ABSTRACT

VOLK, EMMA FRANCES. Soil Disinfestation Through the Application of Steam and alternative chemicals. (Under the direction of Dr. Mark Hoffmann).

Methyl bromide was phased out as a reliable pre-plant fumigant throughout the United States in 2012. In its place, pre-plant chemical fumigants like dazomet, chloropicrin, 1,3-dichloropropene, and metam sodium have been utilized to control soilborne pests, pathogens, and weeds, in annual strawberry plasticulture systems. However, these chemicals can be difficult to use due to cost, managerial complexities, and government regulations. Alternative soil disinfestation practices, such as field-based steam application and biofumigation, have been studied and utilized to control soilborne pests, pathogens, and weeds. Steam application can control soilborne pests and pathogens through exposure to high temperatures. Steam has been utilized as a non-chemical alternative in greenhouse production settings for decades. However, disinfesting large volumes of soil through field-scale steam application is limited by time, labor, and cost.

Biofumigation utilizes crops or seed meals from the *Brassicaceae* family to release isothiocyanates, which are linked to fungal, oomycete, weed, and pest suppression. Synthetically produced isothiocyanates, such as Dominus® (allyl isothiocyanate; AITC), are commercially available and can be used for pre-plant fumigation. However, this product has shown inconsistent efficacy as a sole pre-plant fumigant.

Two studies were conducted to improve the efficacy and efficiency of field-based steam application and biofumigation. Study 1 analyzed soilborne pathogen control through the application of steam and two exothermic substances, quicklime (CaO) and sodium peroxide (Na_2O_2), in a microplot study. Exothermic substances activated by water have the potential to increase soil temperatures more rapidly than steam alone. It was hypothesized that soil-applied steam, in combination with exothermic substances, will lead to improved pathogen and weed

control, compared to steam alone. Six treatments were established in a randomized complete block design with 4 replicates per treatment in Clayton, NC: 1) Non-treated control; 2) Steam for 30 min; 3) Sodium peroxide; 4) Sodium peroxide + steam for 30 min; 5) Quicklime; and 6) Quicklime + steam for 30 min. Steam + quicklime killed *Pythium* sp. at the 2.5 cm and 12.5 cm distances from the steam injection point, but steam alone and quicklime alone did not. Steam + sodium peroxide did not increase *Pythium* sp. control efficacy. The combination of quicklime and steam is a viable method to improve steam application efficiency in the field.

Study 2 investigated pathogen and weed control of AITC co-applied with steam in strawberry plasticulture settings in Clayton and Castle Hayne, NC. It was hypothesized that heat application through steam would increase the efficacy of AITC, making it a more effective soil disinfestant. Nine treatments were applied and replicated 4 times each: 1) Non-treated control; 2) chloropicrin + 1,3-dichloropropene (60:40); 3) AITC; 4) AITC + 60-min steam application; 5) AITC + 30-min steam application; 6) AITC + 10-min steam application; 7) 60-min steam application; 8) 30-min steam application; 9) 10-min steam application. *Pythium* sp. and weed control, as well as strawberry yield, were analyzed. Weeds and *Pythium* sp. were controlled by AITC alone as effectively as 1,3-dichloropropene + chloropicrin. Steam alone did not effectively control weeds or *Pythium* sp. Yields were similar between AITC alone and 1,3-dichloropropene + chloropicrin. These results show the potential for shank-applied AITC to work as a pre-plant fumigant alternative in NC strawberry production.

Investigation of Soilborne Pathogen and Weed Control Efficacy Using Low Pressure Steam in Combination With Allyl Isothiocyanate and Exothermic Substances

by
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DEDICATION

To my parents, for their constant belief, encouragement, and support.

To David Henderson, for everything.

BIOGRAPHY

Emma Volk grew up in Pittsburgh, PA. She received a BS from Cornell University in International Agriculture and Rural Development. After graduation, she moved to Durham, NC, where she began work as a research assistant in the Horticultural Science Department at North Carolina State University. She started pursuing her MS degree in Horticultural Science in 2021.

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CHAPTER 1

Literature Review

1. Strawberry Biology

The strawberry plant (*Fragaria* x *ananassa*) is a perennial plant first cultivated in France in the 18th century. It is a part of the *Rosaceae* family, which encompass many other economically important specialty crops such as apples, peaches, raspberries, almonds, blackberries, and roses (Galletta and Himelrick 1989). The commercialized strawberry is an octoploid, and it is a hybrid between *Fragaria chiloensis* and *Fragaria virginiana* (Darrow 1966). This means that the hybrid has eight complete sets of chromosomes. Many other strawberry species exist as diploids, tetraploids, hexaploids, and octoploids. These species are found naturally, and they are native to areas across the world. For example, *F. nipponica* (diploid) is native to Japan, and *F. moschata* (hexaploid) is native to areas from Northern Europe up to Siberia (Galletta and Himelrick 1989). However, cultivars derived from the octoploid *Fragaria* x *ananassa* hybrid are the most common commercially grown strawberries.

A strawberry plant is composed of a central crown, shallow roots, trifoliate leaves, stolons, and inflorescences (Darrow 1966). The crown, a short, thickened stem, is typically about 2.5 cm tall and covered by leaf bases (stipules). Between the crown and the stipules, axillary buds form, which then develop into stolons or branch crowns. The crown also supports the development of flowers. The base of the crown meets the soil, where strawberry roots develop.

The strawberry root system consists of 20 to 100 primary roots, depending on the cultivar and environmental conditions (Darrow 1966). A primary root's role is to penetrate the soil. Many secondary roots also develop which serve to seek out nutrients. The mass of secondary, tertiary, and higher-order roots make strawberry root complexes appear fibrous.

All strawberry leaves are trifoliate, meaning they are composed of three small leaflets, two of which are pinnate (Darrow 1966). Stolons are elongated stems which grow from the axillary bud of the first leaf developed in the Spring. These stems elongate and form nodes, from which daughter plants grow. Those daughter plants then develop small root nodules, which can be used to directly establish the new plant in the soil.

The strawberry fruit develops from the plant's inflorescences. All strawberry plants have perfect flowers with pistils (female) and stamens (male) (Darrow 1966). Beneath the stamens lies the plant's receptacle which develops into the edible strawberry. A strawberry is not a berry fruit. Berries consist of edible mesocarp, pericarp and/or endocarp. A strawberry fruit is an aggregate and accessory fruit. The berry fruit of a strawberry, meaning the tissue that consists of meso-, endo-, and pericarp, are the individual fruits (often referred to as a 'seed') on the top of a red, enlarged receptacle. The receptacle is the part of the flower that carries single ovaries. The individual fruits are called achenes, which surround and protect a tiny seed. The single strawberry fruit houses, on average, about 200 seeds (Padmanabhan et al. 2016). At the top of the berry is a leafy cap, which is the matured floral calyx. Berries take 20 to 50 days to ripen after initial pollination, depending on the cultivar, temperature, and fertilization.

Strawberry cultivars are divided into different categories based on the photoperiod required for production: short-day, long-day, and day-neutral cultivars (Honjo et al. 2016). Short-day cultivars typically grow in cold regions, and they will bud when the photoperiod is less than 14 hours per day, or when the temperatures are less than 15^{o}C (Padmanabhan et al. 2016). They are typically harvested during late spring and early summer. Long-day cultivars, on the other hand, are less affected by photoperiod, and flower initiation will begin as long as temperatures are moderate (30^{o}C/26^{o}C day/night; Castro et al. 2015; Darrow 1966; Hancock 1999). Long-day

plants can produce fruit throughout summer and into autumn in more temperate regions (Castro et al. 2015). Day-neutral cultivars are less affected by photoperiod.

2. Strawberry Industry

2.a. Overview

The United States strawberry industry was valued at $2.4 billion in 2018 (USDA-NASS 2018). California leads North America in total strawberry production, followed by Mexico (California Strawberry Commission 2022a). Florida is third, contributing approximately 8% of total strawberry production for the U.S., followed by New York, North Carolina, Oregon, and Washington (California Strawberry Commission 2022a, USDA 2021).

The California strawberry crop value was $2.3 billion in 2018. This is quite large in comparison to Florida, which was valued at approximately $300 million, and North Carolina, which was valued at $21.4 million (California Strawberry Commission 2022b; USDA-NASS 2018). The difference in crop value between California and North Carolina is due to many factors, including differences in acreage, climate, and harvest seasons. Strawberry production occurs year-round in California, with peak harvest season between Spring and Fall (USDA 2021). North Carolina's harvest typically starts in April and ends in June, when temperatures climb too high to obtain good fruit quality.

2.b. Strawberry nursery industry

The strawberry nursery industry is an important sector, supplying strawberry growers with bare-root or plug plants for fruit production. Nurseries initially propagate virus free mother plants through tissue culture. These mother plants produce clones called daughter plants which develop from the mother plant's stolon. Nurseries harvest these daughter plants, root them, and

sell them to strawberry producers for fruit production. This process, from tissue culture to strawberry production, takes about 5 years.

During year one, plants are tested for disease and grown in tissue culture. Mother plants are propagated, heat-treated, and tested for disease presence (Foundation Plant Services 2008). From these plants, experts harvest tips and excise their meristems for tissue culture. Once a seedling develops in tissue culture, personnel transplant the seedling into soil medium where it acclimates in a humid chamber for 2-3 weeks. Then plants are transferred to a greenhouse environment before moving on to a low elevation nursery.

Plants remain in a low elevation nursery for one season. During this period, plants grow, propagate, and multiply in field conditions. This process takes place in low elevation climates to expose the plants to high temperatures. High temperatures support more vegetative growth, therefore supporting more daughter plant development. Optimal temperatures for vegetative growth are about 30^{o}C/25^{o}C (day/night), with variability depending on the cultivar (Kadir et al. 2006).

In year four, daughter plants propagated in the low elevation nurseries are transported to a high elevation nursery, or sold as bare-root or plug plants (Foundation Plant Services 2008). The cold temperatures in the high elevation nurseries condition plants for fruit production. A certain amount of chilling hours must be reached before nurseries can mow and harvest the plants for sale to strawberry producers. Chilling hours are defined as the number of hours when temperatures are between 0^{o}C and 7^{o}C (Bigey 2002). Most cultivars need between 200 and 300 chilling hours to reach optimal fruit production (Stafne 2020).

Given the multi-location and multi-year process for strawberry plant production, disease management is crucial to maintain healthy plants that will have high yields. Methyl Bromide

(MB) is a highly effective chemical for controlling soilborne diseases in strawberry nursery systems. Nurseries particularly benefit from the fact that MB is the only fumigant which allows nurseries to avoid plant inspections for nematodes, a time and labor-intensive process. However, MB was phased out of agricultural use in 2005 through the Montreal Protocol, due to its classification as a Class I ozone-depleting substance (US EPA 2022). Strawberry nurseries are still able to apply MB through the grant of a critical use exemption. The U.S. Environmental Protection Agency only grants critical use exemptions if: 1. lack of MB availability would result in a significant market disruption; and 2.) no technically and economically feasible alternatives or substitutes are available (US EPA 2022). Nurseries require alternative fumigants and soil disinfestation practices in the event that the US EPA no longer grants critical use exemptions for MB fumigation for nurseries.

2.c. Strawberry fruit production

The annual hill plasticulture system is the prominent outdoor strawberry production system (Poling et al. 2005). This system involves planting plug plants or bare-root plants into raised, plastic-covered beds. It is an annual system preferred by growers due to the ability to harvest easily and quickly. The annual hill plasticulture strawberry system was developed in the 1950s in California in conjunction with MB soil fumigation. In NC, growers adapted the strawberry plasticulture system in the latter half of the 1960s.

Before plasticulture, the matted row production system was the predominant strawberry production system in the US. Today, it is much less common and primarily used in cooler climates, such as the Northeast U.S. (Black et al. 2002). The matted row system is a perennial system in which mother plants are allowed to produce stolons and form a densely packed field of

strawberry plants. The matted row system has low establishment costs, the ability to harvest from the same plant for more than one year, and low labor intensity. The annual hill system has higher up-front monetary and labor costs due to the annual establishment and time requirement for land preparation, plastic laying, and irrigation setup (Poling et al. 2005). But plasticulture systems reduce labor requirements between plant establishment and harvest. Plasticulture systems can also be correlated with higher yields and income compared to the matted row system in the southeast (Black et al. 2002; Fernandez et al. 2002; Garwood 1998).

In annual hill plasticulture systems, there are multiple types of plastic mulch that strawberry growers can use. Plastic types can vary in regards to their weed control efficacy, soil temperature control, and effect on fruit quality. Black plastic is the most common plastic mulch, given its ability to block sunlight and minimize weed pressure. It also keeps soil warm during the winter months in North Carolina, when it is critical for plants to establish their roots and develop crowns (Poling 2016).

Clear plastic, on the other hand, allows light to penetrate the plastic and reach the soil. This increases soil temperatures, which is particularly important for winter strawberry producers in California (Johnson and Fennimore 2005). But clear plastic also supports weed growth underneath the plastic lining due to photosynthetic wavelength penetration. Johnson and Fennimore (2005) found that weed pressure was minimized by 64.3% in a strawberry production system with clear plastic. This is in contrast to a production system under black plastic where weed pressure was minimized by 100%.

White plastic, like black plastic, is capable of controlling weeds by creating a barrier between the soil and solar radiation (Shiukyh et al. 2015). However, it does not support warm soil temperatures like black plastic because it reflects rather than absorbs solar radiation.

Therefore, white plastic is preferred in hot production areas - particularly regions where strawberries are grown throughout the summer.

In North Carolina, field production begins in the Fall. Fertilizer is broadcasted, and beds are raised to be 2.5-m-wide and about 0.5 m tall. Black plastic mulch is typically laid, fumigants are applied, and strawberry plugs are planted once the restricted-entry interval has concluded. Most cultivars planted in NC under plasticulture conditions are short-days (Poling 2016). These include cultivars such as Camarosa, Chandler, Sweet Charlie, and Ruby June. Albion is another popularly grown cultivar in the region, and it is classified as a day-neutral plant.

In NC, temperatures are still warm (80^{o}C/60^{o}C high/low) at planting time, allowing the plants to establish themselves before going into dormancy over the winter (Poling et al. 2005). It is recommended to remove runners that develop during the establishment period to redirect the plant's energy towards crown formation and floral bud development. Once temperatures start to warm in the spring, the plants will start to grow larger and develop flowers. Early spring frost is a particularly important threat to manage given its ability to damage developing flowers. This can be managed by applying row covers, which help maintain warm air temperatures around the plant. The strawberry season typically ends in June, when strawberry harvests have slowed. A typical strawberry harvest is approximately 24,000 lb ac^{-1}, with variation depending on factors such as plant spacing, cultivar, disease pressure, and rainfall (Poling 2016).

3. Pests, Weeds, and Pathogens

Strawberry production systems rely on chemical pesticides to control pests, weeds, and pathogen. Nematodes cause physical damage and can be vectors for viral diseases. Weeds can acquire nutrients and shade out strawberry plants. In addition, diseases known to affect North

Carolina strawberry growers include, but are not limited to: Anthracnose crown (soilborne) and fruit (soilborne and infected tissue) rot, Phytophthora crown rot (soilborne), Rhizoctonia and Pythium root rot (soilborne), and botrytis gray mold (infected tissue). Many strawberry diseases are often spread through infected nursery planting material. Controlling these diseases within the strawberry nursery system is preferable to prevent disease spread to fruit production fields. Once introduced to fruit production fields, fumigation is a powerful tool to manage soil-borne diseases in the following season.

A particularly troublesome disease for strawberry is Anthracnose crown rot, caused by *Colletotrichum gloeosporioides* (Louws et al. 2014a). Anthracnose crown rot can spread through infected transplant material. Warm, moist conditions favor its dissemination, making it a problem particularly in southeastern U.S. production systems (Rahman and Samtani 2022). Symptoms include red and white marbling of the crown, as well as stunting, wilting, and eventual collapse. Control measures include monitoring for and destroying infected plants, fungicide application, and whole plant dips into the fungicide Switch just prior to planting (Louws et al. 2014a).

Phytophthora crown rot, caused by *Phytophthora cactorum*, is a soilborne strawberry disease (Louws et al. 2014b). The pathogen can spread by either being present in the soil, or it can arrive via infected strawberry transplants. In severe cases, infected plants will turn dark red and collapse. Control measures for Phytophthora crown rot include selection of sites with good drainage, purchase of disease-free plants, and application of chemical fungicides.

Black root rot (BRR) is another soilborne strawberry disease, and it is fairly common and important for growers in the southeast given its spread and ability to reduce yields by 20 to 40% (Louws and Cline 2014). It is a disease complex, which means that a multitude of fungal,

oomycota, and nematode species can cause BRR. However, it has been found that the species *Pythium irregulare* and *Rhizoctonia* sp. are the primary agent of BRR spread in North Carolina. In addition, BRR is a soilborne disease, making it crucial for growers to implement control strategies before strawberries are in the ground.

Nematodes are another soilborne strawberry pest. Of the many plant parasitic nematodes that threaten horticultural crops, *Meloidogyne hapla*, *Pratylenchus penentrans*, and *Belonolaiumus longicaudatus*, are particularly troublesome species for southeastern strawberry production (Desaeger 2022). *M. hapla* causes root knots and galls, and *P. penetrans* causes dark root lesions. *B. longicaudatus* is a sting nematode species which feeds on strawberry roots. Nematodes do not migrate over long distances, meaning that they are typically introduced through infected plant material or soil movement. Pre-plant fumigation can control nematode populations. In addition, non-fumigant nematicides, fluensulfone and fluopyram, can be applied through a foliar spray or drip irrigation system, and have shown promising results for nematode control (Oka et al. 2011; Li et al. 2020). Cover crops which are poor hosts to common nematode species can minimize nematode populations between seasons in annual production systems (Desaegar 2022).

Weeds are additional soil-based strawberry production challenges. While a majority of weed suppression occurs through the integration of black plastic mulching, other weed control tactics are required (Jennings 2006). Weeds can sprout in areas where there is bare soil - whether that's in a plastic tear or the strawberry planting hole. In addition, yellow and purple nutsedge (*Cyperus esculentus* and *Cyperus rotundus*) are ranked the 6th most troublesome weed in vegetable and fruit crops, and they have the ability to poke through plastic (Van Wychen 2019).

In order to manage weeds, pre-plant fumigation is a reliable tactic. Other options include applying postemergence herbicides or hand weeding.

Regardless of the disease, pest, or weed, integrated pest management strategies are the best way to produce healthy strawberry plants. These methods should include crop rotation, cover cropping, pre-plant fumigation, purchasing resistant cultivars, purchasing disease-free transplants, and monitoring (Fennimore and Goodhue 2016; Strand 2008).

4. Soil Disinfestation

4.a. Chemical Fumigation

Strawberry annual hill production systems rely predominantly on chemical soil disinfestation to control soilborne pests, pathogens, and weeds. Biofumigation and heat-induced soil disinfestation practices are additional, alternative methods to chemical fumigation (Zhao et al. 2018). But development and integration for these two practices are in the preliminary stages, compared to chemical soil disinfestation, which has successfully supported the strawberry industry for decades.

Chemical application is often done through drip line or shank injection for strawberry plasticulture systems. Shank-injected chemicals are applied during the process of bed shaping and plastic laying in order to trap the chemical in the soil before it slowly dissipates. Drip line fumigant application is typically done after beds have been shaped and plastic has been laid, but before planting.

Fumigants which do not contain MB are effective at controlling soilborne pests in strawberry fruit production (Kabir et al. 2005; Garcia-Mendez et al. 2008; Fennimore et al. 2003). These chemicals include 1,3-dichloropropene (1,3-D; $C_3H_4Cl_2$), chloropicrin (CCL_3NO_2),

dazomet ($C_5H_{10}N_2S_2$), dimethyl disulfide (DMDS; $C_2H_6S_2$) and metam sodium ($C_2H_4NNaS_2$). All these chemicals are sold as fumigants under various trade names. These fumigants include, but are not limited to: Pic-Clor 60 (39.% 1,3-D and 59.6% chloropicrin), Chloropicrin 100, Paladin® (DMDS), and Vapam® (Metam Sodium).

In one study, chloropicrin (200 L ha^{-1}) controlled weeds in strawberry plasticulture as well as MB + chloropicrin (67:33 w/w) (Fennimore et al. 2003). 1,3-D and MS have been shown to control *Meloidogyne* sp. (root-knot nematode) in late season tomato production (Desaeger et al. 2017). And 1,3-D has shown similar ginger yield and nematode control to MB (Qiao et al. 2012). However, 1,3-D has not controlled weeds as effectively as MB (Qiao et al. 2012).

Studies have shown that a combination of chemicals can effectively control pests and diseases. For example, 1,3-D and dazomet effectively controlled *Fusarium* sp., *Phytophthora* sp., *Meloidogyne* sp., *Digitaria sanguinalis,* and *Abutilon theophrasti*, in a laboratory study (Mao et al. 2012). Another study found that Metam Sodium co-applied with DMDS significantly reduced *Fusarium* sp., compared to Metam Sodium alone (Gerik and Hanson 2011). In addition, strawberries fumigated with iodomethane and chloropicrin had similar yields to strawberries fumigated with MB and chloropicrin (Kabir et al. 2005). However, regional regulations (USEPA 2008a; USEPA 2008b; O'Malley 2010) and limited availability restrict the use of many of these chemicals in the U.S. These issues require effective, alternative methods for soil disinfestation in strawberry production systems.

4.b. Biofumigation

A potential chemical alternative is biofumigation. Biofumigation utilizes crops, seed meals, or glucosinolates within the *Brassicaceae* family to control soilborne pests and pathogens.

Cover cropping with brassicas to control pests and pathogens has been shown to increase pepper yield (Hansen and Keinath 2013), reduce root galls caused by *M. incognita* nematodes (PiedraBuena et al. 2006), and suppress *Rhizoctonia solani* and *Phytophthora nicotianae* (Baysal-Gurel et al. 2020).

Brassica seed meals have been utilized to control soilborne pests and pathogens. Incorporating mustard seed meal (MSM) into soil prior to planting has resulted in similar strawberry yields to MB + chloropicrin (Samtani et al. 2012), reduction in *M. javanica* nematode populations (Rahman and Somers 2005), weed suppression (Earlywine et al. 2010), and control of apple replant disease pathogens (Mazzola et al. 2020).

However, when compared to standard chemical fumigants, brassica green manures and seed meals exhibit varying efficacy as pest and disease controls (Hafez and Sundararaj 2000; Samtani et al. 2012; Ngala et al. 2014). The efficacy of green manures is also dependent on the crop species utilized and their chemical composition (Zasada and Ferris 2003).

Products have been derived from compounds naturally found in the *Brassicaceae* family. For example, the compound allyl-isothiocyanate (AITC) is found naturally in brassica plants, but it is produced synthetically and commercially sold as a biofumigant with the trade name Dominus®. The chemical is considered a biofumigant, and studies have found it capable of controlling weeds such as purple nutsedge, yellow nutsedge, palmer amaranth, large crabgrass, and texas panicum (Bangarwa and Norsworthy 2014, Bangarwa et al. 2012, Norsworthy and Meehan 2005). It has also exhibited control of soilborne pathogens such as *Fusarium* sp., *Meloidogyne* sp., *Pythium* sp., *Phytophthora* sp., and *Rhizoctonia solani* (Ren et al. 2018, Dhingra et al. 2004). However, a trial conducted in California strawberry and cut flower fields found that AITC had inconsistent or no pathogen and weed control (Hoffmann et al. 2020). This

is believed to be due to its low vapor pressure (5 mmHg), which makes it a less ideal alternative to the standard chemical fumigants which have higher vapor pressures, such as chloropicrin (18.3 mmHg).

4.c. Heat-Induced Soil Disinfestation

Another non-chemical alternative to chemical soil disinfestation is heat-induced soil disinfestation (HISD). Through the application of extreme heat, soil pathogens, pests, and weed seeds are controlled. Heat application can occur through soil solarization or steam application. One important component of the HISD method is understanding the importance of the maximum temperatures reached as well as the length of time pests are exposed to high temperatures.

Germination of weed species was prevented by temperatures of 75^{o}C after .5 days, 204^{o}C after 7.5 minutes, and 262^{o}C after 5 minutes (Thompson et al. 2008). Dahlquist et al. (2007) found that as exposure to high temperatures increased, the time that it took to kill 100% of weed seeds decreased. For example, barnyard grass took 16 hours at 46^{o}C, 9 hours at 50^{o}C, .25 hours at 60^{o}C, and .17 hours at 70^{o}C to reach 100% mortality. The exact length and temperatures required for 100% mortality varied among species.

Seed physiology has been studied as one possible explanation for why differences in germination rates between weed species occur. For example, weed species with water-permeable seed coats have lower percent germination in dry conditions compared to the hard-coated seeds (Egley 1990). Seed size and weight are other important factors when it comes to different weed species and their susceptibility to heat treatments. Studies have found that as seed size and weight increase, the temperature required to reach 99% germination reduction also increased (Vidotto et al. 2013; Çağatay and Çatav 2012). Soil moisture also affects heat-induced weed

control. Increasing soil moisture has been correlated with reductions in weed seed viability (Egley 1990).

The relationship between soilborne pathogens and heat has also been studied. It has long been understood that the application of 65°C for 30 minutes is sufficient to kill most soilborne pathogens (Baker and Roistacher 1957). *In vitro* studies have shown that application of 63°C and 49°C for 30 minutes controlled *Pythium* sp. and *Fusarium proliferatum*, respectively (Thiessen et al. 2020; La Placa 2022). In general, as time and temperature increase, pathogen survival has been shown to decrease (La Placa 2022; Yildiz et al. 2010).

However, different environmental factors can affect soilborne pathogen survival other than temperature, such as pH and soil moisture. For example, extremely high or low pH can negatively affect soilborne pathogen populations (Kauraw 1979; Mondal and Hyakumachi 2000). And heat application has been shown to be more effective in moist soils compared to dry soils (Dunn et al. 1985).

Various factors can affect field soil temperature during heat application. For example, soil temperatures can vary depending on soil depth (Gelsomino et al. 2010), and heat transfer can vary depending on soil type (Yang et al. 2019). Heat application methods can also affect soil temperature. For example, maximum soil temperatures during heat treatment differed depending on whether soil was solarized or steamed (Samtani et al. 2012). And mixing soil during steam application improves heat transfer, compared to still soil (Miller et al. 2014).

5. Steam Engineering

5.a. Overview

Steam as a soil disinfestation tool has been utilized for decades (Newhall 1955; Katan 2000; Kim et al. 2021). Initially, research into steam as a chemical alternative primarily took place as a stationary application method. Stationary methods began with the injection of steam into pots and growing substrates (Baker 1962). Other stationary methods include blowing steam under a thermoresistant sheet with hoses attached to a boiler (Dabbene et al. 2003; Fenoglio et al. 2008). Additionally, pipes or hoses can be laid on the soil surface and inject steam into the bed through attached spikes. Pipes can also be buried, where steam is then applied below the soil surface.

Its ability to perform as well as chemical fumigants and its economic practicality are two of the primary components of steam research as it relates to real-life, field application. Prior research shows that net returns in steam-treated strawberry fields are lower compared to fields treated with MB + chloropicrin (Samtani et al. 2012) and 1,3-D + chloropicrin (Xu et al. 2017). Prior research also shows variability in the efficacy of field-applied steam for weed control, pathogen control, and productivity. Steam application has controlled weeds as well as 1,3-D + chloropicrin (Hoffmann et al. 2017; Samtani et al. 2012), and controlled *Fusarium* sp. better than MB (Rainbolt et al. 2013). However, steam application has resulted in significantly higher levels of *Pythium* sp. (Rainbolt et al. 2013) and *Verticillium dahliae* (Samtani et al. 2012) compared to standard chemical fumigants.

The amount of fuel and time required to apply steam depends on the method of application. For example, a tractor-towed steam generator used 14,600 L ha-1 propane

(Fennimore et al. 2014), while a self-propelled machine used 4,484 L ha-1 gas and took 52.5 h ha-1 to apply steam (Peruzzi et al. 2011).

5.b. Steam in combination with biofumigants and alternative chemicals

Studies have been conducted to understand the efficacy of steam, on its own or in combination with biofumigants, to control pests and diseases. Steam in combination with mustard seed meal (MSM) incorporation has been found to control *Verticillium dahliae*, purslane, and nutsedge (Kim et al. 2021). Another study found that steam + MSM had statistically higher yields compared to the NTC, but was statistically similar to plots treated with steam alone (Fennimore et al. 2014). Michuda et al. (2021) analyzed net returns from strawberry field trials that used steam and steam + MSM as pre-plant soil disinfestation methods. In conventional systems, net returns were similar, while in organic systems, steam + MSM had higher net returns. This study also determined that maximum temperatures of 62-63ºC lasting for 41-44 minutes maximized net returns.

In addition to incorporating steam with MSM, steam in combination with AITC has been studied. AITC is known to have weed (Bangarwa and Norsworthy 2014; Bangarwa et al., 2012) and pathogen control properties (Sharma et al. 2008). But it has a low vapor pressure (4 mmHg) compared to other typical fumigants like 1,3-D (34.4 mmHg) which makes it immobile once injected into soil (Dhingra et al. 2004; Kim et al. 2020). Scientists have hypothesized that by co-applying AITC with steam, AITC would vaporize and control soil pests more effectively throughout the soil bed. Kim et al. (2020) compared 4 treatments: NTC, steam alone, AITC alone, and AITC + steam. In regards to pathogen control, they found that AITC + steam was the only treatment that successfully suppressed *V. dahliae*. This is of particular importance when

compared to AITC alone which did not control for *V. dahliae* consistently. This study also found that steam alone and AITC + steam reduced weed seed viability more than AITC alone.

In addition to incorporating biofumigants, inorganic amendments as a steam additive have been studied. For example, studies have looked into the effects that steaming with varying application rates of potassium hydroxide (KOH) and calcium oxide (CaO) had on weed and pathogen control. These two chemicals were studied because they create exothermic reactions with water under standard conditions. These reactions provide an opportunity for soil to reach higher temperatures faster when co-applied with steam, compared to when steam is applied on its own.

Luvisi et al. (2006) found that steam + KOH significantly reduced incidences of Fusarium wilt and improved control of *Sclerotinia minor* in basil production, compared to steam alone. Steam + KOH (1000 kg ha-1) was also found to have greater control of *Rhizoctonia solani* and *Sclerotinia minor* compared to steam alone (Triolo et al. 2004). However, steam + CaO (1000 kg ha-1) did not have a significant difference on *S. minor* or *R. solani* compared to steam alone (Triolo et al. 2004). This is in contrast to an *in vitro* study, where steam + CaO significantly reduced *S. minor* presence compared to steam alone (Luvisi and Triolo 2007). Steam + exothermic chemicals had no effect on lettuce yield, in comparison to steam alone (Triolo et al. 2004). Temperatures in chemical-amended soils have shown higher maximum temperatures after steaming, and they maintained high temperatures for longer, compared to the steam-alone treatments (Barberi et al. 2009).

5.c. Mobile Steam Applicators

One significant improvement in steam applicators within the past two decades is the ability of steam to be applied in a mobile and continuous fashion. In Italy, a self-propelled

machine, Celli Ecostar SC 600 (Celli SpA, Forlì, Italy), was developed and studied for its ability to work in both field and greenhouse settings. Notable features of the machine include: rubber trucks for reduced soil compaction, deep steam injection (20 cm), shallow steam injection (0-10 cm), continuous steam injection, a plastic laying system, and a Bioflash™ system. The Bioflash™ system incorporates exothermically reacting compounds with soil while the Celli Ecostar SC 600 injects steam (Peruzzi et al. 2011). One study used this machine to conduct four experiments to analyze soil temperature values and soil heat persistence: 1) Steam applied to soil with applications of KOH and CaO at three different rates; 2) Steam and CaO applied to plastic mulched and bare soils; 3) Steam applied through three different steam injection bars at four different depths; and 4) Steam applied to optimize heat distribution in the top 150 mm of soil (Peruzzi et al. 2011). They found that, out of the four experiments, the combination of CaO and plastic mulching had the highest temperature values and length of soil heat persistence (Peruzzi et al. 2011).

Other studies also investigated the incorporation of steam and activating compounds with the Celli Ecostar SC 600. One found soil temperatures increased from 70ºC to 82ºC when CaO was incorporated with steam at a rate of 1000 kg/ha (Gelsomino et al. 2010). Others investigating the use of this machine with the Bioflash™ system have also found higher soil temperatures in plots treated with CaO and steam compared to plots treated solely with steam (Barberi et al., 2009). This same study also found a significant increase in NH4-N in the steam + CaO treatment, compared to the steam alone treatment, as well as significant increases in soluble K in both steam + CaO and steam treatments (Gelsomino et al. 2010). Significant increases in K are similar to the findings in other literature after steam application (Lenzi et al. 2004), but contrast with findings elsewhere (Wolf et al. 1989).

Another steam applicator developed in South Korea has been studied for its efficacy as a fumigant alternative. In one study, greenhouse soil was treated with a self-propelled, high-temperature steam disinfester (JS-S002A, JSE Inc., Daegu, Korea). This machine disinfests soil at a rate of 100m2 hr-1, and it sprays steam at temperatures between 120°C and 160°C. Researchers developed a model to determine the optimal operation parameters for steam temperature (150.56°C), running speed (1.69m/min), and spray depth (15 cm) to optimize bacterial removal efficiency (97.49%), nematode removal efficiency (93.99%), and oil consumption (.0749 L/m2) (Huh et al. 2020).

Other steam applicator designs are not self-propelled, but are carried through the field on a tractor. For example, a propane-fueled Clayton 100 HP steam generator (Clayton Industries, City of Industry, CA) was used for steam research in California. The applicator steamed a 1.32 m wide bed at a time, and it used 1.46 L mL-1 of propane (Fennimore et al. 2014). The machine ran at a rate of 169 m hr^{-1}, and maintained a target soil temperature of 70 °C or higher for 30 minutes after steaming. Weed density and *P. ultimum* control varied across time and location, but strawberry fruit yields dramatically increased in the treatments that received steam treatment (Fennimore et al. 2014). While this steam applicator is not as high-tech as others, the steam and steam + MSM treatments from this trial were both found to have significantly higher net returns, in comparison to the non-treated control (Xu et al. 2017).

In addition to stationary vs. mobile steam systems, steam injection methods vary. For example, steam can be applied to still soil, or a machine can simultaneously mix soil and apply steam. Miller et al. (2014) found that temperatures increased more quickly in soil that was mixed during steam application compared to still soil. However, when stationary applications use pipes or hoses, minimizing the distance between lines can increase soil temperatures and pathogen

control efficacy (Yang et al. 2019). Depth of steam injection also varies. Studies have found differences in soil temperatures at different depths, depending on the depth of steam injection (Peruzzi et al. 2011; Huh et al. 2020). Differences in injection depth can alter pathogen control efficacy at different points within the soil profile.

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CHAPTER 2

Weed and pathogen control with steam in combination with calcium oxide and sodium peroxide

Abstract

Steam as a non-chemical alternative for chemical soil disinfestation has been utilized for decades in stationary settings such as greenhouse crop production. However, disinfesting large soil volumes through field-scale steam applications remains an active area of research. Current approaches are limited by time, labor, efficacy, and economic factors. Exothermic substances activated by water have the potential to reduce steam application time under field conditions. A study was conducted under the hypothesis that soil-applied steam, in combination with exothermic substances, will lead to improved pathogen and weed control, compared to steam alone. Therefore, a two-year microplot study was conducted to investigate weed and pathogen control efficacy of steam in combination with two exothermic substances: calcium oxide (quicklime) and sodium peroxide. The following treatments were established in a randomized complete block design with 4 replicates per treatment: 1) Non-treated control; 2) Steam for 30 min; 3) Sodium peroxide; 4) Sodium peroxide + steam for 30 min; 5) Quicklime; and 6) Quicklime + steam for 30 min. Soil-borne pathogen control efficacy was assessed using *Pythium* propagules per gram of soil via a wet plating assay. Steam + quicklime killed *Pythium* sp. at the 2.5 cm and 12.5 cm distances from the steam injection point, and steam or quicklime alone did not. Steam in combination with sodium peroxide did not increase *Pythium* sp. control efficacy. The combination of quicklime with steam is a viable method to improve steam application efficiency in the field.

Introduction:

Crop loss due to soilborne diseases and weeds poses a major problem for specialty crop growers across the United States. For example, black root rot (caused by *Pythium* sp., *Rhizoctonia* sp., *Fusarium* sp., and nematodes) can cause 20-40% yield loss in strawberry (Louws and Cline 2019), and *Phytophthora* crown rot (caused by *Phytophthora cactorum*) can cause up to 50% yield loss (Marin 2018). Common soilborne diseases are controlled through chemical soil fumigation (Holmes et al. 2020). Typical registered soil fumigants are: 1,3-dichloropropene (1,3-D), cloropicrin, methyl-allylisothiocyanate, allyl-isothiocyanate, and di-methyl-di-sulfide. Many of these chemicals are regulated through township caps or local use restrictions in states such as California (California State Legislature 1967). In other areas of the US, the availability of soil fumigants is affected by supply chain issues or production constraints. Moreover, organic certified production guidelines prohibit the use of these chemical soil fumigants.

Therefore, a range of non-chemical soil disinfestation alternatives have been studied: anaerobic soil disinfestation (Mahalingam et al. 2020; Strauss and Kluepfel 2015), biofumigation (Lefebvre et al. 2019; Baysal-Gural et al. 2018), soil solarization (Samtani et al. 2017; Candido et al. 2008), and steam application (Guerra et al. 2022; van Loenen et al. 2003). Steam as a soil disinfestation method has been studied for decades and proven to be effective at controlling soilborne pests and diseases (Newhall 1955; Katan 2000; Kim et al. 2021). Soil temperature, moisture, and heat duration are important factors regarding the efficacy of steam as a soil disinfestant (Pullman et al. 1981; Gay et al. 2010).

Multiple soil steaming application methods have been developed. Stationary steam application is commonly utilized and efficacious in greenhouse settings, where steam is generated by an external unit connected to hoses which blow steam under a sealed thermoresistant covers

(Dabbene et al. 2003; Lu et al. 2010). Additional stationary steam injection methods include buried pipes which eject steam below the soil surface, or pipes or hoses which lay on the soil surface and inject steam through attached spikes. Stationary steam application methods can effectively control pests (Whitehead et al. 1979; van Loenen et al. 2003), weeds (Bitarafan et al. 2021), and pathogens (Samtani et al. 2012).

Mobile field steam applicators are tractor-pulled or self-propelled machines equipped with a steam generator and injection system. Several field applicator models exist, some applying steam via shank injectors connected to steam generators (e.g. SigmaFire Boiler, Clayton Industries,City of Industry, CA), rototillers (e.g. ECOSTAR SC 600, Celli, Forlí, Italy), or steam shields (e.g. Egedal, Tørring, Denmark). Despite advancements in mobile steam application, it is still a challenging technology, demanding large amounts of fuel (e.g. 7598 L ha^{-1} propane), time (e.g. 36 h ha^{-1}), and money [e.g. \$19000 ha^{-1} (Fennimore and Goodhue 2016; Samtani et al. 2012)]. Factors contributing to high inputs include heavy machinery and the time required to reach high soil temperatures throughout a large soil volume (Fennimore and Goodhue 2016).

In order to increase mobile steam application efficiency, exothermic compounds can be applied to the soil. Exothermic compounds release heat in a reaction with another compound or element (e.g. water). Therefore, the application of exothermic compounds to the soil, in combination with steam, has the potential to reach soil temperatures beyond what steam alone can reach. In addition, it could potentially decrease the time required to reach high soil temperatures. This could lead to more efficient weed and disease control, as well as faster steam application times. Previous studies have found that exothermic substances [e.g. quicklime (calcium oxide, CaO) and potassium hydroxide (KOH)] in combination with steam are capable of increasing soil temperature and pathogen control *in vitro* (Luvisi and Triolo 2007), or in combination with

specific soil steamers (Peruzzi et al. 2000; Peruzzi et al. 2011; Luvisi et al. 2006). Questions remain as to whether the combination of steam with exothermic substances will lead to better pathogen control under general field conditions.

Therefore, we tested the hypothesis that control efficacy of soilborne diseases can be improved by the combination of steam with exothermic compounds under field conditions. To test this hypothesis, we followed the experimental microplot trial setup in Kim et al. (2020). The objective of the study was to assess pathogen control of soilborne *Pythium* sp. using steam combined with quicklime and sodium peroxide (Na_2O_2). We further assessed the relationships between temperature and pathogen control.

Materials and Methods:

Microplot Trials

Research was conducted at the Central Crops Research Station (CCRS) in Clayton, NC. In 2021, the trial was conducted in a field that alternates with annual hill strawberry plasticulture and 'brooks' oat cover crop (lat. 35.668361ºN, 78.506261 ºW). In 2022, the trial was conducted in a neighboring field with the same recent crop history (lat. 35.668122ºN, 78.505150ºW). On 1 June 2021 and 1 June 2022, microplots (n=24) were established in a field with soil type Norfolk loamy sand, 1 m wide x .5 m long x 10.2 cm deep and spaced 3 m apart. A total of six treatments were applied in a randomized complete block design (4 replicates per treatment) during both years of the trial: 1) Non-treated control (NTC); 2) 30-min steam (steam); 3) Sodium peroxide (SP); 4) Sodium peroxide + 30-min steam (SPS); 5) Quicklime (QL); 6) Quicklime + 30-min steam (QLS). To evaluate control efficacy of steam combined with exothermic substances,

pathogen control efficacy was evaluated at different distances from the steam injection point (2.5, 12.5, 25, and 38 cm) in each replicate.

Application of steam and exothermic substances

Prior to steam application, exothermic chemicals were incorporated into the soil at the following amounts (Table 1.1): 1% quicklime (w/w) and 0.1% sodium peroxide (w/w). Different application amounts were used due to the high cost of sodium peroxide ($1.82 g^{-1}, Fisher Chemical) compared to quicklime ($0.20 g^{-1}, Thermo Scientific Chemicals). The mass of each chemical applied was based on the approximate soil mass of a microplot at 10.2 cm depth: 152.167 kg (Formula 1).

$$A.\,Number\ of\ soil\ samples\ that\ fit\ in\ one\ microplot = \frac{51000\ cm^3{}_{microplot\ volume}}{1768.16\ cm^3{}_{soil\ sample\ volume}}$$

$$= 28.84\ soil\ samples$$

$$B.\,Microplot\ Soil\ Mass = 28.84_{soil\ samples} * 5274.08_{g\ soil\ sample^{-1}} = 152{,}157.21\ g$$

$$C.\,Mass\ of\ quicklime\ applied = 152{,}157\ _{g,\ \ microplot\ soil\ mass} *\ 0.01$$

$$= 1521.01\ g\ quicklime$$

$$D.\,Mass\ of\ sodium\ peroxide\ applied = 152{,}157\ _{g\ microplot\ soil\ mass} * 0.001$$

$$= 152.10\ g\ sodium\ peroxide$$

Formula 1: Calculations to estimate the mass of a microplot (A and B), and to determine the mass required to apply 1% of quicklime w/w (C), and 0.1% of sodium peroxide w/w (D).

Temperature probes were also inserted at the 2.5, 12.5, 25, and 38 cm distance points from steam injection, to measure soil temperature throughout the steaming process. Black plastic mulch (VIF) was used to cover the microplot area before steam application. Steam was injected using a low-pressure steam generator (Sioux® Steam-Flo 25L Boiler, Sioux, Beresford, SD). This generator injected steam into microplots 10.2 cm deep at a pressure of 5-7 psi through the attachment of a steam-grade spike hose (Fig. 1.1).

Pathogen control evaluation

To evaluate soilborne pathogen control, soil was tested for the presence of *Pythium* sp. colony forming units per gram of soil (propagules per gram = ppg) found naturally in the soil. Soil samples were taken 24 hours after steam application, on 2 June 2021 and 2 June 2022. A soil probe, 2.5 cm in diameter, was used to collect 4 soil cores from each replicate at the 2.5, 12.5, 25, and 38 cm distance points. Each soil core was taken from a 10.2 cm depth. Soil samples were placed in labeled paper bags, mixed, and left to air dry (at room temperature) for two weeks. Dried soil samples were transferred into plastic containers and stored at 7°C. Soil was analyzed for *Pythium* ppg of soil, using a wet plating assay outlined in Klose et al. (2007).

Corn meal agar (17 g L^{-1}, Sigma-Aldrich, St. Louis, MO) were prepared and sterilized at 121°C for 20 minutes in a SterilMatic® Autoclave (Market Forge Industries, Everett, MA). After autoclaving, 1 mL of Tween 20 (Thermo Fisher Scientific, Waltham, MA) was added. This was followed by the addition of prepared antibiotic and antifungal solutions.

Antifungal and antibiotic solutions were added to approximately 50°C corn meal agar at the following concentrations: 0.025 g L^{-1} Rose Bengal (Fisher Chemical, Fair Lawn, NJ), 250 mg L^{-1} ampicillin (Fisher BioReagents™, Fair Lawn, NJ), 22 mg L^{-1} benomyl (Sigma-Aldrich®, St. Louis, MO), 10 mg L^{-1} rifampicin solution (Fisher Chemical), and 50 uL of 2.5% aqueous pimaricin stock solution (Sigma-Aldrich®). The agar was then poured into 100 x 15 mm petri dishes and left in the dark at room temperature for three days before plating soil solution. Soil solution (25 mg mL^{-1}) was spread on a plate (replicated 5x3 times) using a sterile cell spreader (VWR International™, Radnor, PA). Plates were then incubated in the dark at room temperature. *Pythium* sp. colonies were counted 48 and 72 hours after plating. The average number of ppg was then calculated for each of the three replicates.

Soil temperature assessment

Soil temperatures were recorded at 4 distances from the steam injection point (2.5 cm, 12.5 cm, 25 cm, and 38 cm). In the first year, temperature was recorded at a 10.2 cm depth using a PVC probe with an attached Cryopak iMini temperature logger (Part #MX2ES8L, Cryopak, Edison, NJ). In 2022, temperatures were recorded with a HOBO 4-channel thermocouple data logger (Part #UX120-014M, Onset, Bourne, MA), with 4 attached T-type thermocouple wires. Soil temperatures were recorded for the duration of the steaming event (30 min) in both years. In the second year, the thermocouples, as well as the black plastic, were left undisturbed for an additional 15 minutes after steaming ended. Recorded temperatures were compiled using HOBOware software, version 3.7.17.

Soil pH

Because quicklime and sodium peroxide effect soil pH, soil samples were taken to analyze pH before and after treatment. Soil was sampled from each distance point within a replicate to create a sample that was representative of the entire microplot. In the first year (2021), 10 g of the mixed soil sample were placed in an open plastic container and air-dried for 1 week. Once samples were dry, they were placed in a 50 mL beaker with 20 mL of deionized water. Soil solutions were then mixed using a glass stirring rod and left to stand for 10 minutes. pH was measured with a PC800 conductivity meter (Apera Instruments, Columbus, OH).

In the second year of the trial, soil samples were sent to the NC Department of Agriculture and Consumer Sciences Agronomic Division for a soil report. Soil characteristics provided by the report included: pH, HM%, W/V (g cm^{-3}), BS%, Ac (meq 100 cm^{-3}), CEC (meq 100 cm^{-3}), Na (meq 100 cm^{-3}), P (mg dm^{-3}), K (mg dm^{-3}), Ca (mg dm^{-3}), Mg (mg dm^{-3}), S (mg dm^{-3}), Mn (mg dm^{-3}), Cu (mg dm^{-3}), and Zn (mg dm^{-3}).

Statistical Analysis

All data were analyzed using RStudio (RStudio Desktop ver. 2022.07.02, Boston, MA, USA) with R 3.3.3. Pathogen results were tested for normal distribution [Shapiro-Wilk ($\alpha \leq 0.05$)] and number of *Pythium* ppg were $\log_{10}$ transformed prior to further analysis based on this test. Pathogen control was analyzed with an analysis of variance ($\alpha \leq 0.05$). Tukey's honest significant difference post-hoc test was performed when appropriate ($\alpha \leq 0.05$). Data from each year was analyzed separately ($\alpha \leq 0.05$). Regression analyses were performed between average soil temperature during steam application [T_{avg} (x)], maximum soil temperature [T_{max} (x)], and

Pythium ppg (y). Tables and graphs were made using Microsoft® Excel for Mac (Version 16.69, Redmond, WA) and BioRender (Toronto, Ontario, CA).

Results:

Pythium *sp. control with steam and quicklime*

The combination of steam and quicklime (QLS) controlled *Pythium* sp. better than steam alone or quicklime alone (QL). QLS significantly reduced *Pythium* ppg at the 2.5 cm mark (7.4 ppg in 2021; 0 ppg in 2022; Fig. 1.2) and 12.5 cm mark (41ppg in 2021; 0 ppg in 2022). In addition, QLS reduced *Pythium* ppg at the 25 cm mark in the first year (800 ppg; Fig. 1.2). In contrast, steam alone significantly reduced *Pythium* ppg only at the 2.5 cm mark, and only in the second year of the trial (0 ppg; Fig. 1.2). The QL treatment significantly reduced *Pythium* ppg only at the 25 cm mark during the first year (600 ppg). The QLS treatment improved *Pythium* sp. control, compared to steam alone, QL, and steam combined with sodium peroxide (SPS).

Pythium *sp. control with steam and sodium peroxide.*

SPS did not control *Pythium* sp. more effectively than steam alone. But SPS controlled *Pythium* sp. more effectively than sodium peroxide alone (SP). SPS controlled *Pythium* sp. at the 2.5 cm distance point in the first year (0 ppg; Fig. 1.2A). Similarly, the steam alone treatment had one instance of *Pythium* sp. control, at the 2.5 cm distance point in the second year (0 pgg; Fig. 1.2B). SP did not control *Pythium* sp. in either year of the trial (Fig. 1.2). Ultimately, SPS was more effective at pathogen control compared to SP, but not compared to steam alone. These results indicate that steam is the primary control agent in the SPS treatment.

Relationship between maximum soil temperature (T_{max}) and Pythium *sp. control*

Significant exponential relationships ($\alpha \leq 0.05$) between T_{max} and *Pythium* ppg were found in all treatments which included steam in the second year of the trial (steam alone, SPS, and QLS; Fig. 1.3). The steam alone and QLS treatments both had r^2 values of 0.97 (Figs. 3A and C). However, the SPS treatment had a lower r^2 value of 0.50 to describe the relationship between T_{max} and *Pythium* ppg (Fig. 1.3B).

Relationship between average soil temperature (T_{avg}) and Pythium *sp. control*

Significant exponential relationships ($\alpha \leq 0.05$) between T_{avg} and *Pythium* ppg were found in all treatments in the second year of the trial (steam alone, SPS, and QLS). The steam alone and QLS treatments had exponential r^2 values of 0.86 and 0.81, respectively. Complete *Pythium* control (0 ppg) was achieved once T_{avg} reached 58.1°C and 57.8°C in the steam alone and QLS treatments, respectively (Fig. 1.4A and C). In contrast, the SPS treatment had a logarithmic r^2 value of 0.31, and a slightly lower T_{avg} to achieve complete *Pythium* sp. control (52.6°C; Fig. 1.4B).

Soil pH and Pythium *sp. control*

The QL and QLS treatments had the greatest effect on soil pH (Fig. 1.5). The natural pH of the soil in our field study was 5.6 in year 1, and 6.7 in year 2. The QL treatment raised pH to 10.2 in year 1, and 11.7 in year 2. The QLS treatment raised pH to 11.7 in year 1, and 11.1 in year 2 (Fig. 1.5). The SP and SPS treatments also raised the soil pH, although less drastically. The SP treatment measured pH of 7.6 and 9.7 in years 1 and 2, respectively. The SPS treatment raised pH to 8.0 and 9.7 in years 1 and 2, respectively. Many of these measurements exceeded the typical pH range recommended for horticultural crops.

Discussion

Pythium *sp. control with steam and quicklime*

Quicklime in combination with steam (QLS) leads to better *Pythium* sp. control, compared to quicklime alone (QL) and steam alone (steam alone) (Fig. 1.2). These findings are in accordance with prior research, where exothermic substances in combination with steam have improved soilborne pathogen control. Potassium hydroxide and steam showed improved control of *Rhizoctonia solani* (Triolo et al. 2004) and *Fusarium oxysporum* (Luvisi et al. 2006) compared to steam alone.

However, quicklime has shown variable pathogen control efficacy when combined with steam. *Sclerotinia minor* survival decreased significantly when quicklime and steam were applied *in vitro* (Luvisi and Triolo 2007), but not in the field (Triolo et al. 2004). The reaction between quicklime and water to form calcium hydroxide ($Ca(OH)_2$) is exothermic (-64.1 kj mol^{-1}) under standard conditions, and soil temperature increases can enhance pathogen control. Differences in soil type can affect the rate and distance of temperature increases (Yang et al. 2019), and how resistant the soil is to pH changes. This could potentially explain differences between prior field experiments and this study.

Pythium *sp. control with steam and sodium peroxide*

The reaction between sodium peroxide and water to form sodium hydroxide (NaOH) and oxygen (O_2) is exothermic (-278 kj mol^{-1}) under standard conditions. Therefore, we tested the hypothesis that temperature control of soil pathogens would improve when steam and sodium peroxide were applied together, compared to when the components were applied separately. Data results did not

support this hypothesis. To our knowledge, no prior work was conducted on soil-applied steam in combination with sodium peroxide.

The exothermic reaction between quicklime and water is weaker (-64.1 kj mol^{-1}) compared to sodium peroxide and steam. However, the higher application rate of quicklime (1% w/w), compared to sodium peroxide (.1% w/w) could have caused enhanced efficacy in the QLS treatment in regard to temperature and pathogen control.

Changes in abiotic factors can also affect pathogen survival. Increases in sodium content have been shown to inhibit microbial growth (Rietz and Haynes 2003). However, microbial inhibition in response to sodic conditions has been shown to take multiple weeks (Wong et al. 2008). Soil samples were taken only a day after treatment application in this study.

Relationship between maximum temperature (T_{max}) and Pythium *sp. control*

Most studies investigating the effect of short-term, high temperatures on pathogen control were primarily conducted *in vitro*. An older study by Byars and Gilbert (1920) found that exposing *Pythium* sp., *Rhizoctonia* sp., and *Heterodera* sp. in 98ºC water, *in vitro*, for merely five minutes successfully eradicated the pathogens. This indicates that prolonged temperatures above 98ºC are not necessary to control fungal pathogens. Another study found that short exposure (approx. 11 min) to 50ºC *in vitro* was lethal to *Verticillium dahliae*, *Globodera pallida*, and *Sclerotium cepivorum* (van Loenen et al. 2003). The same study also found that short exposure to 60ºC was lethal to *P. ultimum*.

Various factors can affect field soil temperature, including soil depth (Gelsomino et al. 2010), soil type (Miller et al. 2014), and heat application methods (Huh et al. 2020; Miller et al. 2014). Given the variability of temperature control on pathogens, further research on the effect of short,

high temperature field applications could have on pathogen survival would help advance heat-based soil disinfestation practices.

Relationship between average temperature (T_{avg}) and Pythium *sp. control*

The maintenance of high temperatures is understood to be an important factor to control soilborne pathogens with steam (Pullman et al. 1981), and that an average of 65°C for 30 minutes can kill most soilborne pathogens (Baker and Roistacher 1957). Thiessen et al. (2020) found that for *Pythium* sp. specifically, a 30-min *in vitro* incubation at 63°C had effective control on inoculum in Styrofoam float trays. In contrast, in field conditions, temperatures exceeding 70°C for longer than 30 min showed variable *Pythium* sp. control, reducing *Pythium* ppg by 50% to 99.9% (Guerra et al. 2022). Various field conditions can affect fungal pathogen populations other than temperature, including pH (Cruz et al. 2019; Yang et al. 2022; Kauraw 1979; Mondal and Hyakumachi 2000) and soil moisture (Dunn et al. 1985). Therefore, determining an exact average temperature to control pathogens is difficult.

Our findings show that to control *Pythium* sp., an average temperature of about 56.2°C for 30 minutes is beneficial (Fig. 1.4), whether exothermic chemicals are applied or not, under our specific set of field conditions. QLS reached and exceeded an average temperature of 56.2°C more often than SPS and steam alone (Fig. 1.4). These findings indicate that the application of quicklime + steam could improve T_{avg}, compared to steam alone or SPS. This is supported by prior research, where quicklime (1000 kg ha^{-1}) + steam, as well as potassium hydroxide (1000 kg ha^{-1}) + steam, had significantly higher average temperatures compared to steam alone (Bárberi et al. 2009; Peruzzi et al. 2011; Luvisi et al. 2015).

Soil pH

Quicklime and sodium peroxide can raise soil pH, which we observed in this study after chemical application. Studies have found relationships between pH levels, calcium salt levels, and disease resistance. For example, the application of calcium salts can induce disease resistance in the host plant (Corden 1965). In addition, strong alkaline conditions can be detrimental to *Fusarium* sp. growth (Cruz et al. 2019; Yang et al. 2022). Other studies have found that *Pythium* sp. populations decrease when pH is below 5.5 and above 8.0 (Kauraw 1979; Mondal and Hyakumachi 2000). In our study, all four of the SP, SPS, QL, and QLS treatments raised pH above 8.0 in at least one of the years of this study (Fig. 1.5). However, QLS showed more thorough *Pythium* sp. control, compared to the rest of the treatments which increased pH. In addition, the relationship between pH and *Pythium* ppg is weak, with r^2 values of 0.146 and 0.1245 for 2021 and 2022, respectively (Fig. 1.6). This indicates that while pH could play a role in *Pythium* sp. suppression, temperature was the main driver for *Pythium* sp. control in this study.

Conclusion:

Steam in combination with quicklime (1% w/w) controlled *Pythium* sp. better than steam and quicklime alone. Steam in combination with sodium peroxide (.1% w/w) did not significantly improve *Pythium* sp. control. These results indicate that quicklime has the potential to improve field steam application efficiency. However, quicklime application raised soil pH as measured one day after treatment. Additional research is needed to determine the longer-term pH effect on plant productivity and the potential need to optimize quicklime application dosages for steam-based soil disinfestation methods.

Tables and Figures

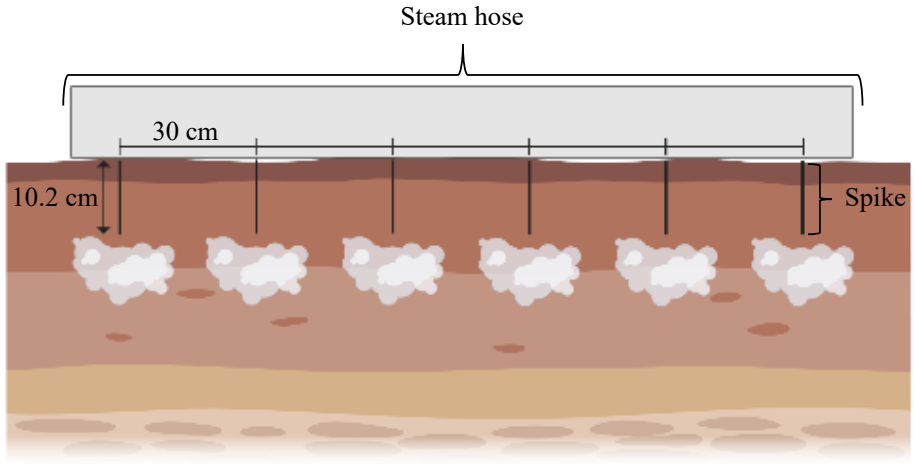
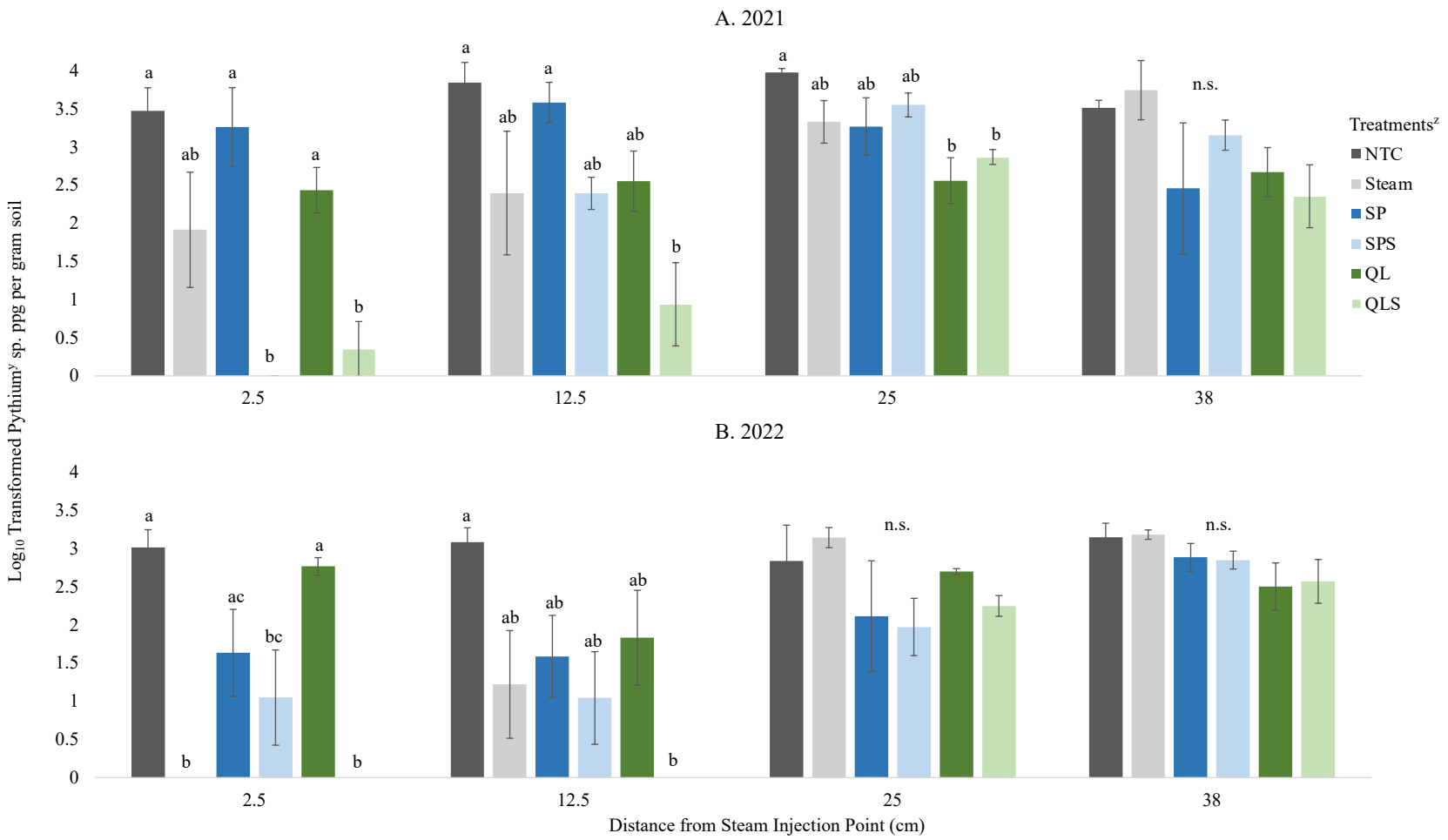


Figure 1.1: Steam hose dimensions. Each spike is 10.2 cm long, so steam is ejected at a 10.2 cm soil depth. Each of the spikes are spaced 30 cm apart.

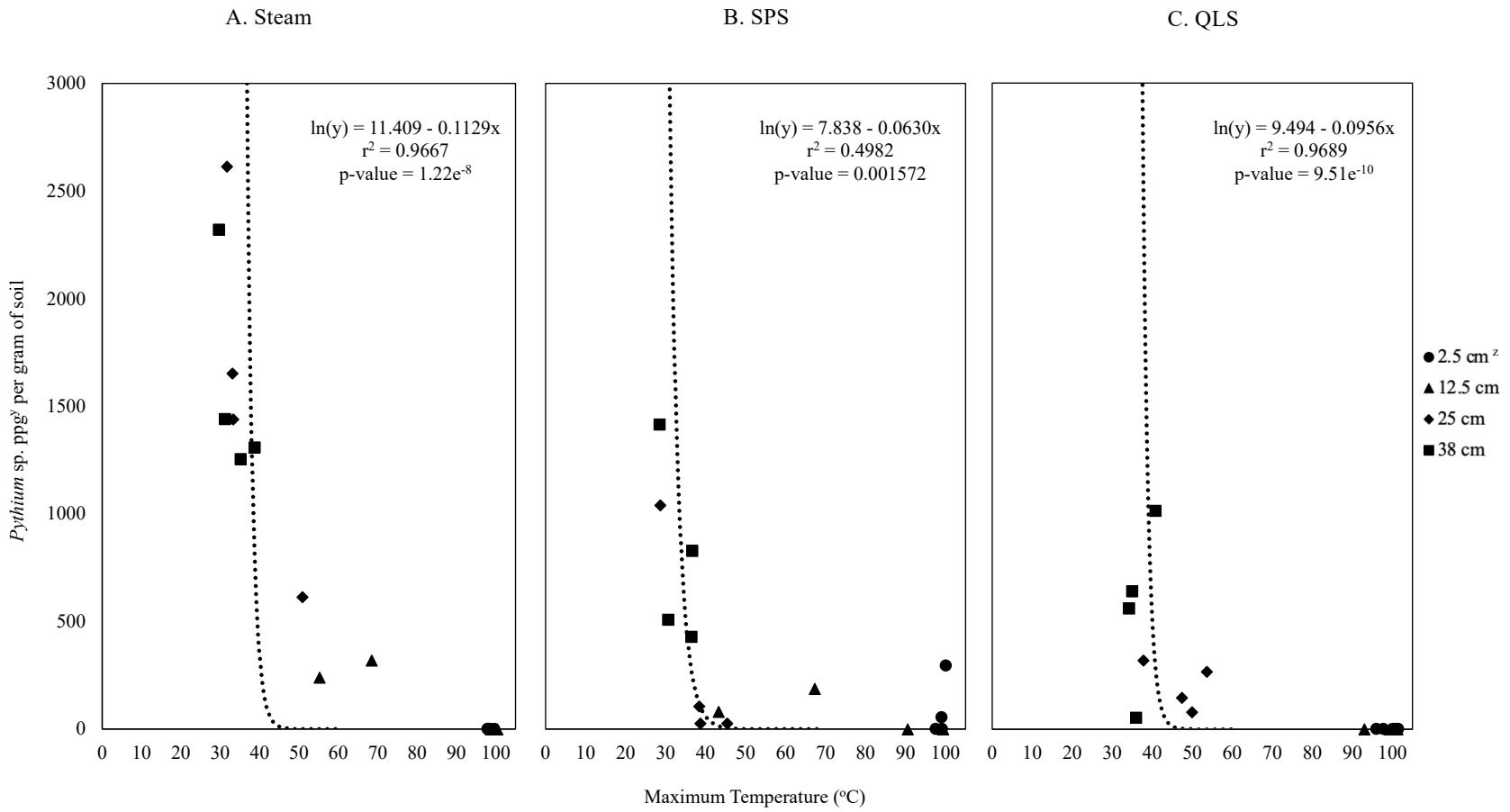
Figure 1.2: *Pythium* propagule per gram of soil separated by treatment and distance from the steam injection point. Values are the averages of $\log_{10}$ transformed *Pythium*. ppg soil values across four replicates for 2021 (A) and 2022 (B). Different letters indicate significant differences between treatments at the distance point from steam injection, according to Tukey's honest significant difference test ($\alpha \leq 0.05$). Error bars show the SE of the mean.



[y]ppg = propagule

[z]Treatments: NTC = Non-treated control; Steam = 30 minute steam application; SP = Sodium peroxide (.1% w/w); SPS = Sodium peroxide (.1% w/w) + 30 minute steam application; QL = Quicklime (1% w/w); QLS = Quicklime (1% w/w) + 30 minute steam application

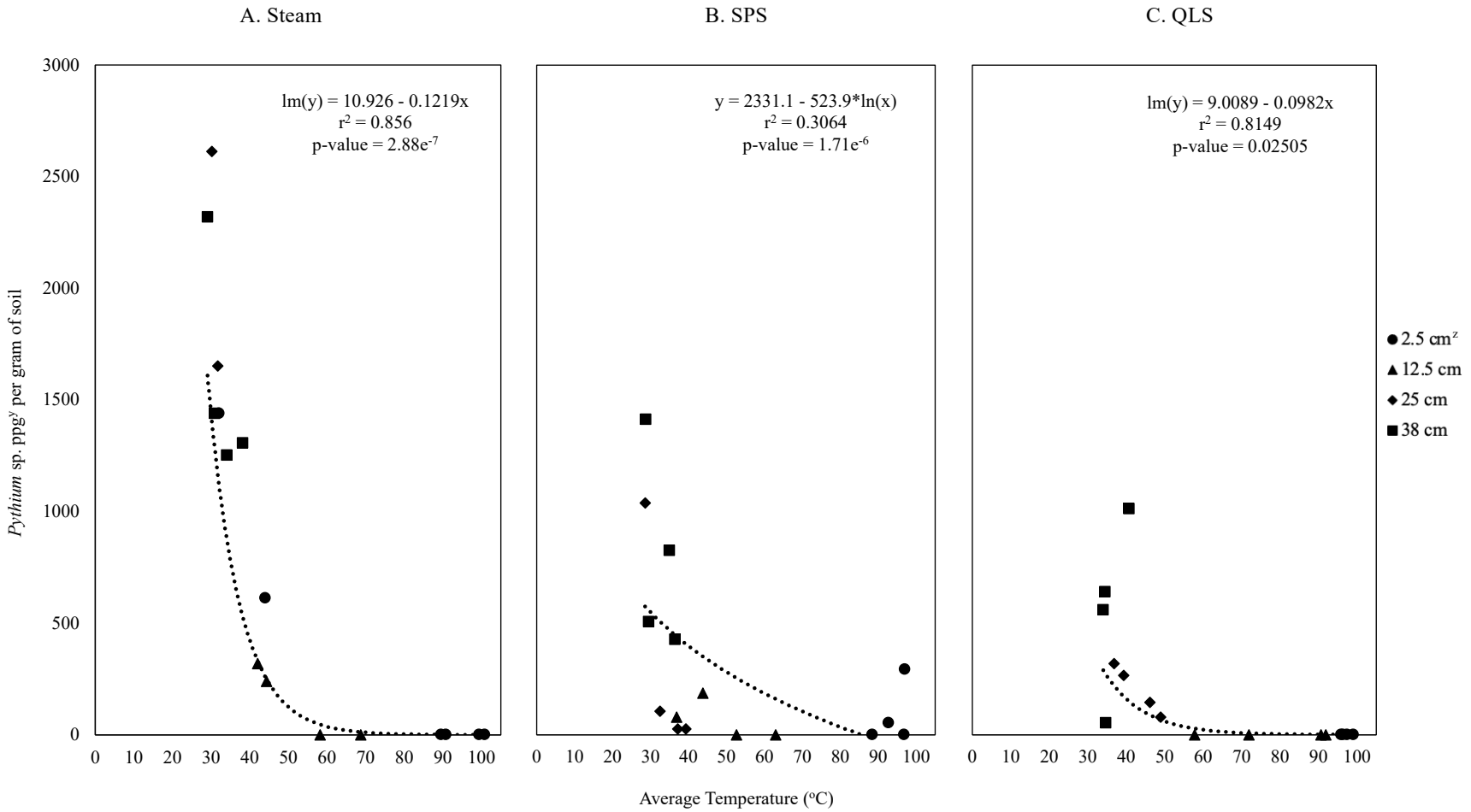
Figure 1.3: *Pythium* propagules of soil as impacted by maximum temperatures for A) steam alone, B) sodium peroxide + steam (SPS), and C) Quicklime + steam (QLS) treatments in the second year of the trial (2021). Shown are the exponential regression curves, regression equations, r^2 values, and p-values for each treatment.



[y]ppg = propagule

[z]Distance from steam injection point

Figure 1.4: *Pythium* propagules of soil as impacted by average temperatures for A) steam alone, B) sodium peroxide + steam (SPS), and C) Quicklime + steam (QLS) treatments in the second year of the trial (2021). Shown are the exponential (Steam and QLS) and logarithmic (SPS) regression curves, regression equations, r^2 values, and p-values for each treatment.



[y]ppg = propagule

[z]Distance from steam injection point

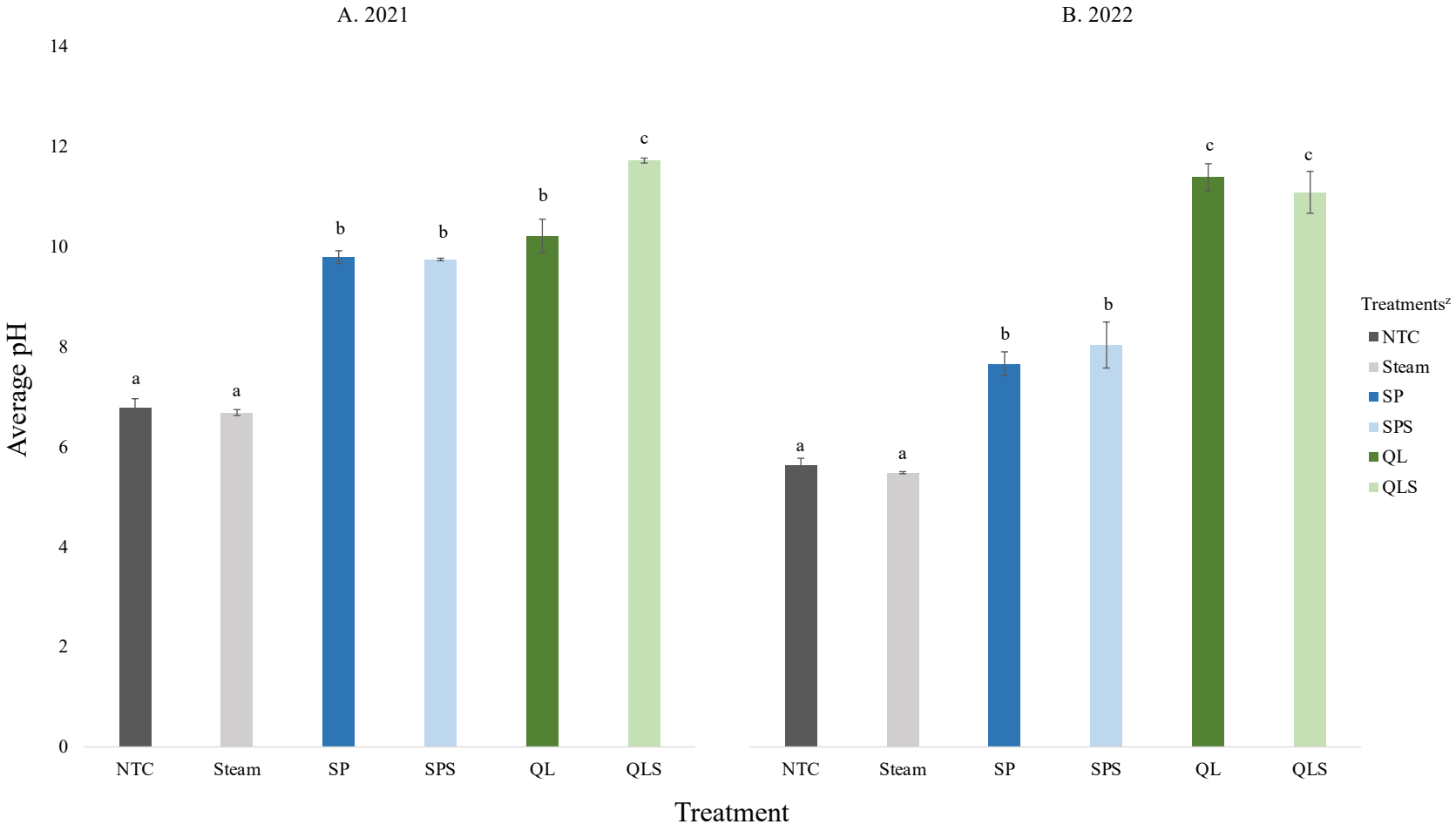


Figure 1.5: Average soil pH for each treatment (n=4) for A) 2021 and B) 2022. Different letters indicate significant differences between treatments according to Tukey's honest significant difference test ($\alpha \leq 0.05$). Error bars show the SE of the mean.

[y]ppg = propagule

[z]Treatments: NTC = Non-treated control; Steam = 30 minute steam application; SP = Sodium peroxide (.1% w/w); SPS = Sodium peroxide (.1% w/w) + 30 minute steam application; QL = Quicklime (1% w/w); QLS = Quicklime (1% w/w) + 30 minute steam application

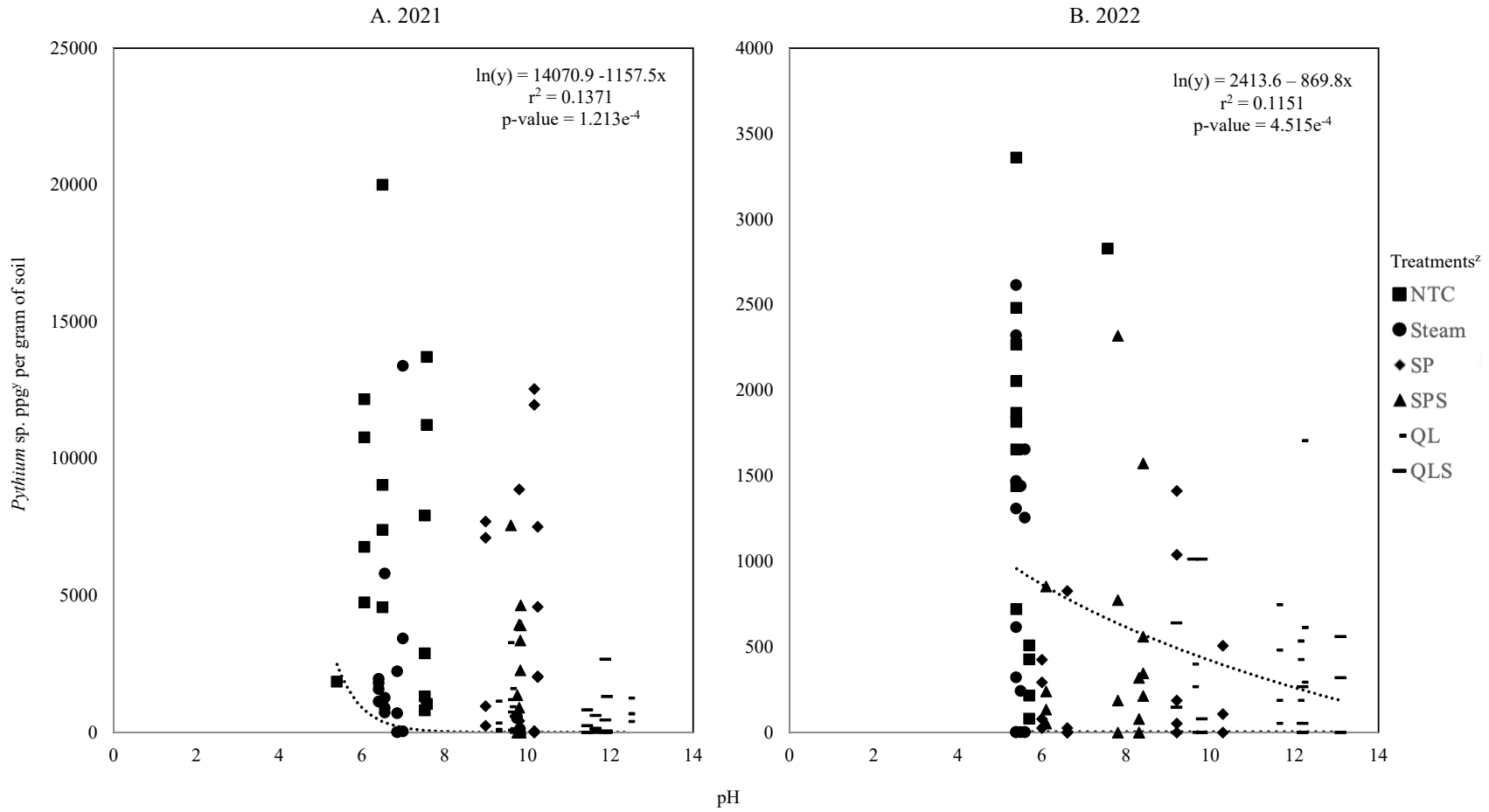


Figure 1.6: Relationship between soil pH and *Pythium* propagules per gram of soil for A) 2021 and B) 2022. Shown are the linear (2021) and exponential (2022) regression curves, regression equations, r^2 values, and p-values for each treatment.

[y]ppg = propagule

[z]Treatments: NTC = Non-treated control; Steam = 30 minute steam application; SP = Sodium peroxide (.1% w/w); SPS = Sodium peroxide (.1% w/w) + 30 minute steam application; QL = Quicklime (1% w/w); QLS = Quicklime (1% w/w) + 30 minute steam application

Table 1.1: List of 6 treatments and their abbreviations, amount of chemical, steam pressure, water and propane used to set up microplots. All numbers are averaged across both years of the trial.

Treatment	Abbreviation	Chemical Applied (%)[w]	Chemical Mass (g)	Pressure (psi)[x]	Water Use (L m^{-2})[y]	Propane Use (L m^{-2})[z]
Non-treated control	NTC	-	-	-	-	-
30-Min Steam	Steam	-	-	5-7	8.0	1.8
Sodium Peroxide	SP	.1	152.10	n/a	8.0	-
Sodium Peroxide + Steam	SPS	.1	152.10	5-7	8.0	1.8
Quicklime	QL	1	1521.01	n/a	8.0	-
Quicklime + Steam	QLS	1	1521.01	5-7	8.0	1.8

[w]Amount of chemical applied, as a percentage of the soil mass of the microplot (w/w).

[x]pound-force per square inch

[y]Liters of water applied to each microplot, averaged over the course of the two-year trial, in the form of steam or as air temperature water.

[z]Liters of propane used to steam each microplot, averaged over the course of the two-year trial.

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CHAPTER 3

Weed and pathogen control through steam and allyl isothiocyanate pre-plant application in a North Carolina strawberry production system

Abstract

Isothiocyanates from the *Brassicaceae* family can control soilborne pathogens, pests, and weeds. Allyl isothiocyanate (AITC) is synthetically produced and sold as a pre-plant biofumigant through the tradename Dominus®, and it has shown variable control efficacy in field settings. We hypothesized that the combination of steam and AITC will increase strawberry yield and control soilborne *Pythium* sp. and weeds more effectively than the components by themselves. Two trials were conducted in Clayton and Castle Hayne, NC, to assess yield, as well as weed and pathogen control efficacy, of steam combined with AITC directly in an annual strawberry plasticulture production system. Nine treatments were applied and replicated 4 times each: 1) Non-treated control; 2) chloropicrin + 1,3-dichloropropene; 3) AITC; 4) AITC + 60-min steam application; 5) AITC + 30-min steam application; 6) AITC + 10-min steam application; 7) 60-min steam application; 8) 30-min steam application; 9) 10-min steam application. Soilborne pathogen control efficacy was assessed using *Pythium* propagules per gram of soil via a wet plating assay. Weed control was assessed using a seed germination assay. Weeds and *Pythium* sp. were controlled by AITC alone as effectively as 1,3-D + chloropicrin. Steam did not effectively control weeds or *Pythium* sp. These results show the potential for AITC to act as a reliable pre-plant biofumigant in NC strawberry production.

Introduction

According to the United States Department of Agriculture's National Agricultural Statistics Service (USDA-NASS), U.S. strawberry fruit production was valued at $2.4 billion in 2018 (USDA-NASS 2018). California leads the country in strawberry fruit production, followed by Florida, New York, North Carolina, and Oregon (USDA-NASS 2018). In 2018, NC's strawberry crop was valued at $21.4 million (USDA-NASS 2018). Most strawberries in the US, including NC, are grown in annual hill plasticulture systems. A typical NC strawberry season begins in August, when beds are raised and fumigated. Plants are then planted between late September and mid-October, depending on the region. Plants go through a dormant season during the winter, and harvest follows in late spring, from early April to early June.

Pre-plant fumigation is a critical component in strawberry plasticulture systems, controlling weeds, pests, and soilborne pathogens. Diseases such as black root rot, *Phytophthora* crown rot, Verticillium wilt, Fusarium wilt, and charcoal rot are caused by soilborne pathogens and can negatively impact yield (Holmes et al. 2020). Black root rot is a common disease complex in NC, caused by multiple pathogens including, but not limited to, *Pythium* sp., *Rhizoctonia* sp., and *Pratylenchus penetrans* (Nemec and Sanders 1970; Heald 1920; Raski 1956; Louws and Cline 2019). Black root rot can decrease strawberry yield by up to 46% (LaMondia 1999). Another important soilborne disease in NC is *Phytophthora* crown rot, commonly caused by *Phytophthora cactorum*, and it has caused up to 50% yield loss in production fields (Marin 2018).

Despite control provided through black plastic mulch, weeds can emerge from the hole around the strawberry plant. Nutsedge species (*Cyperus* sp.) are especially problematic in both West and East Coast strawberry production. Pre-plant fumigation and hand weeding can decrease the weed

seed bank and decrease Nutsedge tuber emergence. However, chloropicrin fumigation at lower rates can also stimulate Nutsedge emergence, a problem often observed in the Southeast, where many growers fumigate under sub-optimal conditions (Santos et al. 2006).

Typical pre-plant fumigants are 1,3-dichloropropene ($C_3H_4Cl_2$), chloropicrin (CCL_3NO_2), dazomet ($C_5H_{10}N_2S_2$), and metam sodium ($C_2H_4NNaS_2$). These chemicals can be effective on their own (Fennimore et al. 2003; Desaeger et al. 2017; Qiao et al. 2015) or when applied in combination (Mao et al. 2019; Gerik and Hanson 2011; Kabir et al. 2005). However, regional regulations (USEPA 2008a; USEPA 2008b; O'Malley 2010) and limited availability restrict the use of many of these chemicals in the U.S.

Alternatives to chemical fumigation include biofumigation, anaerobic soil disinfestation (ASD), and heat-induced soil disinfestation. Biofumigation utilizes crops or seed meals from the *Brassicaceae* family to control soilborne pathogens, pests, and weeds. Brassicas contain glucosinolates which break down to release compounds called isothiocyanates (Morra and Kirkegaard 2002). Isothiocyanates are linked to fungal, oomycete, weed, and pest suppression (Brown and Morra 1997; Baysal-Gurel et al. 2019; Bangarwa et al. 2017; Gao et al. 2021; Ren et al. 2018).

ASD involves the application of organic matter, which decomposes to create anaerobic conditions when the soil is saturated with water. Facultative anaerobes then produce gases which can suppress plant pathogens and nematodes (Shrestha et al. 2016). The process is aided by irrigation as well as laying a clear or gas-impermeable tarp over the amended soil (Rosskopf et al. 2015). The exact techniques, inputs, and success rates vary depending on the crops grown and diseases targeted (Shennan et al. 2014). ASD has been ineffective at controlling weeds in California strawberry production (Shennan et al. 2017). But studies have found that ASD

controlled *V. dahliae* (Muramoto et al. 2014) and increased populations of species which feed on pathogenic fungi (Shennan et al. 2014). Other studies have found effective control of nematodes through ASD (Butler et al. 2012; Shrestha et al. 2016).

Heat application can occur through soil solarization or steam application, or the combination of both (Daugovish et al. 2016; Samtani et al. 2017). Soil solarization utilizes the application of clear plastic and solar energy to heat the soil (Stapleton and DeVay 1986). Steam application as a soil disinfestation tactic has been utilized for decades and uses steam to heat soil and control soilborne pests and pathogens (Baker 1962). Steam has successfully been utilized in greenhouse settings through stationary application methods (Fenoglio et al. 2008; van Loenen et al. 2003). Field-based steam application methods can be either stationary or mobile (Dabbene et al. 2003; Yang et al. 2019; Fennimore et al. 2014; Peruzzi et al. 2011), and it has been shown to control soilborne pests and pathogens in strawberry production systems (Hoffmann et al. 2020; Kim et al. 2022; Samtani et al. 2017).

Soil solarization in combination with the application of biofumigants has controlled soilborne weeds and pathogens, such as *Macrophomina phaseolina*, *Fusarium oxysporum*, and *Rhizoctonia solani* (Israel et al. 2005; Baysal-Gurel et al. 2019; Daugovish et al. 2016). Allyl isothiocyanate (AITC) is naturally found in brassicas, but it is also synthetically produced and sold through the trade name Dominus® (Isagro, Italy). AITC controls pathogens *in vitro* (Vandicke et al. 2020). In a microplot field study, co-applying steam with AITC improved control of *Verticillium dahliae*, common knotweed, and common purslane, compared to AITC alone (Kim et al. 2020). This is believed to be due to an increase in AITC vapor pressure when heat is applied to the soil. This study builds directly on the microplot study of Kim et al. (2020) by assessing weed and pathogen control efficacy of steam combined with AITC directly in an annual strawberry

plasticulture production system. We hypothesized that the combination of steam and AITC will increase strawberry yield and control soilborne *Pythium* sp. and weeds more effectively than the components by themselves. The objective of this study was to assess the impact of AITC combined with several steam treatments, as well as steam alone and AITC alone. Treatment effects were assessed on soilborne pathogen control, weed seed germination, Nutsedge tuber sprouting, and strawberry fruit production.

Materials and Methods

Field Trial Design

Research was conducted at the Central Crops Research Station (CCRS, Clayton, NC) and the Horticultural Crops Research Station (HCRS, Castle Hayne, NC) over the course of two strawberry growing seasons (2020-21, 2021-22). At CCRS, the 2020-21 season took place from 9 Sept. 2020 to 27 May 2021, and the 2021-22 season took place from 15 Sept. 2021 to 26 May 2022. At HCRS, the 2020-21 season took place from 22 Sept. 2020 to 5 May 2021, and the 2021-22 season took place from 29 Sept. 2021 to 19 May 2022. Nine treatments were established in a randomized complete block design (4 replicates, 20 plants per replicate; Table 2.1): 1) Non-Treated Control (NTC); 2) Chloropicrin + 1,3-D (Pic-Clor 60); 3) 60 Minute Steam Injection (Steam60); 4) 30 Minute Steam Injection (Steam30); 5) 10 Minute Steam Injection (Steam10); 6) Allyl Isothiocyanate (AITC); 7) AITC + 60 Minute Steam Injection (AITC60); 8) AITC + 30 Minute Steam Injection (AITC30); 9) AITC + 10 Minute Steam Injection (AITC10)

Four 61-m-long beds were used at each location during both years of the trial. Each plot was comprised of a 3 m x 1.5 m block within each bed, and each replicate was separated by a 3 m

buffer zone. Buffer zones did not include strawberry planting. In addition, there was a 4.6 m buffer zone on both ends of each bed. The field used at CCRS is comprised of a Norfolk Sandy Loam, and the soil at CHRS is comprised of Seagate Fine Sand.

Fumigation

At the start of each season, beds were raised, overlain with black plastic, and fumigants were applied at a depth of 15.2 cm with 2 shanks. In total, 158 m^2 were shank-injected with AITC, and 56 m^2 were injected with Pic-Clor 60 at both locations during both years of the trial (see Table 2.2 for fumigant application dates). Application rates were low during the first season due to a clog in one of the shanks (Table 2.1).

Steam Injection

Steam injection occurred after fumigation, bed shaping, and plastic laying, and before strawberry planting (see Table 2.2 for steam application dates). Steam was generated using the Sioux® Steam-Flo 25L Boiler (Beresford, SD). A 51 m hose with 18 cm spikes spaced 30 cm apart was attached to the boiler. The spikes poked through the plastic, along the area where strawberries would eventually be planted. The spikes were 18 cm long and had small holes at their tips, where steam was released.

Cryopak iMini temperature loggers (Part #MX2ES8L, Cryopak, Edison, NJ) recorded soil temperature in the center of the bed. Each logger recorded temperature at the 18 cm depth. The maximum and average temperatures recorded during steaming were collected (Tables 2.3 and 2.4). ‘Camarosa’ plug plants were planted with 0.3 m spacing (see Table 2.2 for planting dates).

Pre-plant N-P-K fertilizer (6-6-18) was applied at a rate of 1,120 kg N ha^{-1}. Weeds were hand-weeded, as needed, throughout the season.

Harvest

Harvest took place at CCRS from 19 April to 27 May 2021, and 2 April to 26 May 2022. Harvest took place at HCRS from 12 April to 27 May 2021, and 31 Mar to 19 May 2022. Once harvest began, bi-weekly applications of 4-0-8 liquid fertilizer were applied at a rate of 67 kg N ha^{-1}. Marketable and nonmarketable yields were assessed on Mondays and Thursdays throughout the harvest period. Fruit was determined non-marketable due to deformities, disease symptoms, water damage, and small size.

Pythium Sampling and Media Preparation

Five soil samples (2.5 cm diameter and 18 cm depth) were collected from the strawberry planting holes in each replicate. Soil samples were collected on the day of strawberry planting. Soil samples were placed in labeled paper bags, mixed, and left to air dry for one week. Once the soil dried, samples were transferred into sterile plastic containers and kept in the fridge at 7°C. Samples were analyze for *Pythium* colony forming units per gram of soil (propagules per gram = ppg) using a wet plating assay according to Klose et al. (2007). Corn meal agar (17 g L^{-1}, Sigma-Aldrich, St. Louis, MO) was autoclaved at 121°C for 20 min on a liquid/slow cycle using a Sterilmatic® autoclave (Market Forge Industries, Inc., Everett, MA). After autoclaving, Tween 20 (1 mL L^{-1}; Thermo Fisher Scientific, Waltham, MA) was added to the solution. Once the agar cooled to approximately 50°C, antifungal and antibiotic solutions were added at the following rates: 0.025 g L^{-1} rose bengal (Fisher Chemical, Fair Lawn, NJ), 250 mg L^{-1} ampicillin (Fisher

Bioreagents, Fair Lawn, NJ), 22 mg L^{-1} benomyl (Sigma-Aldrich®, St. Louis, MO), 10 mg L^{-1} rifampicin (Fisher Chemical), and 50 uL L^{-1} of 2.5% aqueous pimaricin stock solution (Sigma-Aldrich®).

Afterwards, the agar was poured into 100 mm x 15 mm petri dishes (FisherBrand®, Fair Lawn, NJ). The prepared petri dishes were left in the dark at room temperature for 72 hours before plating soil solutions.

A 0.5 g soil sample was measured and placed into a 50 mL plastic screw cap tube. Under a sterile flow hood, 20 mL of sterile DIW was added to each 50 mL tube and placed on a vortex. Then 0.5 mL of solution was plated across 5 petri dishes using a 100-1000 uL pipette. This process was replicated 3 times for each soil sample. The solution was spread across the agar using a sterile cell spreader (VWR International™, Radnor, PA). Plates were left in the dark at room temperature. *Pythium* colony forming units were counted 48 and 72 hours after plating. The average number of ppg per gram of soil was then calculated.

Weed Germination Assay

Ten soil samples (2.5 cm diameter and 18 cm depth) were collected from each plot. Samples were taken from the planting holes on the same day as planting, just before strawberries were planted. The 10 soil cores were mixed together and used for weed seed survival analysis. In the greenhouse, 12.7 x 12.7 x 5.0 cm plastic pots were lined with a paper towel, labeled, and filled with 10 g of soil medium. Then 400 g of the soil sample were measured and placed on top of the soil medium and watered every other day. Two additional pots were filled with soil medium to test for contamination. Weed seedlings were identified and counted as they germinated. Soil samples were mixed 30 days after establishment. Seedlings which were too difficult to identify at

an early stage were collected and grown out in large pots to ensure accurate identification. Species which germinated < 5 times across all treatments and replicates were not included in data analysis.

Statistical Analysis

All data were analyzed using RStudio (RStudio Desktop ver. 2022.07.02, Boston, MA, USA) with R 3.3.3. Pathogen results, weed germination, and cumulative yield were tested for normal distribution [Shapiro-Wilk ($\alpha \leq 0.05$)]. The number of *Pythium* ppg were $\log_{10}$ transformed prior to further analysis due to Shapiro-Wilk results ($\alpha \leq 0.05$). Pathogen control, weed germination, and cumulative yield were analyzed with an analysis of variance ($\alpha \leq 0.05$). Treatments which received the same steam time (60 min, 30 min, 10 min, or no steam) were only compared to each other (ex. AITC60 vs. Steam60). Fisher's least significant difference post-hoc test was performed when appropriate ($\alpha \leq 0.05$).

Results

Pathogen Control

In both years and at both locations, shank-injected AITC alone (Dominus®) showed similar *Pythium* control, compared to shank-injected Pic-Clor 60 (Table 2.5). AITC + steam treatments provided more efficacious. *Pythium* control compared to steam alone treatments (Table 2.5), but not better control than AITC or Pic-Clor 60 alone. No differences were observed between AITC + steam treatments and steam alone treatments, regardless of steam application time, during both years of the HCRS trial. At CCRS, AITC10 controlled *Pythium* significantly better than Steam10. Generally, AITC controlled *Pythium* sp. as well as Pic-Clor 60. Additional steam

injections (AITC + Steam) did not enhance control of *Pythium* sp., compared to AITC alone or Pic-Clor 60.

Weed Seed Germination Control

In both years and at both locations, AITC controlled weeds similar to Pic-Clor 60 (Table 2.6). Steam alone did not effectively control weeds (Table 2.6). AITC + steam did not result in better weed control, compared to AITC alone treatments. However, AITC + steam treatments did improve weed control, compared to steam alone treatments at 10, 30, and 60 min application time. At CCRS, *Cyperus* sp. germination was significantly lower in AITC10 compared to Steam10. At HCRS, AITC10 had significantly less *Spergula* sp., *Cyperus* sp., and *Portulaca* sp. germination, compared to Steam10. AITC30 had significantly less *Cyperus* sp. and *Trifolium* sp. germination, compared to Steam30. And AITC60 had significantly less *Spergula* sp., *Cyperus* sp., *Trifolium* sp., and *Portulaca* sp. germination, compared to Steam60 (Table 2.6).

Strawberry Yield

In both years and locations, marketable yields in the AITC alone treatment were similar to the Pic-Clor 60 treatment. Generally, steam on its own did not perform as well as when it was combined with AITC (Table 2.7). However, no differences were found between treatments at HCRS in 2020-2021 and CCRS in 2021-2022 (Table 2.7). Highest yields of 1,001 g plant^{-1} were achieved in AITC followed by 60 min of steam injection in the 2021-22 season at HCRS. During the 2020-21 season at CCRS, AITC10 and AITC30 had significantly higher yields, compared to Steam10 and Steam30. During the 2021-22 season at HCRS, AITC10, AITC30, and

AITC60 had higher yields compared to Steam10, Steam30, and Steam60, respectively ($\alpha \leq 0.05$).

Discussion

Pathogen and Weed Control

Our results show that shank-injected AITC (Dominus®) can sufficiently control *Pythium* sp. and weeds in a Southeastern climate. This finding is supported by prior research. In an *in vitro* study, AITC decreased *Fusarium graminearum* and *Fusarium poae* by 100% as AITC concentration increased (Vandicke et al. 2020). A field study found that AITC controlled *Fusarium* sp., *Pythium* sp., and *Phytophthora* sp. as well as, or in some years, better than, chloropicrin (Ren et al. 2018). AITC (183 to 275 kg ha^{-1}) controlled *Fusarium oxysporum* as well as Pic-Clor 60 (337 kg ha^{-1}), and AITC significantly decreased *F. oxysporum* colony forming units when it was applied at a rate of 367 kg ha^{-1} (Yu et al. 2019).

Shank-injected AITC has shown successful control of large crabgrass (*Digitaria sanguinalis*) and yellow nutsedge (*Cyperus esculentus*) when applied as a pre-plant fumigant (Ren et al. 2018; Devkota et al. 2013). And it has effectively controlled purple nutsedge (*Cyperus rotundus*) in tomato production (Yu et al. 2019) and palmer amaranth (*Amaranthus palmeri*) in bell pepper production (Bangarwa et al. 2011). These results come from trials where commercially made biofumigants were applied. In studies where biofumigation is conducted through the incorporation of organic *Brassicaceae* plant material, weed control is more variable (dos Santos et al. 2021). In particular, hard and large seeds are less susceptible to biofumigation treatments (Cauwer et al. 2019).

However, reports of the pathogen control efficacy of AITC vary. AITC on its own did not sufficiently control *V. dahliae* beyond its injection point, and it had high weed densities and weed control compared to chemical fumigants in CA strawberry production (Kim et al. 2020). Another CA study found poor control of *P. ultimum*, in cut flower production (Hoffmann et al. 2020).

One potential cause for the difference in these results is the difference in air temperature. Kim et al. 2020 conducted their trial in June in Salinas, CA, where the average high air temperature is 21.1ºC (U.S. Climate Data 2023). In contrast, the average high air temperature during pre-plant fumigation in September in Clayton and Castle Hayne, NC, are 27.8ºC and 28.9ºC, respectively (U.S. Climate Data 2023). High air temperatures during pre-plant fumigation could support higher soil temperatures that enhance AITC vaporization in the soil bed. The vapor pressure of AITC is 5 mmHg at 23.5ºC, and it doubles to 10 mmHg once temperatures reach 38.2ºC (National Library of Medicine). So small changes in temperature can have large impacts on vapor pressure. Another potential cause for varying pathogen and weed control is the soil type. Hoffmann et al. (2020) and Kim et al. (2020) do not report the soil types in their field studies. However, chemical fumigation can be less effective in clay soils due to smaller particle sizes. Steam applied on its own did not effectively control soilborne *Pythium* sp. or weeds in this study. Soil temperatures of 70ºC for 15 minutes can control most weeds, and 65ºC for 30 minutes can kill most soilborne pathogens (Baker and Roistacher 1957). However, the maximum average temperature achieved during steam application in our trial was 48.86ºC for 60 minutes at 18 cm depth in the center of the raised bed (Table 2.3). Heat transfer through steam application varies depending on soil type (Miller et al. 2014), soil depth (Gelsomino et al. 2010), distance from steam application (Hoffmann et al. 2020), steam application speed (Huh et al. 2020), and

application method (Miller et al. 2014). Steam was injected through spikes every 30 cm through black plastic in this study. While shank-injected, mobile steam application steam can raise soil temperatures to efficacious levels (Guerra et al. 2022), the simpler and more cost-effective method used in this project could not.

Application methods with shank-applied steam and simultaneously mixing the soil have been proven to be more effective at heat transfer, compared to stationary steam application (Miller et al. 2014). Studies found that mobile steam application controlled yellow nutsedge (*Cyperus esculentus*), common purslane (*Portulaca oleraceae*), *P. ultimum*, and *V. dahliae*, as effectively as Pic-Clor 60 in CA strawberry production (Fennimore et al. 2014; Kim et al. 2021). Field-based steam application has also controlled *Pythium* sp. and weeds in lettuce production (Guerra et al. 2022). In addition, the ECOSTAR SC 600 (Celli, Forlí, Italy) has shown the ability of steam, in combination with potassium hydroxide (KOH), to effectively control *Rhizoctonia solani* (Triolo et al. 2004) and *Fusarium oxysporum* (Luvisi et al. 2006). Effective pathogen and weed control through alternative steam application methods suggests that stationary steam injection through a spike hose is insufficient for strawberry plasticulture systems.

Strawberry Yield

Shank-applied AITC had higher marketable yields compared to the NTC, and it had similar yields to shank-applied Pic-Clor 60. Prior research shows varying yield results when AITC is applied. Two studies conducted in China showed its ability to produce similar tomato yields to chloropicrin (99.5%) (Ren et al. 2018) and Pic-Clor 60 (Yu et al. 2019). However, AITC showed no yield improvement, compared to NTC, in California strawberry production (Hoffmann et al. 2020). In addition, a study determined that the ideal application rate of AITC to have similar

tomato yield to methyl bromide-treated plots would be 887 kg ha^{-1} (Bangarwa et al. 2011). This rate is > 500 kg ha^{-1} more than what was applied during our trial, and it exceeds the recommended label rate for Dominus®. Given the yields produced by AITC applied at or below the recommended rate in this study, competitive strawberry yields appear to be attainable in NC sandy and sandy loam soils.

Conclusion

Our study showed that shank-applied AITC (Dominus®) was as effective as shank-applied chloropicrin + 1,3-D (Pic-Clor 60) to control soilborne pathogens and weeds, producing similar marketable yields. The addition of spike-injected, low-pressure steam did not enhance the efficacy of AITC. These results show the potential for shank-applied AITC to work as a pre-plant fumigant alternative for NC strawberry production. However, further research needs to be conducted to verify the efficacy of AITC as a pre-plant fumigant in other NC strawberry production regions.

Tables and Figures:

Table 2.1: Chemical fumigant application rates, steam pressure, and water usage for at the Central Crops Research Station (CCRS) and the Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 growing seasons.

Location	Treatment[w]	AITC Rate (kg ha^{-1}[x])		Pic-Clor 60 Rate (kg ha^{-1})		Steam Pressure (psi[y])		Water (L ha^{-1}[z])		Propane (L ha^{-1})	
		2020-21	*2021-22*	*2020-21*	*2021-22*	*2020-21*	*2021-22*	*2020-21*	*2021-22*	*2020-21*	*2021-22*
	NTC	-	-	-	-	-	-	-	-		
	Pic-Clor 60	-	-	157	325	-	-	-	-		
	Steam60	-	-	-	-	5-7	5-7	244,448	148,541	1,822	1,314
	Steam30	-	-	-	-	5-7	5-7	122,224	74,271	911	657
CCRS	Steam10	-	-	-	-	5-7	5-7	40,074	24,756	304	219
	AITC	168	210	-	-	-	-	-	-		
	AITC60	168	210	-	-	5-7	5-7	244,448	148,541	1,822	1,314
	AITC30	168	210	-	-	5-7	5-7	122,224	74,271	911	657
	AITC10	168	210	-	-	5-7	5-7	40,074	24,756	304	219
	NTC	-	-	-	-	-	-	-	-		
	Pic-Clor 60	-	-	244	523	-	-	-	-		
	Steam60	-	-	-	-	5-7	5-7	141,591	213,164	1,822	1,636
	Steam30	-	-	-	-	5-7	5-7	70,796	106,582	911	818
HCRS	Steam10	-	-	-	-	5-7	5-7	23,599	35,172	303	273
	AITC	130.1	343.0	-	-	-	-	-	-		
	AIT60	130.1	343.0	-	-	5-7	5-7	141,591	213,164	1,822	1,636
	AITC30	130.1	343.0	-	-	5-7	5-7	70,796	106,582	911	818
	AITC10	130.1	343.0	-	-	5-7	5-7	23,599	35,172	304	273

[w]Treatments: NTC = non-treated control; Pic-Clor 60= chloropicrin + 1,3-dichloropropene; Steam60 = 60-min steam application; Steam30 = 30-min steam application; Steam10 = 10-min steam application; AITC = allyl isothiocycanate; AITC60 = allyl isothiocyanate + 60-min steam application; AITC30 = allyl isothiocyanate + 30-min steam application; AITC10 = allyl isothiocyanate + 10-min steam application

[x]kilogram per hectare

[y]pounds per square inch

[z]liters per hectare

Table 2.2: Fumigation application, steam application, and strawberry planting dates at the Central Crops Research Station (CCRS) and Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 growing seasons.

Location	Season	Fumigation Application Date	Steam Application Date	Strawberry Planting Date
CCRS	2020-21	10 Sept 2020	30 Sept & 1 Oct 2020	26 Oct 2020
CCRS	2021-22	15 Sep 2021	5 to 6 Oct 2021	27 Oct 2021
HCRS	2020-21	22 Sep 2020	8 to 9 Oct 2020	23 Oct 2020
HCRS	2021-22	29 Sep 2021	12, 14, & 19 Oct 2021	22 Oct 2021

Table 2.3: Average soil temperature at 18 cm depth in the center of the raised bed during steam application at the Central Crops Research Station (CCRS) and Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 strawberry seasons.

Location	**Season**	**Average Soil Temperature**					
		10 Min Steam[x]		*30 Min Steam*[y]		*60 Min Steam*[z]	
		AITC10	*Steam10*	*AITC30*	*Steam30*	*AITC60*	*Steam60*
CCRS	2020-21	22.84	28.16	22.34	35.89	46.92	48.86
CCRS	2021-22	26.94	34.72	33.33	28.62	30.76	25.87
HCRS	2020-21	25.76	28.16	41.23	44.38	27.96	29.61
HCRS	2021-22	21.33	20.99	44.38	34.55	23.09	25.09

[x]AITC10 = AITC + 10-min steam; Steam10 = 10-min steam

[y]AITC30 = AITC + 30-min steam; Steam30 = 30-min steam

[z]AITC60 = AITC + 60-min steam; Steam60 = 60-min steam

Table 2.4: Maximum soil temperature at 18 cm depth during steam application at the Central Crops Research Station (CCRS) and Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 strawberry seasons.

Location	**Season**	**Maximum Soil Temperature**					
		10 Min Steam[x]		*30 Min Steam*[y]		*60 Min Steam*[z]	
		AITC10	*Steam10*	*AITC30*	*Steam30*	*AITC60*	*Steam60*
CCRS	2020-21	22.93	35.25	25.10	50.21	69.92	54.43
CCRS	2021-22	27.90	35.1	36.57	29.85	33.90	28.50
HCRS	2020-21	26.04	28.42	48.57	52.61	32.53	35.17
HCRS	2021-22	21.40	21.15	32.33	26.75	23.8	26.5

[x]AITC10 = AITC + 10-min steam; steam10 = 10-min steam

[y]AITC30 = AITC + 30-min steam; Steam30 = 30-min steam

[z]AITC60 = AITC + 60-min steam; Steam60 = 60-min steam

Table 2.5: Average *Pythium* ppg g^{-1} soil at the Central crops Research Station (CCRS) and Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 strawberry seasons. Means (n=4 reps) followed by different lowercase letters within the same row, and within the same steam time, indicate significant differences ($\alpha \leq 0.05$) according to Fisher's least significant difference test. Means followed by different uppercase letters indicate significant differences ($\alpha \leq 0.10$) according to Fisher's least significant different test.

Location	**Season**	***Pythium* ppg g^{-1} soil[v]**								
		No Steam[w]			*10 Min Steam[x]*		*30 Min Steam[y]*		*60 Min Steam[z]*	
		NTC	*Pic-Clor 60*	*AITC*	*AITC10*	*Steam10*	*AITC30*	*Steam30*	*AITC60*	*Steam60*
CCRS	2020-21	2406	60	1213	60^{A}	2893^{B}	820	2714	20	3540
CCRS	2021-22	6067^{A}	427^{B}	527^{B}	0^{A}	2740^{B}	61^{a}	2700^{b}	67	1740
HCRS	2020-21	4740^{a}	853^{b}	1460^{b}	1413	2067	907	4044	6560	3607
HCRS	2021-22	8493^{a}	2907^{ab}	87^{b}	3787	5947	7	3333	2060	5580

[v]Data was analyzed using Log_{10} transformation, but data is displayed in its original value (ppg g^{-1} soil)

[w]NTC = Non-treated control; Pic-Clor 60 = chloropicrin +1,3-dichloropropene; AITC = allyl isothiocyanate

[x]AITC10 = AITC + 10-min steam; Steam10 = 10-min steam

[y]AITC30 = AITC + 30-min steam; Steam30 = 30-min steam

[z]AITC60 = AITC + 60-min steam; Steam60 = 60-min steam

Table 2.6: Average weed seeds germinated during a 2-month assay for the 2021-22 season at the Central Crops Research Station (CCRS) and the Horticultural Crops Research Station (HCRS). Means (n=4 reps) followed by different lowercase letters within the same row, and within the same steam time, indicate significant differences ($\alpha \leq 0.05$) according to Fisher's least significant difference test. Means followed by different uppercase letters indicate significant differences ($\alpha \leq 0.10$) according to Fishers least significant different test.

Location	**Weed Species**[v]	**Weed Seed Germination**								
		No Steam[w]			*10-Min Steam*[x]		*30-Min Steam*[y]		*60-Min Steam*[y]	
		NTC	*Pic-Clor*	*AITC*	*AITC*	*Steam*	*AITC*	*Steam*	*AITC*	*Steam*
CCRS	*Spergula arvensis*	2.8	0.0	0.8	0.0	1.8	0.0	0.8	0	0.3
	Cyperus esculentus	7.3^{A}	0.5^{B}	0.5^{B}	0.0^{b}	4.3^{a}	0.0	13.5	0	3.0
	Trifolium repens	2.5	1.8	1.8	2.5	3.0	1.5	2.3	1.8	2.8
	Portulaca amilis	0.8	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
	Lolium multiflorum	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
HCRS	*Spergula arvensis*	21.8^{a}	0.0^{b}	0.5^{b}	0.0^{b}	8.0^{a}	0.0	16.8	0.0^{b}	20.0^{a}
	Cyperus esculentus	37.3^{A}	7.0^{B}	17.0^{AB}	0.5^{b}	98.5^{a}	3.3^{b}	92.0^{a}	0.3^{b}	57.3^{a}
	Trifolium repens	14.5^{a}	0.0^{b}	1.0^{b}	0.0^{B}	12.8^{A}	0.0^{b}	20.8^{a}	0.0^{b}	20.3^{a}
	Portulaca amilis	4.25^{a}	0.5^{b}	0.5^{b}	0.0^{b}	5.3^{a}	0.0	4.5	0.0^{b}	7.8^{a}
	Lolium multiflorum	3.5^{a}	0.0^{b}	0.8^{b}	0.0	2.5	0.0^{B}	4.0^{A}	0.0	4.5

[v]*Spergula arvensis* = corn spurry; Cyp*erus esculentus* = yellow nutsedge; *Trifolium repens* = white clover; *Portulaca amilis* = Paraguayan purslane; *Lolium multiflorum* = annual ryegrass

[w]NTC = Non-treated control; Pic-Clor 60 = chloropicrin +1,3-dichloropropene; AITC = allyl isothiocyanate

[x]AITC10 = AITC + 10-min steam; steam10 = 10-min steam

[y]AITC30 = AITC + 30-min steam; Steam30 = 30-min steam

[z]AITC60 = AITC + 60-min steam; Steam60 = 60-min steam

Table 2.7: Average cumulative yield per plant at Central Crops Research Station (CCRS) and Horticultural Crops Research Station (HCRS) during the 2020-21 and 2021-22 seasons. Means (n=4) and standard error followed by different lowercase letters within the same row, and within the same steam time, indicate significant differences ($\alpha \leq 0.05$) according to Fisher's least significant difference test. Means and standard error followed by different uppercase letters indicate significant differences ($\alpha \leq 0.10$) according to Fishers least significant different test.

Location[v]	**Season**	**Cumulative Yield (g plant^{-1})**								
		No Steam[w]			*10 Min Steam*[x]		*30 Min Steam*[y]		*60 Min Steam*[z]	
		NTC	*Pic-Clor 60*	*AITC*	*AITC10*	*Steam10*	*AITC30*	*Steam30*	*AITC60*	*Steam60*
CCRS	2020-21	226^{B}	274^{AB}	377^{A}	350^{a}	202^{b}	353^{a}	226^{b}	360	286
CCRS	2021-22	513	586	532	536	486	542	478	588	558
HCRS	2020-21	599	723	636	641	539	603	685	617	632
HCRS	2021-22	649^{b}	788^{ab}	894^{a}	956^{a}	668^{b}	960^{a}	753^{b}	1001^{a}	771^{b}

[w]NTC = Non-treated control; Pic-Clor 60 = chloropicrin +1,3-dichloropropene; AITC = allyl isothiocyanate

[x]AITC10 = AITC + 10-min steam; steam10 = 10-min steam

[y]AITC30 = AITC + 30-min steam; Steam30 = 30-min steam

[z]AITC60 = AITC + 60-min steam; Steam60 = 60-min steam

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APPENDICES

Table 1.2: *Pythium* sp. ppg per g soil, divided by treatment, trial year, and distance from steam injection point.

Pythium sp. ppg[w] per gram soil					
Year	Treatment[x]	2.5cm[yz]	12.5cm	25cm	38cm
2021	NTC	5412±2619 a	10447±4072 a	9673±1187 a	3509±697a
	Steam	714±455 ab	1427±736 ab	4280±3044 ab	18967±15958a
	SP	4660±1731 a	6107±2776 a	4427±2691 ab	2547±1776a
	SPS	0±0 b	340±135 ab	4233±1302 ab	1907±769a
	QL	547±359 a	1040±760 ab	600±260 ab	767±266a
	QLS	7.4±6 b	41±25 b	800±185 b	772±633a
2022	NTC	1393±414 a	1600±653 a	1404±607	1747±521
	Steam	0±0 b	141±82 ab	1580±411	1580±250
	SP	147±77 ac	107±45 ab	650±348	1013±449
	SPS	87±70 bc	67±44 ab	300±247	793±224
	QL	647±153 a	247±130 ab	507±45	613±372
	QLS	0±0 b	0±0 b	203±55	567±198

[w]ppg = propagules of *Pythium* sp. per gram of soil

[x]Treatments: NTC = Non-treated control; Steam = 30 minute steam application; SP = Sodium peroxide (.1% w/w); SPS = Sodium peroxide (.1% w/w) + 30 minute steam application; QL = Quicklime (1% w/w); QLS = Quicklime (1% w/w) + 30 minute steam application

[y]Distance from steam injection point. For treatments which did not receive steam application, *Pythium* sp. ppg per gram of soil was still measured at the same locations.

[z]Means (n=4) followed by the same letter within a column are not significantly different ($p < 0.05$) according to Tukey's honest significant difference test.