Section 1: Introduction

*Pythium ultimum* Trow var *ultimum* is one of the causal agents of damping-off and among one of the most important and destructive plant pathogens. *Pythium* species are distributed world-wide and have an extensive host range of over 100 plant species, such as cucumber (*Cucumis sativus*), pea (*Pisum sativum*), pepper (*Capsicum annum*), salvia (*Salvia splendens*), snapdragon (*Antirrhinum majus*), celosia (*Celosia argentea*), impatiens (*Impatiens balsamina*), geraniums (*Pelargonium hortorum*), soybean (*Glycine max*), wheat (*Triticum aestivum*), cotton (*Gossypium hirsutum*), tomatoes (*Lycopersicon esculentum*) and poinsettia (*Euphorbia pulcherrima*) (Anon., 1974; Farr *et al.*, 1989; Stephens and Powell, 1981). Infection by *P. ultimum* in seeds and seedlings usually causes death, whereas older plants infected by *P. ultimum* usually survive. This disease is most noticeable in nurseries, greenhouses and row crops, as symptoms develop suddenly, killing a large number of seedlings and causing low germination in seeds (Hendrix and Campbell, 1973; Stephens and Powell, 1981). Problems associated with severe damping-off of plants in greenhouses, nurseries and in the field have resulted in serious economic loss.

Specific environmental conditions which are favourable to the growth of the pathogen and/or unfavourable to the plant, can lead to infection and disease to a higher extent than otherwise would occur. Some of these factors which promote disease may include high moisture content, low water potential, salinity, extremes of pH, low temperature, low microbial activity and unfavourable nutrient concentration. The physical, chemical and biological characteristics of potting media, therefore, should be unfavourable to the pathogen and favourable to the plant. The following discussion will introduce some of the chemical, physical and biological characteristics of potting media separately and how they may affect potential interactions between the pathogen, host and the environment. Several methods are used to eradicate or reduce plant losses caused by damping-off, including pasteurisation of potting media, chemical application and biological control. The advantages and disadvantages of each method of control will be discussed briefly in the following review also.
1.1 Components of Potting media

Potting media vary in their suppressiveness of the activities of soil-borne plant pathogens (Chen et al., 1988b; Hoitink and Fahy, 1986). The variations in the degree of suppression depend on the chemical, physical and biological properties of potting media. The physical, chemical and biological properties of potting media are determined by the substrates used to prepare the potting media (Bunt, 1976; Handreck and Black, 1993; Jarvis, 1992). Physical and chemical requirements for non-specialist and specialist potting media are listed in the Australian Standards for potting mixes (AS3473-1993, Standards Australia). These requirements are based on factors which are known to assist in achieving maximum plant growth. Jarvis (1992) states that, because of the competitive market between growers, plants are not grown under optimal conditions for production of biomass. These sub-optimal conditions often cause stress in the plant, which often may result in the plant being more susceptible to disease. These conditions aim to maximise productivity in terms of the maximum production of fruit, the highest flower quality, or the most desirable foliage form in the minimum time within greenhouses or nurseries.

Potting media used commercially consist of varying ratios of mineral substrates, such as sand, perlite and vermiculite, and organic matter, such as composted sewage sludge, composted bark or sawdust, and peats, tree barks or sawdusts (Chen and Hadar, 1986; Handreck and Black, 1986; Verdonck, 1983). The most common organic component in potting media is peat. This product is being demanded in increasingly large amounts because of its highly desirable physical properties. Light coloured peat, for example, provides excellent aeration, as well as a moderate water holding capacity, and is low in bulk density. Peat resources are limited, however, and non-renewable.

Forest wastes offer advantages as alternatives to peat, since these wastes can be recycled into useful, non-toxic products, which may be used as component substrates in potting media. Forest wastes used for composting in Australia, such as hardwood and softwood sawdusts and barks, predominantly come from *Eucalyptus* spp. and *Pinus radiata* respectively, and also are inexpensive and abundant in supply.
1.1.1 Some Important Properties of Potting Media

The physical properties of potting media need to provide the plant with an adequate supply of both air and water (Bunt, 1976; Handreck and Black, 1993). The physical properties of potting media are determined by the size, shape and density of particles, which influence the packing of the particles together, the pores interspaced between solid particles and the internal pore space within the particles. These characteristics determine the amount of air and water left in a mix, which are available to plants after drainage, and the bulk density of the medium. Smaller pores tend to be filled with water, whereas larger pores tend to be filled with air. The amount of air and water available to the plant therefore depends on the numbers and relative proportions of different pore sizes in the mix.

Water holding capacity (WHC), air-filled porosity (AFP), moisture content, bulk density and wettability are the only physical measurements determined by nurseries. These measurements are simple to determine by nurseries and considered to be vital for maximum productivity.

**Air-Filled Porosity** is the term given to the proportion of volume of potting media containing air after the medium has been saturated and drained freely. This measurement determines the availability of air to plant roots. Bunt (1991) found that AFP was a good indicator for the oxygen supplying capacity of the growing media to plants. AFP can be varied depending on the component substrates used in the potting media. Incorporation of fine sand for example decreases AFP, whereas large particles of tree bark increase AFP.

**Water Holding Capacity** is a term given for the proportion of volume of water held in the potting medium after drainage. Mixes with high WHC require less frequent watering regimes. WHC of above 50% is recommended for seedlings as they require high water availability (AS3473-1993, Standards Australia). **Moisture content** refers to the percentage of the weight of water over the dry weight of potting medium or soil. Moisture content refers to the moisture status at any given time, whereas WHC refers to the moisture status of a saturated mix that has been allowed to drain for a specified length of time, until it ceases losing water. Increasing WHC simultaneously decreases the AFP.
Water, or moisture potential is the summation of matric and osmotic potential, and relates to the capacity of water to function in biological processes (Bunt, 1976; Griffin, 1972b; Jarvis, 1992). These characteristics describe more accurately the availability of water to plants, rather than just determine the quantity of water in potting media after drainage (Bunt, 1976). For example, heavy clay soil may contain more water content than a light sandy soil, yet heavy clay soil has less water available and lower potential to plants for their uptake than the sandy soil. Matric potential is the negative pressure or suction pressure exerted by the adhesion of water to substrate particles, that is the pressure that must be applied to remove water from media. Matric potential depends on the geometry of the substrate particles and the substrate type, which give rise to forces associated with the interfaces between water and solid, and water and air. Many authors often define matric potential as water potential, since some soils are considered to be salt free (Griffin, 1972b). Osmotic potential is the negative pressure that water is subjected to, in order to reach equilibrium, caused by the presence of dissolved salts. Changes in water potential in potting media should be reported in studies concerning the microbial and plant response to water, as well, as moisture content. These measurements, in isolation, do not accurately describe the physical characteristics of potting media. For example, WHC only describes the capacity of potting media to hold water after it has been saturated and drained for a certain period of time. WHC has little value in interpreting microbial and plant response to water, unless it has been correlated to a moisture potential curve for the particular mix.

Bulk density is defined as the dry mass per unit volume of moist potting medium (Bunt, 1976). Handreck and Black (1993) recommend that a bulk density in the range of 0.3 to 0.6g/mL gives low pot weight, but enough resistance to being tipped over. Wettability refers to the time taken for the water to soak into the dried mix. Organic materials used should be easy to wet without agitation, if not, a wetting agent should be used (Handreck and Black, 1993).

These physical properties can change during degradation of organic substrates in potting media by plants and the microflora in the potting media. Decomposition of potting media substrates decreases the particle size of the substrate. The medium
undergoes compaction, resulting in a decrease in air-filled porosity and an increase in water holding capacity. The substrates used must be stable and with a low rate of decomposition. Compost is mixed with other mineral substrates, which are inert to biological degradation, to improve the physical and chemical properties of media (Chen and Hadar, 1986). These authors recommended that 50% composted hardwood or pine bark be blended with 50% peat for growing plants. In contrast, Handreck and Black (1986) recommended the potting media should contain no more than 30% compost, since composts are fine and decompose quickly. If the compost is sufficiently matured and composted properly, however, it should not undergo high rates of decomposition during utilisation of potting media (Chen et al, 1992; Hoitink et al, 1991; Hoitink and Fahy, 1986).

Chemical properties in potting media refer to the concentrations of nutrients, buffer capacity and cation exchange capacity (CEC). Potting media supply macronutrients, such as nitrogen, phosphorus, potassium, sulphur, calcium and sodium, and micronutrients, such as iron, copper, zinc, manganese and boron, to plants. Potting media often require the application of fertilisers since the substrates do not contain adequate amounts of some of these nutrients (Bunt, 1976; Handreck and Black, 1993; Hoitink and Poole, 1980; Hoitink and Fahy, 1986). Composted sawdust has very little available nitrogen and insufficient Ca, Mg and essential micronutrients for satisfactory plant growth. A high buffering capacity, however, reduces the possibility of extreme changes in pH, which may adversely affect plant growth. High CEC is desirable, as it helps regulate the supply of nutrients to plants. With the application of slow release fertilisers, however, high CEC is less important.

Some raw or immature composted materials may contain toxins which can decrease seed germination and plant growth (DeVleeschauwer et al, 1981; Handreck and Black, 1986). If the compost has not sufficiently matured during the composting process, the presence of phytotoxic compounds can reduce seed germination or plant growth (DeVleeschauwer et al, 1981; Hardy and Sivasithamparam, 1989; Kuter et al, 1988). High salinity, due to total salts in the medium solution of potting media, may also be toxic to plants (Handreck and Black, 1986). These salts are introduced through high concentrations of fertilisers, composts or water supplies.
Biological properties refer to the microorganisms in the potting media. Biological properties of potting media are also important for production of healthy plants (Handreck and Black, 1986; Hoitink and Fahy, 1986). Potting media may not contain soil-borne pathogenic microorganisms, such as *Rhizoctonia solani* Kuhn, *Phytophthora cinnamomii* Rands, *Pythium ultimum* Trow, *Fusarium oxysporum* Schlecht and *Sclerotinia minor* Jagger. These pathogens can cause infection of plants, which may subsequently result in reduced germination of seeds and plant yields. Potting media may contain beneficial microorganisms, which suppress phytopathogenic microorganisms by competing for the nutrients, or by producing undesirable byproducts, such as toxins and antibiotics, which may inhibit activities of the pathogen, or by directly parasitising the pathogen (Hoitink and Fahy, 1986).

1.2 The Pathogen

1.2.1 Taxonomy and Physiology of *Pythium ultimum*

The genus *Pythium* was first established by Pringsheim in 1858 and placed in the Family *Saprolegniasae*. Schroter later placed the genus into the new Family *Pythiaceae* of Class Oomycetes in 1897, as relationships with other Oomycetes became established by the end of the last century (Hendrix and Campbell, 1973). The Oomycete pathogen is a fungus-like organism, which have plant-like features, such as cellulose in their walls (Deacon, 1997). According to the DNA analysis, these group of organisms are closely related to diatoms and brown algae (Deacon, 1997). The Oomycota, however, have a typical fungal lifestyle and it is considered appropriate to regard them as fungi in a broad sense (Deacon, 1997). Separation of the species within the genus *Pythium* is mainly based on morphological structures since these structures are easy to observe. The main characteristics used are the sexual structures, namely oogonia, antheridia and oospores, and asexual propagational structures: sporangia (Plaats-Niterink, 1981).

*Pythium ultimum* was first described by Trow in 1901 (Middleton, 1943). This species is considered to be homothallic, as no mating forms have been found. Mature hyphae in the vegetative growth stages are septate. The mycelium, comprised of many branched hyphae, gives rise to the terminal asexual and sexual propagules. Sporangia, the asexual reproductive propagules, are usually spherical and
terminal with an average diameter of 20 μm. Asexual reproduction occurs when sporangia germinate by means of a germ tube to form new mycelia.

Sexual propagation occurs by gametangial contact. The oogonium (17.0±0.05 μm in diameter) is a female haploid structure, which is spherical with a smooth outer wall (Ali-Shtayeh, 1985). Antheridia, the male haploid structures, consist of single cells which are club shaped and mostly monoclinous, originating from the same hyphae as the oogonia. On contact with the oogonium, antheridia produce a fertilisation tube, which enters the oogonium. A zygote, or oospore (15-18 μm in diameter), is formed after the union of their nuclei. Oospores are aplerotic, where the oospores do not fill the whole oogonium, with a space between the wall of the oospore and the oogonium.

1.2.2 Survival Mechanisms of *P. ultimum* as Sporangia and Oospores in the Environment

*Pythium ultimum* is a necrotroph, and survives in soil by saprophytic growth on dead tissue, and by resting structures such as oospores or sporangia. As nutrients are depleted, mycelium lyses and becomes colonised by competing organisms (Hendrix and Campbell, 1973). Survival mechanisms occur by the formation of sporangia for short and intermediate periods of time or by forming oospores for long term survival (Bruehl, 1987; Lumsden and Ayers, 1975; Hendrix and Campbell, 1973). Oospores are resistant to unfavourable conditions and can survive for a long period of time without losing virulence. Thick walled oospores, which have an average wall thickness of 2.09μm, are generated under unfavourable conditions, such as low nutrient availability (Lockwood, 1990). Thick walled oospores are only killed at a temperature of at least 70°C for 30 minutes (Stasz and Martin, 1988). Exposure at 50°C for 30 minutes reduced the germination of thick walled oospores by 50% as compared to oospores subjected to 25°C for 30 minutes (Stasz and Martin, 1988). Thick walled oospores are similar in appearance to sporangia, but are not detected in plating assays (Bruehl, 1987; Lumsden and Ayers, 1975). Lumsden and Ayers (1975) showed that thin walled oospores and sporangia lost their viability after rapid drying, whereas thick walled oospores survived the drying and remained viable for at least 8 months. Thick walled oospores in flooded conditions or, in response to an
increase in available nutrients, are converted to thin walled oospores (average wall thickness, 0.53μm) after 1 to 10 weeks. Thin walled oospores and sporangia are able to germinate after 2 hours when conditions become favourable (Lumsden and Ayers, 1975; Nelson, 1987; Stranghellini, 1974). The sporangia may develop into oospores if conditions become unfavourable, by developing thick walls, and serve as a reservoir of resistant propagules. Sporangia surviving in suppressive soils are maintained in dormancy, by microbiostasis, and may remain so for several months (Hancock, 1981; Stranghellini, 1974). Microbiostasis restricts the germination of microorganism, possibly because of the energy deficiency imposed by intense microbial competition for available resources, so that a high concentration of nutrients is unavailable to stimulate the germination of sporangia (Lockwood, 1990).

1.2.3 Damping-off of seedlings by *Pythium* spp

In order to cause infection, propagules of *Pythium* must germinate. The Oomycete pathogen germinates in response to seed and root exudates and grows towards the seeds or seedling tissues by chemotaxis (Lockwood, 1990; Lockwood and Filonow, 1981; Nelson, 1987; Nelson, 1991). Infection of seeds or seedlings can lead to pre-emergence or post-emergence damping-off diseases in plants.

Pre-emergence damping-off occurs when the Oomycete pathogen infects the seed, which subsequently fails to germinate. The pathogen penetrates the seed through the moistened swollen seed coat, or through cracks, and enters the embryo. The pathogen utilises many of the plant cell substances and the products of their breakdown. Pectinolytic enzymes dissolve the middle lamella which holds the cells together, proteolytic enzymes break down the protoplasts of invaded cells, and hyphal growth between, and through, the cells result in the breakdown of tissues. These forces and cellulosytic enzymes bring about the total collapse and disintegration of cell walls. The infected seeds are killed and become a rotten mass consisting primarily of pathogen and undecomposable substances. Pre-emergence damping-off may also occur after the seed has germinated, but prior to emergence of the cotyledons above the potting media surface. Initial infection appears as a water soaked spot on the radicle, and progresses as described above, causing death of the plant.
Post-emergence damping-off occurs after the seedling has emerged. Initial infection usually occurs at, or slightly below, the surface of the soil. The mycelium penetrates the epidermal and cortical cells of the stem directly, utilises part or all of the contents and breaks down the cell walls, bringing about the collapse of cells and tissues. In this area, vascular tissues may also be invaded, and become discoloured, even beyond the extent of the cortical lesion and as a result these seedlings die quickly. When invasion is limited to the cortex of the below-ground stem, the seedling may continue to live for a short period of time until the lesion extends above the soil line. The invaded collapsed tissues can no longer support the seedling and eventually the seedling falls over and dies (Agrios, 1978; Hendrix and Campbell, 1973).

1.3 Control of Damping-off of Plants in Potting media


Pasteurisation has been used widely for many years for the control of plant pathogens in potting media and is still recommended by many authors (Handreck and Black, 1986; Strider, 1985). Jarvis (1992) states that pasteurisation kills most plant pathogens and leaves a rich thermotolerant microflora, which can serve as biocontrol agents over the residual pathogens. Pasteurisation of potting media is relatively feasible because small volumes can easily be heat treated. In contrast, other authors state that such treatment serves to enrich the medium for various opportunistic plant pathogens, such as *Pythium*, which may be introduced by contaminated equipment, or, water after pasteurisation. *Pythium* is a poor competitor in the presence of other microorganisms (Hendrix and Campbell, 1973). If introduced into a medium which is low in microbial activity, the pathogen is able to rapidly increase in population numbers and potentially cause disease (Mandelbaum *et al*, 1988).

Chemical control methods is practised by virtually every commercial producer of seedlings (Summerell, pers. comm.). The fungicides propiconazole, metalaxyl, iprodione, etridiazole, and captan, and fumigants methyl bromide, chloropicrin and formalin, are examples of the chemicals used to control plant pathogens in soil or
potting media (Bunt, 1976; Handreck and Black, 1989; Jacobsen and Backman, 1993; Jarvis, 1992; Nelson and Craft, 1992; Stasz and Martin, 1988). Chemical control, although effective, is the least desirable method for controlling plant pathogens. There are adverse environmental effects, such as their toxicity to humans and plants, their costs, and the withholding period, which may vary from several days to several weeks, before planting can proceed. Chemicals are not easily degraded, particularly under greenhouse conditions, which can result in the accumulation of their residues to unacceptable limits (Jarvis, 1992). In addition, non-specific chemicals may kill beneficial microflora in the media, creating a biological vacuum, which allows for the opportunistic invasion and growth of pathogens. Application of chemicals to seeds is inadequate in that it provides local protection only to the germinating seed. Once outside the localised region, the plant tissue is vulnerable to disease. Chemical control is also limited as the pathogen may become resistant to the chemicals. For example, some Pythium and Phytophthora strains have developed resistance to the most commonly used and specific fungicide, metalaxyl (Bruin and Edgington, 1981; Ferrin and Wadsworth, 1992; Jarvis, 1992).

Pasteurisation of potting media, or, the application of chemicals are not necessary for properly prepared compost-amended potting media. Compost is naturally suppressive to soilborne pathogens and can be used as the method of biological control (Chen et al, 1992; Daft et al, 1979; Hoitink, 1980; Stephens and Stebbins, 1985). The disease suppressive properties of compost are largely due to the presence of beneficial microflora, which suppress the development of plant pathogens. While conducive media tend to have low microbial activities, suppressiveness may be restored by introducing antagonistic microflora into the media (Boehm and Hoitink, 1992; Chen et al, 1987; Chen et al, 1988a; Chen et al, 1988b). During the past two decades, the use of composted materials such as sewage sludge, tree bark, and sawdust as potting media constituents have been used more successfully for suppressing disease in plants (Hadar and Mandelbaum, 1986; Hardy and Sivasithamparam, 1991a; Hoitink, 1980; Hoitink et al, 1991; Hoitink and Fahy, 1986; Hoitink and Kuter, 1986; Hoitink and Poole, 1980; Kuter et al, 1988; Schüler et al, 1989; Verdonck, 1988).
Proposed mechanisms of biological control include competition, hyperparasitism and antibiosis (Baker, 1968; Baker, 1990; Hoitink et al., 1991; Lockwood, 1988). Competition occurs when two or more microbial populations are attempting to utilise the same resource. Antibiosis and parasitism may be considered as a form of competition. Antibiosis occurs when inhibition or death of one organism occurs as a result of a byproduct of another organism. Hyperparasitism occurs when the pathogen is itself antagonised by a fungus (Baker, 1990). Some of the biocontrol agents identified in composts are species from the bacterial genera of *Bacillus* and *Pseudomonas* and the fungal genera of *Trichoderma* and *Gliocladium* (Hoitink and Fahy, 1986). The microorganisms, which suppress disease, are able to recolonise compost after the peak heating stage during the composting process. Applications of biocontrol agents to growing media have not been as effective as incorporation of compost with high microbial activity (Fahy, pers. comm).

1.4 Environmental Factors influencing the Incidence and Severity of *Pythium* Damping-off

Incidence of disease refers to the number of plants which show symptoms of disease, as a proportion of the total numbers examined. The severity of disease refers to a rating scheme to determine how severely the plant is affected by disease. Components of the severity and incidence of disease caused by plant pathogenic microorganisms is often expressed in terms of inoculum potential, disease potential or predisposition of the plant to infection (Lockwood, 1988).

Inoculum potential refers to the 'invasive force' of inoculum, which is defined by Garrett (1970) as 'the energy of growth of a fungal parasite available for infection of a host organ to be infected'. The potential for inoculum to cause disease may be dependant on factors such as the inoculum density, the number of propagules, the endogenous and exogenous energy of the propagules, and the biotic and abiotic environment as it affects the inoculum. The exogenous energy required for *Pythium* germination, growth and infection is derived from root leakages of plant exudates or from plant debris. Endogenous energy refers to the concentration of energy substrate produced within the inoculum cell. Disease potential is defined as the ability of a host to become infected and show symptoms of the disease during its life cycle.
Predisposition refers to the abiotic and biotic factors which can lead to disease that otherwise would not occur, or occurs to a lesser extent. In some instances, stressful environments can increase seed exudation and, therefore, increase the incidence and severity of disease (Lockwood, 1988; Nelson, 1991). Environmental factors which may induce stress in plants include salinity, extremes of fertiliser concentration, moisture, temperature and pH (Jarvis, 1992; Kauraw and Singh, 1983; Ko and Kao, 1989; MacDonald, 1984; Moorman, 1986). High pressure watering with cold water stresses seedlings and transplanting damages rootlets, which leaves the plant root system more susceptible to infection by *Pythium* (Jarvis, 1992). High water content accompanied by poor aeration indirectly favours *Pythium* by decreasing the vigour of the host and increasing root exudation and providing a suitable environment for increased diffusion of these exudates. These stressful conditions may cause an increased susceptibility to disease. In addition, the presence of antagonistic microflora can suppress the infection process by *Pythium* (Chen et al, 1987; Chen et al, 1988b; Whipps and Lumsden, 1991). Simultaneously, other conditions, such as high nutrient concentration, may be directly favourable to the pathogen and stimulate germination.

The environment influences the host, pathogen and other microorganisms and each living organism influences other organisms and their environments. The study of environmental factors, which influence predisposition, disease potential and inoculum potential of *P. ultimum*, may be used to modify the properties of growing media, such that the severity and incidence of disease may be prevented or reduced. If any one of these factors is altered, then the severity or incidence of disease may be altered. The following section will focus on some of the environmental characteristics which influence disease. Factors in soil, known to influence *Pythium* damping-off, will be included in the following review, as they may also have reference to potting mixes under similar circumstances or the mixes may contain soil. In cases where literature is even more scarce, studies on the effects on *Phytophthora* will be included as this genus is physiologically similar to *Pythium*. Hardy and Sivasithamparam (1991b) state that it should not be assumed that the effects of one species of *Phytophthora* will happen in relation to another *Phytophthora* species as this genus is a large and diverse one and the same situation may well apply to
Pythium. Optimum matric potentials for production of sporangia, oospores and chlamydomospores varied among 3 species of Phytophthora.

1.4.1 Chemical Factors which Affect Pythium Damping-off in Soil or Potting media

Chemical factors comprise nutrients from seed exudates, fertiliser supplements, including N, P, Ca and Fe, and influence of electrical conductivity (EC) and pH. EC is a measure of the salt concentration in the growing media. Changes in population density of both beneficial and pathogenic microorganisms are likely to be affected by nutrient availability (Hardman et al, 1989). Excess or deficiency of nutrients may reduce the general vitality of the plant and influence its susceptibility to disease (Jarvis, 1992; Kauraw and Singh, 1983).

Soil fertility has been found to influence the effect pathogens have on the induction of disease. Kauraw and Singh (1983) studied the effects of various macro- and micronutrients on Pythium root rot of wheat, using field soils in pot trials. Their studies showed that the addition of 100-400 ppm nitrogen reduced the incidence of disease caused by Pythium graminicola Subramanian. Nitrogen added as urea had an inhibitory effect on Pythium, decreasing the population numbers of the pathogen. The addition of 50-200 ppm phosphorus, or 25 ppm of the micronutrients, zinc, copper, iron, molybdenum and boron, increased the severity of disease. Different ratios of these elements were added also to soil-based media. Applications of 100 ppm nitrogen and 50 ppm phosphorus reduced disease, but 100 ppm nitrogen with 100-200 ppm phosphorus increased the severity of disease. These authors came to the conclusion that the plants required a balanced nutrient supply to reduce their susceptibility to disease.

Srihuttagum and Sivasithaparam (1991) studied the effects of fertiliser treatments on the severity of disease of pea seedlings caused by Pythium vexans. Peas were sown in pots containing soil with four added treatments: N, P and K; N plus P; N plus K; and P plus K. N, P and K were added as 10mg NH₄NO₃, 200mg NaH₂PO₄ and 87mg K₂SO₄ at each application respectively. Their results showed that all fertiliser treatments, with the exception of the N treatment, slightly increased the severity of disease and this increase was mostly affected by the addition of P.
Kauraw and Singh (1983) and Srihuttagum and Sivasithamparam (1991), however, did not measure the possible interaction of pH change, or, salinity, on the ability of the pathogen to cause disease. High salinity, associated with high fertility level, increased the mortality rate of *Poinsettia* plants in three soil-less potting media, consisting of peat, vermiculite, sand, perlite and bark ash and inoculated with *P. ultimum* (Moorman, 1986). Electrical conductivity was used as a measure of salinity and pH was also recorded. Plants in all media showed increased mortality with increasing nutrient salt concentration and this was positively correlated with the increasing fertiliser concentration. The threshold level of fertiliser concentration at which there was significantly increased plant mortality, due to disease, was between 100-200 µg N/g dry weight of potting medium, for peat:perlite, and 150-300 µg N/g dry weight of potting medium for peat:vermiculite:perlite:sand, peat:vermiculite:sand:bark ash and soil:sand:peat. The mortality of plants grown in inoculated peat:vermiculite potting media was not affected by increased fertiliser concentration. Surviving plants from inoculated media were significantly shorter in height than the plants grown in the uninoculated potting media. Plant mortality increased at EC 1.58-1.88 dsm\(^{-1}\) for mixes containing peat:perlite, and at EC 2.72-3.49 dsm\(^{-1}\) for mixes containing peat:vermiculite:perlite:sand, peat:vermiculite:sand:bark ash and soil:sand:peat.

Gladstone and Moorman (1989) tested for the effects of fertiliser treatments, which consisted of concentrations in the range of 10.7-42.8mM of different N forms and 1.6-9.7mM of P forms, on *P. ultimum* root rot of *Geranium* seedlings grown on media containing peat, vermiculite, sand and bark ash. Their experiments showed significant plant losses due to high nutrient salt concentrations. Nutrient concentrations equal to or above 42.8mM N or 9.7mM P increased plant mortality, and if plants were also inoculated, losses were increased further. The effect of NaCl was to increase conductivity, rather than to increase nutrient levels, but did not significantly increase the mortality rates of plants. Plant loss due to disease was associated with total nutrient concentration, rather than total ion concentration. It has been found, however, that the chloride ion is inhibitory to *Pythium* (Martin and Hancock, 1986). Martin and Hancock (1986) studied the ability of *Pythium* to colonise on an organic mixture containing soil and crushed cotton leaves,
supplemented with different chlorine salts, but not the ability of the pathogen to penetrate and colonise seeds or root systems of plants and subsequently cause disease to plants. In Gladstone and Moorman's study (1989) the increase in salinity using chloride ions, therefore, may have masked the effect of increased salinity and mortality rates of plants.

Rasmussen and Stranghellini (1988), however, did find a correlation between increased plant disease and increasing salinity. They exposed creeping bentgrass (*Agrostis palustris*), grown in sand and peat, to achieve salinity levels in the range of 0.5-7.1 ds m$^{-1}$, at temperatures 25-32°C. The range of salinity was altered using CaCl$_2$ and NaCl. They also determined the effect of salinity levels between 1.4-28.4 ds m$^{-1}$ on mycelial growth of *Pythium aphanidermatum* (Edson) Fitz. They found that salinity had little effect on mycelial growth, but accelerated the development of disease, and broadened the temperature range in which severe disease occurred. This is in apparent contradiction with the results reported on the inhibitory effect of salts on inoculum densities of *Pythium* and colonisation of leaf matter by Martin and Hancock (1986). Martin and Hancock used higher levels of salts and exposure times, however, than Rasmussen and Stranghellini (1988). The contradiction in literature, however, on the effects of the addition of nutrients may be due also to the different plant species. These plant species may have had different sensitivities and reaction mechanisms towards high nutrient and salinity levels. High EC kills plant root tips and root hairs, which impedes the uptake of water and nutrients and may adversely affect the pathogen by reducing water uptake (Jarvis, 1992).

Increased salinity was also reported to be associated with increased severity of *Phytophthora* root rot of chrysanthemums and tomato (MacDonald, 1984; Bouchibi *et al.*, 1990). Rooted cuttings of chrysanthemums were severely affected after exposure to nutrient solutions containing 0.2 M NaCl and 0.005 M CaCl$_2$ after inoculation with *Phytophthora cryptogea* Pethybr. and Laff. (MacDonald, 1984). Plants not exposed to salt developed only mild symptoms of disease. Severity of disease increased when plants were exposed to salinity 4-12 hours after inoculation. If the plants were exposed to salinity, either 24 or 48 hours after inoculation the severity of disease decreased. The authors suggested that plants may have developed
a defence mechanism against the pathogen, but exposure to high salinity within 24 hours after inoculation may interfere with this process. Bouchibi et al (1990) also found that tomato seedlings were more severely affected by disease when exposed to increasing concentrations of salts of Na and Ca, before and after inoculation with Phytophthora parasitica Dastur.

Nutrient concentrations also affect the level of microbial activity in growing media. Incorporation of a glucose/asparagine mixture into potting media amended with compost has been shown to increase microbial activity and suppressed Pythium aphanidermatum (Edson) Fitzp (Mandelbaum and Hadar, 1990). The addition of glucose/asparagine did not increase microbial activity in potting media containing peat. It stimulated germination of Pythium oospores, however, and therefore increased the severity of disease. Although there may be other chemical or physical parameters influencing the severity of disease, suppression in composted media was mainly attributed to the presence of beneficial microflora, which increased in numbers, as these microbes competed more successfully for nutrients than the pathogen. Energy source deprivation of the pathogen, through competition from other microorganisms, appears to have been the main cause of suppression of disease. The potting medium was no longer suppressive, however, after several supplements of the nutrient solution, as competition for nutrients between Pythium and other microorganisms was not as severe.

Ko and Kao (1986a, 1986b, and 1989) reported that calcium, in conjunction with other factors, has a role in reducing disease in soils. Damping-off of cucumber, caused by Pythium splendens Braun, was reduced from 78% to 30% when a conducive soil, low in calcium, was supplemented with 6mg CaCO₃ per gram of soil. The suppressiveness of Ca amended soil was not due to change in pH, since addition of CaSO₄ was as effective as CaCO₃. Suppressive soils high in calcium became conducive after sterilisation of soils. Inhibition was restored after reinestation with microorganisms from conducive or suppressive soils. Infestation of conducive soil with microorganisms from the suppressive soil did not make the soil inhibitory to pathogens. Conducive soils high in calcium and low in microbial population were converted to suppressive soils by increasing the microbial population. Ko and Kao
(1989) state that multiple factors may have contributed to the control of diseases caused by *Pythium* spp. Calcium may reduce the pathogen population, suppress spore germination and germ tube growth, stimulate growth of host plants and increase host resistance to invasion by *Pythium*. The observed reduction in sporangial germination rate and germ tube length may have been a result of nutrient deprivation created by greater microbial activity. The addition of Ca may have increased host resistance, since root lesions on plants were smaller relative to those obtained from plants grown in un-amended soils.

The pH of soil influences the severity of *Pythium* damping-off (Bateman, 1962; Griffin, 1958). Bateman (1962) found that the severity of the disease, caused by *P. ultimum*, was reduced in *Poinsettia* at pH 5.5 and below. Neutral or alkaline soil, above pH 6.5, favoured the development of disease.

In conclusion, when applying nutrient salts to potting media, a number of interrelated factors must be considered (Jarvis, 1992). The effect on the plant, pathogen and their interactions depends on the chemical constituents of the nutrient salt and the concentrations applied. This influences the growth and germination of *Pythium*, pH of potting media, the availability of nutrients to both the plants and the pathogen, the osmotic potential of the mix, the physiology and tolerance of the host to high salinity.

1.4.2 Biological Control of Damping-Off caused by *Pythium*

The main mechanism of biological control in potting media amended with compost is based on the interaction of microbial competition, antibiosis and hyperparasitism (Boehm *et al*, 1993; Chen *et al*, 1988a, 1988b; Hardy and Sivasithamparam, 1991a; Mandelbaum and Hadar, 1990). Seed and root exudates released from germinating seeds and seedlings provide the major energy source in the root zone for all microbial activity. Seed and root exudates include carbohydrates, amino acids, organic acids, flavonoids, sterols and proteins. *Pythium* species are among the most responsive pathogen to germinating seeds and developing seedlings. Sporangia of *P. ultimum* germinate within 1-1.5 hr of exposure to germinating seeds, and nearly all seeds can be heavily colonised and rotted within 6-12 hrs after planting in *Pythium* infested soil (Nelson, 1990; Stranghellini and Hancock, 1971).
In compost amended media, however, the germination of propagules of *Pythium* is restricted by the intense competition for available energy resources, due to high microbial activity and biomass. *Pythium* propagules remain dormant and are typically not killed in compost-amended media (Chen *et al.*, 1988a; Mandelbaum and Hadar, 1990). The level of microbial activity in potting media is an important factor which affects the suppressive qualities of the media on the pathogen. Chen *et al.* (1988b) found a negative correlation between the decreasing severity of damping-off of cucumber caused by *Pythium* and an increase of total microbial activity, measured by the hydrolysis of fluorescein diacetate (FDA). Boehm and Hoitink (1992) found that a microbial activity of 3.2μg of fluorescein min⁻¹ g (dry weight) of mix⁻¹ was a minimum threshold value for suppression of *Pythium* damping-off populations of *Pythium*. Fahy and Pike (1991) found a minimum level of 0.8μg hydrolysed FDA min⁻¹mL⁻¹ considered necessary for *Pythium* suppression. Hoitink *et al.* (1996) state that the level of microbial activity in media does not determine how long the suppressive effect will last, but, depends on the concentration of readily degradable carbon in media required to support an active and effective microbial biomass (Boehm and Hoitink, 1992).

Biological control agents which have been shown to be active against *Pythium* spp. in potting media and soil include: *Trichoderma* spp., *Gliocladium* spp., *Pythium nunn Lifshitz, Pythium oligandrum* Drechs and *Pseudomonas fluorescens* Migula (Becker and Cook, 1988; Howell *et al.*, 1988; Kaiser and Hannan, 1989; Kraus and Loper, 1992; Loper, 1988; Martin and Hancock, 1987; Misaghi *et al.*, 1988; Paulitz and Baker, 1987). Boehm *et al.* (1993) isolated bacteria from root tips of cucumber seedlings grown in soils and found that *Pseudomonas* and other *Pythium* suppressive spp. were present in suppressive soils and absent in conducive soils, which were dominated by *Arthrobacter* and *Bacillus* species. Biological control is mainly achieved by means of microbial competition (Hoitink *et al.*, 1997). *Pythium oligandrum* Drechs and *Pythium nunn* Lifshitz have a higher saprophytic ability than *Pythium* and can outgrow and subsequently displace the pathogen (Martin and Hancock, 1987; Paulitz and Baker, 1987). *P. nunn* can also parasitise *Pythium aphanidermatum* (Edson) Fitzp. Other fungi which parasitise the pathogen include species from the genus *Trichoderma* and *Gliocladium*, which antagonise *Pythium* by
producing antibiotics, or, biosynthesis and excrete other inhibitory compounds, which are able to lyse hyphae of *Pythium* (Hoitink and Fahy, 1986; Hoitink *et al.*, 1997). The bacterium *Pseudomonas fluorescens* is an aggressive competitor for sites on the root, and is able to produce antibiotics and other toxic substances, such as cyanide and siderophores (Becker and Cook, 1988; Kaiser and Hannan, 1989; Kraus and Loper, 1992; Loper, 1988). Siderophores are iron chelators which deprive *Pythium* of iron (Misaghi *et al.*, 1988). The availability of iron to siderophores is dependent also on the pH of soil. Garibaldi *et al.* (1993) isolated microorganisms from suppressive soils and tested these microorganisms for antagonistic activity against *P. ultimum*. In their study none of the bacteria were shown to have antagonistic activity, whereas some fungi, *Trichoderma* and *Penicillium* spp., displaced the pathogen and significantly decreased incidence of root rot. Hoitink *et al.* (1997) found that inoculation of a specific strain of *Flavobacterium balustinum* and an isolate of *Trichoderma hamatum* into compost after peak heating during the composting process, but before significant levels of recolonisation, induced consistent levels of suppression to diseases caused by a wide range of plant pathogens.

1.4.3 Physical factors which influence the development of Damping-off

Aeration, water content, matric and osmotic potentials, or a combination of these factors, can have a profound effect on plant growth and soilborne plant pathogens. Table 1.1 summarises some of the effects of these physical factors on colonisation of plant material, germination of propagules, and severity or incidence of disease.

1.4.3.1 Temperature

A temperature favourable to the growth of the pathogen increases the incidence of disease, particularly if it is concurrently undesirable for growth of the plant host, such as low temperature. At temperatures above 30°C, there is low survival of *P. ultimum* in soil (Bateman and Dimock, 1959; Chen *et al.*, 1988a; Hancock, 1977). Maximum vegetative growth of *P. ultimum* in culture occurs at temperatures between 28 and 30°C. According to a review article written by Hendrix and Campbell (1973), *P. ultimum* causes more damping-off at low to moderate temperatures of 12 to 25°C. The optimum temperature for disease caused by *P. ultimum* is shown in Table 1.1,
and varies depending on the host, growth or resting structure of inoculum, or, the environment such as nutrient addition, or sterility of the medium. The optimum temperature for maximum severity of root rot of Poinsettias and Snapbean (Phaseolus vulgaris) occurred at 13-21°C and 15°C, respectively (Bateman and Dimock, 1959; Pieczarka and Abawi, 1977). In Pieczarka and Abawi's study (1978) the soil population counts of P. ultimum did not correlate with the severity of disease, and increased regardless to temperature between 15 and 27°C. Maximum incidence of disease in bentgrass, however, occurred at 28°C (Abad et al., 1994). The maximum incidence of disease in peas grown in soil, naturally infested with P. ultimum and artificially infested with Fusarium solani, occurred in the fluctuating temperature conditions rather than at constant 10 or 30°C (Short and Lacy, 1976). Fluctuating temperature may have provided ideal conditions for both Fusarium and Pythium at different times, resulting in maximum incidence of disease.

In unsterilised soils, collected from 3 different areas and supplemented with dried cotton leaves, the viable population counts of P. ultimum were maximal at temperatures of 16 and 21°C, 18-21°C and 27 to 30°C, respectively and minimal at 32°C. In same soils, but sterilised and supplemented with cotton leaves, however, the optimal temperature for the colonisation of P. ultimum occurred between 27 to 30°C for all 3 soils (Lifshitz and Hancock, 1983). These results indicated that antagonistic microflora may well limit the activities of the pathogen at higher temperatures, so that the optimal temperature for P. ultimum development is reduced to temperatures below 27°C. P. ultimum may have the competitive advantage also over the antagonistic microorganisms in the lower temperature range.

In soil infested with only sporangia, numbers of germinable propagules declined exponentially as the temperature increased from 0 to 27°C (Lifshitz and Hancock, 1984). Death rates of germinable propagules were lower in soil which was inoculated with a mixed culture of sporangia and oospores than in soil infested solely with sporangia. The rates of decline of germinable propagules were higher at 9 and 27°C in soil inoculated with a mixture of oospores and sporangia, but lower rates of decline occurred at 15 and 21°C. The authors suggest that the difference in death rates could reflect differences in the effect of temperature on oospore ripening.
Higher conversions of oospores to germinable propagules could occur between 15 and 21°C, rather than at 9 or at 27°C. Densities of germinable propagules tended to increase in soils incubated at higher temperatures of 21 and 27°C. Soil infested solely with oospores showed no obvious relationship between temperature and oospore conversion to germinable propagules. Soils infested with oospores appeared to have a higher rates of ripening at higher temperatures. Maximum conversion of dormant oospore to germinable oospores occurred at 25°C (Lumsden and Ayers, 1975).

1.4.3.2 Temperature and Flooding

Low soil temperature and flooding increased the incidence of disease in carrot (*Daucus carota*) seedlings (Walker, 1991). *P. ultimum* was isolated only from roots when preincubated at 12°C for 5 days. No *Pythium* was isolated from roots at preincubation temperatures of 25 and 35°C. Flooding increased the percentage of *Pythium* isolated from seedling yields, especially at a preincubation temperature of 12°C.
Table 1.1: Summary of some of the physical factors causing optimal and minimal incidence or severity of disease, colonisation of plants, or, germination of *Pythium*.

Note: * Medium infested with *Phytophthora* rather than *Pythium*

<table>
<thead>
<tr>
<th>Variables tested</th>
<th>optimal</th>
<th>minimal</th>
<th>Host</th>
<th>Medium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature:</td>
<td>13-21°C</td>
<td>above 24°C</td>
<td>Poinsettia</td>
<td>Soil</td>
<td>Bateman and Dimock, 1959</td>
</tr>
<tr>
<td>Temperature: 10, 22, 30 and alternating high, 25-30 and low, 10-15°C temperatures every 12 hrs</td>
<td>alternating temperature</td>
<td>10 and 30°C</td>
<td>Peas</td>
<td>Soil</td>
<td>Short and Lacy, 1976</td>
</tr>
<tr>
<td>Moisture Content: 20 and 37%</td>
<td>37%</td>
<td>20%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature: 15, 21 and 27°C</td>
<td>15°C and 0 to -0.1MPa</td>
<td>27°C and 0 to -0.5MPa</td>
<td>Snapbeans</td>
<td>Pasteurised soil</td>
<td>Pieczarka and Abawi, 1978</td>
</tr>
<tr>
<td>Water potential: 0 to -0.1, 0 to -0.5 and 0 to -1.2MPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature: 16, 28 and 32°C</td>
<td>28°C</td>
<td>32°C</td>
<td>Bentgrass</td>
<td>Soil</td>
<td>Abad <em>et al</em>, 1994</td>
</tr>
<tr>
<td>Temperature: 12, 15, 18, 21, 24, 27, 30 and 33°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature and Matric Potential: 16, 21, 27 and 32°C and 0, -0.025, -0.05, -0.1, -0.3, -0.5, -1.0, -1.5 MPa</td>
<td>i) 27 to 30°C ii) 18 to 21°C, 16 to 21°C and 27 to 30°C C ii) 16 and 21°C at matric potentials -0.025, -0.05MPa</td>
<td>i) 33°C ii) 33°C ii) 32°C at matric potential 0MPa and below -1.0MPa</td>
<td>cotton leaves</td>
<td>i) Sterile soil ii) Non sterile soil</td>
<td>Liftshitz and Hancock, 1983</td>
</tr>
<tr>
<td>Seasonal effects: Soil moisture and temperature</td>
<td>After irrigation</td>
<td>Approx. 15 or more days after irrigation</td>
<td>Cotton roots</td>
<td>Soil</td>
<td>Huisman, 1987</td>
</tr>
<tr>
<td>Water potential: 0, -0.1, -0.4, -0.7, -3.9 and -9.3 MPa</td>
<td>0MPa</td>
<td>-3.9 and -9.3MPa</td>
<td>NA</td>
<td>Soil</td>
<td>Lumsden and Ayers, 1975</td>
</tr>
<tr>
<td>Temperature: 10, 15, 20, 25, 30 and 35</td>
<td>25°C</td>
<td>5 and 10°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature: 9, 15, 21 and 27°C</td>
<td>15-21°C</td>
<td>9°C</td>
<td>NA</td>
<td>Soil</td>
<td>Liftshitz and Hancock, 1984</td>
</tr>
<tr>
<td>Matric Potential: 0.04 and -0.3MPa</td>
<td>-0.04MPa</td>
<td>-0.3MPa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprinkler Irrigation</td>
<td>Irrigated field</td>
<td>Non irrigated field</td>
<td>Peanuts</td>
<td>field</td>
<td>Porter <em>et al</em>, 1987</td>
</tr>
</tbody>
</table>
### Table 1.1: Continued

<table>
<thead>
<tr>
<th>Variables tested</th>
<th>optimal</th>
<th>minimal</th>
<th>Host</th>
<th>Medium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matric potential ranges: -0.04 to -0.02, -0.26 to -0.04 and -1.06 to -0.26 MPA</td>
<td>-0.04 to 0.06 MPA</td>
<td>-1.06 to 0.26 MPA</td>
<td>Chickpeas</td>
<td>Soil</td>
<td>Bhatti and Kraft, 1992</td>
</tr>
<tr>
<td>Initial matric potential: -0.01, -0.03 to -0.05, -0.07 to -0.08 and -0.1 to -0.12 MPA</td>
<td>-0.01 MPA</td>
<td>-0.04 to -0.12 MPA</td>
<td>Wheat seeds</td>
<td>Soil</td>
<td>Hering et al, 1987</td>
</tr>
<tr>
<td>Matric potential: -0.001, -0.01, -0.1 and -1.5 MPA</td>
<td>a) -0.001 to 0.01 MPA</td>
<td>a) -1.5 MPA</td>
<td>Alfalfa seed</td>
<td>a) soil plus arginine b) soil and no arginine</td>
<td>Stranghellini and Burr, 1973</td>
</tr>
<tr>
<td>a) Osmotic potential: -0.5, -1.0, -1.5, -2.0, -2.5, -3.0 and -3.5 MPA</td>
<td>a) below -0.5 MPA</td>
<td>a) -2.5 and -3.0 MPA</td>
<td>NA</td>
<td>Soil extract agar</td>
<td>McQuilkin et al, 1992</td>
</tr>
<tr>
<td>b) Matric potential: -0.5, -0.8, -1.0, -1.5, -2.0 and -2.5 MPA</td>
<td>b) -0.5 MPA</td>
<td>b) -2.0 MPA</td>
<td>NA</td>
<td>Soil extract agar</td>
<td>McQuilkin et al, 1992</td>
</tr>
<tr>
<td>Matric potential: a) &gt;-0.02 to -0.5 MPA b) 0 to -2.2 MPA</td>
<td>NA</td>
<td>NA</td>
<td>Alfalfa rootlets</td>
<td>a) field soil b) potted soil</td>
<td>Hancock and Grimes, 1990</td>
</tr>
<tr>
<td>Matric potential: 0, -0.3, -0.1, -0.3, -0.7 and -1.5 MPA</td>
<td>-0.03 to -0.3 MPA</td>
<td>0 and -1.5 MPA</td>
<td>NA</td>
<td>soil</td>
<td>Johnson et al, 1990</td>
</tr>
<tr>
<td>Moisture holding capacity: 29-35, 41-52, 65-70 and 81-88%</td>
<td>81-88%</td>
<td>29-52%</td>
<td>Poinsettia</td>
<td>soilless, inert potting media</td>
<td>Bateman, 1961</td>
</tr>
<tr>
<td>*AFP: 1, 5, 6, 10 and 20%</td>
<td>1 to 5%</td>
<td>10-20%</td>
<td>cuttings of Toyon</td>
<td>potting media</td>
<td>Filmer et al, 1986</td>
</tr>
</tbody>
</table>

### 1.4.3.3 Temperature and Matric Potential

Lifshitz and Hancock (1983) concurrently studied the effects of matric potential and temperature on the saprophytic development of *P. ultimum* in soil incorporated with dried cotton leaves. Soils containing dried cotton leaf debris were incubated in Petri dishes for 5 days and subjected to the temperatures with matric potential levels as shown in Table 1.1. Optimal population numbers of *P. ultimum* occurred at temperatures between 16 and 27°C with matric potentials of -0.025 and -0.05 MPa and minimal at -1.0 and -1.5 MPA at all temperatures tested.

Lifshitz and Hancock (1984) studied the effects of temperature and matric potential on the survival of *P. ultimum* by infesting soils with two types of propagules, oospores and sporangia. Soils were incubated in Petri dishes at various temperatures and initial matric potential of -0.04 MPa. After 108 days, matric potentials had
decreased to -0.05, -0.05, -0.07 and -0.35 MPa, respectively, for each of the
temperature treatments in the range of 9 to 27°C. The matric potential was then
adjusted to -0.30 MPa by adding water.

After another 108 days, the matric potentials had decreased further to levels of -0.5,
-0.8, -1.5, and -1.5 MPa, respectively. The average death rates of *P. ultimum*,
determined by the half lives of germinable propagules, at each temperature were
about 40% higher at initial matric potentials of wet (-0.04MPa), than at dry
(-0.3 MPa) conditions. Densities of germinable propagules tended to increase, or
were surviving in greater numbers, in soils kept at a higher moisture regime. The
authors suggest these trends over 5-7 months strongly indicate that the longevity of
*P. ultimum* in soil was increased under drier conditions. In soils which were infested
with oospores, there was no obvious relationship between matric potential and
oospore conversion to germinable propagules, but there was a tendency of higher
oospore conversion in moist soils after the 30 day period. The proportion of
oospores which germinated from dormant oospores was always below 5% of total
oospores.

Hancock and Grimes (1990), however, found that infection rates of alfalfa rootlets by
*P. ultimum* were not significantly affected by soil moisture in the field, or under more
controlled conditions in the glasshouse. These authors tested the effects of matric
potential in the range of > -0.02 to -0.5 MPa, in the field, and constant matric
potentials in the range of 0 to -2.2 MPa in the greenhouse. Their results indicated
that the decrease in root density in response to increased irrigation was related to
direct plant growth responses to soil moisture status. Inoculum densities were higher
in the warmer seasons and lower in the colder seasons. In the warmer months,
however, inoculum densities of *P. ultimum* and infection rates tended to be higher in
soils containing a higher water potential. Altering watering regimes did not
therefore, significantly affect the activities of *P. ultimum*. Presumably, temperature
may have influenced the disease development in alfalfa.

**1.4.3.4 Water Potential**

*P. ultimum* has one of the highest water potential requirements for growth; optimal
growth occurs at 0.5MPa and the lower limit for growth occurs at -2.5 to -3.0MPa
(Cook and Duniway, 1985). There was an increase in the severity of disease caused by *P. ultimum* in Snap Beans grown in pasteurised soil, as the soil water potential increased over the 30 day period (Pieczarka and Abawi, 1978). The ranges of soil water potential used in these treatments are stated in Table 1.1. There are ecological, energetic or nutritional reasons why *Pythium* benefits from high water potential. High water potential means that little energy is required for the uptake of water from soil. Water availability may also influence the growth of antagonistic microflora, amount of nutrients exuded by seeds, or increase the diffusion of nutrients. Microbial antagonism is limited at water potentials below -1.0 to -1.5 MPa, while growth of *Pythium* often ceases at a water potential of -1.5 MPa, which is often considered to be also the lower limit required for growth of higher plants in soils (Cook and Papendick, 1970). The variability of some of these studies on the effect of water availability on disease, may be due to the different types of growing media used, types of plants, and methods used for assessing inoculum densities and water potential components.

### 1.4.3.5 Matric Potential

Lumsden and Ayers (1975) and Johnson *et al* (1990) reported that the matric potential influenced the conversion of thick walled oospores to thin walled oospores of *P. ultimum*. Soil samples were adjusted to various matric potentials stated in Table 1.1. Lumsden and Ayers (1975) incubated soil samples in Petri dishes for up to 6 weeks. These authors did not state whether water potentials were maintained constant throughout the experiment, and did not measure the final water potential, which may have decreased significantly. The conversion rate increased as the water potential increased and with time. Maximum conversion occurred at saturation and at the initial water potentials of -3.9 and -9.3 MPa, the conversion of thick walled oospores did not occur during the 6 week incubation. Johnson *et al* (1990) reported that, at matric potential of -0.03 to -0.3MPa, almost all the thick walled oospores converted to thin walled oospores and 91% of the latter lysed. At near saturation and in much drier soils (-1.5MPa), 82 and 62% of oospores remained thick walled oospores respectively. Lumsden and Ayers (1975), however, reported that 96% of their oospores of *P. ultimum* had germinated in moist soils within 6 weeks. These
conflicting results are best explained by the different methodology, *Pythium ultimum* strains and soil characteristics used by the authors.

Bhatti and Kraft (1992) reported that, as matric potential increased, there was also an increase in the severity of disease in chickpeas caused by *P. ultimum*. They used sterile soils adjusted to 3 ranges of matric potentials as presented in Table 1.1. The severity of disease and *Pythium* population counts were determined after 30 days of planting, and were found to increase as the moisture availability increased. Hering *et al* (1987) reported that infection of wheat seeds by *P. ultimum* was maximal at the initial matric potential of -0.01 MPa, but lower in soils drier than -0.04 and -0.05 MPa.

Colonisation of alfalfa seeds by *Pythium aphanidermatum* occurred at soil matric potentials between -0.001 to -0.1 MPa, while oospore germination and germtube length increased at higher water availability in soil amended with arginine (Stranghellini and Burr, 1973). Oospore germination did not occur in soil which did not contain arginine. This indicates that an increase in germination is probably related to an increase in nutrient availability. McQuilken *et al* (1992) demonstrated a similar effect, in that decreases in matric potential in soil extract agar reduced growth of mycelium and oospore germination of *Pythium oligandrum* Drechs. The agar culture system used by McQuilken *et al* (1992) lacked the crucial components of the soil system, the soil/air and soil/water interfaces. These interfaces determine most of the properties of soil. Agar systems are, therefore, often poor settings for ecological experiments (Liddell, 1992).

### 1.4.3.6 Osmotic Potential

A decrease in osmotic potential also reduced growth and oospore germination of *P. ultimum* (McQuilken *et al*, 1992). Germination rate was maximal at osmotic potentials of -0.05 to -2.0 MPa, and ceased at -3.0 and -3.5 MPa, whereas mycelial extension decreased below -0.5 MPa, and ceased between -2.5 and -3.0 MPa. At matric potential -2.0 MPa, mycelial growth and oospore germination ceased. Mycelial growth and oospore germination was affected more by low matric potential than low osmotic potential.
1.4.3.7 Moisture contents

Huisman (1988) reported that colonisation of roots in field grown cotton by *P. ultimum* increased significantly after soil irrigation, and then declined as the soil dried out. Porter et al (1987) reported that sprinkler irrigation increased the incidence and severity of disease caused by *Pythium myriotylum* in peanuts grown in the field, in comparison to peanuts grown in non-irrigated fields. Drying and wetting of soils promotes carbon cycling, which is mainly due to enhanced turnover of microbial products (Van Gestel et al, 1993). Kerr (1964) found a positive correlation between moisture content and increased incidence of disease caused by *P. ultimum* in Peas, grown in each of the 3 soil types of moisture contents 3 to 6% and 7 to 11%. Increased soil moisture only slightly increased *P. ultimum* population counts and indirectly increased incidence of disease, by increasing exudation of seeds. Short and Lacy (1976) found that seed and seedling rot of peas were usually higher in soil with moisture content of 37% than at 20%. WHC was altered by using 2 different pot heights and soils were naturally infested with *P. ultimum* and artificially infested with *Fusarium solani*. Increased percolation rate of water may have increased the diffusion of exudates, which can increase the severity of root rot disease in treatments with greater WHC.

1.4.3.8 Porosity

The air-filled porosity of potting media may affect the severity of disease (Fahy and Pike, 1991; Filmer et al, 1986). An increase in moisture content and water potential has been reported to enhance the severity and incidence of root disease, but the effects of moisture are often dependent on the physical characteristics of the growing medium (Cook and Papendick, 1972; Hendrix and Campbell, 1973, Pieczarka and Abawi, 1978).

Fahy et al (1995) surveyed a wide range of potting mixes and frequently found that suppression of root disease correlated with high AFP, low WHC and high microbial activity. This was not consistent in all their trials and other factors such as nitrogen drawdown index, pH, electrical conductivity and nitrate concentration also interacted to apparently affect the severity of disease caused by *Pythium*. 
There are no definitive studies with potting media which examine the effects of AFP on incidence and severity of disease caused by *Pythium*, but there are some associated with the related pathogen *Phytophthora*.

There has been strong evidence that the percentage of air and water in potting media influence the severity of disease caused by *Phytophthora cinnamomi*. Filmer et al (1986) have shown that as the AFP decreases, the severity of *Phytophthora* root rot of cuttings of Toyon (*Heteromeles arbutifolia*) increased. Disease was most severe at AFP 1% and least severe at AFP of 20%. Their potting media were made up with mineral substrates, which were relatively inert to microbial activity and not typical of commercial potting media. Ownley and Benson (1991) found that container media prepared from pine bark were less conducive to the development of *Phytophthora*, in comparison to the media containing peat. They suggested that host susceptibility to *Phytophthora* is affected by AFP, which moderates root generation and growth, root exudation and plant metabolism related to host resistance, while matric potential influenced sporangium production and zoospore release, movement and chemotaxis, as well as germination and survival of *Phytophthora* spp. These authors proposed that the alteration of particle size distribution and irrigation schedules could be used to increase disease suppression by pine bark media. However, there are also other factors, such as nutrient levels and microbial antagonism, which may have interacted with the severity of disease, as previously discussed. In addition, pine bark is known to have toxic compounds, which reduce the severity of disease. Spencer and Benson (1982) found that the addition of sand to pine bark mixes destroyed the suppressive qualities of pine bark. Physical properties, such as low AFP, may have increased the incidence of *Phytophthora cinnamomi* in Lupins (*Lupinus angustifolius*) in sand amended media.

Tu et al (1992) suggest that manipulation of soil moisture may enable yield to be maximised in bean cultivars susceptible to root rot. These authors found that optimal plant growth and yield for 2 lines of beans, one resistant and the other susceptible to root rot caused by *P. ultimum*, occurred in media of high soil moisture content. The susceptible line was severely affected by disease and reduction of soil moisture also
reduced the incidence of root rot. The resistant strain was not affected by root rot in either moisture content.

Higher moisture holding capacity, of 81-88% compared with 29-52%, increased the incidence of disease caused by *P. ultimum* in poinsettias grown in soilless potting media containing inert ingredients (Bateman, 1961).

Gugino *et al* (1973) compared 3 mixes, 100% sand, bark:sand (1:1, v/v) and 100% bark and proposed that the physical characteristics of potting media at various depths, 4, 10 and 17cm, affected root weights, *Pythium irregulare* and non-pathogenic microbial population numbers. Moisture content increased with increasing depth and percent bark and *Pythium* population numbers was greatest in the bark:sand mix and at depths of 4 and 10cm for all mixes. These authors proposed that moisture was not important in the lifecycle of *P. irregulare*, as many isolates of this species did not form zoospores. Bacteria and root weights increased with increasing bark content. These authors also proposed that bark mixes which have higher particle size distribution will have higher percolation rates and this may increase the movement of exudates. An interaction of factors, such as microbial competition, pH, salinity, phytotoxicity, root exudates and nutrient concentrations, may also have affected *Pythium* numbers.

### 1.4.3.9 Aeration, Oxygen

Lack of aeration may predispose the plant to disease causing damage to plant roots and increasing plant exudation.

Brown and Kennedy (1966) studied the effects of a range of oxygen concentrations, 4 to 21%, on the severity of disease of Chippewa soybean caused by *P. ultimum*. Pre-emergence growth of Chippewa soybean seedlings was significantly retarded, exudation for soybean seed was much greater and *Pythium* severity of seed and root rot were greatest under low oxygen concentrations, 4-8% as compared to higher oxygen concentrations of 11.5, and 21% oxygen. Growth of *Pythium* in yeast broth was reduced at 1.3% oxygen concentration, but not at 4% and above. Plant exudations were higher at 4 and 8% and favoured the growth of *Pythium* than at 11.5 and 21%, while root weights increased as oxygen content increased (Brown and Kennedy, 1966). Growth of *Pythium irregulare* at 1% oxygen concentration was
reduced to less than 20% than at 20.7% oxygen (Mitchell and Mitchell, 1973). Brown and Kennedy (1966) and Mitchell and Mitchell (1973) used culture broth and air for their studies, therefore, plants or the pathogen in growing media may respond differently to low oxygen concentration. These data, however, give some support to the argument that variation in AFP may affect damping-off caused by Pythium as the AFP directly correlates with oxygen concentration in the mix.

1.4.4 Interaction of Biological, Physical and Chemical Factors

Overall, the interrelationships of chemical, physical and biological factors and their effects on the severity of disease are complex. For example, the addition of fertiliser to potting media can significantly alter the nutrient concentration, plant exudation concentration, pH, salinity, osmotic potential and water potential. Any one of these factors, or a combination of these factors, may affect the physiologies of the host, pathogen and antagonistic microflora, nutrient and water availability to plants and microorganisms and influence the severity of disease in any direction depending on the strength of each interrelationship within the complexity. Lower temperatures and neutral or alkaline pH, in addition to increased moisture status, have been shown to increase the severity of disease. Undesirable environmental conditions can increase plant exudation, increase the susceptibility of the plant to disease, decrease microbial activity and increase the growth of the pathogen.
1.5 Aims, Objectives and Applications of this Research study

It has been established in the above review that:

i) Little information exists on the effects of AFP on the suppression of *Pythium* in potting media containing compost, which is now a major component of commercially used media in horticulture in NSW.

ii) Many studies involving the effect of environmental factors on the severity of disease caused by *Pythium* in soils have been conducted. Not much literature, however, is available on these factors in potting media containing compost. The characteristics of soilless potting media are quite different from soils, particularly with respect to their physical characteristics. Currently, commercial acceptance of biological control of soilborne plant pathogens in potting media containing compost is not great, as the degree of the biological control is often variable from time to time in different batches of the product and seemingly uncontrollable.

iii) Other studies, however, varied AFP by using different substrates, altering the chemical characteristics in the potting media, or did not incorporate compost in their potting media. By utilising compost in the medium and altering AFP, there may be altered microbial activity which affects the severity of disease caused by *Pythium*.

iv) Properly composted material offers advantages, as this product has high microbial activity and no phytotoxic compounds, in comparison to unmatured pine barks, while being relatively inexpensive, abundant in supply and reducing pesticide use. This product can also be blended with other components to achieve good physical conditions for plant growth.

With these factors in consideration:

The aim of the work of research now in progress at the Organic Waste Recycling Unit, New South Wales Agriculture, is to produce potting media of premium quality, through potting media formulation which suppresses soilborne plant pathogens. Fahy and Pike (1991) surveyed a wide range of potting media, all containing composted eucalyptus sawdust from different areas in N.S.W. Other organic materials present in the potting media included composts of pine bark, pine sawdust, or sewage sludge, and sedge peat. Most potting media were found to be suppressive
to *P. ultimum* using a cucumber bioassay developed by Chen *et al.*, 1987. A moderate correlation was found between microbial activity and disease suppression. Not all suppressive mixes were high in microbial activity, therefore, potting media were analysed chemically and physically. The authors proposed that *Pythium* suppression may be affected by AFP, salinity levels, Ca levels and percolation rate of water, in addition to microbial activity in the potting media. Fahy *et al.* (1995) surveyed a large range of potting mixes and found a positive correlation (*p*<0.05) between AFP and suppression of *Pythium*, however, the *r* value was not great. Other factors such as nutrient concentration, pH, electrical conductivity and microbial activity may have also affected the severity of disease.

**The main aim of this project**, therefore, was to examine the effects of changes in AFP on the severity of disease caused by *Pythium ultimum*. Other factors such as WHC, microbial activity of potting media and temperature, in combination with AFP, were examined for their combined effect on the severity of disease. These factors were tested such that the part of the mechanism of suppression may be elucidated, concurrently with the effects of plant stress on the severity of disease.

**The objectives** for the following experiments in this thesis were as follows:

In all experiments the effects of AFP of potting media were tested, using plant bioassays, as a measure of the severity of *Pythium* damping-off. In all treatments of each experiment, a consistent amount of compost was blended with various ratios of sand grades to vary AFP and reduce variation of other variables. As well as AFP, other factors such as pot heights, microbial activity levels, plant host, potting media surface humidity and heat treatments to media and/or host were examined for their effects. Microbial activities were also monitored, as a correlating measurement to assist in the explanation of the suppressiveness of the potting media. *Pythium* counts in the potting media were measured to give an indication of the development of *Pythium* population within the media during application of different treatments. Other physical characteristics such as matric potential were monitored on occasion and taken into account in the discussion of the results.

This study may assist in the development of the quality standards for potting media made from Australian components to promote media containing compost which are
consistently suppressive to *Pythium* disease. Ball Seed Company in the USA markets potting media, which are suppressive to *Pythium* disease, to high standards of quality. Research on the physiological factors underlying suppression of *Pythium* may serve as a model for the suppression of other plant pathogens.
Section 2: General Materials and Methods

These experiments were prepared from January 1992 to December 1993. The procedures described below were used for all of the following experiments, unless otherwise specified for the individual experiments and as specified in separate methods for each experiment stated in Sections 3, 4 and 5.

2.1 Preparation of potting media

Potting media were composed of mixtures of composted hardwood sawdust and sand, which were supplied by the Australian Native Landscapes Pty Ltd (Baulkham Hills, Sydney).

Two types of sand grades were prepared, fine grade (212-500 µm) and coarse grade (≥2mm). The sands were dried at 80°C in a fan-forced heating oven (Wessberg and Tulander®, Sydney) before sieving. The fine grade was obtained by sieving Sydney Sand through 500 µm and 212 µm sieves and using material retained on the finer sieve. The coarse grade sand was obtained by using retained material after sieving Propagation Coarse Sand through a 2 mm wire mesh. In experiments 2 to 7, Sydney Sand was not sieved, as approximately 90% of the Sydney Sand was already of particle size 212 to 500 µm, and this was considered satisfactory for the purpose intended. The remaining 10% sand was of size mostly smaller than 212 µm in diameter. Both grades of sand were placed in a 10 L plastic container and washed to remove large pieces of organic matter and scum by flotation. The sand was then acid washed 1:1 v/v with ca. 0.05 M HCl solution to remove the remaining organic matter. The sand was rinsed four times with deionised water to ensure that there were no residues of acid left. This step ensured that the sand was inert and would not influence suppression due to the organic matter present, but only contributed to the physical characteristics of the potting media.

The two grades of sand were combined in different ratios in order to prepare potting media with various air-filled porosities (AFP). The mixed sands were then blended with 33 % compost by volume and mixed thoroughly to give an estimated range of different AFP’s. The exact AFP and WHC were determined and the mixes subsequently used in the cucumber bioassay.
2.1.1 Determination of air-filled porosity (AFP) and water holding capacity (WHC)

AFP and WHC were determined using the then current standard method for potting media (AS3473-1989, Standards Australia). In this method, a volume of the potting medium in a 12cm deep tube is immersed in water for a specified time to allow saturation and then removed and drained for a fixed time. The saturation and drainage cycle is repeated twice more to allow for the mix to be fully saturated and to be fully compacted. At the end of the second cycle, the top half of the cylinder is removed and without applying any force, excess potting media is removed. The lower part of the apparatus is covered with a mesh and provides a known total volume of mix. In the third cycle, the drainage volume is collected from the potting media in the container over a fixed time period. The volume drained is then expressed as a percentage of the total volume of medium to give AFP. WHC was determined after the drained mix was oven dried at 80°C until constant weight and calculated as shown in the following formula:

\[ \text{WHC} = \frac{(W_1 - W_2)}{V_C} \text{ where} \]

\[ W_1 = \text{wet weight of potting media} \]
\[ W_2 = \text{dry weight of potting media} \]
\[ V_C = \text{volume of container} \]

The AFP and WHC, as determined using 12cm containers, will be referred to as the “standard” AFP (AFP_{std}) and WHC (WHC_{std}). In Experiments 4 to 7 the actual AFP and WHC, as well as standard AFP and WHC, were determined as above, except that the containers were equal to the height of the mix in the pot used in the bioassay. The actual AFP (AFP_{act}) were determined, as increasing the pot height also increases the proportion of air in the mix (Handreck, 1993). The AFP_{act}, therefore, describes the aeration of a specific mix at that particular height. The AFP_{std} measures the proportion of air of potting media in 12cm pots and is used to give information about the mix in comparison to other mixes, for which there is already published data (Handreck, 1993). Table 2.1 summarises the AFP and WHC of potting media used in Experiments 1-7.
Table 2.1: Summary of components and physical characteristics of potting media treatments used in Experiments 1-7

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>Potting media components</th>
<th>(s) AFP(_{std}) (%)</th>
<th>(a) AFP(_{act}) (%)</th>
<th>WHC(_{std}) (%)</th>
<th>WHC(_{act}) (%)</th>
<th>Pot height used in bioassay (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>sand (67%) and compost (33%)</td>
<td>4.2, 6.7, 14.0, 18.0 and 25.7</td>
<td>2.4, 3.8, 10, 14 and 25% #</td>
<td>44.1, 41.3, 35.1, 30.1 and 21.2</td>
<td>not determined</td>
<td>8.5</td>
</tr>
<tr>
<td>2</td>
<td>sand (67%) and compost (33%)</td>
<td>2.4, 2.9, 5.2, 12.5, 16.1 and 25.7</td>
<td>1.8, 2.0, 2.6, 8.4, 10.8 and 25.0%#</td>
<td>43.4, 43.1, 42.9, 33.2, 32.8 and 21.2</td>
<td>not determined</td>
<td>8.5</td>
</tr>
<tr>
<td>3</td>
<td>sand (44.5%), compost (33%) and peat (22.5%)</td>
<td>6.3, 12.6, 17.5 and 22.8</td>
<td>2.4, 8.2, 16.0 and 19.1</td>
<td>54.5, 48.6, 43.9 and 42.9</td>
<td>58.2, 51.4, 48.1 and 47.8</td>
<td>4.5</td>
</tr>
<tr>
<td>4</td>
<td>sand (67%) and compost (33%)</td>
<td>5.2 and 16.1</td>
<td>2.6 and 10.8%#</td>
<td>42.9 and 32.8</td>
<td>not determined</td>
<td>8.5</td>
</tr>
<tr>
<td>5.1 &amp; 5.2. sand (67%), wheat bran (1.5%) and compost (31.5%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>3.3, 6.3, 8.3, 18.8 and 21.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.2</td>
<td>3, 10.6 and 22.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>sand (67%), wheat bran (0 to 1.5%) and compost (31.5% to 33%)</td>
<td>3, 6.3 and 8.3</td>
<td>2, 3 and 6</td>
<td>47, 41 and 38</td>
<td>48, 43 and 42</td>
<td>8.5</td>
</tr>
<tr>
<td>7.1</td>
<td>sand (67%), wheat bran (1.5%) and compost (31.5%)</td>
<td>7.1. 3, 7 and 27</td>
<td>7.1. 2, 5 and 19</td>
<td>7.1. 40, 32 and 33%</td>
<td>7.1. 44, 41 and 37</td>
<td>7.1 &amp; 7.2. 4.5</td>
</tr>
<tr>
<td>7.2</td>
<td>sand (44.5%), peat (22.5%), wheat bran (1.5%) and compost (31.5%)</td>
<td>7.2. 3 and 7</td>
<td>7.2. 2 and 5</td>
<td>7.2. 40, 32 and 33%</td>
<td>7.2. 58 and 55</td>
<td></td>
</tr>
</tbody>
</table>

Note: \(s\) Standard AFP and WHC were determined using 12cm cylinder heights, while \(a\) actual values were obtained using pot heights as in bioassays for those experiments. # Estimated values from graphs and were not actually determined.
2.1.2 Determination of Matric potential

As previously discussed, matric potential has been shown to affect the severity or incidence of disease (McQuilkin et al., 1992; Bhatti and Kraft, 1992). The matric potential of potting media of various AFP and WHC used in Experiments 5 & 6 were determined by the filter paper method. This method is simple, rapid and covers a full range of matric potentials encountered in the field as compared to the use of tensiometers, thermocouple psychrometers and the pressure measurement system (Hamblin, 1981). Frequently, only small and unrepresentative volumes of soil are measured, because of the time taken for large samples to equilibrate on pressure plates, or because the geometry of the sample chambers does not permit large volumes.

The filter paper method is based on the assumption that the water potential of moist soil and filter paper in contact with the soil will be the same at equilibrium. Filter paper is used as a standard matrix and is equilibrated with soil water, either through vapour phase, or through combined liquid and vapour phase flow. After equilibration time the water content of the filter paper is determined by gravimetric analysis. With most soil samples the osmotic potential is negligible and some authors define this measurement wholly as water or moisture potential (Papendick and Campbell, 1985). Both matric and osmotic potentials are measured if the paper is supported a small distance from the soil. A moisture release curve, such as determined by Hamblin (1981), describes the relationship between water content and matric potential. The water potential of the soil is determined from the moisture release curve of a series of filter papers in soils of various moisture contents. Gardner (1937) first proposed the water uptake by filter papers as a means of measuring soil-water potential. Fawcett and Collis-George (1967) modified this method by using a stack of 6 Whatman No 42 filter papers. McQueen and Miller (1968) discussed the method using an alternative brand of filter paper and obtained a different calibration curve to that of Fawcett and Collis-George (1967). Hamblin (1981) used Whatman No. 42 filter paper and the calibration curves were in good agreement with that of Fawcett and Collis-George (1967). For precise work, or if filter paper is of a different make than that used by the published method, the
moisture release function for the filter paper should be determined. Hamblin used a single filter paper and equilibration time was under 36 hrs, whereas Fawcett and Collis-George (1967) used 8 filter papers and equilibration time was 7 days.

In practice, a modification of the procedure developed by Hamblin (1981) was used to determine matric potential. Preliminary work showed that removing medium from a single filter paper before weighing was difficult, and led to erroneous results; therefore, three filter papers were used. Water content of the smaller diameter, central filter paper, compared to the diameter of the outer filter papers, was determined. The equilibration time was adjusted to 7 days to ensure for sufficient time for the water to equilibrate between the potting media and the filter paper. Matric potential of media were determined in Experiments 5 and 6 as this measurement may have shown a better relationship, compared to AFP, with severity of disease rating index (SDR), Pythium counts or microbial activity.

A 5.5cm Whatman No 42 filter paper was washed with 0.005% HgCl₂, was dried, weighed and sandwiched between 2 x 9cm Whatman No 42 filter paper also washed with 0.005% HgCl₂. Samples were prepared by pooling the contents of 5 pots into a 10L container. The media were mixed thoroughly, seeds and plant material were removed and three samples collected for each potting mix treatment. A sample (approximately 100g) was immediately placed in the sample container (10cm in diameter x 3cm depth), the three filter papers were placed on the sample and the remainder of the sample was added and pressed down firmly. The container was sealed with electrical tape and incubated at 22°C for 7 days. The water content of the central filter paper was determined after equilibration, followed by determination of matric potential using the wetting curve prepared by Hamblin (1981).

2.1.3 Preparation of potting media for the seedling bioassays

The water content of each mix was adjusted to water holding capacity by addition of deionised water (Refer to Table 3.1). The pH of potting media was determined from an extract of potting media shaken in deionised water at a 2:3 volumetric ratio for 60 min. The pH ranged from 5.5 to 6.0 (data not shown) and, therefore, required no adjustment with lime before utilisation of the potting media. Each potting medium was incubated in a plastic bag at ambient temperature (18-25°C) for at least 4 days to
build up the microbial activity prior to the experiments. This ensured that the potting media were colonised by mesopholic microorganisms, which suppress *Pythium* damping-off (Hoitink *et al*, 1991).

2.2 Preparation of Inoculum of *Pythium ultimum* for Seedling Bioassays

An isolate of *Pythium ultimum* (DAR35790a) from the DAR Herbarium culture collection of the NSW Department of Agriculture at Biological Chemical Research Institute, Rydalmere, was used throughout this study. The isolate of *Pythium ultimum* Trow. var *ultimum* was originally isolated from stem leaf base rot of *Brassica rapa* L. Pekinensis Group, formally known as *Brassica peinensis* Rupr. (Chinese cabbage) at Narromine, NSW in 1980 by D. Trimboli and was subsequently stored in small pieces of potato dextrose vegemite agar in sterile water.

Inoculum of *P. ultimum* was prepared using the method of Chen *et al* (1987), as a modification of the method of Ko and Hora (1971) for preparing *Rhizoctonia* inoculum. Fifty grams of chopped potato and 500 mL of fine, sandy loam soil, collected from the Nepean River at Elizabeth Macarthur Agricultural Institute, Macarthur, were added to a 1L flask. Water was added to give 50% moisture holding capacity to the chopped potato soil medium. The chopped potato soil medium was autoclaved for 1 hr on 2 consecutive days. The medium was inoculated with 1x1x0.25 cm cubes of *Pythium* culture on potato dextrose vegemite agar at 6 cubes per flask, using aseptic techniques. Each flask was incubated for 14 days at 25°C. The inoculum was removed from the flask, placed on a tray and dried in the laminar flow cabinet. In Experiment 1 the inoculum was dried overnight. In Experiments 2 to 7 inoculum was dried in larger clumps for two days, instead of overnight drying as the viability of the inoculum was low. Inoculum was then ground with a mortar and pestle, passed through a 2 mm mesh sieve and collected on a 1 mm sieve. The 1 to 2 mm diameter particles were kept as inoculum in a sealed flask for several months at ambient temperature (18-25°C).

Viability of the Oomycete pathogen decreased gradually over time, but the viability of inoculum was checked for each experiment so that the volume of inoculum could be readjusted to achieve a constant concentration of propagules in the potting media.
In Experiment 1 low viability of the inoculum may have been due to rapid drying, which promotes conversion to thick walled oospores, which do not germinate to cause disease and are not detected in plating assays (Bruehl, 1987; Lumsden and Ayers, 1975). Drying the inoculum more slowly, as compared to rapid drying, has been found to promote the conversion to sporangia or thin walled oospores, which do germinate and cause disease (Tesoriero, pers. comm.).

The population of viable \textit{P. ultimum} propagules in the inoculum was determined using a soil surface dilution plating technique. This method is described in Section 2.4, using the inoculum as the sample for enumeration.

\textbf{2.3 Determination of the Severity of Disease Rating index (SDR) Using Cucumber Bioassays}

A method for determining the suppressiveness of compost amended-potting media to damping-off of seedlings caused by \textit{Pythium} was developed by Chen \textit{et al} (1987) using cucumber seeds. This method has been widely used by various authors, and measures the activity of the pathogen in the mix through a rating scale of the severity of disease caused in the seedlings (Boehm, 1990; Fahy and Pike, 1991; Mandelbaum \textit{et al}, 1988; Mandelbaum and Hadar, 1990).

\textbf{2.3.1 Cucumber bioassay}

Cucumber seeds were planted in the test potting media, which were each inoculated with a specific amount of \textit{Pythium} inoculum, then incubated under controlled conditions. Uninoculated media were used as controls for the determination of germination rates and to ensure that failure of germination was attributed to the disease caused by \textit{Pythium}, rather than some other property in the potting media or seeds. Cucumbers were used because of their sensitivity to \textit{Pythium} damping-off and their fast germination rate, allowing for the determination of the severity of disease at 10 days after sowing. All plants were assessed for the severity of damping-off and rated from 1 to 4.

Bags containing potting medium, prepared as described in Section 2.1.3 at each AFP treatment, were inoculated with a specified quantity of inoculum, prepared as in Section 2.2. Inoculum concentration in each experiment was calculated by obtaining
an SDR range of 3 to 4 on a scale of 4 in peat:perlite mixes which is considered conducive to allowing *Pythium* damping-off (Noble, Pers comm). Preliminary work by Fahy and Pike showed that a concentration of approximately 20 colony forming units (cfu) per mL of potting media was required to cause significant disease rating in a peat-based medium. The bag was vigorously shaken to ensure uniform distribution of inoculum and *Pythium* counts were determined to range from 0 to 5 cfu/mL moist potting medium. Ten centimetre deep pots (400mL) were placed on separate small drainage dishes to avoid drying out of media. At the bottom of each pot, 1cm layer of perlite was applied so as to aid drainage. The inoculated potting media treatments were added to each pot. Five replicate pots were used per potting medium treatment. Each pot was seeded with 8 "Richmond Green Apple" cucumber seeds (Yates®, Milperra NSW) and covered with 1 cm deep potting media. Hydroponic solution (150mL Simple Grow®, Wetherill Park NSW, General Purpose, 1:1 hydroponic solution:deionised water, see Appendix 1) was added to each pot from the bottom and allowed to be absorbed into the media by capillary action. The pots were watered daily from the bottom with deionised water. Pots were arranged in a random complete block design and plants were grown in a constant temperature room at 18°C under 12 hrs of constant illumination (330±60 µE m\(^{-2}\) s\(^{-1}\)) daily. An example of a cucumber bioassay is demonstrated in Figure 2.1.

Figure 2.1: Cucumber Bioassay
2.3.2 Determination of the Severity of Disease Rating Index (SDR)

Plants were rated for symptoms of disease as follows: Symptomless = 1, diseased with a lesion present, but not causing stem collapse “damping off” = 2, post-emergence damping-off = 3, pre-emergence damping off = 4.

The number of plants showing pre-emergence damping-off was determined by comparison to the germination rate of the uninoculated potting media. For example, if the germination rate is 8 in the uninoculated treatment, and in one of the pots of the uninoculated treatment there are 2 healthy plants, 4 plants with symptoms of post-emergence damping-off, none with a lesion but not damped-off, then, by deduction there would be 2 plants with pre-emergence damping-off. Periodic microbial culturing checks were made to ensure symptoms were consistent with *Pythium* infections. Potting media with a severity of disease rating index below 2.5 were considered to be suppressive to *Pythium* damping-off, whereas media which supported plants rated above 2.5 were considered conducive to disease.

The severity of disease rating index (SDR) for each treatment was determined using the following formula:

\[
SDR = \frac{(4xN4)+(3xN3)+(2xN2)+(1xN1)}{N5}
\]

N1 = Number of plants with a rating of 1
N2 = Number of plants with a rating of 2
N3 = Number of plants with a rating of 3
N4 = Number of plants with a rating of 4
N5 = Number of healthy plants in the control treatments

2.4 Enumeration of *Pythium* in potting media by the most probable numbers technique

The number of *Pythium* colony forming units (cfu) were determined in medium for each treatment and may be defined at a level where disease prediction can occur. A positive correlation has been found between SDR and cfu (Chen *et al*, 1987). If cfu is low and SDR is still high, however, this would indicate that the plants were more susceptible to disease, maybe due to stress caused by the environmental conditions.
Isolation techniques and enumeration of *Pythium* from soil have been reviewed in detail by Tesoriero (1989). Quantitative indices of the pathogen were measured by estimates of infection levels, scores of root lesions and the mortality rate. *Pythium* colonies were counted by spreading specified volumes of various dilutions of soil suspension in several aliquots onto the surface of a selective medium in a Petri dish (Tesoriero, 1989). The number of aliquots which yielded growth of *Pythium* colonies were counted and expressed as a proportion of the total number of aliquots plated. This is a most probable numbers method which permits estimation of the population density without actual counts of colonies (Alexander, 1982). The *Pythium* colonies originate from spores or propagules in potting media. According to Tesoriero (1989), soil suspension spread over the surface of agar was a more effective method for recovery and enumeration of *Pythium* than adding molten agar to an aliquot of soil sample. Tesoriero (1989) found that Potato Carrot Agar (PCA, see Appendix 2) amended with 4 antibiotics and $\beta$-sitosterol was the most satisfactory for counting and identifying *Pythium* out of a range of media tested. The modified PCA medium was shown to be highly selective for *Pythium*: antibiotics eliminated growth of bacteria and the $\beta$-sitosterol induced sexual structures which enabled identification. The cultures were examined under a light microscope to confirm the identity of the species.

Samples were collected as described in Section 2.1.2. Each sample of potting media (50mL) was added to a 500 mL Erlenmeyer flask with sterile 0.2 % water agar (150mL) and shaken for 30 min in an orbital shaker (90 rpm, Bio-Line®) at ambient temperature (20-25°C). The contents of each flask were aseptically and serially diluted by 4 fold into 5 McCartney bottles containing 0.2 % water agar (15 mL). Eight replicate drops (100 µL) were pipetted by a automatic pipette (Gilson®) onto a plate of modified PCA medium using one plate for each dilution. All plates were incubated at ambient temperature (18-25°C) for 24 hrs. Incidence of total *Pythium* propagules present was estimated from the frequency of droplets showing growth of mycelium of *Pythium* per plate. The most probable numbers of *Pythium* was determined using Genstat® Primelink version 3.0.5 computer program and expressed as the natural log of colony forming units (log cfu) per mL of potting medium for each treatment.
2.5 Measurement of Microbial activity

Microbial activity is indicative of suppressiveness only when potting media are not stimulatory to population development of *Pythium* (Chen et al, 1988a and b). Most methods that have been developed for analysis of microbial activity and biomass in soil, tend to be cumbersome, time consuming and require expensive equipment (Schnürer and Rosswall, 1982).

Preliminary work by Fahy et al (1995) which involved screening a number of methods for measurement of biological activity, such as luciferase enzyme assay, substrate respiration method and fumigation extraction method, showed that there were no simple assays suitable for a large number of samples and these measurements of biological activity did not correlate with test potting media materials of known levels of microbial activity and suppressiveness to damping-off caused by *Pythium*. ATP analysis, which involves extraction of ATP from cells and is indicative of the level of microbial activity in the mix, has the main advantage in that there is no incubation needed, but specialised equipment for measurement is required and prolonged incubation times may change cell numbers or physiological status. This method is very sensitive, but requires careful extraction to prevent hydrolysis of ATP (Jenkinson and Oades, 1979). Fahy et al (1995) found that measurements obtained using ATP analyses by a luciferase enzyme assay developed by Jenkinson and Oades (1979) related more to potting media type. Peat materials tended to have a high adsorption onto organic materials, leading to erroneous results. Analysis using measurements of respiration involve the measurement of CO₂ or O₂ after incubation of several hours, which is more difficult for large numbers of samples (Van Cleve et al, 1979). The fumigation extraction method, developed by Jenkinson and Powelson (1976), was claimed to be suitable for highly decomposable materials, however; Fahy et al (1995) found that this method did not correlate with microbial activity and suppression of *Pythium*.

Microbial activity in all experiments were determined by the fluorescein diacetate (FDA) hydrolysis method. This method is advantageous since it is rapid, simple and sensitive, and thus useful for comparative studies of microbial activity in natural habitats. This method was first developed by Schnürer and Rosswall (1982).
Microbial activity is measured by determining the concentration of fluorescein released from FDA hydrolysis, by a number of microbial enzymes, such as proteases, lipases and esterases. This method involves incubating a measured quantity of soil sample with FDA for a specified time with termination of the reaction with acetone, which inhibits any further hydrolysis by destroying cell membranes and releasing the fluorescein from cells. Fluorescein is measured spectrophotometrically at 490 nm. Microbial activity of 0.8μgFDA/min/mL of potting media is the minimum level of microbial activity considered necessary for Pythium suppression (Fahy and Pike, 1991 and Fahy et al, 1995).

Inbar et al (1991) found that the microbial activity assay resulted in significant fluorescein adsorption to organic matter fraction of peat based container media. These problems, however, were not encountered for hardwood or compost amended potting media (Chen et al, 1988a & b). Chen et al (1988a) modified the procedure by incubating the reaction mixture for 20min, as the reaction was linear up to at least 20min, and eliminated the centrifugation step prior to the addition of acetone as the medium did not result in a high background absorbance. In the procedure described below, 5mL of sample was used instead of 5g of sample so as to describe the microbial activity occurring in a pot and such that potting media of various densities, but with the same volumes, could be compared with one another. The Australian Standards for Potting Mixes (AS3473-1989) also expresses the results for all tests on a volumetric basis rather than a dry weight basis.

FDA (Sigma Chemicals®) was dissolved in acetone (Ajax Chemicals®, AR grade) and stored as a stock solution (2 mg mL\(^{-1}\)) at -20°C. Four to five replicate samples (5mL) of the already pooled samples, described above in Section 2.1.2, from each treatment, were added to a 250 mL conical flask with 20 mL of Sorensen's 60 mM potassium phosphate buffer, pH 7.6 (Appendix 3: formulation). The reaction was commenced by the addition of 400μg FDA stock solution to the replicate samples, except for one flask, which was used as a blank. These flasks were shaken (90 rpm) for 20 min on an orbital shaker (Bio-Line®) at ambient temperature (20-25°C). After incubation, the reaction was terminated with 20 mL acetone per flask (Ajax Chemicals®, AR grade). The samples were filtered through a Whatman No 1 filter.
paper. The absorbance was measured at 490 nm within 30 minutes after termination of the reaction, using a Cecil Ce 1020® photospectrometer, 100 Series, with a Zippy ATA® Scientific attachment. Hydrolysis of FDA was determined from the standard curve (Appendix 4), which was prepared as described below, and expressed as per mL, rather than per gram, of potting media.

Standard curves were prepared in duplicate for each container medium at each AFP level. Various quantities of FDA (0, 50, 100, 200 and 400ug in the stock solution) were added to 5.0mL of Sorensen's potassium phosphate buffer, pH 7.6 (See Appendix 3) in 5 separate screw capped tubes. These tubes were then placed in a boiling water bath for 1 hr, to chemically hydrolyse all the FDA. The tubes were removed from the water bath and allowed to cool to room temperature. The fluorescein mixtures were then incubated with each of the potting media, such that the absorption of fluorescein into potting media could be accounted for during measurement of the samples. Each concentration of fluorescein was added to a series of 250mL conical flasks each containing 5mL of each potting medium. Another 15 mL of buffer solution was added to each of the tubes to wash out the remaining fluorescein into each flask. The flasks were incubated, the reaction was terminated, filtered and measured, as previously described.

2.6 Measurement of Phytotoxicity

Fahy et al (1995) developed a rapid and sensitive assay, modified from the method described by Zucconi et al (1985), for measurement of phytotoxicity. Preliminary work by these authors showed that the phytotoxicity assay in the Australian Standards for Potting Mixes (AS3473-1989, Standards Australia) was found to be too time consuming and difficult for accurate assessment.

Filtered extract (0.5mL) of potting media shaken in deionised water at a 2:3 volumetric ratio for 60 min was added onto a 4.5cm filter paper (Whatman No 1) placed in a 4.5cm Petri dish. The filter paper was sown with 8 evenly spread cress seeds (Lepidium sativum, Curl Cress). Eight seeds were also placed onto a filter paper containing deionised water (0.5mL) and used as a standard control. Five replicate plates were assayed for each treatment. Petri dishes were incubated in the dark at 26°C for 24 hr. The reaction was stopped with 1.5mL ethanol per dish. Each
seed was scored for the rate of germination and the length of each radicle. A phytotoxicity index for each replicate was calculated as:

\[
\frac{\text{radicle length for each treatment}}{\text{radicle length for the control treatment}} \times \frac{\text{germination rate for each treatment}}{\text{germination rate for the control treatment}} \times 100
\]

Values less than 80 show that there is significant toxicity and values less than 60 also inhibit radish (cv early scarlet Globe) emergence at 26°C.

2.7 Statistical Analysis

Maximum likelihood estimates, logistic regression analysis, analysis of variance, t-tests (LSD) on AFP effects on SDR, *Pythium* counts and microbial activities, means and standard errors of the mean for SDR, *Pythium* counts and microbial activities were determined using Microsoft Excel®, SAS® and Fastat® Programs.
Section 3: Effects of Air-Filled Porosity and its interactions with microbial activity, maintenance of media moisture and temperature on *Pythium* Suppression.

3.1 Introduction

Incorporation of compost in potting media has been shown to be beneficial, due to high microbial activity which suppresses *Pythium* (Chen *et al.*, 1988b; Boehm and Hoitink, 1992). Fahy and Pike (1991), however, suggested that high microbial activity in media of low AFP may induce anoxia in the mix, and increase the susceptibility of the host to disease, as compared to media with low microbial activity.

Low AFP increases the water content and decreases the oxygen diffusion rate of soils (Griffin, 1981) and root and microbial respiration may deplete the available oxygen concentration leading to anaerobic conditions, which may increase the concentration of reduced metal ions, organic acids and volatiles, which are potentially toxic to microorganisms (Lockwood and Filonow, 1981) and the plant (Drew and Lynch, 1980). High soil moisture accompanied by anaerobic conditions may increase the numbers of immature or damaged roots suitable for infection by *Pythium* and thus, increase the severity of disease (Schmitthenner, 1970).

Low AFP also increases the diffusion of solutes and the mobility of bacteria due to concurrent increase in WHC (Griffin, 1981) and these conditions may, thus, increase the ability of bacteria to compete more effectively with *Pythium* for nutrients. The combined effects in media, of low AFP with high microbial activity, may adversely affect the host, pathogen and microbial activity, or, favour specific microorganisms. This may either increase or decrease the severity of disease caused by *Pythium* depending on the balance of these factors.

It may be possible to reduce and/or control the incidence and severity of *Pythium* damping-off in susceptible plants by manipulating the physical characteristics of potting media, such as AFP. Coetzee *et al.* (1993) found that optimal vegetative growth of lemon seedlings occurred at AFP 10 to 15% but recommended an AFP 14
to 20% for citrus nurseries, as Filmer et al (1986) found that the severity of disease caused by *Phytophthora* was greater in media with AFP of 10% or less.

It has been shown that a decrease in AFP, or addition of fine sand to media, increased the incidence and severity, or incidence, of diseases caused by *Pythium* and *Phytophthora* (Fahy et al, 1995; Filmer et al, 1986; Spencer and Benson, 1982; Ownley and Benson, 1991). In studies by Fahy et al (1995) and Spencer and Benson (1982), altering AFP was achieved by changing the organic components of media. Other dependent variables, therefore, such as salinity, nutrient concentration and microbial activity, may have also influenced the severity or incidence of disease. In a study by Filmer et al (1986), the effects of AFP on the severity of disease were studied using inert media and this relationship may be drastically altered by the presence of high microbial activity in compost. The effects of AFP on *Phytophthora* were also determined and, although *Phytophthora* is closely related to *Pythium*, these Oomycota may vary in response to AFP and so this information can be used as a guide only.

A series of four experiments was initially conducted to examine the effect of AFP on *Pythium* damping-off, without altering the organic content of media, but enabling a preliminary study of other factors known to affect the disease.

In Experiment 1, a wide range of AFP<sub>std</sub> values from 4.2 to 25.7% (AFP<sub>act</sub> 2.4 to 25%) was screened in potting media containing compost and sand, to determine their effect on suppression of *Pythium* damping-off of cucumber seedlings. The range of AFP values extended below and above the AFP specifications recommended in the Australian Standard for potting mixes at the time (AS3473-1989, Standards Australia).

In Experiment 2, the range of AFP<sub>std</sub> was extended further to 2.4% (AFP<sub>act</sub> 1.8%) in response to results in Experiment 1. This allowed the results from Experiment 1 to be confirmed, while AFP less than 4.2% was included to determine the effect on the suppressiveness of potting media in relationship to anaerobism. In addition, the unheated and heated subtreatments of media were applied before sowing, to examine the interaction of microbial activity with AFP and its contribution to suppression at various AFP values.
In Experiment 3, the range of AFP_{std} 6.3 to 22.8% (AFP_{act} 2.4 to 19.1%), in combination with WHC_{std} 43 to 55% (WHC_{act} 48 to 58%), were screened in potting media to determine their effect on suppression of *Pythium* damping-off. The range of AFP_{std} values were within the range used in previous experiments, except that WHC_{std} was increased to 43 to 55%, as compared to WHC_{std} 21 to 44% in Experiments 1 and 2, by incorporating spaghnum peat in the potting media and by decreasing the height of the pots from 8.5 to 4.5cm. The objective of decreasing the pot height and increasing WHC was to mimic pots commonly used in the nurseries. Potted plants are also often exposed to very high temperatures, over 30°C in the middle of the day in Australian nursery conditions (Cresswell, pers. comm.). Temperature affects both the medium and the plant and may stress, or, favour the plant, pathogen or microbial activity in various combinations. Cresswell (pers. comm.) conducted a trial using lettuce grown hydroponically and found a higher incidence of *Pythium* root rot in higher solution temperature treatments. Three sub-treatments, therefore, were included in Experiment 3, where seedlings were exposed to heat shock treatments of 18, 35 and 45°C in media of the various AFP and WHC values.

In Experiment 4, the effect of AFP on the suppression of *Pythium* damping-off in five other species of plants was considered, to compare with results from the bioassays, which were used in previous experiments, and from research by Bateman (1961), Fahy *et al* (1995), Filmer *et al* (1986) and Kerr (1964). AFP_{std} 5.2 and 16.1% (estimated AFP_{act} 2.6 and 10.8%) were chosen, as AFP_{std} 5.2% was found most suppressive and AFP_{std} 16.1% was significantly more conducive to *Pythium* damping-off in the previous experiments with cucumbers and also to test the extreme AFP values recommended by the Australian Standard for potting mixes, using species of plant other than cucumbers as severity of damping-off may be affected by the plant itself.

### 3.2 Materials and methods

The potting media were prepared for each experiment, as summarised below, as modifications of the general methods presented in Section 2.1. AFP and WHC were measured on each medium, as in Section 2.1.1. All experiments compared
treatments, with and without inoculation with *Pythium*, as described in Section 2.3.1, except for Experiment 2 as described in Section 3.2.2. The suppressive qualities of media were determined by measurement of microbial activity on the same day as sowing (d0) and on the day of harvesting plants (d10), with *Pythium* counts and SDR on the day of harvesting plants as described in Sections 2.1.3 to 2.5.

### 3.2.1 Experiment 1

Five AFP_{std} treatments were prepared, from 4.2 to 25.7% (estimated AFP_{act} 2.4 to 25%) (See Table 2.1). Potting media were prepared using the following ratios of sand grades: 100 % fine sand, 1:1, 1:3 and 1:6 of fine sand:coarse sand, and 100 % coarse sand. The sand mixtures were then blended with 33% composted hardwood sawdust by volume (See Table 2.1). The inoculum rate of *Pythium* for this bioassay was 7gL^{-1} of potting media.

### 3.2.2 Experiment 2

The AFP treatments consisted of potting media of 6 different AFP_{std} (2.4 to 25.7%) (estimated AFP_{act} 1.8 to 25%), as stated in Table 2.1. For each AFP there were 2 subtreatments, heated (H) and unheated; the unheated treatment consisted of 2 subtreatments, inoculated (U) and uninoculated treatments (C). Preliminary work showed that media germination rates between heated and unheated uninoculated mixes were the same, therefore, one uninoculated treatment was prepared as a set of controls (C). This reduced the workload of measurement to 90 pots total in the experiment, but allowed a comparison of germination rates between uninoculated and inoculated treatments. All other treatments were inoculated with *Pythium*.

The medium of AFP 2.9% was prepared with 33% compost with 2:1 fine sand:coarse sand. Media of AFP 2.4 and 5.2 to 25.7% were prepared as described in Section 2.1, with the ratios of fine to coarse sands and compost as in Experiment 1, except lower AFP values were achieved with unsieved, fine sand. Prior to sowing the cucumber bioassay, all mixtures for the H treatment were placed sealed bags and heated at 60°C for 3 days, in an oven (Qualtex®, Watson Victor Pty Ltd) to reduce the microbial activity of the media.
The inoculum rate of *Pythium* for this bioassay was 0.5 g L\(^{-1}\) of potting medium. In each U treatment there were 7 replicate pots, while there were 5 replicate pots in each of the 2 other treatments. The extra 2 replicates were added in the U treatment to reduce variability. The pots were covered with a plastic sheet during the first 4 days of the bioassay to prevent drying of the surface of the potting media while seeds germinated.

### 3.2.3 Experiment 3

The AFP treatments consisted of potting media of 4 different AFP\(_{\text{std}}\) (6.3 to 22.8%) (measured AFP\(_{\text{act}}\) 2.4 to 19.1%) as stated in Table 2.1. Potting media were composed of mixtures of composted hardwood sawdust, sand supplied from Australian Native Landscapes Pty Ltd (NSW), and Kiwi Spaghnum peatmoss (New Zealand). The two sand grades were prepared as described in Experiment 2. The ratios of sand grades used to achieve 4 different AFP were 100% fine sand, 1:1 and 1:3 fine:coarse sands and 100% coarse sand. Potting media were prepared by combining 33% compost, 22.5% peat and 44.5% premixed sand mixtures. The inoculum rate of *Pythium* for this bioassay was 2.5 g L\(^{-1}\) of potting medium.

Pots were maintained at container capacity by covering the pots in plastic bags during the whole bioassay. Heat subtratments were applied to media at all AFP's after seedling emergence. The temperatures chosen were similar to those used for the lettuce in Cresswell's study (pers. comm.), where higher temperatures resulted in a higher incidence of disease. For 2 of the subtratments, pots were sealed in plastic bags when the seedlings had emerged and were exposed to 2 hour heat treatments (Temp35 and Temp45 treatments) in an oven (Qualtex\(^{\text{R}}\), Watson Victor Pty Ltd), set at temperatures of 35°C±1°C and 45±1°C, respectively. For the third treatment, pots were left in the growth room at a constant 18±1°C (Temp18 treatment).
3.2.4 Experiment 4

Two AFP values (AFP$_{std}$ 5.2 and 16.1%) were used in bioassays with 5 species of plants, namely Celosia (Celosia argentea), Chilli Pepper (Capsicum annuum), Snapdragon (Antirrhinum majus), Salvia (Salvia cv Cleopatra rose) and Impatiens (Impatiens balsamina). These species were chosen as they were known to be sensitive to this pathogen in several NSW nurseries (Fahy, P, pers. comm.; Farr et al, 1989; Parrini and Rumine, 1989).

Plant Bioassay 1 (B1) was prepared using a procedure described in Section 2.3.1, except for changes shown in Table 3.1, to adjust for differences for each species. Each species of plant was grown in potting media of AFP$_{std}$ 5.2 and 16.1% (estimated AFP$_{act}$ 2.6 and 10.8%), prepared as described in Experiment 2. The inoculum rate of *Pythium* for B1 was 0.5gL$^{-1}$ of potting medium. Pots were sown with a specific number of seeds and at various depth levels, depending on the species, as listed in Table 3.1 below. Fourteen days after sowing, SDR, *Pythium* counts and microbial activities were determined as per Sections 2.3.2, 2.4 and 2.5.

Three days after the plants had been harvested, the second bioassay (B2) was prepared and pooled media from each treatment in B1 were placed in pots and resown. Pots were individually placed in plastic bags to prevent drying out. The bags were removed once the seedlings were well developed, being less dependent on high moisture content for growth. Table 3.1 specifies the length of time plants were left in bags, sowing rates for each plant and depths of sowing. SDR, *Pythium* counts and microbial activities were determined 14 days after sowing.

**Table 3.1:** Sowing rate and sowing depths for the 5 plant species and times that pots were left in plastic bags used in B2 for Experiment 4.

<table>
<thead>
<tr>
<th>Species</th>
<th>Planting depth of seeds (mm)</th>
<th>Nos of seeds per pot in B1</th>
<th>Nos of seeds per pot in B2</th>
<th>Time pots were left in plastic bags in B2 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Impatiens</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Celosia</td>
<td>6</td>
<td>20</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Chilli Pepper</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>Salvia</td>
<td>5</td>
<td>12</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Snapdragon</td>
<td>3</td>
<td>70</td>
<td>70</td>
<td>14</td>
</tr>
</tbody>
</table>

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3.3 Results and discussion

3.3.1 Experiment 1 Results

3.3.1.1 Effect of AFP on the Severity of Disease Rating index (SDR)

There was a significant quadratic relationship between AFP\textsubscript{std} and SDR (p=0.001) so that SDR increased as AFP\textsubscript{std} to 14%, then decreased at AFP\textsubscript{std} greater than 18%. The SDR of the cucumbers was significantly lowest for potting media of AFP\textsubscript{std} 4.2% (Figure 3.1a). SDR at AFP\textsubscript{std} 6.7, 18 and 25.7% were not significantly different from each other (p$\leq$0.05), but were significantly higher than for media of AFP\textsubscript{std} 4.2% (1.6 units) and lower than for media of AFP\textsubscript{std} 14% (3.8 units). Emergence of seedlings was delayed by 2 to 3 days at AFP\textsubscript{std} 25.7%, compared to other AFP\textsubscript{std} treatments.

3.3.1.2 Effect of AFP on Pythium counts

AFP accounted for 79% variation of Pythium counts (p=0.003) and a strong quadratic relationship was found on regression between AFP and Pythium counts (r=0.83, p=0.001). The Pythium count was significantly lowest at AFP\textsubscript{std} 4.2% (10cfu/mL potting media) and highest at AFP\textsubscript{std} 14% (3851cfu/mL potting media), but not significantly different to AFP\textsubscript{std} 6.7 to 25.7% (347 to 3851cfu/mL potting media) (Figure 3.1b).

3.3.1.3 Effect of AFP on Microbial activity

AFP accounted for 95% variation in microbial activity (d0) (p=0.0001) and a strong quadratic relationship was found on regression between AFP\textsubscript{std} and microbial activity (d0) (r=0.88, p=0.02). Microbial activity (d0) was lowest at AFP\textsubscript{std} 25.7% (1.15 $\mu$gFDA/min/mL potting media) and highest at AFP\textsubscript{std} 14% (1.625 $\mu$ gFDA/min/mL potting media) (Figure 3.1c).

AFP\textsubscript{std} accounted for 86% variation in microbial activity (d10) (p=0.005), but a strong quadratic relationship was found on regression between AFP\textsubscript{std} and microbial activity (r=0.79, p=0.0003). Microbial activity (d10) was lowest at AFP\textsubscript{std} 25.7% (1.15 $\mu$gFDA/min/mL potting media) and highest at AFP\textsubscript{std} 18% (1.67 $\mu$ gFDA/min/mL potting media) (Figure 3.1c).
Microbial activities were higher on d0 (1.508-1.625 μgFDA/min/mL potting media) than on d10 (1.392-1.408 μgFDA/min/mL potting media) at AFP\text{std} 4.2% and 6.7%, whereas, activities were higher on d10 (1.667 μgFDA/min/mL potting media) than on d0 (1.383 μgFDA/min/mL potting media) at AFP\text{std} 18%, while microbial activities of the other AFP\text{std} treatments were similar on d0 and d10 (1.150-1.650 μgFDA/min/mL potting media).
Figure 3.1: Experiment 1. The effect of Air-Filled Porosity (AFP_{std}) at 4.2, 6.7, 14, 18 and 25.7% on damping-off caused by *Pythium ultimum* in the cucumbers grown in potting media containing composted hardwood and sand. Note: Data points with the same letter(s) and same upper/lower case are not significantly different (p=0.05).

3.1a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

3.1b) Mean *Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing.

3.1c) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium on day of sowing (d0) and day 10 (d10) after sowing.
3.3.2 Experiment 1 Discussion

Suppression of disease occurred only in the potting medium of AFP\textsubscript{std} 4.2%, while all other mixes were conducive to disease as shown in Figure 3.1a.

It was expected media of low AFP (1-6%) would be more conducive to disease than at high AFP as found by Fahy \textit{et al} (1995) and Filmer \textit{et al} (1986). Fahy \textit{et al} (1995) found a moderately negative correlation between AFP\textsubscript{std} and SDR but this relationship was not consistent in all their trials. Materials aged over 9 months, which were consistently suppressive to disease, had an AFP\textsubscript{std} in the range of 13 to 30%, whereas consistently conducive mixes had an AFP\textsubscript{std} range of 6 to 12%. The AFP\textsubscript{std} range used in their study were typically above 6% as compared to the suppressive mix of AFP\textsubscript{std} 4.2% in this experiment.

Filmer \textit{et al} (1986) reported that the incidence of \textit{Phytophthora} disease increased as AFP\textsubscript{act} decreased in media in 13cm pots. Trends in this study were similar to that found by Fahy \textit{et al} (1995), which demonstrated that AFP has a direct effect on a pathogen of similar ecology to \textit{Pythium}, but the species of plant as well as media types were different. Filmer \textit{et al} (1986) used media made up with mineral substrates, which were relatively inert to microbial activity, as compared to compost amended media used by Fahy \textit{et al} (1995). Filmer \textit{et al} (1986) did not statistically determine if AFP treatments caused significantly different effects, but according to the error bars in their graph, only the medium of AFP\textsubscript{act} 1% was significantly more conducive to disease than all other media of AFP\textsubscript{act} 5 to 20%, which did not cause significantly different effects.

Although the severity of disease increased with increasing AFP to a maximum at AFP\textsubscript{std} 14%, microbial activity also increased to a maximum at AFP\textsubscript{std} 14% on day 0 and AFP\textsubscript{std} 18% on day 10 of the bioassay. In conjunction with these increasing microbial activities, \textit{Pythium} counts were also increasing and may not have been sufficiently suppressed by antagonistic microbial activity. This was not expected, as high microbial activity has been previously shown to decrease \textit{Pythium} counts, resulting in a suppressive mix (Chen \textit{et al}, 1988b; Boehm and Hoitink, 1992; Fahy and Pike, 1991).
The microbial activity of all media was above the level recommended by Fahy and Pike (1991) of 0.8 μgFDA/min/mL potting media, considered necessary to achieve suppression. The SDR was still unacceptable, however, at AFP<sub>std</sub> 6.7 to 25.7%. Media of AFP<sub>std</sub> 14 and 18% had the highest microbial activity (d10) (1.625 to 1.667 μgFDA/min/mL potting media), but were the most conducive to disease. It should be remembered that the measurement of microbial activity does not distinguish between Pythium and other microorganisms in media. Pythium counts were highest at AFP<sub>std</sub> 14 and 18% (3210-3851 cfu/mL potting media) and the pathogen may have contributed significantly to the high microbial activities (d10) measured in these samples. At AFP<sub>std</sub> 18%, microbial activity increased over the 10 days and appears most likely due to increasing Pythium counts. Pythium count also was highest at AFP<sub>std</sub> 14% as compared to other AFP treatments, but as microbial activity did not change over 10 days, Pythium perhaps displaced other microbes in this case. At AFP<sub>std</sub> 4.2% and 6.7%, microbial activities decreased over the 10 days, and may have been due to low oxygen concentration and anaerobic conditions. At AFP<sub>std</sub> 25.7%, microbial activity was consistently low on d0 and d10 and may have been a direct result of low moisture conditions (WHC<sub>std</sub> 21.2%), or, indirectly, of decreased plant exudation since the emergence of seedlings was delayed.

Low Pythium counts (10cfu/mL potting media) at AFP<sub>std</sub> 4.2% most likely accounted for low severity of disease, as compared to conducive media of AFP<sub>std</sub> 6.7 to 25.7%, which had Pythium counts in the range of 347-3851cfu/mL potting media. Filamentous fungus are able to grow through air pores, while unicellular organisms, such as bacteria, are dependent upon continuous aqueous pathways to move by Brownian movement, or, flagella motility towards nutrients by chemotaxis (Griffin, 1981). Filamentous-like organisms, such as Pythium, have a competitive advantage, therefore, over bacteria in media of high AFP (Griffin, 1981), which can grow by extending hyphae to the source of nutrients. At AFP<sub>std</sub> 6.7%, plants may have been more susceptible to disease, due to low AFP, or plant injury, due to oxygen deficiency compounded by high microbial activity. At AFP<sub>std</sub> 4.2% growth of Pythium was severely limited, however, and apparently could not take advantage of the already susceptible plant. Plants may be less stressed at AFP<sub>std</sub> 14 and 18%, providing sufficient exudates for attracting Pythium, while media of AFP greater than
18% may be too dry for root exudates to diffuse through the media and/or growth of *Pythium*. Kerr (1964) found that plant exudates from Peas, however, increased as soil moisture increased, but soil moisture in Kerr's study ranged from only 3 to 13% as compared to WHC$_{std}$ 21 to 44% in this experiment and roots of pea plants probably may vary in their type and amount of exudation compared to cucumbers. At AFP$_{std}$ 25.7%, cucumbers may have been more susceptible to disease, due to low moisture content, as the emergence of seedlings was delayed by 2 to 3 days, as WHC$_{std}$ of media was 21.2%, which is less than half of the recommended level for germinating seeds. The slower germination time may have allowed time for the pathogen to infect seeds despite drier conditions.

Unpublished data by Fahy and Pike (pers. comm.) showed that *Pythium* counts of 1100 cfu/mL or greater in potting media, tended to result in an SDR greater than 2.5 index. At AFP$_{std}$ 6.7 and 25.7%, *Pythium* counts of 649 and 347 cfu/mL potting media respectively, resulted in a conducive mix, which may have been due more to plant stress than to the pathogen activities. These counts, however, were not significantly different to counts measured at AFP$_{std}$ 14 to 18% (3210-3851 cfu/mL potting media).

The pathogenic activities of *Pythium* may be limited at AFP$_{std}$ 4.2% due to the interaction of gaseous elements in the mix, causing significantly reduced levels of *Pythium*, compared to other AFP treatments. Brown and Kennedy (1966) and Mitchell and Mitchell (1973) showed that very low O$_2$ content of 1-1.3% reduced the growth of *P. ultimum* and *P. irregulare* by 20-65% compared to O$_2$ content of 20-21%, but this was in axenic, liquid culture. Oxygen content of 4-8% in soil, however, has been shown to increase the incidence of seed rot, plant exudation, the growth of *Pythium* and decreased root weights compared to 21% oxygen content in soil (Brown and Kennedy, 1966). Morard and Silvestre (1996) have shown that the deficiency of oxygen concentration in the nutrient solution interferes with root supply of nutrients, decreases root cell metabolism and decreases plant growth. In both soil and soilless culture, however, it is rare that the absence of oxygen affects the whole root system, but more likely affects localised areas of the root.
Using O2 and CO2 ranges found in soil of 0-20% O2 and 0.03-15% CO2, Mitchell and Mitchell (1973) found that the ratio of CO2 and O2 gases also influenced the growth of Pythium in liquid culture. The addition of 5% CO2 to 1% and 5% O2 was stimulatory to the growth of Pythium, but, the addition of 5% CO2 to 20% O2 had no effect on the growth of the pathogen. The addition of 15% CO2 to 1, 5 and 20% O2, however, was inhibitory to the growth of Pythium compared to media with the same O2 concentration with 0.03% CO2 or less (Mitchell and Mitchell, 1973). At AFPstd 4.2%, Pythium may have been inhibited due to the accumulation of CO2, as well as, low O2 content and high microbial activity. The concentration of gases is influenced by porosity as the rate of diffusion of oxygen through a gas filled pore is 2.1x10^-1cm²/s, while the rate through water is 2.6x10^-5cm²/s (Griffin, 1972a). Groffman and Tiedje (1991) found that the respiration rate, CO2 production, increased as AFP increased in well drained soils (AFP 22 to 80%), while no significant relationship was found between CO2 production and AFP in poorly drained soil (AFP 10 to 60%). These authors have stated that other investigators have shown either a positive, negative, or, no significant relationship between CO2 production and AFP. The relationship between AFP and CO2 production may have been influenced by the presence of microorganisms, which release CO2 during respiration and vary in their optimal conditions for growth. In this experiment, it would be expected that CO2 production in potting media may have increased, as AFPstd increased to 14-18% then decreased, due to the effect of AFP on microbial activity. At AFPstd 6.7 to 25.7%, Pythium counts were high, therefore, CO2 possibly did not increase to inhibitory levels, or, the higher concentration of O2 favoured the growth of Pythium.

The interaction of gaseous factors with other environmental factors in soil is difficult to measure, due to the complexity of soil dynamics (Brown and Kennedy, 1966; Griffin, 1958; Johnson, 1988; Mitchell and Mitchell, 1973). Potting media may be considered to provide a similar environment to soil, therefore, it is difficult to show conclusively here that shortage of oxygen was a limiting factor for a given microbial activity, due to the consequences of the presence of anaerobic micro-organisms, and where fermentation products may accumulate to toxic levels (Drew and Lynch, 1980). It was not done in this and later experiments and, therefore, it is not possible
to determine accurately the effects of aeration in growing media on the severity of disease.

In conclusion the potting medium of AFP$_{\text{std}}$ 4.2% and WHC$_{\text{std}}$ 21.2%, which are both outside their ranges recommended by the Australian Standards for potting mixes, with microbial activity of 1.500 µgFDA/min/mL potting media, was most suppressive to disease caused by *P. ultimum* in this experiment. Potting media of AFP$_{\text{std}}$ 14 and 18% were shown to be most conducive to the disease. The data in this experiment provides strong evidence which supports that AFP affects the suppression of disease. Modification of AFP results in changes to other physical properties of media, which in turn may influence the interactions of the plant, pathogen and/or antagonistic microflora. Perhaps suppression may still occur in potting media of AFP$_{\text{std}}$ lower than 4.2% and at microbial activities below the recommended level of 0.8µgFDA/min/mL potting media, required to achieve suppression. There is need, additionally, to separate the effects of microbial activity from the physical attributes of the potting media on SDR at low AFP, to determine their influence on the suppression of damping-off caused by *Pythium* and determine if physical conditions alone, or in conjunction with high microbial activity, influence suppression of damping-off caused by *Pythium*. 
3.3.3 Experiment 2 Results

3.3.3.1 Effects of AFP and Heated (H) and Unheated (U) Treatments on the Severity of Disease Rating index (SDR)

Significant positive linear relationships were found between $\text{AFP}_{\text{std}}$ and SDR ($p=0.01$ and $p=0.008$, respectively) for the U and H Treatments. The SDR was significantly lower in U Treatments (1.3 to 2.8 units) than in the H Treatments (2.9 to 4.0 units) for each $\text{AFP}_{\text{std}}$ treatment (Figure 3.2a).

The SDR of cucumber plants for the U treatment decreased as $\text{AFP}_{\text{std}}$ increased to 5.2%, then increased as $\text{AFP}_{\text{std}}$ increased above 5.2%. SDR was significantly lower at $\text{AFP}_{\text{std}}$ 2.9 and 5.2 (1.6 and 1.3 units, respectively) as compared to $\text{AFP}_{\text{std}}$ 2.4, 16.1 and 25.7% (2.3 to 2.8 units). SDR at AFP 12.5% (1.8 units) was not significantly different to those at $\text{AFP}_{\text{std}}$ 2.4 to 5.2%, but significantly lower at $\text{AFP}_{\text{std}}$ 16.1 and 25.7%.

The SDR of plants for the H treatment, increased significantly as $\text{AFP}_{\text{std}}$ increased. The SDR was lowest at $\text{AFP}_{\text{std}}$ 2.4 (2.9 units) and highest at $\text{AFP}_{\text{std}}$ 25.7% (4.0 units).

3.3.3.2 Effects of AFP and Heated (H) and Unheated (U) Treatments on Pythium counts

A significant positive linear relationship was found between $\text{AFP}_{\text{std}}$ and Pythium counts for the U treatment ($r=0.82$, $p=0.01$), but no significant relationship was found between $\text{AFP}_{\text{std}}$ and Pythium counts for the H treatment ($p>0.05$). Pythium counts for the U Treatment were significantly lower at $\text{AFP}_{\text{std}}$ 2.9 and 5.2% (352-372 cfu/mL potting media) compared to $\text{AFP}_{\text{std}}$ 2.4, 12.5 and 25.7% (896-1177 cfu/mL potting media) (Figure 3.2b). Pythium counts for the H Treatment were not significantly different at any $\text{AFP}_{\text{std}}$ (1848-2960 cfu/mL potting media) (Figure 3.2b).

Pythium counts were significantly lower in U Treatments (352-1177 cfu/mL potting media) than in the H Treatments (1848-2960cfu/mL potting media) for each $\text{AFP}_{\text{std}}$ treatment. $\text{AFP}_{\text{std}}$ alone does not significantly account for the variation in Pythium counts.
counts ($r=0.89, p>0.05$), but, U and H Treatments had a significant effect on *Pythium* counts ($p=0.0001$).

### 3.3.3.3 Effects of AFP and Heated (H) and Unheated Treatments (U) on Microbial activity

No significant relationships were found between $\text{AFP}_{\text{std}}$ and microbial activity (d0) for the U and H treatments ($p>0.05$). Microbial activity (d0) for the U treatment was not significantly different at any AFP (0.764 to 0.924 $\mu$gFDA/min/mL) ($p \leq 0.05$) (Figure 3.2c). In the H treatment, microbial activities (d0) were not significantly different at each AFP (0.282 to 0.399 $\mu$gFDA/min/mL) ($p \leq 0.05$) (Figure 3.2c).

Microbial activity (d0) was significantly higher in the U treatment (0.764-0.924 $\mu$ gFDA/min/mL potting media) than the H treatment (0.306-0.399 $\mu$ gFDA/min/mL potting media) (Figure 3.2c). AFP and the H and U treatments accounted for 98% variation in microbial activity (d0) ($r=0.99, p=0.03$).

A significant positive linear relationship was found between $\text{AFP}_{\text{std}}$ and microbial activity (d10) for the U treatment ($r=0.96, p=0.001$), but no significant relationships were found between $\text{AFP}_{\text{std}}$ and microbial activity (d10) for the H treatment ($p>0.05$). Microbial activity (d10) for the U Treatment was significantly higher at $\text{AFP}_{\text{std}}$ 5.2% (1.208 $\mu$gFDA/min/mL potting media) than at $\text{AFP}_{\text{std}}$ 12.5 to 25.7% (1.152 $\mu$gFDA/min/mL potting media) ($p \leq 0.05$) (Figure 3.2c). Microbial activities (d10) for the H Treatment were not significantly different at any $\text{AFP}_{\text{std}}$ (0.217 to 0.345 $\mu$gFDA/min/mL) ($p \leq 0.05$) (Figure 3.2c).

Microbial activity (d10) was significantly higher in the U treatment (0.903-1.208 $\mu$ gFDA/min/mL potting media) than in the H treatment (0.240-0.345 $\mu$ gFDA/min/mL potting media). $\text{AFP}_{\text{std}}$ or treatments (H and U) alone account for 96% variation in microbial activity (d10) ($p=0.02$ and $p=0.0001$ respectively).

In the U treatment, microbial activities (d0) ranged from 0.764 to 0.924$\mu$ gFDA/min/mL, while microbial activity (d10) ranged from 0.903-1.208 $\mu$ gFDA/min/mL potting media). In the H treatment, microbial activities (d0) ranged from 0.282 to 0.399$\mu$gFDA/min/mL, while microbial activities (d10) ranged from 0.240-0.345 $\mu$gFDA/min/mL potting media.
Figure 3.2: Experiment 2. The effect of Air-Filled Porosity ($\text{AFP}_{\text{std}}$) at 2.4, 2.9, 5.2, 12.5, 16.1 and 25.7% and Heated (H) and Unheated (U) treatments of media on damping-off caused by *Pythium ultimum* in cucumbers grown in potting media containing composted hardwood and sand. Note: Data points with the same letter(s) and same upper/lower case are not significantly different ($p=0.05$).

3.2a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

3.2b) Mean *Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing.

3.2c) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium on day of sowing (d0) and day 10 (d10) after sowing.
3.3.4 Experiment 2 Discussion

Low AFP_{std} (2.9 and 5.2%) and high microbial activity were apparently necessary in combination to achieve optimal suppression. This data is consistent with the previous experiment, where suppression was optimal at AFP_{std} 4.2% in media with high microbial activity, but still contradicts work by previous authors, which showed that suppression of *Pythium* damping-off was greater at high AFP (13 to 30%) than at low AFP (6 to 12%) (Fahy *et al.*, 1995). Factors such as high water content, growth of specific biocontrol agents, lower production of anaerobic compounds, less plant exudation and lower plant stress may also have a role in reducing disease through manipulation of AFP.

The effect of AFP on the suppression of *Pythium* damping-off was dependent on the interaction with microbial activity, as shown by comparison of the unheated and heated treatments. All unheated media were suppressive to disease, except at AFP 25.7%, whereas all heated treatments were conducive to disease. *Pythium* counts in conducive media (SDR, 2.8 to 4.0 units) ranged from 1177-2960cfu/mL potting media, whereas *Pythium* counts in suppressive media (SDR, 1.3 to 2.4 units) ranged from 352-726cfu/mL.

In the unheated media, *Pythium* counts were higher and microbial activity was lower at AFP_{std} 12.5% to 25.7% than at AFP_{std} 2.9 and 5.2%. Higher *Pythium* counts at AFP_{std} 12.5 to 25.7% may be due to higher plant exudation, higher O$_2$ concentration or *Pythium* may have a competitive advantage compared to bacteria, as filamentous fungus-like organisms are able to grow through air pores towards the food source, while bacteria require water pathways for movement (Griffin, 1981). At AFP_{std} 2.9 and 5.2%, perhaps low O$_2$ concentration compared to AFP_{std} 12.5 to 25.7%, reduced *Pythium* counts. Growth of *Pythium*, however, does not appear to be limited by low aeration at AFP_{std} 2.4%, but a higher concentration of CO$_2$ may have accumulated in this mix, stimulating the growth of *Pythium*. Very high microbial activity at low AFP's may contribute to the production of anaerobic conditions, which may directly cause plant injury (Drew and Lynch, 1980), through the presence of anaerobic metabolites.
In this experiment, trends between AFP and SDR were linear, while in the previous experiment trends were quadratic. The SDR in Experiment 1 was highest at $\text{AFP}_{\text{std}}$ 14%, compared to $\text{AFP}_{\text{std}}$ 4.2, 6.7, 18 and 25.7%, while in this experiment, SDR was highest at $\text{AFP}_{\text{std}}$ 25.7%, compared to $\text{AFP}_{\text{std}}$ 2.4-12.5%. Different trends may have been due to a wider range of AFP tested, or the covering of pots in the first 5 days to prevent the surface of potting media from drying out in Experiment 2. The experimental procedure was modified in Experiment 2 to prevent drying of the potting media surface, which may have contributed to the delayed emergence of seedlings at $\text{AFP}_{\text{std}}$ 25.7% and possibly decreased plant exudates, which may have decreased *Pythium* counts in Experiment 1.

In the heated treatment, SDR becomes more severe as AFP increased, although *Pythium* counts and microbial activities were not significantly different at each AFP. Trends in SDR of the H treatment varies from those observed in the U treatment, as there may have been less competition for $O_2$ and nutrients, or reduced concentrations of anaerobic compounds or $CO_2$ in the H treatment, which had lower microbial activity (<0.4 µgFDA/min/mL potting media). Faster germination, in media of lower AFP, may have assisted the plant to develop resistance against infection by pathogens, before *Pythium* counts increased to large enough numbers to cause disease.

At each AFP, microbial activity may have consisted of different proportions of biocontrol agents, pathogen or other microflora which do not adversely affect *Pythium*. In the unheated treatment, microbial activity (d10) was maximal at AFP 5.2% and microbial activity probably would have been mostly attributable to competitive microbial growth, other than *Pythium*, resulting in lower SDR. At $\text{AFP}_{\text{std}}$ 2.9%, microbial activity was significantly lower than at $\text{AFP}_{\text{std}}$ 5.2%, but the SDR were not significantly different and perhaps both *Pythium* and antagonistic microflora were suppressed. At $\text{AFP}_{\text{std}}$ 12.5 to 25.7%, high *Pythium* counts may have contributed significantly to microbial activity. Alternatively, the growth of specific antagonists may have occurred at $\text{AFP}_{\text{std}}$ 2.9 and 5.2% which were unfavourable to the pathogen but may have not been in great enough numbers to be reflected in significant measures of microbial activities. In the H treatments,
however, microbial activity did not vary at day 0 or day 10 of the bioassay and perhaps microorganisms other than *Pythium* decreased in numbers, while *Pythium* increased significantly without changing the overall microbial activity, i.e. there was a proportional change in numbers of propagules of *Pythium* in relation to numbers of other microorganisms.

One factor which is significantly related to decreased SDR and *Pythium* counts is high microbial activity (d10) (0.8µgFDA/min/mL potting media or greater) and this is in agreement with published data (Boehm and Hoitink, 1992; Fahy and Pike, 1991). In unheated media, *Pythium* counts (352-1177cfu/mL potting media) were significantly lower and microbial activities (d10) (0.764-1.152µgFDA/min/mL potting media) were significantly higher, compared to heated media, with greater *Pythium* counts (1848-2960cfu/mL potting media) and lower microbial activities (d10) (0.217-0.345µgFDA/min/mL potting media). In this experiment, the major limiting mechanism on *Pythium* activity was demonstrated to be microbial activities, such as competition, antagonism and parasitism.

In conclusion, both AFP and microbial activity interacted in this experiment to cause the suppression of *Pythium* damping-off of cucumbers. In media of low microbial activity (0.217 to 0.345µgFDA/min/mL potting media), the SDR was lowest at AFP<sub>std</sub> 2.4%, but, was still considered conducive to disease, at SDR greater than 2.5. Media of high microbial activity (0.764 to 1.208µgFDA/min/mL potting media) were found to be most suppressive at AFP<sub>std</sub> 5.2%. AFP and the level of microbial activity may have interacted to influence factors, such as concentrations of O₂, CO₂ and nutrients, the growth of specific antagonistic microorganisms in the medium and created stresses, which may have affected both the susceptibility of cucumber plants to disease and the relative survival and growth of *Pythium* to remain virulent towards the plants.

It was recognised that WHC<sub>std</sub> of media (21.2-43.4%) used in Experiments 1 and 2 were well below the recommended level of greater than 50% in the Australian Standard for Potting Mixes (1989) and, therefore, may have contributed to the contradiction between the results in the previous experiments and published data. Recommended work for the following experiment was to use 4.5cm pots in order to
represent growing conditions commonly used in nurseries, and determine the effect of AFP on suppression of *Pythium* damping-off under these modified conditions. In nurseries, plants are also commonly exposed to very high temperatures (28-45°C) during the day, and this may also influence the severity of *Pythium* disease. Heat exposure of media and plants together, therefore, was also introduced to try and cause stress to the plant in addition to media and determine if this plant stress affected suppression of *Pythium* damping-off at various AFP values.
3.3.5 Experiment 3 Results

3.3.5.1. Effects of AFP and temperature treatments (Temp18, Temp35 and Temp45 treatments) on the Severity of Disease Rating index (SDR)

No significant relationships were found between AFP\textsubscript{act} and SDR for Temp18 and Temp45 treatments, while a significant positive linear relationship was found between AFP and SDR on regression analysis for Temp35 treatment (p<0.05).

In Temp18 treatment, SDR were significantly lower at AFP\textsubscript{act} 16.0 and 19.1% (1.9 units) than at AFP\textsubscript{act} 8.2% (2.7 units), while SDR at AFP\textsubscript{act} 2.4% (2.0 units) was not significantly different to any other AFP\textsubscript{act} treatments (Figure 3.3a). In Temp35 treatment, SDR was significantly lowest at AFP\textsubscript{act} 8.2% (1.1 units) and highest at AFP\textsubscript{act} 2.4 and 16.0% (2.2 to 2.4 units) (Figure 3.3a). In Temp45 treatment, SDRs were not significantly different between AFP treatments (1.0 to 1.2 units) (Figure 3.3a). Seedlings in the latter treatment were approximately half the size of seedlings in the other 2 treatments at day 10.

SDR’s were below 2.5 units for all treatments, except at AFP\textsubscript{act} 8.2% for Temp18 treatment. At AFP\textsubscript{act} 2.4, 16.0 and 19.1% SDR were significantly lower in Temp45 treatment than in Temp18 and Temp35 treatments. At AFP\textsubscript{act} 8.2% SDR was lower in Temp35 and Temp45 treatments than in Temp18 treatment.

3.3.5.2 Effects of AFP and temperature treatments (Temp18, Temp35 and Temp45 treatments) on Pythium counts

No significant relationships were found between AFP\textsubscript{act} and Pythium counts for Temp35 and Temp45 treatments, while a significant quadratic relationship was found between AFP\textsubscript{std} and Pythium counts on regression analysis for Temp18 treatment (p<0.05).

In Temp18 treatment, Pythium counts were significantly lower at AFP\textsubscript{act} 2.4% (241cfu/mL potting media) than at 8.2 to 19.1% (1491-3570cfu/mL potting media) (Figure 3.3b). In Temp35 treatment, Pythium counts were significantly lower at AFP\textsubscript{act} 8.2% (220cfu/mL potting media), highest at AFP\textsubscript{act} 2.4% (1767cfu/mL potting media), but there were no significant differences between AFP\textsubscript{act} 16.0 and 19.1% (723-900cfu/mL potting media) and any other AFP treatments (Figure 3.3b).
In Temp45 treatment, *Pythium* counts (2-7cfu/mL potting media) were not significantly different at any AFP (Figure 3.3b).

At each AFP, *Pythium* counts were significantly lowest in Temp45 treatment than in Temp18 and Temp35 treatments. At AFP_{act} 2.4%, *Pythium* counts were significantly highest for Temp35 treatment, while at AFP_{act} 8.2%, *Pythium* counts were significantly highest for Temp18 treatment and at AFP_{act} 16.0 and 19.1% *Pythium* counts were significantly highest for both Temp18 and Temp35 treatments.

### 3.3.5.3 Effects of AFP and temperature treatments (Temp18, Temp35 and Temp45 treatments) on microbial activity

Microbial activities (d0) ranged from 0.367-0.517ugFDA/min/mL potting media. No significant relationships were found between AFP_{act} and microbial activity (d10) for Temp18 and Temp35 treatments, while a significant positive linear relationship was found between AFP_{act} and microbial activity (d10) on regression analysis for Temp45 treatment (p<0.05).

Microbial activities (d10) for Temp18 treatment, were not significantly different between AFP treatments (0.498-0.587ugFDA/min/mL potting media) (Figure 3.3c). Microbial activities (d10) for Temp35 treatment were not significantly different between AFP treatments (0.472-0.537ugFDA/min/mL potting media) (Figure 3.3c). Microbial activities (d10) (0.528-0.757ugFDA/min/mL potting media) for Temp45 treatment (2-7cfu/mL potting media) were not significantly different at any AFP (Figure 3.3c).

At AFP_{act} 2.4%, microbial activities (d10) were not significantly different in Temp18, Temp35 and Temp45 treatment. At AFP_{act} 16%, microbial activity (d10) was significantly higher in Temp45 treatment than in Temp18 and Temp35 treatments. At AFP_{act} 19.1%, microbial activity (d10) was higher in Temp45 treatment than Temp18 treatment, whereas microbial activity (d10) in Temp35 treatment was not significantly different to any AFP. At AFP_{act} 8.2, microbial activity (d10) was significantly higher in Temp45 treatment than Temp35 treatment, whereas microbial activity (d10) in Temp18 treatment was not significantly different to the other temperature treatments at that AFP.
Figure 3.3: Experiment 3. The effect of Air-Filled Porosity (AFP_{act}) at 2.4, 8.2,
16.0 and 19.1% (AFP_{std} 6.3, 12.6, 17.5, 22.8%) and temperature Temp18 (18°C),
Temp35 (35°C) and Temp45 (45°C) treatments on damping-off caused by
*Pythium ultimum* in cucumbers grown in potting media containing composted
hardwood and sand. Note: For Figure a) data points with the same letter(s) are not
significantly different between AFP treatments and points with the same number(s)
are not significantly different between heat treatments at each AFP and for Figures b)
& c) data points with the same letter(s) are not significantly different (p=0.05).

3.3a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

3.3b) Mean *Pythium* counts, as calculated from the most probable number per mL of
potting medium, on day 10 after sowing.

3.3c) Microbial activity measured as the mean amount of fluorescein diacetate (FDA)
hydrolysed per mL of potting medium on day of sowing (d0) and day 10 (d10) after
sowing.
Figure 3.3 (a)

Figure 3.3 (b)

Figure 3.3 (c)
3.3.6 Experiment 3 Discussion

All media of $\text{AFP}_{\text{act}}$ 2.4-19.1% were suppressive to \textit{Pythium} damping-off. Optimal suppression varied, depending on AFP and the heat shock treatment. Suppression occurred in all treatments (SDR, 1.0 to 2.4 units), except at $\text{AFP}_{\text{act}}$ 8.2% for Temp18 treatment. Strong suppression (SDR, 1.0 to 1.2 units) occurred at all AFP treatments for Temp45 treatment and at $\text{AFP}_{\text{act}}$ 8.2% Temp35 treatment. A combination of AFP and temperature apparently affects the growth of the pathogen, causes various plant stresses, or influences growth of specific microorganisms which, in turn, may vary in their antagonistic or competitive behaviour against \textit{Pythium}, and thus moderate the effects of AFP on the severity of \textit{Pythium} damping-off.

It was expected that AFP would influence SDR, as found in the previous studies by Filmer \textit{et al} (1986) and Fahy \textit{et al} (1995). In the Temp18 treatment, no relationship was found between AFP and SDR. \textit{Pythium} counts were significantly lowest at $\text{AFP}_{\text{act}}$ 2.4% compared to any other AFP, but, did not result in lowest SDR. Perhaps the plant may have been more susceptible to disease in media with both low $\text{AFP}_{\text{std}}$ (2.4%) and high WHC$_{\text{std}}$ (58.2%) due to root damage by low O$_2$ concentration and anaerobic conditions as discussed previously. Media of $\text{AFP}_{\text{act}}$ 8.2% (2.7 units) was most conducive to disease but this may have been caused by higher plant stresses, than in media of $\text{AFP}_{\text{act}}$ 16.0 and 19.1% (1.9 units), or by higher \textit{Pythium} counts than in media of AFP 2.4% (2.0 units). At high $\text{AFP}_{\text{act}}$ (16.0 and 19.1%), the plant may have been less stressed by conditions of high aeration and moderate water content, compared to media with both low AFP and high (WHC 58.2%), which resulted in less susceptibility to \textit{Pythium} damping-off, even with the presence of higher \textit{Pythium} counts.

The SDR in the previous experiments, however, tended to be lower at $\text{AFP}_{\text{act}}$ 2 to 2.6% than at $\text{AFP}_{\text{act}}$ 1.8 and 3.8 to 25.0%. In this experiment, lower microbial activities (0.472-0.757ugFDA/min/mL potting media) occurred, perhaps due to variable microflora in the new batch of compost and, with higher water contents of potting media, may explain the variation from previous experiments. Other factors, such as the biological and chemical characteristics, may have been different also in this compost and altered the suppressive qualities of media. Fahy \textit{et al} (1995) found
that lack of quality control in composting yards leads to inconsistent batches of compost, with the potential to vary in suppressive qualities.

In Experiments 1 and 2, *Pythium* counts ranged from 10 to 906 cfu/mL potting media in suppressive media, while *Pythium* counts in suppressive media in Temp18 treatment ranged from 240 to 3569 cfu/mL potting media. In this experiment, higher *Pythium* counts may have been due to lower microbial activity compared to previous experiments, or to the differences in batches of inoculum, or their age as used in each experiment. Increasing WHC in this experiment may have reduced plant stress at high AFP$_\text{act}$ (19.1 to 25.0%) or increased the ability of microorganisms to compete for sites on the root zone, by increasing the nutrient availability and mobility of bacteria compared to media of similarly high AFP in previous experiments. Microbial activity in this experiment was not significantly different at each AFP, but tended to be higher at low AFP, as found in previous experiments. *Pythium* counts in previous experiments were also lowest in media of low AFP (AFP$_\text{act}$ 2 to 2.6%) than at any other AFP and may have been due to lower O$_2$ concentration, inhibitory levels of anaerobic compounds or CO$_2$, or growth of specific antagonists.

In Temp35 treatment, however, media of AFP$_\text{act}$ 8.2% was most suppressive to disease and may have been due to the growth of specific aggressive antagonists and unfavourable temperature, which reduced *Pythium* counts and SDR. At AFP$_\text{act}$ 2.4%, *Pythium* growth was not limited, as found in Temp18 treatment, and may have been due to increased plant exudation, which may have been caused by increased plant stress due to low aeration, high water content, as well as unfavourable temperature. At AFP$_\text{act}$ 16.0%, drier conditions may have caused plant stress, whereas there was greater microbial activity at AFP$_\text{act}$ 19.1% and this may have reduced the SDR, compared to AFP$_\text{act}$ 16.0%.

In Temp45 treatment, AFP had no effect on SDR as *Pythium* (counts, 2–7 cfu/mL potting media) was probably killed by high temperature and surviving propagules were perhaps limited by high microbial activity. Microbial activity increased significantly in Temp45 treatment and may have been due to the stimulated growth of heat resistant microorganisms found in compost (Fahy et al, 1986) and favoured higher AFP.
Higher temperature did not result in higher disease, as found in Cresswell's study with lettuce (Cresswell, pers. com), but severely reduced the *Pythium* counts and decreased the SDR. Perhaps lettuce is more susceptible to disease at higher temperatures than cucumber, while heating hydroponic solutions, in conjunction with the physical, chemical and biological properties of the solution, may influence the severity of disease compared to this experiment. Cresswell used a different *Pythium* species, *Pythium aphanidermatum*, which favour higher temperature for growth, rather than *Pythium ultimum*.

The mechanism for suppression appears to be easily altered by applying heat treatments, 35 and 45°C. High temperature, 45°C, may have directly killed the pathogen, converted germinable propagules into dormant oospores, or limited growth of the pathogen through competition with the high microbial activity (0.528-0.757ugFDA/min/mL potting media), resulting in low SDR. Residues of the dead and dying *Pythium* would have provided nutrients to other microorganisms, which were favoured by high temperature. Mandelbaum *et al* (1988) found that a 2hr heat treatment of 55°C of medium alone increased microbial activity, but the pathogen, *Pythium*, could proliferate on the excess of available nutrients released by killing microorganisms and heated organic matter. Under unfavourable conditions, *Pythium* converts to thick walled oospores, which can take 1 to 10 weeks to germinate before being able to cause disease (Lockwood, 1990; Lumsden and Ayers, 1975; Nelson, 1987; Stranghellini, 1974). At AFP<sub>act</sub> 8.2 to 19.1%, *Pythium* growth tended to be favoured by a temperature of 18°C than 35°C, but this was only significant at AFP<sub>act</sub> 8.2%. At AFP<sub>act</sub> 2.4%, *Pythium* counts were significantly higher in Temp35 treatment than in Temp18 treatment and perhaps the plant was stressed or injured at low AFP at 35°C, increasing plant exudation and stimulating the growth of *Pythium*, compared to Temp18 treatment.

In conclusion, there appeared to be an interaction between temperature and AFP and these factors may have affected nutrient or O<sub>2</sub> availability, growth of microorganisms, the concentration of microbial metabolites, plant stress, growth and survival of *Pythium*, mobility of bacteria and microbial antagonism, which influenced SDR. Optimal suppression was achieved at AFP<sub>act</sub> 16.0-19.1%, 8% and
2.4-19.0% for Temp18, Temp 35 and Temp 45 treatments, respectively. Heat treatments of 35 and 45°C tended to be unfavourable to the pathogen, but may have also been unfavourable to the plant, particularly in media with unfavourable AFP.

Different trends found between AFP and SDR in Experiments 1 to 3 may have been due to microbial activity due to a different batch of compost, temperature shock treatment, moisture relationship as modified by covering/uncovering media and different pot heights. Recommended work for the next experiment, therefore, was to select a high and a low AFP and determine their effects on the suppression of *Pythium* damping-off in other species of plant using medium similar to Experiment 2. Suppression was optimal at high AFP for Temp18 treatment in this experiment and at low AFP for previous experiments. It was also considered advisable to use the same batch of compost from Experiments 1 and 2, with higher microbial activities, as it may have increased suppression at AFP_{act} 2.6% compared to AFP_{act} 10.8% compared with Experiment 3.
3.3.7 Experiment 4 Results

3.3.7.1 Effect of AFP on the germination rates, Severity of Disease Rating index (SDR), Pythium counts and microbial activity for media planted with Impatiens in Bioassay 1 and 2

SDR was significantly lower at AFP_{std} 5.2% (1.6 units) than at AFP_{std} 16.1% (3.3 units) in Bioassay 1 (B1) and at AFP_{std} 16.1% (1.3 units) than at AFP_{std} 5.2% (2.9 units) in Bioassay 2 (B2) (p ≤ 0.05) (Figure 3.4a). Germination rates in controls ranged from 83 to 100% at AFP_{std} 5.2% in B1 and B2 and at AFP_{std} 16.1% in B2, whereas the germination rate was only 50% at AFP_{std} 16.1% in B1.

*Pythium* counts were significantly higher at AFP_{std} 5.2% (137 and 2708 cfu/mL potting media) than at AFP_{std} 16.1% (19 and 467 cfu/mL potting media) and *Pythium* counts were significantly higher in B2 than in B1 at each AFP.

In B1, microbial activity (d10) was significantly higher at AFP_{std} 5.2% (0.913 μg FDA/min/mL potting media) than at AFP_{std} 16.1% (0.400 μg FDA/min/mL potting media) (Figure 3.4c) (p ≤ 0.05). In B2, microbial activities (d10) were not significantly different at each AFP (0.867 and 0.770 μg FDA/min/mL potting media). At AFP_{std} 5.2%, microbial activities (d10) were not significantly different in B1 and B2, but at AFP_{std} 16.1%, microbial activity was significantly higher in B2 than in B1.

3.3.7.2 Effect of AFP on the germination rates, Severity of Disease Rating index (SDR), Pythium counts and microbial activity for media planted with Celosia in Bioassay 1 and 2

The SDR at AFP_{std} 16.1% was not significantly different in B1 and B2 (2.4 and 2.6 units, respectively), but was significantly lower than at AFP_{std} 5.2% in B2 (3.2 units) and significantly higher than at AFP_{std} 5.2% in B1 (1.7 units) (Figure 3.4a). Germination rates ranged from 80 to 85% for all controls.

*Pythium* counts were not significantly different at each AFP in B1 (142 and 55 cfu/mL potting media) and in B2 (1767 and 909 cfu/mL potting media) (Figure 3.4b). At each AFP value, however, *Pythium* counts were significantly lower in B1 than in B2 (p ≤ 0.05).
At each AFP, microbial activities (d10) were not significantly different in either bioassay (p≤0.05). Microbial activities (d10) were significantly higher at AFP_{std} 5.2% (0.858-1.000 μgFDA/min/mL potting media) than at AFP_{std} 16.1% (0.617-0.623 μgFDA/min/mL potting media) in both bioassays (Figure 3.4c) (p≤0.05).

3.3.7.3 Effect of AFP on the germination rates, Severity of Disease Rating index (SDR), Pythium counts and microbial activity for media planted with Chilli Pepper in Bioassay 1 and 2

The SDR was not significantly different at AFP_{std} 5.2% in B1 and B2 and at AFP_{std} 16.1% in B2 (1.8-2.1 units), but was significantly lower compared to AFP_{std} 16.1% in B1 (3.9 units) (Figure 3.4a). Germination rates in controls ranged from 67 to 88% at AFP 5.2% in B1 and B2 and at AFP 16.1% in B2, whereas the germination rate was 50% at AFP_{std} 16.1% in B1.

*Pythium* counts were not significantly different in any treatment (41-272 cfu/mL potting media) (Figure 3.4b).

Microbial activities (d10) were significantly higher at AFP_{std} 5.2% (0.803-0.867 μgFDA/min/mL potting media) than at AFP_{std} 16.1% (0.427-0.463 μgFDA/min/mL potting media) for each bioassay. At each AFP, microbial activities (d10) were not significantly different in B1 and B2 (Figure 3.4c) (p≤0.05).

3.3.7.4 Effect of AFP on the germination rates, Severity of Disease Rating index (SDR), Pythium counts and microbial activity for media planted with Salvia in Bioassay 1 and 2

The SDR was significantly highest at AFP_{std} 16.1% in B1 (4.0 units), significantly lower at AFP_{std} 5.2% in B2 and significantly lowest at AFP_{std} 5.2% in B1 and AFP_{std} 16.1% in B2 (1.3-1.6 units) (p≤0.05) (Figure 3.4a). Germination rates in controls ranged from 70 to 75% at AFP_{std} 5.2% in B1 and at AFP 16.1% in B2, whereas the germination rate ranged from 42 to 50% at AFP_{std} 5.2% in B2 and at AFP_{std} 16.1% in B1.

*Pythium* counts were not significantly different in any treatment (26-77 cfu/mL potting media) (p≤0.05) (Figure 3.4b).
In B1, microbial activity (d10) was significantly higher at $\text{AFP}_{\text{std}}$ 5.2% (0.667µ gFDA/min/mL potting media) than at $\text{AFP}_{\text{std}}$ 16.1% (0.450µgFDA/min/mL) (Figure 3.4c) (p≤0.05). In B2, microbial activities (d10) were not significantly different at any AFP (0.575-0.678 µgFDA/min/mL potting media). At $\text{AFP}_{\text{std}}$ 5.2%, microbial activities (d10) were not significantly different in B1 and B2, but at $\text{AFP}_{\text{std}}$ 16.1%, microbial activity (d10) was significantly lower in B1 compared to B2.

3.3.7.5 Effect of AFP on the germination rates, Severity of Disease Rating index (SDR), Pythium counts and microbial activity for media planted with Snapdragon in Bioassay 1 and 2

The SDR was significantly lower at $\text{AFP}_{\text{std}}$ 5.2% (1.0-1.1 units) than at $\text{AFP}_{\text{std}}$ 16.1% (1.7-4.0 units) for B1 and B2 (Figure 3.4a). Within each AFP, the SDR was significantly higher in B1 than in B2 (p≤0.05). Germination rates in controls ranged from 70 to 77% at $\text{AFP}_{\text{std}}$ 5.2% in B1 and at $\text{AFP}_{\text{std}}$ 16.1% in B2, whereas the germination rate was 61% at $\text{AFP}_{\text{std}}$ 5.2% in B2 and 34% at $\text{AFP}_{\text{std}}$ 16.1% in B1.

In B1, *Pythium* counts were significantly higher at $\text{AFP}_{\text{std}}$ 5.2% (121cfu/mL potting media) than at $\text{AFP}_{\text{std}}$ 16.1% (18cfu/mL potting media) (p≤0.05), while in B2, *Pythium* counts were not significantly different at any AFP (45-146cfu/mL potting media) (Figure 3.4b). *Pythium* counts were significantly lower in B1 than in B2 at $\text{AFP}_{\text{std}}$ 16.1%, but at $\text{AFP}_{\text{std}}$ 5.2%, there was no significant difference between B1 and B2 (Figure 3.4b) (p≤0.05).

In B1 and B2, microbial activities (d10) were significantly higher at $\text{AFP}_{\text{std}}$ 5.2% (0.830-0.975µgFDA/min/mL potting media) than at $\text{AFP}_{\text{std}}$ 16.1% (0.350-0.672µ gFDA/min/mL potting media) (Figure 3.4c) (p≤0.05). At $\text{AFP}_{\text{std}}$ 16.1%, microbial activity (d10) was significantly lower in B1 than in B2, but at $\text{AFP}_{\text{std}}$ 5.2%, microbial activities (d10) were not significantly different between B1 and B2.

3.3.7.6 Effect of AFP on microbial activity (d0) in Bioassay 1

The microbial activity (d0) ranged from 0.215-0.330µgFDA/min/mL potting media at $\text{AFP}_{\text{std}}$ 5.2% and 0.230-0.345µgFDA/min/mL potting media at $\text{AFP}_{\text{std}}$ 16.1%.
Figure 3.4: Experiment 4. The effect of Air-Filled Porosity (AFP<sub>std</sub>) at 5.2 and 16.1% on damping-off caused by *Pythium ultimum* in species of Impatiens, Celosia, Chilli Pepper, Salvia and Snapdragon grown in potting media containing composted hardwood and sand in Bioassays 1 and 2. Note 1: Bars with the same letter(s) are not significantly different (p=0.05). Note 2: B1=Bioassay 1 and B2=Bioassay 2).

3.3a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

3.3b) Mean *Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing.

3.3c) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium on day of sowing (d0) and day 10 (d10) after sowing.
3.3.8 Experiment 4 Discussion

AFP and species of plant affect the suppressiveness of media, while covering media and uncovering media may also influence the suppression of *Pythium* damping-off. Media were more suppressive to disease at AFP_{std} 5.2% compared with AFP_{std} 16.1%, when growing 5 species of plants in Bioassay 1 and Snapdragon in Bioassay 2. This data is in agreement with Experiments 1 and 2. When growing Impatiens, Celosia and Salvia in Bioassay 2, however, media were more suppressive at AFP_{std} 16.1% compared with AFP_{std} 5.2%. Suppression was not significantly affected by AFP, when growing Chilli Pepper in Bioassay 2. Each species of plant may release a different quantity or quality of plant exudates in response to the environment and thus affect SDR through effects on *Pythium* counts and microbial activity. The effect of AFP on SDR, *Pythium* counts and microbial activity, therefore will be discussed separately for each plant.

**Impatiens:** Higher SDR in conjunction with lower *Pythium* counts (19cfu/mL potting media) occurred in media of AFP_{std} 16.1% in Bioassay 1 compared to AFP_{std} 5.2% in Bioassay 1 and AFP_{std} 5.2 and 16.1% in Bioassay 2, where *Pythium* counts were higher (137-2708cfu/mL potting media). This was not expected as higher *Pythium* counts would be expected to lead to higher SDR in the absence of any other influence. Other factors, therefore, presumably have played a role in increasing SDR. Higher microbial activity did not apparently suppress *Pythium* counts, as microbial activity increased in conjunction with increased *Pythium* counts. *Pythium* counts would have probably contributed, however, to microbial activity. The activities of microorganisms may be favoured by higher moisture conditions, as microbial activities were higher at AFP_{std} 5.2% than at AFP_{std} 16.1%, but this was only significant in Bioassay 1, while being significantly lowest at AFP_{std} 16.1% in Bioassay 1.

In Bioassay 1, the plant may also be favoured by AFP_{std} 5.2% compared to AFP_{std} 16.1% due to increased moisture availability, resulting in reduced SDR at the low AFP. Higher *Pythium* counts at AFP_{std} 5.2% (138cfu/mL potting media) compared with AFP_{std} 16.1% (19cfu/mL potting media) may have not been sufficient to increase SDR. In Bioassay 2, however, much greater *Pythium* counts
(2708 and 467cfu/mL potting media, at AFP_{std} 5.2 and 16.1% respectively) may have been sufficient to increase the SDR at AFP_{std} 5.2%, than at AFP_{std} 16.1%, but, the counts were not significantly different.

In Bioassay 2, an increase in Pythium counts in both AFP treatments and an increase in microbial activity at AFP_{std} 16.1% since Bioassay 1 suggest some change in the conditions in Bioassay 2 compared with those in Bioassay 1. Perhaps covering media in Bioassay 2 may have created wetter conditions and thus have caused significantly increased Pythium counts and microbial activity compared with circumstances in Bioassay 1. Media in Bioassay 2 may have contained some residual plant exudates to support both Pythium and microbial activities, compared with growing plants in Bioassay 1. Sharma and Gupta (1991) showed that various amino acids and sugars increased the germination of sporangia of P. ultimum. High microbial activity at low AFP’s may have contributed to the production of anaerobic conditions, which may have directly caused plant injury (Drew and Lynch, 1980), and may have reduced the suppressive qualities of potting media through the presence of anaerobic metabolites as previously described. Microbial activity did not increase significantly at AFP_{std} 5.2% in Bioassay 2 compared with Bioassay 1 and perhaps there was sufficient moisture in the media to support the growth of microorganisms.

The changes in AFP, media water content or surface humidity may have affected plant responses, such as seed imbibition, or plant exudation. These combined factors may have resulted in increased production of plant exudates and greater SDR. Germination rates in uncovered media at AFP_{std} 16.1% were reduced by 40% compared to those in covered media. At AFP_{std} 16.1%, a low concentration of the pathogen may then have been able to cause higher SDR under these conditions than with covered media. Covering media of low AFP may have increased the concentration of anaerobic compounds also, or have decreased the O_2 content, promoted by high microbial activity, compared with other treatments and also added to plant stress, Pythium counts and SDR.

Celosia: The low SDR at AFP_{std} 5.2% in Bioassay 1 may have been due to favourable conditions for seed germination and growth of seedlings due to higher
moisture and microbial activity, compared to AFP_{std} 16.1% in both bioassays and lower *Pythium* counts compared to AFP treatments in Bioassay 2. The high SDR, however, at AFP_{std} 5.2% in Bioassay 2 may have been caused by decreased O₂ concentration with concurrent increases in anaerobic compounds, over a 23 day period, due to high microbial activity compared with all other treatments.Covering the media of low AFP may have also contributed to more extensive anaerobic conditions, causing greater plant stress, increased plant exudation, higher *Pythium* counts and SDR.

At AFP_{std} 16.1%, *Pythium* counts of 55-909 cfu/mL potting media, resulted in similar SDR in both bioassays. The increase in *Pythium* counts in Bioassay 2 was not at a high enough concentration, perhaps, to increase the SDR, or plants in uncovered media may have been more susceptible to disease at lower *Pythium* counts, due to an unknown factor. This species germinated and emerged earlier and developed more extensive roots systems than the other plant species and, therefore, may have been able to take up water more efficiently and was not affected by seed imbibition. Germination rates of this species were not affected by AFP or covering and uncovering pots as compared to other species.

**Chilli Pepper:** The lack of differences in *Pythium* counts between treatments (41-272 cfu/mL potting media) may account for similar SDR in all treatments, except at AFP_{std} 16.1% in Bioassay 1. Germination rates at AFP_{std} 16.1% in Bioassay 1 were reduced by 25% compared to Bioassay 2, therefore, Chilli Pepper may have been susceptible to drier conditions at AFP_{std} 16.1% in Bioassay 1, resulting in increased SDR compared with all other treatments.

Higher moisture levels may have significantly increased microbial activity at AFP_{std} 5.2% than at AFP_{std} 16.1% in both bioassays, but does not significantly suppress *Pythium* counts at the lower AFP. In Bioassay 2, there may have been wetter conditions, which may have increased the mobility and activity of antagonistic microflora, which possibly limited the activity of the pathogen. *Pythium* did not increase significantly in Bioassay 2, as found in some of the other plant bioassays, and this may have been due to the lack of change in the quantity or nature of plant exudates caused by various environmental factors. At AFP_{std} 5.2%, SDR did not
increase in Bioassay 2, as compared with Impatiens and Celosia bioassays, and
perhaps Chilli Pepper may not have been susceptible to anaerobic conditions at
AFP_{std} 5.2%.

**Salvia:** As *Pythium* counts were not significantly different in any treatment, they
therefore do not account for the high SDR at AFP_{std} 16.1% in Bioassay 1. At
AFP_{std} 16.1%, Salvia may have been more susceptible to disease, or, the mobility
and competitiveness of microorganisms may have been reduced, due to drying of
media in Bioassay 1, but this effect was negated in Bioassay 2, possibly due to higher
surface humidity caused by covering the media. Seedlings may have been more
stressed at high AFP, but not enough to reduce germination rates. Higher microbial
activity at low AFP, compared with high AFP, probably reduced the *Pythium* counts,
as these tended to be higher at lower AFP for all other species of plant in
Bioassays 1.

Higher disease in media of AFP_{std} 5.2% in Bioassay 2 than in Bioassay 1 may have
been due to greater *Pythium* counts, although the differences were not significant.
Anaerobic compounds may have accumulated in media, with O_2 being depleted by
microorganisms, plant roots and pathogen over the 23 day period, resulting in
increased plant stress and plant exudation.

Salvia in Bioassay 2 may have also possibly exuded inhibitory compounds, as
*Pythium* counts were reduced while microbial activities were higher at AFP_{std} 5.2%
than at AFP_{std} 16.1% for all bioassays, except when growing Salvia in Bioassay 2.
Organic acids have been shown to inhibit the germination of sporangia (Sharma and

**Snapdragon:** This species of plant was more susceptible to disease in drier media,
as SDR was significantly higher at AFP_{std} 16.1% (1.7-4.0 units) than AFP_{std} 5.2%
(1.0-1.1 units) in both bioassays. In Bioassay 1, high microbial activity at the lower
AFP did not appear to suppress *Pythium* counts sufficiently, therefore, some other
factor may have influenced the severely increased SDR at the higher AFP.
Snapdragon appeared to have been more susceptible at AFP_{std} 16.1% in Bioassay 1,
in conjunction with lower *Pythium* counts, possibly due to drier media, causing more
plant stress compared with other treatments. At AFP_{std} 16.1%, the virulence of
Pythium may have been severely enhanced by the drier environment, which appears to have decreased the overall microbial activity. In Bioassay 2, higher SDR at AFP_{std} 16.1% may have been due to lower microbial activity, and increasing Pythium counts, compared with AFP_{std} 5.2%.

Covering media in both AFP treatments may have decreased plant stress, as SDR decreased significantly in Bioassay 2, even though Pythium counts increased significantly in Bioassay 2 compared to Bioassay 1.

Comparison of species: From Table 3.2 it can be seen that each species is affected more or less severely by *Pythium ultimum* under different conditions. It appears that some circumstances are conducive to disease and these are different for each species, as there are complex interactions between the pathogen, the plant and the environment. The environment appears to play a large role in conjunction with plant responses to these changes, such as the air/water balance.

Table 3.2: Treatments shown to be significantly more conducive or suppressive to disease for each plant species (p≤0.05).

<table>
<thead>
<tr>
<th>Species of plant</th>
<th>AFP treatment with significantly lower SDR</th>
<th>SDR</th>
<th>AFP treatment with significantly higher SDR</th>
<th>SDR</th>
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<td>Impatiens</td>
<td>5.2% in Bioassay 1 and 16.1% in Bioassay 2</td>
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<td>16.1% in Bioassay 1 and 5.2% in Bioassay 2</td>
<td>3.3</td>
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<td></td>
<td></td>
<td>1.3</td>
<td></td>
<td>2.9</td>
</tr>
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<td>Celosia</td>
<td>5.2% in Bioassay 1</td>
<td>1.7</td>
<td>5.2% in Bioassay 2</td>
<td>3.2</td>
</tr>
<tr>
<td>Chilli Pepper</td>
<td>5.2% in Bioassays 1 and 16.1% in Bioassay 2</td>
<td>1.8 to 2.1</td>
<td>16.1% in Bioassay 1</td>
<td>3.9</td>
</tr>
<tr>
<td>Salvia</td>
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<td>16.1% in Bioassay 1</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Snapdragon</td>
<td>5.2% in Bioassay 1 and 16.1% in Bioassay 2</td>
<td>1.0</td>
<td>16.1% in Bioassay 1</td>
<td>4.0</td>
</tr>
</tbody>
</table>

When growing Impatiens, Celosia and Salvia, media of AFP_{std} 5.2% were most suppressive to disease in Bioassay 1, but these media became most conducive in Bioassay 2. This conflict of data between bioassays was not expected, as the suppressiveness or conduciveness of media is generally enhanced in the second bioassays, not reversed (Fahy, pers. comm.). In Bioassay 2, Pythium counts were still increasing to very high numbers at both AFP's, therefore, the plant may have been less susceptible to disease at AFP_{std} 16.1%, by reducing plant stress by covering the pots. This change in conditions was intended to reduce drying of the top layer of potting media. It was seen, in some cases, to increase germination rates,
particularly at AFP$_{\text{std}}$ 16.1%. Braunack (1995) also found a greater emergence rate of soybean in covered treatments than uncovered treatments for all water contents and aggregate size of media. Rayner and Arnold (1994) found that a compost mix, with a considerably higher AFP than 2 commercial bark mixes, led to greater drying of the mix, resulting in higher plant stress reflected by lower dry weights. There may have been other toxic effects also, as have been found through use of immature composts (Hoitink and Kuter, 1986).

At AFP$_{\text{std}}$ 5.2%, the SDR of Impatiens, Celosia and Salvia tended to increase in Bioassay 2 compared with Bioassay 1 and may have been due to the accumulation of anaerobic compounds, lower O$_2$ concentration, and/or the increase in Pythium counts. Very low O$_2$ concentration may limit the growth of Pythium (Brown and Kennedy, 1966; Mitchell and Mitchell, 1973), or, conversely may cause plant stress (Morard and Silvestre, 1996) and increase SDR. Minor variations of AFP, microbial activity, WHC, or nutrient concentration may have affected this balance and hence altered the suppressiveness of media. Anaerobic conditions may have been promoted by high microbial activity and/or covering of media. Perhaps these factors may have increased their effect with time, rather than being caused only by covering of media, and by high microbial activities which may have contributed to loss of suitable nutrients for antagonistic microorganisms, the depletion of oxygen and accumulation of anaerobic compounds. Menzies (1970) stated that their unpublished data showed that pathogens were killed from the accumulation of toxic metabolites, when placed under anaerobic conditions and if these substances could not diffuse away.

Microbial activities (d10) of media in all treatments in Bioassay 1 and in the Celosia, Chilli Pepper and Snapdragon treatments in Bioassay 2 were significantly higher at AFP$_{\text{std}}$ 5.2% compared to AFP$_{\text{std}}$ 16.1% and this is in agreement with Experiment 2. High microbial activity did not appear to reduce Pythium counts as stated in the literature (Fahy et al., 1995; Chen et al., 1988a &b) and this was also shown in previous experiments. Pythium presumably contributed to microbial activity and increased the overall microbial activity.

Pythium counts tended to be higher at AFP$_{\text{std}}$ 5.2% than at AFP$_{\text{std}}$ 16.1%, but this was only significant while growing Impatiens and Snapdragon in Bioassay 1. In
Experiment 2, *Pythium* counts were lower at AFP 5.2% compared to AFP 16.1%, but not significantly different. Although these differences were not significant, it may be possible that *Pythium* counts may increase more significantly over a 14 day assay rather than a 10 day period, while plant exudates released by these species of plants may vary compared with cucumbers. Covering the media and/or the change to a different batch of compost, with different biological characteristics to that used in Experiments 1, 2 and 4, would influence *Pythium* counts at each AFP. In this experiment, the range of microbial activity (0.350 to 1.000 μgFDA/min/mL potting media) was lower than the range measured in Experiment 2 (1.152 to 1.208 μgFDA/min/mL potting media) and was not increased at high AFP, as found in Experiment 4. This would have been caused most likely by the different compost component of media.

In conclusion, SDR, *Pythium* counts and microbial activity varied, depending on variables such as AFP and species of plant and these effects were generally not consistent between bioassays, which may be due to covering or the uncovering media. The most surprising part of this experiment was that media of AFP std 16.1% in Bioassay 1, with lower *Pythium* counts, resulted in higher severity of disease, compared to media in Bioassay 2, where higher *Pythium* counts resulted in lower SDR. Covering media of high AFP may have increased microbial activity and enhanced competition with the pathogen for sites on the plant, or, reduced plant stress compared to uncovered media of high AFP. Covering and uncovering media appears to have greatly influenced the suppressiveness of media at various AFP's, but it is not conclusive due to the design of this experiment. Recommended work for further experiments was to thus determine the effect of AFP and covering and uncovering pots, as well as the effects of modifying the levels of microbial activities at each AFP, on *Pythium* damping-off of cucumber seedlings.
3.4 General Discussion of Experiments 1-4

The mechanism by which suppression is achieved is still not elucidated, as these experiments have shown the existence of a dynamic complexity between the interaction of AFP, the host and pathogen, rather than simplify the explanation. Overall, it appears that unfavourably low AFP (AFP_{act} 1.8-3.0%) may cause either the pathogen, or the plant, more stress by i) high compaction of growing media, which may cause a mechanical stress to roots, ii) competition for O_2 at very low AFP, which may be compounded by both the high microbial activity and the seedlings consuming O_2, resulting in iii) accumulation of anaerobic compounds, which are potentially harmful to plants, pathogens and microorganisms in varying degrees, and/or iv) inhibitory or stimulatory effects of CO_2 to the pathogen (Bennie, 1991; Brown and Kennedy, 1966; Drew, 1992; Drew and Stolzy, 1991). Unfavourable high AFP (AFP_{act} 10.8-25.0%) may stimulate the activities of the pathogen by i) favouring production of plant exudates, ii) causing seed imbition, which results in increased plant stress with delayed emergence and/or iii) decrease the mobility of competitive microflora, thereby reducing competition with *Pythium* for nutrients.

In all cucumber bioassays, media with high microbial activities of 0.8-1.7μgFDA/min/mL potting media did not appear to reduce *Pythium* counts, as claimed elsewhere in the literature (Fahy *et al.*, 1995; Chen *et al.*, 1988b). The exception was when the microbial activity at d0 and d10 were approximately three fold greater in the unheated treatment, at 0.9-1.2μgFDA/min/mL potting media, compared with the heated treatment, at 0.3-0.4μgFDA/min/mL potting media, in Experiment 2. Media with such high microbial activities were still conducive to disease, and therefore, other factors must have been increasing the SDR. The contradictions in the literature may have been due to the nature of medium and host in their studies. WHC of media in other studies may have been much higher, as compared to media used in Experiments 1 and 2, which were below the Australian Standards recommended level. This was necessary, however, to achieve a wide range of AFP in experimental potting media amended with 30% compost.
Higher *Pythium* counts (1177-3851 cfu/mL potting media) tended to cause SDR to be greater than 2.5 units, as shown in Table 5.1 (See Section 6), for cucumbers in Experiments 1 (≥347 cfu/mL potting media), Experiment 2 (≥1177 cfu/mL potting media) and Celosia in Experiment 4 (≥909 cfu/mL potting media), but not for Impatiens, Chilli Pepper, Salvia and Snapdragon in Experiment 4 and cucumbers in Experiment 3. Fahy and Pike (pers. comm.) found that *Pythium* counts of 1100 cfu/mL or greater in potting media tended to result in an SDR greater than 2.5 index. Inconsistency between the experiments may have been due to certain uncontrolled factors, such as different inoculum and compost batch used in media, variable microbial activity, as well as covering and uncovering media, altering pot heights and different species of plant. The virulence of inoculum may have also varied slightly, between batches, as inoculum used in each experiment may contain various proportions of sexual or asexual propagational structures, such as sporangia, thin walled oospores and thick walled oospores, which have been found to vary in their response to their environment and in their virulence (Lumsden and Ayers, 1975; Johnson *et al*, 1990; Lifshitz and Hancock, 1984). The viability of inoculum also decreased gradually over time, but it was checked prior to the experiment, so that the volume of inoculum could be readjusted to achieve a constant concentration of viable propagules in the potting media. A different batch of compost used in Experiment 3 had lower initial microbial activity (d0), however, compared with the batch used in Experiment 1, 2 and 4 and this probably contributed to some of the variation between experiments. Fahy *et al* (1995) found that factors, such as microbial activity, nitrogen drawdown, pH, electrical conductivity, ammonium concentration, AFP and WHC varied between batches of compost collected from the same composting yard. Each batch of compost may also contain different types of microorganisms, which may have been more, or less, antagonistic towards *Pythium*, and thus affect its effect on SDR. Covering and uncovering media and altering pot heights are known to affect the moisture content of the media and its surface humidity, and thus influence the effect of AFP_{act} on SDR. Various species of plant also have different susceptibilities or tolerances to the pathogen, as shown in Experiment 4, and may vary in their release of plant exudates, which may stimulate or inhibit *Pythium*, but these exactitudes were not measured and so remain unknown in their effects.
Production of healthy cucumber plants grown at 18°C was optimal at $AFP_{act}$ 2 to 2.6% in Experiment 1 and 2 and at $AFP_{act}$ 16.0-19.1% in Experiment 3 (Table 5.1), which is outside the recommended range of AFP for seedling mixes by the Australian standard. Suppression of *Pythium* damping-off in cucumbers grown at 18 °C was achieved at $AFP_{act}$ 2.4%, 1.8-10.8% and 2.0 and 16.0-19.1% in Experiments 1, 2 and 3 respectively. Suppressive media had *Pythium* counts ranging from 10-3569 cfu/mL potting media and microbial activities (d0) ranging from 0.498-1.392 ugFDA/min/mL potting media; therefore low *Pythium* counts and high microbial activity was not always necessary to achieve suppression.

In Experiment 1, low AFP may have been suppressive due to low *Pythium* counts. In Experiment 2, however, a wider range of AFP were suppressive, in conjunction with moderate levels of *Pythium* counts, which tended to increase at $AFP_{act}$ greater and less than 2.6%. In Experiment 2, cucumbers were less susceptible to higher *Pythium* counts than in Experiment 1. In Experiment 2, covering of pots until seedlings emerged may have reduced the plant's susceptibility to higher *Pythium* counts by increasing surface humidity of potting media and reducing seed imbibition. In Experiment 1, microbial activity was significantly lowest at $AFP_{act}$ 25%, but this did not occur in Experiment 2. This may have been due to the covering of media in Experiment 2, which decreased moisture loss in the mix and favoured microbial activity.

In Experiment 3, a combination of factors, such as good plant vigour, the increased activities of specific antagonists and/or the decreased activities of *Pythium* may have caused suppression of disease. Suppression at low $AFP_{act}$ (2.4%) may have been due to low *Pythium* counts, but high *Pythium* counts were measured in suppressive media of high $AFP_{act}$ (16.0 and 19.1%). Media of high AFP with high WHC, therefore, may provide optimal conditions for growth of the plant, decreasing the susceptibility to *Pythium* disease. The major differences between the first 2 experiments and Experiment 3 are the WHC, pot height and microbial activity (d0). Factors, such as a higher range of WHC and different batch of compost, with lower microbial activity, may have contributed to the contradiction with previous experiments. The microbial activity (d10) of suppressive media in Experiments 1
and 2 ranged from 1.392 and 0.938-1.208ugFDA/min/mL potting media respectively, whereas in Experiment 3 the activity ranged from 0.498 to 0.587ugFDA/min/mL potting media. Plants grown in media of high AFP_{act} (16.0 and 19.1%) appeared more resilient to higher *Pythium* counts in Experiment 3 and perhaps at higher WHC, they may have been less stressed and less susceptible to disease compared to those in media with low WHC mix, as in Experiments 1 and 2.

Media conducive to *Pythium* damping-off in cucumbers at 18°C typically had AFP_{act} of 3.8-25.0, 25.0 and 8% in Experiments 1, 2 and 3 respectively. Higher *Pythium* counts tended to result in high SDR, except in Experiment 3. In Experiment 3, media of AFP_{act} 8.2% (2.7 units) was most conducive to disease and may have been due to a combination of higher plant stress, compared to media of AFP_{act} 16.0 and 19.1% (1.9 units), and higher *Pythium* counts, compared to media of AFP_{act} 2.4% (2.0 units).

Optimal suppression of *Pythium* damping-off in Impatiens, Celosia, Chilli Pepper, Salvia and Snapdragon grown in 8.5cm pots at 18°C also occurred at AFP_{act} 2.6% compared to AFP_{act} 10.8% in Bioassay 1. In Bioassay 2, however, optimal suppression was achieved at AFP_{act} 2.6% only for Snapdragon and at AFP_{act} 10.8% for Impatiens, Celosia and Salvia, whereas AFP did not affect SDR for Chilli Pepper. In Bioassay 1, higher *Pythium* counts did not result in higher disease and perhaps the plants were more susceptible to disease in uncovered media of high AFP, whereas covering media may have prevented the surface of the medium from drying and the plants were less susceptible to disease, despite higher *Pythium* counts. Covering pots may have reduced the SDR, regardless of high *Pythium* counts, either by decreasing plant stress achieved by reducing seed imbibition, or, by reducing moisture loss, particularly from the top layer of potting media. Cucumber plants in previous bioassays had similar germination rates at each AFP and may not have suffered the same degree of stress at high AFP, compared to other species of plant. In Bioassay 2, the SDR tended to be higher at AFP_{act} 2.6% compared to AFP_{act} 10.8% and anaerobic compounds may have accumulated in media at a higher concentration, and/or, the O_{2} content may have decreased. These conditions may have been promoted by high microbial activity over the longer period of time of two bioassays
and by covering the media. Microbial activity and *Pythium* counts tended to be higher at AFP_{act} 2.6% than at AFP_{act} 10.8% in both bioassays and these microorganisms may have been favoured by higher moisture content.

In Experiment 2, significantly higher microbial activity at AFP_{act} 2.6% compared to AFP_{act} 10.8% may have decreased *Pythium* counts and increased suppression, but, in Experiment 4, higher microbial activity at low AFP did not reduce *Pythium* counts, compared with that at high AFP. This variation may have been due to ageing of compost and different species of plant, which influence the quantity and type of plant exudation in their given environment. Ageing of compost may have decreased the microbial activities in Experiment 4 (0.558 to 0.818μgFDA/min/mL) compared with the compost in Experiments 1 and 2 (above 1μgFDA/min/mL). Fahy et al (1995) found that microbial activity, pH, electrical conductivity and nitrate concentration in composted hardwood sawdust tended to increase over a 6 month period. This batch of compost was older than 9 months and may have decreased both in microbial activity and its antagonistic activity towards *Pythium*.

Heating potted plants prior to emergence of seedlings for 2 hrs at 35 and 45°C, appears to have altered the mechanism for suppression of disease in media, with AFP_{act} 2.4-19.1%, compared to treatments maintained at 18°C during the 10 day bioassay. Optimal suppression was achieved at AFP_{act} 16.0-19.1%, 8% and 2.4-19.0% for Temp18, Temp35 and Temp45 treatments, respectively. Heating, as well as unfavourable AFP, may have also increased stresses to the plant and the pathogen and this altered SDR. Optimal suppression in the Temp35 treatment at AFP_{act} 8.2% may have been due to increased the activities of antagonistic microflora, which then reduced *Pythium* counts. Increased microbial activity was not found at this AFP, as *Pythium* counts may have contributed significantly to microbial activities in media with AFP_{act} 2.4 and 16.0-19.1%. High temperature, 45°C, may have reduced *Pythium* counts directly or limited growth of the pathogen through competition with increased microbial activities (0.528-0.757ugFDA/min/mL potting media), resulting in low SDR for media of AFP 2.4-19.1%.

In conclusion, suppression is most likely to be optimal at AFP_{act} 2.0-2.6 in a low WHC_{act} mix (approximately 25-45%) with high microbial activity, whereas in a
high WHC\textsubscript{act} mix (48-58\%) with low microbial activity, suppression is best achieved at AFP\textsubscript{act} 16.1 and 19.1\%. The inconsistency between experiments provides evidence that many other factors, such as microbial activity, WHC and covering and uncovering media, interact with the direct effects of AFP on the suppressive qualities of media.

Further experimentation in the following areas were needed to assist in determining how AFP influences \textit{Pythium} damping-off and identify other interactive factors.

The effects of uncovering and covering media, at various AFP needed to be examined further, using freshly formulated medium, with equal concentrations of \textit{Pythium}, such that fewer uncontrolled variables were introduced. Covering the media, while growing cucumbers, may also result in higher SDR at AFP\textsubscript{std} 5.2\% than at AFP\textsubscript{std} 16.1\%, as found with other species of plants. Data from this experiment would thus indicate whether seeds are drying out during germination and becoming more susceptible to disease through slow emergence. Measurement of moisture potential may also demonstrate the water availability to plants (Griffin, 1981).

The effects of different levels of microbial activity at each AFP also needed to be examined to elucidate if high microbial activity at low AFP increases the severity of \textit{Pythium} damping-off at low AFP, as compared to low microbial activity at low AFP. This was to show whether anaerobic conditions increased the susceptibility of plants to disease, or increased the virulence of \textit{Pythium}, or lessened the effect of microbial activity on \textit{Pythium}. 


Section 4: Effects of surface air humidity interactions with Air-Filled Porosity on \textit{Pythium} Suppression.

4.1 Introduction

Covering potting media during seedling germination, rather than leaving media uncovered, appeared to have altered the suppressive qualities of potting media, although this was moderated by AFP and the species of plant, as shown in Experiment 4. In that experiment, it was proposed that an increase in the suppressiveness of covered media of high AFP$_{\text{std}}$ (16.1%), compared with uncovered media, was achieved by reducing plant stress, as \textit{Pythium} counts were higher in covered media than uncovered media, but SDR was reduced. Covering media of low AFP$_{\text{std}}$ (5.2%) may have reduced water loss of media but may have increased anoxia and the severity of \textit{Pythium} damping-off of plants, compared with uncovered media.

Experiment 5 was designed, therefore, to confirm if covering and uncovering potting media of various AFP would alter the suppressiveness of potting media to damping-off in cucumbers. Matric potential of media were determined in Experiments 5 and 6 to give some indication as to any differences of availability of moisture to the plants that may have been occurring at each AFP. Such differences may confirm the effects that AFP was possibly having on microbial activity and \textit{Pythium} counts through the availability of moisture in the media. Ownley and Benson (1991) suggested that host susceptibility to \textit{Phytophthora} is affected by AFP, which influences root generation and growth, root exudation and plant metabolism related to host resistance. Matric potential, on the other hand, is the primary physical factor which affects sporangium production and zoospore release, movement and chemotaxis, as well as germination and survival of chlamydomospores of \textit{Phytophthora} spp. Pots with a height of 4.5cm were included as an extra uncovered treatment, such that potting media of the same AFP, but of different WHC can be compared, as increasing WHC may alter the effect of AFP on suppression of disease. The microbial activity of this batch of compost was found to be not as high as in Experiments 1, 2 and 4, so therefore, wheat bran
and urea were added to all treatments to increase the content of available C and N. The addition of the C and N sources to media increases microbial activity (Mandelbaum and Hadar, 1990).

4.2 Materials and Methods

The AFP treatments consisted of potting media of 5 different AFP_{act} (2 to 20%), in 8.5cm pots and 3 different AFP_{act} (3 to 20%) in 4.5cm pots, as stated in Table 2.1. For each AFP medium in 8.5cm pots, there were 2 subtratements: inoculated with *Pythium* and uninoculated, and within these subtratements there were covered (CI=Covered and Inoculated treatment and CU=Covered and Uninoculated treatment) and uncovered potting media (UI=Uncovered and Inoculated treatment and UU=Uncovered and Uninoculated treatment). In 4.5cm pots, each level of AFP contained 2 subtratements, which were inoculated and uninoculated, but both left uncovered (ui=uncovered and inoculated treatment and uu=uncovered and uninoculated treatment).

Preparation of potting media, inoculum and bioassay were similar to the procedures described in Section 2, except with the following modifications. Substrates used in the preparation of potting media consisted of 31.5% composted hardwood sawdust, 1.5% wheat bran, and 67% mixed fine and coarse sand. To achieve AFP_{act} 2, 3, 6, 14 and 20% in 8.5cm pots 67, 45, 37, 21 and 14% of fine sand was added to each mix, respectively. Potting media of AFP_{act} 3, 5 and 20% in the 4.5cm pot contained 47, 30 and 0% fine sand, respectively. The remaining proportions to 67% total sand consisted of coarse sand. Unfortunately, a calculation error was discovered after the experiment, resulting in an AFP_{act} 5%, instead of the desired AFP_{act} 6% for the ui treatment. Urea (1.25g/L) was added to all media in plastic bags, then incubated for 2 days in the growth room. All media were flushed with dilute hydroponic solution (10%v/v), pH 4.5 (See Appendix 1), to reduce the mix pH from 8 to the range of 5.5 to 6.0. Potting media were then incubated in open plastic bags for 7 days in the growth room before seeding.

The inoculum rate of *Pythium* was 2gL^{-1} of potting media. During the bioassay, pots in the CU and CI treatments were covered with glass Petri dishes for the first 5 days and then covered with plastic bags for the remaining 5 days to allow room for the
seedlings to grow. Pots in the uu and uu treatments were all uncovered. The suppressive qualities of media were determined by measurement of SDR, *Pythium* counts and microbial activity \((d_0)\) and \((d_{10})\) as described in Sections 2.1.3 to 2.5. Matric potentials were determined for each treatments as described in Section 2.1.2.
4.3 Results

4.3.1 Effect of AFP and covering and uncovering media on the Severity of Disease Rating index (SDR)

No significant relationship was found between AFP and the SDR for the UI and \textit{ui} treatments (p>0.05), but a positive significant linear relationship was found for the CI treatment (p=0.0001). In the UI treatment, the SDR was significantly lower at \textit{AFP}^{act} 2\% (1.3 units) than at \textit{AFP}^{act} 14\% and both were lower than \textit{AFP}^{act} 3, 6 and 20\% (2.8 to 3.1 units) (Figure 4.1a). In the CI treatment, the SDR was significantly lowest at \textit{AFP}^{act} 2 and 6\% (1.8 to 1.9 units) than at \textit{AFP}^{act} 3, 14 and 20\% (2.7 to 2.9 units) (Figure 4.1a). In the \textit{ui} treatment, the SDR was significantly lower at \textit{AFP}^{act} 5 than at \textit{AFP}^{act} 3\% and 20\% (Figure 4.1d).

The SDR for the CI and UI treatments were not significantly different at \textit{AFP}^{act} 2, 3 and 20\%. At \textit{AFP}^{act} 6\%, the SDR was significantly lower in the CI treatment than the UI treatment, while at \textit{AFP}^{act} 14\%, the SDR was higher in the CI treatment than the UI treatment.

4.3.2 Effect of AFP and covering and uncovering media on Pythium counts

Significant positive linear trends were found between AFP and the \textit{Pythium} counts for the UI and CI treatments (p<0.05, r=0.57), while no significant relationship was found for the \textit{ui} treatment. AFP accounts for 81\% variation in \textit{Pythium} population counts in potting media. In the UI treatment, \textit{Pythium} counts were significantly lower at \textit{AFP}^{act} 2\% (39 cfu/mL potting media) than at \textit{AFP}^{act} 6 and 20\% (293-1149 cfu/mL potting media) (Figure 4.1 b). In the CI treatment, \textit{Pythium} counts were not significantly different at any AFP treatment (296-1742 cfu/mL potting media) (Figure 4.1 b). In the \textit{ui} treatment, \textit{Pythium} counts were significantly lowest at \textit{AFP}^{act} 5\% (85 cfu/mL potting media) and highest at \textit{AFP}^{act} 3 and 20\% (726-756 cfu/mL potting media) (Figure 4.1e).

4.3.3 Effect of AFP and covering and uncovering media on Microbial Activity

A significant linear trend was found between AFP and microbial activity (d0) (p=0.001, r=0.76). Microbial activity (d0) for media in 8.5cm pots was significantly lowest at \textit{AFP}^{act} 2\% (0.310 \mu g FDA/min/mL potting media) and significantly highest
at $\text{AFP}_{\text{act}}$ 14% (0.751 $\mu$gFDA/min/mL potting media) (Figure 4.1c). Microbial activity ($d_0$) for media in 4.5cm pots was significantly lowest at $\text{AFP}_{\text{act}}$ 3% and highest at $\text{AFP}_{\text{act}}$ 20% (Figure 4.3f).

No significant trend was found between AFP and microbial activity ($d_{10}$) for the UI ($p>0.05$, $r=0.66$), but a significant linear trend was found in CI treatment ($p<0.01$, $r=0.66$). In the UI treatment, microbial activity ($d_{10}$) was significantly lowest at $\text{AFP}_{\text{act}}$ 2% (0.577 $\mu$gFDA/min/mL) and highest at $\text{AFP}_{\text{act}}$ 6% (0.768 $\mu$gFDA/min/mL potting media) (Figure 4.3f). In the CI treatment, microbial activity ($d_{10}$) was significantly lowest at $\text{AFP}_{\text{act}}$ 2% (0.602 $\mu$gFDA/min/mL potting media) and highest at $\text{AFP}_{\text{act}}$ 6, 14 and 20% (0.820-0.898 $\mu$gFDA/min/mL potting media). In the $\text{ui}$ treatment, microbial activity ($d_{10}$) was not significantly different in any AFP treatment.

4.3.4 Effect of AFP and covering and uncovering media on Matric potential

The matric potential ranged from -0.014 to -0.016 MPa, -0.013 to -0.014 MPa and -0.010 to -0.012 MPa for the UI, CI and $\text{ui}$ treatments, respectively across lowest to highest AFP.
Figure 4.1. Experiment 5. The effect of Air-Filled Porosity ($\text{AFP}_{\text{act}}$) 2, 3, 6, 14 and 20 with low WHC (a, b & c) and AFP 3, 5 and 20% (c, d & e) with high WHC in the covered and uncovered treatments on damping-off caused by *Pythium ultimum* in the cucumbers grown in potting media containing composted hardwood and sand. Note: For Figure a) data points with the same letter(s) are not significantly different between AFP treatments and points with the same number(s) are not significantly different between covered/uncovered treatments at each AFP and for Figures b), c), d), e) & f) data points with the same letter(s) and upper/lower case are not significantly different (p=0.05).

4.1) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

a) 8.5cm pots  
d) 4.5cm pots

4.1) Mean *Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing.

b) 8.5cm pots  
e) 4.5cm pots

4.1) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium on day of sowing (d0) and day 10 (d10) after sowing.

c) 8.5cm pots  
f) 4.5cm pots
4.4 Discussion

AFP was shown to affect the suppressive qualities of potting media and the degree of suppression was enhanced by covering media of AFP<sub>act</sub> 6%, but lessened at AFP<sub>act</sub> 14%. Decreasing the pot height, which increased WHC, did not affect the suppressiveness of media at AFP<sub>act</sub> 3 and 20%. Suppression of disease was achieved at AFP<sub>act</sub> 2 and 14% for the UI treatment, at AFP<sub>act</sub> 2 and 6% for the CI treatment and at AFP<sub>act</sub> 5% for the uI treatment, while all other treatments were conducive to disease.

In the uncovered treatment, suppression at AFP<sub>act</sub> 2% may have been due to very low <i>Pythium</i> counts (39 cfu/mL potting media) compared with other AFP treatments (638-1149 cfu/mL potting media). Such low <i>Pythium</i> counts at AFP<sub>act</sub> 2% may have been caused by very low O<sub>2</sub> and high CO<sub>2</sub> concentration, reduced plant exudations, as the microbial activity (d0 and d10) was also significantly lowest at AFP<sub>act</sub> 2%, there may have been a higher concentration of aggressive antagonistic microflora, however, compared to that at higher AFP. The SDR was highest at AFP<sub>act</sub> 3% and lower O<sub>2</sub> concentration in this mix may have still increased plant stress, but may have been adequate for the growth of <i>Pythium</i> (293 cfu/mL potting media). Media of AFP<sub>act</sub> 14% was suppressive to disease and this may have been due to relatively low <i>Pythium</i> counts (295 cfu/mL potting media) compared with media of AFP<sub>act</sub> 6 and 20% (638-1149 cfu/mL potting media), with higher microbial activity (d0) competing with <i>Pythium</i>, or reduced plant stress compared with media of AFP<sub>act</sub> 3%.

In the covered treatment, higher <i>Pythium</i> counts at AFP<sub>act</sub> 3, 14 and 20% (672-1742 cfu/mL potting media) may account for significantly higher disease compared with any other AFP treatment (296-298 cfu/mL potting media), although these counts were not significantly different from those at other AFPS. At AFP<sub>act</sub> 14 and 20%, higher humidity and higher aeration may have favoured plant exudation and thus the growth of <i>Pythium</i> to cause an increase in SDR. At AFP<sub>act</sub> 3%, however, low oxygen concentration, higher humidity and a higher concentration of anaerobic compounds may have increased plant stress and plant exudation and favoured the growth of <i>Pythium</i>, increasing SDR. At AFP<sub>act</sub> 2%, growth of <i>Pythium</i> may have been limited, however, by lower O<sub>2</sub> concentration and moderate levels of microbial
activity. High microbial activities (d0 and d10) did not reduce *Pythium* counts or SDR and perhaps high *Pythium* counts at AFP<sub>act</sub> 20% may have contributed to high microbial activity (d10). In contrast, high microbial activity (d0) at AFP<sub>act</sub> 14%, remained high to the end of the bioassay, so these more aerated conditions may have increased the growth of *Pythium*, which then displaced other microflora during the bioassay.

Covering media significantly decreased the SDR at AFP<sub>act</sub> 6% and significantly increased SDR at AFP<sub>act</sub> 14%, compared to that of the uncovered media, while all other AFP treatments were not affected by covering or uncovering. At AFP<sub>act</sub> 2%, higher humidity in covered treatments may have increased *Pythium* counts, but both uncovered and covered media were still suppressive to disease. It was proposed that low AFP limited the growth of *Pythium* and reduced SDR, but, its growth was not limited in covered media. Perhaps *Pythium* grew at the surface due to higher humidity and O<sub>2</sub> concentration, particularly at low AFP, which may have contributed to higher *Pythium* counts, but may not have been able to cause as severe disease in this zone. At AFP<sub>act</sub> 3%, *Pythium* may have been stimulated by the accumulation of anaerobic compounds, due to low aeration and high microbial activity, and caused increased SDR in both covered and uncovered media. At AFP<sub>act</sub> 6%, higher humidity may have been most favourable to plant growth and increased plant vigour with reduced exudation, which reduced the growth of *Pythium*, or, favoured the growth of antagonistic microflora, compared with media with lower humidity. At AFP<sub>act</sub> 14%, however, higher humidity may have increased plant exudation and favoured the growth of *Pythium*, compared to uncovered media with lower humidity. At AFP<sub>act</sub> 20%, low WHC may have caused plant stress and increased plant exudation, resulting in both high *Pythium* counts and SDR for both treatments.

The effects of covering and uncovering media on the suppressiveness of media was highly variable and not expected, as data in the previous experiment indicated that covering media with high AFP decreased SDR, while covering media with low AFP increased SDR, as compared with uncovered media. Perhaps cucumbers grown in uncovered media, with high AFP, are not as severely stressed and susceptible to disease, compared with other species. Cucumber plants had similar germination rates.
at each AFP and may not have been subject to high stress at high AFP, as compared with other species of plants, such as Impatiens, Chilli Pepper, Salvia and Snapdragon. In this experiment, lower *Pythium* counts tended to result in lower disease, whereas, in the previous experiment, lower *Pythium* counts resulted in higher SDR and therefore it is suggested that plant stress may have strongly influenced SDR compared to other possible factors.

The activities of microorganisms and *Pythium* may increase in media containing higher moisture contents, as these parameters were higher in the covered treatments than uncovered treatments, except for *Pythium* counts at \( \text{AFP}_{\text{act}} \) 6%. These parameters, however, were not significantly different. In the UI treatment, microbial activity (d10) and *Pythium* counts were lowest at \( \text{AFP}_{\text{act}} \) 2%, increased to a maximum at \( \text{AFP}_{\text{act}} \) 6% and then decreased at the higher AFPs. In the CI treatment, however, microbial activity and *Pythium* counts tended to consistently increase as AFP increased. Perhaps at \( \text{AFP}_{\text{act}} \) 14 and 20%, lower surface humidity in the uncovered treatment limited microbial activity and *Pythium* counts, while competitive microorganisms and *Pythium* may have been favoured by conditions of high AFP, only if there was adequate moisture in the covered mix.

In the UI treatment, suppression was optimal at \( \text{AFP}_{\text{act}} \) 5% compared to \( \text{AFP}_{\text{act}} \) 3 and 20%, possibly due to reduced *Pythium* counts. Very low and high AFP may have been unfavourable to the plant, increasing plant exudation, causing increased *Pythium* counts and SDR. Microbial activity (d0) tended to increase as AFP increased, but did not decrease *Pythium* counts. A similar effect of AFP on the suppression of disease was found in the CI treatment, as suppression was optimal at \( \text{AFP}_{\text{act}} \) 6%, with lower *Pythium* counts, compared with \( \text{AFP}_{\text{act}} \) 3 and 20%. In the UI treatment, however, media of \( \text{AFP}_{\text{act}} \) 3, 6 and 20% were all conducive to disease.

The data from the smaller pots in this experiment was included, despite the unintended \( \text{AFP}_{\text{act}} \) 5%, as it may indicate that suppression may be enhanced at \( \text{AFP}_{\text{act}} \) 5% (WHC\(_{\text{act}}\) 42%) for 4.5cm pots compared with media of \( \text{AFP}_{\text{act}} \) 6% (WHC\(_{\text{act}}\) 42%) in 8.5cm pots, perhaps by altering the percolation rate of water in the media. Perhaps the WHC of media at the depth of seeding was different in the UI and UII treatments, affecting SDR. Increasing WHC of media with \( \text{AFP}_{\text{act}} \) 3 and 20%
(WHC_{act} 44 and 37% respectively) in 4.5cm pots did not alter the suppressiveness compared with the respective AFP treatments (WHC_{act} 43 and 33%) for the uncovered and covered media in 8.5cm pots. To verify whether WHC of media with the same AFP affect the suppressiveness of media, more treatments need to be included to determine the effect of AFP, as well as WHC, on the severity of disease.

*Pythium* counts were significantly lowest at AFP_{act} 2% in the UI treatment and this is consistent with data in previous cucumber bioassays in Experiments 1-3, enhancing the suppression of *Pythium* damping-off in cucumbers. Similar trends were found between AFP and SDR and for AFP and *Pythium* counts in Experiments 1 and 2, but were not in agreement with trends found in the UI treatment in this experiment and Temp18 treatment in Experiment 3. A different batch of compost, with higher microbial activities (d0) (\geq 0.8\mu gFDA/min/mL potting media), was used in Experiments 1 and 2, compared with media in Experiments 3 and 5 (<0.8\mu gFDA/min/mL potting media). The incorporation of peat and a different pot height was used in Experiment 3 compared with this experiment. In Experiment 3, media of AFP_{act} 16 and 19% were suppressive to *Pythium* damping-off, whereas, in this experiment, media of AFP_{act} 20% was conducive to disease. Perhaps high AFP_{act} (20%) with lower WHC (37 to 48%) caused higher plant stress in this experiment, increasing SDR, compared to high AFP_{act} (16.0 and 19.1%), with higher WHC (48 to 58%) in Experiment 3.

In conclusion, covering media of very low and high AFP_{act} (2, 3 and 20%) does not significantly affect SDR, while covering media of moderate AFP significantly does affect suppression. Increasing the potting media surface humidity may not significantly alter the extreme physical parameters of media, with extremely low aeration and high water content or media with high aeration and low water content. Microbial activity and *Pythium* counts, however, tended to be higher in covered treatments, but these did not affect SDR at very low or high AFP. Covering media of moderate AFP resulted in a much more dynamic environment, possibly by favouring the growth of specific antagonists, increasing plant vigour and decreasing the growth of the pathogen at AFP_{act} 6%, but not at AFP_{act} 14%. At AFP_{act} 14%, the increase of potting media surface humidity of media with moderate aeration may have
favoured the growth of *Pythium* and increased SDR. Increasing WHC at AFP<sub>act</sub> 3 and 20% did not alter the suppressiveness of media, but may affect the suppressiveness at AFP<sub>act</sub> 5 and 6%. This experiment emphasises the complexity of media, as AFP, WHC and covering and uncovering media may affect microbial activity, growth and infection of the pathogen and the plant vigour and, therefore, influence SDR. Further studies of the effects of AFP and its interaction with microbial activity on the suppression of *Pythium* is recommended to clarify the mechanism by which suppression is achieved.
Section 5: Effects of microbial activity in potting media of various Air-Filled Porosities on *Pythium* Suppression.

5.1 Introduction

It appears from the previous experiments that the interaction of microbial activity and AFP may affect physical, chemical and microbial characteristics of potting media, and therefore, the suppressive qualities of potting media, to the extent that there were conflicting effects of AFP on the severity of damping-off in cucumbers. The major factor appears to be microbial activity, although this is moderated to the extent of control of surface moisture in the media in pots. Microbial activities in Experiments 1 and 2 were two fold higher compared with Temp18 treatment in Experiment 3 and the uncovered treatment in Experiment 5 and may have altered the effect of AFP on SDR.

Fahy and Pike (1991) generally found that a minimum microbial activity level of 0.8μgFDA/min/mL potting media was required to achieve suppression of damping-off caused by *Pythium*. Boehm and Hoitink (1992) found that the severity of disease and populations of *Pythium* were suppressed when microbial activities were above 3.2μgFDA/min/g dry weight in peat mixes. It is difficult to compare these mixes as potting media substrates used in Experiments 1-5 vary significantly in weight. At each AFP there may be critical levels of microbial activity, e.g., media of low AFP and very high microbial activity may result in anaerobic conditions, which may cause stress to the plant and increase the susceptibility of the plant to disease.

Experiments 6 and 7, therefore, were designed to further study the effects of the interaction between AFP and microbial activity on the suppression of *Pythium* damping-off. One aim of these experiments was to determine the optimum microbial activity for each AFP necessary to achieve suppression. This was achieved by adjusting the microbial activity, either by modifying the C:N ratio of the mixes, or, by combining different ratios of pasteurised and non-pasteurised media. This was used across an $\text{AFP}_{\text{act}}$ range of 2-19% and $\text{WHC}_{\text{act}}$ 42-55% as media with various ratios
of aeration and water content may influence the growth and activity of microorganisms and hence, SDR of damping-off caused by *Pythium*.

In Experiment 6, varying microbial activities were achieved by the addition of C and/or N sources which stimulate microbial activity (Mandelbaum and Hadar, 1990). Media with an AFP\textsubscript{act} range of 2 to 6% in 8.5cm pots were chosen such that anaerobic conditions may be achieved. The range of microbial activities (d0) used, 0.270 to 1.440\(\mu\)gFDA/min/mL potting media, were similar to the ranges used in previous experiments, with the inclusion of a higher and lower range of microbial activity.

In Experiment 7, different ratios of pasteurised and non-pasteurised potting media were combined, to achieve different levels of microbial activity (d0) (0.4 to 2.7\(\mu\)g FDA/min/mL potting media) with minimal changes to nutrient levels, pH and salinity. Equal amounts of nutrients were added to all media, with heated and unheated components combined in different proportions. A wider range of AFP\textsubscript{act} (2 to 19%) values were chosen than in Experiment 6, such that media of AFP\textsubscript{act} 2 and 5%, of WHC\textsubscript{act} 44 and 41%, were compared with media of AFP\textsubscript{act} 2 and 5%, but with high WHC\textsubscript{act} (58 and 55%). Pot heights of 4.5cm were used to simulate pots commonly used in nurseries for germinating seedlings.

### 5.2 Materials and methods

The potting media were prepared for each experiment, as summarised below, as modifications of the general methods presented in Section 2.1. AFP and WHC were measured on each medium, as in Section 2.1.1. All experiments compared treatments with and without inoculation with *Pythium*, as described in Section 2.3.1. The suppressive qualities of media were determined by measurement of SDR, *Pythium* counts and microbial activity on the same day as sowing (d0) and on the day of harvesting plants (d10) as described in Sections 2.1.3 to 2.5.

#### 5.2.1 Experiment 6

The AFP treatments consisted of potting media of AFP\textsubscript{act} 2, 3 and 6% with WHC\textsubscript{act} of 48, 43 and 42% respectively in 8.5cm pots, each treatment being exposed to 4 different levels of microbial activity. For each AFP, there were 2 subtratments:
inoculated and uninoculated, and within these sub-treatments there were 4 levels of microbial activities, M1=lowest microbial level, M2=second lowest microbial level, M3=third lowest microbial level and M4=highest microbial level, achieved by various nutrient combinations.

Preparation of potting media of AFP:\textsubscript{act} 2, 3 and 6% were similar to the procedure in Experiment 5, except organic supplements other than compost were added in the potting media. Preliminary work involving the addition of different amounts of wheat bran, NH\textsubscript{4}NO\textsubscript{3} and glucose to potting media was performed in order to achieve 4 levels of microbial activity. For the lowest microbial level, 33% composted hardwood sawdust was used, instead of 31.5%, and wheat bran was added. At the 3 higher levels of microbial activities, 31.5% compost and the following were added: 1.5% wheat bran (v/v), 1.5% wheat bran (v/v) and NH\textsubscript{4}NO\textsubscript{3} (4g/L of potting media), 1.5% wheat bran (v/v), NH\textsubscript{4}NO\textsubscript{3} (4g/L of potting media) and glucose (4g/L of potting media). The pH of the media was adjusted after 4 days incubation at 18°C by flushing them with General Purpose Hydroponic solution (pH 4.5) (1:1 hydroponic solution:deionised water; see Appendix 1, Simple Grow®, Wetherill Park NSW) followed by free drainage from pots until pH 5.5 was achieved.

The inoculum rate of \textit{Pythium} for this bioassay was 2gL\textsuperscript{-1} of potting medium. These treatments were all uncovered and there were 5 replicates per treatment.

5.2.2 Experiment 7

The AFP treatments consisted of potting media of AFP:\textsubscript{act} 2, 5 and 19%, in the low WHC mix (LW treatment, WHC:\textsubscript{act} 37-44%), and AFP:\textsubscript{act} 2 and 5% only in the high WHC mix (HW treatment, WHC:\textsubscript{act} 55 and 58%). The number of treatments were limited due to the size of the growth chamber. Within LW treatment, there were 4 levels of microbial activity (M1=lowest microbial level, M2=second lowest microbial level, M3=third lowest microbial level and M4=highest microbial level) and in HW treatments there were 3 levels of microbial activity (M5=lowest microbial level, M6=second lowest microbial level and M7=third lowest microbial level).

In the LW treatments, potting media consisted of 31.5% compost, 1.5% wheat bran and 67% sand, while in the HW treatments, potting media consisted of 31.5% compost, 1.5% wheat bran, 44.5% sand and 22.5% spagnum peat. The proportion
of fine sand added to each mix was as follows: 45, 29, 0% to achieve AFPact 2, 5 and 19% respectively in the LW treatments, and 44.4 and 25% for AFPact 2 and 5% respectively in the HW treatments, while the remainder consisted of coarse sand.

A high level of microbial activity was achieved in potting media consisting of compost, wheat bran and sand, by supplementing the mixes with wheat bran (1.5% v/v), D(+) glucose (3.4g/L) and L-asparagine (1.0g/L), followed by incubation and measurement of microbial activity. A proportion of this mix was pasteurised, and the 3 lower levels of microbial activities were achieved by combining different ratios of pasteurised and non-pasteurised potting media. In potting media comprising compost, wheat bran, peat and sand there were also 3 levels of microbial activities, achieved by the above method of combining different ratios of pasteurised and non-pasteurised mixes. The microbial activities (d0) were established at 0.15, 0.50, 0.80 and 1.4μg FDA/min/mL potting media in the LW treatments and 0.15, 0.8 and 2.4μg FDA/min/mL potting media in the HW treatments.

Inoculum of *Pythium* was added at a rate of 2gL⁻¹ of potting media to the inoculated treatments. These treatments were all uncovered and there were 5 replicates per HW treatments and 6 replicates per LW treatments.
5.3 Results and Discussion

5.3.1 Experiment 6 Results

5.3.1.1 Effect of AFP and nutrient supplements on the Severity of Disease Rating Index (SDR)

In the M1 treatment, the SDR was significantly higher at $\text{AFP}_{\text{act}}$ 6% (2.5 units) than at $\text{AFP}_{\text{act}}$ 2 and 3% (1.7 and 1.9 units), whereas in the M4 treatment, the SDR was significantly lower at $\text{AFP}_{\text{act}}$ 6% (1.4 units) than at $\text{AFP}_{\text{act}}$ 2 and 3% (1.5 and 1.7 units) (Figure 5.1a). In the M2 treatment, the SDR (1.8 to 1.9 units) was not significantly different at each AFP treatment (Figure 5.1a). In the M3 treatment, the SDR was significantly lowest at $\text{AFP}_{\text{act}}$ 2% (1.3 units) and highest at AFP 6% (2.0 units), while it was not significantly different at $\text{AFP}_{\text{act}}$ 3% (1.4 units) compared to that at any other AFP (Figure 5.1a).

At $\text{AFP}_{\text{act}}$ 2 and 3%, the SDR was lower in M3 treatments than in M1 and M2 treatments, but was not significantly different to M4 treatments. At $\text{AFP}_{\text{act}}$ 6%, the SDR was significantly lower in M4 treatments than in M1, M2 and M3 treatments.

5.3.1.2 Effect of AFP and nutrient supplements on Pythium counts

In the M1, M3 and M4 treatments, Pythium counts were not significantly different at each AFP, whereas, in the M2 treatment, the Pythium count was lowest at $\text{AFP}_{\text{act}}$ 6% (116cfu/mL potting media), highest at $\text{AFP}_{\text{act}}$ 3% (548cfu/mL potting media) and both of these Pythium counts were not significantly different to that at $\text{AFP}_{\text{act}}$ 2% (381 cfu/mL potting media) (Figure 5.1b).

At $\text{AFP}_{\text{act}}$ 2%, Pythium count was highest in M1 treatment (794cfu/mL potting media) and lower in M3 and M4 treatments (116-123cfu/mL potting media). At $\text{AFP}_{\text{act}}$ 3%, Pythium counts were highest in M1 and M2 treatments (548-1085cfu/mL potting media) and lower in M3 and M4 treatments (157-113). At $\text{AFP}_{\text{act}}$ 6%, Pythium counts were highest in M1 treatment (1059cfu/mL potting media) and lower in M2, M3 and M4 treatments (151-166cfu/mL potting media).
5.3.1.3 Effect of AFP and nutrient supplements on Microbial activity

In the M1 and M2 treatments, microbial activity (d0) was not significantly different at each AFP. Microbial activity (d0) was significantly higher at AFP_{act} 2 and 3% than at AFP_{act} 6% for the M3 treatment and at AFP_{act} 2% than at AFP_{act} 3 and 6% for the M4 treatment (Figure 5.1c).

In the M1 treatment, microbial activity (d10) was not significantly different at each AFP. In the M2 treatment, microbial activity (d10) was lowest at AFP_{act} 2 and 3% (0.270-0.323μgFDA/min/mL potting media) and highest at AFP_{act} 6% (0.495μgFDA/min/mL potting media). In the M3 treatment, microbial activity (d10) was lowest at AFP_{act} 2% (0.897μgFDA/min/mL potting media) and highest at AFP_{act} 3% (1.072μgFDA/min/mL potting media). In the M4 treatment microbial activity (d10) was lowest in potting media of AFP_{act} 3% (1.045μgFDA/min/mL potting media) and highest at AFP_{act} 2 and 6% (1.418-1.440μgFDA/min/mL potting media) (Figure 5.1d).

At AFP_{act} 2%, microbial activity (d10) was lowest for M1 and M2 treatments (0.155-0.270μgFDA/min/mL potting media) and highest for M4 treatment (1.418μgFDA/min/mL potting media). At AFP_{act} 3%, microbial activity (d10) was lower for M1 treatment (0.150μgFDA/min/mL potting media) and highest for M3 and M4 treatments (1.045-1.072μgFDA/min/mL potting media). At AFP_{act} 6%, microbial activity (d10) was lower for M1 treatment (0.248μgFDA/min/mL potting media) and highest for M4 treatment (1.440μgFDA/min/mL potting media). AFP accounted for 95 and 99% variation in microbial activity (d0) and (10), respectively (p=0.0001).
Figure 5.1. Experiment 6. The effect of Air-Filled Porosity (AFP) at AFP$_{act}$ 2, 3 and 6% with various nutrient supplements (M1, M2, M3 and M4 treatments) on damping-off caused by *Pythium ultimum* in the cucumbers grown in potting media containing composted hardwood and sand. Note: In Figure a, data points with the same number(s) and letter(s) are not significantly different at each AFP$_{act}$ treatment and microbial level treatment, respectively and for Figures b, c & d, data points with the same letter(s) are not significantly different at each treatment (p=0.05).

5.1a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

5.1b) Mean * Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing (d10).

5.1c&d) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium (c) on day of sowing (d0) and (d) day 10 (d10) after sowing.
5.3.2 Experiment 6 Discussion

All potting media were suppressive to disease (1.3-2.5 units) and optimal AFP necessary to achieve maximum suppression varied, depending on the microbial activity. Suppression was optimal at \( AFP_{act} 2 \) and 3% for M1 treatment, at \( AFP_{act} 2\% \) for M3 treatment, at \( AFP_{act} 6\% \) for M4 treatment, but SDR's were not significantly different between AFP's for the M2 treatment.

The interaction between AFP and microbial activity appears to be complex and the mechanisms through which suppression occurs are unknown. AFP altered SDR in the M1, M3 and M4 treatment, but only altered *Pythium* counts in the M2 treatment and microbial activities (d0 and/or d10) in the M2, M3 and M4 treatment. Lower AFP may increase the availability and diffusion of the added nutrients, which may increase microbial activity and favour the growth of specific microflora, or affect the physiology of the plant, pathogen and antagonistic microorganisms and affect SDR. The level of microbial activity, as well as AFP, may also alter factors, such as concentration of microbial metabolites or \( O_2 \), which may cause plant stress and increase the plant's susceptibility to the pathogen or influence the growth of specific antagonistic microorganisms. In media of high microbial activity, microorganisms which can compete for nutrients more efficiently and can survive in conditions with high concentration of microbial metabolites at a specific AFP will predominate.

In the M1, M3 and M4 treatments, *Pythium* counts were not significantly different between AFP's and do not account for differences in SDR. Reduced SDR at low \( AFP_{act} 2 \) and 3%, with initial microbial activities of 0.441-0.508\( \mu \)gFDA/min/mL potting media (M1 treatment) and at AFP 2%, with initial microbial activities of 2.260\( \mu \)gFDA/min/mL potting media (M3 treatment), may have been due to a growth of specific antagonists, with increased mobility, or they may have been more effective in competing for sites near the plant roots, compared with microorganisms in media of \( AFP_{act} 6\% \). In the M2 treatment, *Pythium* counts were significantly lower at \( AFP_{act} 6\% \) (166cfu/mL potting media) than at \( AFP_{act} 3\% \) (548cfu/mL potting media), but this did not alter SDR. Perhaps the increased *Pythium* counts were not significant enough to increase disease severity at these specific conditions.
The addition of nutrients did not equally increase microbial activity (d0 and/or d10) between AFPs for M2, M3 and M4 treatments. In the M3 treatment, significantly higher microbial activity (d0) at AFP$_{act}$ 2 and 3%, than at AFP$_{act}$ 6%, may have reduced SDR, but microbial activity (d10) was significantly higher at AFP$_{act}$ 3% than at AFP$_{act}$ 2%. Plant exudation may have varied at each AFP and may have also influenced microbial activity (d10) and growth of specific antagonists. In the M4 treatment, significantly higher microbial activity (d0) at AFP$_{act}$ 2%, than at AFP$_{act}$ 3 and 6%, does not affect SDR. In the M2 treatment, higher microbial activity (d10) at AFP$_{act}$ 6% than at AFP$_{act}$ 2-3% may account for lower Pythium counts, but did not affect SDR.

Adding nutrients affected SDR at AFP$_{act}$ 2-6%, perhaps through increasing microbial activity which suppress the pathogen or by adversely affecting the plant. At AFP$_{act}$ 6%, higher microbial activities (d0) increased suppression possibly due to a decrease in Pythium counts. At AFP$_{act}$ 2 and 3%, higher microbial activities may have decreased Pythium counts, but SDR were significantly lower only for the M3 treatments. Media of low AFP and very high microbial activities in the M4 treatment may give rise to conditions with a high concentration of microbial metabolites, which may affect the physiology of plant roots and increase the susceptibility to disease. Mandelbaum and Hadar (1990) found that the addition of glucose/asparagine mixture into potting media amended with compost increased microbial activity and suppressed Pythium aphanidermatum. The addition of a very high concentration of glucose/asparagine mixture, however, increased the severity of disease. Energy source deprivation, through competition from other microorganisms, appears to be the main cause of suppression of disease. Excess nutrients in the M4 treatment did not significantly increase Pythium counts compared with the M3 treatment, therefore, anaerobic conditions may have increased disease. Microbial activity in this experiment decreased over the 10 days, probably as the nutrients were becoming depleted. The decrease in microbial activity was greater for M2, M3 and M4 treatments than in M1 treatment; therefore, the addition of wheat bran, ammonia and glucose is an artificial way of increasing microbial activity and the long term effects are not stable. Most compost mixes, however, usually have a stable microbial activity over several months (Fahy et al, 1995).
The addition of nutrients may have also altered factors, such as osmotic potential and salinity, or directly inhibited specific microflora. Glucose decreases the osmotic potential, effectively making the medium drier, which may directly decrease Pythium counts, rather than indirectly decreasing Pythium counts through microbial competition (McQuilken et al, 1992). The addition of glucose at AFP<sub>act</sub> 3% did not increase microbial activities as compared with media of AFP<sub>act</sub> 2 and 6%, but, showed similar effects on Pythium counts and SDR as with other AFP's. Glucose probably decreased Pythium counts by decreasing the osmotic potential. There have also been reports that ammonia is inhibitory to Oomycota, such as Phytophthora (Lockwood and Filonow, 1981), and production of ammonia by Enterobacter cloacae is also a possible mechanism which suppresses Pythium (Howell et al, 1988). Nelson and Maloney (1992) stated that the addition of sugars, such as D-glucose, eliminates the volatile inhibitory effect of organisms. The addition of NH<sub>4</sub>NO<sub>3</sub>, therefore, may have directly inhibited Pythium for the M3 treatment, and the addition of glucose may have eliminated this inhibitory effect for the M4 treatment. Kauraw and Singh (1983) stated, however, that P. ultimum could perhaps utilise a wider range of nitrogen sources and that the form of nitrogen did not affect the survival of this species.

This experiment shows that high microbial activity was associated with reduced growth of Pythium, but does not necessarily decrease the severity of disease as the level of suppression varied with the AFP of media. Data from this experiment may explain some of the inconsistencies between Experiments 1 and 2 with Experiments 3 and 5. Microbial activities in Experiments 1 and 2 were similar to the ranges found in the M2 treatment of this experiment, but did not result in similar trends. This variation may have been due to a different batch of compost or inoculum, different pot heights, or the effect of an added nutrient. Microbial activities in Experiment 4 were similar to ranges used in the M1 treatments, but a wider range of AFP was tested.

This experiment indicates that very high microbial activity at low AFP may induce anoxia in the mix and increase the plant's susceptibility to Pythium infection. Suppression may also be influenced, however, by nutrient concentration and type.
Therefore, it is recommended that microbial activity should only be altered using physical means, with minimal changes to nutrient concentration. Inclusion of a high AFP treatment would also be useful for comparison of low and high AFP treatments, as with previous experiments. Measurement of phytotoxicity of media may also assist in determining if AFP and microbial activity increases the concentration of phytotoxic compounds.
5.3.3 Experiment 7 Results

5.3.3.1 Effect of AFP and the ratio of pasteurised and non-pasteurised compost in media on Severity of Disease Rating index (SDR)

The SDR in the M1, M2, M4, M5, M6 and M7 treatments was not significantly different at each AFP, whereas the SDR in the M3 treatment was significantly higher at \( \text{AFP}_{\text{act}} 19\% (3.0 \text{ units}) \) than at \( \text{AFP}_{\text{act}} 2 \) and \( 5\% (1.8 \text{ and } 2.2 \text{ units}) \) (Figure 5.2a).

In the LW treatments, the SDR at \( \text{AFP}_{\text{act}} 2 \) and \( 5\% \) was highest in the M4 treatment (2.9-3.0 units) compared with the M1, M2 and M3 treatments (1.8 to 2.3 units), while at \( \text{AFP}_{\text{act}} 19\% \), the SDR was highest in the M3 treatment (3.0 units) and lowest in the M2 treatment (2.1 units).

In the HW treatments, the SDR was not significantly different in any subtreatment (2.7 to 3.5 units).

5.3.3.2 Effect of AFP and the ratio of pasteurised and non-pasteurised compost in media on Pythium counts

In all M treatments, Pythium counts were not significantly different at any AFP (Figure 5.2b). At each AFP, Pythium counts were not significantly different at any subtreatment.

5.3.3.3 Effect of AFP and the ratio of pasteurised and non-pasteurised compost in media on Microbial activity

AFP did not significantly affect microbial activity (d0), but accounted for 92% variation in microbial activity (d10) (p=0.005). In the M1, M2, M3, M4, M5, M6 and M7 treatments, microbial activity (d0) was not significantly different at each AFP (Figure 5.2c). At each AFP, microbial activity (d0) was higher in media containing a higher proportion of non-pasteurised media.

In the M1, M2 and M4 treatments, microbial activity (d10) was significantly higher at \( \text{AFP}_{\text{act}} 2 \) and \( 5\% \) than at \( \text{AFP}_{\text{act}} 19\% \) and in the M5 treatment, microbial activity (d10) was significantly higher at \( \text{AFP}_{\text{act}} 5\% \) than at \( \text{AFP}_{\text{act}} 2\% \) (Figure 5.2c). In the M3, M6 and M7 treatments, microbial activity (d10) was not significantly different at any AFP (Figure 5.2d).
In the LW treatment, microbial activity (d10) at AFP\textsubscript{act} 2 and 5% were not significantly different in any subtreatment, while at AFP\textsubscript{act} 19%, microbial activities (d10) were significantly higher for M3 and M4 treatments than M2 treatment. In the HW treatment, microbial activities (d10) were significantly higher in media containing a higher proportion of non-pasteurised media at each AFP.

5.3.3.4 Effect of AFP and the ratio of pasteurised and non-pasteurised compost in media on phytotoxicity index

At AFP\textsubscript{act} 2, 5 and 19%, phytotoxicity indices ranged from 42.4 to 71.6, 54.6 to 71.6 and 57.0 to 64.5 respectively (Figure 5.1e). In the M1, M2, M3 and M4 treatments, phytotoxicity indices ranged from 54.6 to 62.8, 57.0 to 71.6, 64.5 to 71.6 and 42.4 to 61.9, respectively.
Figure 5.2: The effect of Air-Filled Porosity (AFP) at AFP_{act} 2, 5 and 19%, with low WHC (41-44%) and containing various ratios of pasteurised and non pasteurised compost (LW treatments: M1, M2, M3 and M4 treatments) and at AFP_{act} 2 and 5%, with high WHC (55-58%) and containing various ratios of pasteurised and non pasteurised compost (HW treatments: M5, M6 and M7 treatments). Note: Data points with the same letter(s) and case (upper/lower) are not significantly different between each treatment. In Figure a) data points with the same letter were not significantly different between treatments at each AFP, while points with the same number are not significantly different between AFP's for each treatment (p=0.05).

5.2a) Severity of disease rating index (SDR) of seedlings on day 10 after sowing.

5.2b) Mean *Pythium* counts, as calculated from the most probable number per mL of potting medium, on day 10 after sowing.

5.2c&d) Microbial activity measured as the mean amount of fluorescein diacetate (FDA) hydrolysed per mL of potting medium (c) on day of sowing (d0) and (d) day 10 (d10) after sowing.

5.2e) Mean Phytotoxicity indices, as calculated from the product of the percentages of germination rates and root length per mL of potting medium extract.
5.3.4 Experiment 7 Discussion

AFP only significantly altered the suppressiveness of media, with moderately high microbial activity (d0) (0.766-0.859\(\mu\)gFDA/min/mL potting media, M3 treatment), but not for media with low and very high microbial activities (d0) (0.150-0.513 and 1.359-1.425\(\mu\)gFDA/min/mL potting media, M1, M2, M4, M5, M6 and M7 treatments). Suppression was optimal at AFP\(_{\text{act}}\) 2 and 5% for the M3 treatment. Within each AFP treatment, the level of microbial activity initially in the mix altered the suppressiveness of media with low WHC\(_{\text{act}}\) (37-44%), but not for media of high WHC\(_{\text{act}}\) (55 and 58%). In the LW treatments, very high microbial activity in media of AFP\(_{\text{act}}\) 2 and 3% decreased suppression, while at AFP\(_{\text{act}}\) 19%, suppression was optimal in media with moderately low and very high microbial activity (M2 and M4 treatments). In the HW treatments, however, suppression was consistently low with increasing microbial activity for each AFP.

*Pythium* counts were not significantly different in any treatment and may account for similar SDRs for M1, M2 and M4 treatments, but not for the M3 treatment. In M1, M2, M3 and M4 treatments, microbial activity (d10) was significantly lowest at AFP\(_{\text{act}}\) 19% than at AFP\(_{\text{act}}\) 2 and 5%, but apparently it reduced SDR only in the M3 treatment. In the M3 treatment, the higher SDR at AFP\(_{\text{act}}\) 19% may have been also due to increased plant stress, caused by a high concentration of microorganisms colonising the plant root, through increased competition for nutrients, water and air and production of metabolites, as well as low WHC, compared with media of AFP\(_{\text{act}}\) 2 and 5%.

Perhaps AFP, with a specific range of microbial activity, does not have a dramatic effect on the suppression of *Pythium* damping-off of cucumbers grown in potted media with a pot height of 4.5cm pots compared with that in 8.5cm pots. Pot heights of 4.5cm pots were used in Experiment 3 (Temp18 treatment), where SDR at AFP\(_{\text{act}}\) 2%, was not significantly different to AFP\(_{\text{act}}\) 8.2, 16.0 and 19%, but SDR at AFP\(_{\text{act}}\) 8.2% was significantly higher than AFP\(_{\text{act}}\) 16.0 and 19.1%, with microbial activities similar to the M1 treatment in this experiment. In Experiment 5 (ui treatment), 4.5cm pots were also used, but SDR was significantly lower at AFP\(_{\text{act}}\) 5% than at AFP\(_{\text{act}}\) 3 and 20%, while microbial activity (d0) was similar to
the range used in the M1 treatment. SDRs were significantly affected by AFP in Experiments 1, 2, 5 and 6, where 8.5cm pots were used. A wider range of AFP needs to be tested to determine whether AFP of media in both 4.5 and 8.5cm pots affects SDR. The contradiction in data may have been also due to ageing and/or a different batch of compost or inoculum. Heating of media may have altered the chemical properties of media and favoured the recolonisation of specific microorganisms for each treatment. Changes observed in the previous experiment may have been, thus, due to changed chemical factors, rather than increased microbial activity.

All HW treatments (M5, M6 and M7 treatments) were conducive to disease and may have been due to high water content, which favoured the growth of *Pythium*. AFP$_{act}$ 2 and 5% of media, with high WHC and microbial activities (d0) from 0.150 to 2.369 μgFDA/min/mL potting media, did not affect the suppressiveness of media, but more AFP treatments are required to verify this hypothesis.

Higher microbial activity did not significantly reduce *Pythium* counts; therefore, other factors probably moderated suppression. In fact, at AFP$_{act}$ 2 and 5%, the M4 treatments were significantly more conducive to disease than the M1, M2 and M3 treatments. At low AFP, an increase in the concentration of anaerobic compounds and microbial metabolites probably occurred due to excessive amounts of easily metabolisable nutrients; this, perhaps in combination with low O$_2$ concentration, may have increased the susceptibility of plant to disease at low AFP. At AFP$_{act}$ 19%, however, the M2 treatment was most suppressive to disease with lower *Pythium* counts than M1, M3 treatments and M4 treatment, but these counts were not significantly different within this AFP. The M2 treatment at high AFP$_{act}$ (19%) may provide ideal conditions for the plant and growth of specific antagonistic microflora.

In media with high microbial activity (d0) (1.359-1.425μgFDA/min/mL potting media, M4 treatment), the phytotoxicity of media increased as AFP decreased, possibly caused by the accumulation of microbial metabolites. The accumulation of microbial metabolites may have related to the increased phytotoxicity of media of AFP$_{act}$ 2 and 5%, with microbial activities (d0) greater than 0.8μgFDA/min/mL potting media. In the M1 and M3 treatments, the phytotoxicity indices were not different between AFP, and, perhaps the lower range of microbial activity did not
alter the concentration of microbial metabolites. In the M2 treatment, the phytotoxicity was highest at AFP 19% compared to AFP 2 and 5% and may have been due to growth of specific microflora. Lower water content of media may also increase the concentration of phytotoxic compounds, compared with media with higher water content. The interaction between water content, microbial activity and AFP, therefore, apparently significantly influences the phytotoxicity of media.

At each AFP, the microbial activities (d10) in this experiment were not significantly different between microbial activity treatments (M1-M4), whereas, in Experiment 5, the range of microbial activity was broader and remained significantly different between microbial activity treatments. Combining different ratios of pasteurised and non-pasteurised compost, therefore, did not successfully alter microbial activity for the full duration of the bioassay, except for peat- and compost-amended media, where the variation in the chemical properties of media was minimised. Mandelbaum et al (1988) found that mixtures of 10 to 20% non-heated composted separated manure (CSM) added to autoclaved CSM was significantly more suppressive than autoclaved medium, but not as suppressive as 30% non-heated CSM added to autoclaved CSM. Mixtures containing 10 to 30% autoclaved CSM were more suppressive than CSM, probably due to enhanced microbial activity resulting from higher levels of nutrients that were released from killed microorganisms and from heated organic matter. Microbial activities remained significantly different when combining different ratios of pasteurised and non-pasteurised compost for the HW treatments (M5-M7 treatments). The incorporation of peat in media, somehow, must have stabilised the level of microbial activity over the 10 day bioassay. Perhaps the microorganisms colonising on the substrate, peat, are heat sensitive and cannot recolonise this medium, or heating peat may have released inhibitory compounds, which may have maintained the microbial activity to similar levels to day 0 of the bioassay. In the previous experiment, microbial activity would most probably have decreased until all the added nutrients had been depleted.

This experiment provides evidence that high microbial activity in media of low AFP may induce conditions which increase disease, however, not through the increase of the growth of *Pythium*. In the media with low WHC_{act} (37-44%), suppression
tended to be optimal at low $AF_P_{act}$ (2-5%), in media at lower microbial activities (d0) ($\leq 0.9 \mu gFDA/min/mL$ potting media), as well as at high $AF_P_{act}$ (19%) in media with moderate and very high microbial activities (d0) (0.5 and 1.5 $\mu gFDA/min/mL$ potting media). Media with high $WHC_{act}$ (55-58%) were all conducive to disease and the level of microbial activity did not moderate the suppressiveness of media. A combination of other factors, such as oxygen stress, water stress and mechanical impedance, may increase the susceptibility of the plant to disease and these factors, including the concentration of anaerobic compounds, in the media may also decrease the growth of potentially antagonistic microflora to $Pythium$. 
5.4 General Discussion for Experiments 6 and 7

The interaction between AFP and microbial activity may induce changes to water content, the availability and diffusion of nutrients, and the concentration of nutrients, $O_2$ and microbial metabolites, which may affect the physiology and growth of the plant, the pathogen, and antagonistic microflora, which subsequently affects SDR. High microbial activities may cause plant or microbial stress, or both, by increasing the competition for nutrients, $O_2$, water and microbial metabolites. Unfavourable AFP and WHC may compound these effects.

Data in Experiments 6 and 7 generally contradict trends found by Boehm and Hoitink (1992), Chen et al (1988b) and Fahy and Pike (1991), where high microbial activity increased the suppressiveness of potting media due to high microbial competition. These experiments show some evidence that anaerobic conditions may increase the severity of disease. In Experiment 6, high microbial activities ($d_0$ $>$2.3 $\mu$gFDA/min/mL potting media) increased SDR at low AFP$_{act}$ (2 and 3%) compared to media with lower microbial activities ($d_0$) $0.5$-$2.2\mu$gFDA/min/mL potting media). Optimal suppression was achieved at microbial activities ($d_0$) $2.222$ to $2.260\mu$ gFDA/min/mL potting media for these AFP treatments. At AFP$_{act}$ 6%, however, increasing the microbial activity ($d_0$) also increased suppression, which is in agreement with published data by Boehm and Hoitink (1992), Chen et al (1988b) and Fahy and Pike (1991).

In Experiment 6, there may have been additive effects, however, due to the variation in nutrient concentration. In Experiment 7, high microbial activity ($d_0$) $1.4\mu$ gFDA/min/mL potting media) also increased SDR at low AFP$_{act}$ (2 and 5%), with low WHC$_{act}$ (41-44%). At high AFP$_{act}$ (19%), with low WHC$_{act}$ (37%), however, suppression was optimal at microbial activity ($d_0$) of 0.5 and 1.4$\mu$gFDA/min/mL potting media, compared with activities of 0.2 and 0.8$\mu$gFDA/min/mL potting media. At AFP$_{act}$ 2 and 5%, with high WHC$_{act}$ (55 and 58%), increased microbial activity ($d_0$) $0.2$-$2.4\mu$gFDA/min/mL potting media) did not significantly affect SDR, possibly due to high water content which favours the activities of the pathogen. There may have also been additive affects in Experiment 7 due to heating of the media.
Data in Experiments 6 and 7 also indicate that variations between experiments may have been due to the variable microbial activity between compost batches, or ageing of compost, but is not conclusive due to the design of the experiments. In Experiment 6, added nutrients in various microbial activity treatments may have altered other factors, such as osmotic potential and salinity, which may affect SDR. In Experiment 7, treatments containing a higher proportion of pasteurised media may have resulted in a higher levels of available nutrients, due to killed microorganisms and heated organic matter. In both experiments, microbial activities were not stable over the 10 day bioassay, where microbial activity in Experiment 6 tended to decrease as nutrients were being depleted, while the combination of pasteurised and non-pasteurised media resulted in microbial recolonisation of media and increased microbial activity to similar levels for all microbial treatments. Overall, microbial activity can be used as a general indicator of suppressiveness of media. High microbial activity (d0) (>1.4μgFDA/min/mL potting media) may also increase SDR, therefore other factors, such as AFP, need to be taken into consideration.
Section 6: General Discussion and Recommendations

A narrow range of AFP required to achieve consistently suppressive media has not been determined, as AFP has been found to interact with many factors such as microbial activity, temperature shock of media and plants and moisture levels modified by covering and uncovering media, or, by using different pot heights to modify WHC. Heating media at 60°C for 3 days significantly decreased the microbial activity and negates the suppressiveness of media as shown in Experiment 2 (See Table 5.1). Higher microbial activity, however, does not consistently lead to a reduction in Pythium counts and SDR, as demonstrated in Experiments 1, 3, 4, 5, 6 and 7, shown in Table 5.1. Increasing microbial activity in peat- and compost-amended media did not alter the suppressiveness of media at AFP_{act} 2 and 5%. Fahy and Pike (1991) also found that microbial activity did not always correlate with suppression of Pythium and the severity of disease therefore, there may have been specific antagonists involved (Hoitink et al, 1997). The development of a simple quantitative assay for the detection of specific antagonists may provide a better indication of the suppressiveness of media against P. ultimum, compared with the measurement of microbial activity by FDA hydrolysis. In Experiments 6 and 7, extremely high microbial activity in compost amended media of low AFP_{act} (2-5%) decreased the suppression of Pythium damping-off, in conjunction with decreasing Pythium counts. Media with high microbial activity at high AFP_{act} (6 and 19%) tended, however, to increase suppression and decrease Pythium counts. These data show evidence that high microbial activity (>1.5μgFDA/min/mL potting media) in media of low AFP may increase the anoxia of the mix and increase the susceptibility of the plant to disease, caused by lower numbers of Pythium propagules than in other situations.

A major concern of this study is the lack of quality control during composting, as it directly affects microbial activity and the resultant potting media and nutrients available for microbial and plant use. Fahy et al (1995) found that composted hardwood sawdusts collected from 4 composting sites were not consistent and found 1-2 out of 5 batches from each site were conducive to Pythium disease. These
authors also found that, in a follow up trial, 5 out of 8 composts aged for 6 months were consistently suppressive. The use of different batches of compost and ageing of the compost in Experiments 1-7 may also account for some of the different effects of AFP on the severity of *Pythium* disease.

Heating media and emerged seedlings for 2 hrs at 35 and 45°C in Experiment 3 also alters suppressiveness of media at AFP\textsubscript{act} 2.4-19.1%. Heating media at 45°C appeared to have killed *Pythium*, resulting in low SDR at all AFP treatments and increased microbial activity, compared with the former treatment, as well as the control treatment kept at 18°C. Heat resistant microorganisms are commonly found in composts. At a high temperature of 45°C, enhanced microbial activity possibly has resulted, therefore, from higher levels of nutrients that were released from killed, heat susceptible microorganisms and from heated organic matter, favoured at AFP\textsubscript{act} 8.2% or greater.

Heating media and the plant prior to emergence of seedlings from the surface of potting media at 45°C for 2hrs significantly decreased *Pythium* counts and the severity of *Pythium* damping-off in cucumbers in media of AFP\textsubscript{act} 2.4-19.1%, while optimum suppression occurred at AFP\textsubscript{act} 8.2% in media heated at 35°C for 2 hrs. Microbial activity does not account for all increased suppression, and therefore, other factors, such as, plant stress, pathogen stress and selection of specific microflora may have influenced SDR.

Increasing WHC appears to cause an increased severity of *Pythium* damping-off, as media of AFP\textsubscript{act} 2 and 5%, WHC\textsubscript{act} 58 and 55%, with microbial activities (d10) of 0.519-2.486µgFDA/min/mL potting media, were more conducive to disease in Experiment 7 compared to media of AFP\textsubscript{act} 2 and 5%, WHC\textsubscript{act} 44 and 41%, with microbial activities of 1.469-1.692µgFDA/min/mL potting media. The incorporation of peat in media may have altered other factors, such as aeration, water and nutrient availability, which may have also influenced SDR. This was expected, as published data state that *Pythium* growth, colonisation and higher severity and incidence of disease caused by *Pythium*, occur at higher water content (Bateman, 1961; Huissman, 1987; Porter \textit{et al}, 1987; Stranghellini and Burr, 1973). Bateman (1961), Fahy \textit{et al} (1995) and Kerr (1964) showed that an increase in WHC increased the incidence and
severity of disease. In Experiment 5, increasing WHC from 33% to 37% in media of AFP$_{\text{act}}$ 20% and from 43% to 44% in media of AFP$_{\text{act}}$ 3%, did not increase SDR. WHC was increased physically by decreasing pot height, so that the organic nutrient contents remained the same in all treatments. Most of the other reported studies, however, compared WHC at much higher values than the range used in these experiments. Bateman (1961) and Fahy et al (1995) tested media with a WHC range of 29 to 88% and 46 to 73%, respectively. WHC$_{\text{std}}$ in these experiments only ranged from 21 to 54% and this range may not have shown an increase in disease severity as WHC increased. WHC in Bateman's study was measured with an impedance comparator and the different methodologies may also account for other differences in results. Kerr (1964) showed that soil moisture content (3-12%) only caused slightly increased Pythium population counts and indirectly increased the incidence of disease by increasing exudation of seedlings. Bateman (1961) and Kerr (1964) used inert potting media and soil which tends to have lower microbial activity, compared to the compost amended media used in these experiments, and so would not have had antagonistic effects of microbiota on the pathogen.

Uncovering and covering media also alters the severity of Pythium damping-off in cucumbers and possibly in Impatiens, Celosia, Chilli Pepper, Salvia and Snapdragon. The mechanisms for suppression of Pythium damping-off, through the manipulation of AFP and uncovering and covering media is unknown, but is probably due to changes in the moisture balance within the medium as well as at its surface and the resultant effects on plant growth and pathogenic and nonpathogenic microbial activities. Covering media increased suppression of Pythium damping-off in cucumbers at AFP$_{\text{act}}$ 6% and decreased suppression at AFP$_{\text{act}}$ 14%, but did not affect the suppressiveness of media of AFP$_{\text{act}}$ 2, 3 and 20%. Higher humidity at AFP$_{\text{act}}$ 6% may have been most favourable to plant growth and increased plant vigour with reduced exudation, which inhibited the growth of Pythium, or favoured the growth of antagonistic microflora, compared to media with lower humidity. Higher humidity at AFP$_{\text{act}}$ 14%, however, may have increased plant exudation and encouraged the growth of Pythium over competitive microbial activities, compared to uncovered media having lower surface humidity.
Each species of plant seems to also influence the severity of *Pythium* damping-off at each of the AFP. At $A_{\text{P act}}$ 10.8%, higher suppression of disease in Impatiens, Chilli Pepper, Salvia and Snapdragon may have been due to the reduction of plant stress by covering media compared to uncovered media. Covering media may lead to wetter conditions, compared to uncovered media. This may have significantly decreased SDR through increased microbial activity, despite increased *Pythium* counts, compared to uncovered media. At $A_{\text{P act}}$ 2.6%, lower suppression of disease in Impatiens, Celosia, and Salvia may have been due to increased anoxia in covered media compared to uncovered media. There is a recent hypothesis from other studies suggesting that compost may activate genes which code for disease resistance (Hoitink *et al*, 1997). To confirm whether covering and uncovering media of various AFP affects SDR, the 2 bioassays for each plant should be repeated as 1 bioassay in order to reduce variations caused by resowing potting medium.

High *Pythium* counts tend to increase the severity of *Pythium* damping-off, as shown in Experiments 1, 2, 5 and 7, with cucumber, and Celosia treatments in Experiment 4 (see Table 5.1). WHC in this study in Experiments 1, 2, 4, and 6 was too low, compared with those recommended by the Australian Standards, and possibly increased the severity of disease at high $A_{\text{P act}}$ (>5%). Low WHC may have decreased the mobility of bacteria and microbial competition at high AFP as well as caused increased plant stress, plant exudation and *Pythium* counts. In Experiment 3, suppression was also optimal at high $A_{\text{P act}}$ (16.0 and 19.1%), perhaps due to a higher WHC range, compared with Experiments 1, 2 and 6.

Fahy *et al* (1995) found that suppressiveness to *Pythium* was achieved by high microbial activity and high AFP, but this was not consistent in all their trials. Materials aged over 9 months and materials reported to have shown good suppression during an ageing period of 9 months were Sunpeat®, pine bark fines, composted hardwood sawdust from yard 2, with an $A_{\text{P std}}$ 23.5 to 28.1%, 12.7 to 21.5% and 20 to 30%, respectively. Consistently conducive mixes were shamrock peat and black peat of AFP in the range of 6 to 12%. Peat materials of $A_{\text{P std}}$ 6 to 26% revealed a moderate positive correlation between AFP and *Pythium* suppression ($r=0.65$, $p=0.01$). Materials which were suppressive initially for the first 0 to 3
months were Eurotof®, Novobalt®, Kiwi-peat® and Moorgold®, which had an 
$AFP_{std}$ range of 15 to 30%, 12 to 26%, 8 to 12% and 12-15%, respectively. Over 
the 9 months, these mixes generally became more compacted, and therefore, the 
decrease in AFP over time may have caused an increase in the conducive ness of 
media. Materials used in trials of Fahy et al (1995) were quite diverse, compared 
with those used in Experiments 1-7, and other factors, such as microbial activity, 
nitrogen drawdown and pH were shown to affect suppression and may explain the 
inconsistency between trials of Fahy et al (1995). Other changes observed over the 9 
months included increases in WHC and pH, and a decrease in electrical conductivity. 
Due to the complexity of media, these correlations do not conclusively show that the 
severity of disease in cucumber plants increases at high AFP. In addition, the AFP 
ranged from 6-30%, in trials of Fahy et al (1995), as compared with media of $AFP_{act}$ 
1.8-25% in Experiments 1-7.

Due to the complexity of interactions occurring in media, the effects of AFP on SDR 
is easily altered by other factors and a consistent range of AFP was not established 
during Experiments 1-7 to achieve suppression. A narrow range of AFP to achieve 
suppression, therefore, cannot yet be included in the Australian Standards from this 
study. Altering AFP to reduce Pythium damping-off is not recommended, unless the 
exact conditions, such as temperature, WHC and microbial activity, may be 
controlled consistently as shown in Table 5.1. This is a difficult objective, but, 
perhaps increasing the quality control for composting may allow for the manipulation 
of AFP to achieve consistently suppressive media. Australian Standards for potting 
mixes are non component based, yet the work conducted by Fahy et al (1995) 
indicates that disease suppression is clearly component based; therefore, it is 
inappropriate to use the Australian Standards as a base to develop specifications for 
disease suppressive potting media. The only recommendation is to give guidelines to 
produce media of specific formulations that are consistently disease suppressive. 
These experiments have shown that high microbial activity does not necessarily lead 
to suppression and, in some conditions, can actually decrease the suppressive 
qualities of media.
<table>
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<th>Experiment and pot height (cm)</th>
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Table 5.1: Severity of disease Rating Index (SDR), microbial activity (d10) and Pythium counts in suppressive and conducive media in Experiments 1-7.

Note 1: NA= no treatment was found to conform with this category; Note 2: B1=Bioassay 1 and B2=Bioassay 2
Variability of compost is one of the principal factors limiting its widespread use; therefore, improving the quality control during the composting process would be the principal factor which should be investigated. Substrates used for composting may vary, but inoculation of specific strains of antagonistic and competitive microorganisms, such as *Trichoderma* spp., into compost after peak heating during the composting process, but before significant levels of recolonisation, has been shown to induce consistent levels of suppression to diseases caused by a wide range of plant pathogens (Hoitink *et al.*, 1997). The following investigations may be pursued in order to formulate a high quality potting medium, after a consistent high quality compost with a microbial activity of 0.8µgFDA/min/mL potting media, or, greater, is produced. Comparison of media of similar AFP, but varying WHC and microbial activities, should be examined as well as a study made of the effects of anaerobic compounds on the severity of disease caused by *Pythium*.

There is need to determine the effect of AFP, as well as WHC, of media in a pot height, which is commonly used in nurseries on the suppressiveness of media. Watering regime may also influence the suppressiveness of media at various AFP. The physical factors which have been shown to be suppressive and conducive to *Pythium* disease may also be tested on a number of species of plant to establish if this is a common phenomenon and perhaps list recommendations for potting media formulation for the major types of plants.

Measurement of other parameters listed below may assist in the explanation of the effect of AFP on the suppressiveness of media.

- Measurements of dry weight of plants and roots and qualitative and quantitative measurement of plant exudates at day 10 of the bioassay may give an indication of plant vigour and the level of plant stress. Stressed plants tend to release higher quantities of plant exudates and decrease dry weights of plant material, thus increasing the plant's susceptibility to *Pythium* disease. Different quantities and type of soluble and volatile plant exudates, such as amino acids, ethanol, organic acids and ammonia, may either inhibit or increase the germination of *Pythium*, which may vary according to the environment and species of plants (Nelson, 1991; Sharma and Gupta, 1991).
• Measurement of anaerobic compounds, such as short chain fatty acids, however, may be difficult as this requires a sensitive method and these compounds may accumulate only in a small area, microniches.

• Measurement of oospore production, germination of sporangia and thin walled oospores and conversion rates of asexual and sexual structures of *Pythium* may assist in describing the mechanism for suppression.

• Matric potential in Experiment 5 was close to saturation and indicates that moisture availability was the same for all media at various AFP. These measurements were made using a pooled sample and perhaps media at the surface may be significantly drier than the pooled sample and may more accurately determine the moisture available to a germinating seed.

• Determination of microbial activities of various species of microflora with antagonistic activity.

• Screening of genes in plants, the activation or deactivation factors, which may play a role in disease resistance upon exposure of the plant to compost may be investigated.
Section 7: PERSONAL COMMUNICATION

“Simple Grow”, Hassel Street, Wetherill Park. NSW.

Cresswell, G. Commodity Storage, Rouse Hill, NSW.

Fahy, P. Organic Waste Recycling Unit, NSW Agriculture, Richmond.

Noble, D. Organic Waste Recycling Unit, NSW Agriculture, Richmond.

Summerell, B. Royal Botanic Gardens, Sydney.

Tesoriero, L. Elizabeth Macarthur Agriculture Institute, NSW Agriculture, Camden, NSW.
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matric potential on growth and oospore germination of the biocontrol agent

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Bentgrass and Annual Bluegrass Turf with Compost-Amended Topdressings.
Plant Disease, 76: 954-958.

biological control mechanisms in bacteria: Studies of the interaction of
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Air-filled Porosity of Container Media to Development of Phytophthora Root
Rot of Rhododendron. Phytopathology, 81: 936-941.


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9.1 Appendix 1: Composition of hydroponic solution, Simple Grow® General Purpose

<table>
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<tr>
<th>Nutrient</th>
<th>Concentration (mgL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃-N</td>
<td>150</td>
</tr>
<tr>
<td>NH₄-N</td>
<td>40</td>
</tr>
<tr>
<td>PO₄-P</td>
<td>40</td>
</tr>
<tr>
<td>K</td>
<td>260</td>
</tr>
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<td>Ca</td>
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</tr>
<tr>
<td>Mg</td>
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<tr>
<td>Mn</td>
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<td>Cu</td>
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<tr>
<td>Zn</td>
<td>0.1</td>
</tr>
<tr>
<td>Fe</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Reference: Cresswell (1991)
9.2 Appendix 2: Method for preparing Potato Carrot Agar amended with antibiotics and β-sitosterol:

Potatoes (20 g) and carrots (20 g) were scrubbed and cut into small pieces (1-2 cm), and added to 500 mL of distilled water in an 1 L Erlenmeyer flask. Agar (Diversey Pty Ltd®) (12 g) and 500 mL of distilled water were added to a separate flask. The flasks containing the ingredients were steamed for 10 min in a steam bath (Thermo-Line Scientific Equipment®, Sydney). The cooked potato and carrot were filtered through a cheesecloth into a clean flask and made up to 500 mL with distilled water. This solution was then added to the flask containing agar. This flask was then covered with a cotton plug and aluminium foil and autoclaved (FCW Scientific Equipment®, Sydney) for 20 min at 15 lbs pressure.

After autoclaving, the contents of the flask were thoroughly mixed and 100 mL aseptically poured into each 150 mL sterile bottles in a laminar flow cabinet (Clemco Ultraviolet Products®, Sydney). Once the agar had cooled to approximately 45°C the following were added: rifampimycin (10 μg mL⁻¹), streptomycin (100 μg mL⁻¹), ampicillin (250 μg mL⁻¹), pimaricin (5 μg mL⁻¹) and β-sitosterol (20 μg mL⁻¹) to the agar in the bottles before it solidified. The bottles were stored in a refrigerator. Before use, the agar was melted in a steam bath (Thermo-Line Scientific Equipment®, Sydney), and then placed in a 60°C water bath (Kenlab®) before it was poured into Petri dishes.

9.3 Appendix 3: Sorenson's Phosphate Buffer Mixture, 60mM Potassium Phosphate buffer, pH 7.6

To achieve a pH of 7.6, 9 mL of primary solution was mixed with 1 mL of secondary solution.

Primary solution: 9.078 g KH₂PO₄ per litre of solution

Secondary solution: 11.876 g Na₂PH₄·2H₂O in 1 L of solution

Reference: Laboratory Manual for the media preparation room at the Biological and Chemical Research Institute, NSW Agriculture.
9.4 Appendix 4: FDA standard Curve

![Graph showing absorbance vs. ugFDA/20min/20mL of buffer.](Image)
9.5 Appendix 5: Regression equations for *Pythium* counts (MPN) and microbial activity at day 0 (MA0) and day 10 (MA10) are as follows:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Relationship between AFP and <em>Pythium</em> counts (MPN)</th>
<th>Relationship between AFP and microbial activity (MA0)</th>
<th>Relationship between AFP and microbial activity (MA10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\text{MPN} = -1.123 + 1.175 \text{AFP} - 0.035 \text{AFP}^2$</td>
<td>$\text{MA0} = 1.421 + 0.0378 \text{AFP} + 0.0022 \text{AFP}^2$</td>
<td>$\text{MA10} = 0.0914 + 0.0956 \text{AFP} - 0.0038 \text{AFP}^2$</td>
</tr>
<tr>
<td>2</td>
<td>$\text{MPN(UU)} = 6.12 + 0.036 \text{AFP}$ [\text{MPN(HI)} = 7.64 + 0.033 \text{AFP}]</td>
<td>$\text{MA0(UU)} = 0.844 - 0.004 \text{AFP}$ [\text{MA0(HI)} = 0.354 - 0.001 \text{AFP}]</td>
<td>$\text{MA10(UU)} = 1.148 - 0.010 \text{AFP}$ [\text{MA10(HI)} = 0.286 - 0.001 \text{AFP}]</td>
</tr>
<tr>
<td>5</td>
<td>$\text{MPN(UU)} = 4.816 + 0.077 \text{AFP}$ [\text{MPN(CD)} = 5.452 + 0.092 \text{AFP}]</td>
<td>$\text{MA0(UU)} = 0.333 + 0.015 \text{AFP}$ [\text{MA0(CD)} = 0.339 + 0.015 \text{AFP}]</td>
<td>$\text{MA10(UU)} = 0.640 + 0.004 \text{AFP}$ [\text{MA10(CD)} = 0.615 + 0.013 \text{AFP}].</td>
</tr>
</tbody>
</table>
9.6 Appendix 6: List of Species Authorities for Plants, Fungi and Bacteria Mentioned in Thesis

Plants

*Agrostis palustris* Huds. Marsh Bent
*also known as Agrostis stolonifera var palustris* (Huds.) Farw.
*Antirrhinum majus* L. Snapdragon
*Brassica rapa* L. Cabbage
*Capsicum annuum* L. Chilli pepper
*Celosia argentea* L. Celosia
*Cucumis sativus* L. Cucumber
*Daucus carota* L. Carrot
*Eucalyptus L’Hérît.* Eucalypts
*Euphorbia pulcherrima* Willd. Poinsetta
*Geranium L.* Geranium see also *Pelargonium hortorum*
*Glycine max* L. Soybean
*Gossypium hirsutum* L. Cotton
*Lepidium sativum* L. Curl Cress
*Lycopersicon esculentum* Miller Tomato
*Lupinus augustifolius* L. Lupins
*Heteromeles arbutilifolia* (Aiton) M.Roemer Toyon
*Impatiens balsamina* L. Impatiens
*Pelargonium hortorum* L. Geranium
*Pinus radiata* D. Don Pine
*Pisum sativum* L. Peas
*Phaseolus vulgaris* L. Snapbeans, Beans
*Poinsettia J.Graham* now *Euphorbia*
*Salvia splendens* Sellow ex Roemer & Schultes Salvia
*Triticum aestivum* L. Wheat

Bacteria

*Arthrobacter* Conn and Dimmick
*Bacillus* Cohn.
*Enterobacter cloacae* (Jordan) Hormaeche & Edwards
*Flavobacterium balustinum* Harrison
*Pseudomonas* Migula
*Pseudomonas fluorescens* Migula

Fungi

*Fusarium oxysporum* Schlecht. emend Snyder & Hansen
*Fusarium solani* (Mart.) Appel & Wollenweber emend. Snyder & Hansen
*Gliocladium* Corda.
*Penicillium* Fries
*Phytophthora cinnamomi* Rands
*Pythium aphanidermatum* (Edson) Fitz.
*Pythium cryptogea* Pethyr. & Laff
*Pythium graminicola* Subramanian
*Pythium irregularare* Buisman
*Pythium nunn* Lifshitz
*Pythium oligandrum* Dreschsler
*Pythium myriotylum* Dreschler
*Pythium parasitica* Dastur.
Pythium splendens Braun.
Pythium ultimum Trow. var ultimum
Pythium vexans de Bary
Rhizoctonia solani Kühn
Sclerotinia minor Jagger
Trichoderma Pers.
Trichoderma hamatum (Bonorden) Bainier

Sources:


SOME EFFECTS OF AIR-FILLED POROSITY ON THE
SUPPRESSION OF DAMPING-OFF OF SEEDLINGS BY
PYTHIUM ULTIMUM IN COMPOST AMENDED POTTING
MEDIA.

by
Rosetta Lainà, BSc

A thesis presented to the University of Western Sydney, Macarthur, in partial
fulfilment of the requirements for the degree of Master of Science (Hons).

December, 1997.
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The opportunity to undertake this research project was provided by the Biological and Chemical Research Institute, Rydalmere. I wish to express my appreciation to some members of the Biological and Chemical Research Institute, Rydalmere for their technical assistance and guidance in this project. Special thanks to Dorothy Noble who provided technical assistance, one of which included the preparation of Pythium inoculum. Also thanks to Michael Hewitt for assisting me in the collection of potting media substrates from the Australian Native Landscapes. Thanks to Lowan Turton for his photographic work and Igris Barcia for the statistical analysis on data.

I thank my dear friend Peter Josh for his assistance and friendship. I thank my brother, Emilio Laina, for his mathematical assistance and for the use of his personal computer. I thank my brother, Chris Laina, for assisting me in setting up a few bioassays, which included counting 70 seeds per pot for the Snapdragon bioassay.

I am grateful to Australian Native Landscapes, Seven Hills, for the sand and composted hardwood sawdust used in the experimental work.

A particular thank you to fellow postgraduates, and lecturers at University of Western Sydney, for their support and good company.

Rosetta Lainà
STATEMENT OF AUTHENTICATION

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in whole or in part, for a degree at this or any other institution.

[Signature]
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<th>FULL TERM</th>
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<tr>
<td>AFP</td>
<td>AIR-FILLED POROSITY</td>
</tr>
<tr>
<td>AFP_{ACT}</td>
<td>AIR-FILLED POROSITY MEASURED IN CYLINDERS WITH THE SAME HEIGHT AS POTS USED IN THE BIOASSAY</td>
</tr>
<tr>
<td>AFP_{STD}</td>
<td>AIR-FILLED POROSITY MEASURED ACCORDING TO THE AUSTRALIAN STANDARDS</td>
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<tr>
<td>CFU</td>
<td>COLONY FORMING UNITS</td>
</tr>
<tr>
<td>D0</td>
<td>MEASURED AT DAY 0</td>
</tr>
<tr>
<td>D10</td>
<td>MEASURED AT DAY 10</td>
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<tr>
<td>FDA</td>
<td>FLUORESCEIN DIACETATE</td>
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<tr>
<td>MPA</td>
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<td>\textit{P. ULTIMUM}</td>
<td>\textit{PYTHIUM ULTIMUM}</td>
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<td>WHC</td>
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<td>WHC_{ACT}</td>
<td>WATER-HOLDING CAPACITY MEASURED IN CYLINDERS WITH THE SAME HEIGHT AS POTS USED IN THE BIOASSAY</td>
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<td>WHC_{STD}</td>
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Abstract

Air-filled porosity (AFP) affected the suppressiveness of organically based plant growth media against *Pythium ultimum* infection. AFP also interacted with microbial activity, moisture levels altered by covering/uncovering media and water holding capacity (WHC) and the interaction of these factors influences the severity of *Pythium* damping-off.

Suppression of *Pythium* damping-off in cucumbers grown in 8.5cm pots at 18°C was optimal at $\text{AFP}_{\text{act}}$ 2.0-2.6% compared to $\text{AFP}_{\text{act}}$ 3.8-25.0%, with microbial activities ranging from 0.779-1.625µg fluorescein diacetate (FDA)/min/mL potting media. Suppression of *Pythium* damping-off of cucumbers in 4.5cm pots at 18°C was optimal at $\text{AFP}_{\text{act}}$ 16.0-19.1%, with microbial activities ranging from 0.356-0.516µgFDA/min/mL potting media.

The severity of *Pythium* damping-off in Impatiens, Celosia, Chilli Pepper, Salvia and Snapdragon was also lower at $\text{AFP}_{\text{act}}$ 2.6% than at $\text{AFP}_{\text{act}}$ 10.8%. When media were resown, however, only Snapdragon was least affected by *Pythium* damping-off at $\text{AFP}_{\text{act}}$ 2.6%, while the severity of *Pythium* damping-off in Impatiens, Celosia and Salvia was lowest at $\text{AFP}_{\text{act}}$ 10.8%. Covering media in the second bioassay, when growing Impatiens, Celosia, Chilli Pepper and Salvia, may have affected the severity of *Pythium* damping-off at $\text{AFP}_{\text{act}}$ 2.6 and 10.8%. Covering media when growing cucumbers increased suppression of *Pythium* damping-off at $\text{AFP}_{\text{act}}$ 6%, but decreased suppression at $\text{AFP}_{\text{act}}$ 14% compared to uncovered media, while suppression at $\text{AFP}_{\text{act}}$ 2, 3 and 20% was not affected by covering or uncovering media.

Heating media at 60°C for 3 days prior to the bioassay negated the suppressiveness of media in cucumber bioassays. Heating media and the plant prior to emergence of seedlings from the surface of potting media at 45°C for 2hrs significantly decreased *Pythium* counts and the severity of *Pythium* damping-off in cucumbers in media of $\text{AFP}_{\text{act}}$ 2.4-19.1%, while optimum suppression occurred at $\text{AFP}_{\text{act}}$ 8.2% in media heated at 35°C for 2 hrs.
At $AFP_{act}$ 2-6%, an increase in microbial activity tended to increase suppression, but very high microbial activities (0.150-1.359$\mu$gFDA/min/mL potting media) decreased the suppression of *Pythium* damping-off in cucumbers, while at $AFP_{act}$ 19%, an increase in microbial activity (0.167-1.425$\mu$gFDA/min/mL potting media) increased suppression.

High microbial activities between AFP treatments do not tend to cause significant decreases in *Pythium* counts, except when microbial activities were 2-6 fold higher than 0.2-0.4$\mu$gFDA/min/mL potting media.

In conclusion, it has not been possible to determine a narrow range of AFP required to achieve consistently suppressive media, as AFP has been found to interact with many factors such as microbial activity, temperature shock of media and plants and moisture levels modified by covering and uncovering media, or using different pot heights to modify WHC. The use of different batches of compost and ageing of compost in the seven experiments reported here may also account for some of the different effects of AFP on the severity of *Pythium* disease.

It is not recommended that AFP be used for controlling *Pythium* damping-off, unless certain conditions, such as microbial activity, can be controlled to a range of specifications also. Variability of compost is one of the principal factors limiting its widespread use, so therefore, improving the quality control during the composting process would be the principal factor which should be investigated in future.