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Mitigation of Replant Disease using Solarization in American Ginseng (Panex quinquefolius)

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Abstract

Ginseng is a perennial plant that is prone to replant disease (GRD), in which ginseng cannot be re-cultivated in a former ginseng garden, largely due to pathogens in the soil. The current mitigation strategy is soil fumigation, but fumigants are being phased out. I assessed the use of solarization as an alternative to fumigation in treating GRD. Two factors, i.e., the timing and duration of solarization, were evaluated, using temperature comparisons, stand counts and root disease as indicators. I found that solarization of raised beds resulted in higher soil temperatures compared with unsolarized beds. While the duration of solarization did not improve the stand count in the first growth year, there was a significant increase in yield of marketable roots, and significant reduction in *Illyonectria mors panacis* root rot. I conclude that solarization is a promising alternative to fumigation to reduce the persistence of GRD in former ginseng gardens.

Keywords

Solarization, *Illyonectria mors panacis*, mitigation, *Panax quinquefolius*, Replant Disease

Summary for Lay Audience

Ginseng is a crop that is grown for its roots and is primarily used in Traditional Chinese Medicine. One major issue is replant disease, a condition in which ginseng cannot be re-planted in the same fields where it was grown previously due to disease-causing organisms in the soil. The current treatment against replant disease involves the use of chemical fumigants, which are hazardous to the environment. Solarization is a safer alternative. This involves harnessing the sun's heat to warm the soil by placing clear plastic tarps over it. The increased soil temperature under the tarp can kill off detrimental disease-causing organisms. To evaluate the effectiveness of solarization on preventing ginseng replant disease, I conducted experiments in a former commercial ginseng garden known to harbor a significant level of replant disease.

My experiment focused on two factors. First, I looked at when to conduct the solarization treatment. Ginseng is usually cultivated in raised bed gardens, which are formed prior to planting by ploughing the soil into mounds. Since solarization typically only affects the top 10-15 cm of soil, forming beds after solarization may introduce un-solarized soil to the surface of the raised beds during their formation. Consequently, for my project, the bed was raised prior to solarization so that seeds would eventually be planted into solarized soil. Second, I considered the duration of the solarization process. I compared the impact of four different durations of solarization: zero, two, four, and six weeks. In a parallel experiment in the same replant garden, some flat ground plots were also solarized (prior to raised bed formation) for comparison.

To evaluate the effectiveness of the solarization treatment, I measured plant survival during the first year of cultivation, and monitored the roots for signs of disease. Initially, plant survival was higher in raised bed treatments. A minimum of Four Weeks of solarization was beneficial to plant quantity. This also reduced signs of disease symptoms especially those that caused replant disease. Overall, I concluded that solarization of raised soil beds could make an effective alternative to fumigation to eliminate replant disease in ginseng gardens.

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List of Abbreviations

ATU	Accumulated Thermal Units
ANCOM	Analysis of Composition of Microbiomes
CITES	Convention of International Trade in Endangered Species
DiBP	Diisobutyl Phthalate
GLM	Generalized Linear Model
GLMM	Generalized Linear Mixed Model
GRD	Ginseng Replant Disease
Imp	*Ilyonectria mors-panacis*
OGGA	Ontario Ginseng Growers Association
OMAFRA	Ontario Ministry of Agriculture, Food, and Rural Affairs
PCR	Polymerase Chain Reaction
PPD	Protopanaxadiol
PPT	Protopanaxatriol
rRNA	Ribosomal RNA
RSD	Reductive Soil Disinfestation
Std. Sol.	Standard Solarization
TCM	Traditional Chinese Medicine
TIF	Total Impermeable Film

1 Introduction

1.1 Ginseng and Commercial Cultivation

Ginseng is a perennial herbaceous plant found in the understory of deciduous forests. (Punja, 2011). Wild ginseng species are distributed across China, parts of Canada (Southern Ontario and Southwestern Quebec), and the central United States, including from the Atlantic coast west to the Mississippi river (Li, 1995). There are two major ginseng species under commercial cultivation in China and North America: *Panax ginseng* C.A. Meyer (Asian ginseng), and *Panax quinquefolius* L. (American ginseng), respectively (Baeg & So, 2013). Due to extensive harvesting, wild American ginseng has been classified as an endangered species under the Convention of International Trade in Endangered Species (CITES). Commercial cultivation, in raised bed, shade gardens, is now the leading source of ginseng produced in North America (Robbins, 2000). As of 2017, approximately 3000 metric tons of ginseng roots (i.e., >90% of the harvest) grown in North America were exported to Asia (Statistics Canada, 2021).

Ginseng has been cultivated for over 2000 years in Asia for its roots, which are primarily used in Traditional Chinese Medicine (TCM) as adaptogens (Brekhman & Dardymov, 1969). Adaptogens are products that help maintain a person's health in equilibrium, such as maintenance and improvement of a healthy metabolism, reduction of stress, as well as improvement in heart health and blood circulation (Qi *et al.*, 2011).

Since it is illegal to harvest American ginseng found in the wild, this species is now commercially cultivated. To replicate growth conditions found in areas with stands of wild ginseng populations, the gardens need to be well drained and with a pH between 5.5 and 6.5 (Li, 1995). The ideal conditions are typically achieved on sandy loam soil sculpted into raised beds (to help with water drainage), under cover of shade canopies that filter out >70% of incident sunlight (to mimic the light conditions found on the floor of deciduous forests). Straw mulch is normally added to maintain a healthy balance of water content to minimize disease, and to maintain raised bed integrity.

American ginseng is typically cultivated for three to four years until roots achieve a marketable size for harvest. According to the Ontario Ministry of Agriculture, Food, and Rural Affairs (OMAFRA), the standard practice for the cultivation of ginseng is based on the three stages of ginseng growth: the seeding year, crop growth in the first to third year and finally harvest and post-harvest which is after the third year (OMAFRA, 2015). In the seeding year ground preparation starts with the application of cattle manure in April/May to the site selected for ginseng cultivation. This provides additional nutrients in the soil to enhance the soil condition for the ginseng crop. Next, the soil is fumigated (typically with either chloropicrin or metam-sodium) to eliminate any potential disease organisms and nematodes that would reduce the quality of the ginseng roots. Once the soil has been fumigated the shade structure is erected, typically in late July/early August. This involves the setting of posts on a 40 × 12 m grid and stringing support wires for the shade tarp that will be used to cover of the gardens in the subsequent growth years. In middle to late August the raised beds are formed in preparation for seeding. Seeds are planted at a density of 90 kg/hectare. At approximately 17,600 seeds per kilogram, this represents a seeding density of 140-150 seeds per m^2. After seeding, straw mulch is applied to help retain soil moisture and protect the seeds from drying out. Ginseng seeds require a two-step stratification before they will germinate. The first step of stratification is done with bulk seeds collected from three-year old plants (see below). Here the seeds are washed of fruit pulp and stored in barrels over winter in an un-heated shed to allow for repeated freeze-thaw cycles according to the weather. The second step of stratification occurs after planting as the seeds will sit in the raised beds over the winter before germinating in the following spring.

In the first year of cultivation, the maintenance of garden starts in April when the shades are pulled over the garden to emulate the optimal growing conditions. Seeds typically germinate in late April/early May. Starting in May until the plant die off in September, irrigation is used to help with the management of heat and water conditions in the garden, both of which are weather dependent. Beside the initial use of either chloropicrin or metam-sodium to fumigate beds prior to seeding, the growers also apply other pesticides depending on the pests present in the gardens. These include nematodes, bacterial and

fungal pathogens, insects, and other pests and lastly weeds. Each requires a different frequency of application as well as duration of application. For example, nematicides can only be applied twice each year where the last application must be seven days prior to the first harvest (in the harvest year). The most common pathogens that require control via pesticides are species of *Pythium* and *Phytophthora* (Oomycota) and *Rhizoctonia* (Fungi), each having their own specific application procedures. For *Pythium* the growers are allowed eight applications prior to first harvest, while for *Phytophthora* and *Rhizoctonia* they can apply pesticides a maximum three and two applications prior to first harvest, respectively. When it comes to insects and other pests the growers are allowed between two and three applications a year, all depending on what kind of infestation or pest they have. Lastly, herbicides are applied as frequently as required to eliminate the presence of weeds (OMAFRA, 2021).

At the end of each growth year (September to October) the shades are pulled back in preparations for the winter. After the first year of growth, a stand count is taken (i.e., the number of plants/m^2 that survived the first growth season) for both first-time gardens as well as gardens suspected of having ginseng replant disease (GRD). The first-year stand count is used to determine whether any intervention is required. For example, if there is a low stand count, growers will determine if it is worth attempting to underseed to help improve the crop in subsequent years. At the same time, using experience as a guide, the growers analyze the disease pressure by assessing the degree of visible pathogen symptoms (such as wilted plants and the reddening of the leaves), environmental factors such as drought, heavy rains, and soil structure and texture in their gardens to help determine the potential value of the crops. In extreme cases, gardens may be abandoned. In the third year of cultivation, other factors such as market value as well as accumulated expenses help determine if the crop will be harvested that year or left to grow one more year, since the presence of disease as well as the size of the roots all determine the value of the crop (OMAFRA, 2015; *pers. comm.* Carl Atkinson, Ginseng Grower, OGGA).

For the second, third and sometimes fourth years of cultivation the management practices used in the first year are repeated with the addition of removing flower buds in June from a portion (or all) of the plants in the garden. This helps to improve the root weight of the

plants. Since, 80-90% of flowering ginseng produce seeds, when the berries ripen the seeds are collected in August, near the end of the third and fourth growth year (OMAFRA, 2015). As noted above, the seeds are allowed to stratify over the winter for planting in the following year at a new site.

At the end of the third year of cultivation, root quality and plant stands are assessed and a decision between allowing an additional growth year or harvest is made. The profitability of the crop is determined in the third growth year by utilizing a deflowering matrix, which compares the economic return of a garden with and without flowers. Where positive results indicates that the garden will provide a greater income from deflowering and only harvesting the roots, while a negative result indicated that the garden would be better to not deflower, and harvest both the roots and seeds (OMAFRA, 2015). These matrix values are all based on the value of the roots and seeds that year; therefore, this matrix is not guaranteed upon harvesting. For ginseng harvest, the process starts in late August/early September with removal of the shade structures, straw mulch, and the senesced foliage, allowing for the easier access to the roots. Roots are dug out of the beds using a modified potato harvester or some sort of similar equipment. Uncleaned roots are placed in cold storage between August and October to improve the root quality by maintaining constant ginsenoside content, as well as reduce starch content while increasing sugar content. The optimal temperature for storage is between 3 and 8°C, since ginsenoside content will decrease by seven percent when stored at lower temperatures. Also, within these optimal temperatures the conversion rate between starch and sugar is best achieved (OMAFRA, 2015). Once conditioned, the roots are washed and graded to remove unmarketable roots. Finally, the roots are dried and packaged in barrels or cardboard boxes lined with plastic and sealed, to prevent re-hydration, and stored in a cool, dry location before being shipped to markets throughout the year (OMAFRA, 2015).

1.2 Replant Disease

One major concern for growers of perennial horticultural crops is an issue referred to as replant disease. Replant disease is a condition in which plants of the same species cannot

be re-cultivated in the same soil after the removal of the initial crop. Replant disease is a complex condition that affects a wide variety of perennial horticultural crops worldwide, including *Prunus* spp. (stone fruits) (Browne *et al.*, 2013, *Malus* spp. (apples), strawberries (Lü & Wu, 2018), asparagus (Blok & Bollen, 1996), and ginseng (Li, 1995). Replant disease conditions emerge through the continuous cultivation of a perennial crop over many years, however the causes and factors that contribute to this condition are poorly understood. The main symptoms are poor seed germination and plant establishment in subsequent crops due to increased pathogen pressure and changes to the soil environment. While root rot is most commonly associated with replant disease, the primary causes of replant disease is unknown (OMAFRA, 2015). Replant disease is crop specific and generally does not prevent the cultivation of unrelated crops.

One critical distinction between crops that are susceptible to replant disease is the duration over which the condition persists. For stone fruits and apples, for example, it is possible to replant in the same field (without any intervention to alter the site) after eight years (Savory, 1969), whereas for asparagus it takes ten years before the crop can be planted again (Hoestra, 1994). Importantly, each of these crop types, once mature, produces marketable product each year. Ginseng, on the other hand, takes four to five years (including the year required to establish the gardens in advance of planting) until it is ready for a single harvest, and replant disease conditions can persist in the soil for over 30 years (Dong *et al*., 2018). With a requirement for well-drained sandy soils for optimal growth, there is limited available land for ginseng production in Ontario. Based on the lengthy persistence of ginseng replant disease, the Ontario Ginseng Growers Association (OGGA) predicts that there may no longer be any suitable land for ginseng production in Ontario within 20-30 years (*pers. comm.* Sean Westerveld, Ginseng Specialist, OMAFRA).

Replant disease is thought to be caused by biotic factors, abiotic factors, or a combination of both. Each perennial crop that experiences replant disease has a different complex of factors that result in the replant disease condition. For example, replant disease in apples is caused by several different biotic factors such as parasitic nematodes and a complex of fungal pathogens found in the genera *Illyonectria* (formerly *Cylindrocarpon*),

Phytophthora, *Pythium* and *Rhizoctonia* (van Schoor *et al.*, 2009), which can be further enhanced through abiotic conditions such as poor soil structure, moisture stress and changes in pH. The most common above ground symptoms that can be observed in orchards are uneven or stunted growth of the trees. Above ground changes are usually associated with the discoloration of the roots, rotting of root tips and the general degradation of root biomass. If the young apple trees manage to survive through the first year, the disease could still cause a reduction of the quality of apples, delay the production of the crop, and even reduce the overall yield from the tree (Mazzola & Manici, 2012).

In other crops, such as strawberries, replant disease is caused by both biotic factors such as the fungus *Fusarium oxysporum* Schltdl. as well as the accumulation of an autotoxic substance known as 4-hydroxybenzoic acid, which inhibits plant growth, photosynthesis as well as cellular protection enzymes required to protect against fungal pathogens (Zhao *et al.*, 2009). Strawberry plants that have been cultivated under replant conditions experience a greater susceptibility to disease, deterioration of root biomass and poor quality and yield.

In the fields of asparagus grown in the Netherlands, Denmark and New Zealand, replant disease has been shown to reduce the quantity and the diameter of stems produced, the development of brown lesions at the base of the stem, the root and even the crown of the plant, and in some cases the degradation of the roots (Grogan & Kimble, 1959). This decline in plant quality and disease symptoms is caused by the accumulation of various organisms, which differs based on geographic locations, but typically it is caused by *F. oxysporum* and *F. fujikuroi* Nirenberg (*F. moniliforme*). In Europe the causal agents also include *Helicobasidium purpureum* (Tul.) Pat., (*Rhizoctonia violacea*) and *F. culmorum* (W.G. Sm.) Sacc., while in New Zealand *Phytophthora sojae* Kaufm. & Gerd. has been shown to be associated with replant disease (Blok & Bollen, 1993). Additionally, the presence of several cinnamic acids released from senescing/dead asparagus roots have been identified to partially enhance the growth of the casual agents listed above.

The causes of ginseng replant disease are still not fully understood; however, it is speculated that a combination of biotic and abiotic factors contribute to the overall condition. For example, the accumulation of autotoxic ginsenosides can inhibit seed germination and alter the soil microbiome, thus decreasing plant establishment and increasing the probability of infection by pathogens from the genera *Illyonectria*, *Phytophthora*, *Pythium* and *Fusarium* (Punja, 1997).

Ginsenosides are triterpene-derived secondary metabolites that, in addition to their medicinal properties, act as defense compounds against some pathogenic fungi. They are classified into two types: protopanaxadiols (PPD) and protopanaxatriols (PPT), based on the parent terpene carbon skeleton. Ginsenosides are found throughout the plant, with over 35 different ginsenosides being isolated from the roots. In mature plants, ginsenosides account for 3 to 7% of the total dry mass of the roots and between 1.9 to 4.2% of the leaves (Li *et al*., 1996). Of the total ginsenosides found the major PPD ginsenosides are Rb1, Rb2, Rc, Rd and gypenoside XVII while the major PPT ginsenosides are Re and Rg1; altogether these major components comprise more then 90% of the total ginsenosides found in ginseng root (Wang *et al.*, 2018).

Ginsenosides accumulate in the soil through root exudates or through the decomposition of plant residues. The accumulation of ginsenosides can lead to growth inhibition of some soil organisms, and growth stimulation of certain pathogenic organisms (Wang *et al.*, 2018). For example, the addition of ginsenosides to culture media led to growth inhibition of *Trichoderma* spp. and *F. oxysporum*, while stimulating the growth of pathogenic organisms such as *Ilyonectria mors-panacis* (A.A. Hildebr.) A. Cabral & Crous and *Pythium irregulare* Briusman, which are known to be a factor in ginseng replant disease (Nicol *et al.*, 2002, 2003). In a more recent study, it was shown that ginsenosides, and more specifically a mixture of Rg1, Rb1 and Rd, could be utilized as an alternative carbon and nitrogen source by both fungi and bacteria for growth, thus modifying the soil microbiome (Luo *et al.*, 2020).

In addition to the stimulation and inhibition of soil borne pathogens in the soil, ginsenosides have demonstrated autotoxic properties. Autotoxicity is the deleterious

allelopathic effect of secondary metabolites from a plant on the same species of plant. The mechanism behind the autotoxicity is the accumulation of Rg1 in root cells, which suppresses the ascorbate-glutathione cycle, and induces cell death in the roots through the accumulation of reactive oxygen species and the subsequent damage of root cell membranes and cell walls (Yang *et al.*, 2018).

1.3 Mitigation Strategies

Since the 1960s fumigation has been used to decontaminate agricultural soils for the production of various crops, with the most common fumigant being methyl bromide. Methyl bromide is a broad-spectrum fumigant that kills microorganisms, nematodes, arthropods, rodents, and certain plants (Duniway, 2002). However, since 1993, Canada (along with other countries) has classified methyl bromide as a class I stratospheric ozone-depleting agent and slowly phased out its use (Canadian Environmental Protection Act, 1999). In modern agricultural practice, chloropicrin and metam-sodium are the primary substitutes for methyl bromide. Chloropicrin is effective as a pre-plant fumigant for strawberry fields infected with *Verticillium dahliae* Kleb. Chloropicrin is normally applied in combination with other fumigants (Martin, 2003). Mai and Abawi (1981) demonstrated that chloropicrin was effective at removing fungal pathogens implicated in apple and stone fruit replant disease, while at the same time improving the yield and vigor of the trees by 109% at treated sites compared to untreated sites. In a more recent study by Spath *et al.* (2015) it was further shown that chloropicrin treatment improved apple shoot growth compared to non-fumigation treatments. By contrast, metam-sodium has been shown to be effective as a fungicide, nematicide and herbicide. Fallahi *et al.* (1998) demonstrated that metam-sodium treatment eliminated parasitic nematodes in apple orchards, while at the same time improving yield and yield efficacy in trees by 50% in the first year (Fallahi *et al.*, 1998). In another study, pre-plant treatment with metam-sodium of apple replant soil led to improved plant yield and tree trunk growth, while reducing infections caused by *Phytophthora cactorum* (Lebert & Cohn) J. Schröt. and *Pythium ultimum* Trow (Utkhede & Smith, 2000). Apple yield was improved by 58% in the first year, while infections were reduced by 39%, compared to untreated orchards. The success of both chloropicrin and metam-sodium in strawberry and apple replant

disease has led to their evaluation by the OGGA and OMAFRA for use in gardens with ginseng replant disease as a means to suppresses the symptoms of replant disease. One major issue with the use of chloropicrin and metam-sodium is their toxicity to animals, which is compounded by their contamination of both the below ground and surface water. Consequently, the usage of these fumigants has been highly regulated and is being slowly phased out, leading to the need to find safer alternatives (Simmons *et al.*, 2016).

Fumigation alternatives include reductive soil disinfestation (RSD), soil steaming, biofumigation and solarization. Reductive soil disinfestation is based on the incorporation of a readily broken-down carbon source into the soil that, when covered with a transparent plastic sheet, causing the soil to become anaerobic through microbial activity, effectively killing a wide variety of aerobic pathogens and nematodes (Shennan *et al.*, 2014). In a recent study, RSD was applied to ginseng replant gardens before Sanqi ginseng was replanted and led to improved plant survival by more than 50%, after only six months (Li *et al.*, 2019). Despite the comparative success of RSD, the approach has some limitations, including the need for large quantities of carbon amendments and frequent incorporation of the amendments through tillage, the latter of which can lead to an increase spread of the pathogens in the fields (Momma *et al.*, 2013).

Soil steaming involves the injection of steam into the soil for a long enough duration that the soil reaches temperatures between 80 and 100°C (Gurtler, 2017). The elevated temperature allows for the elimination of microorganisms, including pathogens, from the soil. The removal of pathogens allows for the soil to be repopulated first by microbes that are more heat tolerant prior to heat-sensitive pathogens. Microorganisms that are less heat tolerant recolonize slower compared to those organisms that are able to survive at higher temperatures (Bollen, 1969). In a study on apple replant disease mitigation, apple trees planted in soil that had been treated with one minute of steaming showed a 68% improvement in tree growth (Moyls & Hocking, 1994). Increasing the duration of the steaming treatment to two minutes resulted in a 120% improvement in tree growth, which was nearly equivalent to that of one minute steam treatment with an 11-55-0 fertilizer added. The disadvantages to steaming, however, are the need for specialized equipment, and the process is labor intensive and costly, with nearly 70% of the expense going

towards fuel consumption (Runia, 2000) as well as the impracticality of steaming large fields.

Biofumigation is the use of cover crops, such as from the *Brassicaceae* family, which are chopped down and incorporated into the soil to allow the formation of the natural allelochemical isothiocyanate (Gimsing & Kirkegaard, 2009). The most common *Brassicaceae* crops used are mustard crops such as *Brassica juncea* (L.) Czern., *Sinapis alba* L. (*Brassica alba*), or *Eruca vesicaria* (L.) Cav. (*E. sativa*) (Edwards and Ploeg, 2014). The accumulation of isothiocyanate in the soil has the capability of killing bacteria, fungi, nematodes and even weeds, since isothiocyanate is similar to the synthetic fumigant metam-sodium (Mattner *et al.*, 2008). Soil microbes are either affected directly by the toxicity of the allelochemical or by increased competition from the growth of some organisms that are not inhibited. For example, in a case of apple replant disease, biofumigation reduced the population of *Rhizoctonia solani* J.G. Kühn, while that of various *Streptomyces* spp. increased (Cohen & Mazzola, 2006). In addition to reducing pathogen load, the altered microbiome also led to an improvement of plant defenses by the release of nitric oxide into the soil by the *Streptomyces* spp. (Cohen & Mazzola, 2006). Similarly, treatment of apple replant disease soil with Brassicaceae seed meal resulted in a decrease in *Pythium* spp. pathogens, while the compositional change of the soil allowed for organisms that have antifungal/antibacterial functions thrived (Wang & Mazzola, 2019). In another study looking at the effect of biofumigation on peach replant disease, pre-plant treatment with *Brassica* crop did not improve initial tree growth; however, there was overall improvement in tree growth and health in both the first and second year (Pokharel & Reighard, 2015). While there has been some success with the use of biofumigation in treating replant disease, one problem with this treatment is that it requires very specific soil conditions to be effective. Moreover, biofumigation is not effective against all pathogens present in the soil (Matthiessen & Shackleton, 2005).

Mulching is the use of organic or inorganic materials to cover the soil surface, which improves plant growth through increased soil moisture and improved soil nutrient availability, all the while protecting against weeds, pests and even disease (Stapleton & DeVay, 1986). Soil solarization (hereafter solarization) is a specialized mulching practice

where the heat from the sun's radiation is trapped by a transparent polyethylene tarp spread over the ground, and can be used as a pest control strategy, especially in moist soil (Katan, 1981). There are two different kinds of solarization: flat ground and raised bed solarization, the difference being that in raised bed solarization the beds are raised prior to the solarization treatment (McSorley & Gill, 2010). Solarization has been shown to be an effective treatment against more then 40 different fungal plant pathogens, many bacterial pathogens, around 25 different nematode species and many kinds of weeds (Stapleton, 1997). The effectiveness of solarization is based on several key factors such as the amount of solar radiation as well as the use of transparent plastic film (tarps) to help maximize heating in the soil. The transparent plastic tarps help with the efficiency of solarization by allowing easy transmission of the solar radiation waves into the soil and trapping the longer wavelengths released under the tarp, thereby creating a greenhouse effect. The greenhouse effect is where the long wavelengths is unable to escape the tarped soil causing the soil to become warmer, since the warm air is unable to escape by convection, therefore the temperature becomes elevated (Stapleton, 2000). For solarization, transparent tarps are better than black tarp or other colored tarps since the latter don't permit the passage of the solar radiation into the soil. Instead, most of the incident radiation is reflected back into the atmosphere (Abu-Gharbieh *et al.*, 1991). However, black, or coloured tarps can be effective at weed suppression and moisture retention. Besides the direct physical changes to the soil microbiome through the heat trapped by the tarp, there are two other mechanisms leading to alterations in both the physical soil environment as well as the soil microbiome caused by solarization: chemical and biological.

Besides the above mentioned direct physical elimination of soil pathogens, solarization is able to change the physical soil environment. The largest change is in the increase in concentration of soluble minerals observed after solarization. The most common consistent increase after solarization being ammonium- (NH_4-) and nitrate-nitrogen (NO_3-N) as well as other minor nutrients such a calcium, magnesium, and potassium (Stapleton, 2000). In a study conducted in California it was found that in a variety of different soil types the concentration of both NH_4-N and NO_3-N in the top 15cm increased by 26-177 kg/ha after solarization (Katan, 1987; Stapleton & DeVay, 1995).

The increase in mineral nutrient availability improves subsequent plant health and growth while also reducing the amount of fertilization required for plant growth. On the other hand, biological changes to the soil environment can also be caused by solarization. That is, the removal of temperature sensitive organisms during solarization creates a "biological void" in which essential nutrients become more readily available for recolonization; however, some organisms, including soil pathogens and parasites, can have a harder time colonizing in the altered environment due to their being unable to compete with other organisms that thrive in the elevated temperature conditions. The result is a shift in the microbiome equilibrium, potentially leading to a healthier soil environment for crops (Katan, 1987).

Solarization has been used in the field since 1974 as a strategy to combat a wide variety of different soil pathogens in different crops and soil conditions. Solarization has been shown to be generally effective in the reduction of *Verticillium* wilt in tomatoes and potatoes, *R. solani* in potatoes, and *Fusarium* diseases in cotton. For example, the presence of *V. dahliae* was reduced by 65% in tomatoes planted in soil that had been solarized for four weeks, as measured 166 days after planting (Katan *et al.*, 1976). Similar results were reported for potatoes in which the incidence of *V. dahliae* was reduced sufficiently to result in a yield improvement of 45% in the first year after the soil was exposed to solarization for approximately 6 weeks (Davis & Sorensen, 1986). Significantly, disease control against *F. oxysporum* in cotton continued for three years after the soil was solarized for seven weeks, while at the same improving the cotton yield by 40-69% compared to the control after the first growth year (Katan *et al.*, 1983).

Solarization has not been used in the commercial production of ginseng in Ontario. However, in a recent unpublished experiment investigating various replant mitigation strategies, a three-week solarization treatment (used as a tarp control for fumigation, biofumigation and RSD treatments in the same experiment) resulted in stand counts approximately 50% higher than no-treatment controls (and equivalent to biofumigation and RSD treatments) in the following growth year (Westerveld & Shi, unpublished data). The improvement in stand count with only a short solarization period prompted further investigation into solarization as a mitigation strategy against GRD.

1.4 Research Objectives

The main objectives of this thesis were: (1) to determine whether soil exposed to solarization reaches temperatures that could affect soil pathogens, (2) to determine the effects of timing and duration of solarization on the stand count in the following growth year and (3) to determine the effects of timing and duration of solarization on root quality and yield. The first objective, evaluating the effect of solarization on soil temperature, was important as most of the pathogens associated with GRD have optimal growth temperatures below 30°C (Cantrell & Dowler, 1971; Cruz *et al.*, 2019; Rahman & Punja, 2005). In the second objective the two factors explored (timing and duration of solarization) were of interest since the standard practice of raised bed formation involves turning the soil, which potentially brings untreated soil to the surface of the newly formed bed. Since seeds are planted in the top few cm of the raised bed, the treatment effect should be most prominent in the seeding zone if the raised beds are solarized directly. For the second factor the duration of solarization was tested. As noted above, previous solarization trials in ginseng replant experiments were maintained for two to three weeks; however, based on results from experiments conducted by Katan *et al.*, 1976; Katan *et al.*, 1983 and Davis & Sorensen, 1986, on tomatoes, potatoes and cotton, longer durations of solarization can have lasting effects on productivity. Lastly the third objective was focused on root quality as the presence of disease symptoms on the roots is a characteristic that reduces the marketability of the roots. I predict that the reduction of pathogens on the roots by solarization would lead to more marketable roots for the growers.

Therefore, I hypothesize that solarization of pre-formed raised beds is an effective alternative to fumigation in reducing the severity of replant disease in *P. quinquefolius*. I predict that the impact of solarization will be greater if the duration of treatment is increased up to six weeks

2 Materials and Methods

2.1 Field Site and Experimental Design

A field trial to test the effect of solarizing raised garden beds was established in a 12 year old replant garden in Norfolk Country, Southwestern Ontario during the 2019 growing season. The soil was classified as sandy loam, with a pH of 6.7. A standard rotation of corn, rye and soybean was employed during the intervening years since the initial ginseng harvest in 2007. Experimental plots were established in one full bay of a commercial ginseng garden and consisted of three raised beds approximately 1.8 m wide by 40 m long, divided into 24 sub-plots measuring 1.8 m x 3.4 m. Treatments were arranged in a randomized plot design with six replications for each treatment (Figure 1). To avoid possible edge effects a 6.8 m buffer area was included at the end of each bed, and the two beds defining the edges of the bay were not used.

The field trial consisted of four separate treatments: (i) Untreated control, (ii) Two weeks of solarization, (iii) Four weeks of solarization, and (iv) Six weeks of solarization. Unlike previous field trials, in which soils were solarized before the formation of raised beds (pers. comm. Sean Westerveld, Ginseng Specialist, 2019), in the present work, raised beds were formed prior to the application of treatments. Accordingly, experimental beds were prepared by the grower in early July 2019. The raised beds were prepared by a bed shaper that formed slightly rounded surface beds measuring 0.30 m high and 1.8 m wide with a 0.25-0.30 m trench between each of the three beds. Posts and wires to support a shade canopy were installed after bed formation, but these were located in beds adjacent to the three experimental beds (Figure 1).

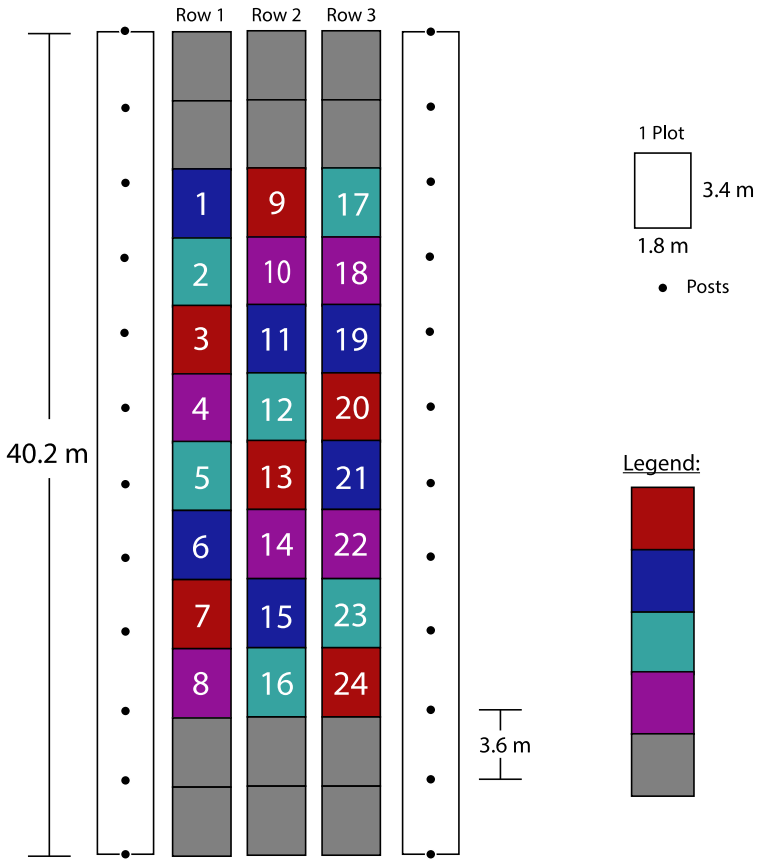

Figure 1: A Random Field Plot Design Showing Solarization Treatments. Each rectangle (block) represents an individual 1.8 m x 3.4 m sub-plot, numbered 1 through 24. The four treatments, Control and Two Weeks, Four Weeks and Six Weeks of Solarization, indicated by colour coding, were assigned to each subplot using a random number generator. The raised beds were arranged in a north-south direction.

2.2 Solarization Treatment

All three beds were initially covered entirely with a 0.03 mm transparent lineal low density polyethylene (LLDPE) VaporSafe Totally Impermeable Film (TIF) barrier (Raven Engineered Films, Sioux Falls, SD) with special care taken to avoid leaving openings or to damage the plastic sheet to prevent moisture or heat loss. For control plots, the film was removed from the surface portion of the beds right after placement, with the

remaining plastic secured with metal stakes to act as erosion protection on the sloped edges of the bed. The soil solarization experiment lasted for six weeks (5 July – 16 August), with the surface portion of the plastic film at each of the corresponding treatments removed every two weeks, and the remaining plastic film secured as above. At the end of the solarization period all remaining plastic film was removed and in late August (i.e., one week after the last solarization period) beds were seeded by the grower using the standard seeding practice of 90 kg of stratified seeds per ha.

At the time of initial plot establishment, HOBO U23 Pro v2 external temperature loggers (Onset Computer Corporation, Bourne, MA) were installed in control and six-week solarization treatment plots at three depths: i) surface, ii) depth of 15 cm and iii) depth of 30 cm. Soil temperature was monitored continuously, and the data was collected weekly, for seven weeks (5 July – 23 August).

2.3 Soil Sampling

At the start of the solarization treatment soil samples were collected and every two weeks with the start of the subsequent solarization treatment. The previous solarization samples were collected with the each new solarization treatment soil collection. At each collection three soil cores (2.5 cm diameter x 30 cm depth) were collected after the exposure of each solarization treatment using a Lamotte model 1055 soil sampling tube (Table 1).

To determine the effect of solarization at different soil depths the soil cores taken was sub-divided into three 10 cm segments prior to being pooled, yielding one pooled sample for each of the three soil depths for each treatment replicate at each collection time.

Table 1: Summary of soil sample collection for the July and August 2019. The three soil samples collected from each of the six plots were pooled and then further sub-divided into three different depths (10 cm, 20 cm and 30 cm).

Time of collection (week)	Treatment duration (week)				
	0	**2**	**4**	**6**	**TOTAL**
0	18	18	18	18	
2	-	18	18	18	
4	-	-	18	18	
6	-	-	-	18	
SUM	18	36	54	72	180

2.4 Soil Microbiome Analysis

A 100 mg soil sample from each of the three depths (10 cm, 20 cm, and 30cm) from each of the treatment durations (Control, Two Weeks, Four Weeks, and Six Weeks) was collected, which DNA was extracted using a ZymoBIOTICS DNA Miniprep Kit (Zymo Research, Irvin, CA, USA) according to the manufacturer's instructions. The quantity and quality of the DNA was measured NanoDrop One spectrophotometer (Thermo Scientific, DE, USA).

Polymerase Chain Reaction (PCR) was conducted on a Biometra TAdvanced thermocycler (Analytik Jena, Germany), to amplify the DNA in the soil using primer pairs for bacteria (V4_515F/ V4_806R) (Gohl, *et al.,* 2016), fungi (5.8S-Fun / ITS4-Fun) (Taylor *et al.*, 2016) and oomycetes (ITS1oo/ ITS3oo) (Riit *et al.*, 2016). A MasterMix solution was used composed of 625 µL of Accustart II ToughMix DNA Taq Polymerase (QuantaBio, MA, USA), 25 µL of gel electrophoresis loading dye and 62.5 µL of both the forward and reverse primers. Two different volumes of DNA (1 µL and 4 µL) was used to get the most amplifications. Lastly Mili-Q water was added to create a final solution volume of 25 µL. The thermocycle conditions were initial denaturing at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, and a final extension at 72°C for 5 min. The amplification was verified using gel electrophoresis.

Illumina sequencing was used to characterize the structure and the composition of the soil microbiome after the solarization treatments. Adapters and barcodes were attached to the amplified DNA. The barcodes Nex.SA501-508, Nex.SB501-508, and Nex.SC501-508 was used as the forward barcode primers and Nex.SA-701-712 was used the reverse barcode primers (Kozich, *et al.*, 2013). The MasterMix solution that was used was 500 µL of Accustart II Tough Mix DNA Taq Polymerase, 345 µL of Mili-Q water and 25 µL of gel electrophoresis loading dye. One µL of the amplified DNA as well as 0.8 µL of both the primers was added to make a total volume of 20 µL. The thermocycle conditions were initial denaturing at 94°C for 5 min, followed by 10 cycles of denaturation at 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 5 min. The amplification was verified using gel electrophoresis and pooled before submission to Roberts Research Institute for next generation Illumina sequencing.

2.5 Stand Count and Percent Survival

Stand count data was collected during the first year of cultivation (June 2020- September 2020) by laying out a 20 cm x 50 cm quadrat in the middle of each sub-plot and counting the number of seedlings in the quadrat. Counts were multiplied by a factor of 10 to determine the plant density per m^2. Stand counts were repeated at the beginning of each month until the end of the growth year. From the collected stand count data the percent survival was calculated for each solarization treatment. For this the initial count in June was taken as a measure of the germination/emergence for each plot. Subsequent counts were made for the months of July, August and September and the percent survival was calculated relative to the June germination/emergence count.

2.6 Ginseng Root Disease Assessment and Root Biomass

A 0.91 m x 1.8 m area (i.e., the first third) was harvested from each sub-plot at the end of the first growing season. All roots from each sub-plot were carefully collected and kept in separate sample bags. The roots from each bag were soaked in water followed by a rinsing under running water to remove access soil prior to disease assessment. The roots were assessed and further separated into three sub-categories: i) healthy, ii) rusty root (soft reddish-brown blemishes), iii) diseased, with *I. mors-panacis* as the likely causal

agent (hard brownish-black blemishes, and/or rotten roots). The disease severity guide used was that established by the OGGA for ginseng root retail quality. Accordingly, roots with no signs of blemish were classified as healthy, whereas roots with ≤ 5% blemish, between 5% and 25% blemish and ≥ 25% blemished were classified as mildly, moderately, and severely infected, respectively. The number of roots in each classification were recorded.

All roots were weighted in bulk, based on each of the disease category severity levels. To determine the root weights on a per plant basis the total weight of each category was divided by the total number of roots found in each of the categories. To make the quality analysis process similar to that used by growers the roots were then re-arranged into the categories of Marketable and Non-marketable, where Marketable included all roots that were found to be healthy or only had a mild case of either *I. mor-panacis* or rusty root. The Non-marketable roots were all the roots that had been categorized as being moderately to severely infected.

2.7 Comparative Data

The experiments described in this thesis are part of a larger collaborative project involving the Ontario Ginseng Growers Association (OGGA) and OMAFRA and aimed at testing alternative mitigation strategies to reduce the impact of GRD. Accordingly, comparative data for stand count and root disease assessment collected for untreated, fumigated, and solarized (prior to bed formation) sub-plots from a separate area in the same replant garden were provided by Amy Shi, research associate with OGGA. These data provide a baseline against which the effectiveness of raised bed formation in advance of solarization could be evaluated.

2.8 Data Analysis

Mean daily temperatures were calculated from the initial temperature data collected separately for all three soil depths. The mean daily number of hours at which the temperature was above 30°C at each of the three soil depths was determined from the raw temperature data. This was achieved by filtering the temperature data so that only

temperatures above 30°C were displayed using the R packages dplyr and tidyr and the data graphed using ggplot2 (R Core Team, 2013). The temperature data were transformed by summing the number of daily hours above 30°C into accumulated temperature units (ATU) over two week intervals for each of the treatment durations (Two Weeks, Four Weeks and Six Weeks). The accumulated temperature data for Control and Solarized plots were analyzed using a linear model (LM) with a Tukeys post hoc test. To compare the impact of solarization on the soil in which seeds would be planted, temperature data were filtered to include only the that for the flat ground solarization treatment at a depth of 30 cm and the raised bed solarization treatment at the surface. The R package dplyr was used to select the required the date (R Core Team, 2013). Stand count data and root disease assessment data were analyzed with a generalized linear model (GLM) using the "glm" and "arm" package in R (R Core Team, 2013). The root biomass, both on a per plant bases as well as the total biomass yield data were analyzed with a generalized linear mixed model (GLMM) with repeated measures using a "glmmTMB" package in R (R Core Team, 2013).

The Raw FASTQ sequencing data were returned after sequencing and initially processed using a QIIME 2 pipeline for Illumina MiSeq demultiplexed single-end sequences. A quality process was used to determine the parameters for denoising the reads using the DADA2 plugin (Callahan *et al.*, 2016). After the detection and correction of the Illumina amplicons, the soil microbiome community diversity would have been analyzed for the Control, Two Weeks, Four Weeks, and Six Weeks of solarization. Lastly, the difference in the abundance of both the fungal and oomycete communities would have been compared between the untreated soil and all the solarization treatments using the analysis of composition of microbiomes (ANCOM).

For all analyses, n = 6, except for stand count and root assessment data for control and four-week solarization treatments. For these, n = 5 since sub-plots 9 and 17 were damaged by a tractor during bed maintenance in mid 2020, rendering the sub-plots unusable.

3 Results and Discussion

Ginseng is a high profit crop; however, ginseng is susceptible to a phenomenon known as replant disease (GRD) in which ginseng cannot be cultivated in the same garden previously used to cultivate ginseng. Unlike replant disease that manifests in other perennial crops (e.g., stone fruits, apples, strawberries, asparagus), anecdotal evidence suggests that GRD persists for decades. The inability to re-use old ginseng gardens has several negative consequences. First, it means that growers cannot use existing infrastructure (poles, wires) for more than one crop without having to dismantle and re-install it; second, it means that growers must rent land further and further away from their home farm if they want to continue growing ginseng; third, and perhaps most negative, it means that eventually, there won't be any land suitable for ginseng cultivation left in Ontario. According to the Ontario Ginseng Growers Association (OGGA), without an effective strategy to combat GRD, the Ontario ginseng industry will cease to exist within 20-30 years.

The current practice to reduce the severity of replant disease (and potentially make the use of former ginseng gardens for additional ginseng crops feasible) is fumigation; however, due to the tightening of restrictions governing the application of fumigants, and the inevitable phasing out of their use, the need to investigate alternative treatments is critical. As part of an ongoing research initiative supported by the OGGA, a solarization treatment used as a "tarp control" for alternative soil fumigation treatments (e.g., RSD, biofumigation), was equally effective as fumigation in reducing GRD in a test garden. This led to the further investigation of using solarization as an alternative to chemical fumigation in treating GRD gardens. In earlier experiments, solarization, which involves covering the soil with a plastic tarp and leaving it for two to three weeks, was applied prior to soil bed formation (i.e., as required for fumigation). After the solarization treatment, the tarps were removed, and the soil prepared for seeding. In the case of ginseng this involves the formation of raised beds. However, bed formation involves turning the soil, effectively placing soil from deeper in the profile that may not have been effectively impacted by the solarization treatment, at the surface. Subsequently, seeds are planted into what is effectively untreated soil. In my thesis, I sought to test the hypothesis

that solarization of replant garden soil after bed formation would be a more effective mitigation strategy. Also, I tested the impact of different durations of solarization treatment, up to six weeks.

3.1 Effect of Solarization on Soil Temperature

The main goal in using solarization as a pre-plant treatment is to raise the soil temperature to reduce disease inoculum density. The application of plastic tarps to pre-formed raised beds resulted in slightly higher soil temperatures, compared to un-tarped beds (Fig. 2). However, there were large fluctuations in the maximum and minimum daily temperature over the course of seven weeks. Daily temperature fluctuations were more extreme at the surface, where the daily maximum temperature reached between 29.5 to 51.1°C during the day and the daily minimum reached between 11.5 to 32.5°C during the night. Solarization treatment generated maximum temperatures that were, on average, 1.24°C higher and minimum temperatures that were 0.85°C higher, compared to the Control plots at the surface. The extremes in daily maximum and minimum temperatures were presumably due to external environmental conditions that increases warming or cooling effects depending on the weather conditions on the day (i.e., sunny vs overcast) as well as the duration of daylight. Deeper in the soil profile, e.g., at 15 and 30 cm, daily temperature fluctuations were of lower amplitude (Fig. 2), where the daily maximum ranged between 26.9 and 35°C at 15 cm and 27.3 and 32.2°C at 30 cm. At the same time the daily minimum ranged between 19.1 and 23.4°C and 20.9 and 24.5°C at 15 and 30 cm, respectively. Again, Solarization treatment yielded a maximum temperature that was on average 1.2 and 0.3°C higher, and minimum temperatures that were on average 0.69 and 1.82°C higher, at 15 and 30 cm, respectively, compared to the Control plots. These trends show that solarization of raised beds resulted in higher temperatures, even to a depth of 30 cm, compared with raised beds alone.

After the tarps were completely removed, it only took one day for the temperatures to converge and be the identical between Control and Solarization plots. This result indicates that there would be no delay in the ginseng cultivation process between the removal of the solarization tarps and the seeding and application of straw in the formation of the ginseng gardens.

Comparing between raised bed solarization and flat bed solarization, temperatures in raised beds during solarization showed a trend of slightly higher temperatures compared to flat ground solarization (Fig. 3). However, there were similar fluctuations in the maximum and minimum daily temperatures at the surface, where daily temperatures ranged between 17.2 and 49.7°C through the course of the three week solarization treatment period, starting from the beginning of July and finishing at the end of July 2019. Meanwhile, at the lower depths in the soil profile (i.e., 15 and 30 cm), the daily temperatures fluctuated less than in raised beds (Fig. 3), where the daily maximum temperature ranged between 18.4 and 38.2°C and 19 and 32.4°C between Control and Solarization treatments, respectively, through the three weeks of solarization. These trends demonstrate that solarization of raised beds influences the temperature in the soil more than that of the solarization of flat ground before bed formation. However, the impact of these small differences can only be assessed through measurements of plant performance (i.e., stand counts, disease assessment).

The daily maximum soil temperatures often exceeded 30°C in both Control and Solarization plots, even at a depth of 30 cm. Since many ginseng pathogens have temperature optima at or below 30°C (Cantrell & Dowler, 1971; Cruz *et al.*, 2019; Rahman & Punja, 2005), this suggests that raised beds alone provide some potential to alter soil microbes relevant to ginseng disease. However, to be effective, elevated temperatures must be maintained long enough to impact pathogen viability.

The practice of tilling the soil is an important factor to the solarization process as tilling improves thermal efficiency due to the change the soil bulk density near the surface (Downie *et al.*, 2015) compared to deeper soil layers (Bottinelli *et al.*, 2017), which allows for the faster movement of solar radiation into the soil. In addition to effects on the soil bulk density, soil porosity is also affected with tillage allowing water to penetrate the soil while preventing evaporation, which would help maintain the temperature consistent throughout the soil profile (Kuzuku & Dökemen, 2005). Since bed formation can have a similar effect on the soil as tilling, bed formation after solarization could be considered deleterious because of the movement of untreated soil that is brought to the surface.

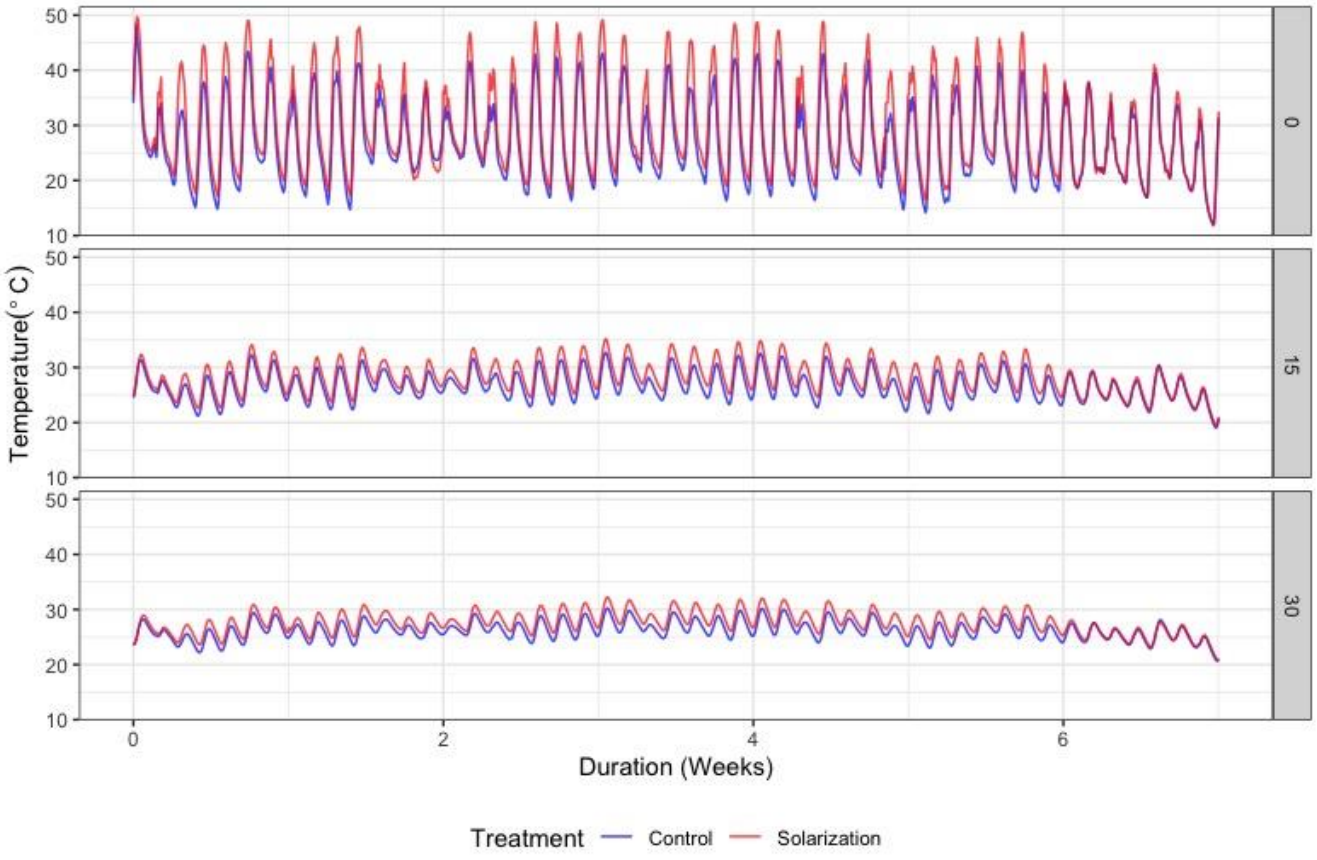

Figure 2: Soil Temperature During Solarization of Raised Beds at Three Different Depths. Temperatures were recorded in un-seeded ginseng garden beds with (Solarization treatment; red line) and without (Control; blue line) plastic tarp cover, over the course of seven weeks. Tarps were removed after six weeks. Temperatures were recorded continuously with HOBO U23 Pro v2 external temperature loggers at the surface (upper panel), 15 cm depth (middle panel) and 30 cm depth (lower panel) starting from July 5th till August 23rd, 2019. Values are the average of six plots for both Control and Solarization treatments. SD error bars removed for clarity.

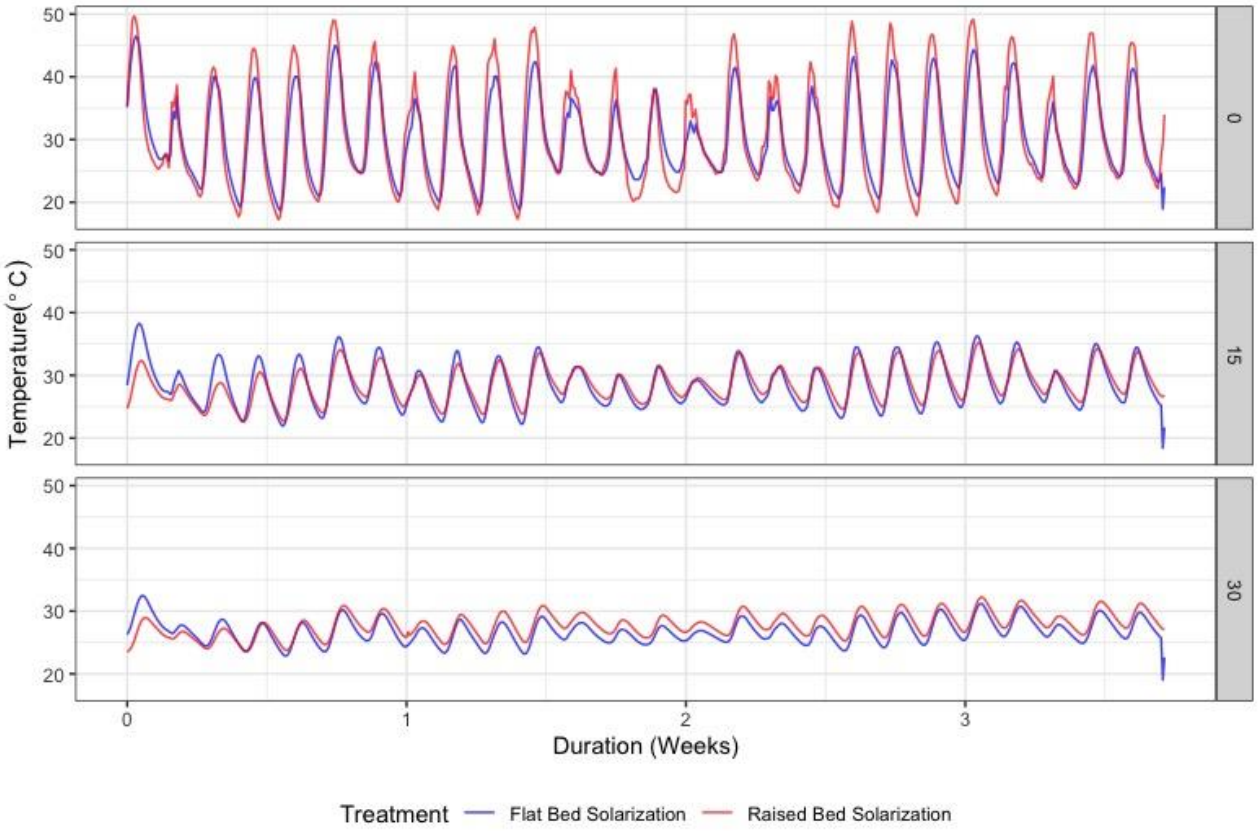

Figure 3: Soil Temperatures Measured at Three Different Depths During Flat Bed Solarization and Raised Bed Solarization. Temperatures were recorded in two separate un-seeded ginseng garden beds with two different solarization protocols (Raised Bed Solarization treatment; red line) and (Flat Bed Solarization; blue line) both covered with plastic tarp cover, over the course of three and a half weeks. Tarps were removed after three and a half weeks. Temperatures were recorded continuously with at the surface (upper panel), 15 cm depth (middle panel) and 30 cm depth (lower panel) starting from July 5th till July 31st, 2019. Flat ground solarization data courtesy of Amy Shi, OGGA, which was assessed in an adjacent bay in the same garden. SD error bars removed for clarity.

Consequently, I probed deeper into the soil temperature data to see whether solarization impacted the duration of elevated temperatures in the soil. For this I arbitrarily chose the

daily number of hours above 30°C as an indicator of treatment impact. The number of hours for which the soil surface temperature was above 30°C was higher in the Solarization treatment, throughout the six weeks of solarization, compared to the non-treatment group (Fig. 4). This was not surprising given the immediate warming effect of the trapped solar radiation under the clear plastic tarp (Stapleton, 2000). Based on the ATU analysis, there was a significant increase in ATU in all, Two, Four and Six Week Solarization treatments, compared to the control (LM, $F_{3,6329} = 272.8$, df = 3, $P<0.001$, Fig. 2). This implies that the solarization generated temperatures above 30°C for extended periods of time with as little as two weeks of solarization. Consequently any duration of solarization of at least two weeks of solarization should be sufficient to affect temperature sensitive pathogens in the soil.

At lower depths in the soil profile, the differential in the duration of temperature above 30°C in solarization plots becomes even more apparent. For example, at 15 cm depth there was a significant difference in the number of hours the soil temperature was above 30°C between the Solarization and Control conditions, over the six-week duration of the experiment. Generally, the number of hours the soil temperature was above 30°C was between 50 and 100 percent longer in the solarization plots compared to the control plots. At 30 cm depth, there was a similar trend with a higher number of hours in which the soil temperature was above 30°C in the solarization plots; however, the differential in duration for solarization was only higher between two and five weeks (Fig. 4). At all soil depths monitored, removal of the tarps after six weeks eliminated the temperature differences in duration of soil temperature above 30°C between solarization and control.

Overall, the soil temperature data indicated that the temperature in the soil during solarization was sustained at higher levels for extended periods of time, even at lower depths (but especially at a depth of 15 cm). Thus, solarization treatment of raised beds has the potential to alter the soil environment, even at a depth of 15 – 30 cm. Solarization has a wide variety of applications from weed control to pathogen management in crops. For example, solarization can be used as an organic form of weed control (Horowitz, *et al.*, 1983). It has been shown that after 4 weeks of solarization the temperature averaged between 44 to 49.5°C with an absolute maximum of 54°C at a depth of 5 cm, which was

12 to 19°C higher compared to the control (Horowitz, *et al.*, 1983). Thus, when solarization of field experiment sites achieved temperatures between 44 to 50°C it was considered an efficient and safe weed control treatment (Horowitz *et al.*, 1983). Solarization can also be used for pathogen management. For example, in strawberry fields in Turkey, solarization of flat-top raised beds for 40 days resulted in temperatures between 44.3 and 46.5°C at a depth of 10 cm. The result was a reduction in the frequency of *Macrophomina phaselina* (Tassi) Goid. as well as *R. solani* by an average of 85% and 42.9% respectively, within the first growth year (Yildiz *et al.*, 2010). Furthermore, when solarization was used for pathogen management of tomatoes in Florida, solarization of flat ground using a clear plastic tarp resulted in temperatures up to 49.2°C at a depth of 10 cm and 38°C at a depth of 25 cm. The outcome from this experiment demonstrated that solarization treatment resulted in tomatoes with similar marketability compared to those grown in methyl bromide-treated soil, while reducing the presence of phytoparastic nematode species as well as *F. oxysporum* f. sp. *lycopersici*, the main cause of wilt root in tomatoes (Chellemi *et al.*,1997).

The key difference between flat ground and raised bed solarization is the effect of the treatment on the soil that will ultimately become the seed bed. Therefore, comparing the temperatures of the flat ground solarization at a depth of 30 cm to that of the surface of the raised bed solarization plots is a way to assess how solarization impacts what is ultimately the seed bed. This is most important as the seeds are planted within the top 5 cm of the soil and where the seedlings would be exposed to the pathogens (OMAFRA, 2015). In general, there was a trend in which the raised bed solarization treatment had higher temperatures at the surface than did soil at 30 cm during flat bed solarization (Fig. 5). The highest temperatures reached at the surface during the raised bed solarization treatment was 49.7°C, while for the flat ground solarization treatment, the highest temperature reached at a depth of 30 cm was 32.5°C. Overall, the raised bed solarization treatment was 34.6% warmer than the flat ground solarization treatment, indicating that at the time of planting, seeds would be placed in soil that had received a more intense temperature treatment (and therefore likely carrying a reduced pathogen load) when planted beds solarized after formation.

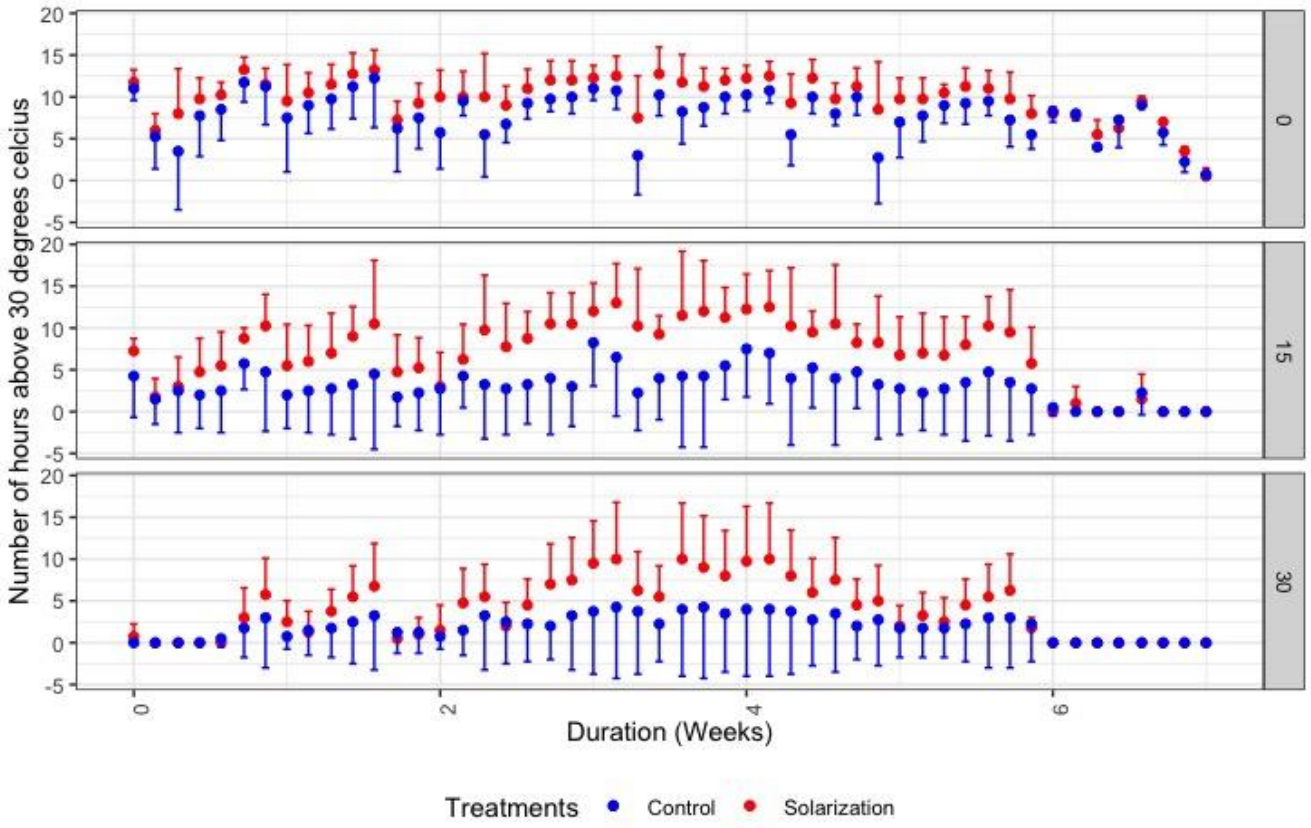

Figure 4: Daily Number of Hours Soil Temperature was Above 30°C During Seven Weeks of Solarization. The mean daily number of hours the soil temperatures was above 30°C were calculated from soil temperature data collected from un-seeded ginseng garden beds with (Solarization treatment; red symbols) and without (Control; blue symbols) plastic tarp cover, over the course of seven weeks. Tarps were removed after six weeks. Temperatures were recorded hourly with HOBO U23 Pro v2 external temperature loggers at the surface (upper panel), 15 cm depth (middle panel) and 30 cm depth (lower panel). Values are the average of six plots for both Control and Solarization treatments. Error bars represent SD, but lower bars on the Solarization and upper bars on the Control were removed for clarity.

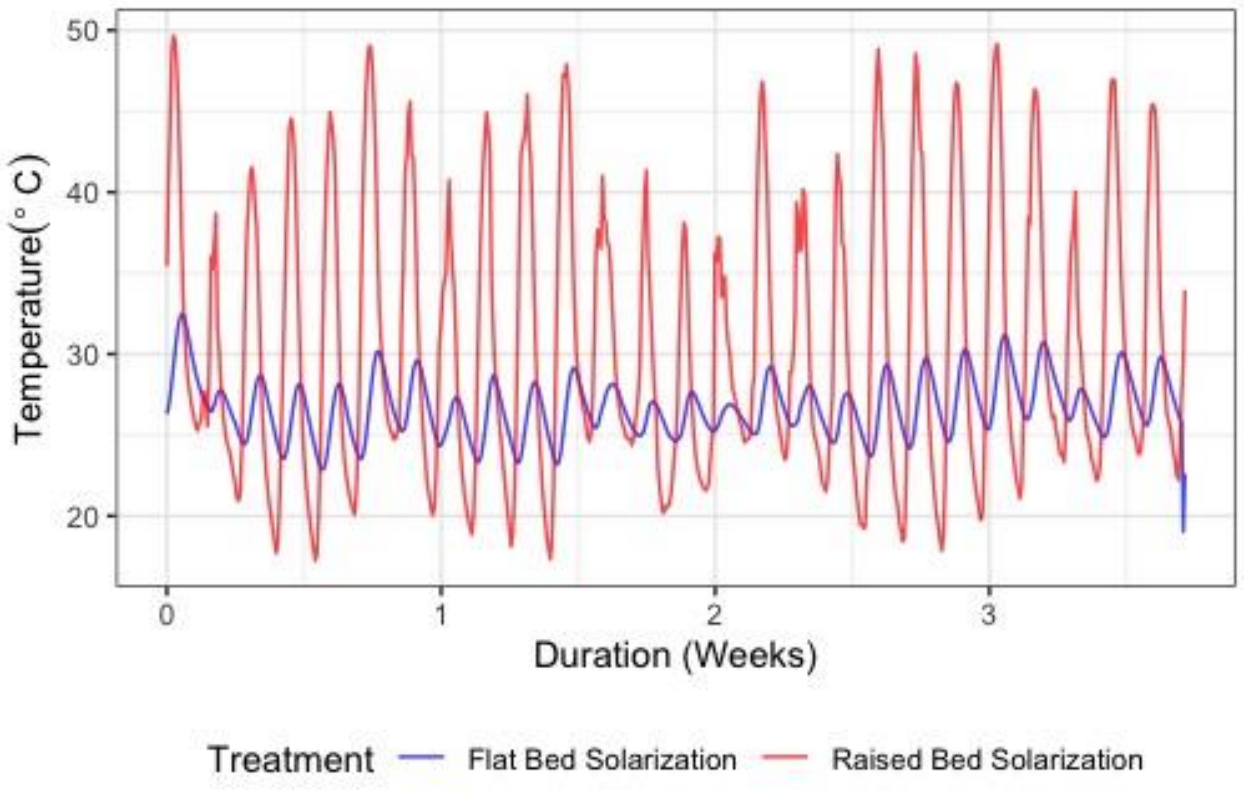

Figure 5: Seed Bed Temperature Profiles for Flat Bed Solarized and Raised Bed Solarized Soils. Temperatures were recorded in two separate un-seeded ginseng garden beds with two different solarization protocols: Raised Bed Solarization treatment (red line) measured at the surface, and Flat Bed Solarization (blue line), measured at 30 cm. In both treatments, soils were covered with plastic tarps over the course of three and a half weeks. Flat ground solarization data courtesy of Amy Shi, OGGA, which was assessed in an adjacent bay in the same garden. SD error bars removed for clarity.

3.2 Stand Count

In Section 3.1 I showed that Solarization treatment elevates the soil temperature, even at a depth of 30 cm in pre-formed raised beds. While this alteration to the soil environment has the potential to impact the soil microbiome, the true impact of the treatment can best be observed by monitoring plant performance. For commercial ginseng production, the critical metrics are the number of plants (i.e., the stand count), root quality and root mass. In this section I present the stand count data for the first year of growth of ginseng

seedlings planted in a replant garden in raised beds that were solarized for 0, 2, 4 or 6 weeks prior to seeding. For comparison, the stand count data for plants seeded in untreated replant soil and replant soil solarized for three weeks using flat ground solarization, were used. These data were provided by Amy Shi (OGGA), who was conducting fumigation and reductive soil disinfestation trials in the same garden as my solarization trials.

In Untreated replant garden soil, in which seeds were planted one week after raised beds were formed, approx. 100 plants per m^2 emerged in the following spring (Fig. 6). By comparison, significantly more plants per m^2 emerged (approx. 150) in Control plots, for which raised beds were formed seven weeks prior to planting (GLMM, $\chi^2 = 11.284$, df= 5, $P<0.001$, Fig. 6). However, as the season progressed, the difference in stand count between Untreated and Control plots declined and were no longer significant. Solarization of soil prior to bed formation did not improve stand counts relative to Untreated soil plots (Fig. 6). Similarly, no amount of solarization of pre-formed raised beds prior to planting resulted in a significant increase in the number of plants per m^2 (Fig. 6).

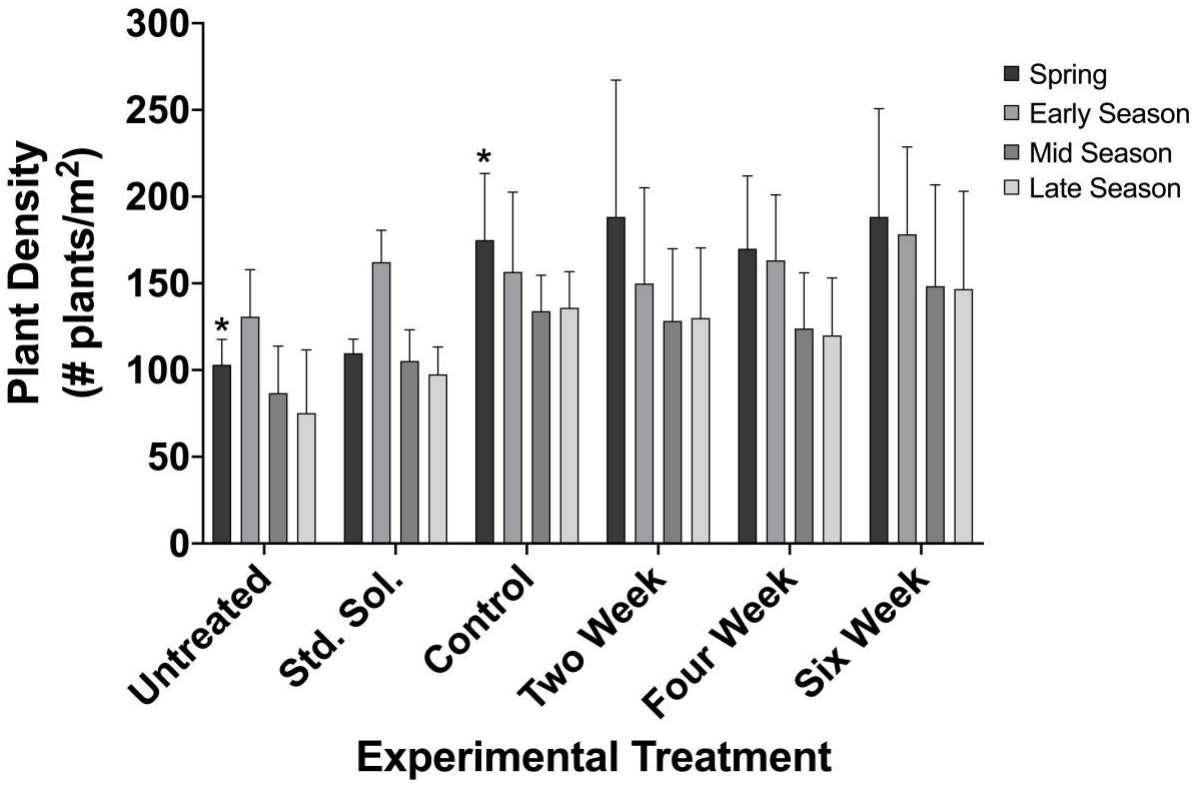

Figure 6: Ginseng Plant Stand Counts in a Replant Garden During the First Year of Cultivation. Ginseng seedlings were counted throughout the first season of growth in a replant garden, starting in the spring post-emergence (June to September). Seeds were planted the previous fall into soil that had no pre-treatment (Untreated), was solarized for three weeks using flat ground solarization (Std. Solar.) as well as solarized for zero (Control), two (Two Week), four (Four Week) or six (Six Week) weeks using raised bed solarization. All plots were seeded at the same density (90 kg of scarified seeds per hectare) with the same batch of scarified seed. Values are the average of six plots for both Control and Solarization treatments and the average of four plots for both Untreated and Std. Solar. treatment. The pair of bars labeled with an asterisk are significantly ($P<0.05$) according to Tukey's HSD test. The plant density data for Untreated and Std. Solar. treatments courtesy of Amy Shi, OGGA.

There was a general, albeit not statistically significant decline in stand count over time throughout the first year of cultivation. This decline in stand count was likely due to the natural self-thinning of the ginseng crop, since in a previous experiment it was shown that a garden seeded with 300 seeds /m^2, 260 plants/m^2 were realized in first growth year and by the end of the fourth growing year at harvest, there were only 68 roots /m^2 (Proctor *et al.*, 2001). Self-thinning is less apparent in wild stands of ginseng since the seeds are dispersed more widely from their origin, forming less dense clusters of a few to hundreds of plants that are several hundred meters away from each other. Furthermore, in wild ginseng patches, the plants are interspersed with other understory plants that potentially prevent the spread of pathogens (even if at the cost of nutrient competition). By comparison, in cultivated fields monoculture and higher plant densities lead to an increased pathogen susceptibility (Anderson *et al.*, 2002). Even though there was no significant difference in stand counts across any of the treatments of the replant garden soil prior to seeding, there was still a general trend indicating a higher stand count across all the plots where the raised beds were formed well in advance of seeding.

Even though after the first year it seems there is no significant difference in the stand counts across any of the solarization treatments, there is the possibility that there would be an improved stand count in the longer term solarized plots (i.e., Four Weeks and Six Week solarization treatments) at the time of harvest . For example, in a study that examined solarization as a method to improve the growth and yield in beans (Ibarra-Jiménez *et al.*, 2012), a 60-day solarization treatment nearly doubled yield to 3.7 tons per hectare compared with 2.1 tons per hectare from un-treated soil. Even a 30 day solarization treatment improved the bean yield to approx. 2.7 tons per hectare. The bean experiment above indicates that the duration of solarization can influence the yield of a crop (Ibarra-Jiménez *et al.*, 2012).

The large variation in my stand count data may explain in part why there was no statistical difference between stand counts from the Two Weeks, Four Weeks and Six Weeks of Solarization and the Untreated controls plots used for comparison. One reason for the high variability may be the presence of disease hot spots in the ginseng garden. These hot spots often form by the preferential flow of water to low spots in the field

(Flury *et al.*, 1994). A second cause would be loss in soil depth due to soil degradation that can cause shifts in the soil microbiome, which was carried away with the eroding soil (Mabuhay *et al.*, 2004). The major feature of preferential flow is the ability of large solutes especially nutrients such as phosphorus to penetrate the soil, which is an important part of plant and microbial growth (Alori *et al.*, 2017; Stamm *et al.*, 1998). In one study, increased nutrients and substrate supply in the soil resulted in an increase in microbial biomass up to 92% higher in areas where preferential flow was present in the soil (Bundt, *et al.*, 2001). These observations provide insight into what may be happening at my ginseng garden site, where in the first two beds there were localized areas of reduced seedling density (e.g., Bed 1 & 2, rows 2/3 and 6/7); however, at the same time there was an instance of an area with higher seedling density (Bed 1, row 5). However, even with this area of higher seedling density, the observed trend of reduced seedling density over time can still be seen over the course of the consecutive months. Overall seedling survival decreased over the course of the four month growing season, regardless of the solarization treatment (Fig. 7).

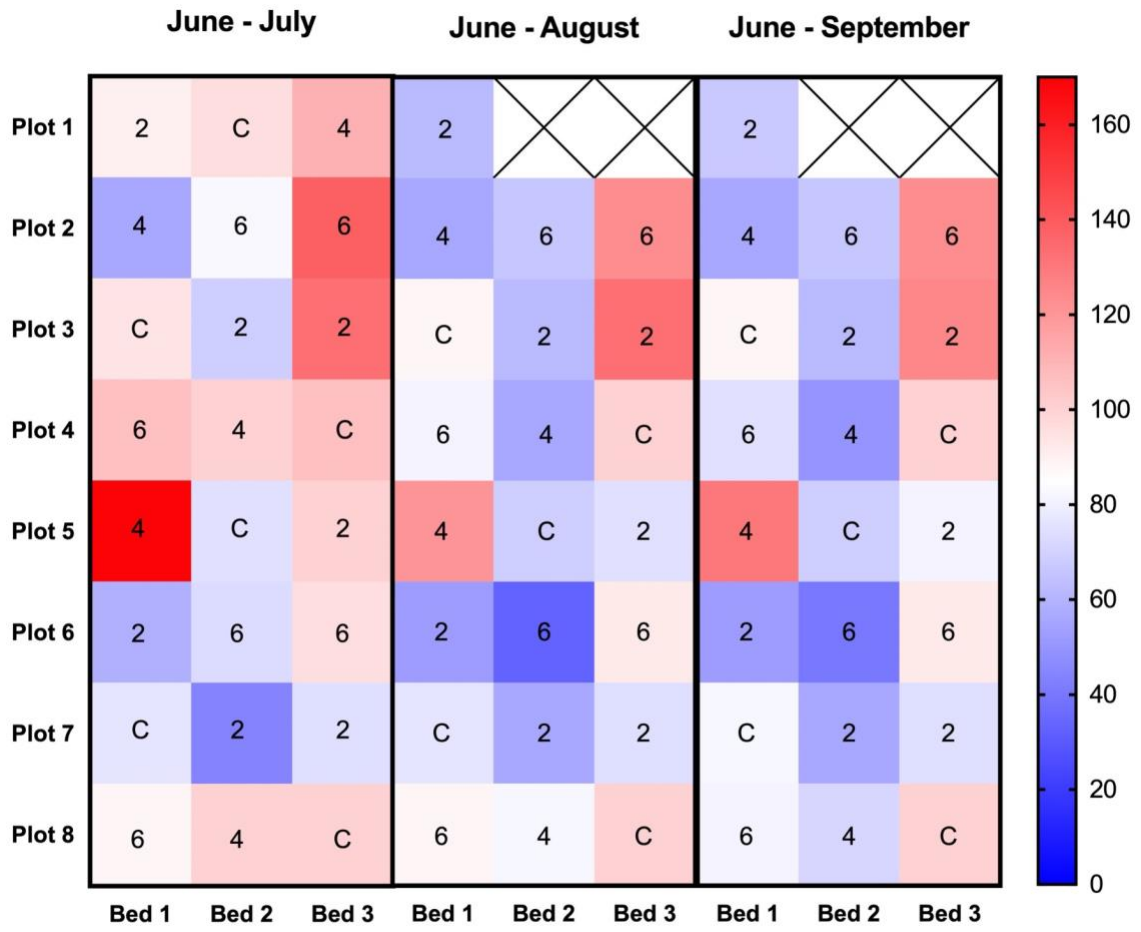

Figure 7: Ginseng Plant Survival in a Replant Garden During the First Year of Cultivation. Ginseng seedlings were counted (plants per m^2) throughout the first season of growth in a replant garden, starting in the spring post-emergence. The number of plants that remained alive at the beginning of July, August and September was compared to the initial count at the beginning of June. Percent survival was calculated relative to the June stand count and is represented as a gradient between high survival (red) and low survival (blue). Survival percentages greater than 100 percent reflect the emergence of additional plants after the initial count was taken in June. The crossed-out plots are the ones damaged by a tractor incident mid-summer.

3.3 Ginseng Root Disease Assessment

Ultimately, the main metric defining the feasibility of a ginseng crop is yield, both in terms of the number of roots, but also their quality and mass. Having addressed the stand count metric in section 3.2., here I address root quality. Ginseng roots are classified as either being marketable or non-marketable, and this is an important factor for ginseng growers to determine if their crop was successful and profitable.

The number of marketable roots increased with the duration of raised bed solarization, such that after Four weeks and the Six weeks of solarization there were significantly more marketable roots compared to Control plots that had raised beds before solarization (GLM, $\chi^2 = 63.2$, df= 5, $P<0.001$, Fig. 8). For comparison, the number of marketable and non-marketable roots from plants seeded in Untreated replant soil or replant soil solarized for three weeks prior to bed formation, were used. As above, these data were provided by Amy Shi (OGGA) and are from the same garden as my solarization trials. While the number of marketable roots was not significantly different between Untreated and Control plots, there were more non-marketable roots recovered from Control plots; however, this difference too was not significant. At the same time, the number of non-marketable roots was not significantly different across all the treatments (Fig. 8).

Ilyonectria mors panacis (Imp), the causal agent of disappearing root rot in ginseng plants is also implicated in GRD, where Imp has been found to be responsible for the average loss of 20 to 30 percent in roots over the course of growing seasons (DesRochers, *et al.*, 2020). Therefore, as part of my root quality assessment I quantified the number of roots showing classic Imp symptoms (i.e., hard brownish-black blemishes, and/or rotten roots). Overall, the proportion of severely infected roots (i.e. > 25% of root surface area showing lesions) was significantly lower in roots harvested from plots given either Four weeks or Six weeks duration of solarization, compared to the Control replant plots (GLM, $\chi^2 = 1503.1$, df= 3, $P<0.001$, Fig. 9). The decrease in the number of severely diseased roots from plots in which raised beds were solarized for Four or Six weeks duration prior to planting shows that solarization treatment of longer durations was more effective at reducing pathogens such as *I. mors panacis* in the soil.

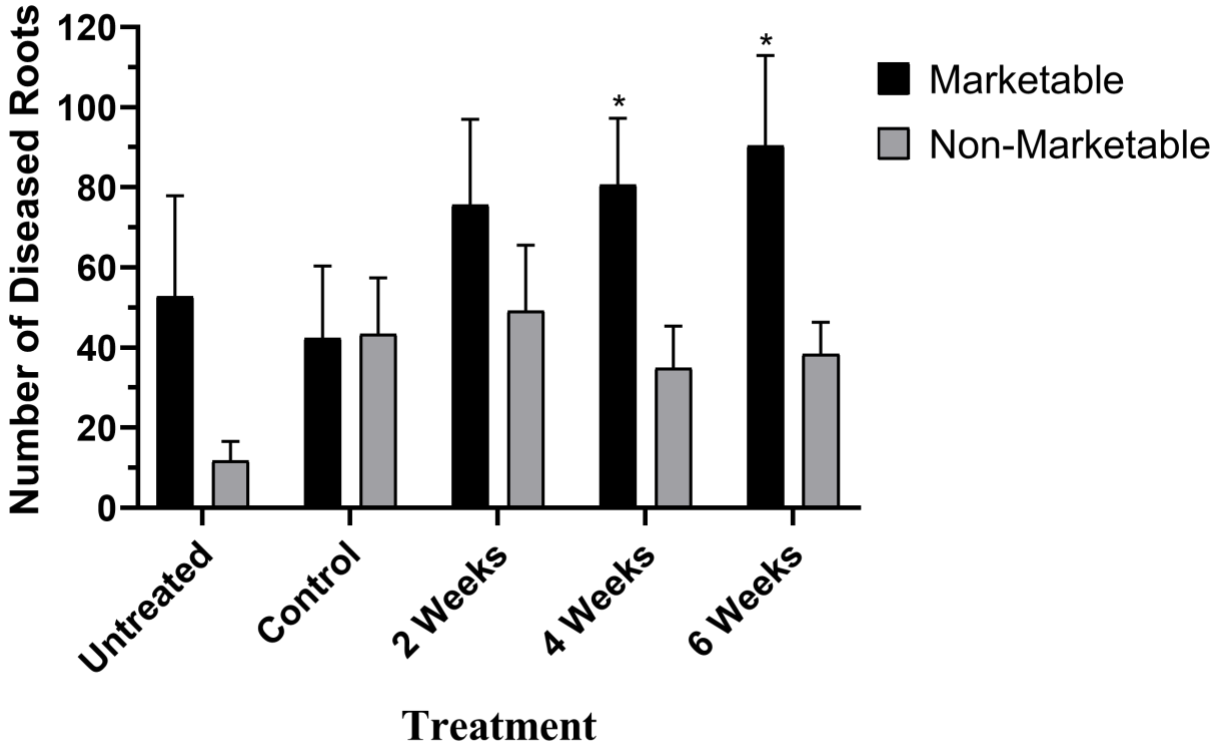

Figure 8: Assessment of Roots from Solarization Plots After One Year of Cultivation. The average number of marketable roots was determined for each solarization treatment. Roots were harvested after one year of cultivation in soil that had no pre-treatment (Untreated) or was solarized for zero (Control), two (2 Weeks), four (4 Weeks) or six (6 Weeks) weeks after bed formation, but prior to planting. Values are the average of five plots for Control and 4 Weeks and six plots for the remaining Solarization treatments and the average of four plots for Untreated treatment (roots per m^2). Bars labeled with an asterisk are significantly different from the corresponding Control bar ($P<0.05$) according to Tukey's HSD test. Data for Untreated plots courtesy of Amy Shi, OGGA.

While there was an increase in stand count and the number of marketable roots in plots from raised beds solarized for four week or six weeks prior to planting, there was still a large proportion of the roots from these plots that displayed signs of disease. The number of non-marketable roots is characterised as roots that display moderate or severe signs of

either Imp or the physiologic characteristics of rusted root disease (Rusty Root). Rusty root is charactered as reddish-brown lesions that are superficial giving the roots a pitted or scabbed appearance. Even though at times the symptoms of Rusty root look severe, it does not reduce the yield of the crop. However, due to the discoloration, deformation, and appearance of the roots the value of the roots is drastically reduced (Reeleder *et al.*, 2006).

Even with improved marketable yield in the first growth year a portion of the roots from raised bed solarization plots still demonstrated symptoms of Imp. The lowest proportion of severe Imp was in the Four Week Solarization treatment roots, indicating that a minimum of four weeks of solarization is necessary to start to reduce the presence of Imp in the soil.

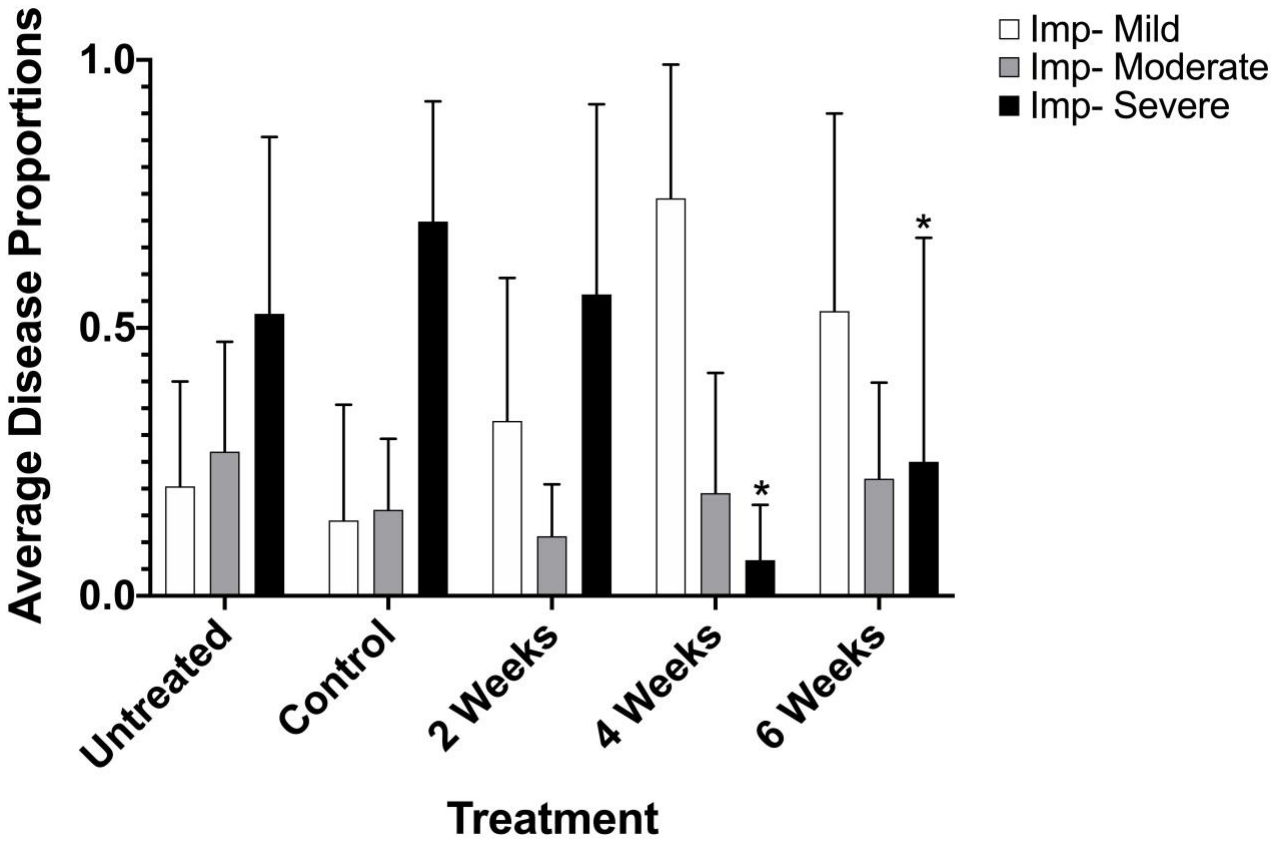

Figure 9: Disease Assessment of Ginseng Roots After One Year of Cultivation. The proportion of mild, moderate, and severe root rot caused by *Illyonectria mors-panacis* (Imp) was determined for roots harvested after one year of cultivation in soil that had no pre-treatment (Untreated) or was solarized for zero (Control), two (2 Weeks), four (4 Weeks) or six (6 Weeks) weeks after bed formation, but prior to planting. Values are the average of five plots for Control and 4 Weeks and six plots for the remaining Solarization treatments and the average of four plots for Untreated treatment. Bars labeled with an asterisk are significantly different based on Imp- Severe compared to Control plots ($P<0.05$) according to Tukey's HSD test. Data for Untreated plots courtesy of Amy Shi, OGGA.

3.4 Marketable Root Biomass

Ultimately, yield (and profit) is dictated by the number of healthy roots and their total biomass. Even though the plants in my solarization plots were only one year old and ginseng is normally cultivated for three to four years before harvest, I wanted to get a

measure of how well the roots were growing. For comparison, the mass of marketable and non-marketable roots from plants seeded in Untreated replant soil or replant soil solarized for three weeks prior to bed formation, were used. As above, these data were provided by Amy Shi (OGGA) and are from the same replant garden as my solarization trials.

Overall, there were no statistically significant differences in root biomass, on a per plant basis, across any treatment, regardless of whether roots were marketable or not (Fig. 10). However, roots harvested from plots treated with raised bed solarization tended to be heavier compared to roots harvested from plots where beds was formed after an application of flat ground solarization. Even though there was a trend indicating that at least Four weeks of solarization of pre-formed raised beds increased the average root biomass in marketable roots compared to non-marketable roots, any amount of solarization less than four weeks had a reduced average root biomass in marketable quality roots in the first growth year (Fig. 10).

Despite their being no significant differences in the root biomass, on a per plant basis, an assessment of the total biomass yield demonstrated that raised bed solarization resulted in a higher marketable root mass. Specifically, while the total biomass yield from flat ground solarization Untreated plots did not differ significantly from that of flat ground Std. Sol. Treated plots or the raised bed solarization Control plots, raised beds solarized for Two Weeks (GLMM, χ^2 =50.437, df= 6, P<0.01, Fig. 11), Four Weeks (GLMM, χ^2 =50.437, df= 6, P<0.01, Fig. 11) or Six Weeks (GLMM, χ^2 =50.437, df= 6, P<0.001, Fig. 11) had significantly higher total biomass yield compared to the raised bed solarization Control. These results suggest that even though the roots are not normally harvested after only one year, at least two weeks of raised bed solarization led to an improvement in the total biomass yield. The increase in total biomass yield after only one year may be a prelude to increased yields at harvest after three years of cultivation.

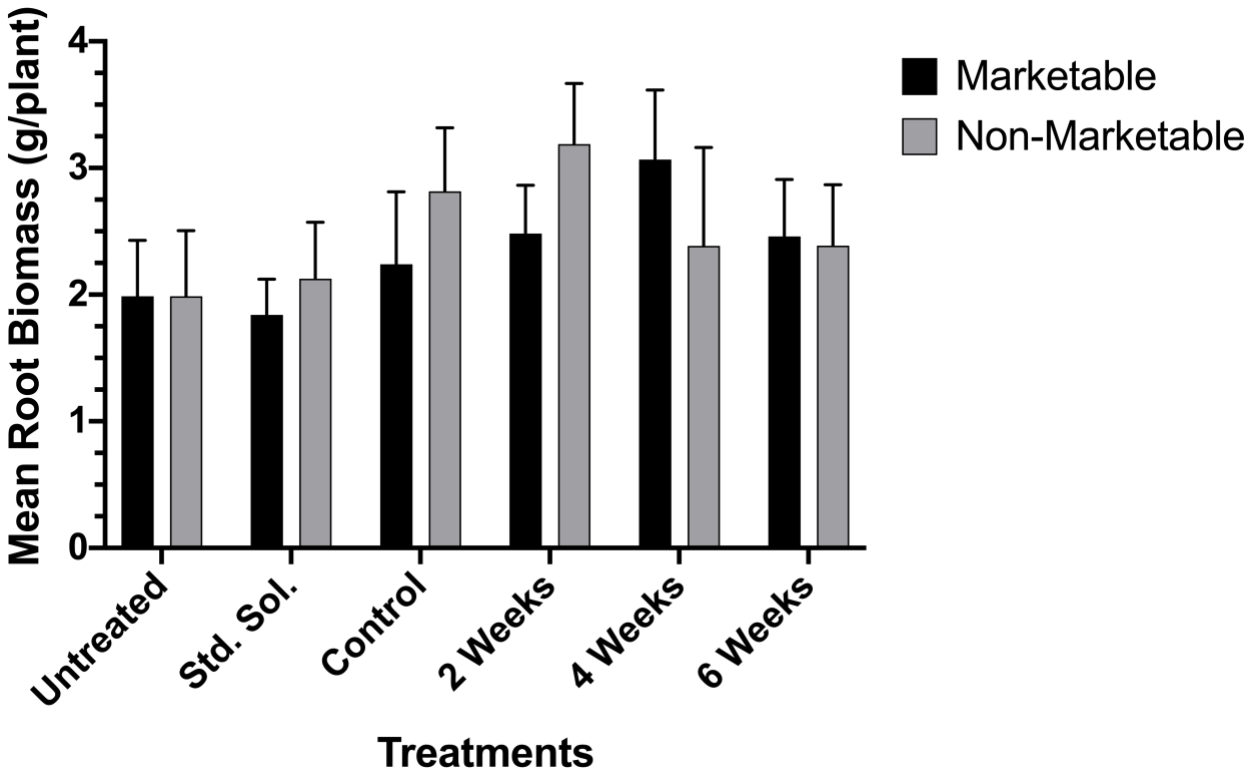

Figure 10: Mean Root Biomass of Marketable Roots per Plant after One Year of Cultivation. Roots were harvested after one year of cultivation in soil that had no pre-treatment (Untreated), was solarized for three weeks prior to bed formation (Std. Solar.) or was solarized for zero (Control), two (Two Week), four (Four Week) or six (Six Week) weeks after bed formation, prior to planting. Values are the average of six plots for both Control and Solarization treatments and an average of four plots for both Untreated and Std. Solar. treatments. Data for Untreated and Std. Solar. plots courtesy of Amy Shi, OGGA.

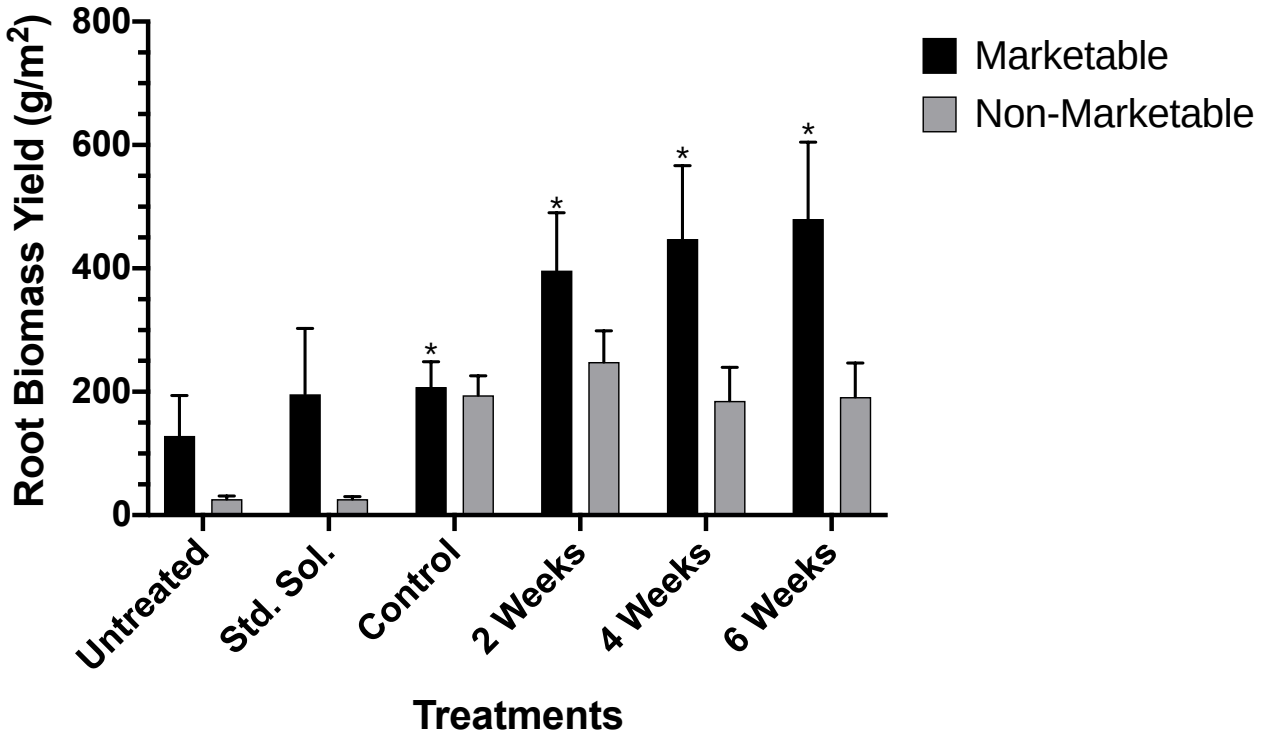

Figure 11: Total Yield of Marketable Roots after One Year of Cultivation. Roots were harvested after one year of cultivation in soil that had no pre-treatment (Untreated), was solarized for three weeks prior to bed formation (Std. Solar.) or was solarized for zero (Control), two (Two Week), four (Four Week) or six (Six Week) weeks after bed formation, prior to planting. Values are the average of six plots of the combined root biomass and number of marketable roots for both Control and Solarization treatments and an average of four plots of the combined root biomass and number of marketable roots for both Untreated and Std. Solar. treatments. Data for Untreated and Std. Solar. plots courtesy of Amy Shi, OGGA.

3.5 Impact of Solarization on the Soil Microbiome

The practice of solarization has been shown to result in the elevation of the temperature in the soil profile at a wide variety of depths. The increase in temperature in the soil profile would likely impact the soil microbiome, particularly the organisms of a pathogenic nature. This idea of changing the abundance of pathogens in the soil

microbiome is what lead me to want to determine whether solarization in a raised bed garden had any impact on the soil microbiome in the garden site.

The exploration into the impact of solarization on the soil microbiome was my initial intent in determine the effectiveness of solarization as an alternative treatment on the mitigation of GRD. Soil samples were collected throughout the six weeks of the experiment when the tarps were removed so that a temporal analysis of the soil microbiome could be achieved. At the same time, the soil samples were further subdivided into three depths of surface, 15 cm, and 30 cm deep to perform a spatial analysis of changes to the soil microbiome.

A metabarcoding (Ruppert *et al.,* 2019) investigation into the abundance of bacterial, fungal and oomycete pathogens in the soil was attempted with three unique primers designed to focus on the 16S ribosomal RNA (rRNA) region in the bacterial microbes (Gohl *et al.,* 2016) and the ITS2 regions for both the fungal (Taylor *et al.*, 2016) and oomycete (Riit *et al.,* 2016) pathogens. However, after months of testing a wide variety of different soil extraction techniques and PCR programs, I could not obtain sufficient quality or quantity of DNA to obtain useable Illumina sequence data. I determined that there was not enough DNA being extracted from the sandy soils typical of ginseng gardens to achieve sufficient reads to complete the soil microbiome abundance analysis. My lack of success in obtaining suitable amounts of DNA for microbiome analysis was probably due to several factors, including (1) using too small an amount of soil, (2) the inability to completely rupture the cells, and (3) high humic acid present in the soil, which can act as an inhibitor of the PCR protocol (Fatima, *et al.*, 2014).

Despite my difficulties in extracting sufficient quality and quantity of DNA from the sandy-loam soils of my ginseng replant garden, microbiome analyses remain an important objective in assessing mechanisms underlying the impact of raised bed solarization as a GRD mitigation strategy. For example, recent articles demonstrate that it would be possible to reduce the prevalence of GRD in ginseng with successful manipulation of the soil microbiome. In one study, it was shown that *Panax ginseng* produces toxic substances such as benzoic acid, diisobutyl phthalate (DiBP), palmitic

acid, p-hydroxybenzoic acid, and cinnamic acid that all have allelopathic potential to inhibit the growth of ginseng seedlings. The results indicated that DiBP alone managed to inhibit seedling shoot growth by 27.16 to 64.17%, while the accumulation of toxic compounds from the continuous monoculture of ginseng leads to the decrease in the soil microbiome further reducing ginseng growth over many years (Dong *et al.*, 2018). The ability of specific bacteria to improve ginseng health over the course of three years suggests that if a mitigation strategy such as solarization resulted in an increase in these beneficial bacteria in the soil profile, then GRD could potentially be reduced. In another study, the impact of solarization on *F. oxysporum* in soils used to grow melons was explored. Specifically, of the five trials in which fields received a solarization treatment (over the course of six years), three of the trials reduced the abundance of *F. oxysporum* by 82 to 90 percent, and thereby the presence of Fusarium wilt in melons (Tamietti & Valentino, 2006). Similarly, four weeks of solarization completely eliminated *V. dahlia* to a depth of 25 cm in a tomato replant field. At the same time *F. oxysporum* was reduced between 94 and 100% at the surface, while at lower depths of 15 cm and 25 cm, the reduction was between 68 to 100% and 54 to 64%, respectively (Katan *et al.*, 1976). These previous studies demonstrate that solarization impacts the soil microbiome, especially those pathogens that have been shown to cause replant disease in crops, thus providing a foundation that solarization could be used to reduce the presence of GRD.

4 Conclusion and Future Work

In summary, the purpose of my thesis experiments was to determine if raised bed solarization compared to flat ground solarization would be an effective alternative treatment practice in reducing the severity of GRD in American ginseng. I found that that the duration of solarization had no major impact on the stand count in the first growth year. However, the timing of solarization treatment did improve the stand count. This result implies that if a grower were to apply a layer of a clear plastic tarp anytime between June and July, but after the formation of the raised beds, there would be an improved stand count at the beginning of the first growth year.

My assessment of the disease status of one year old plants showed that the duration of solarization was related to the amount of Imp in the first growth year, with longer duration of solarization treatment resulting in lower incidence of Imp. At the same time, longer durations of solarization also resulted in an increased number of marketable roots after the first year, especially with four to six weeks of solarization. This shows that a minimum treatment of four weeks of solarization prior to seeding can potentially reduce the severity of Imp on the roots of the ginseng and a replant garden.

Overall, a modification to the standard growing practice of ginseng by potentially starting with the formation of raised beds in June or July followed by a minimum application of four weeks of solarization would benefit ginseng growers and potentially improve the quality of the ginseng crop in a replant garden, while reducing the use of harmful chemicals that is harmful to the environment.

I hope that the promising preliminary results shown in my thesis will encourage a grower to allow this experiment to be repeated; however, this requires a significant time commitment, as the impact of solarization on overall marketable yield of the final crop needs to be assessed. Such an analysis is still plausible in the garden used for my study as it will remain accessible for the next two seasons. This makes it possible to be able to

collect the stand count as well as determine the disease state of the roots over the course of three seasons.

To estimate the impact of solarization on the soil microbiome there is a need to optimize the DNA extraction protocol for sandy-loam soils typical of ginseng gardens. This will allow a global assessment of bacterial, fungal, and oomycete communities in GRD soils, and changes to them during solarization. Optimization will likely be achieved by increasing the amount of soil used for the extraction (Tien *et al.*, 1999), thoroughly grinding the samples, as well as experimenting with the different way to reduce the presence of humic acids in soil extracts as these can interfere with the DNA template amplification (Matheson *et al.*, 2010).

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6 Appendices

Appendix A: R Script used to Create the Temperature Trend Figure. The entire script used to create the temperature trend figure (Fig. 2).

Temperature Trend Figure Code

Andrew Rabas

17/05/2021

Clear RStudio Memory:

```
rm(list = ls())
```

Libraries Used:

```
library(dplyr)
library(ggplot2)
```

Set Working Directory:

Make sure you set your working directory to the same location as where the data is located.

```
setwd("~/Documents/Western Masters/Research Project/Stats")
```

Load and Read Data File:

```
Trend_data <-read.csv("~/Documents/Western Masters/Research
Project/Stats/Temp Trend Data.csv")
glimpse(Trend_data)

## Observations: 7,068
## Variables: 9
## $ Duration..Weeks <dbl> 0.00000, 0.00000, 0.00595, 0.00595, 0.01190,
0.01190,…
## $ Treatment       <fct> Control, Solarization, Control,
Solarization, Control…
## $ Depth           <int> 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
0, 0, 0,…
## $ Mn              <dbl> 34.11450, 35.42775, 39.86050, 40.73650,
43.31825, 44.…
## $ SD              <dbl> 3.654324, 3.666240, 9.261148, 8.987290,
```

```
10.224402, 9.…
## $ SE               <dbl> 0.02179793, 0.02187368, 0.05526607,
0.05364327, 0.061…
## $ X                <lgl> NA, NA, NA, NA, NA, NA, NA, NA, NA, NA, NA,
NA, NA, N…
## $ X.1              <lgl> NA, NA, NA, NA, NA, NA, NA, NA, NA, NA, NA,
NA, NA, N…
## $ X.2              <lgl> NA, NA, NA, NA, NA, NA, NA, NA, NA, NA, NA,
NA, NA, N…
```

####Figure Plot:

```
ggplot(Trend_data) +
   geom_line(aes(x = Duration..Weeks, y = Mn,
                      col = Treatment), alpha = 0.75) +
   labs(x = "Duration (Weeks)",
        y = expression("Temperature" (degree~C))) +
   scale_color_manual(values = colors) +
   facet_grid(Depth ~.) +
  theme_bw() +
  theme(legend.position = "bottom") +
  scale_colour_manual(breaks = c("Control", "Solarization"),
                                  values = c("blue", "red"))

sessionInfo()

## R version 3.6.1 (2019-07-05)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS  10.16
##
## Matrix products: default
## BLAS:
/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.d
ylib
## LAPACK:
/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.d
ylib
##
## locale:
## [1] en_CA.UTF-8/en_CA.UTF-8/en_CA.UTF-8/C/en_CA.UTF-8/en_CA.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggplot2_3.2.1 dplyr_0.8.4
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.3       knitr_1.28       magrittr_1.5
munsell_0.5.0
##  [5] tidyselect_1.0.0 colorspace_1.4-1 R6_2.4.1         rlang_0.4.4
```

```
##  [9] fansi_0.4.1      stringr_1.4.0    tools_3.6.1      grid_3.6.1
## [13] gtable_0.3.0     xfun_0.12        utf8_1.1.4       cli_2.0.1
## [17] withr_2.1.2      htmltools_0.4.0  lazyeval_0.2.2   yaml_2.2.1
## [21] assertthat_0.2.1 digest_0.6.25    tibble_2.1.3
lifecycle_0.1.0
## [25] crayon_1.3.4     purrr_0.3.3      vctrs_0.2.3      glue_1.3.1
## [29] evaluate_0.14    rmarkdown_2.1    stringi_1.4.6
compiler_3.6.1
## [33] pillar_1.4.3     scales_1.1.0     pkgconfig_2.0.3
```

Appendix B: R Script used to Create the Duration Above 30°C Figure. The entire script used to create the duration above 30°C figure (Fig. 4).

Duration Above 30 Figure Code

Andrew Rabas

17/05/2021

Clear RStudio Memory:

```
rm(list = ls())
```

Libraries Used:

```
library(dplyr)
library(ggplot2)
```

Set Working Directory:

Make sure you set your working directory to the same location as where the data is located.

```
setwd("~/Documents/Western Masters/Research Project/Stats")
```

Load and Read Data File:

```
Above_30 <-read.csv("~/Documents/Western Masters/Research
Project/Stats/Duration above 30.csv")
glimpse(Above_30)

## Observations: 150
## Variables: 6
## $ Days      <dbl> 0.00, 0.14, 0.29, 0.43, 0.57, 0.72, 0.86, 1.00,
1.14, 1.29,…
## $ Depth     <int> 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
```

```
0, 0, 0,…
## $ Hours_Sol <dbl> 11.75, 6.00, 8.00, 9.75, 10.25, 13.25, 11.50,
9.50, 10.50, …
## $ Hours_NS  <dbl> 11.00, 5.25, 3.50, 7.75, 8.50, 11.75, 11.25, 7.50,
9.00, 9.…
## $ SD_Sol    <dbl> 1.500000, 2.000000, 5.354126, 2.500000, 1.500000,
1.500000,…
## $ SD_NS     <dbl> 1.414214, 3.862210, 7.000000, 4.856267, 3.696846,
2.362908,…
```

Figure Code:

Way to make specific headers a certain color.

NOTE: Make sure that the color coding matches your header exactly.

```
colors <- c("Solarization" = "red", "Control" = "blue")

pd <- position_dodge(0.1)
```

####Figure Plot:

The `geom_linerange()` code creates the black line between each paired dots.

```
Above30 <- ggplot(data = Above_30, aes(x = Days),
cex.lab=1.25,cex.axis=1.25) +
         geom_point(aes(y = Hours_Sol, color = "Solarization")) +
         geom_point(aes(y = Hours_NS, color = "Control")) +
         geom_linerange(aes(ymax = Hours_Sol, ymin = Hours_NS)) +
         labs(x = "Duration (Weeks)",
              y = "Number of hours above 30 degrees celcius",
              color = "Treatments") +
         scale_color_manual(values = colors) +
         facet_grid(Depth ~.) +
         theme_bw()
```

If you want error bars instead of black lines between each paired dots use code below:

```
Above30 <- ggplot(data = Above_30, aes(x = Days)) +
         geom_errorbar(aes(ymax = Hours_Sol + SD_Sol, ymin= Hours_Sol),
width = 0.05, position = pd, color= "red") +
         geom_point(aes(y = Hours_Sol, color = "Solarization")) +
         geom_errorbar(aes(ymax = Hours_NS , ymin= Hours_NS - SD_NS),
width = 0.05, position = pd, color = "blue") +
         geom_point(aes(y = Hours_NS, color = "Control")) +
         #geom_linerange(aes(ymax = Hours_Sol, ymin = Hours_NS)) +
         labs(x = "Duration (Weeks)",
              y = "Number of hours above 30 degrees celcius",
              color = "Treatments") +
         scale_color_manual(values = colors) +
```

```
          facet_grid(Depth ~.) +
          theme_bw()
```

Axis formatting:

The code below will rotate the axis values. Increase the font size of both the axis values and the axis label. Lastly will place the legend on the bottom of the figure.

```
Above30 + theme(axis.text.x = element_text(angle = 90, hjust = 1)) +
   theme(axis.text = element_text(size = 18)) +
   theme(axis.title = element_text(size = 20)) +
   theme(legend.position = "bottom")

sessionInfo()

## R version 3.6.1 (2019-07-05)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS  10.16
##
## Matrix products: default
## BLAS:
/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.d
ylib
## LAPACK:
/Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.d
ylib
##
## locale:
## [1] en_CA.UTF-8/en_CA.UTF-8/en_CA.UTF-8/C/en_CA.UTF-8/en_CA.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] ggplot2_3.2.1 dplyr_0.8.4
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_1.0.3       knitr_1.28       magrittr_1.5
munsell_0.5.0
##  [5] tidyselect_1.0.0 colorspace_1.4-1 R6_2.4.1         rlang_0.4.4
##  [9] fansi_0.4.1      stringr_1.4.0    tools_3.6.1      grid_3.6.1
## [13] gtable_0.3.0     xfun_0.12        utf8_1.1.4       cli_2.0.1
## [17] withr_2.1.2      htmltools_0.4.0  lazyeval_0.2.2   yaml_2.2.1
## [21] assertthat_0.2.1 digest_0.6.25    tibble_2.1.3
lifecycle_0.1.0
## [25] crayon_1.3.4     purrr_0.3.3      vctrs_0.2.3      glue_1.3.1
## [29] evaluate_0.14    rmarkdown_2.1    stringi_1.4.6
compiler_3.6.1
## [33] pillar_1.4.3     scales_1.1.0     pkgconfig_2.0.3
```

7 Curriculum Vitae

Name: Andrew George Rabas

Post-secondary Education and Degrees:
Brock University
St. Catharines, Ontario, Canada
2005-2010 B.Sc. in Biology

Related Work Experience:
Teaching Assistant for B2290
The University of Western Ontario
2019-2021

Work Shops:

Ginseng Replant Disease Working Group
Winter Meeting 2019
One day workshop where researchers discuss the progress of solutions for ginseng replant disease
hosted by Sean Westerveld, Ginseng Specialist for OMAFRA
January 15, 2019

Ginseng Replant Disease Working Group
Summer Meeting 2019
One day workshop where researchers discuss the progress of solutions for ginseng replant disease
hosted by Sean Westerveld, Ginseng Specialist for OMAFRA
September 4, 2019

Ginseng Replant Disease Working Group
Winter Meeting 2020
One day workshop where researchers discuss the progress of solutions for ginseng replant disease
hosted by Sean Westerveld, Ginseng Specialist for OMAFRA
January 21, 2020

Ginseng Replant Disease Working Group
Winter Meeting 2021
One day workshop where researchers discuss the progress of solutions for ginseng replant disease
hosted by Sean Westerveld, Ginseng Specialist for OMAFRA
February 10, 2021
Presented analyzed data to fellow researchers and growers
Presentation titled: “Mitigating Replant Disease with Soil Solarization”

Non-refereed Publication:

Rabas, A., Atkinson, C. and Bernards, M.A. 2021. SOLARIZATION OF PRE-FORMED GARDEN BEDS: IMPACT ON PLANT STAND COUNT IN YEAR ONE IN A REPLANT GARDEN. Ontario Ginseng Research Report, Ontario Ginseng Growers Association, in press.