CONTROL OF *PHYTOPTHORA PARASITICA* SPREAD UNDER RECIRCULATING IRRIGATION AND OF FUNGICIDE LEACHING FROM SOILLESS MEDIA

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CHAPTER I

INTRODUCTION

The past several decades have witnessed an ever growing environmental awareness which has lead to increasingly tougher federal and state legislation designed to reduce pollution at many levels. Runoff of irrigation water containing fertilizers and pesticides from greenhouse and nursery operations have been targeted as potential sources of contamination of both surface and groundwater. The nursery and greenhouse industries are under increasing scrutiny to reduce their pollutant discharges to the environment, and may soon be required to do so by federal law. Two states, California and Texas already have tough antipollutant legislation for greenhouse and ornamental production. Historically these operations have been categorized along with agriculture as non-point sources of pollution. With reauthorization of the Clean Water Act expected in the near future however, stricter monitoring and limits to the pollutants leaving these operations may be required.

The greenhouse and nursery industries are integral parts of Oklahoma's economy with estimated annual crop sales valued at over $200 million. Oklahoma ranks tenth nationally in nursery and greenhouse crop production. Containerized crop production in these operations is highly intensive and requires constant application of pesticides and

fertilizers to maximize production. One option for these industries to limit the amount of runoff is through the re-use of irrigation water. Recycling irrigation systems, where water and fertilizer solutions are recirculated are therefore an important consideration for greenhouse and nursery industries seeking to limit runoff.

Hydroponic systems, where plants are produced without soil is the classic example of a recirculating irrigation system. The term hydroponic can encompass plant production with or without the use of an artificial substrate (e.g. rock wool, sand, etc.) to provide mechanical support, and can also be open, where nutrient solutions are not recycled or closed, where nutrient solutions are recirculated. In this context however, the term will be used in the more conventional sense: that of recirculated nutrient solution flowing over bare roots.

Hydroponically produced crops have been in production at least since the middle 1930's. These systems are enclosed in greenhouse-type structures that allow temperature control, protection from the elements, and reduced evaporation. Western Europe is considered the center of hydroponic plant production, where lack of land and strict environmental regulation has been instrumental in promulgating this industry. Although small in comparison, the United States has some large facilities with retail crop sales worth millions of dollars. One commonly used hydroponic system is termed Nutrient Film Technique, or NFT, where a thin film of nutrient solution flows through plastic lined channels which contain the plant roots. Nutrient solution is pumped to the top of each channel and flows by gravity past the roots and eventually to a sump where it's chemical composition is monitored and it is readjusted before being recycled.

Hydroponic systems have the advantage of high density crop yield, minimal land use, lack of seasonality, suitability for mechanization, reduced labor, and reduced nutrient and water use. Although originally touted as an end to root disease (because of the lack of soil), hydroponic systems have not performed as promised. In fact, the potential for root infecting pathogens to spread in these closed systems has become a major deterrent to their use. Nutrient solution passes over the roots and pathogens shed by one plant enter the system and can be spread to all others. The situation is further complicated by the lack of chemical products registered for control in these systems. This is primarily due to the small potential market and the high cost of product registration. The majority of what is known about disease spread in recirculating systems is from hydroponic culture.

Another type of recycling irrigation system, is a subirrigation system termed ebb and flow. In an ebb and flow system, nutrient solution is pumped from a reservoir onto a bench with potted plants, the bench is flooded to a certain height and for a certain duration, then allowed to drain back into the reservoir. Theoretically, nutrient solution only flows from the bottom of the pot upwards; therefore pathogens present in the root zone can not be flushed out to infect other plants. This has not been found to be the case, however. Very little research has been done concerning pathogen spread in these systems.

Another strategy for these industries to reduce pollutant discharge is to reduce pollutant loads leaving in the runoff. An important component of potential runoff contaminants in these industries are the fungicides used to prevent or alleviate root diseases. Although fungicide leaching rates have been studied in field soils, no work currently exists which examines the leaching rates of fungicides from the media

components of soilless mixes. With an understanding of the leaching rates of fungicides associated with containerized media and the effect of wetting agents, management plans may eventually be developed which result in efficiently applied fungicides and reduced pesticide loss to the environment.

CHAPTER II

LITERATURE REVIEW

Recirculating irrigation systems have been highly publicized as one way in which industry may seek to reduce fertilizer inputs, water use, labor, and nutrient and pesticide rich runoff (Bauerle, 1990; Elliot 1990, Horticulture Water Quality Alliance, 1992; Roberts, 1991). A major deterrent to their widespread use has been the potential for root-infecting pathogens to spread. Several early researchers felt that pathogen spread in recirculating irrigation systems was not a problem (Jeannequin, 1981; Staunton, 1978; and Staunton and Cormican, 1980). However, others feared rapid dispersal of pathogens (Davies, 1981; Evans, 1977, 1979). Research that has been conducted since this time has shown that fears about pathogen spread were not unfounded.

Much of what is known about disease spread in recirculating systems has been gathered from hydroponic systems in which plants are grown without soil or other organic media. (Bates and Stanghellini, 1982; Daughtrey and Schippers, 1980; Jenkins and Averre, 1983; Gold and Stanghellini, 1985; Stanghellini *et al.*, 1988; Zinnen, 1988; Molitor, 1990). Although hydroponic cultivation has resulted in a decrease in the diversity of root infecting organisms compared with conventional soil culture, research has found that the severity of infectious root diseases is often intensified in hydroponic

systems. Once introduced into the systems root infecting pathogens are favored because of the close culture of a genetically uniform host, a physical environment with favorable water and temperature conditions, and a mechanism for rapid and uniform dispersal (Zinnen, 1988; Stanghellini and Rasmussen, 1994). Some root pathogens which are of little economic importance in the field can be of major importance in hydroponics (Stanghellini and Rasmussen, 1994).

Of most concern in these systems are root infecting pathogens with motile spores, or zoospores, which are favored by an aquatic environment. Zoospores are unicellular propagative bodies which are formed inside a sporangium on an infected root then eventually released into the environment. In a review of *Phytophthora* spp., Carlisle (1983), describes zoospore motility, taxis, and tropism. He states that the distance a zoospore can travel depends on such factors as the duration of swimming, speed, and the path taken. In general however, zoospores typically swim for many hours and may reach a distance of 3-6 cm from their source. Although abundant obstacles may lead to rapid encystment, Carlile details work that has shown that zoospores can swim effectively in many natural soils and can be transported by water movements in soil and still maintain their motility. As stated, zoospores can be moved passively by water currents but are also capable of directly homing in on the host; in the case of root pathogens to root tips, wounds, or individual root cells. The homing sequence is complex and involves the recognition of chemical diffusates and surface components of the host or substrate (Deacon and Donaldson, 1993).

Thompson and Allen (1976), found that zoospores of *Phytophthora parasitica* were present within 10 minutes after water was placed on naturally infested soil. They concluded that the ability of sporangia to germinate rapidly after inundation indicates that *P. parasitica* is stimulated to release zoospores by short periods of flooding. They found in addition, that zoospores or structures produced by them survived for 40-60 days in irrigation water. Work on cyst survival under both field and lab conditions, demonstrated that if the soil is drained to water contents less than saturation, the cysts of several *Phytophthora* spp. may survive for days or weeks (MacDonald and Duniway, 1979). If roots of a host pass close enough to the cysts during the time, it is presumed that the cysts could germinate and infest directly or, under favorable conditions, form microsporangia and release zoospores (Tsao, 1969).

The role of zoospores in hydroponic systems is well defined. With the exception of *Fusarium oxysporum*, most of the destructive root diseases in hydroponics have been attributed to the fungal genera, *Pythium*, *Phytophthora*, *Plasmopara*, and *Olpidium*, all of which produce zoospores (Stanghellini and Rasmussen, 1994). Gold and Stanghellini (1985) studying two *Pythium* spp., reported that zoospores produced from the roots of diseased plants served efficiently as inoculum in pathogen transmission to healthy plants. Recirculating hydroponic systems, they noted provide an ideal environment for the production, unobstructed movement, and rapid dissemination of zoospores. Fry (1992), documents several zoosporic pathogens that have a greater potential for spread by water than non-zoosporic pathogens. Van Voorst *et al.* (1987), demonstrated that the inoculum

of *Phytophthora nicotianae* can circulate freely in a NFT system with the nutrient solution.

Plant losses as high as 100% from *Pythium aphanidermatum* Edson (Fitzp.) have been reported in hydroponically grown spinach (Stanghellini *et al.*, 1984) and tomato seedlings (Stanghellini and Russell, 1971). Jenkins and Averre (1983) listed several plant pathogens which were recovered from North Carolina greenhouses and were readily transmitted in the nutrient solutions of hydroponic systems. *P. aphanidermatum* in particular, caused 100% disease in hydroponically grown cucumber and tomato nine days after inoculation. Stanghellini and Tomlinson (1988) demonstrated that *Pythium intermedium* could be transmitted in the nutrient solutions of hydroponic systems. Other work has shown that temperature of the nutrient solution effects the pathogenicity of *Pythium aphanidermatum* and *Pythium dissotocum* on hydroponically grown spinach (Gold and Stanghellini, 1985). Stanghellini and Kronland (1986), reported significant yield reductions in hydroponically grown lettuce in the absence of visible root or foliar symptoms in the presence of *Pythium dissotocum*.

The problems associated with hydroponic systems have raised fears about increased disease in other types of recirculating systems. Research conducted in this area is more limited and it is unclear what effect organic peat based media used in container plant production has on spread of plant pathogens in recirculated nutrient solution. Hockenhull and Funk-Jensen (1983) hypothesized that disease would be greater in containerized plant production with organic media than in hydroponics, because the constant flow of nutrient solution in the latter would sweep away root exudates and

zoospores away from the root zone. In addition, Kiplinger *et al.* (1975) suggested that disease would not be a factor in subirrigation systems because water can only move up in the capillary pores, leaving sufficient oxygen in the non-capillary pores to discourage disease development. Other researchers have suggested that there would be a protection effect of the pots and peat medium to disease spread and disease would be lessened (Staunton and Cormican, 1978). Koch and Holcomb (1983), witnessed no increase in disease development in a subirrigation capillary mat system either with or without re-use of irrigation solutions, despite the constant wetness of the media. In Europe, research has shown that pathogen spread in recirculating subirrigation systems is relatively low, with the caveat that the risk could increase if unsuitable flooding, inadequate technical equipment, or poor hygiene is employed (Molitor, 1990). Additionally, George (1989) reports that a limited number of growers in the United States have reported no problems with soilborne disease in their flood subirrigation systems as long as suitable sanitation was practiced. However, other researchers have reported that root diseases are more serious in greenhouse production in the United States because of warmer temperatures (Atmatjidou *et al.*, 1991).

Spread of the zoosporic fungus, *Pythium aphanidermatum*, between potted geraniums was shown to be greater in a scaled down flood irrigation system using recirculating water than for hand watering (George, 1989). A non-zoosporic species, *P. ultimum* was studied in the same system and no spread was documented (George and Stephens, 1990). However, other researchers found both *P. aphanidermatum* and *P. ultimum* were spread from inoculated to healthy poinsettias on the same bench in ebb and

flow systems, but *P. ultimum* was much less readily transmitted (Atmatjidou *et al.*, 1991). Sanogo and Moorman (1993) reported the transmission of *P. aphanidermatum* in an ebb and flow irrigation system with potted cucumber plants. In the Netherlands, Stelder (1993) studied the spread of the non-zoosporic root pathogen *Fusarium oxysporum f. sp. cyclaminis* in an ebb and flow system, and found that when artificially infected plants were placed between healthy plants the density of propagules increased with time. Stelder also reported that inoculum (chlamydospores) of *F. oxysporum* can survive in nutrient solution at least one year without loss of pathogenicity.

In states where tailwater discharge restrictions have been in place, many nurseries have installed reservoirs to hold and collect effluents. Recirculation of this wastewater can occur as needed to prevent release of runoff, but this introduces the potential risk of pathogen spread. Shokes and McCarter (1979) studied the occurrence, survival, and dissemination on plant pathogens in Georgia surface irrigation ponds and found primarily *Pythium* spp., but also *Phytophthora*, *Fusarium*, and *Rhizoctionia* with a wide range of virulence. *Pythium aphanidermatum* was able to overwinter as oospores and remain pathogenic, but zoospores were not recovered. Studies of the occurrence of *Phytophthora* spp. in recirculated nursery irrigation water in California found propagule numbers and trends differed among several nursery sites (MacDonald *et al.*, 1994). Additional studies of the occurrence of pythiaceous fungi in nursery ebb and flow systems have been conducted in Europe (Thinggaard and Middelboe, 1989).

Although not in recirculating irrigation systems, other researchers have added some pertinent information to the disease development in these systems. Results from field

work with *Phytophthora parasitica* and tomatoes indicates that the inoculum of *P. parasitica*, probably in the form of zoospores, is formed more rapidly and abundantly when previous irrigations have been frequent and have not allowed the soil to dry extensively (Hoy, *et al.*, 1984). Wilcox and Mircetich (1985) demonstrated that Phytophthora root and crown rot disease severity progressed from mild in treatments that were not flooded to extreme with 48-hr flooding periods. In work with *Phytophthora capsici* and field grown peppers, researchers found that plant to plant spread resulted in severe disease in uninfested plots in high rainfall fields, whereas less plant to plant spread occurred in low rainfall fields. In addition, both rainfall and irrigation had greater effects on the time of onset and final incidence of disease than the range of inoculum densities evaluated and final disease incidence was independent of inoculum densities (Ristaino, 1991). Bowers and Mitchell (1991) working with *Phytophthora capsici* and peppers demonstrated that when zoospores where added to free water above flooded soil, 75 and 95% of the plants exposed to 10 and 25 zoospores per plant, respectively, died. In Denmark, researchers have found that it is possible to considerably reduce the attacks of *Phytophthora* in an ebb and flow system by lowering the watering frequency and raising the electrical conductivity of the nutrient solution, either alone or in combination (Thinggaard and Anderson, 1995).

In work with soil matric potentials, Bernhardt and Grogan (1982), demonstrated that while nutrition of CO_2 and O_2 levels may interact to determine the optimum soil matric potential (ψ_m) value for initiation of sporangium formation, once sporangia are initiated, other ψ_m values may be conducive to the completion of the process.

Phytophthora parasitica formed large numbers of sporangia within 24 hr at -300 mb ψ_m and no indirect germination (zoospore discharge) occurred when sporangia were saturated. Researchers concluded that *Phytophthora parasitica* in infected tissues can probably form sporangia under two sets of circumstances: when the soil dries from saturation to values near field capacity, and when the soil is subsequently rewetted by irrigation. Zoospore release by *P. parasitica* appears to require longer periods of saturation than the relatively short time (5-6 hrs) required by *P. capsici*.

An additional problem faced by users of recirculating irrigation systems in the United States is the lack of products registered for introduction into irrigation systems as a means of control. This is due in part to the high cost of registration to manufacturers and the small potential market. Various forms of control have been investigated for use in these systems from the chemical to the biological. Several researchers have investigated the effects of fungicides. Early research by Price (1978) and Cook (1979) indicated that phytotoxicity problems could arise at quite low concentrations of fungicide. Investigations by Price and Dickinson (1980) into the effects of fungicide in NFT indicated that metalaxyl prevented zoospore formation at all concentrations tested. They found in addition, that mycelial growth rates were reduced after 24 hr at the lowest concentration tested (10 ppm) but that 48 hr exposure to metalaxyl at 50 ppm was required to kill *Phytophthora parasitica*. Growth rates in response to metalaxyl differed with species as did the concentrations required for lethality. They indicated however, that concentrations above 20 ppm of metalaxyl were phytotoxic to either young cucumber or tomato plants.

Gold and Stanghellini (1985) found that metalaxyl at 5 μg a.i./ml in the nutrient solution of a hydroponic system was enough to control Pythium root rot, but noted that the pathogen could be reisolated from the roots and that metalaxyl works as a fungistatic compound. Stuanton and Cormican (1980) indicated that fungicides both alone and in combination can be safely used at low levels in NFT. They found, however, that metalaxyl caused marginal leaf scorch at all rates tested with severe scorch at the higher rates. Work with the fungicide furalaxyl in NFT has shown effective suppression of *Phytophthora cryptogea*; however, researchers report increased sodium concentrations, increased iron uptake, and decreased zinc, and reduced yield (Price and Fox, 1986). Other work with use of benomyl to control Fusarium crown and root rot in a rock wool hydroponic system indicates that benomyl may provide adequate control, but researchers caution against widespread or intensive use for fear of selection of benomyl-tolerant strains (Mihuta-Grimm *et al.*, 1990). The fear of fungicide resistance in hydroponic culture is echoed by other researchers (Stanghellini and Rasmussen, 1994; Gold and Stanghellini, 1985; Price and Fox (1986)). A metalaxyl resistant strain of a nursery isolate of *Phytophthora parasitica* from vinca (*Catharanthus roseus*) has been reported in California, where researchers note that the recycling of runoff in some nurseries greatly increases the risk of selection for fungicide resistant populations (Ferrin and Rohde, 1992).

A variety of methods other than fungicides have been investigated as means to control disease in recirculating irrigation systems. There is a diversity of environmental laws in operation in other countries which have encouraged the development and marketing of additional methods. Daughtrey and Schippers (1980) investigated the use of

ultraviolet light in a hydroponic system as a method to prevent root disease in an artificially infested system, and found no direct phytotoxic effects; but found however, that the light caused precipitation of iron from chelate which led to foliar iron deficiency systems. An analysis of disease control was not made due to the absence of symptoms in inoculated plants. Other work with ultraviolet irradiation, has demonstrated 100% disease suppression of *Pythium aphanidermatum* in a recirculating hydroponic system compared to complete seedling losses for untreated water (Stanghellini *et al.*, 1984). Ewart and Chrimes (1980) investigated the effects of chlorine and ultraviolet light in disease control in NFT and found that both treatments were effective in reducing numbers of potentially pathogenic types of bacteria in the nutrient solution; however, chlorination when used at dosage rates which were as effective as ultraviolet was found to cause root damage. In addition, the treatments were found to have coincidentally increased dissolved oxygen concentration.

Other research has indicated that disinfection of recirculated irrigation water by heat treatments at high temperatures (more than 90 C) for short periods (approx. 10 sec) was effective in controlling tobacco mosaic virus and *Verticillium dahliae*, but was not effective at eliminating *Fusarium oxysporum* at exposure times used (Runia, *et al.*, 1988). Goldberg *et al.* (1992) investigated filtration as a method for controlling Pythium root rot of hydroponically grown cucumbers and found a 7-μm filter effectively removes zoospores of *P. aphanidermatum* from nutrient solution. Although plants in the tank receiving water passed through the 7-μm filter eventually became infected, shore flies were suspected of introducing the pathogen.

Research into the effect of using potassium silicate amendments in recirculating nutrient solutions to suppress *Pythium ultimum* on cucumber found that 100 or 200 ppm silicate additions significantly reduced mortality, root decay, and yield losses (Cherif and Belanger, 1992). Matheron and Matejka (1988) investigated the in vitro activity of sodium tetrathiocarbonate (STCC) on six *Phytophthora* spp. and found that 12 μg/ml reduced the duration of zoospore motility 94% in aqueous solutions, as well as reduced cyst and sporangium production. Other research has focused on the use of biological control agents. Rankin and Paulitz (1994) investigated the control of Pythium root rot of hydroponically grown cucumbers using selected rhizosphere bacteria and found significantly reduced cull rates, and in some instances, increased marketable fruit production and total fruit weight. Most of the antagonistic organisms studied are not registered for use in commercial hydroponic systems. Resistant cultivars are not available for most of the pathogens of import in these systems (Stanghellini and Rasmussen, 1994; Jenkins and Averre, 1983).

Surfactants have also been investigated as a means to control root diseases caused by zoosporic fungi. Tomlinson and Faithfull (1979) found that when recirculated nutrient solution was adjusted to 20 ppm Agral (a complex, non-ionic surfactant) every four days, it was effective in controlling lettuce big vein disease, in which the causal virus is vectored by the zoosporic fungus *Olpidium brassicae.* Other work has shown that zoospores of *Pythium* and *Phytophthora* spp. ceased motility within one minute after addition of 20 μg/ml of Agral but otherwise remained motile for 14 hr or more and was effective in inhibiting vesicle formation and zoospore production (Stanghellini and Tomlinson, 1986).

Mode of action is believed to be the disruption of the integrity and/or permeability of the plasma membrane of fungus structures lacking a cell wall. Von Broembsen (1992), in addition has investigated the inhibitory effects of the surfactant Aqua-Gro on *Phytophthora parasitica*.

Leaching. Currently very little information exists which demonstrates the influence of media and wetting agents on the amount of fungicides leaching from container grown crops. Although research has been conducted on the leaching rates for many fungicides in field soil, it is not applicable to the greenhouse or nursery environment since the media used to grow containerized crops differs markedly from field soils and may not even contain soil. No literature could be found on the interaction between pesticides and the media in which containerized crops are grown; however, general characteristics of pesticide behavior in soil and water are well documented (Rao and Hornsby, 1989; Stiegler *et al.*, 1993; Buttler *et al.*, 1991).

Characteristics of the pesticide alone may give an indication of its inherent leaching potential. To produce a qualitative assessment of pesticide losses from soils or growing media and consequently their potential to contaminate surface water or groundwater, it is important to consider both persistence as measured by half-life (t 1/2) and sorption as measured by the organic carbon adsorption coefficient (Koc). For the purposes of this research, a fungicide commonly drenched into containerized media to control root diseases in the greenhouse and nursery industries, metalaxyl, has been chosen. The Koc of metalaxyl is 50 ml/g indicating relatively low binding to organic material and therefore high leaching potential (Buttler *et al.*, 1991). In studying Ontario field soils, Sharom and

Edgington (1982) found that the mobility of metalaxyl was inversely related to the adsorption capacity and organic matter contents of the soils. Wetting agents commonly used in the production of containerized crops to improve the water retention of media, may affect the actual amounts of the fungicides leaching from the media. Additionally, wetting agents have been shown to have some fungicidal activity against zoosporic root pathogens (Stanghellini and Tomlinson, 1987; Von Broembsen, 1992).

Media components which will be investigated include: peat, perlite, vermiculite, bark, sand, and a commercial soilless mix, Fafard No. 2. An understanding of the composition of these components is important to the understanding of their leaching potentials. In the text *Growing Media*, the authors describe several of the comonents of soilless mixes (Handreck and Black, 1984). Peat is described as the partly decomposed remains of plants (most commonly sphagnum mosses and sedges) that grow in swampy conditions. Lignified cell walls and humus are the primary remnants of decomposition. The cellular structure of the plant is more or less preserved; the many holes in the cells walls allow entry and exit of water, giving sphagnum peats some unique properties. Because of their heterogeneous nature it is impossible to report the exact amount of organic matter, but it is estimated that peats contain between 30 and 90% organic matter. Perlite is a porous siliceous material produced by rapidly heating a natural volcanic glass to 1200 C, and vermiculite is a flaky mineral that occurs naturally in many parts of the world.

CHAPTER III

PATHOGEN SPREAD

I. Effects of irrigation method and inoculum level on spread of *Phytophthora parasitica* during vinca production

MATERIALS AND METHODS

The influence of irrigation method and inoculum level on the spread of the root pathogen, *Phytophthora parasitica*, and the development of root disease caused by the pathogen was evaluated using vinca (*Catharanthus roseus*). A treatment unit consisted of a block of fifteen pots arranged three by five. Spread of the pathogen from plant to plant was determined by inoculating only the central plant of a fifteen pot block and then isolating from the surrounding plants at the end of the experiment, or weekly as death occurred to detect the presence of the pathogen. Inoculated plants were produced by transplanting healthy seedlings in potting media artificially infested with the pathogen. Control blocks were not inoculated but otherwise similarly handled. These treatments were compared for each of three irrigation methods. Symptom development was recorded at weekly intervals throughout the six week trial. The height of plants in all treatments was measured at the termination of the trial.

Gree*nhouse, Trial I.* 'Grape Cooler' vinca (*Catharanthus roseus*) seeds were sown 11 January 1994 in 288 plug flats (13.5 ml/cell) filled with a commercial soilless medium (Redi-Earth, Peat-lite mix; Grace Sierra Horticultural Products, Milpitas, CA). Seeds were germinated in the dark then grown under both fluorescent and incandescent lighting upon emergence in a growth chamber (27C 12 hr (light); 24C 12 hr (dark); 80% RH). On 3 February 1994 seedlings were transplanted into 2.5 in square plastic pots (American Plant Products, Oklahoma City, OK) containing a commercial peat: perlite: vermiculite medium (Fafard Growing Mix no. 2; Conrad Fafard, Springfield, MA). The trial was initiated on 4 February and plants were placed in a fiberglass-reinforced plastic greenhouse averaging 68/84 F (high/low) temperatures located at the Oklahoma State University research facility, Stillwater, OK.

Inoculum Production. Phytophthora parasitica (isolate P068 obtained from previously diseased vinca) was used in this trial. Plants were inoculated by transplanting into Fafard no. 2 growing media in which fungal inoculum had been incorporated at 0.1, 1.0, or 10.0% (v/v) levels. Inoculum of *P. parasitica* was produced by inoculating sterile corn meal/vermiculite media with the fungus and allowing the fungus to completely colonize the media. The colonized corn meal/vermiculite media was produced in a 500 ml bottle by filling each bottle to 200 ml mark with vermiculite then adding 60 g of corn meal. The mixture was shaken vigorously, then 70 ml of distilled water was added, shaken again and autoclaved for 15 min at 121 C. Once out of the autoclave, the media was shaken again to prevent it from setting up. The bottles containing media were re-autoclaved following the same procedure 24 hr later to kill any organisms which may have

germinated. Five 2 mm blocks of *P. parasitica* growing on V8 juice agar were placed on top of the corn meal/vermiculite media and incubated for four weeks at 27 C until fully colonized. V8 Juice Agar is composed of a mixture of 5.0 g of $CaCO_3$, 340 ml of V8 juice, 15.0 g of Diftco Bacto agar, that is brought to 1L with distilled water and is sterilized by autoclaving for 20 min at 121 C.

Irrigation Methods. Plants were grown with one of three irrigation systems: 1) modified small-scale ebb and flow, 2) capillary mat, or 3) hand-watering. Plants were watered on demand with 75 mg N/liter from a commercial water soluble fertilizer with 20N-4.3P-16.6K (Peters 20-10-20 PLS; Sierra Chemical Co., Milpitas, CA).

The modified ebb-and-flow subirrigation system consisted of plastic trays (American Plant Products, Oklahoma City, OK) (52.5 x 25.5 x 3.0 cm) with a drain hole at one end connected to a segment of plastic tubing approximately 24 cm long. The reservoir used to deliver the nutrient solution consisted of a one gallon (3.875 L) plastic milk jug with a side-mounted spigot approximately 8 cm from the bottom of the jug, to which a piece of tubing had been connected. This reservoir was suspended from a hook above the tray. Nutrient solution was delivered by connecting the tubing from the reservoir to the tubing under the tray and opening the spigot. Solution would flow by gravity through the tubing and flood the tray in similar fashion to the large scale ebb and flow benches with the water level gradually rising until the pots were in approximately 0.5 cm of nutrient solution. Solution would only flow to the level just above the spigot so that the volume required to flood trays to the appropriate depth was determined. Therefore, once the spigot was opened, trays would fill to the appropriate level automatically. Trays

were flooded until the media at the top of the pots was wetted (approximately 10 minutes). The connection was then disassembled, the reservoir placed on the floor, and the back of the tray raised with a piece of wood so that the excess solution would gradually drain back into the reservoir. The reservoirs were topped up with fresh nutrient solution prior to each watering. Plants were placed approximately 5 cm back from the drain hole to prevent contact with the turbulence created by filling and spaced evenly throughout the rest of the tray.

The capillary mat system utilized a plastic tray (same dimensions as above) on which a piece of capillary mat [6 mm black plastic bottom layer, mat, and black, perforated plastic covering (Vorttex Capillary Watering System; OS Plastic, Norcross, GA)] was placed. Plants were arranged in the same manner as the ebb and flow trays. The mat at the front of the tray was lifted and sufficient nutrient solution was slowly poured under the mat to allow for the wetting of all plants in the tray (approximately 700 ml).

The hand-watering system consisted of trays and pots, as described above. Hand-watering was accomplished by top-watering each individual plant with nutrient solution (approximately 700 ml per tray) with a small watering pitcher until runoff occurred.

Each combination of irrigation method and inoculum level was replicated three times as were the uninoculated controls, for a total of thirty-six observation units. Trays were randomly arranged and were spaced approximately 15 x 60 cm along two benches with two rows per bench, for a total of four rows.

Detection in plants. A visual rating of plant health was recorded weekly for the duration of the experiment. Plant deaths were recorded weekly and dead plants were removed (pots were left on the trays) and taken back to the lab and assayed for the presence of *Phytophthora parasitica*. Roots were prepared by washing in reverse osmosis (RO) water and plating on P10VPH selective media (Tsao and Guy 1977). Plates were incubated for 48 hr at 27 C and presence of *P. parasitica* was determined microscopically. The trial was terminated on 18 March 1994, 6 weeks after initiation, and all remaining plants were removed and assayed for the presence of *P. parasitica* as described above. Additionally, plant heights were recorded by measuring from the base of the soil line to the base of the tallest leaf at that time.

Detection in nutrient solution. Nutrient solution from the inoculated trays was sampled at the end of the third, fourth, fifth, and sixth week of the trial for the presence of *P. parasitica*. Reservoirs for the ebb and flow trays were taken back to the lab. Plants from the capillary mat trays were placed to the side, and each capillary system was rinsed with 180 ml of distilled de-ionized (DDI) water. The rinsate was collected in a glass bottle. A similar rinsing was followed for the hand-watered trays. In the lab, four 100 ml aliquots from each of the ebb and flow reservoirs, and two approximately 90 ml aliquots of each of the capillary mat and hand-watered samples were filtered using Nucleopore 47 mm 3.0 μm filters (Costar Scientific Corp., Cambridge, MA). Filters were inverted, placed on P10VP media (Tsao and Ocana, 1967) in Week 3, and on P10VPH selective media for the remainder of the samplings, and incubated at 27 C for 24 hours. At this

time, the filter was removed and plates were incubated for an additional 48 hr at 27 C. Presence of *P. parasitica* was determined microscopically.

Greenhouse, Trial II. Seeds were sown on 20 May 1994 and transplanted on 26 June 1994 following the same procedure. The trial ran from 27 June 1994 - 8 July 1994 and was a repeat of the earlier trial with a few differences. Greenhouse temperatures averaged 77/99 F (high/low) for the duration of the trial. Instead of two benches with four rows, the trays were placed along the same two benches which had been pushed together to create two rows at another location in the same greenhouse. The modified ebb and flow system was changed from spigots to in-line siphon pumps. In this system, the reservoirs would sit on the bench alongside the tray and an in-line siphon was placed in the nutrient solution and then connected to the piece of tubing entering from below the tray. In this way, the solution would flood the tray and drain in a similar manner as before. With this siphon system flow would not stop automatically; to stop the system the tubing into the reservoir had to be removed. Hand-watered plants were watered using a watering can with a breaker nozzle that dispersed into the nutrient solution into many droplets. Plants were sprayed with Enstar II growth regulator to control fungus gnats during the fourth week of the trial on 21 June 1994. Water was sampled at three, four, and six weeks for *P. parasitica* as described above.

RESULTS

Greenhouse, Trial I. Disease was greatest in the ebb and flow system when compared to the capillary mat and hand-watered systems (Table 3.1). In the ebb and flow system disease occurred at all three levels of initial inoculum density, with spread being

greatest at the highest inoculum density and lowest at the lowest density. Spread occurred only at the highest (10%) inoculum level for the capillary mat and hand-watered irrigation methods, but at a very low level compared to that of the ebb and flow (Table 3.1). There was no significant difference between spread for the highest inocululm densities between the capillary mat and the hand-watering systems. All other differences were significant at the $p = 0.05$ level.

Heights of plants that were remaining at the end of the trial were significantly different from one another across irrigation methods, with ebb and flow plants being tallest on average when compared to capillary mat and hand-watered plants (Table 3.2) *P. parasitica* was recovered from all three watering systems at points other than potted plants (Table 3.3). Recovery was greatest during Week 5, where *P. parasitica* was recovered from all irrigation systems. *P. parasitica* was not recovered in Week 6 from any of the systems.

Greenhouse, Trial II. No significant effect of initial inoculum density was found in this trial (Table 3.4). Spread was greatest in the hand-watered system with the revised watering method, with 78.6 % of the total surrounding plants becoming infected during the trial. Spread was 15.9 % in the ebb and flow system and 8.7 % in trays irrigated by capillary mat.

There was no statistical difference in plant heights for plants irrigated by ebb and flow or hand-watered, other combinations were statistically different (Table 3.5).

P. parasitica was only detected once from any of the irrigation systems (Table 3.6). One hand-watered sample was positive during Week 6 of the trial.

DISCUSSION

In Trial I, spread of *P. parasitica* in the ebb and flow system was seen at all inoculum densities and the values were significantly different from each other; however, these results were not repeatable in the second trial. Field studies with peppers (Ristaino, 1991) have demonstrated that both rainfall and irrigation had greater effects on disease then inoculum density, and final disease incidence was independent of inoculum density. Further studies would be necessary before a conclusion could be drawn about the effect of inoculum density and disease spread. Irrigation by ebb and flow showed consistently significant spread in all findings to date, especially when compared to irrigation by capillary mat

Spread of the pathogen was much higher in the hand-watered trays in the second trial where plants were irrigated with a watering can with a breaker nozzle, as opposed to the lower levels of spread in Trial I where plants were hand-watered with a pitcher. The irrigation method was changed to more likely approximate what would be found in a commercial practice. In Trial I, very little splash was encountered with use of a pitcher, where the nutrient solution was applied in a very directed manner. In Trial II, splash was more evident. Splash appears to be an important factor in disease spread in this system. For vinca seedlings grown in flats (Fallon, *et al.*, 1994) and exposed to a 5 % inoculum level, spread of *P. parasitica* from the center four inoculated plants to the surrounding 32 plants was found to be 100% for hand-watered plants (with a watering can and breaker nozzle) , agreeing with the results of Trial II. In research with hand-watered pot-grown poinsettias that were well spaced, thus minimizing splash, spread of *Pythium*

aphanidermatum occurred at low levels (Von Broembsen and Dole, personal communication).

One surprising observation is that not all center plants died by the end of the trial or even had *P. parasitica* detected. However, spread from these central inoculated plants did occur, so the inoculum in the media was effective as a source.

During Trial I, the pathogen was recovered in only one ebb and flow sample in Week 4, from at least one sample for all the irrigation methods at Week 5, and for none in Week 6. *P. parasitica* was only recovered in one hand-watered sample in Week 6 of Trial II. Temperatures in the greenhouse had been on the rise in the greenhouse during Trial I, with very high temperatures present in the summer when Trial II was conducted. These high temperatures were most likely unfavorable to the survival of *P. parasitica* propagules in the irrigation water or exposed parts of the system, resulting in the lack of recovery at the end of Trial I and during most of Trial II.

II. Investigations into the infectivity of motile and encysted zoospores of *Phytophthora parasitica* in vinca

MATERIALS AND METHODS

The infectivity of encysted versus motile zoospores of *Phytophthora parasitica* was investigated under controlled conditions in a growth chamber. A treatment unit consisted of eight approximately two week old vinca seedlings arranged in two parallel rows of plants in a plastic tray (Rubbermaid, Servin' Saver ™) 14 x 14 x 4.8 cm

containing Fafard no. 2. A central groove was hollowed out approximately 4 cm deep between the two rows and 2.5 cm away from the rows of seedlings. A suspension of either encysted or motile zoospores was applied and the groove was re-covered with media. Controls consisted of seedlings treated in a similar fashion, but with DDI water alone. Disease progress was recorded daily and dead plants removed and taken to the lab for confirmatory isolation.

Growth Chamber, Experiment I Seeds were sown on 21 February 1995 as previously described (Chapter III). Seedlings were transplanted on 7 March 1995 into the plastic trays containing 500 ml of Fafard no. 2 and placed back in the growth chamber. Each treatment was replicated three times, and trays were randomly arranged in growth chamber. Trial was initiated on 12 March 1995. After treatments, the trays were watered with RO water as needed using a hose equipped with a mister nozzle.

Zoospore production. Ten approximately 2 mm blocks were cut from the margin of a three to four day old *Phytophthora parasitica* culture on V8 Juice Agar (V8JA) and placed in a petri dish containing 10 ml of 10 % V8 Broth (340 ml V8 Juice, plus 0.5g $CaCO_3$, centrifuged for 20 min 4000 rpm, 1:10 dilution) and incubated for 48 hr at 27 C to produce mycelial mats. The V8 broth was removed and replaced with MSS (a mineral salt solution: 3.08g $Ca(NO_3)_2$ /4 H_2O; 1.49 g $MgSO_4$; 0.51 g KNO_3 ; 1000 ml, water warmed to dissolve; autoclaved 30 minutes 121 C) and the dish placed 40 cm under fluorescent lighting at room temperature (20-21 C) for 24 hr. The MSS was removed and replaced with 10 ml of fresh MSS and placed back under the light for an additional 24 hr. To produce synchronous zoospore release the MSS was replaced with 10 ml of sterile

distilled water and plates placed in the refrigerator at 4 C for thirty minutes then returned to room temperature for an additional 30 minutes. Zoospores were harvested by collecting this solution, and concentrations were estimated by encysting a small aliquot and counting using a hemocytometer (Baxter Healthcare Corp., McGaw Park, IL). Zoospores were encysted, by shaking solution vigorously for several minutes.

Inoculation. Trays were brought back into the lab and RO water was applied until media was at field capacity. This was achieved by applying a total of 100 to 150 ml of RO water in small volumes until runoff was produced when the tray was tilted. Grooves were produced between rows by parting media with a spatula. Several petri dishes of zoospores were produced in the manner described above and zoospore suspensions were combined in a graduated cylinder. A small aliquot was removed, encysted, and a count taken. A dilution was made resulting in an estimated 1,110 zoospores per ml. The solution was divided and placed into two bottles and the bottle to contain the encysted zoospores was shaken for several minutes. Forty ml of either motile zoospores, encysted zoospores, or DDI water was applied to the appropriate tray with a 10 ml pipette and caution exercised to apply solution evenly across the tray. Once the groove was covered with growing medium, trays were placed back into the growth chamber.

Plants which had died by 24 March 1995 were removed and assayed for the presence of *P. parasitica* by plating on P10VPH selective media. Presence of the fungus was determined microscopically. The trial was ended on 3 April 1995 at which time all remaining plants were removed and taken to lab for confirmatory isolation.

Growth Chamber, Experiment II. Repeated Trial I, all methods used were the same with the following exceptions. Seeds were sown on 26 April 1995 and transplanted into trays on 7 May 1995 to allow seedlings to harden off. The trial was initiated on 8 May 1995 and plants which had died by 22 May 1995 were removed and assayed for the presence of the pathogen. The trial ended on 3 June 1995 and all remaining plants were removed and taken back to the lab for confirmatory isolation.

RESULTS

Growth Chamber, Experiment I. Plant death was much more rapid in trays inoculated with motile when compared to encysted zoospores (Table 3.7). The first deaths in motile trays occurred within three days of inoculation whereas the first deaths in the encysted trays did not occur until ten days post inoculation. Total plants infected by the end of the trial however was 100% for both the motile and encysted treatments. Control plants were not infected but appeared to be chlorotic, as did the other plants that were still alive.

Growth Chamber, experiment II. Plant death for motile trays was rapid and evident as early as the second day after treatment; however, no plants treated with encysted zoospores died by the termination of the experiment (Table 3.6). *P. parasitica* was detected, however, in plants in both motile and encysted trays by the end of the trial. More plants exposed to motile zoospores (19) became infected in this experiment when compared to encysted zoospores (13).

DISCUSSION

Plant death was more rapid when plants were challenged with motile compared to encysted zoospores; however, the end result of treatment with the levels tested for both forms of zoospores was a high rate of infection (100 % for both motile and encysted trays in Trial I, and 79 % for motile and 54 % for encysted trays in Trial II). This supports the fact that motile zoospores have the ability to home in on root sites and therefore infect plants more rapidly than encysted zoospores (Deacon and Donaldson, 1993). The exact method of infection of plants treated with encysted zoospores has not been well studied. Research has shown that zoospore cysts may either germinate directly and form a germ tube, or form a microsporagium and then release 1-2 zoospores which are capable of causing infections (Thomson and Allen, 1976). Either method of infection might account for the additional delay. By the end of both trials roots had grown out to the area where the encysted zoospores had been applied, which would allow germinated cyst germ tubes to come in contact with the roots.

Table 3.1. Greenhouse, Trial I. Effect of inoculum level and irrigation method on spread of *Phytophthora parasitica* to pot- grown uninoculated 'Grape Cooler' vincas from a central inoculated plant.

Irrigation Method	% Inoculum 0	0.1	1.0	10.0	
	% Spread to uninoculated plants[z]				Total %
Hand-watering	0	0	0	2.4	2.4**
Capillary mat	0	0	0	4.8	4.8**
Ebb and Flow	0	9.5	14.3	54.8	78.6

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 0, 0.1, 1.0, or 10.0% *P. parasitica* inoculum. The percentage of the surrounding 14 plants which became infected during the trial is given as % spread.
** Two-sample binomial test - figures are not significantly different at $p = 0.05$

Table 3.2 Greenhouse, Trial I. Effect of irrigation method and inoculum level on pot-grown vinca plant heights taken from soil-line to apex at the termination of the trial (Week 6).

Irrigation Method	% Inoculum 0	0.1	1.0	10.0	
	Height[x] (cm)				Average
Hand-watering	3.5	4.1	3.7	3.6	3.7 *
Capillary mat	4.2	3.9	4.4	4.1	4.2 *
Ebb and Flow	5.1	5.0	5.2	4.3	4.9 *

[z] The average of the heights taken for all plants remaining in trays at the end of the trial for each combination of irrigation method and inoculum density.
* LS mean test - all numbers are significantly different from each other at p = .05

Table 3.3 Greenhouse, Trial 1. Number of water samples, out of 3 samples, testing postive for *Phytophthora parasitica* from ebb and flow reservoirs, capillary mats or hand-watering trays of pot-grown 'Grape Cooler' vincas in systems inoculated with 10% inoculum[z].

Irrigation Method	Number of positive[y] water samples		
	Week 4	Week 5	Week 6
Hand-watering (trays)	0	1	0
Capillary mat (mats)	0	2	0
Ebb and Flow (reservoirs)	1	3	0

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 10.0% (v/v) *P.parasitica* inoculum.
[y]Water samples from which *P. parasitica* could be isolated are given as the number positive for presence of the pathogen out of three replicate treatments.

Table 3.4 Greenhouse, Trial II. Effect of inoculum level and irrigation method on spread of *Phytophthora parasitica* to pot- grown uninoculated 'Grape Cooler' vincas from a central inoculated plant.

Irrigation Method	% Inoculum 0	0.1	1.0	10.0	
	% Spread to uninoculated plants[z]				Total
Hand-watering	0	95.2	81.0	59.5	78.6 *
Capillary mat	0	9.5	2.4	14.3	8.7 *
Ebb and Flow	0	11.9	21.4	14.3	15.9 *

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 0, 0.1, 1.0, or 10.0% *P.parasitica* inoculum. The percentage of the surrounding 14 plants which became infected during the trial is given as % spread.
* Two-sample binomial test - all totals significantly different at $p = 0.05$

Table 3.5 Greenhouse, Trial II. Effect of irrigation method and inoculum level on pot-grown vinca plant heights taken from soil-line to apex at the termination of the trial (Week 6).

Irrigation Method	% Inoculum 0	0.1	1.0	10.0	
	Height[x] (cm)				Average
Hand-watering	18.2	18.0	18.8	18.2	18.3**
Capillary mat	22.7	21.3	21.1	20.3	21.3
Ebb and Flow	19.4	18.7	19.7	18.4	19.0**

[z] The average of the heights taken for all plants remaining in trays at the end of the trial for each combination of irrigation method and inoculum density.
** LS mean test - figures are not significantly different from each other at $p = 0.05$, but are significantly different from capillary mat

Table 3.6 Greenhouse, Trial II. Number of water samples, out of 3 samples, testing postive for *Phytophthora parasitica* from ebb and flow reservoirs, capillary mats or hand-watering trays of pot-grown 'Grape Cooler' vincas in systems inoculated with 10% inoculum[z].

Irrigation Method	Number of positive[y] water samples		
	Week 4	Week 5	Week 6
Hand-watering (trays)	0	0	1
Capillary mat (mats)	0	0	0
Ebb and Flow (reservoirs)	0	0	0

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 10.0% (v/v) *P.parasitica* inoculum.
[y]Water samples from which P. parasitica could be isolated are given as the number positive for presence of the pathogen out of three replicate treatments.

Table 3.7 Growth Chamber Experiments I and II. The effect of adding either motile or encysted zoospores[y] to media adjacent to where vinca seedlings were growing.

	Motile	Encysted	Uninoculated Control
Average No. of infected plants @ 23 days [z]			
Experiment I	8.0	8.0	0
Experiment II	6.3	4.3	0
Total No. dead @ 10 days			
Experiment I	10	0	0
Experiment II	9	0	0
Total No. dead @ 23 days			
Experiment I	20	9	0
Experiment II	14	0	0

[y] 1,100 zoospores/ml for a total of 40 ml applied to soilless mix at field capacity approximatel 2.5 cm away from root zone

[z] Out of eight plants per replicate, three replicates per treatment

CHAPTER IV

DISEASE CONTROL

I. Effect of disease control methods and irrigation method on spread of *Phytophthora parasitica* during greenhouse production of vinca

MATERIALS AND METHODS

The influence of irrigation method and disease control treatments on the spread of root disease caused by *Phytophthora parasitica* was evaluated using vinca cultured as previously described in Chapter III. The experimental design was also similar to that previously described in Chapter III. However, only ebb and flow and hand-watered systems were investigated, plants were inoculated with 10% (v/v), and three disease control treatments were applied. Plants surrounding the inoculated plant were treated with one of three disease control treatments, i.e. fungicide, surfactant, or a combination of both. Inoculated and uninoculated control treatments were also included.

Disease Control, Trial I. Vinca seeds were sown on 10 January 1995, transplanted and placed in the greenhouse on 2 February 1995, and treated on 3 February 1995. Procedures followed were the same as in irrigation method and inoculum density, Trial II (Chapter III), unless otherwise noted. Control treatments consisted of: 1) Subdue

2E fungicide at the 2.0 fl.oz. ornamental soil drench application rate, 0.8 ml/5 L, (Ciba Geigy, Greensboro, NC; active ingredient - metalaxyl), 2) Aqua-Gro 2000 L, media wetting agent at the ornamental application rate, 6.45 ml/ 5L, (Aquatrols, Cherry Hill, NJ), or 3) both fungicide and surfactant. Aqua-Gro is a proprietary blend of non-ionic surfactants and is composed of 80 % ethoxylated alkyl phenols, 5 % fatty acid esters, and 15 % inert solvent (water).

Controls consisted of both a centrally located untreated uninoculated plant in an untreated tray as well as a centrally located inoculated plant in an untreated tray. Each combination of disease control treatment and irrigation method or controls was replicated three times (for a total number of 30 observation units). Trays were placed along both sides of one long bench for a total of two rows and were spaced approximately 15 x 90 cm.

Symptoms were recorded and dead plants assayed at the end of every week for the duration of the six week trial. Water samples were taken at the end of Weeks 2, 4, and 6 for all inoculated trays. In this case, ebb and flow reservoirs were not taken back to the lab; instead, approximately 180 ml of nutrient solution was poured out of the reservoirs into glass bottles. Two 90 ml aliquots were filtered, making the total volume filtered for each irrigation method/treatment equal. The trial was ended on 17 March 1995 at which time remaining plants were assayed for the presence of *P. parasitica* and height measurements were recorded. The average daily low temperature was 68 F and the average daily high was 79 F.

Disease Control, Trial II. Seeds were sown on 14 March 1995, transplanted on 11 April 1995, and treatments applied on 12 April 1995. The trial ran from 13 April 1995 to 25 May 1995 and was a repeat of the earlier trial. Inoculum in this instance was only approximately one and a half weeks old (instead of four weeks); however, media was still fully colonized. This was achieved by inoculating several smaller bottles of corn meal/vermiculite media instead of one large bottle. Plants were placed in a similar but different greenhouse with an average temperatures of 66/82 F (high/low). Trays were spaced approximately 15 x 60 cm. Reservoirs were covered with aluminum foil at the beginning of Week 5 in an attempt to prevent algal growth.

RESULTS

Disease Control, Trial I. Metalaxyl effectively controlled the spread of *Phytophthora parasitica* in both irrigation systems even under the high levels of inoculum encountered (Table 4.1). No spread was found in uninoculated plants. For the inoculated controls, spread occurred at similar levels in both hand-watered (9.5 %) and ebb and flow trays (11.9 %), and values were not significantly different from one another. Surprisingly, spread was highest in the ebb and flow systems treated with surfactant (23.8 %), but relatively low in the hand-watered trays treated with surfactant (2.4 %). *P. parasitica* was also found to have spread in the ebb and flow trays treated with both fungicide and surfactant (7.1 %), but not in hand-watered trays treated with both. The pathogen was not detected in any of the water samples during the course of the trial.

Plants irrigated by ebb and flow were significantly taller than hand-watered plants at p = .0001 (Table 4.3). Although there were significant differences in heights between treatments, height differences were not correlated to infection.

Disease Control, Trial II. Complete disease control by metalaxyl was not observed in this trial, although spread was held at a moderate level (9.5 %) for ebb and flow irrigated trays, and no spread was found in hand-watered trays (Table 4.2). No spread occurred with surfactant in plants that were hand-watered, but spread of *P. parasitica* was again highest in trays irrigated by ebb and flow and treated with surfactant (33.3 %) and higher than the levels found in the inoculated ebb and flow controls. Levels of spread in inoculated treatments were higher than the previous trial, with 23.8 % spread in ebb and flow systems and 21.4 % in hand-watered trays (values are not significantly different from each other). No spread was found for either irrigation method in trays treated with both fungicide and surfactant, or in the uninoculated controls.

Although plants irrigated by ebb and flow were taller than those hand-watered, differences were not significant (Table 4.4) and no correlation was found between plant height and disease.

DISCUSSION

Metalaxyl was shown to be an effective tool for controlling disease spread in recirculating irrigation systems with high levels of inoculum. This has been documented in other systems as well (Gold and Stanghellini, 1985, and Staunton and Cormican, 1980). However, it is not a legitimate disease control option for recirculating irrigation systems as it is only labeled for use as a soil drench. In addition, its low binding, and thus high

mobility, make it a potential contaminant of both surface and groundwater. One of the most surprising results was the high levels of disease spread encountered in the ebb and flow system in plants treated with the surfactant Aqua-Gro, where spread was found to be higher than in the inoculated controls. This suggests that the surfactant somehow increased spread in this system. The exact reason for this enhanced spread is not clear but is possible that the increased wettability of the media and the flooded trays, aided zoospore movement to the root zone and increased infection rates. Previous work had shown that surfactants could be used to effectively control fungi with motile spores; however, surfactant was continually applied to the irrigation water in this system (Tomlinson and Faithfull, 1979). Like metalaxyl, surfactants are only registered for use as a soil drench.

II. The effect of different compounds on the motility of zoospores of *Phytophthora parasitica* using a videomicroscope system

MATERIALS AND METHODS

The effect of different compounds on the motility of zoospores of *Phytophthora parasitica* was evaluated using a videomicroscope system equipped with a video cassette recorder. Zoospores were produced as previously described. A test unit consisted of a 50 µl drop of zoospore suspension placed in a well on a glass slide with circular raised edges to which a 50 µl drop of test solutions was applied. Zoospores were first filmed for five minutes to get basal counts, test solutions were added, and the resulting interactions

filmed for an additional five minutes. No coverslip was used. Test solutions included: 1) Subdue 2E fungicide, 0.5 fl oz (final conc. .0396 ml/L) and 2.0 fl. oz. (final conc. .159ml/L) application rates, 2) Aqua-Gro 2000L wetting agent at ornamental application rate (12.50 ml/L) and a 1:100 dilution (.125 ml/L), 3) hydrogen peroxide (Phar-Mor brand, final conc., 1.5 %), and 4) DDI water. Each treatment was performed eight times. Tapes were viewed in slow motion, and qualitative and quantitative data on lysis and immobilization of zoospores was gathered.

Microscopy and video recording. The system used was similar to one described by Laing and Deacon (1991). A color video camera (Sony, Hyper HAD, CCD-IRIS/RGB) was attached to a photographic extension tube of a research light microscope (Nikon, Labophot, Japan). The camera was attached with a cable to a camera adapter (Sony, CMA-2) connected to a VCR (Sony, stereo video cassette recorder, SVO-1610) and also to a Sony Trinitron 13 inch color monitor. A Panasonic time-date generator (WJ- 810) was also included in the system. Observations were made with transmitted light from a halogen lamp (Bellaphot 6V 20W), using a 4x objective, and experiments were recorded on Polaroid videocassette tapes (T-120, High Grade). Times in 0.01 second intervals were superimposed on the recordings by the time-date generator, and in conjunction with the VCR allowed the experiment to be viewed one frame at a time (one frame = approx. 0.03 second). Counts of zoospores (either motile or not) were obtained most often for every one minute by freezing a frame, overlaying clear plastic wrap over the video monitor, and marking the wrap with a pen. Some segments were filmed for intervals which precluded counting every minute so counts were made more frequently

with an effort made toward uniform time intervals. The pieces of plastic wrap served as a temporary record of zoospore numbers and location. After positions were marked, counts were made, and the plastic wrap was discarded.

RESULTS

Videomicroscopy. The results of these experiments do not lend themselves to statistical analysis; however, some descriptive analysis has been possible. When zoospores were treated with the application rate of Aqua-Gro, loss of motility occurred almost instantaneously (Table 4.5). Treatment with a 1:100 dilution of Aqua-Gro however, did not result in complete motility loss, but in an apparent decrease of zoospore number when compared to the water treatment. Hydrogen peroxide greatly reduced motility. Metalaxyl treatments, at both application rates tested, still had motile zoospores after five minutes. Treatment with metalaxyl at the 2.0 fl. oz. rate also decreased the number of motile zoospores.

DISCUSSION

The experiments described here represent the first attempt to use videomicroscopy as a tool to measure and evaluate the immediate effects of fungicide application to zoospores. Though, this method has been used to document fungal interactions and mycoparasitism (Laing and Deacon, 1991, and Deacon and Donaldson, 1993). Although the data obtained in this case was not statistically analyzable, such data may be obtained with modifications to the procedures.

Aqua-Gro at the application rate effectively immobilized zoospores. Whether these zoospores would function afterwards is not known, but it is evident from greenhouse

Trials I and II that a single application does not ensure effective control of pathogen spread. Hydrogen peroxide warrants further investigations into its use as a control agent in recirculating irrigation systems. Metalaxyl, a fungistatic compound, did not markedly effect zoospore motility. This was as expected based on its mode of action of slowing hyphal growth by interfering with RNA synthesis (Fisher and Hayes, 1982). It is important to note that the pure compound metalaxyl was not used in these experiments, but rather the fungicide Subdue of which metalaxyl is the active ingredient. Subdue contains other components such as emulsifiers, and it is possible that one of these components was responsible for the loss of zoospores at the 2.0 fl. oz. rate.

Table 4.1. Disease Control, Trial I. Effect of inoculum level and disease control treatments on spread of *Phytophthora parasitica* to uninoculated treated 'Grape Cooler' vincas from a central inoculated plant.

Disease Control Treatment	% Spread to uninoculated plants[z]	
	Ebb and Flow	Hand-watered
Fungicide (Subdue)	0	0
Surfactant (Aqua-Gro)	23.8*	2.4*
Fungicide plus Surfactant	7.1*	0
Inoculated Control	11.9[N/S]	9.5[N/S]
Uninoculated Control	0	0

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 10.0% *P.parasitica* inoculum. The percentage of the surrounding 14 plants which were treated with control methods and became infected during the trial is given as % spread.

* Two value binomial test - values are significantly different at p = 0.005

N/S values are not significantly different at p = 0.05

Table 4.2 Disease Control, Trial II. Effect of inoculum level and disease control treatment on spread of *Phytophthora parasitica* to uninoculated treated 'Grape Cooler' vincas from a central inoculated plant.

Disease Control Treatment	% Spread to uninoculated plants[z]	
	Ebb and Flow	Hand-watered
Fungicide (Subdue)	9.5*	0
Surfactant (Aqua-Gro)	33.3*	0
Fungicide plus Surfactant	0	0
Inoculated Control	23.8[N/S]	21.4[N/S]
Uninoculated Control	0	0

[z]The center plant of 15 potted plants arranged 5 x 3 was inoculated by transplanting into soil mix infested with 10.0% *P.parasitica* inoculum. The percentage of the surrounding 14 plants which were treated with control methods and became infected during the trial is given as % spread.

* Two sample binomial test - all values are significantly different at $p = 0.0001$

N/S values are not significantly different at $p = 0.05$

Table 4.3 Disease Control, Trial I. Effect of irrigation method and disease control treatment on pot-grown vinca plant heights taken from soil-line to apex at the termination of the trial (Week 6)

Disease Control	Height[x] (cm)		
	Ebb and Flow	Hand-watered	Average
Fungicide	8.3	7.1	7.7 [b]
Surfactant	7.2	5.9	6.5 [a, d]
Fungicide plus Surfactant	7.8	5.1	6.5 [a]
Inoculated Control	8.3	7.1	7.7 [c, d]
Uninoculated Control	8.0	6.5	7.3 [a, b, c, d]
Totals	7.9*	6.3*	

[z] The average of the heights taken for all plants remaining in trays at the end of the trial for each combination of irrigation method and inoculum density

[a, b, c, d] Heights with the same letter combination i.e. " a , a " are not statistically different at p = 0.05

* Values are significantly different at p = 0.05

Table 4.4 Disease Control, Trial II. Effect of irrigation method and disease control treatment on pot-grown vinca plant heights taken from soil-line to apex at the termination of the trial (Week 6)

Disease Control	Height[x] (cm)		
	Ebb and Flow	Hand-watered	Average
Fungicide	8.3	7.1	7.8 [a, b]
Surfactant	7.2	5.9	7.6 [a, b]
Fungicide plus Surfactant	7.8	5.1	8.0 [a]
Inoculated Control	8.3	7.1	8.6 [a, c]
Uninoculated Control	8.0	6.5	8.8 [c]
Totals	8.3[N/S]	7.9[N/S]	

[z] The average of the heights taken for all plants remaining in trays at the end of the trial for each combination of irrigation method and inoculum density

[a, b, c, d] Heights with the same letter combination i.e. "a , a" are not statistically different at p = 0.05

N/S Values are not significantly different at p = 0.05

Table 4.5 Videomicroscopy. Effects of selected compounds on number and motility of *Phytophthora parasitica* zoospores

Treatments	Average ratio of zoospores[z] post-treatment:pre-treatment	Average time (min)[y] until cessation of motility
DDI Water	0.655	> 5.00
Aqua-Gro 2000-L (1.25 ml/L)	0.000	0.00
Aqua-Gro 2000-L (.0125 ml/L)	0.122	2.44
Metalaxyl 2.0 fl. oz. (.159 ml/L)	0.086	> 5.00
Metalaxyl 0.5 fl. oz. (.0396 ml/L)	0.389	> 5.00
Hydrogen peroxide (1.5 %)	0.063	0.61

[z] Ratio of the average number of motile zoospores after treatment to before treatment based on five counts each for eight replicates (the ideal ratio be 0.500 after the 1:2 dilution)

[y] Average of eight replicates

CHAPTER V

LEACHING

I. Metalaxyl leaching potential of selected components of soilless mixes and the effect of a surfactant on leaching

MATERIALS AND METHODS

The effect of surfactant and containerized media composition on metalaxyl leaching was investigated with a growth chamber study and with batch equilibration. In the growth chamber study, a one time application of fungicide or fungicide plus surfactant was applied to vinca plants grown in either peat, vermiculite, or a 50:50 mixture of both. Plants were top-watered and leachate collected. Combined weekly samples were analyzed for fungicide content using an ELISA (Enzyme Linked Immunosorbent Assay) method. Batch equilibration studies were conducted to provide an indirect measure of leaching potential. These studies involve equilibration of soilless media with solution containing dissolved fungicide. The amount of fungicide left in solution after equilibration was measured by analyzing the amount of fungicide adsorbed by media components with HPLC (High Performance Liquid Chromatography). Fungicide or fungicide plus surfactant was equilibrated with various containerized media components and analyzed for the amount of fungicide adsorbed by the different media types.

Growth Chamber Study. Seeds of vinca were sown on 3 September 1993 as previously described. On 24 September 1993 seedlings were transplanted, one plant per four inch pot, containing 400 ml of either peat, vermiculite, or a 50:50 mixture of both. Pots were placed in disposable plastic bowls to collect runoff. The trial was conducted in a growth chamber (27 C, 12 hr (light) 24 C, 12 hr (dark); 80% RH) with both incandescent and fluorescent lights. 100 ml of Subdue 2E fungicide (Ciba Geigy Corp., Greensboro, NC, active ingredient metalaxyl) was applied either alone at the 0.5 fl. oz. ornamental application rate (.0396 ml/L), or with Aqua-Gro wetting agent (Aquatrols, Cherry Hill, NJ) at the ornamental application rate (12.50 ml/L) to the media on 4 October 1993. Each media type and treatment application combination was replicated three times and pots were arranged in a complete random block design within the growth chamber. The trial was conducted for eight weeks and was terminated on 29 November 1993.

Plants were watered with 75 mg/L solution of fertilizer (Peters 20-20-20 PLS; Sierra Chemical Co., Milpitas, CA). Watering frequency was determined with a Instamatic™ moisture meter (Model DG -495, Medical Electronics, Inc., Ronkonkoma, NY). When the media dried, pots were top-watered to runoff (100-200 ml) and the amount of water used was recorded for each pot. The collected runoff was poured into a small glass bottle (total volume 180 ml) and placed in a cardboard box in a cold room (4 C) until analysis. Each week a new set of bottles was used in order to measure differences in metalaxyl concentration present on a weekly basis. The leachate was analyzed using a commercially available ELISA kit (Ohmicron RaPID assays) which had been designed for metolachlor analysis but which was also effective for metalaxyl. Pesticide immunoassays

are based on an antibody that selectively binds to a pesticide. Use of ELISA methods combines selective antibodies with enzymatic reactions to measure low pesticide concentrations.

Leachate solutions were prepared for analysis by vacuum filtration and volumes of leachate were recorded prior to filtration. Filtered leachate was then diluted 1:100 with an automatic dilutor and samples analysis performed in duplicate. A repeat pipetor was utilized for consistency of measurement. Analysis was performed with procedures and materials that were provided with the ELISA Rapid Assay Kit. After the assay was run, the absorbance of the solution at 450 nm was determined. It was determined early on that Aqua-Gro interfered with the test and produced a false positive, therefore analysis was limited, and only focused on the results of the non- surfactant containing samples.

The percent (B/B_o) values for the samples was calculated where B is the sample absorbance and B_o is the absorbance of a negative control that does not contain metalaxyl. Metalaxyl concentration was determined from percent (B/B_o) vs. log metalaxyl calibration plots constructed from metalaxyl standards which had previously been determined with reagent grade metalaxyl. Sample absorbance is inversely related to metalaxyl content in a nonlinear fashion. However, linear calibration curves were obtained by plotting percent (B/B_o) vs. log metalaxyl concentration. Metalaxyl concentration (in ppb) was determined by plotting the standard curve using the linear regression formula $\% B/B_o = - 18.09 \log(m) + 76.1$ ($r^2 = .977$).

Batch Equilibration Study. The time required for the equilibration of metalaxyl (Subdue 2E) with selected media types was determined by equilibration of media at 1, 3,

6, and 18 hr. Equilibration was reached at three hours. In addition, adsorption isotherms were developed for the selected media types. Half gram samples of peat, perlite, vermiculite, bark, sand, and Fafard no. 2 were combined with 20 ml of Subdue 2E fungicide, or fungicide plus surfactant, in test tubes and shaken on a tube rotator for three hours. Fungicide was mixed up at the 0.5 fl. oz. application rate, and solutions older than one week were never used. Two samples of each combination of media type and fungicide alone or fungicide plus surfactant were analyzed. Supernatant was filtered from the test tubes through a syringe equipped with a .45 μm filter into 50 μl HPLC sample vials. Standards were prepared from reagent grade metalaxyl in acetonitrile with each sample run. Metalaxyl analysis was performed with a Dionex DX - 500 HPLC using a C_{18} NovaPak 4 mm x 25 cm column. The mobile phase consisted of a 40:60 ratio of water: acetonitrile (AN), with addition of 0.1% TFA (trifluoroacetic acid) in the water and 1% TFA added to the acetonitrile. Wavelength for analysis was 220 nm, retention time was approximately 3.17 min. Metalaxyl analysis in samples that contained Aqua-Gro was similar except a gradient program was utilized. The mobile phase gradient program utilized was: 0 min, 40:60 water: AN, 1.0 ml/min; 4 - 9 min, ramp to 20:80 water: AN, 1.5 ml/min; 9 - 14 min, ramp to 40:60 water: AN, 1.0 ml/min; 14-25 min, 40:60 water: AN, 1.0 ml/min. Retention time was approximately 10.0 min and analysis time was 25.0 min.

RESULTS

Growth Chamber Study. Only the first four weeks of samples were analyzed and only the samples from treatments without surfactant could be analyzed because the surfactant interfered with some aspect of the ELISA. The overall pattern of leaching

(after four weeks), when looking at total amount of metalaxyl leached, shows that the pots containing vermiculite were least able to retain the fungicide, whereas pots containing peat were most able to retain the fungicide (Table 5.1). Pots containing the 50:50 mixture of peat and vermiculite leached an intermediate amount of metalaxyl. An analysis of leaching rates over four weeks demonstrated different patterns for the media types studied. Pots with vermiculite leached the greatest initial concentration of metalaxyl when compared to the other components (Figure 5.2). This amount was statistically different from both peat and the 50:50 combination ($p=0.05$). With the exception of Week 2, levels of metalaxyl in leachate from pots containing vermiculite were always higher, and statistically different ($p=0.05$), than pots containing peat. The trend for vermiculite containing pots showed a high level of fungicide leaching in the first week with less leaching in following weeks. Peat demonstrated a more or less uniform level of leaching. Pots containing the combination showed a leaching pattern in between the two parent components for Week 1 with less leaching in Week 3 and Week 4. Levels in the fourth week approached levels in Week 1.

Batch Equilibration Study. Analysis of adsorption of metalaxyl by various media components demonstrated the highest levels of adsorption for those components with highest levels of organic matter, namely peat, bark, and Fafard no. 2, with the latter two being statistically similar (Table 5.2). Sand, vermiculite, and perlite adsorbed at much lower levels, with sand being statistically similar to both vermiculite and perlite. The addition of the surfactant Aqua-Gro decreased the adsorption of metalaxyl in all components, however decreases were not statistically significant at $p = 0.05$ in sand,

perlite, and bark (Table 5.3). Peat still adsorbed the most metalaxyl, however, and bark adsorbed slightly more than Fafard no. 2. The addition of the surfactant made differences between vermiculite and perlite less apparent as sand, vermiculite, and perlite were all statistically similar. Statistical analysis using orthogonal contrasts of the overall amounts of fungicide adsorbed in tests with surfactant shows a significant effect of the surfactant. Based on this data, there appears to be an interaction between media and surfactant; however, this is only significant at $p = 0.09$ level.

DISCUSSION

The use of solely peat or vermiculite as potting mixes in the ELISA experiment is not what would be encountered in a commercial setting but was used to produce clear cut comparisons in the preliminary stages of this type of research. The data from the Growth Chamber Study confirms the hypothesis that media containing high levels of organic matter such as peat, would retain fungicide better than media types with very low levels, if any, of organic matter such as vermiculite. The 50:50 combination had levels very close to midway between the two parent components demonstrating the possibility of manipulating media to reduce fungicide leaching rates. In addition, metalaxyl has a low Koc, thus low binding, when compared to the spectrum of available fungicides; therefore, the fact that metalaxyl produced clear cut leaching rates in these experiments demonstrates that similar research with fungicides with higher Koc values could be explored.

The data gathered from the Batch Equilibration Study also confirmed these results and added a wider range of available media components to the list of those studied. Although the exact amount of organic carbon is unknown for most of the components

tested, it is more than likely a safe assumption to say that peat (30-90% organic matter) and bark from trees contain significantly higher levels of organic carbon than do sand (primarily silica), the mineral vermiculite, or perlite (a siliceous material). The exact composition of Fafard no. 2 is not available, but a visual inspection shows that it consists primarily of peat (certainly greater than 50 %), with lesser amounts of perlite, vermiculite and small pieces of wood. Soilless media mix constituents with high levels of organic matter such as peat, bark, and Fafard no. 2 adsorbed twenty to thirty percent of the applied fungicide, significantly more than vermiculite, sand, and perlite which adsorbed in the range of two to eight percent of the metalaxyl applied. Surfactants such as Aqua-Gro are used as wetting agents to allow easier spreading of water across surfaces. This increased wettability increased fungicide leaching *in vitro*. Aqua-Gro lowered the amount of fungicide adsorbed for all media components tested in all cases. For peat, bark, and Fafard no. 2, the amount of fungicide adsorbed was lowered approximate 10 % in all cases. Very little if any adsorption occurred for those components (sand, vermiculite, perlite) which adsorbed less than 10 % of the applied fungicide originally.

Adsorption isotherms were developed for each media type based on two replications; however, results were variable (data not presented). Additional replications would be necessary for meaningful results.

The overall goal of this work was to identify management options which might reduce fungicide loss to the environment by manipulating media. Based on these results, it is evident that media components which have a high organic matter content are best suited to retain fungicide and those which contain little if any organic matter are least able to

retain fungicide. Surfactant increases leaching in all cases. There is a necessity for surfactants, however, but their use is most likely to occur with peats and bark which are hard to wet, but as has been demonstrated have the better ability to retain fungicide. There are of course plant nutritional requirements that may not be conducive to certain mix combinations and this has not been explored. Future studies might focus on identifying the effect of split applications of fungicides on leaching in a plant system as well as the leaching rates of commercial mixes and the effect of various combinations of the media components tested.

Figure 5.1 Growth Chamber Study. Metalaxyl calibration curve (generated using reagent grade metalaxyl).

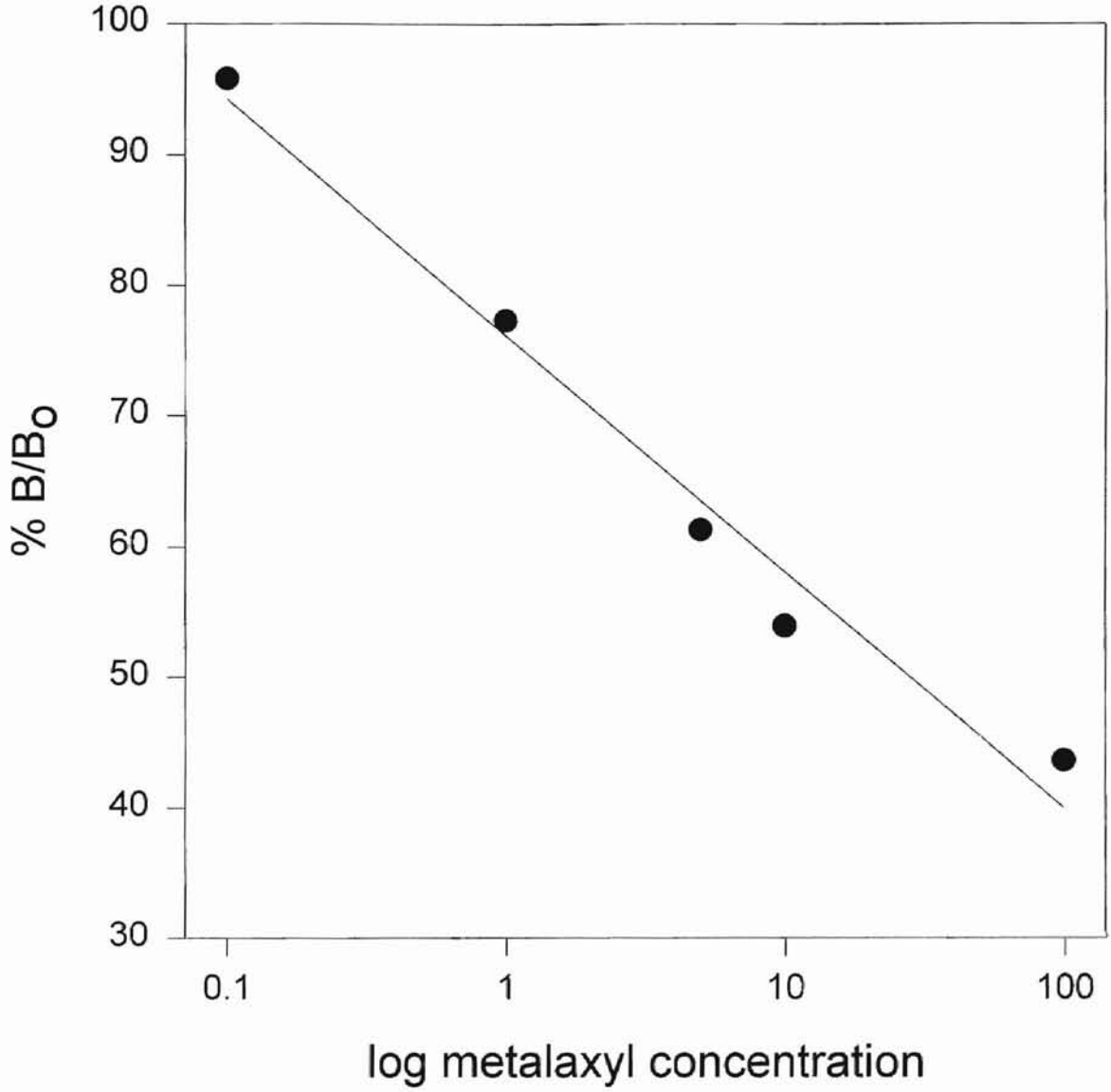
100
90
80
70
60
50
40
30
% B/B0
0.1
1
10
100
log metalaxyl concentration

Table 5.1 Growth Chamber Study. Metalaxyl (μg)[z] in leachate collected from growth chamber grown vinca that were treated with a one time application of metalaxyl (2000 μg).

Media	Metalaxyl (μg)				
	Week 1	Week 2	Week 3	Week 4	Total
Vermiculite	200.3 [a]	18.3 [a, b]	62.2 [a]	65.0 [a]	345.8 [a]
50:50 Peat/ Vermiculite	86.6 [b]	9.3 [b]	35.7 [b]	82.3 [a]	213.9 [b]
Peat	33.4 [b]	59.7 [a]	26.7 [b]	18.9 [b]	138.7 [b]

[z]Numbers represent the average amount from four replicate treatments. Two aliquots per replicate were analyzed by ELISA and averaged for each replicate.
[a, b] LSD- letter pairs, i.e. (a, a) represent values which are statistically similar at p=0.05

Table 5.2 Batch Equilibration Study. Mean adsorption values of metalaxyl for selected media components of soilless mixes.

Media	Mean metalaxyl[z] adsorbed (μg/g)	% metalaxyl adsorbed
Peat	241.3	32.58 [a]
Fafard	165.2	24.02 [b]
Bark	148.5	20.04 [b]
Vermiculite	59.8	8.10 [c]
Sand	50.7	6.71 [c, d]
Perlite	15.7	2.36 [d]

[z] Values represent the average amount of metalaxyl adsorbed from three replicate runs. Two samples per run were analyzed and averaged for each replicate.

[a, b, c, d] Duncan Groupings - letter pairs i.e. (a, a) represent values which are statistically similar at p=0.05

Table 5.3. Batch Equilibration Study. Mean adsorption values of metalaxyl plus surfactant for selected media components of soilless mixes.

Media	Mean metalaxyl[z] adsorbed (μg/g)	% metalaxyl adsorbed
*Peat	137.9	22.06 [a]
[N/S]Bark	100.6	16.05 [b]
*Fafard	67.7	10.78 [c]
[N/S]Sand	12.3	1.9 [d]
*Vermiculite	6.0	0 [d]
[N/S]Perlite	5.4	0 [d]

[z] Values represent the average amount of metalaxyl adsorbed from three replicate runs. Two samples per run were analyzed and averaged for each replicate.

* Significant effect within media identified and surfactant using orthogonal contrasts at p = .05

[N/S] Effect not significant

[a, b, c, d] Duncan Groupings - letter pairs i.e. (a, a) represent values which are statistically similar at p=0.05

CHAPTER VI

SUMMARY AND CONCLUSIONS

Two strategies for reducing fungicides in runoff from containerized crops were investigated. The first strategy involved the use of a recirculating irrigation method, ebb and flow, in comparison with two non-recirculating methods, capillary mat and hand-watering. The potential for spread of *Phytophthora parasitica* , a typical zoosporic fungal root pathogen, from a central infestation to surrounding potted vincas was examined.

Pathogen spread was considerable with the ebb and flow system in all trials. Spread with hand-watering was high when a method which resulted in an increased splash effect was used but not when irrigation water was carefully applied with little or no splash. In subirrigated treatments, pathogen spread was always less in trays irrigated by capillary mat than in trays irrigated by ebb and flow. Pathogen spread appeared to be density dependent in the first greenhouse trial but this result was not repeated in the second trial.

The fungus was recovered from all three irrigation systems at parts remote from the central infestation, but was recovered more often from the ebb and flow systems. Motile zoospores were shown to cause more rapid infection and symptom development in comparison to encysted zoospores when placed at a distance from the root zone in growth chamber experiments.

In greenhouse trials, metalaxyl was shown to control or suppress pathogen spread completely in systems with artificially high inoculum levels suggesting that metalaxyl remains an important tool in controlling root rot caused by pythiaceous fungi. The fact that the fungicide was only applied once is an important point. Other work in recycling irrigation systems has often used repeated or continual application of fungicides in the water itself; a potentially dangerous situation which favors the selection of fungicide resistance strains (Ferrin and Rohde, 1992). In both trials, spread was high in ebb and flow trays treated with surfactant (23.8 and 33.3 %), surprisingly even higher than the inoculated controls (11.9 and 23.8 %). The exact cause of this increased spreading is unclear but it is possible that the increased wettability of the media aided zoospore movement to the root zone and increased infection rates. Previous work which demonstrated control used continual applications of surfactant to the water system (Tomlinson and Faithfull, 1979). Therefore these results should not discourage the use of surfactants as a means of control. Unlike the use of toxic fungicides in irrigation water, surfactants may represent an environmentally benign option for fungal control given appropriate application.

Videomicroscopy methods allowed visualization of the effects of fungicidal compounds on zoospores immediately after application. Aqua-Gro and a 1.5 % solution of hydrogen peroxide were shown to immobilize zoospores very quickly, but the ability of immobilized zoospores to subsequently cause infection was not evaluated. Not surprisingly, metalaxyl did not markedly effect motility, since its primary mode of action is to affect RNA synthesis.

The studies of pathogen spread confirm that disease is an important factor that must be managed if recirculating irrigation systems are to be used as a strategy for limiting contaminated runoff to the environment. Although metalaxyl has been shown to be quite effective in overcoming this problem, it is not registered for introduction into recirculating systems and its high mobility raises concerns regarding surface and groundwater contamination. These studies have not shown the surfactant Aqua-Gro to be a good control compound when applied as a single application. But the possibility of introducing this or other surfactants continually into the recirculating irrigation systems should be further investigated. The videomicroscope studies demonstrated that Aqua-Gro strongly interferes with zoospore function, at least initially. Hydrogen peroxide also markedly affected zoospore function in these experiments and may be a potential means of control.

The effect of inoculum density was not clearly shown in the greenhouse trials. The issue is an important one, since if spread is density dependent, cultural methods such as early detection and removal of diseased plants would be more useful in controlling disease. Another potential control method not investigated in these studies was the use of biological control agents. Biological control will not be possible until fungicidal control, which is usually harmful to biocontrol agents, is abandoned by growers.

Another approach to reducing contaminated runoff is to reduce fungicide leaching from individual containers instead of trying to contain polluted water by recycling. No information exists about fungicide leaching from soilless mixes which are used in containerized crop production. Therefore this possibility was explored using growth chamber experiments and batch equilibration to measure leaching of the fungicide,

metalaxyl, from various components of soilless mixes. Both studies confirmed the hypothesis that metalaxyl would bind most highly to mix constituents which contained organic matter and least to components which contained little if any organic matter. The batch equilibration studies demonstrated that surfactant decreases the binding of metalaxyl. The leaching research is significant in that it indicates that manipulation of media components could be a method of limiting contaminated runoff from containerized crops.

This work is an attempt to evaluate two strategies to reduce contaminated runoff from containerized nurseries and greenhouse production. Greenhouses and nurseries have been identified as potential sources of pollution of both surface and ground water, and in fact, monitoring in Oklahoma has shown that contamination of surface water from nurseries has occurred (Oklahoma Department of Agriculture, 1993). Attention must be given to finding solutions to the practical problems which are faced by these industries.

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