Stanley Graham
Dr. Yoshimura
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The *Pythium* suppressive ability of *Glomus intraradices* in cherry tomato propagation

Introduction

This experiment aims at establishing the ability of the endomycorrhizal fungus *Glomus intraradices* to suppress the plant pathogen *Pythium ultimum* in cherry tomato. A study published in Canadian Journal of Plant Pathology in 1994 showed that *Glomus intraradices* suppresses *Pythium ultimum* in *Tagetes patula* (french marigold) (St-Arnaud, Fortin, Caron, & Hamel, 1994). *Pythium ultimum* and other *Pythium* species are fungi-like organisms which cause *Pythium* damping-off, a serious disease of seeds and seedlings (Hartmann, Kester, Davies, & Geneve, 2011, p.217). *Pythium* species resemble parasitic brown algae and are not considered to be true fungi (Dreistadt, 2004, p.268). *Pythium ultimum* is one of the three most commonly encountered species of *Pythium,* the other two being *P. aphanidermatum* and *P. irregulare* (Moorman, 2014).

Endomycorrhizal fungi belong to one of two major types of mycorrhizae: endomycorrhizae and ectomycorrhizae. Mycorrhizae are intimate and mutually beneficial symbiotic associations between fungi and roots, and they occur in the vast majority of vascular plants, both wild and cultivated. Endomycorrhizal fungi (also referred to as vesicular arbuscular or VA mycorrhizae) penetrate plant root cells and increase the uptake of water and nutrients by both the plant and fungal cells. Ectomycorrhizal fungi also increase water and nutrient uptake but do so by surrounding plant roots not penetrating plant root cells. Of the two types, endomycorrhizae are by far the more common, occurring in about 80 percent of all vascular plants. The hyphae of *Glomus intraradices* penetrate the cortical cells of the plant root, where they form highly branched structures called arbuscules, and in some cases terminal swellings called vesicles. The arbuscules greatly invaginate the plasma membrane of the cortical cell, increasing its surface area and facilitating the transfer of metabolites and nutrients between the plant cells and the fungus. Most, or possibly all, exchange between plant and fungus occurs at the arbuscules. Vesicles may also occur between the host plant cells and are thought to function as storage compartments for the fungus. The fungal hyphae extend out into the surrounding soil for several centimeters, greatly increasing the potential for the absorption of water and the uptake of phosphates and other essential nutrients. In addition to increased water and nutrient uptake, mycorrhizae have also been shown to provide resistance to plant pathogens and nematodes. In

return for these benefits, the fungal partner receives from the host plant carbohydrates and vitamins essential for its growth (Raven, Evert, Eichhorn, 2005, p.291).

Fungicides have been used to manage *Pythium* damping-off, however concern over pesticide safety and the increasing popularity of organically produced crops has stimulated the need for further development of microbial biocontrol. Inoculating propagation media with mycorrhizal fungus is a relatively low input preventative measure for growers (Jack & Nelson, 2010). UC IPM states that prevention is the most effective disease management technique (Dreistadt, 2004, p.4). Furthermore, by 2050, 75% of the world's population will live in cities (The Endless City, 2007, p.9) and urban agriculture will become increasingly implemented and important for a sustainable food supply (Lim & Liu, 2010, p.14). Growing food in urban areas presents more restrictions on the use of fungicides, adding more value to alternative management options like the inoculation of growing media with beneficial organisms.

Materials and Methods

Glomus intraradices inoculum was obtained from Reforestation Technologies International (RTI), 1341 Dayton St., Suite G, Salinas, CA 93901, in their granular product Mykos PRO 100 which contains 80 spores of *Glomus intraradices* per gram. Two cultures of *Pythium ultimum* were obtained from the plant pathology lab at Cal Poly San Luis Obispo. The cultures were grown on 10% V-8 juice agar in petri plates measuring 8.9 cm. in diameter.

The experiment consisted of two treatment groups and a control group. Cherry tomato (*Lycopersicon lycopersicum*), seeds were sown into six plastic 72 plug trays, two plug trays for each group, 144 seeds per group. A 72 plug tray contains 72 cells measuring 1.5 in. wide at the top tapering down to 1 in. wide at the bottom by 2.25 in. deep. The entire plug tray is 21 in. long by 10.5 in. wide by 2.25 in. deep.

The media used was a 1:1:1 mix of sphagnum peat moss, perlite, and aged fir bark. The media for the treatment group labeled "Pythium" was inoculated with *Pythium ultimum* only. The media for the treatment group labeled "Mykos + Pythium" was inoculated with *P. ultimum* and *Glomus intraradices*.

The mycorrhizal inoculation was accomplished by mixing one pound of Mykos PRO 100 with the propagation media prior to filling the plug trays, which equates to 1/2 lb./plug tray. Since there are 80 spores per gram of Mykos PRO 100, it is calculated that there was approximately 252 spores per cell.

The pathogen inoculation was accomplished by cutting the agar plates into 1/4 in. by 1/4 in. pieces. One piece was then inserted into each cell approximately 1/2 in. from the bottom of the cell, 288 pieces in total. This was done after the trays had been filled with media. Cherry tomato seeds were then sown into each group, one seed per cell, 432 seeds in total.

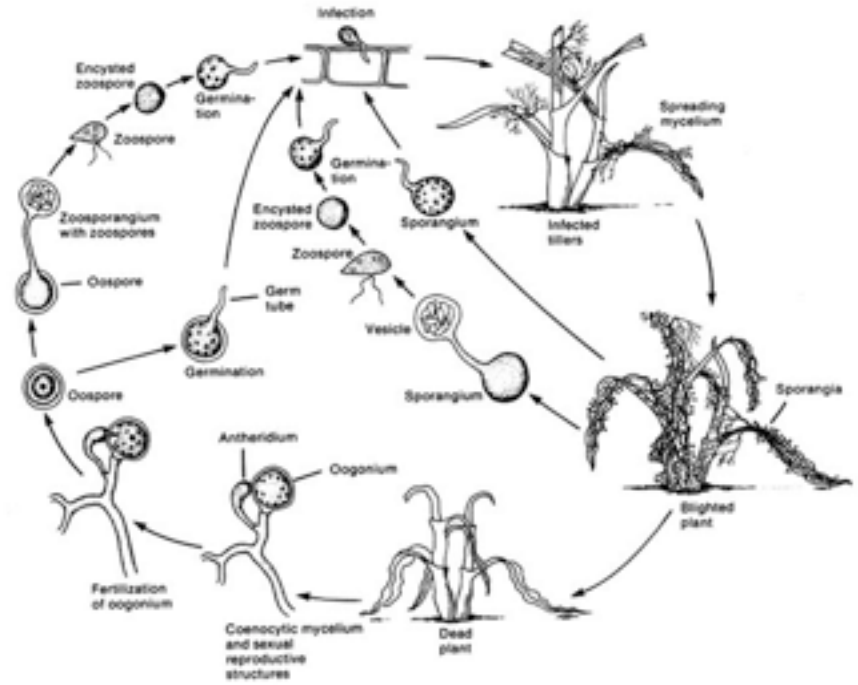
Due to a lack of availability of seed, five cultivars of cherry tomato were used in order to acquire the amount of seed needed to conduct this experiment. The cultivars used were 'Sweetie' (7 packs, 30 seeds ea.), 'Gardener's Delight' (4 packs, 30 seeds ea.), 'Rainbow Blend' (1 pack, 30 seeds), 'Yellow Pear' (2 packs, 30 seeds ea.), and 'Supersweet 100' (1 pack, 20 seeds). The seed was produced by Botanical Interests, Inc., 660 Compton St., Broomfield, Colorado. The seed is certified organic with no seed treatments. The seed was purchased in packs of 30 seeds/pack at a local home and garden store. The seed packs were emptied into a dish and mixed thoroughly prior to sowing. Of all the seeds in the dish 47.73% were 'Sweetie', 27.27% were 'Gardener's Delight', 13.64% were 'Yellow Pear', 6.8% were 'Rainbow Blend', and 4.55% were 'Supersweet 100'. There are no *Pythium* resistant varieties of cherry tomato (British Columbia Ministry of Agriculture, 2012).

The medium was kept wet by sub-irrigation. Each plug tray was kept in a water holding tray which was slightly larger than the plug tray. The water holding tray served as a means of sub-irrigation and also as a way to contain the pathogen. When the water level in the trays approached or reached zero, approximately 1 in. of water was added to the trays.

The plug trays were kept in a greenhouse designated for propagation. The experiment was checked on every 1-8 days, between 10:00 am and 4:00 pm. Temperature and humidity readings were recorded each time the experiment was checked on, starting at 29 days into the experiment. Readings were recorded close to the trays (within 1 ft.). The heating system for the greenhouse was programmed to maintain nighttime temperatures between 62 - 65 degrees F. Bottom heat with warm water tubes was also used. Readings were obtained using a RadioShack wired indoor/outdoor thermometer/hygrometer, cat. no. 6300334. Percent germination for all three groups and observations of seedling health were also recorded each time the experiment was checked on.

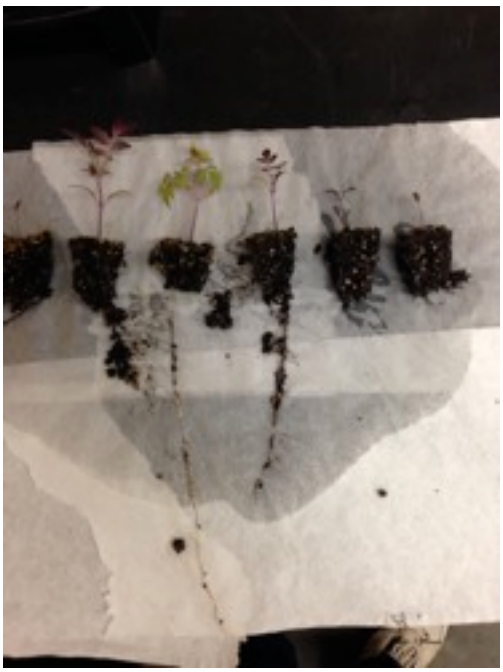
The seeds were sown on December 13, 2013 and the trays/seedlings were removed from the greenhouse to be analyzed on February 3, 2014. Fifty-two days after sowing seed, visual observations for the aseptate hyphae and oospores of *Pythium* (see figure 1) were conducted using a compound microscope at 40X. Visual observations for the arbuscules and vesicles of Glomus intraradices were also conducted using a compound microscope at 40X.

Re-isolation of *Pythium ultimum* was attempted by surface sterilizing 1" root sections with a 10% bleach 90% water solution for one minute, blotting them dry on a clean paper towel, then placing the root sections on water agar petri plates. Root sections from five representative seedlings from each plug tray (30 seedlings total, see figures 2-7) were placed in petri plates, five approximately 1" root sections per petri plate (see figure 8). One petri plate was made for each seedling (30 petri plates total). Root sections were taken from the entire root system. One week later, two additional plates were made with seedlings from the Pythium group. This time, root sections were taken only from the root tips. Also, for the last two plates, instead of choosing two representative seedlings, the two least healthy appearing seedlings were chosen.

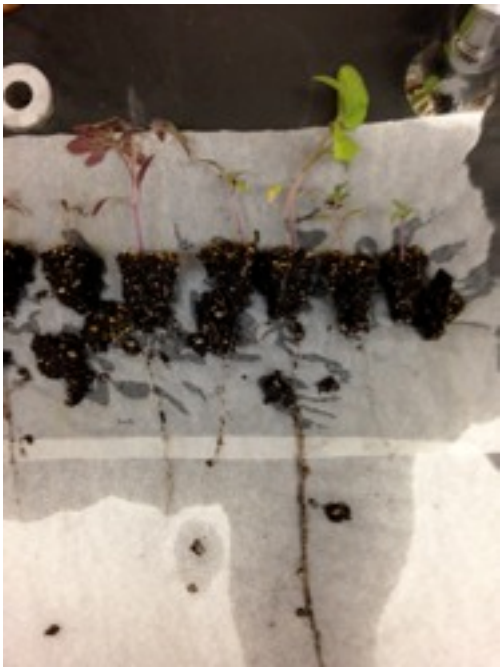


(Courtesy R.W. Smiley, P.H. Dernoeden, and B.B. Clarke), Compendium of Turfgrass diseases, 2nd edition, Page 47.)

Figure 1. Life cycle of *Pythium ultimum* (Allen, Martinez, & Burpee, 2004).



Figures 2-3. Five representative seedlings from plug tray #1 (top left) and five representative seedlings from plug tray #2 (top right) in the control group.

Figures 4-5. Five representative seedlings from plug tray #1 (top left) and five representative seedlings from plug tray #2 (top right) in the Pythium group.


Figures 6-7. Five representative seedlings from plug tray #1 (top left) and five representative seedlings from plug tray #2 (top right) in the Mykos + Pythium group.

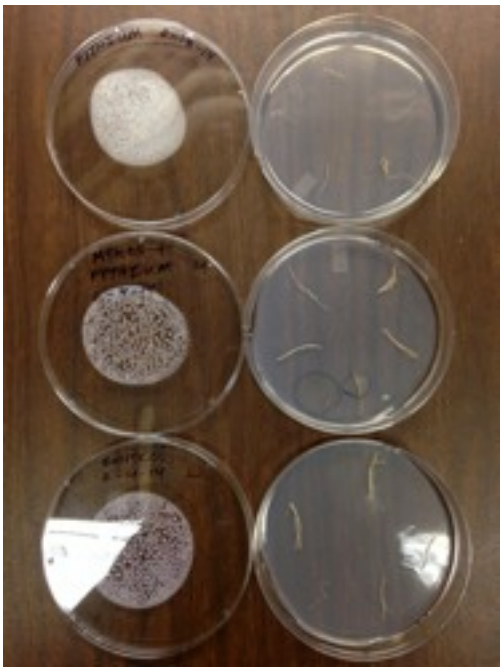


Figure 8. Three of the thirty-two petri plates, showing the placement of the root sections on water agar.

Results

Percent germination at 52 days for each group is as follows: Control = 54.86 % (see figures 9-10), Mykos + Pythium = 50.00 % (see figures 11-12), Pythium = 33.33 % (see figures 13-14). There was no visually obvious difference in seedling vigor between the control group and the Mykos + Pythium group. At least two seedlings died soon after emergence in the Pythium group. This is evidence of damping-off, the death of seedlings that collapse at the soil line under damp conditions (Dreistadt, 2004, p.268). One plant in that group grew malformed leaves and another appeared chlorotic, but still reached the fourth true leaf stage. Tomato seedlings are only susceptible to *Pythium spp*. until the two or three true leaf stage (UC IPM, 2014). These symptoms were not seen in the other two groups.

The temperature range from day 29 through day 52 was 73.8 - 88 degrees F. The heating system for the greenhouse was programmed to maintain nighttime temperatures between 62 - 65 degrees F. The humidity range from day 29 through day 52 was 21 - 94 %.

Figures 9-10. Plug trays #1 (top left) and #2 (top right) in the control group. Three seedlings had already been removed for analysis prior to taking the photos.

Figures 11-12. Plug trays #1 (top left) and #2 (top right) in the Mykos + Pythium group.


Figures 13-14. Plug trays #1 (top left) and #2 (top right) in the Pythium group.

Re-isolation of *Pythium ultimum* was not successful. Aseptate hyphae and oospores were not found on the roots observed at 40X. Also, arbuscules and vesicles were not found on the roots observed at 40X.

The data were analyzed using the chi-square test for the 2 X 2 contingency table to test for a significant difference in percent germination between all three groups. Significance level: alpha = 0.01. A statistically significant difference in percent germination was observed between the Pythium group and the Mykos + Pythium group with a P value between 0.0005 and 0.005. No statistically significant difference was observed between the control group and the Mykos + Pythium group, P value > 0.1. A statistically significant difference in percent germination was also observed between the control group and the Pythium group, P value between 0.0005 and 0.005.

Table 1. Date, temperature, and humidity data from day 8 through day 52 for all three groups.

Date, Temperature, RH	Control % germination	Pythium % germination	Mykos + Pythium % germination
12-21-13	3.47%	0%	9.72%
12-24-13	9.72%	0%	12.5%
12-27-13	11.81%	2.08%	14.58%

Date, Temperature, RH	Control % germination	Pythium % germination	Mykos + Pythium % germination
12-31-13	14.58%	4.17%	15.97%
1-6-14	21.53%	7.64%	20.83%
1-8-14	24.31%	9.03%	25.69%
1-10-14	27.78%	10.42%	29.17%
1-11-14 Temp. = 74-75 F, RH = 70%	29.17%	15.97%	33.33%
1-13-14	34.72%	20.14%	38.19%
1-17-14 Temp. = 88 F RH = 21%	40.97%	25.69%	43.06%
1-18-14 Temp. = 80.8 F RH = 30%	40.97%	26.39%	43.75%
1-21-14 Temp = 82.8 F RH = 33%	42.36%	27.78%	47.22%
1-24-14 Temp. = 79.2 F RH = 53%	43.75%	28.47%	49.31%
1-27-14 Temp.= 79.9-80.3 F RH = 44-78%	47.22%	27.78%	50.00%
1-29-14 Temp. = 83.2 F RH = 31%	49.31%	29.17%	50.00%
2-3-14 Temp. = 73.8 F RH = 94%	54.86%	33.33%	50.00%

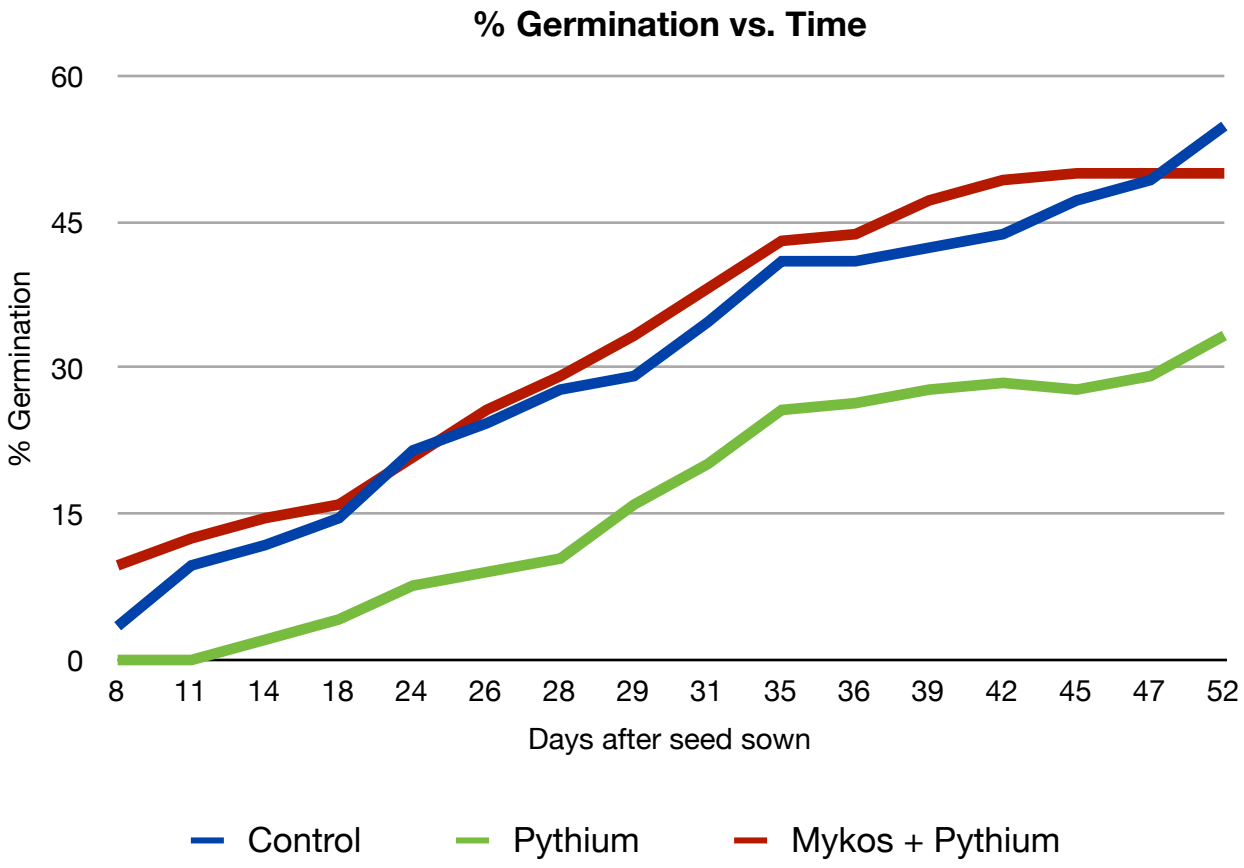


Figure 15. Percent germination vs. time from day 8 through day 52 for all three groups.

Table 2. Chi-square test for the 2 X 2 contingency table to test the null hypothesis P(germination | Pythium) = P(germination | Mykos + Pythium) against the alternative hypothesis P(germination | Pythium) < P(germination | Mykos + Pythium). Significance level: alpha = 0.01. The P value is between 0.0005 and 0.005, indicating a statistically significant difference in percent germination between the Pythium group and the Mykos + Pythium group.

	Germination		
	Yes	**No**	**Total**
Pythium	48 (60)	96 (84)	144
Mykos + Pythium	72 (60)	72 (84)	144
Total	120	168	288

Chi-Square Statistic = 8.228
0.0005 < P value < 0.005

Table 3. Chi-square test for the 2 X 2 contingency table to test the null hypothesis P(germination | Mykos + Pythium) = P(germination | Control) against the alternative hypothesis P(germination | Mykos + Pythium) < P(germination | Control). Significance level: alpha = 0.01. The P value is greater than 0.1, indicating that there is no statistically significant difference in percent germination between the control group and the Mykos + Pyhtium group.

Germination

	Yes	No	Total
Mykos + Pythium	72 (75.5)	72 (68.5)	144
Control	79 (75.5)	65 (68.5)	144
Total	151	137	288

Chi-Square Statistic = 0.682
P value > 0.1

Table 4. Chi-square test for the 2 X 2 contingency table to test the null hypothesis P(germination | Control) = P(germination | Pythium) against the alternative hypothesis P(germination | Control) > P(germination | Pythium). Significance level: alpha = 0.01. The P value is between 0.0005 and 0.005, indicating a statistically significant difference in percent germination between the control group and the Pythium group.

Germination

	Yes	No	Total
Control	79 (63.5)	65 (80.5)	144
Pythium	48 (63.5)	96 (80.5)	144
Total	127	161	288

Chi-Square Statistic = 13.536
0.0005 < P value < 0.005

Discussion

The results of this experiment show that *Glomus intraradices* suppresses *Pythium ultimum* in cherry tomato. Other experiments have also shown that *Glomus intraradices* suppresses *Pythium ultimum* (St-Arnaud, Fortin, Caron, & Hamel, 1994) and *Pythium aphanidermatum* (Larsen, Graham, Cubero, & Ravnskov, 2011). The mechanisms of suppression are unclear but there are some current hypotheses. Research suggests that these effects could be the result of increased salicylic acid in *Glomus intraradices* colonized roots which activates plant defense mechanisms through the salicylic acid and jasmonate signaling pathways (Larsen et al., 2011), also known as induced systemic acquired resistance. Evidence suggests that in addition to induced systemic acquired resistance, pathogen suppression provided by vesicular arbuscular mycorrhizae is related to improved plant growth, changes in root morphology (Whipps, 2000), competition for plant photosynthates, antagonism from mycorrhiza-associated bacteria (Larsen et al., 2011), and competition for access sites (Pal & Gardener, 2011).

The environmental conditions prevailing during the germination period will affect the growth rate of both *Pythium ultimum* and the cherry tomato seedlings. The optimum temperature for the growth of *Pythium ultimum* is between approximately 68 and 86 degrees F, with a decrease in activity at both higher and lower temperatures (Hartmann et al., 2011, p.218). UC IPM states that *Pythium ultimum* infection is most severe at temperatures below 68 degrees F (UC IPM, 2014). Seeds that have a high minimum temperature for germination (warm-season plants) are particularly susceptible to damping-off, because at lower or intermediate temperatures (less than 75 degrees F), their growth rate is low at a time when the activity of the attacking fungi is high. At high temperatures, not only do the seeds germinate faster, but the activity of the attacking fungi is less, allowing the seedlings to quickly grow past their initial vulnerable stage of growth (Hartmann et al., 2011, p.218). Tomatoes are warm season plants, meaning they require warm temperatures (>50 degrees F) to germinate (Hartmann et al., 2011, p.212), and grow best between 70-80 degrees F (Relf, McDaniel, & Morse, 2009).

The overall environmental conditions during this experiment were favorable to the growth of *Pythium ultimum* and less than ideal for the growth of cherry tomato. The daytime temperature range was between approximately 73.8 and 88 degrees F, with nighttime temperatures between 62 and 65 degrees F. Both the daytime and nighttime temperature ranges were not warm enough for optimal germination and seedling growth of cherry tomato. Although bottom heat with warm water tubes was used it is unlikely that enough heat was provided to raise the temperature to an ideal range. The relative humidity range was far below optimal for germination, optimal being 95% RH (Hartmann et al., 2011, p.269). Irrigating by means of sub-irrigation kept the growing media wet and all *Pythium* species favor wet conditions (Beckerman).

Seedlings affected by damping-off fail to emerge or fall over and die soon after emergence (UC IPM, 2014). During the experiment, at least two seedlings in the Pythium group emerged then rapidly declined and died before development of the first true leaves. Re-isolation

of the pathogen could have been a success if samples were taken from these dying seedlings. Nothing like this symptom was seen in the other two groups.

This experiment should be repeated with three improvements made to the experimental design. First, only one cultivar of cherry tomato should be used, not five. Second, pathogen re-isolation should be attempted with any seedlings showing symptoms of *Pythium ultimum* infection during the experiment, not just at the end of the experiment. Third, this experiment should be repeated under ideal conditions for cherry tomato seed germination and seedling growth. It is possible that the difference in percent germination between the Pythium group and the Mykos + Pythium group would not be as drastic if conditions were ideal. It would also be valuable to conduct the experiment until all of the seedlings have reached the fourth true leaf stage. Approximately 91.5% of the seedlings were still susceptible to *Pythium ultimum* at the time the experiment was ended due to time constraints.

$20 worth of Mykos PRO 100 was used to inoculate two plug trays in this experiment, or $10 per tray. This is very expensive and would definitely be cost prohibitive for a commercial propagator. However, this product was purchased online through a home gardening focused branch of Reforestation Technologies International, it is probable that the price could be reduced for large orders. Also, it is possible for growers to produce their own mycorrhizal inoculum, as it is a relatively low tech and low input process. Nevertheless, research should be done to determine the minimum rate of *Glomus intraradices* inoculum at which adequate suppression of *Pythium ultimum* occurs (lower rates of inoculum would lower the cost).

Conclusion

The applicability of mycorrhizal fungi to the plant propagation industry is immense. The benefits of inoculating propagation media with mycorrhizal fungi exceed pathogen suppression. Other benefits include improved plant health as well as improved tolerance of adverse conditions and transplanting (Davies). Taking into account the possible future restrictions on chemical use further highlights the importance of *Glomus intraradices* for use in the control of *Pythium ultimum*.

References

Allen, T.W., Martinez, A., and Burpee, L.L. (2004). Pythium blight of turfgrass. *The Plant Health Instructor.* DOI: 10.1094/PHI-1-2004-0929-01

Beckerman, J. Purdue University. Purdue Extension. *Disease Management Strategies for Horticultural Crops: Pythium Root Rot of Herbaceous Plants.* Web site: http://www.extension.purdue.edu/extmedia/BP/BP-181-W.pdf

British Columbia Ministry of Agriculture. (2012, May). *Pythium Diseases of Greenhouse Vegetable Crops.* Web site: http://www.agf.gov.bc.ca/cropprot/pythium.htm

Davies, F. T. Texas A&M University. *Mycorrhizal Effects on Host Plant Physiology.* Web site: *http://aggie-horticulture.tamu.edu/faculty/davies/research/mycorrhizae.html*

Dreistadt, S. (2004). *Pests of Landscape Trees and Shrubs: An Integrated Pest Management Guide*. University of California Division of Agriculture and Natural Resources. Publication 3359

Hartmann, T., Kester, D., Davies, F., Geneve, R. (2011) *Hartmann & Kester's Plant Propagation: Principles and Practices*. Eighth Edition. Pearson Education, Inc., Upper Saddle River, NJ.

Larsen, Graham, Cubero, & Ravnskov. (2011). Biocontrol traits of plant growth suppressive arbuscular mycorrhizal fungi against root rot in tomato caused by Pythium aphanidermatum. *European Journal of Plant Pathology.* 10.1007/s10658-011-9909-9

Lim, CJ., & Ed Liu. (2010). smartcities + ecowarriors. Oxfordshire: Routledge.

Moorman, G. (2014) Penn State College of Agricultural Sciences. Penn State Extension. *Pythium.* Web site: http://extension.psu.edu/pests/plant-diseases/all-fact-sheets/pythium

Pal, K., & Brian, G. (2011). Biological Control of Plant Pathogens. *The Plant Health Instructor.* 10.1094/PHI-A-2006-1117-02

Raven, Evert, & Eichhorn. (2005). *Biology of Plants.* New York: W. H. Freeman and Company.

Relf, D., McDaniel, A., & Morse, R. (2009, May) Virginia Cooperative Extension. Virginia Tech. Virginia State University. *Tomatoes.* Web site: http://pubs.ext.vt.edu/426/426-418/426-418.html

St-Arnaud, M., Fortin, J.A., Caron, M., & Hamel, C. (1994, Sept.). Inhibition of Pythium ultimum in roots and growth substrate of mycorrhizal Tagetes patula colonized with Glomus intraradices. *Canadian Journal of Plant Pathology*. v. 16 (3), p. 187-194. 16 3.

The Endless City. The Urban Age Project by the London School of Economics and Deutsche Bank's Alfred Herrhausen Society. (2007). London: Phaidon Press Ltd.

University of California Agriculture and Natural Resources. UC IPM Online. Statewide Integrated Pest Management Program. (2014, January). *UC IPM Pest Management Guidelines Tomato Damping-Off.* UC ANR Publication 3470. Web Site: http://www.ipm.ucdavis.edu/PMG/ r783103111.html

Whipps, J. M. (2000). Microbial interactions and biocontrol in the rhizosphere. *Journal of Experimental Botany.* Volume 52, Pp. 487-511