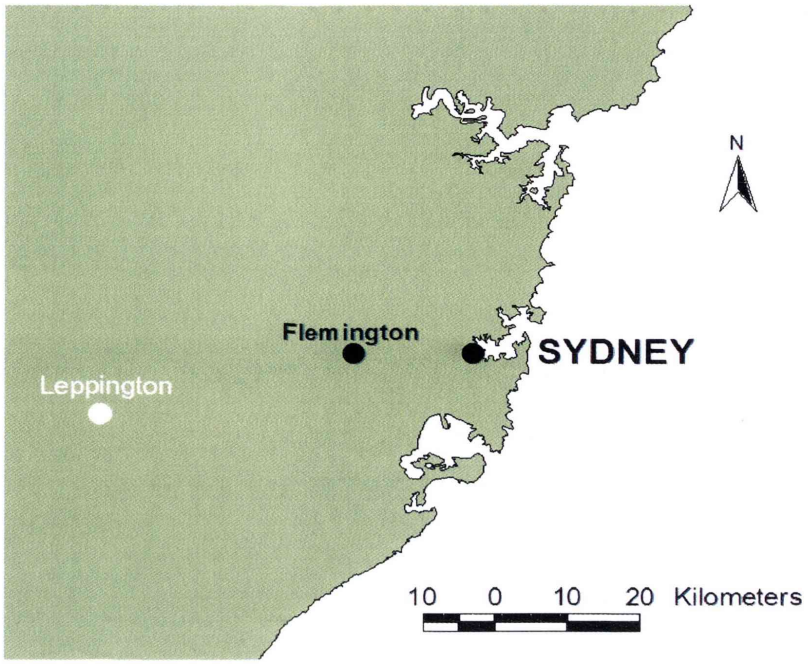

Figure 2-2 Map of the Sydney area showing Leppington (location of Manicaro's Lettuce Farm), Flemington (location of main Sydney fruit and vegetable market) and the Sydney Central Business Direct (CBD).

Figure 2-3 Lettuce cultivars examined for root rot diseases.
(a) Brown Mignonette, mature;
(b) Red Oak, mature;
(c) Baby Cos, mature; and
(d) Brown Mignonette, young.

Figure 2-4 Scale used to assess root rot disease severity on hydroponic lettuce roots:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour; and
(4) dead roots and/or black roots.
Root rot ratings 1 or 2 are considered indicative of healthy roots, whilst ratings of 3 or 4 are considered indicative of diseased roots.

2.2.2 Lettuce sampling, isolation of fungi and oomycetes from lettuce roots and identification of these microorganisms

Lettuce plants were sampled from the same farm where the survey was done at five times during the 11 month period: May 2003, August 2003, November 2003, January 2004 and March 2004. Three lettuce cultivars (Brown Mignonette, Baby Cos and Red Oak) (Figure 2-3) at two ages (mature and young) were sampled to isolate any potential pathogens in their roots. Five lettuce plants were randomly sampled from each cultivar and at each age (mature and young) for the August 2003 through to March 2004 sampling times; three lettuce plants from each cultivar and age were sampled in May 2003. Samples were placed separately in labelled plastic bags and kept at 4°C until isolations were undertaken in the plant pathology laboratory of the Botanic Gardens Trust (BGT), Sydney, Australia, normally within 24 hours.

Root systems were washed under running tap water for 5 minutes. Ten small (1 cm) root pieces of mature lettuces and five root pieces of young lettuces were excised and plated in 90 mm Petri dishes onto four different selective media. Young lettuces have a small root system and five pieces will include most of their root system compared with the larger root system of mature lettuces.

The three selective media initially used were *Phytophthora* selective agar (PSA), *Fusarium* selective agar (Peptone PCNB Agar (PPA)) and ¼ strength PDA with lactic acid for general fungal growth (Appendix 1). A fourth medium of *Pythium* selective agar (PYSA) was used from November 2003 onwards (Tesoriero, pers. comm.) (Appendix 1). Initially it was thought that *Pythium* spp. would be isolated on some of the first three media used but when few *Pythium* spp. were isolated on these media, the fourth selective medium was included.

Petri dishes of all four media (PSA, PPA, ¼ strength PDA with lactic acid and PYSA) were incubated under the recommended environmental conditions noted in the keys for the corresponding fungal genera. Petri dishes containing PSA and PYSA were incubated in the dark at 25°C ± 1°C (Stamps *et al.* 1990; van der Plaats-Niterink 1981, respectively) as were Petri dishes with ¼ strength PDA with lactic acid. Petri dishes containing PPA were incubated under continuous fluorescent light (TLD 36/54 Power Miser, Philips, Australia) at room temperature (25°C ± 1°C) with 35 cm distance between the light source and Petri dishes (Burgess *et al.* 1994).

All cultures were incubated for two days. All root pieces were then examined microscopically (Olympus CX41RF, Olympus Optical, Philippines) for oomycete and fungal growth (generally 100 root pieces for young lettuces of each of three cultivars and 200 root pieces for mature lettuces of each of three cultivars at each sampling time). All growth found was transferred to new media. The growth from PPA was transferred to Carnation Leaf Agar (CLA) media (Appendix 1). The growth from PSA, PYSA and ¼ strength PDA with lactic acid was transferred to Potato Carrot Agar (PCA) media (Appendix 1). All cultures were incubated at 25°C ± 1°C for 5-7 days. Cultures on CLA were incubated under continuous fluorescent light (as described previously for isolation on PPA) while cultures on PCA were incubated in the dark at 25°C ± 1°C. Keys of *Pythium* (van der Plaats-Niterink 1981), *Phytophthora* (Stamps *et al.* 1990) and

Fusarium (Burgess *et al.* 1994) were used to identify pathogen genus and species where possible.

2.2.3 Pathogenicity tests

Pathogenicity tests (to determine Koch's Postulates) were carried out for the main fungi and oomycetes isolated from lettuce plants sampled at different times. Koch's Postulates or rules were set up in 1887 by Robert Koch to investigate diseases on different types of organisms such as animals and plants. This method is followed to confirm that a pathogen isolated from a diseased plant is indeed the cause of the disease (Agrios 2005). In this method, healthy plants are inoculated with a pure culture of the presumed pathogens isolated previously from diseased plants and incubated under similar environmental conditions to the original plants. For Koch's Postulates to be satisfied, symptoms on the inoculated plants should be observed similar to those on the original diseased plant and the pathogens should be re-isolated from the inoculated plants and its identify confirmed. However, in the present pathogenicity tests, three temperatures (15, 25 and 35°C) were used to study whether pathogens isolated at a particular temperature could cause root disease not only at that temperature, but also at other temperatures likely to be found in the lettuce farm throughout the year. Of particular interest was whether pathogens isolated at a low temperature (e.g. during winter samples) could cause disease at a high temperature (e.g. under summer conditions).

Two trays of healthy lettuce seedlings, Mignonette Ember or Brown Mignonette (198 seedlings /tray), were obtained from Leppington Speedy Seedlings® and Supplies Pty. Ltd. (35 Riley Road, Leppington, NSW). In the absence of an experimental hydroponics system at this stage of the research, seedlings were transferred from trays into individual 225 mL Styrofoam cups (5 holes punched in base) containing potting mix and kept in the glasshouse at the University of Technology Sydney (UTS) (Gore Hill campus) for two weeks to allow them to grow to a size suitable for pathogenicity testing. The potting mix used was Lithuanian peat : perlite (1:1) from Elders Ltd (282 New Line Road, Dural, NSW). Irrigation was provided twice a day for 3 minutes each time in the morning and afternoon, using a fine spray. Nutrient solution (Crystal Dew Hydroponic Solutions, Greenlite, 252 Oxford Street, Bondi Junction, NSW; see Appendix 2 for

details of chemical composition) was provided once a week, at the rate of 20 mL per plant.

After two weeks in the glasshouse, plants were inoculated. The experiments were designed to test the pathogenicity of two to four fungi and/or oomycetes that had previously been isolated from the roots of lettuces collected from Manicaro's Lettuce Farm (Table 2-1). The fungi and/or oomycetes chosen were the main ones isolated from lettuce roots at each sample time.

Twenty-eight plants were used in each pathogenicity test. The inoculation treatment was applied to 21 plants and a corresponding control treatment without a microorganism was applied to seven plants. The inoculum was prepared in one of two ways (Table 2-1):

1. Agar disc - a 5 mm disc was cut from the margin of an actively growing fungal and/or oomycete culture (5 to 7 days old) on PCA media incubated in the dark at 25°C ± 1°C in the case of the 21 test plants and from a corresponding non-inoculated agar plate in the case of the 7 control plants. The agar disc was placed on the lettuce seedling root system, near the stem, and under the surface of the potting mix to prevent the agar drying out.

2. Macerated agar culture - PCA plate cultures of the fungi and/or oomycetes were blended (Model HR2817/A, 50-60 Hz, Philips, Mexico) in 500 mL sterile distilled water with three pulses each of two seconds, for inoculating the 21 test plants. Macerated agar inoculum was prepared at two concentrations - a normal concentration used two agar plates per 500 mL sterile distilled water, while a high concentration used 10 plates per 500 mL sterile distilled water. Corresponding non-inoculated PCA agar plates were blended in 500 mL sterile distilled water as above for use with control plants. The macerated agar was added at the rate of 20 mL per plant when a single isolate was tested. When two isolates were tested together (combination of *Pythium* and *Phytophthora*), 20 mL of macerated agar inoculum (at normal concentration), consisting of 10 mL of each isolate, was added.

Table 2-1 Fungi and oomycetes isolated from hydroponic lettuce roots obtained at five sample times and tested in pathogenicity tests.

Sample time	Isolates tested	Isolate code	Inoculation method
May-03	*Fusarium oxysporum*	F.o M03	Agar disc
	Fusarium avenaceum	F.av M03	Agar disc
	Rhizoctonia sp.	Rh M03	Agar disc
	Phytophthora sp.	Ph M03	Agar disc
Aug-03	*Pythium* sp.	Py A03	Agar disc
	Pythium sp.	Py A03	Macerated agar culture (normal)*
	Fusarium oxysporum	F.o A03	Agar disc
	Fusarium oxysporum	F.o A03	Macerated agar culture (normal)
Nov-03	*Pythium* sp. (at normal concentration)	Py N03	Macerated agar culture (normal)
	Pythium sp. (at high concentration)	Py N03	Macerated agar culture (high)^
	Phytophthora sp.	Ph N03	Macerated agar culture (normal)
	Combination of *Pythium* sp.	Py N03	Macerated agar culture (normal)
	and *Phytophthora* sp.	Ph N03	Macerated agar culture (normal)
Jan-04	*Pythium* sp. isolate 1	Py J04 1	Macerated agar culture (normal)
	Pythium sp. isolate 2	Py J04 2	Macerated agar culture (normal)
	Phytophthora sp.	Ph J04	Macerated agar culture (normal)
	Combination of *Pythium* sp. 1	Py J04 1	Macerated agar culture (normal)
	and *Phytophthora* sp.	Ph J04	Macerated agar culture (normal)
Mar-04	*Pythium* sp. (at normal concentration)	Py Mr04	Macerated agar culture (normal)
	Pythium sp. (at high concentration)	Py Mr04	Macerated agar culture (high)
	Phytophthora sp.	Ph Mr04	Macerated agar culture (normal)
	Combination of *Pythium* sp.	Py Mr04	Macerated agar culture (normal)
	and *Phytophthora* sp.	Ph Mr04	Macerated agar culture (normal)

*normal = normal concentration; 2 agar plates /500 mL sterile distilled water.
^high = high concentration; 10 agar plates /500mL sterile distilled water.

After inoculation, plants were transferred to three environmental cabinets at UTS (Environ Air Controlled Environment Equipment, S.R.J. Cabinets Wholesale Pty. Ltd., 23-29 Beresford Avenue, Greenacre, NSW). Each environmental cabinet was set at a different temperature i.e. 15, 25 and 35°C.

The 28 plants used to test one fungal species or oomycete species were kept on one shelf and each Styrofoam cup containing a lettuce seedling and potting mix was placed in a separate clear cup to retain any water and prevent cross contamination between individual plants. The contents of each shelf were rotated amongst the four shelf positions every 2-3 days to randomise any shelf-position effects. All plants were kept in the environmental cabinets for 16 days. Irrigation water was added every two days to maintain the potting mix at field capacity. Nutrient solution was added once a week at the rate of 20 mL per plant (Crystal Dew Hydroponic Solutions, as described previously, see Appendix 2 for details of chemical composition).

The plants on each shelf were illuminated by a single fluorescent tube (TLD 18W/33 Cool White, Philips, Australia) with 1477 lux (lumens/m^2) intensity at plant level, with a 12 hours photoperiod, for 16 days.

Symptoms on leaves and roots were assessed every 2-3 days. After 16 days, all plants were harvested and roots were washed under tap water for 5 minutes. Two 1 cm pieces of lettuce roots were placed on PSA, PPA, ¼ strength PDA with lactic acid and PYSA, in order to reisolate any fungi and/or oomycetes present. Shoots and roots of all plants were harvested and individual weights for each plant were obtained for wet weight of shoots and wet weight of roots. Shoots and roots of all plants were then placed separately in pre-weighed individual paper bags which were subsequently dried in a hot air oven at 80°C (± 5°C) for 4 days to dry to constant weight. Individual plant dry weights for both shoots and roots were then measured.

2.2.4 Temperature responses in culture of fungi and oomycetes

Nine temperatures from 5°C to 45°C (5°C intervals) were used to incubate PCA Petri dish (90 mm diameter) cultures of the main fungi and oomycetes isolated in May 2003, August 2003, November 2003, January 2004 and March 2004 to find out the optimum temperature for each fungus and oomycete, as well as the lowest and the highest temperatures at which fungal and oomycete growth occurred. All incubation was carried out in the dark. The oomycetes and fungi studied were *Pythium* spp. (5 isolates), *Phytophthora* spp. (5 isolates), *Fusarium oxysporum* (3 isolates) and *Fusarium avenaceum* (3 isolates) (Table 2-2). All cultures were incubated for 2 to 6 days, to allow sufficient time for the different fungi and oomycetes to reach the edge of the Petri dishes. Three replicates were used for each fungus or oomycete at each temperature. Growth was measured at 24 h, 48 h, 72 h and 120 h, using two diameter measurements perpendicular to each other. Colony radius was calculated for each of these times.

Table 2-2 Fungi and oomycetes isolated from hydroponic lettuce roots obtained at five sample times and tested for temperature responses in culture.

Isolates tested	Sample time	Isolate code
Pythium spp.	Aug-03	Py A03
	Nov-03	Py N03
	Jan-04	Py J04 1
	Jan-04	Py J04 2
	Mar-04	Py Mr04
Phytophthora spp.	May-03	Ph M03
	Aug-03	Ph A03
	Nov-03	Ph N03
	Jan-04	Ph J04
	Mar-04	Ph Mr04
Fusarium oxysporum	May-03	F.o M03
	Aug-03	F.o A03
	Mar-04	F.o Mr04
Fusarium avenaceum	May-03	F.av M03
	Aug-03	F.av A03
	Nov-03	F.av N03

2.2.5 Data analysis

General Linear Model (GLM) ANOVA (MiniTab VER 13) was used to find the differences ($P \leq 0.05$) in root rot rating based on the scale used (1, 2, 3, and 4). Percentages of diseased plants (pooled data from root rot ratings 3 and 4) were used to present the results in graphs. GLM ANOVA (MiniTab VER 13) was used to find the differences ($P \leq 0.05$) between the mean wet weight and dry weight for shoots and roots for inoculated and non-inoculated (control) plants from the pathogenicity tests. GLM ANOVA (MiniTab VER 13) was also used to find the differences ($P \leq 0.05$) between the isolates of each species tested for their temperature responses. Heteroscedastic data were transformed as required using log or square root transformations. Microsoft Excel XP 2003 was used to prepare graphs and tables.

2.3 Results

2.3.1 Root rot disease survey

Mean monthly maximum air temperatures for May 2003 and August 2003 were less than 22°C while mean monthly maximum air temperatures for November 2003, January 2004 and March 2004 were greater than 25°C (Figure 2-5). For the five survey times, the highest mean monthly maximum temperature occurred in January 2004, while the lowest occurred in August 2003. The measurement of the nutrient solution temperature on the survey day showed that the lowest temperatures were recorded in cooler season samples (May 2003 (17.5°C) and August 2003 (14.5°C)) compared with temperatures recorded in warmer seasons (November 2003 (30.5°C), January 2004 (26°C) and March 2004 (25°C)).

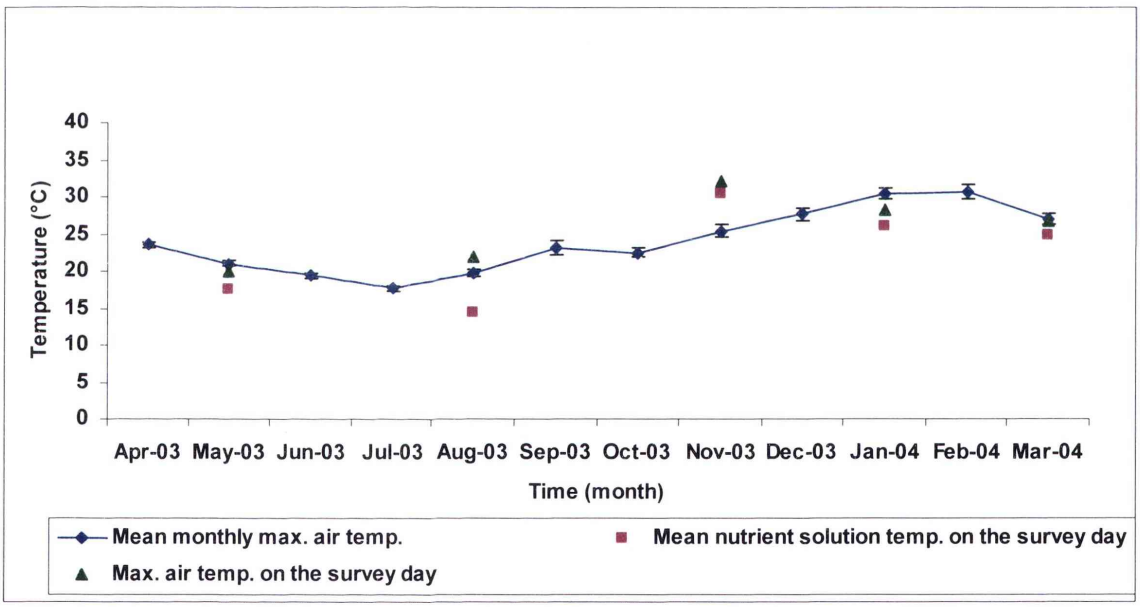

Figure 2-5 Mean monthly maximum air temperature at Leppington, NSW (Bureau of Meteorology, NSW, station 67020, mean of daily maxima) from April 2003 to March 2004, hydroponic nutrient solution temperature on five survey days from May 2003 to March 2004 and maximum air temperature on the survey days.

Although the root rot disease severity scale of 1 to 4 could be used successfully to assess hydroponic lettuce roots (see section 2.2.1, including Figure 2-4), ratings of 1 and 2 were pooled to indicate generally healthy roots and ratings of 3 and 4 were pooled to indicate diseased roots.

Pooled data on the percentage of diseased roots (rated at 3 and 4) at different sample times are shown for Brown Mignonette in Figure 2-6. The root rot disease level of the Brown Mignonette ranged from 5% in May 2003 when the nutrient solution temperature was 17.5°C on the sample day to 45% in January 2004 when the nutrient solution temperature was 26°C. Root assessment of this cultivar (Figure 2-6) showed an increase in brown, dead and/or black roots (root rot ratings of 3-4) in the warmer temperature samples of November 2003 (43% diseased roots at nutrient solution temperature 30.5°C) and January 2004 (45% diseased roots at 26°C), compared with the cooler temperature samples of May 2003 (5% diseased roots at 17.5°C) and August 2003 (18% diseased roots at 14.5°C). However, the sample in March 2004 showed an unusually low disease incidence (15%) when the nutrient solution temperature was relatively high (25°C).

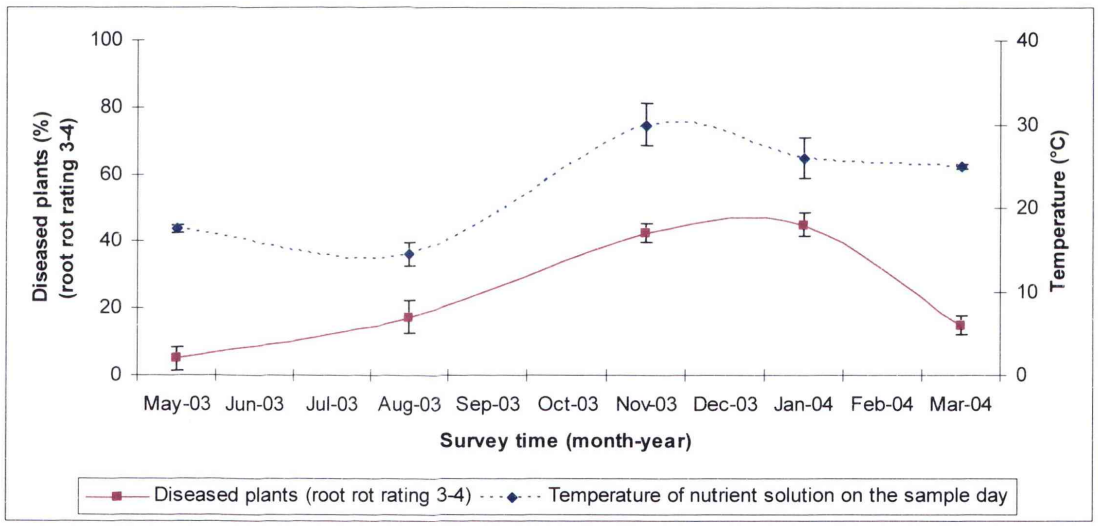

Figure 2-6 Percentage of diseased Brown Mignonette lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. (See Figure 2-4 for details of scale rating.) Temperature of the nutrient solution was measured on the survey day. Error bars are standard error of the mean.

There were no significant differences in root rot disease between the two warm temperature samples of November 2003 and January 2004 (P=0.96), and between the two cool temperature samples of May 2003 and August 2003 (P=0.23) (Appendix 3 a). However, the March 2004 sample was found to be not significantly different from the May 2003 (P=0.83) and the August 2003 (P=0.83) samples, but significantly different from the November 2003 sample (P=0.04) and the January 2004 (P=0.01) sample. No significant differences were found in root rot assessment between young and mature

Brown Mignonette in four of the five samples i.e. in May 2003 sample (P=0.95), August 2003 (P=0.95), January 2004 (P=0.85) and March 2004 (P=0.95) (Appendix 3 a). However significant differences were found in root rot assessment between young and mature Brown Mignonette in the November 2003 sample (P=0.00).

Pooled data on the percentage of diseased roots (rated at 3 and 4) at different sample times are shown for Baby Cos in Figure 2-7. In the Baby Cos cultivar, the root rot disease level ranged from 5% in May 2003 when the nutrient solution temperature was 17.5°C to 30% in January 2004 when the nutrient solution temperature was 26°C. Root assessment of this cultivar showed higher disease incidence in the November 2003 sample (23% diseased roots) when the nutrient solution temperature was 30.5°C and 30% diseased roots in January 2004 at nutrient solution temperature of 26°C, compared with the May 2003 sample (5% diseased roots at 17.5°C). However, a relatively high level of diseased roots appeared in August 2003 (20% diseased roots) when the nutrient solution temperature was the lowest (14.5°C). Moreover, a sample in March 2004 showed an unusually low disease incidence (5%) when the nutrient solution temperature was relatively high (25°C).

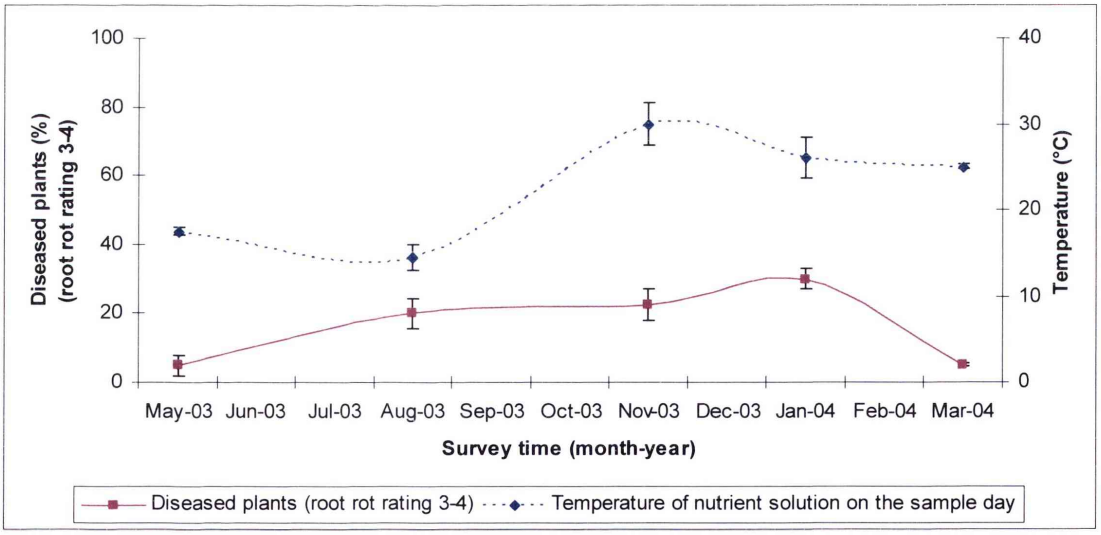

Figure 2-7 Percentage of diseased Baby Cos lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. (See Figure 2-4 for details of scale rating.) Temperature of the nutrient solution was measured on the survey day. Error bars are standard error of the mean.

Root rot disease in the May 2003 sample was significantly less than root rot disease in the August 2003 (P=0.04), November 2003 (P=0.02) and January 2004 (P=0.02)

samples (Appendix 3 a), but not significantly different from root rot disease in the March 2004 sample (P=0.50). No significant differences in root rot disease were found between the November 2003 sample and the January 2004 sample (P=0.98). No significant differences were found in root rot assessment between young and mature Baby Cos in the five samples (Appendix 3 a).

Pooled data on the percentage of diseased roots (rated at 3 and 4) at different sample times are shown for Red Oak in Figure 2-8. The root rot disease level of the Red Oak ranged from 3% in August 2003 when the nutrient solution temperature was 14.5°C on the sample day to 48% in both January 2004 and March 2004 when the nutrient solution temperature was 26°C and 25°C, respectively. Root assessment of this cultivar (Figure 2-8) showed an increase in brown, dead and/or black roots (root rot ratings of 3-4) in the warmer temperature samples of November 2003 (20% diseased roots at nutrient solution temperature 30.5°C), January 2004 (48% diseased roots at 26°C) and March 2004 (48% diseased roots at 25°C), compared with the colder temperature samples of May 2003 (8% diseased roots at 17.5°C) and August 2003 (3% diseased roots at 14.5°C).

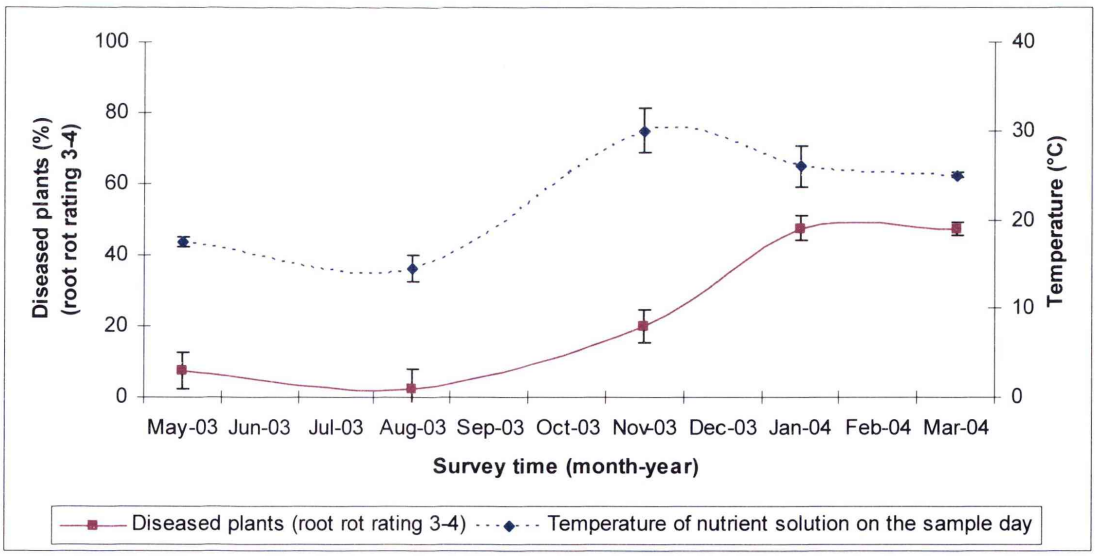

Figure 2-8 Percentage of diseased Red Oak lettuce (both mature and young) from the five time periods (May-03, Aug-03, Nov-03, Jan-04 and Mar-04) with root rot ratings of 3 and 4. (See Figure 2-4 for details of scale rating.) Temperature of the nutrient solution was measured on the survey day. Error bars are standard error of the mean.

There were no significant differences in root rot disease between the January 2004 and March 2004 samples (P=0.28) (Appendix 3 a). However, root rot disease incidence in the May 2003 sample was found to be significantly different from the four other sample times (P=0.00). Moreover, root rot disease in the August 2003 sample was found to be significantly different from the November 2003 sample (P=0.04), the January 2004 sample (P=0.00) and the March 2004 sample (P=0.02). Root rot disease in the November 2004 sample was found to be significantly different from the January 2004 sample (P=0.05) and the March 2004 sample (P=0.04). No significant differences were found in root rot assessment between young and mature Red Oak in the five samples (Appendix 3 a).

Statistical analysis also showed that there were no significant differences found in root rot rating between the three lettuce cultivars examined at the two ages, mature (P=0.26) and young (P=0.23) (Appendix 3 a).

2.3.2 Isolation of fungi and oomycetes from lettuce roots and identification of these microorganisms

The oomycetes *Pythium* and *Phytophthora* were the main pathogens isolated when the root rot disease symptoms were most evident in the disease survey (section 2.3.1) i.e. in the January 2004, November 2003, and (to some extent) March 2004 samples (Tables 2-3, 2-4, 2-5 and 2-6). *Pythium* spp. were isolated from all plants sampled (100% of plants sampled) in November 2003, January 2004 and March 2004 (Table 2-3), when PYSA was available as the isolation medium. When PYSA was not available, only 17% of plants sampled in August 2003 yielded *Pythium* isolates, while no plants in May 2003 yielded *Pythium* isolates. Data from the 2004-2005 survey (see Chapter 3) at the same farm (Table 2-3, columns in italics) show that 100% of the mature plants sampled in August 2004 and May 2004 yielded *Pythium* isolates on PYSA. There were no significant differences in the number of *Pythium* isolates found between the six combinations of cultivar and age (P=0.57), but there were significant differences between the sample times (P=0.00) (Appendix 3 b).

Phytophthora spp. were isolated from most plants sampled in November 2003 (93% of plants sampled), January 2004 (87%), March 2004 (90%) and May 2003 (83%) (Table

2-4). However, limited numbers of plants collected in August 2003 yielded *Phytophthora* (30%); this was the sampling time with the lowest nutrient solution temperature (14.5°C). There were no significant differences in the number of *Phytophthora* isolates found between the the six combinations of cultivar and age (P=0.07), but there were significant differences between the sample times (P=0.00) (Appendix 3 b).

Fusarium spp. were isolated most often in August 2003 (63% of plants sampled) (Table 2-5), when the temperature of the nutrient solution was lowest (14.5°C). *Fusarium* spp. were isolated infrequently (ranging from 0% to 7%) at the other four sampling times which included the higher nutrient solution temperatures.

Rhizoctonia spp. were isolated most often in May 2003 (44% of plants sampled) (Table 2-6) when the nutrient solution temperature was 17.5°C. However, *Rhizoctonia* spp. were isolated infrequently (ranging from 0% to 7%) at the other four sampling times, which included both the higher nutrient solution temperatures (25-30°C) and the lowest temperature (14.5°C).

Table 2-3 Numbers of young and mature lettuce plants yielding *Pythium* spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Subsequent data from May 2004 and August 2004 are also given (in italics). Nutrient solution temperature was measured on the sample day.

Lettuce cultivar and age^	Sample time and temperature of nutrient solution							Totals (%) [without *May-04* and *Aug-04* data]
	May-03 (17.5°C)	*May-04 (14.5°C)*	Aug-03 (14.5°C)	*Aug-04 (14.5°C)*	Nov-03 (30.5°C)	Jan-04 (26°C)	Mar-04 (25°C)	
B. Mignonette y.	0/3	*N**	0/5	*N*	5/5	5/5	5/5	15/23 (65%)
B. Mignonette m.	0/3	*5/5*	1/5	*5/5*	5/5	5/5	5/5	16/23 (70%)
Baby Cos y.	0/3	*N*	1/5	*N*	5/5	5/5	5/5	16/23 (70%)
Baby Cos m.	0/3	*5/5*	0/5	*5/5*	5/5	5/5	5/5	15/23 (65%)
Red Oak y.	0/3	*N*	2/5	*N*	5/5	5/5	5/5	17/23 (74%)
Red Oak m.	0/3	*5/5*	1/5	*5/5*	5/5	5/5	5/5	16/23 (70%)
Totals (%)	0/18 (0%)	*15/15 (100%)*	5/30 (17%)	*15/15 (100%)*	30/30 (100%)	30/30 (100%)	30/30 (100%)	

*N= No samples; only mature lettuces were sampled in the second survey (May 2004- March 2005).
^age: y = young; m = mature.

Table 2-4 Numbers of young and mature lettuce plants yielding *Phytophthora* spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.

Lettuce cultivar and age	Sample time and temperature of nutrient solution					Totals
	May-03 (17.5°C)	Aug-03 (14.5°C)	Nov-03 (30.5°C)	Jan-04 (26°C)	Mar-04 (25°C)	(%)
B. Mignonette young	1/3	1/5	3/5	5/5	5/5	15/23 (65%)
B. Mignonette mature	3/3	1/5	5/5	4/5	5/5	18/23 (78%)
Baby Cos young	3/3	0/5	5/5	3/5	2/5	13/23 (57%)
Baby Cos mature	3/3	4/5	5/5	5/5	5/5	22/23 (96%)
Red Oak young	3/3	1/5	5/5	4/5	5/5	18/23 (78%)
Red Oak mature	2/3	2/5	5/5	5/5	5/5	19/23 (83%)
Totals (%)	15/18 (83%)	9/30 (30%)	28/30 (93%)	26/30 (87%)	27/30 (90%)	

Table 2-5 Numbers of young and mature lettuce plants yielding *Fusarium* spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.

Lettuce cultivar and age	Sample time and temperature of nutrient solution					Totals
	May-03 (17.5°C)	Aug-03 (14.5°C)	Nov-03 (30.5°C)	Jan-04 (26°C)	Mar-04 (25°C)	(%)
B. Mignonette young	1/3	3/5	0/5	0/5	0/5	4/23 (17%)
B. Mignonette mature	0/3	3/5	1/5	0/5	0/5	4/23 (17%)
Baby Cos young	0/3	3/5	0/5	0/5	0/5	3/23 (13%)
Baby Cos mature	0/3	4/5	0/5	0/5	1/5	5/23 (22%)
Red Oak young	0/3	3/5	1/5	0/5	0/5	4/23 (17%)
Red Oak mature	0/3	3/5	0/5	0/5	0/5	3/23 (13%)
Totals (%)	1/18 (6%)	19/30 (63%)	2/30 (7%)	0/30 (0%)	1/30 (3%)	

Table 2-6 Numbers of young and mature lettuce plants yielding *Rhizoctonia* spp. from their roots compared with total number of plants sampled at five times from May 2003 to March 2004. Nutrient solution temperature was measured on the sample day.

Lettuce cultivar and age	Sample time and temperature of nutrient solution					Totals
	May-03 (17.5°C)	Aug-03 (14.5°C)	Nov-03 (30.5°C)	Jan-04 (26°C)	Mar-04 (25°C)	(%)
B. Mignonette young	0/3	0/5	0/5	0/5	0/5	0/23 (0%)
B. Mignonette mature	2/3	1/5	0/5	0/5	0/5	3/23 (13%)
Baby Cos young	1/3	0/5	0/5	0/5	0/5	1/23 (4%)
Baby Cos mature	3/3	1/5	0/5	0/5	0/5	4/23 (17%)
Red Oak young	1/3	0/5	0/5	0/5	0/5	1/23 (4%)
Red Oak mature	1/3	0/5	0/5	2/5	0/5	3/23 (13%)
Totals (%)	8/18 (44%)	2/30 (7%)	0/30 (0%)	2/30 (7%)	0/30 (0%)	

2.3.3 Pathogenicity tests

All *Pythium* spp. isolated from lettuces in November 2003, January 2004 and March 2004 were pathogenicity tested using healthy young lettuce plants, resulting in disease symptoms of wilting and brown roots on inoculated plants but not on control plants kept at 25°C (Figure 2-9) and 35°C but not at 15°C. Significant differences in wet weight and/or dry weight of shoots and/or roots were found between inoculated and control (non-inoculated) plants for pathogenicity tests involving *Pythium* spp. isolated from these three sample times (i.e. November 2003, January 2004 and March 2004) and particularly at the pathogenicity test temperatures of 25°C and 35°C (Table 2-7 and Appendix 3 c 1). On the other hand, *Pythium* sp. isolated from lettuces sampled in August 2003 and tested using macerated agar inoculum (as in November, January and March pathogenicity tests), caused no disease symptoms at all temperatures tested and there were no significant differences between inoculated and control plants in wet weight and dry weight of shoots and roots (Table 2-7).

Generally, no significant differences were found between the two inoculation methods (agar disc and macerated agar culture) used in the August 2003 test involving *Pythium* sp. at all temperatures used (Appendix 3 c 1). For only two of the twelve parameters measured were significant differences found between the inoculation methods used; the two parameters involved were wet root weight at 15°C and dry shoot weight at 35°C.

No significant differences were found between the two inoculum concentrations (normal and high concentration) tested at all temperatures used in November 2003 and March 2004 pathogenicity tests (Appendix 3 c 1). No significant differences were found between the two *Pythium* isolates tested in January 2004 at all temperatures used (Appendix 3 c 1).

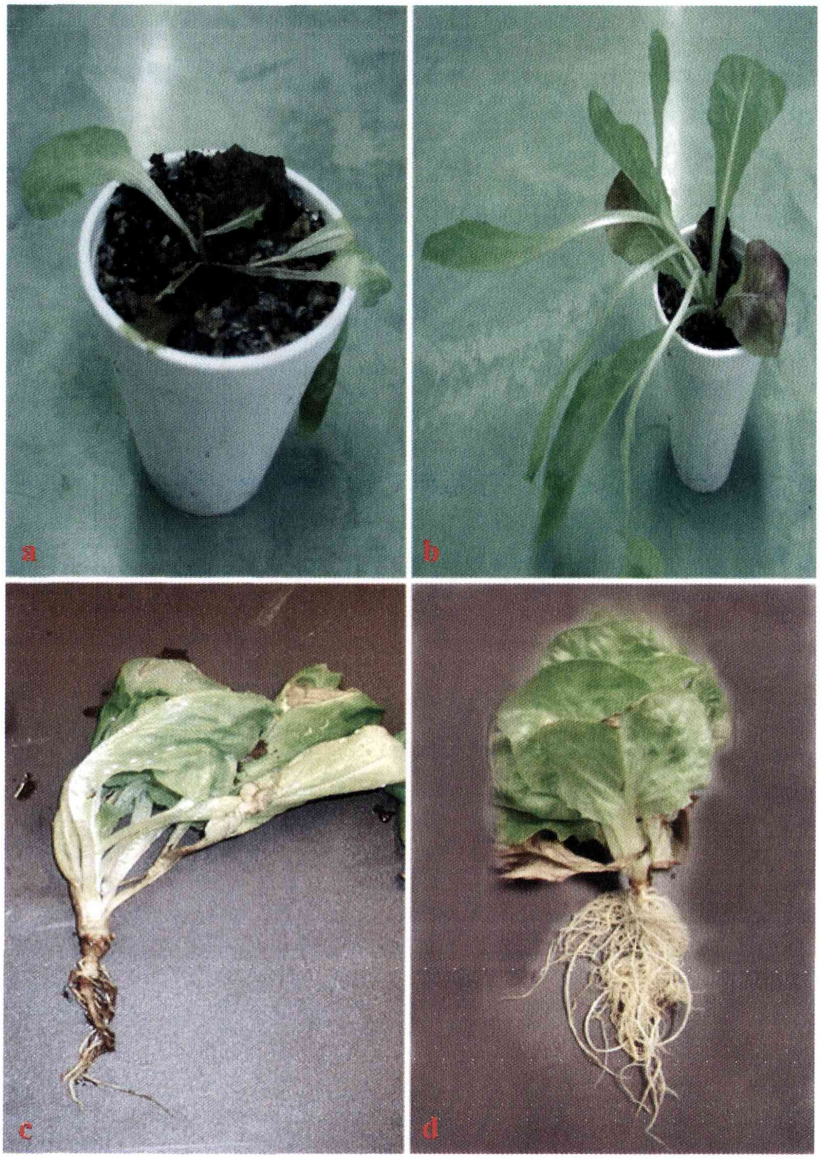

Figure 2-9 Lettuce plants from November 2003 pathogenicity test involving an isolate of *Pythium* sp. tested at 25°C. Inoculated plants ((a) and (c)) show wilting of leaves (in (a)) and a small root system/brown roots (in (c)). Control plants ((b) and (d)) show no disease and a larger root system with healthy white roots.

Table 2-7 *P* value of the mean difference between control plants and plants inoculated with *Pythium* isolates for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (August 2003, November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C). Means for the control plants were greater than the means for the inoculated plants. *P* values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 1.)

Sample time (temp. (°C) of nutrient solution at farm) and type of inoculum used#	Temperature for Pathogenicity tests (°C)	Shoot weight		Root weight	
		Wet	Dry	Wet	Dry
Aug-03	15	0.99	0.85	0.67	0.37
(14.5)	25	0.82	0.45	0.38	0.52
Macerated agar, normal conc.	35	1.00	0.81	0.97	0.83
Aug-03	15	0.18	**0.04**	0.27	0.88
(14.5)	25	0.15	**0.00**	0.16	0.75
Agar disc	35	0.43	0.22	0.21	**0.05**
Nov-03	15	0.09	0.14	**0.01**	0.10
(30.5)	25	**0.00**	0.75	**0.00**	**0.00**
Macerated agar, normal conc.	35	0.31	0.60	0.69	0.80
Nov-03	15	0.21	0.10	0.06	0.47
(30.5)	25	0.20	**0.00**	0.06	**0.00**
Macerated agar,high conc.	35	0.08	0.24	**0.00**	**0.04**
Jan-04	15	0.95	0.98	1.00	0.99
(26)	25	**0.00**	**0.00**	**0.00**	**0.00**
Macerated agar, normal conc., isolate 1	35	**0.00**	**0.02**	0.30	**0.05**
Jan-04	15	0.24	0.33	0.11	0.22
(26)	25	**0.01**	**0.00**	**0.01**	**0.03**
Macerated agar, normal conc., isolate 2	35	**0.01**	**0.00**	0.09	**0.01**
Mar-04	15	1.00	1.00	0.99	0.99
(25)	25	**0.00**	0.84	**0.02**	0.91
Macerated agar, normal conc.	35	0.63	**0.00**	**0.01**	0.63
Mar-04	15	0.43	0.69	0.75	0.60
(25)	25	**0.00**	0.10	0.21	**0.00**
Macerated agar,high conc.	35	0.32	**0.00**	**0.00**	0.08

see Table 2-1 for further details of type of inoculum used.

All *Phytophthora* spp. isolated from lettuces in November 2003, January 2004 and March 2004 and tested caused disease symptoms of wilting and brown roots on inoculated plants but not on control plants at 25°C (Figure 2-10) and 35°C but not at 15°C. Significant differences in wet weight and/or dry weight of shoots and/or roots were found between inoculated and control (non-inoculated) plants, particularly for the pathogenicity tests involving *Phytophthora* spp. isolated from November 2003 and March 2004 (Table 2-8; Figure 2-10 and Appendix 3 c 2). However, the isolate of *Phytophthora* sp. isolated from May 2003 caused no disease symptoms at all temperatures tested (15, 25 and 35°C) and there were no significant differences between inoculated and control plants in wet weight of shoots and roots. However, significant differences were found between inoculated and control (non-inoculated) plants in dry weight of roots at 15°C and in dry weight of shoots at 25°C.

Figure 2-10 Lettuce plants from November 2003 pathogenicity test involving an isolate of *Phytophthora* sp. tested at 25°C. Inoculated plants ((a) and (c)) show wilting of leaves (in (a)) and a small root system/brown roots (in (c)). Control plants ((b) and (d)) show no disease and a larger root system with healthy white roots.

Table 2-8 *P* value of the mean difference between control plants and plants inoculated with *Phytophthora* isolates for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003, November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C). Means for the control plants were greater than the means for the inoculated plants. *P* values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 2.)

Sample time (temp. (°C) of nutrient solution at farm)	Temperature for pathogenicity tests (°C)	Shoot weight		Root weight	
		Wet	Dry	Wet	Dry
May-03 (17.5)	15	0.40	0.26	0.31	**0.02**
	25	0.41	**0.01**	0.77	0.63
	35	0.55	0.59	0.90	0.79
Nov-03 (30.5)	15	0.12	0.72	0.47	0.68
	25	**0.00**	**0.00**	**0.00**	**0.00**
	35	0.37	0.66	**0.01**	**0.01**
Jan-04 (26)	15	0.50	0.56	0.28	0.46
	25	0.08	**0.01**	0.14	0.15
	35	0.06	0.23	**0.01**	0.08
Mar-04 (25)	15	0.81	0.60	0.51	0.69
	25	0.10	0.07	**0.04**	**0.00**
	35	0.48	**0.01**	**0.04**	0.11

Fusarium spp. and *Rhizoctonia* sp. sampled in May 2003 and/or August 2003 caused no disease symptoms at all temperatures tested (15, 25 and 35°C). Moreover, no significant differences in wet weight and/or dry weight of shoots and/or roots were found between inoculated and control (non-inoculated) plants (Table 2-9 and Appendix 3 c 3 and 4 for *Fusarium* spp., and Table 2-10 and Appendix 3 c 5 for *Rhizoctonia* sp.).

Table 2-9 *P* value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003 and August 2003) under different temperatures (15°C, 25°C and 35°C) for *Fusarium avenaceum* and *Fusarium oxysporum* isolates. Means for the control plants were greater than the means for the inoculated plants. (Appendix 3 c 3 and 4.)

Sample time (temp. (°C) of nutrient solution at farm)	Temperature for pathogenicity tests (°C)	Shoot weight		Root weight	
		Wet	Dry	Wet	Dry
May-03 *Fusarium avenaceum* (17.5)	15	0.44	0.09	0.46	0.56
	25	0.29	0.14	0.26	0.93
	35	0.23	0.34	0.41	0.48
May-03 *Fusarium oxysporum* (17.5)	15	0.11	0.74	0.12	0.06
	25	0.88	0.30	0.36	0.69
	35	0.16	0.21	0.46	0.31
Aug-03 *Fusarium oxysporum* (14.5)	15	0.56	0.45	0.29	0.69
	25	0.08	0.31	0.23	0.13
	35	0.18	0.90	0.14	0.90

Table 2-10 *P* value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (May 2003 and August 2003) under different temperatures (15°C, 25°C and 35°C) for *Rhizoctonia* sp. isolate. Means for the control plants were greater than the means for the inoculated plants. (Appendix 3 c 5.)

Sample time (temp. (°C) of nutrient solution at farm)	Temperature for pathogenicity tests (°C)	Shoot weight		Root weight	
		Wet	Dry	Wet	Dry
May-03 (17.5)	15	0.87	0.14	0.16	0.30
	25	0.08	0.41	0.08	0.43
	35	0.09	0.54	0.30	0.52

The combination of an isolate of *Pythium* sp. and an isolate of *Phytophthora* sp. caused disease symptoms on infected lettuce at temperatures 25°C and 35°C, but not at 15°C in November 2003, January 2004 and March 2004 pathogenicity tests. Significant differences were found between inoculated and control plants in wet weight and/or dry weight of shoots and/or roots for the November 2003 test at all three temperatures and for the March 2004 test at 25°C and 35°C (Table 2-11 and Appendix 3 c 6).

All oomycetes and fungi tested (*Pythium* spp., *Phytophthora* spp., *Fusarium* spp. and *Rhizoctonia* sp.) were re-isolated from the respective inoculated plants but not from the control (non-inoculated) plants at the conclusion of all pathogenicity test experiments.

Table 2-11 *P* value of the mean difference between control plants and inoculated plants for wet shoot weight, dry shoot weight, wet root weight and dry root weight from different pathogenicity test experiments (November 2003, January 2004 and March 2004) under different temperatures (15°C, 25°C and 35°C) for the combination of *Phytophthora* sp. and *Pythium* sp. isolates. Means for the control plants were greater than the means for the inoculated plants. *P* values indicating significant differences ($P \leq 0.05$) are shown in bold. (Appendix 3 c 7).

Sample time (temp. (°C) of nutrient solution at farm)	Temperature for pathogenicity tests (°C)	Shoot weight		Root weight	
		Wet	Dry	Wet	Dry
Nov-03 (30.5)	15	**0.00**	**0.00**	**0.00**	0.09
	25	0.27	0.15	**0.01**	**0.00**
	35	0.37	0.27	**0.01**	**0.04**
Jan-04 (26)	15	0.99	0.99	0.99	0.95
	25	0.76	0.57	0.83	0.71
	35	0.98	0.96	0.99	**0.00**
Mar-04 (25)	15	0.99	1.00	0.99	0.98
	25	**0.02**	0.13	**0.00**	**0.00**
	35	0.36	**0.03**	0.15	0.30

2.3.4 Temperature responses in culture of fungi and oomycetes

Isolates of *Pythium* have an optimum temperature of 30°C (Figure 2-11) which is the highest of all organisms obtained from lettuce roots in this study (Figures 2-12, 2-13 and 2-14). They also grew well at both 25°C and 35°C. *Pythium* isolates grew at 40°C (but not at 45°C) and 5°C. Moreover, there were no significant differences in colony growth between the five *Pythium* isolates tested at each temperature (P=0.56) (Appendix 3 d). *Phytophthora* isolates had an optimum temperature of 25°C and grew well at both 20°C and 30°C (Figure 2-12); they also grew slightly at 35°C but not at 40°C. There were no significant differences in colony growth between the five isolates tested at each temperature (P=0.49) (Appendix 3 d). *Fusarium oxysporum* isolates have an optimum temperature between 25°C and 30°C; they grew at 10°C (but not at 5°C) and at 40°C (but not at 45°C) (Figure 2-13). There were no significant differences in colony growth between the three isolates tested at each temperature (P=0.13) (Appendix 3 d). Isolates of *Fusarium avenaceum* have an optimum temperature of 25°C (Figure 2-14). Moreover, *Fusarium avenaceum* grew slightly at 5°C and at 35°C but not at 40°C; there were no significant differences in colony growth between the three isolates tested at each temperature (P=0.11) (Appendix 3 d).

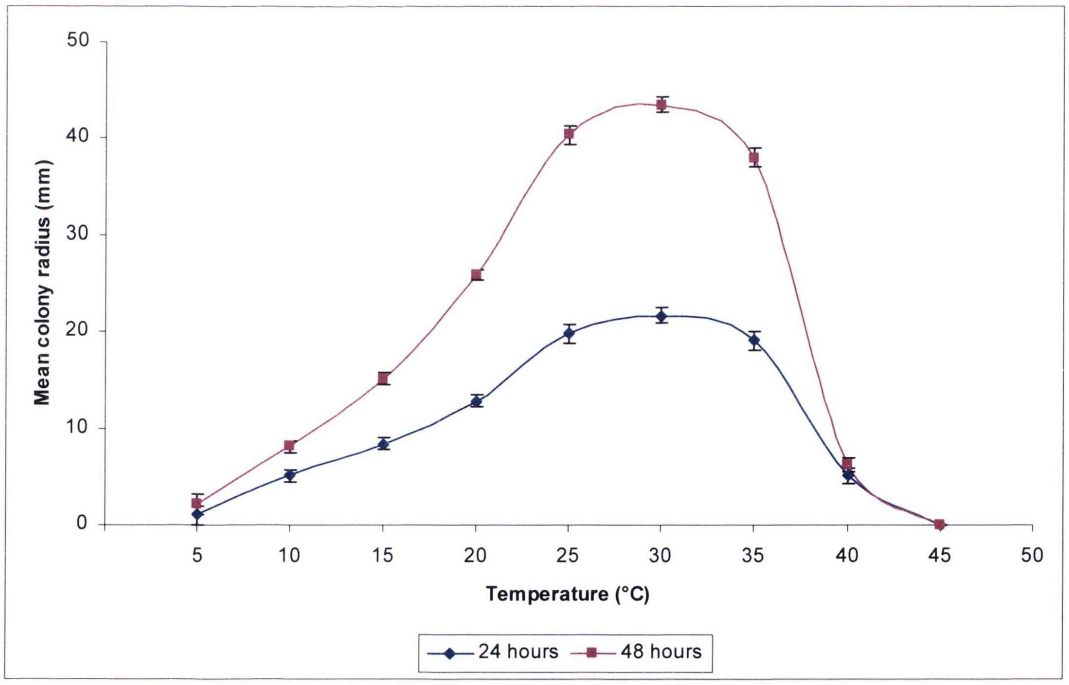

Figure 2-11 Mean colony radius (mm) of the main *Pythium* isolates from lettuce roots grown on PCA media and measured at 24h and 48h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C. Bars are standard error of the mean.

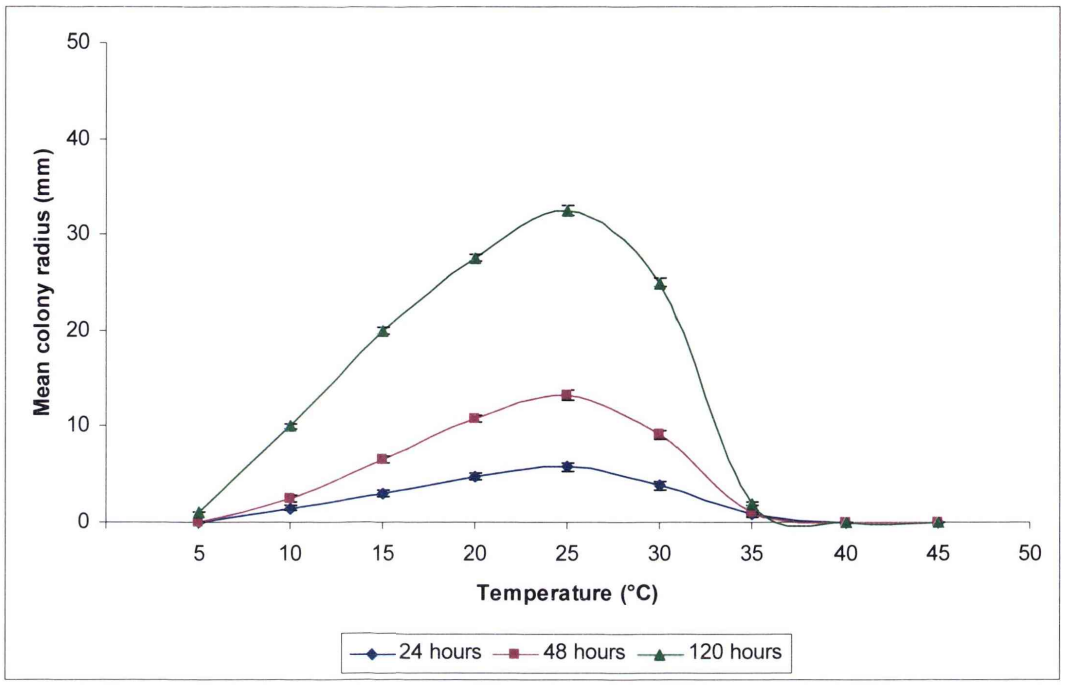

Figure 2-12 Mean colony radius (mm) of the main *Phytophthora* isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 120h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C. Bars are standard error of the mean.

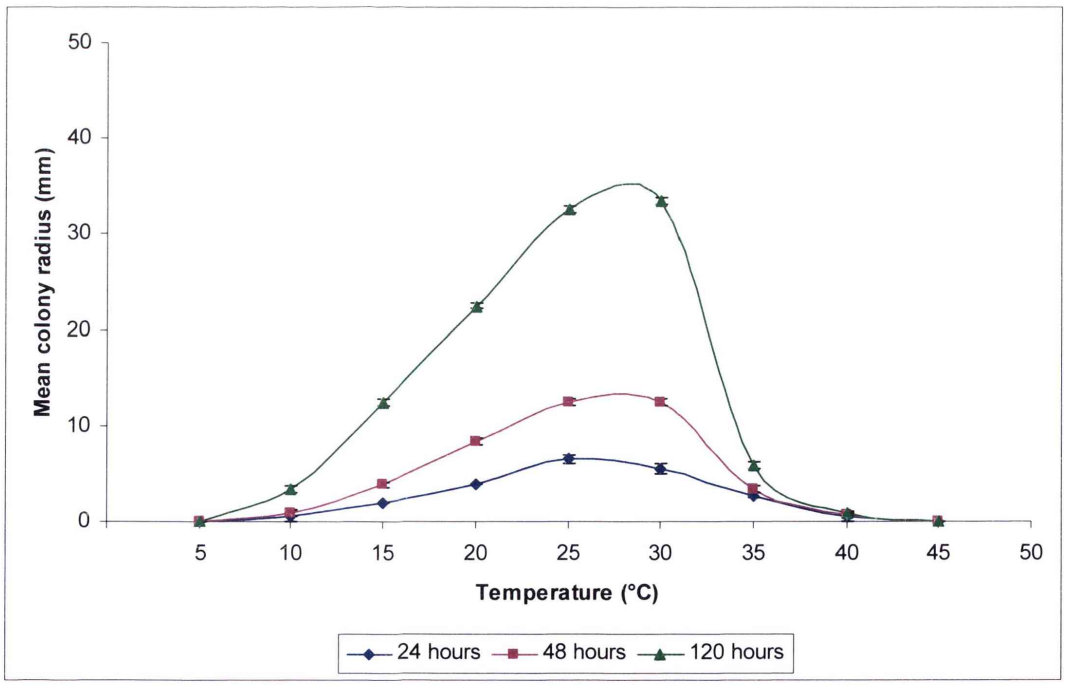

Figure 2-13 Mean colony radius (mm) of the main *Fusarium oxysporum* isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 120h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C. Bars are standard error of the mean.

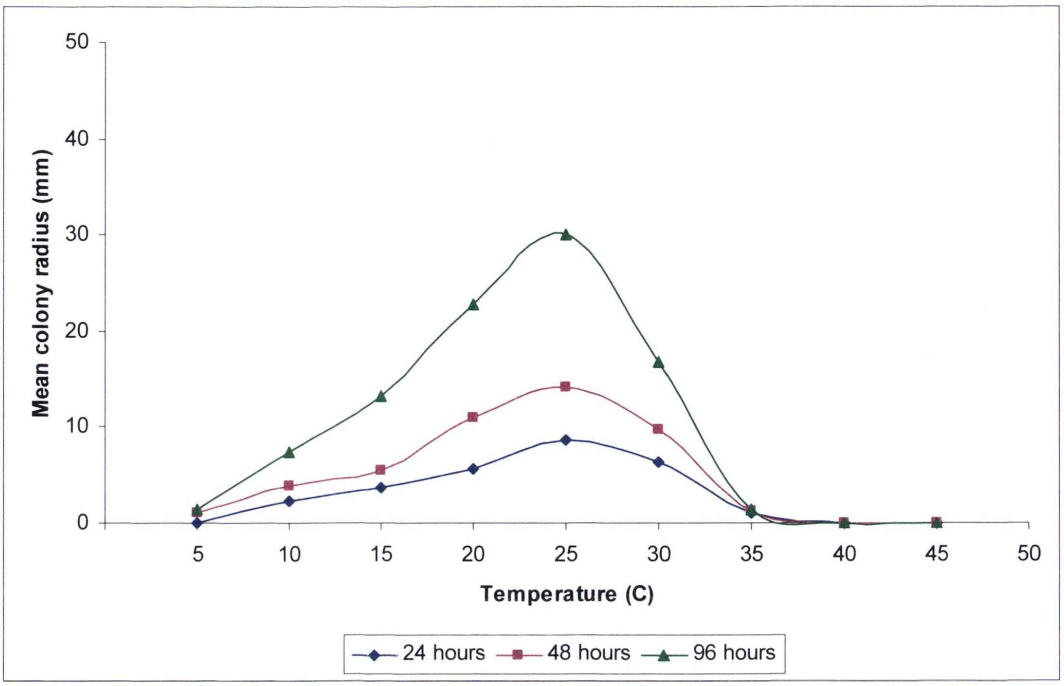

Figure 2-14 Mean colony radius (mm) of the main *Fusarium avenaceum* isolates from lettuce roots grown on PCA media and measured at 24h, 48h and 96h after inoculation and incubation in the dark at nine temperatures from 5°C to 45°C. Bars are standard error of the mean.

2.4 Discussion

This study demonstrated that root rot disease of hydroponic lettuce from a farm in the Sydney area was most severe under conditions of high temperature (Figures 2-6, 2-7 and 2-8). Based on five survey and sampling dates over an 11 month period, significant lettuce root damage was found in all three cultivars studied (Brown Mignonette, Baby Cos and Red Oak) when the temperature of the nutrient solution was 25°C or higher – that is, in the surveys undertaken in November 2003 (nutrient solution 30.5°C), January 2004 (26°C) and (to lesser extent) March 2004 (25°C). By contrast, healthy lettuce roots were found when the temperature of the nutrient solution was 17.5°C or lower in the surveys undertaken in May 2003 (17.5°C) and August 2003 (14.5°C). Bates and Stanghellini (1984) found similar results for hydroponically grown spinach, in that the incidence of root rot disease was lowest when the nutrient solution temperatures were below 20°C; the highest disease incidence occurred when the temperatures of the nutrient solution were greater than 20°C.

Based on the findings of this survey and temperature data from the Bureau of Meteorology, NSW, for Leppington (Figure 2-5), hydroponic lettuce growers in the area could expect to have few root rot disease problems for the period from April through to September (or perhaps October), when the air temperature remains below approximately 23°C. On the other hand, root disease problems could be expected to occur between November and March, when the air temperature is above 25°C. This finding would appear to support the claims made by hydroponic lettuce farmers that they do indeed suffer greater losses to root rot disease in summer.

An unusual finding of the present survey was a low incidence of root rot disease in March 2004 in the lettuce cultivars Brown Mignonette and Baby Cos (Figures 2-6 and 2-7) even though the nutrient solution temperature was high (25°C) and similar to the temperature in January 2004 (26°C) when a high incidence of root rot disease was recorded in these same cultivars. There could be several explanations for this finding, such as heavy culling of diseased lettuce prior to the March 2004 sampling date or variation amongst cultivars as regards root rot disease development in relation to temperature. There are anecdotal reports from farmers concerning differences between lettuce cultivars and root rot disease development. This aspect deserves further

investigation using a larger range of lettuce cultivars, over a range of different nutrient solution temperatures, under experimental conditions which can be controlled by the researcher.

It is claimed that there are no lettuce cultivars available with confirmed resistance to root rot pathogens in hydroponic systems (Stanghellini & Rasmussen 1994). In the present study, there were no significant differences in root rot rating found between the three lettuce cultivars examined at both young and mature ages. Moreover, there were generally no significant differences in root rot assessment between young and mature lettuces for each cultivar. These findings suggest that if the pathogen is present in the system and the environmental conditions are favorable, the pathogen will affect lettuce of both ages and conversely, if there are no pathogens in the system and/or the environmental conditions are not favorable, lettuce of both ages will not be affected. However, on one occasion (in the November 2003 sample) roots of young Brown Mignonette appeared healthier than roots of the mature plants. The reason for this finding could be that the young Brown Mignonette lettuces had been placed in the system shortly before the survey day and there was insufficient time for root rot disease to develop. Future studies could focus on the development of lettuce cultivars resistant to root rot pathogens, especially for those grown in hydroponic systems. There is some evidence for the existence of plant cultivars resistant to *Pythium* (McCarter & Littrell 1970).

Pythium and *Phytophthora* were the main microorganisms isolated from lettuce roots when root rot disease was greatest i.e. in the warmer season samples of January 2004, November 2003 and (to some extent) March 2004 (Tables 2-3 to 2-6; Figures 2-6 to 2-8). *Pythium* spp. were isolated from all plants sampled (100%) in late spring, summer and early autumn (November 2003, January 2004 and March 2004) when the nutrient solution temperature was 25°C or higher. In late autumn (May 2003) when the nutrient solution temperature was 17.5°C, there were no *Pythium* spp. isolated, but this could be attributed to the lack of PYSA as an isolation medium or inexperience on the part of the researcher. However, in the winter sample (August 2003), *Pythium* spp. were isolated from five plants on another medium (*Fusarium* selective agar).

There were no significant differences found in the number of *Pythium* isolates obtained from the six combinations of cultivar and age. This is not surprising, since it is known that, if *Pythium* spp. are present in a hydroponic system, they will spread quickly throughout the entire system (Stanghellini & Rasmussen 1994). Nevertheless, these findings suggest that the three cultivars examined, as well as both young and mature lettuces of each cultivar, can all be infected by *Pythium* spp. Various studies have found *Pythium* spp. to be the most common pathogen in hydroponic systems and *Pythium* spp. have been isolated from lettuce roots grown in hydroponic systems (Jenkins & Averre 1983; Zinnen 1988; Labuchagne *et al.* 2002; Stanghellini & Rasmussen 1994; Stanghellini & Kim 1998). Moreover, *Pythium* spp. were reported on lettuce grown in soilless systems in the past in NSW (Tesoriero *et al.* 1991). However, the failure to isolate *Pythium* spp. from lettuce roots sampled at low temperatures in May 2003 (Table 2-3) and the small number of isolates obtained in August 2003 (Table 2-3) suggests that a more comprehensive study using PYSA medium needs to be undertaken in cooler conditions at both this farm and other hydroponic farms. The results of the present study indicate that *Pythium* spp. are able to survive and grow at typical summer temperatures of 25°C to 30.5°C (Table 2-3).

Phytophthora spp. were also isolated from most plants (83% - 93% of plants) sampled in late spring, summer and early to late autumn (November 2003, January 2004, March 2004 and May 2003), when the nutrient solution temperature was 17.5°C or higher. However limited numbers of plants collected in August 2003, when the nutrient solution was the lowest (14.5°C), yielded *Phytophthora* species. It may be that a temperature as low as 14.5°C does not favour the growth of *Phytophthora* species. Forster *et al.* (1998) recorded *Phytophthora* root and crown rot on green pepper plants grown in hydroponic culture at a nutrient solution temperature between 22.6°C and 28.1°C. In previous study in NSW, *Phytophthora* sp. was recovered from lettuce roots grown in a hydroponic system (Tesoriero *et al.* 1991). Sitepu and Bumbieris (1981) reported that *Phytophthora porri* caused stem rot of lettuce grown in soil at a temperature of 15°C. Erwin and Ribeiro (1996) reported that optimum growth temperature for the majority of *Phytophthora* spp. was between 25 and 27°C.

Fusarium spp. and *Rhizoctonia* spp. were isolated much less frequently than *Pythium*

spp. and *Phytophthora* species. *Pythium* spp. were isolated from 100% of lettuce plants when PYSA medium was used and *Phytophthora* spp. were isolated from 83%-93% of plants, but *Fusarium* spp. were isolated from a maximum of 63% of plants in one sample and generally from only 7% of plants or less at other times (Table 2-5). *Rhizoctonia* spp. were isolated from only 44% of plants in one sample and generally from only 7% of plants or less at other times (Table 2-6). The high number of *Fusarium* spp. isolates was obtained in the August 2003 sample when the temperature of the nutrient solution was low (14.5°C). *Rhizoctonia* spp. were isolated most frequently in May 2003 when the nutrient solution temperature was 17.5°C. Both *Fusarium* spp. and *Rhizoctonia* spp. were isolated in the two coldest sampling times and were not associated with hydroponic lettuce root rot diseases in the farm, which were more pronounced in the three warmer sampling times. It is likely that the lettuce plants grown in hydroponic systems are not a specific host for *Fusarium* spp. and *Rhizoctonia* spp., although they are pathogenic to lettuce grown in soil (Davis *et al.* 1997).

In the pathogenicity tests carried out, the *Pythium* spp. isolated from lettuce roots growing in nutrient solution with a temperature of 25°C or more were shown to be pathogenic at temperatures 25°C and 35°C but not at 15°C (Figure 2-9). Symptoms of wilting and brown roots were recorded on inoculated plants but not on non-inoculated plants. *Pythium* spp. were reisolated from roots of inoculated plants, but not from roots of non-inoculated plants. Hence the pathogenicity tests satisfy Koch's Postulates for the *Pythium* isolates obtained from the warm temperature sampling times (November 2003, January 2004 and March 2004).

Significant differences in the wet weight and/or dry weight of shoots or roots between plants inoculated with *Pythium* spp. and the control plants were found for the isolates of *Pythium* spp. obtained from warmer sample times of November 2003, January 2004 and March 2004 (Table 2-7). However, when *Pythium* sp. was isolated from lettuce roots grown in nutrient solution with a temperature 14.5°C (August 2003), it was found to be not pathogenic using both inoculation methods (agar disc or macerated agar culture) at all of the temperatures tested (15°C, 25°C and 35°C). Furthermore, there were very few instances of significant differences between inoculated and control plants for wet weight and/or dry weight of shoots and/or roots (Table 2-7). It is possible that the *Pythium* isolate obtained at this low temperature is different from those isolated at high

temperatures. Also, the isolate obtained at this low temperature did not appear to be pathogenic at the farm as there was very little evidence of root rot disease symptoms at the farm in August 2003.

For the pathogenicity tests involving *Pythium*, no significant differences were found between the two inoculum concentrations used (normal and high) (Appendix 3.c.1). Generally, no significant differences were found between the two inoculum methods used (agar disc and macerated agar culture) (Appendix 3.c.1). These results indicate that, when *Pythium* inoculum is introduced into the potting mix at different concentrations of macerated agar culture or by agar disc, *Pythium* will spread and infect the lettuce plants if the conditions are favourable for pathogen growth and infection. No significant differences were found between the two *Pythium* isolates tested in January 2004 (Appendix 3.c.1). This could be attributed to the fact that they are same species, or even the same strain, and/or that they have the same level of pathogenicity towards lettuce.

Isolates of *Phytophthora* spp., like those of *Pythium* spp., were pathogenic on lettuce at temperatures of 25°C and 35°C but not at 15°C when they were isolated from lettuce roots grown in nutrient solution with a temperature 25°C or more (Figure 2-10). Significant differences between plants inoculated with *Phytophthora* spp. and the control plants in wet weight and/or dry weight of shoots and/or roots were found, particularly for *Phytophthora* isolates from November 2003 and March 2004 (Table 2-8). However, *Phytophthora* showed no pathogenicity at all temperatures tested when it was isolated from lettuce roots grown in nutrient solution with a temperature of 17.5°C on the sampling day. Furthermore, there were few significant differences between inoculated and control plants for growth parameters when this isolate was tested (Table 2-8). It is possible that the isolate of *Phytophthora* obtained in the cooler sampling period had lost its pathogenicity or was not pathogenic at all. This finding agrees with that of Hutton and Forsberg (1991) who reported that *Phytophthora* sp. was pathogenic on lettuce plants when the trays of lettuce seedlings were placed in town water for three days in a glasshouse at a temperature 25-30°C.

The combination of an isolate of *Pythium* and an isolate of *Phytophthora* was also found to be pathogenic to lettuce plants at temperatures of 25°C and 35°C but not at 15°C in the November 2003, January 2004 and March 2004 pathogenicity tests. Since

the same isolates of *Pythium* and *Phytophthora* were found to be pathogenic to lettuce plants when each was tested alone, it might be expected that they would be pathogenic when tested together.

Fusarium spp. and *Rhizoctonia* sp. were not pathogenic to lettuce plants at all three of the temperatures tested. It may be that lettuce plants are not a host of these fungal species, or it could be that the isolates obtained from the hydroponic farm are not pathogenic. The optimum growth temperatures of *Fusarium* spp. were between 25 and 30°C (Figures 2-13 and 2-14) and those for *Rhizoctonia* spp. are reported to be 25°C (Harikrishnan & Yang 2004), so the pathogenicity test carried out at 25°C would have been favorable for the growth of these fungi.

The optimum growth temperatures of *Pythium* isolates tested in this study were 30°C; they also grew quite well at 35°C and there was some growth at 40°C. However, the optimum growth temperatures of *Phytophthora* isolates tested were 25°C and, although they grew quite well at 30°C, they grew very little at 35°C and not at 40°C. In view of the much greater growth at 35°C shown by *Pythium* isolates in comparison *Phytophthora* isolates, it is likely that *Pythium* will be more active than *Phytophthora* in the warmer times of the year.

The interaction between temperature and pathogen seems to be very important in relation to root rot disease incidence in lettuce. It appears that during periods of warm temperature when the nutrient solution temperate is high (25°C or above), root rot diseases will affect lettuces more severely and significant yield losses are likely to occur. Also, oomycetes like *Pythium* and *Phytophthora* are highly favoured by aquatic conditions that prevail in hydroponic systems (Agrios 2005). High temperatures in the nutrient solution will favour more growth and the production of greater numbers of zoospores of *Pythium* and *Phytophthora*. As well, the optimum temperature range for lettuce growth is 20°C to 25°C (Davis *et al.* 1997), so it is likely that higher nutrient solution temperatures than this will affect plant growth and could stress their roots. Several studies have found that root rot diseases are more severe when the nutrient solution temperatures are higher than 25°C (Zinnen 1988; Stanghellini & Rasmussen 1994; Stanghellini & Kim 1998).

In summary, root rot disease of lettuce grown in hydroponic systems in the Sydney area occurs during the period from November to March (late spring, summer and early autumn) when the nutrient solution temperatures are 25°C or higher. These temperatures also seem to favour the growth of pathogens isolated from lettuce roots, especially *Pythium* and *Phytophthora*. *Pythium* and *Phytophthora* show pathogenicity in tests carried out at temperatures of 25°C and 35°C but not at 15°C. A nutrient solution temperature of 25°C or more has two effects – firstly, on the pathogens, favoring their growth; and secondly, on the lettuce plants, where high temperatures could stress the roots such that pathogens can more easily enter these roots. Further studies into root rot disease of hydroponic lettuce should include surveys of more than one farm, and preferably farms at different locations, to confirm the results found in this study. Also, other studies should investigate the pathogens *Pythium* and *Phytophthora* to find out more about the variation within each pathogen and to identify these pathogens to species where possible by using morphological characterisation and molecular methods.

3 SURVEY OF ROOT ROT DISEASE OF LETTUCE GROWN IN HYDROPONIC SYSTEMS IN SYDNEY AND THE CENTRAL COAST

3.1 Introduction

New South Wales (NSW) has 190 ha of land dedicated to hydroponic lettuce, making it the state with the largest production area in Australia, as estimated in 1996 (Hassall & Associates 2001). The area of hydroponic crop production in Australia increased from 155 to 500 ha in just 6 years between 1990 and 1996. However, no recent statistical information is available for hydroponic production areas in Australia (Australian Bureau of Statistics, pers. comm.).

Most hydroponic lettuce farms in NSW are concentrated in the Sydney basin, especially in south-west Sydney which is close to the market and on the Central Coast of NSW (Hassall & Associates 2001). Most hydroponic lettuce production is consumed in NSW but some farms export their production. Hydroponic lettuce sells as whole lettuce or in loose-leaf packaged mixes.

Root diseases of lettuce grown in hydroponic systems have become a serious problem in Australia and worldwide. Various studies investigating the causes of root diseases in hydroponically grown vegetables, including lettuce, have found that *Pythium* and *Phytophthora* were the main causes of root diseases (Stanghellini & Kronland 1986; Hutton & Forsberg 1991; Jamart 1999).

In Australia, farmers claim that they suffer heavy yield losses of hydroponic lettuce in summer. Symptoms of stunted and wilted lettuces with brown to black roots were recorded on lettuce grown in hydroponic systems in summer (Tesoriero & Cresswell 1995). A year round survey of hydroponic lettuce in south-west Sydney (Chapter 2) showed that root rot disease was more obvious in the warmer season samples of November 2003, January 2004 and March 2004 than in the cooler season samples of May 2003 and August 2003. *Pythium* and *Phytophthora* were found associated with

root rot diseases and yield losses of lettuce grown in hydroponic systems in the Sydney area (Tesoriero *et al.* 1991; Tesoriero & Cresswell 1995; Chapter 2).

The aims of the research reported in this chapter were developed from Chapter 2 and include the following:

- to carry out a disease survey over a year on four hydroponic lettuce farms in the Sydney and Central Coast areas of NSW, to assess the incidence and severity of root rot disease in several lettuce cultivars at the mature stage, with a view to establishing whether root rot disease is a summer-specific problem;

- to isolate and identify the oomycetes *Pythium* and *Phytophthora* associated with root rot diseases at the four hydroponic lettuce farms surveyed in the Sydney and Central Coast areas of NSW; and

- to carry out pathogenicity testing for these oomycetes to confirm that they are the cause(s) of root rot diseases.

3.2 Materials and methods

3.2.1 Root rot disease survey

A disease survey was carried out over an 11 month period between May 2004 and March 2005 at four hydroponic lettuce farms, two of which were at Leppington in south-west Sydney and two of which were on the Central Coast of NSW (Table 3-1; Figures 3-1 and 3-2). In these four farms, lettuce was the main crop grown continuously during the year with several different cultivars produced.

Table 3-1 The four hydroponic lettuce farms surveyed in this study, including location, seedling sources and water source for nutrient solution.

Farm	Location	Seedlings	Water for nutrient solution
Manicaro's Farm (L1)	216 Rickard Road, Leppington NSW (South-west Sydney)	Bought from various suppliers	Town water
Camilleri's Farm (L2)	700 15th Avenue, Leppington NSW (South-west Sydney)	Bought from various suppliers	Town water
Borg Hydroponics (CC1)	77 Sparks Road, Warnervale NSW (Central Coast)	Produced own seedlings	Town water
Pacific Hydroponics (CC2)	The Grove 120 Pacific Highway, Lake Munmorah NSW (Central Coast)	Produced own seedlings	Town water (and dam water sometimes)

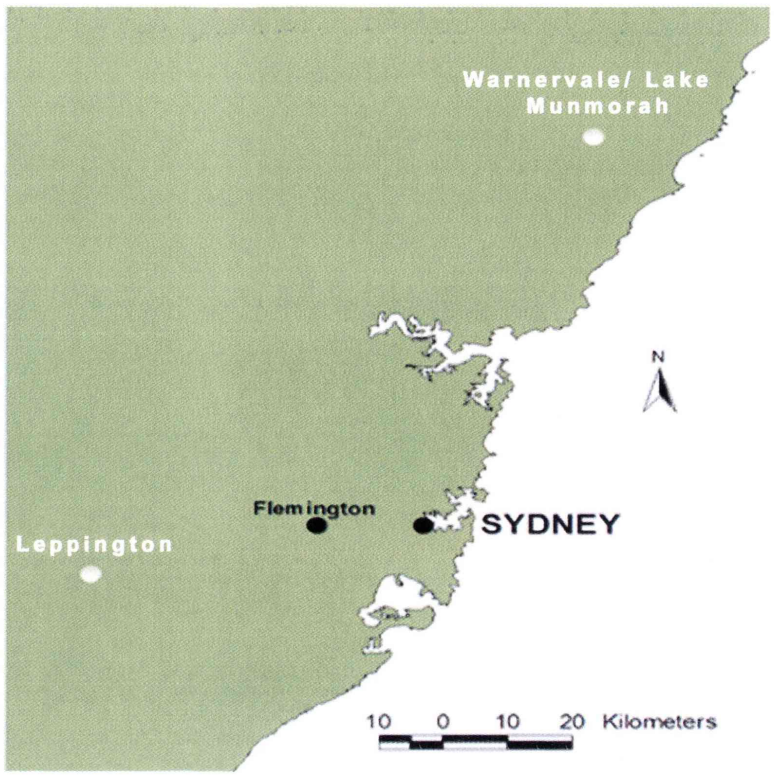

Figure 3-1 Map of Sydney area and Central Coast of NSW. Two farms were surveyed in the Leppington area (South-west Sydney) and two at Warnervale/ Lake Munmorah area (Central Coast). The main fruit and vegetable market is located at Flemington not far from the Sydney CBD.

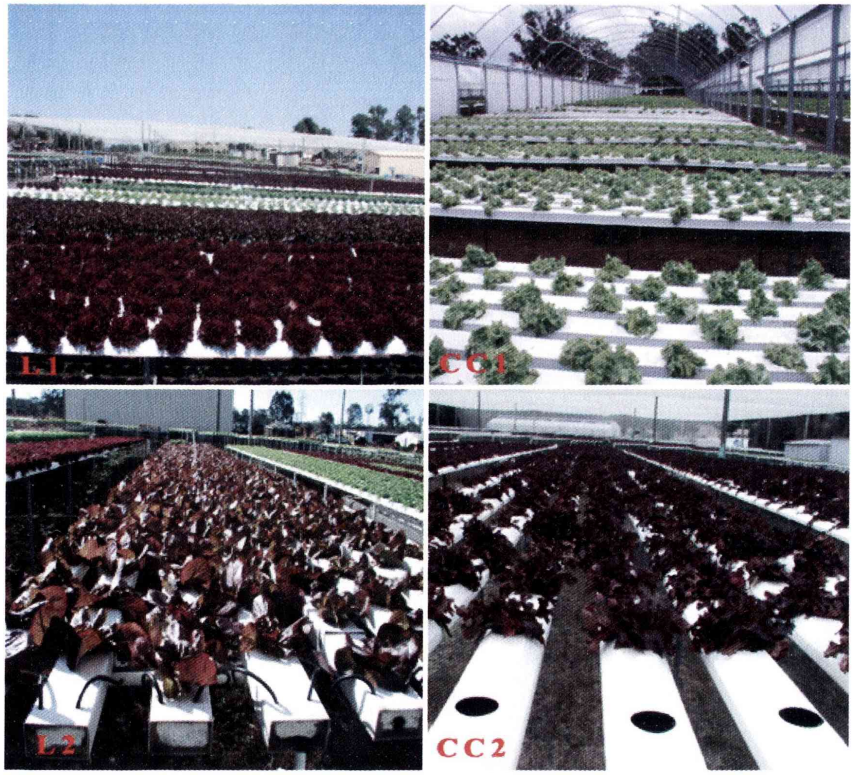

Figure 3-2 The four hydroponic lettuce farms surveyed in this study.
(L1) Manicaro's Farm (Leppington 1)
(L2) Camilleri's Farm (Leppington 2)
(CC1) Borg Hydroponics (Central Coast 1)
(CC2) Pacific Hydroponics (Central Coast 2)
(See Table 3-1 for further details.)

Mature lettuces (with 15-25 leaves) of four cultivars (Brown Mignonette, Baby Cos, Green Oak and Red Oak) (Figure 3-3) were surveyed five times during 2004-2005 to cover a range of seasons i.e. in May 2004, August 2004, November 2004, January 2005 and March 2005. The temperature of the nutrient solution in the growing channels was measured on each survey day. Additional information on air temperatures (daily maxima and mean monthly maxima) were obtained from the Bureau of Meteorology, NSW (pers. comm.).

For each cultivar, disease severity ratings on roots were assessed in 30 randomly selected plants. A scale of 1 to 4 was used to assess disease severity (Figure 3-4) as follows:

1. healthy white roots;
2. generally healthy white roots, but with some brown colouration;
3. unhealthy roots, with most roots brown in colour; and
4. dead roots and/or black roots.

For each cultivar, symptoms on leaves were assessed on 100 randomly chosen plants. Four categories of plant symptoms were used for leaf symptoms assessment (Figure 3-5):

1. plant with healthy leaves and neither wilting nor dead leaves apparent;
2. plant with small leaves and/or signs of wilting;
3. dead plant; and
4. no plant present (empty planting hole); the plant may have died and been removed by the farmer or, less likely, the planting hole was overlooked at planting time.

Figure 3-3 The four lettuce cultivars surveyed and sampled.
(a) Brown Mignonette **(b) Baby Cos**
(c) Green Oak **(d) Red Oak.**

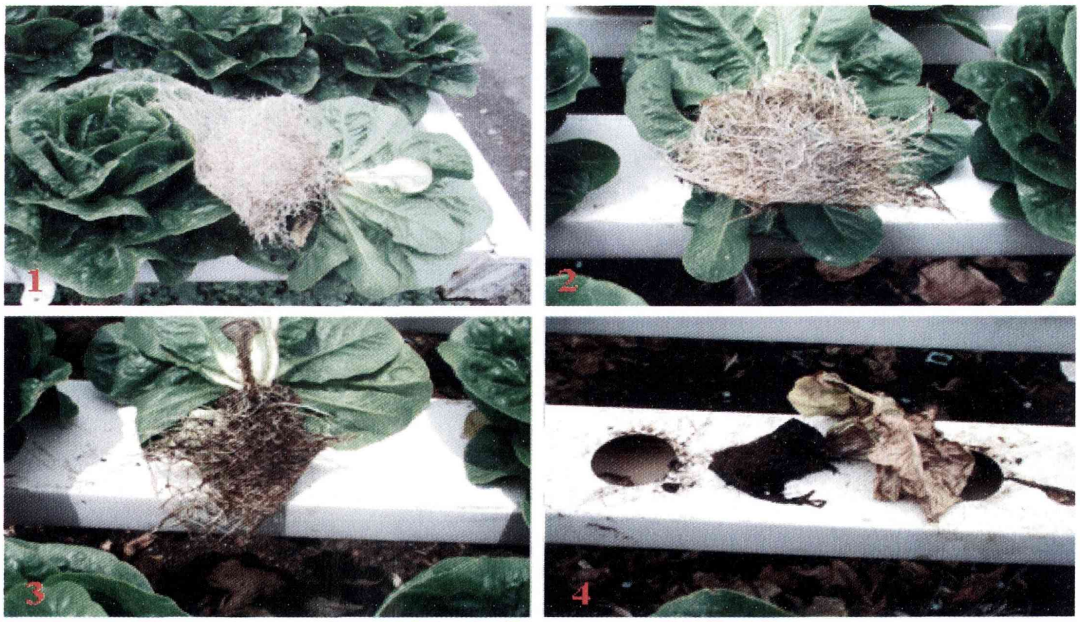

Figure 3-4 Scale used to assess root rot disease severity on hydroponic lettuce roots:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour;
(4) dead roots and/or black roots.

Figure 3-5 Scale of one to four used to assess disease symptoms on lettuce leaves affected by root rot pathogens:
(1) plant with healthy leaves and neither wilting nor dead leaves apparent;
(2) plant with small leaves and/or signs of wilting;
(3) dead plant; and
(4) no plant present (empty planting hole).

3.2.2 Isolation and identification of oomycetes from lettuce roots

Mature lettuce plants were collected five times from the same four farms where the survey was done at five times during the year: May 2004, August 2004, November 2004, January 2005 and March 2005. Four lettuce cultivars (Brown Mignonette, Baby Cos, Green Oak, Red Oak) were sampled to isolate *Pythium* and *Phytophthora* from their roots. The cultivar Brown Mignonette, however, was not available in November 2004, January 2005 and March 2005 at the second Central Coast farm (CC2). Five lettuces were randomly sampled for each cultivar. The root systems of a total of 385 lettuces were examined during the course of this survey. Samples were placed separately in plastic bags and kept at 4°C until isolations were carried out at the plant pathology laboratory of the Botanic Gardens Trust, Sydney (BGT), normally within 24 hours.

Root systems were washed under running tap water for 5 minutes. Ten root pieces from each plant were excised and plated onto *Pythium* selective agar (PYSA) and *Phytophthora* selective agar (PSA) in 90 mm Petri dishes (Appendix 1). Petri dishes were incubated at the recommended environmental conditions in the keys used for identification of each genus. Petri dishes containing *Pythium* selective agar and

identification of each genus. Petri dishes containing *Pythium* selective agar and *Phytophthora* selective agar were incubated in the dark at room temperature (25°C ± 1°C) (van der Plaats-Niterink 1981 and Stamps *et al.* 1990, respectively).

All cultures were incubated for two days. All root pieces (200/ cultivar/ sampling time) were then examined microscopically (Olympus CX41RF, Olympus Optical, Philippines) for *Pythium* and *Phytophthora* growth. All growth found was transferred to Potato Carrot Agar (PCA) media (Appendix 1). All cultures were incubated in the dark at 25°C ± 1°C for 5-7 days. Keys for *Pythium* (van der Plaats-Niterink 1981) and *Phytophthora* (Stamps *et al.* 1990) were used to identify the genus and species where possible.

3.2.3 Pathogenicity tests

Pathogenicity tests were carried out to confirm Koch's Postulates for isolates of *Pythium* spp. that were different from those isolated in the previous survey (see Chapter 2). These differences were confirmed by using morphological features. One *Pythium* sp. was found to be different from the previous isolates. No pathogenicity tests were carried out for *Phytophthora* isolates since all were determined to be the same as the *Phytophthora* sp. isolated in the previous survey (see Chapter 6).

3.2.3.1 Hydroponic units

Four independent recirculating experimental hydroponic units were set up in a non-temperature-controlled glasshouse in the Botanic Gardens Trust, Sydney (BGT), supplied by a commercial hydroponic supplier (Quik Grow Hydroponic Specialists, 672 Parramatta Road, Croydon NSW). Each hydroponic unit contained a covered, rectangular, white plastic channel (250 cm long × 16 cm wide × 7 cm deep) with 10 holes (5 cm diameter and 20 cm apart). The upper end of the channel was raised approximately 5 cm above the lower end, to allow nutrient flow in the channel under gravity.

Each channel was supplied with nutrient solution from a 50 L black plastic tank (60 cm long × 40 cm wide × 27 cm deep) which contained 40 L of nutrient solution (Quik Grow Hydroponic Specialists, 672 Parramatta Road, Croydon NSW; see Appendix 2 for details of chemical composition). Two channels were placed on each of two tables. The

two tanks supplying two channels on one table were placed under the table and at opposite ends to reduce the likelihood of cross infection between units.

Different sizes of black plastic poly-pipe (6, 13, 19 or 32 mm) were used to transfer nutrient solution from the tank to the upper end of the channel then back to the tank from the lower end of the channel. A submersible pump (SunSun HJ-1502, output 1500 L/h, Zhejiang Sensen Industry Co., Ltd, China, supplied by Quik Grow Hydroponic Specialists, 672 Parramatta Road, Croydon NSW) was used in each unit to pump the nutrient solution to the upper end of the channel.

3.2.3.2 Plant materials

Seeds of Brown Mignonette lettuce (Terranova Seeds Pty Ltd, 13/19 Chifley Street, Smithfield NSW) were sown in a seedling tray (53 cm long × 27 cm wide, with 98 square planting holes, each hole being 3.5 cm wide × 6 cm deep), to produce individual lettuce seedlings. The potting mix contained two parts composted pine bark fines (Australian Native Landscape Supplies, 317 Mona Vale Rd, Terrey Hills NSW) and one part crushed quartz sand (Cable Sand, 12 Carmichael Street, Raymond Terrace NSW). Seedlings were grown for 6 weeks before being transplanted into the hydroponic system. During these 6 weeks, the seedlings were watered daily via sprayers and fertilised once a week with the same nutrient solution as used in the hydroponic system. Seedlings were produced in the same glasshouse as was used for the hydroponic system.

3.2.3.3 Inoculation

Pythium inoculum was produced as follows. Five-day-old *Pythium* cultures growing on PCA at 25°C ± 1°C in the dark were flooded with 20 mL sterile distilled water and incubated at 4°C for two hours, then kept at room temperature overnight. Zoospores, oospores and/or sporangia were counted using a haemocytometer slide. Three of the four nutrient solution tanks were inoculated with a suspension of zoospores, oospores and sporangia to give a final concentration 500–1000 zoospores, oospores and/or sporangia per mL (Funck-Jensen & Hockenhull 1983). The fourth nutrient solution tank was not inoculated and this hydroponic unit served as a control, receiving an equivalent amount of sterile distilled water.

3.2.3.4 Data collection

Root rot disease symptoms were assessed by using the same scale of one to four (Figure 3-4) used to assess root rot disease at the farms. Assessments were made at 3, 6, 9, 12 and 14 days after the start of the pathogenicity test. After 14 days, reisolation of the pathogen tested was attempted from the root systems of four plants from each of the four hydroponic units (three inoculated units and one control unit). Wet weight and dry weight of shoots and roots were obtained for all plants after 14 days.

Thermometers were used to record daily minimum and maximum nutrient solution temperature. Nutrient solution pH was tested every three days by taking three samples of 10 mL nutrient solution from each tank and measured by using a pH meter (Basic pH Meter, Denver Instrument Company, Colorado, USA).

At the conclusion of the pathogenicity test, approximately 250 mL of chlorine (2%) was added to the nutrient solution and pumped into the system for 10 min. Then all surfaces were cleaned with chlorine and left to dry for one week or more.

3.2.4 Data analysis

Results from root rot disease severity assessments and leaf symptom assessments were summarised by calculating a disease index (DI) for each treatment using the following formula (Merrin 1998):

$DI = [(n4*4) + (n3*3) + (n2*2) + n1] / Tn$

Where n4 = number of lettuces rated 4

n3 = number of lettuces rated 3

n2 = number of lettuces rated 2

n1 = number of lettuces rated 1

Tn = total number of lettuces examined in each treatment

The DI was used for graphical representation of results.

General Linear Model (GLM) ANOVA (MiniTab VER 13) was used to find the differences ($P \leq 0.05$) between the four categories of both scales (root rot disease severity scale and leaf symptom scale). Moreover, GLM ANOVA was used to find the differences ($P \leq 0.05$) between inoculated and non-inoculated (control) plants as regards the mean wet weight and mean dry weight for shoots and roots from the pathogenicity

tests. Heteroscedastic data were transformed as required using log or square root transformations.

Microsoft Excel XP 2003 was used to prepare graphs and tables.

3.3 Results

3.3.1 Root rot disease survey

Mean monthly maximum air temperatures provided by the Bureau of Meteorology, NSW showed that the air temperature in the Leppington area was always higher than the air temperature in the Central Coast area (Figure 3-6). Mean monthly maximum air temperatures in May 2004 and August 2004 were less than 21°C for both the Leppington and Central Coast areas while mean monthly maximum air temperatures in November 2004, January 2005 and March 2004 were greater than 22°C for Leppington and greater than 21°C for the Central Coast (Figure 3-6). For the five survey times, the highest mean monthly maximum temperature occurred in January 2005, while the lowest occurred in August 2004. The measurement of the nutrient solution temperature on the survey day showed that the lowest temperatures were recorded in cooler season samples (May 2004 (15-16°C) and August 2004 (14-15°C)) compared with temperatures recorded in warmer seasons (November 2004 (21-22°C), January 2005 (23-29°C) and March 2005 (20-27°C)).

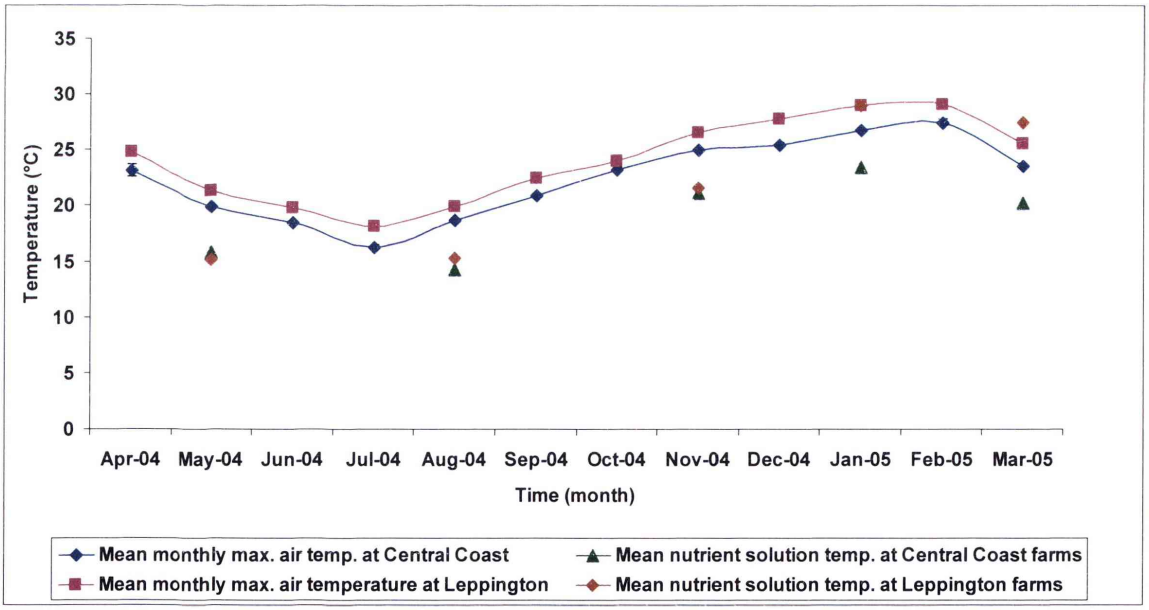

Figure 3-6 Mean monthly maximum air temperature in the Leppington area (station 67020) and Central Coast area (station 61351) of NSW from April 2004 to March 2005 (Bureau of Meteorology) and mean hydroponic nutrient solution temperature on five survey days from the both farms in the two areas.

Statistical analysis indicated significant differences between the five sample times as regards both root rot disease severity (P=0.00) (Appendix 4 a) and leaf symptom assessment (P=0.00) (Appendix 4 a) as well as between the four farms in both root disease (P=0.00) (Appendix 4 a) and leaf symptoms (P=0.00) (Appendix 4 a). However,

there were no significant differences between the four lettuce cultivars examined in root rot disease severity (P=0.06) and leaf symptom assessment (P=0.84) (Appendix 4 a).

3.3.1.1 Baby Cos

Baby Cos showed the lowest root rot disease severity (generally rated 1-2) in May 2004 and August 2004 samples from all farms, when the nutrient solution temperature was also lowest (13.5 to 17°C) (Figure 3-7). The highest root rot disease severity (> 2, with many rated 3-4) was found in November 2004, January 2005 and March 2005 samples from all farms when the nutrient solution temperature was also highest (20-30°C) (Figure 3-7).

Generally, roots of lettuces surveyed in cooler sample times (May 2004 and August 2004) appeared to be significantly healthier than roots of lettuces surveyed in warmer sample times (November 2004, January 2005 and March 2005) (Table 3-2; Figure 3-7; Appendix 4 b 1). In three of the four farms, no significant differences were found between root disease assessments made at the cooler sample times (May 2004 and August 2004) (Table 3-2). Moreover, there were generally no significant differences between root disease assessments made in November 2004 in comparison with those made in both January 2005 and March 2005. Root rot assessment of Baby Cos was found to be significantly different among the four farms surveyed (P=0.00) (Appendix 4 b 1). In relation to the two areas surveyed (Leppington and Central Coast areas), roots of lettuces in Leppington farms appeared to be healthier than roots of lettuces in Central Coast farms (P=0.00) (Appendix 4 b 1).

Generally, leaves of Baby Cos appeared healthy in both cooler and warmer sample times with ratings of 1-2 (Figure 3-8). However, leaf symptom assessment at the January 2005 survey time, when the nutrient solution temperature was 23-30°C, was often significantly greater than leaf symptom assessment at other survey times (Table 3-3; Figure 3-8). No significant differences were found in leaf symptom assessment between the two cooler survey times (May 2004 and August 2004) (Table 3-3) when the nutrient solution temperature was 13.5-17°C (Figure 3-8). Significant differences in leaf assessment were found among the four farms surveyed (P=0.00) (Appendix 4 b 2). Leaves of Baby Cos appeared healthier in Leppington farms than in Central Coast farms (P=0.00) (Appendix 4 b 2).

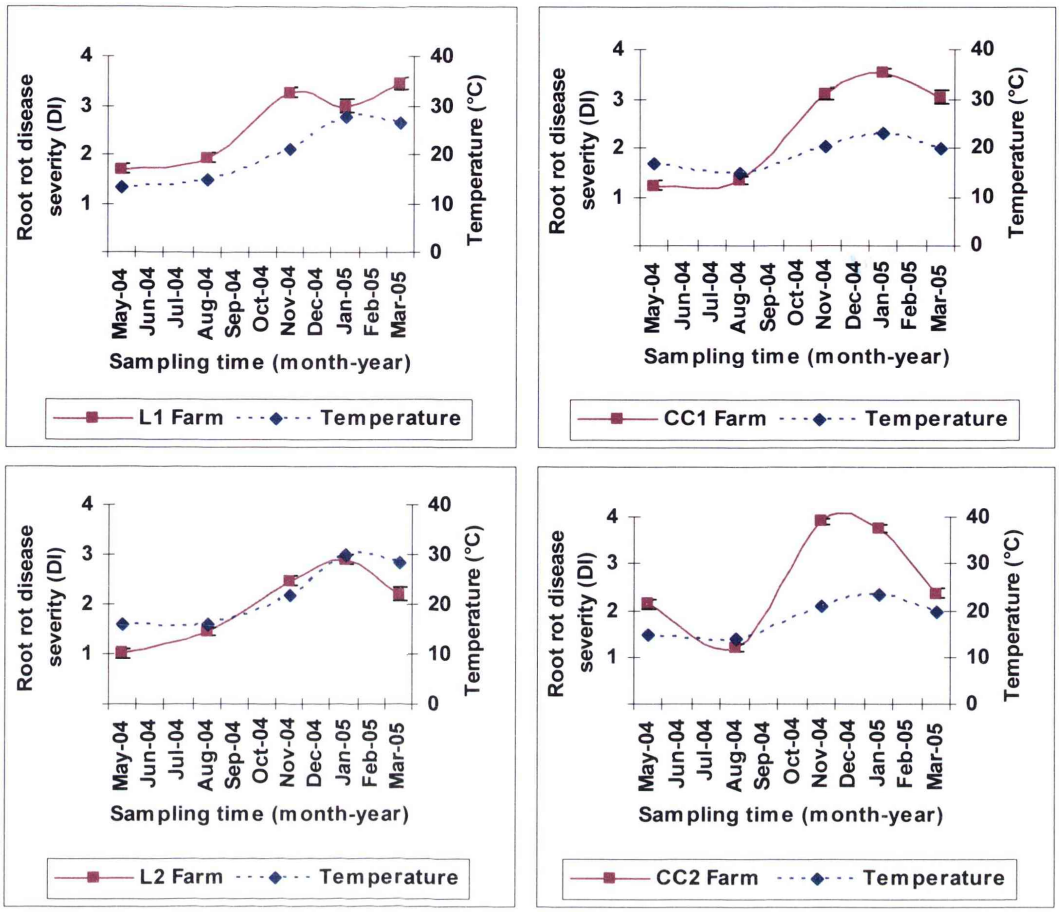

Figure 3-7 Disease severity on roots of Baby Cos lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour; and
(4) dead roots and/or black roots.
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-2 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Baby Cos at four farms. (Appendix 4 b 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.53
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	0.68	0.40		
Jan-05	**0.02**			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.96
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	0.99	**0.03**		
Jan-05	**0.01**			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.15
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	0.62	0.15		
Jan-05	**0.00**			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.30	**0.00**	**0.00**	**0.00**
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	**0.00**	0.80		
Jan-05	**0.00**			

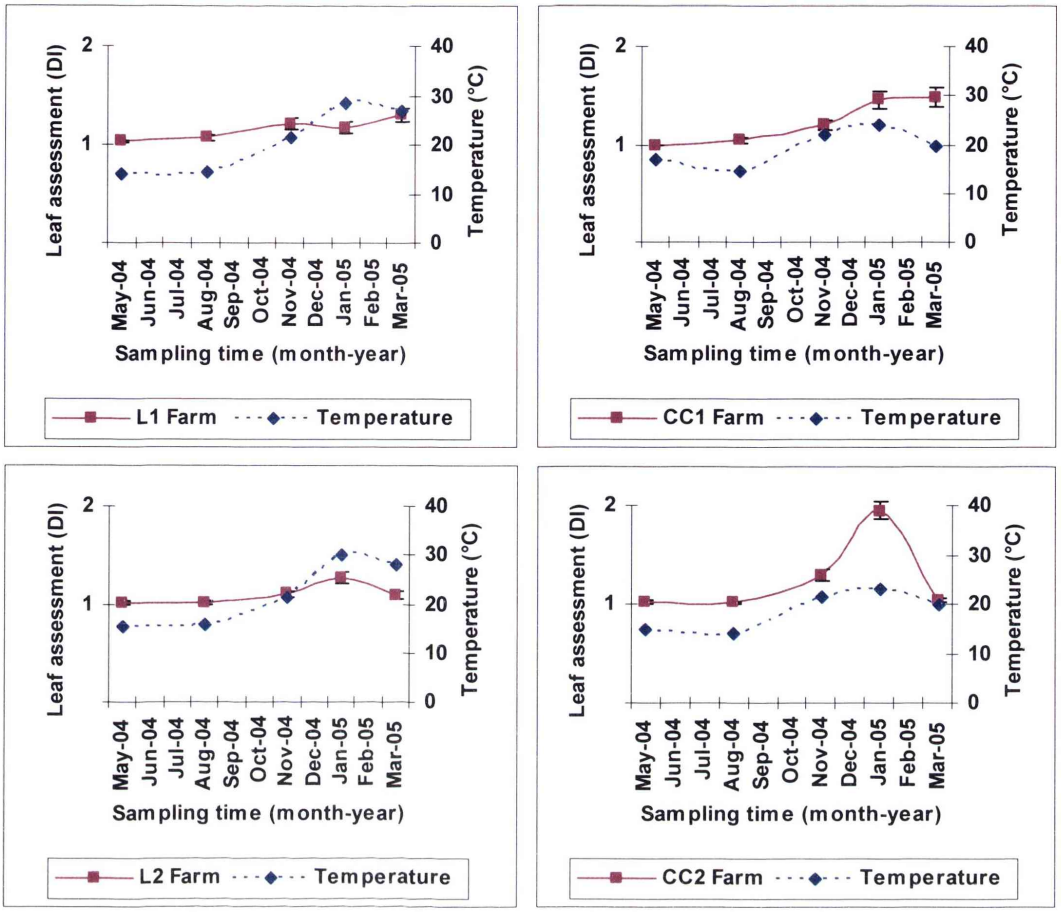

Figure 3-8 Leaf symptom assessment for Baby Cos lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) plant with healthy leaves;
(2) plant with small leaves and/or signs of wilting;
(3) dead plant; and
(4) no plant present (empty planting hole).
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-3 *P* values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Baby Cos at four farms. (Appendix 4 b 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	0.19	**0.05**	0.98
Aug-04	**0.00**	0.51	0.19	
Nov-04	0.69	0.98		
Jan-05	0.33			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	0.18	0.99
Aug-04	**0.00**	**0.00**	0.39	
Nov-04	**0.01**	**0.03**		
Jan-05	1.00			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.49	**0.00**	0.37	1.00
Aug-04	0.49	**0.00**	0.37	
Nov-04	1.00	**0.01**		
Jan-05	**0.00**			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	1.00	**0.00**	**0.00**	1.00
Aug-04	0.99	**0.00**	**0.00**	
Nov-04	**0.00**	**0.00**		
Jan-05	**0.00**			

3.3.1.2 Red Oak

The lowest root rot disease severity in the lettuce cultivar Red Oak was recorded in May 2004 and August 2004 samples from all farms, when the nutrient solution temperature was also lowest (14-17°C) (Figure 3-9). These ratings were generally 1-2. The highest root rot disease severity was recorded in November 2004, January 2005 and March 2005 samples, when the nutrient solution temperature was also highest (20-30°C) (Figure 3-9). These ratings were generally greater than 2, with many rated 3-4.

Roots of Red Oak appeared to be significantly healthier in the May 2004 and August 2004 samples than in the warmer survey times (November 2004, January 2005 and March 2005) (Table 3-4; Figure 3-9; Appendix 4 c 1). Generally, roots of Red Oak in May 2004 and August 2004 were not significantly different in disease assessment (Table 3-4). As well, root disease assessments in the November 2004 and January 2005 samples were not significantly different (Table 3-4). However, root disease in the March 2005 samples was significantly different from the other two warm temperature samples (Table 3-4); root disease was significantly greater at the Leppington farms but significantly less at the Central Coast farms (Figure 3-9). Root assessment shows significant differences among the four farms surveyed (P=0.00) (Appendix 4 c 1). In relation to the two areas surveyed (Leppington and Central Coast areas), no significant differences were found in root rot assessment between the two areas surveyed (P=0.26) (Appendix 4 c 1).

Leaves of the lettuce cultivar Red Oak generally appeared healthy at both the cooler and warmer survey times with ratings of 1-2 (Figure 3-10). However, for the Central Coast farms, leaves in the November 2004 and January 2005 survey times often appeared to show more symptoms compared with the May 2004 and August 2004 survey times (Figure 3-10; Table 3-5; Appendix 4 c 2). Leaves of Red Oak, however, appeared to be similar in May 2004 and August 2004 (Table 3-5). Among the four farms surveyed, significant differences in leaf assessment were found (P=0.00) (Appendix 4 c 2). No significant differences were found in leaf assessment between the two areas surveyed (Leppington and Central Coast areas) (P=0.19) (Appendix 4 c 2).

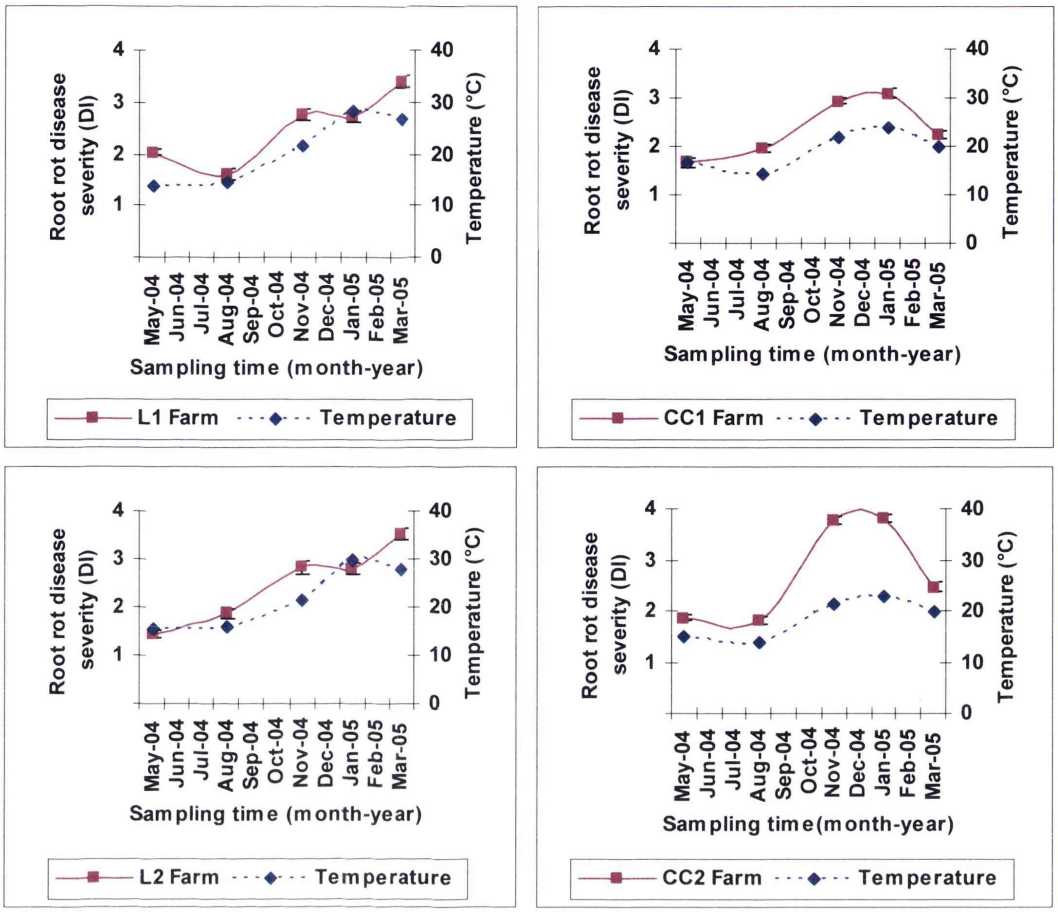

Figure 3-9 Disease severity on roots of Red Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour; and
(4) dead roots and/or black roots.
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-4 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Red Oak at four farms. (Appendix 4 c 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	**0.04**
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	**0.00**	1.00		
Jan-05	**0.00**			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.10
Aug-04	0.18	**0.00**	**0.00**	
Nov-04	**0.00**	0.64		
Jan-05	**0.00**			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.08
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	**0.00**	1.00		
Jan-05	**0.00**			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.97
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	**0.00**	1.00		
Jan-05	**0.00**			

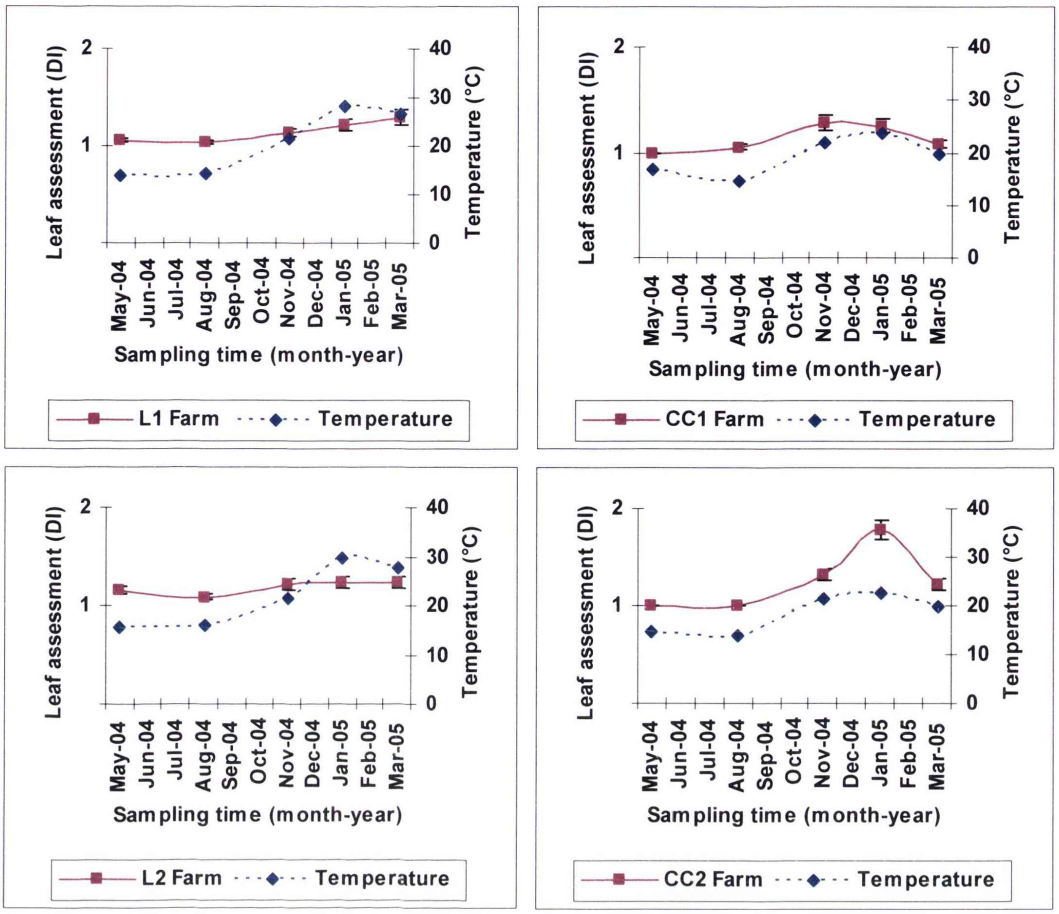

Figure 3-10 Leaf symptom assessment for Red Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) plant with healthy leaves;
(2) plant with small leaves and/or signs of wilting;
(3) dead plant; and
(4) no plant present (empty planting hole).
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-5 *P* values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Red Oak at four farms. (Appendix 4 c 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	0.11	0.74	1.00
Aug-04	**0.00**	**0.05**	0.55	
Nov-04	0.11	0.74		
Jan-05	0.74			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.76	**0.00**	**0.00**	0.95
Aug-04	0.99	**0.03**	**0.01**	
Nov-04	**0.03**	0.99		
Jan-05	0.09			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.87	0.81	0.96	0.81
Aug-04	0.24	0.18	0.38	
Nov-04	1.00	0.99		
Jan-05	1.00			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.03**	**0.00**	**0.00**	1.00
Aug-04	**0.03**	**0.00**	**0.00**	
Nov-04	0.67	**0.00**		
Jan-05	**0.00**			

3.3.1.3 Green Oak

The lowest root rot disease severity in the lettuce cultivar Green Oak was reported in the May 2004 and August 2004 samples from all farms, when the nutrient solution temperature was also lowest (14-18°C) (Figure 3-11). These ratings were generally 1-2, but slightly higher (>2) for the Central Coast 2 farm. The highest root rot disease severity was recorded in November 2004, January 2005 and March 2005 with samples rated higher than 2, with many rated 3-4. At these times, the nutrient solution temperature was also highest (20-30°C) (Figure 3-11).

Roots of the lettuce cultivar Green Oak appeared significantly healthier in May 2004 and August 2004 compared with November 2004, January 2005 and sometimes March 2005 (Table 3-6; Appendix 4 d 1). Significant differences in root rot assessment were found among the four farms surveyed (P=0.00) (Appendix 4 d 1). In relation to the two areas surveyed (Leppington and Central Coast), roots of Green Oak in the Leppington farms appeared healthier than roots of Green Oak in the Central Coast farms (P=0.00) (Appendix 4 d 1).

Generally, leaves of Green Oak appeared healthy in both cooler and warmer samples, with ratings of 1-2 (Figure 3-12). However, for all four farms, leaf symptom assessment in January 2005 appeared to be significantly greater than that in November 2004, which was another warm-season survey time (Table 3-7; Figure 3-11; Appendix 4 d 2). No significant differences were found in leaf assessment between the cooler survey times (May 2004 and August 2004) (Table 3-7) when the nutrient solution temperature was 14-18°C (Figure 3-11). Significant differences in leaf assessment were found among the four farms surveyed (P=0.00) (Appendix 4 d 2). Leaves of Green Oak appeared healthier at Leppington farms than at farms at the Central Coast (P=0.00) (Appendix 4 d 2).

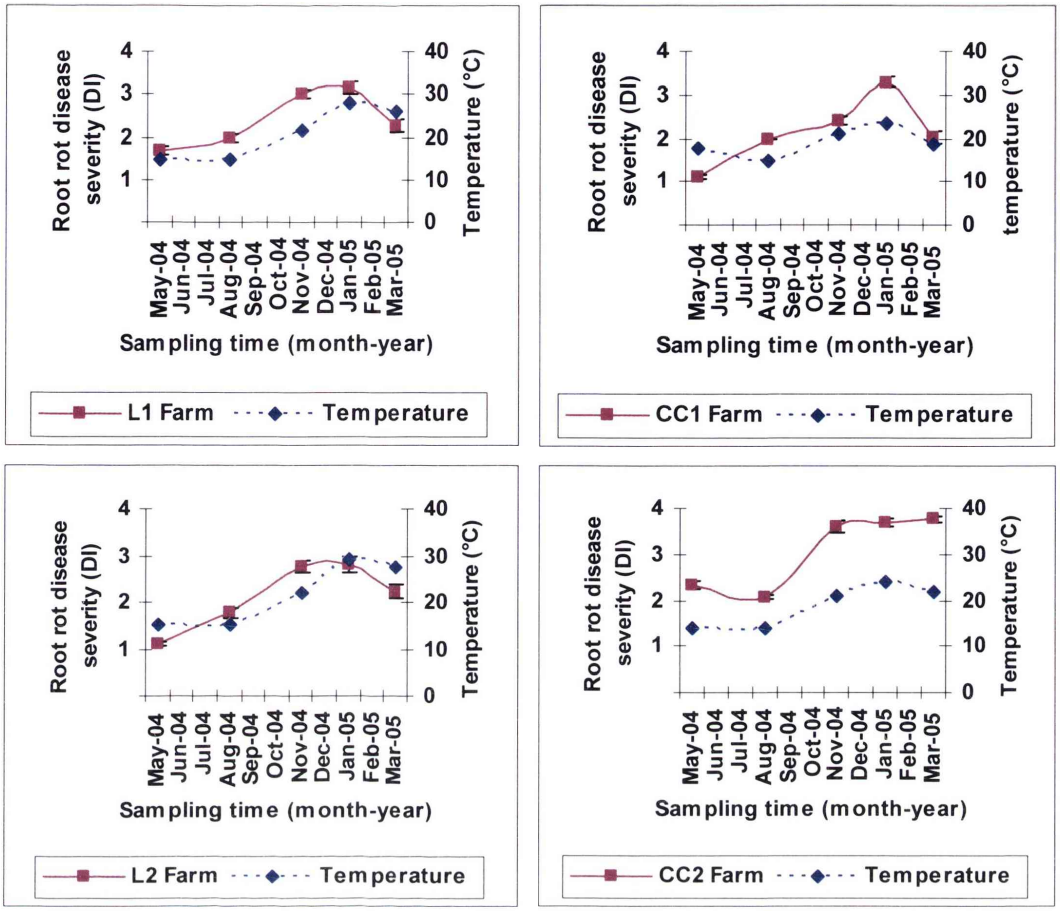

Figure 3-11 Disease severity on roots of Green Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour; and
(4) dead roots and/or black roots.
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-6 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Green Oak at four farms. (Appendix 4 d 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.37
Aug-04	0.37	**0.00**	**0.00**	
Nov-04	**0.00**	0.85		
Jan-05	**0.00**			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	**0.00**
Aug-04	1.00	**0.00**	**0.03**	
Nov-04	**0.05**	**0.00**		
Jan-05	**0.00**			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	**0.00**
Aug-04	0.10	**0.00**	**0.00**	
Nov-04	**0.04**	1.00		
Jan-05	**0.02**			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.20
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	0.66	0.93		
Jan-05	0.98			

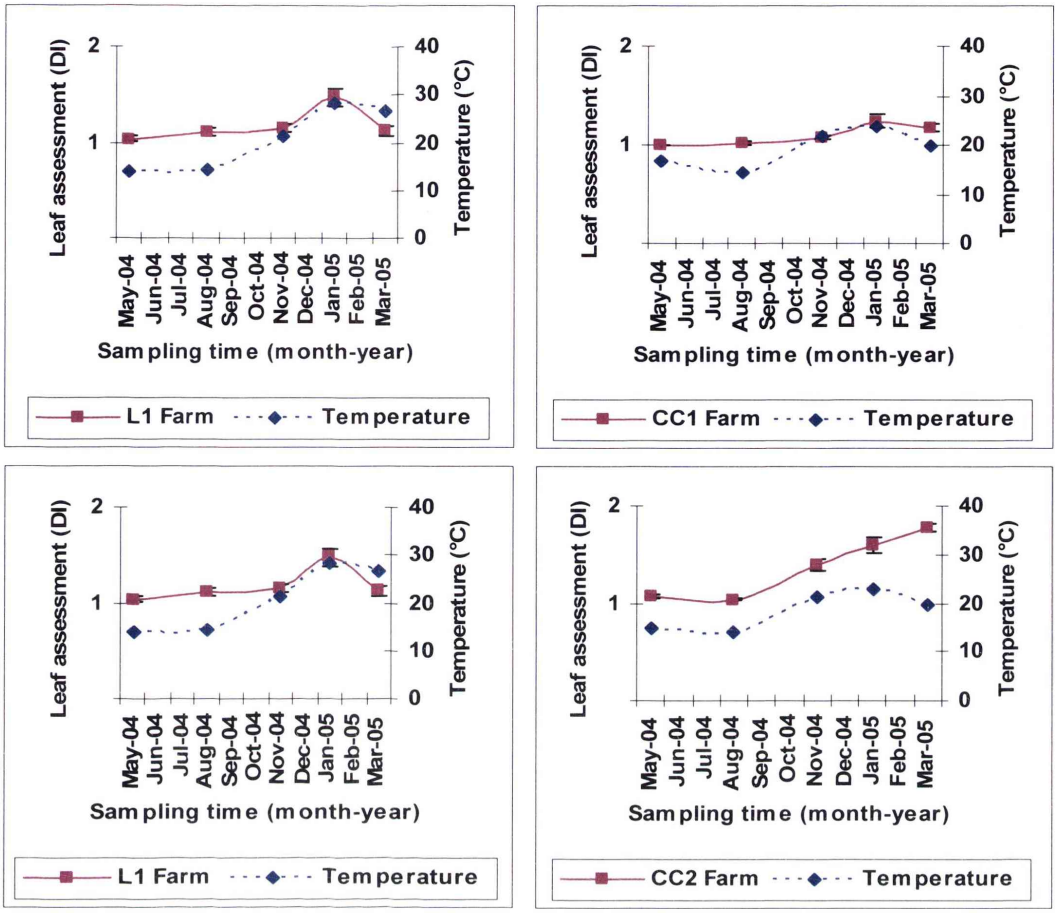

Figure 3-12 Leaf symptom assessment for Green Oak lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) plant with healthy leaves;
(2) plant with small leaves and/or signs of wilting;
(3) dead plant; and
(4) no plant present (empty planting hole).
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-7 *P* values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Green Oak at four farms. (Appendix 4 d 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.77	**0.00**	0.52	0.89
Aug-04	1.00	**0.00**	0.97	
Nov-04	1.00	**0.00**		
Jan-05	**0.00**			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	0.53	1.00
Aug-04	**0.02**	**0.00**	0.77	
Nov-04	0.30	**0.02**		
Jan-05	0.77			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.25	**0.00**	0.33	1.00
Aug-04	0.43	**0.00**	0.54	
Nov-04	1.00	**0.01**		
Jan-05	**0.01**			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	0.99
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	**0.00**	**0.04**		
Jan-05	0.11			

3.3.1.4 Brown Mignonette

Brown Mignonette showed the lowest root rot disease severity (generally rated 1-2) in the May 2004 and August 2004 samples from the three farms with complete data sets; this was when the nutrient solution temperature was also lowest (14-17°C) (Figure 3-13). The highest root rot disease severity (>2, with many rated 3-4) was found in November 2004, January 2005 and March 2005 samples from these three farms when the nutrient solution temperature was also highest (20-30°C) (Figure 3-13).

Generally, roots of lettuces surveyed in cooler sample times (May 2004 and August 2004) appeared to be significantly healthier than roots of lettuces surveyed in warmer times (November 2004, January 2005 and March 2005) (Table 3-8; Figure 3-13; Appendix 4 e 1). Moreover, commonly there were no significant differences in root disease severity between the warmer sample times (November 2004, January 2005 and March 2005) (Table 3-8). Root disease severity of Brown Mignonette was found to be not significantly different among the four farms surveyed (P=0.79) (Appendix 4 e 1). In relation to the two areas surveyed (Leppington and Central Coast areas), root disease severity in Brown Mignonette was found to be similar in both areas (P=0.19) (Appendix 4 e 1).

For the three farms with complete data sets, leaves of Brown Mignonette generally appeared healthy in both cooler and warmer samples with ratings of 1-2 (Figure 3-14). Leaf symptom assessments in the warmer survey times of November 2004, January 2005 and March 2005 appeared to be similar; at these times, the nutrient solution temperature was 20-30°C (Table 3-9; Appendix 4 e 2). Moreover, generally no significant differences were found in leaf symptom assessment between the cooler survey times (May 2004 and August 2004) (Table 3-9) when the nutrient solution temperature was 13.5-17°C. When comparisons of leaf symptom assessment were made between different survey times, symptoms were often significantly greater in the warmer survey times than in the cooler survey times (Table 3-9; Figure 3-14). Significant differences in leaf assessment were found among the four farms surveyed (P=0.00) (Appendix 4 e 2). Leaves of Brown Mignonette appeared to be similar between the two areas (Leppington and Central Coast) surveyed (P=0.18) (Appendix 4 e 2).

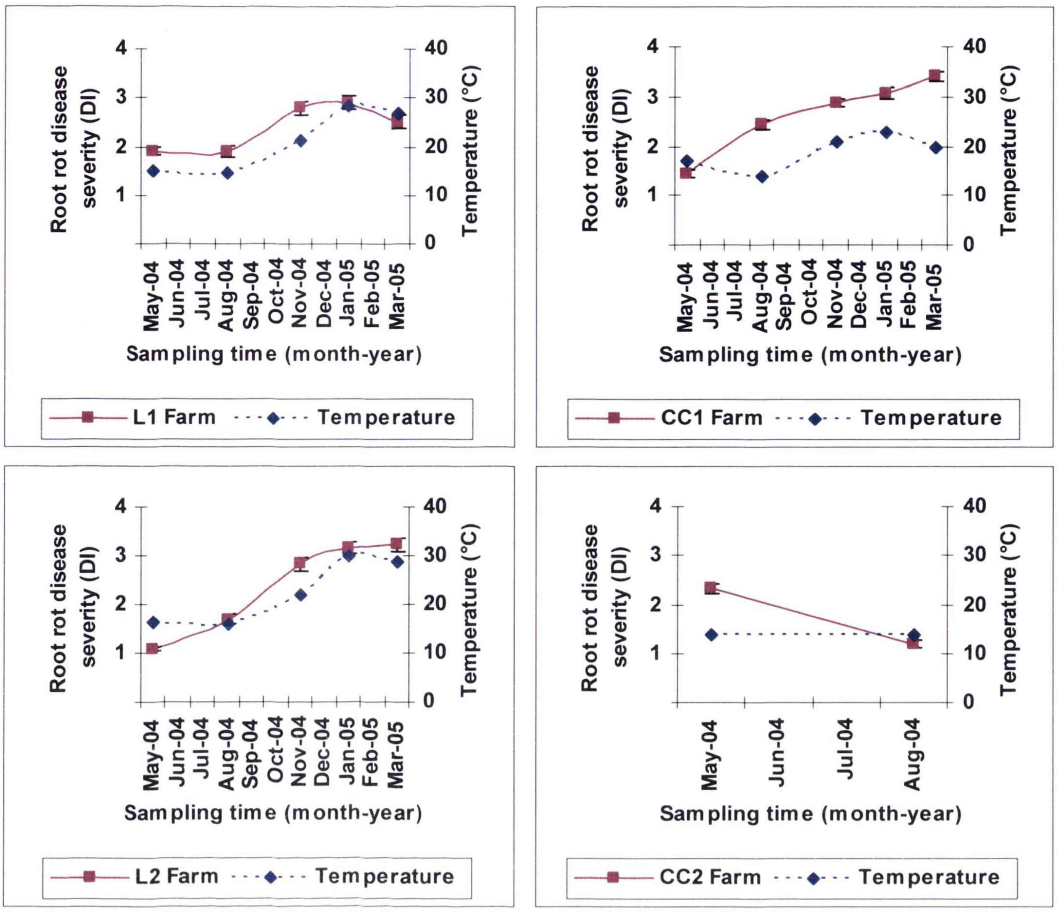

Figure 3-13 Disease severity on roots of Brown Mignonette lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) healthy white roots;
(2) generally healthy white roots, but with some brown colouration;
(3) unhealthy roots, with most roots brown in colour; and
(4) dead roots and/or black roots.
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-8 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between survey times, for the lettuce cultivar Brown Mignonette at four farms. (Appendix 4 e 1.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.01**	**0.00**	**0.00**	1.00
Aug-04	**0.01**	**0.00**	**0.00**	
Nov-04	0.43	0.98		
Jan-05	0.15			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	**0.00**
Aug-04	**0.00**	**0.00**	**0.02**	
Nov-04	**0.00**	0.62		
Jan-05	0.13			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.00**	**0.00**
Aug-04	**0.00**	**0.00**	**0.00**	
Nov-04	0.13	0.28		
Jan-05	0.99			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04				**0.00**
Aug-04				
Nov-04				
Jan-05				

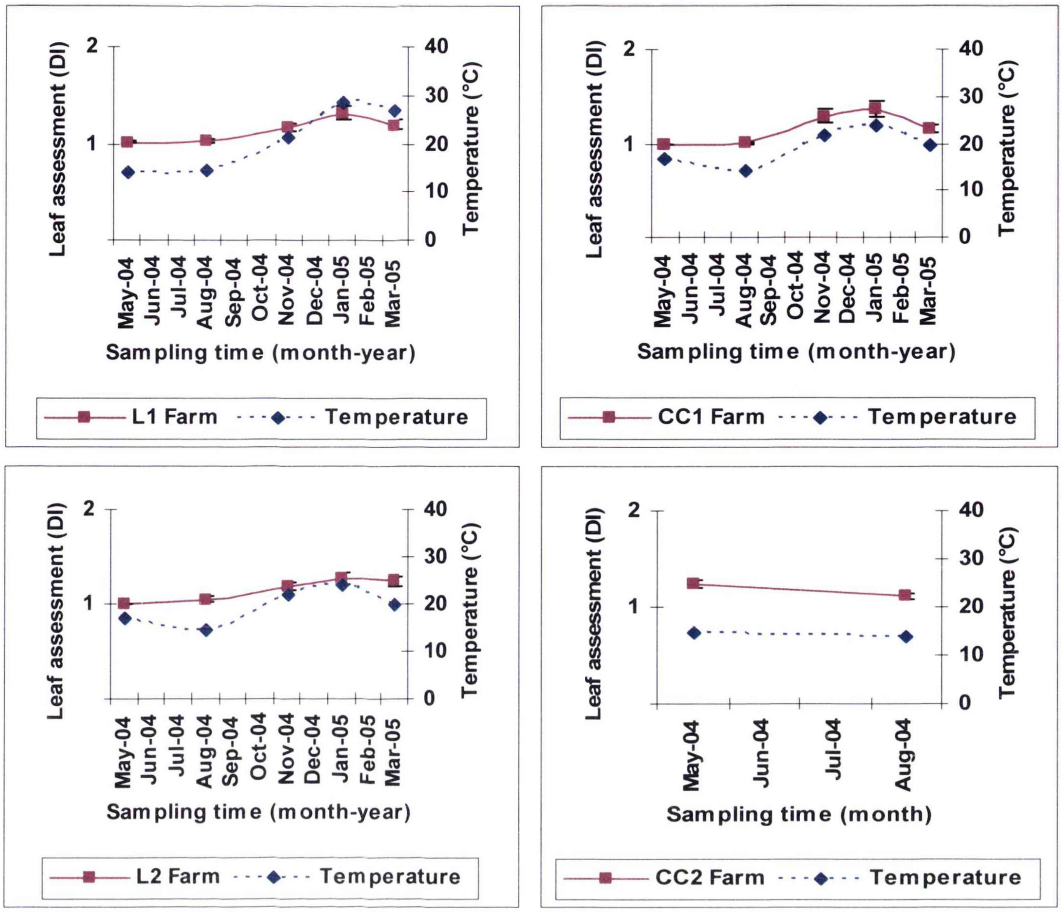

Figure 3-14 Leaf symptom assessment for Brown Mignonette lettuce cultivar from the four farms surveyed five times during the 11 month period as indicated by disease index (DI) based on a scale of 1 to 4 as follows:
(1) plant with healthy leaves;
(2) plant with small leaves and/or signs of wilting;
(3) dead plant; and
(4) no plant present (empty planting hole).
Temperature of the nutrient solution on the survey day was measured. Error bars are the standard error of the mean.

Table 3-9 *P* values ($P \leq 0.05$ in bold) of the mean differences in leaf symptom assessment between survey times, for the lettuce cultivar Brown Mignonette at four farms. (Appendix 4 e 2.) Shaded areas represent comparisons between cool (May-Aug) and warm (Nov-Jan-Mar) times.

L1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.03**	**0.00**	0.11	1.00
Aug-04	**0.05**	**0.00**	0.16	
Nov-04	0.99	0.11		
Jan-05	0.30			

CC1 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	0.25	**0.00**	**0.00**	1.00
Aug-04	0.31	**0.00**	**0.00**	
Nov-04	0.46	0.85		
Jan-05	0.06			

L2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04	**0.00**	**0.00**	**0.04**	0.94
Aug-04	**0.03**	**0.01**	0.25	
Nov-04	0.88	0.62		
Jan-05	0.99			

CC2 farm Sample time	Mar-05	Jan-05	Nov-04	Aug-04
May-04				**0.01**
Aug-04				
Nov-04				
Jan-05				

3.3.2 Isolation and identification of oomycetes from lettuce roots

Isolations were attempted from five plants of each of 77 combinations of sample time (5 times), hydroponic farm (4 farms) and cultivar (generally 4 cultivars, but 3 cultivars for one farm at three sample times).

Pythium spp. were isolated from all lettuce plants sampled in 75 of these 77 combinations (Table 3-10). The only two exceptions, where not all lettuce plants yielded *Pythium* spp., occurred in the August 2004 sample at one farm (CC2), where *Pythium* isolation levels were 80% for Baby Cos and 60% for Red Oak. This sample was taken when the nutrient solution temperature was the lowest (14-16°C). The isolation rate for the total number of plants sampled (385) was 99% (Table 3-10). No significant differences in the number of *Pythium* isolates obtained were found between sample times (P=0.13), between cultivars (P=0.52) or between farms (P=0.10) (Appendix 4 f).

Phytophthora spp. were isolated from at least some lettuce plants at each of the four farms at all five sample times (Table 3-11). However, *Phytophthora* spp. were isolated less frequently than *Pythium* spp. (Table 3-10), being isolated from some (but not necessarily all) plants in 63 of the 77 combinations of time, farm and cultivar. *Phytophthora* spp. were isolated least often from roots of lettuce plants sampled in August 2004 (from 15 plants out of 80 sampled) (Table 3-11) when the nutrient solution temperature was the lowest (14-16°C). *Phytophthora* spp. were isolated most often in the samples of November 2004 (63/75) and March 2005 (63/75), when the nutrient solution was higher than 20°C (Table 3-11). Significant differences in the number of *Phytophthora* isolates obtained from lettuce roots were found between sample times (P=0.00) and between farms (P=0.00) (Appendix 4 f). However, no significant differences in the number of *Phytophthora* isolates obtained from lettuce roots were found between the four cultivars sampled (P=0.85) (Appendix 4 f).

Table 3-10 Number of lettuce plants sampled yielding *Pythium* species (n=5) at five sampling times from four cultivars at four hydroponic farms (except for CC2 farm where three cultivars were sampled in November 2004, January 2005 and March 2005). Nutrient solution temperature was measured in hydroponic channels on the sampling day. Totals show number of plants yielding *Pythium* compared with total numbers of plants sampled.

Sample time	Cultivar	Nutrient solution temperature (°C)	Farm					Grand
			L1	L2	CC1	CC2	Totals	Totals
May-04	Baby Cos	13.5-17	5	5	5	5	**20/20**	**80/80**
	Red Oak	15-17	5	5	5	5	**20/20**	
	Green Oak	14-18	5	5	5	5	**20/20**	
	B. Mignonette	14-17	5	5	5	5	**20/20**	
Aug-04	Baby Cos	14-16	5	5	5	4	**19/20**	**77/80**
	Red Oak	14-16	5	5	5	3	**18/20**	
	Green Oak	14-15.5	5	5	5	5	**20/20**	
	B. Mignonette	14-16	5	5	5	5	**20/20**	
Nov-04	Baby Cos	20.5-22	5	5	5	5	**20/20**	**75/75**
	Red Oak	21.5-22	5	5	5	5	**20/20**	
	Green Oak	21-22	5	5	5	5	**20/20**	
	B. Mignonette	21-22	5	5	5	n/a	**15/15**	
Jan-05	Baby Cos	23-30	5	5	5	5	**20/20**	**75/75**
	Red Oak	23-30	5	5	5	5	**20/20**	
	Green Oak	23.5-29.5	5	5	5	5	**20/20**	
	B. Mignonette	23-30	5	5	5	n/a	**15/15**	
Mar-05	Baby Cos	20-28.5	5	5	5	5	**20/20**	**75/75**
	Red Oak	20-28	5	5	5	5	**20/20**	
	Green Oak	20-27.5	5	5	5	5	**20/20**	
	B. Mignonette	20-29	5	5	5	n/a	**15/15**	
Totals			**100/100**	**100/100**	**100/100**	**82/85**		**382/385 (99%)**

Table 3-11 Number of lettuce plants sampled yielding *Phytophthora* species (n=5) at five sampling times from four cultivars at four hydroponic farms (except for CC2 farm where three cultivars were sampled in November 2004, January 2005 and March 2005). Nutrient solution temperature was measured in hydroponic channels on the sampling day. Totals show number of plants yielding *Phytophthora* compared with total numbers of plants sampled.

Sample time	Cultivar	Nutrient solution temperature (°C)	Farm					Grand
			L1	L2	CC1	CC2	**Totals**	**Totals**
May-04	Baby Cos	13.5-17	5	2	5	3	**15/20**	**54/80**
	Red Oak	15-17	4	5	3	5	**17/20**	
	Green Oak	14-18	4	0	2	2	**8/20**	
	B. Mignonette	14-17	5	4	0	5	**14/20**	
Aug-04	Baby Cos	14-16	1	0	0	1	**2/20**	**15/80**
	Red Oak	14-16	0	0	3	0	**3/20**	
	Green Oak	14-15.5	0	0	1	3	**4/20**	
	B. Mignonette	14-16	0	1	2	3	**6/20**	
Nov-04	Baby Cos	20.5-22	5	0	5	4	**14/20**	**63/75**
	Red Oak	21.5-22	5	5	5	5	**20/20**	
	Green Oak	21-22	5	4	5	5	**19/20**	
	B. Mignonette	21-22	3	4	3	n/a	**10/15**	
Jan-05	Baby Cos	23-30	4	3	5	4	**16/20**	**50/75**
	Red Oak	23-30	3	0	4	4	**11/20**	
	Green Oak	23.5-29.5	5	0	5	4	**14/20**	
	B. Mignonette	23-30	5	0	4	n/a	**9/15**	
Mar-05	Baby Cos	20-28.5	5	4	5	5	**19/20**	**63/75**
	Red Oak	20-28	4	3	4	4	**15/20**	
	Green Oak	20-27.5	5	4	4	4	**17/20**	
	B. Mignonette	20-29	3	4	5	n/a	**12/15**	
Totals			**71/100**	**43/100**	**70/100**	**61/85**		**245/385 (64%)**

3.3.3 Pathogenicity tests

One isolate of *Pythium* was obtained that appeared to be different from all previous isolates obtained and tested for pathogenicity (Chapter 2). This isolate was obtained from roots of Red Oak cultivar in March 2005 from the second Central Coast hydroponics farm (CC2). This isolate was found to be pathogenic to lettuce plants tested in an experimental hydroponic system, in which the minimum and the maximum temperature of the nutrient solution ranged from 22°C to 26°C and the pH was 5.5 to 6.2. Root rot disease and wilted plants were observed in plants inoculated with *Pythium*; however, no symptoms appeared in the non-inoculated plants (Figure 3-15). Root systems of inoculated plants were unhealthy and most roots were brown in colour compared with the root systems of the control plants which were healthy and white with only a few brown roots (Figures 3-15 and 3-16).

Root systems of inoculated plants had higher root rot disease severity ratings than root systems of control (non-inoculated) plants at all five times (3, 6, 9, 12 and 14 days) after the start of the experiment (Figure 3-16). Furthermore, the root rot disease severity of inoculated plants increased over time (Figure 3-16). Significant differences in root rot severity were found between inoculated and non-inoculated plants (P=0.00) (Appendix 4 g). In addition, significant differences were found between times after inoculation (P=0.00) (Appendix 4 g). These significant differences, however, were found between times after inoculation in inoculated plants (Table 3-12) but not in control plants (Table 3-13) (Appendix 4 g). Disease severity in inoculated plants was significantly greater at 12 and 14 days compared with that at 3 and 6 days (Table 3-12).

Significant differences were found between inoculated and control plants for both wet and dry weight of both roots and shoots (Table 3-14). Both wet weight and dry weight of both roots and shoots of inoculated plants were significantly less than all corresponding weights of control (non-inoculated) plants (Table 3-12).

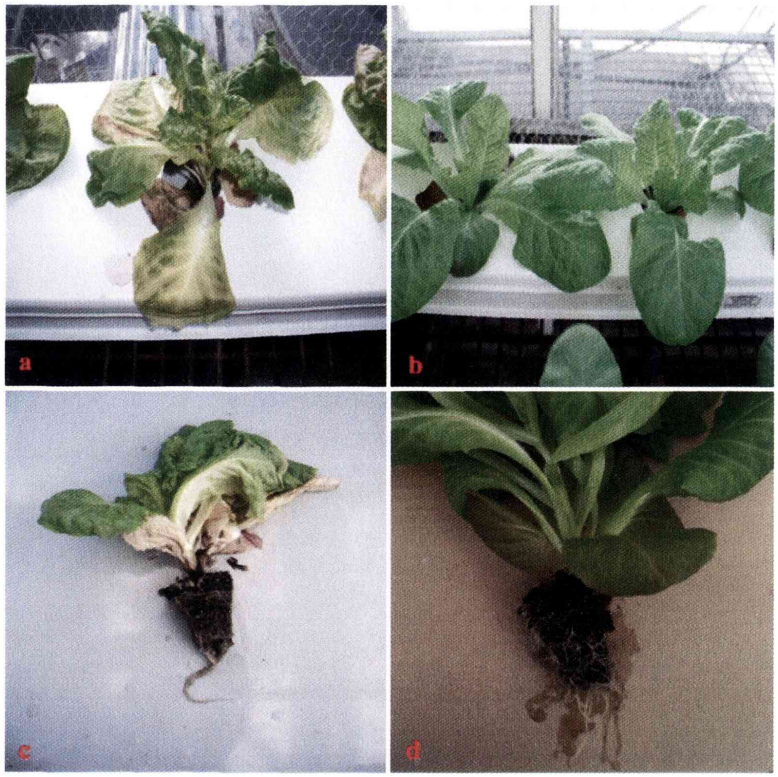

Figure 3-15 Pathogenicity test involving an isolate of *Pythium* obtained from farm CC2 in March 2005 that appeared different from all previous isolates of *Pythium*.
(a) Inoculated lettuce showing wilted leaves; (b) Non-inoculated (control) lettuce with no wilt apparent; (c) Inoculated lettuce showing unhealthy brown roots; (d) Non-inoculated lettuce with white roots.

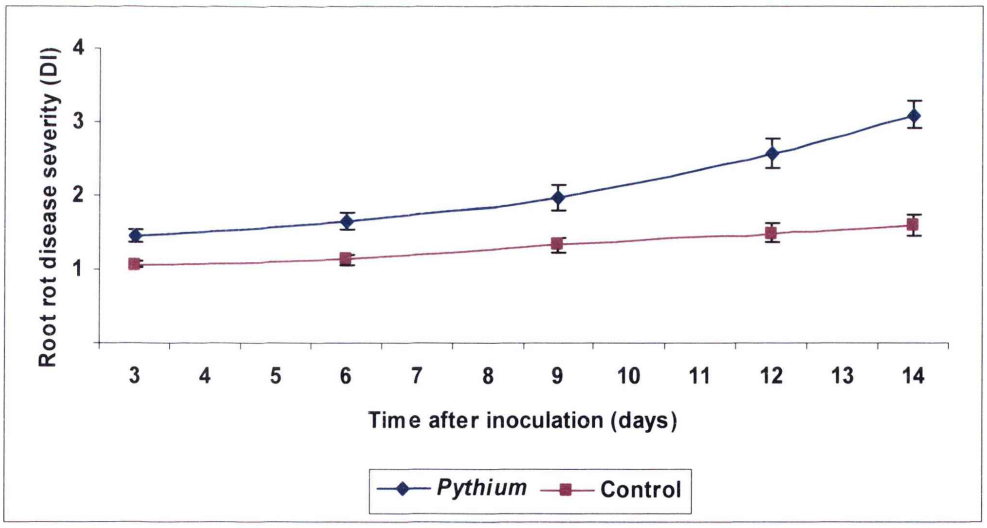

Figure 3-16 Disease severity on roots of Brown Mignonette cultivar inoculated with *Pythium* sp. and non-inoculated as control using a scale of 1 to 4: (1) healthy white roots; (2) generally healthy white roots, but with some brown colouration; (3) unhealthy roots, most roots brown in colour and (4) dead roots and/or black roots (Figure 3-4). Error bars are the standard error of the mean.

Table 3-12 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between five observation times (days after inoculation) for Brown Mignonette lettuce inoculated with *Pythium* sp. (See also Figure 3-16.) (Appendix 4 g.)

Days after inoculation	14	12	9	6
3	**0.00**	**0.00**	0.06	0.95
6	**0.00**	**0.01**	0.72	
9	**0.00**	0.83		
12	0.34			

Table 3-13 *P* values ($P \leq 0.05$ in bold) of the mean differences in root disease assessment between five observation times (days after inoculation) for non-inoculated (control) Brown Mignonette lettuce. (See also Figure 3-16.) (Appendix 4 g.)

Days after inoculation	14	12	9	6
3	0.10	0.34	0.91	1.00
6	0.24	0.59	0.98	
9	0.91	0.99		
12	1.00			

Table 3-14 Mean wet and dry weights (g) of roots and shoots of Brown Mignonette lettuces inoculated with *Pythium* and non-inoculated (control) lettuces. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 4 g.)

Treatment	Mean weight (g) of growth parameter			
	Wet root	Dry root	Wet shoot	Dry shoot
Pythium	1.0502a	0.0674a	15.5191a	0.2508a
Control	1.5594b	0.1349b	24.3683b	0.6249b

3.4 Discussion

The more extensive survey reported here, involving four hydroponic lettuce farms, confirms the finding from the previous survey on one hydroponic lettuce farm (Chapter 2) that root rot disease on lettuce grown in hydroponic systems in the study area is more severe in the warmer months than in the cooler months (Figures 3-7, 3-9, 3-11 and 3-13). Based on five survey dates over an 11 month period, significantly more lettuce root damage tended to occur in the warmer months (November 2004, January 2005 and March 2005) when the temperature was 20-30°C (Table 3-10) than in the cooler months (May 2004 and August 2004) when the temperature was 13.5-18°C (Figures 3-7, 3-9, 3-11 and 3-13, plus corresponding Tables 3-2, 3-4, 3-6 and 3-8). There was a similar tendency for more wilted and dead lettuces to be observed in all cultivars examined in the warmer temperature samples than in the cooler temperature samples (Figures 3-8, 3-10, 3-10 and 3-14, plus corresponding Tables 3-3, 3-5, 3-7 and 3-9). Nevertheless, it appears that information on disease severity in roots is a more sensitive indicator of the link between root rot disease and temperature than is information on leaf symptoms.

There is some support from the literature for this main finding in the present study. *Pythium irregulare* was recorded causing root rot diseases on lettuce grown in hydroponic systems when the temperature of the nutrient solution was 21°C (Labuschagne *et al.* 2002). Moreover, Stanghellini and Kim (1998) reported that *Pythium myriotylum* caused severe root rot disease and wilt of hydroponic lettuce occurred when the nutrient solution temperature was 27 to 29°C. It would be worthwhile to study root rot disease severity in more samples over the period from August to November, as temperatures increase, in order to define more clearly the temperature at which root rot disease starts to become a significant problem in hydroponic lettuce farms.

In this study, four lettuce cultivars were examined from four farms representing two different areas of hydroponic lettuce production compared with three lettuce cultivars from one farm in the previous study. The present study found that there were no significant differences between the four cultivars surveyed in the four farms in relation to both root disesase assessment and leaf symptom assessment. It is claimed that there are no lettuce cultivars available with resistance to root rot pathogens such as *Pythium*

spp. and *Phytophthora* spp. (Stanghellini & Rasmussen 1994). The present study confirms that the four cultivars surveyed, namely Baby Cos, Red Oak, Green Oak and Brown Mignonette, are not resistant to root rot pathogens and that root rot disease commonly occurs in the warmer months in of the year.

Based on the findings of this survey and temperature data from the Bureau of Meteorology, NSW, for the Leppington and Central Coast areas (Figure 3-6), hydroponic lettuce growers in the two areas could expect to have few root rot disease problems for the period from May through to September, when the air temperature remains below approximately 23°C. On the other hand, root disease problems could be expected to occur between November and March, when the air temperature is above 25°C in the Leppington area and above 23°C in the Central Coast area. This finding supports the finding of Chapter 2 and the claims made by hydroponic lettuce farmers that they do indeed suffer greater losses to root rot disease in summer.

From the results reported in the present study, it may be possible to manage root rot disease in hydroponic lettuce if a cost-effective way can be found to decrease the temperature of the nutrient solution, especially during the warmer months of the year. It appears that a nutrient solution temperature of 20-23°C or lower reduces root rot disease. As mentioned before, further studies are needed to determine the critical temperature at which root rot disease starts to become a significant problem over the August-November period. For this approach to be effective, farmers must also routinely monitor nutrient solution temperatures in their hydroponic channels all year round to become aware of any increases in temperature likely to cause root rot disease.

This study demonstrated that *Pythium* spp. are endemic in hydroponic systems in which lettuce are grown in the Sydney and Central Coast areas based on the five time samples taken at four farms and from four cultivars (Table 3-10). *Pythium* spp. were isolated from 75 of 77 combinations of time, cultivar and farm with an overall isolation rate of 99%. There was also 100% recovery from roots of the four lettuce cultivars sampled from the four farms in the May 2004, November, 2004, January 2005 and March 2005 samples (Table 3-10). Several studies have considered *Pythium* spp. to be the most common pathogens in hydroponic lettuce systems around the world (Jenkins & Averre 1983; Zinnen 1988; Stanghellini & Rasmussen 1994; Stanghellini & Kim 1998;

Labuschagne *et al.* 2002). Nevertheless, it would be useful to sample hydroponic systems for *Pythium* spp. in both the coldest month of the year (July; see Figure 3-6) and the hottest month of the year (February; see Figure 3-6) to determine whether *Pythium* spp. are present in hydroponic systems at these extreme temperatures. Moreover, it would also be worthwhile to sample hydroponic systems immediately after systems are emptied and cleaned with bleach to determine whether this process significantly reduces the occurrence of *Pythium* species.

Phytophthora spp. were isolated regularly from 63 of 77 combinations of time, cultivar and farm (Table 3-11). They were, however, isolated less frequently than *Pythium* spp. having an overall isolation rate of just 64% (Table 3-11). As well, in the August 2004 sample, *Phytophthora* spp. were isolated just from 15 of the 80 lettuces. Significant differences in the number of *Phytophthora* isolates obtained from lettuce roots were found between sample times and between farms but not amongst the four cultivars sampled. There could be several different explanations for the lowest isolation rate in the coldest of the five samples. Firstly, *Phytophthora* has an optimum temperature for growth of 25°C and, while it also grows well at 20°C, its growth is slow at 10-15°C (Erwin & Ribeiro 1996; Chapter 2, Figure 2-12). Secondly, zoospore production in *Phytophthora* spp. requires a temperature of 25°C ± 2°C, at least in culture (Erwin & Ribeiro 1996). It may be that warm temperatures are also required for zoospore production when *Phytophthora* is in hydroponic systems and so zoospore production was limited at temperatures of 14 to 16°C of the August 2004 sample. Although the nutrient solution temperature in May 2004 was low and similar (13.5-18°C) to that in August 2004, *Phytophthora* was more readily isolated in May 2004 than in August 2004. It is likely that the warmer temperatures preceding the May 2004 sample (Figure 3-6) lead to a higher isolation rate in May 2004 than in August 2004, which followed several months of cooler temperatures (Figure 3-6).

The presence of *Pythium* in hydroponic systems all year round, as well as *Phytophthora* (to a lesser extent), indicates the problems farmers face in attempting to manage root rot disease in hydroponic lettuce. There is no information in the literature as to how *Pythium* is introduced into hydroponic systems, but potential sources of *Pythium* could include both the water supply and planting material. It appears that, in the farms surveyed, no methods are currently in use that result in effective, long-term elimination

of these pathogens. It would, therefore, appear that disease management is likely to be more successful through control of nutrient solution temperatures. Nevertheless, various techniques to disinfect recirculating nutrient solution are worth investigating as are populations of rhizosphere microorganisms that may offer protection to roots against root pathogens.

This study indicated that the *Pythium* isolate tested was pathogenic on lettuce plants grown in a hydroponic system. Wilted and dead lettuces with brown roots were observed when plants were inoculated with *Pythium* sp. compared with the control lettuces (Figure 3-15). Several studies have reported that *Pythium* spp. were pathogenic to lettuce plants grown in hydroponic systems (Stanghellini & Kronland 1986; Tesoriero *et al.* 1991; Tesoriero & Cresswell 1995; Amer & Utkhede 2000; Chapter 2). For the inoculated plants, significant differences were found between the times after inoculation, especially between the later times (12 and 14 days after inoculation) and the earlier times (3 and 6 days) (Table 3-12). The reason for this could be that the number of zoospores and sporangia of *Pythium* sp. increased over time and the infection increased with the increasing inoculum. Gold and Stanghellini (1985) reported that zoospores of *Pythium aphanidermatum* and *Pythium dissotocum* infected spinach roots within 15 minutes after inoculation at a temperature of 23°C and that the inoculum accumulated over time.

4 EFFECTS OF TEMPERATURE ON ROOT ROT DISEASE OF HYDROPONIC LETTUCE

4.1 Introduction

Environmental conditions are very important factors affecting plant disease severity. Temperature and moisture are probably the most important environmental factors influencing plant disease development (Strange 2003). These factors affect disease development through their influence on the growth and susceptibility of the host, on the multiplication and activity of the pathogen, or on the interaction of host and pathogen as it relates to the severity of symptom development (Agrios 2005). Moreover, all pathogens and plants require a specific temperature and moisture regime for their growth and reproduction. Diseases caused by *Pythium* and *Phytophthora* are favoured by warm and wet conditions (Agrios 2005). These pathogens produce zoospores which can swarm for a few minutes before germination commences and new plants are subsequently infected.

In hydroponic systems, especially Nutrient Film Technique (NFT), nutrient solution recirculates in a flat-bottomed channel and roots grow in a shallow nutrient film. *Pythium* and *Phytophthora* require wet conditions to release their zoospores and to continue their life cycle. The NFT system provides excellent conditions for these pathogens to release their zoospores. Gold and Stanghellini (1985) reported that the optimum temperature for zoospore release in *Pythium aphanidermatum* and *Pythium dissotocum* was between 20 and 30°C when these cultures were flooded with sterile distilled water. For the majority of *Phytophthora* spp., a temperature of 25°C appears to be the optimum temperature for zoospore release (Erwin & Ribeiro 1996).

Pythium spp. cause seed rot, seedling damping-off and root rot of most plants (Agrios 2005; Deacon 2006). *Phytophthora* spp. also cause root rot and blights of many plants. *Pythium* spp. have been reported as the most common and destructive root pathogens of vegetable crops in hydroponic systems and of general nursery production in glasshouses as well (Zinnen 1988; Stanghellini & Rasmussen 1994; Stanghellini *et al.* 1996b). Various studies have isolated *Pythium* spp. and *Phytophthora* spp. from plants grown in hydroponic systems including lettuce (Zinnen 1988; Labuschagne *et al.* 2002) and

cucumber, tomato and pepper (Stanghellini *et al.* 1984, 1988 & 1996a; Cherif *et al.* 1994; Amer & Utkhede 2000; Labuschagne *et al.* 2003).

Temperature seems to be an important factor in root rot disease incidence and severity in vegetable production in soil (Agrios 2005) and in hydroponics (Zinnen 1988; Stanghellini & Rasmussen 1994; Chapters 2 and 3). There are a few studies examining the relationship between temperature and disease incidence and severity in hydroponic lettuce. A single study in Australia (Tesoriero & Cresswell 1995) examined the relationship between disease severity and temperature in hydroponic lettuce and found that the severity of yield loss and damage to lettuce plants was in general greater when roots had been dipped in a water bath for two hours at temperatures between 30°C and 40°C and then inoculated with *Pythium* sp. or *Phytophthora* sp. before being returned to the hydroponic system. A contrasting result, however, came from the study of Funck-Jensen & Hockenhull (1983) who found that no significant disease symptoms occurred after lettuce roots were dipped in a water bath for 6 minutes at 42°C and inoculated with two *Pythium* species before being returned to the hydroponic system, located in a growth chamber where the air temperature was 20°C. The lack of disease obtained in the latter study may be attributable to the shorter time of exposure to high temperatures and/or the use of less virulent *Pythium* isolates.

A study using spinach plants found that they died within 3-4 days after inoculation with *Pythium aphanidermatum* at nutrient solution temperatures of 21°C and 27°C, although the pathogen was more aggressive at 27°C. However, when spinach plants were inoculated with *Pythium dissotocum* at nutrient solution temperatures of 21°C and 27°C they wilted but did not die. Stanghellini and Kronland (1986) reported that *Pythium dissotocum* was responsible for yield reduction in lettuce plants grown in hydroponic systems in the absence of any visible root rot symptoms at glasshouse temperatures of 23 to 36°C (with a mean of 28°C).

Following on from the results of the disease surveys reported in Chapters 2 and 3 of this thesis, the inconsistencies reported in the literature regarding temperature and disease development and the lack of information available for lettuce in Australia, it was decided that further investigation of the effects of temperature on root rot disease development in hydroponic lettuce would be worthwhile. Furthermore, the use of an

experimental hydroponic system allows close monitoring of disease development under controlled conditions, without complications arising from the intervention of farmers.

The aim of this study was to examine the effects of the temperature of the nutrient solution on root disease of lettuce caused by *Pythium* and *Phytophthora* in an experimental hydroponic system.

4.2 Materials and methods

4.2.1 Hydroponic system

Four independent recirculating experimental hydroponic units, each containing holes for 10 replicate lettuce seedlings, were set up in a non-temperature-controlled glasshouse at the Botanic Gardens Trust, Sydney (BGT) (see Chapter 3, section 3.2 Materials and methods, sub-section 3.2.3.1 for details).

4.2.2 Experimental design, glasshouse conditions and nutrient solution temperatures

Four experiments were carried out, each using different nutrient solution temperature regimes (as described below). Each experiment was repeated three times. To obtain the different temperature regimes, experiments were carried out in different seasons. Submersible heaters were used to obtain nutrient solution temperatures higher than the ambient air temperature (ViaAqua stainless steel 300W, made in China, supplied by Quik Grow Hydroponic Specialists, 672 Parramatta Road, Croydon NSW). During the experiments, data loggers were used to record air temperatures in the glasshouse and thermometers were used to record daily minimum and maximum nutrient solution temperatures. The four nutrient solution temperature regimes were:

1. 24-27°C (minimum and maximum, respectively): experiments were carried out in late spring and early summer (November 2004, December 2004 and January 2005) to replicate farm temperatures in summer;
2. 34°C (continuously heated): heaters were used to obtain this temperature, with experiments carried out in late January 2005, February 2005 and March 2005;
3. 16-17°C (minimum and maximum, respectively): experiments were carried out in winter (July 2004, August 2004 and August 2005) to replicate winter temperatures at the farms; and
4. 34°C for 10 hours (obtained by using heaters), followed by 18-20°C (minimum and maximum, respectively): these experiments were carried out in autumn and early winter (April 2005, May 2005 and June 2005).

Each experiment ran for three weeks. At the conclusion of each experiment, approximately 250 mL chlorine (2%) was added to the nutrient solution and pumped

through the system for at least 10 minutes. All surfaces were then cleaned with chlorine and left to dry for at least one week. New nutrient solution was used for each experiment. Nutrient solution pH was tested every three days by taking three samples of 10 mL nutrient solution from each tank and measuring them with a pH meter (Basic pH Meter, Denver Instrument Company, Colorado, USA).

4.2.3 Plant materials

The lettuce cultivar Brown Mignonette (Terranova Seeds Pty Ltd, 13/19 Chifley Street, Smithfield NSW) was used for all experiments. Lettuce seedlings were produced as described in Chapter 3 (see section 3.2 Materials and methods, sub-section 3.2.3.2 for details).

4.2.4 Inoculum preparation and inoculation

The isolates used were those obtained in January 2004 from lettuce plants at Manicaro's Farm, Leppington (Table 4-1), when root rot disease was most severe (see Chapter 2). Four different treatments were used to inoculate Brown Mignonette lettuce seedlings in all four experiments - *Pythium* sp., *Phytophthora* sp., a combination of *Pythium* sp. and *Phytophthora* sp., or sterile distilled water as control (uninoculated).

Table 4-1 Pathogens used in the three treatments involving inoculation of Brown Mignonette lettuce in the experiments at four different nutrient solution temperatures (with three experiments per temperature regime).

Pathogen	Sample time	Code
Pythium sp.	Jan-04	PyJ04 1
Phytophthora sp.	Jan-04	PhJ04
combined *Pythium* sp. and *Phytophthora* sp.	Jan-04	PyJ04 1 and PhJ04

Pythium inoculum was prepared as follows. Five to 7-day-old cultures on Potato Carrot Agar (PCA), incubated in the dark at 25°C ± 1°C, were flooded with sterile distilled water and incubated at 4°C for 1-3 hours. Cultures were then placed at room temperature (25°C ± 2°C) for 2-5 hours until substantial zoospore release occurred. Zoospores and/or sporangia were collected and counted using a haemocytometer.

Phytophthora inoculum was prepared as follows. Five to 7-day-old cultures on PCA, incubated in the dark at 25°C ± 1°C, were flooded with sterile distilled water and incubated 4°C for one hour after which they were moved to room temperature (25°C ±

2°C) for 30 minutes or more until enough zoospores were released. Then zoospores and/or sporangia were collected and counted using a haemocytometer.

Inoculum of *Pythium* or *Phytophthora* was added to give a final concentration of 500–1000 zoospores and/or sporangia per mL of nutrient solution in each tank (Funck-Jensen & Hockenhull 1983). The combined treatment of *Pythium* and *Phytophthora* was made up using half-strength individual inoculum to give a final combined concentration of 500–1000 zoospores and/or sporangia per mL.

Six-week old lettuce seedlings were transplanted to the hydroponic system and kept for 3-5 days before inoculum was added. This was to ensure that seedlings were not suffering from transplanting shock at the time of inoculation. Inoculum was added to the nutrient solution tank as described above.

4.2.5 Data collection

Plants were kept for three weeks from the time of inoculation to harvest. Symptoms of root rot disease were assessed on a scale of 1 to 4 (Figures 2-4 and 3-4, Chapters 2 and 3 respectively) every three days. This scale was the same as that used in the disease surveys at the hydroponic farms. At the conclusion of each experiment, wet weight and dry weight of shoots and roots were obtained individually for all plants. Re-isolation of the pathogens was attempted. When no root rot disease symptoms were apparent macroscopically, lettuce roots were examined microscopically (Olympus CX41RF, Olympus Optical, Philippines) to determine whether the pathogen(s) were present on or in root cells.

4.2.6 Data analysis

General Linear Model (GLM) ANOVA (MiniTab VER 13) was used to find the differences ($P \leq 0.05$) between inoculated and non-inoculated (control) plants as regards the mean wet weight and dry weight for shoots and roots. Data from root assessments (using ratings of 1 to 4) were also analysed by GLM ANOVA to find the difference ($P \leq 0.05$) between inoculated and control plants as regards root disease levels. Heteroscedastic data were transformed as required using log or square root transformations.

Results from root rot assessments were summarised by calculating a disease index (DI) for each treatment using the following formula (Merrin 1998):

$DI = [(n4*4) + (n3*3) + (n2*2) + n1] / Tn$

Where n4 = number of lettuces rated 4

n3 = number of lettuces rated 3

n2 = number of lettuces rated 2

n1 = number of lettuces rated 1

Tn = total number of lettuces examined in each treatment

The DI was used for graphical representation of results. Microsoft Excel XP 2003 was used to prepare graphs and tables.

4.3 Results

4.3.1 Nutrient solution temperature 24-27°C

Root rot disease and wilted plants occurred at nutrient solution temperatures of 24-27°C (Figure 4-1) in plants inoculated with *Pythium* (Figure 4-1 a) and the combination of *Pythium* and *Phytophthora* (Figure 4-1 c). However, no disease symptoms were evident on plants inoculated with *Phytophthora* alone (Figure 4-1 b) or the control plants (Figure 4-1 d) at this temperature regime. Root disease symptoms appeared to be more severe on the plants inoculated with *Pythium* alone (Figure 4-1 a) than on plants inoculated with the combination of *Pythium* and *Phytophthora* (Figure 4-1 c).

Significant differences in wet weight and dry weight of roots, as well as wet weight of shoots, were found at nutrient solution temperatures of 24-27°C between plants inoculated with *Pythium*, the combined *Pythium* sp. and *Phytophthora* sp., and the control (Table 4-2; Appendix 5 a). However, no significant differences in wet weight and dry weight of roots and shoots were found between plants inoculated with *Phytophthora* alone and the control plants (Table 4-2). Plants inoculated with *Pythium* alone had the lowest mean weight for the three parameters of wet root weight, dry root weight and wet shoot weight, whilst control (uninoculated) plants had the highest mean weight for these three parameters. Plants inoculated with the combination of *Pythium* and *Phytophthora* had mean weights that were intermediate between those measured for *Pythium*-inoculated plants and control plants. For the growth parameter wet shoot weight, which is the best indicator of marketable lettuces, the reduction in growth brought about by inoculation with *Pythium* was 29%, whilst inoculation with the combination of *Pythium* and *Phytophthora* led to a 14% reduction.

Significant differences in root rot disease severity were found between roots of plants inoculated with *Pythium* and roots of the control plants, as well as between roots of plants inoculated with the combination treatment and roots of the control plants (Table 4-3; Appendix 5 a). Significant differences were also found in root rot disease severity between *Phytophthora*-inoculated plants and control plants. However, no significant differences in root rot severity were found between plants inoculated with *Phytophthora* and plants inoculated with the combination of *Pythium* and *Phytophthora* (Table 4-3 and Figure 4-2) (Appendix 5 a).

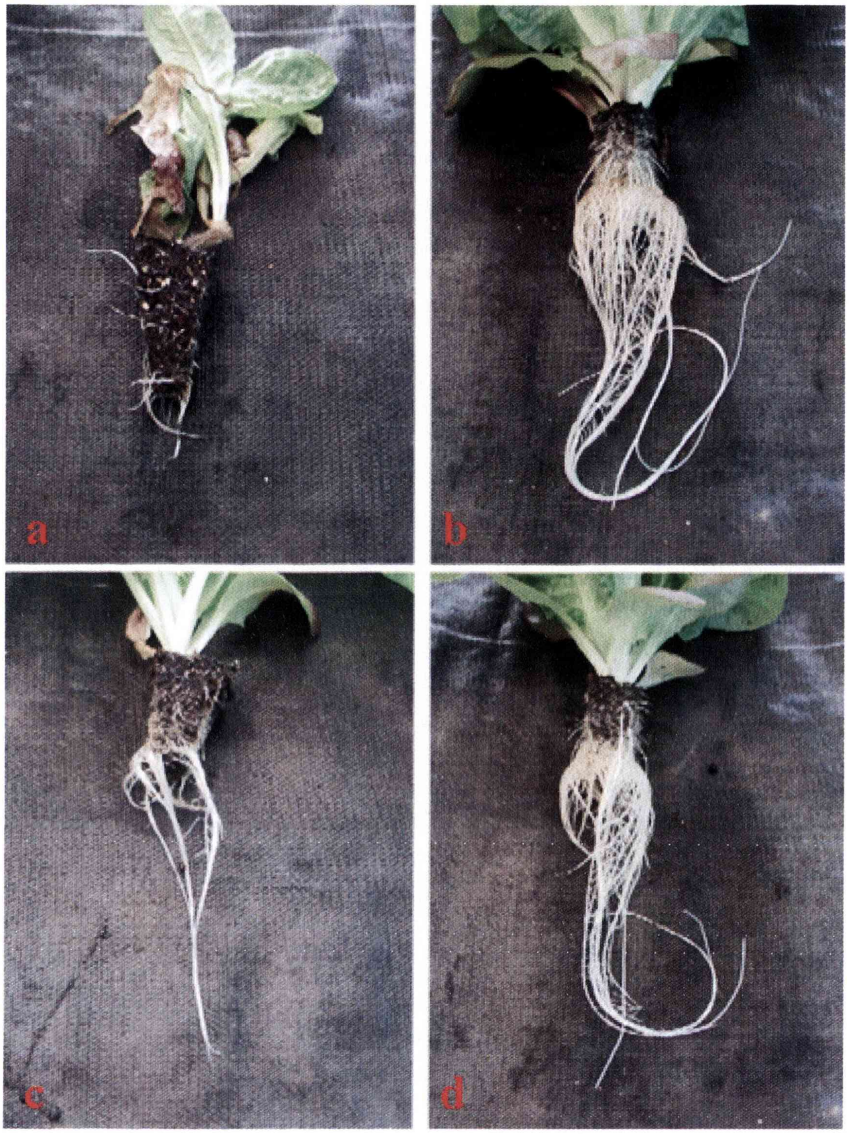

Figure 4-1 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 24-27°C for 21 days. Plants were inoculated with one of four treatments.
(a) *Pythium*;
(b) *Phytophthora*;
(c) *Pythium* & *Phytophthora*; and
(d) sterile distilled water (control).

Table 4-2 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 24-27°C after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 a.)

Treatment	Mean weight (g) of growth parameter			
	Wet root	Dry root	Wet shoot	Dry shoot
Pythium	1.73a	0.10a	24.63a	0.89a
Phytophthora	2.82bc	0.23c	32.07bc	1.48b
Pythium & *Phytophthora*	2.31b	0.17b	29.65b	1.11a
Control	3.13c	0.27c	34.65c	1.61b

Table 4-3 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 24-27°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 a.)

Treatment	Disease index
Pythium	1.95a
Phytophthora	1.64b
Pythium and *Phytophthora*	1.63b
Control	1.40c

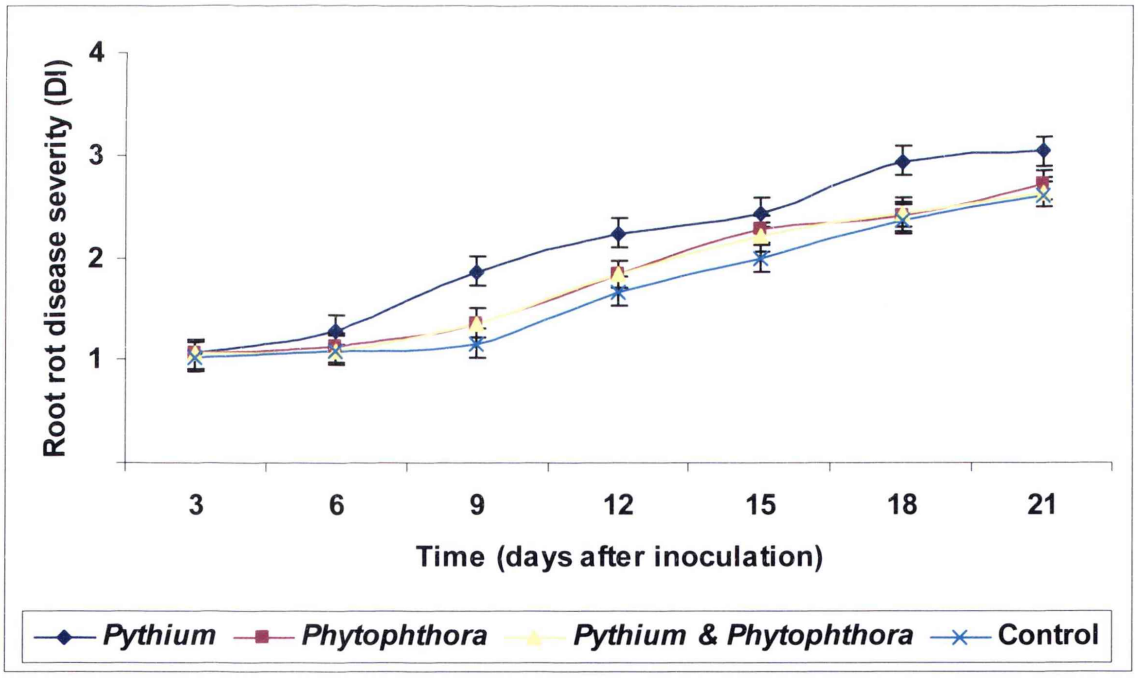

Figure 4-2 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 24-27°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.

4.3.2 Nutrient solution temperature 34°C

Lettuce plants grown at a nutrient solution temperature of 34°C (continuously heated) showed poor root development in all four treatments (Figure 4-3). The plants showed wilting when they were inoculated with *Pythium* (Figure 4-3 a) and the combination of *Pythium* and *Phytophthora* (Figure 4-3 c). However, plants inoculated with *Phytophthora* alone (Figure 4-3 b) showed little wilting and they looked similar to the control plants (Figure 4-3 d).

Significant differences in all four growth parameters measured (both wet and dry weights of both roots and shoots) were found between plants inoculated with *Pythium* and the control (uninoculated) plants (Table 4-4; Appendix 5 b). Furthermore, there were significant differences in all four parameters between plants inoculated with combination of *Pythium* and *Phytophthora* and the control plants. However, for only two parameters (dry root weight and dry shoot weight) were the weights significantly different between plants inoculated with *Pythium* alone and plants inoculated with the combination of *Pythium* and *Phytophthora*. The mean weights of the growth parameters were always significantly less in *Pythium*-inoculated plants compared with control plants (Table 4-4). In the case of wet shoot weight, inoculation with *Pythium* led to a 45% reduction in weight, whilst inoculation with the combination of *Pythium* and *Phytophthora* led to a 29% reduction in weight.

No significant differences were found between plants inoculated with *Phytophthora* and control plants as regards the three growth parameters dry root weight, wet shoot and dry shoot weight (Table 4-4). However, the wet root weight of *Phytophthora*-inoculated plants was significantly less than that of control plants.

Significant differences in root rot severity were found between roots of plants inoculated with *Pythium* and roots of the control plants, as well as between roots of plants inoculated with the combination treatment and roots of the control plants (Table 4-5; Appendix 5 b). However, no significant differences in root rot severity were found between plants inoculated with *Phytophthora* and the control plants (Table 4-5 and Figure 4-4) (Appendix 5 b).

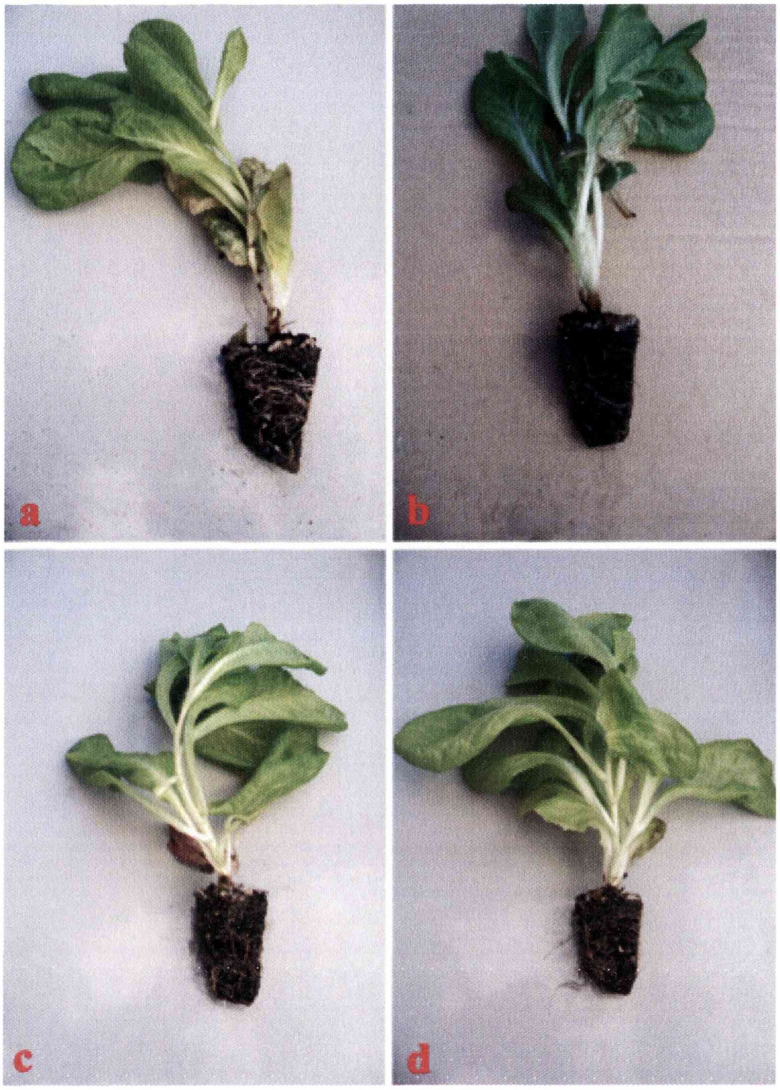

Figure 4-3 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 34°C (continuously heated) for 21 days. Plants were inoculated with one of four treatments.
(a) *Pythium*;
(b) *Phytophthora*;
(c) *Pythium* & *Phytophthora*; and
(d) sterile distilled water (control).

Table 4-4 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 34°C (continuously heated) after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 b.)

Treatment	Mean weight (g) of growth parameter			
	Wet root	Dry root	Wet shoot	Dry shoot
Pythium	0.98a	0.08a	12.66a	0.49a
Phytophthora	2.18b	0.20c	22.39b	1.04c
Pythium & *Phytophthora*	1.05a	0.14b	16.42a	0.79b
Control	2.38c	0.20c	22.97b	1.08c

Table 4-5 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 34°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 b.)

Treatment	Disease index
Pythium	2.07a
Phytophthora	1.67b
Pythium and *Phytophthora*	1.91a
Control	1.62b

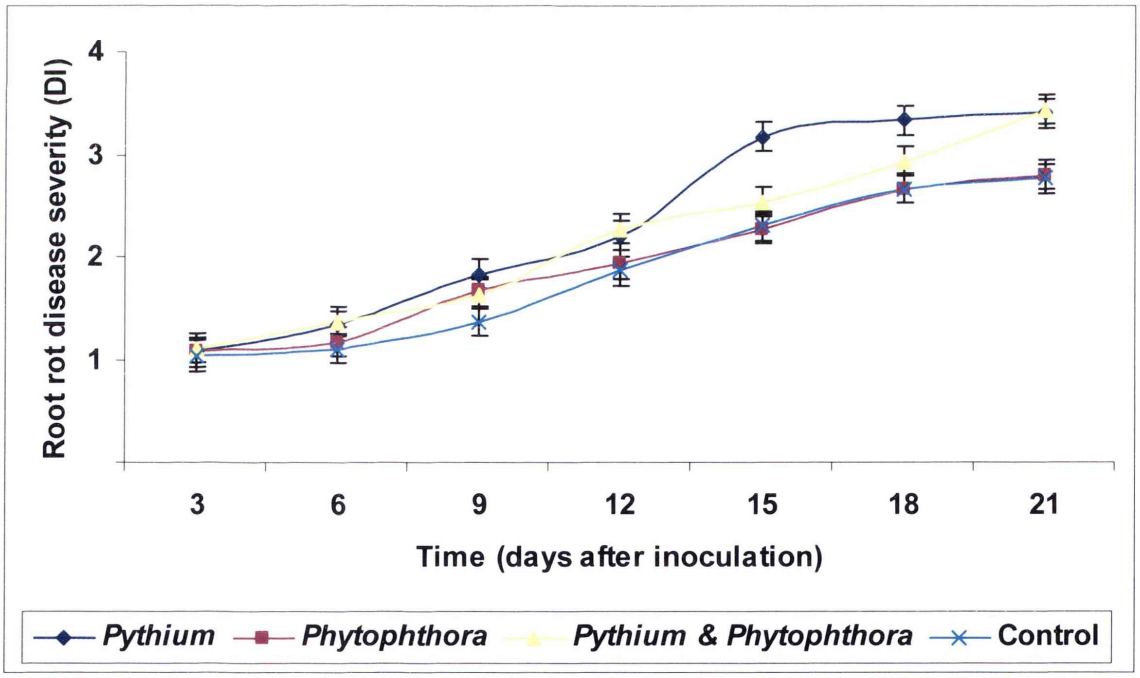

Figure 4-4 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 34°C (continuously heated) temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.

4.3.3 Nutrient solution temperature 16-17°C

No obvious root rot disease and no wilting occurred in any plants inoculated with the four treatments at nutrient solution temperatures of 16-17°C (Figure 4-5). Nevertheless, zoospores and other structures of *Pythium* and *Phytophthora* were found in or on root cells of lettuce grown at these temperatures (Figure 4-6). No significant differences were found in wet weight and dry weight of roots and shoots between plants inoculated with *Pythium*, *Phytophthora*, the combination of *Pythium* and *Phytophthora*, and the control plants (Table 4-6; Appendix 5 c). In the case of wet shoot weight, inoculation with *Pythium* lead to only 8% reduction in weight, whilst inoculation with the combination of *Pythium* and *Phytophthora* lead to just 7% reduction in weight.

No significant difference in the disease index for root assessment was found between plants inoculated with *Phytophthora* and the control plants, or between plants inoculated with the combination of *Pythium* and *Phytophthora* and the control plants (Table 4-7; Appendix 5 c). Although roots of lettuce plants inoculated with *Pythium* were generally healthy (Figure 4-5), they tended to have more brown roots compared with the other three treatments (Figure 4-7), particularly towards the end of the experiments. A significant difference was found in the root rot disease severity rating of *Pythium*-inoculated plants compared with the control, but this inoculated treatment was not significantly different from the other inoculated treatments (Figure 4-7 and Table 4-7) (Appendix 5 c).

Figure 4-5 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 16-17°C for 21 days. Plants were inoculated with one of four treatments.
(a) *Pythium*;
(b) *Phytophthora*;
(c) *Pythium* & *Phytophthora*; and
(d) sterile distilled water (control).

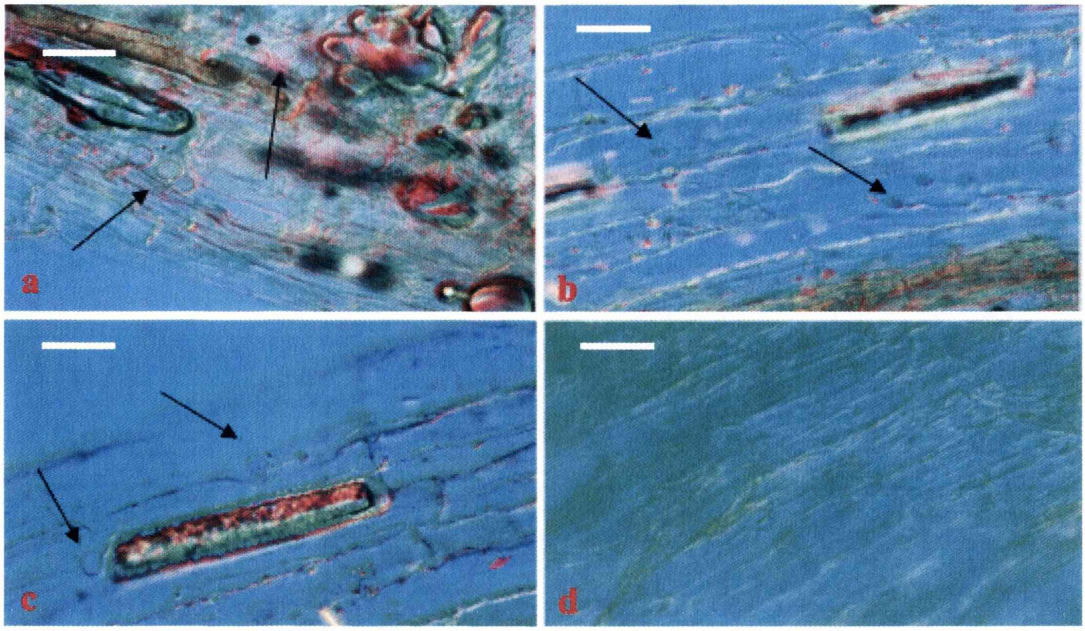

Figure 4-6 Root cells of Brown Mignonette lettuce cultivar grown at a nutrient solution temperature of 16-17°C. Plants were inoculated with:
(a) *Pythium*; (b) *Phytophthora*;
(c) a combination of *Pythium* and *Phytophthora*; and (d) sterile distilled water (control).
Arrows indicate zoospores or other pathogen structures. Bars are equal to 49 µm.

Table 4-6 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 16-17°C after 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 c.)

Treatment	Mean weight (g) of growth parameter			
	Wet root	Dry root	Wet shoot	Dry shoot
Pythium	1.99a	0.17a	21.16a	0.74a
Phytophthora	2.15a	0.19a	22.98a	0.85a
Pythium & *Phytophthora*	2.04a	0.17a	21.47a	0.75a
Control	2.19a	0.19a	23.05a	0.85a

Table 4-7 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 16-17°C with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 c.)

Treatment	Disease index
Pythium	1.60a
Phytophthora	1.45ab
Pythium and *Phytophthora*	1.55ab
Control	1.43b

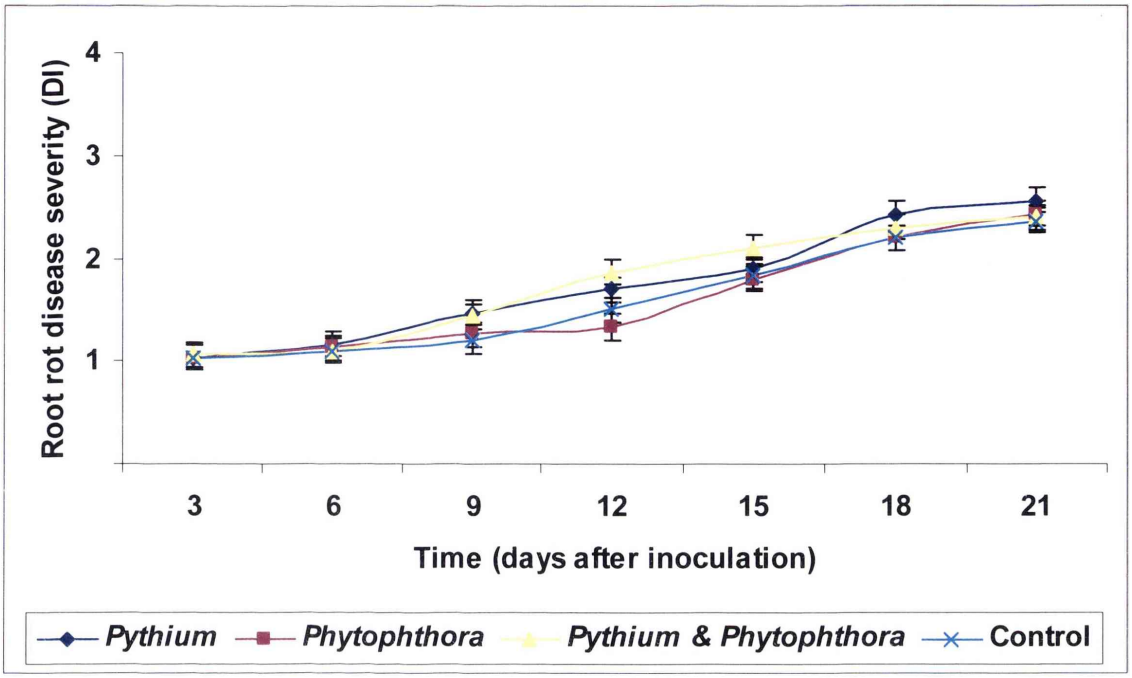

Figure 4-7 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 16-17°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each value, n = 30.

4.3.4 Nutrient solution temperature 34°C for 10 h followed by 18-20°C

No obvious root rot disease or wilting occurred in plants inoculated with the four treatments at nutrient solution temperatures of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days (Figure 4-8). Root system development, however, appeared to be less extensive in the two inoculation treatments involving *Pythium* (Figures 4-8 a and 4-8 c) compared with the control (Figure 4-8 d).

Significant differences in all four growth parameters (both wet and dry weights of both roots and shoots) were found between plants inoculated with *Pythium* and the control (uninoculated) plants (Table 4-8; Appendix 5 d). Furthermore, there were significant differences in all four parameters between plants inoculated with the combination of *Pythium* and *Phytophthora* and the control plants (Table 4-8). However, for only one parameter (dry shoot weight) was the weight significantly different between plants inoculated with *Pythium* and plants inoculated with the combination of *Pythium* and *Phytophthora*. Plants inoculated with *Phytophthora* were not significantly different from the controls as regards two growth parameters measured (wet shoot weight and dry shoot weight) (Table 4-8). The mean weights of the growth parameters were always significantly less in *Pythium*-inoculated plants compared with control plants (Table 4-8). For the growth parameter wet shoot weight, which is the best indicator of marketable lettuces, the reduction in growth brought about by inoculation with *Pythium* was 26%, whilst that brought about by inoculation with the combination of *Pythium* and *Phytophthora* was 15%.

Based on the average disease index for root assessments made over 3 to 21 days, significant differences in root rot disease severity were found between roots of plants inoculated with *Pythium* and roots of the control plants, as well as between roots of plants inoculated with the combination treatment and roots of the control plants (Table 4-9; Appendix 5 d). Significant differences were also found in root rot disease severity between *Phytophthora*-inoculated plants and control plants. However, no significant differences in root rot severity were found between plants inoculated with *Pythium* and plants inoculated with the combination of *Pythium* and *Phytophthora* (Table 4-9; Figure 4-9) (Appendix 5 d).

Figure 4-8 Root rot disease on Brown Mignonette lettuce cultivar grown in an experimental hydroponic system under a temperature regime of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days. Plants were inoculated with one of four treatments.
(a) *Pythium*; **(b) *Phytophthora*;**
(c) *Pythium* & *Phytophthora*; and **(d) sterile distilled water (control)**

Table 4-8 Mean wet and dry weights (g) of roots and shoots at nutrient solution temperatures of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. For each mean value, n = 30. (Appendix 5 d.)

Treatment	Mean weight (g) of growth parameter			
	Wet root	Dry root	Wet shoot	Dry shoot
Pythium	1.05a	0.11a	18.10a	0.49a
Phytophthora	1.92b	0.22b	24.10bc	1.16c
Pythium & *Phytophthora*	1.14a	0.13a	20.82ab	0.86b
Control	2.15c	0.30c	24.60c	1.24c

Table 4-9 Average disease index over 3 to 21 days for root assessment of Brown Mignonette lettuce cultivar at nutrient solution temperatures of 34°C for 10 h followed by 18-20°C for the remainder of the 21 days with four treatments (three inoculated and one control). Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. (Appendix 5 d.)

Treatment	Disease index
Pythium	1.97a
Phytophthora	1.51b
Pythium and *Phytophthora*	1.86a
Control	1.28c

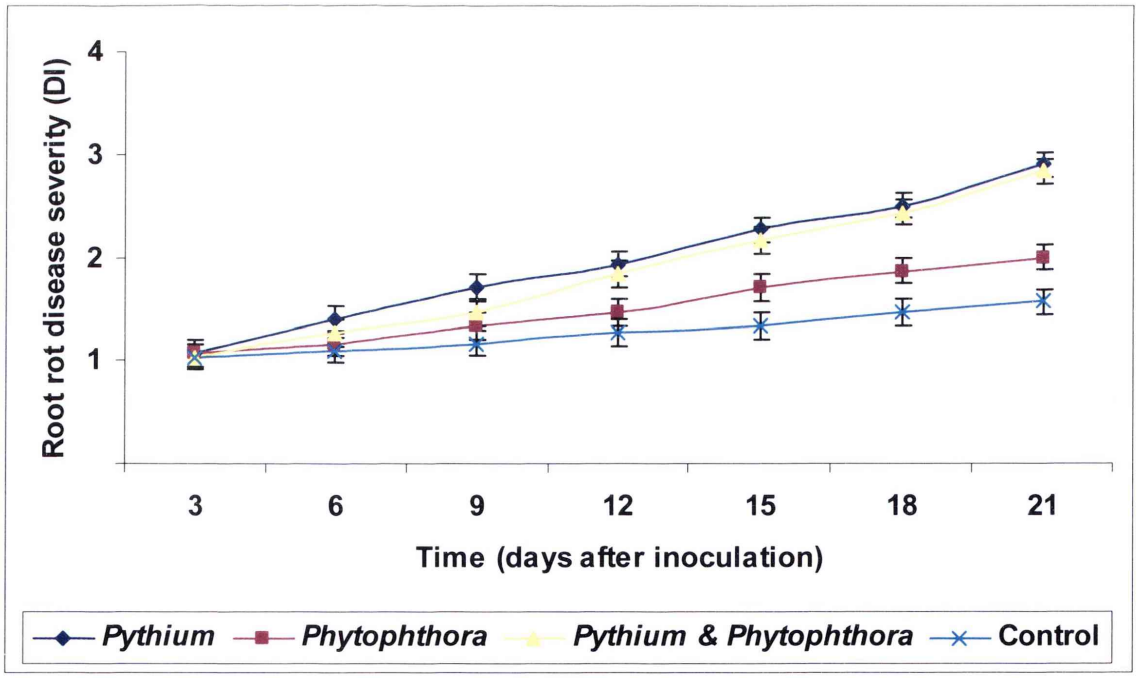

Figure 4-9 Disease severity on roots of Brown Mignonette lettuce cultivar inoculated with four treatments and grown at 34°C for 10 h followed by 18-20°C temperature regime for 21 days indicated by disease index (DI) based on a scale of 1 to 4 (Figure 2-4 in Chapter 2). Error bars are standard error of the mean. For each mean value, n = 30.

4.4 Discussion

This study demonstrated that root rot disease occurred in lettuce grown in an experimental hydroponic system, following inoculation with *Pythium* or a combination of *Pythium* and *Phytophthora* under a temperature regime of 24-27°C (minimum and maximum) (Figure 4-1) but not at 16-17°C (minimum and maximum) (Figure 4-5). This finding is consistent with the results of a year-round survey of hydroponic lettuce farms (Chapters 2 and 3) in which it was found that root rot disease was prevalent in warm weather when the nutrient solution temperature was 20°C or higher, but not when the nutrient solution temperature was below 18°C.

Significant differences in wet and dry weight of roots, as well as wet weight of shoots, were found at nutrient solution temperatures of 24-27°C between plants inoculated with *Pythium*, with *Pythium* and *Phytophthora* combined, and the control (Table 4-2). Other researchers have found that root rot disease on spinach plants caused by *Pythium aphanidermatum* is most destructive at nutrient solution temperatures above 25°C but that it is not economically important at temperatures below 20°C (Bates & Stanghellini 1984; Gold & Stanghellini 1985). No disease symptoms were evident on plants inoculated with *Phytophthora* alone (Figure 4-1 b) or the control plants (Figure 4-1 d) at the 24-27°C temperature regime. The reason for this finding could be that the *Phytophthora* isolate used in this experiment was not pathogenic to lettuce grown in NFT and may have been isolated from the hydroponic farm as a secondary pathogen. However, this isolate appeared to be pathogenic to lettuce in the pathogenicity tests carried out in individual cups and reported in Chapter 2. As well, the disease index data comparing Phytophthora with the control (Table 4-3) suggests pathogenicity, which is not evident in Figure 4-1. At 24-27°C, yield reduction in wet shoot weight occurred on plants inoculated with *Pythium* alone (29% reduction) and the combination of *Pythium* and *Phytophthora* (14% reduction); however, *Phytophthora* alone did not cause a significant reduction in wet shoot weight (Table 4-2). The fact that reduction in wet shoot weight was less in the *Pythium-Phytophthora* combination treatment than in the *Pythium*-alone treatment may reflect the lower initial *Pythium* inoculum concentration in the former treatment. In any case, the combination of pathogens at the concentrations tested does not cause greater yield losses than *Pythium* alone at the equivalent combined concentration.

The severity of root rot disease at 24-27°C compared with that at 16-17°C could be at least partly explained by findings regarding the optimum temperature for growth of the pathogens involved. In the present study, the optimum temperatures were found to be 30°C for *Pythium* sp. and 25°C for *Phytophthora* sp. (Chapter 2, Figures 2-11 and 2-12). Both are regarded as being aggressive pathogens when all conditions are favourable (Zinnen 1988; Linde *et al.* 1990; Agrios 2005; Deacon 2006).

The present study, using an experimental hydroponic system, found that lettuce root rot severity increased and growth measurements decreased when lettuce, inoculated with *Pythium* (or a combination of *Pythium* and *Phytophthora*), were grown at nutrient solution temperatures of 24-27°C in the months of November, December and January compared with nutrient solution temperatures of 16-17°C in the months of July and August. These small scale experiments using hydroponic units in a glasshouse reflect, to some extent, the conditions likely to be encountered in hydroponic farms in these months. Nevertheless, there are other seasonal differences that occur in these months, including those associated with light duration and the angle of the sun in the sky. Thus, weight data and root rot assessment data were not compared amongst the four nutrient temperature regimes. Both lettuce seedling production and lettuce growth in the treatments (three inoculated and one control treatment) were subject to these other seasonal differences, as well as temperature.

A nutrient solution temperature maintained continuously at 34°C appeared to be too high for satisfactory lettuce growth (Figure 4-3 compared with Figure 4-5). Unhealthy lettuces were observed in all treatments, including the control plants. However, lettuces inoculated with *Pythium* and the combination of *Pythium* and *Phytophthora* at 34°C were badly infected (Table 4-5). Davis *et al.* (1997) reported that the optimum temperature for germination and growth of lettuce is 20-25°C. The optimum growth temperature of *Pythium* sp. in agar culture was found to be 30°C, although it still grew at 40°C (Chapter 2, Figure 2-11). The optimum temperature for zoospore production in *Pythium* is 25°C; however, some *Pythium* spp. can release zoospores at 35°C (Gold & Stanghellini 1985). The present study indicates that a nutrient solution temperature of 34°C provides a favourable environment for *Pythium* infection of lettuce roots and the development of root rot disease.

Phytophthora appeared to be not pathogenic at temperatures of 34°C (continuously heated) (Table 4-5). This temperature could be too high for the growth and reproduction of *Phytophthora*. The optimum growth temperature of *Phytophthora* sp. in agar culture was found to be 25°C, but only a very small amount of growth could occur at 35°C (Chapter 2, Figure 2-12). Several studies have reported that the optimum growth temperature of *Phytophthora* is 24-25°C and 25°C appears to be the optimum temperature for release of zoospores for the majority of species (Hutton & Forsberg 1991; Erwin & Ribeiro 1996).

It is important to note that no obvious disease symptoms on leaves and roots appeared on any inoculated or control plants at 16-17°C (Figure 4-5) even when zoospores of *Pythium* and *Phytophthora* were found in or on the cells of the root systems of inoculated plants (Figure 4-6). Furthermore, there were no significant differences between any of the treatments as regards the four growth parameters measured (Table 4-6). The disease index for root assessment was significantly greater for *Pythium*-inoculated plants compared to control plants (Table 4-7), but this did not cause a significant reduction in lettuce growth. Studies have found that root rot disease of spinach plants grown in hydroponic systems was not economically important at temperatures below 20°C (Bates & Stanghellini 1984; Stanghellini & Rasmussen 1994). *Pythium* and *Phytophthora* can be isolated from healthy lettuce roots at nutrient solution temperatures less than 20°C (Chapters 2 and 3) and grown in culture. At these temperature regimes, however, zoospore production or the number of zoospores released and the subsequent level of infection were not enough to cause disease symptoms. However, temperatures less than 20°C are more favourable for lettuce growth (Davis *et al.* 1997) than pathogen growth, so plants could resist infection given the smaller number of pathogen propagules and/or slower pathogen growth (Agrios 2005). A study on cucumber plants grown in soilless culture found that an isolate belonging to *Pythium* group F was a minor pathogen and that roots could recognise the pathogen and start to produce defence structures, such as numerous papillae at sites of attempted penetration, so that the pathogen could not go further than the endodermis (Rey *et al.* 1996). However, other evidence was provided that *Pythium* group F was capable of colonising cucumber roots and damaging the cell walls (Rey *et al.* 1996). Favrin *et al.* (1988) reported that, under stress conditions and physiological changes,

minor pathogens, such as *Pythium* spp. causing crown rot of cucumber, could multiply and spread causing cell damage and severe root degradation.

Significant yield reduction was found in all four growth parameters (both wet and dry weights of both roots and shoots) in plants inoculated with *Pythium* and the combination of *Pythium* and *Phytophthora* at a nutrient solution temperature of 34°C for 10 hours followed by 18-20°C (Table 4-8). Tesoriero and Cresswell (1995) similarly showed that the severity of yield loss and damage to lettuce plants was in general greater when roots had been dipped in a water bath for two hours at temperatures between 30°C and 40°C and then inoculated with *Pythium* sp. or *Phytophthora* sp. before being returned to the hydroponic system. However, unlike Tesoriero and Cresswell (1995), the present study found that plants inoculated with *Phytophthora* were not significantly different from the controls as regards two growth parameters measured (wet shoot weight and dry shoot weight) (Table 4-8). Another study from Europe showed that there was no significant increase in disease symptoms after lettuce roots were dipped in a water bath for 6 minutes at 42°C and then inoculated with two *Pythium* species before being returned to the hydroponic system (Funck-Jensen & Hockenhull 1983). These differences between the three studies could be attributable to the different times of exposure to high temperatures and/or the use of *Pythium* and *Phytophthora* isolates of differing virulence. Plants inoculated with *Pythium* had a 26% reduction in yield (wet shoot weight) (Table 4-8) without showing visible symptoms such as wilt and/or root rot when grown at a nutrient solution temperature of 34°C for 10 hours followed by 18-20°C (Figure 4-8). Stanghellini and Kronland (1986) reported that *Pythium dissotocum* was responsible for yield reduction in the absence of any visible root rot symptoms when they inoculated lettuce plants in a glasshouse at 23 to 36°C (mean 28°C). The yield reduction in the present study could have occurred because roots exposed initially to a high temperature (34°C) could have allowed the pathogen to enter the roots and damage them, but when the temperature was decreased to 18-20°C, the plants could have recovered to some extent.

In this study, only four selected temperature regimes were investigated. Further studies should investigate the effect of nutrient solution temperatures between 17°C and 24°C to determine the critical temperature at which infection and disease development occurs. This recommendation is similar to the suggestion made previously in Chapter 3 that

further studies are needed in hydroponic farms over the August-November period, when mean monthly maximum air temperatures start to increase above 18°C. Based on the findings of the study reported in the present chapter, lettuce grown at 16-17°C are not significantly affected by root rot disease, even in the presence of *Pythium*. However, it may be that a nutrient solution temperature slightly higher than this, but less than 24-27°C, would also be satisfactory for avoiding root rot disease, with the added advantage of being closer to the 20-25°C optimum temperature for lettuce growth.

To investigate further the links between temperature and root rot disease development, it would be useful to study temperature regimes incorporating typical day-night fluctuations in late spring and early summer. This would enable more precise predicitions as to when root rot disease is likely to first develop as a significant problem in hydroponic lettuce farms, as temperatures start to increase in spring and summer. Moreover, to assist hydroponic farms in their management of root rot disease, it would be worthwhile to examine, in greater detail than in the present study, the effects on disease of development various periods of high temperature exposure, followed by a returen to cooler temperatures.

The present study used only one representive isolate of *Pythium* and one of *Phytophthora* to provide inoculum for experiments. Other isolates of *Pythium* and *Phytophthora* obtained from hydroponic lettuce farms should be investigated to determine whether similar results are obtained across several isolates of both genera.

5 CHARACTERISATION AND IDENTIFICATION OF *PYTHIUM* SPP. ISOLATED FROM LETTUCE ROOTS BY MORPHOLOGICAL AND MOLECULAR TECHNIQUES

5.1 Introduction

The genus *Pythium* was created by Pringsheim in 1858 with the description of *Pythium monospermum* Pringsh. as the type species (Martin 1992). More than 200 species have been described worldwide (Mathew *et al.* 2003; Abdelghani *et al.* 2004), but only 120 species have been given names (Dick 1990) and the rest were placed in five groups (F, T, G, P and HS) (van der Plaats-Niterink 1981; Dick 1990). Members of the genus *Pythium* are now classified as fungal-like organisms in the phylum Oomycota in the kingdom Chromista and not in the kingdom Fungi. The fungal-like organisms have mycelium lacking septa and both molecular and biochemical studies suggest that they are closer to algae (Abdelghani *et al.* 2004) than true fungi. However, these organisms infect plants in similar ways to true fungi (Agrios 2005). The phylum Oomycota contains some of the most economically important plant pathogens such as those belonging to the genera *Pythium*, *Phytophthora* and *Aphanomyces* (Deacon 2006).

The main and traditional methods for identifying *Pythium* species are based on morphological and physiological studies (van der Plaats-Niterink 1981). The most widely used keys for identification of *Pythium* spp. are those by Middleton (1943), Waterhouse (1967 & 1968), van der Plaats-Niterink (1981), who described 87 species, and Dick (1990), who described 120 species. The main features used for morphological identification are sporangial size and shape, production of zoospores, formation and morphological features of oospores, the number and shape of antheridia and the way in which antheridia attach to oogonia (van der Plaats-Niterink 1981; Dick 1990; Martin 1992). *Pythium* isolates that fail to produce oogonia are identified to group level as belonging to group F, T, G, P or HS based on their sporangial shape and form, as well as the presence of hyphal swelling. However, morphological identification becomes difficult and time consuming because of the similarity in morphological features amongst the different groups of species and intraspecific morphological variation

frequently observed in different field isolates (van Os 2003). Incorrect identification could lead to control strategies and methods that are ineffective. The main physiological features used for identification of *Pythium* species are those based on cardinal temperatures (minimum, optimum and maximum) (van der Plaats-Niterink 1981).

More recently, molecular techniques based on several DNA methods have been developed to identify *Pythium* spp. and also to understand the relationships between species (Bruns *et al.* 1991; Levesque *et al.* 1998; Levesque & De Cock 2004). Most of these methods involve amplifying a fragment from the internal transcribed spacer (ITS) region of rDNA by polymerase chain reaction (PCR). This is a highly variable region and has a large number of characters that can be used to identify organisms, including Oomycetes, to the species level (Matsumoto *et al.* 1999; Paul & Masih 2000; Paul 2001; Mathew *et al.* 2003).

In recent years, numerous papers have described PCR using short arbitrary primers, Random Amplified Polymorphic DNA (RAPD) (Williams *et al.* 1990), as a useful method for genetic mapping and identification. This method has been used successfully to distinguish between 20 isolates of *Pythium aphanidermatum* obtained from cucumber or water from cucumber greenhouses and four isolates of *P. deliense*, two isolates of *P. ultimum*, two isolates of *P. irregulare* and one isolate of *P. paroecandrum* (Herrero & Klemsdal 1998).

Restriction fragment length polymorphism (RFLP) analysis of amplified ribosomal DNA has been widely used and is a powerful technique to identify *Pythium* species and study the populations within a species or between different species (Chen 1992; Wang & White 1997; Harvey *et al.* 2000 & 2001). Chen (1992) was able to distinguish between three heterothallic *Pythium* species by using RFLPs. Moreover, RFLPs were used to study the variation among 34 isolates of *Pythium irregulare* obtained from cereal, medic and sub-clover hosts; it was found that isolates formed groups based on their host but not on their geographical origins (Harvey *et al.* 2001). Recently, Scott *et al.* (2005) studied 130 *Pythium* isolates obtained from soil and diseased table beet (*Beta vulgaris* var. *vulgaris*) plants using RFLP analysis with *Hha*I, *Hinf*I and *Mbo*I restriction enzymes of the PCR amplified ITS region of rDNA. They found that this method was able to group these isolates into three genotype groups.

Sequence analysis of the ITS region of rDNA has also been used to identify and study the relationships among isolates of *Pythium* species (Paul 2000; Bailey *et al.* 2002; Mathew *et al.* 2003; Schurko *et al.* 2003; Levesque & De Cock 2004; Paul 2004). Scott *et al.* (2005) sequenced the ITS 1, 5.8S rDNA and ITS 2 for three groups of *Pythium* isolates obtained from table beet in the Lockyer Valley in Queensland and compared these sequences to the databases in GenBank. They recorded that group Lockyer Valley *Pythium* A (LVP A) was identical to the corresponding sequence from *P. aphanidermatum*, group LVP B was identical to the corresponding sequence from *P. ultimum* and group LVP C was identical to the corresponding sequence from *P. dissotocum.*

The technique of reverse dot blot hybridization, based on an array of species-specific amplified fragments or oligonucleotides derived from the ITS region, has also been used for molecular studies. This technique was used to identify *Pythium aphanidermatum*, *P. ultimum*, *P. acanthicum* and *Phytophthora cinnamomi* (Levesque *et al.* 1998).

Other methods have been used to study the variation among populations within a species. Garzon *et al.* (2005) used amplified fragment length polymorphism (AFLP) fingerprinting to characterise plant pathogenic *Pythium* spp. and intraspecific populations. As well, the method of inter simple sequence repeat (ISSR) markers has been used to study population variation in plant species (Archak *et al.* 2003) and some fungi (Grunig *et al.* 2001; Meng & Chen 2001). However, the ISSR technique was found to be more effective and economical than the AFLP technique in detecting genetic variation in *Phialophora gregata* (Meng & Chen 2001) and more effective than the RAPD technique as well (Tymon & Pell 2005). Recently, the ISSR technique was used to study the variation amongst populations of *Phytophthora cinnamomi* in New South Wales, Australia (Pongpisutta 2005). To date, no studies have been reported using the ISSR technique to study intraspecific variation in *Pythium* species.

Pythium spp. are very important pathogens causing disease and yield losses in vegetables (Agrios 2005; Deacon 2006), with the main diseases being seed rot, seedling damping-off and root rot. *Pythium* spp. can be found in most hydroponic systems around the world, including those producing lettuce (Zinnen 1988; Tesoriero & Cresswell 1995), tomato (Vanachter *et al.* 1983), cucumber (Stanghellini *et al.* 1988;

Cherif & Belanger 1992), capsicum (Harris 1999) and spinach (Stanghellini *et al.* 1984). Most vegetables grown hydroponically appear to be affected by *Pythium* spp., especially as regards their root systems (Stanghellini & Rasmussen 1994). *Pythium* spp. were reported causing root disease on tomato grown in hydroponic systems (NFT) in Jersey (UK) (Davies 1980). Stanghellini *et al.* (1988) provided the first report of *Pythium intermedium* as a pathogen of hydroponically grown cucumber in England.

In hydroponic lettuce, *Pythium* spp. are the main pathogens that can affect seedlings and root systems (Stanghellini & Rasmussen 1994). Stanghellini and Kim (1998) first reported root rot of hydroponically grown lettuce caused by *Pythium myriotylum* in a commercial production system in the Virgin Islands, USA. In South Africa, *Pythium irregulare* and other *Pythium* spp. were isolated from roots of lettuce grown in hydroponic systems (Labuschagne *et al.* 2002). Other researchers have shown that *Pythium* spp. cause damping-off and root rot in lettuce grown in hydroponic systems (Stanghellini & Kronland 1986; Funck-Jensen & Hockenhull 1983; Hockenhull & Funck-Jensen 1983).

In Australia, *Pythium* spp. were reported on crops grown in hydroponic systems. Tesoriero *et al.* (1991) reported that *Pythium aphanidermatum* was found on cucumber and capsicum crops grown in soilless systems in New South Wales (NSW). Moreover, they also noted that *Pythium afertile*, *P. coloratum, P. myriotylum* and *P. vexans* were recorded on lettuce grown in soilless systems.

The aims of this study were to:

- group and identify all *Pythium* isolates obtained from roots of hydroponically grown lettuce during two farm-based surveys (see Chapters 2 and 3) by using morphological, physiological and molecular techniques, specifically PCR-RFLP analysis of the ITS region of rDNA;

- sequence the ITS region of rDNA for selected isolates to confirm the identifications based on morphological and physiological studies; and

- determine the variation within populations of *Pythium* isolated from lettuce roots at different sample times, from different cultivars and at different hydroponic farms in the Sydney and Central Coast areas by using the ISSR technique.

5.2 Materials and methods

5.2.1 Isolation of *Pythium* spp. from lettuce roots

5.2.1.1 Recovery of *Pythium* cultures

Pythium species were isolated from roots of apparently healthy and diseased lettuce grown in hydroponic systems from two major lettuce-producing areas in and near Sydney (Leppington and the Central Coast of NSW). *Pythium* isolates were obtained from three different lettuce cultivars (Baby Cos, Red Oak and Brown Mignonette) at four sampling times (out of a possible five) over an 11 month period (May 2003 to March 2004) from one farm (Leppington 1). Isolates were also obtained from four different lettuce cultivars (Baby Cos, Red Oak, Green Oak and Brown Mignonette) sampled five times over an 11 month period (May 2004 to March 2005) from four different farms (Leppington 1 and 2, and Central Coast 1 and 2). Root systems were washed under tap water and root pieces were plated on *Pythium* selective media (PYSA) (Appendix 1). Petri dishes were incubated in the dark at 25°C ± 1°C for 2 days (van der Plaats-Niterink 1981). Cultures were identified as *Pythium* spp. by morphological examination using a light microscope (Olympus CX41RF, Olympus Optical, Philippines).

5.2.1.2 Hyphal tip isolation

Pythium cultures were transferred to water agar (WA) (Appendix 1) and incubated at 25°C ± 1°C in the dark for 1 day. Hyphal tips were carefully transferred under a stereomicroscope and subcultured onto potato carrot agar (PCA) (Appendix 1) and incubated at 25°C ± 1°C for 5 days in the dark prior to further study. Working stocks of the cultures were maintained in sterile distilled water at 4°C. Representative cultures of each of the 81 isolates obtained were stored in sterile distilled water in McCartney bottles at room temperature.

5.2.2 Morphological studies

5.2.2.1 Colony characteristics on different agar media

Colony characteristics of all *Pythium* spp. isolates (81) were investigated. Petri dishes (90 mm) containing 20 mL of corn meal agar (CMA), PCA and potato dextrose agar (PDA) (Appendix 1) were inoculated with a 5 mm diameter core taken from the edge of

an actively growing 3-day-old culture on PCA incubated at 25°C ± 1°C in the dark. The core was placed upside down in the centre of each plate and the plates were incubated in the dark at 25°C ± 1°C. Colony morphology was noted after 3 to 5 days incubation and compared to the colony morphology types outlined in van der Plaats-Niterink (1981). Five replicates per isolate were examined for this study. Isolates were separated into three groups based on the colony characteristics.

5.2.2.2 Morphological characteristics using light microscopy

For further study, 15 *Pythium* isolates were chosen, based on colony characteristics, to represent all *Pythium* isolates. Microscopic characteristics, including sexual structures, appressoria and hyphal swellings, were studied in samples from cultures grown at 25°C ± 1°C on PCA. Petri dish agar cultures were scanned by light microscopy (Olympus CX41RF, Olympus Optical, Philippines) with bright field illumination at 100x and 200x total magnification. Slides were made from these cultures, stained with lacto-phenol cotton blue (20 g of phenol crystals, 20 mL of lactic acid, 40 mL of glycerol, 0.05 g of cotton blue stain, and 20 mL of distilled water, according to Parija and Prabhakar (1995)) and examined using the same microscope. Measurements were made of hyphal diameters, oogonia, oospores and oospore wall thicknesses at 400x total magnification using an eyepiece micrometer. Thirty to fifty measurements per isolate were made of these structures. Other features studied were: the number, shape and arrangement of antheridia; the size and shape of oogonial projections (spines); and the shape and abundance of appressoria forming at the points of contact with the Petri dish.

Sporangia and zoospores were induced from 2- to 4-day-old cultures growing on PCA. Rectangular pieces of agar culture (20 mm square) were placed in plastic Petri dishes and flooded with 20 mL sterile distilled water. The dishes were incubated at 4°C for 1-3 hours, then at room temperature (25°C ± 1°C); the water was changed hourly for the first three hours. Sporangial development was observed using light microscopy at 100x and 200x total magnification at each water change. If no zoospores were observed after the first 24 hours, cultures were left for 5 days at room temperature (25°C ± 1°C) and re-examined periodically. If no zoospore production was observed in this period they were treated as such in the taxonomic keys used.

5.2.3 Physiological studies

5.2.3.1 Colony growth rates at different temperatures and optimum, minimum and maximum temperatures

Fifteen selected isolates from the three groups were inoculated centrally on a Petri dish (90 mm diameter) containing 20 mL PCA with a 5 mm diameter plug taken from the edge of actively growing 3-day-old cultures on PCA incubated at 25°C ± 1°C in the dark. The growth temperatures were 5, 10, 15, 20, 25, 30, 35, 40 and 45°C. Five replicates were used for each isolate at each temperature. The colony diameters of the cultures were recorded at 24 and 48 h in two directions perpendicular to each other. The colony growth in millimetres over a 24 h period was calculated using the mean colony diameters at 24 and 48 h. The data for growth at different temperatures were analysed with Tukey's Highest Significant Difference (HSD) tests using a one-way analysis of variance (ANOVA).

5.2.4 Molecular studies

5.2.4.1 DNA extraction

The mycelium of all isolates obtained for *Pythium* (81 isolates) was cultured on Spezieller Nährstoffarmer Agar (SNA) medium (Appendix 1) by transferring a small section of an actively growing 3-day-old culture on PCA incubated at 25°C ± 1°C in the dark. Cultures for DNA extraction were incubated at 25°C ± 1°C in the dark. Once the mycelial mat had grown to 3-4 cm in diameter (approximately 1 g), it was harvested for genomic DNA extraction. DNA was purified using a variation of the FastPrep (QBiogene, USA) system. Approximately 1 g of mycelium was taken and placed in a multimix FastDNA tube containing a spherical ceramic ball and lysing garnet; 1 mL of extraction buffer (Appendix 6) was added. The tubes were placed in the FastPrep system and shaken at 5.5 speed setting for 35 s.

The cellular debris was pelleted by centrifugation (Model EBA 12, Rotor 1412, Hettich, Germany) at 12,000 revolutions per minute (rpm) for 5 min and the supernatant (750 µL) was transferred to a sterile 1.5 mL tube containing 125 µL of protein precipitation solution (PPS) (Appendix 6) and then centrifuged at 12,000 rpm for a further 5 min.

The supernatant (700 µL) was then transferred to a sterile tube containing 700 µL binding matrix (Appendix 6) and mixed gently on a rotator for 10 min followed by centrifugation for 15 s at 10,000 rpm. The supernatant was discarded without disturbing the pellet.

The resulting pellet was washed with 800 µL salt ethanol wash solution (SEWS) (Appendix 6). The wash solution was gently pipetted up and down to resuspend the pellet. The DNA/binding matrix was then re-pelleted by spinning at 10,000 rpm for 10 s. The SEWS was decanted and the pellet was allowed to dry at room temperature before being resuspended in 200 µL sterile Tris-EDTA buffer (TE buffer) (Appendix 6) to elute the DNA. The mixture was centrifuged at 14,000 rpm for 2 min and 160 µL of the DNA solution was transferred to a new tube. The DNA was stored at -20°C until needed.

5.2.4.2 Gel electrophoresis of genomic DNA

Genomic DNA was evaluated using agarose gel electrophoresis to assess approximate concentration and purity. Two percent agarose gel (w/v) was prepared by dissolving 2 g agarose (Agarose 1, AMRESCO, USA) in 98 mL 1 × Tris-Borate-EDTA (1x TBE) buffer (Appendix 6) and melting in a microwave until a clear solution was observed. The gel was loaded with 5 µL of each DNA sample and 1 µL of dye (Appendix 6). A molecular weight marker was added to one lane as a size standard (New England Biolabs (NEB), USA). Five microlitres of sterile distilled water were added to one lane as a control.

Electrophoresis was carried out at 80 V, in 1 × TBE buffer (1x TBE) for approximately 90 min after which the gel was stained in an ethidium bromide solution (according to Sambrook and Russell (2001)) before being viewed on a UV transilluminator and photographed using a digital camera.

5.2.4.3 PCR amplification of ITS region of ribosomal DNA

Seven *Pythium* isolates, selected to represent the three groups found on the basis of colony characteristics, were compared using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the ITS region of ribosomal

DNA. The ITS region was PCR amplified (PCR model CG-1, Corbett Research, Australia) to ensure sufficient template for one digestion reaction.

The PCR mix was prepared to a final volume of 50 μL containing reaction buffer [10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 0.1% Triton X-100 (NEB)], 0.20 mM dNTPs (50 μM each of dATP, dCTP, dTTP and dGTP), 100 μg bovine serum albumin (BSA), 0.5 μM DLH64 primer (Table 5-1), 0.5 μM DLH65 primer (Table 5-1), and 1 U Taq Polymerase (NEB). The components were added in the order given above, mixed well, and 49 μL aliquots of this mixture was distributed into sterile 0.5 mL Eppendorf tubes. After this, 100 ng of total genomic template was added to the mixture. A negative control (PCR water blank, no DNA template) was used in every experiment to test for the presence of DNA contamination of reagents and reaction mixtures.

The PCR was performed using the following parameters: initial denaturation period at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and terminated with a final extension at 72°C for 10 min. After amplification, the samples were used immediately. The length of the PCR amplification products was estimated by comparing them to a standard 100 bp DNA ladder (NEB) using gel electrophoresis as in section 5.2.4.2.

Table 5-1 Sequences of primers used for PCR amplification of the ITS region of rDNA.

Primer	Sequence
DLH64	5'-ACTTTCCACGTGAACCGTTTC-3'
DLH65	5'-ATATCAGGTCCAATTGAGATG-3'

5.2.4.4 Restriction enzyme digestion for restriction fragment length polymorphism (RFLP) analysis

The PCR-amplified ITS region of rDNA was digested with the restriction enzymes *Eco*RI, *Mse*I, *Msp*I and *Xmn*I. The digested DNA fragments were prepared by adding each restriction enzyme to a 1.5 mL Eppendorf tube on ice. Each digestion reaction had

a final volume of 30 µL, with the mixture for restriction enzyme *Eco*RI comprising 1x NE Buffer 2 (NEB) (Appendix 6) and 10 U *Eco*RI, for *Mse*I consisting of 1x NE Buffer 2, 1x BSA and 3 U of *Mse*I, for *Msp*I comprising 1x NE Buffer 2 and 5 U *Msp*I, and for *Xmn*I containing 1x NE Buffer 2, 1x BSA and 6 U *Xmn*I.

The tubes were incubated in a water bath for all four restriction enzymes (Table 5-2). Each digested product (15 µL) was electrophoresed on 2% agarose, stained with ethidium bromide and observed under UV light. The resulting RFLP profiles were used to compare the isolates of *Pythium*.

Table 5-2 The restriction enzymes used for digestion of the ITS region of rDNA of *Pythium*, together with their recognition sequences and the incubation temperature and time.

Enzyme	Recognition sequence*	Incubation temperature (°C)	Incubation time (h)
*Eco*RI	G▼AATTC	37	3
*Mse*I	T▼TAA	37	3
*Msp*I	C▼CGG	37	3
*Xmn*I	GAANN▼NNTTC	37	3

*N= A, C, G or T

5.2.4.5 Sequencing the ITS region of ribosomal DNA

In order to identify selected *Pythium* isolates based on morphological description and the ITS-RFLP technique used above, the ITS region of a selection of thirteen isolates was sequenced. PCR products were purified using a JET Quick PCR Purification Kit (Genomed, Germany) as per the manufacturers instructions with DNA being eluted using the maximum concentration method. One microlitre of the cleaned PCR products was electrophoresed in a 1.5% agarose gel in 1x TBE (pH 8.0) buffer and stained with ethidium bromide. The amount of the cleaned PCR products was estimated by comparing them to a standard 100 bp DNA ladder (NEB).

Dideoxyterminators were incorporated into the cleaned products by PCR amplification using a BigDye Terminator v1.1/3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sixty nanograms of cleaned PCR product was added to a mixture of 3.5 µL of 5× BigDye Sequencing Buffer, 1 µL Ready Reaction Premix and 0.5 µL of 20 µM primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′). Sterile water was added to give a final

volume of 20 µL. PCR conditions were as follows: 25 cycles of denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec and extension at 60°C for 4 minutes. Samples were held at 4°C until further use. Unincorporated dideoxyterminators were removed by cleaning the product of this PCR as follows: 16 µL sterile water, 20 µL of the PCR product and 64 µL 95% ethanol were added together in a tube. Tubes were vortexed and left at room temperature for 15 min before centrifugation at 14,000 rpm for 20 min. Then supernatant was removed and 250 µL of 70% ethanol was added to wash the pellet. Tubes were vortexed and centrifuged at 14,000 rpm for 10 min. The supernatant was removed and the alcohol wash was repeated. The DNA pellet was left to dry for 1-2 hours in a laminar flow cabinet and samples were sent for analysis (UNSW Automated Sequencing Facility, NSW Australia).

Obtained sequences were edited using the program BioEdit 7.0.5.2 (Hall 1999) and were compared to known GenBank sequences using BLAST (basic local alignment search tool) analysis (Altschul *et al.* 1990).

5.2.4.6 Inter Simple Sequence Repeat (ISSR) analysis for population studies

Eighty-one isolates of *Pythium* spp. were used for the population study. Four anchored oligonucleotide primers, AC, AG, CGA, and GT (Table 5-3), were used for amplification. PCR was performed in a total volume of 15 µL containing 1x Thermo Pol Buffer [20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8], 1 mM each dNTP, 2 µM primer (Table 5-3), 0.5 U Taq DNA polymerase and 20 ng template DNA. Cycling conditions were as follows: 94°C for 5 min, 30 cycles of 94°C for 20 s, annealing temperature for 30 s (different annealing temperatures were tested to determine the optimum temperature) (Table 5-3) and 72°C for 3 min. The reaction was completed with a 10 min extension at 72°C and samples were held at 25°C.

Table 5-3 Sequences of primers used for PCR amplification for the ISSR analysis of *Pythium* isolates.

Primer	Sequence A	Annealing temperature
AC – primer	5′ DBD (AC)7 3′	49°C
AG – primer	5′ (AG)8G 3′	55°C
CGA – primer	5′ DHB (CGA)5 3′	58°C
GT – primer	5′ VHV (GT)7G 3′	57°C

A The following designations are used for the degenerate sites: B (C, G or T), D (A, G or T), H (A, C or T) and V (A, C or G).

Seven microlitres of the PCR samples were separated on 1.5% agarose gels and stained with ethidium bromide for visualisation as above. Electrophoresis was performed at constant power, 80 V, for 90 min. Data on the ISSR markers were scored as the presence (1) or absence (0) of a band. The combined ISSR data was created containing all scorable bands observed. Dice's coefficient of similarity was calculated, and the similarity matrix was used to group species by cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) with the numerical taxonomy system (NTSYSpc) version 2.1 software (Adams & Rohlf 2000).

5.3 Results

5.3.1 Recovery of *Pythium* spp. cultures

Eighty-one *Pythium* isolates were recovered from hydroponic lettuce roots during the two farm-based surveys carried out from May 2003 to March 2004 (first survey, see Chapter 2) and May 2004 to March 2005 (second survey, see Chapter 3) (Table 5-4). Four isolates were recovered in the first survey from a single farm at Leppington (see Chapter 2) identified as Leppington 1. In the second survey, 77 isolates were recovered from four farms, including two farms at Leppington (Leppington 1 and Leppington 2) and two farms on the Central Coast of NSW (Central Coast 1 and Central Coast 2). These 77 isolates comprised 20 from the farm Leppington 1, 20 from Leppington 2, 20 from Central Coast 1 and 17 from Central Coast 2 (see Chapter 3; Table 5-4). Isolates of *Pythium* spp. were obtained from all times (when appropriate selective media was used) and all four lettuce cultivars sampled in the second survey.

Table 5-4 indicates the isolates used for various types of study, the results of which are presented in following sections. Also shown is the group to which each isolate belongs, based on colony characteristics on three media (see following section 5.3.2.1).

Table 5-4 Details of isolates of *Pythium* obtained from roots of different hydroponic lettuce cultivars during two farm-based surveys carried out in and near Sydney involving one farm in the first survey (Leppington 1; see Chapter 2) and four farms in the second survey (Leppington 1 and 2, and Central Coast 1 and 2; see Chapter 3).

Isolate no.	Isolate code*	Isolation time	Hydroponic farm	Lettuce cultivar	Types of study^	Group†
1	PA03L1	Aug-03	Leppington 1	Not recorded	C, I, M, S, P, R	i
2	PN03L1	Nov-03	Leppington 1	Not recorded	C, I	ii
3	PJ04L1	Jan-04	Leppington 1	Not recorded	C, I, R	i
4	PMr04L1	Mar-04	Leppington 1	Not recorded	C, I	i
5	PM04L1Mig	May-04	Leppington 1	Mignonette	C, I, R	ii
6	PM04L1GO	May-04	Leppington 1	Green Oak	C, I	i
7	PM04L1BC	May-04	Leppington 1	Baby Cos	C, I	i
8	PM04L1RO	May-04	Leppington 1	Red Oak	C, I	i
9	PM04L2GO	May-04	Leppington 2	Green Oak	C, I	ii
10	PM04L2BC	May-04	Leppington 2	Baby Cos	C, I	iii
11	PM04L2Mig	May-04	Leppington 2	Mignonette	C, I	ii
12	PM04L2RO	May-04	Leppington 2	Red Oak	C, I	i
13	PM04CC1BC	May-04	Central Coast 1	Baby Cos	C, I	iii
14	PM04CC1RO	May-04	Central Coast 1	Red Oak	C, I	i
15	PM04CC1Mig	May-04	Central Coast 1	Mignonette	C, I	i
16	PM04CC1GO	May-04	Central Coast 1	Green Oak	C, I	ii
17	PM04CC2Mig	May-04	Central Coast 2	Mignonette	C, I	i
18	PM04CC2RO	May-04	Central Coast 2	Red Oak	C, I, R	ii
19	PM04CC2BC	May-04	Central Coast 2	Baby Cos	C, I, M, S, P	i
20	PM04CC2GO	May-04	Central Coast 2	Green Oak	C, I	i
21	PA04L1Mig	Aug-04	Leppington 1	Mignonette	C, I, R	iii
22	PA04L1RO	Aug-04	Leppington 1	Red Oak	C, I	ii
23	PA04L1GO	Aug-04	Leppington 1	Green Oak	C, I	i
24	PA04L1BC	Aug-04	Leppington 1	Baby Cos	C, I	iii
25	PA04L2GO	Aug-04	Leppington 2	Green Oak	C, I	i
26	PA04L2Mig	Aug-04	Leppington 2	Mignonette	C, I	ii
27	PA04L2BC	Aug-04	Leppington 2	Baby Cos	C, I, M, S, P	i
28	PA04L2RO	Aug-04	Leppington 2	Red Oak	C, I	iii
29	PA04CC1BC	Aug-04	Central Coast 1	Baby Cos	C, I	ii
30	PA04CC1GO	Aug-04	Central Coast 1	Green Oak	C, I	i
31	PA04CC1Mig	Aug-04	Central Coast 1	Mignonette	C, I	ii
32	PA04CC1RO	Aug-04	Central Coast 1	Red Oak	C, I	i
33	PA04CC2Mig	Aug-04	Central Coast 2	Mignonette	C, I, M, S, P	iii
34	PA04CC2RO	Aug-04	Central Coast 2	Red Oak	C, I	i
35	PA04CC2BC	Aug-04	Central Coast 2	Baby Cos	C, I	i
36	PA04CC2GO	Aug-04	Central Coast 2	Green Oak	C, I	ii
37	PN04L1GO	Nov-04	Leppington 1	Green Oak	C, I	iii
38	PN04L1RO	Nov-04	Leppington 1	Red Oak	C, I	i
39	PN04L1Mig	Nov-04	Leppington 1	Mignonette	C, I	ii
40	PN04L1BC	Nov-04	Leppington 1	Baby Cos	C, I	iii
41	PN04L2BC	Nov-04	Leppington 2	Baby Cos	C, I	i
42	PN04L2GO	Nov-04	Leppington 2	Green Oak	C, I, M, S, P	ii
43	PN04L2Mig	Nov-04	Leppington 2	Mignonette	C, I	i
44	PN04L2RO	Nov-04	Leppington 2	Red Oak	C, I	iii
45	PN04CC1GO	Nov-04	Central Coast 1	Green Oak	C, I	i
46	PN04CC1RO	Nov-04	Central Coast 1	Red Oak	C, I, M, S, P	ii
47	PN04CC1Mig	Nov-04	Central Coast 1	Mignonette	C, I, M, P	iii
48	PN04CC1BC	Nov-04	Central Coast 1	Baby Cos	C, I	i

49	PN04CC2RO	Nov-04	Central Coast 2	Red Oak	C, I	iii
50	PN04CC2GO	Nov-04	Central Coast 2	Green Oak	C, I	i
51	PN04CC2BC	Nov-04	Central Coast 2	Baby Cos	C, I	ii
52	PJ05L1GO	Jan-05	Leppington 1	Green Oak	C, I	ii
53	PJ05L1RO	Jan-05	Leppington 1	Red Oak	C, I	iii
54	PJ05L1Mig	Jan-05	Leppington 1	Mignonette	C, I	ii
55	PJ05L1BC	Jan-05	Leppington 1	Baby Cos	C, I	iii
56	PJ05L2RO	Jan-05	Leppington 2	Red Oak	C, I, M, S, P	ii
57	PJ05L2BC	Jan-05	Leppington 2	Baby Cos	C, I, M, S, P	i
58	PJ05L2Mig	Jan-05	Leppington 2	Mignonette	C, I, M, S, P	i
59	PJ05L2GO	Jan-05	Leppington 2	Green Oak	C, I	i
60	PJ05CC1RO	Jan-05	Central Coast 1	Red Oak	C, I, M, S, P	i
61	PJ05CC1BC	Jan-05	Central Coast 1	Baby Cos	C, I, R	ii
62	PJ05CC1GO	Jan-05	Central Coast 1	Green Oak	C, I	i
63	PJ05CC1Mig	Jan-05	Central Coast 1	Mignonette	C, I	ii
64	PJ05CC2BC	Jan-05	Central Coast 2	Baby Cos	C, I	iii
65	PJ05CC2RO	Jan-05	Central Coast 2	Red Oak	C, I	i
66	PJ05CC2GO	Jan-05	Central Coast 2	Green Oak	C, I	i
67	PMr05L1RO	Mar-05	Leppington 1	Red Oak	C, I	i
68	PMr05L1Mig	Mar-05	Leppington 1	Mignonette	C, I	i
69	PMr05L1BC	Mar-05	Leppington 1	Baby Cos	C, I, M, P	ii
70	PMr05L1GO	Mar-05	Leppington 1	Green Oak	C, I	iii
71	PMr05L2RO	Mar-05	Leppington 2	Red Oak	C, I, M, S, P	i
72	PMr05L2GO	Mar-05	Leppington 2	Green Oak	C, I	ii
73	PMr05L2Mig	Mar-05	Leppington 2	Mignonette	C, I	iii
74	PMr05L2BC	Mar-05	Leppington 2	Baby Cos	C, I	i
75	PMr05CC1Mig	Mar-05	Central Coast 1	Mignonette	C, I, M, S, P	ii
76	PMr05CC1BC	Mar-05	Central Coast 1	Baby Cos	C, I	ii
77	PMr05CC1RO	Mar-05	Central Coast 1	Red Oak	C, I	i
78	PMr05CC1GO	Mar-05	Central Coast 1	Green Oak	C, I	i
79	PMr05CC2RO	Mar-05	Central Coast 2	Red Oak	C, I, M, S, P, R	i
80	PMr05CC2BC	Mar-05	Central Coast 2	Baby Cos	C, I	i
81	PMr05CC2GO	Mar-05	Central Coast 2	Green Oak	C, I	iii

*Components of isolate code are genus/ month/ year/ farm/ cultivar as follows –
P: *Pythium*
M: May; A: August; N: November; J: January; Mr: March
03: 2003; 04: 2004; 05: 2005
L1: Leppington 1; L2: Leppington 2; CC1: Central Coast 1; CC2: Central Coast 2
BC: Baby Cos; GO: Green Oak; Mig: Brown Mignonette; RO: Red Oak

^Types of study are as follows –
Morphological studies: C: Colony shape; M: Microscopic
Physiological studies: P: Growth at different temperatures
Molecular studies: R: RFLP; S: Sequencing; I: ISSR

†Group refers to isolates belonging to one of three groups (i, ii, or iii) based on colony characteristics on three media (section 5.3.2.1)

5.3.2 Morphological studies

5.3.2.1 Colony characteristics on different agar media

Eighty-one *Pythium* isolates were divided into three groups based on colony characteristics on three media (PCA, PDA and CMA):

(i) Forty isolates were found with submerged mycelium and a somewhat radiate pattern with white mycelia on PCA, aerial mycelium with tufted chrysanthemum rosette on PDA and submerged mycelium with a rosette pattern on CMA (Figure 5-1).

(ii) Twenty four isolates had submerged mycelium pattern with white mycelia and a distinct rosette on PCA, submerged mycelium without a special pattern on PDA and aerial white mycelia on CMA (Figure 5-1).

(iii) Seventeen isolates had colonies with submerged mycelium a vaguely radiate and rosette to chrysanthemum pattern on PCA, submerged mycelium with a rosette pattern on PDA and submerged mycelium without a special pattern on CMA (Figure 5-1).

Eight isolates from the first group, five isolates from the second group and two isolates from the last group were selected for further morphological characterisation using light microscopy and physiological studies.

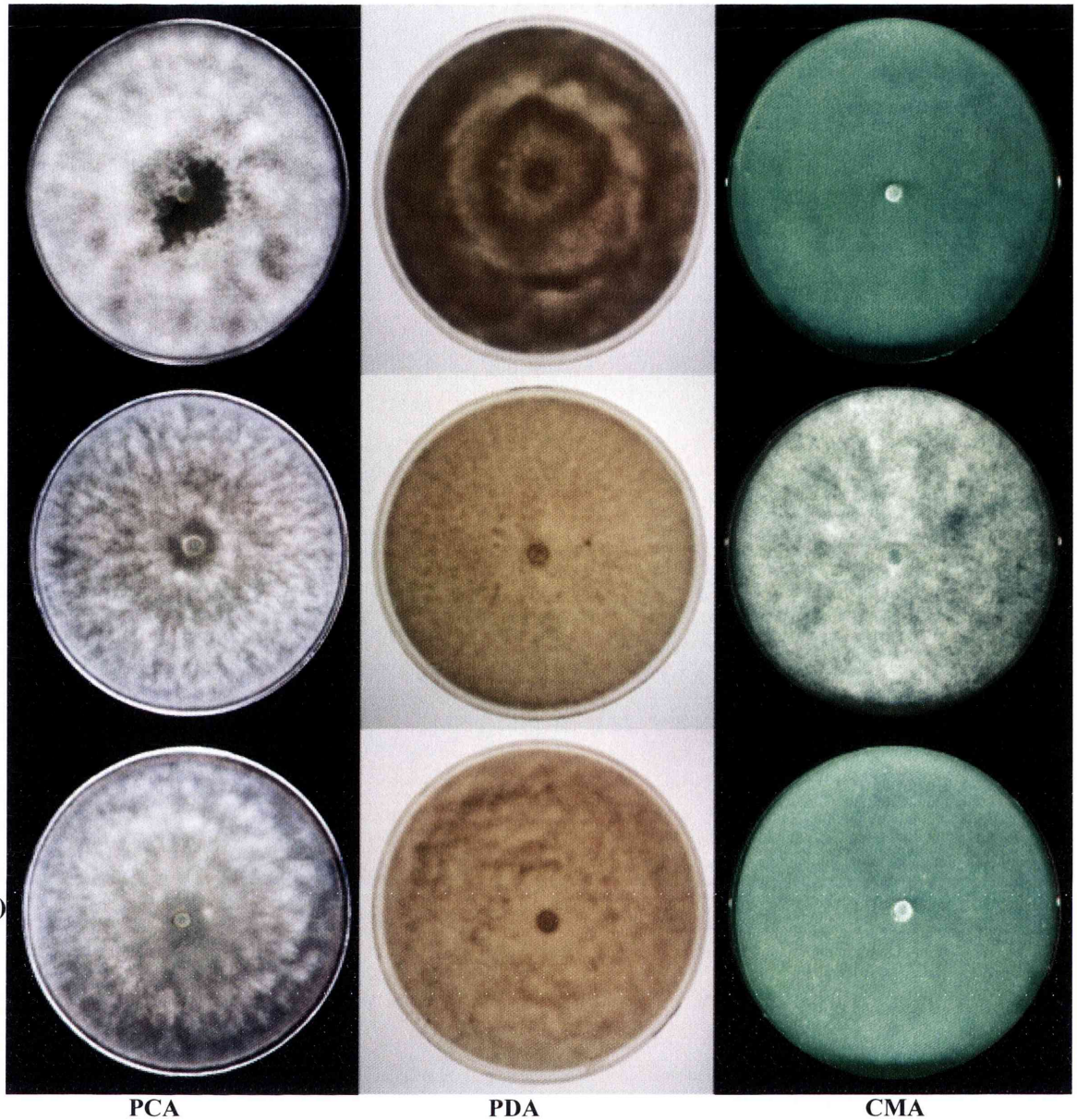

Figure 5-1 Representative cultures of each of the three major groups of isolates of *Pythium* spp. (group (i) top row; (ii), middle row; and (iii), bottom row) grown on three different media (column 1, PCA; column 2, PDA; and column 3, CMA). Cultures were grown in the dark at 25°C ± 1°C for 5 days.

5.3.3 Morphological characteristics using light microscopy

5.3.3.1 Group (i)

Hyphae were up to 8 μm wide (Figure 5-2 a). Appressoria were club or sausage shaped (Figure 5-2 b). Sporangia were filamentous, non-inflated and forming dendroid structures (Figure 5-2 c). Vesicles up to 60 μm were formed on cultures flooded with water (Figure 5-2 d and e). Released zoospores encysted (10-14 μm diameter) and germinated within 24 h. Oogonia were produced in one isolate (PMr05CC2RO) and had smooth walls, were in both terminal and intercalary positions, and were 25-30 μm in diameter (average 26.4 μm) (Figure 5-2 f, g and h). Antheridia were 1(-2) mostly stalked and diclinous (Figure 5-2 f, g and h). Oospores were aplerotic (with a space between the oogonial wall and oospore wall) and slightly plerotic (without a space between the oogonial wall and oospore wall), 22-25 μm in diameter (average 23.5 μm). Oospore walls were lilac in colour and 3-4 μm thick.

Thirty nine isolates of *Pythium* in this group were identified as belonging to *Pythium* group F according to the key of van der Plaats-Niterink (1981). Isolate PMr05CC2RO was identified as *Pythium coloratum* according to the same key.

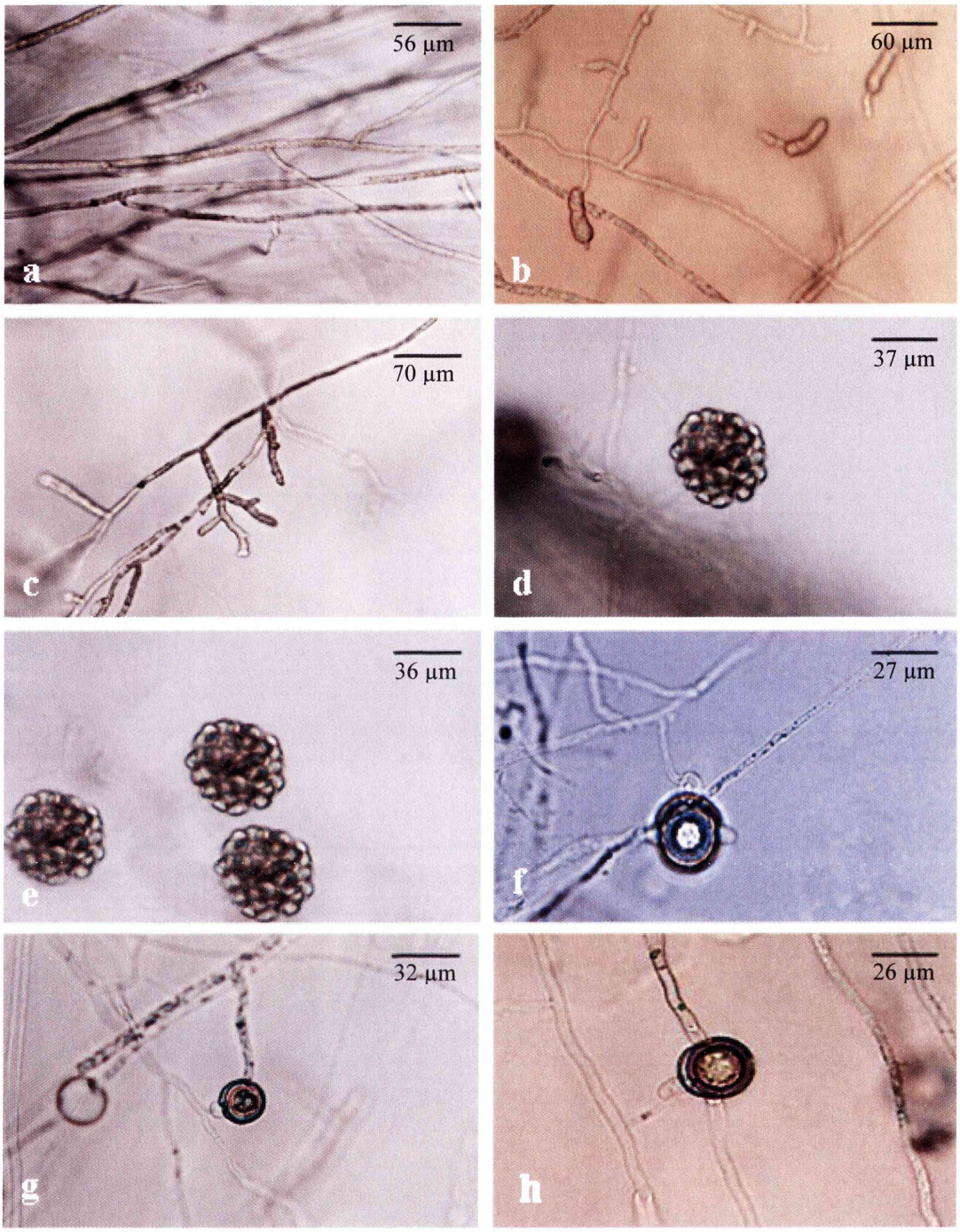

Figure 5-2 Structures of *Pythium* from group (i) isolates.

(a) hyphae;
(b) appressoria;
(c) sporangia;
(d, e) mature vesicles;
(f, g, h) oospores with antheridia.

5.3.3.2 Group (ii)

Hyphae were up to 6 μm wide (Figure 5-3 a). Appressoria were club shaped (Figure 5-3 b). Sporangia were filamentous, non-inflated and forming dendroid structures (Figure 5-3 c). Vesicles up to 55 μm were formed on cultures flooded with water (Figure 5-3 d and e). Released zoospores encysted (10-12 μm diameter) (Figure 5-3 f) and germinated within 24 h. Oogonia were not produced in this group. All isolates of *Pythium* in this group were identified as belonging to *Pythium* group F according to the key of van der Plaats-Niterink (1981).

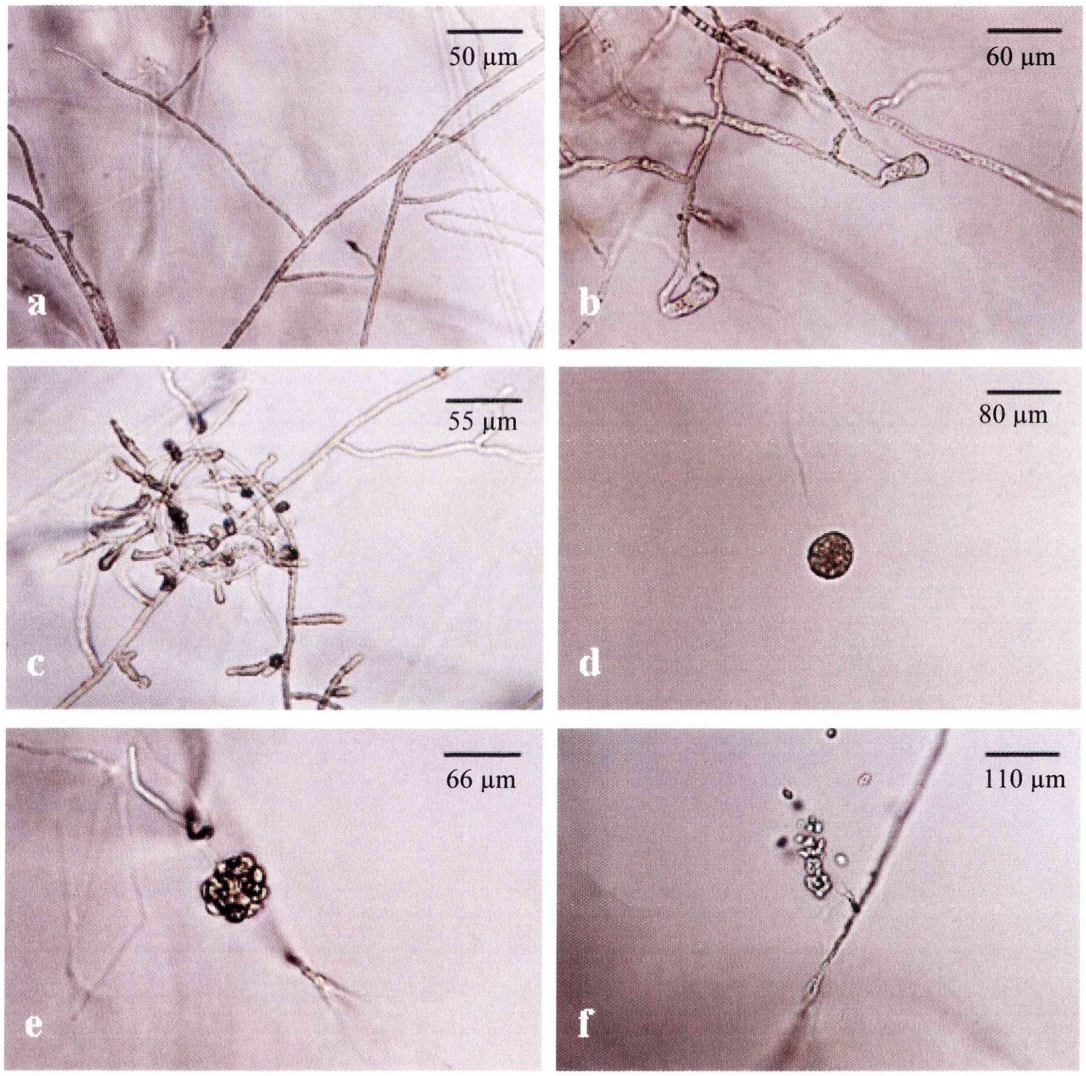

Figure 5-3 Structures of *Pythium* from group (ii) isolates.

(a) hyphae;
(b) appressoria;
(c) sporangia;
(d) young vesicle;
(e) mature vesicle;
(f) vesicle releasing zoospores.

5.3.3.3 Group (iii)

Hyphae were up to 8 µm wide (Figure 5-4 a). Appressoria were club shaped and more often they were attached to the walls of the Petri dishes (Figure 5-4 b). Sporangia were filamentous, non-inflated and forming dendroid structures (Figure 5-4 c). Vesicles up to 60 µm were formed on cultures flooded with water (Figure 5-4 d). Released zoospores encysted (10-14 µm diameter) and germinated within 24 h. Oogonia were not produced in this group. All isolates of *Pythium* in this group were identified as belonging to *Pythium* group F according to the key of van der Plaats-Niterink (1981).

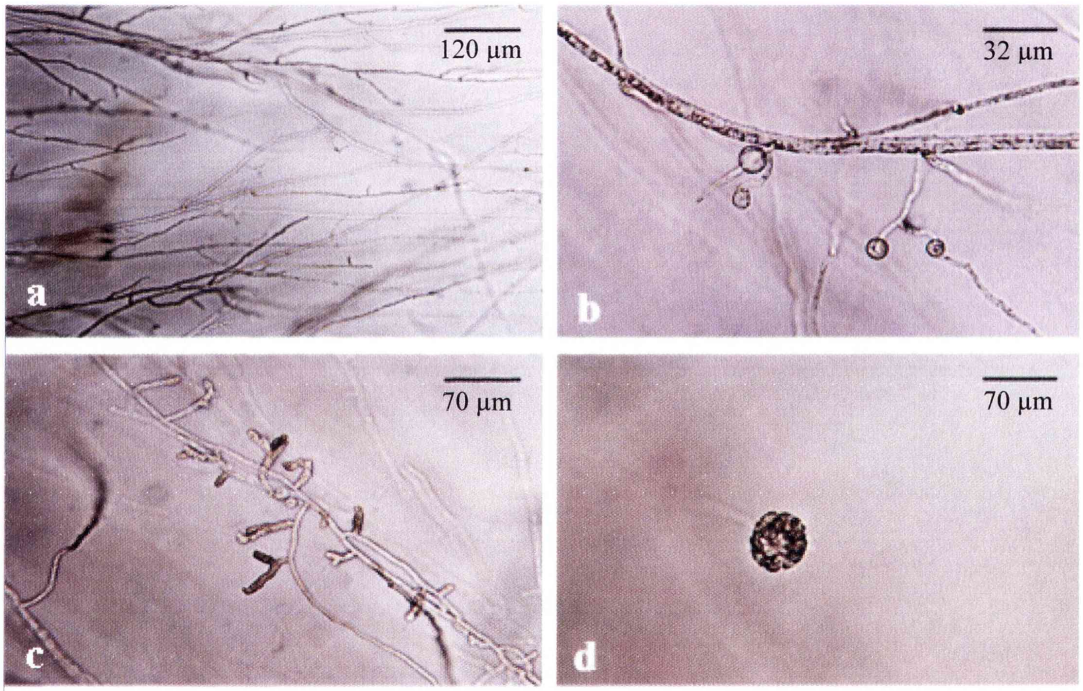

Figure 5-4 Structures of *Pythium* from group (iii) isolates.

(a) hyphae;
(b) appressoria;
(c) sporangia;
(d) almost mature vesicle.

5.3.4 Physiological studies

5.3.4.1 Colony growth rates at different temperatures and optimum, minimum and maximum temperatures

Colonies of all 15 isolates, from all three groups (section 5.3.3), grew approximately 20-21 mm in 24 hours (from 24 to 48 hours) on PCA at 25°C ± 1°C (Table 5-5). No significant differences (at $P \leq 0.01$) were found in growth rate amongst isolates at temperatures between 10°C and 45°C (Table 5-5) (Appendix 7 a). However, significant differences in growth rate were found between isolates at a temperature of 5°C ($P \leq 0.01$). Isolate PMr05CC1Mig (from group (ii)) had the lowest growth rate of all isolates tested at 5°C (Table 5-5). The highest growth rate at 5°C occurred in isolate PJ05L2RO (from group (ii)) (Table 5-5). These significant differences in growth at 5°C were not linked to sampling times, farms or cultivars.

All of the 15 isolates of *Pythium* tested had an optimum growth temperature of 30°C and grew well at temperatures of 25°C and 35°C (Figure 5-5). Within the limits of the temperatures available for testing, the lowest temperature at which growth occurred for 15 isolates tested was 5°C (Figure 5-5). All 15 isolates tested grew at a temperature of 40°C but not at 45°C (Figure 5-5).

Table 5-5 Mean growth (mm) in 24 h of 15 isolates of *Pythium* incubated at nine different temperatures from 5°C to 45°C on PCA in the dark. Growth was measured between 24 and 48h. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.01$. n = 5 for each isolate at each temperature. (Appendix 7 a.)

Isolate code* and group†	Temperature (°C)								
	5	10	15	20	25	30	35	40	45
PMr05CC1Mig (ii)	0.96 a	3.62 a	7.05 a	14.49 a	20.72 a	22.05 a	18.89 a	1.00 a	0
PMr05L2RO (i)	1.02 ab	3.84 a	7.23 a	14.45 a	20.78 a	22.14 a	19.14 a	1.08 a	0
PMr05L1BC (ii)	1.07 abc	3.75 a	6.94 a	13.67 a	20.78 a	22.22 a	19.18 a	1.04 a	0
PJ05CC1RO (i)	1.08 abc	3.69 a	6.98 a	13.48 a	20.67 a	22.22 a	19.18 a	1.04 a	0
PA04L2BC (i)	1.09 abc	3.69 a	6.99 a	13.32 a	20.69 a	22.01 a	18.61 a	1.06 a	0
PMr05CC2RO (i)	1.09 abc	3.50 a	6.98 a	13.32 a	20.23 a	21.87 a	18.64 a	1.08 a	0
PN04L2GO (ii)	1.12 abc	3.68 a	7.06 a	14.75 a	20.48 a	22.19 a	19.02 a	1.00 a	0
PN04CC1RO (ii)	1.12 abc	3.72 a	7.04 a	13.57 a	20.41 a	21.99 a	18.78 a	1.02 a	0
PA04CC2Mig (iii)	1.13 abc	3.64 a	7.15 a	14.22 a	20.56 a	21.91 a	18.94 a	1.06 a	0
PN04CC1Mig (iii)	1.13 abc	3.82 a	6.98 a	13.95 a	20.56 a	22.19 a	19.02 a	1.00 a	0
PJ05L2Mig (i)	1.18 abc	3.86 a	6.99 a	14.14 a	20.79 a	22.22 a	18.64 a	1.10 a	0
PM04CC2BC (i)	1.19 abc	3.64 a	6.99 a	14.49 a	20.68 a	22.13 a	19.10 a	1.00 a	0
PA03L1 (i)	1.19 abc	3.50 a	7.34 a	14.39 a	20.23 a	21.92 a	18.78 a	1.06 a	0
PJ05L2BC (i)	1.24 bc	3.68 a	7.20 a	14.45 a	20.34 a	22.10 a	18.97 a	1.08 a	0
PJ05L2RO (ii)	1.30 c	3.72 a	7.12 a	13.73 a	20.69 a	22.06 a	19.08 a	1.06 a	0

* Isolate code – see footnotes to Table 5-4

† Isolate group – see footnotes to Table 5-4

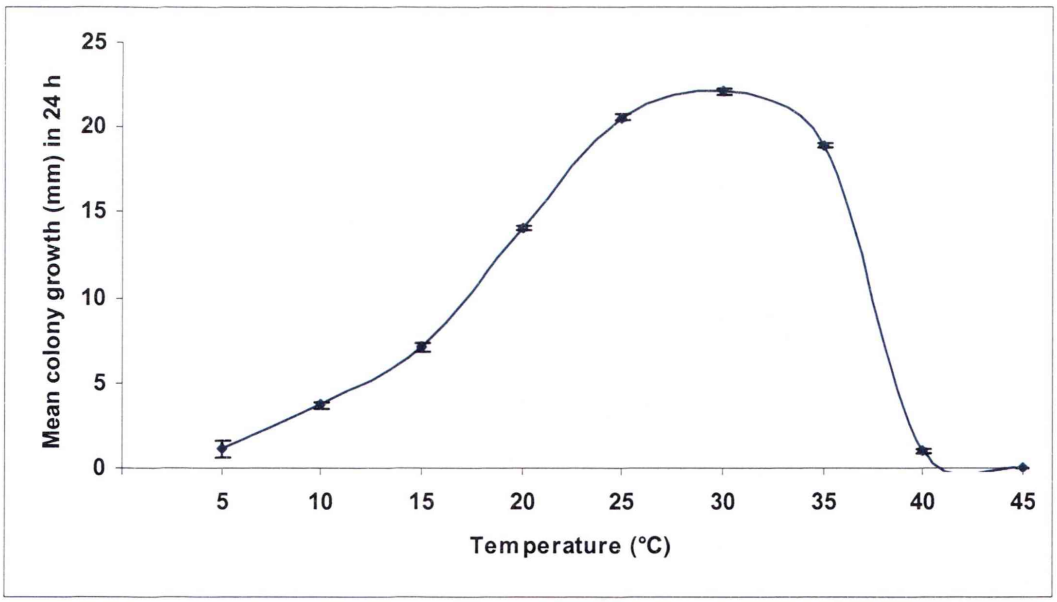

Figure 5-5 Mean colony growth (mm) in 24 h of 15 isolates of *Pythium* grown on PCA media at a range of temperatures from 5°C to 45°C in the dark. Colony growth was measured between 24h and 48h. Bars are standard error of the mean. n=5 for each isolate at each temperature.

5.3.5 Molecular studies

5.3.5.1 Comparison of isolates by PCR-RFLP of ITS region of rDNA

The technique of PCR-RFLP of the ITS region of rDNA was used to compare seven selected isolates from the three different groups of *Pythium* (i, ii, iii). The ITS PCR amplified fragment was observed to be about 850bp for *Pythium* isolates (Figure 5-6).

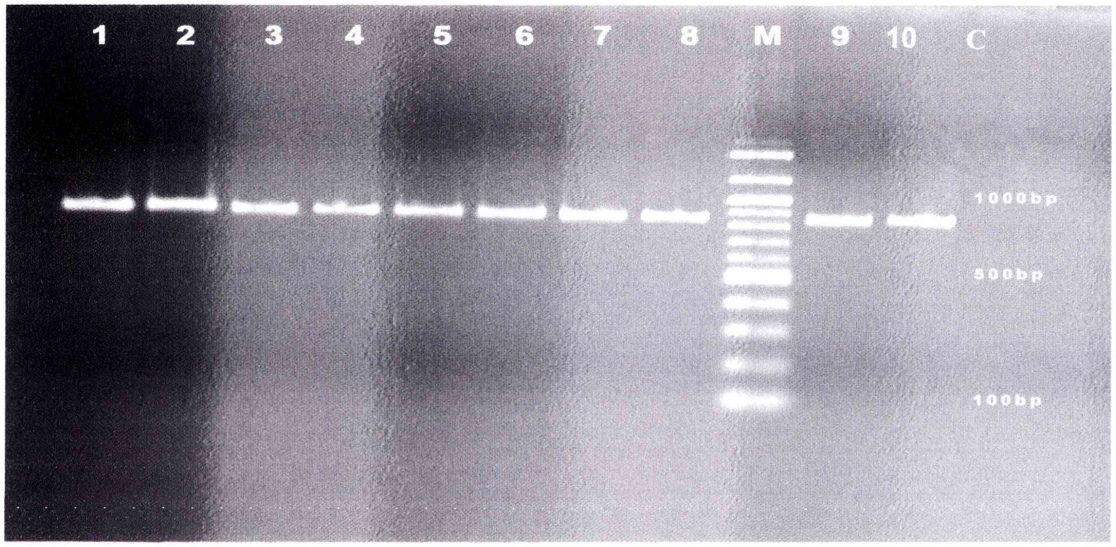

Figure 5-6 Representative gel of PCR amplification products of ITS region of rDNA for ten *Pythium* isolates.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) –

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PM04L1Mig (ii)
(4) PM04CC2RO (ii)
(5) PA04L1Mig (iii)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(8) PN04L2RO (iii)
(9) PMr05L1GO (iii)
(10) PN04CC1RO (ii)
(C) control reactions
(M) 100 bp ladder

The different restriction enzymes *Eco*RI, *Mse*I, *Msp*I and *Xmn*I yielded different numbers of bands, ranging between 1 and 4 bands (Figure 5-7 and Figure 5-8). No differences were found amongst the isolates tested with the same four restriction enzymes. The *Eco*RI enzyme yielded four bands (900, 890, 600 and 300 bp) (Figure 5-7 a), *Xmn*I yielded one band (885 bp) (Figure 5-7 b), *Mse*I yielded four bands (290, 160, 120 and one band less than 100 bp) (Figure 5-8 c) and *Msp*I yielded one band (875 bp) (Figure 5-8 d). No PCR products were obtained in any of the control reactions omitting template DNA (see "C" lane in Figure 5-7 a and b and Figure 5-8 a and b).

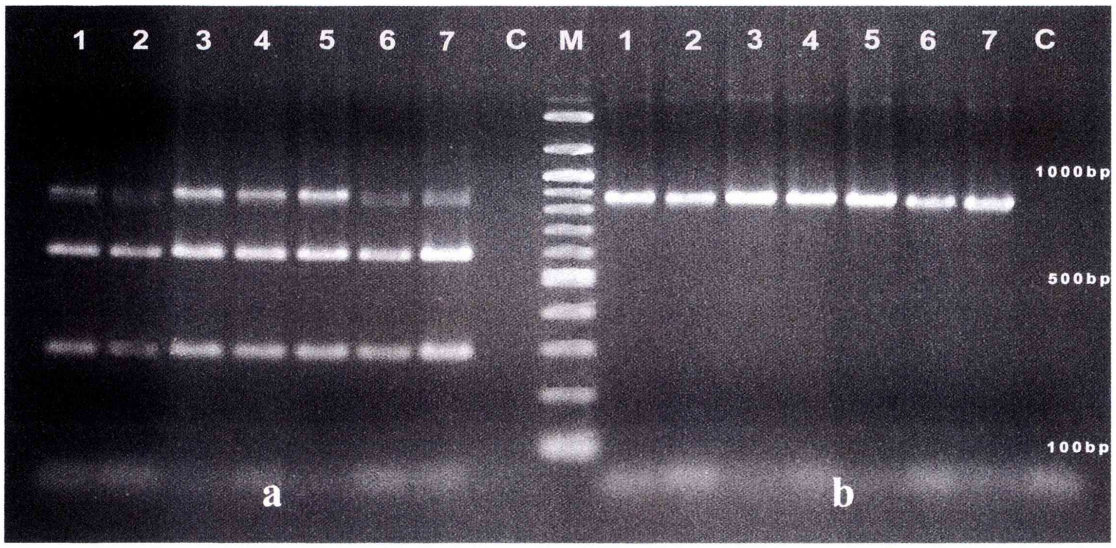

Figure 5-7 Gel photo of digested products of ITS amplification with restriction enzymes *Eco*RI (a) and *Xmn*I (b) for seven *Pythium* isolates.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) –

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PM04L1Mig (ii)
(4) PM04CC2RO(ii)
(5) PA04L1Mig (iii)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(C) control reaction
(M) 100 bp ladder

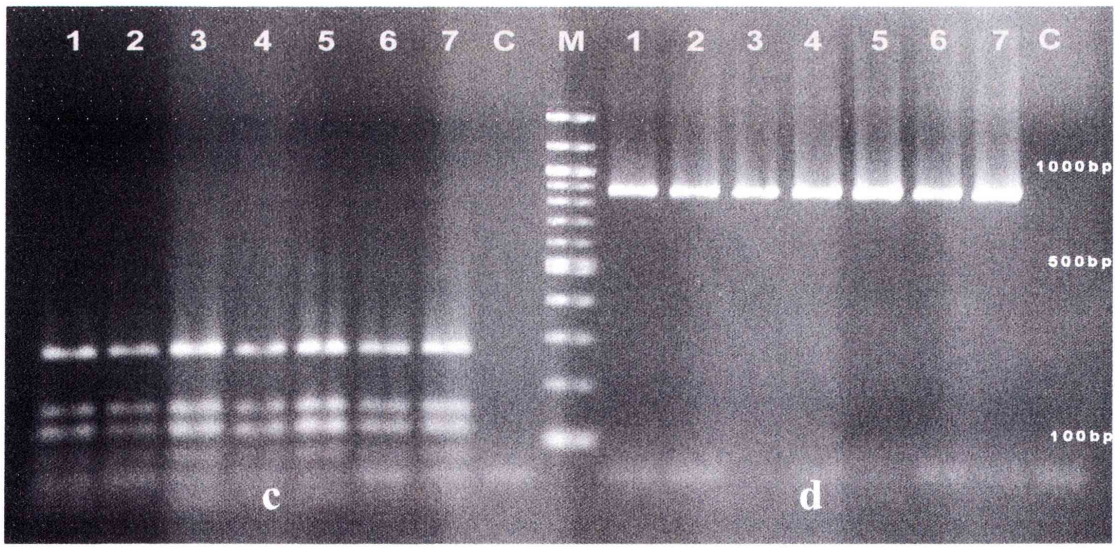

Figure 5-8 Gel photo of digested products of ITS amplification with restriction enzymes *Mse*I (c) and *Msp*I (d) for seven *Pythium* isolates.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) –

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PM04L1Mig (ii)
(4) PM04CC2RO(ii)
(5) PA04L1Mig (iii)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(C) control reaction
(M) 100 bp ladder

5.3.5.2 Sequence of ITS region of rDNA

Sequences of nine isolates obtained in this study matched *Pythium* sp. PTS 2003 isolates (AY445122) from GenBank (Table 5-6). The sequence of isolate PMr05L2RO was 833 bp in length and was 99% identical to the corresponding sequence from *Pythium* sp. PTS 2003 (AY445122) (Scott *et al.* 2005) with 1 nucleotide gap (see Table 5-6 for the other eight isolates). However, the sequence of isolate PJ05L2Mig was 657 bp in length and was 99% identical to the corresponding sequence from *Pythium* sp. JN-7a (DQ235226) with no nucleotide gaps. Moreover, the sequence of isolate PJ05L2RO was 724 bp in length and was 99% identical to the corresponding sequence from *Pythium* sp. JN-6 (DQ232767) with no nucleotide gaps. The sequence of one isolate (isolate PJ05CC1RO) was 503 bp in length and was 100% identical to the corresponding sequence from *Pythium dissotocum* (AF271228) with no nucleotide gaps. The sequence of another isolate (isolate PN04CC1RO) was 471 bp in length and was 97% identical to the corresponding sequence from four *Pythium* spp. (Table 5-6) with no nucleotide gaps. The sequence of one isolate (isolate PN04L2GO) was 303 bp in length and was 99% identical to the corresponding sequence from two *Pythium* spp. (Table 5-6).

Table 5-6 Sequence length (bp) of ITS region of rDNA for 13 isolates of *Pythium* (Appendix 7 b) from hydroponic lettuce roots and comparison with sequences in GenBank.

Isolate code* and group†	Sequence length (bp)	Match from GenBank (Location)^	GenBank accession number	Identities (%)	Gaps
PMr05L2RO (i)	833	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	1
PA03L1 (i)	1075	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	2
PMr05CC2RO (i)	1060	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PA04CC2Mig (iii)	1056	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PMr05CC1Mig(ii)	961	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PJ05L2BC (i)	811	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PA04L2BC (i)	817	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PM04CC2BC (i)	773	*Pythium* sp. PTS 2003 (LPVC) (Aus)	AY445122	99	0
PJ05L2Mig (i)	657	*Pythium* sp. JN-7a (Ger)	DQ235226	99	0
PJ05L2RO (ii)	724	*Pythium* sp. JN-6 (Ger)	DQ232767	99	0
PJ05CC1RO (i)	503	*Pythium dissotocum* (Aus)	AF271228	100	0
PN04CC1RO (ii)	471	*Pythium lutarium* (UK)	AY598688	97	0
		Pythium dissotocum (USA)	AY598634	97	0
		Pythium coloratum (Aus)	AY598633	97	0
		Pythium diclinum (Mor)	AY666087	97	0
		Pythium PTS 2003 (LPVC) (Aus)	AY445122	97	0
PN04L2GO (ii)	303	*Pythium* sp. JN-7a (Ger)	DQ235226	99	0
		Pythium sp. JN-6 (Ger)	DQ232767	99	0

* Isolate code – see footnotes to Table 5-4

† Isolate group – see footnotes to Table 5-4

^ Aus: Australia; Ger: Germany; UK: United Kingdom and Mor: Morocco.

5.3.5.3 Variation among populations of *Pythium* isolates by using Inter Simple Sequence Repeat (ISSR) analysis

The multiple bands obtained using four primers varied in size from 250 bp to more than 2000 bp. The AC-, AG-, CGA- and GT- primers separated the 81 *Pythium* spp. isolates into 7, 5, 4 and 7 groups respectively. Designation of each fragment was done in order to get a clear identification from DNA fingerprinting when these bands were compared to the marker sizes with the same primers.

5.3.5.3.1 Variation observed using the AC-primer

There were 1-6 polymorphic fragments of AC-amplified products and these divided the isolates into 7 groups (Table 5-7; Figure 5-9). These fragments ranged between 250 and 1300 bp. There were no correlations found between groups and farms or cultivars. However, a slight correlation was found between groups and sampling time, when considered on the basis of cooler season sample times (May and August) compared with warmer season sample times (November, January and March). Group AC1 contains 26 isolates, 17 of which were obtained in May and August. Group AC3 contains eight isolates, five of which were obtained from cold season samples (May and August). On the other hand, group AC4 contains eight isolates, all of which came from warm season samples (November, January and March). As well, group AC5 contains 17 isolates, 14 of which were from warm samples (November, January and March). Other groups contained isolates from both seasons.

Table 5-7 Groups of isolates based on analysis of polymorphic fragments generated by AC ISSR primer. Isolates in bold are shown in Figure 5-9.

Group	Isolate code*	No. of isolates
AC1	**PA03L1**, PM04L2GO, PM04L1RO, PM04L2Mig, PM04L2RO, PM04CC2Mig, PA04L2GO, PA04L2Mig, **PM04CC2RO**, PA04CC1BC, **PJ05CC1Mig**, PA04CC1Mig, **PM04L1Mig**, PN04L1RO, **PJ05CC2BC,** PN04L2Mig, **PJ04L1**, PM04L1BC, PN04L2BC, **PA04CC2GO,** PJ05L1GO, PM04L2BC, PM04L1GO, **PMr05CC1GO**, **PA04L1Mig,** PN04CC1GO	26
AC2	PN04L1BC, PA04L1RO, **PA04L1BC**, **PJ05CC2GO, PMr05CC2RO**	5
AC3	PM04CC1Mig, PMr05CC1BC, PA04CC2RO, PMr05CC2BC, PA04CC1RO, PA04L2RO, PMr05L2Mig, PA04L2BC	8
AC4	PN03L1, **PJ05CC1BC**, PMr05L1Mig, PN04CC2GO, PJ05L1Mig, PJ05L1BC, **PJ05L1RO**, **PJ05L2GO**	8
AC5	PM04CC1RO, PMr05CC2GO, PN04L1GO, PJ05CC1GO, PMr05CC1Mig, PJ05L2RO, PM04CC2GO, PMr05L1BC, PN04CC1BC, PJ05L2Mig, PN04CC2BC, PA04CC2Mig, PJ05CC1RO, PMr05L1RO, PN04L1Mig, **PN04L2GO,** PJ05L2BC	17
AC6	PA04L1GO, PA04CC1GO, PN04CC2RO, PA04CC2BC, PN04CC1Mig, PN04CC1RO, **PN04L2RO**	7
AC7	PMr04L1, PM04CC1BC, **PMr05L1GO**, PMr05L2GO, PM04CC2BC, PJ05CC2RO, PMr05L2BC, PMr05CC1RO, PM04CC1GO, **PMr05L2RO**	10

* Isolate codes as in Table 5-4.

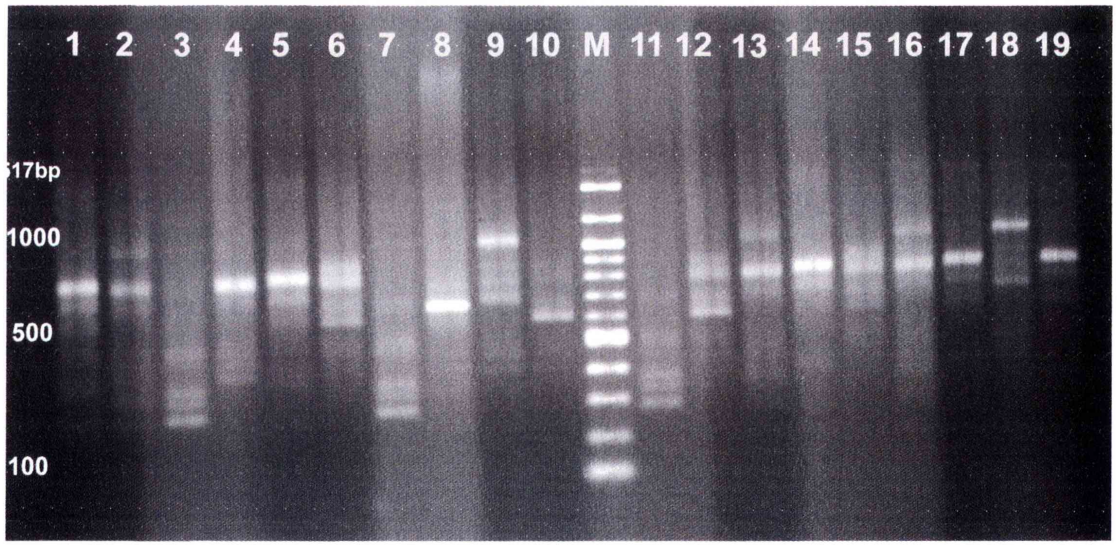

Figure 5-9 ISSR Profiles observed for isolates of *Pythium* using AC-primer.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) -

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PA04L1BC (iii)
(4) PM04CC2RO (ii)
(5) PMr05CC1GO (i)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(8) PN04L2RO (iii)
(9) PMr05L1GO (iii)
(10) PN04L2GO (ii)
(11) PJ05CC2GO (i)
(12) PJ05L2GO (i)
(13) PM04L1Mig (ii)
(14) PA04CC2GO (ii)
(15) PJ05L1RO (iii)
(16) PJ05CC2BC (iii)
(17) PJ05CC1Mig (ii)
(18) PMr05L2RO (i)
(19) PA04L1Mig (iii)
(M) 100 bp ladder

5.3.5.3.2 Variation observed using the AG-primer

The AG primer divided *Pythium* isolates into 5 groups (Table 5-8; Figure 5-10). There were 2-14 polymorphic fragments produced ranging between 250 and 2000 bp. All groups contained isolates from different cultivars, from different sample times or different places (Leppington and Central Coast) (Table 5-8). No correlation was found between groups and cultivars, farms or sample times.

Table 5-8 Groups of isolates based on analysis of polymorphic fragments generated by AG ISSR primer. Isolates in bold are shown in Figure 5-10.

Group	Isolate code*	No. of isolates
AG1	**PA03L1**, PM04L2RO, PM04CC2Mig, PA04L2Mig, PA04L2BC, PN04L1RO, PN04CC1BC, PM04L2G.O, **PMr05CC1GO,** PA04L2GO, **PA04CC2GO**, PA04L2RO, **PA04L1Mig**, PA04CC1Mig, PN04CC1Mig, PN04CC1GO, PA04CC1BC, PN04L1GO, PMr05L1RO, PJ05CC1RO, PN04L2Mig, PJ05L2RO	22
AG2	**PJ04L1**, **PM04L1Mig**, PN04L1BC, **PJ05L1RO**, PJ05L1Mig, PMr05CC2GO, PA04L1RO, PJ05L1GO, PM04L1RO, PA04CC2Mig, PA04CC2RO, PN04L2BC, PM04L2BC, PA04CC1RO, PMr05CC1BC, PMr05CC2BC, PJ05CC1GO, PMr05L1BC, PMr05CC1RO, **PN04L2GO,** PM04CC2BC, PN04CC2RO, PM04CC2GO, **PM04CC2RO**, PA04CC1GO, PM04L1BC, PMr05L2Mig, PN04CC2BC, PM04CC1RO, PM04CC1Mig, PJ05L1BC, PM04CC1BC, PJ05CC2RO, **PJ05L2GO,** PMr05L2GO, **PJ05CC1Mig**, **PMr05L2RO**, **PJ05CC2BC**, **PMr05L1GO**, PM04CC1GO, **PJ05CC1BC**, PMr05L1Mig, PA04CC2BC, PMr05L2BC	44
AG3	PMr04L1, **PJ05CC2GO,** PA04L1GO, PJ05L2Mig, **PMr05CC2RO**, PN04L1Mig, PJ05L2BC, **PA04L1BC**	8
AG4	PN03L1, PN04CC1RO, PN04CC2GO, PM04L1GO, **PN04L2RO**	5
AG5	PM04L2Mig, PMr05CC1Mig	2

* Isolate codes as in Table 5-4.

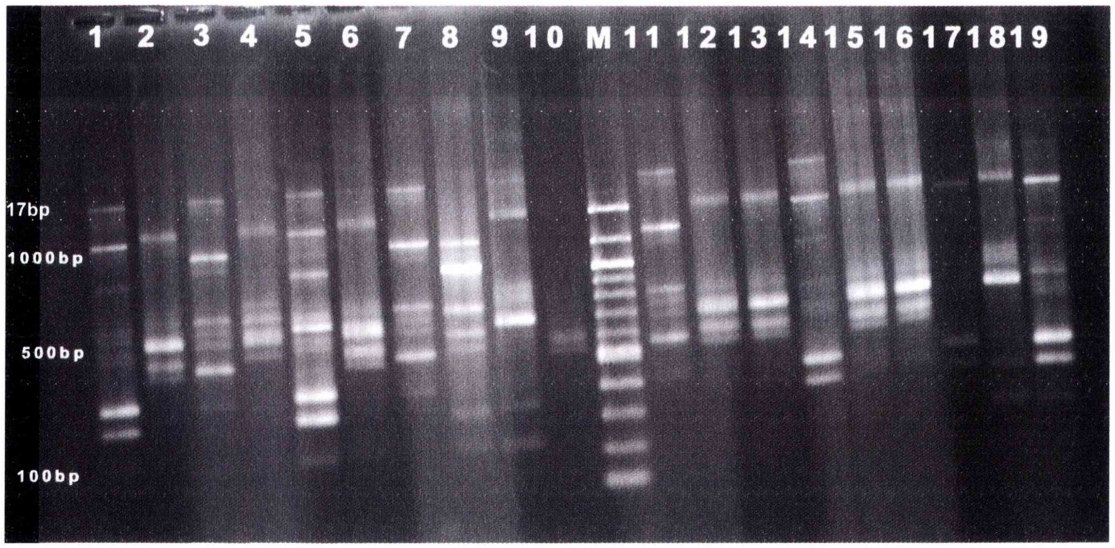

Figure 5-10 ISSR profiles observed for isolates of *Pythium* using AG-primer.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) -

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PA04L1BC (iii)
(4) PM04CC2RO (ii)
(5) PMr05CC1GO (i)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(8) PN04L2RO (iii)
(9) PMr05L1GO (iii)
(10) PN04L2GO (ii)
(11) PJ05CC2GO (i)
(12) PJ05L2GO (i)
(13) PM04L1Mig (ii)
(14) PA04CC2GO (ii)
(15) PJ05L1RO (iii)
(16) PJ05CC2BC (iii)
(17) PJ05CC1Mig (ii)
(18) PMr05L2RO (i)
(19) PA04L1Mig (iii)
(M) 100 bp ladder

5.3.5.3.3 Variation observed using the CGA-primer

Four groups were formed by using the CGA primer (Table 5-9; Figure 5-11). Two to eleven bands were found ranging between 370 and 1700 bp. The first three groups contained 79 isolates collected from different cultivars, at different sample times or different places (Leppington and Central Coast) (Table 5-9). However, one group contained two isolates collected from Central Coast farms (1 and 2) in March 2005 and November 2004.

Table 5-9 Groups of isolates based on analysis of polymorphic fragments generated by CGA ISSR Isolates in bold are shown in Figure 5-11.

Group	Isolate code*	No. of isolates
CGA1	PN04L1RO, PM04L2RO, PA04L2GO, PA04L2BC, PM04CC2Mig, PA04L2Mig, PA04CC1BC, PN04L1GO, **PMr05L2RO,** PN04L2Mig, PM04L2Mig, **PN04L2GO**, PJ05L2RO, PM04L1GO, PA04L2RO, PN04L2BC, **PA04L1Mig,** PMr04L1, PA04L1GO, **PN04L2RO,** PA04CC1GO, **PJ05CC1Mig,** PA04CC1Mig, PN04CC1Mig, PN04CC1BC, PN04CC1GO	26
CGA2	**PA03L1,** PN03L1, **PJ04L1**, PJ05L1Mig, **PJ05L1RO**, PMr05CC1RO, PJ05L1BC, PMr05CC1Mig, PM04L1BC, PMr05CC2BC, PM04CC1BC, **PJ05L2GO**, **PA04L1BC,** PMr05L2GO, **PA04CC2GO**, **PJ05CC2GO,** PJ05CC2RO, **PMr05L1GO**, PM04CC1GO, **PJ05CC1BC**, PMr05L1Mig, **PJ05CC2BC**, PN04CC1RO, PA04L1RO, PN04CC2BC, **PM04L1Mig**, PJ05L1GO, **PM04CC2RO**, PM04L2GO, PMr05L1RO, PJ05CC1RO, PJ05CC1GO, PMr05L1BC	33
CGA3	PN04L1BC, PM04CC1Mig, PA04CC1RO, PJ05L2BC, PM04L2BC, PM04L1RO, PMr05L2Mig, **PMr05CC1GO,** PA04CC2Mig, PA04CC2RO, PJ05L2Mig, PMr05L2BC, PA04CC2BC, **PMr05CC2RO,** PN04CC2RO, PMr05CC2GO, PM04CC2BC, PM04CC2GO, PM04CC1RO, PN04L1Mig	20
CGA4	PN04CC2GO, PMr05CC1BC	2

* Isolate codes as in Table 5-4.

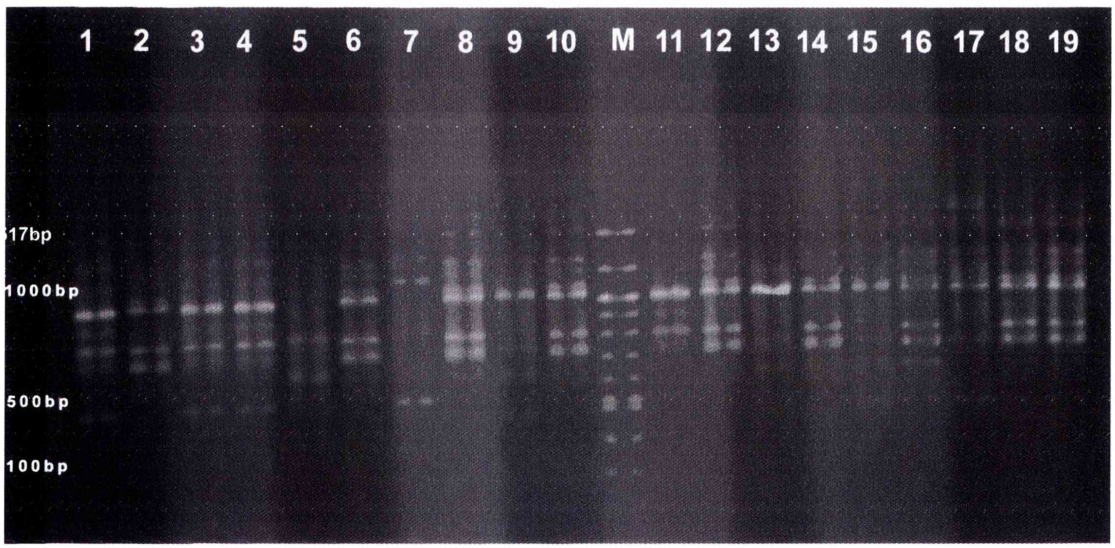

Figure 5-11 ISSR profiles observed for isolates of *Pythium* using CGA-primer.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) -

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PA04L1BC (iii)
(4) PM04CC2RO (ii)
(5) PMr05CC1GO (i)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(8) PN04L2RO (iii)
(9) PMr05L1GO (iii)
(10) PN04L2GO (ii)
(11) PJ05CC2GO (i)
(12) PJ05L2GO (i)
(13) PM04L1Mig (ii)
(14) PA04CC2GO (ii)
(15) PJ05L1RO (iii)
(16) PJ05CC2BC (iii)
(17) PJ05CC1Mig (ii)
(18) PMr05L2RO (i)
(19) PA04L1Mig (iii)
(M) 100 bp ladder

5.3.5.3.4 Variation observed using the GT-primer

One to eight polymorphic fragments of GT-amplified products divided isolates into 7 groups (Table 5-10)with bands ranging between 200 and 1100 bp (Figure 5-12). Two isolates collected from Leppington 1 farm in November 2004 and January 2005 were found in one group (group 7). Group GT6 contains four isolates, three of which were obtained in August. Other isolates were spread amongst six groups without correlation between different cultivars, different sample times or different places (Leppington and Central Coast).

Table 5-10 Groups of isolates based on analysis of polymorphic fragments generated by GT ISSR primer. Isolates in bold are shown in Figure 5-12.

Group	Isolate code*	No. of isolates
GT1	**PA03L1**, PM04L2RO, PM04CC2Mig, PA04L2Mig, **PA04CC2GO**, PA04L2BC, PN04L1RO, PM04L2Mig, **PJ05CC1Mig**, PA04L2GO, **PMr05CC1GO**	11
GT2	**PJ04L1**, PMr05CC1RO, PJ05L1Mig, PM04CC1RO, PJ05L1BC, PMr05L1BC, **PM04CC2RO, PJ05L1RO**, PJ05CC1GO, **PJ05L2GO,** PM04CC1Mig, PM04CC1GO, **PMr05L2RO**, **PJ05CC2BC**, **PMr05L1GO**, **PJ05CC1BC,** PJ05L2BC, PM04CC2BC, PM04CC2GO, PN04L2BC, **PM04L1Mig**, PN04CC2RO, **PN04L2GO**	23
GT3	PN03L1, PM04L1BC, PMr05CC2GO, PN04CC2BC, PMr05L2BC, PM04L1RO, PM04CC1BC, **PA04L1BC**, PMr05CC2BC, PMr05CC1Mig, PMr05CC1BC, **PMr05CC2RO,** PJ05CC2RO, PMr05L2GO, PA04CC1RO, PMr05L1Mig, PM04L2GO, **PJ05CC2GO**	18
GT4	PMr04L1, **PN04L2RO**, PMr05L2Mig, PMr05L1RO, PJ05CC1RO, PA04L1RO, PA04CC2BC	7
GT5	PM04L1GO, **PA04L1Mig**, PA04CC1Mig, PN04L2Mig, PN04CC1RO, PN04CC2GO, PA04CC1BC, PA04CC1GO, PN04CC1GO, PN04CC1Mig, PN04L1GO, PN04CC1BC, PA04L1GO, PN04L1Mig, PJ05L2Mig, PM04L2BC	16
GT6	PA04L2RO, PA04CC2Mig, PA04CC2RO, PJ05L2RO	4
GT7	PN04L1BC, PJ05L1GO	2

* Isolate codes as in Table 5-4.

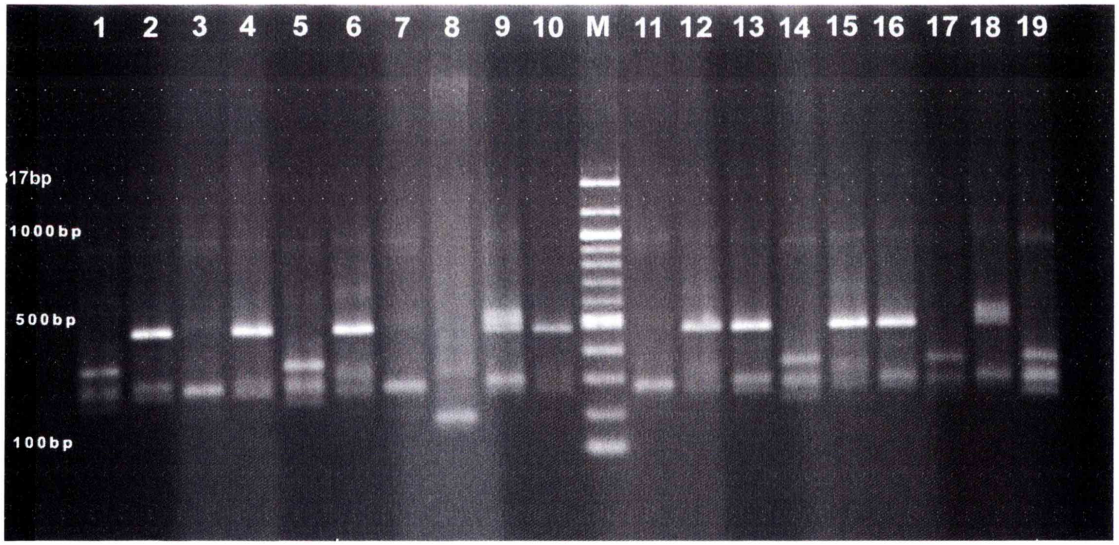

Figure 5-12 ISSR profiles observed for isolates of *Pythium* using GT-primer.

Key to lane numbers, indicating isolate code and group based on colony characteristics (Table 5-4 and section 5.3.2.1) -

(1) PA03L1 (i)
(2) PJ04L1 (i)
(3) PA04L1BC (iii)
(4) PM04CC2RO (ii)
(5) PMr05CC1GO (i)
(6) PJ05CC1BC (ii)
(7) PMr05CC2RO (i)
(8) PN04L2RO (iii)
(9) PMr05L1GO (iii)
(10) PN04L2GO (ii)
(11) PJ05CC2GO (i)
(12) PJ05L2GO (i)
(13) PM04L1Mig (ii)
(14) PA04CC2GO (ii)
(15) PJ05L1RO (iii)
(16) PJ05CC2BC (iii)
(17) PJ05CC1Mig (ii)
(18) PMr05L2RO (i)
(19) PA04L1Mig (iii)
(M) 100 bp ladder

5.3.5.3.5 Variation observed using the four ISSR primers

Data described by the four primers were used to construct an UPGMA dendrogram (Figure 5-13). The estimated similarity by Dice's coefficient within isolates of *Pythium* was based on 78 combined ISSR markers from the four primers. The UPGMA dendrogram showed that there were eleven groups at 50% Dice similarity (Figure 5-13). Group 1 contains 12 isolates, 11 of which were isolated in the May and August 2004 sampling times, which are both in cool seasons (see Chapters 2 and 3). Groups 3 and 4 together contain five isolates obtained from lettuce roots sampled in November 2004 and January 2005, which are both warm season sampling times. Moreover, 13 of the 15 isolates found in group 6 were obtained from January and March 2005 sampling times, which are both in warm seasons. All of the three isolates in groups 10 and 11 were from the November 2004 sampling time. However, group 5, which is the largest group, contains 29 isolates obtained from roots of lettuce cultivars sampled from all five sampling times, which therefore includes both cool season and warm season sampling times.

5.3.6 Storage of representative isolates

Representative isolates belonging to *Pythium* group F and the isolate of *P. coloratum* obtained in the present study have been stored in the collection of NSW Agriculture at Orange, NSW (see Appendix 7 c for details).

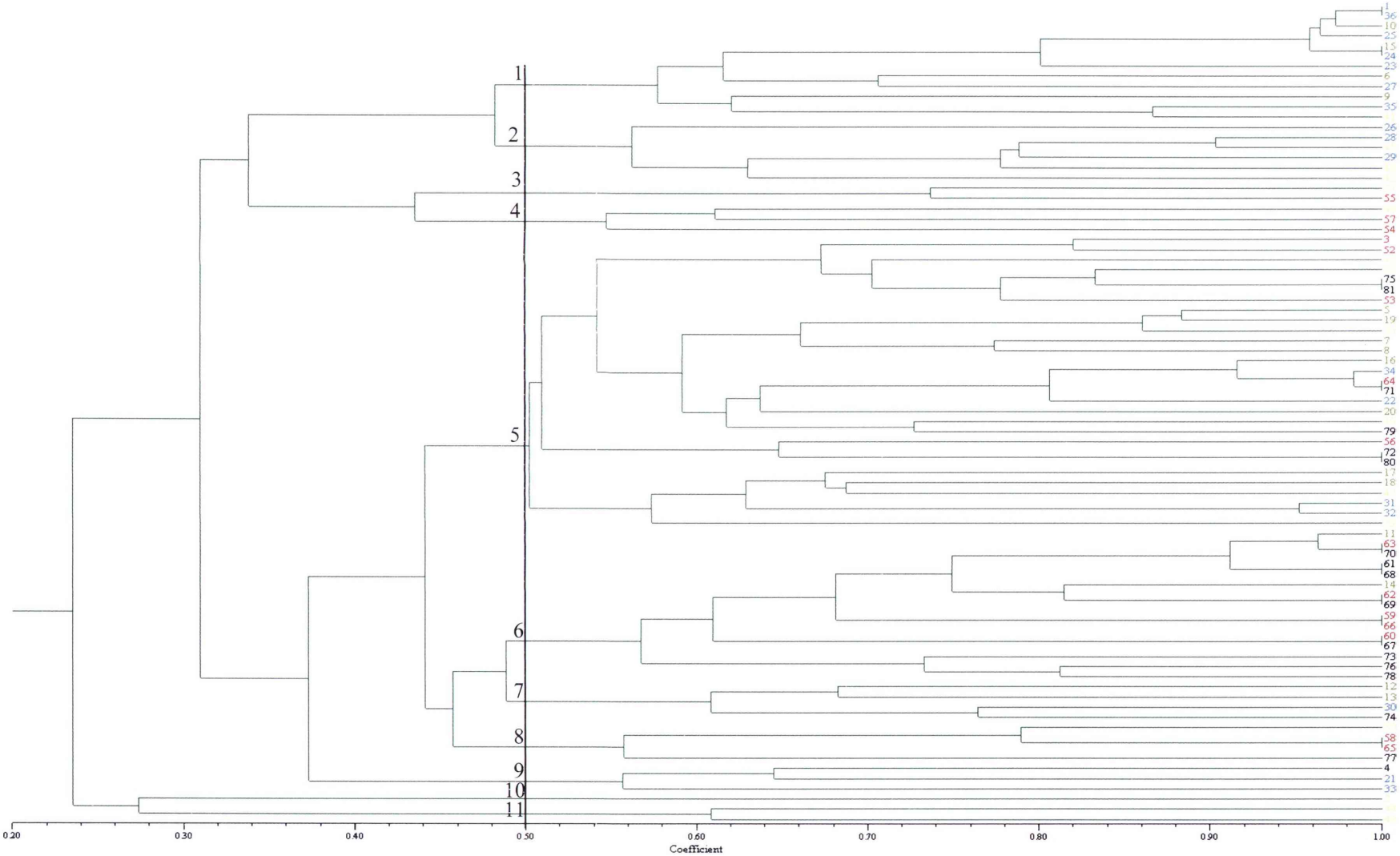

Figure 5-13 Dendrogram of ISSR similarity using DICE Coefficient based on UPGMA clustering of *Pythium* spp. from different cultivars, different sample times or different farms. See Table 5-4 for isolate codes corresponding to isolate numbers shown at right. Colors are indicated sample time as follows: green: May samples; blue: August samples; yellow: November samples; red: January samples; and black: March samples.

5.4 Discussion

The results presented in this study include the first comprehensive survey of hydroponic lettuce farms in the Sydney and Central Coast areas of NSW to obtain isolates of *Pythium* from a range of lettuce cultivars and at different sampling times throughout the year. In this study, 80 isolates of *Pythium* group F and one isolate of *P. coloratum*, identified according to the key of van der Plaats-Niterink (1981), were recovered from roots of diseased and apparently healthy lettuce (see Chapters 2 and 3).

Pythium spp. are known as pathogens of seeds and root systems, particularly of seedling root systems (Agrios 2005; Deacon 2006). They can be found in most hydroponic systems around the world including those used for the production of lettuce (Zinnen 1988; Tesoriero & Cresswell 1995), tomato (Vanachter *et al.* 1983), cucumber (Stanghellini *et al.* 1988; Cherif & Belanger 1992) and spinach (Stanghellini *et al.* 1984). In NSW, *Pythium afertile*, *P. coloratum, P. myriotylum* and *P. vexans* were recorded on lettuce grown in soilless systems (Tesoriero *et al.* 1991). The present study confirms the occurrence of *P. coloratum* in hydroponic lettuce; however, only one of the 81 isolates obtained produced sexual structures which led to its identification as *P. coloratum*. In this study, nearly all isolates were identified as belonging to *Pythium* group F, a group which contains species lacking sexual structures.

In order to identify and characterise the 81 *Pythium* isolates collected in this study, colony appearance on PCA, PDA and CMA media were used initially to divide the isolates into groups, as recommended by van der Plaats-Niterink (1981). Three groups were identified – group (i) contained approximately half of the isolates (40), group (ii) contained 24 isolates and group (iii) contained 17 isolates. Each of the three groups contained isolates obtained from different hydroponic farms and different lettuce cultivars at different sampling times. For further identification and characterisation, 15 representative isolates, including two to eight from each group, were studied in greater depth. All 15 isolates appeared similar to each other based on the observations that they all had the same optimum temperature for growth (30°C) and showed no significant differences in growth rate at temperatures between 10°C and 45°C. However, significant differences in growth rate at 5°C were found, but only between 3 pairs of isolates. The occurrence of these differences could be related to the very small amount

of growth in all isolates tested (maximum 1.30 mm in 24 hours) or to no growth occurring in one or more of the five replicates that were used for each isolate tested. The status of the three groups identified on the basis of colony appearance remains unresolved. The grouping was not supported by the results obtained regarding growth at different temperatures. Further studies could investigate more temperatures between 25°C and 35°C to determine more precisely the optimum growth temperature for *Pythium* spp. isolated from roots of hydroponic lettuce. Temperatures less than 5°C and between 40°C and 45°C could also be investigated to determine the lowest and highest growth temperatures for *Pythium* isolates obtained from lettuce grown in hydroponic systems.

Isolates in group (i) were found to have hyphae up to 8 μm in diameter, club- or sausage-shaped appressoria, filamentous non-inflated sporangia, vesicles up to 60 μm formed in water and zoospores released within 24 h (10-14 μm diameter). Sexual structures were not produced in 39 of the 40 isolates. Using the key of van der Plaats-Niterink (1981), these isolates were identified as belonging to *Pythium* group F based on morphological characterisation. However, the only isolate (PMr05CC2RO) in this group producing oospores, which were aplerotic and slightly plerotic, was identified as *P. coloratum* by using the same key. It is possible that the 39 identified as *Pythium* group F isolates belong to *P. coloratum* but that they have lost their ability to produce sexual structures, a feature which could, in turn, be related to their occurrence in hydroponic systems rather than in soil. Rafin *et al.* (1995) found that *Pythium* from group F were identical to *P. coloratum* and *P. aquatile* when restriction analysis of amplified ribosomal DNA was used for *Pythium* spp. isolated from soilless culture systems.

Isolates in group (iii) were similar to isolates in group (i) as regards hyphal diameter (up to 8 μm), shape of appressoria, sporangial shape, vesicle size (up to 60 μm) and time needed for zoospore release. They were, however, different in colony type on three agar media (Figure 5-1) and they did not produce sexual structures. Isolates in this group were identified as belonging to *Pythium* group F based on the key of van der Plaats-Niterink (1981).

On the other hand, isolates in group (ii) produced similar structures to those of isolates in groups (i) and (iii) but with differences in the sizes of their hyphae, vesicles and zoospores. Hyphae were up to 6 μm in diameter, vesicles up to 55 μm in diameter and zoospores were 10-12 μm in diameter. Isolates in this group did not produce sexual structures and the colony type was found to be different from isolates in groups (i) and (iii). These isolates, however, were also identified as belonging to *Pythium* group F, based on the key of van der Plaats-Niterink (1981). This is because the key grouped the non-sexual-structure-producing isolates into five groups (F, T, G, P and HS) based on sporangia shape and form, and presence of hyphal swellings. By using these criteria, nearly all the isolates from the present study could be included in *Pythium* group F.

It would be worthwhile to attempt to obtain sexual structures from the isolates that failed to produce them under the culture conditions used in this study. To achieve this, the isolates could be grown on other media and/or under different environmental conditions. As well, the isolates could be used to re-infect lettuce plants and be freshly re-isolated before culturing again. If sexual structures were obtained, further studies on their morphological characteristics could enable identification to species level using the key of van der Plaats-Niterink (1981).

Since morphological and physiological characteristics could not enable the identification of the majority of the isolates obtained in this study to particular species of *Pythium*, molecular techniques were investigated in order to find out more about these isolates and the relationships between the isolates within each group and between groups. PCR-RFLP of the ITS region of rDNA was used to study the relationships among selected isolates from the three groups identified on the basis of colony features. Four different restriction enzymes were used (*Eco*RI, *Mse*I, *Msp*I and *Xmn*I). Results from this technique applied to seven of the 81 isolates collected in this study confirmed that these isolates were similar to each other. Furthermore, the isolate identified as *P. coloratum* (isolate code PMr05CC2RO and present in lane seven in the gels shown in Figures 5-7 and 5-8) was indistinguishable from the other six isolates identified as belonging to *Pythium* group F (lane one to six of the gels shown in Figures 5-7 and 5-8). The results of the present study thus support the finding of Rafin *et al.* (1995) that isolates of *Pythium* from group F were identical to *P. coloratum* when restriction analysis of amplified ribosomal DNA was applied to these organisms.

The technique of PCR-RFLP of the ITS region of rDNA has been successfully used in many studies to differentiate between several *Pythium* species. Chen (1992) indicated that PCR-RFLP was a simple and speedy taxonomical technique for ecological studies of *Pythium* species. He distinguished between five different *Pythium* spp. (*P. arrhenomanes, P. graminicola, P. irregulare, P. spinosum* and *P. ultimum*) by using this technique. Furthermore, the RFLP technique was used successfully to differentiate between 36 plant pathogenic *Pythium* spp. by using four restriction enzymes (Wang & White 1997); intraspecific variability was found to be low. More recently, 130 *Pythium* isolates obtained from soil and diseased table beet plants were compared and placed into three genotype groups by using RFLP analysis with *Hha*I, *Hinf*I and *Mbo*I restriction enzymes of the PCR amplified ITS region of rDNA (Scott *et al.* 2005). The inability of this technique to indicate the occurrence of several different *Pythium* spp. in the hydroponic systems investigated in the present study could be attributable to the fact that there is indeed only one *Pythium* sp. present in the hydroponic lettuce systems sampled. However, the low number of isolates studied by this technique (seven of the 81 available) reduced the likelihood of finding different *Pythium* spp., even though care was taken to include members of all three groups identified on morphological features as well as both the isolate of *P. coloratum* and isolates belonging to *Pythium* group F. It would be possible to study more isolates obtained in the present investigation using this technique and to use additional restriction enzymes as well. Such studies may yield additional *Pythium* spp.

The sequences of the ITS region of rDNA for 13 *Pythium* isolates, selected to represent the three groups, were obtained for identification to species level. Nine isolates, six from group (i), including the isolate *P. coloratum* (PMr05CC2RO), two from group (ii) and one from group (iii) were found to be identical to *Pythium* sp. PTS 2003 (Lockyer Valley *Pythium* C (LVP C) (AY445122); Table 5-6) which was isolated from roots of table beet (*Beta vulgaris* var. *vulgaris*) in the Lockyer Valley, Queensland, Australia (Scott *et al.* 2005). This isolate, *Pythium* sp. PTS 2003, was found to be 98% identical to *P. dissotocum* when these researchers compared it with the GenBank database. Moreover, *Pythium* PTS 2003 (LVP C) isolates were genetically related to both *P. dissotocum* and *P. coloratum* as shown in the phylogenetic tree of the *Pythium* isolates from their study based on DNA sequence analysis of the region encompassing ITS 1, 5.8S rDNA and ITS 2.

Three isolates of *Pythium* obtained in the present study matched unclassified *Pythium* species (*Pythium* sp. JN-7a and *Pythium* sp. JN-6) (Table 5-6). This finding is consistent with these isolates being identified as belonging to *Pythium* group F, rather than as a particular species. One isolate (PJ05CC1RO, group (i)) matched *P. dissotocum* and one isolate (PN04CC1RO, group (i)) matched five different *Pythium* species including *P. dissotocum*, *P. coloratum*, *P. lutarium, P. diclinum* and *Pythium* sp. PTS 2003. Levesque and De Cock (2004), studying 116 species and varieties of *Pythium* through analysis of the ITS region of rDNA, found that the four species *P. dissotocum*, *P. coloratum*, *P. lutarium* and *P. diclinum* and several others belonged to the same subclade (B2). As well, they suggested that the species concept in this important group needed further investigation, ideally in a study with several highly variable genes.

The results obtained in the present study using the ISSR technique (Figure 5-13) indicated that there were no correlations between the 11 groups of 81 *Pythium* isolates and either the farm or the geographic area from which isolates were obtained. Furthermore, there were no correlations between these groups and the lettuce cultivars yielding the isolates. Moreover, no correlations were found between the groups established by the ISSR technique and the three groups identified on the basis of colony characteristics. On the other hand, there appeared to be a slight correlation between the 11 groups of *Pythium* isolates and the sampling times. Nearly all the isolates in group 1 were from cool season samples (May and August), whereas groups 3, 4, 6, 10 and 11 contained isolates that were predominantly or exclusively from warm season samples (November, January and March). Nevertheless, the interpretation of the results from the ISSR study is complicated by the fact that there remains some uncertainty as to the number of different *Pythium* spp. isolated in the present study. *P. coloratum* was found in group 5 (Figure 5-13), along with other *Pythium* isolates belonging to group F which were obtained from different farms, different sample times, different lettuce cultivars and different isolates from the three groups based on colony features.

The variation amongst the 81 isolates obtained using the ISSR technique contrasts with the lack of variation found with both the PCR-RFLP technique applied to the ITS region (Figures 5-7 and 5-8) and sequencing of the ITS region (Table 5-6). The likely reason for this is that the ITS region may not have enough differences to enable separation of the isolates, whereas the entire DNA, as scanned by the primers in the ISSR technique,

allows comparison of a large number of markers, so that the potential to differentiate variability between isolates in greatly enhanced. It is difficult to determine if this variability indicates the presence of different taxa of *Pythium*. Comparative studies using the data obtained for such an ISSR study and sequence data from a variety of genes, including both mitochondrial and nuclear genes, may provide insight into the taxonomic status of these isolates. Such a study was outside the scope of the research presented in this thesis but the results reported here and in Scott *et al.* (2005) indicate that a major taxonomic study on *Pythium* in Australia is now needed.

6 CHARACTERISATION AND IDENTIFICATION OF *PHYTOPHTHORA* SPP. ISOLATED FROM LETTUCE ROOTS BY MORPHOLOGICAL AND MOLECULAR TECHNIQUES

6.1 Introduction

The genus *Phytophthora* was named for the first time by Anton de Bary in 1876 when he described the potato late blight fungus, *Phytophthora infestans* de Bary (Zentmyer 1983). This genus contains some of the world's most important and destructive plant pathogens. More than 70 species of *Phytophthora* have been described worldwide. Forty-eight species and varieties were described in 1978 by Newhook *et al.* (1978) and Stamps *et al.* (1990) revised them and described more to give a total of 67 species and varieties. The genus *Phytophthora* is classified now as belonging to the fungal-like organisms or oomycetes in the phylum Oomycota within the kingdom Chromista. The phylum Oomycota contains some of the most economically important plant pathogens, such as *Pythium*, *Phytophthora* and *Aphanomyces*.

Morphological characteristics alone have traditionally been used to distinguish the genus *Phytophthora* from *Pythium*. For further identification of *Phytophthora*, morphological characteristics, supplemented by several physiological features, were used to separate *Phytophthora* species into six groups (Waterhouse 1970; Newhook *et al.* 1978; Stamps *et al.* 1990). The parameters assessed for determining *Phytophthora* groups and identification to species include the branching patterns of the sporangiophores, the nature of the sporangial apex (non-papillate, semi-papillate or papillate), the abundance of sporangia on solid media, the non-caducous or caducous nature of the sporangia, internal proliferation of sporangia, production of oogonia and oospores in a single culture (homothallic), production of oospores and oogonia only when opposite mating types are paired in a culture (heterothallic), nature of the antheridium, and the abundance or absence of oospores on host tissue or in culture (Waterhouse 1970; Newhook *et al.* 1978; Stamps *et al.* 1990; Erwin & Ribeiro 1996).

Identifying *Phytophthora* species by using only morphological characteristics becomes difficult because many species produce overlapping characteristics which are often hard to differentiate. Moreover, this difficulty may be exacerbated by environmental factors, such as temperature, and physiological factors, such as nutrient (Bates *et al.* 1993). However, morphological characteristics of *Phytophthora* species can still be useful tools and may often provide the basis for species identification. Molecular techniques have supplied several tools with the potential to confirm the identification of species (Erwin & Ribeiro 1996), particularly closely related species with few morphological differences (Klich & Mullaney 2004). Ribosomal DNA is regularly used for PCR-based diagnostics (Drenth *et al.* 1999 & 2006).

The internal transcribed spacer (ITS) region has been shown to be sufficient to distinguish different species in many different genera such as in *Beauveria* (Neuveglise *et al.* 1994) and in the oomycetes *Pythium* (Rafin *et al.* 1995) and *Phytophthora* (Cooke & Duncan 1997; Cooke *et al.* 2000). This is because sequence changes in the non-coding ITS region of the nuclear rDNA occur at a rate that often allows species to be distinguished within a genus (White *et al.* 1990). The techniques developed by Drenth *et al.* (1999) and Cooke *et al.* (2000) have shown great applicability to the identification of a wide range of *Phytophthora* species and are likely to be used in a range of studies on this genus in the future. Also, the restriction fragment length polymorphism (RFLP) method, based on the ITS region, was used successfully to identify *Phytophthora megasperma* and study the variation amongst isolates within the species (Forster & Coffey 1993).

Study of the population genetics of *Phytophthora* species is relatively recent and started in the mid-1980s when the first studies on isozyme variation in *P. cinnamomi* (Old *et al.* 1984) and *P. infestans* (Tooley *et al.* 1985) were published. Since that time, reports outlining the use of molecular tools to determine genetic variation or diversity within *Phytophthora* species have used a number of different techniques.

Two of the most commonly used molecular techniques for assessment of variation of this nature are analysis of the internal transcribed spacer region (ITS) of rDNA and microsatellite or inter simple sequence repeats (ISSR). Drenth *et al.* (1993), using DNA fingerprinting patterns developed using Southern blot techniques, were able to estimate

the genotypic diversity of *P. infestans* isolated from potato and tomato from different locations in the Netherlands. DNA fingerprinting of isolates with probe RG57 revealed significantly more diversity in the *P. infestans* population than had been detected previously. They found that genotypic diversity varied among regions. Isolates collected from community gardens in west and central regions were different from each other as well as from isolates collected from commercial potato fields in other regions (Drenth *et al.* 1993). Recently, the ISSR technique was used to study the variation among populations of *P. cinnamomi* isolated from soil in different geographic areas of New South Wales (NSW), Australia (Pongpisutta 2005).

Phytophthora species cause several diseases, including root rot, rots of buds or fruits and rot of lower stems and tubers, on different types of plants ranging from seedlings to fully developed fruit and forest trees (Agrios 2005). Root rot disease caused by *Phytophthora* spp. can be found in almost all parts of the world, where the soil is too wet for good growth of susceptible plants and temperatures are between 15 and 23°C (Mitchell & Kannwischer-Mitchell 1992; Agrios 2005).

In hydroponic systems, *Phytophthora* spp. cause root rot disease on most vegetables, including tomato (Snapp & Shennan 1992; Forster *et al.* 1998), pepper (Stanghellini *et al.* 1996a) and both lettuce seedlings and mature lettuce (Hutton & Forsberg 1991; Jamart 1999). Hydroponic system conditions are likely to favour the development of root disease caused by *Pythium* and *Phytophthora* species since free water is available for zoospore movement.

The aims of this study were to:

- identify all *Phytophthora* isolates obtained from roots of hydroponically grown lettuce during two farm-based surveys (see Chapters 2 and 3) by using morphological, physiological and molecular techniques, specifically PCR-RFLP analysis of the ITS region of rDNA;

- sequence the ITS region of rDNA for selected isolates to confirm the identifications based on morphological and physiological studies; and

- determine the variation within populations of *Phytophthora* isolated from lettuce roots at different sample times, from different cultivars and at different hydroponic farms in the Sydney and Central Coast areas by using the ISSR technique.

6.2 Materials and methods

6.2.1 Isolation of *Phytophthora* spp. from lettuce roots

6.2.1.1 Recovery of *Phytophthora* cultures

Phytophthora species were isolated from roots of apparently healthy and diseased lettuce grown in hydroponic systems from two major lettuce-producing areas in and near Sydney (Leppington and the Central Coast of NSW). *Phytophthora* isolates were obtained from three different lettuce cultivars (Baby Cos, Red Oak and Brown Mignonette) at five sampling times over an 11 month period (May 2003 to March 2004) from one farm (Leppington 1). Isolates were also obtained from four different lettuce cultivars (Baby Cos, Red Oak, Green Oak and Brown Mignonette) sampled five times over an 11 month period (May 2004 to March 2005) from four different farms (Leppington 1 and 2, and Central Coast 1 and 2). Root systems were washed under tap water and root pieces were plated on *Phytophthora* selective media (PSA) (Appendix 1). Petri dishes were incubated in the dark at 25°C ± 1°C for 2 days (Stamps *et al.* 1990; Erwin & Ribeiro 1996). A light microscope (Olympus CX41RF, Olympus Optical, Philippines) was used to identify cultures as belong to the genus *Phytophthora*, by morphological examination (Stamps *et al.* 1990).

6.2.1.2 Hyphal tip isolation

Phytophthora cultures were transferred to water agar (WA) (Appendix 1) and incubated at 25°C ± 1°C in the dark for 2 days. Hyphal tips were carefully transferred under a stereomicroscope and subcultured onto potato carrot agar (PCA) (Appendix 1) and incubated at 25°C ± 1°C for 7 days in the dark prior to further study. Working stocks of the cultures were maintained in sterile distilled water at 4°C. Representative cultures of each of the 68 isolates obtained were stored in sterile distilled water in McCartney bottles at room temperature.

6.2.2 Molecular studies

6.2.2.1 DNA extraction

DNA was extracted from the mycelium of all *Phytophthora* isolates obtained (68 isolates) by using the same protocol as described for *Pythium* (Chapter 5, section 5.2.4.1).

6.2.2.2 Gel electrophoresis of genomic DNA

Genomic DNA was evaluated using agarose gel electrophoresis as described for *Pythium* (Chapter 5, section 5.2.4.2).

6.2.2.3 PCR amplification of ITS region of ribosomal DNA

Sixty-eight isolates of *Phytophthora* were compared and identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of the ITS region of ribosomal DNA (rDNA). PCR amplification of the ITS region of rDNA was carried out using the same protocol as described for *Pythium* (Chapter 5, section 5.2.4.3).

6.2.2.4 Restriction enzyme digestion for restriction fragment length polymorphism (RFLP) analysis

The restriction enzymes *Msp*I, *Rsa*I and *Taq*I are able to distinguish between *Phytophthora* spp. following the protocols of Drenth *et al.* (1999 & 2006). The digested products were prepared by adding each restriction enzyme to a 1.5 mL Eppendorf tube on ice. Each digestion reaction had a final volume of 30 µL, with the mixture for restriction enzyme *Msp*I comprising 1x NE Buffer 2 (NEB) (Appendix 6) and 5 U *Msp*I, for *Rsa*I consisting of 1x NE Buffer 2 and 5 U of R*sa*1, and for *Taq*I containing 1x NE Buffer 2, 1x BSA and 3 U T*aq*I.

The tubes were incubated in a water bath for the three restriction enzymes (Table 6-1). Each digested product (15 µL) was electrophoresed on 2% agarose, stained with ethidium bromide and observed under UV light. The resulting RFLP profiles were compared to profiles in the "Diagnostic Laboratory Manual: PCR based detection and identification of *Phytophthora* species" (Drenth *et al.* 1999).

Table 6-1 The restriction enzymes used for digestion of the ITS region of rDNA of *Phytophthora*, together with their recognition sequences and the incubation temperatures and time.

Enzyme	Recognition sequence	Incubation temperature (°C)	Incubation time (h)
*Msp*I	C▼CGG	37	3
*Rsa*I	GT▼AC	37	3
*Taq*I	T▼CGA	65	3

6.2.2.5 Sequencing the ITS region of ribosomal DNA

In order to confirm correct species identification and verify the PCR-RFLP technique used above, the ITS region of a selection of four *Phytophthora* isolates was sequenced using the same technique as described for *Pythium* (Chapter 5, section 5.2.4.5).

6.2.2.6 Inter Simple Sequence Repeat (ISSR) analysis for population studies

All 68 isolates of *Phytophthora* were used to study the variation among the populations. The same protocol was used as for *Pythium* (Chapter 5, section 5.2.4.6), but with modifications to the annealing temperatures for three of the four primers, as shown in Table 6-2.

Table 6-2 Sequences of primers used for PCR amplification for the ISSR analysis of *Phytophthora* isolates.

Primer	Sequence A	Annealing temperature
AC – primer	5′ DBD (AC)7 3′	46°C
AG – primer	5′ (AG)8G 3′	44°C
CGA – primer	5′ DHB (CGA)5 3′	58°C
GT – primer	5′ VHV (GT)7G 3′	48°C

A The following designations are used for the degenerate sites: B (C, G or T), D (A, G or T), H (A, C or T) and V (A, C or G).

6.2.3 Morphological studies

6.2.3.1 Colony characteristics on different agar media

For morphological studies, including both colony characteristics on different agar media and morphological characteristics observed using light microscopy, 15 cultures of *Phytophthora* were systematically chosen from the major groups revealed by the ISSR results to represent all *Phytophthora* isolates. Petri dishes (90 mm) containing 20 mL of corn meal agar (CMA), PCA and potato dextrose agar (PDA) (Appendix 1) were inoculated with a 5 mm diameter core taken from the edge of an actively growing 3-day-old culture on PCA incubated at 25°C ± 1°C in the dark. The core was placed upside down in the centre of each plate and the plates were incubated in the dark at 25°C ± 1°C. Colony morphology was noted after 5 days incubation and compared to the colony morphology types outlined in Erwin and Ribeiro (1996). Five replicates per isolate were examined for this study.

6.2.3.2 Morphological characteristics using light microscopy

The morphological characteristics assessed were microscopic features (Olympus CX41RF, Olympus Optical, Philippines) and included presence or absence of hyphal swellings, morphology of the sporangia (shape and size) and chlamydospore production from cultures grown on PCA at 25°C ± 1°C for 7 days in the dark. Thirty to fifty measurements of each characteristic were made for each isolate. Mycelial mats were mounted in distilled water and sporangia measured at 200x total magnification under the light microscope. These characters were compared to earlier published descriptions in Stamps *et al.* (1990) and Erwin and Ribeiro (1996). The data on sporangial size was analysed with Tukey's Highest Significant Difference (HSD) tests using a one-way analysis of variance (ANOVA).

6.2.4 Physiological studies

6.2.4.1 Colony growth rates at different temperatures and optimum, minimum and maximum temperatures

The same 15 isolates as used for morphological studies (section 6.2.3 above) were used for physiological studies. Petri dishes (90 mm diameter) containing 20 mL PCA were inoculated with a 5 mm diameter plug taken from the edge of actively growing 3-day-old cultures on PCA incubated at 25°C ± 1°C in the dark. The growth temperatures

were 5, 10, 15, 20, 25, 30, 35, 40 and 45°C and cultures were incubated in the dark. Five replicates were used for each isolate at each temperature. The colony diameters of the cultures were recorded at 24, 48, 72 and 120 h in two directions perpendicular to each other. The colony growth in millimetres over 24 h periods was calculated for the mean colony diameters at 24 and 48 h. The data for growth at different temperatures were analysed with Tukey's HSD tests using a one-way analysis of variance (ANOVA).

6.2.5 Determination of mating type

By the time the research into mating types was undertaken, the identity of all *Phytophthora* isolates obtained in the present study had been determined as *P. drechsleri* (see 6.3 Results, sections 6.3.2 and 6.3.3). In heterothallic *Phytophthora* species, the direct interaction of two mating types (A_1 and A_2) is required to initiate sexual reproduction. No identified mating type cultures (A_1 and A_2) for *P. drechsleri* were available, so mating type cultures of *P. cryptogea* were used instead. The 68 *P. drechsleri* isolates, whose mating types were unknown, were paired opposite A_1 and A_2 tester cultures of *P. cryptogea* strains (UQ759-A_1 and UQ763-A_2), supplied by Dr. Andre Drenth (CRC for Tropical Plant Protection, Indooroopilly, Qld), on 90 mm Petri dishes containing thinly poured (15 mL per plate) Kidney Bean Extract Agar (KBEA) (Duncan 1988) (Appendix 1). A 5 mm diameter inoculum plug from a 5-day-old culture growing on KBEA incubated at 25°C ± 1°C in the dark of either an A_1 or A_2 tester culture was placed 10 mm in from one side of the Petri dish, and a plug taken from a 3-day-old culture growing on PCA incubated at 25°C ± 1°C in the dark of the unknown isolate placed in a similar position on the opposite side. Reciprocal crosses using the other mating type were also prepared. The plates were kept in an incubator at 25°C ± 1°C in the dark for 2-4 weeks, after which oospore production was confirmed by observation under the light microscope.

6.3 Results

6.3.1 Recovery of *Phytophthora* cultures

Sixty-eight *Phytophthora* isolates were recovered from hydroponic lettuce roots during the two farm-based surveys carried out from May 2003 to March 2004 (first survey, see Chapter 2) and May 2004 to March 2005 (second survey, see Chapter 3) (Table 6-3). Five isolates were recovered in the first survey from a single farm at Leppington (see Chapter 2) identified as Leppington 1. In the second survey, 63 isolates were recovered from four farms, including two farms at Leppington (identified as Leppington 1 and Leppington 2) and two farms on the Central Coast of NSW (identified as Central Coast 1 and Central Coast 2). These 63 isolates comprised 17 from the farm Leppington 1, 12 from Leppington 2, 18 from Central Coast 1 and 16 from Central Coast 2 (see Chapter 3; Table 6-3). Isolates of *Phytophthora* were obtained from all time samples in both surveys and from all four lettuce cultivars sampled in the second survey.

Table 6-3 indicates the isolates used for various types of study, the results of which are presented in the following sections.

Table 6-3 Details of isolates of *Phytophthora* obtained from roots of different hydroponic lettuce cultivars during two farm-based surveys carried out in and near Sydney involving one farm in the first survey (Leppington 1; see Chapter 2) and four farms in the second survey (Leppington 1 and 2, and Central Coast 1 and 2; see Chapter 3).

Isolate no.	Isolate code*	Isolation time	Hydroponic farm	Lettuce cultivar	Types of study^
1	PhM03L1	May-03	Leppington 1	Not recorded	T, R, I
2	PhA03L1	Aug-03	Leppington 1	Not recorded	T, R, I
3	PhN03L1	Nov-03	Leppington 1	Not recorded	T, R, I, M, C, P, S
4	PhJ04L1	Jan-04	Leppington 1	Not recorded	T, R, I
5	PhMr04L1	Mar-04	Leppington 1	Not recorded	T, R, I
6	PhM04L1BC	May-04	Leppington 1	Baby Cos	T, R, I, S
7	PhM04L1RO	May-04	Leppington 1	Red Oak	T, R, I, M, C, P
8	PhM04L1GO	May-04	Leppington 1	Green Oak	T, R, I
9	PhM04L1Mig	May-04	Leppington 1	Mignonette	T, R, I, M, C, P
10	PhM04L2BC	May-04	Leppington 2	Baby Cos	T, R, I, M, C, P
11	PhM04L2RO	May-04	Leppington 2	Red Oak	T, R, I
12	PhM04CC1Mig	May-04	Leppington 2	Mignonette	T, R, I
13	PhM04CC1BC	May-04	Central Coast 1	Baby Cos	T, R, I
14	PhM04CC1RO	May-04	Central Coast 1	Red Oak	T, R, I, M, C, P
15	PhM04CC1GO	May-04	Central Coast 1	Green Oak	T, R, I
16	PhM04CC2BC	May-04	Central Coast 2	Baby Cos	T, R, I
17	PhM04CC2RO	May-04	Central Coast 2	Red Oak	T, R, I
18	PhM04CC2GO	May-04	Central Coast 2	Green Oak	T, R, I
19	PhM04CC2Mig	May-04	Central Coast 2	Mignonette	T, R, I, M, C, P
20	PhA04L1BC	Aug-04	Leppington 1	Baby Cos	T, R, I
21	PhA04L2Mig	Aug-04	Leppington 2	Mignonette	T, R, I
22	PhA04CC1RO	Aug-04	Central Coast 1	Red Oak	T, R, I
23	PhA04CC1GO	Aug-04	Central Coast 1	Green Oak	T, R, I
24	PhA04CC1Mig	Aug-04	Central Coast 1	Mignonette	T, R, I
25	PhA04CC2BC	Aug-04	Central Coast 2	Baby Cos	T, R, I, M, C, P
26	PhA04CC2GO	Aug-04	Central Coast 2	Green Oak	T, R, I
27	PhA04CC2Mig	Aug-04	Central Coast 2	Mignonette	T, R, I, M, C, P, S
28	PhN04L1BC	Nov-04	Leppington 1	Baby Cos	T, R, I, M, C, P
29	PhN04L1RO	Nov-04	Leppington 1	Red Oak	T, R, I
30	PhN04L1GO	Nov-04	Leppington 1	Green Oak	T, R, I, M, C, P
31	PhN04L1Mig	Nov-04	Leppington 1	Mignonette	T, R, I
32	PhN04L2RO	Nov-04	Leppington 2	Red Oak	T, R, I
33	PhN04L2GO	Nov-04	Leppington 2	Green Oak	T, R, I
34	PhN04L2Mig	Nov-04	Leppington 2	Mignonette	T, R, I
35	PhN04CC1BC	Nov-04	Central Coast 1	Baby Cos	T, R, I
36	PhN04CC1RO	Nov-04	Central Coast 1	Red Oak	T, R, I
37	PhN04CC1GO	Nov-04	Central Coast 1	Green Oak	T, R, I
38	PhN04CC1Mig	Nov-04	Central Coast 1	Mignonette	T, R, I, M, C, P
39	PhN04CC21BC	Nov-04	Central Coast 2	Baby Cos	T, R, I
40	PhN04CC2RO	Nov-04	Central Coast 2	Red Oak	T, R, I
41	PhN04CC2GO	Nov-04	Central Coast 2	Green Oak	T, R, I
42	PhJ05L1BC	Jan-05	Leppington 1	Baby Cos	T, R, I
43	PhJ05L1RO	Jan-05	Leppington 1	Red Oak	T, R, I
44	PhJ05L1GO	Jan-05	Leppington 1	Green Oak	T, R, I
45	PhJ05L1Mig	Jan-05	Leppington 1	Mignonette	T, R, I
46	PhJ05L2BC	Jan-05	Leppington 2	Baby Cos	T, R, I, M, C, P
47	PhJ05CC1BC	Jan-05	Central Coast 1	Baby Cos	T, R, I
48	PhJ05CC1RO	Jan-05	Central Coast 1	Red Oak	T, R, I

49	PhJ05CC1GO	Jan-05	Central Coast 1	Green Oak	T, R, I, M, C, P, S
50	PhJ05CC1Mig	Jan-05	Central Coast 1	Mignonette	T, R, I
51	PhJ05CC2BC	Jan-05	Central Coast 2	Baby Cos	T, R, I
52	PhJ05CC2RO	Jan-05	Central Coast 2	Red Oak	T, R, I
53	PhJ05CC2GO	Jan-05	Central Coast 2	Green Oak	T, R, I
54	PhMr05L1BC	Mar-05	Leppington 1	Baby Cos	T, R, I, M, C, P
55	PhMr05L1RO	Mar-05	Leppington 1	Red Oak	T, R, I
56	PhMr05L1GO	Mar-05	Leppington 1	Green Oak	T, R, I
57	PhMr05L1Mig	Mar-05	Leppington 1	Mignonette	T, R, I
58	PhMr05L2BC	Mar-05	Leppington 2	Baby Cos	T, R, I, M, C, P
59	PhMr05L2RO	Mar-05	Leppington 2	Red Oak	T, R, I
60	PhMr05L2GO	Mar-05	Leppington 2	Green Oak	T, R, I
61	PhMr05L2Mig	Mar-05	Leppington 2	Mignonette	T, R, I
62	PhMr05CC1BC	Mar-05	Central Coast 1	Baby Cos	T, R, I
63	PhMr05CC1RO	Mar-05	Central Coast 1	Red Oak	T, R, I
64	PhMr05CC1GO	Mar-05	Central Coast 1	Green Oak	T, R, I
65	PhMr05CC1Mig	Mar-05	Central Coast 1	Mignonette	T, R, I
66	PhMr05CC2BC	Mar-05	Central Coast 2	Baby Cos	T, R, I
67	PhMr05CC2RO	Mar-05	Central Coast 2	Red Oak	T, R, I
68	PhMr05CC2GO	Mar-05	Central Coast 2	Green Oak	T, R, I

*Components of isolate code are genus/ month/ year/ farm/ cultivar as follows –
Ph: *Phytophthora*
M: May; A: August; N: November; J: January; Mr: March
03: 2003; 04: 2004; 05: 2005
L1: Leppington 1; L2: Leppington 2; CC1: Central Coast 1; CC2: Central Coast 2
BC: Baby Cos; GO: Green Oak; Mig: Brown Mignonette; RO: Red Oak

^Types of study are as follows –
Morphological studies: C: Colony shape; M: Microscopic
Physiological studies: P: Growth at different temperatures
Molecular studies: R: RFLP; S: Sequencing; I: ISSR
Mating type studies: T: mating type

6.3.2 Molecular studies

6.3.2.1 Comparison of isolates by PCR-RFLP of ITS region of rDNA

The technique of PCR-RFLP of the ITS region of rDNA was used to compare identify all *Phytophthora* isolates (68) obtained in this study. The ITS PCR amplified fragment was observed to be about 850-880 bp for *Phytophthora* isolates (Figure 6-1).

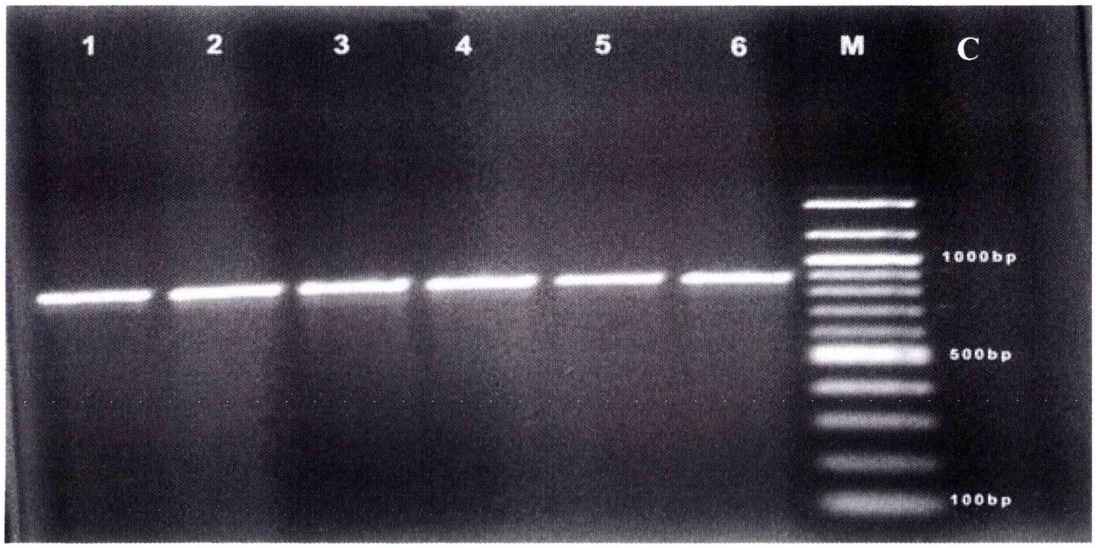

Figure 6-1 Representative gel of PCR amplification products of ITS region of rDNA for six *Phytophthora* isolates.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhN03L1	**(4) PhM04L1BC**
(2) PhA04CC2Mig	**(5) PhMr05CC2RO**
(3) PhJ05CC1GO	**(6) PhN04L2RO**
(M) 100 bp ladder	**(C) control reactions**

Five bands of 258, 158, 122, 119 and 100 bp in length were produced when the PCR amplification products of the ITS region of *Phytophthora* isolates were digested with the *Msp*I enzyme (Figure 6-2). When digested with *Rsa*I (Figure 6-3), three bands of 355, 345 and 110 bp in length were produced and when digested with *Taq*I (Figure 6-4), three bands of 235, 200 and 185 bp in length were produced, with a further two bands less than 100 bp. All 68 isolates obtained in the present study were identified as belonging to the one species, *P. drechsleri*, on the basis of comparison of these gels with those in Drenth *et al.* (1999 & 2006) who used the same methods.

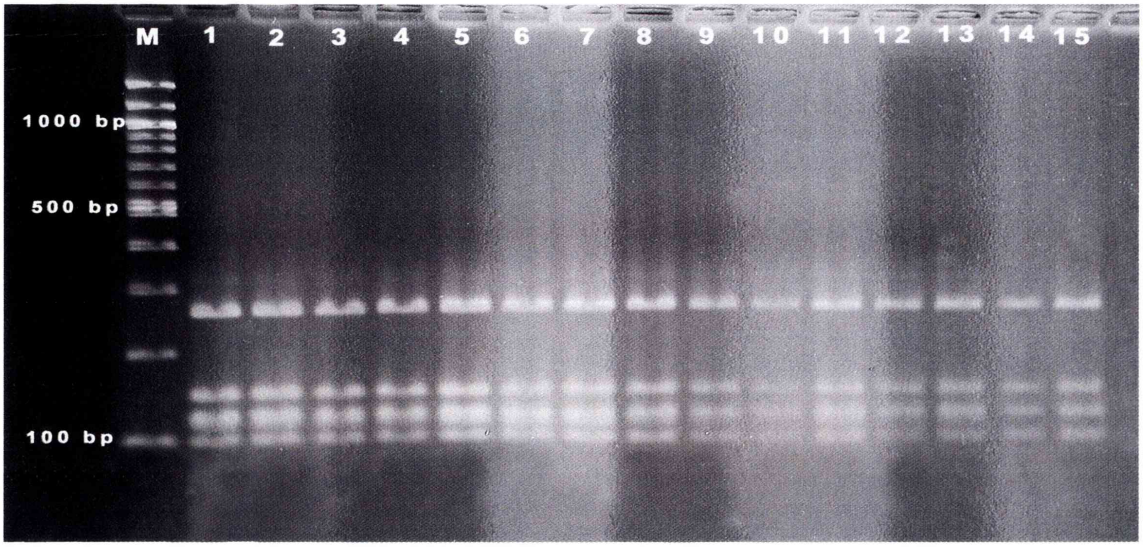

Figure 6-2 Gel photo of digested products of ITS amplification followed by *Msp*I restriction enzyme for 15 different *Phytophthora* isolates.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhM03L1
(2) PhMr04L1
(3) PhM04CC2GO
(4) PhM04CC1BC
(5) PhM04L2Mig
(6) PhA04L1RO
(7) PhA04CC1GO
(8) PhA04L2RO
(9) PhN04CC2BC
(10) PhN04L1Mig
(11) PhJ05CC1BC
(12) PhJ05L2RO
(13) PhMr05CC2BC
(14) PhMr05L1Mig
(15) PhMrL2BC
(M) 100 bp ladder

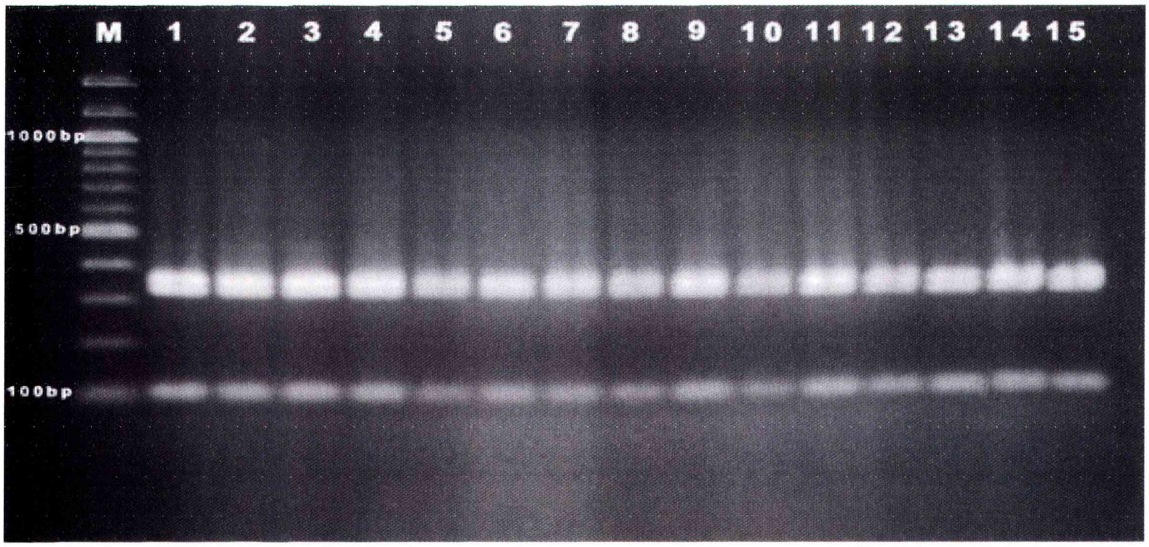

Figure 6-3 Gel photo of digested products of ITS amplification followed by *Rsa*I restriction enzyme for 15 different *Phytophthora* isolates.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhM03L1
(2) PhMr04L1
(3) PhM04CC2GO
(4) PhM04CC1BC
(5) PhM04L2Mig
(6) PhA04L1RO
(7) PhA04CC1GO
(8) PhA04L2RO
(9) PhN04CC2BC
(10) PhN04L1Mig
(11) PhJ05CC1BC
(12) PhJ05L2RO
(13) PhMr05CC2BC
(14) PhMr05L1Mig
(15) PhMrL2BC
(M) 100 bp ladder

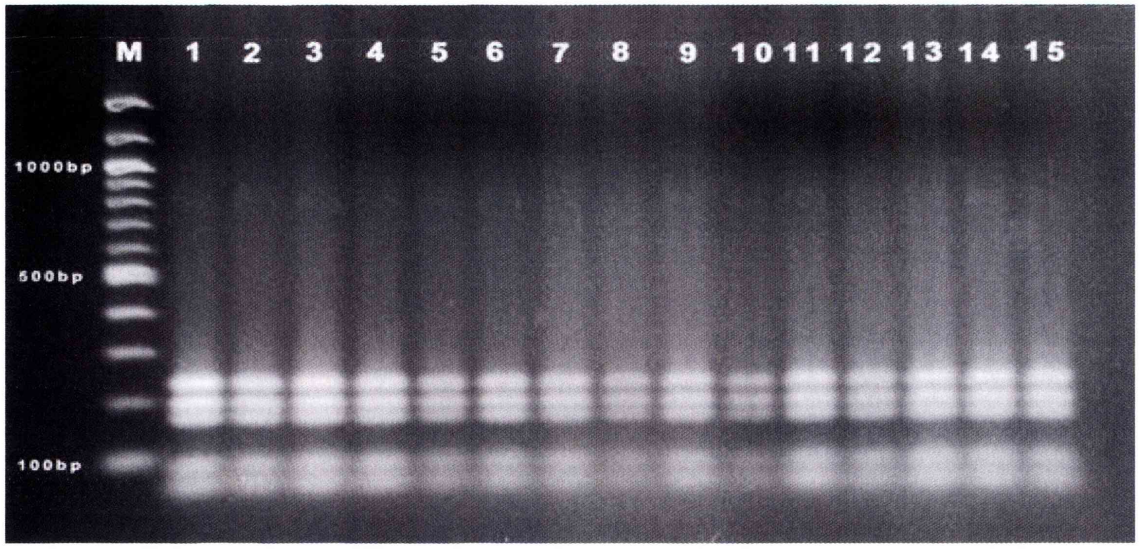

Figure 6-4 Gel photo of digested products of ITS amplification followed by *Taq*I restriction enzyme for 15 different *Phytophthora* isolates.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhM03L1
(2) PhMr04L1
(3) PhM04CC2GO
(4) PhM04CC1BC
(5) PhM04L2Mig
(M) 100 bp ladder
(6) PhA04L1RO
(7) PhA04CC1GO
(8) PhA04L2RO
(9) PhN04CC2BC
(10) PhN04L1Mig
(11) PhJ05CC1BC
(12) PhJ05L2RO
(13) PhMr05CC2BC
(14) PhMr05L1Mig
(15) PhMrL2BC

6.3.2.2 Sequence of ITS region of rDNA

On the basis of sequencing of the ITS region of rDNA of four isolates and comparing the sequences obtained with the GenBank databases using BLAST search, the four isolates were found to match two different *P. drechsleri* isolates (Table 6-4). The sequence of isolate PhN03L1 was 757 bp in length and was 99% identical to the corresponding sequence from *P. drechsleri* (AF228091) with no nucleotide gaps (Table 6-4). The sequence from this isolate (PhN03L1) also matched *P. cryptogea* (AF087476) with 99% identity and no nucleotide gaps.

The two isolates with the longer sequences (isolate PhA04CC2Mig with 934 bp and isolate PhM04L1BC with 900 bp) matched *P. drechsleri* isolate AF087471 (Table 6-4). However, the two isolates with the shorter sequences (isolate PhN03L1 with 757 bp and isolates Ph J05CC1GO with 900 bp) matched *P. drechsleri* isolate AF228091 (Table 6-4).

Table 6-4 Sequence length (bp) of ITS region of rDNA for four isolates of *Phytophthora* (Appendix 8 a) from hydroponic lettuce roots and comparisons with sequences in GenBank.

Isolate code*	Sequence length (bp)	Match from GenBank (Location)^	GenBank accession number	Identities (%)	Gaps
PhN03L1	757	*Phytophthora drechsleri* (Kor)	AF228091	99	0
		Phytophthora cryptogea (Kor)	AF087476	99	0
PhA04CC2Mig	934	*Phytophthora drechsleri* (Kor)	AF087471	99	0
PhJ05CC1GO	712	*Phytophthora drechsleri* (Kor)	AF228091	99	0
PhM04L1BC	900	*Phytophthora drechsleri* (Kor)	AF087471	99	0

*Isolate code – see footnotes to Table 6-3.
^Kor: Korea

6.3.2.3 Variation among populations of *Phytophthora drechsleri* by using Inter Simple Sequence Repeat (ISSR) analysis

The multiple bands varied in size from 250 to more than 1600 bp. The AC-, AG-, CGA and GT-primers separated the 68 *P. drechsleri* isolates into 5, 4, 5 and 4 groups respectively. Designation of each fragment was done in order to get a clear identification from DNA fingerprinting when these bands were compared to the marker sizes with the same primers.

6.3.2.3.1 Variation observed using the AC-primer

There were 1-8 polymorphic fragments of AC-amplified products and these divided the isolates into 5 groups (Table 6-5; Figure 6-5). These fragments ranged between 800 and 1400 bp. There were no correlations found between groups and farms or cultivars. However, quite good correlations were found between groups and sampling time, when considered on the basis of cooler season sample times (May and August) compared with warmer season sample times (November, January and March). Group AC1 contains 29 isolates, 25 of which were obtained in warm season samples (November, January and March). Group AC3 contains five isolates, all of which were from warm season samples (January and March). Moreover, group AC4 contains 13 isolates, 11 of which were from warm season samples (November and January). On the other hand, group AC2 contains 19 isolates, 16 of which were from cool season samples (May and August).

Table 6-5 Groups of isolates based on analysis of polymorphic fragments generated by AC ISSR primer. Isolates in bold are shown in Figure 6-5.

Group	Isolate code*	No. of isolates
AC1	PhM03L1, PhN03L1, PhMr04L1, **PhJ04L1**, **PhJ05L1RO**, PhJ05L1Mig, PhMr05CC1GO, **PhMr05L2GO,** PhMr05CC2BC, **PhM04L2BC**, PhMr05CC2GO, PhMr05L1BC, PhMr05L1Mig, PhMr05L2RO, PhMr05L2Mig, **PhM04CC2RO**, PhMr05CC1Mig, PhJ05CC2GO, PhJ05L1BC, **PhMr05CC1BC**, **PhN04L1RO**, **PhJ05CC2RO, PhJ05CC2GO,** PhJ05CC1RO, PhJ05L2BC, **PhA03L1**, **PhN04L2GO**, PhN04L2RO, **PhJ05L1GO**	29
AC2	PhM04L1BC, PhM04L1RO, PhM04L1GO, PhM04L1Mig, PhM04CC1Mig, PhM04CC1RO, **PhMr05L1GO**, PhM04CC1GO, PhM04CC2BC, PhM04CC2GO, **PhA04L2Mig**, PhM04CC2Mig, **PhMr05L2BC**, PhA04CC1GO, PhA04CC1Mig, **PhA04L1BC,** PhA04CC2BC, PhA04CC2GO, PhN04L2Mig	19
AC3	PhJ05CC2BC, PhMr05CC2RO, PhJ05CC1GO, PhMr05CC1RO, **PhMr05L1RO**	5
AC4	PhN04CC1BC, PhN04CC1RO, PhN04CC1GO, PhN04CC1Mig, PhN04CC21BC, PhN04CC2RO, PhN04CC2GO, PhA04CC2Mig, PhN04L1BC, PhN04L1GO, PhN04L1Mig, PhJ05CC1Mig, **PhM04CC1BC**	13
AC5	PhM04L2RO, **PhJ05CC1BC**	2

*Isolate codes as in Table 6-3.

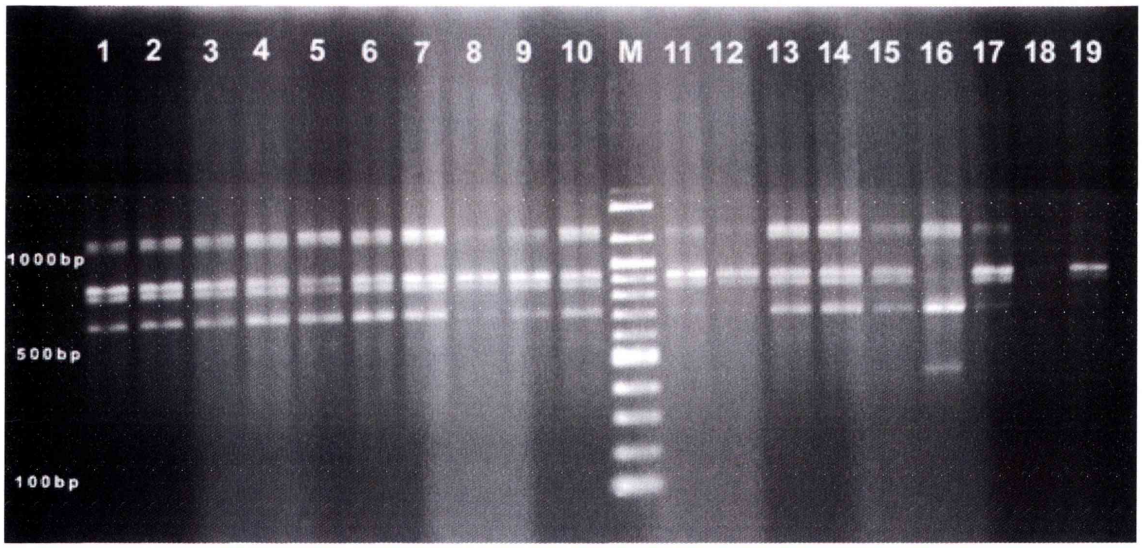

Figure 6-5 ISSR profiles observed for isolates of *Phytophthora drechsleri* using AC-primer.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhA03L1	**(6) PhJ04L1**	**(11) PhMr05L1GO**	**(16) PhJ05CC1BC**
(2) PhN04L2GO	**(7) PhM04L2BC**	**(12) PhA04L2Mig**	**(17) PhMr05L2BC**
(3) PhJ05L1RO	**(8) PhA04L1BC**	**(13) PhJ05L1GO**	**(18) PhMr05L1RO**
(4) PhMr05CC1BC	**(9) PhM04CC2RO**	**(14) PhN04L1RO**	**(19) PhM04CC1BC**
(5) PhMr05L2GO	**(10) PhJ05CC2RO**	**(15) PhJ05CC2GO**	**(M) 100 bp ladder**

6.3.2.3.2 Variation observed using the AG-primer

The AG-amplification products divided the isolates into two main groups containing the majority of the isolates and two minor groups, with only one isolate each (Table 6-6; Figure 6-6). There were 3-8 polymorphic fragments produced ranging between 290 and 1500 bp. In the two main groups, there was no correlation found between groups and sample times, farms or cultivars.

Table 6-6 Groups of isolates based on analysis of polymorphic fragments generated by AG ISSR primer Isolates in bold are shown in Figure 6-6.

Group	Isolate code*	No. of isolates
AG1	PhM03L1, PhN03L1, PhMr04L1, PhM04L1BC, PhM04L1RO, PhM04CC1GO, PhM04CC2BC, PhM04CC2GO, PhM04CC2Mig, PhN04CC2RO, PhN04CC2GO, PhJ05L1BC, PhM04CC1RO, PhMr05CC1Mig, PhMr05L2RO, **PhMr05L2GO, PhJ05CC1BC**	17
AG2	PhM04L1GO, **PhJ04L1, PhA03L1,** PhM04L1Mig, **PhM04L2BC**, PhM04L2RO, PhM04CC1Mig, **PhM04CC1BC**, PhMr05CC2BC, PhMr05CC2RO, PhMr05CC2GO, PhMr05L1BC, **PhMr05L1GO**, PhMr05L1Mig, **PhMr05L2BC**, PhMr05L2RO, **PhM04CC2RO**, **PhJ05CC2RO**, PhMr05L2Mig, **PhMr05CC1BC**, PhMr05CC1RO, PhMr05CC1GO, PhJ05CC1Mig, PhJ05CC2BC, **PhJ05CC2GO**, PhA04CC1RO, PhA04CC1GO, PhA04CC1Mig, PhA04CC2BC, PhA04CC2GO, PhA04CC2Mig, PhN04L1BC, **PhN04L1RO**, PhN04L1GO, PhN04L1Mig, PhN04L2RO, **PhN04L2GO**, PhN04L2Mig, PhN04CC1BC, PhN04CC1RO, PhN04CC1GO, PhN04CC1Mig, PhN04CC21BC, **PhJ05L1RO**, **PhJ05L1GO**, PhJ05CC1RO, PhJ05CC1GO, **PhA04L1BC, PhA04L2Mig**	49
AG3	**PhMr05L1RO**	1
AG4	PhJ05L2BC	1

*Isolate codes as in Table 6-3.

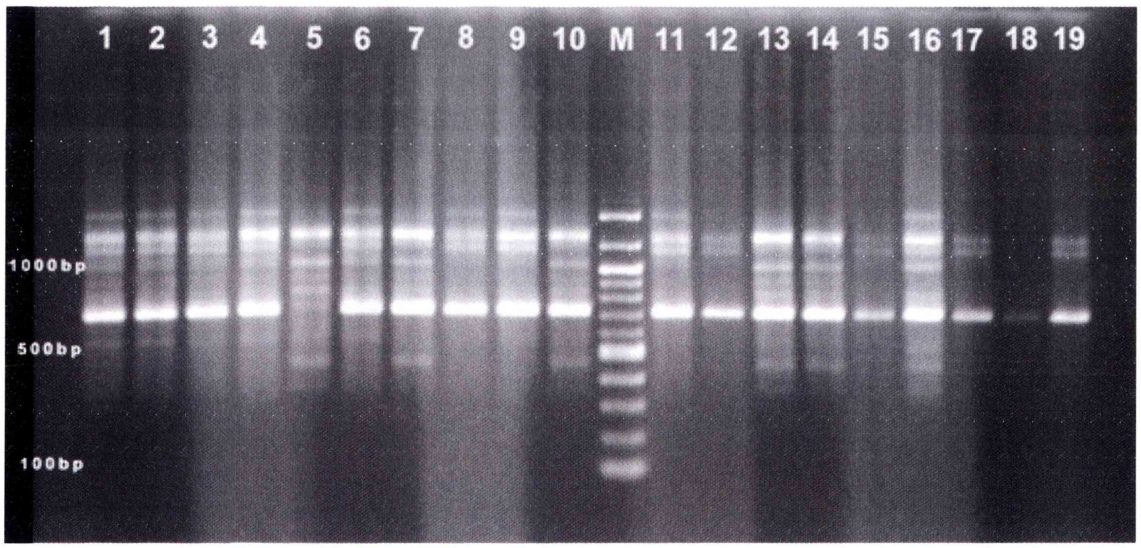

Figure 6-6 ISSR profiles observed for isolates of *Phytophthora drechsleri* using AG-primer.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhA03L1	**(6) PhJ04L1**	**(11) PhMr05L1GO**	**(16) PhJ05CC1BC**
(2) PhN04L2GO	**(7) PhM04L2BC**	**(12) PhA04L2Mig**	**(17) PhMr05L2BC**
(3) PhJ05L1RO	**(8) PhA04L1BC**	**(13) PhJ05L1GO**	**(18) PhMr05L1RO**
(4) PhMr05CC1BC	**(9) PhM04CC2RO**	**(14) PhN04L1RO**	**(19) PhM04CC1BC**
(5) PhMr05L2GO	**(10) PhJ05CC2RO**	**(15) PhJ05CC2GO**	**(M) 100 bp ladder**

6.3.2.3.3 Variation observed using the CGA-primer

Five groups were formed by using this primer (Table 6-7; Figure 6-7). Two to nine bands were found ranging between 250 and 1350 bp. Two main groups were found containing the majority of isolates; there were no correlations found between these groups and sample times, farms or cultivars. However, group CGA1, one of the minor groups, contains only isolates from the farm Leppington 1; there were three isolates from the May 2003 and May 2004 samples, one isolate from the March 2004 sample and one isolate from the November 2003 sample. Group CGA2 contains just two isolates obtained in the May 2004 sample from the farm Leppington 1 but from different cultivars. However, group CGA4 contains three isolates obtained from the May 2004 sample from both farms located in the Central Coast area, but from different cultivars.

Table 6-7 Groups of isolates based on analysis of polymorphic fragments generated by CGA ISSR primer. Isolates in bold are shown in Figure 6-7.

Group	Isolate code*	No. of isolates
CGA1	PhM03L1, PhN03L1, PhMr04L1, PhM04L1BC, PhM04L1RO	5
CGA2	PhM04L1GO, PhM04L1Mig	2
CGA3	PhM04CC1RO, **PhJ05L1RO,** PhMr05CC2GO, PhM04L2RO, PhN04L1Mig, **PhMr05L2GO**, PhMr05L2Mig, **PhMr05CC1BC**, PhMr05CC1RO, PhMr05CC1GO, PhMr05CC1Mig, **PhM04CC2RO,** PhMr05CC2BC, **PhA03L1,** PhMr05CC2RO, PhMr05L2RO, **PhA04L1BC,** PhN04CC1BC, **PhJ04L1,** PhN04CC1Mig, **PhJ05L1GO**, PhJ05L1Mig, PhJ05L2BC, PhJ05CC1RO, PhJ05CC1GO, **PhN04L2GO, PhM04L2B, PhJ05CC2RO, PhN04L1RO**	29
CGA4	PhM04CC1Mig, PhM04CC1GO, PhM04CC2BC	3
CGA5	**PhMr05L2BC**, PhM04CC2GO, PhM04CC2Mig, **PhMr05L1GO**, **PhA04L2Mig**, PhA04CC1RO, PhA04CC1GO, PhA04CC1Mig, PhA04CC2BC, PhA04CC2GO, PhA04CC2Mig, PhN04L1BC, **PhJ05CC2GO**, PhN04L1GO, PhN04L2RO, **PhM04CC1BC**, PhN04L2Mig, PhN04CC1RO, PhN04CC1GO, PhN04CC2RO, PhN04CC2GO, **PhJ05CC1BC**, PhJ05CC1Mig, PhJ05CC2BC, PhMr05L1BC, **PhMr05L1RO**, PhMr05L1Mig, PhJ05L1BC, PhN04CC21BC	29

*Isolate codes as in Table 6-3.

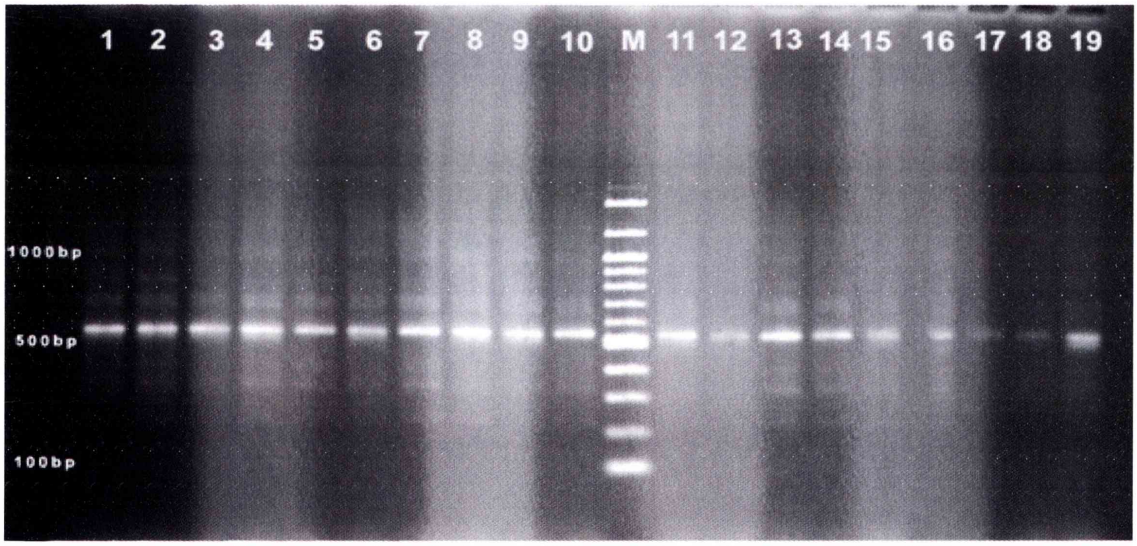

Figure 6-7 ISSR profiles observed for isolates of *Phytophthora drechsleri* using CGA-primer.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhA03L1	**(6) PhJ04L1**	**(11) PhMr05L1GO**	**(16) PhJ05CC1BC**
(2) PhN04L2GO	**(7) PhM04L2BC**	**(12) PhA04L2Mig**	**(17) PhMr05L2BC**
(3) PhJ05L1RO	**(8) PhA04L1BC**	**(13) PhJ05L1GO**	**(18) PhMr05L1RO**
(4) PhMr05CC1BC	**(9) PhM04CC2RO**	**(14) PhN04L1RO**	**(19) PhM04CC1BC**
(5) PhMr05L2GO	**(10) PhJ05CC2RO**	**(15) PhJ05CC2GO**	**(M) 100 bp ladder**

6.3.2.3.4 Variation observed using the GT-primer

One to eight polymorphic fragments of GT-amplified products divided isolates into four groups with bands ranging between 250 and 1600 bp. Two isolates collected from the farm Central Coast 1 in the May 2004 sample were found in one group (GT4). Other isolates were spread amongst the three other groups without correlation between groups and sample times, farms or cultivars.

Table 6-8 Groups of isolates based on analysis of polymorphic fragments generated by GT ISSR primer. Isolates in bold are shown in Figure 6-8.

Group	Isolate code*	No. of isolates
GT1	PhM03L1, **PhA03L1**, PhN03L1, **PhJ04L1**, PhMr04L1, PhM04L1BC, PhM04L1RO, PhM04L1GO, PhM04L1Mig, **PhM04L2BC**, PhM04CC1RO, PhM04CC1GO, PhM04CC2BC, PhM04CC2GO, PhM04CC2Mig, PhA04CC1GO, PhA04CC1Mig, PhA04CC2BC, PhA04CC2GO, PhN04L1BC, PhN04L2RO, **PhN04L2GO**, PhN04L2Mig, **PhJ05L1GO,** PhN04CC1RO, PhN04CC1GO, PhN04CC1Mig, PhN04CC21BC, PhN04CC2RO, PhJ05L1BC, **PhJ05L1RO**, PhJ05L2BC, **PhMr05CC1BC**, PhMr05CC1GO, PhMr05CC2BC, PhMr05L1BC, **PhMr05L2GO**, PhJ05CC1GO, PhN04CC2GO	39
GT2	PhM04L2RO, PhMr05L2RO, PhMr05CC2RO, PhA04CC1RO, **PhMr05L1GO**, **PhA04L2Mig**, PhA04CC2Mig, PhJ05L1Mig, PhN04L1GO, PhN04L1Mig, PhN04CC1BC, **PhJ05CC2RO, PhM04CC2RO, PhA04L1BC**	14
GT3	**PhN04L1RO**, PhMr05CC2GO, PhMr05CC1Mig, PhMr05CC1RO, **PhJ05CC2GO**, PhJ05CC2BC, **PhJ05CC1BC**, PhMr05L1Mig, **PhMr05L2BC**, PhJ05CC1Mig, PhJ05CC1RO, **PhMr05L1RO**, PhMr05L2Mig	13
GT4	PhM04CC1Mig, **PhM04CC1BC**	2

*Isolate codes as in Table 6-3.

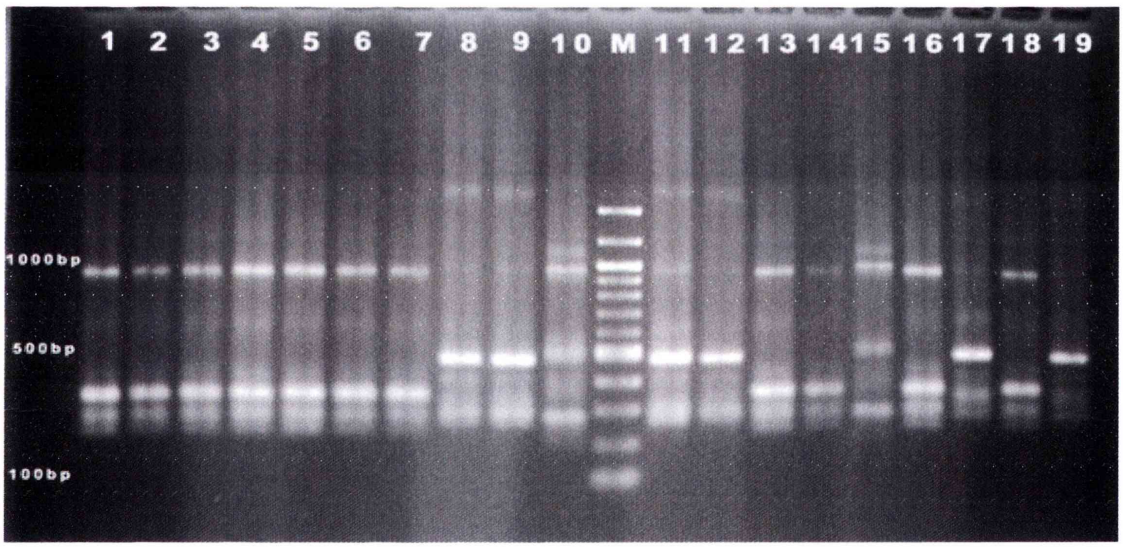

Figure 6-8 ISSR profiles observed for isolates of *Phytophthora drechsleri* using GT-primer.

Key to lane numbers, indicating isolate codes (Table 6-3) –

(1) PhA03L1	**(6) PhJ04L1**	**(11) PhMr05L1GO**	**(16) PhJ05CC1BC**
(2) PhN04L2GO	**(7) PhM04L2BC**	**(12) PhA04L2Mig**	**(17) PhMr05L2BC**
(3) PhJ05L1RO	**(8) PhA04L1BC**	**(13) PhJ05L1GO**	**(18) PhMr05L1RO**
(4) PhMr05CC1BC	**(9) PhM04CC2RO**	**(14) PhN04L1RO**	**(19) PhM04CC1BC**
(5) PhMr05L2GO	**(10) PhJ05CC2RO**	**(15) PhJ05CC2GO**	**(M) 100 bp ladder**

6.3.2.3.5 Variation observed using the four ISSR primers

Data described by the four primers were used to construct an UPGMA dendrogram (Figure 6-9). The estimated similarity by Dice's coefficient within isolates of *Phytophthora drechsleri* was based on 66 combined ISSR markers. The UPGMA dendrogram showed that there were 6 groups at 78% of Dice similarity (Figure 6-9).

There appeared to be a correlation between the groups and the sample times, particularly when considered on the basis of cooler season samples (May and August) compared with warmer season samples (November, January and March). The majority of the cooler season isolates were located in the last two groups, 5 and 6 (Figure 6-9). These two groups contained a total of 18 isolates and all were from either the May or August sample times. Group 2 also contained only cooler season isolates and both members were from May sample times. Four isolates from cooler season samples were found in other groups (one isolate of the 19 in group 1 and three isolates of the 23 in group 3).

The majority of the warmer season isolates were located in groups 1, 3 and 4 (Figure 6-9). In group 1, 18 of the 19 isolates were from warmer season samples, with 17 of the 18 being isolates from the January or March sample times, and one isolates from the November sample time. In group 3, 20 of the 23 isolates were from warmer season samples, with 14 of the 20 isolates being from the November sample, 4 of the 20 isolates being from the March sample and 2 of the 20 isolates being from the January sample. In group 4, all 6 isolates were from the warmer season, January sample time (Figure 6-9).

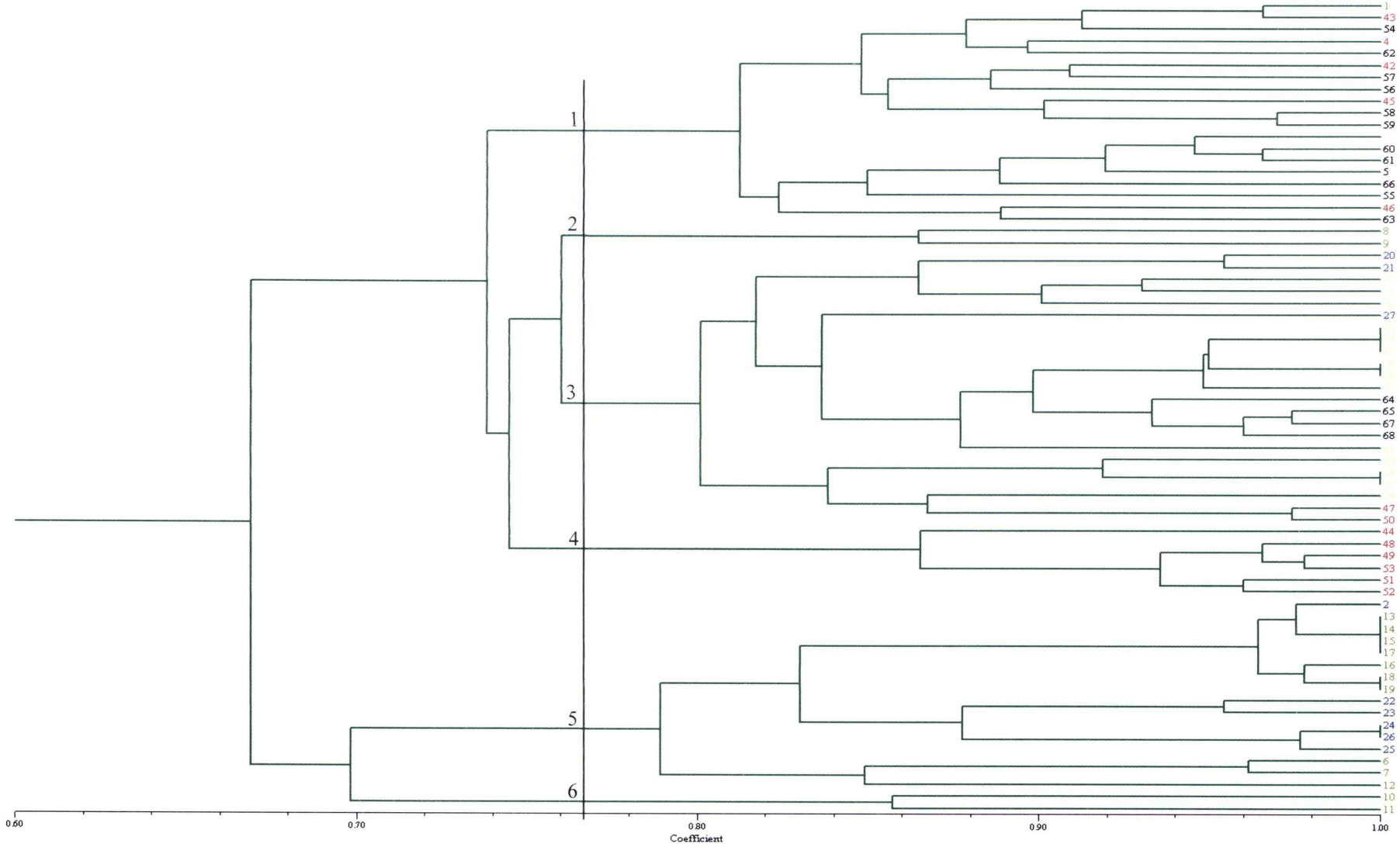

Figure 6-9 Dendrogram of ISSR similarity using DICE Coefficient based on UPGMA clustering of *Phytophthora drechsleri* from different times, farms and lettuce cultivars. See Table 6-4 for isolate codes corresponding to isolate number shown at right. Colors are indicated sample time as follows: green: May samples; blue: August samples; yellow: November samples; red: January samples; and black: March samples.

6.3.3 Morphology studies

6.3.3.1 Colony characteristics on different agar media

Colonies of the 15 *P. drechsleri* isolates tested formed a stellate to rosaceous pattern on PDA (Figure 6-10 a and b). On PCA, colonies were stellate to petallate in pattern (Figure 6-10 c), while on CMA, were colonies without any special pattern (Figure 6-10 d).

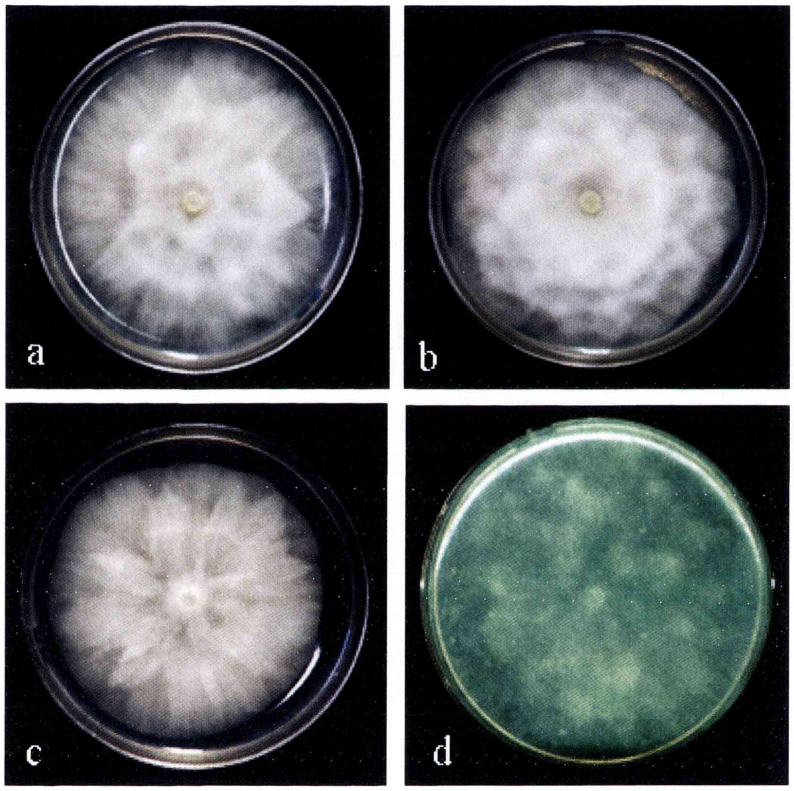

Figure 6-10 Colonies of *Phytophthora drechsleri* on different media – PDA (a and b), PCA (c) and CMA (d).
(a) stellate to rosaceous pattern;
(b) rosaceous colony;
(c) stellate to petallate colony; and
(d) colony without special pattern.

6.3.3.2 Morphological characteristics

In all isolates studied hyphae were quite uniform and fine (4 to 8 µm; average diameter 6 µm) (Figure 6-11 a and b), with hyphal swellings forming in groups of angular or round chains or clusters (Figure 6-11 c). Chlamydosopres were not produced in any cultures examined.

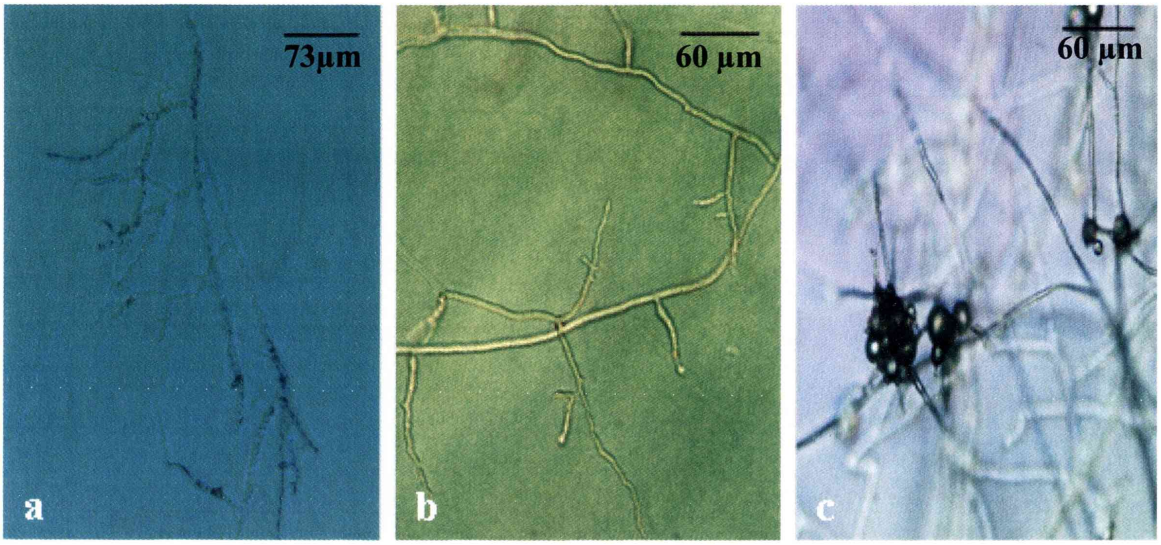

Figure 6-11 *Phytophthora dreschleri* hyphae (a and b) and hyphal swellings (c) on PCA.

All isolates studied produced an abundant number of sporangia on PCA media (Figure 6-12). Sporangiophores were narrow, tending to widen slightly below the sporangium, usually unbranched with a single terminal sporangium, with growth continuing by internal proliferation through the empty sporangium with further sporangia produced inside or beyond the first one. Sporangia were variable in size and shape (Figure 6-12). Shapes were ovoid-ovate, elongated obpyriform, sometimes asymmetrical and tapering at the base, non-papillate, noncaducous, and with apical thickening barely detectable. Sporangial size was variable in both length and breadth, ranging between 18–76 × 10–40 µm (Table 6-9). Significant differences in sporangial length, breadth and length : breadth ratio were found between the 15 isolates examined (Appendix 8 b). However, these differences were not associated with sample time, farm or cultivar (Table 6-9).

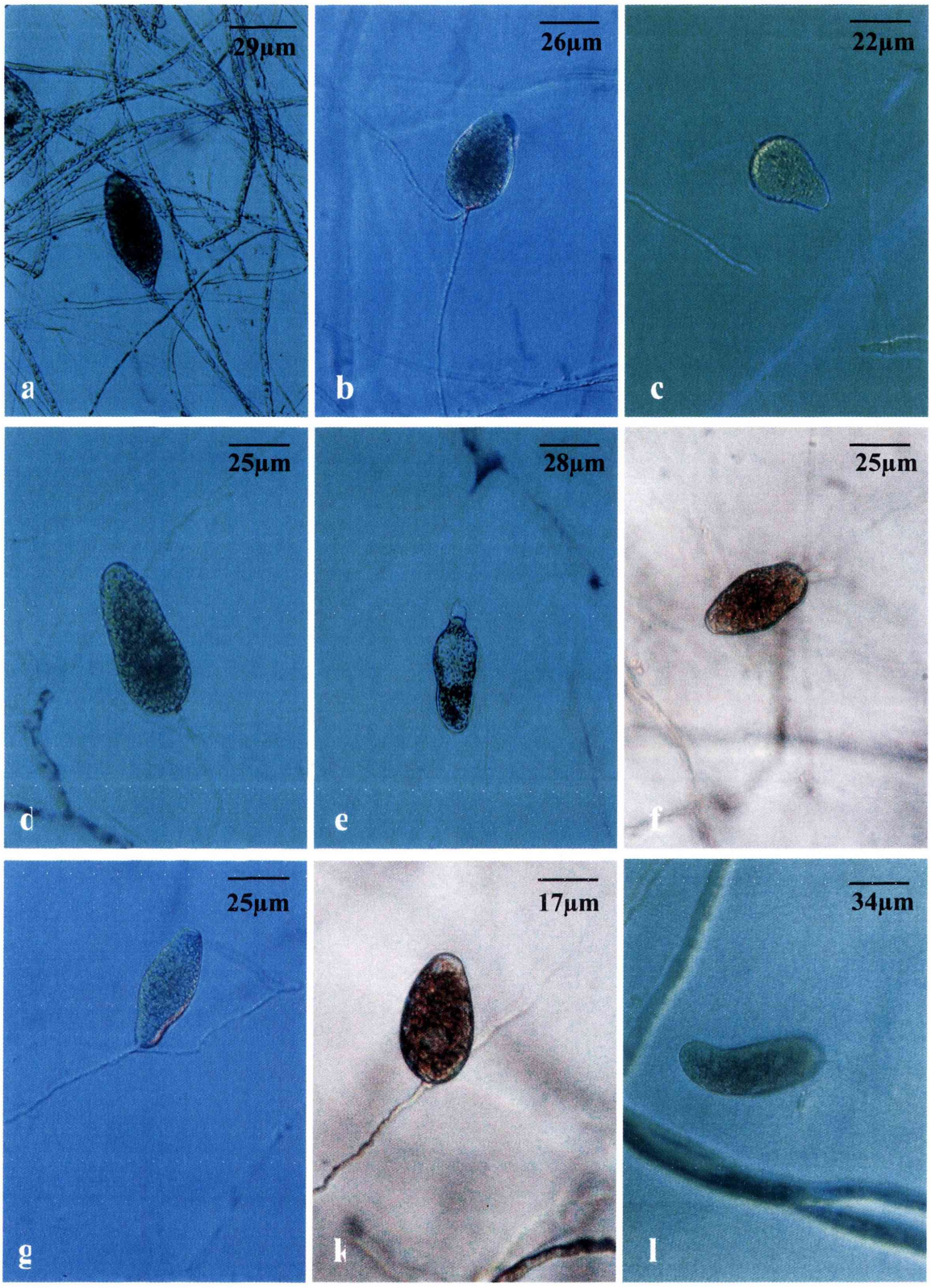

Figure 6-12 A nonpapillate and noncaducous sporangium of *Phytophthora drechsleri* with different shapes. a, limoniform or naviculate to ovoid. b, ovoid-ovate. c, ovoid-obpyriform. d, obpyriform to ellipsoid. d, obpyriform to ellipsoid, tapered at the base. f, ellipsoid to obpyriform with shape varying. g, limoniform or naviculate to ovoid, tapered at the base. k, ovoid-ovate. l, obpyriform with curved shape.

Table 6-9 Sporangial size (length, breadth and length : breadth ratio) in 15 isolates of *Phytophthora drechsleri* isolated from roots of hydroponic lettuce. Within a column, means followed by the same letter are not significantly different from each other at $P \leq 0.05$. n = 5 for each isolate at each temperature. (Appendix 8 b.)

	Length (µm)			Breadth (µm)			L:B ratio ±
Isolate code*	Min.	Max.	Mean ± Std. Error	Min.	Max.	Mean ± Std. Error	Std. Error
PhMr05L2BC	18	70	32.04 ± 1.97 a	10	40	20.27 ± 1.05 abc	2.30 ± 0.20 b
PhN04L1GO	19	70	32.79 ± 2.08 ab	11	40	20.69 ± 1.02 abc	2.27 ± 0.15 b
PhN03L1	20	70	32.88 ± 1.91 ab	10	38	19.60 ± 0.92 a	2.07 ± 0.16 ab
PhN04L1BC	19	72	34.06 ± 1.80 abc	10	33	18.38 ± 0.86 a	2.14 ± 0.14 ab
PhN04CC1Mig	20	72	35.50 ± 1.66 abcd	10	37	19.33 ± 0.94 a	2.35 ± 0.13 b
PhM04L2BC	18	76	38.81 ± 2.07 abcde	12	37	19.96 ± 0.90 ab	2.60 ± 0.18 b
PhJ05L2BC	18	76	39.00 ± 2.06 abcde	11	38	20.17 ± 0.79 abc	2.05 ± 0.12 ab
PhM04CC1RO	20	70	39.29 ± 1.99 abcde	10	40	24.63 ± 0.99 bc	2.60 ± 0.18 b
PhMr05L1BC	19	76	41.75 ± 2.08 bcde	11	40	29.85 ± 1.13 d	1.45 ± 0.07 a
PhM04L1RO	20	76	42.52 ± 1.94 de	11	37	24.48 ± 1.79 bc	2.60 ± 0.18 b
PhM04L1Mig	20	76	42.60 ± 2.05 cde	13	40	24.85 ± 1.09 c	2.21 ± 0.14 ab
PhM04CC2Mig	19	76	44.25 ± 2.25 de	11	37	19.29 ± 0.94 a	2.60 ± 0.18 b
PhA04CC2BC	20	76	44.93 ± 2.16 de	11	37	19.17 ± 0.95 a	2.60 ± 0.18 b
PhJ05CC1GO	20	76	45.00 ± 2.18 de	11	37	19.15 ± 0.95 a	2.60 ± 0.18 b
PhA04CC2Mig	20	76	45.23 ± 2.08 e	10	40	19.29 ± 1.06 a	2.39 ± 0.14 b

* Isolate codes as in Table 6-3.

6.3.4 Physiological studies

6.3.4.1 Colony growth rates at different temperatures and optimum, minimum and maximum temperatures

All of the 15 isolates of *Phytophthora drechsleri* tested had an optimum growth temperature of 25°C and grew well at temperatures of 20°C and 30°C (Figure 6-13). The lowest growth temperature for the 15 isolates tested, recorded within the limit of temperatures available for testing, was 10°C (Figure 6-13). All of the 15 isolates tested grew at 35°C but not at 40°C (Figure 6-13). No significant differences in growth rate were found between the 15 isolates tested at each temperature (Appendix 8 c).

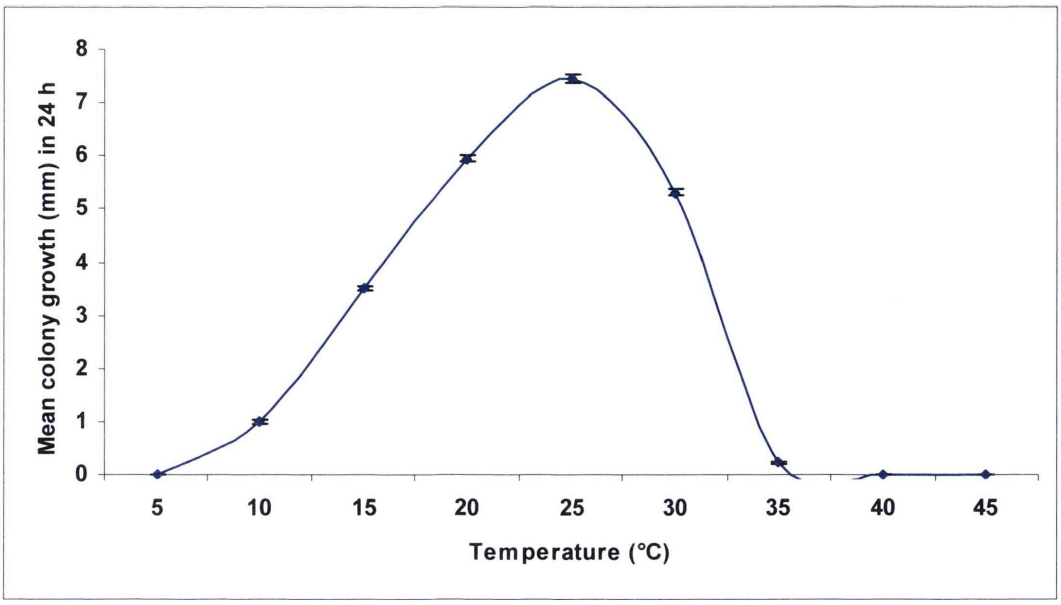

Figure 6-13 Mean colony growth (mm) in 24 h for 15 isolates of *Phytophthora drechsleri* grown on PCA media at range of temperatures from 5°C to 45°C in the dark. Colony growth was measured between 24h and 48h. Bars are standard error of the mean.

6.3.5 Mating type

All isolates were found to be heterothallic and of the A_1 mating type. They produced oogonia with antheridia when paired with the A_2 mating type of *P. cryptogea*. Most isolates produced antheridia between 14 and 18 days after inoculation; a few isolates, however, produced antheridia after 25 days. Antheridia were amphigynous (Figure 6-14). Oogonia (20 to 55 µm in diameter, average 36 µm) were globose with a tapered base and the oogonial walls were smooth, hyaline and thick. Oospores were plerotic (Figure 6-14).

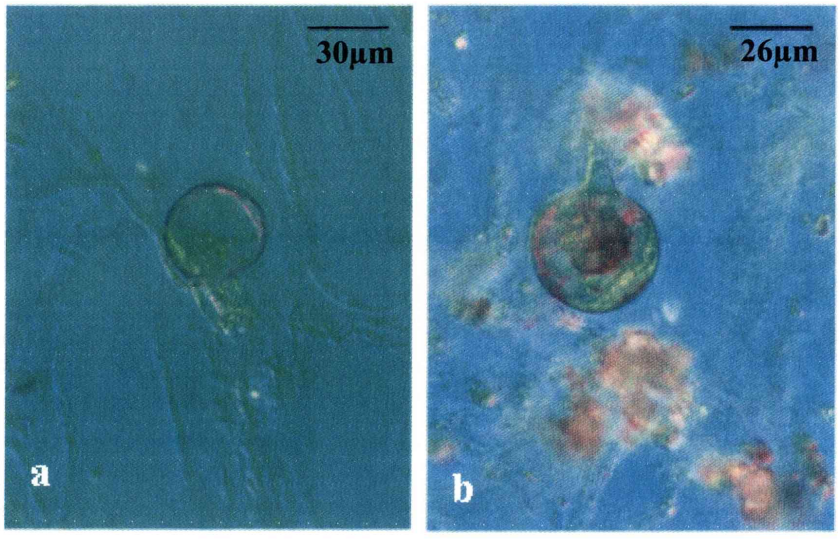

Figure 6-14 Globose oogonia with amphigynous antheridia in *P. drechsleri*.

6.3.6 Storage of representative isolates

Representative isolates of *Phytophthora drechsleri* from the present study have been stored in the collection of NSW Agriculture at Orange, NSW (see Appendix 8 d for details).

6.4 Discussion

The results presented in this study include the first comprehensive survey of hydroponic lettuce farms in the Sydney and Central Coast areas of NSW to obtain isolates of *Phytophthora* from several lettuce cultivars and at different sampling times throughout the year. This study reports, for the first time, the occurrence of *Phytophthora drechsleri* in hydroponic lettuce in NSW. Sixty-eight isolates of *P. drechsleri* were recovered from roots of diseased and apparently healthy hydroponic lettuce in the Sydney and Central Coast areas of NSW at different times throughout the year. The number of isolates recovered was greater in samples taken when the nutrient solution temperature was higher than 20-25°C (see Chapter 2, Table 2-4 and Chapter 3, Table 3-11).

Phytophthora sp. has been recorded on lettuce grown in soilless culture in NSW (Tesoriero *et al.* 1991), but identification to species level was not determined. As well, *Phytophthora* spp. have been isolated only infrequently from hydroponic lettuce samples sent by farmers in recent years to the Plant Health Diagnostic Service of NSW Department of Primary Industries (Tesoriero, pers. comm.). Moreover, none of these isolates has been identified as *P. drechsleri* (Tesoriero, pers. comm.).

P. drechsleri does not appear to have been reported from hydroponic farms in other states of Australia. *Phytophthora* was isolated from hydroponic lettuce roots in Queensland, Australia (Hutton & Forsberg 1991), but was not identified to species level. To date, *P. cryptogea* but not *P. drechsleri* has been reported on lettuce grown hydroponically around the world (Linde *et al.* 1990; Jamart 1999). Other *Phytophthora* spp. have been isolated from roots of crops grown in hydroponic systems including *P. cryptogea* from root rot of tomato (Pegg & Jordan 1991) and *P. capsici* from roots and crown rot of pepper plants (Forster *et al.* 1998).

In soil, *P. porri* was reported causing stem rot of lettuce plants at low temperatures (15°C) in South Australia (Sitepu & Bumbieris 1981). Most diseases caused by *P. drechsleri* are root rots and stem rots (Erwin & Ribeiro 1996). These diseases include root and stem rot of safflower (*Carthamus tinctorius* L.), root rot of sugar beet (*Beta vulgaris* L.), root rot of cucurbits (e.g. cucumber, *Cucumis sativus* L.), stem rot of

sunflower (*Helianthus annuus* L.) and stem rot and blight of pigeon pea (*Cajanus cajan* L.).

Traditionally, identification of *Phytophthora* spp. is based on morphological characteristics. Even though there are several comprehensive keys available (Waterhouse 1970; Stamps *et al.* 1990; Erwin & Ribeiro 1996), the identification of *Phytophthora* species is still considered to be difficult, mostly because there are relatively few morphological features by which species are distinguished and significant variability and overlap of features exist between some species (Mills *et al.* 1991; Bates *et al.* 1993; Erwin & Ribeiro 1996). Nevertheless, there appear to be few problems with many of the species. With closely related species, however, such as *P. drechsleri* and *P. cryptogea*, the identification of a particular isolate varies, depending on the person carrying out the identification and the host plant. For example, *Phytophthora* isolated from sunflower, cucurbits and potato tubers is identified as *P. cryptogea* by some researchers, but as *P. drechsleri* by others (Ho & Jong 1986).

In this study, morphological characteristics of the isolates obtained were within the ranges for both *P. drechsleri* and *P. cryptogea* which are located in group VI of *Phytophthora* keys (Waterhouse 1970; Stamps *et al.* 1990; Erwin & Ribeiro 1996). These characteristics include sporangial size (18–76 × 10–40 µm) (Table 6-9) and appearance (ovoid-ovate to elongated obpyriform, sometimes asymmetrical and tapering at the base, non-papillate, noncaducous, apical thickening barely detectable, and proliferating internally), hyphal diameter (4 to 8 µm, average 6 µm), and the presence of hyphal swellings (section 6.3.3.2, including Figures 6-11 and 6-12). Moreover colony characteristics on three different media (PDA, PCA and CMA) were similar to those reported for both *P. drechsleri* and *P. cryptogea.*

Studies on the cardinal temperatures of 15 representative isolates indicated that the optimum temperature for growth was 25°C, whilst the minimum and maximum temperatures at which growth occurred were 10°C and 35°C, respectively (Figure 6-13). It should be noted, however, that the growth measured at 35°C was less than 0.5 mm over a 24 hour period and much less than the growth measured at 30°C for the same period. Based on these results, isolates of *Phytophthora* spp. obtained in this study were *P. drechsleri* because of their growth at 35°C, according to the majority of the keys

(Waterhouse 1970; Stamps *et al.* 1990; Erwin & Ribeiro 1996). Guzman *et al.* (2005), however, found that *P. cryptogea* isolated from peach trees did not grow at 35°C. Ho and Jong (1986) found that just five of 14 *P. drechsleri* isolates grew at 35°C; they also found one of 19 *P. cryptogea* isolates grew at 35°C. Thus, whilst the occurrence of growth at 35°C for the isolates obtained in this study suggests that these isolates are *P. drechsleri*, by reference to the main keys on *Phytophthora*, conflicting information in the literature suggests that this characteristic alone is not a reliable basis for distinguishing between this species and *P. cryptogea*.

This study determined that all of the *P. drechsleri* isolates collected from two different geographic areas of NSW, in different seasons and from four lettuce cultivars were of the A_1 mating type. It seems that there is no need for *P. drechsleri* isolates to produce sexual structures in hydroponic systems especially NFT. The reasons for this could be the simplicity of the NFT system and the fact that pathogens can survive without the sexual structures. *P. drechsleri* was reported as being heterothallic and producing oogonia when paired with either the A_1 or A_2 mating type (Kannaiyan *et al.* 1980; Ho & Jong 1986). Tucker (1931) and Klisiewicz (1977), however, reported that *P. drechsleri* could produce oogonia in single cultures. Nevertheless, Erwin and Ribeiro (1996) noted that only a few isolates of *P. drechsleri* had been reported as producing oogonia in single cultures and that the majority needed to pair with mating type A_1 or A_2 in order to produce oogonia.

In order to identify the isolates obtained in the present study and to investigate the relationships among them, molecular techniques were used. PCR-RFLP analysis of the ITS region of rDNA, following the protocols of Drenth *et al.* (1999), was used to identify the 68 *Phytophthora* isolates obtained in this study. By comparing the fragments produced from the isolates in this study (Figures 6-2, 6-3 and 6-4) with the fragments of 27 *Phytophthora* species in the manual produced by Drenth *et al.* (1999), all 68 *Phytophthora* isolates were identified as *P. drechsleri*. In this method, restriction enzymes were able to differentiate between 27 *Phytophthora* species (Drenth *et al.* 1999) including between the two related species *P. drechsleri* and *P. cryptogea*, which many researchers consider to be one species or one species complex. However, this method failed to distinguish between the closely related species *P. erythroseptica* and *P. cryptogea*. This technique was found to be a useful and time saving method for

scanning and identifying a large number of *Phytophthora* spp. compared with morphological methods, especially for investigating those species with related morphological features. Other studies using the technique of PCR-RFLP analysis to distinguish between different isolates of *Phytophthora* have found that *P. drechsleri* and *P. cryptogea* were closely related and always located in the one group (Ho & Jong 1986; Mills *et al.* 1991; Forster *et al.* 1995; Cooke *et al.* 2000; Kroon *et al.* 2004; Guzman *et al.* 2005).

The identification of the *Phytophthora* isolates as *P. drechsleri* was further confirmed through sequencing of the ITS region for four selected isolates. When these sequences were compared with the GenBank databases by using BLAST search, they matched *P. drechsleri* (Table 6-4). However, the sequence of one isolate (PhN03L1) matched both *P. drechsleri* and *P. cryptogea* at the same level of identity (99%) and with no nucleotide gaps. Sequencing of ITS region has been found to be effective in identifying species and determining groupings of species within the genus *Phytophthora* (Lee & Taylor 1992; Cooke & Duncan 1997; Kroon *et al.* 2004).

The results of this study demonstrated that a DNA fingerprint profile of *P. drechsleri* can be generated using the ISSR protocol. The ISSR clustering, based on the use of four primers which generated 66 markers, gathered the 68 isolates of *P. drechsleri* into six clonal lineages (Figure 6-9). A correlation was found between the six groups and the sample times from which isolates were obtained. Three of the groups (groups 5, 6 and 2) contained isolates that were obtained only from sampling in the cooler seasons (May and August samples) whilst the other three groups (groups 1, 3 and 4) contained isolates that were obtained predominantly from sampling in the warmer seasons (November, January and March samples). Isolates from cooler seasons were found to have less variation than isolates from warmer seasons. The reason for this variation could be that fewer isolates were obtained in the cooler seasons (24 isolates) than in the warmer seasons (44 isolates). It would be worthwhile to obtain and study more isolates in the cooler seasons (from May and August) to confirm the finding of this ISSR analysis.

No correlation was found between the six groups established by ISSR clustering and either the farms or the geographic areas from which the isolates were obtained. Furthermore, there was no correlation between the groups and the lettuce cultivars

yielding the isolates. It appeared that within either the cooler times of the year (May and August) or warmer times of the year (November, January and March), the types of isolates of *P. drechsleri* present in hydroponic lettuce are similar for different farms, different geographic areas and different cultivars. It may be that *P. drechsleri* is introduced via water, seeds or seedlings and that these potential sources are similar for all four farms studied. The farms examined in this study used town water to dissolve the nutrient solution and, whilst this supply was different for the Sydney and Central Coast areas, both supplies could have contained *P. drechsleri*. Furthermore, many hydroponic farms in the Sydney and Central Coast areas access the same seed and seedling sources. Unfortunately, no records could be found regarding the distribution of *P. drechsleri* in Australi.

Further research should investigate the relationships among the populations of *Phytophthora* isolated from roots of hydroponic lettuce collected from more farms in NSW and from other states in Australia to determine if *P. drechsleri* is widespread in hydroponic systems producing lettuce in Australia and whether other *Phytopthora* spp. are present elsewhere. Comparisons should also be made between these isolates and *Phytophthora* spp. obtained from roots of hydroponic lettuce overseas. This research should include both the molecular techniques used in the present study, as well as morphological characteristics, physiological studies to establish the cardinal temperatures and determination of mating type.

To clarify the distinction between *P. drechsleri* and *P. cryptogea* and to enable accurate identification of these species in the future, it would also be useful to study these species further, with molecular techniques involving different region(s) of DNA.

7 GENERAL DISCUSSION

This study reports several key findings on root rot disease of hydroponic lettuce in the Sydney and Central Coast areas of New South Wales (NSW). This study is the first comprehensive, systematic survey of hydroponic lettuce at different farms (four farms) in different lettuce-production areas (two areas) for fungi and oomycetes causing root disease. In this study, hydroponic lettuce farms were surveyed in both the warmer times of the year, when root disease is often reported by farmers to the NSW Department of Primary Industries, and the cooler times of the year when root disease is not reported. This study confirmed that more root disease of hydroponic lettuce occurred in warmer times of the year than in cooler times of the year.

Results obtained in this study indicated that two oomycetes, *Pythium* and *Phytophthora*, were the main microorganisms isolated from lettuce roots grown in hydroponic systems. Most isolates of *Pythium* obtained in this study were identified as belonging to *Pythium* group F according to van der Plaats-Niterink (1981) and were found to be present in the hydroponic lettuce systems all year round. One isolate of *Pythium* (PMr05CC2RO) was identified as *P. coloratum* according to the same key. In the literature, including literature from Australia, *Pythium aphanidermatum* is the main *Pythium* species present in hydroponic systems and especially on cucumber. However, in the present study, this species was not isolated from lettuce.

Isolates of *Phytophthora* spp. were identified as *P. drechsleri* according to Stamps *et al.* (1990), Erwin and Ribeiro (1996) and Drenth *et al.* (1999 & 2006), and were found to be present in hydroponic lettuce systems all year round. This is the first report of the isolation and identification of *P. drechsleri* from lettuce roots grown in hydroponic systems in NSW. *Phytophthora drechsleri* was isolated less frequently than *Pythium* spp.

Other fungi were isolated, such as *Fusarium* spp. and *Rhizoctonia* spp., but these appeared to be not pathogenic to lettuce grown in hydroponic systems. Both fungi, however, were reported causing diseases on lettuce grown in soil. One of the advantages of hydroponic systems, compared with soil-based production systems, is that they reduce the number of pathogens that can affect plants.

Nutrient solution temperature seems to be the most important factor in relation to root rot disease severity on hydroponic lettuce. As reported in this study, root rot disease severity at hydroponic lettuce farms was high when the nutrient solution temperature was 20-30.5°C; at temperatures of 13.5-18°C, however, disease severity was low. It should be noted that these temperatures were measured in the growth channels on the survey days; daily temperature fluctuations would have occurred and so precise monitoring of nutrient solution temperature is needed to further describe the relationship between temperature and root rot disease severity.

To study the effect of the nutrient solution temperature on root rot disease development, an experimental hydroponic system was employed. It was found that root rot disease occurred in lettuce plants inoculated with an isolate belonging to *Pythium* group F or a combination of an isolate of *Pythium* group F and an isolate of *Phytophthora drechsleri*, when the nutrient solution temperature was 24-27°C (minimum and maximum). There was, however, no disease at 16-17°C (minimum and maximum) when the same inoculations were carried out. This finding is consistent with reports from surveys of hydroponic lettuce farms, in which it was found that root rot disease was prevalent in warm weather when the nutrient solution temperature was 20°C or higher, but not when the nutrient solution temperature was below 18°C.

Other experiments further studied the effects of high nutrient solution temperature. A nutrient solution temperature maintained continuously at 34°C appeared to be too high for satisfactory lettuce growth; unhealthy lettuces were observed in all treatments, including the control plants. However, lettuces inoculated with an isolate belonging to *Pythium* group F and the combination of isolates of *Pythium* group F and *Phytophthora drechsleri* were more severely infected. Experiments were also carried out at a nutrient solution temperature of 34°C for ten hours followed by 18-20°C to test the effect of exposure to a short period of high temperature on root rot disease development. Unhealthy roots appeared in plants inoculated with an isolate of *Pythium* group F and the combination of *Pythium* and *Phytophthora drechsleri*; yield reduction occurred in plants inoculated with an isolate of *Pythium* group F and with the combination of *Pythium* and *Phytophthora drechsleri*.

The findings of this study increase our understanding of root rot diseases of hydroponic lettuce and provide valuable information in relation to disease management. Ultimately these results could help farmers to reduce their losses and provide consumers with high quality crops. In relation to disease management, nutrient solution temperature seems to be the main factor affecting the severity of the disease. One possibility for reducing the losses in warmer seasons is to try to control the temperature of the nutrient solution. As shown in this study, the potential pathogens are in hydroponic systems all year round and when the temperature reaches a certain level, which is closer to the optima for these pathogens, they will start damaging plants. One way of controlling the nutrient solution temperature is to place hydroponic systems in temperature-controlled glasshouses to keep the air temperature, and hence the nutrient solution temperature, under control, especially in hotter weather. Another possibility, which a few farmers are trying already, is that of using dam water to cool the nutrient solution temperature in a heat-exchange system, by pumping the nutrient solution in pipes to the bottom of the dam, where water is colder, and then pumping it back to the nutrient solution supply tank. This method, if designed well, can be an effective way to reduce the nutrient solution temperature. However, such heat-exchange systems need to be implemented and monitored to assess the extent of their effectiveness.

Other possibilities for root disease management lie with the pathogens themselves, such as reducing the inoculum level in the nutrient solution in the warmer seasons. If the inoculum level of *Pythium* and/or *Phytophthora* in the nutrient solution is low, the disease severity level could be low as well. Cleaning the hydroponic systems before nutrient solution temperatures start to rise above approximately 20°C could help to reduce crop losses. The main reason for cleaning the systems is that starting the warmer seasons with a very low level of inoculum of *Pythium* and/or *Phytophthora* may reduce the losses. As well there may be times during lettuce production in summer when inoculum levels are so high that lettuce production is not feasible and systems need to be thoroughly cleaned before introducing a new batch of lettuce seedlings.

Generally, isolates of *Pythium* group F and *Phytophthora drechsleri* obtained in this study were found to be pathogenic to lettuce plants grown in potting mix and incubated at 25°C and 35°C, but not at 15°C. Moreover, the isolate of *Pythium coloratum* was

found to be pathogenic to lettuce plants grown in an experimental hydroponic system when the nutrient solution temperature was between 22°C and 26°C. However, *Phytophthora drechsleri* was found to be not pathogenic to lettuce grown in an experimental hydroponic system when the nutrient solution temperature was 24°C or higher. The reason for the difference in pathogenicity of *Phytophthora drechsleri* in potting mix compared with the experimental hydroponic system could be related to the use of different media (potting mix compared with liquid nutrient solution alone) or to the conditions where these experiments were carried out (environmental cabinets compared with the glasshouse). Based on the findings of several studies within this research project, it would appear that *Pythium* is more important than *Phytophthora* in causing severe root rot disease in hydroponic lettuce.

The results of physiological studies in this project, for the main pathogens isolated, showed that the optimum growth temperature of isolates of *Pythium* group F and *Pythium coloratum* was 30°C. They also grew well at temperatures of 25°C and 35°C and could still grow at 40°C and 5°C. The optimum growth temperature for *Phytophthora drechsleri* isolates was 25°C but they were still able to grow at temperatures of 10°C and very slightly at 35°C. These results could help to explain why root rot disease in lettuce is more severe under conditions of high temperature (such as above 20-24°C) than low temperature (such as below 18°C).

The interaction between temperature, pathogens and plants is very important in relation to root rot disease severity. The pathogens isolated in this study, *Pythium* belonging to group F, *Pythium coloratum* and (possibly) *Phytophthora drechsleri*, have an optimum growth temperature of 25-30°C and prefer aquatic conditions provided by the nutrient film technique (NFT). However, the optimum temperature for growth of lettuce is 20°C to 25°C (Davis *et al.* 1997). Increased root rot disease severity in the warmer months of the year could be explained by the high temperatures of the nutrient solution causing stress in plant roots and allowing the pathogens to enter the roots more easily. Furthermore, a temperature of 30°C is optimal for the growth of *Pythium* and its growth is also quite good at 35°C.

Physiological characteristics and morphological features were used to try to identify 81 *Pythium* isolates obtained in this study. The isolates were placed in three groups (groups i, ii and iii) but these techniques could not identify the majority of the isolates obtained in this study to particular species of *Pythium*. Molecular techniques were also used in order to try to identify these isolates to species level and to find out the relationships between the isolates within each group and between groups. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the internal transcribed spacer (ITS) region of ribosomal DNA technique, using four restriction enzymes, was not able to differentiate between isolates from the three groups (i, ii and iii). Sequencing of the ITS region of rDNA was also used for possible identification of *Pythium* isolates by comparing the sequences of the isolates obtained in the present study with GenBank sequences. The majority of *Pythium* isolates sequenced in this study were found to be identical to *Pythium* sp. PTS 2003 (AY445122; LPV C) which was isolated from roots of table beet (*Beta vulgaris* var. *vulgaris*) in the Lockyer Valley, Queensland, Australia (Scott *et al.* 2005). This isolate, *Pythium* sp. PTS 2003, was found to be 98% identical to *P. dissotocum* when these researchers compared it with the GenBank database. Moreover, *Pythium* PTS 2003 isolates were genetically related to both *P. dissotocum* and *P. coloratum* as shown in the phylogenetic tree of the *Pythium* isolates from their study based on DNA sequence analysis of the region encompassing ITS 1, 5.8S rDNA and ITS 2. The sequence of the ITS region of rDNA also showed that there were few or no differences between the isolates sequenced from the three groups (i, ii and iii).

Isolates belonging to *Pythium* group F should be studied in more depth using both morphological and molecular techniques in order to identify them to particular species. It would be worthwhile to attempt to obtain sexual structures from the isolates that failed to produce them under the culture conditions used in this study. To achieve this, the isolates could be grown on other media and/or under different environmental conditions. As well, the isolates could be used to re-infect lettuce plants and be freshly re-isolated before culturing again. Molecular techniques such as PCR-RFLP could be used to study more isolates and additional restriction enzymes could be used as well. Comparative studies involving many *Pythium* isolates from hydroponic systems and sequence data from a variety of genes (including both mitochondrial and nuclear genes)

may resolve the taxonomic status of these isolates. A major taxonomic study on *Pythium* in Australia is needed.

The inter simple sequence repeat markers (ISSR) technique investigated the relationships between the 81 isolates of *Pythium* obtained in this study. No correlations were found between the 11 groups of *Pythium* isolates and either the farm or the geographic area from which isolates were obtained. Furthermore, there were no correlations between these groups and the lettuce cultivars yielding the isolates. Moreover, no correlations were found between the groups established by the ISSR technique and the three groups identified on the basis of colony characteristics. On the other hand, there appeared to be a slight correlation between the 11 groups of *Pythium* isolates and the sampling times. The variation amongst the 81 isolates obtained using the ISSR technique contrasts with the lack of variation found with both the PCR-RFLP technique applied to the ITS region and sequencing of the ITS region. The likely reason for this is that the ITS region may not have enough differences to enable separation of the isolates, whereas the entire DNA, as scanned by the primers in the ISSR technique, allows comparison of a large number of markers, so that the potential to differentiate variability between isolates in greatly enhanced.

Physiological characteristics, morphological features and molecular techniques were used to try to identify 68 isolates of *Phytophthora* obtained in this study. Morphological features of isolates of *Phytophthora* can be used to distinguish between many different *Phytophthora* spp., but not between *P. drechsleri* and *P. cryptogea* (Stamps *et al.* 1990; Erwin & Ribeiro 1996). However, in many keys, these two *Phytophthora* species are distinguished by the ability of *P. drechsleri* to grow at 35°C whereas *P. cryptogea* does not grow at 35°C. In the present study, all *Phytophthora* isolates that were tested were able to grow at 35°C, but only to a very small extent. Nevertheless, this growth indicates that they were *P. drechsleri*. Moreover, all 68 isolates in this study produced sexual structures when paired with the A_2 mating type of *P. cryptogea*, indicating that they belonged to the A_1 mating type.

Molecular techniques confirmed that the 68 isolates of *Phytophthora* obtained in this study were *P. drechsleri* based on the PCR-RFLP technique of the ITS region of rDNA

and sequencing of the ITS region. The bands reported for the 68 isolates by using three restriction enzymes were identical to those reported in the manual by Drenth *et al.* (1999 & 2006) for *P. drechsleri*. Moreover, ITS sequences for representative isolates from this study were 99% identical to the corresponding sequence from *P. drechsleri*; one sequence was 99% identical to both *P. drechsleri* and *P. cryptogea*. The relationships between *Phytophthora drechsleri* and *P. cryptogea* should be investigated in more detail by using the latest molecular techniques combined with morphological and physiological studies. It would be worthwhile to sequence a range of different genes in these studies.

The ISSR technique was used to carry out a population study of 68 *Phytophthora drechsleri* isolates. It was found that there was a correlation between the six groups and the sample times from which isolates were obtained. As well, isolates from cooler seasons were found to have less variation than isolates from warmer seasons. The reason for this reduced variation could be that fewer isolates were obtained in the cooler seasons (24 isolates) than in the warmer seasons (44 isolates). It would be worthwhile to obtain and study more isolates in the cooler seasons (from May and August) to confirm the findings of this ISSR analysis. No correlation was found between the six groups established by ISSR clustering and either the farms or the geographic areas from which the isolates were obtained. Furthermore, there was no correlation between the groups and the lettuce cultivars yielding the isolates.

The results reported in this study are valuable for producers of hydroponic lettuce and for researchers who study the management of root diseases of hydroponic lettuce. However, further investigations are required to extend the findings of the present study. Nutrient solution temperature appeared to be an important factor in root rot disease severity in hydroponic lettuce. Results obtained in the present study show that root rot disease was significant when the nutrient solution temperature was 20-30°C. It would be worthwhile to study root rot disease severity in more samples over the period from August to November in order to define more clearly the temperature at which root rot disease starts to become a significant problem as temperatures start to increase. Moreover, it would be useful to monitor the nutrient solution temperature in growth channels all year round.

Further studies should focus on finding economic ways to reduce the nutrient solution temperature in the warmer seasons to the level where root rot disease is not significant. Moreover, different methods of disinfection of nutrient solution, such as UV light, using filtering systems or using chemical disinfection solutions, should be investigated. Given that *Pythium* and *Phytopthora* (to a lesser extent) are present in the hydroponic channels all year round, it would also be worthwhile to investigate biological control mechanisms, such as populations of rhizosphere microorganisms that may offer protection to roots against root rot pathogens.

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9 APPENDIX

1. Growth media

1. Carnation Leaf Agar (CLA)

Pieces of sterilized carnation leaf were placed in Petri dishes containing water agar medium. This medium is composed of 2% agar in distilled water and autoclaved at 121°C for 20 min (Burgess *et al.* 1994).

2. Corn Meal Agar (CMA)

To make up this medium, 40 g of maize meal were placed in 1 L distilled water and kept at 58°C (never over 60°C) for 1 h. This suspension was then filtered through filter paper, after which 15 g agar was added to the filtrate and this was then made up to 1 L. The medium was autoclaved for 20 min at 121°C.

3. Kidney Bean Extract Agar (KBEA)

To make up this medium, 120 g of dry red kidney beans were steamed for 1 h in 190 mL distilled water. Then the broth was made up to 100 mL and autoclaved at 121°C for 20 minutes. To 40 mL of this solution, the following were added - 200 mL V8 juice, 800 mL distilled water, 4 g $CaCO_3$, 20 g agar, 0.05 g B-sitosterol. This was then autoclaved at 121°C for 20 min (Duncan 1988).

4. Peptone PCNB Agar (PPA)

This medium was used for *Fusarium* spp. isolation. PPA (Burgess *et al.* 1994) is comprised of a sugar-free basal medium supplemented with antibiotics and fungicides. The base medium contained the following – 15 g peptone, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 1 g Terrachlor and 20 g agar. It was made up to 1 L with distilled water. The medium was then autoclaved at 121°C for 20 min. After the medium had cooled to 60°C, 1 g Streptomycin sulfate and 0.12 g Neomycin sulfate were added.

5. Potato carrot agar (PCA)

To make up this medium, 20 g of sliced potatoes and 20 g of sliced carrots were boiled in 500 mL of distilled water until just soft, then filtered through cheesecloth. To the filtrate, 20 g of agar was added and this was then made up to 1 L with distilled water, after which it was autoclaved at 121°C for 20 min.

6. Potato dextrose agar (PDA)

To make up this medium, 200 g of potatoes were washed and sliced (unpeeled) and then boiled in 500 mL of distilled water until just tender. The boiled potatoes were filtered through cheesecloth after which 20 g of dextrose and 20 g of agar were added to the broth and this was adjusted with distilled water to 1 L. It was then autoclaved at 121°C for 20 min.

7. ¼ strength PDA with Lactic acid

This medium uses 80 mL of the potato stock made as for PDA. To the broth were added 5 g dextrose and 20 g agar; this was adjusted with distilled water to 1 L then autoclaved at 121°C for 20 min. Just before pouring, 3 mL of 25% lactic acid was added.

8. *Phytophthora* selective agar (PSA)

PSA is composed of 8 mL carrot puree, 32 mL potato puree, and 8 g agar, made up to 400 mL with distilled water, then autoclaved at 121°C for 20 min. The anti-fungal chemicals and antibiotic reagents Pimaricin (160 µL) and of Hymexazol (1.5 mL) in water and Rifampicin (1.5 mL) were added to the agar medium after it cooled to 60°C.

9. *Pythium* selective agar (PYSA)

PYSA is composed 8 mL carrot puree, 32 mL potato puree, and 8 g agar, made up to 400 mL with distilled water, then autoclaved at 121°C for 20 min. The anti-fungal chemicals and antibiotic reagents Pimaricin (160 µl) in water and Rifampicin (1.5 mL) were added to the agar medium after it cooled to 60°C.

10. Spezieller Nährstoffarmer Agar (SNA)

This medium was prepared without agar to use it for mycelial growth for DNA extraction. SNA was prepared by autoclaving, in 1 L of distilled water, the following – 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose. The medium was then autoclaved at 121°C for 20 min.

11. Water agar (WA)

This medium consists of 2% agar in distilled water, autoclaved at 121°C for 20 min.

2. Nutrient solution for growth of lettuce plants

Crystal Dew Hydroponic Solutions

Nutrient solution part A and B for growing lettuce

Analysis (%w/v)	Pack A	Pack B
Nitrogen as nitrate	3.660	0.900
Nitrogen as ammonium	0.250	0.000
Nitrogen as total nitrogen	3.910	0.900
Phosphorus water soluble	0.000	0.800
Potassium as nitrate	2.500	2.500
Potassium as phosphate	0.000	1.000
Potassium as total potassium	2.500	3.510
Calcium as nitrate	3.600	0.000
Sulfur as sulfate	0.000	2.000
Magnesium as sulfate	0.000	1.500
Iron as EDTA chelate	0.100	0.000
Manganese as EDTA chelate	0.000	0.020
Zinc as EDTA chelate	0.000	0.004
Cooper as EDTA chelate	0.000	0.003
Boron as EDTA borax	0.000	0.007
Molybdenum as sodium molybdate	0.000	0.001
Water (w/w)	85.100	87.200

3. Data analysis for Chapter 2: Survey of root rot disease in hydroponic lettuce in the Sydney area, isolation of fungi and oomycetes, and their temperature responses in culture.

a. Root assessment (section 2.3.1)

Brown Mignonette: General Linear Model: root rot severity versus month, age -

```
Factor     Type Levels Values
Month      fixed      5 May-03 Aug-03 Nov-03 Jan-04 Mar-04
age        fixed      2 Mature Young

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Month       4    10.6700    10.6700     2.6675    6.48  0.000
age         1     6.8450     6.8450     6.8450   16.62  0.056
Error     194    79.8800    79.8800     0.4118
Total     199    97.3950

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Month

Month = May-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Aug-03         0.3000      0.1435     2.091     0.2282
Nov-03         0.5250      0.1435     3.659     0.0030
Jan-04         0.6250      0.1435     4.356     0.0002
Mar-04         0.1500      0.1435     1.045     0.8338

Month = Aug-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Nov-03         0.2250      0.1435     1.568     0.0714
Jan-04         0.3250      0.1435     2.265     0.1609
Mar-04        -0.1500      0.1435    -1.045     0.8338

Month = Nov-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Jan-04         0.1000      0.1435     0.697     0.9570
Mar-04        -0.3750      0.1435    -2.614     0.0428

Month = Jan-04 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Mar-04        -0.4750      0.1435    -3.310     0.0097

Tukey Simultaneous Tests
Response Variable root rot
All Pairwise Comparisons among Levels of Month*age

Month = May-03
age   = Mature subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
May-03 Young         -0.2500      0.1879    -1.331     0.9453
Aug-03 Mature         0.3000      0.1879     1.597     0.8482
Aug-03 Young          0.0500      0.1879     0.266     1.0000
Nov-03 Mature         1.0500      0.1879     5.589     0.0000
Nov-03 Young         -0.2500      0.1879    -1.331     0.9453
Jan-04 Mature         0.6500      0.1879     3.460     0.0229
Jan-04 Young          0.3500      0.1879     1.863     0.6937
Mar-04 Mature        -0.1000      0.1879    -0.532     0.9999
Mar-04 Young          0.1500      0.1879     0.798     0.9985
```

Month = May-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03 Mature	0.55000	0.1879	2.92779	0.1052
Aug-03 Young	0.30000	0.1879	1.59698	0.8482
Nov-03 Mature	1.30000	0.1879	6.92023	0.0000
Nov-03 Young	-0.00000	0.1879	-0.00000	1.0000
Jan-04 Mature	0.90000	0.1879	4.79093	0.0002
Jan-04 Young	0.60000	0.1879	3.19395	0.0512
Mar-04 Mature	0.15000	0.1879	0.79849	0.9985
Mar-04 Young	0.40000	0.1879	2.12930	0.5093

Month = Aug-03
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03 Young	-0.2500	0.1879	-1.331	0.9453
Nov-03 Mature	0.7500	0.1879	3.992	0.0037
Nov-03 Young	-0.5500	0.1879	-2.928	0.1052
Jan-04 Mature	0.3500	0.1879	1.863	0.6937
Jan-04 Young	0.0500	0.1879	0.266	1.0000
Mar-04 Mature	-0.4000	0.1879	-2.129	0.5093
Mar-04 Young	-0.1500	0.1879	-0.798	0.9985

Month = Aug-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03 Mature	1.0000	0.1879	5.323	0.0000
Nov-03 Young	-0.3000	0.1879	-1.597	0.8482
Jan-04 Mature	0.6000	0.1879	3.194	0.0512
Jan-04 Young	0.3000	0.1879	1.597	0.8482
Mar-04 Mature	-0.1500	0.1879	-0.798	0.9985
Mar-04 Young	0.1000	0.1879	0.532	0.9999

Month = Nov-03
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03 Young	-1.300	0.1879	-6.920	0.0000
Jan-04 Mature	-0.400	0.1879	-2.129	0.5093
Jan-04 Young	-0.700	0.1879	-3.726	0.0095
Mar-04 Mature	-1.150	0.1879	-6.122	0.0000
Mar-04 Young	-0.900	0.1879	-4.791	0.0002

Month = Nov-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-04 Mature	0.9000	0.1879	4.7909	0.0002
Jan-04 Young	0.6000	0.1879	3.1940	0.0512
Mar-04 Mature	0.1500	0.1879	0.7985	0.9985
Mar-04 Young	0.4000	0.1879	2.1293	0.5093

Month = Jan-04
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-04 Young	-0.3000	0.1879	-1.597	0.8482
Mar-04 Mature	-0.7500	0.1879	-3.992	0.0037
Mar-04 Young	-0.5000	0.1879	-2.662	0.1973

Month = Jan-04
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-04 Mature	-0.4500	0.1879	-2.395	0.3345
Mar-04 Young	-0.2000	0.1879	-1.065	0.9874

```
Month = Mar-04
age   = Mature subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Mar-04 Young          0.2500      0.1879     1.331     0.9453
```

Baby Cos: General Linear Model: root rot severity versus month, age -

```
Factor     Type Levels Values
Month      fixed      5 May-03 Aug-03 Nov-03 Jan-04 Mar-04
age        fixed      2 Mature Young

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Month       4    13.3300    13.3300     3.3325    8.42  0.000
age         1     0.8450     0.8450     0.8450    2.14  0.146
Error     194    76.7800    76.7800     0.3958
Total     199    90.9550

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Month

Month = May-03 subtracted from:

Level     Difference       SE of             Adjusted
Month       of Means  Difference   T-Value    P-Value
Aug-03        0.4000      0.1407     2.843     0.0392
Nov-03        0.4250      0.1407     3.021     0.0236
Jan-04        0.3500      0.1407     2.488     0.0176
Mar-04       -0.2250      0.1407    -1.599     0.4996

Month = Aug-03 subtracted from:

Level     Difference       SE of             Adjusted
Month       of Means  Difference   T-Value    P-Value
Nov-03        0.0250      0.1407     0.178     0.9998
Jan-04       -0.0500      0.1407    -0.355     0.9966
Mar-04       -0.6250      0.1407    -4.443     0.0002

Month = Nov-03 subtracted from:

Level     Difference       SE of             Adjusted
Month       of Means  Difference   T-Value    P-Value
Jan-04       -0.0750      0.1407    -0.533     0.9838
Mar-04       -0.6500      0.1407    -4.621     0.0001
Month = 4 subtracted from:

Level     Difference       SE of             Adjusted
Month       of Means  Difference   T-Value    P-Value
Mar-04      -0.5750      0.1407    -4.088     0.0006

Tukey Simultaneous Tests
Response Variable Scal
All Pairwise Comparisons among Levels of age

age = Mature subtracted from:

Level   Difference       SE of             Adjusted
age       of Means  Difference   T-Value    P-Value
Young     -0.1300     0.08897    -1.461     0.1456


Tukey Simultaneous Tests
Response Variable Scal
All Pairwise Comparisons among Levels of Month*age

Month = May-03
age   = Mature subtracted from:
Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
May-03 Young        -0.2500      0.1969    -1.270     0.9591
Aug-03 Mature        0.5000      0.1969     2.540     0.2546
Aug-03 Young         0.0500      0.1969     0.254     1.0000
```

Nov-03 Mature	0.1500	0.1969	0.762	0.9990
Nov-03 Young	0.4500	0.1969	2.286	0.4033
Jan-04 Mature	0.2500	0.1969	1.270	0.9591
Jan-04 Young	0.2000	0.1969	1.016	0.9910
Mar-04 Mature	-0.2500	0.1969	-1.270	0.9591
Mar-04 Young	-0.4500	0.1969	-2.286	0.4033

Month = May-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03 Mature	0.7500	0.1969	3.809	0.0071
Aug-03 Young	0.3000	0.1969	1.524	0.8811
Nov-03 Mature	0.4000	0.1969	2.032	0.5779
Nov-03 Young	0.7000	0.1969	3.555	0.0168
Jan-04 Mature	0.5000	0.1969	2.540	0.2546
Jan-04 Young	0.4500	0.1969	2.286	0.4033
Mar-04 Mature	-0.0000	0.1969	-0.000	1.0000
Mar-04 Young	-0.2000	0.1969	-1.016	0.9910

Month = Aug-03
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03 Young	-0.4500	0.1969	-2.286	0.4033
Nov-03 Mature	-0.3500	0.1969	-1.778	0.7484
Nov-03 Young	-0.0500	0.1969	-0.254	1.0000
Jan-04 Mature	-0.2500	0.1969	-1.270	0.9591
Jan-04 Young	-0.3000	0.1969	-1.524	0.8811
Mar-04 Mature	-0.7500	0.1969	-3.809	0.0071
Mar-04 Young	-0.9500	0.1969	-4.825	0.0001

Month = Aug-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03 Mature	0.1000	0.1969	0.508	1.0000
Nov-03 Young	0.4000	0.1969	2.032	0.5779
Jan-04 Mature	0.2000	0.1969	1.016	0.9910
Jan-04 Young	0.1500	0.1969	0.762	0.9990
Mar-04 Mature	-0.3000	0.1969	-1.524	0.8811
Mar-04 Young	-0.5000	0.1969	-2.540	0.2546

Month = Nov-03
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03 Young	0.3000	0.1969	1.524	0.8811
Jan-04 Mature	0.1000	0.1969	0.508	1.0000
Jan-04 Young	0.0500	0.1969	0.254	1.0000
Mar-04 Mature	-0.4000	0.1969	-2.032	0.5779
Mar-04 Young	-0.6000	0.1969	-3.047	0.0769

Month = Nov-03
age = Young subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-04 Mature	-0.2000	0.1969	-1.016	0.9910
Jan-04 Young	-0.2500	0.1969	-1.270	0.9591
Mar-04 Mature	-0.7000	0.1969	-3.555	0.0168
Mar-04 Young	-0.9000	0.1969	-4.571	0.0004

Month = Jan-04
age = Mature subtracted from:

Level Month *age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-04 Young	-0.0500	0.1969	-0.254	1.0000
Mar-04 Mature	-0.5000	0.1969	-2.540	0.2546
Mar-04 Young	-0.7000	0.1969	-3.555	0.0168

```
Month = Jan-04
age   = Young subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Mar-04 Mature        -0.4500      0.1969    -2.286     0.4033
Mar-04 Young         -0.6500      0.1969    -3.301     0.0373

Month = Mar-04
age   = Mature subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Mar-04 Young         -0.2000      0.1969    -1.016     0.9910
```

Red Oak: General Linear Model: root rot severity versus month, age -

```
Factor     Type Levels Values
Month      fixed      5 May-03 Aug-03 Nov-03 Jan-04 Mar-04
age        fixed      2 Mature Young

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Month       4    22.5200    22.5200     5.6300   17.81  0.000
age         1     1.2800     1.2800     1.2800    4.05  0.046
Error     194    61.3200    61.3200     0.3161
Total     199    85.1200

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Month

Month = May-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Aug-03         0.4500      0.1257     3.580     0.0039
Nov-03         0.6500      0.1257     5.170     0.0000
Jan-04         1.0000      0.1257     7.955     0.0000
Mar-04         0.7500      0.1257     5.966     0.0000

Month = Aug-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Nov-03         0.2000      0.1257     1.591     0.0350
Jan-04         0.5500      0.1257     4.375     0.0002
Mar-04         0.3000      0.1257     2.386     0.0234

Month = Nov-03 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Jan-04         0.3500      0.1257    2.7841     0.0461
Mar-04         0.1000      0.1257    0.7955     0.0377

Month = Jan-04 subtracted from:

Level      Difference       SE of             Adjusted
Month        of Means  Difference   T-Value    P-Value
Mar-04        -0.2500      0.1257    -1.989     0.2754

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of age

age = Mature subtracted from:

Level      Difference       SE of             Adjusted
age          of Means  Difference   T-Value    P-Value
Young         -0.1600     0.07951    -2.012     0.0456

Tukey Simultaneous Tests
Response Variable root rot
All Pairwise Comparisons among Levels of Month*age
```

Month = May-03
age = Mature subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
May-03	Young	-1.100	0.1497	-7.346	0.0570
Aug-03	Mature	-0.050	0.1497	-0.334	1.0000
Aug-03	Young	-0.150	0.1497	-1.002	0.9919
Nov-03	Mature	-0.000	0.1497	-0.000	1.0000
Nov-03	Young	0.200	0.1497	1.336	0.9441
Jan-04	Mature	0.700	0.1497	4.675	0.0003
Jan-04	Young	0.200	0.1497	1.336	0.9441
Mar-04	Mature	-0.150	0.1497	-1.002	0.9919
Mar-04	Young	0.550	0.1497	3.673	0.0114

Month = May-03
age = Young subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03	Mature	1.0500	0.1497	7.012	0.0000
Aug-03	Young	0.9500	0.1497	6.344	0.0000
Nov-03	Mature	1.1000	0.1497	7.346	0.0000
Nov-03	Young	1.3000	0.1497	8.682	0.0000
Jan-04	Mature	1.8000	0.1497	12.021	0.0000
Jan-04	Young	1.3000	0.1497	8.682	0.0000
Mar-04	Mature	0.9500	0.1497	6.344	0.0000
Mar-04	Young	1.6500	0.1497	11.019	0.0000

Month = Aug-03
age = Mature subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-03	Young	-0.1000	0.1497	-0.6678	0.9997
Nov-03	Mature	0.0500	0.1497	0.3339	1.0000
Nov-03	Young	0.2500	0.1497	1.6696	0.8111
Jan-04	Mature	0.7500	0.1497	5.0088	0.0001
Jan-04	Young	0.2500	0.1497	1.6696	0.8111
Mar-04	Mature	-0.1000	0.1497	-0.6678	0.9997
Mar-04	Young	0.6000	0.1497	4.0070	0.0035

Month = Aug-03
age = Young subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03	Mature	0.150000	0.1497	1.00176	0.9919
Nov-03	Young	0.350000	0.1497	2.33744	0.3701
Jan-04	Mature	0.850000	0.1497	5.67663	0.0000
Jan-04	Young	0.350000	0.1497	2.33744	0.3701
Mar-04	Mature	0.000000	0.1497	0.00000	1.0000
Mar-04	Young	0.700000	0.1497	4.67488	0.0003

Month = Nov-03
age = Mature subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-03	Young	0.2000	0.1497	1.336	0.9441
Jan-04	Mature	0.7000	0.1497	4.675	0.0003
Jan-04	Young	0.2000	0.1497	1.336	0.9441
Mar-04	Mature	-0.1500	0.1497	-1.002	0.9919
Mar-04	Young	0.5500	0.1497	3.673	0.0114

Month = Nov-03
age = Young subtracted from:

Level Month	*age	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-04	Mature	0.5000	0.1497	3.339	0.0333
Jan-04	Young	0.0000	0.1497	0.000	1.0000
Mar-04	Mature	-0.3500	0.1497	-2.337	0.3701
Mar-04	Young	0.3500	0.1497	2.337	0.3701

```
Month = Jan-04
age   = Mature subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Jan-04 Young         -0.5000      0.1497    -3.339     0.3333
Mar-04 Mature        -0.8500      0.1497    -5.677     0.0000
Mar-04 Young         -0.1500      0.1497    -1.002     0.9919

Month = Jan-04
age   = Young subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Mar-04 Mature        -0.3500      0.1497    -2.337     0.3701
Mar-04 Young          0.3500      0.1497     2.337     0.3701

Month = Mar-04
age   = Mature subtracted from:

Level             Difference       SE of             Adjusted
Month *age          of Means  Difference   T-Value    P-Value
Mar-04 Young          0.7000      0.1497     4.675     0.0803
```

One-way ANOVA: root rot severity between the three mature cultivars

```
Analysis of Variance for Scale
Source     DF        SS        MS        F        P
Cultivar    2     0.540     0.270     0.63    0.257
Error     297   126.430     0.426
Total     299   126.970
```

One-way ANOVA: root rot severity between the three young cultivars

```
Analysis of Variance for Scale
Source     DF        SS        MS        F        P
Cultivar    2     1.047     0.523     1.16    0.233
Error     297   133.950     0.451
Total     299   134.997
```

b. Isolation (section 2.3.2)

***Pythium* spp. General Linear Model: isolate numbers versus month, cultivar – age combination**

```
Factor     Type Levels Values
month      fixed      5 May-03 Aug-03 Nov-03 Jan-04 Mar-04
Cultivar   fixed      6 Mature Baby Cos          Mature Brown Mignonette
                       Mature Red Oak           Young Baby Cos
                       Young Brown Mignonette   Young Red Oak

Analysis of Variance for Isolates, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
month       4    25.4348    25.4348     6.3587  201.29  0.000
Cultivar    5     0.1232     0.1232     0.0246    0.78  0.566
Error     128     4.0435     4.0435     0.0316
Total     137    29.6014

Tukey Simultaneous Tests
Response Variable Isolates
All Pairwise Comparisons among Levels of Time

month = May-03 subtracted from:

Level      Difference       SE of             Adjusted
month         of Means  Difference   T-Value    P-Value
Aug-03          0.1667     0.05299     3.145     0.0173
Nov-03          1.0000     0.05299    18.871     0.0000
Jan-04          1.0000     0.05299    18.871     0.0000
Mar-04          1.0000     0.05299    18.871     0.0000
```

```
month = Aug-03 subtracted from:
Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Nov-03          0.8333     0.04589     18.16     0.0000
Jan-04          0.8333     0.04589     18.16     0.0000
Mar-04          0.8333     0.04589     18.16     0.0000

month = Nov-03 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Jan-04        0.000000     0.04589  0.000000      1.000
Mar-04        0.000000     0.04589  0.000000      1.000

month = Jan-04 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Mar-04        0.000000     0.04589  0.000000      1.000
```

Phytophthora spp. General Linear Model: isolate numbers versus month, cultivar – age combination

```
Factor     Type Levels Values
month      fixed      5 May-03 Aug-03 Nov-03 Jan-04 Mar-04
Cultivar   fixed      6 Mature Baby Cos          Mature Brown Mignonette
                       Mature Red Oak           Young Baby Cos
                       Young Brown Mignonette   Young Red Oak

Analysis of Variance for Isolates, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
month       4    15.2454    15.2454     3.8114   28.97  0.000
Cultivar    5     1.3696     1.3696     0.2739    2.08  0.072
Error     128    16.8415    16.8415     0.1316
Total     137    33.4565
Tukey Simultaneous Tests
Response Variable Isolates
All Pairwise Comparisons among Levels of Time

month = May-03 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Aug-03          0.1444     0.1081     1.336     0.6696
Nov-03          0.6444     0.1081     5.959     0.0000
Jan-04          0.8444     0.1081     7.808     0.0000
Mar-04          0.8111     0.1081     7.500     0.0000

month = Aug-03 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Nov-03          0.5000     0.09366     5.339     0.0000
Jan-04          0.7000     0.09366     7.474     0.0000
Mar-04          0.6667     0.09366     7.118     0.0000

month = Nov-03 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Jan-04          0.2000     0.09366     2.135     0.2116
Mar-04          0.1667     0.09366     1.780     0.3900

month = Jan-04 subtracted from:

Level      Difference       SE of              Adjusted
month         of Means  Difference   T-Value    P-Value
Mar-04       -0.03333     0.09366   -0.3559     0.9965
```

c. Pathogenicity tests (section 2.3.3)

1. *Pythium* Pathogenicity tests

August 2003 (macerated agar inoculum) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      1.415      1.415    0.37  0.993
Error       26     98.901      3.804
Total       27    100.316

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.03044    0.03044    2.26  0.847
Error       26    0.35044    0.01348
Total       27    0.38088

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.5526     0.5526    3.22  0.668
Error       26     4.4585     0.1715
Total       27     5.0111


Analysis of Variance for Dry root
Source      DF         SS         MS       F      P
Treatment    1  0.0004132  0.0004132    2.76  0.370
Error       26  0.0038952  0.0001498
Total       27  0.0043084
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatment fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      7.354      7.354    0.85  0.824
Error       26    224.145      8.621
Total       27    231.500

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.03803    0.03803    3.18  0.454
Error       26    0.31057    0.01194
Total       27    0.34860

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.9489     0.9489    2.58  0.383
Error       26     9.5547     0.3675
Total       27    10.5036

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1  0.0008557  0.0008557    2.59  0.516
Error       26  0.0085957  0.0003306
Total       27  0.0094514
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      0.179      0.179    0.08  0.999
Error       26     54.932      2.113
Total       27     55.112

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1   0.017938   0.017938    2.04  0.812
Error       26   0.228630   0.008793
Total       27   0.246568
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.1429     0.1429    0.68  0.971
Error       26     5.4588     0.2100
Total       27     5.6018

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.001539   0.001539    0.57  0.832
Error       26   0.070470   0.002710
Total       27   0.072009
```

August 2003 (agar disc inoculum) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type   Levels  Values
Treatment  fixed       2  Control, Infected

Analysis of Variance for Wet shoot

Source         DF       SS      MS     F      P
Treatment       1   13.043  13.043  1.92  0.179
Error          25  170.174   6.807
Total          26  183.217

Analysis of Variance for Dry shoot

Source     DF        SS        MS     F      P
Treatment   1  0.045490  0.045490  4.89  0.036
Error      25  0.232793  0.009312
Total      26  0.278283

Analysis of Variance for Wet root

Source         DF      SS      MS     F      P
Treatment       1  0.2471  0.2471  1.26  0.273
Error          25  4.9106  0.1964
Total          26  5.1577

Analysis of Variance for Dry root

Source     DF        SS         MS     F      P
Treatment   1 0.0000048  0.0000048  0.02  0.881
Error      25 0.0052221  0.0002089
Total      26 0.0052269
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type   Levels  Values
Treatment  fixed       2  Control, Infected

Analysis of Variance for Wet shoot

Source         DF       SS      MS     F      P
Treatment       1   21.884  21.884  2.25  0.146
Error          26  253.024   9.732
Total          27  274.909

Analysis of Variance for Dry shoot

Source     DF        SS        MS     F      P
Treatment   1  0.057677  0.057677  9.90  0.004
Error      26  0.151521  0.005828
Total      27  0.209198

Analysis of Variance for Wet root

Source         DF      SS      MS     F      P
Treatment       1  0.3512  0.3512  2.10  0.160
Error          26  4.3558  0.1675
Total          27  4.7071

Analysis of Variance for Dry root

Source         DF      SS      MS     F      P
Treatment       1  0.0150  0.0150  0.10  0.753
Error          26  3.8517  0.1481
Total          27  3.8667
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type   Levels  Values
Treatment  fixed       2  Control, Infected

Analysis of Variance for Wet shoot

Source     DF      SS     MS     F      P
Treatment   1   1.407  1.407  0.63  0.434
Error      26  57.895  2.227
Total      27  59.302

Analysis of Variance for Dry shoot

Source     DF        SS        MS     F      P
Treatment   1  0.005907  0.005907  1.59  0.218
Error      26  0.096462  0.003710
Total      27  0.102368

Analysis of Variance for Wet root

Source     DF      SS      MS     F      P
Treatment   1  0.2742  0.2742  1.63  0.214
Error      26  4.3848  0.1686
Total      27  4.6590

Analysis of Variance for Dry root

Source     DF        SS        MS     F      P
Treatment   1  0.008553  0.008553  4.13  0.052
Error      26  0.053830  0.002070
Total      27  0.062383
```

November 2003 (macerated agar inoculum, normal concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in November 2003 experiment versus treatment for normal concentration

```
Factor     Type Levels Values
Treatment fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     9.3514     9.3514   10.16  0.094
Error       26    23.9292     0.9204
Total       27    33.2806

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.08551    0.08551    3.22  0.144
Error       26    0.69070    0.02657
Total       27    0.77621

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     1.1728     1.1728   27.50  0.009
Error       26     1.1090     0.0427
Total       27     2.2818

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1  0.0044066  0.0044066    4.69  0.103
Error       26  0.0244198  0.0009392
Total       27  0.0288264
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in November 2003 experiment versus treatment for normal concentration

```
Factor     Type Levels Values
Treatment fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     22.267     22.267   10.45  0.003
Error       26     55.389      2.130
Total       27     77.656

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1   0.015341   0.015341    4.53  0.754
Error       26   0.088044   0.003386
Total       27   0.103385

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     2.8983     2.8983   13.58  0.001
Error       26     5.5489     0.2134
Total       27     8.4472

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.013158   0.013158   20.05  0.000
Error       26   0.017060   0.000656
Total       27   0.030218
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in November 2003 experiment versus treatment for normal concentration

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     5.6429     5.6429    6.35  0.308
Error       26    23.1117     0.8889
Total       27    28.7546

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1   0.023300   0.023300    4.09  0.603
Error       26   0.147987   0.005692
Total       27   0.171287

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1    0.21796    0.21796    4.39  0.694
Error       26    1.29066    0.04964
Total       27    1.50862

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1  0.0010171  0.0010171    3.20  0.804
Error       26  0.0082632  0.0003178
Total       27  0.0092803
```

November 2003 (macerated agar inoculum, high concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in November 2003 experiment versus treatment for high concentration

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     1.5792     1.5792    1.63  0.213
Error       26    25.1412     0.9670
Total       27    26.7204

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.03669    0.03669    2.98  0.096
Error       26    0.32035    0.01232
Total       27    0.35704

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1    0.20996    0.20996    3.86  0.060
Error       26    1.41581    0.05445
Total       27    1.62577

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.000783   0.000783    0.53  0.472
Error       26   0.038292   0.001473
Total       27   0.039075
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in November 2003 experiment versus treatment for high concentration

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      4.368      4.368    1.70  0.204
Error       26     66.830      2.570
Total       27     71.198

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1   0.050529   0.050529   15.47  0.001
Error       26   0.084939   0.003267
Total       27   0.135468

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.6914     0.6914    3.98  0.057
Error       26     4.5168     0.1737
Total       27     5.2082

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.016663   0.016663   32.29  0.000
Error       26   0.013416   0.000516
Total       27   0.030079
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in November 2003 experiment versus treatment for high concentration

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.9557     1.9557    3.39  0.077
Error       26    14.9843     0.5763
Total       27    16.9400

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.001975   0.001975    1.48  0.235
Error       26   0.034770   0.001337
Total       27   0.036745

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.21223    0.21223   14.95  0.001
Error       26    0.36907    0.01419
Total       27    0.58129
Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0005878  0.0005878    4.90  0.036
Error       26  0.0031185  0.0001199
Total       27  0.0037063
```

January 2004 (*Pythium* 1) (macerated agar inoculum, normal concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in January 2004 experiment versus treatment (*Pythium* 1) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     21.682     21.682   11.32  0.952
Error       26     49.808      1.916
Total       27     71.491

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.14947    0.14947    2.16  0.983
Error       26    1.79534    0.06905
Total       27    1.94481

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     1.3404     1.3404    6.58  0.996
Error       26     5.2959     0.2037
Total       27     6.6363

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.005974   0.005974    3.12  0.994
Error       26   0.049767   0.001914
Total       27   0.055742
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in January 2004 experiment versus treatment (*Pythium* 1) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      4.896      4.896    1.55  0.002
Error       26     82.318      3.166
Total       27     87.214

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1     0.5373     0.5373    5.24  0.000
Error       26     2.6655     0.1025
Total       27     3.2028
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.6733     0.6733    5.11  0.000
Error       26     3.4234     0.1317
Total       27     4.0967

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.007904   0.007904    5.27  0.001
Error       26   0.038987   0.001500
Total       27   0.046891
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in January 2004 experiment versus treatment (*Pythium* 1) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     0.5605     0.5605    0.60  0.004
Error       26    24.2459     0.9325
Total       27    24.8064

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.02097    0.02097    0.61  0.021
Error       26    0.88862    0.03418
Total       27    0.90959

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.3646     0.3646    2.17  0.301
Error       26     4.3647     0.1679
Total       27     4.7294

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.002989   0.002989    2.18  0.052
Error       26   0.035642   0.001371
Total       27   0.038631
```

January 2004 (*Pythium* 2) (macerated agar inoculum, normal concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in January 2004 experiment versus treatment (*Pythium* 2) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     1.2275     1.2275    1.47  0.236
Error       26    21.6936     0.8344
Total       27    22.9211
Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.04995    0.04995    0.97  0.333
Error       26    1.33514    0.05135
Total       27    1.38508

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1    0.57177    0.57177    9.56  0.105
Error       26    1.55579    0.05984
Total       27    2.12757

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1  0.0067950  0.0067950    9.37  0.215
Error       26  0.0188494  0.0007250
Total       27  0.0256444
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in January 2004 experiment versus treatment (*Pythium* 2) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      8.666      8.666    4.03  0.005
Error       26     55.928      2.151
Total       27     64.594

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1     2.1216     2.1216   21.63  0.000
Error       26     2.5502     0.0981
Total       27     4.6718

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     3.4922     3.4922   14.23  0.011
Error       26     6.3793     0.2454
Total       27     9.8715

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.012315   0.012315    2.06  0.032
Error       26   0.155495   0.005981
Total       27   0.167811
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in January 2004 experiment versus treatment (*Pythium* 2) (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     1.2325     1.2325    1.34  0.008
Error       26    23.9924     0.9228
Total       27    25.2249

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.87761    0.87761    9.82  0.004
Error       26    2.32255    0.08933
Total       27    3.20017

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.4260     0.4260    3.63  0.089
Error       26     3.0528     0.1174
Total       27     3.4788

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.009385   0.009385    7.65  0.010
Error       26   0.031889   0.001227
Total       27   0.041274
```

March 2004 (macerated agar inoculum, normal concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in March 2004 experiment versus treatment for normal concentration (inoculated or control)

```
Factor      Type Levels Values
Treatment   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     0.0138     0.0138    0.02  0.997
Error       26    22.5226     0.8663
Total       27    22.5364

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1     0.1023     0.1023    0.24  1.002
Error       26    11.3102     0.4350
Total       27    11.4125

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1    0.02879    0.02879    0.92  0.987
Error       26    0.81581    0.03138
Total       27    0.84460
Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1  0.0006789  0.0006789    1.68  0.993
Error       26  0.0105361  0.0004052
Total       27  0.0112149
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in March 2004 experiment versus treatment for normal concentration (inoculated or control)

```
Factor      Type Levels Values
Treatment   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     23.472     23.472    7.87  0.004
Error       26     77.549      2.983
Total       27    101.020

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1     0.2514     0.2514    1.45  0.840
Error       26     4.5153     0.1737
Total       27     4.7667

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     1.4533     1.4533    6.14  0.020
Error       26     6.1530     0.2367
Total       27     7.6062

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1    0.24552    0.24552   12.51  0.907
Error       26    0.51039    0.01963
Total       27    0.75590
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in March 2004 experiment versus treatment for normal concentration (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1      3.898      3.898    3.81  0.632
Error       26     26.569      1.022
Total       27     30.467

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1     1.5530     1.5530   18.64  0.000
Error       26     2.1660     0.0833
Total       27     3.7190
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     0.7861     0.7861    3.94  0.008
Error       26     5.1882     0.1995
Total       27     5.9744

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.013130   0.013130    7.02  0.628
Error       26   0.048637   0.001871
Total       27   0.061767
```

March 2004 (macerated agar inoculum, high concentration) (analysis for Table 2-7)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in March 2004 experiment versus treatment for high concentration (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     0.2184     0.2184    0.64  0..432
Error       26     8.9191     0.3430
Total       27     9.1374

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatment    1    0.00708    0.00708    0.16  0.693
Error       26    1.15896    0.04458
Total       27    1.16604

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1    0.00134    0.00134    0.10  0.752
Error       26    0.34200    0.01315
Total       27    0.34334

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1    0.00886    0.00886    0.29  0.595
Error       26    0.79506    0.03058
Total       27    0.80392
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in March 2004 experiment versus treatment for high concentration (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     31.354     31.354   10.49  0.003
Error       26     77.684      2.988
Total       27    109.037

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.6548     1.6548   13.20  0.101
Error       26     3.2604     0.1254
Total       27     4.9152

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     2.7892     2.7892   10.87  0.213
Error       26     6.6742     0.2567
Total       27     9.4634

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.098352   0.098352   15.12  0.001
Error       26   0.169138   0.006505
Total       27   0.267490
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in March 2004 experiment versus treatment for high concentration (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     6.7466     6.7466   12.08  0.322
Error       26    14.5208     0.5585
Total       27    21.2674

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.0698     1.0698   17.25  0.000
Error       26     1.6128     0.0620
Total       27     2.6826

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     1.5490     1.5490   15.03  0.001
Error       26     2.6801     0.1031
Total       27     4.2291

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.017049   0.017049   26.32  0.083
Error       26   0.016841   0.000648
Total       27   0.033890
```

Comparison between the two inoculation methods (agar disc and macerated agar) (section 2.3.3)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in August 2003 experiment versus methods

```
Factor  Type   Levels  Values
method  fixed       2  Disk, Macerated

Analysis of Variance for Wet shoot

Source  DF       SS     MS     F      P
method   1    0.044  0.044  0.01  0.928
Error   54  287.099  5.317
Total   55  287.143

Analysis of Variance for Dry shoot

Source  DF       SS       MS     F      P
method   1  0.00322  0.00322  0.26  0.610
Error   54  0.65962  0.01222
Total   55  0.66284

Analysis of Variance for Wet root

Source  DF       SS      MS     F      P
method   1   0.9297  0.9297  4.58  0.037
Error   54  10.9611  0.2030
Total   55  11.8907

Analysis of Variance for Dry root

Source  DF         SS         MS     F      P
method   1  0.0007898  0.0007898  1.43  0.237
Error   54  0.0298173  0.0005522
Total   55  0.0306071
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in August 2003 experiment versus methods

```
Factor  Type   Levels  Values
method  fixed       2  Disk, Macerated

Source  DF       SS      MS     F      P
method   1   16.630  16.630  1.77  0.189
Error   54  506.408   9.378
Total   55  523.038

Analysis of Variance for Dry shoot

Source  DF       SS       MS     F      P
method   1  0.03050  0.03050  2.95  0.091
Error   54  0.55780  0.01033
Total   55  0.58829

Analysis of Variance for Wet root

Source  DF       SS      MS     F      P
method   1   0.7671  0.7671  2.72  0.105
Error   54  15.2106  0.2817
Total   55  15.9778

Analysis of Variance for Dry root
Source  DF       SS       MS     F      P
method   1  0.06702  0.06702  0.93  0.339
Error   54  3.88195  0.07189
Total   55  3.94897
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in August 2003 experiment versus methods

```
Factor  Type   Levels  Values
method  fixed       2  Disk, Macerated

Analysis of Variance for Wet shoot

Source  DF       SS     MS     F      P
method   1    5.419  5.419  2.56  0.116
Error   54  114.414  2.119
Total   55  119.832

Analysis of Variance for Dry shoot

Source  DF       SS       MS      F      P
method   1  0.10769  0.10769  16.67  0.000
Error   54  0.34894  0.00646
Total   55  0.45662

Analysis of Variance for Wet root

Source  DF       SS      MS     F      P
method   1   0.4648  0.4648  2.45  0.124
Error   54  10.2608  0.1900
Total   55  10.7256

Analysis of Variance for Dry root

Source  DF        SS        MS     F      P
method   1  0.000001  0.000001  0.00  0.985
Error   54  0.134393  0.002489
Total   55  0.134393
```

Comparison between the two concentrations of *Pythium* sp. inoculum (normal and high) (section 2.3.3)

November 2003

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C of *Pythium* between the two concentrations for November 2003

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      4.708      4.708    3.64  0.063
Error       40     51.683      1.292
Total       41     56.392

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.027056   0.027056    2.80  0.102
Error       40   0.386091   0.009652
Total       41   0.413147

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.00599    0.00599    0.16  0.690
Error       40    1.48275    0.03707
Total       41    1.48873

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.000465   0.000465    0.41  0.523
Error       40   0.044925   0.001123
Total       41   0.045390
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C of *Pythium* between the two concentrations for November 2003

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      7.201      7.201    2.71  0.108
Error       40    106.385      2.660
Total       41    113.586

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.004505   0.004505    0.65  0.425
Error       40   0.277331   0.006933
Total       41   0.281837

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.6394     0.6394    2.98  0.092
Error       40     8.5931     0.2148
Total       41     9.2325
Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0001283  0.0001283    0.21  0.651
Error       40  0.0247146  0.0006179
Total       41  0.0248429
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C of *Pythium* between the two concentrations for November 2003

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.3218     0.3218    0.44  0.509
Error       40    29.0485     0.7262
Total       41    29.3704

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.000868   0.000868    0.23  0.635
Error       40   0.151803   0.003795
Total       41   0.152671

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.10252    0.10252    2.87  0.098
Error       40    1.43009    0.03575
Total       41    1.53261

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0001321  0.0001321    0.61  0.439
Error       40  0.0086648  0.0002166
Total       41  0.0087970
```

March 2004

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C of *Pythium* between the two concentrations for March 2004

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.2189     1.2189    1.83  0.184
Error       40    26.6316     0.6658
Total       41    27.8505

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.00466    0.00466    0.39  0.535
Error       40    0.47560    0.01189
Total       41    0.48026
Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.6509     0.6509    2.17  0.149
Error       40    12.0070     0.3002
Total       41    12.6579

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1    0.02900    0.02900    1.45  0.236
Error       40    0.80160    0.02004
Total       41    0.83060
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C of *Pythium* between the two concentrations for March 2004

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      6.651      6.651    2.10  0.155
Error       40    126.905      3.173
Total       41    133.556

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.5578     0.5578    2.13  0.152
Error       40    10.4722     0.2618
Total       41    11.0300

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.0961     0.0961    0.69  0.411
Error       40     5.5682     0.1392
Total       41     5.6643

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.017951   0.017951    2.26  0.141
Error       40   0.318264   0.007957
Total       41   0.336215
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C of *Pythium* between the two concentrations for March 2004

```
Factor     Type Levels Values
Treatmen  fixed      2  High   Low

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.0954     1.0954    1.56  0.218
Error       40    28.0303     0.7008
Total       41    29.1257

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.1843     0.1843    1.10  0.300
Error       40     6.6959     0.1674
Total       41     6.8802

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.05561    0.05561    0.99  0.325
Error       40    2.24258    0.05606
Total       41    2.29819

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.004368   0.004368    3.03  0.089
Error       40   0.057628   0.001441
Total       41   0.061995
```

Comparison between the two *Pythium* isolates (1 and 2) (section 2.3.3)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in January 2004 experiment versus isolates

```
Factor   Type   Levels  Values
Isolate  fixed       2  1, 2

Analysis of Variance for Wet shoot

Source   DF      SS     MS     F      P
Isolate   1   0.139  0.139  0.08  0.781
Error    40  70.991  1.775
Total    41  71.130

Analysis of Variance for Dry shoot

Source   DF         SS       MS     F      P
Isolate   1    3511131  3511131  1.00  0.323
Error    40  140464101  3511603
Total    41  143975232

Analysis of Variance for Wet root

Source   DF       SS       MS     F      P
Isolate   1  0.10131  0.10131  1.52  0.225
Error    40  2.67121  0.06678
Total    41  2.77252

Analysis of Variance for Dry root

Source   DF       SS       MS     F      P
Isolate   1  0.00000  0.00000  0.00  0.988
Error    40  0.72456  0.01811
Total    41  0.72457
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in January 2004 experiment versus isolates

```
Factor   Type   Levels  Values
Isolate  fixed       2  1, 2

Analysis of Variance for Wet shoot

Source   DF       SS     MS     F      P
Isolate   1    0.017  0.017  0.01  0.940
Error    40  114.115  2.853
Total    41  114.131

Analysis of Variance for Dry shoot

Source   DF      SS      MS     F      P
Isolate   1  0.0114  0.0114  0.08  0.773
Error    40  5.4196  0.1355
Total    41  5.4311

Analysis of Variance for Wet root

Source   DF      SS      MS     F      P
Isolate   1  0.0135  0.0135  0.12  0.729
Error    40  4.4253  0.1106
Total    41  4.4388

Analysis of Variance for Dry root

Source   DF       SS       MS     F      P
Isolate   1  0.01213  0.01213  0.60  0.444
Error    40  0.81044  0.02026
Total    41  0.82257
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in January 2004 experiment versus isolates

```
Factor   Type   Levels  Values
Isolate  fixed       2  1, 2

Analysis of Variance for Wet shoot

Source   DF      SS     MS     F      P
Isolate   1   0.034  0.034  0.01  0.904
Error    40  90.491  2.262
Total    41  90.524

Analysis of Variance for Dry shoot

Source   DF      SS      MS     F      P
Isolate   1  0.0043  0.0043  0.02  0.888
Error    40  8.6146  0.2154
Total    41  8.6189

Analysis of Variance for Wet root

Source   DF       SS     MS     F      P
Isolate   1    1.958  1.958  0.71  0.404
Error    40  110.184  2.755
Total    41  112.142

Analysis of Variance for Dry root

Source   DF        SS        MS     F      P
Isolate   1  0.002047  0.002047  1.16  0.288
Error    40  0.070579  0.001764
Total    41  0.072626
```

2. *Phytophthora* sp. pathogenicity tests (section 2.3.3) (analysis for Table 2-8)

May 2003 (analysis for Table 2-8)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F       P
Treatmen     1      2.315      2.315    0.74   0.397
Error       26     81.068      3.118
Total       27     83.383

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F       P
Treatmen     1    0.01414    0.01414    1.34   0.258
Error       26    0.27499    0.01058
Total       27    0.28913

Analysis of Variance for Wet root

Source      DF         SS         MS       F       P
Treatmen     1    0.08704    0.08704    1.09   0.306
Error       26    2.07885    0.07996
Total       27    2.16589

Analysis of Variance for Dry root

Source      DF         SS         MS       F       P
Treatmen     1  0.0052503  0.0052503    6.17   0.020
Error       26  0.0221395  0.0008515
Total       27  0.0273898
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F       P
Treatmen     1      1.127      1.127    0.71   0.407
Error       26     41.300      1.588
Total       27     42.427

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F       P
Treatmen     1    0.10096    0.10096    6.88   0.014
Error       26    0.38130    0.01467
Total       27    0.48225

Analysis of Variance for Wet root

Source      DF         SS         MS       F       P
Treatmen     1    0.00282    0.00282    0.08   0.774
Error       26    0.86951    0.03344
Total       27    0.87233

Analysis of Variance for Dry root

Source      DF         SS         MS       F       P
Treatmen     1  0.0000253  0.0000253    0.24   0.626
Error       26  0.0027016  0.0001039
Total       27  0.0027269
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.2492     0.2492    0.37  0.548
Error       26    17.5084     0.6734
Total       27    17.7577

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.000449   0.000449    0.30  0.586
Error       26   0.038326   0.001474
Total       27   0.038776

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1   0.000076   0.000076    0.02  0.898
Error       26   0.118626   0.004563
Total       27   0.118702

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1 0.00000520 0.00000520    0.07  0.792
Error       26 0.00190615 0.00007331
Total       27 0.00191135
```

November 2003 (analysis for Table 2-8)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in November 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      4.883      4.883    2.63  0.117
Error       26     48.200      1.854
Total       27     53.083

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.00164    0.00164    0.14  0.716
Error       26    0.31519    0.01212
Total       27    0.31682

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.01044    0.01044    0.54  0.471
Error       26    0.50651    0.01948
Total       27    0.51694

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.000201   0.000201    0.17  0.681
Error       26   0.030186   0.001161
Total       27   0.030386
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in November 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     20.245     20.245   14.12  0.001
Error       26     37.273      1.434
Total       27     57.519

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.23179    0.23179   25.02  0.000
Error       26    0.24085    0.00926
Total       27    0.47263

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.69677    0.69677   15.77  0.001
Error       26    1.14901    0.04419
Total       27    1.84578

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0071799  0.0071799   23.86  0.000
Error       26  0.0078229  0.0003009
Total       27  0.0150028
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in November 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.6527     0.6527    0.84  0.369
Error       26    20.2819     0.7801
Total       27    20.9346

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.000891   0.000891    0.20  0.655
Error       26   0.113143   0.004352
Total       27   0.114034

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.15241    0.15241    7.44  0.011
Error       26    0.53269    0.02049
Total       27    0.68510

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0043024  0.0043024    9.18  0.005
Error       26  0.0121831  0.0004686
Total       27  0.0164856
```

January 2004 (analysis for Table 2-8)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      0.711      0.711    0.47  0.499
Error       26     39.427      1.516
Total       27     40.139

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.02432    0.02432    0.35  0.561
Error       26    1.82135    0.07005
Total       27    1.84567

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.07767    0.07767    1.21  0.282
Error       26    1.67135    0.06428
Total       27    1.74903

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.003563   0.003563    0.57  0.457
Error       26   0.162738   0.006259
Total       27   0.166301
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     14.065     14.065    3.38  0.077
Error       26    108.097      4.158
Total       27    122.163

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.8793     0.8793    8.20  0.008
Error       26     2.7883     0.1072
Total       27     3.6676

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.2793     0.2793    2.28  0.143
Error       26     3.1908     0.1227
Total       27     3.4701

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.003535   0.003535    2.19  0.151
Error       26   0.041885   0.001611
Total       27   0.045420
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.8307     1.8307    3.98  0.056
Error       26    11.9449     0.4594
Total       27    13.7756

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.02105    0.02105    1.52  0.229
Error       26    0.36064    0.01387
Total       27    0.38169
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.77121    0.77121    8.52  0.007
Error       26    2.35351    0.09052
Total       27    3.12472

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0026985  0.0026985    3.32  0.080
Error       26  0.0211356  0.0008129
Total       27  0.0238340
```

March 2004 (analysis for Table 2-8)

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 15°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      0.210      0.210    0.06  0.814
Error       26     96.872      3.726
Total       27     97.082

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.02741    0.02741    0.29  0.596
Error       26    2.46953    0.09498
Total       27    2.49694

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.1048     0.1048    0.45  0.510
Error       26     6.0910     0.2343
Total       27     6.1958

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.000529   0.000529    0.16  0.693
Error       26   0.086265   0.003318
Total       27   0.086794
```

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 25°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     17.757     17.757    8.95  0.10
Error       26     51.612      1.985
Total       27     69.368

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.64520    0.64520   11.22  0.070
Error       26    1.49490    0.05750
Total       27    2.14010

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     1.9373     1.9373   14.66  0.041
Error       26     3.4349     0.1321
Total       27     5.3722

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1    0.11754    0.11754   28.38  0.000
Error       26    0.10768    0.00414
Total       27    0.22522
```

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 35°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatment  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatment    1     6.7463     6.7463    9.89  0.478
Error       26    17.7307     0.6820
Total       27    24.4771

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatment    1     1.2036     1.2036   17.56  0.011
Error       26     1.7823     0.0685
Total       27     2.9858

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatment    1     1.5072     1.5072   11.99  0.039
Error       26     3.2693     0.1257
Total       27     4.7765

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatment    1   0.018322   0.018322   23.46  0.112
Error       26   0.020309   0.000781
Total       27   0.038632
```

3. *Fusarium avenaceum* pathogenicity tests (section 2.3.3) (analysis for Table 2-9)

May 2003 (analysis for Table 2-9)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F       P
Treatmen     1      1.080      1.080    0.62   0.438
Error       26     45.292      1.742
Total       27     46.372

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F       P
Treatmen     1   0.012819   0.012819    3.10   0.090
Error       26   0.107376   0.004130
Total       27   0.120195

Analysis of Variance for Wet root

Source      DF         SS         MS       F       P
Treatmen     1    0.04641    0.04641    0.56   0.459
Error       26    2.13891    0.08227
Total       27    2.18532

Analysis of Variance for Dry root

Source      DF         SS         MS       F       P
Treatmen     1  0.0000631  0.0000631    0.35   0.562
Error       26  0.0047508  0.0001827
Total       27  0.0048139
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in May 2003 experiment versus treatment (inoculated or control)

```
actor      Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F       P
Treatmen     1      4.970      4.970    1.18   0.286
Error       26    109.120      4.197
Total       27    114.090

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F       P
Treatmen     1    0.05348    0.05348    2.38   0.135
Error       26    0.58486    0.02249
Total       27    0.63835

Analysis of Variance for Wet root

Source      DF         SS         MS       F       P
Treatmen     1     0.2067     0.2067    1.31   0.263
Error       26     4.1014     0.1577
Total       27     4.3081

Analysis of Variance for Dry root

Source      DF         SS         MS       F       P
Treatmen     1  0.0000009  0.0000009    0.01   0.931
Error       26  0.0030968  0.0001191
Total       27  0.0030978
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      2.432      2.432    1.55  0.225
Error       26     40.865      1.572
Total       27     43.297

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.008020   0.008020    0.96  0.336
Error       26   0.217206   0.008354
Total       27   0.225227

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.03875    0.03875    0.69  0.412
Error       26    1.45047    0.05579
Total       27    1.48922

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0003713  0.0003713    0.52  0.478
Error       26  0.0186457  0.0007171
Total       27  0.0190170
```

4. *Fusarium oxysporum* pathogenicity tests (section 2.3.3) (analysis for Table 2-9)

May 2003 (analysis for Table 2-9)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     2.5949     2.5949    2.82  0.105
Error       26    23.9214     0.9201
Total       27    26.5164

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.000226   0.000226    0.11  0.742
Error       26   0.053295   0.002050
Total       27   0.053521

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.11181    0.11181    2.64  0.116
Error       26    1.09941    0.04228
Total       27    1.21122

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0034817  0.0034817    3.97  0.057
Error       26  0.0228243  0.0008779
Total       27  0.0263061
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in May 2003 experiment versus treatment (inoculated or control)

Factor	Type	Levels	Values
Treatmen	fixed	2	1 2

Analysis of Variance for Wet shoot

Source	DF	SS	MS	F	P
Treatmen	1	0.073	0.073	0.03	0.875
Error	26	74.871	2.880		
Total	27	74.944			

Analysis of Variance for Dry shoot

Source	DF	SS	MS	F	P
Treatmen	1	0.02696	0.02696	1.10	0.303
Error	26	0.63439	0.02440		
Total	27	0.66135			

Analysis of Variance for Wet root

Source	DF	SS	MS	F	P
Treatmen	1	0.08643	0.08643	0.87	0.359
Error	26	2.57266	0.09895		
Total	27	2.65909			

Analysis of Variance for Dry root

Source	DF	SS	MS	F	P
Treatmen	1	0.00001475	0.00001475	0.17	0.687
Error	26	0.00230431	0.00008863		
Total	27	0.00231906			

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      2.656      2.656    2.11  0.158
Error       26     32.721      1.258
Total       27     35.377

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.012774   0.012774    1.66  0.209
Error       26   0.199966   0.007691
Total       27   0.212739

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.04527    0.04527    0.58  0.455
Error       26    2.04620    0.07870
Total       27    2.09146

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0005075  0.0005075    1.08  0.309
Error       26  0.0122728  0.0004720
Total       27  0.0127803
```

August 2003 (analysis for Table 2-9)

ANOVA: Log wet shoot weight, dry shoot weight, Log wet root weight, dry root weight at temperature 15°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for logWet shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.0697     0.0697    0.35  0.561
Error       26     5.2256     0.2010
Total       27     5.2953

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.00599    0.00599    0.59  0.448
Error       26    0.26200    0.01008
Total       27    0.26799

Analysis of Variance for logWet root

Source      DF         SS         MS       F      P
Treatmen     1    0.02968    0.02968    1.19  0.286
Error       26    0.65113    0.02504
Total       27    0.68081

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0000808  0.0000808    0.16  0.690
Error       26  0.0129585  0.0004984
Total       27  0.0130393
```

ANOVA: log wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for logWet shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.8235     0.8235    3.31  0.081
Error       26     6.4756     0.2491
Total       27     7.2992

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.01528    0.01528    1.06  0.312
Error       26    0.37337    0.01436
Total       27    0.38865

Analysis of Variance for logWet root

Source      DF         SS         MS       F      P
Treatmen     1    0.10316    0.10316    1.50  0.232
Error       26    1.79333    0.06897
Total       27    1.89648

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0005565  0.0005565    2.47  0.128
Error       26  0.0058565  0.0002253
Total       27  0.0064130
```

ANOVA: log wet shoot weight, dry shoot weight, log wet root weight, dry root weight at temperature 35°C in August 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for logWet shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.15945    0.15945    1.87  0.183
Error       26    2.21332    0.08513
Total       27    2.37277

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.000082   0.000082    0.02  0.902
Error       26   0.137532   0.005290
Total       27   0.137614

Analysis of Variance for logWet root

Source      DF         SS         MS       F      P
Treatmen     1    0.13440    0.13440    2.37  0.135
Error       26    1.47139    0.05659
Total       27    1.60579

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0000054  0.0000054    0.02  0.897
Error       26  0.0081768  0.0003145
Total       27  0.0081821
```

5. *Rhizoctonia* sp. pathogenicity tests (section 2.3.3) (analysis for Table 2-10)

May 2003 (analysis for Table 2-10)

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 15°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      0.099      0.099    0.03  0.870
Error       26     94.775      3.645
Total       27     94.874

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.012046   0.012046    2.36  0.136
Error       26   0.132485   0.005096
Total       27   0.144531

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.3845     0.3845    2.09  0.160
Error       26     4.7892     0.1842
Total       27     5.1738

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0002343  0.0002343    1.11  0.302
Error       26  0.0055037  0.0002117
Total       27  0.0057380
```

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 25°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      7.880      7.880    3.35  0.079
Error       26     61.146      2.352
Total       27     69.026

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.00769    0.00769    0.72  0.405
Error       26    0.27914    0.01074
Total       27    0.28683
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.4125     0.4125    3.24  0.084
Error       26     3.3117     0.1274
Total       27     3.7241

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1 0.00004815 0.00004815    0.63  0.434
Error       26 0.00198663 0.00007641
Total       27 0.00203478
```

ANOVA: wet shoot weight, sq-dry shoot weight, wet root weight, dry root weight at temperature 35°C in May 2003 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     2.4036     2.4036    3.09  0.090
Error       26    20.2131     0.7774
Total       27    22.6167

Analysis of Variance for Sq-dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.002050   0.002050    0.40  0.535
Error       26   0.134862   0.005187
Total       27   0.136913

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1   0.004028   0.004028    1.11  0.303
Error       26   0.094741   0.003644
Total       27   0.098769

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0000614  0.0000614    0.42  0.524
Error       26  0.0038224  0.0001470
Total       27  0.0038837
```

6. The combination of *Pythium* sp. and *Phytophthora* sp. (section 2.3.3) (analysis for Table 2-11)

November 2003 (analysis for Table 2-11)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in November 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     18.855     18.855   24.69  0.000
Error       26     19.855      0.764
Total       27     38.710

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.1105     1.1105   46.85  0.000
Error       26     0.6163     0.0237
Total       27     1.7269

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.66883    0.66883   39.06  0.000
Error       26    0.44518    0.01712
Total       27    1.11400

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0083581  0.0083581   19.11  0.093
Error       26  0.0113689  0.0004373
Total       27  0.0197270
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in November 2004 experiment versus treatment (inoculated or control)

```
actor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     3.4896     3.4896    4.10  0.267
Error       26    22.1057     0.8502
Total       27    25.5953

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.039087   0.039087    8.15  0.148
Error       26   0.124676   0.004795
Total       27   0.163763

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.33457    0.33457    8.01  0.009
Error       26    1.08613    0.04177
Total       27    1.42070

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.017638   0.017638   48.39  0.000
Error       26   0.009477   0.000364
Total       27   0.027115
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in November 2004 experiment versus treatment (inoculated or control)

```
Factor    Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     2.9332     2.9332    4.49  0.374
Error       26    16.9788     0.6530
Total       27    19.9120

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1   0.029670   0.029670    9.91  0.267
Error       26   0.077815   0.002993
Total       27   0.107485

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.36420    0.36420   14.29  0.011
Error       26    0.66256    0.02548
Total       27    1.02677

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0039292  0.0039292    7.58  0.041
Error       26  0.0134701  0.0005181
Total       27  0.0173992
```

January 2004 (analysis for Table 2-11)

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 15°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      0.404      0.404    0.14  0.990
Error       26     72.956      2.806
Total       27     73.360

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.00539    0.00539    0.07  0.988
Error       26    2.12551    0.08175
Total       27    2.13090
Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.0223     0.0223    0.10  0.994
Error       26     5.5134     0.2121
Total       27     5.5356

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.000865   0.000865    0.66  0.952
Error       26   0.034283   0.001319
Total       27   0.035148
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 25°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen   fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      5.022      5.022    1.15  0.764
Error       26    113.680      4.372
Total       27    118.702

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     0.2321     0.2321    2.17  0.569
Error       26     2.7864     0.1072
Total       27     3.0185

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.2293     0.2293    1.98  0.832
Error       26     3.0152     0.1160
Total       27     3.2445

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0023490  0.0023490    2.88  0.710
Error       26  0.0212385  0.0008169
Total       27  0.0235874
```

ANOVA: wet shoot weight, dry shoot weight, wet root weight, dry root weight at temperature 35°C in January 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      1.355      1.355    1.09  0.980
Error       26     32.371      1.245
Total       27     33.726

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.05732    0.05732    0.84  0.959
Error       26    1.78224    0.06855
Total       27    1.83956

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     0.0071     0.0071    0.05  0.992
Error       26     3.7187     0.1430
Total       27     3.7258

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.000749   0.000749    0.55  0.000
Error       26   0.035332   0.001359
Total       27   0.036082
```

March 2004 (analysis for Table 2-11)

ANOVA: wet shoot weight, dry shoot weight, sq-wet root weight, dry root weight at temperature 15°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1      0.676      0.676    0.47  0.990
Error       26     37.504      1.442
Total       27     38.181

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.00001    0.00001    0.00  0.998
Error       26    1.37444    0.05286
Total       27    1.37444

Analysis of Variance for Sq-Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.00885    0.00885    0.88  0.986
Error       26    0.26065    0.01003
Total       27    0.26950

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1  0.0014617  0.0014617    1.69  0.975
Error       26  0.0224904  0.0008650
Total       27  0.0239521
```

ANOVA: wet shoot weight, dry shoot weight, sq-wet root weight, dry root weight at temperature 25°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     24.964     24.964    5.59  0.024
Error       26    116.136      4.467
Total       27    141.101

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1     1.0900     1.0900    7.37  0.127
Error       26     3.8432     0.1478
Total       27     4.9332

Analysis of Variance for Sq-Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.30487    0.30487    8.13  0.000
Error       26    0.97474    0.03749
Total       27    1.27961

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.058128   0.058128    8.45  0.004
Error       26   0.178752   0.006875
Total       27   0.236880
```

ANOVA: wet shoot weight, dry shoot weight, sq-wet root weight, dry root weight at temperature 35°C in March 2004 experiment versus treatment (inoculated or control)

```
Factor     Type Levels Values
Treatmen  fixed      2     1     2

Analysis of Variance for Wet shoot

Source      DF         SS         MS       F      P
Treatmen     1     8.7598     8.7598   11.31  0.362
Error       26    20.1332     0.7744
Total       27    28.8930

Analysis of Variance for Dry shoot

Source      DF         SS         MS       F      P
Treatmen     1    0.84443    0.84443   11.55  0.028
Error       26    1.90127    0.07313
Total       27    2.74570

Analysis of Variance for Sq-Wet root

Source      DF         SS         MS       F      P
Treatmen     1    0.15268    0.15268    7.15  0.153
Error       26    0.55484    0.02134
Total       27    0.70752
Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.012755   0.012755   12.96  0.301
Error       26   0.025583   0.000984
Total       27   0.038338
```

d. Temperature responses in culture of fungi and oomycetes (section 2.3.4)

General Linear Model: Growth of *Pythium* versus isolate, temp. (section 2.3.4)

```
Factor     Type Levels Values
Isolate   fixed      5 1 2 3 4 5
temp.     fixed      8 5 10 15 20 25 30 35 40

Analysis of Variance for growth, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Isolate     4       1.23       1.23       0.31    0.75  0.562
temp.       7    5654.45    5654.45     807.78 1958.60  0.000
Error     108      44.54      44.54       0.41
Total     119    5700.23
```

General Linear Model: Growth of *Phytophthora* versus isolate, temp. (section 2.3.4)

```
Factor   Type   Levels  Values
Isolate  fixed       5  1, 2, 3, 4, 5
temp.    fixed       6  10, 15, 20, 25, 30, 35


Analysis of Variance for Growth, using Adjusted SS for Tests

Source          DF   Seq SS   Adj SS  Adj MS       F      P
Isolate          4    0.956    0.956   0.239    0.87  0.486
temp.            5  289.967  289.967  57.993  211.10  0.000
Error           80   21.978   21.978   0.275
Total           89  312.900
```

General Linear Model: Growth of *Fusarium oxysporum* versus isolate, temp. (section 2.3.4)

```
Factor     Type Levels Values
Isolate   fixed      3 1 2 3
temp.     fixed      6 10 15 20 25 30 35

Analysis of Variance for growth, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Isolate     2      6.164      6.164      3.082    2.14  0.129
temp.       5    186.133    186.133     37.227   25.90  0.000
Error      46     66.116     66.116      1.437
Total      53    258.413
```

General Linear Model: Growth of *Fusarium avenaceum* versus isolate, temp. (section 2.3.4)

```
Factor     Type Levels Values
Isolate   fixed      3 1 2 3
temp.     fixed      6 10 15 20 25 30 35

Analysis of Variance for growth, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Isolate     2      6.496      6.496      3.248    2.33  0.109
temp.       5    191.134    191.134     38.227   27.43  0.000
Error      46     64.117     64.117      1.394
Total      53    261.748
```

4. Data analysis for Chapter 3: Survey of root rot disease of lettuce grown in hydroponic systems in Sydney and the Central Coast

a. Comparsions between times, farms and cultivars (section 3.3.1)

General Linear Model: Root assessment versus time, cultivar and farm (section 3.3.1)

```
Factor     Type   Levels  Values
Time       fixed       5  May-04, Aug-04, Nov-04, Jan-05, Mar-05
Cultivar   fixed       4  Baby Cos, Geen Oak, Mignonette, Red Oak
Farm       fixed       4  CC1, CC2, L1, L2


Analysis of Variance for Scale, using Adjusted SS for Tests

Source       DF   Seq SS   Adj SS  Adj MS       F      P
Time          4   997.14  1025.54  256.39  532.54  0.000
Cultivar      3     2.50     4.28    1.43    2.96  0.061
Farm          3    87.29    87.29   29.10   60.44  0.000
Error      2299  1106.83  1106.83    0.48
Total      2309  2193.76
```

General Linear Model: Leaf assessment versus time, cultivar and farm (section 3.3.1)

```
Factor     Type   Levels  Values
Farm       fixed       4  CC1, CC2, L1, L2
Time       fixed       5  May-04, Aug-04, Nov-04, Jan-05, Mar-05
Cultivar   fixed       4  Cos, G. Oak, Mig., R. Oak


Analysis of Variance for Scale, using Adjusted SS for Tests

Source       DF    Seq SS    Adj SS  Adj MS       F      P
Farm          3    24.114    30.213  10.071   40.72  0.000
Cultivar      3     0.205     0.205   0.068    0.28  0.842
Time          4   143.822   143.516  35.879  145.05  0.000
Error      7689  1901.857  1901.857   0.247
Total      7699  2069.998
```

b. Baby Cos (section 3.3.1.1) (analyses for Tables 3-2 and 3-3)

1. Root assessment (section 3.3.1.1 and Table 3-2)

General Linear Model: Root rot severity from L1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     77.493     77.493     19.373   56.75  0.000
Error     145     49.500     49.500      0.341
Total     149    126.993

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.2333      0.1509     1.547     0.5340
Nov-04         1.5667      0.1509    10.385     0.0000
```

```
Jan-05          1.3000       0.1509      8.617      0.0000
Mar-05          1.7667       0.1509     11.711      0.0000

Time = Aug-04 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Nov-04           1.333      0.1509      8.838      0.0000
Jan-05           1.067      0.1509      7.071      0.0000
Mar-05           1.533      0.1509     10.164      0.0000

Time = Nov-04 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Jan-05         -0.2667      0.1509     -1.768      0.3965
Mar-05          0.2000      0.1509      1.326      0.6757

Time = Jan-05 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Mar-05          0.4667      0.1509      3.093      0.0198
```

General Linear Model: Root rot severity from L2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     68.573     68.573     17.143   32.12  0.000
Error     145     77.400     77.400      0.534
Total     149    145.973

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Aug-04          0.4333      0.1886      2.297      0.1518
Nov-04          1.4333      0.1886      7.598      0.0000
Jan-05          1.8667      0.1886      9.895      0.0000
Mar-05          1.1667      0.1886      6.185      0.0000

Time = Aug-04 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Nov-04          1.0000      0.1886      5.301      0.0000
Jan-05          1.4333      0.1886      7.598      0.0000
Mar-05          0.7333      0.1886      3.887      0.0014

Time = Nov-04 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Jan-05          0.4333      0.1886      2.297      0.1518
Mar-05         -0.2667      0.1886     -1.414      0.6199

Time = Jan-05 subtracted from:

Level       Difference        SE of              Adjusted
Time          of Means  Difference    T-Value     P-Value
Mar-05         -0.7000      0.1886     -3.711      0.0027
```

General Linear Model: Root rot severity from CC1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    139.907    139.907     34.977  112.29  0.000
Error     145     45.167     45.167      0.311
Total     149    185.073

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.1000      0.1441    0.6939     0.9575
Nov-04         1.8667      0.1441   12.9535     0.0000
Jan-05         2.3000      0.1441   15.9606     0.0000
Mar-05         1.8000      0.1441   12.4909     0.0000

Time = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04          1.767      0.1441     12.26     0.0000
Jan-05          2.200      0.1441     15.27     0.0000
Mar-05          1.700      0.1441     11.80     0.0000

Time = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05        0.43333      0.1441    3.0071     0.0255
Mar-05       -0.06667      0.1441   -0.4626     0.9905

Time = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.5000      0.1441    -3.470     0.0061
```

General Linear Model: Root rot severity from CC2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    157.693    157.693     39.423  182.63  0.000
Error     145     31.300     31.300      0.216
Total     149    188.993

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04        -0.9333      0.1200    -7.780     0.0000
Nov-04         1.7667      0.1200    14.727     0.0000
Jan-05         1.6333      0.1200    13.615     0.0000
Mar-05         0.2333      0.1200     1.945     0.2985

Time = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04          2.700      0.1200    22.507     0.0000
```

```
Jan-05          2.567      0.1200    21.396      0.0000
Mar-05          1.167      0.1200     9.725      0.0000

Time = Nov-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Jan-05          -0.133      0.1200     -1.11      0.8003
Mar-05          -1.533      0.1200    -12.78      0.0000

Time = Jan-05 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Mar-05          -1.400      0.1200    -11.67      0.0000
```

General Linear Model: Root rot severity versus time, farms

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms      fixed      4 CC1 CC2 L1  L2

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    378.073    378.073     94.518  208.04  0.000
Farms       3     43.565     43.565     14.522   31.96  0.000
Error     592    268.960    268.960      0.454
Total     599    690.598
```

General Linear Model: Root rot severity versus time, two places

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two plac   fixed      2 Central Coast leppington

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    378.073    378.073     94.518  183.79  0.000
Two plac    1      7.042      7.042      7.042   13.69  0.000
Error     594    305.483    305.483      0.514
Total     599    690.598


Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Two plac

Two plac = Central subtracted from:

Level         Difference       SE of              Adjusted
Two plac        of Means  Difference   T-Value     P-Value
leppington       -0.2167     0.05855    -3.700      0.0002
```

2. Leaf symptom assessment (section 3.3.1.1 and Table 3-3)

General Linear Model: Leaf symptom assessment from L1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     5.1480     5.1480     1.2870    5.37  0.000
Error     495   118.7300   118.7300     0.2399
Total     499   123.8780

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time
```

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	0.04000	0.06926	0.5775	0.9784
Nov-04	0.19000	0.06926	2.7432	0.0479
Jan-05	0.15000	0.06926	2.1657	0.1928
Mar-05	0.28000	0.06926	4.0426	0.0005

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.1500	0.06926	2.166	0.1928
Jan-05	0.1100	0.06926	1.588	0.5050
Mar-05	0.2400	0.06926	3.465	0.0048

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	-0.04000	0.06926	-0.5775	0.9784
Mar-05	0.09000	0.06926	1.2994	0.6916

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	0.1300	0.06926	1.877	0.3298

General Linear Model: Leaf symptom assessment from L2 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	4.5120	4.5120	1.1280	9.17	0.000
Error	495	60.8800	60.8800	0.1230		
Total	499	65.3920				

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time
Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	0.000000	0.04960	0.00000	1.0000
Nov-04	0.090000	0.04960	1.81465	0.3650
Jan-05	0.260000	0.04960	5.24232	0.0000
Mar-05	0.080000	0.04960	1.61302	0.4889

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.09000	0.04960	1.815	0.3650
Jan-05	0.26000	0.04960	5.242	0.0000
Mar-05	0.08000	0.04960	1.613	0.4889

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	0.17000	0.04960	3.4277	0.0055
Mar-05	-0.01000	0.04960	-0.2016	0.9996

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	-0.1800	0.04960	-3.629	0.0026

General Linear Model: Leaf symptom assessment from CC1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    21.0080    21.0080     5.2520   12.76  0.000
Error     495   203.6700   203.6700     0.4115
Total     499   224.6780

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Aug-04         0.04000     0.09071    0.4409      0.9922
Nov-04         0.20000     0.09071    2.2047      0.1778
Jan-05         0.46000     0.09071    5.0709      0.0000
Mar-05         0.49000     0.09071    5.4016      0.0000

Time = Aug-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Nov-04          0.1600     0.09071     1.764      0.3949
Jan-05          0.4200     0.09071     4.630      0.0000
Mar-05          0.4500     0.09071     4.961      0.0000

Time = Nov-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Jan-05          0.2600     0.09071     2.866      0.0338
Mar-05          0.2900     0.09071     3.197      0.0121

Time = Jan-05 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Mar-05         0.03000     0.09071    0.3307      0.9974
```

General Linear Model: Leaf symptom assessment from CC2 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     64.548     64.548     16.137   75.26  0.000
Error     495    106.130    106.130      0.214
Total     499    170.678

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Aug-04        -0.01000     0.06548   -0.1527      0.9999
Nov-04         0.27000     0.06548    4.1232      0.0004
Jan-05         0.93000     0.06548   14.2021      0.0000
Mar-05         0.02000     0.06548    0.3054      0.9981

Time = Aug-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value     P-Value
Nov-04         0.28000     0.06548    4.2759      0.0002
```

```
Jan-05        0.94000      0.06548   14.3548      0.0000
Mar-05        0.03000      0.06548    0.4581      0.9909

Time = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.6600     0.06548    10.079     0.0000
Mar-05        -0.2500     0.06548    -3.818     0.0013

Time = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.9100     0.06548    -13.90     0.0000
```

General Linear Model: Leaf symptom assessment versus time, farms

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms      fixed      4 CC1 CC2 L1  L2

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    53.2070    53.2070    13.3017   49.86  0.000
Farms       3     8.5535     8.5535     2.8512   10.69  0.000
Error    1992   531.4190   531.4190     0.2668
Total    1999   593.1795
```

General Linear Model: Leaf symptom assessment versus time, two places

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two plac   fixed      2 Central Coast leppington

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     53.207     53.207     13.302   49.80  0.000
Farms       1      7.321      7.321      7.321   27.40  0.000
Error    1994    532.652    532.652      0.267
Total    1999    593.179

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Farms

Farms = Central Coast subtracted from:

Level      Difference       SE of             Adjusted
Farms        of Means  Difference   T-Value    P-Value
Leppington  -0.1210     0.02311    -5.235     0.0000
```

c. Red Oak (section 3.3.1.2 and Tables 3-4 and 3-5)

1. Root assessment (section 3.3.1.2 and Table 3-4)

General Linear Model: Root rot severity from L1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     58.893     58.893     14.723   42.19  0.000
Error     145     50.600     50.600      0.349
Total     149    109.493

Tukey Simultaneous Tests
Response Variable Root rot
```

All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.4333	0.1525	-2.841	0.0405
Nov-04	0.7333	0.1525	4.808	0.0000
Jan-05	0.7000	0.1525	4.589	0.0001
Mar-05	1.3667	0.1525	8.960	0.0000

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	1.167	0.1525	7.649	0.0000
Jan-05	1.133	0.1525	7.430	0.0000
Mar-05	1.800	0.1525	11.801	0.0000

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	-0.03333	0.1525	-0.2185	0.9995
Mar-05	0.63333	0.1525	4.1523	0.0005

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	0.6667	0.1525	4.371	0.0002

General Linear Model: Root rot severity from L2 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	84.227	84.227	21.057	49.83	0.000
Error	145	61.267	61.267	0.423		
Total	149	145.493				

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	0.4333	0.1678	2.582	0.0791
Nov-04	1.4000	0.1678	8.342	0.0000
Jan-05	1.3667	0.1678	8.143	0.0000
Mar-05	2.1000	0.1678	12.512	0.0000

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.9667	0.1678	5.760	0.0000
Jan-05	0.9333	0.1678	5.561	0.0000
Mar-05	1.6667	0.1678	9.930	0.0000

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	-0.03333	0.1678	-0.1986	0.9996
Mar-05	0.70000	0.1678	4.1708	0.0005

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	0.7333	0.1678	4.369	0.0002

General Linear Model: Root rot severity from CC1 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	45.773	45.773	11.443	52.56	0.000
Error	145	31.567	31.567	0.218		
Total	149	77.340				

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	0.3000	0.1205	2.490	0.0985
Nov-04	1.2667	0.1205	10.514	0.0000
Jan-05	1.4333	0.1205	11.898	0.0000
Mar-05	0.5667	0.1205	4.704	0.0001

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.9667	0.1205	8.024	0.0000
Jan-05	1.1333	0.1205	9.407	0.0000
Mar-05	0.2667	0.1205	2.214	0.1806

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	0.1667	0.1205	1.383	0.6392
Mar-05	-0.7000	0.1205	-5.810	0.0000

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	-0.8667	0.1205	-7.194	0.0000

General Linear Model: Root rot severity from CC2 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	116.960	116.960	29.240	163.70	0.000
Error	145	25.900	25.900	0.179		
Total	149	142.860				

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.06667	0.1091	-0.6109	0.9732
Nov-04	1.90000	0.1091	17.4114	0.0000
Jan-05	1.93333	0.1091	17.7168	0.0000
Mar-05	0.60000	0.1091	5.4983	0.0000

```
Time = Aug-04 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         1.9667      0.1091    18.022     0.0000
Jan-05         2.0000      0.1091    18.328     0.0000
Mar-05         0.6667      0.1091     6.109     0.0000

Time = Nov-04 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05          0.033      0.1091      0.31     0.9981
Mar-05         -1.300      0.1091    -11.91     0.0000

Time = Jan-05 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05         -1.333      0.1091    -12.22     0.0000
```

General Linear Model: Root rot severity versus time, farms

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms      fixed      4 CC1  L2 L1  CC2

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    228.460    228.460     57.115  137.04  0.000
Farms       3     10.273     10.273      3.424    8.22  0.000
Error     592    246.727    246.727      0.417
Total     599    485.460
```

General Linear Model: Root rot severity versus time, two places

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two plac   fixed      2 Central coast  Leppington

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    228.460    228.460     57.115  132.29  0.000
Two plac    1      0.540      0.540      0.540    1.25  0.264
Error     594    256.460    256.460      0.432
Total     599    485.460

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Two plac

Two plac = Central Coast subtracted from:

Level         Difference       SE of              Adjusted
Two plac        of Means  Difference   T-Value    P-Value
leppington       -0.06000     0.05365    -1.118     0.2634
```

2. Leaf symptom assessment (section 3.3.1.2 and Table 3-5)

General Linear Model: Leaf symptom assessment from L1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     4.7680     4.7680     1.1920    5.48  0.000
Error     495   107.6800   107.6800     0.2175
Total     499   112.4480
```

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.02000	0.06596	-0.3032	0.9982
Nov-04	0.08000	0.06596	1.2129	0.7439
Jan-05	0.16000	0.06596	2.4257	0.1084
Mar-05	0.24000	0.06596	3.6386	0.0025

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.1000	0.06596	1.516	0.5519
Jan-05	0.1800	0.06596	2.729	0.0498
Mar-05	0.2600	0.06596	3.942	0.0008

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	0.08000	0.06596	1.213	0.7439
Mar-05	0.16000	0.06596	2.426	0.1084

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	0.08000	0.06596	1.213	0.7439

General Linear Model: Leaf symptom assessment from L2 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	1.7320	1.7320	0.4330	1.63	0.165
Error	495	131.3400	131.3400	0.2653		
Total	499	133.0720				

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.08000	0.07285	-1.098	0.8076
Nov-04	0.05000	0.07285	0.686	0.9595
Jan-05	0.08000	0.07285	1.098	0.8076
Mar-05	0.07000	0.07285	0.961	0.8726

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.1300	0.07285	1.785	0.3826
Jan-05	0.1600	0.07285	2.196	0.1810
Mar-05	0.1500	0.07285	2.059	0.2381

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	0.03000	0.07285	0.4118	0.9940
Mar-05	0.02000	0.07285	0.2745	0.9988

Time = Jan-05 subtracted from:

Level	Difference	SE of	Adjusted

Time	of Means	Difference	T-Value	P-Value
Mar-05	-0.01000	0.07285	-0.1373	0.9999

General Linear Model: Leaf symptom assessment from CC1 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	6.2680	6.2680	1.5670	6.86	0.000
Error	495	113.0200	113.0200	0.2283		
Total	499	119.2880				

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	0.05000	0.06758	0.7399	0.9471
Nov-04	0.28000	0.06758	4.1435	0.0003
Jan-05	0.25000	0.06758	3.6996	0.0020
Mar-05	0.08000	0.06758	1.1839	0.7607

Time = Aug-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.23000	0.06758	3.4036	0.0060
Jan-05	0.20000	0.06758	2.9596	0.0256
Mar-05	0.03000	0.06758	0.4439	0.9920

Time = Nov-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	-0.0300	0.06758	-0.444	0.9920
Mar-05	-0.2000	0.06758	-2.960	0.0256

Time = Jan-05 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	-0.1700	0.06758	-2.516	0.0871

General Linear Model: Leaf symptom assessment from CC2 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	41.072	41.072	10.268	36.28	0.000
Error	495	140.080	140.080	0.283		
Total	499	181.152				

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.000000	0.07523	-0.0000	1.0000
Nov-04	0.320000	0.07523	4.2535	0.0002
Jan-05	0.780000	0.07523	10.3680	0.0000
Mar-05	0.220000	0.07523	2.9243	0.0285

```
Time = Aug-04 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.3200     0.07523     4.254     0.0002
Jan-05         0.7800     0.07523    10.368     0.0000
Mar-05         0.2200     0.07523     2.924     0.0285

Time = Nov-04 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.4600     0.07523     6.114     0.0000
Mar-05        -0.1000     0.07523    -1.329     0.6729

Time = Jan-05 subtracted from:

Level      Difference       SE of              Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.5600     0.07523    -7.444     0.0000
```

General Linear Model: Leaf symptom assessment versus time, farms

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms      fixed      4 CC1  L2 L1  CC2

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    30.4851    30.3791     7.5948   29.45  0.000
Farms       3     5.0856     5.0856     1.6952    6.57  0.000
Error    2000   515.7181   515.7181     0.2579
Total    2007   551.2888
```

General Linear Model: Leaf symptom assessment versus time, two places

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two plac   fixed      2 Central coast  Leppington

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    30.2420    30.2420     7.5605   28.97  0.000
Two plac    1     0.4500     0.4500     0.4500    1.72  0.189
Error    1994   520.3300   520.3300     0.2609
Total    1999   551.0220

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Two plac

Two place = Central Coast subtracted from:

Level         Difference       SE of              Adjusted
Two plac        of Means  Difference   T-Value    P-Value
Leppington     -0.03000     0.02285    -1.313     0.1891
```

d. Green Oak (section 3.3.1.3) (analysis for Table 3-6 and 3-7)

1. Root assessment (section 3.3.1.3 Table 9-4)

General Linear Model: Root rot severity from L1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     50.707     50.707     12.677   30.81  0.000
Error     145     59.667     59.667      0.411
```

```
Total     149    110.373

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.3000      0.1656     1.811     0.3711
Nov-04         1.3333      0.1656     8.050     0.0000
Jan-05         1.5000      0.1656     9.056     0.0000
Mar-05         0.6000      0.1656     3.623     0.0037

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         1.0333      0.1656     6.239     0.0000
Jan-05         1.2000      0.1656     7.245     0.0000
Mar-05         0.3000      0.1656     1.811     0.3711

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.1667      0.1656     1.006     0.8521
Mar-05        -0.7333      0.1656    -4.428     0.0002

Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.9000      0.1656    -5.434     0.0000
```

General Linear Model: Root rot severity from L2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     61.733     61.733     15.433   29.60  0.000
Error     145     75.600     75.600      0.521
Total     149    137.333

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.6667      0.1864     3.576     0.0043
Nov-04         1.6667      0.1864     8.940     0.0000
Jan-05         1.7000      0.1864     9.118     0.0000
Mar-05         1.1333      0.1864     6.079     0.0000

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         1.0000      0.1864     5.364     0.0000
Jan-05         1.0333      0.1864     5.543     0.0000
Mar-05         0.4667      0.1864     2.503     0.0956

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.0333      0.1864     0.179     0.9998
Mar-05        -0.5333      0.1864    -2.861     0.0384
```

```
Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.5667      0.1864    -3.039     0.0232
```

General Linear Model: Root rot severity from CC1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     75.667     75.667     18.917   70.03  0.000
Error     145     39.167     39.167      0.270
Total     149    114.833

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.9000      0.1342     6.707     0.0000
Nov-04         1.3000      0.1342     9.688     0.0000
Jan-05         2.2000      0.1342    16.394     0.0000
Mar-05         0.9333      0.1342     6.955     0.0000

Time = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04        0.40000      0.1342    2.9808     0.0275
Jan-05        1.30000      0.1342    9.6876     0.0000
Mar-05        0.03333      0.1342    0.2484     0.9992

Time = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.9000      0.1342     6.707     0.0000
Mar-05        -0.3667      0.1342    -2.732     0.0541
Time = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05         -1.267      0.1342    -9.439     0.0000
```

General Linear Model: Root rot severity from CC2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time_1      4     81.293     81.293     20.323   88.23  0.000
Error     145     33.400     33.400      0.230
Total     149    114.693

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04        -0.2667      0.1239    -2.152     0.2042
Nov-04         1.2667      0.1239    10.222     0.0000
Jan-05         1.3667      0.1239    11.029     0.0000
Mar-05         1.4333      0.1239    11.567     0.0000
```

```
Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04          1.533      0.1239     12.37     0.0000
Jan-05          1.633      0.1239     13.18     0.0000
Mar-05          1.700      0.1239     13.72     0.0000

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.1000      0.1239    0.8070     0.9281
Mar-05         0.1667      0.1239    1.3449     0.6636

Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        0.06667      0.1239    0.5380     0.9832
```

General Linear Model: Root rot severity versus time, farms (section 3.3.1.3)

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms     fixed      4 CC1      L2 L1  CC2

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    233.290    233.290     58.322  141.54  0.000
Farms       3     89.365     89.365     29.788   72.29  0.000
Error     592    243.943    243.943      0.412
Total     599    566.598
```

General Linear Model: Rot rot severity versus time, two places (section 3.3.1.3)

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two plac  fixed      2 Central coast leppington

Analysis of Variance for Root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    233.290    233.290     58.322  110.25  0.000
Two plac    1     19.082     19.082     19.082   36.07  0.000
Error     594    314.227    314.227      0.529
Total     599    566.598

Tukey Simultaneous Tests
Response Variable Root rot
All Pairwise Comparisons among Levels of Two plac

Two plac = Central subtracted from:

Level        Difference       SE of             Adjusted
Two plac       of Means  Difference   T-Value    P-Value
leppingt        -0.3567     0.05939    -6.006     0.0000
```

2. Leaf symptom assessment section 3.3.1.3 Table 3-7)

General Linear Model: Leaf symptom assessment from L1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 1 2 3 4 5

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    11.7320    11.7320     2.9330    9.99  0.000
Error     495   145.3400   145.3400     0.2936
Total     499   157.0720
```

```
Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = 1 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
2             0.07000     0.07663    0.9135     0.8919
3             0.12000     0.07663    1.5659     0.5194
4             0.44000     0.07663    5.7418     0.0000
5             0.09000     0.07663    1.1745     0.7661

Time   = 2 subtracted from:
Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
3             0.05000     0.07663    0.6525     0.9662
4             0.37000     0.07663    4.8283     0.0000
5             0.02000     0.07663    0.2610     0.9990

Time   = 3 subtracted from:

Level      Difference       SE of             Adjusted
Time        of Means  Difference   T-Value    P-Value
4             0.32000     0.07663    4.1759     0.0003
5            -0.03000     0.07663   -0.3915     0.9950

Time   = 4 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
5             -0.3500     0.07663    -4.567     0.0000
```

General Linear Model: Leaf symptom assessment from L2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 1 2 3 4 5

Analysis of Variance for Scale_3, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     6.0280     6.0280     1.5070    8.72  0.000
Error     495    85.5300    85.5300     0.1728
Total     499    91.5580

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = 1 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
2             0.02000     0.05879    0.3402     0.9971
3             0.11000     0.05879    1.8712     0.3330
4             0.31000     0.05879    5.2734     0.0000
5             0.12000     0.05879    2.0413     0.2462

Time   = 2 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
3             0.09000     0.05879      1.531     0.5422
4             0.29000     0.05879      4.933     0.0000
5             0.10000     0.05879      1.701     0.4332

Time   = 3 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
4             0.20000     0.05879    3.4022     0.0060
5             0.01000     0.05879    0.1701     0.9998

Time   = 4 subtracted from:

Level      Difference       SE of             Adjusted
```

```
Time         of Means  Difference   T-Value    P-Value
5             -0.1900     0.05879    -3.232     0.0108
```

General Linear Model: Leaf symptom assessment from CC1 farm versus ttime

```
Factor     Type Levels Values
Time       fixed      5 1 2 3 4 5

Analysis of Variance for leaf assessment, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     4.2720     4.2720     1.0680    7.97  0.000
Error     495    66.3200    66.3200     0.1340
Total     499    70.5920

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time = 1 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
2           0.02000     0.05176    0.3864     0.9953
3           0.08000     0.05176    1.5455     0.5327
4           0.24000     0.05176    4.6364     0.0000
5           0.18000     0.05176    3.4773     0.0046

Time = 2 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
3           0.06000     0.05176     1.159     0.7747
4           0.22000     0.05176     4.250     0.0002
5           0.16000     0.05176     3.091     0.0171

Time = 3 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
4             0.1600     0.05176     3.091     0.0171
5             0.1000     0.05176     1.932     0.3003

Time = 4 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
5          -0.06000     0.05176    -1.159     0.7747
```

General Linear Model: Leaf symptom assessment severity from CC2 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     42.012     42.012     10.503   37.87  0.000
Error     495    137.300    137.300      0.277
Total     499    179.312

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time_1

Time  = 1 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
2           -0.03000     0.07448   -0.4028     0.9945
3            0.32000     0.07448    4.2964     0.0002
4            0.53000     0.07448    7.1159     0.0000
5            0.71000     0.07448    9.5326     0.0000

Time  = 2 subtracted from:
```

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
3	0.3500	0.07448	4.699	0.0000
4	0.5600	0.07448	7.519	0.0000
5	0.7400	0.07448	9.935	0.0000

Time = 3 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
4	0.2100	0.07448	2.819	0.0387
5	0.3900	0.07448	5.236	0.0000

Time = 4 subtracted from:

Level Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
5	0.1800	0.07448	2.417	0.1108

General Linear Model: Leaf symptom assessment versus time, farms (section 3.3.1.3)

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farms	fixed	4	CC1 L2 L1 CC2

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	42.6480	42.6480	10.6620	46.59	0.000
Farms	3	23.2415	23.2415	7.7472	33.85	0.000
Error	1992	455.8860	455.8860	0.2289		
Total	1999	521.7755				

General Linear Model: Leaf symptom assessment versus time, two places (section 3.3.1.3)

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05
Two Plac	fixed	2	Central coast leppington

Analysis of Variance for Leaf ass, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	42.6480	42.6480	10.6620	44.73	0.000
Two Plac	1	3.7845	3.7845	3.7845	15.88	0.000
Error	1994	475.3430	475.3430	0.2384		
Total	1999	521.7755				

Tukey Simultaneous Tests
Response Variable Leaf ass
All Pairwise Comparisons among Levels of Two Plac

Two Plac = Central Coast subtracted from:

Level Two Plac	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Leppington	-0.08700	0.02184	-3.984	0.0001

e. Brown Mignonette (section 3.3.1.4) (analysis for Table 3-8 and 3-9)

1. Root assessment (section 3.3.1.4 and Table 3-8)

General Linear Model: Root rot severity from L1 farm versus time

Factor	Type	Levels	Values
Time	fixed	5	May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	27.6000	27.6000	6.9000	15.07	0.000
Error	145	66.4000	66.4000	0.4579		

```
Total     149    94.0000

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04      -0.00000      0.1747  -0.00000     1.0000
Nov-04       0.90000      0.1747   5.15096     0.0000
Jan-05       1.00000      0.1747   5.72329     0.0000
Mar-05       0.60000      0.1747   3.43397     0.0069

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.9000      0.1747     5.151     0.0000
Jan-05         1.0000      0.1747     5.723     0.0000
Mar-05         0.6000      0.1747     3.434     0.0069

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.1000      0.1747     0.572     0.9789
Mar-05        -0.3000      0.1747    -1.717     0.4268

Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        -0.4000      0.1747    -2.289     0.1543
```

General Linear Model: Root rot severity from L2 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    112.133    112.133     28.033   65.70  0.000
Error     145     61.867     61.867      0.427
Total     149    174.000

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         0.6333      0.1687     3.755     0.0023
Nov-04         1.7667      0.1687    10.475     0.0000
Jan-05         2.1000      0.1687    12.451     0.0000
Mar-05         2.1667      0.1687    12.847     0.0000

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04          1.133      0.1687     6.720     0.0000
Jan-05          1.467      0.1687     8.696     0.0000
Mar-05          1.533      0.1687     9.092     0.0000

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.3333      0.1687     1.976     0.2828
Mar-05         0.4000      0.1687     2.372     0.1291
```

```
Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05        0.06667      0.1687    0.3953     0.9948
```

General Linear Model: Root rot severity from CC1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for root rot severity, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     69.293     69.293     17.323   58.06  0.000
Error     145     43.267     43.267      0.298
Total     149    112.560

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04          1.000      0.1410     7.090     0.0000
Nov-04          1.433      0.1410    10.162     0.0000
Jan-05          1.633      0.1410    11.581     0.0000
Mar-05          1.967      0.1410    13.944     0.0000

Time = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.4333      0.1410     3.072     0.0211
Jan-05         0.6333      0.1410     4.490     0.0001
Mar-05         0.9667      0.1410     6.854     0.0000

Time = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05         0.2000      0.1410     1.418     0.6171
Mar-05         0.5333      0.1410     3.781     0.0021

Time = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05         0.3333      0.1410     2.363     0.1315
```

General Linear Model: Root rot severity from CC2 farm versus time

```
Factor     Type Levels Values
Time      fixed      2 May-04 Aug-04

Analysis of Variance for Scal_1, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        1     19.267     19.267     19.267   82.98  0.000
Error      58     13.467     13.467      0.232
Total      59     32.733

Tukey Simultaneous Tests
Response Variable root rot severity
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04         -1.133      0.1244    -9.109     0.0000
```

General Linear Model: Root rot severity versus time, farms (section 3.3.1.4)

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farm       fixed      4 CC1 CC2 L1  L2

Analysis of Variance for Scal, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    195.289    168.385     42.096   86.29  0.000
Farm        3      5.795      5.795      1.932    3.96  0.788
Error     502    244.908    244.908      0.488
Total     509    445.992
```

General Linear Model: Root rot severity versus time, places (section 3.3.1.4)

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farm       fixed      2 CC L

Analysis of Variance for Scal, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    195.289    200.417     50.104  102.84  0.000
Farm        1      5.139      5.139      5.139   10.55  0.190
Error     504    245.564    245.564      0.487
Total     509    445.992
```

2. Leaf symptom assessment (section 3.3.1.4 and Table 3-9)

General Linear Model: Leaf symptom assessment from L1 farm versus time

```
Factor     Type Levels Values
Time       fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for leaf assessment, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     6.3080     6.3080     1.5770    8.24  0.000
Error     495    94.7400    94.7400     0.1914
Total     499   101.0480

Tukey Simultaneous Tests
Response Variable leaf assessment
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04        0.01000     0.06187    0.1616     0.9998
Nov-04        0.15000     0.06187    2.4244     0.1088
Jan-05        0.30000     0.06187    4.8489     0.0000
Mar-05        0.18000     0.06187    2.9093     0.0298

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.1400     0.06187     2.263     0.1571
Jan-05         0.2900     0.06187     4.687     0.0000
Mar-05         0.1700     0.06187     2.748     0.0473

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05        0.15000     0.06187    2.4244     0.1088
Mar-05        0.03000     0.06187    0.4849     0.9888

Time_2 = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time_2       of Means  Difference   T-Value    P-Value
Mar-05        -0.1200     0.06187    -1.940     0.2963
```

General Linear Model: Leaf symptom assessment from L2 farm versus time

```
Factor     Type Levels Values
Time    fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for leaf assessment, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4     5.5880     5.5880     1.3970    6.82  0.000
Error     495   101.4600   101.4600     0.2050
Total     499   107.0480

Tukey Simultaneous Tests
Response Variable leaf assessment
All Pairwise Comparisons among Levels of Time

Time   = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04        0.05000     0.06403    0.7809     0.9362
Nov-04        0.18000     0.06403    2.8113     0.0396
Jan-05        0.27000     0.06403    4.2170     0.0002
Mar-05        0.24000     0.06403    3.7484     0.0017

Time   = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.1300     0.06403     2.030     0.2513
Jan-05         0.2200     0.06403     3.436     0.0053
Mar-05         0.1900     0.06403     2.968     0.0250

Time   = Nov-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Jan-05        0.09000     0.06403    1.4057     0.6240
Mar-05        0.06000     0.06403    0.9371     0.8825

Time   = Jan-05 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Mar-05       -0.03000     0.06403   -0.4686     0.9901
```

General Linear Model: Leaf symptom assessment from CC1 farm versus time

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for leaf assessment, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4    10.8920    10.8920     2.7230    8.85  0.000
Error     495   152.3300   152.3300     0.3077
Total     499   163.2220

Tukey Simultaneous Tests
Response Variable leaf assessment
All Pairwise Comparisons among Levels of Time
Time = May-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Aug-04        0.01000     0.07845    0.1275     0.9999
Nov-04        0.29000     0.07845    3.6965     0.0020
Jan-05        0.37000     0.07845    4.7162     0.0000
Mar-05        0.16000     0.07845    2.0395     0.2471

Time = Aug-04 subtracted from:

Level      Difference       SE of             Adjusted
Time         of Means  Difference   T-Value    P-Value
Nov-04         0.2800     0.07845     3.569     0.0033
Jan-05         0.3600     0.07845     4.589     0.0000
```

```
Mar-05         0.1500     0.07845     1.912     0.3108

Time = Nov-04 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value    P-Value
Jan-05          0.0800     0.07845     1.020     0.8464
Mar-05         -0.1300     0.07845    -1.657     0.4608

Time = Jan-05 subtracted from:

Level       Difference       SE of              Adjusted
Time          of Means  Difference   T-Value    P-Value
Mar-05         -0.2100     0.07845    -2.677     0.0574
```

General Linear Model: Leaf symptom assessment from CC2 farm versus time

```
Factor     Type Levels Values
Time      fixed      2 May-04 Aug-04

Analysis of Variance for leaf assessment, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        1     1.2800     1.2800     1.2800    7.86  0.006
Error     198    32.2400    32.2400     0.1628
Total     199    33.5200
```

General Linear Model: Leaf symptom assessment versus farms, time (section 3.3.1.4)

```
Factor     Type Levels Values
Farm      fixed      4 CC1 CC2 L1  L2
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Scale, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Farm        3     0.1858     3.7897     1.2632    5.61  0.001
Time        4    21.0460    21.0460     5.2615   23.36  0.000
Error    1692   381.1470   381.1470     0.2253
Total    1699   402.3788
```

General Linear Model: Leaf symptom assessment versus two places, time (section 3.3.1.4)

```
Factor     Type Levels Values
Farm      fixed      2 CC L
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05

Analysis of Variance for Scale, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Farm        1     0.1743     1.1736     1.1736    5.18  0.183
Time        4    18.4415    18.4415     4.6104   20.35  0.000
Error    1694   383.7631   383.7631     0.2265
Total    1699   402.3788
```

f. Isolation (section 3.3.2)

***Pythium*: General Linear Model: isolates versus time, farm, cultivar**

```
Factor     Type Levels Values
Time      fixed      5 May-04 Aug-04 Nov-04 Jan-05 Mar-05
Farm      fixed      4 CC1 CC2 L1  L2
Cultivar  fixed      4 Baby Cos   Green Oak  Mignonette Red Oak

Analysis of Variance for Data, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Time        4   0.088816   0.085210   0.021303    2.82  0.133
Farm        3   0.076964   0.071608   0.023869    3.16  0.100
Cultivar    3   0.023634   0.023634   0.007878    1.04  0.518
Error     369   2.786902   2.786902   0.007553
Total     379   2.976316
```

Phytophthora: General Linear Model: isolates versus time, farm, cultivar

Factor	Type	Levels	Values
Time	fixed	5	May-04, Aug-04, Nov-04, Jan-05, Mar-05
Farm	fixed	4	CC1, CC2, L1, L2
Cultivar	fixed	4	Baby Cos, Green Oak, Mignonette, Red Oak

Analysis of Variance for Data, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Time	4	22.8150	22.8150	5.7037	35.29	0.000
Farm	3	6.0675	6.0675	2.0225	12.51	0.000
Cultivar	3	0.1275	0.1275	0.0425	0.26	0.852
Error	389	62.8675	62.8675	0.1616		
Total	399	91.8775				

Tukey Simultaneous Tests
Response Variable Data
All Pairwise Comparisons among Levels of Time
Time = May-04 subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Aug-04	-0.4875	0.06356	-7.669	0.0000
Nov-04	0.1625	0.06356	2.556	0.0786
Jan-05	-0.0000	0.06356	-0.000	1.0000
Mar-05	0.1625	0.06356	2.556	0.0786

Time = Aug-04 subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Nov-04	0.6500	0.06356	10.226	0.0000
Jan-05	0.4875	0.06356	7.669	0.0000
Mar-05	0.6500	0.06356	10.226	0.0000

Time = Nov-04 subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Jan-05	-0.1625	0.06356	-2.556	0.0786
Mar-05	0.0000	0.06356	0.000	1.0000

Time = Jan-05 subtracted from:

Time	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Mar-05	0.1625	0.06356	2.556	0.0786

Tukey Simultaneous Tests
Response Variable Data
All Pairwise Comparisons among Levels of Farm
Farm = CC1 subtracted from:

Farm	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
CC2	0.0300	0.05685	0.528	0.9524
L1	0.0100	0.05685	0.176	0.9981
L2	-0.2700	0.05685	-4.749	0.0000

Farm = CC2 subtracted from:

Farm	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
L1	-0.0200	0.05685	-0.352	0.9851
L2	-0.3000	0.05685	-5.277	0.0000

Farm = L1 subtracted from:

Farm	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
L2	-0.2800	0.05685	-4.925	0.0000

g. Pathogenicity tests (section 3.3.3)

General Linear Model: Root assessment versus treatment, time (day)

```
Factor      Type Levels Values
treatmen    fixed      2 Control Pythium
time (day)  fixed      5  3  6  9 12 14

Analysis of Variance for scale, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
treatmen     1    14.0833    14.0833    14.0833   27.34  0.000
time (day)   4    32.9200    32.9200     8.2300   15.98  0.000
Error      294   151.4333   151.4333     0.5151
Total      299   198.4367
```

General Linear Model: Root assessment versus time (day) for *Pythium* (analysis for Table 3-12)

```
Factor      Type Levels Values
Time (day   fixed      5  3  6  9 12 14

Analysis of Variance for scale, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Time (day    4    31.6933    31.6933     7.9233   11.08  0.000
Error      145   103.6667   103.6667     0.7149
Total      149   135.3600

Tukey Simultaneous Tests
Response Variable scale
All Pairwise Comparisons among Levels of time (day)

time (day) = 3 subtracted from:

Level          Difference       SE of              Adjusted
Time (day)        of Means  Difference   T-Value    P-Value
 6                0.2333      0.2183     1.069     0.9482
 9                0.5667      0.2183     2.596     0.0645
12                0.8667      0.2183     3.970     0.0011
14                1.3000      0.2183     5.955     0.0000

Time (day) = 6 subtracted from:

Level          Difference       SE of              Adjusted
Time (day)        of Means  Difference   T-Value    P-Value
 9                0.3333      0.2183     1.527     0.7238
12                0.6333      0.2183     2.901     0.0144
14                1.0667      0.2183     4.886     0.0000

Time (day) = 9 subtracted from:

Level          Difference       SE of              Adjusted
Time (day)        of Means  Difference   T-Value    P-Value
12                0.3000      0.2183     1.374     0.8342
14                0.7333      0.2183     3.359     0.0017

Time (day) = 12 subtracted from:

Level          Difference       SE of              Adjusted
Time (day)        of Means  Difference   T-Value    P-Value
14                0.4333      0.2183     1.985     0.3423
```

General Linear Model: Root assessment versus time (day) for control (analysis for Table 3-13)

```
Factor      Type Levels Values
Time        fixed      5  3  6  9 12 14

Analysis of Variance for Root ass, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Time         4     6.2933     6.2933     1.5733    5.34  0.100
Error      145    42.7000    42.7000     0.2945
Total      149    48.9933
```

```
Tukey Simultaneous Tests
Response Variable Root ass
All Pairwise Comparisons among Levels of Time

Time = 3 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
 6          0.06667      0.1401    0.4758     0.9894
 9          0.26667      0.1401    1.9032     0.9120
12          0.43333      0.1401    3.0927     0.3419
14          0.53333      0.1401    3.8064     0.1019

Time = 6 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
 9           0.2000      0.1401     1.427     0.9811
12           0.3667      0.1401     2.617     0.5872
14           0.4667      0.1401     3.331     0.2396

Time = 9 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
12           0.1667      0.1401     1.189     0.9942
14           0.2667      0.1401     1.903     0.9103

Time = 12 subtracted from:

Level    Difference       SE of             Adjusted
Time       of Means  Difference   T-Value    P-Value
14           0.1000      0.1401    0.7137     0.9951
```

ANOVA: Wet root weight, dry root weight, wet shoot weight, dry shoot weight versus treatment (analysis for Table 3-14)

```
Factor     Type Levels Values
Treatmen  fixed      2 Control Pythium

Analysis of Variance for Wet shoo

Source      DF         SS         MS       F      P
Treatmen     1     587.31     587.31   42.89  0.000
Error       38     520.37      13.69
Total       39    1107.68

Analysis of Variance for Wet root

Source      DF         SS         MS       F      P
Treatmen     1     1.9447     1.9447   10.83  0.002
Error       38     6.8214     0.1795
Total       39     8.7661

Analysis of Variance for Dry shoo

Source      DF         SS         MS       F      P
Treatmen     1     1.0502     1.0502   81.59  0.000
Error       38     0.4891     0.0129
Total       39     1.5393

Analysis of Variance for Dry root

Source      DF         SS         MS       F      P
Treatmen     1   0.034189   0.034189   29.18  0.000
Error       38   0.044527   0.001172
Total       39   0.078716
```

5. Data analysis for Chapter 4: Effects of temperature on root rot disease of hydroponic lettuce

a. Nutrient solution temperature 24-27°C (section 4.3.1)

General Linear Model for weight at 24-27°C: wet root weight, dry root weight, wet shoot weight, dry shoot weight versus treatment (Table 4-2)

Wet root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3     33.928     33.928     11.309   19.62  0.000
Error     116     66.869     66.869      0.576
Total     119    100.797

Tukey Simultaneous Tests
Response Variable Wet root
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht         -0.311      0.1960    -1.586     0.3908
Py and P         -0.819      0.1960    -4.179     0.0003
Pythium          -1.402      0.1960    -7.153     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -0.508      0.1960    -2.593     0.0518
Pythium          -1.091      0.1960    -5.568     0.0000

Treatmen = Py and P subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium         -0.5831      0.1960    -2.974     0.0185
```

Dry root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    0.46470    0.46470    0.15490   39.79  0.000
Error     116    0.45158    0.45158    0.00389
Total     119    0.91628

Tukey Simultaneous Tests
Response Variable Dry root
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht       -0.0316     0.01611     -1.96     0.2088
Py and P       -0.0914     0.01611     -5.68     0.0000
Pythium        -0.1631     0.01611    -10.12     0.0000
```

```
Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P       -0.0599     0.01611    -3.715     0.0018
Pythium        -0.1315     0.01611    -8.162     0.0000

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium        -0.07164    0.01611    -4.447     0.0001
```

Wet shoot weight

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet shoo, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    1637.49    1637.49     545.83   10.67  0.000
Error     116    5933.92    5933.92      51.15
Total     119    7571.41

Tukey Simultaneous Tests
Response Variable Wet shoo
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht          -2.58       1.847    -1.400     0.5022
Py and P          -5.00       1.847    -2.708     0.0385
Pythium          -10.02       1.847    -5.425     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -2.417       1.847    -1.309     0.5592
Pythium          -7.434       1.847    -4.025     0.0006

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium          -5.017       1.847    -2.717     0.0376
```

Dry shoot weight

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry shoo, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    10.0452    10.0452     3.3484   14.00  0.000
Error     116    27.7526    27.7526     0.2392
Total     119    37.7978

Tukey Simultaneous Tests
Response Variable Dry shoo
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht       -0.1278      0.1263    -1.012     0.7427
Py and P       -0.5035      0.1263    -3.987     0.0007
Pythium        -0.7240      0.1263    -5.733     0.0000

Treatmen = Phytopht subtracted from:
```

```
Level          Difference       SE of              Adjusted
Treatmen         of Means  Difference   T-Value     P-Value
Py and P          -0.3757      0.1263    -2.975      0.0185
Pythium           -0.5962      0.1263    -4.721      0.0000

Treatmen = Py and P subtracted from:

Level          Difference       SE of              Adjusted
Treatmen         of Means  Difference   T-Value     P-Value
Pythium           -0.2205      0.1263    -1.746      0.3050
```

General Linear Model: root rot severity at 24-27°C versus treatment, time (day) (Table 4-3)

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora   Py + Phy     Pythium
time       fixed      7  3  6  9 12 15 18 21

Analysis of Variance for root rot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS        F      P
Treatmen    3     30.562     30.562     10.187   17.47  0.000
time        6    184.740    184.740     30.790   52.81  0.000
Error     830    483.955    483.955      0.583
Total     839    699.257

Tukey Simultaneous Tests
Response Variable root rot
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level          Difference       SE of              Adjusted
Treatmen         of Means  Difference   T-Value     P-Value
Phytopht           0.2143     0.07452     2.876      0.0210
Py + Phy           0.2048     0.07452     2.748      0.0306
Pythium            0.5333     0.07452     7.157     -0.0000

Treatmen = Phytopht subtracted from:

Level          Difference       SE of              Adjusted
Treatmen         of Means  Difference   T-Value     P-Value
Py + Phy       -0.009524     0.07452    -0.1278      0.9993
Pythium         0.319048     0.07452     4.2814      0.0001

Treatmen = Py + Phy subtracted from:

Level          Difference       SE of              Adjusted
Treatmen         of Means  Difference   T-Value     P-Value
Pythium            0.3286     0.07452     4.409      0.0001
```

b. Nutrient solution temperature 34°C continuously heated (section 4.3.2)

General Linear Model for weight at 34°C continuously heated: wet root weight, square dry root weight, wet shoot weight, square dry shoot weight versus treatment (Table 4-4)

Wet root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS        F      P
Treatmen    3     48.761     48.761     16.254  192.83  0.000
Error     116      9.778      9.778      0.084
Total     119     58.539

Tukey Simultaneous Tests
Response Variable Wet root
All Pairwise Comparisons among Levels of Treatmen
```

```
Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht         -0.203     0.07496     -2.71     0.0382
Py and P         -1.334     0.07496    -17.80     0.0000
Pythium          -1.401     0.07496    -18.68     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -1.131     0.07496    -15.09     0.0000
Pythium          -1.197     0.07496    -15.97     0.0000

Treatmen = Py and P subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium        -0.06605     0.07496    -0.8811     0.8146
```

Dry root weight

```
Factor     Type   Levels  Values
Treatment  fixed       4  Control, Phytophthora, Py+Phy, Pythium

Analysis of Variance for Dry Root, using Adjusted SS for Tests

Source      DF   Seq SS   Adj SS   Adj MS      F      P
Treatment    3  0.31660  0.31660  0.10553  13.80  0.000
Error      116  0.88728  0.88728  0.00765
Total      119  1.20389

Tukey Simultaneous Tests
Response Variable Dry Root
All Pairwise Comparisons among Levels of Treatment
Treatment = Control  subtracted from:

             Difference       SE of             Adjusted
Treatment      of Means  Difference  T-Value    P-Value
Phytophthora     0.0000     0.02258    0.001     1.0000
Py+Phy          -0.0629     0.02258   -2.784     0.0315
Pythium         -0.1237     0.02258   -5.478     0.0000

Treatment = Phytophthora  subtracted from:

           Difference       SE of             Adjusted
Treatment    of Means  Difference  T-Value    P-Value
Py+Phy        -0.0629     0.02258   -2.785     0.0314
Pythium       -0.1237     0.02258   -5.479     0.0000

Treatment = Py+Phy  subtracted from:

           Difference       SE of             Adjusted
Treatment    of Means  Difference  T-Value    P-Value
Pythium      -0.06083     0.02258   -2.694     0.0400
```

Wet shoot weight

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet shoo, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    2205.89    2205.89     735.30   23.04  0.000
Error     116    3701.63    3701.63      31.91
Total     119    5907.52

Tukey Simultaneous Tests
Response Variable Wet shoo
All Pairwise Comparisons among Levels of Treatmen
```

```
Treatmen = Control subtracted from:
Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht          -0.58       1.459    -0.399     0.9784
Py and P          -6.56       1.459    -4.495     0.0001
Pythium          -10.31       1.459    -7.070     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -5.973       1.459    -4.095     0.0005
Pythium          -9.730       1.459    -6.671     0.0000

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium          -3.757       1.459    -2.576     0.0541
```

Square root of dry shoot weight

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Sqdry sh, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3     6.7572     6.7572     2.2524   62.59  0.000
Error     116     4.1743     4.1743     0.0360
Total     119    10.9315

Tukey Simultaneous Tests
Response Variable Sqdry sh
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        -0.0399     0.04898     -0.82     0.8472
Py and P        -0.2901     0.04898     -5.92     0.0000
Pythium         -0.5941     0.04898    -12.13     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P        -0.2502     0.04898     -5.11     0.0000
Pythium         -0.5542     0.04898    -11.32     0.0000

Treatmen = Py and Phy subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium         -0.3040     0.04898    -6.207     0.0000
```

General Linear Model: root rot severity at 34°C continuously heated versus treatment, time (day) (Table 4-5)

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py + Phy     Pythium
time      fixed      7  3  6  9 12 15 18 21

Analysis of Variance for root ass, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3     27.362     27.362      9.121   14.85  0.000
time        6    275.229    275.229     45.871   74.67  0.000
Error     830    509.905    509.905      0.614
Total     839    812.495
```

```
Tukey Simultaneous Tests
Response Variable root ass
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        0.04762     0.07649    0.6225     0.9249
Py + Phy        0.29048     0.07649    3.7975     0.0008
Pythium         0.44286     0.07649    5.7897    -0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py + Phy         0.2429     0.07649     3.175     0.0082
Pythium          0.3952     0.07649     5.167     0.0000

Treatmen = Py + Phy subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium          0.1524     0.07649     1.992     0.1909
```

c. Nutrient solution temperature 16-17°C (section 4.3.3)

General Linear Model for weight at 16-17°C: wet root weight, dry root weight, wet shoot weight, dry shoot weight versus treatment (Table 4-6)

Wet root weight

```
Factor     Type Levels Values
Treatmen  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    0.75431    0.75431    0.25144    2.93  0.057
Error     116    9.94988    9.94988    0.08577
Total     119   10.70419

Tukey Simultaneous Tests
Response Variable Wet root
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        -0.0356     0.07562    -0.471     0.9653
Py and P        -0.1446     0.07562    -1.912     0.2285
Pythium         -0.1957     0.07562    -2.588     0.0525

Treatmen = Phytopht subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P        -0.1090     0.07562    -1.441     0.4764
Pythium         -0.1600     0.07562    -2.116     0.1540

Treatmen = Py and P subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium         -0.05106     0.07562   -0.6752     0.9063
```

Dry root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3   0.009710   0.009710   0.003237    2.39  0.073
Error     116   0.157376   0.157376   0.001357
Total     119   0.167086
Tukey Simultaneous Tests
Response Variable Dry root
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht       -0.00620    0.009510    -0.652     0.9148
Py and P       -0.01981    0.009510    -2.083     0.1649
Pythium        -0.02123    0.009510    -2.232     0.1207

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P       -0.01361    0.009510    -1.431     0.4827
Pythium        -0.01503    0.009510    -1.581     0.3936

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium       -0.001423    0.009510   -0.1497     0.9988
```

Wet shoot weight

```
Factor     Type Levels Values
Treatment  fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet shoot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatment   3     87.619     87.619     29.206    3.07  0.061
Error     116   1103.211   1103.211      9.510
Total     119   1190.830

Tukey Simultaneous Tests
Response Variable Wet shoo
All Pairwise Comparisons among Levels of Treatment

Treatment = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht         -0.072      0.7963    -0.091     0.9997
Py and P         -1.576      0.7963    -1.979     0.2018
Pythium          -1.885      0.7963    -2.367     0.0893

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -1.504      0.7963    -1.888     0.2386
Pythium          -1.812      0.7963    -2.276     0.1096

Treatment = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatment      of Means  Difference   T-Value    P-Value
Pythium         -0.3087      0.7963   -0.3877     0.9801
```

Dry shoot weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry shoot, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen     3    0.35976    0.35976    0.11992    2.74  0.057
Error      116    5.08272    5.08272    0.04382
Total      119    5.44248

Unusual Observations for Dry shoot

Obs  Dry shoot      Fit      SE Fit  Residual   St Resid
  1    1.51210   0.74395     0.03822   0.76815      3.73R
 30    0.29810   0.74395     0.03822  -0.44585     -2.17R
 82    1.19680   0.75131     0.03822   0.44549      2.16R
 93    1.76610   0.86028     0.03822   0.90582      4.40R

Tukey Simultaneous Tests
Response Variable Dry shoo
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        -0.0067     0.05405    -0.125     0.9993
Py and P        -0.1090     0.05405    -2.016     0.1880
Pythium         -0.1163     0.05405    -2.153     0.1429

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P        -0.1022     0.05405    -1.891     0.2374
Pythium         -0.1096     0.05405    -2.028     0.1839

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium       -0.007363     0.05405   -0.1362     0.9991
```

General Linear Model: root rot severity at 16-17°C treatment, time (day) (Table 4-7)

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora    Py + Phy     Pythium
time       fixed      7  3  6  9 12 15 18 21

Analysis of Variance for Scale, using Adjusted SS for Tests

Source      DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen     3      4.127      4.127      1.376    3.18  0.023
time         6    118.750    118.750     19.792   45.75  0.000
Error      830    359.064    359.064      0.433
Total      839    481.942

Tukey Simultaneous Tests
Response Variable Scale
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        0.01429     0.06419     0.2226     0.9961
Py + Phy        0.11905     0.06419     1.8547     0.2479
Pythium         0.16667     0.06419     2.5965     0.0465

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py + Phy          0.1048     0.06419      1.632     0.3605
```

```
Pythium          0.1524     0.06419     2.374     0.0821

Treatmen = Py + Phy subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value     P-Value
Pythium         0.04762     0.06419    0.7419      0.8801
```

d. Nutrient solution temperature 34°C for 10 h followed by 18-20°C

General Linear Model for weight at 34°C for 10h followed by 18-20°C: square wet root weight, dry root weight, wet shoot weight, dry shoot weight versus treatment (Table 4-8)

Square Wet root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for SWet root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3     4.3571     4.3571     1.4524   86.70  0.000
Error     116     1.9432     1.9432     0.0168
Total     119     6.3004

Tukey Simultaneous Tests
Response Variable SWet root
All Pairwise Comparisons among Levels of Treatmen
Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value     P-Value
Phytopht        -0.0867     0.03342     -2.59      0.0517
Py and P        -0.3960     0.03342    -11.85      0.0000
Pythium         -0.4403     0.03342    -13.18      0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value     P-Value
Py and P        -0.3093     0.03342     -9.26      0.0000
Pythium         -0.3537     0.03342    -10.58      0.0000

Treatmen = Py and P subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value     P-Value
Pythium        -0.04433     0.03342    -1.327      0.5480
```

Dry root weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry root, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    0.65919    0.65919    0.21973   23.83  0.000
Error     116    1.06960    1.06960    0.00922
Total     119    1.72878

Tukey Simultaneous Tests
Response Variable Dry root
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of              Adjusted
Treatmen       of Means  Difference   T-Value     P-Value
Phytopht        -0.0796     0.02479    -3.209      0.0092
Py and P        -0.1625     0.02479    -6.555      0.0000
Pythium         -0.1888     0.02479    -7.615      0.0000
```

```
Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P        -0.0830     0.02479    -3.346     0.0060
Pythium         -0.1092     0.02479    -4.405     0.0001

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium         -0.02626    0.02479    -1.059     0.7149
```

Wet shoot weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Wet shoot, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3     819.29     819.29     273.10   10.57  0.000
Error     116    2998.47    2998.47      25.85
Total     119    3817.76

Tukey Simultaneous Tests
Response Variable Wet shoo
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht         -0.418       1.313    -0.318     0.9888
Py and P         -3.702       1.313    -2.820     0.0285
Pythium          -6.418       1.313    -4.889     0.0000

Treatmen = Phytopht subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Py and P         -3.284       1.313    -2.501     0.0650
Pythium          -6.000       1.313    -4.571     0.0001

Treatmen = Py and P subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Pythium          -2.717       1.313    -2.069     0.1693
```

Dry shoot weight

```
Factor     Type Levels Values
Treatmen   fixed      4 Control      Phytophthora Py and Phy   Pythium

Analysis of Variance for Dry shoo, using Adjusted SS for Tests

Source     DF     Seq SS     Adj SS     Adj MS       F      P
Treatmen    3    10.4279    10.4279     3.4760   22.39  0.000
Error     116    18.0051    18.0051     0.1552
Total     119    28.4329

Tukey Simultaneous Tests
Response Variable Dry shoo
All Pairwise Comparisons among Levels of Treatmen

Treatmen = Control subtracted from:

Level        Difference       SE of             Adjusted
Treatmen       of Means  Difference   T-Value    P-Value
Phytopht        -0.0745      0.1017    -0.732     0.8840
Py and P        -0.3807      0.1017    -3.742     0.0016
Pythium         -0.7475      0.1017    -7.348     0.0000

Treatmen = Phytopht subtracted from:
```

Level Treatmen	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Py and P	-0.3062	0.1017	-3.010	0.0167
Pythium	-0.6730	0.1017	-6.616	0.0000

Treatmen = Py and P subtracted from:

Level Treatmen	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Pythium	-0.3668	0.1017	-3.606	0.0026

General Linear Model: root rot severity at 34°C for 10h followed by 18-20°C treatment, time (day) (Table 4-9)

Factor	Type	Levels	Values
Treatment	fixed	4	Control, Phytophthora, Py + Phy, Pythium
Time	fixed	7	3, 6, 9, 12, 15, 18, 21

Analysis of Variance for Scsle, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Treatment	3	63.681	63.681	21.227	42.93	0.000
Time	6	151.764	151.764	25.294	51.15	0.000
Error	830	410.436	410.436	0.495		
Total	839	625.881				

Tukey Simultaneous Tests
Response Variable Scsle
All Pairwise Comparisons among Levels of Treatment
Treatment = Control subtracted from:

Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Phytophthora	0.2381	0.06863	3.469	0.0029
Py + Phy	0.5857	0.06863	8.535	0.0000
Pythium	0.6905	0.06863	10.061	0.0000

Treatment = Phytophthora subtracted from:

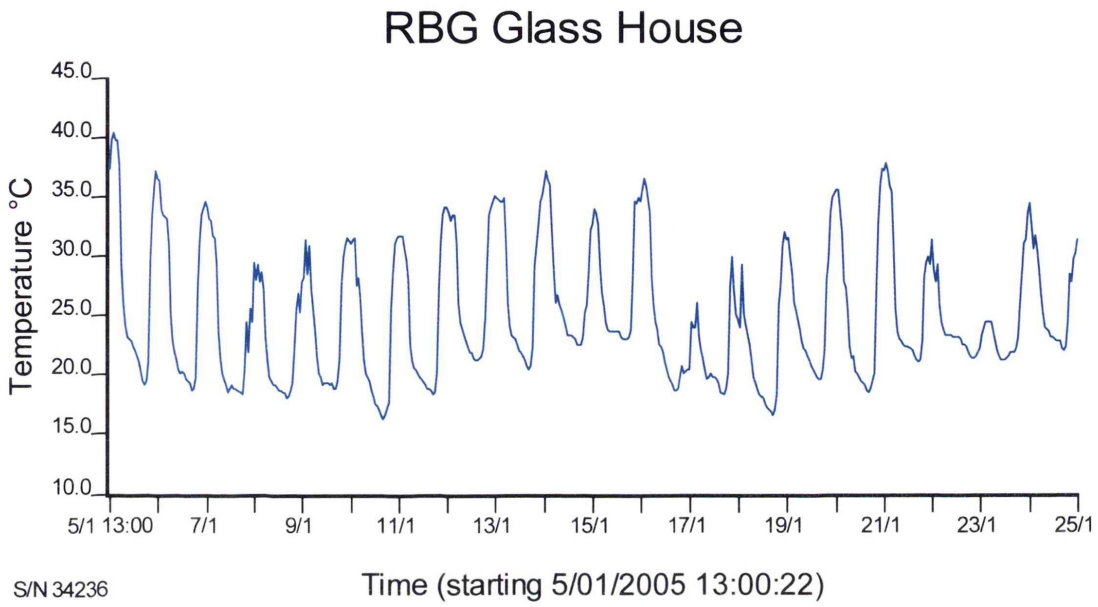
Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Py + Phy	0.3476	0.06863	5.065	0.0000
Pythium	0.4524	0.06863	6.592	0.0000

Treatment = Py + Phy subtracted from:

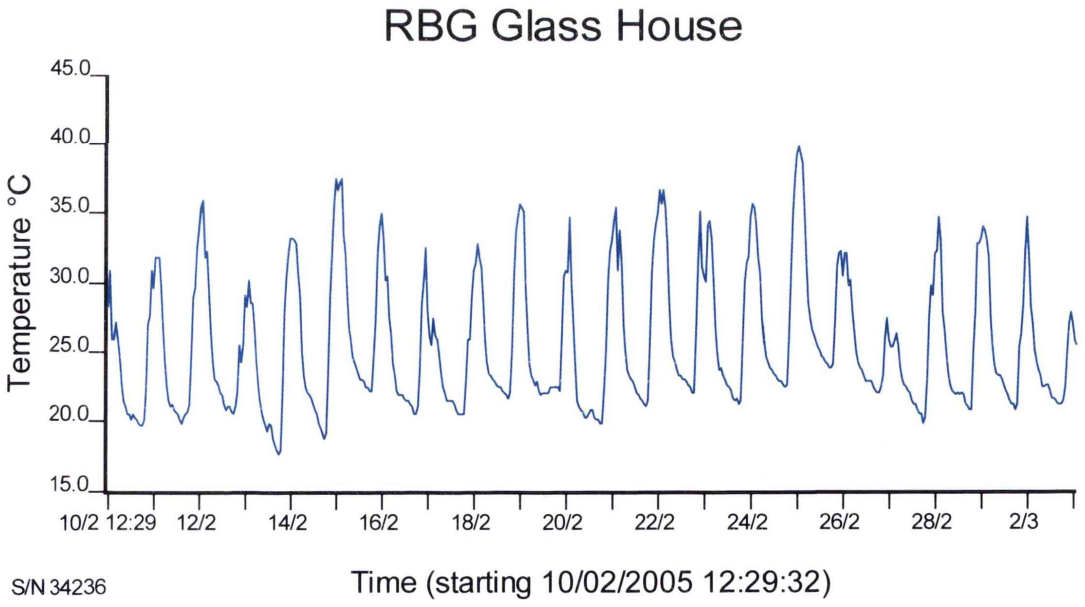
Treatment	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
Pythium	0.1048	0.06863	1.527	0.4215

e. Typical air temperatures in the glasshouse (measured using Tiny Tags) during experiments at each of four temperature regimes.

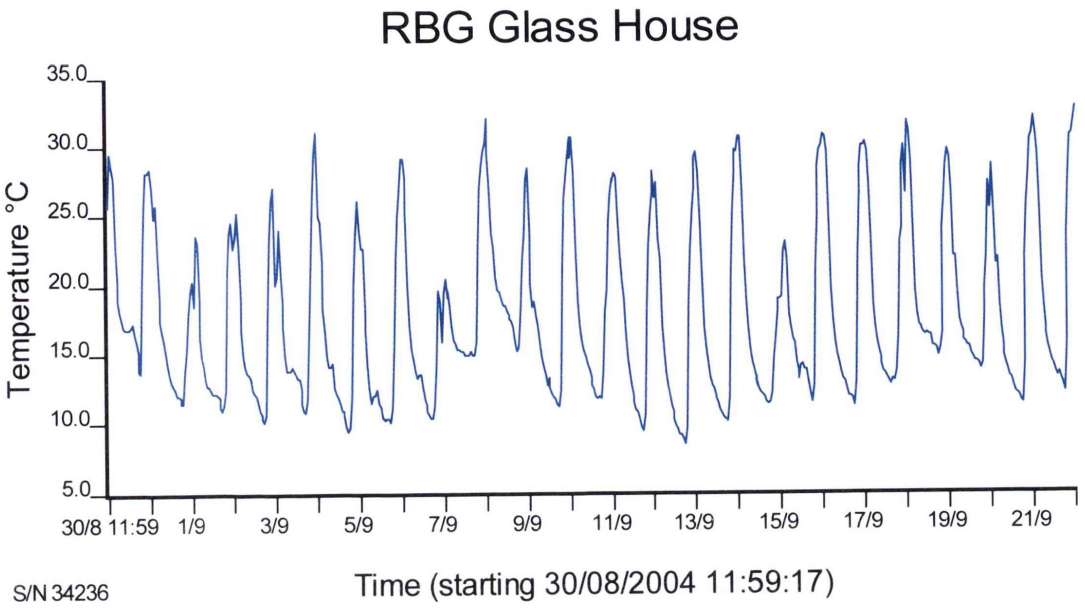
1. Experiment at nutrient solution temperature 24-27°C measured using thermometer



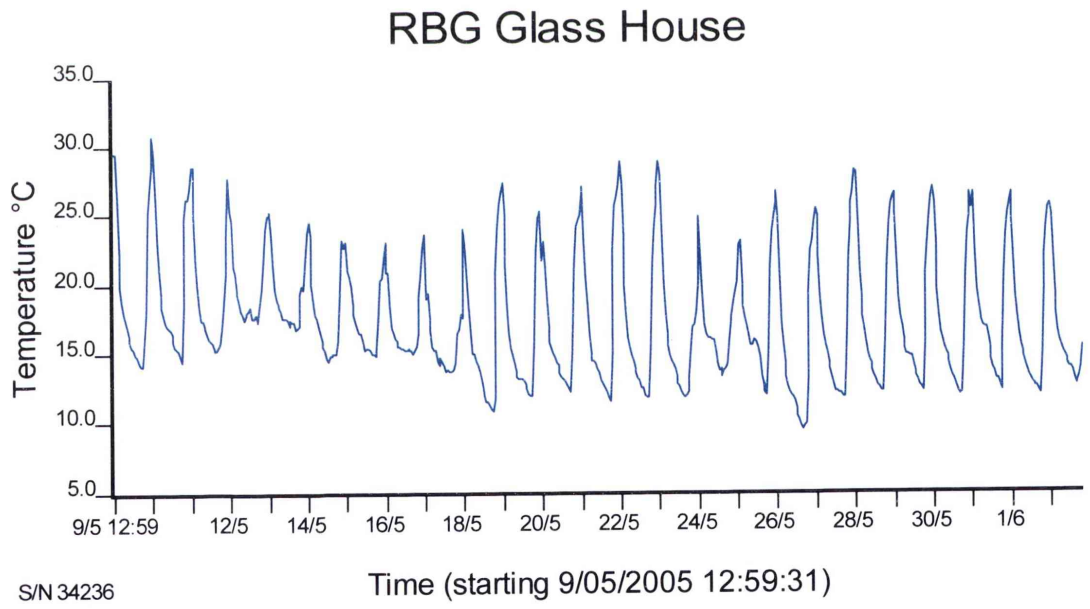
2. Experiment at nutrient solution temperature 34°C continuously heated (heater in nutrient solution determined temperature) measured using thermometer



3. Experiment at nutrient solution temperature 16-17°C measured using thermometer



4. Experiment at nutrient solution temperature 34°C for 10 hours followed by 18-20°C measured using thermometer



6. Solution for DNA extraction and molecular biology reagents

1. Binding matrix

This solution is composed of Glass milk in 6M Guanidine thiocyanate (GT, MW 118.16; 187.2 g) and 66 mL non-diluted binding matrix put into a sterilised empty 500 mL bottle. Then 264 mL water was added.

2. Extraction buffer

2.42 g Tris HCl was placed in 100 mL distilled water with pH 8.0. Then the following were added - 8.0 mL of 0.5M EDTA, 5.8 g NaCl, and 2.0 g of SDS. The solution was adjusted to 200 mL, after which it was autoclaved. This solution may sometimes develop a precipitate with storage. If so, place in a water bath at 60°C for a few minutes before use to resuspend.

3. NE Buffer 2

10 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 1 mM dithiothreitol (pH 7.9 at 25°C).

4. Protein precipitation solution (PPS)

PPS is comprised of 29.44 g K-acetate (MW 98.14) mixed into 90 mL distilled water while stirring. If it did not dissolve in 10 min, then 5-10 mL of distilled water was added. The pH was adjusted to 5.5 with glacial acetic acid. The volume was made up to 100 mL, then autoclaved at 121°C for 20 min.

5. Salt ethanol wash solution (SEWS)

SEWS was prepared by weighing 3.896 g Na-acetate (CH_3COONa) which was dissolved in 100 mL distilled water, then autoclaved at 121°C for 20 min. After the solution cooled down, 375 mL of 95% ethanol was added. The final concentration of CH_3COONa in ethanol is 100 mM.

6. Tris-EDTA buffer (TE)

1 mL of 1M Tris-HCl and 200 µl 0.5M EDTA were made up to 100 mL with 98.8 mL of water and stored in the freezer in Eppendorf tubes at –20°C.

7. 5x Tris-Borate-EDTA buffer (TBE)

54 g Tris base and 27.5 g Boric acid were added to 20 mL of 0.5 M EDTA (pH 8.0) or 3.71 g EDTA, then dissolved in 800 mL distilled water and mixed well.

7. 1x TBE

100 mL of 5x TBE was diluted with 400 mL distilled water and mixed well.

8. Loading dye

Loading dye is contained 0.1 mg EDTA, 0.1 mg Xylene cyanol FF and 0.1 mg Bromophenol Blue. Then 50 mL glycerol was added and adjusted to 100 mL with distilled water.

7. Data analysis for Chapter 5: Characterisation and identification of *Pythium* spp. isolated from lettuce roots by morphological and molecular techniques.

a. *Pythium* growth at different temperatures (section 5.3.4.1 and Table 5-5)

One-way ANOVA: 5°C versus isolate

```
Analysis of Variance for 5
Source     DF        SS        MS        F        P
Isolate    14    0.5007    0.0358     2.35    0.012
Error      60    0.9138    0.0152
Total      74    1.4145

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  -------+---------+---------+---------
 1          5    1.0900    0.0843          (------*------) (PMr05CC2RO)
 2          5    0.9600    0.0548  (------*------) (PMr05CC1Mig)
 3          5    1.0200    0.0447      (------*------) (PMr05L2RO)
 4          5    1.0800    0.0837          (------*-----) (PJ05CC1RO)
 5          5    1.1320    0.1205             (------*------) (PA04CC2Mig)
 6          5    1.1200    0.1095           (------*------) (PN04L2GO)
 7          5    1.3000    0.0752                       (------*------) (PJ05L2RO)
 8          5    1.0900    0.0822          (------*------) (PA04L2BC)
 9          5    1.1860    0.1698                (------*------) (PM04CC2BC)
10          5    1.2400    0.2191                    (-----*------) (PJ05L2BC)
11          5    1.1200    0.1095           (------*------) (PN04CC1RO)
12          5    1.0720    0.0657         (------*------) (PMr05L1BC)
13          5    1.1860    0.1698                (------*------) (PA03L1)
14          5    1.1320    0.1807             (------*------) (PN04CC1Mig)
15          5    1.1820    0.1256                (------*------) (PJ05L2Mig)
                                    -------+---------+---------+---------
Pooled StDev =    0.1234                 0.96      1.12      1.28


Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.000791

Critical value = 5.00

Intervals for (column level mean) - (row level mean)

                1           2           3           4           5           6

       2     -0.1459
              0.4059

       3     -0.2059     -0.3359
              0.3459      0.2159

       4     -0.2659     -0.3959     -0.3359
              0.2859      0.1559      0.2159

       5     -0.3179     -0.4479     -0.3879     -0.3279
              0.2339      0.1039      0.1639      0.2239

       6     -0.3059     -0.4359     -0.3759     -0.3159     -0.2639
              0.2459      0.1159      0.1759      0.2359      0.2879

       7     -0.4859     -0.6159     -0.5559     -0.4959     -0.4439     -0.4559
              0.0659     -0.0641     -0.0041      0.0559      0.1079      0.0959

       8     -0.2759     -0.4059     -0.3459     -0.2859     -0.2339     -0.2459
              0.2759      0.1459      0.2059      0.2659      0.3179      0.3059

       9     -0.3719     -0.5019     -0.4419     -0.3819     -0.3299     -0.3419
              0.1799      0.0499      0.1099      0.1699      0.2219      0.2099

      10     -0.4259     -0.5559     -0.4959     -0.4359     -0.3839     -0.3959
```

	0.1259	-0.0041	0.0559	0.1159	0.1679	0.1559
11	-0.3059	-0.4359	-0.3759	-0.3159	-0.2639	-0.2759
	0.2459	0.1159	0.1759	0.2359	0.2879	0.2759
12	-0.2579	-0.3879	-0.3279	-0.2679	-0.2159	-0.2279
	0.2939	0.1639	0.2239	0.2839	0.3359	0.3239
13	-0.3719	-0.5019	-0.4419	-0.3819	-0.3299	-0.3419
	0.1799	0.0499	0.1099	0.1699	0.2219	0.2099
14	-0.3179	-0.4479	-0.3879	-0.3279	-0.2759	-0.2879
	0.2339	0.1039	0.1639	0.2239	0.2759	0.2639
15	-0.3679	-0.4979	-0.4379	-0.3779	-0.3259	-0.3379
	0.1839	0.0539	0.1139	0.1739	0.2259	0.2139

	7	8	9	10	11	12
8	-0.0659					
	0.4859					
9	-0.1619	-0.3719				
	0.3899	0.1799				
10	-0.2159	-0.4259	-0.3299			
	0.3359	0.1259	0.2219			
11	-0.0959	-0.3059	-0.2099	-0.1559		
	0.4559	0.2459	0.3419	0.3959		
12	-0.0479	-0.2579	-0.1619	-0.1079	-0.2279	
	0.5039	0.2939	0.3899	0.4439	0.3239	
13	-0.1619	-0.3719	-0.2759	-0.2219	-0.3419	-0.3899
	0.3899	0.1799	0.2759	0.3299	0.2099	0.1619
14	-0.1079	-0.3179	-0.2219	-0.1679	-0.2879	-0.3359
	0.4439	0.2339	0.3299	0.3839	0.2639	0.2159
15	-0.1579	-0.3679	-0.2719	-0.2179	-0.3379	-0.3859
	0.3939	0.1839	0.2799	0.3339	0.2139	0.1659

	13	14
14	-0.2219	
	0.3299	
15	-0.2719	-0.3259
	0.2799	0.2259

One-way ANOVA: 10°C versus isolate

Analysis of Variance for 10

Source	DF	SS	MS	F	P
Isolate	14	0.775	0.055	0.27	0.995
Error	60	12.380	0.206		
Total	74	13.155			

One-way ANOVA: 15°C versus isolate

Analysis of Variance for 15

Source	DF	SS	MS	F	P
Isolate	14	0.895	0.064	0.49	0.931
Error	60	7.869	0.131		
Total	74	8.764			

One-way ANOVA: 20°C versus isolate

Analysis of Variance for 20

Source	DF	SS	MS	F	P
Isolate	14	16.197	1.157	1.89	0.046
Error	60	36.711	0.612		
Total	74	52.908			

One-way ANOVA: 25°C versus isolate

```
Analysis of Variance for 25
Source     DF        SS        MS        F        P
Isolate    14     2.653     0.190     0.91    0.549
Error      60    12.446     0.207
Total      74    15.099
```

One-way ANOVA: 30°C versus isolate

```
Analysis of Variance for 30
Source     DF        SS        MS        F        P
Isolate    14    0.9892    0.0707     0.75    0.719
Error      60    5.6713    0.0945
Total      74    6.6605
```

One-way ANOVA: 35°C versus isolate

```
Analysis of Variance for 35
Source     DF        SS        MS        F        P
Isolate    14     2.808     0.201     1.14    0.341
Error      60    10.521     0.175
Total      74    13.330
```

One-way ANOVA: 40°C versus isolate

```
Analysis of Variance for 40
Source     DF        SS        MS        F        P
Isolate    14    0.0809    0.0058     0.56    0.886
Error      60    0.6200    0.0103
Total      74    0.7009
```

b. *Pythium* sequences (section 5.3.5.2 and Table 5-6)

> PMr05L2RO
TTCCCGTAGGTGAACCCTGCGGAATGATCATTTCCACATCAAAAAAACTCTCCACGTGAACC
GATGTAACTATCTTCTGTGCTCTCTTCTCGGAGAGAGCTGAACGAAGGTGGGCTGCTTAATT
GTAGTCTGCCGATGTACTTTTAAACCCATTAAACTAATACTGAACTATACTCCGAAAACGAA
AGTCTTTGGTTTTAATCAATAACAACTTTCAGCAGTGGATGTCTAGGCTCGCACATCGATGA
AGAACGCTGCGAACTGCGATACGTAATGCGAATTGCAGAATTCAGTGAGTCATCGAAATTTT
GAACGCACATTGCACTTTCGGGTTATGCCTGGAAGTATGCCTGTATCAGTGTCCGTACATCA
AACTTGCCTTTCTTTTTTTGTGTAGTCAAGAAGAGAGATGGCAGACTGTGAGGTGTCTCGCTG
ACTCCCTCTTCGGAGGAGAAGACGCGAGTCCCTTTAAATGTACGTTCGCTCTTTCTTGTGTTT
AAGATGAAGTGTGACTTTCGAACGCAGTGATCTGTTTGGATCGCTTTGCTCGAGTGGGCGAC
TTCGGTTAGGACATTAAAGGAAGCAACCTCTATTGGCGGTATGTTAGGCTTCGGCCCGACTT
TGCAGCTGACTGGAGTTGTTTTCTGTTCTTTCCTTGAGGTGTACCTGTCTTGTGTGAGGCAAT
GGTCTGGGCAAATGGTTATTGTGTAGTAGGAAGTTGCTGCTCTTAAGCGCTCTAGCTTCGGTT
AGAGTAAAGGAGGCAACACCAATTTGGGATAGTCGTTGATTTATCAATGGCGCTCTTTCTAA
TTGGACCTGATATCAGGCAAGAC

>PA03L1
CCGAAATGGGAGTCTGCCTGATATCAGGTCCAATTAGAAAGAGCGCCATTGATAAATCAAC
GACTATCCCAAATTCGGTGAAGCCTCCTTTACTCTAACCGAAGCTAGAGCGTTTAACAGCAG
CAACTTCCTACTACACAATAACCATTTGCCCAGACCATTGCCTCACACAAGACAGGTACACC
TCAAGGAAAGAACAGAAAACAACTCCAGTCAGCTGCAAAGTCGGGCCGAAGCCTAACATAC
CGCCAATAGAGGTTGCTTCCTTTAATGTCCTAACCGAAGTCGCCCACTCGAGCAAAGCGATC
CAAACAGATCACTGCGTTCGAAAGTCACACTTCATCTTAAACACAAGAAAGAGCGAACGTA
CATTTAAAGGGACTCGCGTCTTCTCCTCCGAAGAGGGAGTCAGCGAGACACCTCACAGTCTG
CCATCTCTCTTCTTGACTACACAAAAAAAGAAAGGCAAGTTTGATGTACGGACACTGATACA
GGCATACTTCCAGGCATAACCCGAAAGTGCAATGTGCGTTCAAAATTTCGATGACTCACTGA
ATTCTGCAATTCGCATTACGTATCGCAGTTCGCAGCGTTCTTCATCGATGTGCGAGCCTAGAC
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> PMr05CC2RO
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> PJ05L2BC
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> PA04L2BC
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> PJ05L2RO
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> PN04L2GO
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CACTGTCAGCTGCAAAGTCGGGCCGAAGCCTAACATACCGCCAATAGAGGTTGCTTCCTTTA
ATGTCCTAACCGAAGTCGCCCACTCGAGCAAAGCGATCCAAATATATCGGTGCGAC

c. Representative isolates of the 81 isolates of *Pythium* obtained in the present study strored in the collection of the Agricultural Institute at NSW Agriculture Department (Orange, NSW, Australia) (DAR number).

Isolate	Code used in the present study	DAR number
Pythium Group F	PJ04L1	77477
Pythium Group F	PNov04CC1GO	77478
Pythium Group F	PJ05CC1RO	77479
Pythium Group F	PMar05L2Mig	77480
Pythium coloratum	PMar05CC2RO	77481

8. Data analysis for Chapter 6: Characterisation and identification of *Phytophthora* spp. isolated from lettuce roots by morphological and molecular techniques.

a. *Phytophthora* sequences (section 6.3.2.2 and Table 6-4)

> PhN03L1
CTCGTCTGGCCGGCCGGTTTTCGGCTGGCTGGGTGGCGGCTCTATCATGGCGACCGCTTGGG
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AGGCTTGGCGTTTGAACCGGCGGTGTGGTGCCAAATAAGGTGTCTGTTCCGGCGTAAGCTGG
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ACCACTGAATTA

> PhA04CC2Mi
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CCTTCATTCGAAATATCTGGGGACAA

b. Morphological characteristics (section 6.3.3.2)

One-way ANOVA: Sporangia Length versus Isolate (section 6.3.3.2 and Table 6-9)

```
Analysis of Variance for Length
Source     DF        SS        MS        F        P
Isolate    14     15823      1130     5.91    0.000
Error     705    134764       191
Total     719    150587

                                    Individual 95% CIs For Mean
                                     Based on Pooled StDev
Level       N      Mean     StDev  ----+---------+---------+---------+--
 1         48     41.75     14.41                    (------*-----) (PhMr05L1B)
 2         48     32.04     13.67   (-----*------) (PhMr05L2BC)
 3         48     32.87     13.23    (------*-----) (PhN03L1)
 4         48     39.00     14.27              (------*------) (PhJ05L2BC)
 5         48     42.60     14.20                    (------*------) (PhM04L1Mig)
 6         48     32.79     11.18    (------*-----) (PhN04L1GO)
 7         48     45.23     14.41                         (-----*------) (PhA04CC2Mig)
 8         48     35.50     11.47         (-----*------) (PhN04CC1Mig)
 9         48     34.06     12.50      (------*-----) (PhN04L11BC)
10         48     45.00     15.09                       (------*------) (PhJ05CC1GO)
11         48     39.29     13.82               (-----*------) (PhM04CC1RO)
12         48     44.25     15.60                      (------*-----) (PhM04CC2Mig)
13         48     44.94     14.98                       (------*-----) (PhA04CC2BC)
14         48     42.52     13.43                     (------*-----) (PhM04L1RO)
15         48     38.81     14.33              (------*-----) (PhM04L2BC)
                                   ----+---------+---------+---------+--
Pooled StDev =    13.83             30.0      36.0      42.0      48.0


Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.000727

Critical value = 4.80

Intervals for (column level mean) - (row level mean)

                1           2           3           4           5           6

       2        0.13
               19.29

       3       -0.70      -10.41
               18.45        8.75

       4       -6.83      -16.54      -15.70
               12.33        2.62        3.45
```

5	-10.43	-20.14	-19.31	-13.18		
	8.72	-0.98	-0.15	5.97		
6	-0.62	-10.33	-9.50	-3.37	0.23	
	18.54	8.83	9.66	15.79	19.39	
7	-13.06	-22.77	-21.93	-15.81	-12.20	-22.02
	6.10	-3.61	-2.78	3.35	6.95	-2.86
8	-3.33	-13.04	-12.20	-6.08	-2.47	-12.29
	15.83	6.12	6.95	13.08	16.68	6.87
9	-1.89	-11.60	-10.77	-4.64	-1.04	-10.85
	17.27	7.56	8.39	14.52	18.12	8.31
10	-12.83	-22.54	-21.70	-15.58	-11.97	-21.79
	6.33	-3.38	-2.55	3.58	7.18	-2.63
11	-7.12	-16.83	-16.00	-9.87	-6.27	-16.08
	12.04	2.33	3.16	9.29	12.89	3.08
12	-12.08	-21.79	-20.95	-14.83	-11.22	-21.04
	7.08	-2.63	-1.80	4.33	7.93	-1.88
13	-12.77	-22.47	-21.64	-15.52	-11.91	-21.72
	6.39	-3.32	-2.48	3.64	7.25	-2.57
14	-10.35	-20.06	-19.22	-13.10	-9.50	-19.31
	8.81	-0.90	-0.07	6.06	9.66	-0.15
15	-6.64	-16.35	-15.52	-9.39	-5.79	-15.60
	12.52	2.81	3.64	9.77	13.37	3.56

	7	8	9	10	11	12
8	0.15					
	19.31					
9	1.59	-8.14				
	20.75	11.02				
10	-9.35	-19.08	-20.52			
	9.81	0.08	-1.36			
11	-3.64	-13.37	-14.81	-3.87		
	15.52	5.79	4.35	15.29		
12	-8.60	-18.33	-19.77	-8.83	-14.54	
	10.56	0.83	-0.61	10.33	4.62	
13	-9.29	-19.02	-20.45	-9.52	-15.22	-10.27
	9.87	0.14	-1.30	9.64	3.93	8.89
14	-6.87	-16.60	-18.04	-7.10	-12.81	-7.85
	12.29	2.56	1.12	12.06	6.35	11.31
15	-3.16	-12.89	-14.33	-3.39	-9.10	-4.14
	16.00	6.27	4.83	15.77	10.06	15.02

	13	14
14	-7.16	
	12.00	
15	-3.45	-5.87
	15.70	13.29

One-way ANOVA: Sporangia breadth versus Isolate (section 6.3.3.2 and Table 6-9)

```
Analysis of Variance for Width
Source     DF        SS        MS        F        P
Isolate    14    6913.7     493.8    10.66    0.000
Error     705   32661.4      46.3
Total     719   39575.1

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev --------+---------+---------+---------
 1         48    29.854     7.817                            (---*---) (PhMr05L1BC)
 2         48    20.271     7.287       (---*--) (PhMr05L2BC)
 3         48    19.604     6.407     (---*---) (PhN03L1)
 4         48    20.167     5.498      (---*---) (PhJ05L2BC)
 5         48    24.854     7.560                (--*---) (PhM04L1Mig)
 6         48    20.688     7.072        (--*---) (PhN04L1GO)
 7         48    19.292     7.322     (---*--) (PhA04CC2Mig)
 8         48    19.333     6.544     (---*---) (PhN04CC1Mig)
 9         48    18.375     5.949   (---*---) (PhN04L11BC)
10         48    19.146     6.608    (---*---) (PhJ05CC1GO)
11         48    24.625     6.850                (---*---) (PhM04CC1RO)
12         48    19.292     6.500     (---*--) (PhM04CC2Mig)
13         48    19.167     6.583    (---*---) (PhA04CC2BC)
14         48    24.479     7.469                (---*---) (PhM04L1RO)
15         48    19.958     6.206      (---*---) (PhM04L2BC)
                                  --------+---------+---------+---------
Pooled StDev =    6.806               20.0      25.0      30.0

Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.000727

Critical value = 4.80

Intervals for (column level mean) - (row level mean)

                1           2           3           4           5           6

      2       4.868
             14.299

      3       5.534      -4.049
             14.966       5.382

      4       4.972      -4.612      -5.278
             14.403       4.820       4.153

      5       0.284      -9.299      -9.966      -9.403
              9.716       0.132      -0.534       0.028

      6       4.451      -5.132      -5.799      -5.237      -0.549
             13.882       4.299       3.632       4.195       8.882

      7       5.847      -3.737      -4.403      -3.841       0.847      -3.320
             15.278       5.695       5.028       5.591      10.278       6.112

      8       5.805      -3.778      -4.445      -3.882       0.805      -3.362
             15.237       5.653       4.987       5.549      10.237       6.070

      9       6.763      -2.820      -3.487      -2.924       1.763      -2.403
             16.195       6.612       5.945       6.507      11.195       7.028

     10       5.993      -3.591      -4.257      -3.695       0.993      -3.174
             15.424       5.841       5.174       5.737      10.424       6.257
     11       0.513      -9.070      -9.737      -9.174      -4.487      -8.653
              9.945       0.362      -0.305       0.257       4.945       0.778

     12       5.847      -3.737      -4.403      -3.841       0.847      -3.320
             15.278       5.695       5.028       5.591      10.278       6.112

     13       5.972      -3.612      -4.278      -3.716       0.972      -3.195
             15.403       5.820       5.153       5.716      10.403       6.237

     14       0.659      -8.924      -9.591      -9.028      -4.341      -8.507
```

```
          10.091       0.507      -0.159       0.403       5.091       0.924

15         5.180      -4.403      -5.070      -4.507       0.180      -3.987
          14.612       5.028       4.362       4.924       9.612       5.445


             7           8           9          10          11          12

 8      -4.757
         4.674

 9      -3.799      -3.757
         5.632       5.674

10      -4.570      -4.528      -5.487
         4.862       4.903       3.945

11     -10.049     -10.007     -10.966     -10.195
        -0.618      -0.576      -1.534      -0.763

12      -4.716      -4.674      -5.632      -4.862       0.618
         4.716       4.757       3.799       4.570      10.049

13      -4.591      -4.549      -5.507      -4.737       0.743      -4.591
         4.841       4.882       3.924       4.695      10.174       4.841

14      -9.903      -9.862     -10.820     -10.049      -4.570      -9.903
        -0.472      -0.430      -1.388      -0.618       4.862      -0.472

15      -5.382      -5.341      -6.299      -5.528      -0.049      -5.382
         4.049       4.091       3.132       3.903       9.382       4.049


            13          14

14     -10.028
        -0.597

15      -5.507      -0.195
         3.924       9.237
```

One-way ANOVA: Sporangia l:b versus Isolate (section 6.3.3.2 and Table 6-9)

```
Analysis of Variance for l:b
Source     DF        SS        MS        F        P
Isolate    14     68.53      4.90     4.07    0.000
Error     705    848.56      1.20
Total     719    917.09

                                  Individual 95% CIs For Mean
                                  Based on Pooled StDev
Level       N      Mean     StDev  --------+---------+---------+--------
 1         48     1.453     0.505   (-----*-----) (PhMr05L1BC)
 2         48     2.303     1.410                   (-----*-----) (PhMr05L2BC)
 3         48     2.066     1.099              (-----*------) (PhN03L1)
 4         48     2.054     0.855              (-----*-----) (PhJ05L2BC)
 5         48     2.206     0.940                 (-----*-----) (PhM04L1Mig)
 6         48     2.265     1.059                  (-----*------) (PhN04L1GO)
 7         48     2.387     0.965                     (-----*-----) (PhA04CC2Mig)
 8         48     2.349     0.926                    (-----*-----) (PhN04CC1Mig)
 9         48     2.138     0.987                 (-----*-----) (PhN04L11BC)
10         48     2.605     1.232                          (-----*-----) (PhJ05CC1GO)
11         48     2.605     1.232                          (-----*-----) (PhM04CC1RO)
12         48     2.605     1.232                          (-----*-----) (PhM04CC2Mig)
13         48     2.605     1.232                          (-----*-----) (PhA04CC2BC)
14         48     2.605     1.232                          (-----*-----) (PhM04L1RO)
15         48     2.605     1.232                          (-----*-----) (PhM04L2BC)
                                  --------+---------+---------+--------
Pooled StDev =    1.097               1.50      2.00      2.50


Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.000727
```

Critical value = 4.80

Intervals for (column level mean) - (row level mean)

	1	2	3	4	5	6
2	-1.610 -0.089					
3	-1.373 0.147	-0.524 0.997				
4	-1.360 0.160	-0.511 1.009	-0.747 0.773			
5	-1.512 0.008	-0.663 0.857	-0.899 0.621	-0.912 0.608		
6	-1.572 -0.052	-0.723 0.798	-0.959 0.561	-0.972 0.548	-0.820 0.700	
7	-1.694 -0.173	-0.844 0.676	-1.081 0.440	-1.093 0.427	-0.941 0.579	-0.882 0.639
8	-1.655 -0.135	-0.806 0.714	-1.042 0.478	-1.055 0.465	-0.903 0.617	-0.843 0.677
9	-1.444 0.076	-0.595 0.925	-0.831 0.689	-0.844 0.676	-0.692 0.828	-0.632 0.888
10	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421
11	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421
12	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421
13	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421
14	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421
15	-1.912 -0.391	-1.062 0.458	-1.298 0.222	-1.311 0.209	-1.159 0.361	-1.099 0.421

	7	8	9	10	11	12
8	-0.722 0.798					
9	-0.511 1.009	-0.549 0.971				
10	-0.978 0.542	-1.016 0.504	-1.227 0.293			
11	-0.978 0.542	-1.016 0.504	-1.227 0.293	-0.760 0.760		
12	-0.978 0.542	-1.016 0.504	-1.227 0.293	-0.760 0.760	-0.760 0.760	
13	-0.978 0.542	-1.016 0.504	-1.227 0.293	-0.760 0.760	-0.760 0.760	-0.760 0.760
14	-0.978 0.542	-1.016 0.504	-1.227 0.293	-0.760 0.760	-0.760 0.760	-0.760 0.760
15	-0.978 0.542	-1.016 0.504	-1.227 0.293	-0.760 0.760	-0.760 0.760	-0.760 0.760

	13	14

```
14      -0.760
         0.760

15      -0.760      -0.760
         0.760       0.760
```

c. ***Phytophthora*** **growth at different temperatures (section 6.3.4.1)**

Isolate code*	Temperature (°C)					
	10	15	20	25	30	35
PhMr05L1BC	0.80 a	3.23 a	5.59 a	6.78 a	4.95 a	0.04 a
PhMr05L2BC	0.84 a	3.40 a	5.77 a	7.00 a	5.03 a	0.04 a
PhN03L1	0.88 a	3.41 a	5.80 a	7.10 a	5.11 a	0.08 a
PhJ05L2BC	0.96 a	3.42 a	5.86 a	7.16 a	5.13 a	0.08 a
PhM04L1Mig	1.00 a	3.46 a	5.86 a	7.28 a	5.24 a	0.08 a
PhN04L1GO	1.00 a	3.46 a	5.89 a	7.31 a	5.26 a	0.12 a
PhA04CC2Mig	1.00 a	3.49 a	5.94 a	7.42 a	5.27 a	0.12 a
PhN04CC1Mig	1.02 a	3.50 a	5.96 a	7.45 a	5.31 a	0.22 a
PhN04L11BC	1.04 a	3.50 a	5.97 a	7.51 a	5.33 a	0.24 a
PhJ05CC1GO	1.04 a	3.50 a	6.00 a	7.53 a	5.35 a	0.30 a
PhM04CC1RO	1.06 a	3.54 a	6.08 a	7.69 a	5.39 a	0.34 a
PhM04CC2Mig	1.08 a	3.57 a	6.10 a	7.72 a	5.42 a	0.38 a
PhA04CC2BC	1.08 a	3.63 a	6.11 a	7.89 a	5.44 a	0.38 a
PhM04L1RO	1.10 a	3.68 a	6.12 a	7.93 a	5.56 a	0.42 a
PhM04L2BC	1.10 a	3.74 a	6.17 a	7.99 a	5.81 a	0.50 a

* Isolate codes in Table 6-3.

One-way ANOVA: 10 versus isolate

```
Analysis of Variance for 10
Source      DF        SS         MS        F        P
isolate     14    0.6080     0.0434     0.81    0.654
Error       60    3.2120     0.0535
Total       74    3.8200
```

One-way ANOVA: 15 versus isolate

```
Analysis of Variance for 15
Source      DF        SS         MS        F        P
isolate     14     1.055      0.075     0.74    0.730
Error       60     6.141      0.102
Total       74     7.195
```

One-way ANOVA: 20 versus isolate

```
Analysis of Variance for 20
Source      DF        SS         MS        F        P
isolate     14     1.754      0.125     0.84    0.626
Error       60     8.965      0.149
Total       74    10.719
```

One-way ANOVA: 25 versus isolate

```
Analysis of Variance for 25
Source      DF        SS         MS        F        P
isolate     14     8.795      0.628     1.75    0.068
Error       60    21.509      0.358
Total       74    30.304
```

One-way ANOVA: 30 versus isolate

```
Analysis of Variance for 30
Source      DF        SS         MS        F        P
isolate     14     3.136      0.224     1.21    0.292
Error       60    11.099      0.185
Total       74    14.235
```

One-way ANOVA: 35 versus isolate

```
Analysis of Variance for 35
Source      DF        SS         MS         F         P
isolate     14    1.6715     0.1194      1.79     0.061
Error       60    4.0000     0.0667
Total       74    5.6715
```

d. Representive isolates of 68 isolates of *Phytophthora drechsleri* obtained in the present study in the collection of the Agricultural Institute at NSW Agriculture Department (Orange, NSW, Australia) (DAR number).

Isolate	Code used in the present study	DAR number
Phytophthora drechsleri	PhJ04L1	77482
Phytophthora drechsleri	PhM04CC2RO	77483
Phytophthora drechsleri	PhJ05CC1Mig	77484
Phytophthora drechsleri	PhMar05L2GO	77485