UTILIZATION OF CHLORINATION AND SOILLESS MEDIA FOR MANAGEMENT OF *PYTHIUM APHANIDERMATUM* (EDSON) FITZP. IN GREENHOUSE PRODUCTION OF *CAPSICUM ANNUUM* L. IN A CLOSED SOILLESS SYSTEM

By

SHUBIN KUMAR SAHA

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

To my family

ACKNOWLEDGMENTS

I would like to thank Daniel J. Cantliffe for his guidance and support throughout the completion of my degree. His support has helped to propel me into the next stage of my career with an excellent foundation for working within horticulture. Additionally I would like to thank the other members of my graduate committee including Dorota Z. Haman, Jerry A. Bartz, George J. Hochmuth and Eric H. Simonne for their guidance and thoughts. I want to also thank Peter J. Stoffella for all his assistance with understanding and implementation of statistical analysis throughout my research. I would also like to extend my appreciation to all members and participants of the University of Florida Protected Agriculture Project whom I have interacted with including Elio Jovicich, Elizabeth Thomas, Jeanmarie Mitchell, Dzingai Rukuni, Nicole Shaw, Cecil Shine, John Thomas, and Gene Hannah. Lastly I would like to thank my wife and family for being supportive through this long venture in pursuit of knowledge. It has truly been a gift to have such an opportunity for which I am forever grateful.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

UTILIZATION OF CHLORINATION AND SOILLESS MEDIA FOR MANAGEMENT OF *PYTHIUM APHANIDERMATUM* (EDSON) FITZP. IN GREENHOUSE PRODUCTION OF *CAPSICUM ANNUUM* L. IN A CLOSED SOILLESS SYSTEM.

By

Shubin Kumar Saha

August 2009

Chair: Daniel J. Cantliffe
Major: Horticultural Science

Water sanitation by chlorination was evaluated in growth chamber and greenhouse studies for management of *Pythium aphanidermatum* (Edson) Fitzp. in recycled effluent used for production of 'Legionnaire' bell pepper (*Capsicum annum* L.) in pine bark, sand, and perlite. Results of growth chamber studies supported that chlorination (source: 5.25% sodium hypochlorite) rates as low as 2 mg/L resulted in reduced plant growth in all media. Shoot growth and plant height were less affected in plants growing in pine bark relative to plants growing in sand. This was likely a result of the organic pine bark reacting with the chlorine. In greenhouse experiments conducted in spring and summer of 2007, plant growth such as height and shoot and root fresh weights decreased linearly as chlorination rate of nutrient solution increased from 0 to 4 ppm. In greenhouse experiments in fall 2007 and spring 2008, plants chlorinated and grown in pine bark had greater height and shoot fresh weight relative to those chlorinated and grown in perlite. Effects of inoculation were similar to the previous studies in that reduction in growth variables such as plant height and shoot and root fresh weights were a result of *P. aphanidermatum* infection. In spring 2007, the marketable number of fruit per square meter decreased with increasing chlorine concentration. In fall 2007, plants grown in pine bark that were chlorinated had greater marketable fruit weight relative to plants grown in perlite, treated

by chlorination. The pathogen was isolated from roots in chlorinated and nonchlorinated plots indicating the pathogen was not eliminated via the chlorination treatment in addition to causing reduced yield. In spring 2008, plants grown in pine bark had greater marketable fruit numbers and weight relative to plants grown in perlite. Overall the pine bark would be the better choice for soilless media which can reduce phytotoxic effects from chlorination as compared to inorganic sand and perlite. 'Legionnaire' peppers are sensitive to chlorination rates as low as 2 mg/L and the use of an organic media such as pine bark can be beneficial in limiting phytotoxic effects and also has some pathogen suppressive characteristics.

CHAPTER 1
INTRODUCTION

1.1 Protected Cultivation of Vegetables

Cultivation of vegetables in protected structures is a method which is increasingly being utilized throughout the world including areas with mild winter climates such as Spain and Mexico (Cantliffe and VanSickle, 2003). Increased yield, quality, and price are some of the benefits of producing vegetables in a protected structure. Relative to field production, the extended season in the greenhouse and greater control over environmental conditions allows for the increased production and reduction in pesticide usage (Shaw and Cantliffe, 2008). Protected structures can vary greatly in initial investment and from very basic functions to the most technologically advanced facilities. High tunnels are an example of a protected structure which is merely a Quonset plastic covered structure which provides added cold protection for crops and to extend the production season. The majority of crops produced in protected structures utilize traditional soil cultures. More technologically advanced facilities allow for greater environmental control and increased overall production (Figure 1-1). In these types of greenhouse facilities, the use of soilless media is one method of growing which allows for increased productivity. Currently, many soilless systems are termed “open” because the nutrient solution passes through the root zone of the plant and is allowed to drain, and is not recycled or reused in the greenhouse. Allowing this effluent outside the protected structure may have negative environmental impacts.

The use of closed irrigation systems is increasingly becoming important to greenhouse producers as laws and regulations are now requiring the capture of effluent from such production facilities (James and van Iersel, 2001; Kluepfel and Stanghellini, 2006). The rise in regulations has been prompted by heightened environmental concern for water quality and quantity. Effluent from greenhouses and other agricultural operations are typically high in fertilizer materials and

pesticide residues which can adversely affect ground water and surrounding aquatic ecosystems (Kabashima, 1993; Hanan, 1997; Norman et al., 2003). Additionally, closed irrigation systems are increasing in use in various regions of the world as further strains are placed upon fresh water resources. Agriculture currently accounts for 87% of fresh water usage worldwide and as the world population is set to increase by 65% by 2050, the demand for agricultural water usage will only increase with rising food demand (Postel, 1992; Wallace, 2000). One means of addressing the increasing demand for agricultural irrigation water is to increase the overall water use efficiency. Utilizing closed systems has the potential to greatly increase water use efficiency relative to an open system, in which the effluent from the plants is not reused. Reusing the irrigation water is a beneficial means of conserving fresh water resources (Postel et al., 1996). Closed irrigation systems can result in up to 30% in water savings and 40% in fertilizer savings (Van Os, 1999). However, growers have avoided using closed irrigation systems until now largely because of the potential of crop loss as the probability of encountering a rootborne disease caused by such oomycete fungi as various *Pythium* spp. and *Phytophthora* spp. greatly increases (Stanghellini and Rasmussen, 1994).

1.2 *Pythium* spp. and Impacts on Crop Production

Pythium spp. are fungal-like chromistans in the phylum Oomycota all of which are facultative parasites. A facultative parasite is an organism which survives typically as a saprotroph on dead organic materials, but can also establish a parasitic relationship as a plant pathogen (Agrios, 1997). *Pythium* spp. cause some of the most significant losses in plant production relative to other pathogens (Plaats-Niterink, 1981). Diseases caused by *Pythium* spp. occur throughout the world and on any number of crop settings including vegetables, ornamentals, turf, grains, field, greenhouse, and forestry industry (Liu, 1976; Deep and Lipps, 1996; Feng et al., 1999; Spies et al., 2007; Bryla et al., 2008; Ceja-Torres et al., 2008; de Cara et

al., 2008; Nordskog et al., 2008; Platt, 2008; Serrano et al., 2008). *Pythium* spp. are typically associated with the root zone and lead to diseases such as root rots and damping off of seedlings. Oomycetes in general are associated with wet and aquatic environments as the asexual reproducing structure is a flagellated zoospore which is dispersed readily in water. Some *Pythium* spp. proliferate very well in soilless and hydroponic production systems due to the favorable environment, with potential for inoculum increase in recycling systems (Bates and Stanghellini, 1984; Favrin et al., 1988; Moulin et al., 1994).

Pythium aphanidermatum (Edson) Fitzp. is one of the more commonly isolated species in closed irrigation systems and has led to entire crop loss in greenhouse tomatoes and cucumbers in North Carolina (Jenkins and Averre, 1983). *P. aphanidermatum* is found in many locations in the world and has also led to significant crop loss of tomatoes, lettuce, and cucumbers grown in greenhouses with closed irrigation systems in Norway (Herrero et al., 2003). Infection with *P.aphanidermatum* led to mortality of mature cucumber plants in a commercial greenhouse production facility in Okeechobee, FL (Figure 1-2). Pathogenicity of *Pythium* spp. varies with respect to temperature (Plaats-Niterink, 1981). *P. aphanidermatum* is considered a warm season species as it is most pathogenic from 30°C - 35°C, however infection does occur at temperatures as low as 20° (Gold and Stanghellini, 1985). Additionally, the ability of the inoculum to effectively disseminate is dependent on the type of irrigation utilized (Stanghellini et al., 2000). These facts support the increasing need for information regarding the disinfection of nutrient effluent if reused as in a closed irrigation system.

1.3 Managing Recycled Nutrient Effluent

Although there are various methods of sanitizing recycled greenhouse effluent, there is currently a lack of research-based information to support the use of the various technologies (Postel, 1992; Uva et al., 1998; Hong and Moorman, 2005). The majority of the techniques for

disinfection of nutrient solution have been adapted from municipal water treatment facilities, including the use of UV radiation, ozone, and chlorination. Slow sand filtration, pesticides, surfactants, and biological control are also other methods that have been considered in the horticultural industry. Heating of nutrient solution has also been adopted from pasteurization facilities utilizing heat exchangers but also has a large initial capital investment (Ehret et al., 2001). UV radiation and ozone can all be effective for pathogen management ,but require large capital investment (Postma et al., 2000). UV radiation has also been shown to cause precipitation of iron from the nutrient solution which increases the need for iron fertilization management (Ewart and Chrimes, 1980). This increases the complexity of the management of nutrients within the irrigation solution. Additionally, UV radiation is not sufficient alone as a disinfection tactic as filtration is required. UV radiation cannot penetrate any solid particulate matter in the recycled nutrient effluent. Slow sand filters have not proven to eliminate all potential pathogens in recycled effluent as some plant viruses and bacteria can also proliferate in irrigation systems (Pategas et al., 1989; Schnitzler, 2004). Slow sand filters are also rather slow to process the water and could become an issue in a commercial setting from the perspective of water demand. Surfactants function by causing lysis of the zoospores but is not effective if they are encysted (Stanghellini et al., 1996). When the zoospores encyst, the motility of the zoospore is lost and a protective adhesive coating and cyst wall are secreted by the plasma membrane prior to formation of an infection tube to penetrate the plant root tissue (Estradagarcia et al., 1990). Additionally, detecting encysted zoospores in the nutrient solution does not seem to be a practical resolution for the problem. Pesticides are often not an option for management as there are a limited number of registered products available (Shaw and Cantliffe, 2008). Limited chemical options also increases the difficulty from the perspective of resistance management as

can frequently occur with oomycetes (Cohen and Coffey, 1986). Additionally, pesticides must be used preventatively as there are few to no curative fungicides. In the Netherlands, 15% of pesticides applied in greenhouse vegetable crops are for the prevention of root rot caused by *Pythium* spp., indicating the potential negative impacts that can result from inoculum dispersal and disease development (Postma et al., 2000).

Research to further understanding and utilization of biological control for rootborne pathogens is undergoing much study. Currently, however, large scale production and usage of such products is not in use (Paulitz and Belanger, 2001; Chave et al., 2008). One main issue with regards to biocontrol is that the antagonistic or parasitic organism is generally highly specific to a pathogen and will only manage one species. Interestingly, one biocontrol, a mycoparasitic organism being studied for management of phytopathogenic *Pythium* spp. is *Pythium oligandrum* Drechsler (Wulff et al., 1998; Benhamou et al., 2001). However, biocontrol via suppression of pathogen development is a promising option. Specifically, the organic substrates such as pine bark have been colonized by microorganisms which can either affect the pathogen antagonistically or by competition for nutrients. *Bacillus* spp. and *Pseudomonas* spp. are examples of beneficial bacteria which are commonly associated with recolonization of composting organic substrates such as pine bark (Zinati, 2005). Pine bark is considered an organic substrate in that it originates from living plants and is typically colonized with beneficial microorganisms. The organic nature of pine bark will be discussed and is important when dealing with chlorination which will be addressed at a later time. Pine bark has been widely adopted in the ornamental industry for management of diseases caused by *Pythium* spp. and *Phytophthora* spp. (Mandelbaum and Hadar, 1990). Although it has been adopted in the ornamental industry, there is currently limited use of pine bark as a soilless substrate. Perlite and

rockwool are two more commonly utilized in soilless production of vegetable crops. It has been reported that similar harvest results were obtained from Beit Alpha cucumber plants growing in either perlite or pine bark (Shaw et al., 2004). One component of my studies is the effects of pine bark in management of *P. aphanidermatum* in soilless production of peppers.

Chlorination is a potential option for disinfection of recycled nutrient solution as it has a high sanitizing efficiency and low capital input (Benoit and Ceustermans, 2002; Kim et al., 2006). Use of chlorine can also be phytotoxic to plants but there is very little information regarding what concentrations of free chlorine causes phytotoxicity (Teoh and Chuo, 1978; Ewart and Chrimes, 1980; Premuzic et al., 2007). The concentration at which phytotoxicity occurs will also be dependent on crop and production system. A nutrient film technique (NFT) system is not a likely candidate to utilize chlorination for disease management due to phytotoxicity (Ewart and Chrimes, 1980). Phytotoxicity can occur more readily in an NFT system as the substrate for the roots is water, thus offering little chlorine demand capacity relative to an organic substrate such as coconut coir or pine bark as free chlorine reacts with any organic material. Tomato transplants produced in a floatation irrigation system did not exhibit reduced growth until free chlorine concentrations exceeded 20 ppm, where tomatoes produced in an NFT system had reduced root growth when exposed to chlorine concentrations less than 0.5 ppm (Ewart and Chrimes, 1980; Saha et al., 2008). These results further suggest that chlorination rates at which phytotoxicity occurs can be dependent on type of production system. One of the components of my studies is to evaluate the buffering capacity of different substrates with respect to chlorine concentration in the nutrient solution. Information regarding effective and economic sanitization of greenhouse effluent will better facilitate the adoption of closed irrigation systems in greenhouse vegetable production operations. Thus, ultimately achieving the

goal of reducing negative environmental impact and increasing efficiency of both water and fertilizer use.

The objective of these first experiments were to collect various Pythiacious isolates from greenhouse vegetable production settings and to determine relative pathogenicity on bell pepper for selection of an isolate to utilize in future studies regarding sanitizing nutrient effluent for reuse in closed irrigation systems. The objective of the other four experiments was to evaluate media and chlorination rate impacts on disease development of *Pythium aphanidermatum* on bell pepper grown in a growth chamber. Results from these experiments were utilized to provide supplemental information for the development of greenhouse studies which focuses on the interaction of the different components of the whole production system. Specifically, the objective is to observe if any pathogen suppression occurs in an organic substrate versus and inorganic substrate. Another objective to the studies is to evaluate phytotoxic effects from chlorination at different rates to bell peppers growing in perlite, sand, pine bark, and steamed pine bark. Lastly, the goal of the entire project is to determine how chlorination might be used in conjunction with an organic substrate such as pine bark for the management of *P. aphanidermatum* in production of bell peppers grown in a closed soilless production system.

Figure 1-1. Eurofresh greenhouse facility in Wilcox, AZ, USA.

Figure 1-2. Wilting caused by disease development of Pythium aphanidermatum (Edson) Fitzp. on greenhouse grown 'Logica' cucumber (Cucumis sativus L.), Okeechoobee, FL. Photo taken by S. Saha.

CHAPTER 2
PATHOGENICITY OF SELECTED *PYTHIUM* SPP. ISOLATES AND THE DISEASE MITIGATION EFFECTS OF MEDIA AND CHLORINATION ON GROWTH OF BELL PEPPER (*CAPSICUM ANNUUM* L.) TRANSPLANTS IN A CONTROLLED ENVIRONMENT CHAMBER

2.1 Introduction

Heightened environmental concern for water quality and quantity has led to increase in regulations. Such regulations are now requiring the capture of effluent from such production facilities (James and van Iersel, 2001; Kluepfel and Stanghellini, 2006). Effluent from greenhouses and other agricultural operations are typically high in fertilizers and pesticide residues. The contaminants within the effluent can degrade ground water quality and surrounding aquatic ecosystems (Kabashima, 1993; Hanan, 1997; Norman et al., 2003). As a result of all these factors the use of closed irrigation systems is increasingly becoming important to greenhouse producers. Additionally closed irrigation systems are increasing in use in various regions of the world as further strains are placed upon fresh water resources with rising population and food demand (Postel, 1992; Wallace, 2000). Utilizing closed systems may greatly increase water use efficiency relative to an open system, in which the effluent from the plants drains to waste. Reusing the irrigation water is a beneficial means of conserving fresh water resources (Postel et al., 1996). However commercial producers are still hesitant to utilizing closed irrigation systems.

Growers have avoided using closed irrigation systems until now largely because of the potential of crop loss due to a rootborne disease caused by such oomycete fungi as various *Pythium* spp. and *Phytophthora* spp. greatly increases (Stanghellini and Rasmussen, 1994). *Pythium* spp. are fungal-like chromistans in the phylum Oomycota all of which can survive on dead organic material between hosts. *Pythium* spp. cause some of the most significant losses in plant production relative to other pathogens in a variety of crops throughout the world (Plaats-

Niterink, 1981). *Pythium* spp. can be associated with above ground plants parts but are most frequently associated with the root zone. Infection with *Pythium* spp. can lead to diseases such as root rots. Some *Pythium* spp. proliferate very well in soilless and hydroponic production systems due to the favorable environment, with potential for large inoculum increase in recycling systems (Jenkins and Averre, 1983; Bates and Stanghellini, 1984; Favrin et al., 1988; Moulin et al., 1994). Oomycetes in general are associated with wet and aquatic environments as the asexual reproducing structure is a flagellated zoospore which is dispersed readily in water. However the ability of the inoculum to effectively disseminate can be dependent on the type of irrigation utilized (Stanghellini et al., 2000). These facts support the increasing need for information regarding the disinfestation of nutrient effluent if reused as in a closed irrigation system.

Chlorination is a potential option for disinfestation of recycled nutrient solution as it has a high sanitizing efficiency and low capital input (Benoit and Ceustermans, 2002; Kim et al., 2006). When using sodium hypochlorite for purposes of disinfection it is added to water to create a solution. Free chlorine that reacts with the pathogen or other materials is in the form of hypochlorous acid. The percentage of available hypochlorous acid is dependent upon pH and is most available ($\geq$ 90%) in the range of 5-7 (White, 1992). Chlorine can be phytotoxic to plants but is rate dependent. However there is very little information regarding what concentrations of free chlorine causes phytotoxicity (Teoh and Chuo, 1978; Ewart and Chrimes, 1980; Premuzic et al., 2007).

The concentration of free chlorine that results in phytotoxicity is dependent on crop species and the type of production system utilized. A nutrient film technique (NFT) system is not a likely candidate for chlorination due phytotoxicity (Ewart and Chrimes, 1980). Phytotoxicity can occur more readily in an NFT system as the substrate for the roots is water, thus offering

little buffering capacity relative to an organic substrate such as coconut coir or pine bark as free chlorine reacts with any organic material. Information regarding effective and economic sanitization of greenhouse effluent is needed to allow for the implementation of closed irrigation systems in greenhouse vegetable production operations. The use of closed systems will help attain the goal of reducing negative environmental impact from agricultural operations. Utilization of such systems will also lead to improvements in both water and fertilizer use efficiency.

In Florida, wilting pepper (UF research facility-Citra) and cucumber (commercial producer Ft. Pierce) plants with compromised root systems have been observed in both open and closed irrigation systems. In both cases the rootborne disease led to plant mortality with potential for spread of inoculum. The initial identification of the causal agent of the wilting was that of a *Pythium* sp., which is one of two fungi commonly associated with closed irrigation systems (Armitage, 1993). Collection of isolates from these settings is necessary to further the studies and understanding of the management of such rootborne diseases. Specifically one must utilize pathogenic isolates of the causal agent of the disease in studies with any of a variety of sanitizing methods. The objectives of the first experiment were to collect various Pythiacious isolates from greenhouse vegetable production settings and to determine relative pathogenicity on bell pepper (*Capsicum annuum* L.) for selection of an isolate to utilize in future studies regarding sanitizing nutrient effluent for reuse in closed irrigation systems. The objective of the other four experiments was to evaluate media and chlorination rate impacts on disease development of *Pythium aphanidermatum* (Edson) Fitzpatrick on bell pepper growth and development.

2.2 Materials and Methods

2.2.1 Collection of *Pythium* spp.

Two general collection methods were utilized for obtaining the various isolates. The first method was done by obtaining root tissue samples from symptomatic plants in two greenhouses. One isolate collected by this method was from bell pepper roots in a passively ventilated greenhouse located near Citra, FL at the Plant Science Research and Education Unit (PSREU). Two other isolates were collected from a commercial greenhouse cucumber producer located in Ft. Pierce, FL. The second method of collection was done in both April and March of 2006 by collecting water samples from the nutrient effluent collection tanks at the PSREU greenhouse.

Root samples were cut into 1 cm pieces, surface disinfected for 2 minutes in a 1:100 dilution of sodium hypochlorite (5.25% bleach) and deionized water, rinsed with sterile deionized water, dried, and plated on agar gel in petri dishes with PART media (Shurtleff and Averre, 1997). Cultures were incubated for 2 days at 28°C in the dark. The two isolates collected from the commercial grower were able to be speciated utilizing the pond water and grass blade technique (Plaats-Niterink, 1981). Transfers of the isolates were made onto V8 juice agar media and incubated for an additional two days at 28°C in the dark (Shurtleff and Averre, 1997). Five 5-mm plugs of the V8 culture were taken from the expanding growing edge of the mycelia and placed in a petri dish full of water (1 part sterilized pond water:1 part deionized water). The plugs were incubated at 25°C in continuous light for 24 hours utilizing 40 watt fluorescent tube lights. Reproductive structures were observed after 24 hours and with the use of the Van der Plaats-Niterink key, the two isolates were speciated as *P. aphanidermatum and Pythium myriotylum* Drechsler (1981). The other isolate obtained from root tissue was described as *Pythium* 'isolate P' for the purposes of the pathogenicity study.

The two *pythiacious* isolates collected from water samples from the PSREU were obtained using a filtration method. The 1-liter samples were processed through a 9-cm Whatman No. 4 filter paper disk utilizing a Millipore Sterifil aseptic vacuum filtration system unit and a vacuum pump. Square, 1-cm long pieces of the filter paper were cut and plated on PART media (Shurtleff and Averre, 1997). Cultures were incubated at 28°C in the dark for two days to allow for identification as *Pythium* spp. The isolate from the March and April water sample will be referred to as *Pythium* 'isolate M' and 'isolate A' respectively, for the purposes of the pathogenicity study.

2.2.2 Experiments and Treatments

Five experiments were conducted in growth chambers located on the main campus of the University of Florida in Gainesville, FL. The first of five experiments was a pathogenicity study utilizing the 5 collected *Pythium* spp. isolates and bell pepper. Along with the 5 isolates an uninoculated control made up six treatments for this experiment. The additional four experiments conducted in the growth chamber were similar to one another with the exception of the concentration of free chlorine utilized in the studies. The second and third experiments were identical in that they both utilized a chlorination rate of 2 ppm. The fourth experiment utilized a chlorine concentration of 1 ppm and the fifth experiment utilized 4 ppm. Media was also another variable in these studies which included the use of composted pine bark, steam sterilized pine bark, and sand. The treatment combinations included an uninoculated control, a chlorinated control, an inoculated treatment and an inoculated and chlorinated treatment for each of the three substrates, which was a total of twelve treatments.

2.2.3 Inoculum Production

Inoculum for the growth chamber studies was produced utilizing a wheat berry technique (Chellemi et al., 2000). 20 g of hard red wheat seeds were soaked in 25-ml of deionized water for

24 hours in 250-ml Erlenmeyer flasks. The wheat berries were autoclaved on two occasions with 24 hours in between after the initial soaking. Two day old V8 agar cultures of the isolates were cut into five 5-mm plugs utilizing a No. 2 cork borer and placed into the flasks containing the wheat berries. The plugs were then allowed to grow for 2-3 weeks with a periodic shaking to ensure uniform mycelia growth.

2.2.4 Transplant Production

Seeds of bell pepper cv 'Legionnaire' (Rogers-Syngenta Seeds, Boise, Idaho) were planted in 128-cell polystyrene Speedling® float tray. The media utilized for germination was a combination of 75% peat moss: 25% vermiculite (v/v) seedling mix (Pro-mix PGX, Premier, Quakertown, PA) with a 25% increase (v/v) with coarse perlite (Airlite Processing Corp. of Florida, Vero Beach, FL). Seedlings were grown in a reach-in controlled environment chamber (width x depth x height: 183 x 76 x 102 cm^3; Conviron, Controlled Environments Limited, Winnepeg, Manitoba, Canada). The chamber was fitted with fifteen 1.8-m long fluorescent bulbs (Sylvania cool white, Danvers, MA; 160W). The photoperiod was maintained at 16:8 hours and 25°C/22°C day/night. The speedling flats were irrigated overhead once per day with water to maintain moist media for germination. After emergence seedlings were irrigated to maintain moist media with hydroponic nutrient solution with concentrations (mg/L) of NO_3-N: 80, P: 50, K: 120, Ca: 150, Mg: 50, S: 66, B: 0.7, Cu: 0.2, Fe: 2.8, Mn: 0.8, Mo: 0.05, and Zn: 0.3 (Hochmuth and Hochmuth, 1996). Seedlings were grown in the reach-in controlled environment chamber for 6 weeks prior to transplanting for the start of the various experiments.

2.2.5 Growing System, Conditions, and Inoculation

All growth chamber studies were conducted in a modified walk-in refrigeration unit (width x depth x height: 2.1 x 3.6 x 1.4 m^3; Model # 17601-S, Vollrath Refrigeration Division, Sheboygan, WI) which was equipped with four 2.4 m long fluorescent bulbs (Sylvania

supersaver cool white, Danvers, MA; 40W) and two HID metal halide bulbs (Sylvania metalarc, Danvers, MA; 1000W). The photoperiod was maintained throughout all experiments at 16/8 and the temperature was set to 35°C/25°C day/night. Plants were grown in 4-L black polyethylene nursery pots with 5 drain holes (diameter: 18.4 cm, height: 21.6 cm, model C475, Nursery Supplies Inc., Kissimmee, FL) and utilized in conjunction with a clear polyethylene saucer to provide a small reservoir of water (diameter: 20.3 cm, height: 3.8 cm, Lowes, Gainesville, FL). Aged pine bark was utilized as the substrate for the first experiment. However, media was a variable in the other four growth chamber studies which included composted pine bark, pure sand (Play sand, Quikrete, Atlanta, GA), and steam sterilized pine bark. At planting of each experiment, 20 g of wheat berries inoculated with *P. aphanidermatum* was incorporated into the various media in pots of the designated treatments. Additionally, 20 g of noninoculated wheat berries was incorporated into the media of the designated treatments without the addition of *P. aphanidermatum*. Plants were irrigated daily with 400 ml of nutrient solution containing concentrations (mg/L) of NO_3^-N: 150, P: 50, K: 200, Ca: 150, Mg: 60, S: 70, B: 0.7, Cu: 0.2, Fe: 2.8, Mn: 0.8, Mo: 0.05, and Zn: 0.3 (Hochmuth and Hochmuth, 1996) for all growth chamber studies. All growth chamber experiments, with the exception of the pathogenicity experiment, also included chlorination treatments. Sodium hypochlorite (5.25% bleach) was utilized to chlorinate the nutrient solution to the appropriate concentration including 1, 2, and 4 mg/L of free chlorine. Chlorine demand of nutrient solution was established in a laboratory setting by comparison to amount of sodium hypochlorite necessary to generate the designated concentration of free chlorine. Nutrient solution chlorine demand was 20 times that of deionized water. Free chlorine was measured using a DPD-colorimetric test kit (Kit 5910, Lamotte Co., Chestertown, MD).

2.2.6 Data Collection

Data collected for each of the experiments included plant height and stem diameter growth once per week. Other data collected at the termination of the experiments included shoot fresh weight, shoot dry weight, root ratings, and presence-absence sampling for *Pythium* spp. The root ratings were based on visual analysis for the percentage of damaged roots either as a result of the pathogen or chlorination treatment. Root ratings were on a scale of 0-5 with: 0 = no damage, 1 = 1 – 25% damage, 2 = 26 – 50% damage, 3 = 51-75% damage, 4 = > 75% damage, 5 = plant mortality. Presence-absence sampling was conducted in the same manner as when collecting the isolates. Root tissue was sampled from each plant and surface sterilized for plating on gel agar PART media. The cultures were then incubated at 28°C in the dark for up to one week in order to check for reisolation of the pythium isolate.

2.2.7 Experimental Design and Analysis

All five growth chamber studies were a randomized complete block design. However, based on the number of treatments and floor space in the growth chamber, the pathogenicity study had 4 replicates and the other four experiments had three replicates. Each experiment was conducted for six weeks beginning on 2 Aug. 2006, 2 Oct. 2006, 13 Nov. 2006, 1 Mar. 2007, and 7 May 2007. Statistical Analysis System was used for all the data analysis (SAS Institute, Cary, NC). The data from all the experiments were subjected to analysis of variance (ANOVA). Means were separated utilizing the least significant difference (LSD).

2.3 Results and Discussion

2.3.1 Pathogenicity of Various *Pythiacious* Isolates on Bell Pepper

The average day/night temperatures were 34.6°C and 26.4°C respectively. The maximum daily irradiance was 194.9 µmols/m^2s (PAR). Temperatures within this growth chamber and the following ones are more consistent and it was possible to maintain temperatures within the

optimum range for pathogen development. In a greenhouse experiment it would be more difficult to control the environment relative to a growth chamber. Light levels in the growth chamber are reduced relative to a greenhouse environment; however it is sufficient for crop growth. There was no significant difference with regards to height amongst the isolates relative to the uninoculated control. There were some significant differences regarding stem diameter growth in that the two speciated isolates, *P. aphanidermatum* and *P. myriotylum*, both had significantly less stem diameter growth ($P \leq 0.05$) when compared to the uninoculated control (Table 2 -1). All isolates except *Pythium* 'isolate M' had significantly less shoot fresh and dry weight ($P \leq 0.05$) relative to the uninoculated control (Table 2-1). Additionally, *P. aphanidermatum* had less fresh and dry shoot weight ($P \leq 0.05$) than all isolates with the exception of *P. myriotylum* (Table 2-1). Root ratings of plants inoculated with *P. aphanidermatum* and *P. myriotylum* were greater ($P \leq 0.05$) as compared to the uninoculated control and all other isolates (Table 2-1). Presence-absence sampling of plants inoculated with *P. aphanidermatum* and *P. myriotylum* had greater reisolation percentage ($P \leq 0.05$) as compared to the uninoculated control and all other isolates excluding *P.* 'isolate M' (Table 2-1).

When observing the results of this pathogenicity, it was important for the overall future development of experiments to select the most pathogenic isolate. This would allow for adequate testing and information to be utilized in commercial production settings utilizing closed irrigation systems. Plants inoculated with *P. aphanidermatum* consistently had diminished overall growth as the pathogen was efficient at the parasitism of the pepper roots. Additionally, *P. aphanidermatum* is continually being utilized for similar types of experiments and is one of the most commonly encountered species in commercial vegetable settings (Stanghellini and Rasmussen, 1994; Herrero and Klemsdal, 1998). Based on these initial findings and past

research, the decision was made to utilize the isolate identified as *P. aphanidermatum* for the following growth chamber and greenhouse experiments.

2.3.2 Effects of Media, Chlorination (2 mg/L), and *P. aphanidermatum* on Bell Pepper Growth

The results of the second and third growth chamber experiments were analyzed together as they were identical. The average day/night temperatures were 34.7°C and 28.1°C respectively. The maximum daily irradiance was 245.4 µmols/m^2s (PAR). All the data parameters collected throughout the two experiments had significant ($P \leq 0.05$) two -way interaction effects with the exception of presence-absence sampling. The significant interactions were between amount of free chlorine and presence of *P. aphanidermatum* as well as presence of *P. aphanidermatum* and type of media (Tables 2-3 and 2-4). However, the main effects will only be discussed as the percent of the sums of squares of the significant interactions account for less than 10% of the variability (Table 2-5). The main effects of media, chlorination, and inoculation account for the majority of the percent of the sums of squares indicating that the impacts of the main effects was greater than that of the significant interactions (Table 2-2).

The effects of media were significant in all data parameters collected with the exception of the presence-absence sampling (Table 2-2). The lack of significance of media with regards to presence –absence could be a result that regardless of the media, *P. aphanidermatum* was detected during attempts for reisolation. Detection does not however indicate one media to be better at suppression of root pathogens as quantification would be necessary. However it has been noted in one study that isolation of *P. aphanidermatum* from healthy roots is possible (Rafin et al., 1995). Pathogens can be and many times are present in various settings but can only proliferate well when conditions are conducive to rapid development. *Pythium* sp. proliferate well in flooded conditions as the zoospores are easily disseminated in water and the roots of

plants are also vulnerable in these anaerobic conditions (Saha et al., 2005). This was merely a qualitative test as a check to ensure presence of pathogens throughout the experiments. Plants grown in steamed pine bark had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to plants grown in pine bark and sand (Table 2-2, Figure 2-1). Broadbent et al. reported that steamed pine bark can increase the substrate's ability to suppress pathogens as compared to pine bark (1971). Steaming allows the heat tolerant bacteria such as *Bacillus* species to proliferate. These bacteria are antagonistic to rootborne pathogens and can tolerate temperatures up to 60°C (Zinati, 2005). Additionally, plants grown in pine bark had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to sand (Table 2-2). These results were consistent with other studies suggesting one potential reason for enhanced growth in pine bark as compared to sand was the better aeration (Hoitink et al., 1975). Zoosporic pathogens such as *Pythium* spp. proliferate well in wet poorly aerated environments. Sand had a significantly higher ($P \leq 0.05$) root rating as compared to steamed pine bark and pine bark (Table 2-2). The higher root rating could potentially be attributed to the fact that there was a lack of competition in the root zone due the lack of microflora in the sand (Davis et al., 1992).

The effects of chlorination were negatively associated with all the growth parameters (Table 2-2). The 'Legionnaire' bell peppers used in these experiments were not tolerant of irrigation solutions containing 2 mg/L of free chlorine (Figure 2-2). Under different conditions, various other cultivars of pepper and other crops tolerated higher levels of free chlorine (Teoh and Chuo, 1978; Saha et al., 2008). Teoh and Chuo reported that 'Green-giant' peppers were tolerant of free chlorine concentrations up to 25 mg/L when utilizing sub-irrigation (1978). The media utilized in these greenhouse experiments was granite chips and plants were irrigated four

times per day. This is more exposure to chlorine than in these current growth chamber experiments, indicating that there may be varietal differences in chlorine sensitivity amongst *Capsicum annuum* L. Saha et al. reported that 'Sunny' tomato transplants were tolerant of free chlorine concentrations up to 20 mg/L when utilizing floatation irrigation (2008). However, there is an overall lack of information regarding the phytotoxic effects of free chlorine (Hong, 2005). The nonchlorinated treatments had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to the chlorinated (2 mg/L) treatments (Table 2-2). Additionally, the average root ratings of chlorinated treatments were higher ($P \leq 0.05$) relative to the nonchlorinated treatments (Table 2-2). Root ratings are potentially higher in all chlorinated treatments due to root damage caused by the bleach (Ewart and Chrimes, 1980). Presence absence sampling was not affected by chlorination. This supports the theory that there are not many curative measures for pathogens in general and pathogen suppression should be approached from a preventative standpoint (Schnitzler, 2004).

The effects of inoculation with *P. aphanidermatum* were the greatest relative to media and chlorination as it had the highest significance based on the contribution to the total percentage of the sums of squares (Table 2-5). Presence of *P. aphanidermatum* negatively affected all the data parameters analyzed (Figure 2-3). Uninoculated treatments had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to the inoculated treatments (Table 2-2). The inoculated treatments had a significantly higher ($P \leq 0.05$) root ratings as compared to the uninoculated treatments (Table 2-2). These data support the fact that this isolate of *P. aphanidermatum* is pathogenic to bell peppers (Alfieri et al., 1994).

There was a significant two-way interaction between free chlorine and *P. aphanidermatum* for height, stem diameter growth, shoot fresh weight, and shoot dry weight. Treatments with chlorination and inoculation had reduced ($P \leq 0.05$) height, stem diameter growth, shoot fresh weight, and shoot dry weight relative to treatments with only chlorination or those without either chlorination or inoculation (Table 2-3, Figure 2-4). Additionally, plants growing in control plots without chlorination or inoculation had greater ($P \leq 0.05$) height, stem diameter growth, shoot fresh weight, and shoot dry weight relative to the uninoculated/chlorinated treatment (Table 2-3). These results indicate that there are some negative effects of the chlorination treatment which are separate from those associated with the root pathogen. There was also a two-way interaction between media and *P. aphanidermatum* for height and root rating. Plants grown in sand which were inoculated had less ($P \leq 0.05$) height growth and a greater ($P \leq 0.05$) root rating relative to inoculated plants grown in pine bark and steamed pine bark (Table 2-4). Inoculated plants grown in steamed pine bark had the greatest ($P \leq 0.05$) height growth of inoculated treatments. These results support the position that pine bark (organic) media provides better conditions for disease suppression relative to sand (inorganic) media.

2.3.3 Effects of Media, Chlorination (1 mg/L), and *P. aphanidermatum* on Bell Pepper Growth

The average day/night temperatures throughout the experiment were 33.9°C and 24.3°C respectively. The maximum daily irradiance was 263.2 µmols/m^2s (PAR). The only parameter in which there was a two and three-way interaction was the presence-absence sampling data (Table 2-6 and 2-7). However, the main effects will be the focus of discussion as in the previous experiment the presence of pathogen accounts for the majority (86 %) of the percentage of the total sums of squares (Table 2-6). Additionally, the main effects of chlorination were not

significant ($P \leq 0.05$) for any data parameters collected (Table 2-6). This is an indication that bell pepper cv. 'Legionnaire' transplants are tolerant to free chlorine at concentrations of 1 mg/L in these types of controlled environments.

The effects of media were significant in all variables measured (Table 2-6). Plants growing in either pine bark or steamed pine bark had greater ($P \leq 0.05$) height, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to sand (Table 2-6). Plants grown in sand had significantly higher ($P \leq 0.05$) root ratings as compared to plants grown in steamed pine bark and pine bark (Table 2-6). Presence-absence had the lowest ($P \leq 0.05$) re-isolation rate for treatments utilizing steamed pine bark (Table 2-6). The overall effects of media in this experiment were consistent with the two previous experiments (chlorination at 2mg/L).

The main effects of inoculation with *P. aphanidermatum* were significant for shoot fresh and dry weight, root rating, and presence absence sampling (Table 2-6). Treatments without inoculation had significantly greater ($P \leq 0.05$) shoot fresh and dry weights relative to inoculated treatments (Table 2-6). As expected, the average root rating of treatments without inoculum was lower ($P \leq 0.05$) relative to inoculated treatments (Table 2-6). Additionally, the average presence-absence re-isolation percentage of treatments with inoculum was higher ($P \leq 0.05$) relative to uninoculated treatments (Table 2-6). As in the previous experiment, these data also support the fact that this isolate of *P. aphanidermatum* is pathogenic to bell peppers (Alfieri et al., 1994).

2.3.4 Effects of Media, Chlorination (4 mg/L), and *P. aphanidermatum* on Bell Pepper Growth

The average day/night temperatures throughout the experiment were 33.2°C and 22.4°C respectively. The maximum daily irradiance was 196.4 µmols/m^2s (PAR). The main effects of media, chlorination (4 mg/L), and inoculum were significant ($P \leq 0.05$) for height and stem

growth, shoot fresh and dry weight, and root rating (Table 2-8). These results are consistent with all of the previous experiments. There are two and three-way interactions, however the focus will be on the main effects based on the interactions accounting for a small percentage of the total sums of squares relative to the main effects as in previous experiments (Tables 2-10, 2-11, 2-12).

Pine bark and steamed pine bark had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to sand (Table 2-8). Sand had a significantly higher ($P \leq 0.05$) root rating as compared to steamed pine bark and pine bark (Table 2-8). The main effect of media was not significant ($P \leq 0.05$) for presence-absence samples (Table 2-8). The overall effects of media in this experiment were consistent with the three previous experiments (chlorination 1 and 2 mg/L).

The effects of chlorination were negatively linked with all the growth parameters (Table 2-8). These data support that bell pepper cv. 'Legionnaire' is not tolerant of irrigation solution containing 4 mg/L of free chlorine in these conditions. This was expected based on the results of the two experiments which utilized a concentration of 2 mg/L. Plants growing in the nonchlorinated treatments had greater ($P \leq 0.05$) height growth, stem diameter growth, shoot fresh weight, and shoot dry weight as compared to the chlorinated (4 mg/L) treatments (Table 2-8). The effects of chlorination were not significant ($P \leq 0.05$) with regards to presence -absence sampling and root rating (Table 2-8). The main effects of chlorination (4 mg/L) in this experiment are consistent with the previous studies at a concentration of 1 and 2 mg/L.

The main effects of inoculation with *P. aphanidermatum* were significant for all data parameters collected (Table 2-8). Treatments without inoculation had significantly greater ($P \leq 0.05$) height and stem diameter growth and shoot fresh and dry weights relative to inoculated treatments (Table 2-8). As expected, the average root rating of treatments without inoculum was

lower ($P \leq 0.05$) relative to inoculated treatments (Table 2-8). Additionally, the average presence-absence re-isolation percentage of treatments with inoculum was higher ($P \leq 0.05$) relative to uninoculated treatments (Table 2-8). As in the previous experiments, these data also support the fact that this isolate of *P. aphanidermatum* is pathogenic to bell peppers. *P. aphanidermatum* has been observed to express pathogenicity and cause significant losses in hydroponic and field settings of a variety of crops including bell peppers, beans, strawberries, tall fescue, lettuce, and cucumbers (Harter and Whitney, 1927; Stanghellini et al., 1996; Chellemi et al., 2000; Utkhede et al., 2000; Settle et al., 2001; Ceja-Torres et al., 2008).

There was a significant two-way interaction between media and inoculation with regards to stem diameter growth and root rating (Table 2-8). Uninoculated plants grown in both sand and steamed pine bark had the greatest ($P \leq 0.05$) stem diameter growth relative to other treatments (Table 2-10). Inoculated plants grown in sand had the least ($P \leq 0.05$) stem diameter growth (Table 2-10). Sand is not a living substrate and does not initially contain microbes which allow the root pathogen to proliferate, unlike in a substrate with antagonistic microbes. There was a significant two-way interaction between media and chlorination with regards to stem diameter growth (Table 2-8). Plants growing in sand, pine bark, and steamed pine bark that were irrigated with chlorinated nutrient solution had less ($P \leq 0.05$) stem diameter growth relative to a plant growing in the same media that was irrigated with un-chlorinated nutrient solution (Table 2-11). Additionally, of the plants that were not irrigated with chlorinated solution, those growing in sand had the least ($P \leq 0.05$) stem diameter growth (Table 2 -11).

There was a significant interaction between media, chlorination, and inoculation with regards to height growth, shoot fresh weight, and shoot dry weight (Table 2-8). Height growth of plants grown in steamed pine bark control did not differ ($P \leq 0.05$) from inoculated plants grown

in steamed pine bark (Table 2-12). However, plants growing in steamed pine bark that were irrigated with chlorinated nutrient solution had less ($P \leq 0.05$) height growth relative plants growing in the steamed pine bark control plots (Table 2-12). A similar trend was also observed with regards to plants growing in sand and pine bark that were not inoculated. Shoot fresh and dry weight was less ($P \leq 0.05$) for plants growing in either media when irrigated with chlorinated versus no-chlorinated nutrient solution (Table 2-12). This indicated that the chlorination rate of 4 mg/L had significant negative impacts on crop growth. Specifically 'Legionnaire' bell peppers are susceptible to phytotoxicity associated with chlorination in the irrigation solution.

2.4 Summary

Water quality and availability are increasingly becoming an issue in many locations throughout the world. Considering agriculture accounts for a large majority of fresh water usage it becomes necessary to improve the water use efficiency to help conserve water as a natural resource (Postel, 1992). The use of closed irrigation systems in which the effluent is collected and re-used is one of the best means of conserving water, but the management level increases due to issues with pathogens which can thrive in soilless and hydroponic production systems (Jenkins and Averre, 1983; Bates and Stanghellini, 1984; Favrin et al., 1988; Moulin et al., 1994). Through these experiments the effects of media and chlorination were evaluated for the mitigation of disease development of *P. aphanidermatum*.

The initial pathogenicity experiment showed that the isolate collected and identified was *P. aphanidermatum*. Relative to the other isolates, it had some of the most negative impacts on plant shoot growth. Identifying a pathogenic isolate is important for all future studies as it is a critical component to conduct research regarding disinfestation of nutrient effluent for re-use. The other four experiments conducted consistently supported that pine bark or steamed pine bark lead to better crop growth than sand (Figure 1-4). In some cases plants grown in steamed pine

bark had greater shoot weight and plant height. Bell pepper cv 'Legionnaire' is sensitive to constant free chlorine concentrations as low as 2 mg/L in the nutrient solution in a controlled growth chamber environment. There was no adverse growth affects associated with a free chlorine concentration of 1 mg/L. However, *Pythium* spp. are not mitigated at this low of a concentration. As at a concentration of 2 mg/L, there were negative impacts associated with chlorination at rates of 4 mg/L. Plant height growth, stem diameter growth, shoot fresh weights, and shoot dry weights were all reduced in treatments chlorinated at 4 mg/L. Additionally, plant roots in treatments chlorinated at 2 and 4 mg/L were observed to be stubby as if they had been damaged by the free chlorine. All the negative effects of chlorination were more drastic in plants growing in sand relative to those growing in either pine bark or steamed pine bark. Main effects of inoculation with pathogen consistently supported through all experiments that treatments with pathogen were negatively impacted with regards to growth. The pathogen was re-isolated in inoculated plots indicating that chlorination cannot be utilized as a curative measure but must be utilized as a preventative within the irrigation solution. These initial studies provided a foundation develop the following studies which would be conducted in a greenhouse under near-commercial settings. In these experiments, each irrigation of specified treatments contained the designated chlorination rate consistently exposing the plant to the oxidizer. In a commercial setting the irrigation solution is what is treated and would have a period of time before it was all applied to the crop to allow some dissipation of free chlorine. The data from these growth chamber experiments has provided a base line level at which phytotoxicity is observed and will help with the design of experiments conducted in a greenhouse under realistic conditions of production. It is necessary to see if similar results are observed in a greenhouse setting as growth chambers allow for much more control of environmental factors which can affect the results of

these types of experiments. Greenhouse studies will allow further investigation into the effects of media and chlorination as it relates to rootborne disease in soilless production systems ultimately to develop management strategies for recycling nutrient effluent.

Table 2-1. Effects of *pythiacious* isolates on growth parameters of *Capsicum annuum* L. grown in a growth chamber

Isolate	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root rating[z]	Presence – absence sampling for pathogen
Control	20.5	5.8	155.3	24.5	0	0
Pythium aphanidermatum	15.6	2.4	45.6	9.5	3.0	1.0
Pythium myriotylum	16.0	3.4	75.5	12.9	3.0	1.0
Pythium 'isolate M'	18.9	5.5	145.2	23.0	0.5	0.5
Pythium 'isolate A'	17.5	4.9	115.6	21.8	0.5	0.5
Pythium 'isolate P'	16.4	4.1	105.4	16.6	2.3	1.0
LSD[y]	7.2	1.7	38.3	4.6	0.6	0.6
Significance	NS	**	**	**	**	*
CV	27.1	26.2	23.8	17.0	28.3	54.8

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Data collected 56 days after transplanting.

Table 2-2. Media, chlorination (2 ppm), and *Pythium aphanidermatum* (Edson) Ftizp. Effects on growth variables of *Capsicum annuum* grown in a growth chamber.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root rating[z]	Presence – absence sampling for pathogen
Pine Bark	11.8	2.6	54.1	6.6	0.9	0.5
Steamed Pine Bark	13.4	3.4	63.3	8.4	0.8	0.5
Sand	8.2	1.9	35.3	4.8	2.5	0.5
LSD[y]	1.7	0.4	7.3	1.0	0.5	0
Chlorine (C) (ppm)						
0	12.8	3.2	68.4	8.7	1.1	0.5
2	9.5	2.0	33.4	4.4	1.6	0.5
Significance	** (7.6)[x]	** (11)	** (16.5)	** (15.5)	* (2.3)	NS
Pythium (P)						
-	15.1	4.0	83.3	10.7	0.3	0
+	7.1	1.3	18.5	2.5	2.4	1.0
Significance	** (45.4)	** (58)	** (56.5)	** (56)	** (41.1)	** (100)
M	** (13.5)	** (10)	** (7.3)	** (7.2)	** (21.1)	NS
M x C	NS	NS	NS	NS	NS	NS
M x P	* (2.6)	NS	NS	NS	* (3.1)	NS
C x P	* (2.5)	**(6)	** (9.9)	** (9.2)	NS	NS
M x C x P	NS	NS	NS	NS	NS	NS
CV	26.8	28.9	24.8	26.9	67.8	0

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

[x] Percent of the total sums of squares.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Data collected 56 days after transplanting.

Table 2-3. Interaction effects of chlorination and *Pythium aphanidermatum* (Edson) Ftizp. on growth variables of *Capsicum annuum* L. grown in a growth chamber.

Free Chlorine (mg/L)	Pythium Inoculation	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)
0	-	17.7	5.1	114.3	14.5
0	+	7.8	1.4	22.4	2.9
2	-	12.6	2.9	52.3	6.9
2	+	6.4	1.1	14.6	1.9
LSD[z]		1.4	0.4	5.9	0.8

[z]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 2-4. Interaction effects of media and *Pythium aphanidermatum* (Edson) Ftizp. on growth variables of *Capsicum annuum* L. grown in a growth chamber.

Media	Pythium Inoculation	Height (cm)	Root rating[z]
Pine bark	-	15.8	0
Pine bark	+	7.8	1.8
Steamed pine bark	-	16.2	0
Steamed pine bark	+	10.6	1.6
Sand	-	13.3	1.0
Sand	+	3.0	3.9
LSD[y]		1.7	0.2

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 2-5. Sums of squares for growth chamber experiment with chlorination (2 ppm).

Source	Height	Stem diameter growth	Shoot fresh weight	Shoot dry weight	Root rating[z]	Presence – absence sampling for pathogen
Replicate	233.1[y]	0.47	3,164.9	76.5	5.61	0
Media (M)	341.5	24.2	9,801.6	155.5	41.4	0
Chlorination (C)	193.4	26.2	21,976.6	334.9	4.5	0
Pythium (P)	1,152.0	139.4	75,537.4	1,206.2	80.2	18
M x C	0.72	0.08	106.0	2.02	5.1	0
M x P	65.4	1.7	161.5	0.98	6.0	0
C x P	62.3	14.4	13,243.8	199.0	0.5	0
M x C x P	0.76	0.9	738.1	7.04	3.1	0
Error	487.2	32.0	8,773.9	172.2	48.7	0
Total	2,536.4	239.5	133,503.8	2,154.5	195.1	18

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Type III sums of squares.

Table 2-6. Effects of media, chlorination (1 ppm), and *P. aphanidermatum* (Edson) Fitzp. on growth variables of *Capsicum annuum* L. grown in a growth chamber.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root rating[z]	Presence – absence sampling for pathogen
Pine bark	19.5	3.7	119.1	12.5	0.9	0.5
Steamed pine bark	18.6	3.8	127.9	13.4	0.3	0.3
Sand	5.1	0.9	20.2	2.2	2.8	0.5
LSD[y]	4.4	0.9	29.0	3.3	0.8	0.1
Chlorine (C) (ppm)						
0	15.3	2.9	94.7	10.0	1.6	0.5
1	13.6	2.6	83.5	8.7	1.1	0.4
Significance	NS	NS	NS	NS	NS	NS
Pythium (P)						
-	15.3	3.1	103.1	11.2	0.7	0
+	13.5	2.5	75.1	7.5	1.9	0.9
Significance	NS	NS	*	**	**	** (86)[x]
M x C	NS	NS	NS	NS	NS	* (2.6)
M x P	NS	NS	NS	NS	NS	* (2.6)
C x P	NS	NS	NS	NS	NS	NS
M x C x P	NS	NS	NS	NS	NS	* (2.6)
CV	36.2	39.3	38.5	42.1	74.7	37.5

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).
[x] Percent of the total sums of squares.
[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2-7. Interaction effects of media, chlorination (1 ppm), and *Pythium aphanidermatum* (Edson) Fitzp. on presence-absence samples.

Media	Chlorine (mg/L)	Pythium Inoculation	Presence – absence sampling for pathogen
Pine bark	0	-	0
Pine bark	0	+	1
Pine bark	1	_	0
Pine bark	1	+	1
Sand	0	-	0
Sand	0	+	1
Sand	1	_	0
Sand	1	+	1
Steamed pine bark	0	-	0
Steamed pine bark	0	+	1
Steamed pine bark	1	_	0
Steamed pine bark	1	+	0.3
	LSD[z]		0.2

[z]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 2-8. Effects of media, chlorination (4 ppm), and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of *Capsicum annuum* L. grown in a growth chamber.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root rating[z]	Presence – absence sampling for pathogen
Pine bark	18.9	4.7	96.5	10.3	0.4	0.4
Steamed pine bark	18.5	4.9	89.3	10.1	0.5	0.3
Sand	8.6	3.6	46.3	5.5	2.0	0.5
LSD[y]	2.3	0.6	12.8	1.3	0.5	NS
Chlorine (C) (ppm)						
0	18.9	5.5	114.4	13.2	0.8	0.3
4	11.8	3.3	40.4	4.1	1.1	0.5
Significance	**	**	**	**	NS	NS
Pythium						
-	19.6	5.5	105.9	11.9	0.1	0
+	11.1	3.3	48.9	5.3	1.9	0.8
Significance	**	**	**	**	**	**
M x C	**	**	**	**	NS	NS
M x P	**	**	**	**	**	NS
C x P	NS	NS	**	**	NS	NS
M x C x P	**	NS	**	*	NS	NS
CV	17.4	14.8	19.5	18.1	57.8	59.1

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 2-9. Sums of squares for growth chamber experiment with chlorination (4 mg/L).

Source	Height	Stem Diameter Growth	Shoot Fresh Weight	Shoot Dry Weight	Root rating[z]	Presence – absence sampling for pathogen
Media (M)	814.1[y]	12.1	17,698.8	177.9	19.1	0.2
Chlorine (C)	458.7	41.4	49,217.4	748.9	0.7	0.3
Pythium (P)	654.5	43.6	29,166.9	392.0	30.3	0.3
M x C	101.4	5.9	6,163.0	63.7	0.7	0.2
M x P	245.4	19.7	3,692.6	40.6	15.2	0.2
C x P	0.56	1.2	4,110.9	95.4	0.3	0.3
M x C x P	106.2	2.4	2,653.0	19.8	1.5	0.2
Error	156.1	9.2	5,021.6	53.9	6.9	1.3
Total	2,547.9	136.1	117,936.9	1,603.1	74.9	8.8

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Type III sums of squares.

Table 2-10. Interaction effects of media and *Pythium aphanidermatum* (Edson) Fitzp. on stem diameter and root ratings of *Capsicum annuum* L. grown in a growth chamber.

Media	Pythium	Stem diameter growth (mm)	Root rating[z]
Pine Bark	-	5.1	0
Pine Bark	+	4.4	0.8
Sand	-	5.7	0.2
Sand	+	1.5	3.8
Steamed pine bark	-	5.7	0
Steamed pine bark	+	4.1	1.0
LSD[y]		0.6	0.5

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 2-11. Interaction effects of media and chlorine on stem diameter and root ratings *Capsicum annuum* L..

Media	Free Chlorine (mg/L)	Stem diameter growth (mm)
Pine Bark	0	6.2
Pine Bark	4	3.3
Sand	0	4.1
Sand	4	3.1
Steamed pine bark	0	6.2
Steamed pine bark	4	3.6
LSD[z]		0.6

[z] Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Table 2-12. Interaction effects of media, chlorination (4 ppm), and *Pythium aphanidermatum* (Edson) Fitzp. on plant height, shoot fresh weight and dry weight of *Capsicum annuum* L..

Media	Free Chlorine (mg/L)	Pythium	Height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Pine Bark	0	-	25.8	191.8	21.0
Pine Bark	0	+	21.8	105.5	11.1
Pine Bark	4	-	15.5	60.4	6.2
Pine Bark	4	+	12.3	28.5	2.8
Sand	0	-	19.5	126.2	15.3
Sand	0	+	0.2	6.8	1.1
Sand	4	-	13.5	47.0	4.8
Sand	4	+	1.3	5.30	0.7
Steamed pine bark	0	-	23.8	142.5	18.0
Steamed pine bark	0	+	22.3	113.4	12.6
Steamed pine bark	4	-	19.5	67.2	6.2
Steamed pine bark	4	+	8.5	34.1	3.6
LSD[z]			3.2	18.1	1.9

[z] Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Figure 2-1. Plant controls growing in each of the three substrates. Steamed pine bark, sand, and pine bark from left to right.

Figure 2-2. Plants irrigated with chlorinated (2 mg/L) nutrient solution growing in each of the three substrates. Steamed pine bark, sand, and pine bark from left to right.

Figure 2-3. Plants inoculated with *P. aphanidermatum* growing in each of the three substrates. Steamed pine bark, sand, and pine bark from left to right.

Figure 2-4. Plants irrigated with chlorinated (2 mg/L) nutrient solution and inoculated with P. aphanidermatum growing in each of the three substrates. Steamed pine bark, sand, and pine bark from left to right.

CHAPTER 3
IMPACTS OF MEDIA AND CHLORINATION ON THE MANAGEMENT OF PYTHIUM APHANIDERMATUM ON GROWTH OF BELL PEPPER IN GREENHOUSE ENVIRONMENT

3.1 Introduction

Increased efficiency in the production of food has steadily remained important on a global level and will continue as world population and food demand increases (Postel, 1992; Wallace, 2000). In many parts of the world the use if irrigation has increased the efficiency in production of food crops many times over (Wallace and Batchelor, 1997; Van Cooten and Borrell, 1999). To further the efficiency of food production the recycling of water in greenhouses has become significant as a result of reduced water quality because of pollution. The use of closed irrigation systems is increasingly becoming imperative to greenhouse producers as laws and regulations are now requiring the capture of effluent from such production facilities (James and van Iersel, 2001; Kluepfel and Stanghellini, 2006). Effluent from greenhouses are typically high in fertilizer and pesticide residues both which can negatively affect ground water and surrounding aquatic ecosystems (Kabashima, 1993; Hanan, 1997; Norman et al., 2003).

Adoption of closed irrigation systems by commercial growers has been slow due to the potential of crop losses associated with rootborne pathogens. The probability of encountering a rootborne disease caused by such oomycete fungi as various *Pythium* spp. and *Phytophthora* spp. greatly increases when effluent is captured and reused on another crop (Stanghellini and Rasmussen, 1994). *Pythium* spp. are the causal agent of diseases that lead to some of the most significant losses in crop production relative to other pathogens (Plaats-Niterink, 1981). Soilless and hydroponic production systems are ideal environments for *Pythium* spp. to proliferate due to the favorable aquatic environment. The asexual reproducing structure of oomycetes is a flagellated zoospore which is dispersed readily in water, hence the proliferation in hydroponic

and soilless production systems. All the aforementioned issues are reasons to support the need for research and development of managing closed irrigation systems.

There are many methods of managing pathogens in the recycled greenhouse effluent; however there is currently a lack of research and information to support the use of the various technologies (Postel, 1992; Uva et al., 1998; Hong and Moorman, 2005). Techniques for sanitizing nutrient effluent including the use of heating, UV radiation, ozone, chlorination, slow sand filtration, pesticides, surfactants, and biological control have all been adapted from municipal water treatment facilities. Large capital investment is an obstacle with regards to the adoption of some of the sanitizing techniques including UV radiation, heating, and ozone (Postma et al., 2000).

Another obstacle for UV radiation is that it is not sufficient alone as a disinfection tactic as filtration is required. UV radiation cannot penetrate solid particulate matter in the recycled nutrient effluent, thus reducing the sanitizing efficiency. Additionally UV radiation has also been shown to cause precipitation of iron from the nutrient solution which increases the need for iron fertilization management (Ewart and Chrimes, 1980). Nonionic surfactants such as those utilized in tank mixes for pesticide applications (ex. Agral 90 - nonylphenoxy polyethyoxyethanol) can be applied to recycled nutrient solution as another option for rootborne pathogen management via lysis of the zoospores. When the zoospores encyst, the motility of the zoospore is lost and a protective adhesive coating and cyst wall are secreted by the plasma membrane (Estradagarcia et al., 1990). The protective coating renders the surfactants ineffective (Stanghellini et al., 1996). Detecting encysted zoospores in the nutrient solution does not seem to be a practical resolution for the problem, thus limiting the practical usage of surfactants for management of nutrient effluent for pathogens.

High sanitizing efficiency and low capital input makes chlorination a good possibility as an option for disinfestation of recycled nutrient solution (Benoit and Ceustermans, 2002; Kim et al., 2006). There is very little information regarding what concentrations of free chlorine causes phytotoxicity which is critical for implementation of this sanitization method (Teoh and Chuo, 1978; Ewart and Chrimes, 1980; Premuzic et al., 2007). Phytotoxicity caused by treatment with chlorinated irrigation solution varies based on crop (Teoh and Chuo, 1978; Premuzic et al., 2007; Saha et al., 2008). A nutrient film technique (NFT) system is not a likely candidate for chlorination due to phytotoxicity from free chlorine (Ewart and Chrimes, 1980). Plants grown in NFT systems are more susceptible to phytotoxicity due to the water substrate that the roots are surrounded by. Water does not offer any buffering capacity relative to an organic substrate such as pine bark. Free chlorine reacts with any organic material such as the pine bark, however in the NFT system the only organic material in the root zone is the roots themselves. This leaves the roots very vulnerable to toxic effects of the chlorinated irrigation solution. Information regarding effective and economic sanitization of greenhouse effluent is necessary to facilitate the adoption of closed irrigation systems in commercial greenhouse vegetable production operations.

The objective of the following experiments was to further the overall understanding of managing pathogens in closed irrigation systems. The objective of the first two experiments was to observe effects of chlorination rate on the proliferation of *P. aphanidermatum* and the ability to move within the closed system. The third and fourth greenhouse experiments were designed to further explore the interactions of chlorination, inoculation with a rootborne pathogen, and the effects of inorganic substrates vs. organic substrates. Lastly the final experiment was to corroborate in the greenhouse results from the growth chamber experiments.

3.2 Materials and Methods

3.2.1 Pythium Isolate and Inoculum Production

The results from the initial growth chamber pathogenicity study and past research supported the use of the isolate from greenhouse cucumbers (*Cucumis sativus* L.) identified as *Pythium aphanidermatum*. This isolate was maintained on PART media with weekly transfers and incubation at 25° C in the dark (Shurtleff and Averre, 1997). At the termination of each greenhouse experiment it was reisolated and root samples were cut into 1-cm long pieces, surface disinfected for 2 minutes in a dilution of 1:100 sodium hypochlorite (5.25% bleach) to distilled deionized water, rinsed with sterile deionized water, dried, and plated on agar gel in Petri dishes with PART media (Shurtleff and Averre, 1997). The isolate was reidentified utilizing the pond water and grass blade technique (Plaats-Niterink, 1981). Transfers of the isolates were made onto V8 juice agar media and incubated for an additional two days at 28°C in the dark (Shurtleff and Averre, 1997). 5-mm plugs of the V8 culture were taken from the expanding growing edge of the mycelia and place in shallow petri dish of water (1 part sterilized pond water:1 part deionized water). The plugs were incubated at 25°C in continuous light for 24 hours utilizing 40 watt fluorescent tube lights. Reproductive structures were observed after 24 hours and with the use of the Van der Plaats-Niterink key, the two isolates were speciated as *P. aphanidermatum and Pythium myriotylum* Drechsler (1981).

The inoculum was prepared in two different ways for the 5 greenhouse experiments. The location of inoculation in the system varied amongst the experiments. This was necessary as the plants were directly inoculated in their pot at planting for the first two greenhouse experiments to observe if there was movement of the pathogen within the system. However in the third and fourth greenhouse experiments the irrigation reservoir was inoculated in an attempt to see if there were differences in management regarding the source of the initial inoculum. Inoculum utilized

in the first two experiments in spring and in summer of 2007 was prepared in the same manner as in the growth chamber experiments. Inoculum was produced utilizing a wheat berry technique (Chellemi et al., 2000). 20 g of hard red wheat seeds were soaked in 25-ml of deionized water for 24 hours in 250-ml Erlenmeyer flasks. The wheat berries were autoclaved on two occasions with 24 hours in between after the initial soaking. Two-day old V8 agar cultures of the isolates were cut into five 5-mm plugs utilizing a No. 2 cork borer and placed into the flasks containing the wheat berries. The plugs are then allowed to grow for 2-3 weeks with a periodic shaking to ensure uniform mycelia growth.

The other three greenhouse experiments utilized a different inoculum production method for the generation of zoospores specifically. The technique is as described in Wulff et al. ((Wulff et al., 1998). 5-mm plugs of *P. aphanidermatum* cultures growing on PART media were transferred to petr-dishes with V8 agar media. The cultures were then allowed to incubate in constant light under 4-20W fluorescent bulbs at 35° C for around 24 hours or until the fungal growth had reached the edge of the petri-dish. The cultures were then cut into 5-mm strips and divided in half in two separate 250-ml Erlenmeyer flasks. 20-ml of sterilized pond water was then added to the strips to soak for 30 minutes. The fluid was then poured off retaining the strips and an additional 20-ml of fresh sterilized pond water was added. The strips were then allowed to incubate in constant light near 35° C for 24 hours. The water was then changed again with 20-ml of fresh sterilized pond water and the strips were then incubated at 20°C for 4 hours. At this point utilizing a hemacytometer the number of zoospores could be estimated and was typically in the range of 10^8 zoospores/ml in the inoculum solution.

3.2.2 Experiments and Treatments

Five experiments were conducted in two University of Florida greenhouse facilities. The first four experiments (Spring 2007, Summer 2007, Fall 2007, and Spring 2008) were conducted

at the Protected Agriculture Project passively ventilated greenhouses (Top Greenhouses Ltd., Barkan, Israel) located at the University of Florida's Plant Science Research and Education Unit, in Marion Co., near Citra, FL. The last experiment was conducted in an evaporative-cooled fan and pad glasshouse at the University of Florida Horticultural Sciences greenhouse facility located in Gainesville, FL. The first two experiments were conducted in spring and summer of 2007 and differed only in season with all treatments being identical. The experiments had randomized complete block designs with six treatments, three replicates, and were eight weeks in length. The treatments consisted of 3 levels of free chlorine (0, 2, 4 ppm derived from 5.25% sodium hypochlorite) and two levels of inoculation (with or without *P. aphanidermatum*). Treatments included: 0 ppm uninoculated, 0 ppm inoculated, 2 ppm uninoculated, 2 ppm inoculated, 4 ppm uninoculated, and 4 ppm inoculated. In these experiments the inoculum utilized was that produced from the wheat berry technique. The objective was to inoculate individual plants growing in a closed growing system to observe movement of the pathogen within a closed system as well as observing disease development.

The two experiments conducted in fall 2007 and spring 2008 in Citra only differed in season and all treatments were identical. The experiments were of a randomized complete block design with 8 treatments, three replicates, and were eight weeks in length. The treatments consisted of two types of media (pine bark and perlite), two levels of chlorination (0 and 2 ppm, derived from sodium hypochlorite, 5.25% bleach (Pride Enterprises, St. Petersburg, FL)), and two levels of inoculation (with and without *P. aphanidermatum*). The treatments included: 0 ppm uninoculated perlite, 0 ppm inoculated perlite, 2 ppm uninoculated perlite, 2 ppm inoculated perlite, 0 ppm uninoculated pine bark, 0 ppm inoculated pine bark, 2 ppm uninoculated pine bark, and 2 ppm inoculated pine bark. In these experiments the inoculum utilized was zoospores

produced as described previously. In this case the irrigation reservoirs were inoculated to simulate contaminated leachate and effects of the chlorination.

The final experiment was conducted in the glasshouse and was similar to the growth chamber experiments described in Chapter 2. The experiment was a randomized complete block design with 18 treatments, 3 replicates, and was six weeks in length. The treatments included three levels of chlorination (0, 2, and 4 ppm), three types of media (pine bark, steamed pine bark, and sand), and two levels of *P. aphanidermatum* (inoculated and uninoculated). The treatments included pine bark 0 ppm uninoculated, pine bark 0 ppm inoculated, pine bark 2 ppm uninoculated, pine bark 2 ppm inoculated, pine bark 4 ppm uninoculated, pine bark 4 ppm inoculated, steamed pine bark 0 ppm uninoculated, steamed pine bark 0 ppm inoculated, steamed pine bark 2 ppm uninoculated, steamed pine bark 2 ppm inoculated, steamed pine bark 4 ppm uninoculated, steamed pine bark 4 ppm inoculated, sand 0 ppm uninoculated, sand 0 ppm inoculated, sand 2 ppm uninoculated, sand 2 ppm inoculated, sand 4 ppm uninoculated, and sand 4 ppm inoculated. As in the fall 2007 and spring 2008 experiments, the inoculum utilized was zoospores. The objective of this experiment was to observe any similarities with regards to results of the growth chamber experiments and this one.

3.2.3 Transplant Production

Seeds of bell pepper cv. 'Legionnaire' (Rogers-Syngenta Seeds, Boise, Idaho) were planted in 128-cell polystyrene Speedling® float tray. The media utilized for germination was a combination of 75% peat moss: 25% vermiculite (v/v) seedling mix (Pro-mix PGX, Premier, Quakertown, PA) with a 25% increase (v/v) with coarse perlite (Airlite Processing Corp. of Florida, Vero Beach, FL). Seedlings were grown in a reach-in controlled environment chamber (width x depth x height: 183 x 76 x 102 cm; Conviron, Controlled Environments Limited, Winnepeg, Manitoba, Canada). The chamber was fitted with fifteen 1.8-m fluorescent bulbs

(Sylvania cool white, Danvers, MA; 160W). The photoperiod was maintained at 16:8 hours and 25°C/22°C day/night. Seedlings flats were irrigated overhead once daily with water to maintain moist media for germination. After emergence seedlings were irrigated to maintain moist media with hydroponic nutrient solution with concentrations (mg/L) of NO_3-N: 80, P: 50, K: 120, Ca: 150, Mg: 50, S: 66, B: 0.7, Cu: 0.2, Fe: 2.8, Mn: 0.8, Mo: 0.05, and Zn: 0.3 (Hochmuth and Hochmuth, 1996). Seedlings were grown in the reach-in growth chamber for 6 weeks prior to transplanting for the start of the various experiments.

3.2.4 Growing System and Inoculation

The four experiments (Spring 2007, Summer 2007, Fall 2007, and Spring 2008) were conducted at the Protected Agriculture Project passively ventilated greenhouses (Top Greenhouses Ltd., Barkan, Israel) located at the University of Florida's Plant Science Research and Education Unit, in Marion Co., near Citra, FL. Each experiment was established in one bay (8 m-wide x 32 m-long x 9 m-height) of the greenhouse. Seedlings were transplanted 10 March (Spring 2007), 13 May (Summer 2007), 17 Sept. (Fall 2007), and 7 March (Spring 2008). All plants were trellised in a modified 'Spanish' system as the plants were a determinate growth structure (Jovicich et al., 2004). In spring 2007 and summer 2007, the four middle plants were inoculated with 40 g of wheat berries per plant in each plot at planting. Only the designated plots were treated with inoculated wheat berries while the other plots were inoculated with autoclaved wheat berries. In fall 2007 and spring 2008, the zoospore suspension was added at a rate of 40 ml per irrigation reservoir at planting. Each plot of the four experiments consisted of a 10-plant closed hydroponic unit (Figure 3-1).

The hydroponic units were constructed and designed utilizing various components. The irrigation reservoirs utilized were two 55-gal drums (Industrial Containers, Zellwood, FL) which were attached at the base utilizing a ¾" threaded hose barb and a 15-cm section of ¾" contractor-

grade water hose (Swan Hose, Bucyrus, OH) (Figure 3-2). A third 55-gal drum was utilized in a horizontal position to allow the leachate to be collected for reuse (Figure 3-3). The irrigation reservoirs required a lid to prevent algae growth in the nutrient solution. One lid for the drum located in the rear simply was a 63.5 cm square piece of ½" plywood. The lid for the other drum in the front consisted of a plywood constructed box (63.5 cm cube with one open side) which also served to house the pump and relay (Figure 3-4). Each unit utilized a pony pump (Little Giant PP-1S, Oklahoma City, OK) (Figure 3-5) and a pump start relay (Model PSR-22, Hunter Industries, San Marcos, CA) (Figure 3-6). The pump start relay was activated via a computer timer using a 24-volt signal (Eldar Shaney, Elgal Fertimix, Israel). The 24 volt signal was initially intended to activate the misting system, but was amplified utilizing transformers and additional relays to initiate the irrigation event in each of the wired pump/relay boxes for each unit. The irrigation frequency ranged from 15-20 minutes for duration of 45-90 seconds depending on the crop age and environmental conditions. For the two weeks post-transplanting irrigation frequency was maintained at 20 minutes as the water demand was low due to crop age. As the crop increased with age and water demand the irrigation frequency was reduced to 15 minutes. The irrigation duration was also managed in a similar fashion in that the length of the irrigation event was 45 seconds in the first two weeks post-transplanting as the demand for water was low due to crop age. As the crop increased with age and water demand similarly the irrigation duration was increased to 90 seconds. The suction side of the pump utilized a 1.2 m long piece of ¾" hose which went down into the first reservoir. The flow side of the pump was connected to a back flow preventer (Figure 3-7) and then to 20 mm diameter black poly pipe (Netafim-USA, Fresno, CA) and at the end was connected to a 20 mm ball valve (Netafim, Tel-Aviv, Israel) and pressure gauge with a return line back to the irrigation reservoir (Figure 3.8).

This was done to reduce stress on the pump and to allow the pressure compensated drip emitters (Flow: 1.5 L/min, Netafim, Tel-Aviv, Israel) to work properly. There were two emitters installed into the poly pipe for each plant. The specifications on the emitters required the pressure to be in a range of 7-12 psi. Each plot contained 10 plants established in 12.1-L bato buckets (22 cm-length x 22 cm-width x 25 cm-height, Bato Plastics B.V., Zevenbergen, Netherlands). Bato buckets are designed with one drain which allows for drainage into a 1 ½ " PVC pipe obtained locally (NIBCO, Elkhart, IN). To allow for drainage into the horizontal leachate reservoir the pots had to be placed on a stand (0.25 m-wide x 0.60 m-tall x 3 m-long) welded from ¾" electrical conduit obtained locally (Republic Conduit, Cedar Springs, GA). The 3.5 m drainage pipe was prepared by drilling 2-cm holes for the drain on the bato buckets and was then attached to the stand with electrical ties (Gardner Bender, Milwaukee, WI). As needed, typically weekly, the leachate was transferred back to the irrigation reservoirs utilizing a sump pump (Model RES, Water Ace Pump Company, Ashland, OH). The pump was rinsed and flushed between treatments to avoid contamination. As an additional precaution all uninoculated plots' leachate was transferred prior to inoculated plots. At this point the specified plots were treated with the appropriate levels of sodium hypochlorite at 5.25%.

The final greenhouse experiment (GNV-Spring 2008) was conducted in an evaporative-cooled fan and pad rigid-frame glasshouse located in the Horticultural Sciences greenhouse complex on main campus at the University of Florida in Gainesville, FL. The dimensions of the glass house are 12.2 x 10.0 x 9.2 m^3. This experiment as previously mentioned was conducted in much the same way as the growth chamber experiments. Seedlings were transplanted and inoculated on 29 Feb (GNV-Spring 2008). Designated plants were treated with 20 ml of the zoospore solution and those designated as uninoculated were treated with 20 ml of autoclaved

pond water. Plants were grown in 4-L black polyethylene nursery pots with 5 drain holes (diameter: 18.4 cm, height: 21.6 cm, model C475, Nursery Supplies Inc., Kissimmee, FL) and utilized in conjunction with a clear polyethylene saucer to provide a small reservoir of water (diameter: 20.3 cm, height: 3.8 cm, Model VS6L, Woodstream Corporation, Lititz, PA). Plants were grown on a raised bench (1 m – wide x 10 m-long x 0.9 m – high). The media utilized included composted pine bark, pure sand (Play sand, Quikrete, Atlanta, GA), and steamed pine bark.

Plants were irrigated daily utilizing two dosatron injectors (Dosatron DI210, Clearwater, FL) and a timer clock every 20 minutes for 45 seconds. This was necessary to keep up with the crop's water demand which is greater in the greenhouse relative to the previous growth chamber experiments. One pressure compensated drip emitter (Flow: 1.5 L/min, Netafim, Tel-Aviv, Israel) was utilized for each plant and was connected to 20 mm black poly pipe (Netafim-USA, Fresno, CA). The final nutrient solution contained concentrations (mg/L) of NO_3-N: 150, P: 50, K: 200, Ca: 150, Mg: 60, S: 70, B: 0.7, Cu: 0.2, Fe: 2.8, Mn: 0.8, Mo: 0.05, and Zn: 0.3 (Hochmuth and Hochmuth, 1996). The plants were irrigated with 400 ml of nutrient solution, once daily. This was done to apply the chlorination treatments to the appropriate plants as it was not practical to set up three different irrigation systems for each of the chlorination levels. Sodium hypochlorite (5.25% bleach) was utilized to chlorinate the nutrient solution to the appropriate concentration including 0, 2, and 4 ppm of free chlorine. Chlorine demand of nutrient solution was established in a laboratory setting by comparison to amount of sodium hypochlorite necessary to generate the designated concentration of free chlorine. Nutrient solution chlorine demand is twenty times that of deionized water. Free chlorine was measured using a DPD-colorimetric test kit (Kit 5910, Lamotte Co., Chestertown, MD).

3.2.5 Pest Management

An integrated approach to pest management was utilized in all greenhouse experiments. One plant within each plot was scouted on a weekly basis for arthropod pests and diseases. There were no incidences of foliar disease within any of the experiments. Arthropod pests however were encountered in some of the experiments. Arthropods found included two-spotted spider mite (*Tetranychus urticae* (Koch)), silver-leaf whitefly (*Bemisis tabaci* (Gennadius)), flower thrips (*Frankliniella tritici* (Fitch)), mealy bugs (*Phenacoccus solani* (Ferris.)), and green peach aphids (*Myzus persicae* (Sulzer)). All arthropods were successfully managed below damaging levels with biological control. Various organisms and organisms/complex system were utilized. *Neoseiulus californicus* (McGregor) (Biotactics Inc., Romoland, CA) is a predatory mite which was released for management of two-spotted spider mite. Amblysius swirskii (McGregor) (Swirski-mite, Koppert Biological Systems-USA, Romulus, MI) is a predatory mite that was released for the management of thrips larvae and whitefly eggs and larvae. *Orius insidiosus* (Say) (Thripor-I, Koppert Biological Systems-USA, Romulus, MI) is a predator that was released for management of flower thrips. *Aphidius colemani* (Aphipar, Koppert Biological Systems-USA, Romulus, MI) is a parasitoid wasp that was utilized for management of aphids and was also incorporated into a sorghum banker plant system utilizing *Rhopalosiphum maidis* (Fitch) as an alternative host.

3.2.6 Data Collected and Analysis

Data collected for each of the experiments included plant height and stem diameter growth over time from transplanting until experiment termination. Other data collected at the termination of the experiments included shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and root ratings. In the spring 2008 experiment SPAD readings were also collected. The root ratings were based on visual analysis for the percentage of damage roots

either as a result of the pathogen or chlorination treatment. Root ratings were on a scale of 0-5 with: 0 = no damage, 1 = 1 – 25% damage, 2 = 26 – 50% damage, 3 = 51-75% damage, 4 = > 75% damage, 5 = plant mortality. Additionally harvest data was collected from 4 plants from each plot at the termination of the experiment (8 weeks post transplanting). Fruit were counted and graded using diameter which is utilized for greenhouse peppers produced in North America (De Ruiter, 1987; Jovicich et al., 2005). The sizes included extra-large (> 84.0 mm), large (76.0 – 83.9 mm), medium (64.0 – 75.9 mm), small (56.0 – 63.9 mm), and unmarketable (blemishes, misshapen, blossom end rot, sunscald). Environmental conditions were also monitored for all five greenhouse experiments. Solar radiation and temperatures were collected every 15 minutes utilizing a HOBO data logger (UA-002-08, Onset, Bourne, MA) suspended 10 cm above the plants.

Statistical Analysis System was used for all the data analysis (SAS Institute, Cary, NC). The data from all the experiments was subjected to analysis of variance (ANOVA). Means were separated utilizing the least significant difference (LSD) function. In some cases where there was a range of chlorination rates the contrast procedure was utilized as LSD is not an appropriate means of evaluating rates. The contrast procedure is utilized to determine differences amongst multiple means as affected by a rate variable (Littell et al., 2002). Specifically orthogonal contrasts were utilized for the preplanned comparisons of different rates of chlorination.

3.3 Results and Discussion

3.3.1 Impacts of Chlorination Rate on *P. aphanidermatum* Spring (March – May) 2007

The average temperature throughout the experiment was 25.1°C, with a maximum of 47.6°C and a minimum of 10.3°C (Figure 3-9). Infection of the host by *P.* aphanidermatum is optimal between 30°C and 35°C (Plaats-Niterink, 1981). In the first two weeks of the experiment the temperature only exceeded 30°C one day. The significance of this is that in a season such as

this the temperatures were not optimal in the initial critical two weeks for potential infection post inoculation. In a season with warmer temperatures at the timing of inoculation, it would be possible to observe a greater amount of negative growth effects such as reduced root and shoot weights associated with the pathogen. This has been observed in similar experiments with both bell peppers and spinach in that disease incidence was greater in warmer temperatures (Gold and Stanghellini, 1985; Chellemi et al., 2000). The average light levels in the greenhouse were 436.8 µmols/m^2s and the average light levels outside the structure were 932.6 µmols/m^2s (Figure 3-10). Average light levels in the greenhouse were reduced (53%) based on the polyethylene covering in addition to dust and dirt on the surface of the covering.

The effect of chlorination rate was significant ($P \leq 0.05$) with respect to shoot fresh weight, root fresh weight, and root dry weight (Table 3-1). There is an inverse linear relationship between rate of chlorination and to shoot fresh weight, root fresh weight, and root dry weight (Table 3-1). This is consistent with the growth chamber studies in that generally the higher the concentration of free chlorine, the greater the chance of negative effects associated with phytotoxicity. Similar trends with regards to chlorination concentration were also observed in tomato transplant production although they were tolerant to higher levels relative to peppers (Saha et al., 2008). Increasing the chlorination rate in irrigation water for lettuce produced in soilless culture was correlated with increased symptoms of phytotoxicity (Premuzic et al., 2007). Cucumbers, onions, citrus are other crops in which increasing exposure to chlorine is associated with increased phytotoxicity (Bennett, 1993). Chlorination did not affect plant height and shoot dry weight (Table 3-1). Inoculation with *P. aphanidermatum* led to reduced height growth, shoot fresh weight and shoot dry weight (Table 3-1). This trend was also observed in the previous growth chamber experiments discussed in Chapter 2. Root borne diseases caused by such

pathogens as *Pythium* sp. adversely affect some aspects of crop growth (Teoh and Chuo, 1978; Premuzic et al., 2007; Saha and Cantliffe, 2007). In greenhouse-grown peppers, *P. aphanidermatum* has been documented for causing reduced overall growth including shoot mass, leaf area, and root mass (Sutton et al., 2006). Long English cucumbers produced hydroponically are another crop in which inoculation with *P. aphanidermatum* has been associated as the cause of reduced growth and yield (Menzies et al., 1996).

There was a significant two-way interaction of chlorination rate and presence/absence of *P. aphanidermatum* with regards to stem diameter growth and root rating (Table 3-1). Treatments with 0 ppm/uninoculated, 2 ppm/uninoculated, and 4 ppm uninoculated had greater ($P \leq 0.05$) stem diameter growth relative to treatments with 4 ppm/inoculated and 0 ppm/inoculated (Table 3-2). These results are similar to other studies in which pathogenicity of *P. aphanidermatum* was high in various crops. *P. aphanidermatum* causes significant disease and losses in a variety of crops including grains and vegetables (Hendrix and Campbell, 1973). Inoculation with *P. aphanidermatum* stunted or caused mortality of hydroponically produced spinach (*Spinacea oleracea* L.) under commercial settings (Bates and Stanghellini, 1984). In extreme cases with conducive conditions for disease development led to mortality of mature English cucumber plants (Stanghellini et al., 1996). Additionally, stem diameter of plants in the treatment that was inoculated and chlorinated at 2 ppm was not significantly ($P \leq 0.05$) different from uninoculated plots indicating control of the pathogen. Yield was generally unaffected by chlorination treatments and inoculation with *P.* aphanidermatum (Table 3-3).

The main effect of chlorination as led to reduced number of marketable fruit, number of total fruit, and weight of the total fruit. As the concentration of free chlorine increased, there was an inverse linear relationship in which the marketable and total number of fruit decreased (Table

3-3). Total weight of fruit responded with a negative quadratic response relative to the concentration of free chlorine (Table 3-3). Total fruit weight did not decrease until the chlorination rate was at 4 ppm. One potential explanation for the limited amount of significant impacts of treatments can be associated with the fact that the plants were only harvested one time. Similarly, chlorination did not affect the harvest of lettuce in a hydroponic production system although there was foliar damage associated with the phytotoxicity (Premuzic et al., 2007). Lettuce differs in this way that the plant is only harvest once. In a true commercial setting a pepper crop would be grown for up to 8 months and harvested multiple times and in this setting one could potentially see more significant impacts over time. Further, pepper greenhouse growers would normally use indeterminate cultivars and not a determinant cultivar used in the present experiments. Field producers use determinate pepper cultivars, and only harvest two or three times. Additionally it must also be noted that in a commercial production settings the planting density is typically as high as 3 plants/m^2. Due to constraints the plant density used in these experiments was much lower (1.1 plants/m^2) and thus the yields could have potentially been three times higher if space allowed for it.

3.3.2 Impacts of Chlorination Rate on *P. aphanidermatum* Summer (May – July) 2007

The average temperature throughout the experiment was 29.9°C, with a maximum of 53.9°C and a minimum of 11.9°C (Figure 3-11). Temperatures in the summer crop were greater than in the spring crop. Implications of this are with regards to the optimal temperature for proliferation of the pathogen which is between 30°C and 35°C. The increased temperatures should lead to more disease development and negative effects on crop growth associated with *P. aphanidermatum*. Increased temperatures can also be beneficial to vigorous growth of the pepper plants which could potentially offset the increased disease development. Additionally in a soilless culture plants are given high levels of nutrients multiple times per day could offset a

compromised root system. The average light levels were 487.4 µmols/m^2s and the average light levels outside the structure were 984.36 µmols/m^2s (Figure 3-12). Average light levels in the greenhouse were reduced (50%) based on the polyethylene covering in addition to dust and dirt on the surface of the covering.

Shoot fresh weight decreased with increasing chlorine concentration in the irrigation solution (Table 3-4). Shoot fresh weight was also negatively affected by higher chlorination rates in the growth chamber studies in Chapter 2. Tomato transplants had reduced shoot growth as free chlorine concentrations in the floatation irrigation were increased from 20 ppm to 80 ppm (Saha et al., 2008). The negative impacts of chlorination can vary based on a number of conditions however media can be on important factor. For example, in an NFT production system chlorination rates as low 3 ppm reduced root growth of tomatoes (Ewart and Chrimes, 1980). In an NFT system the substrate is water so there is limited buffering capacity relative to an organic substrate such as pine bark which can help to neutralize free chlorine in the root zone to avoid damage. Treatments without inoculum had greater height growth, stem diameter growth, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, and root rating relative to those with inoculum (Table 3-4). These results are indicative of the pathogen causing negative impacts on growth as would be expected from a root pathogen. In field peppers, *Pythium* sp. associated with hurricane flood events were determined to be the cause of reduced yield and plant mortality (Saha et al., 2005). Foliar chlorosis and root discoloration of tomatoes occurred with inoculation of tomatoes grown hydroponically with rockwool as the substrate (Calvo-Bado et al., 2006).

Harvest data in the summer was similar to that of spring in that none of the harvest parameters were affected by the pathogen. Impacts of the pathogen might have been more

significant if more than one harvest had been collected as greenhouse pepper crops are harvest several times, especially during an eight month production season. Yields of marketable fruit were not affected by the chlorination or inoculation with *P. aphanidermatum* (Table 3-5). The weight of culls and fruit with blossom end rot, and the number of blossom end rot fruit decreased with increasing free chlorine concentration (Table 3-5). In this particular type of production system the pine bark substrate, acted as a buffer to reduce negative impacts on marketable fruit weight and number associated with chlorination of the nutrient solution. Also, pine bark appeared to be able to suppress the pathogen sufficiently to avoid negative effects on harvest of marketable fruit weight and number. Trying to determine validity of this reasoning is support for conducting the following studies in which an inorganic substrate (perlite) will be compared with an organic substrate (pine bark).

3.3.3 Impacts of Chlorination and Media on *P. aphanidermatum* Fall (September–November) 2007

The average temperature throughout the experiment was 25.7°C, with a maximum of 49.9°C and a minimum of 10.3°C (Figure 3-13). Average temperature of the fall crop was similar to the spring crop. This is significant as the ideal range for disease development of *P. aphanidermatum* is between 30°C and 35°C. The average greenhouse light levels were 511.8 µmols/m^2s and outside the light levels were 733.18 µmols/m^2s (Figure 3-14). In the fall the day length gets shorter over time from August to October, which would explain the reduced solar radiation outside during the fall crop relative to longer day lengths in the late spring and summer. Average light levels in the greenhouse were reduced (30%) based on the polyethylene covering which is significantly greater light penetration through the poly ethylene as compared to previous experiments. This is a result of replacing the poly ethylene covering with new clean material. The additional light penetration has potential to increase crop growth.

Shoot fresh weight was greater in plants grown in pine bark compared to perlite (Table 3-6, Figure 3-15). Use of perlite or pine bark resulted in no growth and harvest differences of Beit Alpha cucumbers (Shaw et al., 2004). Pine bark is organic as compared to the inorganic perlite and due to that property it has the ability to neutralize the free chlorine through chemical reactions before it can have a negative effect on the roots via oxidation (White, 1992).

Plants not treated with chlorination had greater stem growth, shoot fresh weight, and root dry weight as compared to those grown with chlorination (Table 3-6, Figure 3-16). Negative impacts of chlorination on plants have been observed with a variety of crops including peppers (Saha and Cantliffe, 2007). Increased chlorination ranging from 0 ppm to 25 ppm of irrigation solution for production of bell pepper var. Green-giant grown in granite chips led to decreased shoot weight (Teoh and Chuo, 1978). Sensitivity to chlorination can depend on various factors such as crop and type of substrate. Chlorination was not recommended for use in production of tomatoes in NFT as it led to reduction in root development in concentrations as low as 3 ppm (Ewart and Chrimes, 1980). In tomato transplant production utilizing peat based organic substrate there were no adverse affects associated with growth and development until concentrations of 20 ppm free chlorine were utilized in the irrigation solution (Saha et al., 2008).

Inoculation with *P. aphanidermatum* led to reduced height growth and root fresh weight (Table 3-6, Figure 3-17). The earlier greenhouse experiments led to similar trends with regards to negative impacts on growth parameters associated with inoculation. This further supports that in fact the species being utilized in the experiment is in fact maintaining its pathogenicity. In the Spring 2007 and Summer 2007 experiments, inoculation with *P. aphanidermatum* also led to reduced height growth and root fresh weight. This is significant because there are isolates and species that can lose pathogenicity. *P. aphanidermatum* inoculation led to root rot and reduced

root growth of bell peppers in a greenhouse pathogenicity study (Chellemi et al., 2000). Plant height was reduced in cucumbers inoculated with *P. aphanidermatum* produced in an ebb and flow hydroponic system (Sanogo and Moorman, 1993). In a survey of Norwegian greenhouse cucumber producers, *P. aphanidermatum* was the only species causing significant production losses (Herrero et al., 2003).

There were significant two-way interactions with regards to media and chlorination plus media and pythium (Table 3-6). Height growth and root fresh weight had significant two-way interactions between media and chlorination (Table 3-7). Plants grown in pine bark with chlorination had greater height growth and root fresh weight relative to perlite with chlorination (Table 3-7). This further suggests that pine bark as an organic substrate has increased buffering capacity regarding the negative effects associated with chlorination relative to the inorganic perlite media. Pine bark has been observed to have suppressive properties with regards to some rootborne pathogens such as *Pythium* sp and *Phytophthora* sp. and has been adopted widely in the ornamental industry to replace methyl bromide for disease suppression (Hoitink et al., 1997). Beneficial microbes such as *Bacillus* spp. and *Enterobacter* spp. often recolonize pine bark after composting (Hoitink and Fahy, 1986). Use of pine bark medium reduced damping off of pea (*Pisum sativum* L.) caused by *P. ultimum* Trow (Steinmetz and Schonbeck, 1994).

Pine bark is not the only composted material which has led to suppression of *P. aphanidermatum*. Inoculum load was decreased in potting mix containing composted manure as compared to a potting mix without compost (Mandelbaum and Hadar, 1990). However there were some negative impacts of chlorination as pine bark with chlorination had less ($P \leq 0.05$) height growth and root fresh weight as compared to the pine bark treatments without chlorination (Table 3-7). Stem diameter and root rating had significant two-way interactions with media and

pythium (Table 3-6). In this case pine bark treatments with pythium were not different as compared to pine bark without pythium (Table 3-7). Pine bark with pythium had greater ($P \leq 0.05$) stem diameter growth relative to perlite with pythium (Table 3-7). This can also be attributed to the fact that in a living media such as pine or hardwood bark compost there are other organisms which are potential antagonists or competitors with pathogens for nutrient sources (Davis et al., 1992). There are various ways for beneficial microbes to reduce pathogen development. *Pseudomonas flourescens* inhibited development of *P. aphanidermatum* through direct antagonism and induced production of defense related enzymes in tomato plants (Ramamoorthy et al., 2002). Many of the beneficial microbes suppress *Pythium* sp. directly through biochemical signals to prevent spore germination and subsequent infection of host tissue (Chen et al., 1988).

Medium fruit number, weight of medium fruit, number of small fruit, weight of small fruit, number of culls, and total number of fruit were significantly ($P \leq 0.05$) affected by media (Table 3-8). The aforementioned harvest parameters were all greater in treatments with perlite (Table 3-8). However, marketable fruit weight produced was similar from both media treatments and would suggest that there are not significant production differences between perlite and pine bark (Table 3-8). Results such as this have been observed with various vegetable crops grown in pine bark and perlite. There were no differences in 'Galia' muskmelon (*Cucumis melo* L.) quality or quantity when produced in perlite or pine bark (Rodriguez et al., 2006). There was no difference in fruit yield of Beit Alpha cucumbers grown in either pine bark or perlite (Cantliffe et al., 2003). Results from these experiments, as well as the aforementioned studies, it appears that a variety of vegetable species can be successfully produced in pine bark or perlite with no differences in yield.

The main effects of chlorination were significant with regards to number of small fruit, weight of small fruit, and weight of blossom end rot fruit. Treatments without chlorination had less total small fruit and small fruit weight relative to treatments with chlorination (Table 3-8). Similarly as in the main effects of media, it is not possible to clearly state that chlorinated treatments produced greater fruit and weight as these were only a few of the total harvest parameters. Yields did not differ for production of 'Galia' muskmelon grown in pine bark or perlite (Shaw et al., 2007). There was no difference in production for bell peppers grown in perlite or pine bark (Jovicich et al., 2007). The main effects of inoculation were not significant with regards to any of the harvest parameters collected (Table 3-8). Results from the spring 2007 and summer 2007 experiments were also the same in that inoculation had no effect on harvest. As in the other experiments only one harvest was collected and differences could have been observed if harvest multiple times as in a commercial setting.

Two-way interactions were significant including media x chlorine, media x pythium, and chlorine x pythium with regards to some of the growth parameters (Table 3-8). Plants grown in pine bark with and without chlorine in addition to plants grown in perlite without chlorine did not differ in production of extra-large fruit number and extra-large fruit weight (Table 3-9). Extra-large fruit number and weight produced from plants grown in perlite without chlorination were greater than those produced from those grown in perlite with chlorination (Table 3-9). Marketable fruit weight of plants grown in perlite with chlorination was also the lowest of all treatment combinations between media and chlorination (Table 3-9).This is indicative of the inorganic nature of perlite in that it may not buffer the oxidizing of root tissue as pine bark or any organic substrate. However, treatments with perlite and no chlorination produced greater number and weight of marketable fruit relative to treatments with pine bark and no chlorination

(Table 3-8). This is different from past studies previously mentioned conducted by Rodriguez et al. and Jovicich et al. in that there was a difference in yield between plants grown in perlite and pine bark. It is important to consider that this is within the interaction between two variables within the experiment. However, when directly comparing the main effects of perlite and pine bark on pepper yield, there was no difference in total marketable weight (Table 3-8).

The effect of the interaction between chlorination and inoculation significantly affected numbers and weight of extra-large fruit (Table 3-8). Treatment media controls without chlorination or inoculation had greater extra-large fruit weight and number (Table 3-10). Extra-large fruit number of treatments with chlorination and pythium, chlorination without pythium, and no chlorination with pythium were not different (Table 3-10). This supports that in addition to damage from rootborne pathogens, there can also be damage incurred from the chlorination treatment as in the growth chamber studies in chapter 2, as well as another study. Chlorination of nutrient solution at concentrations less than 0.5 ppm led to root damage and no disease control in tomatoes produced in an NFT system (Ewart and Chrimes, 1980). Bennett also reported symptoms of phytotoxicity associated with chlorine in cucurbit crops (1993).

Pine bark treatments with inoculation had the lowest cull weight relative to the other treatments (Table 3-10). Increased cull weight in pine bark with pythium is indicative of the negative impacts on crop growth associated with rootborne diseases (Table 3-10) (Herrero et al., 2003). Weight of fruit with blossom end rot was the lowest in the treatment with pine bark without pythium and perlite with pythium (Table 3-10). However, this parameter alone is not sufficient to determine which media yields the greatest amount. Most importantly for yield data are results of marketable fruit weight as this is directly linked with profitability in a commercial setting. However since chlorination is the factor having the greatest impact on marketable fruit

weight in these experiments pine bark would be the better option as there was not a reduction in marketable fruit regardless of whether the treatment was chlorinated (Table 3-9). Plants grown in perlite with chlorination led to reduced total marketable fruit weight relative to treatments with perlite and no chlorination.

Total cull weight was affected by the three-way interaction between media, chlorination, and inoculation. The treatment with pine bark – no chlorination – pythium and the perlite control both had the greatest ($P \leq 0.05$) total cull weight relative to the other treatments (Table 3 -11). These data are not sufficient alone to determine which media is superior between the two if either. Further repeated studies in similar seasons would be beneficial for obtaining additional information to make this determination. Additionally it would be beneficial if the system could be altered to allow chlorinated solution to rest for a period to allow dissipation of free chlorine to avoid phytotoxicity problems further isolating the variables to determine which is having most impact on the system. Additionally it would be closer to a commercial setting as larger reservoir tanks allow the potential to allow recycled irrigation solution to rest post chlorination.

3.3.4 Impacts of Chlorination and Media on *P. aphanidermatum* Spring (March – May) 2008

The average temperature throughout the experiment was 25.8°C, with a maximum of 52.6°C and a minimum of 8.5°C (Figure 3-18). Average temperature in this experiment was similar to the Spring 2007 and Fall 2007 experiments. Averages of this experiment as well as the others were within the range of temperatures (20-35°C) of pathogenicity for *P. aphanidermatum*. This would indicate that conditions were suitable for some disease development as optimal occurs between 30°C and 35°C. However maximum daily temperatures in this experiment exceeded 40°C six days including the days 1-6 after transplanting (Figure 3-19). The significance

of this is that this would be the critical infection period, but infection by *P. aphanidermatum* does not occur above 40°C. Infection of tomatoes with *P.butleri,* a synonym of *P. aphanidermatum,* did not occur above 40°C (Tripathi and Grover, 1974). Temperatures in this experiment could potentially lead to reduced infection by the pathogen. The average light levels in the greenhouse were 528.6 µmols/m^2s and outside light levels were 1070.59 µmols/m^2s (Figure 3-9). Average light levels were reduced (51%) which is similar to experiments in spring 2007 and summer 2007. This is indication of problems of light penetration through polyethylene which is has been covered in dust and dirt over time and in this case occurred after only one fall crop. Even though the averages of solar radiation are similar in the fall 2007 and spring 2008 experiments, the amount of light outside during this spring experiment exceeded that of available light in the fall 2007 experiment so there was potential for increased solar radiation in this experiment. The benefits of increased radiation based on season could not be realized as the condition of the polyethylene would not allow for the additional light penetration.

Shoot fresh and dry weights of plants grown in pine bark were greater relative to plants grown in perlite (Table 3-12, Figure 3-20). Some studies with bell peppers have shown some differences in growth of vegetable crops grown in different soilless media. Peppers grown in paper mill compost had less shoot fresh weight compared to plants grown in a peat-perlite mix in greenhouse studies (Evanylo and Daniels, 1999). This could be attributed to the type of production system and media. In more recent studies utilizing soilless production and comparing pine bark with perlite it was reported that there was no difference between the two substrates for production of Beit Alpha cucumbers and peppers (Cantliffe et al., 2003; Shaw et al., 2004). This could be indicative of the ability of the media to interact with the other treatments such as chlorination and inoculation. Pine bark is organic as compared to the inorganic perlite and due to

that property it has the ability to neutralize the free chlorine through chemical reactions before it can have a negative effect on the roots via oxidation (White, 1992). Additionally it has been reported that plants inoculated with *Pythium* sp. grown in composted substrates such as pine bark led to increased root weights in cucumbers relative to inoculated plants grown in a peat-perlite substrate (Zhang et al., 1996).

Chlorination significantly led to reduced height growth, shoot fresh weight, and shoot dry weight (Table 3-12). Similarly, chlorination reduced shoot fresh weight in the fall 2007 experiment. Chlorination has led to reduction in various growth parameters throughout all four greenhouse experiments conducted in Citra. Treatments without chlorination had greater height growth, shoot fresh weight, and shoot dry weight. Negative effects of chlorination were possible as observed in other studies regarding vegetable crops and management of rootborne disease In a study regarding management of bacterial wilt of peppers utilizing chlorination for management, it was reported that plant height was reduced with increasing chlorine concentrations ranging from 0 – 25 ppm (Teoh and Chuo, 1978). In NFT culture of tomato plants root growth was reduced in treatments chlorinated at concentrations less than 3 ppm as compared to plants grown in nonchlorinated nutrient solution (Ewart and Chrimes, 1980).

Stem diameter growth was reduced ($P \leq 0.05$) on plants in treatments with inoculation (Table 3-12). Some reductions in crop growth parameters such as height or stem diameter would be expected from inoculation with a pathogenic isolate. Infection with *P. aphanidermatum* led to root rot of chrysanthemums (*Dendranthema grandiflorum* (Ramat.) Kitamura) (Hendrix and Campbell, 1973). Inoculation with *Pythium* spp. resulted in plant mortality of tobacco seedlings (Liu, 1976). It has been reported that inoculation with *P. aphanidermatum* leads to root dysfunction in creeping bent grass in growth chamber experiments (Feng and Dernoeden, 1999).

From these experiments it is apparent that *P. aphanidermatum* can lead to reduction in growth in a variety of crops. *P. aphanidermatum* has also been associated with losses in melon production in Guatemala as a result of root and crown rot (de Cara et al., 2008). *P. aphanidermatum* has been identified as the causal agent of wilting and root necrosis in greenhouse production of beans (*Phaseolus vulgaris* cv. Festival) (Serrano et al., 2008).

There were also two-way interactions with respect to media x pythium, media x chlorine, and chlorine x pythium (Table 3-12). Height growth of inoculated plants grown in pine bark was greater relative to plants grown in perlite (Table 3-13). There was no effect ($P \leq 0.05$) of pythium on height growth of plants grown in pine bark (Table 3-13). The contrary was true for perlite however in that inoculated plants had less height growth relative to uninoculated plants grown in perlite (Figure 3-22). This is indicative of pine bark being an organic living substrate with organisms which can compete and antagonize rootborne pathogens (Davis et al., 1992; Hoitink et al., 1997). *P. aphanidermatum* led to less stem diameter growth in inoculated plants grown in perlite compared to inoculated plants grown in pine bark in the fall 2007 experiment. Although not the same from one season to the next, consistently in both experiments at least one growth parameter was more affected in plants grown in perlite as compared to plants grown in pine bark as a result of pathogen infection.

Plants inoculated and grown in perlite had a higher root rating further supporting findings in these studies and others previously mentioned (Table 3-13). This same result occurred in a similar experiment conducted in fall 2007. Since inoculated plants grown in pine bark had a lower root rating compared to those in inoculated and grown in perlite it would indicate that pine bark is again better at mitigating root damage associated with *P. aphanidermatum.* Treatments with plants growing in pine bark and treated with chlorine had greater stem diameter growth

relative to plants growing in perlite that were treated with chlorine (Table 3-14). In addition to buffering negative growth affects associated with *P.* aphanidermatum, the fact that the pine bark is organic media potentially allows it to also better buffer the negative aspects associated with chlorination relative to perlite, an inorganic substrate (Davis et al., 1992; White, 1992). Treatments with plants that were inoculated and chlorinated had less root growth relative to inoculated and nonchlorinated plants (Table 3-14). Additionally, since plants uninoculated and chlorinated did not differ in root dry weights from plants that were inoculated and treated with chlorine it would suggest that in this experiment and conditions that the negative effects of chlorination were greater relative to effects of inoculation with respect to root dry weight. These results are consistent with the previous experiments in that there have been negative effects on growth parameters such as height and root weights. This is consistent with findings of other studies in which chlorination was associated with phytotoxicity of various vegetable crops. Bell peppers grown in granite chips had less height growth at increasing chlorine concentrations (Teoh and Chuo, 1978). Chlorination rates utilized in NFT production of tomatoes which were sufficient for management of Verticillium wilt (*Verticillium tricorpus* Isaac) led to significant reductions in root growth (Ewart and Chrimes, 1980).

The main effects of media significantly affected some of the harvest parameters (Table 3-16). Plants grown in pine bark had greater numbers of extra-large fruit, extra-large fruit weight, numbers of marketable fruit, marketable fruit weight, numbers of fruit with blossom end rot, blossom end rot fruit weight, numbers of total fruit, and total fruit weight. From this study involving chlorination and *P. aphanidermatum*, pine bark was observed to be better for overall production as there was greater marketable fruit weight from plants grown in pine bark relative to perlite (Table 3-16). This result is different from the fall 2007 experiment and can potentially

be attributed to temperatures. The average daily temperatures in the spring 2008 experiment were lower than in the fall 2007. Additionally the spring 2008 experiment had 14 more days where minimum temperatures were below 20°C relative to the fall 2007 experiment in the first few weeks. The implications from this are that the pathogen was exposed to lower temperatures during the infection period in the spring 2008 experiment, therefore its pathogenicity was limited, thus having less of a chance to infect plants and thus affect yield. It has been reported that pathogenicity of *P. aphanidermatum* decreases significantly below 20°C (Plaats-Niterink, 1981).

From a practical view, a commercial grower's ultimate goal is to produce the largest amount of marketable fruit weight economically as peppers are marketed by size and how many can fit in a 1-1/9 bushel ultimately leading to weight. The lower cost of pine bark relative to perlite also increases the economic feasibility from a grower perspective. The cost of pine bark is approximately 5 times less than the cost of perlite and today's agricultural producer is being pressured to continually find ways to increase profitability (Cantliffe et al., 2003). Reducing input costs is one of the better mechanisms of increasing overall economic productivity in any business. Aside from the increased cost, perlite can also become a disposal issue from an environmental perspective as well as an economic issue if producers have to pay for removal. Utilizing pine bark is a better choice also from this perspective as it is organic material which a producer could potentially compost and will not be burdened with the environmental issues or expenses associated with removal.

The main effects of chlorination significantly affected harvest parameters similar to media (Table 3-16). Plants grown without chlorination had greater numbers of extra-large fruit, extra-large fruit weight, numbers of small fruit, small fruit weight, numbers of cull fruit, cull

fruit weight, numbers of marketable fruit, marketable fruit weight, numbers of fruit with blossom end rot, numbers of total fruit, and total fruit weight (Table 3-16). These data relate that certain species are sensitive to chlorination, as has been observed in other studies. In NFT production of tomatoes, root damage associated with chlorination occurred at low concentrations (3 ppm) and it was concluded that chlorination was not a good option for disease management (Ewart and Chrimes, 1980). It is important to consider this, as substrate and crop species are important factors in determining crop sensitivity. It was also reported that growth of tomato transplants in floatation irrigation utilizing a peat based substrate had less shoot fresh weight and more negative effects associated with chlorine as concentrations increased above 20 ppm (Saha et al., 2008). Both experiments utilized different substrates; however tomatoes were the species under investigation, but sensitivity in the NFT system was observed at lower concentrations relative to those grown in a floatation system.

The main effects of inoculation with *P. aphanidermatum* did not affect as many harvest parameters when compared to chlorination and media (Table 3-16). Cull fruits were greater in treatments that were inoculated and marketable fruit number was greater in treatments that were not inoculated (Table 3-16). Negative effects on crop growth associated with rootborne disease have been found in many other studies including the preceding greenhouse and growth chamber experiments. Yield of corn (*Zea mays* L.) produced under field conditions had reduction in yield associated with inoculation with *P. arrhenomanes* (Drechs.) Sidaris (Deep and Lipps, 1996). Plant mortality of peppers associated with hurricane flood events was greatest in plots where *Pythium* spp. were isolated from roots of infected plants (Saha et al., 2005). Infection of muskmelons with *P. aphanidermatum* lead to mortality in greenhouse pathogenicity studies and it has been linked with root and crown rot of field melon crops in Guatemala (de Cara et al.,

2008). It was also reported that 15% of all pesticides utilized in production of cucumbers in Dutch greenhouses is for management of *Pythium* spp. indicating that the problem exists in commercial production systems (Postma et al., 2000).

The two-way interactions between media x chlorine, chlorine x pythium, and media x pythium significantly affected some of the harvest parameters (Table 3-16). The numbers of large fruit, large fruit weight, and number of fruit with sun scald were the parameters affected by the interaction between media and chlorination (Table 3-17). Plants grown in perlite without chlorination had the greatest number of and weight of large fruit (Table 3-17). However plants grown in both pine bark and perlite that were chlorinated had a greater number of fruit with sunscald as compared to the nonchlorinated treatments. Plants grown in perlite which were chlorinated had greater number of fruit with sunscald relative to all treatments. This may be indicative of the reduced shoot growth observed in plants grown in perlite (Table 3-12). Plants with less foliage will allow for more penetration of solar radiation leading to sun scald (Cerkauskas, 2008). Reduction in shoot growth as a result of chlorination allows less foliage to develop. With the reduced foliage there is greater light penetration into the canopy which in turn damages the fruit. In production of field peppers increased irrigation led to greater shoot development which reduced incidence of sunscald (Madramootoo and Rigby, 1991). Relative to this experiment the same phenomenon occurred in that shoot growth was reduced by chlorination which led to increased incidence of sunscald. The importance of light penetration to fruit development has also been observed in other studies. This concept can be observed between the selections of trellising method for greenhouse bell peppers depending on location. Specifically producers in northern latitudes such as Canada and Holland utilize a “V” trellis system in which plants are pruned to two main stems to allow for adequate light penetration to allow for fruit

development. In mild winter climate producing regions such as in Florida, solar radiation is not limiting as in northern latitudes. In these latitudes a "Spanish" trellis system is utilized as in these experiments. This is a system in which pruning is minimal and it can lead to reduction in labor cost (Jovicich et al., 2004). However in regions with high solar radiation, it could be inferred that the extra canopy within the "Spanish" system provides some protection from sunscald.

The interaction effects between chlorine and pythium affected weight of fruit with blossom end rot, number of fruit with sun scald, and weight of fruit with sun scald (Table 3-16). Treatments that were inoculated but not chlorinated had the greatest of blossom end rot fruit weight (Table 3-17). The chlorinated and inoculated treatment had no blossom end rot fruit (Table 3-17). This could be attributed to the fact that chlorinated treatments had less total weight of fruit (Table 3-16). Sun scald weight and number was greatest in the treatments with both inoculation and chlorination (Table 3-17). This could also be attributed to the fact that chlorinated treatments with less shoot fresh weight provided less canopy protection for fruit (Table 3-12). The two-way interaction between media and pythium affected the number and weight of large fruit (Table 3-16). Treatments with plants grown in perlite and uninoculated had the largest number and weight of large fruit. As previously mentioned, one harvest parameter is not sufficient to determine which media performs better. From a practical view, the total marketable fruit weight is more important than the individual sizes. Based on the results of this experiment plants grown in pine bark had greater total marketable weight relative plants grown in perlite and would be a better choice when utilizing chlorination for management of *P. aphanidermatum*.

3.3.5 Impacts of Chlorination rate and Media on *P. aphanidermatum* GNV-Spring 2008

The average temperature throughout the experiment was 31.3℃, with a maximum of 49.9°C and a minimum of 24.1°C (Figure 3-23). The average temperature of this experiment was

similar to the previous greenhouse experiments. However, the minimum temperature was much higher in this experiment. Overall temperatures were more consistent in this experiment relative to the others. The temperature was within the optimal range for disease development more consistently throughout this experiment as this facility allowed for increase management of heating resources. This could potentially lead to increased effect of inoculation with *P. aphanidermatum* relative to the other greenhouse experiments. The average light levels in the greenhouse were 372.2 µmols/m^2s and outside the average light levels were 946.45 µmols/m^2s (Figure 3-24). Although this was a glasshouse which should allow for greater light penetration relative to polyethylene, the condition of the roof (i.e. dirt and residue) led to a reduction (61) in light penetration which was similar to what was observed at the greenhouses the other experiments were conducted in. The main effect of media was significant with regards to many of the growth parameters collected. Consistently plants grown in the two types of pine bark media had greater stem diameter growth, shoot fresh weight, shoot dry weight, and root fresh weight relative to plants grown in sand (Table 3-18). Steamed pine bark had the greatest ($P \leq 0.05$) shoot fresh and dry weight (Table 3-18). Other researchers have reported that steaming pine bark increased the substrate's ability to suppress pathogens as compared to pine bark (Broadbent et al., 1971). Steaming allows the heat tolerant bacteria such as *Bacillus* species to proliferate. These bacteria are antagonistic to rootborne pathogens and can tolerate temperatures up to 60°C (Zinati, 2005).

Chlorination reduced height growth, stem diameter growth, shoot fresh weight, and shoot dry weight (Table 3-18). Height growth, stem diameter, and shoot dry weight were observed to have inversely proportional linear relationships with the chlorination rate. Shoot fresh weight was reduced in plants at either chlorination rate relative to those plants not chlorinated. Shoot

fresh weight was observed to have an inverse quadratic relationship with regards to rate of chlorination (Table 3-18). Both the inverse linear and inverse quadratic relationship exhibit that there are reductions in growth including plant height, stem diameter, shoot fresh weight, and shoot dry weight associated with the chlorination treatment. This has been observed through all the previous experiments conducted in the greenhouse and growth chamber. The importance of this is that consistently in greenhouse experiments there were reductions in growth parameters such as height and shoot fresh weight which could ultimately impact harvest of marketable fruits. In the GNV-spring 2008 experiment there was a reduction in plant height and shoot fresh weight in plants treated with chlorine. Additionally plants that were chlorinated had a reduction in marketable fruit weight relative to the nonchlorinated plants. This indicates chlorine sensitivity is one of the obstacles to overcome when trying to utilize chlorination for management of rootborne disease in soilless production systems.

The main effects of inoculation were significant for some of the same growth parameters as chlorination including stem diameter growth, shoot fresh weight, and shoot dry weight (Table 3-18). The response of all growth parameters was negatively associated with inoculation. Plants that were inoculated had reduced stem diameter growth, shoot fresh weight, and shoot dry weight relative to uninoculated plants. The treatments that were inoculated exhibited lower ($P \leq 0.05$) growth values (Table 3-18). This was consistent with previous findings from earlier experiments and was expected when working with a pathogenic isolate. The significance of this was that pathogens such as *P. aphanidermatum* can reduce growth of various crops including bell peppers and that the successful management of this pathogen with chlorination will be determined by several factors, including, species, production system, and type of substrate. The properties of media such as organic vs. inorganic can also dictate the sensitivity of the crop to free chlorine.

Chlorination of tomatoes at 3 ppm in an NFT system caused root damage, where it required concentrations greater than 20 ppm before reduction in growth was observed in tomato transplants grown in a floatation irrigation system (Ewart and Chrimes, 1980; Saha et al., 2008).

There were growth parameters that had significant two-way interactions with regards to media x chlorine, media x pythium, and chlorine x pythium (Table 3-18). Height growth and root ratings were significant for two-way interaction between media and pythium (Table 3-19). Of the inoculated treatments, the plants growing in sand exhibited the lowest height growth and plants growing in steamed pine bark exhibited the greatest height growth (Table 3-19). Pine bark has been observed to have suppressive properties with regards to some rootborne pathogens such as *Pythium* sp and *Phytophthora* sp. and has been adopted widely in the ornamental industry to replace methyl bromide for disease suppression (Hoitink et al., 1997). Beneficial microbes such as *Bacillus* spp. and *Enterobacter* spp. often recolonize pine bark after composting (Hoitink and Fahy, 1986). The two-way interaction between chlorination and inoculation affected the root fresh weight (Table 3-18). Nonchlorinated and uninoculated treatments exhibited the greatest root fresh weight (Table 3-19). All other treatment combinations had less root development as has been observed in these studies as well as others (Table 3-19) (Ewart and Chrimes, 1980). The two-way interaction between media and chlorination affected the root dry weight of plants (Table 3-18). Plants growing in sand chlorinated at 2 and 4 ppm both had the lowest ($P \leq 0.05$) root dry weight relative to other treatment combinations (Table 3-20). This indicated that there was a profound affect on crop growth of plants in sand as a result of chlorination. Sand, like water, has minimal buffering capacity for phytotoxicity as was reported in the use of chlorination for disease management in and NFT system (Ewart and Chrimes, 1980). This was similar to what was observed with regards to perlite as it is inorganic as well as sand. Pine bark is organic

which allows for buffering of some of the phytotoxic effects of chlorination. Similar results were observed with regards to the three-way interaction between media, chlorination, and inoculation (Table 3-21). The poor performance of sand as a substrate relative to either pine bark media utilized is potentially attributable to the limited aeration properties of sand.

3.4 Summary

Water as a natural resource and the quality and availability of it will continue to be an important issue to crop production in many locations throughout the world. Considering agriculture accounts for a large majority of fresh water usage It has become necessary to improve the water use efficiency to help conserve water as a natural resource with the large agricultural dependence tied to the increasing world population (Postel, 1992). The use of closed irrigation systems in which the effluent is collected and reused is one of the best means of conserving water, but the management level increases due to issues with pathogens which can thrive in soilless and hydroponic production systems (Jenkins and Averre, 1983; Bates and Stanghellini, 1984; Favrin et al., 1988; Moulin et al., 1994). Through these experiments the effects of media and chlorination rate were evaluated for the mitigation of disease development of *P. aphanidermatum*. In the spring 2007 and summer 2007 experiments, chlorination rates of 2 and 4 ppm were evaluated for their effect on crop growth as well as management of *P. aphanidermatum*. In both seasons, as chlorination rate increased there was a linear decrease in shoot fresh weight. In the spring 2007 experiment, there was also a linear decrease in shoot and root dry weight as the chlorination rate increased. The GNV-spring 2008 experiment had similar results in that shoot fresh weight decreased with presence of chlorination as it was an inverse quadratic relationship. In the fall 2007 and spring 2008, plants grown in chlorinated treatments had less shoot fresh weight relative to those that were not chlorinated. Additionally in the fall 2007 experiment, chlorination led to reduced stem diameter growth and root dry weight. In the

spring 2008 experiment plant height and shoot dry weight was reduced in plants grown in chlorinated plots.

The data from all five greenhouse experiments indicated that 'Legionnaire' bell peppers grown in soilless culture are sensitive to chlorination rates as low as 2 ppm which can lead to reductions in crop growth such as height, stem diameter, shoot fresh, and root fresh weights. In the spring 2007 experiment, as chlorination rate increased from 0-4 ppm, the number of marketable fruit decreased linearly. In the spring 2008 experiment the use of chlorination led to reduced weight and number of marketable fruit as compared to plants that were not chlorinated. The reduction in yield associated with chlorination indicates phytotoxicity as a concern when trying to manage *P. aphanidermatum* or other rootborne pathogens in closed soilless production systems. Consistently results from these greenhouse experiments related negative associations with crop growth and presence of a rootborne disease. In the spring 2007 and summer 2007 experiments there was a reduction in height, shoot fresh weight and shoot dry weight caused by infection of *P. aphanidermatum*.

In the fall 2007 and spring 2008 experiments inoculation also led to reduced height growth, root fresh weight. Although inoculation reduced various growth parameters in the experiments, there was no reduction in marketable fruit weight or number as a consequence of infection with *P. aphanidermatum*. However, as mentioned previously, there could have been potential to see greater effect from the pathogen had the plants been harvested multiple times as in commercial greenhouse production. The experiments conducted in fall 2007, spring 2008, and GNV-spring 2008 were different from the previous two experiments to evaluate the effect of media on phytotoxicity associated with chlorination and the effect of media on pathogen suppression. In the fall 2007, spring 2008, and GNV-spring 2008 experiments, use of pine bark

media led to greater shoot fresh weights relative to plants grown in sand or perlite. Plants grown in sand had reduced growth, likely a result of its minimal aeration properties. Plants grown in perlite and treated with chlorine had the lowest marketable fruit weight relative to plants grown in perlite without chlorination as well as plants grown in pine bark with or without chlorination in the fall 2007 experiment. Additionally in the spring 2008 experiment plants grown in pine bark had greater extra-large fruit weight and number as well as greater marketable fruit weight and number.

Based on these findings, when utilizing a closed-irrigation system in soilless culture, an organic substrate such as pine bark is better at buffering negative effects associated with chlorination and rootborne pathogens relative to an inorganic substrate such as sand or perlite. However, chlorination did cause some reduction in growth of bell peppers in pine bark indicating phytotoxicity as a concern when trying to manage rootborne diseases, but the problem could be addressed by allowing a holding period for the free chlorine to dissipate prior to crop application. Further work will be necessary to develop protocol and more understanding of the complex interactions for management of rootborne pathogens in nutrient solution.

Table 3-1. Chlorination and *Pythium aphanidermatum* (Edson) Fitzp. effects on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2007.

Chlorine (C) (ppm)	Height (cm)	Stem diameter (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root rating[z]
0	45.4	10.5	565	81.8	251	28.1	0.38
2	41.7	10.7	522	76.8	199	27.2	0.08
4	40.8	9.6	464	70.1	163	20.2	0.17
	L[y]	NS	L	L	L	L	Q
Pythium (P)							
-	45.5	11.0	564	84.0	203	26.7	0
+	39.7	9.51	470	68.5	205	23.6	0.42
Significance	**	**	**	**	NS	NS	**
C x P	NS	*	NS	NS	NS	NS	*
CV	18.0	14.9	24.3	26.1	34.9	37.8	170.0

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]L, Q, significant linear and quadratic effect, respectively.
[NS,*,**]Nonsignificant (NS) and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-2. Interaction effects of chlorination and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2007.

Chlorine (ppm)	Pythium	Stem diameter (mm)	Root rating[z]
0	-	10.94	0.0
0	+	9.99	0.75
2	-	11.06	0.0
2	+	10.38	0.17
4	-	11.01	0.0
4	+	8.17	0.33
LSD[y]		0.88	0.20

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-3. Main effects of chlorination and *Pythium aphanidermatum* (Edson) Fitzp. on harvest of greenhouse-grown *Capsicum annuum* L. Spring 2007.

Chlorine (C)(ppm)	XL[z] (no.)[y]	XL (g/m^2)	L (no.)	L (g/m^2)	M (no.)	M (g/m^2)	S (no.)	S (g/m^2)	Cull (no.)	Cull (g/m^2)	Mrkt (no.)	Mrkt (g/m^2)	BER (g/m^2)	BER (no.)	Total (g/m^2)	Total (no.)
0	4.3	750	4.8	565	5.2	410	2.5	125	10.3	770	16.7	1850	595	5.8	2620	27.0
2	5.4	990	4.4	530	4.8	405	1.4	70	7.7	650	15.9	1995	540	4.8	2645	23.6
4	3.9	694	3.4	440	2.0	185	1.0	55	6.3	560	10.3	1375	475	4.4	1935	16.6
	NS	NS	NS	NS	L[x]	NS	NS	NS	NS	NS	L	NS	NS	NS	Q	L
Pythium (P)																
-	4.4	800	4.0	495	4.1	355	1.7	90	8.7	720	14.2	1735	610	5.7	2460	22.9
+	4.6	823	4.4	525	3.8	315	1.6	80	7.5	600	14.4	1740	460	4.3	2340	21.9
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	36.7	38.3	33.1	29.5	61.1	60.5	84.0	82.8	55.9	60.2	27.4	27.3	73.2	77.7	12.4	14.2

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Number of fruit harvested per square meter.

[x]L, Q, significant linear and quadratic effect respectively.

[NS,*,**]Nonsignificant (NS) and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-4. Chlorination and *Pythium aphanidermatum* (Edson) Fitzp. effects on growth variables of greenhouse-grown *Capsicum annuum* L., Summer 2007.

Chlorine (C) (ppm)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root Rating[z]
0	52.9	10.5	665	88	153	18	0.5
2	50.3	10.7	590	81	145	15	0.5
4	46.1	10.1	504	73	150	16	0.6
	L[y]	NS	L	L	NS	NS	NS
Pythium (P)							
-	58.2	11.9	740	97	169	19	0
+	41.4	9.0	433	64	129	14	1.1
Significance	**	**	**	**	**	**	**
C	NS	NS	*	NS	NS	NS	NS
C x P	NS	NS	NS	NS	NS	NS	NS
CV	22.6	21.0	31.9	30.2	38.7	33.0	153.7

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]L, Q, significant linear and quadratic effect, respectively.

[NS,*,**]Nonsignificant (NS) and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-5 Main effects of chlorination and *Pythium aphanidermatum* (Edson) Fitzp. on harvest of greenhouse grown *Capsicum annuum* L., Summer 2007.

Chlorine (C) (ppm)	XL[z] (no.)[y]	XL (g/m^2)	L (no.)	L (g/m^2)	M (no.)	M (g/m^2)	S (no.)	S (g/m^2)	Cull (no.)	Cull (g/m^2)	Mrkt (no.)	Mrkt (g/m^2)	BER (g/m^2)	BER (no.)	Total (g/m^2)	Total (no.)
0	2.8	495	3.0	385	5.5	495	4.9	280	12.9	355	16.3	1595	130	2.2	1950	29.1
2	1.9	320	3.8	485	5.8	495	5.0	275	10.4	270	16.5	1505	35	0.8	1800	26.8
4	1.7	265	3.2	410	4.5	465	3.9	215	26.4	190	13.3	1510	40	0.9	1700	39.7
	NS	NS	NS	NS	NS	NS	NS	NS	NS	L[x]	NS	NS	L	L	NS	NS
Pythium (P)																
-	2.8	490	3.8	500	5.1	465	4.3	255	24.2	295	16.0	1670	70.0	1.2	1950.0	40.2
+	1.5	225	2.8	350	5.5	470	5.0	260	8.9	250	14.7	1405	65.0	1.4	1650.0	23.6
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV	66.9	73.4	54.5	56.9	25.7	27.4	45.3	45.1	174.2	45.3	18.3	23.7	93.1	56.1	19.5	89.2

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Number of fruit harvested per square meter.

[x]L, Q, significant linear and quadratic effect respectively.

[NS,*,**]Nonsignificant (NS) and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-6. Media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. effects on growth variables of greenhouse-grown *Capsicum annuum* L., Fall 2007.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root Rating[z]
Pine bark	56.0	8.2	788	98	86	9	0.1
Perlite	44.6	6.7	519	88	75	9	0.4
Significance	**	**	**	NS	**	NS	**
Chlorine (C) (ppm)							
0	57.2	8.1	777	98	89	10	0.3
2	43.1	6.9	530	88	72	9	0.2
Significance	**	**	**	NS	**	**	NS
Pythium (P)							
-	52.3	7.9	675	105	84	9	0
+	48.2	7.1	633	80	76	9	0.5
Significance	**	**	NS	NS	*	NS	**
M x C	**	NS	NS	NS	*	NS	NS
M x P	NS	*	NS	NS	NS	NS	**
C x P	NS	NS	NS	NS	NS	NS	NS
M x C x P	NS	NS	NS	NS	NS	NS	NS
CV	14.5	12.5	16.1	85.9	20.4	17.6	137.7

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-7. Interaction effects of chlorination, media, and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of greenhouse-grown *Capsicum annuum* L., Fall 2007.

Media	Chlorine (ppm)	Height (cm)	Root fresh weight (g)
Pine bark	0	61.04	90.59
Pine bark	2	50.85	81.11
Perlite	0	53.88	86.50
Perlite	2	35.33	62.64
	LSD[z]	2.95	6.63
Media	Pythium	Stem diameter growth (cm)	Root rating[y]
Pine bark	-	8.37	0
Pine bark	+	8.05	0.13
Perlite	-	7.33	0
Perlite	+	6.13	0.83
	LSD	0.38	0.13

[z]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

[y]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

Table 3-8. Media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. effects on harvest of greenhouse-grown *Capsicum annuum* L., Fall 2007.

Media (M)	XL[z] (no.)[y]	XL (g/m^2)	L (no.)	L (g/m^2)	M (no.)	M (g/m^2)	S (no.)	S (g/m^2)	Cull (no.)	Cull (g/m^2)	Mrkt (no.)	Mrkt (g/m^2)	BER (no.)	BER (g/m^2)	Total (no.)	Total (g/m^2)
Pine bark	6.6	1365	4.1	695	4.2	485	0.7	55	2.3	150	15.6	2595	1.0	115.0	17.9	2745
Perlite	4.8	945	5.3	840	5.6	675	1.8	145	3.5	205	17.5	2610	1.2	135.0	21.0	2810
Significance	**	**	NS	NS	*	*	**	**	*	NS	*	NS	NS	NS	**	NS
Chlorine (C) (ppm)																
0	6.5	1330	5.2	870	4.5	560	0.8	60	3.2	240	16.9	2815	1.5	190.0	20.1	3050
2	4.9	980	4.2	665	5.3	605	1.8	140	2.6	120	16.2	2390	0.8	65.0	18.8	2505
Significance	*	**	NS	NS	NS	NS	**	*	NS	*	NS	**	NS	*	NS	**
Pythium (P)																
-	6.1	1235	4.4	720	4.7	540	0.9	75	2.6	160	16.0	2570	1.2	130.0	18.7	2735
+	5.3	1075	5.0	815	5.1	620	1.6	125	3.2	195	17.0	2630	1.0	125.0	20.3	2825
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M x C	**	**	NS	NS	NS	NS	NS	NS	NS	NS	*	**	NS	NS	NS	**
M x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	*	NS	NS
C x P	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
M x C x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS
CV	23.0	20.1	39.4	39.3	26.9	32.2	64.4	70.7	42.0	58.3	10.5	9.2	80.2	84.1	9.9	7.8

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Number of fruit harvested per square meter.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-9. Interaction effects of media and chlorination on harvest of greenhouse grown *Capsicum annuum* L., Fall 2007.

Media	Chlorine (ppm)	XL[z] (no./m^2)	XL weight (g/m^2)	Mrkt (no./m^2)	Mrkt weight (g/m^2)	Total weight (g/m^2)
Pine bark	0	6.4	1345	15.2	2615	2825
Pine bark	2	6.8	1380	16.0	2575	2665
Perlite	0	6.5	1215	18.7	3015	3275
Perlite	2	3.1	575	16.3	2200	2345
LSD[y]		1.1	205	1.5	210	190

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-10. Interaction effects of media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. on harvest of greenhouse-grown *Capsicum annuum* L., Fall 2007.

Chlorine (ppm)	Pythium	XL[z] (no./m^2)	XL weight (g/m^2)
0	-	7.5	1545.0
0	+	5.4	1120.0
2	-	4.7	925.0
2	+	5.2	1030.0
LSD[y]		1.1	205.0
Media	Pythium	Cull weight (g/m^2)	BER weight (g/m^2)
Pine bark	-	70.0	60.0
Pine bark	+	230.0	170.0
Perlite	-	250.0	195.0
Perlite	+	155.0	75.0
LSD		85.0	85.0

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-11. Interaction effects of media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp.on cull weight of greenhouse grown *Capsicum annuum* L., Fall 2007.

Media	Chlorine (ppm)	Pythium	Cull[z] weight (kg)
Pine bark	0	-	85
Pine bark	0	+	345
Pine bark	2	-	60
Pine bark	2	+	120
Perlite	0	-	375
Perlite	0	+	150
Perlite	2	-	125
Perlite	2	+	160
LSD[y]			125

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-12. Media, chlorination, and *Pythium aphanidermatum*(Edson) Fitzp. effects on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Root Rating[z]	SPAD[y]
Pine bark	45.5	9.3	767	119	132	10	0.1	68
Perlite	29.1	6.4	394	67	83	10	0.4	65
Significance	**	**	**	**	NS	NS	**	**
Chlorine (C) (ppm)								
0	49.0	9.1	754	112	135	10.96	0.25	76
2	25.5	6.6	406	73	80	9.08	0.23	57
Significance	**	**	**	**	NS	**	NS	**
Pythium (P)								
-	39.1	8.2	587	93	128	10.23	0	68
+	35.5	7.6	573	92	87	9.82	0.48	65
Significance	*	*	NS	NS	NS	NS	**	**
M x C	NS	**	NS	NS	NS	NS	NS	**
M x P	**	NS	NS	NS	NS	NS	**	**
C x P	NS	NS	NS	NS	NS	*	NS	**
M x C x P	NS	NS	NS	NS	NS	NS	NS	**
CV	23.1	15.0	23.8	24.9	142.0	24.9	137.7	5.7

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Reading of chlorophyll in units of SPAD with higher values indicating higher crop nitrogen status relative to lower SPAD values.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-13. Interaction effects of media and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Pythium	Height (cm)	Root rating[z]
Pine bark	-	44.8	0
Pine bark	+	46.1	0.1
Perlite	-	33.4	0
Perlite	+	24.8	0.8
LSD[y]		3.5	0.1

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
[y]Means in columns separated by Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-14. Interaction effects of media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Chlorine (ppm)	Stem diameter growth (mm)
Pine bark	0	10.2
Pine bark	2	8.4
Perlite	0	8.0
Perlite	2	4.8
LSD[z]		0.5
Chlorine (ppm)	Pythium	Root dry weight (g)
0	-	10
0	+	12
2	-	9
2	+	9
LSD		1

[z] Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-15. Interaction effects of media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. on SPAD readings of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Free chlorine (ppm)	Pythium	SPAD[z]
Pine bark	0	-	87
Pine bark	0	+	72
Pine bark	2	-	56
Pine bark	2	+	59
Perlite	0	-	74
Perlite	0	+	72
Perlite	2	-	56
Perlite	2	+	58
	LSD[y]		2

[z]Reading of chlorophyll in units of SPAD with higher values indicating higher crop nitrogen status relative to lower SPAD values.
[y] Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-16. Media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. effects on harvest of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media (M)	XL[z] (no.)[y]	XL (g/m^2)	L (no.)	L (g/m^2)	M (no.)	M (g/m^2)	S (no.)	S (g/m^2)	Cull (no.)	Cull (g/m^2)	Mrkt (no.)	Mrkt (g/m^2)	BER (no.)	BER (g/m^2)	Sun Scald (no.)	Sun Scald (g/m^2)	Total (no.)	Total (g/m^2)
Pine bark	12.1	2690	3.0	470	2.3	245	2.2	135	8.7	660	19.5	3545	3.5	410	0.5	80	28.3	4200
Perlite	6.3	1335	4.2	655	3.6	360	2.3	150	8.4	470	16.4	2500	1.7	165	1.6	140	24.8	2950
Sig.	**	**	**	**	NS	NS	NS	NS	NS	NS	**	**	*	*	*	NS	*	**
Chlorine (C) (ppm)																		
0	13.5	2990	3.8	580	3.7	350	3.5	215	12.0	735	24.3	4130	4.5	485	0.1	5	36.3	4865
2	4.9	1035	3.4	550	2.3	255	1.0	70	5.2	400	11.6	1910	0.7	95	2.0	215	16.8	2305
Sig.	**	**	NS	NS	NS	NS	**	**	**	*	**	**	**	**	**	**	**	**
Pythium (P)																		
-	9.9	2120	3.9	585	3.9	400	2.1	135	6.5	450	19.8	3240	2.5	270	0.7	65	26.4	3685
+	8.5	1905	3.3	540	2.0	210	2.4	150	10.6	685	16.1	2800	2.7	305	1.4	160	26.7	3485
Sig.	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	**	NS	NS	NS	NS	NS	NS	NS
M x C	NS	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS
M x P	NS	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	*	NS	NS
M x C x P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Sun Scald = unmarketable fruits with sun damage due to excessive light exposure, Total = all fruit including marketable and culls.

[y]Number of fruit harvested per square meter.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-17 Two-way interaction effects of media, chlorination, and *Pythium aphanidermatum* on harvest of greenhouse grown *Capsicum annuum*.

Media	Chlorine (ppm)	XL[z] (no./m^2)	XL weight (g/m^2)	Sun Scald (no./m^2)
Pine bark	0	2.3	330	0.0
Pine bark	2	3.7	610	1.0
Perlite	0	5.3	825	0.2
Perlite	2	3.1	485	3.0
LSD[y]		0.8	130	0.8
Chlorine (ppm)	Pythium	BER weight (g/m^2)	Sun Scald (no./m^2)	Sun Scald weight (g/m^2)
0	-	355	0.2	15
0	+	610	0.0	0
2	-	185	1.2	115
2	+	0	2.8	315
LSD		220	0.8	100
Media	Pythium	L (no./m^2)	L weight (g/m^2)	
Pine bark	-	2.8	420	
Pine bark	+	3.2	525	
Perlite	-	5.0	755	
Perlite	+	3.3	555	
LSD		0.8	130	

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Cull = undersize, blemished, misshapen, and blossom end rot, Mrkt = marketable fruit including XL, L, M, and S sizes, BER = Blossom end rot fruits, Sun Scald = unmarketable fruits with sun damage due to excessive light exposure, Total = all fruit including marketable and culls.

[y]Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Planting density was 1.1 plants/m^2, which is lower than in a commercial setting (3 plants/m^2), resulting in overall lower yields per square meter compared to a commercial facility.

Table 3-18. Media, chlorination, and *Pythium aphanidermatum* (Edson) Fitzp. effects on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media (M)	Height (cm)	Stem diameter growth (mm)	Shoot fresh weight (g)	Shoot Dry weight (g)	Root fresh weight (g)	Root Dry weight (g)	Root Rating[z]
Pinebark	38.2	6.4	205	40	119	15	0.4
Steamed Pinebark	47.3	7.0	263	51	124	16	0
Sand	23.3	3.4	74	13	48	9	1.9
LSD[y]	6.3	0.9	41	8	19	4	0.4
Chlorine (C) (ppm)							
0	43.0	7.0	234	45	103	15	0.4
2	32.6	5.2	157	31	66	9	0.8
4	33.1	4.7	151	29	68	8	1.1
	L[x]	L	Q	L	Q	L	L
Pythium (P)							
-	70.8	6.1	203	39	89	13	0.5
+	31.7	5.2	159	31	69	9	1.1
Significance	**	*	**	*	*	*	**
M x C	NS	NS	NS	NS	NS	*	**
M x P	*	NS	NS	NS	NS	*	**
C x P	NS	NS	NS	NS	*	*	NS
M x C x P	NS	NS	NS	NS	NS	*	NS
CV	25.8	24.5	33.3	34.5	35.3	50.6	67.0

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]LSD: means in columns separated using Fisher's least significant difference test.

[x]L, Q, significant linear and quadratic effect respectively.

[NS,*,**]Nonsignificant and significant at $P \leq 0.05$ and $P \leq 0.01$, respectively.

Table 3-19. Interaction effects of chlorination, media, and *Pythium aphanidermatum* (Edson) Fitzp. on growth variables of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Pythium	Height (cm)	Root rating[z]
Pine Bark	-	41.6	0.2
Pine Bark	+	34.8	0.7
Sand	-	32.4	1.2
Sand	+	14.2	2.6
Steamed Pine Bark	-	48.3	0
Steamed Pine Bark	+	46.2	0
LSD[y]		6.3	3.7
Chlorine (ppm)	Pythium	Root fresh weight (g)	
0	-	128	
0	+	78	
2	-	72	
2	+	60	
4	-	68	
4	+	69	
LSD		19	

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-20. Interaction effects of chlorination and media on root dry weight of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Chlorine (ppm)	Root dry weight (g)
Pine Bark	0	18
Pine Bark	2	15
Pine Bark	4	14
Sand	0	17
Sand	2	6
Sand	4	3
Steamed Pine Bark	0	18
Steamed Pine Bark	2	14
Steamed Pine Bark	4	17
LSD[z]		5

[z]Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Table 3-21. Interaction effects of chlorination, media, and *Pythium aphanidermatum* (Edson) Fitzp. on root dry of greenhouse-grown *Capsicum annuum* L., Spring 2008.

Media	Chlorine (ppm)	Pythium	Root dry weight (g)
Pine Bark	0	-	19
Pine Bark	0	+	18
Pine Bark	2	-	18
Pine Bark	2	+	16
Pine Bark	4	-	17
Pine Bark	4	+	17
Sand	0	-	20
Sand	0	+	5
Sand	2	-	9
Sand	2	+	3
Sand	4	-	3
Sand	4	+	3
Steamed Pine Bark	0	-	183
Steamed Pine Bark	0	+	173
Steamed Pine Bark	2	-	163
Steamed Pine Bark	2	+	12
Steamed Pine Bark	4	-	15
Steamed Pine Bark	4	+	18
LSD[z]			7

[z]Means in columns separated using Fisher's least significant difference test, ($P \leq 0.05$).

Figure 3.1 Ten plant closed system soilless production research unit.

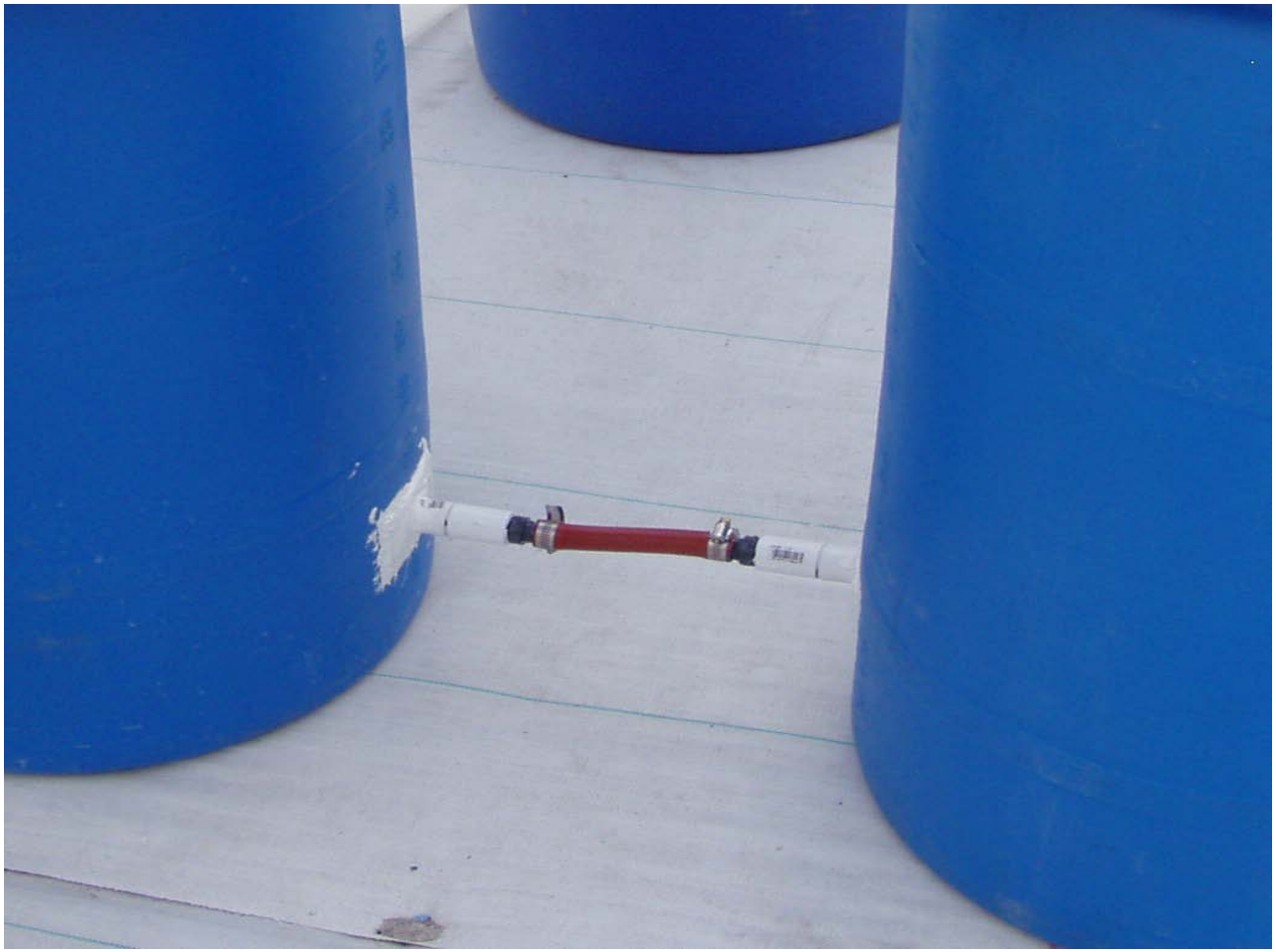

Figure 3-2. Hose barbs and hose connecting two 55 – gallon drum reservoirs for the closed soilless production units.

Figure 3-3. 55 – gallon drum for irrigation reservoirs as well as collection of effluent.

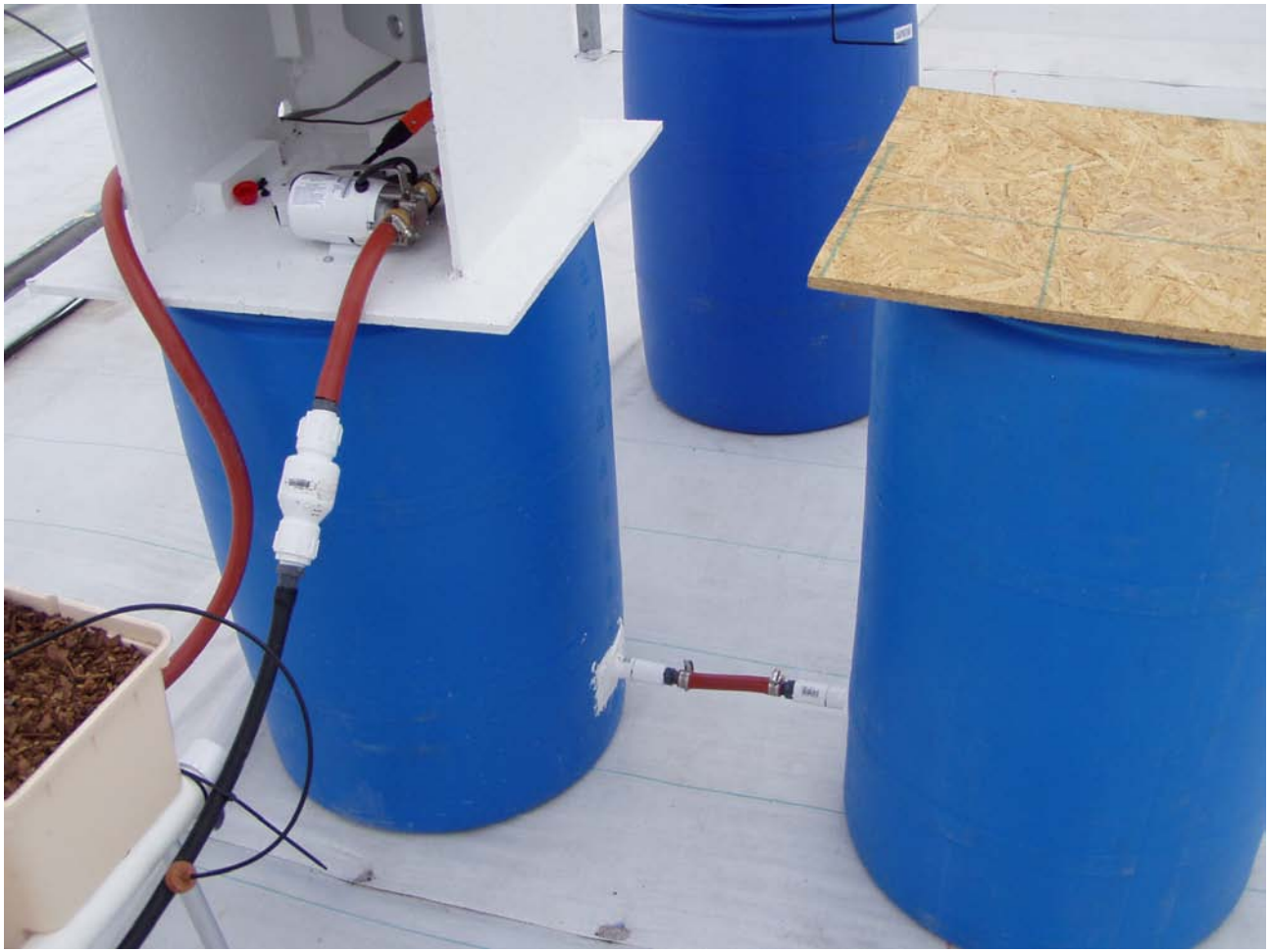

Figure 3-4. Box serving as a lid and housing for pump and the pump start relay.

Figure 3-5. Pony pump utilized for irrigation in the closed soilless production units.

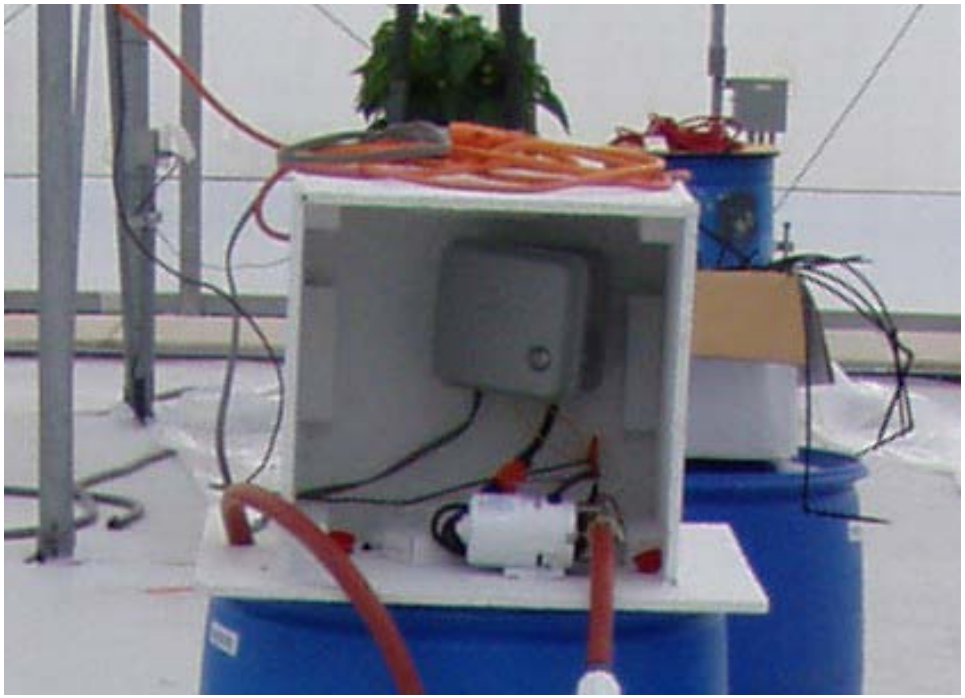

Figure 3-6. Pump start relay to allow for pony pump to be controlled via computer for irrigation event initiation.

Figure 3-7. Back flow preventer to allow for proper pump operation.

Figure 3-8. Pressure gauge, ball valve, and return line to allow for proper operation of drip emitters.

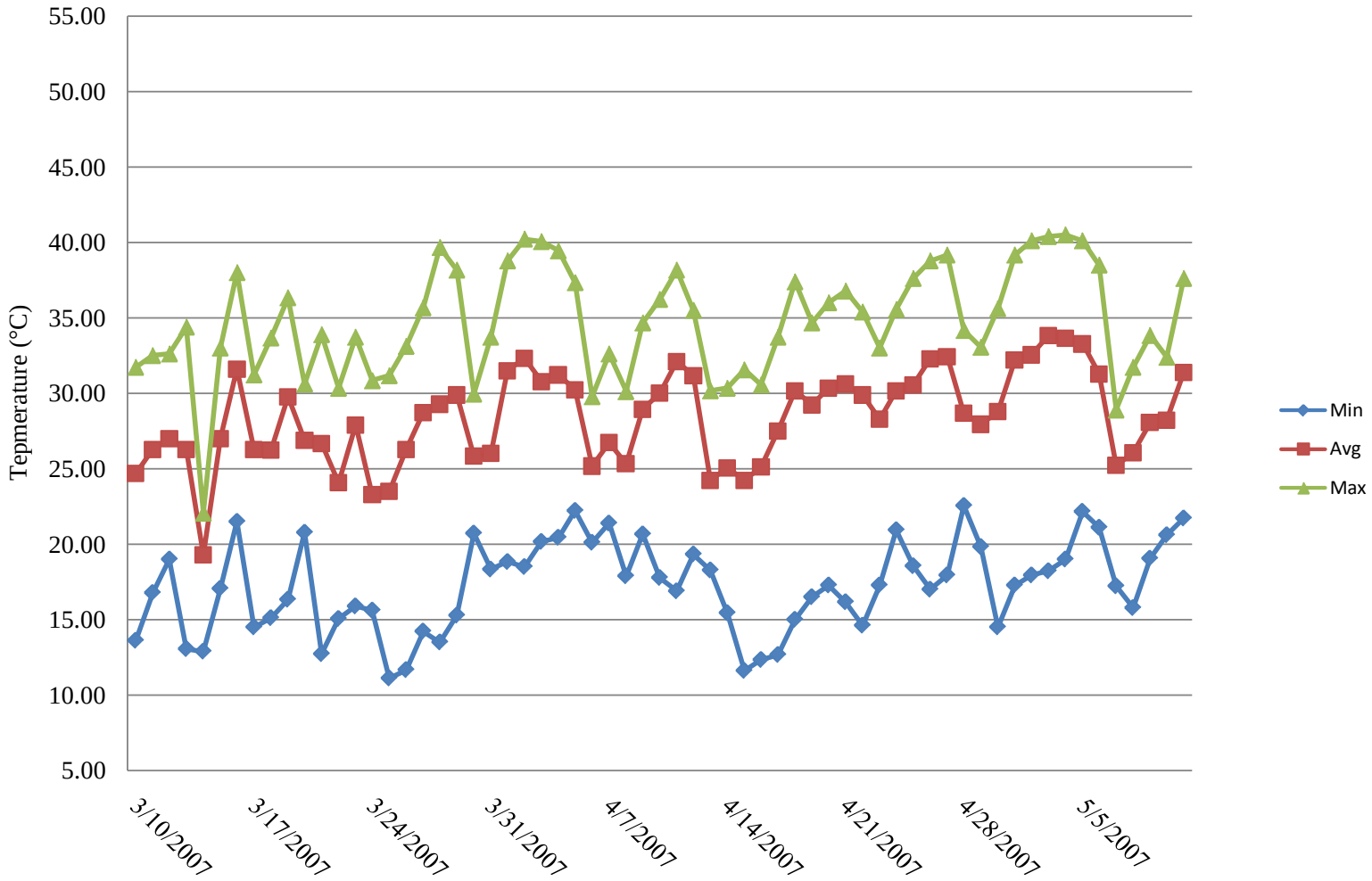

Figure 3.9 Greenhouse temperatures from an experiment Spring 2007, March 10 – May 5.

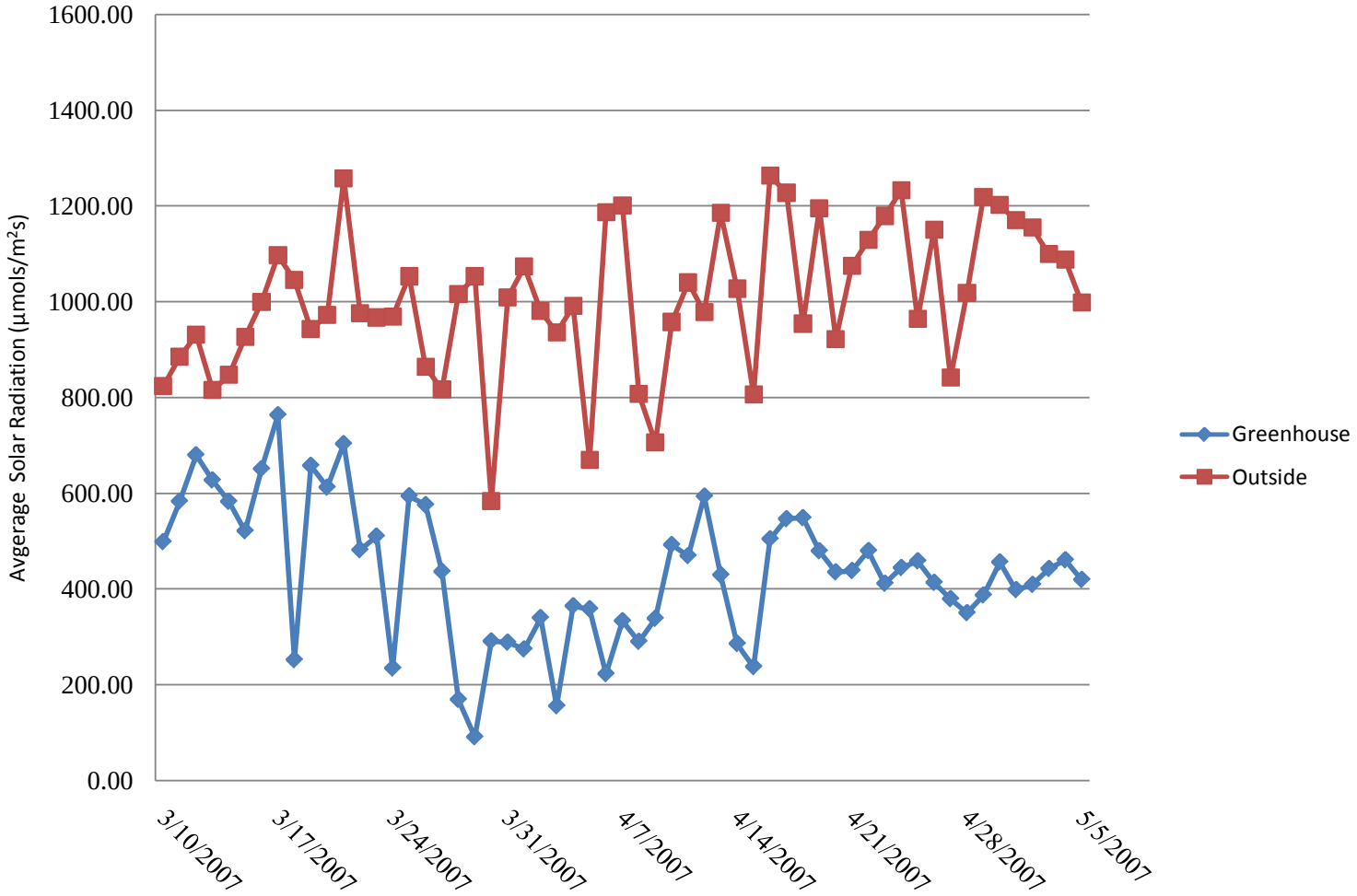

Figure 3.10 Solar radiation from greenhouse experiment in Spring 2007, March 10 – May 5.

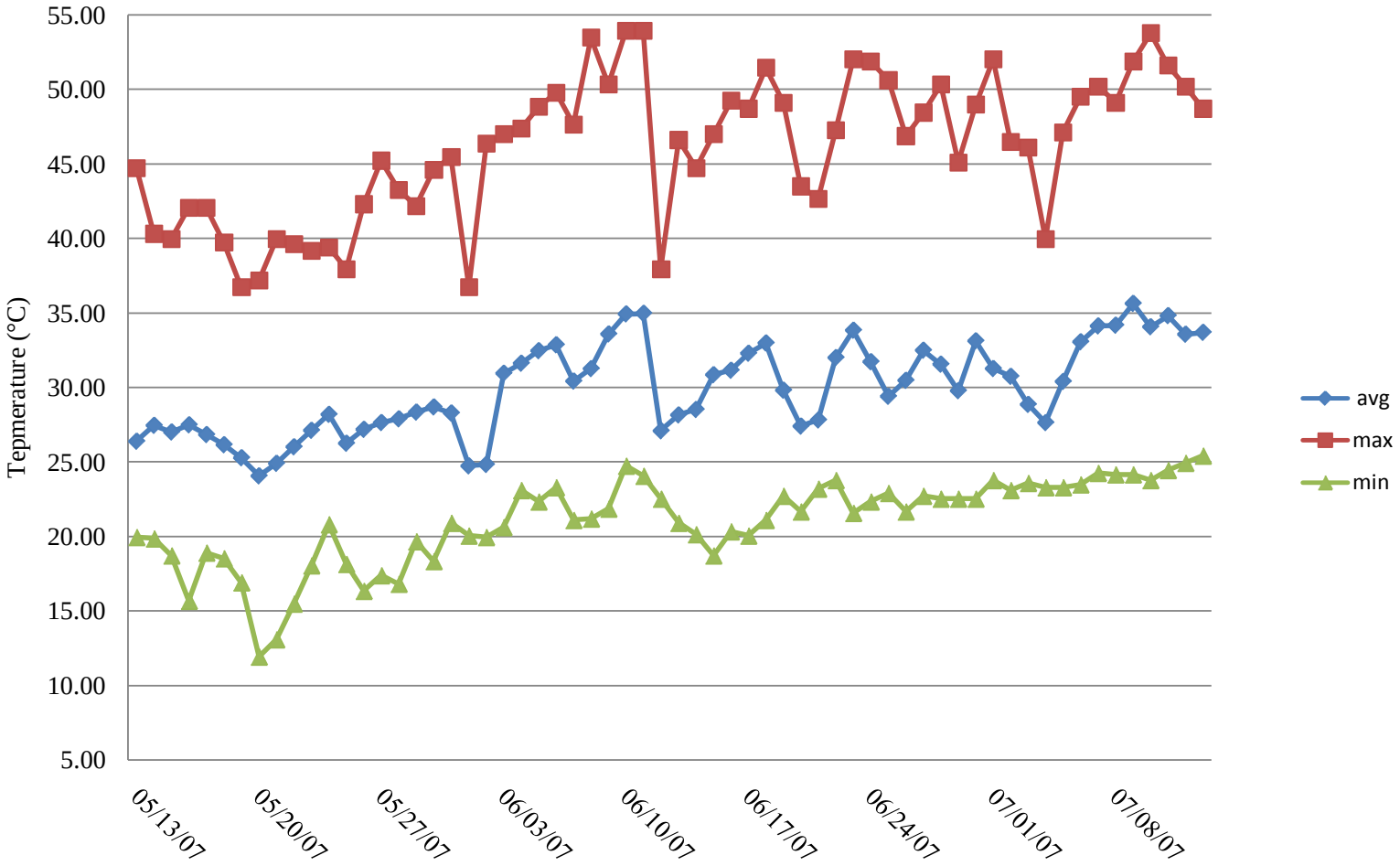

Figure 3.11 Temperatures from a greenhouse experiment Summer 2007, May 13 – July 13.

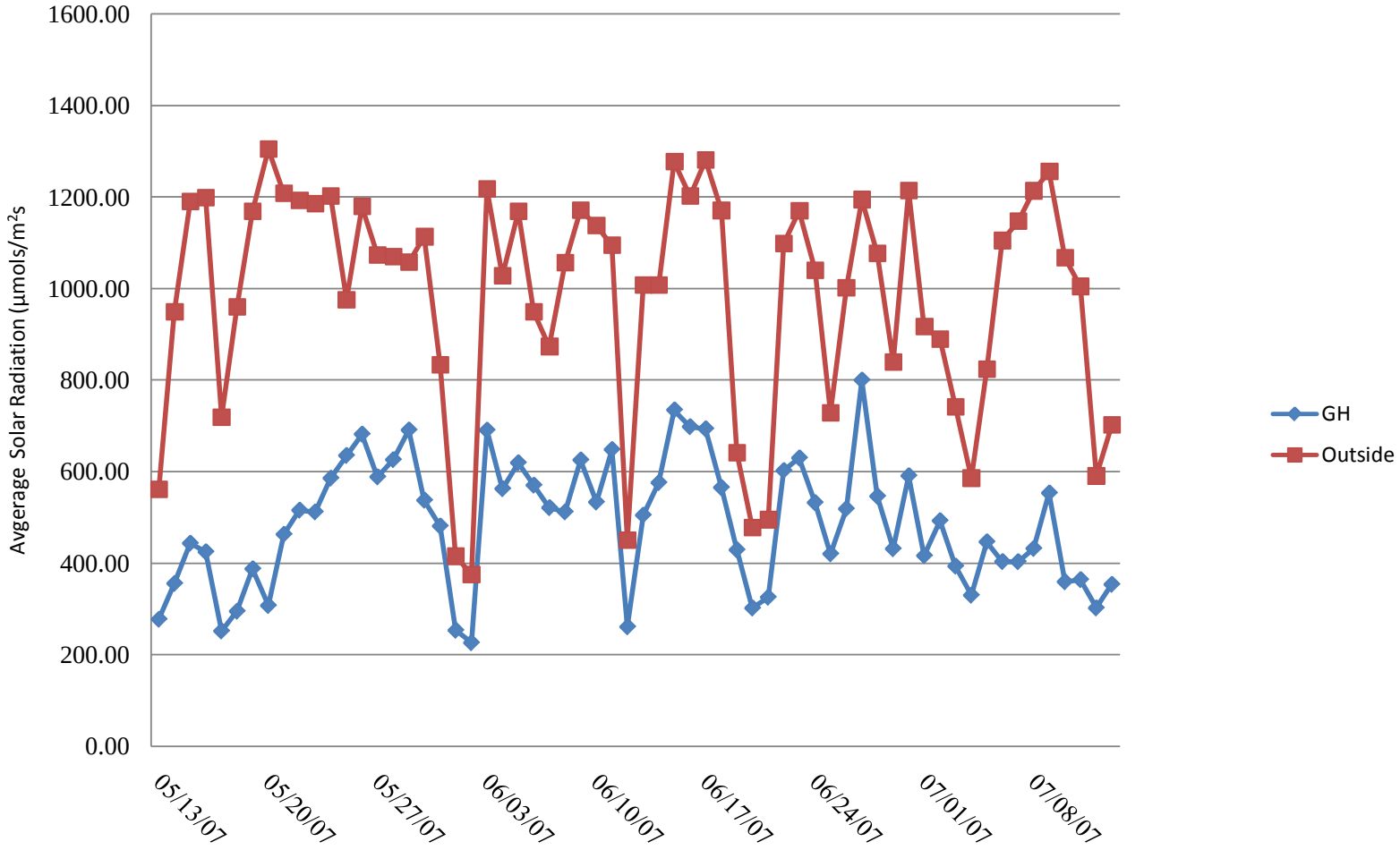

Figure 3.12 Solar radiation for a greenhouse experiment Summer 2007, May 13 – July 13.

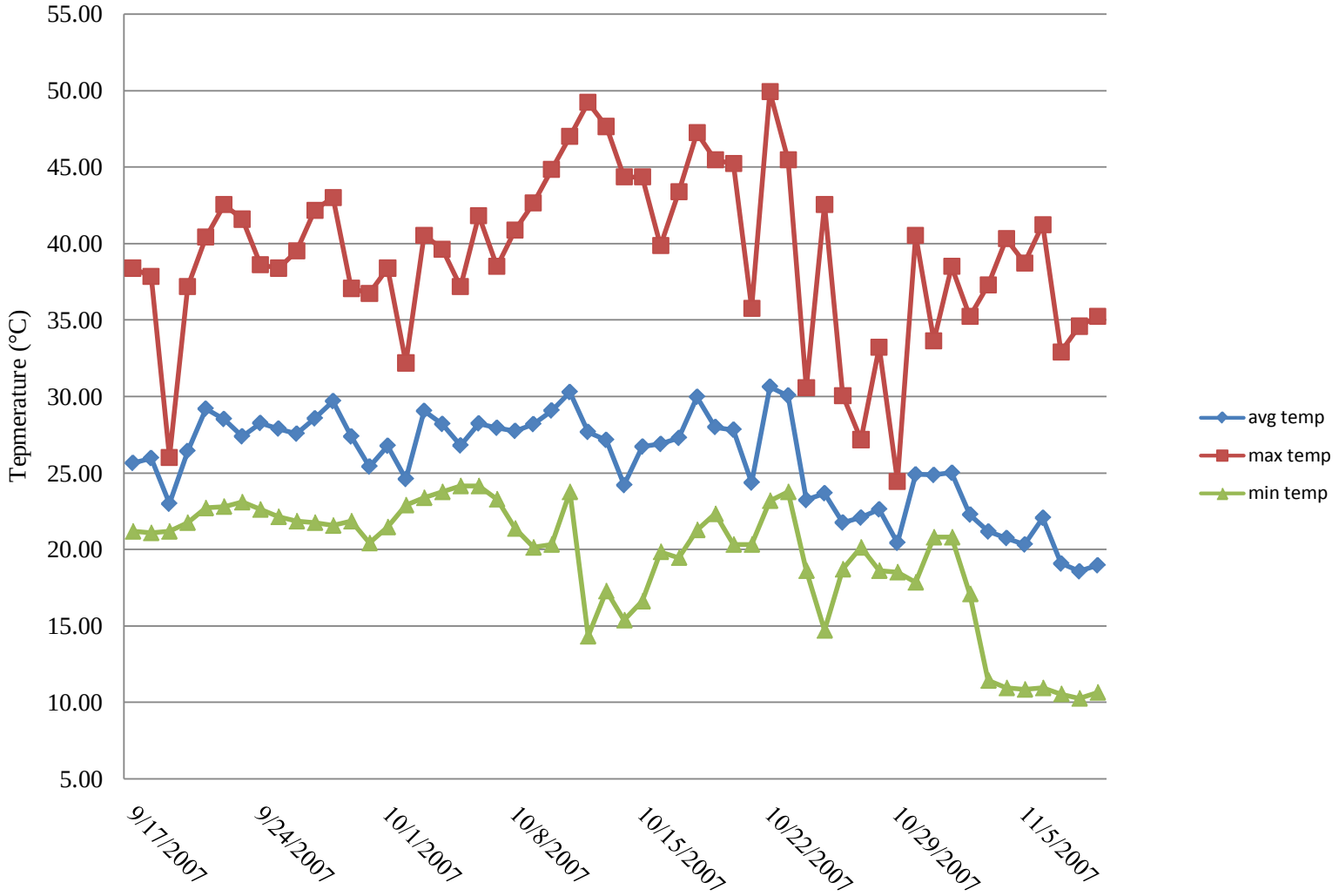

Figure 3.13 Temperatures from a greenhouse experiment Fall 2007, Semptember 17 – November 9.

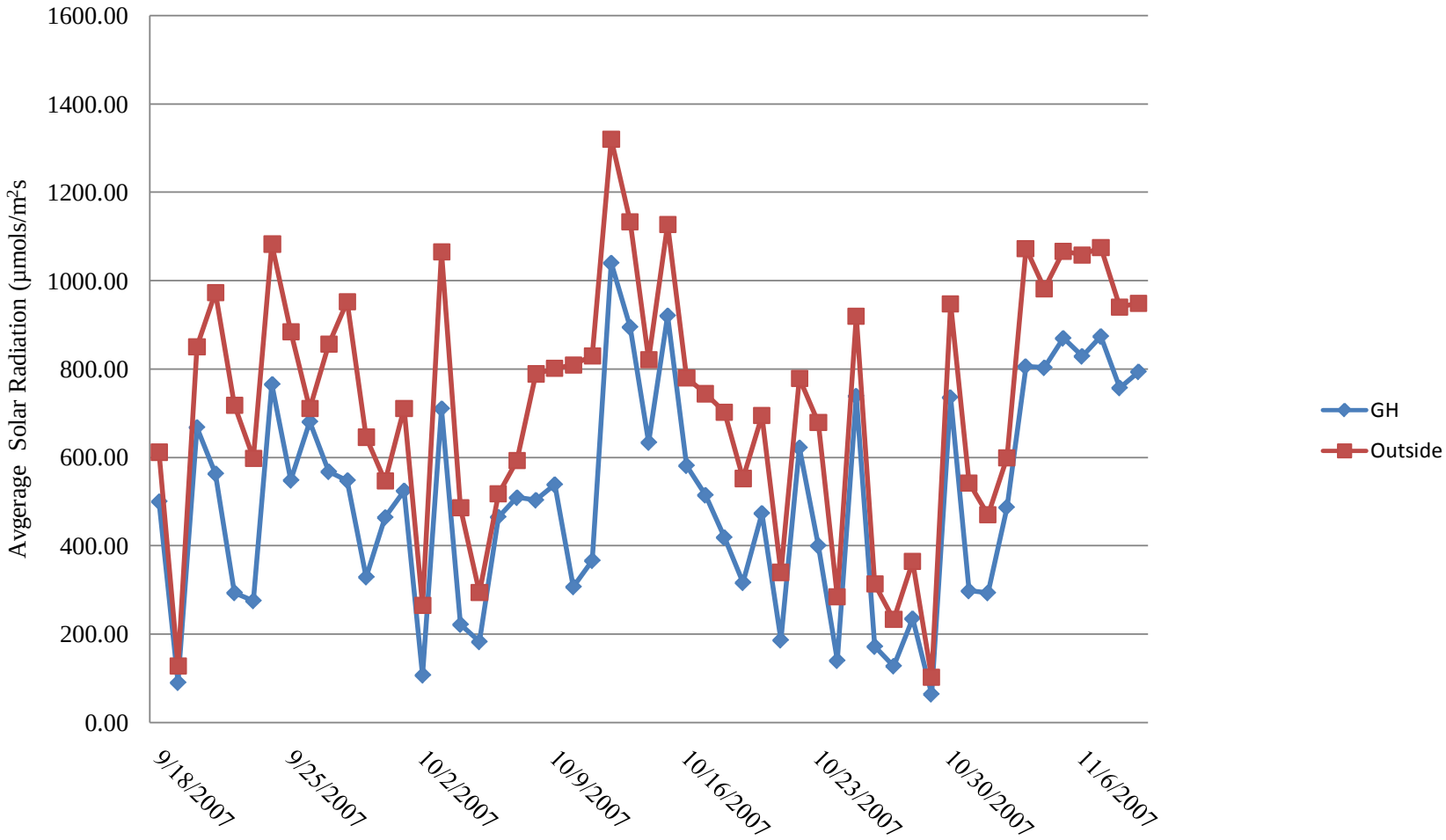

Figure 3.14 Solar radiation froma a greenhouse experiment Fall 2007, Semptember 17 – November 9.

Figure 3-15. Plants growing in control plots in two different substrates. (Pine bark on left, Perlite on right)

Figure 3-16. Plants growing in chlorinated plots in two different substrates. (Pine bark on left, Perlite on right)

Figure 3-17. Plants growing in plots inoculated with *P. aphanidermatum* in two different substrates. (Pine bark on left, Perlite on right)

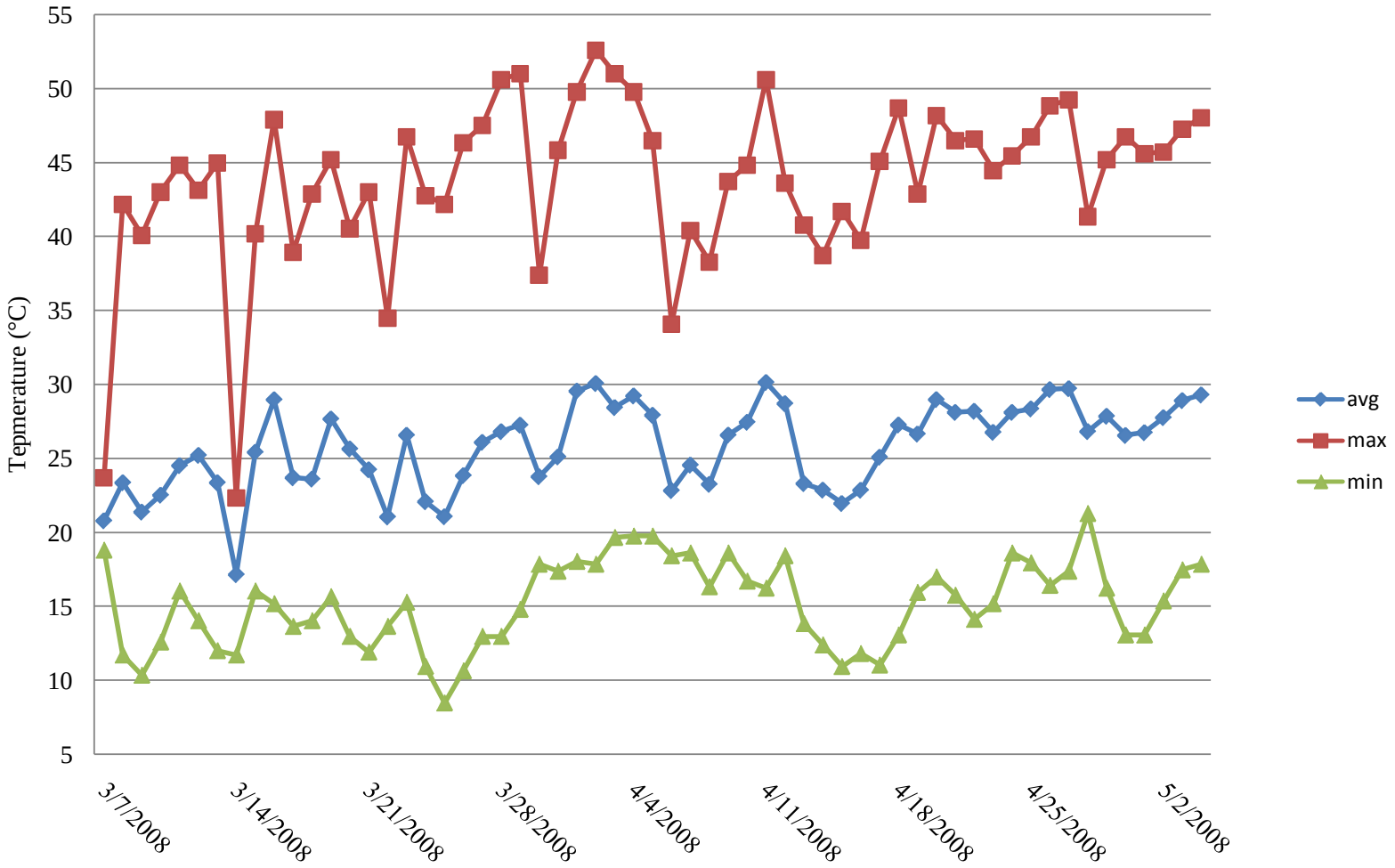

Figure 3.18 Temperatures from a greenhouse experiment Spring 2008, March 7 – May 4.

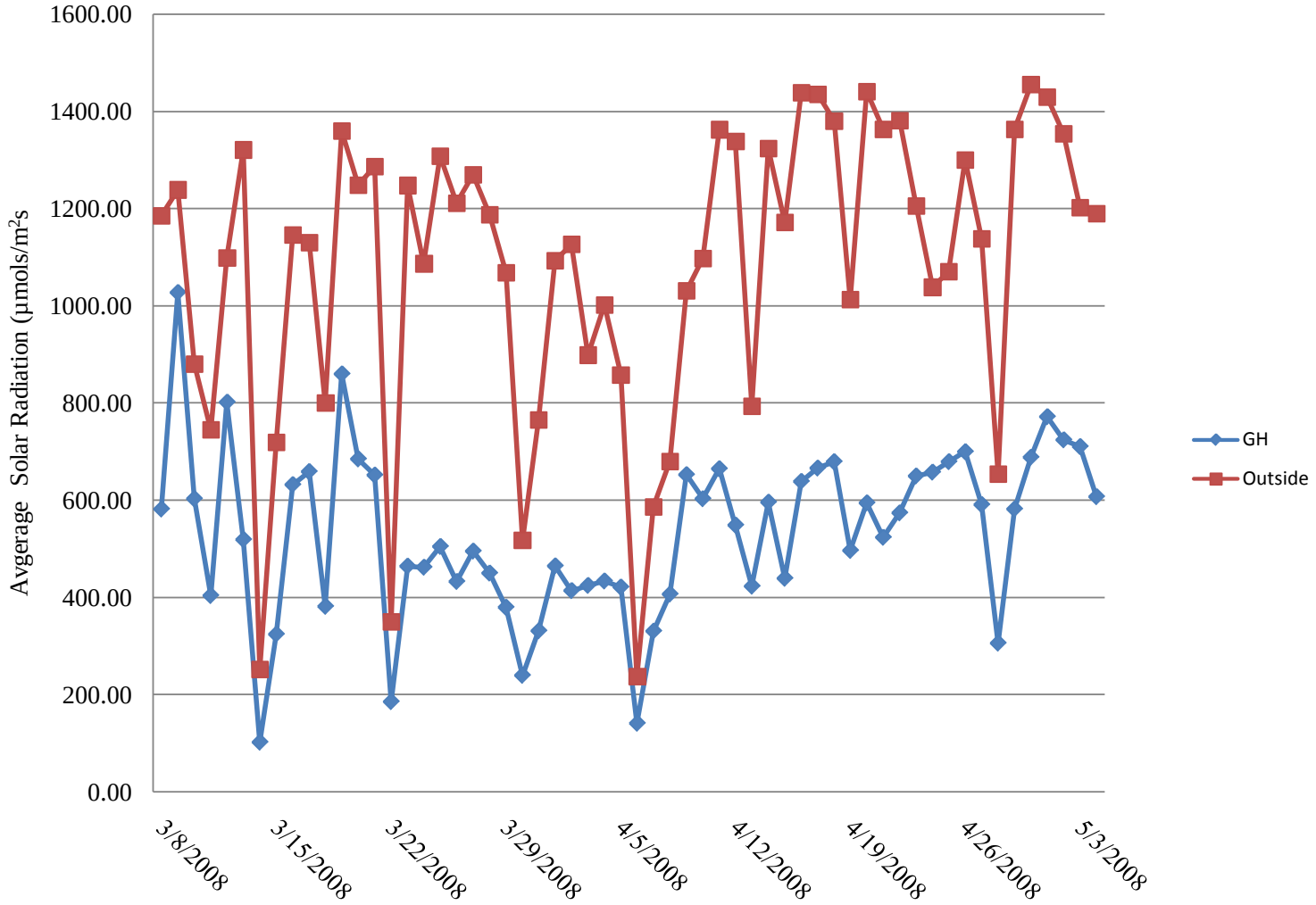

Figure 3.19 Solar radiation from a greenhouse experiment Spring 2008, March 7 – May 4.

Figure 3-20. Plants growing in control plots in two different substrates. (Pine bark on left, Perlite on right)

Figure 3-21. Plants growing in chlorinated plots in two different substrates. (Pine bark on left, Perlite on right)

Figure 3-22. Plants growing in plots inoculated with P. aphanidermatum in two different substrates. (Pine bark on left, Perlite on right)

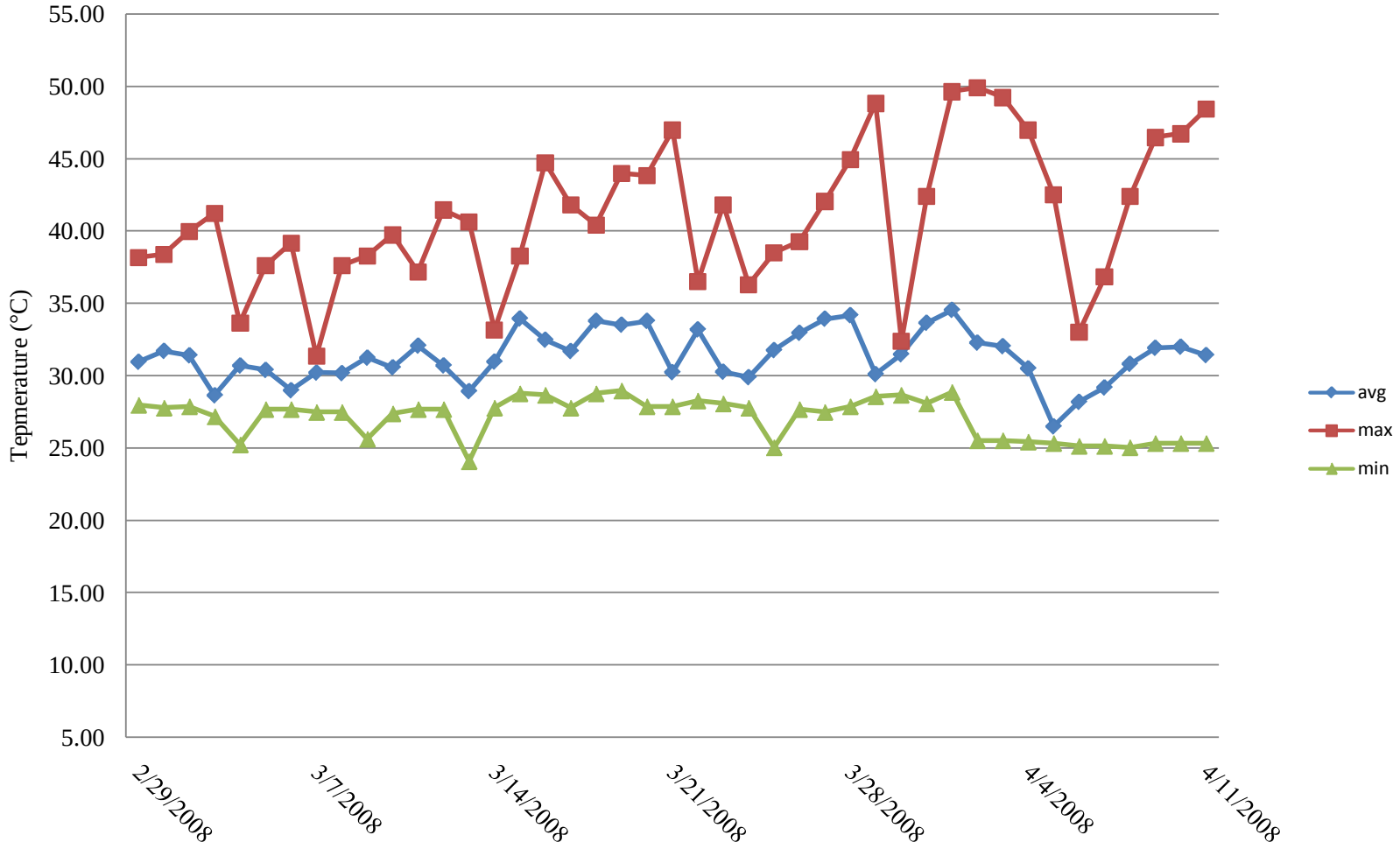

Figure 3.23 Temperatures from glasshouse experiment in spring 2008, February 29 – April 11.

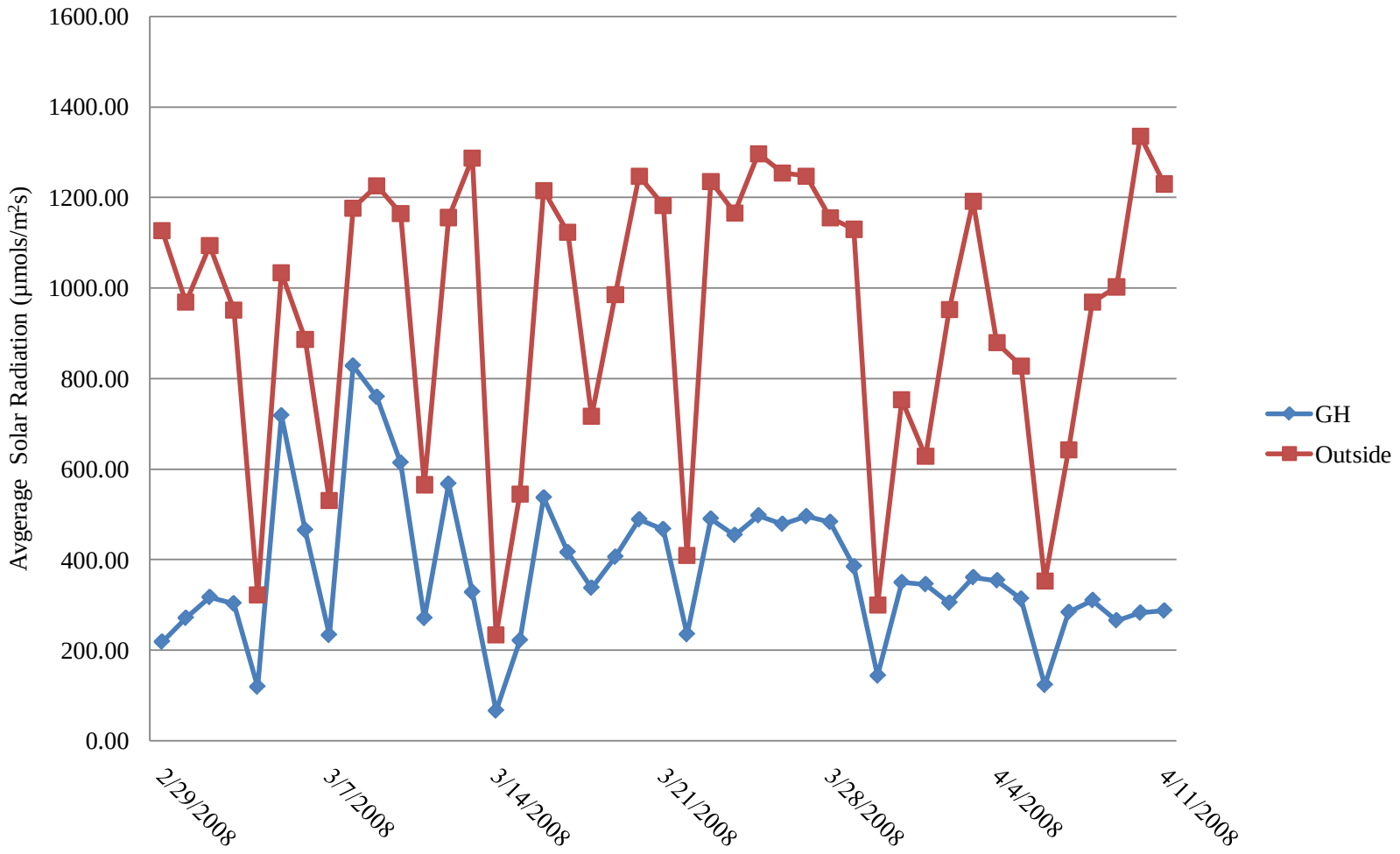

Figure 3.24 Solar radiation from glasshouse experiment Spring 2008, February 29 – April 11.

CHAPTER 4
SUMMARY

Production of vegetable crops in protected structures is an expanding sector in horticulture throughout the world (Cantliffe et al., 2001). Protected structures such as greenhouses and high tunnels allow for an increase in environmental control relative to production within the field. Relative to field production, the extended season in the greenhouse and greater control over conditions allows for the increased production and reduction in pesticide usage (Shaw and Cantliffe, 2008). Greenhouses allow for the most environmental control relative to all protected structures. The majority of greenhouse production still utilizes a traditional soil culture as in the field. Problems caused by rootborne pathogens associated with field soil are also found in greenhouse facilities. Use of methyl bromide, a broad-spectrum fumigant, has been the industry standard in Florida for production of field vegetables (Saha et al., 2007). Additionally methyl bromide was also used in soils in greenhouse facilities. The use of methyl bromide is nearing its end as it has been phased out due to its ozone depleting properties, as required by the Montreal Protocol(United Nations Environment Programme. Ozone Secretariat., 1996). Production of vegetables in protected structures utilizing soilless culture is a potential alternative to using methyl bromide in either the field or protected structure (Smither-Kopperl and Cantliffe, 2004).

Soilless production systems utilize a substrate such as coconut coir, rock wool, perlite, peat, or pine bark and plants are grown in a container such as a polyethylene bag, trough, or pot. This type of system utilizes fertigation through which a hydroponic nutrient solution is applied in regular intervals throughout the day. Currently many of these systems are considered open as the effluent coming from the plants is allowed to drain to waste. The implications of this are negative with respects to the environment and increasingly to the economics of profitable

production. Nutrient effluent in these settings contains large amounts of fertilizer material due to the nature of the media in these types of systems being extremely well-drained. When this solution is allowed to drain to waste there is the possibility of contamination of ground water and surrounding aquatic ecosystems (Kabashima, 1993). In addition to the negative effects of the excess fertilizer materials in the environment, it is also an economic waste in the form of fertilizer. This is increasingly becoming an issue as the profit margins for horticultural businesses are getting smaller. Soilless systems commonly utilize several irrigation events per day which also results in a loss of water as a natural resource if not utilized efficiently. There has been an increase in rules and regulations in management of greenhouse effluent (James and van Iersel, 2001; Kluepfel and Stanghellini, 2006). New regulations in 2001 in the Netherlands required horticultural production facilities to collect all greenhouse effluent (van der Gaag and Wever, 2005). As water quality and availability increasingly becomes an issue, there will be an increase in regulation on water usage and discharge throughout the world.

Closed irrigation systems in which the nutrient effluent is collected and reused can address all the previous mentioned issues. However, closed systems have largely been avoided by commercial producers in the past as there are frequently rootborne disease problems as a result of recycling the nutrient solution. The pathogens causing the disease problems in closed soilless production systems are the same as those that producers were trying to escape when shifting from field to greenhouse production. Examples of such pathogens commonly associated with closed irrigation systems include *Pythium* spp. and *Phytophthora* spp. (Stanghellini and Rasmussen, 1994). *Pythium aphanidermatum* (Edson) Fitzp. is one of the more commonly isolated species in closed irrigation systems and has led to entire crop loss in greenhouse tomatoes and cucumbers (Jenkins and Averre, 1983; Stanghellini and Rasmussen, 1994;

Schnitzler, 2004).There have been various approaches to sanitizing nutrient solution, the majority of which were adopted from municipal water treatment facilities. Such methods include the use of UV- radiation, ozonation, and chlorination. Other methods also include heat pasteurization, slow sand filtration, surfactants, pesticides, and biological control. Although there are methods of sanitizing recycled greenhouse effluent, there is currently a lack of research and information to support the use of the various technologies (Postel, 1992; Uva et al., 1998; Hong and Moorman, 2005).

The method of sanitizing recycled nutrient effluent evaluated in these experiments was chlorination. Chlorination was selected as it requires less initial capital investment as compared to some of the other methods. Additionally chlorination was selected as the concept is simple and the implementation into a commercial operation facility would be a practical option. As the other methods of sanitization, chlorination has the potential to also have undesired effects, specifically in the form of phytotoxicity. There is limited information with regards to utilization of chlorination in a closed production system. Research has been conducted with regards to use of chlorination in peppers (Teoh and Chuo, 1978), lettuce (Premuzic et al., 2007), and tomatoes (Ewart and Chrimes, 1980; Saha et al., 2008) produced in a greenhouse. Results from these studies indicate that levels of chlorination associated with phytotoxicity vary amongst species but also within species when produced utilizing different substrates. Teoh and Chuo reported phytotoxicity occurring in 'Green-giant' bell peppers, grown hydroponically with granite chips as media, when exposed to chlorination rates greater than 20 ppm in the irrigation solution (1978). Premuzic et al. reported foliar leaf damage due to phytotoxicity occurring in lettuce, produced in soilless culture with perlite media, from the chlorination of irrigation solution at concentrations as low as 0.5 ppm (2007). Foliar damage of the lettuce also increased as the

concentration was raised to 11 ppm. Ewart and Chrimes reported reduced root development as a result of phytotoxicity of tomatoes, produced hydroponically in an NFT system, occurring at concentrations as low as 0.5 ppm (1980). However, Saha et al. reported phytotoxicity not occurring in tomato seedlings, produced in floatation irrigation with peat based media, until concentrations exceeded 20 ppm (2008). This would indicate that not only is phytotoxicity dependent on species but also what type of production system and media is utilized. Additionally the majority of research has focused on crops growing in an NFT system or perlite based soilless system which supports the need for further research in other types of substrates. Prior to these experiments, there were no studies which had utilized pine bark in soilless production of vegetables with respect to chlorination and the effect of inoculation with *P. aphanidermatum* (Edson) Fitzp..

It has been reported that pine bark is a less expensive viable alternative to other soilless media such as peat and perlite with similar results in production for strawberries, 'Galia' muskmelons, 'Beit Alpha' cucumbers, and bell peppers (Cantliffe et al., 2003). Pine bark has already been largely adopted for use in ornamental plant production in part as a replacement for methyl bromide as a management tool for disease suppression (Hoitink et al., 1997). The overall objective of these experiments was to look at disease suppression using pine bark in addition to chlorination in the production of bell pepper. Another objective was to compare sand and perlite with pine bark in regards to disease suppression and how they interact with the chlorination treatment of the irrigation solution. Growth chamber studies were conducted initially to observe differences in pine bark, steamed pine bark, and sand with respects to the inoculation treatment as well as the chlorination treatment. Plants in these experiments were irrigated daily with nutrient solution that was chlorinated (0, 1, 2, 4 ppm) to the designated plots. The pots were also

placed in a saucer to provide a reservoir for the plants as they were only irrigated once per day. Additionally plants were inoculated with 20 grams of wheat berries infected with *P. aphanidermatum* in designated plots to observe differences in plant growth response in the different substrates.

In growth chamber studies, consistently plants grown in sand had reduced growth relative to plants grown in pine bark when irrigated with chlorinated (2 ppm) nutrient solution. All the negative effects of chlorination were more drastic in plants growing in sand relative to those growing in either pine bark or steamed pine bark. Under these conditions 'Legionnaire' bell peppers exhibited reduced growth. The significance of this is that free chlorine reacts with organic materials, thus plants growing in an organic substrate such as pine bark are more buffered against the negative effects of chlorination as compared to plants growing in an inorganic substrate such as sand. This further confirms the need for research on sanitation of nutrient solution is needed for different production systems.

Main effects of inoculation with pathogen consistently supported through all experiments that treatments with pathogen were negatively impacted with regards to growth. Plants that were inoculated had reduced height, stem diameter, shoot fresh weight, and shoot dry weight as compared to plants that were not inoculated. The pathogen was reisolated in inoculated plots indicating that chlorination cannot be utilized as a curative measure but must be utilized as a preventative within the irrigation solution.

Plants irrigated with chlorinated nutrient solution (2 ppm) that were inoculated showed no difference in plant height, stem diameter, shoot fresh, and shoot dry weight as compared to plants that were inoculated but did not receive chlorinated nutrient solution. However individual plants can be possible point sources of inoculum to contaminate the entire recycling system and

was evaluated in the later greenhouse studies by inoculating individual plants within 10 plant closed loop system and checking for presence of *P. aphanidermatum* in the recycled water. The pathogen was isolated in the irrigation water indicating that in fact the pathogen can spread in this type of closed loop production system. Additionally plants grown in sand relative to plants grown in pine bark that were inoculated with wheat berries infected with *P. aphanidermatum* had reduced growth with regards to height, stem diameter, and shoot fresh weight.

These data suggested that pine bark as an organic substrate has suppressive properties associated with beneficial microbes acting as competitors and antagonists to rootborne pathogens. Plants grown in sand, an inorganic substrate, that were irrigated with chlorinated (Cl_2 ppm) nutrient solution and inoculated had less height, shoot fresh weight, and shoot dry weight as compared to plants grown in pine bark or steamed pine bark. These data indicated that not only is the organic substrate better at suppressing negative growth affects associated with infection but also at reducing the negative effects of the chlorination treatment.

Results from the first two greenhouse experiments in spring and summer of 2007 had similar results to some of the findings from the growth chamber experiments. Pine bark was the only substrate utilized in these experiments. Plant growth such as height, shoot fresh weight, and root fresh weight decreased linearly as chlorination rate of the nutrient solution increased from 0 to 4 ppm. These data provide further evidence that chlorination rates as low as 2 ppm can cause reduction in growth of 'Legionnaire' bell pepper produced in a closed soilless production system in a greenhouse. Results from the fall 2007 and spring 2008 experiments were similar also to the growth chamber studies and previous greenhouse studies with respect to reduction in growth parameters associated with *P. aphanidermatum* infection and the chlorination of the recycled nutrient effluent. In these experiments two substrates were compared for effects within these

conditions. The two substrates were pine bark (organic) and perlite (inorganic). Plants chlorinated and grown in pine bark had greater height and shoot fresh weight relative to those chlorinated and grown in perlite. Again this emphasizes the inactivation of the free chlorine that occurs when it comes in contact with organic material. Plants grown in pine bark were better buffered against the negative effects on growth associated with chlorination of the nutrient solution as compared to plants grown in perlite (inorganic).

Chlorination is commonly used in post harvest operations in dump tanks to remove dirt and potential post harvest pathogens. In these settings the chlorination rate is affected by organic matter in the solution as a chemical reaction occurs that inactivates the free chlorine (Sapers, 2001). In municipal water treatment facilities which utilize chlorination the same issue is a concern as the organic matter content in the water is one factor which determines the amount of oxidant to add to reach the desired concentration of chlorine (White and NetLibrary Inc., 1999). Chlorination is utilized in these settings as the chemistry of chlorine is such that it results in oxidation of organic materials. The main effects of inoculation were similar to the previous studies in that reduction in some growth variables such as height, shoot fresh weight, and root fresh weight were associated with infection with *P. aphanidermatum* (Edson) Fitzp..

'Legionnaire' bell pepper is a determinant variety and as with these types of varieties they would typically be harvest 2-3 times depending on conditions. However for these experiments based on time and projected results only one harvest was obtained from the four greenhouse experiments conducted in Citra, FL. Results from these experiments were variable with respect to harvest parameters. In the spring 2007 experiment, marketable number of fruit per square meter decreased with increasing chlorine concentration. In the fall 2007 experiment plants grown in pine bark that was chlorinated had greater total marketable fruit weight as

compared to plants grown in perlite that was also treated with chlorination. This is additional evidence that pine bark (organic) is better at managing negative effects of chlorination as compared to perlite. The chlorinated irrigation water entering the pots with perlite (inorganic) only had the organic material in form of roots to interact with, thus leaving potential for root damage or reduction in overall growth. Plants growing in pine bark (organic) were less likely to have oxidation of the root tissue as they were surrounded by an organic substrate which the chlorinated irrigation water could react with.

In the spring 2008 experiment plants that were grown in pine bark had greater marketable fruit numbers and weight relative to plants grown. Additionally plants treated with chlorinated nutrient solution had less marketable fruit (weight and number) as compared to plants treated with nonchlorinated nutrient solution. These data suggest that chlorination in irrigation solution at rates as low as 2 ppm can have adverse affects on crop growth including yield. In these experiments a determinant variety was utilized, however in a commercial greenhouse production system the crop is harvested many times as indeterminant varieties are typically used. Over several harvests there may have been differences observed as overall shoot growth can be linked with yield (Hall, 1977). The average marketable yield from these experiments with one harvest and lower planting density was similar to field grown yield averages (3 kg/m^2) for bell peppers harvested two to three times (Jovicich et al., 2005). This exemplifies the benefits of protected cultivation over field production.

Results from these studies would support the use of pine bark (organic) over perlite (inorganic). Since the pine bark was most likely to better act as a buffer to the chlorination treatment. Additionally some of the data suggests benefits from the organic substrate as a source for beneficial microbes to help with the mitigation of rootborne diseases caused by pathogens

such as *P. aphanidermatum* (Edson) Fitzp. (Mandelbaum and Hadar, 1990; Steinmetz and Schonbeck, 1994; Zhang et al., 1996; Hoitink et al., 1997; Zhang et al., 1998). Since in most cases plants grown in pine bark performed as well as or better relative to plants grown in perlite or sand, it would be the better option for media. Cost is also another significant factor as perlite is five times more costly per cubic meter as compared to pine bark (Rodriguez et al., 2006). With respect to the use of chlorination as management tactic for rootborne diseases in closed soilless production systems utilizing pine bark media; 'Legionnaire' bell peppers had reductions in growth and yield when exposed to chlorination rates as low as 2 ppm. Based on past studies and results of earlier studies in this work it was clear that chlorination rates at which growth reduction occurred was species dependent as well as media dependent (Teoh and Chuo, 1978; Saha and Cantliffe, 2007; Saha et al., 2008).

Based on the results from all these experiments it could be concluded that the rate of chlorination in the irrigation must be less than 2 ppm at the emitter to avoid reductions in crop growth of 'Legionnaire' bell pepper in this type of closed soilless system. However for effective pathogen management concentrations utilized should be greater than or equal to 2 ppm which increases the complexity of management of effluent. Additional studies to evaluate different media and different crop sensitivities in these types of settings will be beneficial in helping commercial producers to implement such management tactics. Further studies should focus on allowing a holding period prior to irrigation application to the plants. The holding period will be dependent on the concentration of free chlorine utilized in addition to temperature. The thought is that it would be possible to chlorinate nutrient solution at 2 ppm or higher if there was a time period to allow the levels to dissipate below 2 ppm to avoid negative effects on crop growth. The amount of chlorine in the solution will reduce over time. Chlorination as a management tactic for

rootborne pathogens has potential but still requires some more understanding and establishment of safe levels to avoid unwanted negative effects on crop growth.

APPENDIX A
ADDITIONAL TABLES FOR CHAPTER 2

Table A-1. Anova table for pathogenicity growth chamber experiment.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root rating[z]
Rep	3	19.4	0.6	2,711.1	32.8*	0.2
Pythium (P)	5	14.3	6.7**	10.8**	146.0**	6.9**
Error	15	22.5	1.3	644.4	9.4	0.2
Total	23	56.2	8.6	3,36.3	188.2	7.3

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table A-2. Anova table for growth chamber experiments with chlorination (2 ppm).

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root rating[z]
Rep	5	233.1**	0.47	3,164.9**	76.6**	5.6**
Media (M)	2	170.8**	12.1**	4,900.8**	77.8**	20.7**
Chlorine (C)	1	193.4**	26.2**	21,976.6**	335.0**	4.5*
Pythium (P)	1	1,152.0**	139.4**	75,537.4	1,206.2**	80.2**
M x C	2	0.4	0.0	53.0	1..0	2.5
M x P	2	32.7*	0.9	80.8	0.5	3.0
C x P	1	62.3*	14.4**	13,243.8**	199.0**	0.5
M x C x P	2	0.4	0.5	369.1	3.5	1.5
Error	55	8.6	0.6	159.5	3.1	0.9
Total	71	1,853.7	194.6	195,462.5	1,902.7	119.4

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table A-3. Anova table for growth chamber experiments with chlorination (1 ppm).

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root rating[z]
Rep	2	72.1	3.9	4,035.4	53.1	0.2
Media (M)	2	780.2**	31.6**	42,983.2**	463.6**	21.6**
Chlorine (C)	1	25.1	1.2	1,135.7	16.5	1.8
Pythium (P)	1	30.3	3.5	7,056.0*	127.7**	13.4**
M x C	2	29.4	2.2	348.3	8.8	1.9
M x P	2	52.2	0.9	246.2	5.5	1.7
C x P	1	36.1	2.0	4,422.3	37.2	1.0
M x C x P	2	39.3	1.7	1,545.4	12.0	0.8
Error	22	27.3	1.2	1,173.3	15.4	1.0
Total	35	1092	48.2	62,945.8	43.4	

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table A-4. Anova table for growth chamber experiments with chlorination (4 ppm).

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root rating[z]
Rep	2	11.0	0.7	212.4	10.9	0.4
Media (M)	2	407.0**	6.1**	8,849.4**	88.9**	9.5**
Chlorine (C)	1	458.7**	41.4**	49,217.4**	748.9**	0.7
Pythium (P)	1	654.5**	43.6**	29,166.9**	392.0**	30.3**
M x C	2	50.7**	3.0**	3,081.5**	31.8**	0.4
M x P	2	122.7**	9.8**	1,846.3**	20.3**	7.6**
C x P	1	0.6	1.2	4,110.9**	95.4**	0.3
M x C x P	2	53.1**	1.2	1,326.5**	9.9*	0.8
Error	22	7.1	0.4	228.3	2.5	0.3
Total	35	1,765.4	107.4	98,039.6	1,400.6	50.3

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

APPENDIX B
ADDITIONAL TABLES FOR CHAPTER 3

Table B-1. Anova table for growth parameters of bell peppers grown in a greenhouse, spring 2007.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root fresh weight	Mean Square Root dry weight	Mean Square Root rating[z]
Rep	2	7.3	7.5*	38,043.1	1,205.5	8,851.4	72.8	0.3
Chlorine (C)	2	140.5	8.5*	61,505.6*	822.2	46,851.4**	453.6**	0.5*
Pythium (P)	1	608.4**	39.9**	155,868.1**	4,355.6**	138.9	177.7	3.1**
C x P	2	60.6	8.3*	39,938.9	288.9	9,443.1	255.6	0.5*
Error	64	58.9	2.3	15,772.7	396.7	5,072.1	90.5	0.1
Total	71	877	67	311,129	7,070	70,356	1,052	5

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-2. Anova table for harvest data of bell pepper grown in a greenhouse, spring 2007.

Source	df	Mean Square XL[z] (quantity)	Mean Square XL (weight)	Mean Square L (quantity)	Mean Square L (weight)	Mean Square M (quantity)	Mean Square M (weight)	Mean Square S (quantity)	Mean Square S (weight)	Mean Square Mrkt (quantity)	Mean Square Mrkt (weight)
Rep	2	9.4	0.37	22.4	0.28	19.4	0.15	14.4	0.04	156.7	1.1
Chlorine (C)	2	14.4	0.6	11.6	0.1	71.1	0.4	14.4	0.0	295.4*	2.5
Pythium (P)	1	0.5	0.0	2.7	0.0	1.4	0.0	0.1	0.0	0.9	0.0
C x P	2	6.5	0.2	20.2	0.2	3.4	0.0	22.1	0.1	63.7	0.0
Error	10	10.8	0.4	7.7	0.1	23.6	0.2	7.6	0.0	61.4	0.9
Total	17	41.6	1.57	64.6	0.68	118.9	0.75	58.6	0.14	578.1	4.5

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Mrkt = marketable fruit including XL, L, M, and S sizes.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-3. Anova table for harvest data of bell pepper grown in a greenhouse, spring 2007.

Source	df	Mean Square Cull[z] (quantity)	Mean Square Cull (weight)	Mean Square BER (weight)	Mean Square BER (quantity)	Mean Square Total (weight)	Mean Square Total (quantity)
Rep	2	19.1	0.05	0.02	8.2	1.2	193.4*
Chlorine (C)	2	99.6	0.3	0.1	11.2	3.9**	676.7**
Pythium (P)	1	56.9	0.3	0.4	32.0	0.3	18.0
C x P	2	38.9	0.2	0.1	16.2	0.4	148.2
Error	10	82.3	0.6	0.6	60.3	0.4	40.5
Total	17	296.8	1.45	1.22	127.9	6.2	1,076.8

[z]Cull = undersize, blemished, misshapen, and blossom end rot, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-4. Anova table for growth parameters of bell peppers grown in a greenhouse, summer 2007.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root fresh weight	Mean Square Root dry weight	Mean Square Root rating[z]
Rep	2	333.7	25.7**	80,607	1,490	9,256	198	0.68
Chlorine (C)	2	283.6	1.9	155,417.7*	1,357.1	398.4	41.5	0.1
Pythium (P)	1	5,050.1**	146.2**	1,697,096.1**	20,395.3**	28,916.1**	364.1**	20.1**
C x P	2	50.5	3.2	11,027	0.5	3,704.3	76.9	0.1
Error	64	126.4	4.8	35,031.0	589.4	3,343.8	29.8	0.7
Total	71	5,844.3	181.8	1,979,178.8	23,832.3	45,618.6	710.3	21.68

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-5. Anova table for harvest data of bell pepper grown in a greenhouse, summer 2007.

Source	df	Mean Square XL[z] (quantity)	Mean Square XL (weight)	Mean Square L (quantity)	Mean Square L (weight)	Mean Square M (quantity)	Mean Square M (weight)	Mean Square S (quantity)	Mean Square S (weight)	Mean Square Mrkt (quantity)	Mean Square Mrkt (weight)
Rep	2	18.4	0.59	1.06	0.01	76.2**	0.59**	28.4	0.09	127.2	0.20
Chlorine (C)	2	9.1	0.3	3.7	0.1	11.6	0.0	8.7	0.0	78.5	0.1
Pythium (P)	1	34.7	1.3	20.1	0.4	3.6	0.0	8.0	0.0	32.0	1.3
C x P	2	2.1	0.0	5.4	0.1	0.9	0.0	0.2	0.0	1.2	0.0
Error	10	8.2	0.3	13.0	0.2	7.4	0.1	17.5	0.1	31.6	0.5
Total	17	72.5	2.49	43.26	0.81	99.7	0.69	62.8	0.19	270.5	2.1

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Mrkt = marketable fruit including XL, L, M, and S sizes.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-6. Anova table for harvest data of bell pepper grown in a greenhouse, summer 2007.

Source	df	Mean Square Cull[z] (quantity)	Mean Square Cull (weight)	Mean Square BER (weight)	Mean Square BER (quantity)	Mean Square Total (weight)	Mean Square Total (quantity)
Rep	2	3,201.7	0.04	0.04	8.7*	0.37	2,341.7
Chlorine (C)	2	1,797.7	0.2	0.1	14.4*	0.4	1,127.1
Pythium (P)	1	4,201.4	0.0	0.0	0.9	1.8	4,996.7
C x P	2	3,032.7	0.0	0.0	7.4	0.1	3,222.7
Error	10	3,315.7	0.1	0.0	2.1	0.5	3,230.6
Total	17	15,549.2	0.34	0.14	33.5	3.17	14,918.8

[z]Cull = undersize, blemished, misshapen, and blossom end rot, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-7. Anova table for growth parameters of bell peppers grown in a greenhouse, fall 2007.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root fresh weight	Mean Square Root dry weight	Mean Square Root rating[z]
Rep	2	266.9**	10.1**	111,050**	11,692	2,025	8.08*	0.20
Media (M)	1	3088.3**	52.5**	1,733,437.5**	2,187.9	3,055.5	0.8	3.0**
Chlorine (C)	1	4952.2**	32.7**	1,465,204.2**	2,532.8	6,670.0**	29.4**	0.0
Pythium (P)	1	410.4**	13.8**	42,504.2	14,937.6	1,393.9	3.9	5.5**
M x C	1	418.8**	2.5	14,504.2	2,772.4	1,239.8	7.1	0.1
M x P	1	13.9	4.5*	21,004.2	11,794.9	534.9	0.3	3.0**
C x P	1	76.1	2.5	17,604.2	2,290.3	515.2	0.3	0.0
M x C x P	1	65.8	0.2	42,504.2	9,015.2	798.1	7.4	0.1
Error	86	52.7	0.9	11,095.3	6354.8	266.1	2.6	0.1
Total	95	9345.1	119.7	3,458,908	63,577.9	16,498.5	59.88	12

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-8. Anova table for harvest data of bell pepper grown in a greenhouse, fall 2007.

Source	df	Mean Square XL[z] (quantity)	Mean Square XL (weight)	Mean Square L (quantity)	Mean Square L (weight)	Mean Square M (quantity)	Mean Square M (weight)	Mean Square S (quantity)	Mean Square S (weight)	Mean Square Mrkt (quantity)	Mean Square Mrkt (weight)
Rep	2	21.4	1.1*	7.0	0.14	7.3	0.05	9.9*	0.08*	28.3	1.1*
Media (M)	1	77.0**	4.2**	32.7	0.5	51.0*	0.9*	28.1**	0.2**	88.2*	0.0
Chlorine (C)	1	57.0*	3.0**	24.0	1.0	15.0	0.1	24.0**	0.1*	13.5	4.3**
Pythium (P)	1	15.0	0.6	10.7	0.2	5.0	0.1	10.7	0.1	24.0	0.1
M x C	1	84.4**	3.6**	1.5	0.0	2.0	0.0	1.5	0.0	60.1*	3.6**
M x P	1	0.0	0.0	4.2	0.1	5.0	0.2	1.5	0.0	2.7	0.0
C x P	1	40.0*	1.7*	0.2	0.0	18.4	0.5	0.0	0.0	2.7	0.2
M x C x P	1	3.4	0.1	54.0	1.3	26.0	0.4	0.2	0.0	0.0	0.0
Error	14	6.9	0.2	13.8	0.41	6.9	0.1	2.6	0.0	12.0	0.2
Total	23	605.7	10.9	148.1	3.65	136.6	2.35	78.5	0.48	231.5	9.5

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Mrkt = marketable fruit including XL, L, M, and S sizes.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-9. Anova table for harvest of bell peppers grown in a greenhouse, fall 2007.

Source	df	Mean Square Cull[z] (quantity)[y]	Mean Square Cull (weight)	Mean Square BER (weight)	Mean Square BER (quantity)	Mean Square Total (weight)	Mean Square Total (quantity)
Rep	2	2.7	0.01	0.03	0.88*	1.1*	23.3
Media (M)	1	32.7*	0.1	0.7	0.0	0.1	228.2**
Chlorine (C)	1	8.2	0.35*	13.5	0.4*	7.1**	42.7
Pythium (P)	1	8.2	0.0	0.7	0.0	0.2	60.2
M x C	1	8.2	0.0	0.0	0.0	3.6**	24.0
M x P	1	8.2	0.4**	13.5	0.3*	0.4	1.5
C x P	1	2.7	0.0	0.7	0.0	0.3	10.7
M x C x P	1	24.0	0.3*	4.2	0.2	0.4	24.0
Error	14	6.0	0.0	3.3	0.0	0.2	14.9
Total	23	100.9	1.16	36.63	1.78	13.4	429.5

[z]Cull = undersize, blemished, misshapen, and blossom end rot, BER = Blossom end rot fruits, Total = all fruit including marketable and culls.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-10. Anova table for growth parameters of bell peppers grown in a greenhouse, spring 2008.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root fresh weight	Mean Square Root dry weight	Mean Square Root rating[z]	Mean Square SPAD[y]
Rep	2	162.3	2.3	11,631	48.7	25,153	2.7	0.20	17.8
Media (M)	1	6,430.5**	206.2**	3,337,604.2**	64,320.1**	57,545.6	1.7	3.0**	274.7**
Chlorine (C)	1	13,270.5**	148.3**	2,898,150.0**	36,025.9**	72,369.2	85.9**	0.0	8,445.0**
Pythium (P)	1	317.1*	9.6*	4,266.7	12.1	41,691.7	4.1	5.5**	232.5**
M x C	1	17.8	11.3**	8,437.5	245.4	25,291.5	2.7	0.1	204.8**
M x P	1	574.8**	0.2	42,504.2	988.8	36,169.4	0.1	3.0**	175.0**
C x P	1	1.4	0.0	416.7	1,140.6	13,814.4	34.6*	0.0	861.6**
M x C x P	1	126.2	0.9	33,004.1	517.5	40,573.9	1.9	0.1	293.3**
Error	86	74.0	1.4	18,984.1	534.6	23,355.4	6.2	0.1	14.3
Total	95	201.6	380.2	6,354,998.5	103,833.7	335,964.1	139.9	12	10,519

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.

[y]Reading of chlorophyll in units of SPAD with higher values indicating higher crop nitrogen status relative to lower SPAD values.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-11. Anova table for harvest data of bell pepper grown in a greenhouse, spring 2008.

Source	df	Mean Square XL[z] (quantity)	Mean Square XL (weight)	Mean Square L (quantity)	Mean Square L (weight)	Mean Square M (quantity)	Mean Square M (weight)	Mean Square S (quantity)	Mean Square S (weight)	Mean Square Mrkt (quantity)	Mean Square Mrkt (weight)
Rep	2	6.1	0.57	34.1**	0.84**	50.5	0.38	9.0	0.04	130.7*	3.0
Media (M)	1	805.0**	44.0**	35.0**	0.8**	40.0	0.3	0.4	0.0	240.7**	26.2**
Chlorine (C)	1	1,751.0**	91.4**	3.4	0.0	45.4	0.2	145.0**	0.5**	3,901.5**	118.7**
Pythium (P)	1	51.0	1.1	9.4	0.1	84.4	0.9	2.0	0.0	322.7**	4.6
M x C	1	26.0	0.6	77.0**	2.3**	5.0	0.0	7.0	0.0	80.7	3.4
M x P	1	45.4	1.2	26.0*	0.6*	18.4	0.1	26.0	0.0	60.2	0.1
C x P	1	15.0	0.1	0.4	0.1	22.0	0.1	5.0	0.0	32.7	0.0
M x C x P	1	0.0	0.0	12.0	0.4	30.3	0.2	1.0	0.0	8.2	0.0
Error	14	24.7	1.4	3.1	0.1	23.6	0.2	8.9	0.0	23.7	1.1
Total	23	2,724.2	140.37	200.4	5.24	319.6	2.38	204.3	0.54	4,801.1	157.1

[z]XL = extra large fruit (Diameter > 84 mm), L = large fruit (76.0 – 83.9 mm), M = medium fruit (64.0 – 75.9 mm), S = small fruit (56.0 – 63.9 mm), Mrkt = marketable fruit including XL, L, M, and S sizes.

*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-12. Anova table for harvest data of bell pepper grown in a greenhouse, spring 2008.

Source	df	Mean Square Cull[z] (quantity)[y]	Mean Square Cull (weight)	Mean Square BER (quantity)	Mean Square BER (weight)	Mean Square Total (quantity)	Mean Square Total (weight)	Mean Square Sun Scald (quantity)	Mean Square Sun Scald (weight)
Rep	2	47.6	0.07	2.8	0.03	23.3	2.2	0.30	0.01
Media (M)	1	2.0	0.8	77.0*	1.5*	287.0*	36.4**	28.2*	0.0
Chlorine (C)	1	1,107.0**	2.7*	345.0**	3.7**	9,165.0**	157.2**	88.2**	1.0**
Pythium (P)	1	392.0*	1.3	1.0	0.0	3.4	1.0	13.5	0.2
M x C	1	187.0	0.7	18.4	0.3	22.0	1.0	20.2*	0.1
M x P	1	12.0	0.0	0.0	0.0	126.0	0.4	0.2	0.0
C x P	1	117.0	0.7	63.4	1.2*	26.0	0.4	20.2*	0.3*
M x C x P	1	100.0	0.6	18.4	0.4	165.4	0.7	0.2	0.0
Error	14	56.4	0.4	14.4	0.3	43.8	0.6	3.3	0.0
Total	23	2,021	7.27	540.4	7.43	9,861.9	199.9	174.3	1.61

[z]Cull = undersize, blemished, misshapen, and blossom end rot, BER = Blossom end rot fruits, Total = all fruit including marketable and culls, Sun Scald = unmarketable fruits with sun damage due to excessive light exposure.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

Table B-13. Anova table for growth parameters of bell peppers grown in a greenhouse, spring 2008-GNV.

Source	df	Mean Square Height	Mean Square Stem diameter	Mean Square Shoot fresh weight	Mean Square Shoot dry weight	Mean Square Root fresh weight	Mean Square Root dry weight	Mean Square Root rating[z]
Rep	2	260.2	3.3	7,687.9	598.0*	1,573.5	47.2	0.1
Media (M)	2	2,629.1**	69.1**	167,896.2**	6,834.5**	28928.5**	402.6**	17.6**
Chlorine (C)	2	619.7**	26.3**	38,373.7**	1,321.9**	7957.0**	198.3**	1.7**
Pythium (P)	1	1,102.5**	10.6*	26,942.9*	1,032.3*	5669.3*	218.8**	4.7**
C x P	2	105.6	4.8	7,596.5	315.9	3183.2*	116.0*	0.4
M x P	2	309.2*	1.9	2,356.1	79.9	2321.4	147.0*	2.1**
M x C	4	134.1	0.7	2,194.5	85.6	1444.8	91.4*	1.3**
M x C x P	4	88.5	1.4	1,092.9	12.3	1337.5	84.0*	0.2
Error	34	87.7	1.9	3,621.2	144.1	781.6	29.4	0.3
Total	53	5,202.5	120.0	257,761.9	9,235.5	53,193.3	1332.0	28.4

[z]Root rating: 0 = no damage/discoloration, 1 = 1 – 25% damage/discoloration, 2 = 26 – 50% damage/discoloration, 3 = 51-75% damage/discoloration, 4 = > 75% damage/discoloration, 5 = plant mortality.
*,** Significant F-test at 5% (*) or 1% (**), respectively.

LIST OF REFERENCES

Agrios, G. N. 1997. Plant pathology. San Diego, Academic Press.

Alfieri, S. A., K. R. Langdon, J. W. Kimbrough, N. E. El-Gholl, and C. Wehlburg 1994. Diseases and disorders of plants in Florida. Gainesville, FL, Florida Dept. of Agriculture and Consumer Services, Division of Plant Industry.

Armitage, P. 1993. Chemical control of *Phytopthora cinnamomi* in irrigation water. Australian Horticulture 91(10): 30-36.

Bates, M. L., and M. E. Stanghellini 1984. Root-Rot of Hydroponically Grown Spinach Caused by Pythium-Aphanidermatum and Pythium-Dissotocum. Plant Disease 68(11): 989-991.

Benhamou, N., R. R. Belanger, P. Rey, and Y. Tirilly 2001. Oligandrin, the elicitin-like protein produced by the mycoparasite Pythium oligandrum, induces systemic resistance to Fusarium crown and root rot in tomato plants. Plant Physiology and Biochemistry 39(7-8): 681-696.

Bennett, W. F. 1993. Nutrient deficiencies & toxicities in crop plants. St. Paul, Minn., APS Press.

Benoit, F., and N. Ceustermans 2002. Consequences of Closed Soilless Growing Systems for the Recirculating Nutrient Solution and the Production Techniques. Acta Horticulturae 633: 331-340.

Broadbent, P., K. F. Baker, and Y. Waterworth 1971. Bacteria and Actinomycetes Antagonistic to Fungal Root Pathogens in Australian Soils. Australian Journal of Biological Sciences 24(5): 925-944.

Bryla, D. R., R. G. Linderman, and W. Q. Yang 2008. Incidence of Phytophthora and Pythium infection and the relation to cultural conditions in commercial blueberry fields. Hortscience 43(1): 260-263.

Calvo-Bado, L. A., G. Petch, N. R. Parsons, J. A. W. Morgan, T. R. Pettitt, and J. M. Whipps 2006. Microbial community responses associated with the development of oomycete plant pathogens on tomato roots in soilless growing systems. Journal of Applied Microbiology 100(6): 1194-1207.

Cantliffe, D. J., N. L. Shaw, J. Funes, E. Jovicich, A. Rodriguez, and J. C. Rodriguez 2003. Media and containers for greenhouse soilless grown cucumbers, melons, peppers, and strawberries. VI International Symposium on Protected Cultivation in Mild Winter Climate: Product and Process Innovation, Ragusa-Sicily, Italy, International Society for Horticulture Science.

Cantliffe, D. J., N. L. Shaw, E. Jovicich, J. C. Rodriguez, I. Secker, and Z. Karchi 2001. Passive ventilated high-roof greenhouse production of vegetables in a humid mild-winter climate. Acta Horticulturae 559: 195-201.

Cantliffe, D. J., and J. J. VanSickle 2003. Mexican Competition: now from the grenhouse. Florida Tomato Inst., Naples, FL.

Ceja-Torres, L. F., G. Mora-Aguilera, D. Teliz, A. Mora-Aguilera, P. Sanchez-Garcia, C. Munoz-Ruiz, B. Tlapal-Bolanos, and R. De la Torre-Almaraz 2008. Fungi prevalence and etiology of strawberry dry wilt under different crop management systems. Agrociencia 42(4): 451-461.

Cerkauskas, R. 2008. "Pepper Disorders: Sun scald." The World Vegetable Center Fact Sheet Retrieved November 1, 2008, from http://www.avrdc.org/LC/pepper/sunscald.pdf.

Chave, M., P. Dabert, R. Brun, J. J. Godon, and C. Poncet 2008. Dynamics of rhizoplane bacterial communities subjected to physicochemical treatments in hydroponic crops. Crop Protection 27(3-5): 418-426.

Chellemi, D. O., D. J. Mitchell, M. E. Kannwischer-Mitchell, P. A. Rayside, and E. N. Rosskopf 2000. Pythium spp. associated with bell pepper production in Florida. Plant Disease 84(12): 1271-1274.

Chen, W., H. A. J. Hoitink, A. F. Schmitthenner, and O. H. Tuovinen 1988. The Role of Microbial Activity in Suppression of Damping-Off Caused by Pythium-Ultimum. Phytopathology 78(3): 314-322.

Cohen, Y., and M. D. Coffey 1986. Systemic Fungicides and the Control of Oomycetes. Annual Review of Phytopathology 24: 311-338.

Davis, C. L., C. J. Donkin, S. A. Hinch, and P. Germishuizen 1992. The Microbiology of Pine Bark Composting - an Electron-Microscope and Physiological Study. Bioresource Technology 40(3): 195-204.

de Cara, M., V. Lopez, M. Santos, and J. C. T. Marquina 2008. Association of Pythium aphanidermatum with root and crown rot of melons in Honduras. Plant Disease 92(3): 483-483.

de Cara, M., M. Santos, M. L. Herrero, M. B. Brurberg, and J. C. T. Marquina 2008. First report of Pythium deliense as a pathogen of melon in Honduras and Guatemala. Plant Pathology 57(4): 776-776.

De Ruiter, S. 1987. Guidelines for culture of greenhouse peppers in North America. News Letter no. 8, De Ruiter Seeds Inc., Columbus, Ohio.

Deep, I. W., and P. E. Lipps 1996. Recovery of Pythium arrhenomanes and its virulence to corn. Crop Protection 15(1): 85-90.

Ehret, D. L., B. Alsanius, W. Wohanka, J. G. Menzies, and R. Utkhede 2001. Disinfestation of recirculating nutrient solutions in greenhouse horticulture. Agronomie 21(4): 323-339.

Estradagarcia, T., T. C. Ray, J. R. Green, J. A. Callow, and J. F. Kennedy 1990. Encystment of Pythium-Aphanidermatum Zoospores Is Induced by Root Mucilage Polysaccharides, Pectin and a Monoclonal-Antibody to a Surface-Antigen. Journal of Experimental Botany 41(227): 693-699.

Evanylo, G. K., and W. L. Daniels 1999. Paper Mill Sludge Composting and Compost Utilization. Compost Science & Utilization 7(2): 30.

Ewart, J. M., and J. R. Chrimes 1980. Effects of chlorine and ultra-violet light in disease control in NFT. Acta Horticulturae 98: 317-323.

Favrin, R. J., J. E. Rahe, and B. Mauza 1988. Pythium Spp Associated with Crown Rot of Cucumbers in British-Columbia Greenhouses. Plant Disease 72(8): 683-687.

Feng, Y., and P. H. Dernoeden 1999. Pythium species associated with root dysfunction of creeping bentgrass in Maryland. Plant Disease 83(6): 516-520.

Feng, Y., P. H. Dernoeden, and A. P. Grybauskas 1999. A simple Pythium aphanidermatum field inoculation technique for perennial ryegrass. Hortscience 34(2): 301-304.

Gold, S. E., and M. E. Stanghellini 1985. Effects of Temperature on Pythium Root-Rot of Spinach Grown under Hydroponic Conditions. Phytopathology 75(3): 333-337.

Hall, A. J. 1977. Assimilate Source-Sink Relationships in Capsicum-Annuum-L. .1. Dynamics of Growth in Fruiting and Deflorated Plants. Australian Journal of Plant Physiology 4(4): 623-636.

Hanan, J. J. 1997. Greenhouses: Advanced Technology for Protected Horticulture. Boca Raton, FL, CRC Press.

Harter, L. L., and W. A. Whitney 1927. A transit disease of snap beans caused by Pythium aphanidermatum. Journal of Agricultural Research 34: 0443-0447.

Hendrix, F. F., and W. A. Campbell 1973. Pythiums as Plant Pathogens. Annual Review of Phytopathology 11: 77-98.

Herrero, M. L., A. Hermansen, and O. N. Elen 2003. Occurrence of Pythium spp. and Phytophthora spp. in Norwegian greenhouses and their pathogenicity on cucumber seedlings. Journal of Phytopathology-Phytopathologische Zeitschrift 151(1): 36-41.

Herrero, M. L., and S. S. Klemsdal 1998. Identification of Pythium aphanidermatum using the RAPD technique. Mycological Research 102: 136-140.

Hochmuth, G. J., and R. C. Hochmuth 1996. Nutrient Solution Formulation for Hydroponic (Perlite, Rockwool, NFT) Tomatoes in Florida. U. o. F. Extension. Gainesville, FL, IFAS Publications.

Hoitink, H. A. J., and P. C. Fahy 1986. Basis for the Control of Soilborne Plant-Pathogens with Composts. Annual Review of Phytopathology 24: 93-114.

Hoitink, H. A. J., A. F. Schmitthenner, and L. J. Herr 1975. Composted Bark for Control of Root Rot in Ornamentals. Journal of Arboriculture 1(11): 217-218.

Hoitink, H. A. J., A. G. Stone, and D. Y. Han 1997. Suppression of plant diseases by composts. Hortscience 32(2): 184-187.

Hong, C. X., and G. W. Moorman 2005. Plant pathogens in irrigation water: Challenges and opportunities. Critical Reviews in Plant Sciences 24(3): 189-208.

Hong, J. C. 2005. Detection of Ralstonia solanacearum in irrigation ponds and semi-aquatic weeds, and its chemical treatment in water. Department of Plant Pathology. Gainesville, University of Florida. Masters.

James, E. C., and M. W. van Iersel 2001. Fertilizer concentration affects growth and flowering of subirrigated petunias and begonias. Hortscience 36(1): 40-44.

Jenkins, S. F., and C. W. Averre 1983. Root Diseases of Vegetables in Hydroponic Culture Systems in North-Carolina Greenhouses. Plant Disease 67(9): 968-970.

Jovicich, E., D. J. Cantliffe, and P. J. Stoffella 2004. Fruit yield and quality of greenhouse-grown bell pepper as influenced by density, container, and trellis system. Horttechnology 14(4): 507-513.

Jovicich, E., D. J. Cantliffe, P. J. Stoffella, and D. Z. Haman 2007. Bell pepper fruit yield and quality as influenced by solar radiation-based irrigation and container media in a passively ventilated greenhouse. Hortscience 42(3): 642-652.

Jovicich, E., J. J. VanSickle, D. J. Cantliffe, and P. J. Stoffella 2005. Greenhouse-grown colored peppers: A profitable alternative for vegetable production in Florida? Horttechnology 15(2): 355-369.

Kabashima, J. N. 1993. Innovative Irrigation Techniques in Nursery Production to Reduce Water Usage. Hortscience 28(4): 291-293.

Kim, H., S. Kwon, S. Han, M. Yu, J. Kim, S. Gong, and M. F. Colosimo 2006. New ORP/pH based control strategy for chlorination and dechlorination of wastewater: pilot scale application. Water Science and Technology 53(6): 145-151.

Kluepfel, D., and M. Stanghellini 2006. Annual report on Research Project: Control of soil borne pathogens in greenhouse production. U. S. D. o. Agriculture. Washington, D.C., Agricultural Research Service.

Littell, R. C., W. W. Stroup, R. J. Freund, and Books24x7 Inc. 2002. SAS for linear models, fourth edition. Wiley series in probability and statistics. Cary, N.C., USA, SAS Institute.

Liu, C. 1976. Species and Distribution of Pythium Causing Damping-Off of Tobacco Seedlings in Taiwan. Plant Protection Bulletin 18(2): 199-206.

Madramootoo, C. A., and M. Rigby 1991. Effects of Trickle Irrigation on the Growth and Sunscald of Bell Peppers (Capsicum-Annuum-L) in Southern Quebec. Agricultural Water Management 19(2): 181-189.

Mandelbaum, R., and Y. Hadar 1990. Effects of Available Carbon Source on Microbial Activity and Suppression of Pythium-Aphanidermatum in Compost and Peat Container Media. Phytopathology 80(9): 794-804.

Menzies, J. G., D. L. Ehret, and S. Stan 1996. Effect of inoculum density of Pythium aphanidermatum on the growth and yield of cucumber plants grown in recirculating nutrient film culture. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 18(1): 50-54.

Moulin, F., P. Lemanceau, and C. Alabouvette 1994. Pathogenicity of Pythium Species on Cucumber in Peat-Sand, Rockwool and Hydroponics. European Journal of Plant Pathology 100(1): 3-17.

Nordskog, B., R. Naerstad, M. L. Herrero, L. Sundheim, and A. Hermansen 2008. Fungal pathogens in Norwegian lettuce fields. Acta Agriculturae Scandinavica Section B-Soil and Plant Science 58(3): 230-235.

Norman, D. J., J. M. F. Yuen, R. Resendiz, and J. Boswell 2003. Characterization of Erwinia populations from nursery retention ponds and lakes infecting ornamental plants in Florida. Plant Disease 87(2): 193-196.

Pategas, K. G., A. C. Schuerger, and C. Wetter 1989. Management of Tomato Mosaic-Virus in Hydroponically Grown Pepper (Capsicum-Annuum). Plant Disease 73(7): 570-573.

Paulitz, T. C., and R. R. Belanger 2001. Biological control in greenhouse systems. Annual Review of Phytopathology 39: 103-133.

Plaats-Niterink, A. J. V. D. 1981. Monograph of the Genus Pythium. Studies in Mycology 21: 1-242.

Platt, R. 2008. Potato diseases caused by oomycete species pathogens. Cahiers Agricultures 17(3): 361-367.

Postel, S. 1992. Last oasis:facing water scarcity. New York, W.W. Norton and Co.

Postel, S. L., G. C. Daily, and P. R. Ehrlich 1996. Human appropriation of renewable fresh water. Science 271(5250): 785-788.

Postma, J., M. J. E. I. M. Willemsen-de Klein, and J. D. van Elsas 2000. Effect of the indigenous microflora on the development of root and crown rot caused by Pythium aphanidermatum in cucumber grown on rockwool. Phytopathology 90(2): 125-133.

Premuzic, Z., H. E. Palmucci, J. Tamborenea, and M. Nakama 2007. Chlorination: Phytotoxicity and effects on the production and quality of Lactuca sativa var. Mantecosa grown in a closed, soil-less system. Phyton-International Journal of Experimental Botany 76: 103-117.

Rafin, C., Y. Brygoo, and Y. Tirilly 1995. Restriction Analysis of Amplified Ribosomal DNA of Pythium Spp Isolated from Soilless Culture Systems. Mycological Research 99: 277-281.

Ramamoorthy, V., T. Raguchander, and R. Samiyappan 2002. Enhancing resistance of tomato and hot pepper to Pythium diseases by seed treatment with fluorescent pseudomonads. European Journal of Plant Pathology 108(5): 429-441.

Rodriguez, J. C., D. J. Cantliffe, N. L. Shaw, and Z. Karchi 2006. Soilless media and containers for greenhouse production of 'Galia' type muskmelon. Hortscience 41(5): 1200-1205.

Saha, S., and D. Cantliffe 2007. Impacts of media and chlorination on the growth of bell pepper (Capsicum annuum L.) inoculated with Pythium aphanidermatum. Hortscience 42(4): 932-933.

Saha, S. K., R. McSorley, K.-H. Wang, and R. J. McGovern 2005. Impacts of extreme weather and soil management treatments on disease development of *Pythium* spp. in field grown pepper. Annual Meeting of the Florida State Horticultural Society, Tampa, FL, Florida State Horticulture Society.

Saha, S. K., C. S. Vavrina, and D. J. Cantliffe 2008. Chlorination impacts on tomato transplants in production with floatation irrigation. Hortscience 43(4): 1125.

Saha, S. K., K. H. Wang, R. McSorley, R. J. McGovern, and N. Kokalis-Burelle 2007. Effect of solarization and cowpea cover crop on plant-parasitic nematodes, pepper yields, and weeds. Nematropica 37(1): 51-63.

Sanogo, S., and G. W. Moorman 1993. Transmission and Control of Pythium-Aphanidermatum in an Ebb-and-Flow Subirrigation System. Plant Disease 77(3): 287-290.

Sapers, G. M. 2001. Efficacy of washing and sanitizing methods for disinfection of fresh fruit and vegetable products. Food Technology and Biotechnology 39(4): 305-311.

Schnitzler, W. H. 2004. Pest and Disease Management of Soilless Culture. South Pacific Soilless Culture Conference, Palmertson North, New Zealand, International Society of Horticultural Science.

Serrano, Y., M. L. Guirado, M. P. Carmona, J. Gomez, and J. M. Melero-Vara 2008. First report of root and crown necrosis of bean caused by Pythium aphanidermatum in Spain. Plant Disease 92(1): 174-174.

Serrano, Y., J. M. Melero-Vara, M. L. Guirado, E. Segundo, and J. Gomez 2008. Pythium myriotylum, a new pathogen of green beans in Spain. Plant Pathology 57(4): 776-776.

Settle, D. M., J. D. Fry, and N. A. Tisserat 2001. Development of brown patch and Pythium blight in tall fescue as affected by irrigation frequency, clipping removal, and fungicide application. Plant Disease 85(5): 543-546.

Shaw, N. L., and D. J. Cantliffe 2008. Greenhouse protected agriculture of vegetable crops at the University of Florida: an overview. The 34th National Agricultural Plastics Conference, Plasticulture, Bellefonte, PA, American Society for Plasticulture.

Shaw, N. L., D. J. Cantliffe, J. Funes, and C. Shine 2004. Successful beit alpha cucumber production in the greenhouse using pine bark as an alternative soilless media. Horttechnology 14(2): 289-294.

Shaw, N. L., D. J. Cantliffe, J. C. Rodriguez, and Z. Karchi 2007. Alternative Use of Pine Bark Media for Hydroponic Production of 'Galia' Muskmelon Results in Profitable Returns. III Internation Symposium on Cucurbits, Townsville, Australia, International Society for Horticultural Science.

Shurtleff, M. C., and C. W. Averre 1997. The plant disease clinic and field diagnosis of abiotic diseases. St. Paul, MN, APS Press.

Smither-Kopperl, M. L., and D. J. Cantliffe 2004. Protected agriculture as a methyl bromide alternative? Current reality and future promise. Proceedings of the Florida State Horticulture Society 117: 21-27.

Spies, C. F. J., M. Mazzola, and A. McLeod 2007. Characterization of Pythium species associated with grapevine decline. South African Journal of Science 103(1-2): Xii-Xii.

Stanghellini, M. E., C. J. Nielsen, D. H. Rim, S. L. Rasmussen, and P. A. Rorbaugh 2000. Influence of sub- versus top-irrigation and surfactants in a recirculating system on disease

incidence caused by Phytophthora spp. in potted pepper plants. Plant Disease 84(10): 1147-1150.

Stanghellini, M. E., and S. L. Rasmussen 1994. Hydroponics - a Solution for Zoosporic Pathogens. Plant Disease 78(12): 1129-1138.

Stanghellini, M. E., S. L. Rasmussen, D. H. Kim, and P. A. Rorabaugh 1996. Efficacy of nonionic surfactants in the control of zoospore spread of Pythium aphanidermatum in a recirculating hydroponic system. Plant Disease 80(4): 422-428.

Steinmetz, J., and F. Schonbeck 1994. Conifer Bark as Growth-Medium and Carrier for Trichoderma-Harzianum and Gliocladium-Roseum to Control Pythium-Ultimum on Pea. Zeitschrift Fur Pflanzenkrankheiten Und Pflanzenschutz-Journal of Plant Diseases and Protection 101(2): 200-211.

Sutton, J. C., C. R. Sopher, T. N. Owen-going, W. Liu, B. Grodzinski, J. C. Hall, and R. L. Benchimol 2006. Etiology and epidemiology of Pythium root rot in hydroponic crops:current knowledge and perspectives. Summa Phytopathologica 32(4): 307-321.

Teoh, T. S., and S. K. Chuo 1978. The possible use of sodium hypochloride for bacterial wilt control in the hydroponic cultivation of sweet pepper. Singapore Journal of Primary Industries 6(2): 102-112.

Tripathi, N. N., and R. K. Grover 1974. Inoculum potential and soil factors affecting the pathogenesis of *Pythium butleri* in causing damping-off of tomato. Symposium on "Ecology of Soil Microorganisms". New Delhi, India. 41**:** 466-474.

United Nations Environment Programme. Ozone Secretariat. 1996. Handbook for the International Treaties for the Protection of the Ozone Layer : the Vienna Convention (1985), the Montreal Protocol (1987). Nairobi, Kenya, Ozone Secretariat, United Nations Environment Programme.

Utkhede, R. S., C. A. Levesque, and D. Dinh 2000. Pythium aphanidermatum root rot in hydroponically grown lettuce and the effect of chemical and biological agents on its control. Canadian Journal of Plant Pathology-Revue Canadienne De Phytopathologie 22(2): 138-144.

Uva, W. F. L., T. C. Weiler, and R. A. Milligan 1998. A survey on the planning and adoption of zero runoff subirrigation systems in greenhouse operations. Hortscience 33(2): 193-196.

Van Cooten, D. E., and A. K. Borrell 1999. Enhancing food security in semi-arid eastern Indonesia through permanent raised-bed cropping: a review. Australian Journal of Experimental Agriculture 39(8): 1035-1046.

van der Gaag, D. J., and G. Wever 2005. Conduciveness of different soilless growing media to Pythium root and crown rot of cucumber under near-commercial conditions. European Journal of Plant Pathology 112(1): 31-41.

Van Os, E. A. 1999. Closed soilless growing systems: A sustainable solution for Dutch greenhouse horticulture. Water Science and Technology 39(5): 105-112.

Wallace, J. S. 2000. Increasing agricultural water use efficiency to meet future food production. Agriculture Ecosystems & Environment 82(1-3): 105-119.

Wallace, J. S., and C. H. Batchelor 1997. Managing water resources for crop production. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 352(1356): 937-946.

White, G. C. 1992. The handbook of chlorination and alternative disinfectants. New York, Van Nostrand Reinhold.

White, G. C., and NetLibrary Inc. 1999. The handbook of chlorination and alternative disinfectants. New York, J. Wiley**:** xxi, 1569 p.

Wulff, E. G., A. T. H. Pham, M. Cherif, P. Rey, Y. Tirilly, and J. Hockenhull 1998. Inoculation of cucumber roots with zoospores of mycoparasitic and plant pathogenic Pythium species: Differential zoospore accumulation, colonization ability and plant growth response. European Journal of Plant Pathology 104(1): 69-76.

Zhang, W., W. A. Dick, and H. A. J. Hoitink 1996. Compost-induced systemic acquired resistance in cucumber to Pythium root rot and anthracnose. Phytopathology 86(10): 1066-1070.

Zhang, W., D. Y. Han, W. A. Dick, K. R. Davis, and H. A. J. Hoitink 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology 88(5): 450-455.

Zinati, G. M. 2005. Compost in the 20th century: A tool to control plant diseases in nursery and vegetable crops. Horttechnology 15(1): 61-66.

BIOGRAPHICAL SKETCH

Shubin Kumar Saha was born in 1976 in Lexington, Kentucky. He received his B.S. degree in plant and soil science from the University of Kentucky in 2001. That same year he left for Gainesville, Florida to begin his graduate studies in the newly created Doctor of Plant Medicine Program at the University of Florida. Throughout the completion of this degree he was involved in interdisciplinary studies in entomology, plant pathology, weed science, horticulture, agronomy, and soil science. While pursuing his degree he was awarded a research assistantship and worked with methyl bromide alternatives including soil solarization and cover cropping for use in field production of vegetables. As a plant medicine student, he also had the opportunity to work with two commercial greenhouse vegetable producers in north and central Florida. In 2005 he was awarded a D.P.M. and then continued his graduate studies at the University of Florida. In August 2005 he began pursuing a PhD in the Horticultural Sciences Department working in greenhouse production of bell peppers in closed soilless systems evaluating methods of managing root diseases. He received his PhD from the University of Florida in August 2009.