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# Micronutrient Concentration Effects on Lettuce Growth and Susceptibility to Pythium

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Micronutrient Concentration Effects on Lettuce Growth and Susceptibility to *Pythium*

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by

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**Abstract**

In hydroponic production waterborne pathogens such as *Pythium* are ubiquitous and continually threaten a wide range of Controlled Environment Agriculture (CEA) crops in hydroponic production, including but not limited to: lettuce, spinach, basil, arugula, cucumber, tomato, sweet pepper, roses, chrysanthemums, and cannabis (Sutton et al., 2006; Gull, 2002; McGehee and Raudales, 2021; Gillespie, 2020). Despite extensive sanitation measures, disease control in hydroponics is fallible and requires constant surveillance and management to minimize outbreaks (Sutton et al., 2006). A potential disease suppression strategy is to increase micronutrient concentrations within hydroponic systems to naturally strengthen plant defenses against pathogens such as *Pythium*. This thesis combines previous literature and research that looks at the effects of nutrient solution management and *Pythium* root rot disease on hydroponic lettuce. A series of preliminary studies were conducted to determine the correct *Pythium* species and strain, environmental parameters, and dosing methods in order to induce disease in hydroponic 'Rex' lettuce. These studies found that the *Pythium* strain and species *P. myriotylum* 'PM1` had increased pathogenicity to cultivar 'Rex' lettuce and effectively caused root browning at concentrations of $1.80 \times 10^4$ oospores per L of solution or greater. Experiments in which preliminary findings were implemented looked at increasing Si and metal micronutrients iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) concentrations above standard hydroponic nutrient formulations for the effects on plant growth and susceptibility to *P. myriotylum* with hydroponic 'Rex' lettuce. Metal micronutrient/Si concentration and *Pythium* effects were measured with leaf SPAD chlorophyll content, shoot height and width, total plant fresh mass, percent reduction in lettuce growth, and root disease severity. It was found that increasing metal micronutrient and Si concentrations above standard hydroponic formulations resulted in

decreased plant growth and yield. Overall, *Pythium* reduced plant growth and yield, however, increasing metal micronutrient and Si concentrations did not reduce *Pythium* disease severity compared to the standard solution, except for Cu at 10 $mg \cdot L^{-1}$. High concentrations of Cu have known fungicide and algaecide effects, however, can also be phytotoxic and reduce plant yield. Ultimately, combining proper sanitation, best management and cultural practices, appropriate hydroponic system design, and implementation of water treatment technologies will be the most effective strategy in controlling waterborne pathogens for hydroponic growers.

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**Epigraph**

*“If you don’t take risks, you can’t create a future!” — Monkey D. Luffy*

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# CHAPTER 1. LITERATURE REVIEW ON HYDROPONIC PRODUCTION AND WATERBORNE PATHOGENS


## Abstract

Hydroponic food crop production in controlled environments is an increasingly important sector of U.S. agriculture. Despite the many benefits of hydroponic production, controlling waterborne pathogens such as *Pythium* is notoriously difficult in recirculating solutions and can result in major crop losses for growers. This chapter provides an overview of hydroponic production, common hydroponic systems and cultural practices, and strategies for reducing water-borne pathogens. In addition, this thesis discusses the potential and need to research novel nutrient management strategies to assist in mitigating pathogen risks in hydroponics.


## Introduction to controlled environment production

Food crop production in controlled environments is an increasingly important sector of U.S. agriculture (Coyle and Ellison, 2017; Eigenbrod and Gruda, 2015; Graamans et al, 2017; Agrilyst, 2017; Lensing, 2018). The global trends of increasing human population, urbanization, desertification, diminishing freshwater supply, and continuing climate change have contributed to the depletion of arable land per person (Benke and Tomkins, 2017). As arable land resources decrease, agriculturists are now faced with addressing sustainability concerns and feeding a rapidly growing world population which is projected to reach roughly 9.7 billion by 2050 (Benke and Tomkins, 2017). Controlled environment agriculture (CEA) takes advantage of technology and automation in order to modify the production climate, protect crops from biotic and abiotic stressors, and optimize environmental factors (light for photosynthesis, air and root temperatures, fertilizer nutrients, water, and gases) to maximize plant yield and quality (Hamrick, 2003; Sonneveld and Voogt, 2009; Resh, 2013; Jones, 2014; Nelson, 2012).

Common types of CEA structures include greenhouses, high-tunnels, and indoor warehouses, such as vertical farming. The ability to control environmental factors and crop inputs allows growers to extend the growing season and produce crops in extreme climates and urban settings. In addition, CEA allows for increased resource use efficiency of water, nutrients, labor, and energy while decreasing carbon- and water-footprints and ensuring consistent crop quality (Bar-Yosef, 2008; Al-Kodmany, 2002; Blok et al., 2017; Eaves and Eaves, 2018; Hodges et al., 1968; Hoekstra et al., 2011; Mundler and Rumpus, 2012).

**Overview of hydroponic production**

Hydroponics is a common CEA production method used in almost every country globally (Resh, 2013) and has recently become more profitable in the U.S. as a result of increased consumer demand for fresh, year-round, and locally produced food (Gillespie, 2019; Kozai et al., 2016a; Kozai et al., 2016b). Hydroponics refers to the practice of growing plant roots directly in solution or water-based culture without the use of soil (Resh, 2013).

In commercial production, the term “hydroponics” also applies to crops grown in a “soilless” substrate or growing media. Growing plants in substrate that does not contain soil is called “soilless culture” and is similar to hydroponics in the sense that the root zone is characterized by a high availability of nutrients and water. Soilless substrates can be formulated using various organic and inorganic materials but do not contain a mineral or field soil component (such as sand, clay, loam, silt, etc.). Soilless substrates typically do not supply or retain large quantities of nutrients. However, soilless substrates such as peat moss, coconut coir, and bark, do hold relatively large volumes of water and are irrigated with nutrient solutions. Soilless substrates still perform the basic functions of a soil, which are to (1) anchor plant roots,

(2) hold water and nutrients for root uptake, and (3) allow the exchange of important gases (i.e., oxygen, carbon dioxide) between the roots and the environment.

Additionally, hydroponic crop production systems take advantage of cutting-edge technology and can be adapted to provide fresh food through intensive production in locations ranging from remote places in developing countries to space stations orbiting the Earth (Resh, 2013). In the 1980's, global hydroponic production was estimated at approximately 5,000−6,000 ha (12,500−15,000 acres) and by 2001 increased to 20,000−25,000 ha (50,000−62,500 acres) (Resh, 2013). In 2014, the United States Department of Agriculture reported that 63% of total food crops grown under protection (controlled environment agriculture) were grown in hydroponic systems (USDA, 2014).

**Common types of commercial hydroponic systems for leafy greens**

The two major types of hydroponic systems used for leafy greens and herb production (Gómez et al., 2019; Resh, 201) include nutrient film technique (NFT) and deep water culture (DWC) systems. Various soilless substrate systems are also used but are beyond the scope of this review. These systems can be designed to be "closed" systems where the nutrient solution that flows through the system is recirculated and reused. Alternatively, systems can be an "open" design where the solution that flows through systems is discarded at the end of each cycle and new solution is pumped into the systems.

*Nutrient film technique (NFT).* Nutrient film technique is a form of water culture in which plants are placed in a small channel through which a thin "film" of solution passes over the roots and is continually recirculated (Doty, 2020; Resh, 2013). In order to achieve optimal growth, the system needs to provide proper root aeration through either forced aeration or through recirculation. Roots must be shaded from sunlight to reduce algae growth and root burn.

In addition, plant shoots need support from plastic covers or closed-cell extruded polystyrene foam (XPS) sheets to float on top of nutrient solution in these systems which also prevent light from leaking into the systems (Resh, 2013).

Common designs of NFT systems include A-frame systems and gutter-and-pipe NFT systems (Resh, 2013). The A-frame systems were originally designed for testing low-profile plants such as lettuce, arugula, and herbs in Taiwan in the late 1980's (Resh, 2013). The A-frame, however, was designed for small-scale production, rather than commercial. An A-frame design consists of light-weight metal tubing that supports PVC pipes where plants are inserted. Irrigation is provided through drip tubes inserted into each PVC pipe that drains through a cap that can be removed at the ends. These systems are typically not recirculated, and the solution is pumped through the system from a reservoir (Resh, 2013).

Throughout the history of NFT designs, the systems went through many modifications in an effort to resolve oxygen deficiency and ethylene buildup in plant roots (Resh, 2013). Commercial NFT systems often involve growing plants in mineral wool media made from spun basalt rock. Gutter-and-pipe NFT channel systems make up the majority of commercial NFT systems. Construction consists of plastic eaves troughs typically used for homes, or prefabricated systems that use rigid PVC extruded growing channels (Resh, 2013). These NFT channels come in a variety of lengths, however, the maximum length should not exceed 15 m, as uptake competition between plants can result in oxygen and nutrient deprivation zones throughout the channel (Resh, 2013). As plant roots develop within the channel the roots can accumulate into a 'dam' that blocks the even flow of nutrient solution down the channel, resulting in plants further down the channel struggling to uptake water, nutrients, and oxygen. Therefore, to prevent oxygen and nutrient uptake competition between plants in a channel the maximum recommended

length for channels is 4.6 m (Resh, 2013). Nutrient solution circulates from a reservoir that pumps solution to the top end of the gutters, and a slight slope in the gutters (2%-3%) directs solution down the channel to a catch where water is fed back to the reservoir and recirculated. These gutters are supported by metal benches that are typically waist-high for worker convenience (Walters and Currey, 2015).

*Deep water culture (DWC).* Deep water culture (DWC), or deep flow technique (DFT), systems expose plant roots to a larger volume of solution compared to NFT systems typically in the range from 15 to 20 cm in depth. Plant roots are completely submerged and float within this nutrient solution (Doty, 2020). DWC systems support plants through floating rafts or boards laid across the solution surface that exclude light to prevent algae growth (Blok et al., 2017). DWC systems are designed as either closed or open systems. Newer DWC system designs leave a small space of air between plant roots and solution to increase oxygen uptake in plants (Blok et al., 2017).

The systems mentioned above differ fundamentally in the delivery of water and nutrients to the root zone, the root zone volume, and crop management and maintenance (Walters and Currey, 2018; Resh, 2013). These differences can impact growth as well as overall plant health and performance. Little research has been conducted on the persistence of pathogens in hydroponic systems (Riggio et al., 2019) and the potential of hydroponic systems to influence disease susceptibility.

## Management of hydroponic nutrient solution and water treatment technologies

*Nutrient management in hydroponics.* Management of the nutrient solution is an important and sometimes challenging aspect of growing plants hydroponically. Critical nutrient solution parameters, such as temperature, pH, E.C., and available nutrients for plant uptake, are

constantly changing and must be maintained within optimal growing ranges to produce healthy plants (Mattson and Peters, 2014; Resh, 2013). Applied nutrient solutions are often the primary, if not the sole supply, of mineral nutrients essential for plant growth in hydroponic production (Gómez et al., 2019; Resh, 2013; Sonneveld and Voogt, 2009). There are 12 mineral nutrients required for plant growth, which can be divided into macronutrients and micronutrients. Macronutrients accumulate in relatively large quantities in plant tissues, often ranging between 0.2% to 6.0% of leaf tissue dry weight and include: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S). Micronutrients accumulate in lower quantities often in the part-per-million range of leaf tissue dry weight and include: iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu), and molybdenum (Mo) (Resh, 2013; Sonneveld and Voogt, 2009).

When formulating hydroponic nutrient solutions, growers often customize the concentration of individual nutrients for the crop species. Straver and Sonneveld (1994) have provided a review of nutrient solution formulations standardized for individual crop species grown hydroponically and in soilless substrate; however, it is important to note that the concentration of nutrients in standardized solutions still require adjusting depending on cultural and management practices, as well as climate conditions (Sonneveld and Voogt, 2009). A general-purpose nutrient solution recommended by Mattson and Peters (2014) for hydroponic leafy greens consists of (in mg/L) 150 N, 16 P, 132 K, 38 Ca, 14 Mg, 2.10 Fe, 0.47 Mn, 0.49 Zn, 0.21 B, 0.13 Cu, and 0.08 Mo.

*Solution pH in hydroponics.* In addition to supplied nutrient concentrations**,** the solution pH is the next most important factor affecting plant nutrient uptake because of its effects on nutrient solubility (Islam et al. 1980; Mengel et al., 2001; Voogt, 1995; Voogt and Sonneveld,

1997). In hydroponic production the conventional pH range typically considered for nutrient solution is 5.5−6.5 (Savvas and Gruda, 2018). The optimum pH range for maximum growth will differ not only between plant species, but also between cultivar, and environmental, substrate, or nutrient solution conditions (Islam et al., 1980; Mengel et al., 2001). Solution pH values measure the proportion of hydrogen ions [$H^+$] or hydroxide ions [$OH^-$] present in solution, with a higher concentration of hydrogen ions indicating a solution is acidic, while increasing hydroxide ions signals a basic solution.

Nutrient solution pH influences the solubility of nutrients, particularly Fe, Mn, Zn, B, and Cu, and the availability for uptake by plant roots (Lindsay, 1979; Sonneveld and Voogt, 2009). An increasing pH decreases the solubility of metal micronutrients and can result in deficiency whereas a decreasing pH increases micronutrient availability and can result in toxicity (Lindsay, 1979; Sonneveld and Voogt, 2009). In solution, the microelements that become insoluble at a pH of >6.5 are Fe, Cu, Zn, B, Mo, and Mn. Chelating agents and specific nutrient solubility can play a factor in nutrient availability, however, outside the typical pH range for growing plants (pH 5.5 to 6.5), plants tend to exhibit growth inhibition and display specific nutrient disorders due to pH-dependent factors such as nutrient availability, ion antagonism, and/or precipitation of fertilizer salts. Still, research suggests that the direct effects of pH on plant growth is found only at the extreme ends of acidity and alkalinity, and that decreased plant growth outside of the typical pH range can usually be attributed to one or more of the aforementioned pH-dependent factors (Arnon and Johnson, 1942; Islam et al., 1980; Mengel et al., 2001; Vlamis, 1953).

*Water treatment technologies.* In addition to managing nutrients, controlling water-borne pathogens is another major aspect of managing the hydroponic solution. Water treatment technologies and extensive sanitation measures help but do not necessarily exterminate or

exclude pathogens from hydroponic systems, and once pathogens are in contact with plants, epidemics are difficult to contain (Sutton et al., 2006). A range of water treatment technologies are commercially available and commonly used to control water-borne pathogens and other microbes in irrigation lines (Fisher et al., 2009; Raudales et al., 2014). For maintaining hydroponic systems, sanitation measures should prioritize multiple control strategies to reduce outbreaks, such as filtration, chemical applications (chlorine, hydrogen peroxide and activated peroxygens, mefenoxam, biosurfactants, etc.), ultraviolet (UV) radiation, and heat sterilization (Raudales et al., 2014). Most technologies are suitable for ornamental greenhouse and nursery production, whereas fewer options are available for hydroponic production of food crops (Raviv et al, 2019; Riggio et al, 2019). The efficacy and adoption of water treatment technologies is still limited by factors such as a lack of *in vivo* pathogen studies, phytotoxicity thresholds, understanding of the relationship between pathogen inoculum level and disease incidence in irrigation water, and cost (Raudales et al., 2014).

The amount of water treatment needed may be influenced by hydroponic system type and design. In a study comparing hydroponic systems including DWC and NFT, incidental DNA checks on diseases revealed high levels of waterborne pathogens present, enough to require sanitization in all water-based growing systems (Blok et al., 2017). Systems that require lower volumes of water (e.g., NFT) can be disinfected through UV sterilization, however, this method does not result in complete pathogen control. This is due to the presence of particulate matter or debris in systems that spores can harbor in and hide from the UV rays. When using UV radiation, it is important to have proper filtration of solution at multiple points throughout the systems. Studies have shown that in hydroponic systems with recirculating nutrient solution, treatment of

the solution with UV radiation at doses sufficient to destroy *Pythium* spores gave little to no suppression of root rot (Sutton et al., 2006).

**Waterborne pathogens in hydroponics**

Waterborne plant pathogens are a major cause of reduced crop yield in hydroponics (Stanghellini et al., 1986; Hong et al., 2005) and have even prevented the successful production of certain leafy greens in parts of the U.S. (Albright, 2007). Waterborne pathogens can enter controlled-environment systems in the same manner as field-grown crops (Resh, 2013; Raviv et al, 2019), such as through contaminated ground or municipal water, non-sterilized equipment, contaminated seeds or plant material from other locations, employees and staff, as well as insects, animals, and wind (Sutton et al., 2006). Hydroponic nutrient solutions are typically recirculated (Bugbee, 2004; Pardossi et al., 2011), and once contaminated with pathogens, disease development is imminent. Symptoms of diseases caused by waterborne pathogens vary depending on the specific pathogen and plant host (Raudales et al., 2014), yet typically consist of root and stem rot and overall reduced crop growth and yield.

The waterborne pathogen *Pythium* is commonly present in irrigation water sources, including municipal and natural water sources. *Pythium* is also highly resilient to a wide range of pesticides and sanitizing chemicals, and spores can lay dormant for long periods of time until favorable environmental conditions occur (Martin and Loper, 1999). *Pythium* belongs to the family *Pythiaceae* of the class Oomycota, which are commonly described as fungus-like due to their filamentous growth, spore production, and detritivore diet (Sutton et al., 2006).

Most *Pythium* species are homothallic and do not require an opposite mating type to reproduce, making them quick root colonizers in aquatic environments (Lévesque, n.d.). During vegetative or asexual life cycle of *Pythium*, the pathogen produces thick-walled resting spores,

chlamydospores, sporangia (which can produce zoospores), and hyphal swellings (do not produce zoospores) (Lévesque, n.d.). The sexual life cycle of *Pythium* begins when an antheridium fertilizes an oogonium to produce a thick-walled oospore. These oospores then produce a sporangium, where asexual zoospores are produced within, and are released as motile zoospores once exposed to moisture. These zoospores are single cells with two lateral flagella that allow them to swim in solution and seek plant hosts. Zoospores display a preference for plant root zones, a mechanism known as chemotaxis, where a motile organism moves in a directed cellular migration along the concentration gradient of a chemoattracting substance (Fisher, 2009; Benjamin and Maheshwari, 2020). With *Pythium* zoospores, infection begins when a zoospore loses its lateral flagella, becomes a cyst, then encysts or adheres to the root surface, produces a germ tube, that will finally penetrate the cell wall of the root surface (Sutton et al., 2006). Sufficient post-penetration development of hyphae and mycelium allow the colony to function independently of the germinated spore (Sutton et al., 2006). Once the zoospores have infected the host, they lose their flagella and encyst to complete the life cycle.

The colonization of infected hydroponic plants by *Pythium aphanidermatum* and *Pythium dissotocum* is typically biotrophic in early stages and later develops into necrotrophic (Sutton et al., 2006). In the biotrophic stage, plant roots are colonized yet lack overt symptoms, a condition sometimes referred to as subclinical (Sutton et al., 2006). Yet in the necrotrophic stage, symptoms begin to appear as roots become discolored, generally in hues of brown, red, and yellow depending upon plant and pathogen species or isolate (Sutton et al., 2006).

The discoloration of tissue is associated with the accumulation of phenolic polymers, which can become bound to cell walls of plant root tissue (Sutton et al., 2006). Shoots of *Pythium*-infected plants may not immediately show symptoms of infection, however, they may

display stunted growth. Over time, plants may become severely stunted, leaves chlorotic, and in severe cases, complete plant death (Mattson, 2018). *Pythium* targets root tips, elongation zones, and young root hairs, yet are able to infect both un-wounded and wounded tissues, such as sites of lateral root emergence and tissue attacked by insects (Sutton et al., 2006). Therefore, monitoring insect and animal pest levels is a component of managing factors that increase plant disease susceptibility.

**Crop susceptibility and management of *Pythium* in hydroponics**

In recirculating hydroponic systems, all plants essentially share the same nutrient solution, which increases the spread and risk of water-borne plant pathogens. Water-mold pathogens such as *Pythium* are especially adapted to thrive in hydroponic conditions, and infection can cause significant crop losses in a relatively short time frame (Stanghellini and Kronland, 1986; Stanghellini and Rasmussen, 1994; Menzies et al., 1996; Stanghellini and Kim, 1998). Many species of *Pythium* are considered generalists, meaning they colonize a wide variety of plant species (Mattson, 2018). *Pythium* is opportunistic and takes advantage of plants under stress.

Environmental conditions favorable to *Pythium* include excessively high fertility (i.e., high solution EC), waterlogged substrates, low dissolved oxygen levels, and extreme temperatures (Mattson, 2018). *Pythium* is known to cause severe symptoms of root rot in a variety of crops when root zone temperatures are moderate to high (appx. 23-27°C) (Sutton et al., 2006). Temperature also has an effect on the life cycle of *Pythium*, such as the production, dispersal, and germination of zoospores, oospore germination, and infection processes (Sutton et al., 2006). During the warm summer months, growers may struggle to maintain adequate dissolved oxygen levels (above 4 $mg \cdot L^{-1}$ $O_2$), making *Pythium* a common threat to production in

hotter climates nation-wide. Low $O_2$ concentrations in hydroponic nutrient solution can result in decreased root growth, leading to limited nutrient absorption, and a weaker, disease-susceptible plant.

Common water treatments to manage *Pythium* in horticulture include the use of sanitizing chemicals such as bromine, chlorine gas, sodium hypochlorite, calcium hypochlorite, chlorine dioxide, ozone, activated peroxygen, and copper ionization, as well as filtration, ultraviolet (UV) radiation, and heat treatment or pasteurization (Fisher, 2009; Sutton et al., 2006). Examples of pesticides include propamocarb hydrochloride (Previcur®N), metalaxyl (Ridomil®), and electrolytically generated copper and cupric sulfate (Sutton et al., 2006). Although effective, these treatment options do not provide reliable control over water-borne pathogens like *Pythium*.

Reducing the hydroponic solution temperature is an effective strategy to reduce root infection by warm-weather *Pythium* species, such as *P. aphanidermatum*. A target root zone temperature of 68 to 75 ºF (20 to 24 ºC) is recommended for the control of *Pythium aphanidermatum* which is known to cause severe symptoms of root rot in a range of crops when root zone temperatures are above 25 ºC (Sutton et al., 2006). Lower solution temperatures influence pathogenicity of *Pythium* by disrupting the life cycle stages of *Pythium*, the germination and dispersal of zoospores and oospores, and infection processes (Sutton et al., 2006). Decreasing solution temperature also increases the amount of dissolved oxygen, which promotes root health and also reduces infection.

Recent research with hydroponic basil suggests maintaining a low solution pH can reduce *Pythium* infection of roots. Current recommendations for hydroponic crops suggest maintaining a solution pH of ~ 6 to maintain adequate nutrient solubility and uptake. However, growing plants at solution pH below 5.0 resulted in decreased *Pythium* infection of roots, but plants grown at pH

below 4.5 had stunted growth (Gillespie, 2019). In addition to recent research on pH, further work is needed to understand the role of nutrient concentration in plant disease resistance.

**Role of plant nutrition in prevention of diseases**

Researchers generally agree that certain micronutrients have a role in preventing disease (Bugbee, 2004; Marschner, 2012; Dordas, 2008), but research on this topic is limited and nutrient roles are not well-established, particularly for hydroponic production (Sonneveld and Voogt, 2009). Of the micronutrients that stimulate plant defense mechanisms, those involved in phenolics and lignin biosynthesis are perhaps best understood (Marschner, 2012)—mainly manganese (Mn), boron (B), and copper (Cu). However, others such as iron (Fe) and zinc (Zn) may also be directly or indirectly involved with resistance to pathogenicity. Copper has also been a known fungicide since the 1880's (Agrios, 2005; Raudales et al., 2014) and an algaecide since the early 1900's (Flemming and Trevors, 1989; Thurman et al., 2009; Raudales et al, 2014), however, is phytotoxic at high concentrations and can reduce plant growth (Raudales et al., 2014). All micronutrients at excessive concentrations in the root zone can be toxic to plants, from either toxic accumulation in tissues or competing with uptake of other nutrient ions (Langenfeld et al., 2022).

Silicon (Si) is not considered an essential plant nutrient, yet is abundant in soils and is known to reduce pathogenicity from foliar diseases for a range of crop species (Bugbee, 2004; Cherif et al., 1994; Winslow, 1992; Samuels, 1991; Marschner, 2012). However, little is known regarding Si effects on infection of root rot pathogens like *Pythium*. Overall, the effects of micronutrient and Si concentrations on plant health, and their role in disease prevention, are understudied in CEA hydroponic production with leafy greens.

**Knowledge gaps and future directions**

Plant nutrition has a role in the prevention of pathogen infection in horticulture (Duffy and Défago, 1999; Marschner, 2013; Sonneveld and Voogt, 2009), although the potential for controlling nutrients to suppress disease in hydroponics is generally not well-understood. Past research has evaluated macronutrient effects on disease suppression in soilless culture (Duffy and Défago, 1999; Sonneveld and Voogt, 2009), particularly nitrogen (ammoniacal and nitrate nitrogen), phosphorus, and calcium. However, hydroponic growers would likely control macronutrient concentrations to optimize crop yield and quality rather than manage disease risks.

Metal micronutrients and the beneficial element silicon (Si) are known to influence the pathogenicity of certain diseases (Bugbee, 2004; Duffy and Défago, 1999; Langenfeld et al., 2022; Sonneveld and Voogt, 2009; Zhang et al., 2021), where the effects of root zone copper (Cu) and Si are best understood. Raudales et al. (2014) reported Cu can be increased to 5 $mg \cdot L^{-1}$ to control *Pythium* and without causing phytotoxicity for some species. Bugbee (2004) found evidence suggesting insufficient manganese (Mn) supply increased disease severity from *Pythium* with hydroponic lettuce. Supplementing hydroponic solutions with Si is a well-established strategy for suppressing foliar diseases such as powdery mildews and leaf spot (Langenfeld et al., 2022; Voogt and Sonneveld, 2009). In addition to mitigating disease, research from Langenfeld et al. (2022) as well as anecdotal evidence from industry also suggest increases in metal micronutrient concentrations [including iron (Fe), Mn, Cu, and zinc (Zn)] and supplementation with Si can substantially increase nutrient uptake and promote plant growth in soilless culture/hydroponics.

Langenfeld et al. (2022) suggested increasing micronutrient concentrations in solution as well as supplementing with silicon (Si) as an effective and low-risk strategy to suppress *Pythium*

in hydroponic lettuce. However, few studies have investigated the effects of increasing micronutrients other than Cu. Also, the effects of Si on suppression of root rot pathogens such as *Pythium* are not well understood. To better determine whether the Langenfeld et al. (2022) recommendation will be effective for commercial hydroponic growers, future research directions should focus on evaluating metal micronutrient and Si concentrations on plant growth and susceptibility to *Pythium* root rot.

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# CHAPTER 2. *PYTHIUM* CULTURE AND INOCULATION IN DEEP WATER CULTURE HYDROPONIC SYSTEMS


## Abstract

*Pythium* root rot is a waterborne pathogen ubiquitous in hydroponics and is a primary grower concern in production. The study of *Pythium* species (spp.) is commonly contained to *in vitro* research, leaving knowledge gaps in the practical application of *in vivo Pythium* effects on controlled environment crops. Modern protocols for *Pythium* spp. inoculum strategies exist, however, are not always applicable or successful in hydroponic research when implemented. *Pythium* spp. pathogenicity differs depending upon plant host and environmental conditions, therefore, the selection of *Pythium* spp. strain is of great importance when screening plants for disease development. The objective of this review is to provide a detailed standardized protocol for *Pythium* culture and inoculation in hydroponic deep-water culture with leafy green vegetables.


## Introduction

The most destructive pathogens in hydroponic production belong in the Oomcoyta class, which produces motile zoospores that thrive in aquatic environments. The oomycete *Pythium* spp. is of particular concern to CEA growers as the pathogen seeks out roots through chemotaxis and after colonization, can spread throughout a hydroponic system, resulting in mass root-rot and stunted root rot. Several factors make the development of standardized methods for testing *Pythium* effects on plant growth in hydroponics challenging. Multiple *Pythium* species are often found in commercial hydroponic systems, with the most widely reported being *P. aphanidermatum, P. ultimum, P. dissotocum,* and *P. myriotylum* (Raudales and McGehee, 2016; Gillespie, et al., 2020). *Pythium* species and strains differ in pathogenicity towards certain crops

(Raudales and McGehee, 2016; unpublished data *R. Dickson*), and therefore it is important to match the appropriate species and strain with the target crop being tested. In addition, several environmental and cultural parameters are known to influence plant susceptibility to *Pythium* pathogenicity in hydroponics including nutrient solution and air temperature, pH, dissolved oxygen, solution flow rate, and root zone electrical conductivity (Gillespie, et al., 2020; Mattson, 2018; Raudales and McGehee, 2016).

A series of protocol development studies were conducted with objectives to identify (1) the appropriate *Pythium* species, strain, and oospore concentration needed for pathogenicity towards 'Rex' lettuce, (2) the effects of certain hydroponic cultural practices on pathogenicity, and (3) appropriate techniques for culturing *Pythium* in the laboratory, preparing inoculum solution, and inoculating hydroponically grown lettuce in the greenhouse. The goal was to develop standardized protocols for use in small-scale hydroponic research by conducting protocol development studies and modifying existing *Pythium* methodologies from other research groups.

**Summary of *Pythium* species and strain protocol development in the laboratory**

*P. myriotylum* 'PM1'and *P. aphanidermatum* 'KOP8' isolates were obtained from Dr. Rosa Raudales program at the University of Connecticut, where experimentation showed the cultivar 'Rex' lettuce was especially susceptible to *Pythium* strains 'PM1' and 'KOP8' (Raudales et al., 2016) (Figure 2-1). The species strains *P. aphanidermatum* 'S18' and 'M18' isolates were received from Dr. Sean Toporek and Dr. Anthony Keinath from Clemson University. The strains *P. aphanidermatum* '196-1' and '197-2' isolates were received from UC Davis from Dr. Johanna del Castillo and Dr. Cassandra Swett. The remaining species *P. aphanidermatum* 'AZ3' was

received from the University of Arkansas from Dr. Jim Correll and was selected for experimentation due to disease severity in leafy greens.

Protocols for *Pythium* dosing and culture were modified from Dan Gillespie's graduate research at The Ohio State University and from Gillespie et al. (2020), as well as from Dr. Rosa Raudales and Cora McGhee's experiments with *Pythium* species and lettuce at the University of Connecticut. Gillespie's protocol called for culturing *Pythium* using grass blades to encourage mycelial growth, however, it was found that by using a liquid V8 rich media for cultures, *Pythium* mycelium readily grew and produced thick mats. Sterilized liquid V8 media allowed for the standardization of a medium for growing *Pythium* cultures in, without relying on the need for plant clippings. Additionally, in Gillespie's work, zoospores were counted on the hemocytometer for determining *Pythium* spore concentration. However, under the microscope, zoospores were motile, reducing counting accuracy and were not readily produced. Therefore, viable *Pythium* oospores, the sexual spores that can germinate and produce sporangia, were counted using a microscope and hemocytometer to calculate spore concentration in experiments (Figure 2-2).

It was found that *Pythium* grows most readily on Potato Dextrose Agar (PDA) and PDA + Ampicillin, compared to CMA-PARP+B (corn meal agar - pimaricin, ampicillin, rifampicin, pentachloronitrobenzene + benomyl) media. After *Pythium* plugs were plated onto V8 media, it was found that final spore concentrations increased as incubation periods increased. It was observed that plates grown for 14 days in nutrient rich V8 media and then shocked with a replacement of V8 media with deionized water for 7 days produced thicker mycelial mats than plates grown for only 7 days in V8 media and shocked for 7 days with deionized water. Plates were then grown for 21-28 days after plating and produced ample mycelial mats and oospores. From Dr. Raudales and McGhee's protocol, it was found that blending and incorporating

mycelium into *Pythium* inoculum, rather than only using liquid from *Pythium* plates resulted in increased oospore concentrations. Root cultures and isolations are needed in order to determine the presence of *Pythium* in or on the root hair. Through observation, it was found that cleaning root tissue samples with ethanol reduced *Pythium* growth on PDA plates. This is possibly due lettuce's hair-like root structure and ethanol over-sterilizing root contents (*data not shown*). Therefore, ethanol washes were switched to sterilized deionized water washes prior to plating. Furthermore, it was found that burrowing root tissue cultures into PDA media (~1 cm) with a scalpel and forceps lessens bacterial infection, yet still allows adequate *Pythium* growth. This is likely due to a larger portion of the root being influenced by the antibacterial properties of PDA media, resulting in minimized bacterial contamination.

**Summary of hydroponic protocol development in the greenhouse**

Several *Pythium* species and strains were evaluated at different concentrations for effects on pathogenicity towards 'Rex' lettuce. *Pythium* pathogenicity towards spinach was also evaluated for comparison to lettuce, as severe *Pythium* infection and crop loss in hydroponic leafy green crops is a nation-wide commercial problem and widely reported in literature (Raudales and McGehee, 2016; Gillespie, et al., 2020; Stanghellini et al., 1986; Hong et al., 2005).

*Experiment 1: Pythium strain and species effects on 'Rex' lettuce.* An experiment looked at evaluating *Pythium* species/strain oospore production and concentration on plant growth in hydroponic 'Rex' lettuce. Seven *Pythium* species and strains were used in the experiment: *P. myriotylum* 'PM1' and 'M18', and *P. aphanidermatum* 'S18', 'KOP8', 'AZ3', '196-1', and '197-2'. One hydroponic unit consisted of one *Pythium* dose treatment, with six observational units (lettuce plants) each. Data was recorded on each individual plant in a system and averaged

per treatment. Initially, mixtures of *Pythium* species in the inoculum were tested in a previous experiment as a strategy to increase *Pythium* pathogenicity in lettuce, however, difficulty discerning *Pythium* species effects at the end of the experiment resulted in the decision of using only one species when inoculating plants. Spore concentrations were optimized for the highest concentration practically achievable, using the same *Pythium* inoculum methods and procedures between treatments. This resulted in uneven spore concentrations across treatments, however, reflected *Pythium* species and strain culture viability and spore production in the laboratory.

Concentrations tested for each *Pythium* strain and species are as follows in Figure 2-3: $3.75 \times 10^3$ zoospores·mL$^{-1}$ (*P. aphanidermatum* 'KOP8'), $1.25 \times 10^3$ oospores·mL$^{-1}$ (*P. aphanidermatum* 'S18'), $5.38 \times 10^4$ oospores·mL$^{-1}$ (*P. myriotylum* 'M18'), $7.5 \times 10^3$ oospores·mL$^{-1}$ (*P. aphanidermatum* 'AZ3a'), $2.88 \times 10^4$ oospores·mL$^{-1}$ (*P. myriotylum* 'PM1'), and $3.75 \times 10^3$ zoospores·mL$^{-1}$ (*P. myriotylum* 'PM1'). The dominant spore type present during hemocytometer counts were used to calculate spore concentration (oospores or zoospores).

Data collected on each of the six plants per treatment and averaged included leaf SPAD chlorophyll index, fresh leaf mass, and fresh root mass. It was found that *P. myriotylum* strains tended to increase leaf SPAD compared to *P. aphanidermatum* strains and control (Figure 2-4). An increase in leaf SPAD or darkening of leaves has been found in previous research to be a primary aboveground symptom identified in infected crops (Bowden and Rouse, 1991a; Johnstone, M.B., 2001). Root and shoot biomass were reduced by both species, *P. myriotylum* and *P. aphanidermatum*, however, *P. myriotylum* treatments had a greater reduction in root mass (Figure 2-5) (Jatho et al., 2022). Compared to the control, shoot biomass was not significantly reduced by *P. aphanidermatum*, while *P. myriotylum* caused a significant reduction (Figure 2-5) (Jatho et al., 2022). Based on Experiment 1, it was decided that *P. myriotylum* 'PM1' would be

the selected strain of interest for experiments with hydroponic lettuce, due to consistent spore production, culture viability, and pathogenicity to lettuce cultivar 'Rex'.

*Experiment 2: Pythium myriotylum 'PM1' effects on hydroponic lettuce and spinach.* An experiment looked at the effects of *Pythium myriotylum* 'PM1' spore concentration based on Experiment 1 findings on hydroponic lettuce and spinach growth and disease susceptibility. A 2 × 5 factorial experiment with two factors plant species ('Rex' lettuce and spinach) and *Pythium* spore concentration was conducted using a split plot design. *Pythium* treatments were the whole plot factor and plant species a split-plot factor. *Pythium* spore concentration was $4.5 \times 10^3$ oospores·mL$^{-1}$ and was increased at levels 0× (control solution, or no *Pythium*), 1×, 2×, 3×, and 4× of the base *Pythium* inoculum solution. This study was largely unsuccessful, as complete plant death was observed in *Pythium* levels as low as the 1× spore concentration in spinach, while lettuce plants treated with *Pythium* displayed little to no visible growth stunting at the 1× level (Figure 2-6). This indicated that at *Pythium* spore concentrations that resulted in total plant death in spinach (Figure 2-6B), minimal to no disease symptomology was observed in lettuce (Figure 2-6A). For this reason, a final experiment was conducted that looked at the effects of multiple *Pythium* species/strains on lettuce and spinach plant growth and disease susceptibility, in order to determine a strain and concentration that could be standardized between both plant species.

*Experiment 3: Effects of Pythium species and strain on hydroponic lettuce and spinach.* In order to conduct a rapid screening, hydroponic systems were replaced with saucers containing a standard hydroponic nutrient solution and *Pythium* treatment to determine *Pythium* species and strain effects on hydroponic lettuce and spinach plant growth and susceptibility. A 2 × 4 × 4 factorial experiment with factors of two plant species (lettuce and spinach), four *Pythium* strains

(*P. aphanidermatum* 'KOP8', *P, myriotylum* 'PM1', *P. myriotylum* 'M18', and *P. aphanidermatum* '197-2'), at four levels (0, 2.25 × $10^3$, 2.5 25 × $10^4$, and 2.5 25 × $10^5$ oospores·$mL^{-1}$). Treatments were arranged using a split-plot design with *Pythium* treatments as the whole-plot factor and plant species as a split-plot factor. There were two replications for each treatment, with two lettuce and two spinach plants in one saucer, for a total of four plants per saucer. This experiment had similar results to Experiment 2, where spinach had greater susceptibility to *P. myriotylum* 'PM1' compared to lettuce. Spinach plants experienced permanent wilting and complete plant death for all *Pythium* treatment combinations, while lettuce showed mild to no symptoms of disease infection. Due to the significant differences in *Pythium* pathogenicity and plant disease susceptibility between plant species, experimentation proceeded only on lettuce, with spinach being removed entirely.

*Additional research findings*. Through experimentation, we found dosing *Pythium* into hydroponic solution resulted in decreased disease infection compared to dosing *Pythium* into the rockwool growing substrate (Grodan AO Plugs, 24mm x 40mm, 10 x 10 hole, 1" plug, 200 cubes/sheet, Rockwool, Hedehusene, Denmark). One experiment looked at the differences between dipping rockwool cubes (Grodan AO Plugs, 24mm x 40mm, 10 x 10 hole, 1" plug, 200 cubes/sheet, Rockwool, Hedehusene, Denmark) into *Pythium* inoculum solution versus dosing into systems, where neither was found more conducive to disease infection.

Growing 'Rex' lettuce plants in a cold (20.0 – 22.8°C) versus hot (22.8 – 25.6°C) greenhouse also revealed that increased ambient air and solution temperature in systems resulted in increased overall plant stress and greater *Pythium* infection in plants. Previous literature has reported increased temperatures in greenhouses can result in increased disease symptomology,

likely due to decreased dissolved oxygen levels and plant stress from environmental parameters (Gillespie, et al., 2020; Mattson, 2018; Raudales and McGehee, 2016).

Initially, a recirculating DWC system was used for experimentation, however, after comparison studies between the recirculating DWC with two reservoirs versus a non-recirculating DWC with one reservoir, it was found that the non-recirculating DWC system resulted in increased *Pythium* infection and root browning, possibly due to issues is spore distribution within the system and increased solution volume and buffering capacity. It was found that 'Rex' lettuce plants transplanted into DWC hydroponic systems most susceptible to *Pythium* infection at a younger stage of development (<14 days from germination).

**Protocol for culturing *Pythium* and inoculation**

*Laboratory Pythium culture.* To create stock plates, *Pythium myriotylum* 'PM1' isolates were obtained from Dr. Rosa Raudales and Cora McGehee (University of Connecticut, CT), and isolate plugs were plated on potato dextrose agar (PDA) media in a sterile petri dish and sealed with parafilm. Plates were placed in an incubator (25°C) and allowed to develop mycelia (3 days colony development). After colonies were formed, stock plates were used to replicate *Pythium* by transferring plugs to plates with PDA media needed for the inoculum (Figure 2-7A).

From the stock plates, PDA plugs (4 cm × 4 cm) were transferred into 60 sterile 100 x 15mm polystyrene disposable Petri dishes (VWR International, Radnor, PA) at six plugs per dish. 10% liquid V8 (Campbell Soup Company, Camden, NJ) media solution was then dispensed into each of the 60 petri dishes at 10 mL per dish, sealed with parafilm, and allowed to incubate under LED lights (Figure 2-7B).

After 14 days and when mycelial mats had formed, plates were washed by pipetting out old V8 liquid media from the plates, and solution was replaced with 10 mL of sterile deionized

water per plate. Washes were repeated three times for each plate. Once all plates were washed, plates were sealed with parafilm and placed back under the LED lights to incubate for 7 days inducing starvation to promote spore development. After 24 days of total culture preparation, plates are ready to be made into *Pythium* inoculum.

*Pythium inoculum solution preparation.* After the incubation period, from the 60 Petri dishes, mycelial mats and solution were transferred into 50 mL centrifuge tubes with 3mm sterilized glass beads for maceration. Tubes were placed on a vortex and mycelial mats were macerated for 2-3 minutes each, or until mats were blended. Mycelium were then strained from the blended *Pythium* solution and cumulated into a large glass container. The container was placed on a stir plate with a magnetic stir bar and set to stir at a low rotation. From the top of the *Pythium* inoculum, 10µL of oospore solution was pipetted and dispensed into a sterile hemocytometer with glass cover slip.

A 20× microscope was used to count the oospores from the four corner grid sections (1-$mm^2$ diameter) in both wells of the hemocytometer (i.e., total oospores in eight grids). This was repeated 20 times, averaged, and divided by eight to estimate total oospore count per grid section. Total oospore count per grid section was multiplied by $10^4$ to calculate oospores per mL. Between Thesis experimental runs, final inoculum concentrations were $7.60\times10^3$ [Expt. 1], $7.5\times10^3$ [Expt. 2], and $9.5\times10^3$ [Expt. 3] oospores·$mL^{-1}$, for an average spore concentration of $8.2\times10^3$ oospores·$mL^{-1}$.

*Isolating Pythium from root cultures***.** During experimentation, lettuce root samples were collected in order to re-isolate *Pythium* and confirm the presence of *Pythium* in roots. This allowed us to confirm the symptomology seen in plants was due to *Pythium* infection. For root isolations, a 2.5 cm section of root showing symptomology approximately 5.0 - 7.5 cm from the

root tip was obtained from an infected plant, at one root section per plant. The root was placed into a sterile plastic bag and transported to laminar flow hood for isolation. There, sterile deionized water was dispensed into a clean petri dish, and sterile PDA plates were collected. Using sterilized tools, the root was cut into 1-2 cm sections, and washed for 30 seconds in sterile water. Washed root cuttings were then placed on sterile paper towels and allowed to dry for 10-15 minutes, meanwhile checking that roots did not overly dry. Using a clean PDA plate and scalpel, a 2-4cm incision was made in the center of the media, and root was buried within the incision to decrease contamination and bacterial growth, as opposed to placing root directly on top of media. PDA plates were then closed, sealed with film, and allowed to incubate in a 25ºC growth chamber, with daily checks for mycelium growth (Figure 2-8). Once *Pythium* mycelium was observed, it would be placed under a microscope to confirm *Pythium* growth from root isolation.

*Procedures for eliminating contamination and purifying Pythium isolation.* *Pythium* readily grows in the absence of competing pathogens and bacteria, however, has slower growth compared to certain bacteria, and is often outcompeted, resulting in cultures becoming contaminated if not monitored (*data not published*). In the case of bacterial contamination (Figure 2-7C.), plugs or mycelium were transferred from clean sections of a contaminated *Pythium* plate to a media containing anti-bacterial solutions, such as PDA + Ampicillin or PDA + Streptomycin. Plates were allowed to grow in an incubator at 25ºC and checked within 12 hours. At the first sign of mycelial growth, plugs or mycelium were transferred to a new PDA plate until contamination was eliminated. The most common type of contamination encountered was fungal contamination with *Fusarium*. To remove or suppress contaminants on plates, the steps above were followed, with media changed to CMA-PARP+B.

*Pythium inoculation and dosing into hydroponic systems.* Before dosing, solution in hydroponic systems were adjusted to a pH of 5.5-6.5±0.05 using 1 *N* $H_2SO_4$ and 1 *N* NaOH and allowed to equilibrate. *Pythium* inoculum was placed in the experimental greenhouse with stir bar and plate set to a low circulation for one hour to bring solution to ambient temperatures before dosing into systems. After solution has reached equilibrium with ambient temperatures, 10mL of stock *Pythium* inoculum was dosed into mineral wool substrate for each plant to assure oospores delivered to each plant. A target of $4.5x10^3$ oospores per plant was used for experiments. For more information on hydroponic systems, see chapter 3.

**Conclusions**

In order to adequately analyze plants for disease susceptibility to *Pythium* root rot disease, it is vital to have a developed approach when selecting a pathogenic and reliable *Pythium* species and strain per plant species. Research and protocol development over the effects of *Pythium* spp. on hydroponic lettuce indicate *P. myriotylum* species have an increased pathogenicity to lettuce cultivar 'Rex'. Additionally, environmental conditions such as temperature, dissolved oxygen levels, and hydroponic nutrient solution can alter plant disease susceptibility. When screening plants for disease susceptibility or tolerance, it is important to understand the interactions between plant species, environmental conditions, *Pythium* spp. or strain, and spore concentration.

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Figure 2-1**.** ‘Rex’ lettuce (*Latuca sativa* L.) roots infected with *Pythium.* Severity of *Pythium* infection and root lesions increases from left to right.

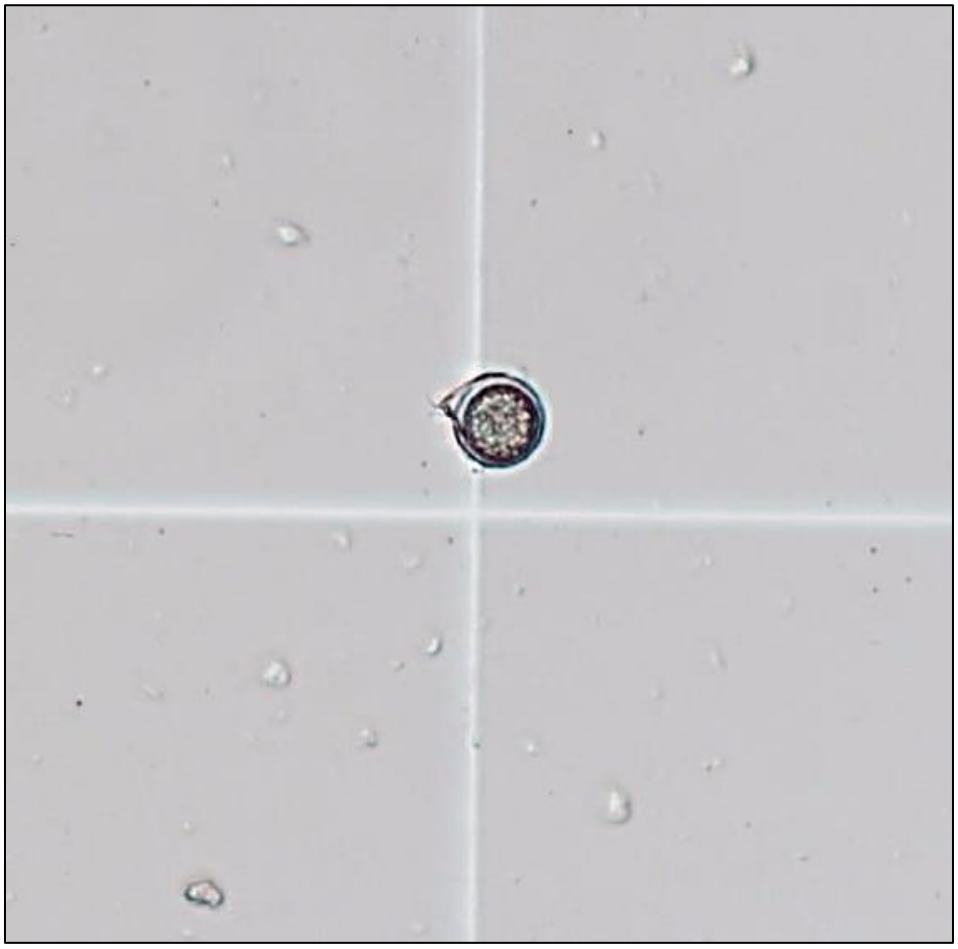


Figure 2-2**.** A *Pythium myriotylum* ‘PM1’ oospore under a microscope on a hemocytometer grid during spore concentration counts. The oospore is a highly resilient sexual structure that produces sporangia and finally zoospores

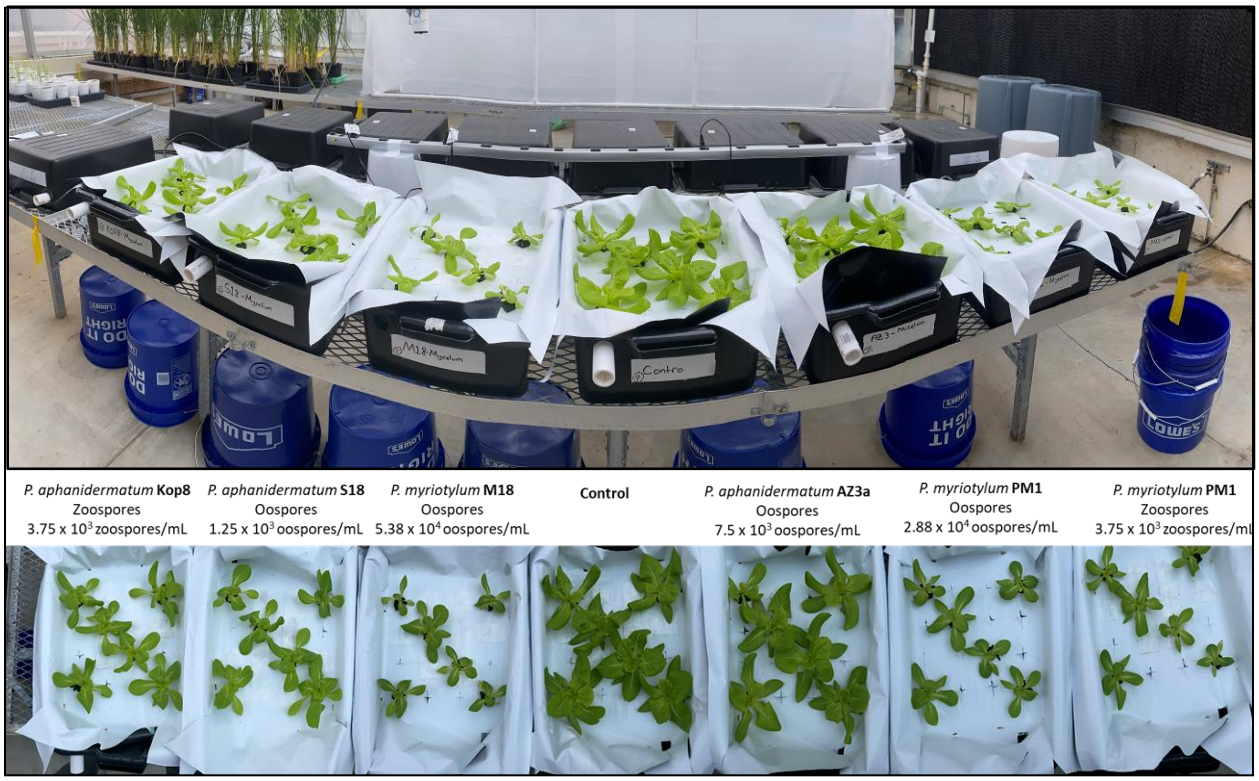



Figure 2-3. Effects of *Pythium* species and strain on plant growth in hydroponic 'Rex' lettuce. *Pythium* species and strains from left to right: *P. aphanidermatum* 'KOP8', *P. aphanidermatum* 'S18', *P. myriotylum* 'M18', *P. aphanidermatum* 'AZ3a', *P. myriotylum* 'PM1' (oospores and zoospores). The dominant spore type present during hemocytometer counts were used to calculate spore concentration (oospores or zoospores).

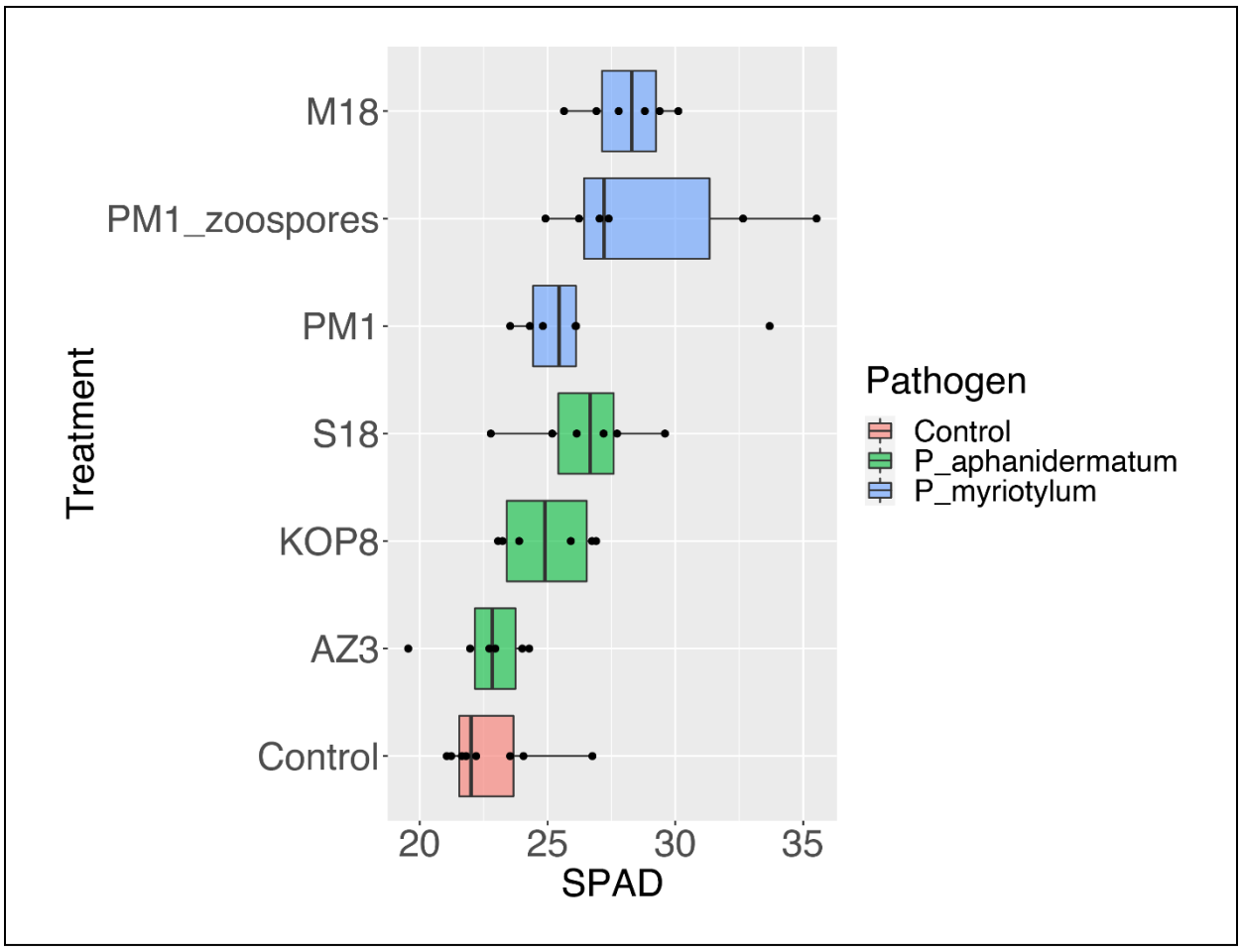



Figure 2-4. Effects of *Pythium* species and strain on hydroponic 'Rex' lettuce leaf SPAD chlorophyll content. *Pythium* species or control indicated by legend on the right where red color indicates the control, green color *P. aphanidermatum*, and blue color *P. myriotylum*. *Pythium* species and strains from top to bottom: *P. myriotylum* 'M18', *P. myriotylum* 'PM1' (oospores and zoospores), *P. aphanidermatum* 'S18', *P. aphanidermatum* 'KOP8', and *P. aphanidermatum* 'AZ3'.

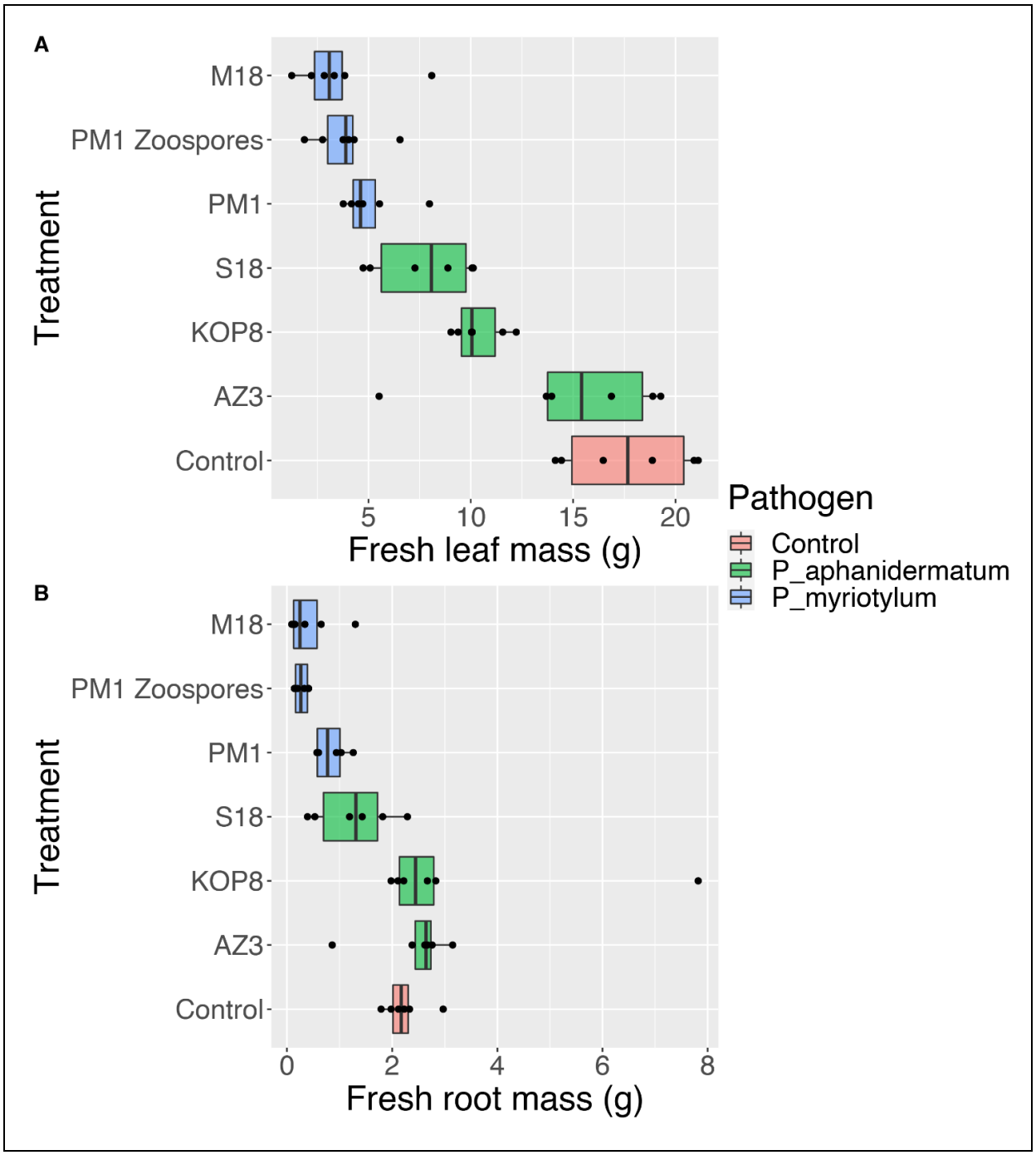
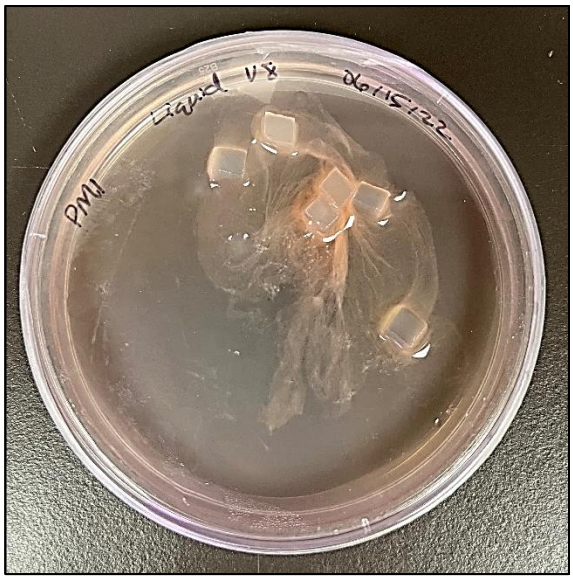



Figure 2-5. Effects of *Pythium* species and strain on hydroponic 'Rex' lettuce fresh leaf and root mass. *Pythium* species or control indicated by legend on the right where red color indicates the control, green color *P. aphanidermatum*, and blue color *P. myriotylum*. *Pythium* species and strains from top to bottom: *P. myriotylum* 'M18', *P. myriotylum* 'PM1' (oospores and zoospores), *P. aphanidermatum* 'S18', *P. aphanidermatum* 'KOP8', and *P. aphanidermatum* 'AZ3'.


Figure 2-6. *Pythium myriotylum* strain ‘PM1’ effects on hydroponic ‘Rex’ lettuce and spinach at 1× spore concentration level ($4.5 \times 10^3$ oospores·mL$^{-1}$). Figure 2-6A depicts lettuce plants dosed with *Pythium* (1× concentration) with green foliage and little to no growth stunting, while Figure 2-6B depicts spinach plants dosed with *Pythium* (1× concentration) with permanently wilted shoots and severely stunted growth.

**A.**

**B.**




**C.**

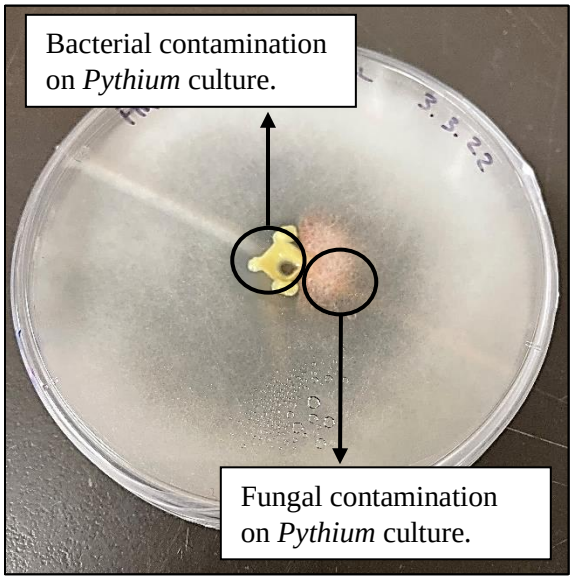



Figure 2-7**.** Healthy *Pythium myriotylum* 'PM1' growth on PDA media (a) and the liquid media stage (b). Bacterial and fungal growth in *Pythium myriotylum* 'PM1' mycelium (c). The circle on the left of Figure 2-7C. outlines bacterial contamination on a 'PM1' culture. The circle on the right of Figure 2-7C. outlines fungal contamination of *Fusarium* on a 'PM1' culture.

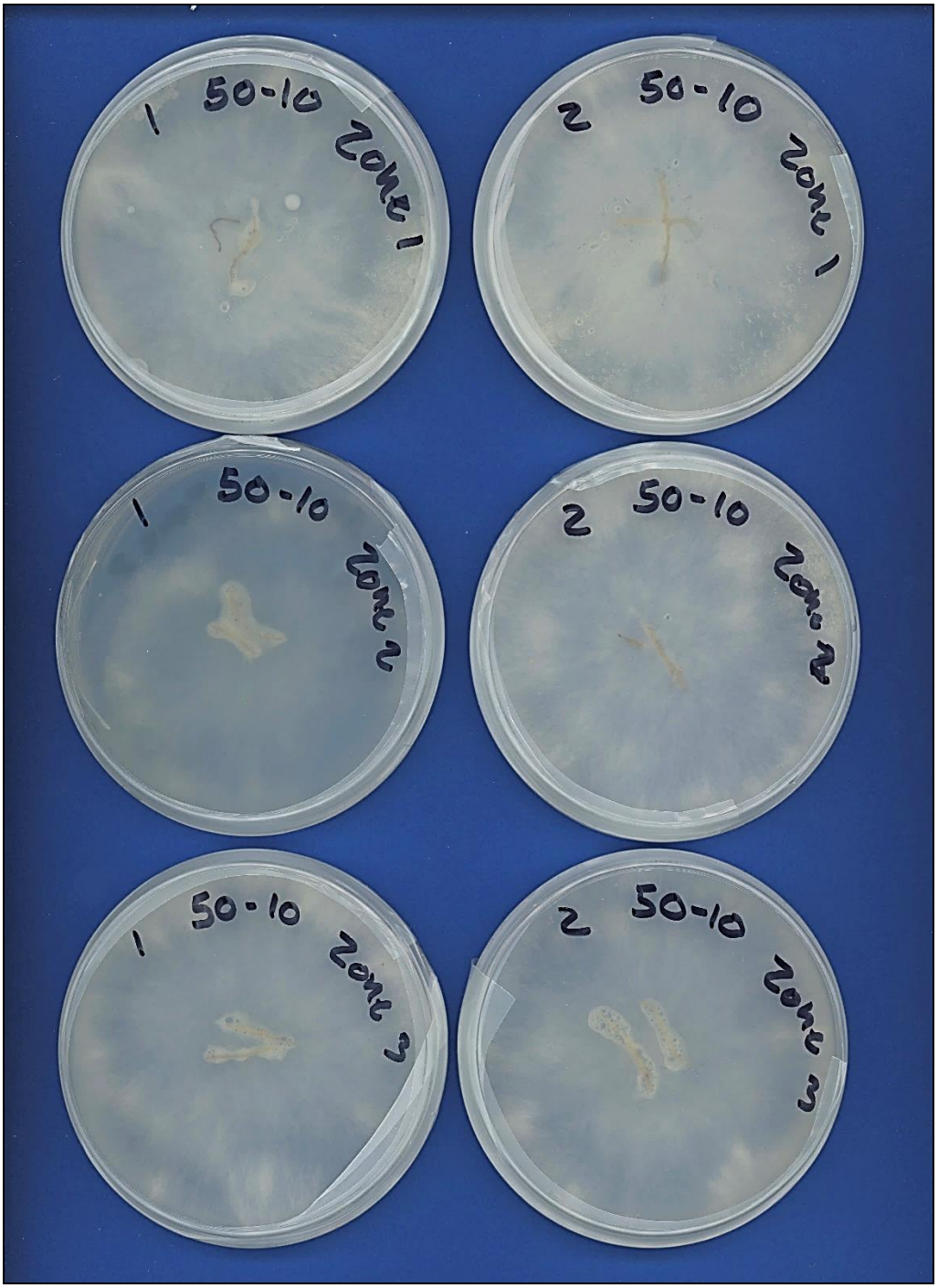



Figure 2-8. Examples of 'Rex' lettuce root isolations with *Pythium* grow out on PDA plates to confirm *Pythium* presence in roots. Root cuttings were taken from plants infected with *Pythium myriotylum* 'PM1'.

# CHAPTER 3. EFFECTS OF MICRONUTRIENT AND SILICON CONCENTRATION ON LETTUCE GROWTH AND SUSCEPTIBILITY TO *PYTHIUM*


## Abstract

Objectives were to evaluate the effects of increasing metal micronutrient (Experiment 1) and silicon (Si) (Experiment 2) concentrations on 'Rex' lettuce growth and susceptibility to *Pythium* root rot in hydroponics. In the first experiment, lettuce was grown in hydroponic solutions with metal micronutrients iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) supplied at either 0, 2.5, 5, and 10 $mg \cdot L^{-1}$. A standard commercial hydroponic solution was also included as a control, with metal micronutrients supplied at (in $mg \cdot L^{-1}$) 0.2 for Cu, 2.0 for Fe, 1.0 for Mn, and 0.5 for Zn. In the second experiment, hydroponic lettuce was grown with Si at 0, 7, 14, 28, and 56 $mg \cdot L^{-1}$. Hydroponic treatments solutions for both experiments were either dosed with *Pythium myriotylum* (*Pythium* treatment) at $1.80 \times 10^4$ oospores per L or deionized water as a non-*Pythium* control. Data were collected on leaf SPAD chlorophyll content, shoot height and width, total plant fresh mass, percent change in lettuce growth, and root disease severity. Increasing solution Cu decreased *Pythium* disease severity, but reduced lettuce growth and yield, likely from Cu being both an effective fungicide and phytotoxic when supplied at higher concentrations. Increasing the concentration of other metal micronutrients also tended to reduce lettuce growth and increase disease severity. Metal micronutrients supplied at 0 $mg \cdot L^{-1}$, particularly Fe and Zn, resulted in the greatest reduction of plant growth in this experiment, especially in the presence of *Pythium*. Results of this study showed that metal micronutrients supplied at concentrations near those found in conventional hydroponic solutions for leafy greens would result in near optimal lettuce growth, and substantial deviations in micronutrient concentrations can reduce yield. For example, a full-strength Hoagland's solution (in $mg \cdot L^{-1}$) 0.5

for Cu, 2.0 for Fe, 1.0 for Mn, and 0.5 for Zn would provide optimal growth. In contrast to previous reports in the literature, increasing solution Cu was not shown to be a commercially viable strategy to mitigate *Pythium* root disease in hydroponic lettuce as disease control did not outweigh yield reduction. However, supplementing with 14 $mg \cdot L^{-1}$ Si may reduce risks of foliar diseases and micronutrient toxicity in lettuce. Overall, successful mitigation of root rot pathogens in commercial hydroponic production requires a combination of proper sanitation, best management and cultural practices, appropriate hydroponic system design, and implementation of a water treatment system with proper design and multi-barrier approach.

## Introduction

Water-borne pathogens such as *Pythium* spp. are major causes of root rot disease and crop loss in hydroponic production (Gillespie, 2020; Gull, 2002; McGehee and Raudales, 2021; Sutton et al., 2006). *Pythium* spp. are oomycete pathogens that produce highly resilient oospores as well as motile zoospores (Sutton et al., 2006; McGehee and Raudales, 2021), which have the ability to persist in and spread rapidly through recirculating hydroponic solutions. Commercial growers aim to mitigate pathogen entry into hydroponic systems using a range of preventative measures and proper sanitation (Jensen and Collins, 2011; Stranghellini, 1996), as well as limit pathogen distribution in nutrient solutions using multi-barrier water treatment approaches involving combinations of chemical (chlorine, bromine, chlorine dioxide, ionized copper, copper salts, ionized silver, ozone, hydrogen peroxide, and peroxyacetic acid), non-chemical or physical (filtration, heat, and ultraviolet radiation), or ecological (constructed wetlands, biosurfactants, and slow sand filtration) control strategies (Raudales et al., 2014). Although proper prevention and water treatment is critical to reducing pathogen risk in commercial horticulture, these

strategies alone do not guarantee disease-free production, and preventing crop losses from *Pythium* outbreaks remains a challenging aspect of hydroponic production.

Certain hydroponic solution and root zone parameters can be manipulated as cultural strategies to reduce oomycete pathogen risks (Albright et al., 2007; Bugbee, 2004; Gillespie et al., 2020; Langenfeld et al., 2022). For example, a common commercial practice is to reduce the hydroponic solution temperature for crops such as lettuce (*Lactuca sativa* L.) to increase root health and resistance to *Pythium* infection (Albright et al., 2007; Mattson, 2018). Langenfeld et al. (2022) emphasized that high dissolved oxygen levels in the root zone, where dissolved oxygen also increases as solution temperature decreases, and adequate solution flow rate were critical to root health and avoiding *Pythium* root rot. The same authors also recommended a root zone solution volume to cultivation area ratio (V:CA ratio) ranging from 10 to 30 to ensure adequate diffusion of dissolved oxygen to root surfaces. In addition, Gillespie et al. (2020) found maintaining a relatively acidic hydroponic solution ($pH \leq 5$), compared to a conventional target pH of ~6, decreased the viability of *Pythium* zoospores and root infection for basil (*Ocimum basilicum* L.) without compromising plant nutrient uptake and yield. Improved root zone management strategies would complement the use of water treatment technologies and improve risk management of water-borne pathogens.

Plant nutrition has a role in the prevention of pathogen infection in horticulture (Duffy and Défago, 1999; Marschner, 2013; Sonneveld and Voogt, 2009). Past research has evaluated macronutrient effects on disease suppression in soilless culture (Duffy and Défago, 1999; Sonneveld and Voogt, 2009), particularly nitrogen (ammoniacal and nitrate nitrogen), phosphorus, and calcium. However, hydroponic growers would likely control macronutrient concentrations to optimize crop yield and quality rather than manage disease risks.

Metal micronutrients and the beneficial nutrient silicon (Si) are known to have roles in prevention of disease (Bugbee, 2004; Duffy and Défago, 1999; Langenfeld et al., 2022; Sonneveld and Voogt, 2009; Zhang et al., 2021), where the effects of root zone copper (Cu) and Si are best understood. Raudales et al. (2014) reported Cu can be increased to 5 $mg \cdot L^{-1}$ to control *Pythium* and without causing phytotoxicity for some species. Bugbee (2004) found an insufficient manganese (Mn) supply significantly increased disease severity from *Pythium* with hydroponic lettuce. In addition to mitigating disease, research from Langenfeld et al. (2022) and anecdotal evidence from industry also suggests metal micronutrient concentrations [including iron (Fe), Mn, Cu, and zinc (Zn)] may be increased substantially to promote plant nutrient uptake in soilless culture and hydroponics. Supplementing hydroponic solutions with Si is a strategy for suppressing foliar diseases such as powdery mildews and leaf spot (Langenfeld et al., 2022; Voogt and Sonneveld, 2001).

Langenfeld et al. (2022) suggested increasing micronutrient concentrations in solution as well as supplementing with silicon (Si) as an effective and low-risk strategy to suppressing *Pythium* in hydroponic lettuce. However, few studies have investigated the effects of increasing micronutrients other than Cu and supplementing Si on *Pythium* suppression and plant growth.

Objectives were to evaluate the effects of increasing metal micronutrient and Si concentrations on plant growth and susceptibility to *Pythium* root rot for hydroponic lettuce, with emphasis on comparing the effects of micronutrient/Si treatments to a standard hydroponic control solution used in commercial production. This thesis hypothesizes that increasing micronutrient concentrations may increase *Pythium* suppression for certain nutrients, particularly Cu, but would also result in decreased plant growth.

## Materials and Methods

Two studies were conducted concurrently to evaluate metal micronutrient and silicon (Si) concentration in the hydroponic solution for effects on plant growth and resistance to *Pythium* root rot disease with lettuce (*Lactuca sativa* L.). The metal micronutrients included iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu). Both experiments were conducted concurrently in a controlled-environment polycarbonate greenhouse at the University of Arkansas in Fayetteville, AR (36.0687º N, 94.1748º W). Average daily temperature (ADT) and daily light integral (DLI) during the experiments were (mean ± standard deviation) 27.0±4.7°C and 12.2±8.6 $mol \cdot m^{-2} \cdot d^{-1}$ of photosynthetically active radiation, respectively. Hydroponic solution temperature was 26.8±3.0°C. DLI was measured using a QCOM environmental sensor (QCOM, Micro Grow Control Systems Inc., Temecula, CA) and ADT was measured using a portable data logger (WatchDog 2475 Plant Growth Station; Spectrum Technologies, Aurora, IL). Solution temperature was measured using portable battery-powered data loggers (HOBO; Onset Computer Corporation, Bourne, MA).

*Pythium culture and inoculum preparation. Pythium myriotylum* 'PM1' isolate plugs were obtained from Dr. Rosa Raudales and Cora McGehee (University of Connecticut, CT) and recovered from hydroponically grown 'Rex' lettuce. These 'PM1' isolate plugs were then plated on potato dextrose agar (PDA) in a sterile petri dish and sealed with parafilm. Plates were incubated at 25°C and allowed to develop mycelium for 3 d. The mycelium-dense petri dishes served as stock plates used to propagate *Pythium* in 20-30 plates for experimentation.

From stock plates, PDA plugs (4 cm × 4 cm) of mycelium were transferred to sterile petri dishes at six plugs per dish. Liquid V8 (Campbell's, Camden, NJ) media solution was dispensed into petri dishes at 10 mL per dish, sealed with parafilm, and allowed to incubate at 25°C under

light-emitting diode (LED) lights. After 14 d of incubation under LED lights, once thick mats of mycelial tissue had formed, plates were rinsed three times each with 10 mL deionized water in order to remove the remaining V8 liquid media and induce sporulation. Plates were then re-sealed with parafilm, placed back under LED lights, and incubated at 25°C for 7 d. After 24 days of total culture preparation, plates are ready to be harvested for preparing *Pythium* inoculum.

*Pythium* mycelial mats were transferred to 50 mL centrifuge tubes and were macerated for 2-3 min using a vortex and 3-mm sterilized glass beads, forming a well-blended solution. Mycelia were strained from the blended *Pythium* solution to ensure a homogeneous dose concentration per plant, and the remaining solution was aliquoted into a sterilized glass beaker and stirred. The oospore concentration in solution was measured and adjusted to $9.0\times10^6$ oospores/L ($9.00\times10^6$ oospores/mL) using a hemocytometer. Each hemocytometer measurement consisted of 20 10-µL samples of spore solution and averaging the oospore counts using a 20× microscope lens. The *Pythium* inoculum solution was prepared the same day the solution was used in experimentation.

*Plant culture.* Seed of 'Rex' lettuce (Johnny's Selected Seeds, Fairfield, ME) were sown into 2.5-cm diameter rockwool cubes at one seed per cube (AO plugs 200 counts, 2.5-cm height; Grodan, Roermond, The Netherlands), and irrigated with a nutrient solution consisting of a commercial 13N-0.9P-10.8K (Jack's Professional LX, JR Peters, Inc., Allentown, PA) water-soluble fertilizer mixed at 150 ppm-N with tap water. The tap water was low in soluble salts with an electrical conductivity (EC) of <0.3 $mS\cdot cm^{-1}$ and <60 ppm bicarbonate alkalinity. Seeded rockwool cubes were transferred to the greenhouse and sub-irrigated with nutrient solution as needed. After 14 d, or when plants displayed at least two true leaves, seedlings were transferred into hydroponic systems.

Hydroponic culture vessels were 20-L black plastic containers (45.5 × 34.0 × 17.5 cm). A 2.6-cm thick polystyrene foam board (Styrofoam Utilityfit R- 10; Dow, Midland, MI) cut to 37.5 × 26.0 cm, covered with white-black plastic (Black and White Panda Film; Vivosun, Ontario, CA) with the white side facing upwards, was used as a raft on top of the nutrient solution in each culture vessel. The white-black plastic extended over and down the culture vessel sides, preventing light from directly entering the nutrient solution. Each culture vessel contained a submersible fountain pump (Low Water Shut-off 80-GPH Submersible Fountain Pump; Smartpond, Niedersachsen, Germany) used to continually circulate the hydroponic solution. A clear plastic tube fitted with an aquarium air-stone was inserted between the raft and side of the culture vessel, underneath the white-black plastic, and provided continuous aeration of the nutrient solution. Plastic containers, rafts, plastic films, pumps, and aeration tubes were washed with a phosphate-free detergent and rinsed with deionized water before use in this experiment.

Each hydroponic culture vessel contained four lettuce plants. During seedling transplant, rockwool cubes were inserted into 2.5-cm square holes cut in the rafts, allowing roots to grow into the nutrient solution. Spacing consisted of 2 × 2 configurations for each vessel, where each lettuce plant was 15.5 cm from the adjacent plant.

The standard hydroponic nutrient solution used in both experiments was a modified Hoagland's solution with macronutrient concentrations supplied at (in $mg \cdot L^{-1}$) 210 nitrate nitrogen ($NO_3$-N), 32 phosphorus (P), 234 potassium (K), 200 calcium (Ca), 48 magnesium (Mg), and 71 sulfate sulfur ($SO_4$-S). Micronutrient concentrations were (in $mg \cdot L^{-1}$) 2 iron (Fe), 1 manganese (Mn), 0.5 boron (B), 0.5 copper (Cu), 0.5 zinc (Zn), and 0.1 molybdenum (Mo). Macronutrients were derived from reagent-grade calcium nitrate, potassium nitrate, potassium phosphate, potassium chloride, and magnesium sulfate. Micronutrients were derived from

commercial grade iron-EDDHA, Mn-EDTA, Zn-EDTA, Cu-EDTA, boric acid, and sodium molybdate (JR Peters, Inc, Allentown, PA). Fertilizer salts were mixed in tap water dechlorinated with 2.5 $mg \cdot L^{-1}$ of sodium thiosulfate. For the formulation of the treatment solutions below, individual micronutrients and silicon were either added to or omitted from this standard hydroponic solution recipe.

*Experiment 1: Effects of metal micronutrient concentration*. An augmented (4 × 2) + 1 factorial experiment was conducted for each metal micronutrient (Fe, Mn, Cu, and Zn) using a randomized split-plot design. The first factor consisted of metal micronutrient concentration (0.0, 2.5, 5.0, and 10.0 $mg \cdot L^{-1}$) in the hydroponic solution and the second factor consisted of hydroponic systems dosed either with *Pythium* or deionized water (non-*Pythium* control). The *Pythium* dose treatment was the whole-plot factor, and metal micronutrient concentration was the split-plot factor. The experimental design for each micronutrient experiment was augmented with the addition of a standard hydroponic control solution (previously mentioned standard hydroponic solution) which received both *Pythium* and non-*Pythium* doses, indicated by the +1 in factorial experiment design.

The experiment was set up in the greenhouse so metal micronutrient experiments ran concurrently, and each experiment utilized the same hydroponic control solution replicates. Climate data were collected prior to experimentation to ensure homogenous conditions across each *Pythium* and non-*Pythium* experimental block. Each hydroponic culture system with four lettuce plants was considered one observational unit and treatment replicate. There was one replicate culture vessel per micronutrient concentration treatment and two replicates per standard hydroponic control solution treatment for each *Pythium* and non-*Pythium* plot, with the

exception of the standard hydroponic control solution. Three replications were achieved with three experimental runs starting on 27 Apr 2022, 22 Jun 2022, and 10 Aug 2022.

Each experimental run started with the transfer of lettuce seedlings into the hydroponic culture vessels. At this time, each culture vessel was filled to 20.00±0.05 L of the respective treatment solution, with pH adjusted to 6.00±0.05 and EC averaging 1.33 $mS \cdot cm^{-1}$ across treatment solutions. Hydroponic culture vessels were placed on two adjacent and identical benches within the same greenhouse. Solution pH in each vessel was monitored every 2-3 d and maintained within a pH of 5.5 to 6.0 by titrating with $H_2SO_4$ and KOH at 1 *N*.

The *Pythium* inoculum solution was dosed into the respective nutrient solution treatments 5 d after seedlings were transferred to the hydroponic culture vessels. The prepared inoculum solution was covered and placed in the greenhouse and allowed to equilibrate to ambient temperature for 1 hour prior to dosing. *Pythium* inoculum solution was dosed into culture vessels to supply $9.0 \times 10^4$ oospores per plant ($1.80 \times 10^4$ oospores per L of solution). Equivalent volumes of deionized water were dosed into non-*Pythium* control vessels.

The following data were collected 14 d after inoculation with *Pythium* (28 d after sowing seed). Leaf SPAD chlorophyll content, shoot height and width, shoot and root fresh and dry mass, and severity of *Pythium* root lesions were measured for each treatment replicate. Data reflects the average of four plants per metal micronutrient/Si treatment replicate (one replicate) and control units at eight plants per treatment (two replicates).

Leaf SPAD chlorophyll content was measured for each treatment replicate using a Minolta SPAD-502 Plus chlorophyll meter (Konica Minolta, Tokyo, Japan), which measures the ratio of light transmitted through leaves at 650- and 940-nm wavelengths (Uddling et al., 2007). SPAD data per replicate consisted of averaging three SPAD measurements taken from randomly

selected leaves on each of the four lettuce plants per replicate. Data reflects the average SPAD measurement (n=3) of four plants per treatment replicate.

Shoot height was measured (in centimeters (cm)) from the substrate line of each plant to the highest leaf for each lettuce plant in a hydroponic unit and recorded as an average amongst four plants per replicate. Shoot width measurements (in cm) consisted of averaging two perpendicular width measurements taken on each lettuce plant per replicate, averaged amongst four plants in a treatment replicate.

Root disease severity was analyzed as the percentage of roots showing brown discoloration using a modified method of Chiang et al (2017), which emphasized root disease severities of ≤50%. The severity of *Pythium* root lesions and damage was first measured per plant using a six-point visual index with one rater, where index values of 0, 1, 2, 3, 4, and 5 corresponded to no root damage (0% damaged), 1 to 10% damaged roots, 11 to 25% damaged roots, 26 to 50% damaged roots, 51 to 75% damaged roots, and 76 to 100% damaged roots, respectively. Root disease severity was then estimated on a midpoint percentage basis (Chiang et al., 2017), where disease severity percentage (DS%) goes as follows:

$$DS\% = \left( \frac{sum\ (class\ frequency\ \times score\ of\ rating\ class)}{(total\ number\ of\ plants) \times (maximal\ disease\ index)} \right) \times 100$$

For each replicate, root tips measuring 5-cm in length (approximately 0.003 g fresh mass) collected from each lettuce plant were combined and placed in petri dishes with fresh PDA media. Petri dishes were then incubated in the laboratory at 25°C, monitored daily for mycelial growth, and data were collected on whether *Pythium* was re-isolated from the root samples. From the total 256 root isolations made from plants dosed with *Pythium*, 243 were successfully re-isolated, indicating 94.9% of plants treated with *Pythium* contained *Pythium* within their root system by the end of the experiment.

Plant growth was measured at the end of the experiment (28 d after sow) by harvesting shoot and root tissues from each lettuce plant per treatment replicate. Fresh mass was determined for shoots and roots, and tissues were oven-dried at 60°C for 72 hours for dry mass determination. Treatment effects on plant dry mass followed similar trends to those observed for plant fresh mass, although there were generally fewer significant treatment effects on plant dry mass across metal micronutrient and Si experiments, and therefore only results for treatment effects on fresh mass were reported. Fresh root and shoot data were combined and reported as total fresh mass on a per replicate basis. The effects of growing lettuce in hydroponic solutions with metal micronutrient concentrations of 0, 2.5, 5, and 10 $mg \cdot L^{-1}$ and Si concentrations of 7, 14, 28, and 56 $mg \cdot L^{-1}$ was determined by calculating the percent change in total fresh mass per plant and treatment replicate from the average plant fresh mass per replicate for the standard hydroponic control solutions.

*Experiment 2: Effects of silicon concentration*. A 5 × 2 factorial experiment was conducted using a randomized split-plot design. The first factor consisted of Si concentrations of 0, 7, 14, 28, and 56 $mg \cdot L^{-1}$ in the hydroponic solution. The second factor consisted of hydroponic systems dosed either with *Pythium* or deionized water (non-*Pythium* control). The *Pythium* dose treatment was the whole-plot factor, and Si concentration was the split-plot factor. Silicon was added to hydroponic solutions as potassium silicate, which supplied 0, 25.4, 50.8, 101.5, and 203.0 $mg \cdot L^{-1}$ of additional K, respectively. Each hydroponic culture system with four lettuce plants was considered one experimental unit and treatment replicate. There was one replicate culture vessel per silicon concentration for each *Pythium* and non-*Pythium* plot, with the exception of the 0 $mg \cdot L^{-1}$ Si treatment (same as the standard hydroponic control solution in Expt.

1), for which there were two replicate vessels per plot. Treatment replication, dosing with *Pythium*, and data collection were identical to methods described in the first experiment.

*Statistical analysis*. Analysis of variance (ANOVA) with PROC GLIMMIX from SAS 9.4 (SAS Institute, Cary, NC) was used to evaluate the fixed effects of metal micronutrient/Si concentration and *Pythium* dose and interaction effects on leaf SPAD chlorophyll content, shoot height and width, total plant fresh mass, and root disease severity. Random effects included the replication or block. The selection of mixed-model statistics using PROC GLIMMIX was made partially because the factorial design in the first experiment was augmented with the addition of a standard hydroponic control solution, and also because of the additional replication for the hydroponic control solutions in both studies. Means separation used Tukey's honestly significant difference (HSD) at $\alpha=0.05$, with the exception of root disease severity, where the percentage of diseased roots at each metal micronutrient/Si concentration were compared to values observed with the standard hydroponic control solution using Dunnett's T ($\alpha=0.05$). Metal micronutrient/Si concentration effects on percent change in total plant fresh mass compared to the hydroponic control solution was also analyzed using PROC GLIMMIX, where *Pythium* and non-*Pythium* treatments were analyzed separately. Significant percent changes in total plant fresh mass were identified when 95% confidence intervals for treatment means did not overlap 0%.

**Results and Discussion**

Lettuce plants without the *Pythium* dose treatment appeared healthy with green foliage color and a primarily white root system (Figure 3-1A). In contrast, the majority of lettuce plants dosed with *Pythium* developed symptoms commonly associated with *Pythium* root infection in hydroponics including reduced shoot growth, brown discolored roots, root lesions, sloughing of root cortexes, and root tissue necrosis as shown in Figure 3-1B (Mattson, 2018; Raudales and

McGehee, 2016). *Pythium* was not reisolated from any of the root samples collected from the no *Pythium* treatments, whereas *Pythium* was reisolated from 94.9% of root samples from lettuce plants dosed with *Pythium* (*data not shown*). Reduced growth for lettuce plants dosed with *Pythium* compared to the no *Pythium* control plants was likely the result of *Pythium* infection based on the combination of visual shoot and root symptoms and successful *Pythium* re-isolation from roots. Tables 3-1, 3-2, and 3-3 list *P*-values for main (concentration and *Pythium*) and interaction (concentration**Pythium*) effects for response variables leaf SPAD chlorophyll content, shoot height, shoot width, and total fresh mass.

*Leaf SPAD chlorophyll content*. Concentration had significant main effects leaf SPAD chlorophyll content on Cu, Fe, and Zn (Tables 3-1 and 3.2), and *Pythium* treatments had significant main effects on leaf SPAD chlorophyll content for metal micronutrients Cu, Mn, and Si as shown in (Tables 3-1, 3-2, and 3-3). There were significant interaction effects between concentration and *Pythium* on leaf SPAD chlorophyll content dose for Mn only (Figure 3-2). Most notably, leaf SPAD was statistically similar between *Pythium* and non-*Pythium* dose for each Mn concentration, however, Mn concentrations of 0 and 5 mg $L^{-1}$ in the plants dosed with *Pythium* resulted in a statistically significant drop in leaf SPAD compared to the *Pythium* control solution at 1 mg $L^{-1}$ Mn. This indicates that omitting or increasing Mn concentration above a standard hydroponic solution can result in a decrease in leaf SPAD when *Pythium* is present.

Lettuce dosed with *Pythium* had slightly increased leaf SPAD chlorophyll for Cu and Si (Tables 3-4 and 3-6) and decreased leaf SPAD for Mn when compared to no *Pythium* treatments (Table 3-4), whereas *Pythium* dose had no significant main effect for Fe and Zn (Tables 3-4). It is to be noted that for elements Cu, Mn, and Si, *Pythium* and non-*Pythium* treatments in the control solution were statistically similar in leaf SPAD chlorophyll content (*data not shown*),

indicating that if ‘Rex’ lettuce is grown in a standard hydroponic solution growers will see little to no difference in leaf SPAD between plants infected with *Pythium* and not.

Supplying 0 mg·$L^{-1}$ Fe resulted in early visual symptoms of interveinal leaf chlorosis and a decreased leaf SPAD compared to the control and remaining Fe treatments (Table 3-4), likely caused by the start of iron deficiency. In contrast, 0 mg·$L^{-1}$ Zn resulted in a slightly increased leaf SPAD compared to the control (Table 3-4).

Previous research has found that an increase in leaf SPAD or darkening of infected leaves is a primary aboveground symptom identified in infected crops (Bowden and Rouse, 1991a; Johnstone, M.B., 2001). A study by Fukada et al. (2000) found that darker green leaf color in potato plants was temporary, whereas the dark green leaf color in lettuce (unspecified cultivar) plants infected with *Pythium* persisted until the termination of the experiment. The darker green leaf color is theorized to be increased chlorophyll concentration in infected leaves as a morphological response to compensate for reduced leaf area, or to facilitate plant stress responses or defense mechanisms (Johnstone, M.B., 2001).

Overall, treatment effects on leaf SPAD were relatively minor, and except for the start of iron deficiency symptoms at 0 mg·$L^{-1}$ Fe, all plants had visually green foliage color by the end of the experiment. Based on results of this experiment, increasing metal micronutrient and Si concentrations above the standard concentrations in the control had minimal or no effects of green leaf color for lettuce. Leaf chlorosis is sometimes a symptom of severe root rot infection and is often associated with metal micronutrient deficiency, and it is likely additional leaf chlorosis would have occurred in more treatments after a longer duration of *Pythium* infection and/or with-holding of Fe, Mn, Cu, and Zn.

*Plant shoot height and width.* There were significant concentration main effects on either plant shoot height or width for each of the metal micronutrients (Table 3-1 and 3-2), but not for Si (Table 3-3). *Pythium* treatment also had a significant main effect on either shoot height or width for Fe, Mn, Zn, and Si (Tables 3-1, 3-2, and 3-3), but not for Cu. Concentration effects on shoot dimensions were not consistent between the different metal micronutrients, but shoot height and width tended to decrease when micronutrients were omitted (0 $mg \cdot L^{-1}$) or supplied at the highest concentration (10 $mg \cdot L^{-1}$) compared to the control solution. Similarly, dosing with *Pythium* tended to reduce shoot height and width (Tables 3-2 and 3-3). The only interaction between concentration and *Pythium* treatment occurred for Fe, where shoot width was further decreased at 0 $mg \cdot L^{-1}$ Fe compared to the control solution in the presence of *Pythium* (Figure 3-3). This finding indicates that plant stress can compound, often stemming from inadequate environmental parameters such as hydroponic nutrient solution.

Although the ANOVA indicated statistical differences between treatments for certain micronutrients and Si, the overall effects on lettuce shoot height and width were relatively small, and noticeable visual differences in shoot dimensions occurred primarily between plants grown in the control solution and 0 $mg \cdot L^{-1}$ Fe and Zn.

*Total shoot and root fresh mass.* The concentration of each metal micronutrient and Si significantly influenced total plant fresh mass at the end of the experiment (Tables 3-1, 3-2, and 3-3), and dosing with *Pythium* tended to decrease total fresh mass across all metal micronutrient and Si treatments (Tables 3-1, 3-2, 3-3 and Figure 3-4). Compared to non-*Pythium* controls, dosing with *Pythium* decreased total fresh mass by 11.5 g per plant when averaged across micronutrient treatments (*data not shown*). There was an interaction between concentration and *Pythium* treatments for Cu and Zn (Table 3-1 and 3-2), but not for the remaining metal

micronutrients or Si. Data on dry shoots and roots were collected but followed a similar trend to that observed for total fresh shoots and roots mass, and therefore only total fresh mass data are shown and discussed.

Total fresh mass per plant decreased as Cu concentration increased above that of the control solution (0.2 mg·$L^{-1}$ Cu) for lettuce treated with and without *Pythium* (Figure 3-4A). Compared to the control solution, supplying 0 mg·$L^{-1}$ Cu reduced total fresh mass when *Pythium* was present in solution (Figure 3-4A), but appeared to have no effect without *Pythium*.

Increasing Fe concentration above that of the control solution (2 mg·$L^{-1}$ Fe) had no effect on total fresh mass, although omitting Fe (0 mg·$L^{-1}$ Fe) from solution decreased total fresh mass for both *Pythium* and non-*Pythium* plants (Figure 3-4B). Total fresh mass decreased as Mn concentration increased above 1 mg·$L^{-1}$ (control solution) for lettuce treated with and without *Pythium* (Figure 3-4C). Total fresh mass at 0 mg·$L^{-1}$ Mn was not affected compared to the control solution, regardless of *Pythium* treatment. Similar to the trend observed with Fe in Figure 3-4B, Tukey's HSD mean separation ($\alpha$=0.05) indicated a possible significant difference in total fresh mass between *Pythium* and non-*Pythium* at 2.5 mg·$L^{-1}$ Mn (Figure 3-4C), although overall there was no significant interaction effect (*P*=0.3544).

Increasing Zn concentration above that of the control solution (0.5 mg·$L^{-1}$ Zn) did not influence total fresh mass in lettuce without *Pythium* but reduced total fresh mass for lettuce treated with *Pythium* at 5 and 10 mg·$L^{-1}$ Zn (Figure 3-4D). In addition, supplying 0 mg·$L^{-1}$ Zn reduced total fresh mass for lettuce plants treated with and without *Pythium*.

Increasing Si concentration from 0 to 56 mg·$L^{-1}$ decreased total fresh mass from 82.9 to 75.7 g per plant, respectively (Figure 3-3A). Although ANOVA indicated Si concentration had a significant main effect (*P*=0.0396), it was not possible to determine differences between Si

concentration treatments using Tukey's HSD means separation (α=0.05), likely because of variability in the data. Across Si concentration treatments, dosing with *Pythium* decreased total fresh mass from 84.5 to 71.9 g per plant (Figure 3-5B).

*Percent change in total fresh mass.* When compared to lettuce grown in the standard control solution, plants showed significant percent changes in total fresh mass when Cu was increased to 5 $mg \cdot L^{-1}$ (-16.2%), Fe to 2.5 $mg \cdot L^{-1}$ (-11.9%), and Mn to 10 $mg \cdot L^{-1}$ (-17.0%) without the presence of *Pythium* (Table 3-7). For hydroponic systems dosed with *Pythium*, plants had significant reductions in total fresh mass when Cu was increased to 10 $mg \cdot L^{-1}$ (-14.1%), Mn to 2.5 $mg \cdot L^{-1}$ (-21.1%), and Zn to 10 $mg \cdot L^{-1}$ (-19.8%) (Table 3-7). Overall, results in Table 3-7 indicated that increasing metal micronutrient concentration to ≥2.5 $mg \cdot L^{-1}$ would likely reduce lettuce plant growth.

The greatest percent changes in total fresh mass occurred with 0 $mg \cdot L^{-1}$ of Fe and Zn, which resulted in a -24.8% and -30.6% change in fresh mass without *Pythium* and a -33.7% and -26.5% change with *Pythium*, respectively (Table 3-7). Copper at 0 $mg \cdot L^{-1}$ reduced total fresh mass (-12.6%) only when *Pythium* was dosed into solution (Table 3-7), whereas 0 $mg \cdot L^{-1}$ Cu without *Pythium* resulted in a slight increase in fresh mass (3.9%) not statistically different from 0%. Regardless of *Pythium* treatment, supplying 0 $mg \cdot L^{-1}$ of Mn did not result in a significant increase or decrease of total fresh mass compared to the control solution.

Supplementing Si also appeared to result in slight reductions in total fresh mass when compared to the 0 $mg \cdot L^{-1}$ Si control solution (Table 3-8), although percent reductions in lettuce growth with and without *Pythium* were not significant in this experiment.

*Root disease severity.* Data on root disease severity in Table 3-9 only include treatments dosed with *Pythium* at the end of the experiment, since the percentage of the root system which

appeared brown and discolored (potential symptoms of root rot) was relatively low (≤6%) for metal micronutrient and Si treatments not dosed with *Pythium* (*data not shown*). Lettuce grown in the standard hydroponic control solution and dosed with *Pythium* resulted in a root disease severity of 24.2% (Table 3-9).

Increasing Cu concentration above that of the control solution (0.2 $mg \cdot L^{-1}$ Cu) tended to decrease root disease severity (Table 3-9), and only root disease severity at 10 $mg \cdot L^{-1}$ Cu (6.3%) was significantly lower compared to the control solution. Although not significantly different from the control, it appeared supplying 2.5 $mg \cdot L^{-1}$ of Zn decreased root disease severity to 10.1% whereas supplying 0 $mg \cdot L^{-1}$ of Cu and Fe as well as 10 $mg \cdot L^{-1}$ Zn increased disease severity (Table 3-9).

Results of Experiment 1 indicated that increasing the concentration of Cu in the hydroponic solution had potential to reduce root disease severity caused by *Pythium* (Table 3-9), but at the consequence of reducing plant growth and yield (Figure 3-4A, Table 3-5 and 3-7). Increasing the remaining metal micronutrients (Fe, Mn, and Zn) to greater than 2.5 $mg \cdot L^{-1}$ did not significantly impact root disease severity (Table 3-9) and tended to reduce lettuce growth as well as increase the negative effects of *Pythium* on growth (Figure 3-4). Increasing the concentration of Zn to 10 $mg \cdot L^{-1}$ had no impact of total fresh mass without *Pythium* (Figure 3-4D), but reduced fresh mass with *Pythium*, suggesting excess Zn may have resulted in greater root stress and susceptibility to root disease. Metal micronutrients also have a divalent cationic charge and compete for root uptake and increasing the concentration of one metal micronutrient likely decreased the uptake of the others in this experiment. For example, increasing the concentration of Zn may have reduced the uptake of Cu, possibly resulting in reduced growth and increased disease severity.

Hydroponic solutions used in commercial production would typically supply all plant essential nutrients, including metal micronutrients. However, Table 3-7 suggests a sub-optimal supply of metal micronutrients would increase root disease risks and reduce plant growth. For example, omitting metal micronutrients Fe and Zn resulted in the greatest reductions in lettuce growth both with and without *Pythium* (Table 3-7). Compared to lettuce grown in the control solution, plant growth was reduced with 0 $mg \cdot L^{-1}$ Cu only in the presence of *Pythium* (Table 3-7), which also suggested lettuce was more susceptible to *Pythium* when Cu concentration was insufficient.

Even though lettuce growth was not significantly influenced when Mn was omitted from solution, or when Cu was omitted without *Pythium*, plants were likely still able to absorb sufficient quantities of these micronutrients during the experiment. Trace amounts of Mn, Cu, and Zn can leach into solution over time from the various plastics and metal components used in hydroponic systems (Bugbee, 2004; Sonneveld and Voogt, 2009), sometimes in sufficient quantities to meet plant nutritional requirements. Although data for nutrient concentrations at the end of the experiment were not available at the time of this publication, the authors suspect near sufficient quantities of Mn and Cu leached into the hydroponic solution.

Supplementing the hydroponic solution with Si had no influence on lettuce resistance to *Pythium* root disease, and there was evidence that higher Si concentrations reduced lettuce growth. It was not possible to determine the cause of reduced lettuce growth at high Si concentrations, however Si toxicity in plants is unlikely (Marschner, 2013). Reduced growth at 56 $mg \cdot L^{-1}$ Si may have resulted from an imbalance in root zone macronutrients caused by the large addition of K from the potassium silicate. Voogt and Sonneveld (2002) reported no Si effects on lettuce yield at 14 $mg \cdot L^{-1}$ Si but recommended this concentration to help prevent Mn

toxicity since Si increased the distribution of Mn within lettuce tissues. Langenfeld et al. (2022) also recommended 14 $mg \cdot L^{-1}$ Si in solution as a general strategy to prevent foliar diseases in lettuce.

Silicon uptake and accumulation in shoot tissues has been reported to reduce susceptibility to foliar diseases for a range of plant species (Sonneveld and Voogt, 2009; Voogt and Sonneveld, 2002), however Si may be less effective at mitigating root diseases in lettuce for several reasons. Silicon is taken up and translocated passively through plant tissues via the bulk flow of water (i.e., transpiration), generally resulting in a greater accumulation in leaf compared to root tissues (Marschner, 2013; Voogt and Sonneveld, 2002). Marschner (2013) suggests the accumulation of Si in the epidermal leaf cells and deposition under the cuticle may provide a physical barrier against the penetrating hyphae arising from spores, although the mechanism by which Si mitigates fungal diseases is not well-researched and may differ between foliar and root rot diseases. Voogt and Sonneveld (2002) also labeled lettuce as a "Si non-accumulator" species characterized by low Si uptake and relatively few benefits from supplemental Si compared to "Si accumulator" species such as cucumber (*Cucumis sativus* L.).

Historically, target micronutrient concentrations in recirculating solutions have primarily been determined by interpreting nutrient analyses from harvested plant tissues and solution samples as well as from grower experience (Langenfeld et al., 2022; Sonneveld and Voogt, 2009). Langenfeld et al. (2022) suggested increasing micronutrient concentrations, particularly Cu, as a low-risk approach to mitigating *Pythium* root disease in hydroponics; however, micronutrient concentrations above 2.5 $mg \cdot L^{-1}$ appeared to reduce lettuce growth in this experiment. Based on the results of these experiments, it is suggested that hydroponic lettuce growers aim to maintain metal micronutrient concentrations near those found in more traditional

and standard hydroponic solutions, for example a full-strength Hoagland's solution (in $mg \cdot L^{-1}$) 0.5 for Cu, 2.0 for Fe, 1.0 for Mn, and 0.5 for Zn. Increasing Cu may reduce root disease risks but has potential to also decrease growth and yield. Although Si did not increase yield or resistance to *Pythium* for lettuce, supplying 14 $mg \cdot L^{-1}$ Si may provide alternative benefits such as decreased risk of foliar diseases and micronutrient toxicity. Successful mitigation of root rot pathogens for hydroponics likely depends more on good sanitation practices combined with the proper design of a water treatment system using a multi-barrier approach (Raudales et al. 2014).

**Conclusions**

Increasing the concentration of solution Cu decreased *Pythium* disease severity, but at the consequence of lettuce growth and yield. Increasing the concentration of other metal micronutrients also tended to reduce lettuce growth and increase disease severity. Metal micronutrients supplied at 0 $mg \cdot L^{-1}$, particularly Fe and Zn, resulted in the greatest reduction of plant growth in this experiment, especially in the presence of *Pythium*. Supplementing the hydroponic solution with Si had no effect on *Pythium* disease severity.

Results of this experiment suggest metal micronutrients supplied at concentrations near those typically supplied in conventional hydroponic solutions for leafy greens would likely result in adequate growth of lettuce. For example, a full-strength Hoagland's solution at (in $mg \cdot L^{-1}$) 0.5 for Cu, 2.0 for Fe, 1.0 for Mn, and 0.5 for Zn. This experiment also emphasized the relatively low and narrow concentration ranges for which micronutrients must be maintained in hydroponic solutions, where small deviations in micronutrient concentration can potentially result in significant decreases in crop growth.

Successful mitigation of root rot pathogens in commercial hydroponic production requires the combination of proper sanitation, best management and cultural practices,

appropriate hydroponic system design, and implementation of a water treatment system with proper design and multi-barrier approach. Supplementing the hydroponic solution with 14 $mg \cdot L^{-1}$ Si may be an additional nutrient management strategy to help growers decrease the risk of foliar diseases and micronutrient toxicity in lettuce.

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Table 3-1. Metal micronutrient concentration main and interaction effects on response variables leaf SPAD, shoot height, shoot width, and total fresh mass for hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

| Element | Response Variable | [a]Num DF | [b]Den DF | F Value | Pr > F[c] |
|---|---|---|---|---|---|
| Copper | Leaf SPAD | | | | |
| | *Concentration* | 1 | 132 | 4.93 | 0.0281 |
| | *Pythium* | 4 | 132 | 2.48 | 0.0472 |
| | *Concentration*Pythium* | 4 | 132 | 0.69 | 0.6034 |
| Copper | Shoot height | | | | |
| | *Concentration* | 1 | 128 | 2.29 | 0.1325 |
| | *Pythium* | 4 | 128 | 2.74 | 0.0315 |
| | *Concentration*Pythium* | 4 | 128 | 1.81 | 0.1304 |
| Copper | Shoot width | | | | |
| | *Concentration* | 1 | 128 | 0.05 | 0.8215 |
| | *Pythium* | 4 | 128 | 4.85 | 0.0011 |
| | *Concentration*Pythium* | 4 | 128 | 1.22 | 0.3059 |
| Copper | Total fresh mass | | | | |
| | *Concentration* | 1 | 132 | 23.75 | <.0001 |
| | *Pythium* | 4 | 132 | 7.40 | <.0001 |
| | *Concentration*Pythium* | 4 | 132 | 2.72 | 0.0325 |
| Iron | Leaf SPAD | | | | |
| | *Concentration* | 1 | 132 | 3.51 | 0.0634 |
| | *Pythium* | 4 | 132 | 6.71 | <.0001 |
| | *Concentration*Pythium* | 4 | 132 | 1.34 | 0.2593 |
| Iron | Shoot height | | | | |
| | *Concentration* | 1 | 132 | 4.31 | 0.0397 |
| | *Pythium* | 4 | 132 | 2.29 | 0.0628 |
| | *Concentration*Pythium* | 4 | 132 | 0.94 | 0.4434 |
| Iron | Shoot width | | | | |
| | *Concentration* | 1 | 132 | 6.60 | 0.0113 |
| | *Pythium* | 4 | 132 | 3.99 | 0.0043 |
| | *Concentration*Pythium* | 4 | 132 | 2.82 | 0.0275 |
| Iron | Total fresh mass | | | | |
| | *Concentration* | 1 | 132 | 29.45 | <.0001 |
| | *Pythium* | 4 | 132 | 14.57 | <.0001 |
| | *Concentration*Pythium* | 4 | 132 | 1.65 | 0.1661 |

[a] Num DF is the number of degrees of freedom in the model

[b] Den DF is the number of degrees of freedom associated with the model error
[c] Probability of greater F value, P values were considered significant with a=0.05.

Table 3-2. Metal micronutrient concentration main and interaction effects on response variables leaf SPAD, shoot height, shoot width, and total fresh mass for hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level.

| Element | Response Variable | [a]Num DF | [b]Den DF | F Value | Pr > F [c] |
|---|---|---|---|---|---|
| Manganese | Leaf SPAD | | | | |
| | *Concentration* | 1 | 132 | 4.68 | 0.0322 |
| | *Pythium* | 4 | 132 | 2.28 | 0.0637 |
| | *Concetration*Pythium* | 4 | 132 | 4.38 | 0.0024 |
| Manganese | Shoot height | | | | |
| | *Concentration* | 1 | 132 | 1.74 | 0.1901 |
| | *Pythium* | 4 | 132 | 2.33 | 0.0595 |
| | *Concentration*Pythium* | 4 | 132 | 1.02 | 0.3974 |
| Manganese | Shoot width | | | | |
| | *Concentration* | 1 | 132 | 14.49 | 0.0002 |
| | *Pythium* | 4 | 132 | 1.36 | 0.2507 |
| | *Concentration*Pythium* | 4 | 132 | 1.38 | 0.2447 |
| Manganese | Total fresh mass | | | | |
| | *Concentration* | 1 | 132 | 35.50 | <.0001 |
| | *Pythium* | 4 | 132 | 9.88 | <.0001 |
| | *Concentration*Pythium* | 4 | 132 | 1.11 | 0.3544 |
| Zinc | Leaf SPAD | | | | |
| | *Concentration* | 1 | 132 | 1.48 | 0.2266 |
| | *Pythium* | 4 | 132 | 7.90 | <0.0001 |
| | *Concentration*Pythium* | 4 | 132 | 1.62 | 0.1723 |
| Zinc | Shoot height | | | | |
| | *Concentration* | 1 | 132 | 8.44 | 0.0043 |
| | *Pythium* | 4 | 132 | 10.05 | <0.0001 |
| | *Concentration*Pythium* | 4 | 132 | 1.78 | 0.1365 |
| Zinc | Shoot width | | | | |
| | *Concentration* | 1 | 132 | 1.27 | 0.2614 |
| | *Pythium* | 4 | 132 | 10.10 | <0.0001 |
| | *Concentration*Pythium* | 4 | 132 | 2.37 | 0.0562 |
| Zinc | Total fresh mass | | | | |
| | *Concentration* | 1 | 132 | 23.69 | <0.0001 |
| | *Pythium* | 4 | 132 | 13.56 | <0.0001 |
| | *Concentration*Pythium* | 4 | 132 | 3.36 | 0.0118 |

[a] Num DF is the number of degrees of freedom in the model
[b] Den DF is the number of degrees of freedom associated with the model error

[c] Probability of greater F value, P values were considered significant with a=0.05.

Table 3-3. Metal micronutrient concentration main and interaction effects on response variables leaf SPAD, shoot height, shoot width, and total fresh mass for hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the α=0.05 significance level.

| Element | Response Variable | [a] Num DF | [b] Den DF | F Value | Pr > F[c] |
|---|---|---|---|---|---|
| Silicon | Leaf SPAD | | | | |
| | *Concentration* | 1 | 132 | 10.48 | 0.0015 |
| | *Pythium* | 4 | 132 | 1.35 | 0.2561 |
| | *Concentration*Pythium* | 4 | 132 | 1.00 | 0.4094 |
| Silicon | Shoot height | | | | |
| | *Concentration* | 1 | 124 | 4.36 | 0.0388 |
| | *Pythium* | 4 | 124 | 1.32 | 0.2651 |
| | *Concentration*Pythium* | 4 | 124 | 1.06 | 0.3788 |
| Silicon | Shoot width | | | | |
| | *Concentration* | 1 | 124 | 5.46 | 0.0211 |
| | *Pythium* | 4 | 124 | 1.36 | 0.2520 |
| | *Concentration*Pythium* | 4 | 124 | 1.03 | 0.3965 |
| Silicon | Total fresh mass | | | | |
| | *Concentration* | 1 | 132 | 32.88 | <0.0001 |
| | *Pythium* | 4 | 132 | 2.59 | 0.0396 |
| | *Concentration*Pythium* | 4 | 132 | 0.38 | 0.8232 |

[a] Num DF is the number of degrees of freedom in the model
[b] Den DF is the number of degrees of freedom associated with the model error
[c] Probability of greater F value, P values were considered significant with a=0.05.

Table 3-4. Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) concentration effects on leaf SPAD chlorophyll content in hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

| | Leaf SPAD chlorophyll content | | | |
|---|---|---|---|---|
| Concentration | Cu | Fe | Mn | Zn |
| Control | 27.7 a | 27.7 a | 27.7 a | 27.7 bc |
| 0 mg·L$^{-1}$ | 28.2 a | 25.2 b | 26.5 a | 29.5 a |
| 2.5 mg·L$^{-1}$ | 27.3 a | 27.9 a | 27.0 a | 26.3 c |
| 5 mg·L$^{-1}$ | 26.8 a | 28.4 a | 26.9 a | 28.0 ab |
| 10 mg·L$^{-1}$ | 28.1 a | 27.9 a | 27.6 a | 27.7 bc |
| *p-value* | 0.0472 | <0.0001 | 0.0637 | <0.0001 |
| | | | | |
| No Pythium | 27.2 b | 27.0 a | 27.5 a | 27.6 a |
| Pythium | 28.0 a | 27.8 a | 26.8 b | 28.1 a |
| *p-value* | 0.0281 | 0.0634 | 0.0322 | 0.2266 |

Table 3-5. Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) concentration effects on plant shoot height and width (cm) for hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

| | Shoot height (cm) | | | | Shoot width (cm) | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | Cu | Fe | Mn | Zn | Cu | Fe | Mn | Zn |
| Control | 11.0 ab | 11.0 a | 11.0 a | 11.0 a | 22.3 a | 22.3 a | 22.3 a | 22.3 a |
| 0 mg·L$^{-1}$ | 11.1 ab | 9.9 b | 10.8 ab | 8.8 b | 22.2 a | 20.7 b | 21.8 a | 18.3 b |
| 2.5 mg·L$^{-1}$ | 11.5 ab | 10.8 ab | 10.5 ab | 11.3 a | 22.7 a | 21.7 ab | 21.6 a | 22.6 a |
| 5 mg·L$^{-1}$ | 11.0 ab | 10.6 ab | 10.3 ab | 10.9 a | 23.1 ab | 22.1 ab | 22.5 a | 21.7 a |
| 10 mg·L$^{-1}$ | 10.2 b | 10.7 ab | 9.7 b | 10.3 a | 20.9 b | 21.1 ab | 21.6 a | 21.7 a |
| *p-value* | 0.0315 | 0.0628 | 0.0595 | <0.0001 | 0.0011 | 0.0043 | 0.2507 | <0.0001 |
| | | | | | | | | |
| No Pythium | 11.1 a | 10.9 a | 10.7 a | 10.8 a | 22.1 a | 22.0 a | 22.6 a | 21.0 a |
| Pythium | 10.8 a | 10.3 b | 10.3 a | 10.1 b | 22.4 a | 21.2 b | 21.4 b | 21.6 a |
| *p-value* | 0.1325 | 0.0397 | 0.1901 | 0.0043 | 0.8215 | 0.0113 | 0.0002 | 0.2614 |

Table 3-6. Silicon (Si) concentration effects on SPAD, shoot height and width (cm) for hydroponic lettuce. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

| Silicon Concentration | SPAD | Shoot height (cm) | Shoot width (cm) |
|---|---|---|---|
| Control (0 mg·L$^{-1}$) | 27.7 a | 11.0 a | 22.3 a |
| 7 mg·L$^{-1}$ | 27.3 a | 11.0 a | 22.5 a |
| 14 mg·L$^{-1}$ | 27.4 a | 10.3 a | 22.0 a |
| 28 mg·L$^{-1}$ | 27.2 a | 10.7 a | 22.6 a |
| 56 mg·L$^{-1}$ | 28.7 a | 10.3 a | 21.6 a |
| *p-value* | 0.2561 | 0.2651 | 0.2520 |
| | | | |
| No Pythium | 27.0 b | 10.9 a | 22.2 a |
| Pythium | 28.4 a | 10.5 b | 21.5 b |
| *p-value* | 0.0015 | 0.0388 | 0.0211 |

Table 3-7. Percent change in total fresh mass (roots and shoots) per lettuce plant when solution copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) were supplied at (in 0 $mg \cdot L^{-1}$) 0, 2.5, 5, and 10 compared to the standard hydroponic control solution. Fresh mass data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications.

| | Percent change in fresh mass | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration | Cu | | Fe | | Mn | | Zn | |
| *No Pythium* | | | | | | | | |
| 0 $mg \cdot L^{-1}$ | 3.9% | | -24.8% | * | -1.2% | | -30.6% | * |
| 2.5 $mg \cdot L^{-1}$ | -8.6% | | -11.9% | * | -5.6% | | -6.4% | |
| 5 $mg \cdot L^{-1}$ | -16.2% | * | 2.7% | | -3.0% | | 0.7% | |
| 10 $mg \cdot L^{-1}$ | -15.4% | * | -11.8% | * | -17.0% | * | -0.2% | |
| ±Std. Error | 5.9% | | 5.8% | | 4.6% | | 8.0% | |
| | | | | | | | | |
| *Pythium* | | | | | | | | |
| 0 $mg \cdot L^{-1}$ | -12.6% | * | -33.7% | * | 3.4% | | -26.5% | * |
| 2.5 $mg \cdot L^{-1}$ | -6.7% | | -12.0% | | -21.1% | * | 5.1% | |
| 5 $mg \cdot L^{-1}$ | -7.4% | | -12.1% | | -9.6% | | -10.7% | |
| 10 $mg \cdot L^{-1}$ | -14.1% | * | -7.3% | | -23.7% | * | -19.8% | * |
| ±Std. Error | 4.6% | | 8.3% | | 8.7% | | 9.9% | |

Negative values indicate a percent reduction in fresh mass. Values represent least-square means of three replicates per treatment. Dunnett's T was used to determine which treatment means were significantly different from the control solution (six replicates) at $\alpha$=0.05. Asterisks denote a significant difference from the control. Standard solution micronutrient concentrations were (in $mg \cdot L^{-1}$) 2.1 Fe, 0.19 Cu, 0.99 Mn, and 0.5 Zn.

Table 3-8. Percent change in total fresh and dry mass (roots and shoots) per lettuce plant when solution silicon (Si) was supplied at (in $mg \cdot L^{-1}$) 0, 2.5, 5, and 10 compared to the standard hydroponic control solution. Fresh mass data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications.

| Silicon | Percent change in fresh mass |
|---|---|
| *No Pythium* | |
| 7 $mg \cdot L^{-1}$ | 0.3% |
| 14 $mg \cdot L^{-1}$ | -4.7% |
| 28 $mg \cdot L^{-1}$ | -6.6% |
| 56 $mg \cdot L^{-1}$ | -3.8% |
| ±Std. Error | 4.3% |
| | |
| *Pythium* | |
| 7 $mg \cdot L^{-1}$ | -1.9% |
| 14 $mg \cdot L^{-1}$ | -7.5% |
| 28 $mg \cdot L^{-1}$ | -12.7% |
| 56 $mg \cdot L^{-1}$ | -9.7% |
| ±Std. Error | 8.1% |

Negative values indicate a percent reduction in fresh mass. Values represent least-square means of three replicates per treatment. Dunnett's T was used to determine which treatment means were significantly different from the control solution (six replicates) at $\alpha$=0.05. No treatments were significantly different from the control. Standard solution micronutrient concentrations contained 0 $mg \cdot L^{-1}$ Si.

Table 3-9. Copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and silicon (Si) effects on disease severity percentage for lettuce grown hydroponically for 28 d and inoculated with *Pythium*. Lettuce grown in the standard hydroponic control solution and inoculated with *Pythium* had a disease severity percentage of 24.2% (*data not shown*). Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications.

| | *Pythium* Disease Severity (%) | | | | | *Pythium* Disease Severity (%) | |
|---|---|---|---|---|---|---|---|
| Concentration | Cu | | Fe | Mn | Zn | Concentration | Si |
| 0 $mg \cdot L^{-1}$ | 36.5% | | 33.8% | 13.1% | 16.3% | 7 $mg \cdot L^{-1}$ | 25.4% |
| 2.5 $mg \cdot L^{-1}$ | 12.6% | | 27.7% | 29.0% | 10.1% | 14 $mg \cdot L^{-1}$ | 28.0% |
| 5 $mg \cdot L^{-1}$ | 12.5% | | 24.2% | 27.4% | 29.2% | 28 $mg \cdot L^{-1}$ | 26.4% |
| 10 $mg \cdot L^{-1}$ | 6.3% | * | 23.4% | 18.8% | 34.4% | 56 $mg \cdot L^{-1}$ | 20.2% |
| ± Std. Error | 7.1% | | 11.8% | 7.2% | 7.8% | ± Std. Error | 11.8% |

Values represent least-square means of three replicates per treatment. Dunnett's T was used to determine which treatment means were significantly different from the control solution (six replicates) at $\alpha=0.05$. A value of 0% indicates no disease symptoms and 100% severe disease symptoms (treatments averaged across 4 plants on a 6-point rater scale). Asterisks denote a significant difference from the control. Standard solution micronutrient concentrations were (in mg/L) 2.1 Fe, 0.19 Cu, 0.99 Mn, 0.5 Zn, and 0 Si.

**Fig. 3-1A.**

**Fig. 3-1B.**

Figure 3-1. The left most image, Fig. 3-1A depicts a healthy white root system from a ‘Rex’ lettuce plant with no apparent lesions or necrosis. Alternatively, Fig. 3-1B from left to right, shows increasing root lesions and necrosis of root tissue caused by *Pythium myriotylum* ‘PM1’.

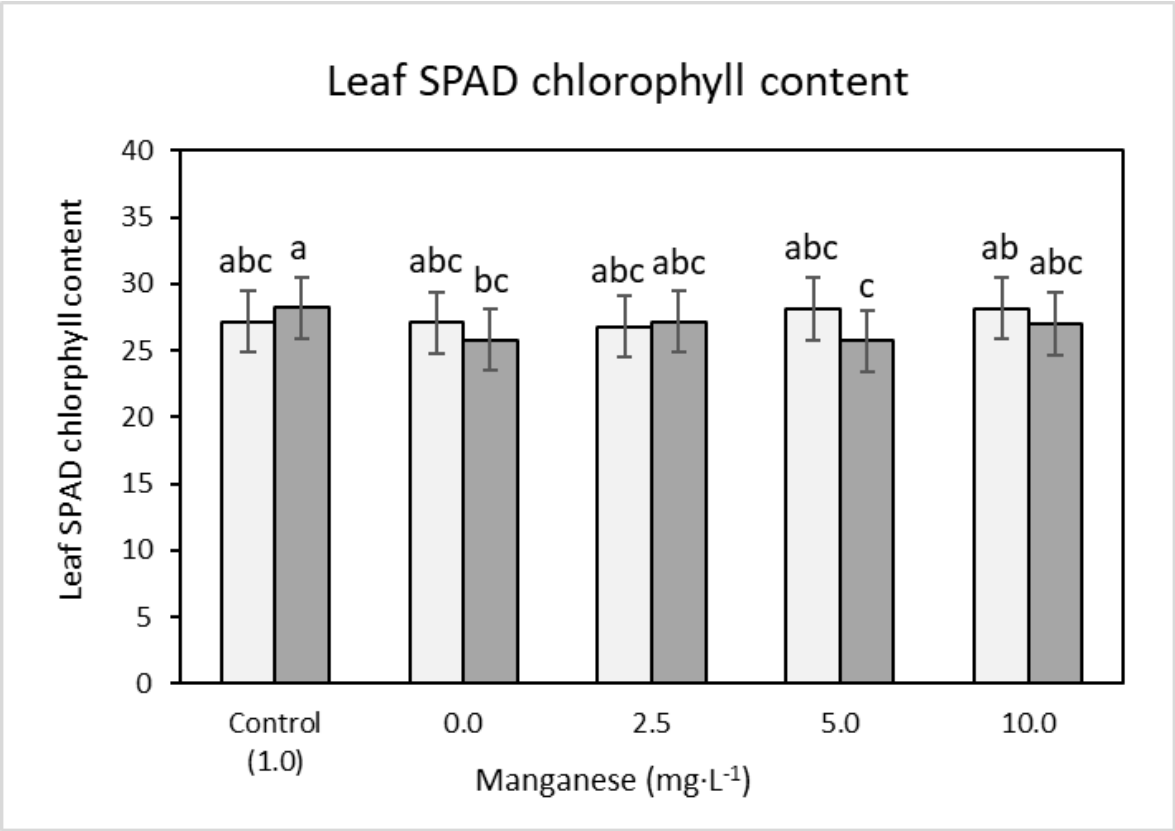


Figure 3-2. Effects of manganese concentration and *Pythium* dose on Leaf SPAD chlorophyll content with plant species 'Rex' lettuce. White bars indicate control plants not dosed with *Pythium*, and grey bars indicate plants dosed with *Pythium*. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

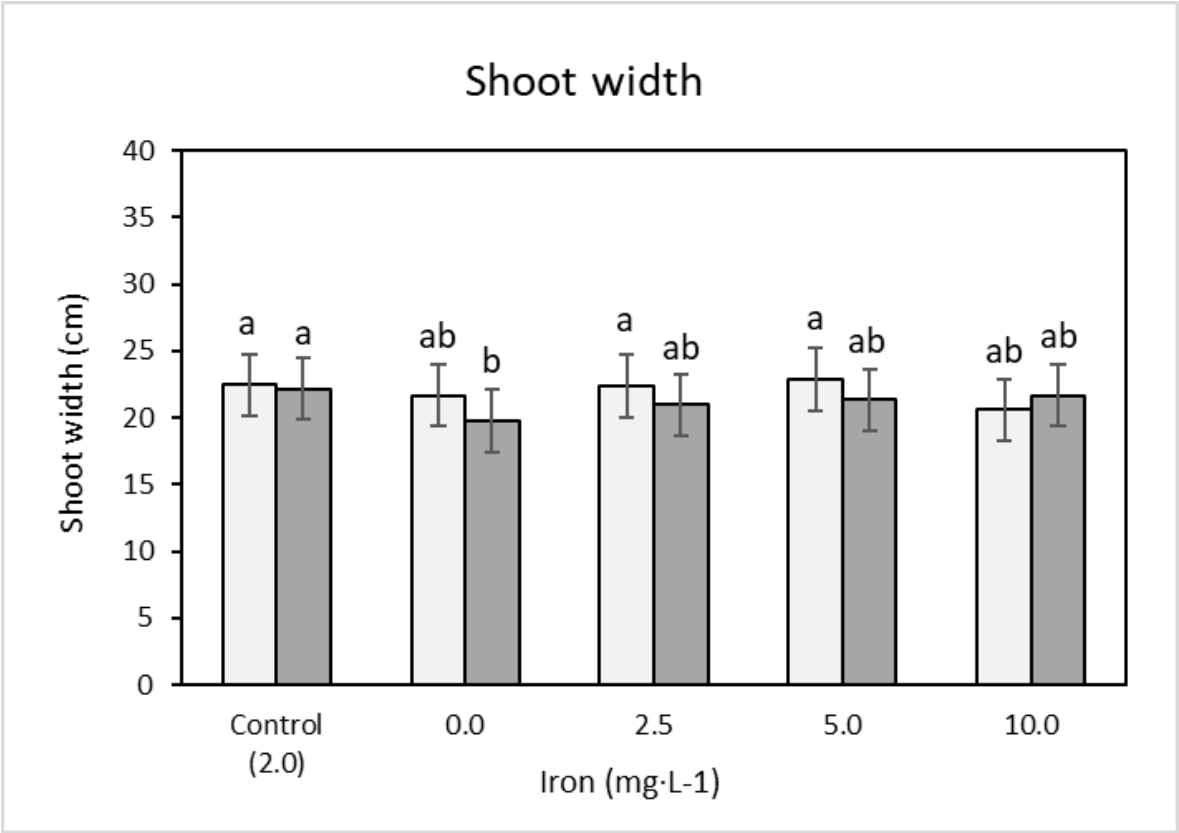


Figure 3-3. Effects of iron concentration and *Pythium* dose on plant shoot width with plant species 'Rex' lettuce. White bars indicate control plants not dosed with *Pythium*, and grey bars indicate plants dosed with *Pythium*. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications. Data are least-square means of at least six and 15 replicates per concentration and *Pythium* dose treatment, respectively. Mean separation for analysis of variance (ANOVA) used Tukey's honestly significant difference (HSD) at the $\alpha$=0.05 significance level.

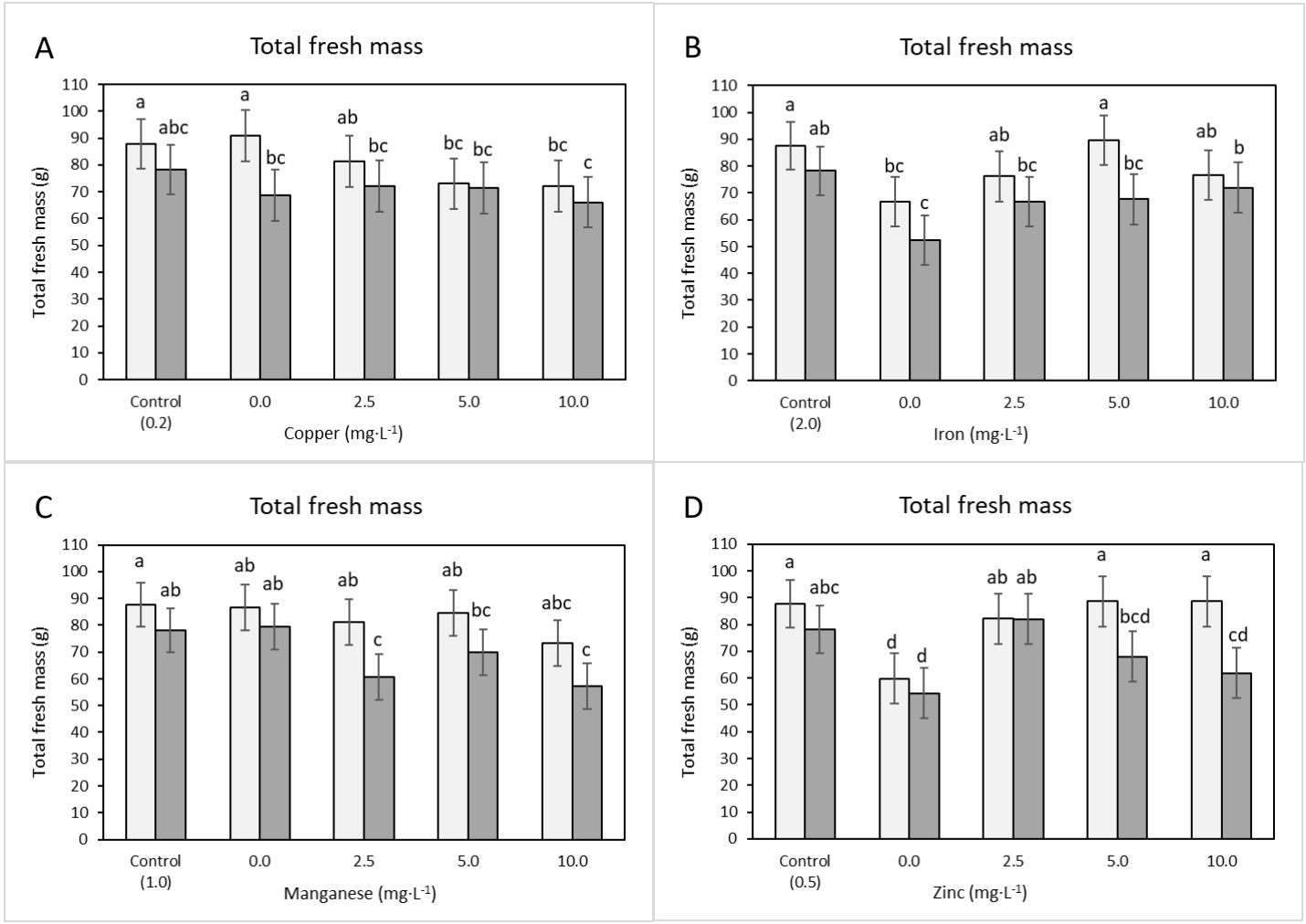


Figure 3-4. Effects of microelement concentration and *Pythium* dose on total fresh mass with plant species 'Rex' lettuce. Microelements listed from left to right: copper (a), iron (b), manganese (c), zinc (d). White bars indicate control plants not dosed with *Pythium*, and grey bars indicate plants dosed with *Pythium*. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications

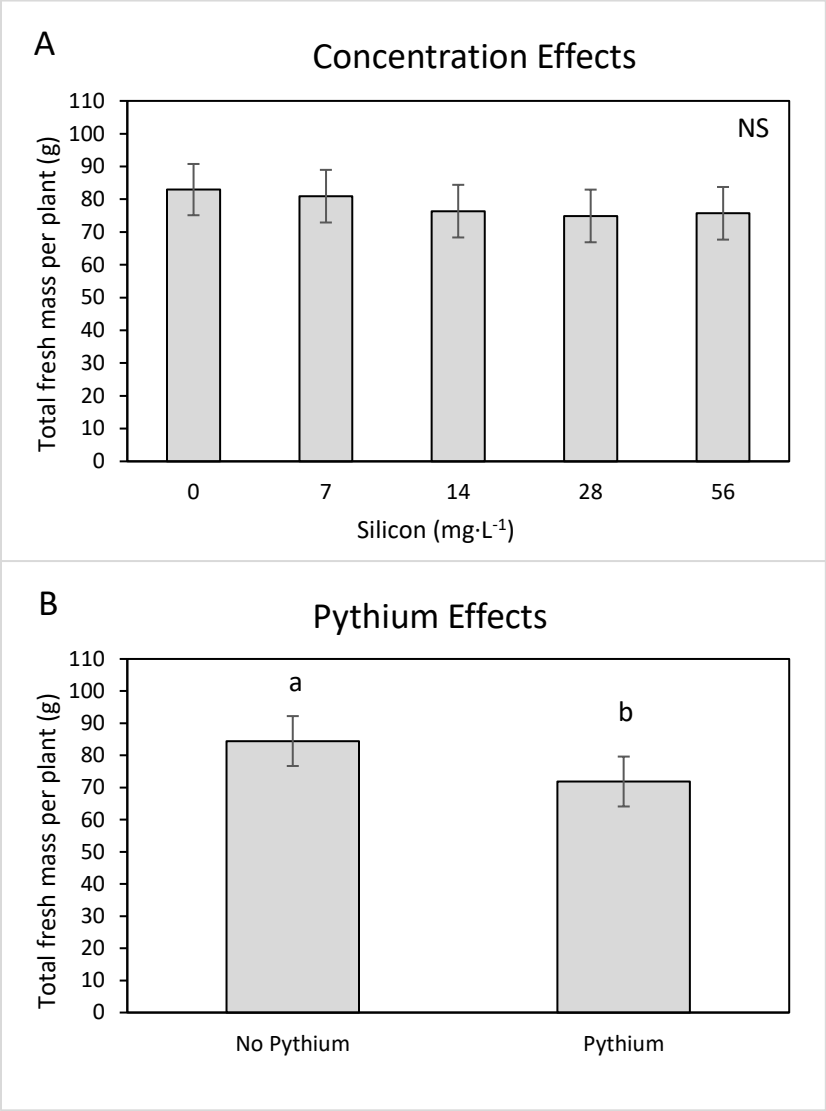


Figure 3-5. Effects of silicon concentration (a) and Pythium treatment (b) on final total fresh mass per plant with 'Rex' lettuce. White bars indicate control plants not dosed with *Pythium*, and grey bars indicate plants dosed with *Pythium*. Data were collected at the end of the experiment (28 d after sow) on each of the four plants in a hydroponic unit and averaged between three treatment replications.

# CHAPTER 4: CONCLUSIONS

The objectives of this study were to evaluate the effects of increasing metal micronutrient and silicon (Si) concentrations on disease susceptibility to *Pythium* in hydroponic 'Rex' lettuce. Although not statistically significant compared to the control, increasing metal micronutrient concentrations from 0 to 10 $mg \cdot L^{-1}$ and Si 0 to 56 $mg \cdot L^{-1}$ resulted in a reduction of disease severity from *Pythium* root rot disease. The only element to significantly reduce disease severity by half or more compared to the control was copper (Cu) at 10 $mg \cdot L^{-1}$, however, plant growth was reduced at the higher concentrations. In hydroponic nutrient solution, Cu is supplied at relatively low concentrations (0.03 – 0.5 $mg \cdot L^{-1}$) and has known effects on suppressing plant growth at higher concentrations (Raudales et al., 2014). High concentrations of Cu are typically applied as fungicides and algaecides, however, is phytotoxic to plants (Agrios, 2005; Flemming and Trevors, 1989; Raudales et al., 2014; Thurman et al., 2009). Therefore, increasing Cu concentration effects on suppressing plant growth and disease align with previous literature, however, do not align with current recommendations for growers to increase concentrations as suggested by Langenfeld et al. (2022).

The effects of omitting micronutrients on plant growth differed between elements. Plant growth for Fe and Zn at 0 $mg \cdot L^{-1}$ was stunted in comparison to the other concentrations, both Cu and Mn saw no reductions in plant growth when omitted. We theorize this is either due to: 1.) residual metal micronutrients leftover in growing media or pre-transplant production absorption or 2.) hydroponic parts leaking elements into solution for plant uptake (Bugbee, 2004; Langenfeld et al., 2022; Sonneveld and Voogt, 2009).

Additionally, increasing Mn concentrations from 0 to 10 $mg \cdot L^{-1}$ resulted in a reduction in plant growth, likely due to toxic levels of Mn. Supplying Si into solution has shown to alleviate toxic levels of certain metal micronutrients (Fe, Mn, Cu, and Zn) in lettuce and other crops and increase plant vigor against plant pathogens such as *Botrytis* and *Pythium* (Flora et al., 2019; Hein et al., 2007; Ma, 2004; Mattson and Leatherwood, 2010; Pozo et al., 2015; Stamatakis et al., 2003; Voogt and Sonneveld, 2001). Langenfeld et al. (2022) recommend increasing micronutrient and Si concentrations to increase nutrient uptake and promote vigorous plants in hydroponic production.

Plants were more susceptible to *Pythium* infection at higher nutrient concentrations, indicating that compound stress from nutrient toxicity or uptake competition can increase plant disease susceptibility. Overall, *Pythium* reduced total fresh shoot mass by approximately 14% (11.5 g) across all micronutrient and Si treatments (*data not shown*). Visual observations of *Pythium* included mild to severe root browning, where in severe cases, it was noticed that remaining white roots were thicker in diameter compared to the necrotic roots in the root system (Fig. 4-1A and Fig, 4-1B). This indicates a possible morphological response to *Pythium* infection in hydroponic lettuce plant roots.

Based on these results, we would recommend that growers maintain nutrient concentrations following conventional hydroponic nutrient formulations, such as a full-strength or modified Hoagland's solution. Additionally, it is vital to combine proper sanitation measures with a multi-barrier of water treatment technologies such as chemical (chlorine, bromine, chlorine dioxide, ionized copper, copper salts, ionized silver, ozone, hydrogen peroxide, and peroxyacetic acid), non-chemical or physical (filtration, heat, and ultraviolet radiation), or ecological (constructed wetlands, biosurfactants, and slow sand filtration) control strategies

(Raudales et al., 2014) in order to mitigate disease development. For example, it was reported that Oomycete zoospores were controlled with 2 $mg \cdot L^{-1}$ residual free chlorine (Hong et al., 2003; Raudales et al., 2014). Future studies of interest include the effects of micronutrients in various hydroponic system designs (nutrient film technique, aeroponics, etc.) on disease susceptibility in a variety of crop species grown in hydroponic production.

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**Figure 4-1A.** **Figure 4-1B.**

Figure 4-1A and B. Noticeable thickening of remaining white roots in plants infected with *Pythium* root rot disease. Root browning indicates necrosis of root tissue caused by *Pythium* symptoms.