STRAWBERRY GROWTH, YIELD, FRUIT NUTRITION, AND CONTROL OF
VERTICILLIUM WILT WITH PRE-PLANT SOIL FUMIGANTS,
OZONE, AND BIOLOGICAL CONTROL

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Justin J. Scurich

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COMMITTEE MEMBERSHIP

TITLE: Strawberry growth, yield, fruit nutrition, and control of Verticillium wilt with pre-plant soil fumigants, ozone, and biological control

AUTHOR: Justin J. Scurich

DATE SUBMITTED: April, 2012

COMMITTEE CHAIR: Dr. Terry Smith, Professor Earth and Soil Sciences

COMMITTEE MEMBER: Dr. Michael Yoshimura, Professor Biological Sciences

COMMITTEE MEMBER: Dr. Shaun Kelly, Professor BioResource and Agricultural Engineering

COMMITTEE MEMBER: Dr. Tom Ruehr (deceased), Professor Earth and Soil Sciences

ABSTRACT

Strawberry growth, yield, fruit nutrition, and control of Verticillium wilt with pre-plant soil fumigants, ozone, and biological control

Justin J. Scurich

Verticillium wilt is a widespread soilborne disease of strawberry historically controlled by soil fumigation with methyl bromide (MB). MB was banned by the United Nations in 1995 and will be completely phased out by 2015. Research has concentrated on alternative methods of disease control without finding a single alternative able to replace MB in widespread disease control and yield increase. For the current study, strawberries were greenhouse grown in container pots filled with soil from both infested and non-infested areas of a commercial strawberry field in Watsonville, CA. Treatments included pre-plant soil fumigation with commercially available formulations of methyl bromide, chloropicrin, and 1, 3-Dichloropropene. Additional treatments included ozone gas (six treatments) and biological control (three treatments). Collected data included total plant yield, individual berry weight, number of fruit produced per plant, plant vegetative weight, infection status, and mineral concentration of fruit (calcium, magnesium, potassium, iron, zinc, manganese, carbon, and nitrogen). Plants grown in 'clean' soil were less likely than plants grown in 'infested' soil to be infected with Verticillium. Plants grown in soil treated with MB had higher plant weight and yield than did non-treated control. Ozone and biological control treatments did not have statistically higher yield than non-treated control plants nor statistically lower yield than plants grown in soil treated with MB. Individual berry weights had a narrow range while the number of berries produced per treatment had a wide range. Data suggests strawberry yield is dependent on the number of berries produced per plant. Plants with high vegetative weight produced the highest yield suggesting large plants produce many berries resulting in higher yield.

Keywords: strawberry, fumigation, methyl bromide, chloropicrin, 1,3-D, Telone, InLine, ozone, biological control, mychorrhizae, *Bacillus* spp., rhizobacteria, fruit nutrition, Verticillium

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Introduction

Statement of Problem

Methyl bromide (MB) gained popularity as an agricultural fumigant in the 1960s. MB is biocidal providing excellent control of soil borne plant pathogens, nematodes, and weed seeds (Gullino et al., 2005). The fumigant essentially sterilizes soil before planting allowing crop plants to establish with reduced pest pressure. No single alternative fumigant, chemical, or other technology readily substitutes for MB in efficacy, low cost, ease of use, wide availability, worker safety, and environmental safety at ground level (Recamales et al., 2007). Commercially viable production systems (combinations of crops, variety, satisfactory yield, and rotations) evolved with the advantage of ‘sterilized’ soil provided by MB. Early application of MB was rudimentary compared to current standards designed to control and reduce emissions.

Widespread use and high emission percentage of applied MB created the downfall of the chemical. Nearly half of MB applied to agricultural soils was found to escape soil to the atmosphere. Structural and commodity fumigation require venting the vapors to the outside of the structure before reentry. Increasing concentration of bromine in the upper atmosphere was attributed to anthropogenic uses of MB (agricultural soil, stored commodity, and structural fumigation). A documented and naturally occurring global MB cycle exists between decomposing organic matter, ocean waters, and the stratosphere. The bromine ion is more active than any other halogen gas at destroying atmospheric ozone molecules (Hines et al., 1998).

The ozone destructing ability of methyl bromide was considered in 1995 at the Montreal Protocol on substances depleting stratospheric ozone. The United Nations

passed a worldwide treaty requiring developed nations to eliminate MB use by 2005 (2015 for developing nations) (Gullino et al., 2005).

Developed nations became concerned developing nations were placed in an advantageous position and the Critical Use Exemption (CUE) was created. Critical use exemptions must demonstrate no technically and economically feasible alternatives exist or significant market or regulatory disruptions would result without use of the fumigant (Martin, 2003).

Growers of strawberries (and other crops) in the United States file annual CUE petitions to use methyl bromide as a pre-plant soil fumigant (US EPA, 2008; 2011). Approximately one third of California strawberry growers used MB in 2009 (CSC, 2011a; DPR, 2011) accounting for 45 % of agriculturally applied MB in California in 2009 (DPR, 2011).

Extensive research has been dedicated to finding viable alternatives to MB. Gas impermeable plastic films and other application innovations have reduced MB application rates and nearly eliminated emissions (Gullino et al., 2005). Alternative methods of soil fumigation, fungicides, and biological control have been thoroughly studied for crops and respective diseases controlled by MB.

Overall Goal

- To control Verticillium Wilt of strawberry using pre-plant soil fumigants, ozone, and biological control.

Sub-goals tested

- Determine if treatments prevent plant infection
- Measure yield
- Measure plant growth
- Measure fruit nutrition

Importance

The California strawberry crop is an integral part of both the California and American agricultural economy. Strawberry growers spend approximately \$30,000 per acre in production costs (UCCE, 2006) directly injecting money into local economies via employees, supplies, and services. California strawberries are primarily grown in Watsonville / Salinas (41.2 %), Santa Maria (29 %), and Oxnard (24.1 %) (CSC, 2011a) creating agricultural communities dependent on successful strawberry production systems. California strawberries were grown on 35,915 acres in 2009 producing 88 % of United States strawberry crop. California strawberries generated \$316,394,000 in export revenues mainly from Canada, Mexico, and Japan (CSC, 2011b). One study estimated California strawberry growers would have 25 % reduced revenue after MB phase-out (attributed to increased disease and decreased yield) (Goodhue et al., 2005).

Verticillium Wilt is a widespread soil borne diseases of strawberry primarily controlled by pre-plant soil fumigation with MB. Alternative control methods for soil borne diseases have been studied and compared to systems utilizing MB. The economic and social value of the California strawberry crop warrants continuous study of production using alternative methods to soil fumigation with MB. The literature review and findings of the current study could be of benefit to strawberry producers and researchers in California and elsewhere.

General Approach

Pathogen infested soil was obtained and treated with fumigants, ozone gas, or biological control organisms. Strawberry plants were container grown in a greenhouse. Plants were harvested and measured. Growing practices were held constant among plants.

Scope

- Single growing season (November 26, 2008 – July 22, 2009)
- Conducted in greenhouse without climate control
- Measure yield
- Measure plant growth
- Analyze fruit
- Measure pathogen infection

Objectives

- Record individual berry weight and harvest date
- Measure vegetative height and weight
- Isolate pathogen from plants
- Measure fruit elemental concentration of C, N, Ca, Mg, K, Fe, Zn, Mn

Literature Review

Disease description: Verticillium Wilt

Verticillium spp. (Phylum: Ascomycota, Subphylum: Peziomycotina, Class: Sordariomycetes, Order: Phyllachorales, Genus: *Verticillium*) infect over 200 species of plants. *Verticillium* spp. are soil borne pathogens infecting plants through root tissues. *V. dahliae* and *V. albo-atrum* are the most agronomically important *Verticillium* spp. (Agrios, 2005). *V. dahliae* Kleb is causal agent of Verticillium wilt of strawberry (Wilhelm and Koch, 1956; Maas, 1998).

Mycelium is hyaline, simple or branched, septate and multinucleate. Conidia are ovoid to elongate and are produced on long phialides positioned in a whorl (spiral-like shape) around the conidiophores. Branching of conidiophores occurs in whorls at several levels (Fradin and Thomma, 2006).

V. dahliae produces microsclerotia while *V. albo-atrum* does not produce microsclerotia (Isaac, 1967). Microsclerotia and melanized mycelium are source inoculum and long-term survival structures. Large amounts of microsclerotia are produced in decomposing plant material capable of surviving 10–15 years without host plants (Wilhelm, 1955). The pathogen grows quickly within decomposing crop residue to produce microsclerotia (Gordon and Subbarao, 2008).

Each individual cell of microsclerotium can germinate giving one survival structure multiple germination opportunities (Fradin and Thomma, 2006). Mycostasis (resting stage of the fungus) is hypothesized to break when excessive carbon and nitrogen (from root exudates) is released into the rhizosphere (Mol et al., 1995). Germinating hyphae possibly grow toward high nutrient concentration (Fradin and Thomma, 2006;

Gordon and Subbarao, 2008). Exposure of microsclerotia to root exudates of tomato resulted in 75 % germination compared to 5 % in non-host soil (Schreiber and Green, 1969). Germinating hyphae growth is estimated to be within a 300 µm sphere of influence (Huisman, 1982). Germination of microsclerotia in non-amended soil was measured after 2 (45 %), 5 (50 %), and 9 (7 %) rewetting periods where soil was mock irrigated (Farley et al., 1971). Root exudates provide energy and nutrients to stimulate microsclerotia germination; limited germination occurs in soil without host plants (Heitefuss and Williams, 1976).

The general detection level of one to two microsclerotia per gram of soil is higher than the threshold level of disease occurrence in several crops (Harris and Yang, 1996; Grogan et al., 1979). Less than one microsclerotium per gram soil can cause disease in strawberry. Two microsclerotia per gram soil was found in plots with 50 % strawberry plant death (Harris and Yang, 1996).

Plant infection by *V. dahliae* is acropetal (from base to apex). Hyphae must penetrate the root endodermis acting as a physical barrier to infection. The pathogen infects plants at the root tip or at nodes of newly emerged lateral roots where the endodermis is not developed. Once past the endodermis, conidia are formed and released into the xylem. Conidia in the xylem become trapped in pit cavities, vessel end walls, or other trapping points (a place without turbulence) for germination. Hyphae from germinated conidia grow into adjacent vessel elements to produce conidia completing the first fungal life cycle (Bishop and Cooper, 1983). The fungus possibly suppresses host plant defense response initiated near trapping sites for several hours, providing time for the fungus to grow into adjacent vessels (Lee et al., 1992).

V. dahliae is found in agricultural soils throughout California. Infectious strains are capable of infecting artichoke, cotton, eggplant, mint, lettuce, potato, watermelon, tomato, and strawberry (Gordon and Subbarao, 2008).

Verticillium Wilt symptoms of strawberry could include wilting, chlorosis, stunting, necrosis, or vein clearing. Cross-sections of stem tissues could reveal brown discolored vascular tissue (Frandin and Thomma, 2006). Marginal and inter-veinal browning occurs on outer leaves first. Inner leaves are stunted but tend to remain green and turgid until plant death (Maas, 1998).

Hyphal growth of *Verticillium* spp. within xylem tissues reduces water flow creating water stress. Transpirational demand cannot be met when reduced water flow in the xylem occurs and plants wilt in response. If cavitation (breaking of the water column) occurs, the plant may suffer irreparable damage and may never recover (Misaghi, 1982).

Initial symptoms appear in late spring during periods of water stress (high temperature, high light intensity, low soil moisture). Symptoms tend to be most severe in fruiting plants. Individual plants could recover from wilting damage but may never regain economic productivity. Symptoms could appear on single plants or in clusters with healthy plants often appearing near infected plants (Maas, 1998). Late appearing symptoms could be indicative of low inoculum levels (Gordon and Subbarao, 2008). Strawberry roots have several flushes of root growth occurring throughout the season offering repeated opportunity for infection (Martin and Bull, 2002). Root system may develop extensively before growing into areas of soil containing microsclerotium.

Fumigants

Pre-plant soil fumigation was originally investigated for control of *V. dahliae* infection of strawberry (Wilhelm and Koch, 1956). Subsequent studies demonstrated control of weeds, lethal pathogens, and nematodes and a general growth and yield response appearing independent of specific host–pathogen interactions (Wilhelm et al., 1961, 1974). Pre-plant soil fumigation with MB:CP (67:33, 390 kg ha⁻¹) became standard practice in California strawberry fields in the 1970s for control of weeds, nematodes, and soilborne plant pathogens (Shaw and Larson, 1999). Chloropicrin and 1, 3-dichloropropene (1,3-D) are two alternatives to methyl bromide commonly used in production and research settings.

Soil fumigation improves plant production and quality in the absence of identifiable pests or pathogens (Larson and Shaw, 1995; 2000). Field plots without detected *V. dahliae* had 25–85 % reduced yield in non-fumigated compared to MB:CP fumigated plots. Yield decline was attributed to *Pythium* spp., *Rhizoctonia* spp., and *Cylindrocarpon* spp. (Martin and Bull, 2002). Soil fumigation suppresses sublethal and competitive microorganisms giving crop plants a competitive advantage (Wilhelm and Paulus, 1980).

Application methods

Fumigation can either be shank injected (total area) or drip irrigation applied (area specific). Shank applications are applied via tractor with chisel points set to a specified depth (generally 30–60 cm) (Ruzo, 2006). Pressurized canisters containing the fumigant are connected to a hose system ending on the back of the chisel points.

A secondary bar on the back of the tractor carries a plastic roll and soil implements. As the tractor passes, a 'shovel' implement on one side (outside of the application area) of the tractor cuts a trough and the plastic is tightly packed into the trough with pressure of a rubber tire from above. A disc blade behind the plastic roll pushes soil into the trough burying the plastic. The other side (inside of application area) of the plastic roll is glued to the plastic of the previous pass via pressurized rolling glue dispenser. Plastic films are chosen considering current regulation and price. Films are HDPE (high density polyethylene) or VIF (virtually impermeable film) and are supplied by the application company.

Drip fumigation involves injecting fumigant into drip irrigation system. Strawberries are generally grown on raised beds with 2 or more drip tape per bed (beds are covered with plastic film) allowing for selective fumigation of the planted bed. Fumigants are injected at rates approximately 0.2 % of system flow rate. Fumigant distribution is largely dependent on distribution uniformity of the irrigation system. Polyvinyl chloride (PVC) (common piping used in underground irrigation mainlines) can be exposed to fumigants for short periods of time without risk of degradation. Systems must be flushed after fumigant application. In general, drip applications are cheaper than shank injected applications (Ajwa and Trout, 2004).

Environmental fate of alternative fumigants

Chloropicrin degrades rapidly in sunlight and soil into environmentally benign products (EXTOXNET, 2001). A five-year study sampling California groundwater wells (1300 wells) found no incidents of chloropicrin contamination near applications. Three

groundwater wells in Florida (1500 wells sampled) were contaminated with CP (US EPA, 1992).

1, 3-D degrades into a multitude of compounds (1, 2-dichloroethane, vinyl chloride, ethylene glycol, 3-chloroallyl alcohols, and carbon dioxide). 1, 3-D has relatively high water solubility (Table 1) creating risk of groundwater contamination (Ruzo, 2006). Application amount per township (93 km²), buffer zones, and minimum application interval is strictly regulated in California to minimize human exposure to 1, 3-D (Duniway, 2002).

Methyl bromide and chloropicrin

CP is always added to MB in commercial settings; MB is never applied alone. CP was originally mixed with MB as a warning agent. CP (a lacrymator commonly known as tear gas) is extremely irritating to the skin and eyes. Applications vary in the percentage mixture (weight basis) of MB and CP and in the amount applied (MB is almost always shank applied in commercial settings). Ratios are commonly expressed as MB:CP indicating the percentage of each material. Common ratios include 98:2, 87:13, 80:20, 67:33, and 57:43. MB and CP act synergistically in destroying propagules of weed seeds, fungal pathogens, and plant parasitic nematodes (Hutchinson et al., 2000).

The mode of action of MB is attributed to the methylation of functional groups in proteins, peptides, and nucleic acids making the chemical biocidal (Becker et al., 1998). The mode of action of CP is less understood despite the observation MB and CP act synergistically (Hutchinson et al., 2000). Chloropicrin is generally regarded as more toxic to fungal pathogens than MB (Fennimore et al., 2008).

Combining MB and CP decreased dosage need 9.6 times compared to MB alone for destruction of *Fusarium oxysporum* (Hutchinson et al., 2000). Synergism mechanisms for MB and CP have variable explanations. One hypothesis suggests the first fungicide changes cell wall permeability increasing the absorption or decreasing the efflux of a second fungicide (Cohen and Levy, 1990). Another hypothesis suggests sub-lethal exposure of fungi to one fungicide may alter cell processes enough to make the second fungicide more effective (Samoucha and Gisi, 1987).

The primary mechanism of shank injected fumigant movement is vapor diffusion. The efficiency of vapor diffusion determines the amount of pest control achieved (Lembright, 1990). MB has high vapor pressure and water solubility (Table 1). MB will instantly convert into the gas phase after exiting pressurized canisters and begin to diffuse through soil. Soil moisture will slow the diffusion of MB (due to high water solubility). CP has low water solubility suggesting soil water content will not slow soil diffusion.

Table 1. Physiochemical properties of soil fumigants (Ruzo, 2006).

Fumigant	Boiling point (°C)	Water solubility (mg liter ⁻¹)	Vapor pressure (mm Hg)
Methyl bromide	3.6	13400	1600 (20 °C)
Chloropicrin	112	1.62	18.3 (20 °C)
1,3-D (<i>cis</i>)	104	2320	34.3 (25 °C)
1,3-D (<i>trans</i>)	113	2180	23.0 (25 °C)

Chloropicrin and 1, 3-D

1, 3-D has been used as a pre-plant fumigant to control plant parasitic nematodes and fungi since the 1950's (Ruzo, 2006). The biocidal activity of 1, 3-D and CP was increased when combined suggesting synergism. An optimal formulation of 61 % 1, 3-D and 33 % CP (essentially equal to InLine[®], Dow AgroSciences) was suggested (Stromberger et al., 2005). Combining 1, 3-D and CP may not achieve acceptable control of weeds (Duniway, 2002).

Chloropicrin and 1, 3-D are slightly soluble in water and will convert to the gas phase in soil at rates slower than MB (Table 1). Chloropicrin moves rapidly by diffusion within 30-cm of injection point and has been observed to diffuse to a depth of 120 cm in sandy soils (Ruzo, 2006). The half-life of 1, 3-D in soil has been reported to be between 2 and 17 days while the half-life of CP is between a 0.2 and 4.5 days (Ruzo, 2006). The limited diffusion distance and short half-life of 1, 3-D and CP in soil places importance on the application method (Ajwa and Trout, 2004).

Combinations of 1, 3-D and CP are applied as either broadcast shank injection or with drip irrigation water. InLine[®] is applied with drip irrigation water as an emulsified concentrate formulation. PicClor60 (TriCal Inc., Hollister, CA) (67 % CP, 33 % 1, 3-D) is applied as broadcast shank injection.

Shank fumigation can lead to rapid release of chemicals through soil cracks created by the shank passing through soil. Drip fumigation under standard HDPE films had more uniform distribution of 1, 3-D in soil than shank injected application in sandy loam soils. Greatest concentrations of 1, 3-D and CP in soil gas were found 24 to 36 hours after application and were below detection levels 14 days later when drip applied.

Uniform distribution of fumigants within a strawberry bed occurred 72 hours after application. Drip applied fumigants had more uniform distribution and less volatilization loss compared to shank injected fumigants (Ajwa and Trout, 2004).

Case studies

Mixtures of MB and CP control *V. dahliae* (the most difficult strawberry pathogen to control) (Wilhelm et al., 1961). Soil artificially inoculated with microsclerotia of *V. dahliae* was treated with CP injected 15 cm deep at 537 kg ha⁻¹. *Verticillium* wilt incidence decreased from 14.1 % in non-treated soil to 1.6 % in treated soil. Fungi from the genera *Trichoderma*, *Gliocladium*, *Melanospora*, *Stysanus*, *Chaetomium*, *Ceratostoma*, and *Graphium* survived all treatments (Wilhelm et al., 1961).

V. albo-atrum was approximately 11 times more susceptible to CP than MB when soil was 'moist' (45 % water by weight) and approximately 500 times more susceptible to CP than MB when soil was 'saturated' (150 % water by weight) (Munnecke et al., 1982). Moist and saturated conditions would occur during drip fumigation.

Soils were determined to contain 3–7 viable microsclerotia gram⁻¹ soil (*V. dahliae*). Soils were fumigated with MB:CP (67 % MB, 33 % CP; 420 kg ha⁻¹), CP (96 % CP, drip applied; 336 kg ha⁻¹), and InLine[®] (448 kg ha⁻¹). No viable microsclerotia were detected in all fumigated soils throughout the following growing season (Stromberger et al., 2005).

Sachets of *V. dahliae* inoculum (fungal structure not specified) were buried before soil fumigation. Soil was fumigated with MB:CP (57 % MB, 43 % CP; 448 kg ha⁻¹) and InLine[®] (3665 L ha⁻¹). MB:CP destroyed inoculum to 91 cm depth while InLine[®] did not control at any depth (Fennimore et al., 2008).

Soil containing 30–45 microsclerotia g⁻¹ soil was fumigated with increasing rates of InLine[®]. A rate of 1030 kg InLine[®] ha⁻¹ was required to kill 90 % of microsclerotia (*V. dahliae*) while 420 kg InLine[®] ha⁻¹ was required to kill 90 % of *Phytophthora cactorum* inoculum. InLine[®] fumigation at a rate of 380 kg ha⁻¹ destroyed over 90 % weed seeds and propagules of pathogens (*P. cactorum*, *F. oxysporum*, *P. ultimum*), with the exception of *V. dahliae* (Klose et al., 2007).

Case studies: comparing fumigants

Soils fumigated with MB:CP (67:33, 392 kg ha⁻¹), CP (336 kg ha⁻¹, with metam sodium added), and 1,3-D:CP (327 L ha⁻¹, percentage mixture not specified) was studied. Strawberry yield in all fumigant treatments was statistically equal and were nearly 5-fold greater than non-fumigated control soils (Gilreath et al., 2008).

Strawberry plantings in two areas of Spain were grown two consecutive seasons and pre-plant fumigated with MB:CP (50:50, 400 kg ha⁻¹), CP (300 kg ha⁻¹), and 1,3-D:CP (65:35, 300 kg ha⁻¹). Total fruit yield and average fruit weights were statistically equal in all fumigation treatments. Fumigated treatments produced approximately 33 % more fruit and 20 % larger fruit compared to non-fumigated control (Lopez-Aranda et al., 2009).

A minimum rate of 336 kg ha⁻¹ InLine[®] per treated bed area was required to achieve yield equivalent of 390 kg ha⁻¹ MB:CP (67:33) (Ajwa and Trout, 2004).

A review of research between 1987 and 1997 (45 studies) was compiled and data synthesized into generalizations. Strawberry yield was predicted to increase 59.2, 100.2, and 148.4 % when soil is fumigated with MB:CP after one, two, or three non-fumigated growing cycles. Strawberry yield was predicted to increase 9.6 % with MB:CP compared

to CP soil fumigation. Strawberry yield was predicted to increase 14.4 % with MB:CP compared to 1,3-D:CP soil fumigation (Shaw and Larson, 1999).

Ozone

Ozone (O₃) is commonly used to remediate hydrocarbon-contaminated soils and to sterilize drinking water (Shin et al., 2004). Direct application of ozone to soil is potentially useful for remediation after bioterrorist attacks to eradicate infectious microorganisms (Pawlat et al., 2010). Ozone gas could potentially be used as an agricultural soil fumigant. Ozone gas can be created *in situ* in soil by applying high voltage to dielectric barrier electrodes (Takayama et al., 2006). Ozone is relatively soluble in aqueous phase, suggesting ozone could be applied with drip irrigation water (Pawlat et al., 2010). Ozone created *in situ* could diffuse in the aqueous and gas phase through soils.

Ozone is a highly reactive and unstable molecule capable of oxidizing and quickly destroying insects, bacteria, and fungi (Ebihara et al., 2006). Oxidative species destroy, volatilize, and etch cells at the molecular level by breaking elemental bonds (Pawlat et al., 2010).

Ozone has a short half-life in soil (minutes) decomposing into diatomic oxygen. Onsite generation, low human toxicity, and low chemical persistence are benefits of ozone soil fumigation (Takayama et al., 2006).

High reactivity of ozone makes uniform soil distribution difficult to achieve. Ozone has a constant rate of self-decomposition. Soil ozone is ‘consumed’ by available soil organic matter (humus) and soil inorganic matter (mineral component of soil) (Shin et al., 2004).

Humus is comprised of extremely large organic molecules created from decomposing organic material (Alexander, 1977). Approximately 25–40% of soil

organic matter was hypothesized to be available for ozone consumption (Shin et al, 2004).

Ozone oxidation of humus produced small molecular fragments split from larger molecules. Humic substances can range from 10^3 to 10^5 g mol⁻¹ in size. Main molecules remained intact possibly due to the large number of double bonds on the internal structure of the molecules. Broken bonds of internal structures could decrease molecule stability. Small molecular fragments split from larger molecules were mostly alcoholic, carbonyl, carboxyl, and ester functional groups (Jansen et al., 2006).

Destruction of humus molecules and subsequent release of organic functional groups decreases soil pH and increases electrical conductivity. Soil pH decreased from approximately 6.0 to 5.5 during ozone exposure while soil electrical conductivity (EC) increased from approximately 50 to 70 mS m⁻¹ (Ebihara et al., 2006) consistent with findings of other researchers (Jansen et al., 2006; Pawlat et al., 2010). Creation and release of carboxylic and other organic acids explain decreasing soil pH during ozone exposure (Pawlat et al., 2010). Soil pH increased to equilibrium after soil ozonation while EC remained high (Ebihara et al., 2006). Oxidation of microbial cells and humus is synonymous with demineralization releasing unassimilated ions into the soil solution thereby increasing soil EC.

Little research has been focused on using ozone as an agricultural soil fumigant. Fungal pathogen *F. oxysporum* spores were fully destroyed by 0.3 g ozone per 50 g soil. Root nematode *Meloidogyne incognita* was destroyed to 50 mm depth when 45 g ozone m⁻³ was injected into soil for 20 minutes at a flow rate of 3 L min⁻¹ (Ebihara et al., 2006).

Irradiation with dielectric barrier discharge created radial ozone diffusion of 4 cm over 60 minutes (maintaining 1 g ozone cm⁻³) (Takayama et al., 2006).

Biological control

Biological control is the reduction of inoculum density or disease producing activities of a pathogen or parasite in the active or dormant state by one or more organisms. Biological control is accomplished naturally or through manipulation of the environment, host, antagonist, or by mass introduction of one or more antagonists (Cook and Baker, 1983). Modes of action of biological control agents can range from enzyme and antibiotic production, induction of plant defense response, and competition for nutrients. Current research fields of biological control for soil borne disease include (but are not limited to) mycorrhizal fungi and rhizobacteria. Successful biological control can be achieved by using a mixture of agents providing disease protection under a wider array of environmental conditions than would be achieved using a single agent (Baker and Cook, 1974). Combining biological control agents can become counterproductive when the introduced species compete with each other.

Mycorrhizal fungi

Mycorrhizae (root fungus) refers to a mutualistic symbiosis between fungus and plant. In general, mycorrhizae fungi obtain carbohydrate nutrition from the host while providing improved mineral absorption. Two types of mycorrhizal fungi exist: ectotrophic mycorrhizae and vesicular arbuscular mycorrhizae (VAM) (Bruehl, 1987).

Ectotrophic mycorrhizae form fungal sheaths several cells thick around roots. Roots are deformed and appear diseased. Within the mycorrhizal root are living epidermal and cortical cells surrounded by hyphae (termed Hartig net) possibly extending into the endodermis. Mutual growth is most prominent on the terminal meristem

(without secondary wall thickening). Ectotrophic mycorrhizae are able to defend plant roots from pathogen infection by physical exclusion (Bruehl, 1987).

VAM fungi do not deform roots. Vesicles (enlarged hyphae swellings) form intra- or intercellularly. Arbuscules (highly branched haustoria) form intracellularly. Vesicles and arbuscules are connected to hyphae outside the plant. No fungal sheath or mantle forms on roots. VAM (AM if no vesicles) fungi are not able to physically exclude pathogens (Bruehl, 1987).

Trichoderma spp.

Trichoderma spp. are opportunistic, avirulent plant symbionts functioning as parasites and antagonists of pathogenic fungi. *Trichoderma* spp. colonize the first few cell layers of root epidermis and root outer cortex trading nutrients for protection (ectotrophic mycorrhizae). *Trichoderma* spp. are able to mobilize and absorb mineral nutrients increasing plant health (Vinale et al., 2008). *Trichoderma* spp. produce and secrete organic acids decreasing soil pH potentially increasing plant mineral nutrition (Harman et al., 2004). Elicitors produced by the fungus activate expression of genes involved in plant defense response and promote growth of the plant root system. Increasing the plant root system provides increased resources for the fungus thereby increasing overall antagonism potential (Yedidia et al., 2003).

Metabolites isolated from *Trichoderma* spp. were produced during different growth stages suggesting multiple modes of action. Multiple biosynthetic pathways could lead to a range of antifungal agents produced through a range of environmental conditions allowing for greater adaptability of a biological control program (Dickinson et al., 1995). ‘Long distance’ activity of *Trichoderma* spp. is dominated by non-polar,

volatile compounds concentrating in soil and acting as antibiotics. 'Short distance' control by *Trichoderma* spp. is dominated by polar antibiotics and peptaibols acting in close proximity to producing hyphae (Vinale et al., 2008).

Three phases of antagonism by *Trichoderma* spp. exist. First, a volatile metabolite is produced and secreted and subsequently permeates through soil inhibiting the growth of other fungi ('long range'). Secondly, antibiotics to suppress fungal growth are secreted inhibiting organisms in close proximity ('short range'). Thirdly, *Trichoderma* spp. produce enzymes invading and destroying other organisms ('short range') (Dennis and Webster, 1971a,b).

Trichoderma polysporum and *Trichoderma sporulosum* produced the sesquiterpenoid trichothecenes and the antibiotic trichodermol. Harzianum A (an ester of trichodermol) is produced by *Trichoderma harzianum* and some strains of *T. harzianum* produce the diterpenoid harziandione (Dickinson et al., 1995).

A bioactive strain of *T. harzianum* (IMI 275950) produced a semi-volatile metabolite identified as 6-pentyl-2-pyrone active against *Rhizoctonia solani* and *Gaeumannomyces graminis* at 10^{-5} M. The chemical was sufficiently volatile to permeate through soil as a mild fumigant. *T. harzianum* (IMI 298371) produced harzianopyridone, an antifungal pyridine active against *Botrytis cinerea* and *R. solani* (Dickinson et al., 1995). *Trichoderma* spp. produce the antibiotic trichodermin (Ishikawa et al., 1976).

Active growth of *Trichoderma* spp. is associated with production of large quantities of extracellular β -(1,3)-glucanase and chitinase (Raziq and Fox, 2005). Chitinase and glucanase dissolve cell walls of fungal pathogens.

Case studies

T. harzianum, *V. dahliae*, and *R. solani* were grown on PDA (potato dextrose agar) medium in 15 cm petri plates singly and in combination. Plates were designed to have *V. dahliae* and *R. solani* 4.5 cm to the sides of *T. harzianum*. All fungi were deemed hydrophilic and had decreased growth rates when temperature was decreased from 25 to 15° C. *T. harzianum* grew 12 times faster than did *V. dahliae* at 25° C and high water availability. *T. harzianum* had an Index of Dominance (I_d) value of 4 (considered highly dominant) when growing with *V. dahliae* and *R. solani*. A mode of action for suppressed pathogen growth by *T. harzianum* was not identified (Santamarina and Rosello, 2006).

T. harzianum strains Th2 and Th1 were applied alone or in combination to strawberry plants for protection from *Armillaria* root rot. The Th1 strain did not provide protection while the Th2 strain provided 75 % protection. Combining strains Th2 and Th1 did not provide synergistic (or additive) protection (Raziq and Fox, 2005).

Trichoderma spp. applications and soil solarization were combined factorially in an experiment studying control of Anthracnose crown rot (causal agent *Colletotrichum* spp.). *T. harzianum* and *Trichoderma viride* were applied at 10^8 conidia m^{-2} through drip irrigation 7 days before planting (total application amount not specified). Strawberry transplant roots were dipped in a 10^6 conidia mL^{-1} solution prior to planting. *Trichoderma* spp. application increased *Trichoderma* spp. population 9.0 % (year 1), 403.1 % (year 2), and 277.8 % (year 3) compared to the non-inoculated control. *Trichoderma* spp. application increased year 2 yield 84.9 % compared to the non-inoculated control. Yield increases of 6.5 % (year 1) and 17.6 % (year 2) were recorded

but were not statistically different from the non-inoculated control. Combining soil solarization with application of *Trichoderma* spp. increased *Trichoderma* spp. population, root colonization, and yield suggesting a potential control measure for anthracnose crown rot. Non-inoculated–non-infested control plants did not exist (Porrás et al., 2007). Data suggests increasing *Trichoderma* spp. colonization increases yield.

Mycelium of *Trichoderma aureoviride* T122, *T. harzianum* T66 and T334, and *T. viride* T114 and T228 did not grow in soil extract medium with pH values greater than 7. Maximum mycelia growth rates occurred at pH 4; mycelia growth occurred from pH 2 to pH 6. Enzyme activity of cellulolytic β -glucosidase and cellobiohydrolase was optimal between pH 4 and 6. N-acetyl- β -glucosaminidase and the protease-like enzymes trypsin and chymotrypsin were operational through a wide range of pH values including pH values of 8 to 9 (Kredics et al., 2004). The species used were isolated from rhizosphere samples taken in the Ásotthalom forest in southern Hungary and were possibly adapted to low pH values found in forest soils. The enzyme stability and activity existing after mycelia growth cessation suggests potential to use *Trichoderma* spp. extracts in lieu of applying *Trichoderma* spp. into an environment less suitable for mycelia growth.

(Vesicular) arbuscular mycorrhizal fungi

Glomus spp. are widely used in research and production applications for increased nutrient and water absorption and for protection from plant pathogens. *Glomus* spp. infect host plants (but are not deemed parasitic) and are characterized as being vesicular arbuscular mycorrhize (VAM) or arbuscular mycorrhizal (AM) fungi.

Plant Growth Parameters

Strawberry seedlings inoculated with *Glomus intraradices* were approximately 50 % taller at 51 and 68 days after sowing compared to the non-inoculated control. *G. intraradices* inoculated plants had more leaves, larger leaf area, and more runners (stolons) than did non-mycorrhizal plants. Inoculating with 750 spores plant⁻¹ produced the highest total dry matter per plant. Plant survival ranged from 85 to 95 % and was not increased or decreased by mycorrhizal infection (de Silva et al., 1996).

Strawberry plants ('Senga sengana' cv.) inoculated with *Glomus etunicatum* (0.48 g) and *Glomus fasciculatum* (0.52 g) had higher shoot dry weight than did non-mycorrhizal control (0.28 g) plants (Gryndler et al., 2002).

Non-mycorrhizal plants tended to have better growth and productivity than did some mycorrhizal plants (using *Glomus macrocarpum*, *Glomus versiform*, and *Glomus* sp. CPH-23 in combinations with 'Aiko', 'Doublas', 'Pajaro', and 'Tioga' cvs.). Endophytes (AM and VAM fungi) can become parasites inhibiting growth of the host plant when fungal biomass is large or when conditions for photosynthesis are below optimal (Chavez and Ferrera-Cerrato, 1990).

Red raspberry plants (*Rubus idaeus* cv. 'Malling Autumn Bliss') inoculated with *Glomus mosseae* had lower total soluble solid concentrations in fruit (representative of sugar accumulation) than did non-mycorrhizal plants. *G. mosseae* and fruit could be viewed as photoassimilate (sugars and other hydrocarbons) sinks in competition with one another (Campos-Motá et al., 2004).

Strawberry runner (stolon; vegetative reproductive structure) production was reduced after inoculation with *G. intraradices* suggesting the export of carbon chains to

the fungus outweighed growth potential from increased nutrient and water absorption (Stewart et al., 2005).

Root dry weights decreased linearly as spore density of inocula was increased from 750 to 12,000 spores per plant. Increased hyphal colonization of roots (via higher inoculation rates) could have provided essential nutrients and water thereby decreasing need for root growth (de Silva et al., 1996). Contrarily, root dry weights (0.40 g) were higher in cucumber (*Cucumis sativus* L. cv. 'Aminex' ; F1 hybrid) inoculated with *G. intraradices* than in non-mycorrhizal plants (0.32 g). Cucumber plants inoculated with *G. intraradices* had lower shoot dry weight (3.33 g) than did non-mycorrhizal (3.55 g) plants (Green et al., 1999).

Winter wheat (*Triticum aestivum* cv. Tarso) seeds were infested with spores of *Microdochium nivale* (causal agent of pink snow mold) before sowing. Application of *G. mossea* to soil prior to sowing produced higher root dry weights than did applying *G. mosseae* + either *Paenibacillus brasilensis* PB177 or *Pseudomonas fluorescens* SBW25 suggesting increased root colonization (leading to decreased root growth) was achieved by combining *G. mosseae* with bacteria (Jäderlund et al., 2008).

Stomatal conductance, transpiration rate, and leaf water potential are often higher in mycorrhizal plants under drought conditions. Thus, mycorrhizal plants maintain higher rates of photosynthesis and higher water contents than do non-mycorrhizal plants. Increased ability to absorb water reduces damage from *V. dahliae* (Hernandez-Sebastia et al., 1999).

Relative water content of whole strawberry plants was 11 % higher in 'Kent' cv. plants inoculated with *G. intraradices* (91 ± 2.77 %) than in non-mycorrhizal plants ($80 \pm$

2.77 %) . Dry weights of roots, shoots, and the root : shoot ratio were not statistically different between plants inoculated with *G. intraradices* and non-mycorrhizal control plants (Hernandez–Sebastia et al., 1999). American Elm (*Ulmus Americana*) seedlings inoculated with *Glomus deserticola* and *V. dahliae* had higher leaf relative water content than did plants inoculated with *V. dahliae* suggesting mycorrhizal plants have increased hydraulic conductance capacity (Mushin and Zwiazek, 2002).

Plant disease parameters

Strawberry plants (‘Elsanta’ and ‘Rhapsody’ cvs.) inoculated with *G. fasciculatum* grew fifth order roots (branched 5 times) while non-mycorrhizal plants had no fifth order roots. A positive correlation existed between tertiary and higher order roots and disease occurrence for non-mycorrhizal plants while a negative correlation existed between tertiary and higher order roots and disease occurrence for *G. fasciculatum* and / or *G. etunicatum* colonized plants (Norman et al., 1996). Data suggests *Glomus* spp. provide better disease protection when more sites for colonization exist. Contrarily, mycorrhizal inoculation neither increased nor decreased root branching (Hernandez–Sebastia et al., 1999).

Root system necrosis was reduced approximately 60 % in strawberry (‘Cambridge Favourite’ cv.) when inoculated with either *G. fasciculatum* or *G. etunicatum*. Root system necrosis in strawberry (‘Elsanta’ cv.) was reduced by 30 % when colonized by *G. etunicatum* while *G. fasciculatum* provided no disease protection (Norman et al., 1996). Data suggests protection by *G. fasciculatum* or *G. etunicatum* is dependent upon the specific host–AM combination.

Strawberry plants ('Selva' cv.) inoculated with either *G. mosseae* or *G. intraradices* were later challenge inoculated with *V. dahliae*. Yield of mycorrhizal plants was higher than yield of non-mycorrhizal plants (94.7 g) after challenge inoculation with *V. dahliae*. No significant differences existed between plants inoculated with *G. mossae* / *G. intraradices* and either challenged (345 g) or not challenged (389.5 g) with *V. dahliae*. No significant differences existed between non-mycorrhizal–non-challenged plants (285.2 g) and mycorrhizal–non-challenged plants (389.5 g) suggesting *G. mossae* and / or *G. intraradices* did not reduce plant growth in absence of *V. dahliae* pressure (Tahmatsidou et al., 2006).

Pepper plants (*Capsicum annuum* Piquillo cv.) were inoculated with *Glomus desertica* and subsequently challenge inoculated with *V. dahliae* (3.6×10^7 conidia) during the vegetative growth stage. All non-mycorrhizal control plants displayed foliar disease symptoms within 3 weeks of challenge inoculation while 15 % of *G. desertica* inoculated plants displayed foliar disease symptoms three weeks after challenge inoculation (Idoia et al., 2004). Data suggests *G. desertica* reduces virulence of *V. dahliae*.

Fruit production

V. dahliae inoculated pepper plants produced axillary buds while non-*V. dahliae* plants did not produce axillary buds. Pepper plants inoculated with *G. desertica* did not have reduced fruit length and dry mass after being challenge inoculated with *V. dahliae* while non-mycorrhizal plants had reduced fruit length and dry mass. Inoculation with *G. deserticola* delayed pepper fruit maturation suggesting hormonal levels were different in mycorrhizal and non-mycorrhizal plants (Idoia et al., 2004). Increased cytokinin

concentration in mycorrhizal roots was observed (Danneberg et al., 1992). Red color development in pepper fruit is delayed by cytokinin production in roots (Agustí, 2000).

Red raspberry (*Rubus idaeus* ‘Malling Autumn Bliss’ cv.) was grown on a crushed volcanic soil substrate using hydroponic nutrient solution. Plants were inoculated with 300 spores of *G. mosseae*. Inoculation with *G. mosseae* increased total fruit count and count of small fruit per plant compared to non-mycorrhizal control plants (Campos–Motá et al., 2004). *G. mosseae* produced gibberellic acid in host roots (Jaen et al., 1997). Gibberellic acid (a plant hormone) promotes fruit set (more fruit but smaller size).

Nutrient absorption

Concentration of N, P, K, Ca, Mg, and Na were not significantly different in the leaves and roots of strawberry plants (‘Kent’ cv) inoculated with *G. intraradices* and non-inoculated control plants (Hernandez–Sebastia et al., 1999), in agreement with other researchers (de Silva et al., 1996). Strawberry plants (‘Chandler’, ‘Tudla’, and ‘Selva’ cvs) inoculated with *G. macrocarpum* absorbed 11 to 50 % more P than did non-mycorrhizal plants. Absorption of Cu and Zn (‘Chandler’, ‘Tudla’, and ‘Selva’ cvs.) and Mn (‘Tudla’ cv.) was slightly decreased by inoculation with *G. macrocarpum*. Boron and Fe absorption was slightly higher in *G. macrocarpum* inoculated plants compared to non-mycorrhizal control plants (Paraskevopoulou–Paroussi et al., 1997). Mycorrhizal induced differences in nutrient acquisition were slight and not likely relevant with exception to P absorption.

‘Tioga’ strawberry plants did not flower in non-mycorrhizal plants (data not given) while mycorrhizal plants did flower (*Glomus macrocarpum*, *G. versiform*, and

Glomus CPH-23 as inocula). Low P availability suppressed flowering of strawberry plants suggesting mycorrhizal inoculation increased P absorption (Chavez and Ferrera-Cerrato, 1990). Inoculation with *G. fasciculatum* increased P (1.73 mg P plant⁻¹) and K (9.02 mg K plant⁻¹) absorption compared to non-mycorrhizal control plants (0.92 mg P plant⁻¹ and 5.88 mg K plant⁻¹). No significant differences in P absorption existed in *G. etunicatum* inoculated and non-mycorrhizal control plants. Magnesium absorption was not statistically different between non-mycorrhizal plants (0.75 mg Mg plant⁻¹) and plants inoculated with either *G. fasciculatum* (0.93 mg Mg plant⁻¹) or *G. etunicatum* (0.70 mg Mg plant⁻¹) (Gryndler et al., 2002). Cucumber plants (*Cucumis sativus* L. cv. 'Aminex' ; F1 hybrid) infected with *G. intraradices* absorbed 5.4 times more ³³P than did non-mycorrhizal plants (Green et al., 1999).

Soil containing 498 mg P kg⁻¹ soil (Mehlich-3 extract) was used to grow strawberry plants with and without mycorrhizal inoculation. *G. intraradices* was applied singly or in a mixture with *G. mossea* and *G. etunicatum* (at least 1 viable AM propagule g⁻¹ potting mix). 'Chambly', 'Kent', and 'Sweet Charlie' cultivars were grown and colonization percentages ranged from 9 to 12 %. *G. intraradices* produced higher colonization rates of daughter plants at 7 and 14 weeks after transplanting than did the mixed inocula suggesting *G. intraradices* has a higher ability to resist high soil P concentrations (or is able to spread extraradically over longer distances than the mixed inocula species). Colonization levels of 25–27 % existed in previous studies with lower soil P concentration (Stewart et al., 2005). Strawberry plants ('Senga sengana' cv.) inoculated with *G. etunicatum* (15.6 %) and *G. fasciculatum* (12.2 %) became colonized

in soil with 55 mg available P kg⁻¹ soil using Olsen extracting solution (Gryndler et al., 2002).

Raspberry plants inoculated with *G. mosseae* (approximately 143 g plant⁻¹) had higher yield than did non-mycorrhizal plants (approximately 107 g plant⁻¹) when the nutrient solution was adjusted to 200 % more di-hydrogen phosphate than the standard solution (2 moles H₂PO₄⁻ m⁻³). *G. mosseae* could have been increasing P absorption resulting in higher production (Campos–Motá et al., 2004).

Interactions of *Trichoderma* spp. and VAM / AM

Trichoderma pseudokoningii strains 453, 511, 741A, 741B, and 2212 decreased the germination percentages of *G. mossae* spores when physically present and when exudates of *T. pseudokoningii* were applied. *T. pseudokoningii* strains 511, 741A, 741B, and 2212 decreased the percentage germination of *Gigispora rosea*. Volatile compounds produced by the *T. pseudokoningii* strains did not increase or decrease (statistically significant) germination percentages of *G. mossae* or *G. rosea* (Martinez et al., 2004). Data suggests short range activity of *Trichoderma* spp.

Shoot dry weight and percentage root length of soybean colonized with *G. mosseae* was decreased by *T. pseudokoningii* 2212 application compared to non-*T. pseudokoningii* inoculated plants (Martinez et al., 2004).

Application of *T. harzianum* reduced colonization of cucumber (*Cucumis sativus* L. cv. ‘Aminex’ ; F1 hybrid) by *G. intraradices*. *T. harzianum* reduced hyphal length density of *G. intraradices* when wheat bran (a carbohydrate source) was added to soil (Green et al., 1999) suggesting *T. harzianum* increases growth and antagonism when provided with a carbohydrate food source.

***Bacillus* spp.**

Bacillus spp. are soil dwelling spore forming bacteria (Alexander, 1977). Spores of *Bacillus* spp. are highly resistant to the dryness needed to formulate commercially stable products (Ongena and Jacques, 2007). *Bacillus* spp. are highly competitive bacteria capable of colonizing the rhizosphere thereby inhibiting the growth of plant pathogens.

Bacillus subtilis can differentiate into subpopulations of phenotypically distinct but genetically identical cells (bimodality). Subpopulations are specialized cells capable of producing and responding to separate signals serving different functions within the community. Subpopulation cells can sense one particular signal and discard the rest (Lopez and Kolter, 2010). Bimodality allows the bacterial community to perform necessary functions with minimal cost of energy (Kearns, 2008).

Bacillus spp. respond to natural products secreted by other soil dwelling organisms (Lopez and Kolter, 2010). Antimicrobial peptides produced by *Bacillus* spp. have been identified (subtilin, subtilosin A, iturin A, bacilysin, rhizocticin, and surfactin) (Benitez et al., 2010).

Bacillus spp. produce biosurfactins (lipopeptides) capable of disrupting and solubilizing protective lipid layers of cells. Biosurfactins increase motility of flagella driven *Bacillus* spp. spores allowing rapid root colonization. *Bacillus* spp. colonies produce 'biofilm' coating the colony (and root surface) providing physical exclusion from pathogens (Ongena and Jacques, 2007).

Subpopulations of *Bacillus* spp. create extracellular matrix material (protective covering essential for 'biofilm' formation to encase the entire community) in response to

release of surfactin (Chai et al., 2008). Another subpopulation (miners) produces and secretes proteases (in response to amino acid starvation) to degrade extracellular proteins into small peptides. Small peptides serve as food for the entire community (Veening et al., 2008). *B. subtilis* secreted approximately 20 volatile organic compounds with no known effect on plant growth (Kai et al., 2007).

Biosurfactins have strong emulsifying and foaming properties weakening or destroying cell walls of other fungi or bacteria. Secondary chemicals produced by *Bacillus* spp. are able to permeate weakened cell walls. *B. subtilis* and *B. amyloliquefaciens* produce iturins. Iturins are heptapeptides with strong antifungal properties for yeast and fungi and weak antibacterial properties (Ongena and Jacques, 2007).

B. subtilis was found to secrete plant growth stimulating molecules (2,3-butanediol and acetoin) (Kai et al., 2007). *B. subtilis* produces siderophores (metal chelates) to bind iron (and other metals) with high specificity and affinity potentially increasing plant metal nutrition or decreasing fungal pathogen nutrition (Yu et al., 2010).

Case Studies

Bacillus spp. are generally applied as pre-plant, root-dip inoculations, or soil drench applications. *Bacillus* spp. inoculations containing multiple species had increased plant growth and treatment efficacy compared to single species inoculations (Jiang et al., 2006) in agreement with a theory proposed by researchers using *Pseudomonas* spp. (Pierson and Weller, 1994).

Phytophthora capsici infection and disease of peppers was reduced approximately 80 to 85 % by *Bacillus* spp. inoculations compared to non-inoculated control.

Inoculating compost with *Bacillus* spp. before soil amending increased disease control approximately 8 % compared to soil drench method (Jiang et al., 2006).

B. subtilis reduced *F. oxysporum* infection and disease of pepper when plants were co-inoculated (44.4 %) and when soil was pre-plant inoculated with *B. subtilis* and plants later challenge inoculated with *F. oxysporum* (56.9 %). Inoculated plants produced fewer and bigger fruit. Yield increased approximately 50 % in *B. subtilis* treated plants compared to non-treated control (Yu et al., 2010). *F. oxysporum* infection of cucumber was slightly decreased by *B. subtilis* soil drench (Chung et al., 2008).

B. amyloliquefaciens (strain G-V1) reduced infection of strawberry by *P. cactorum* (32.7 %) and *P. fragariae* var. *fragariae* (37.6 %) compared to non-treated control. Transplants were dipped 15 minutes in bacterial solution (10^9 – 10^{11} colony forming units mL⁻¹) prior to planting (Anandhakumar and Zeller, 2008). *B. subtilis* reduced the amount of oospores on strawberry roots compared to non-treated control (Vestburg et al., 2004). *B. amyloliquefaciens* did not reduce in vitro petri dish growth of *V. albo-atrum* (Benitez et al., 2010).

Strawberry plant height and fresh weight had a tendency to increase (not significant) when transplants were dipped in *B. velezensis* prior to planting into soil infested with *F. oxysporum* (Mycong et al., 2009).

Plant nutrition

Lack of soil fumigation is a major component of organic strawberry production. Organic strawberry production is the only system currently adopted by growers not using pre-plant soil fumigation (Martin and Bull, 2002). Surveys indicate consumers purchase organic produce because of the belief organic foods have higher nutrition than conventionally grown food (Winter and Davis, 2006).

Nutritious foods are defined as having high nutrient density relative to energy content. No clear evidence exists organic foods are more nutritious than ‘conventional’ foods (Reganold et al., 2010). Genetics, climate, irrigation, cultural practices, and time of season were identified as factors determining strawberry fruit quality (Wold and Opstad, 2007; Hargreaves et al., 2008).

Strawberry fruit contain approximately 95 % water and 5 % dry matter. Dry matter is comprised of proteins, lipids, sugars, vitamins, and minerals (Recamales et al., 2007). Organic strawberries lost less water weight on ‘the shelf’ than did conventional berries. Slower dehydration (and rotting from *Botrytis cinerea*) was attributed to thicker cuticular and epidermal cell walls of the organic fruit. Organic fruit was 13.4 % smaller in size, but contained 8.3 % more dry matter content (Reganold et al., 2010).

Plant elemental concentrations (N, P, K, Ca, Mg) were not significantly different using variable fertilization and at different sample times in the growing season. No correlations existed between fruit, leaf, or sap elemental concentrations (Wold and Opstad, 2007). Fruit elemental content was not statistically different in organic or conventionally grown strawberry fruit and leaf elemental concentration did not predict fruit elemental content (Hargreaves et al., 2008).

Methods and Materials

Soil preparation

Soil was obtained in July, 2008 from the Redman Ranch in Watsonville, CA located at 36°53'43.13''N, 121°46'02.42''W. Strawberries being grown on the ranch displayed symptoms of Verticillium Wilt; plants within the fumigation buffer zone (area of a field within a defined distance of occupied structure that cannot be fumigated) were mostly dead from the disease (Figure. 1). Raised bed soil was taken from both inside and outside the fumigation buffer zone (minimum application distance from an occupied structure). 'Clean' soil samples were taken before 'infested' soil samples minimizing cross-contamination.

Figure 1. Fumigation buffer zone at sample site.

Soil was classified as Fine-loamy, mixed, superactive, thermic Pachic Haploxerolls named Conejo clay loam (30.2 % clay, 35.8 % sand, 40.0 % silt). The Conejo series consists of very deep and well drained soils formed in alluvium from basic igneous or sedimentary rocks. Conejo soils form on alluvial fans and stream terraces (NRCS, 2011).

Cultivation

The concrete floor of a warehouse building was swept, cleaned with a squeegee, and sterilized with bleach solution (0.6 % v / v NaOCl). Soil samples were spread on the concrete slab and spayed with shovels and rakes to break any clods to an approximate size of 10-cm diameter. Soil was allowed to dry, and was thoroughly mixed. ‘Clean’ soil samples were processed before ‘infested’ soil samples minimizing cross contamination. After tillage, soil was packed into appropriate containers for transfer. Soil samples (approximately 454 g) were taken and sent to A & L Western Agricultural Laboratories (Modesto, CA 95351) (Table 2).

Table 2. Soil chemical properties of sample site.

Soil property	Clean	Infested
Ca (cmol _c kg soil ⁻¹)	14.00	8.34
Mg (cmol _c kg soil ⁻¹)	5.69	3.82
K (cmol _c kg soil ⁻¹)	1.04	0.77
Na (cmol _c kg soil ⁻¹)	0.40	0.26
H (cmol _c kg soil ⁻¹)	0.70	0.20
CEC (cmol _c kg soil ⁻¹)	21.12	13.18
Zn (mg kg ⁻¹)	8.50	11.20
Mn (mg kg ⁻¹)	4.00	2.00
Fe (mg kg ⁻¹)	22.00	22.00
Cu (mg kg ⁻¹)	2.20	3.20
B (mg kg ⁻¹)	1.00	1.00
P† (mg kg ⁻¹)	74.00	78.00
NO ₃ ⁻ - N (mg kg ⁻¹)	51.00	45.00
SO ₄ ²⁻ - S (mg kg ⁻¹)	392.00	50.00
OM (%)	2.00	1.70
pH	6.80	6.90
Ca : Mg Ratio	2.46	2.18

† Olson sodium bicarbonate method used.

Pre-plant treatment application

Fumigation

Soil to be fumigated was placed into either ‘Ten gallon’ (38 dm³) or ‘Twenty gallon’ (76 dm³) steel drums with removable gasket sealed lids. All soil fumigation was conducted at TriCal Inc. facilities (8770 California 25, Hollister CA, 95023).

TriCon[®] 57:43 [57 % v/v methyl bromide (CH₃Br), 43% v/v chloropicrin (CCl₃NO₂)] (Appendix E) was applied at a rate of 392 kg ha⁻¹. PicClor60[®] (56.7 % v/v chloropicrin, 37.1 % v/v 1, 3-D) (Appendix E) was applied at a rate of 233.75 L ha⁻¹. InLine[®] (60.8 % 1, 3 – D, 33.3 % chloropicrin) (Appendix E) was applied at a rate of 168.3 L ha⁻¹.

Micro-fumigation apparatus was fabricated and calibrated to create desired rates. Steel drums were sealed after fumigant application for approximately 10 days. Lids were subsequently removed allowing the soil to vent for approximately 2 days. Soil was mixed to ensure homogenous fumigant distribution. All fumigation was conducted by Matt Gillis (laboratory technician, TriCal Inc.).

Ozone

Ozone was applied via two methods; all ozone application was outsourced. Ozone application was overseen by Dr. Shaun Kelly (California Polytechnic State University, San Luis Obispo, Department of BioResources and Agricultural Engineering).

In situ

Ozone gas can be created within soil air-pores via high-energy particle beams (irradiation) (Kelly, 2008). Soil samples were packaged into plastic bags and radiation

sensors placed in representative bags. Samples were taken to the Radiation Effects Facility (The Cyclotron Institute, Texas A & M University, College Station TX). An electron beam scanned the samples one, two, or three times within the radiation chamber. Each 'scan' produced 1.5kGy radiation. Thus, radiation dosage rates were 1.5 (1-scan), 3.0 (2-scan), and 4.5 (3-scan) kGy. Soil samples were homogenized before potting.

Injection

Small volumes of soil [approximately $.056 \text{ m}^3$ (two cubic feet)] were placed into soil columns. Ozone gas was externally generated and pumped through soil columns. Ozone concentration was measured at the inlet and outlet and throughout the middle of the soil columns. Estimated rates were 45, 112, and 897 kg ozone ha-15 cm^{-1} (Hurtado, 2009). Soil samples were homogenized before potting.

Planting and biological control application

Potting

Prior to potting the soil, bench tops were washed and sterilized with bleach solution (0.6 % v/v NaOCl). Fiber cloth was added to the bottom of each pot to contain soil. Soil was added to ‘One gallon’ (3.8 dm^3) plastic pots within 5 cm of the rim. A drip irrigation system was installed; a single drip emitter was added to each pot.

Planting

Strawberry (*Fragaria ananassa* Duchesne cv. ‘San Juan’) plants were planted November 26, 2008 in the Earth and Soil Sciences Department greenhouse (California Polytechnic University, San Luis Obispo, CA) located at $35^{\circ}18'35.41''$ N, $120^{\circ}39'48.42''$ W. Bare root transplants were planted 14 days after suggested varietal plant date for the Santa Maria, CA area. Planting date was determined by strawberry breeder Phil Stewart (Driscoll Strawberry Associates, Watsonville, CA). Matt Wilson (Driscoll Strawberry Associates, Santa Maria, CA) supplied a small research work crew for planting.

Biological control application

Mycormax[®] (22.0 g) (Appendix E) was added to each treated pot prior to planting. A small spade hole was made, powder poured into the hole, and the soil mixed. Strawberry plants were transplanted into the inoculated soil.

Promot[®] MZM (Appendix E) was made into a root dip solution (30.0 g Promot[®] MZM 3.8 L water^{-1}). Plants were submerged in inoculation solution prior to planting, simulating a field application method. A fertigation solution resulting in $0.1 \text{ mL Promot}^{\text{®}}$ MZM plant^{-1} was applied after planting.

BioStartTM RhizoBoost (*Bacillus* spp.) and BioStartTM Defender (*Bacillus* spp.) (Appendix E) were applied after planting. A fertigation solution containing 1.15 mL of both BioStartTM RhizoBoost and BioStartTM Defender per plant was applied five times at seven day interval beginning at planting.

Statistical design

A bench-top grid (30.5 cm x 30.5 cm) was made to simulate field spacing of strawberry plants. Bench tops of the greenhouse were oriented on a northwest – southeast axis. Tall trees to the west of the greenhouse created unequal shading.

Five blocks of 32 plants were created to account for sunlight and climatic variability within the greenhouse. Each end row of the bench had ‘buffer’ rows of non – experimental plants. Three rows of ‘buffer’ plants were added between ‘Block 3’ and ‘Block 4’ of each bench to accommodate irrigation pipes.

Each treatment was applied to plants in both ‘clean’ and ‘infested’ soil and appeared in each block once, resulting in five replicates. Rows contained either ‘Clean’ or ‘Infested’ soil placing a restriction on complete randomization.

Alphabetical letters were assigned to treatments, and numbers assigned to soil type. Treatment combinations were randomized throughout each block via random number generation (Minitab15, Minitab Inc[®]; State College, PA). Random number generation determined which soil type started a block and the order of blocks. Blocks were randomized until treatment combinations had equal distribution across the four columns of the bench top. Three fictitious treatments (‘Filler 1’, ‘Filler 2’, and ‘Filler 3’) were added to the treatment list to allow for permutation in multiples of four (Appendix A, B). Soil used for ‘Filler’ plants was either fumigated or from ‘Clean’ soil.

Growing the plants

Irrigation

A drip irrigation system was designed to apply water to each greenhouse bench in one irrigation set allowing for equal timing of irrigation. 'Tap' water (not chemically tested) was available for irrigation and deionized water was available for use during fertigation. Drip emitters were pressure compensated with 1.89 L hr^{-1} flow rate. Drip feeder tubes were cut to equal length. Distribution uniformity of the irrigation system was not assessed.

Soil would shrink and crack upon drying making subsequent irrigations less efficient (water running through the cracks). Frequent irrigations were required to meet plant water needs.

Irrigation frequency and duration was adjusted as plants grew and was determined by the author. One tensiometer was placed in each block (Soil Probe 2100F; Soilmoisture Equipment Inc., Goleta, CA). Tensiometers were installed in pots of 'Filler' plants.

Fertigation

Fertilizer was applied with drip irrigation water (Table 3, Appendix C). Equal fertilizer distribution was attempted by momentarily opening flush valves installed at mainline ends at the beginning of fertilizer injection. All fertilizer solutions contained approximately 10 % v/v humic acid (Liquid Humus[®], Acragro[®] LLC, Biola, CA) giving irrigation water a brown colored tint. Flush valves were closed when the tinted water appeared. A secondary utility of humic acid was to supply soil microbes with organic molecules for use as carbon and energy sources.

Fertilizer injection was measured from the time the flush valve was closed. The fertilizer pump intake hose was placed into a 2 L graduated cylinder containing fertilizer solution. In general, fertilizer injection was completed in less than three minutes.

Table 3. Applied nutrient amounts.

Nutrient	Rate	
	lbs acre ⁻¹	kg ha ⁻¹
Nitrate - N	106.33	119.18
Ammonium, urea, organic - N	56.06	62.83
Total N	162.39	182.01
P ₂ O ₅	86.64	97.11
K ₂ O	100.14	112.24
Ca	105.20	117.91

Cultural practices

Pest management

Powdery mildew, two-spotted spider mite, western flower thrip, whitefly, and aphid were managed with chemical pest control. Yellow sticky cards were added to help reduce aphid and whitefly pressure.

Pollination

Strawberries are self-pollinating needing a vector to move pollen (Chagnon et al., 1989). The greenhouse did not have adequate air flow for self-pollination. Bumble bees (*Bombus terrestris*) were used for pollination (Fisher and Pomeroy, 1989). A total of three hives (Koppert Biological Sciences US, Howell, MI) were placed in the greenhouse. One hive would remain viable approximately five weeks.

A high amount of flower abortion was observed (Figure 3). Bruising of flower tissues after bumble bee pollination has been observed (Higo et al., 2004). Excessive flower visitation was hypothesized to cause aborted flowers and supplemental pollen was

placed near the hive to reduce flower visitation. Hive population and rate of supplemental feeding were advised by Chrissie Davis (Koppert Biological Sciences).

Figure 2. Appearance of typical aborted flower.

Stolon removal

Strawberries tend to reproduce via vegetative stolons (runners). Runners were cut on an as needed basis; the amount of runners per plant was recorded for use as potential statistical covariate.

Plant data Collection

Harvest

Fruit was harvested on an as needed basis averaging 4.5-day intervals (Table 3). Berries were harvested when ripe (subjective). Ripe berries have red colored tips, and red / dark orange coloring below the calyx. Every harvested berry was weighed to the nearest 0.1 g accuracy (Mettler Toledo PM 34, Columbus, OH). Berries were frozen (temperature not recorded) for later processing. The first half of fruit (February–April) was stored in separate bags from the second half of fruit (May–July).

Table 4. Strawberry harvest dates for 2009.

Harvest	Date	Interval (days)	Harvest	Date	Interval (days)
1	31-Jan	*	21	9-May	4
2	4-Feb	4	22	15-May	6
3	10-Feb	6	23	20-May	5
4	13-Feb	3	24	27-May	7
5	18-Feb	5	25	29-May	2
6	21-Feb	3	26	3-Jun	5
7	22-Feb	1	27	5-Jun	2
8	26-Feb	4	28	10-Jun	5
9	4-Mar	6	29	15-Jun	5
10	5-Mar	1	30	18-Jun	3
11	12-Mar	7	31	23-Jun	5
12	18-Mar	6	32	29-Jun	6
13	27-Mar	9	33	1-Jul	2
14	31-Mar	4	34	4-Jul	3
15	8-Apr	8	35	7-Jul	3
16	16-Apr	8	36	9-Jul	2
17	24-Apr	8	37	12-Jul	3
18	29-Apr	5	38	18-Jul	6
19	1-May	2	39	22-Jul	4
20	5-May	4	Average		4.5

Vegetative measures

Plant production was ended July 22, 2009 (Table 3). Pots were brought inside for processing. Any dead and dried plant matter was removed to standardize the weights; plant handling was likely to break unequally sized pieces of dead and dried leaves. Plants were subsequently cut at soil level and weighed to the nearest 0.1 g accuracy (Mettler Toledo PM 34, Columbus, OH).

Immature fruit past the flower stage was removed and total weight and count of immature fruit were recorded for use as a potential statistical covariate.

Four full-length petiole samples per plant (leaf blade attached) were removed with razor blades and placed into plastic bags. Samples were stored in ice chests and processed within 24 hours.

Fruit elemental analysis

Sample preparation

All glassware and utensils were acid washed with 2.0 *M* HCl, rinsed three times with deionized water, and allowed to air dry before use.

Approximately 100.0 grams of berries per sample were oven dried approximately 56 hours at 83° C (Isotemp oven, Fisher–Scientific; Pittsburg, PA). Oven dry fruit were homogenized with mortar and pestle. Plants not producing 100 grams of fruit were processed in smaller amounts. Homogenized sample material was stored in plastic flip top vials.

Atomic absorption spectrophotometry (Ca, Mg, K, Fe, Zn, Mn)

Approximately 1000.0 milligrams homogenized dry fruit was weighed into ceramic crucibles using an analytical balance (Mettler Toledo, Columbus, OH). Crucibles were dry ashed in a muffle furnace (Isotemp programmable muffle furnace Model 14, Fisher–Scientific; Pittsburg, PA) for two hours at 200° C and increased for five hours at 550° C (Onyeike and Acheru, 2002).

Dry ash in crucibles was dissolved into 5.0 mL 2.0 *M* HCl. Samples were heated without boiling and stirred periodically with glass stirring rods for 15 minutes. Dissolved solution was passed through Whatman number 42 filter paper into 100 mL volumetric flasks (Class A) by gravity flow. Crucible, glass stirring rod, and filter paper were rinsed five times with deionized water into volumetric flasks. Volumetric flasks were brought to 100 mL total liquid volume with deionized water, covered with Parafilm[®] wax, and inverted five times to ensure homogenization. Analyte was diluted by pipetting 1000 μ L

solution into a new 100 mL volumetric flask and brought to volume with deionized water. Original and diluted samples were stored in plastic flip top vials.

Samples were analyzed for Ca, Mg, K, Fe, Mn, and Zn using a SpectrAA 55B Atomic Absorption Spectrometer (Varian Inc., Palo Alto, CA). Analyte intake tube was purged with deionized water between readings. Each data point is the mathematical mean of three consecutive measurements internally taken and calculated by the machine.

Carbon and Nitrogen by combustion method

Approximately 300.00 mg oven dry homogenized fruit was weighed into graphite crucibles for Carbon and Nitrogen analysis using an analytical balance (Mettler Toledo, Columbus, OH). Samples were combusted at 1150° C and the output measured for percentage concentration of C and N (Elementar vario MAX CNS, Elementar Analysensysteme GmbH; Hanau, Germany).

Quality Assurance and Quality Control

Quality control measures were implemented through each step of sample processing. Thresholds of 20 % error are acceptable for duplicate samples while 10 % error is acceptable for machine measurement error (Klesta and Bartz, 1996).

Calcium, Magnesium, Potassium, Iron, Manganese, and Zinc

Once fruit samples were dehydrated and homogenized, duplicate samples were prepared for elemental concentration analysis. One duplicate per ten samples was created (Klesta and Bartz, 1996). Each muffle furnace batch contained 24 crucibles including at least two duplicate pair. In general, percentage recovery of duplicate samples was within 20 % error (Table 4). Appropriate procedure would be to reanalyze muffle furnace

batches with greater than 20 % duplicate recovery error (Klesta and Bartz, 1996).

Reanalysis was not performed due to time and material constraints.

Table 5. Range of percent recovery of duplicate samples.

Ca	Mg	K	Fe	Mn	Zn
%					
87.19	72.91 †	89.19	66.93	75.21	62.49
93.71	84.38	95.21	80.23	78.65	78.96
93.97	87.40	95.26	81.27	87.62	79.60
95.83	88.86	96.84	88.12	87.97	86.80
96.11	89.23	97.24	88.38	88.38	90.08
96.79	90.11	97.41	89.55	92.04	90.17
97.46	92.47	97.73	93.28	93.55	91.39
98.38	93.64	97.88	93.40	95.16	93.49
98.47	93.66	98.58	97.51	95.26	94.91
99.15	94.74	98.66	98.74	95.36	96.03
99.19	95.43	98.66	99.13	95.79	96.25
99.51	95.51	99.10	99.25	95.81	96.89
99.69	97.80	99.21	99.59	96.41	98.75
99.99	99.22	99.29	100.22	99.94	100.00
100.13	100.07	99.44	100.41	100.45	100.42
100.18	101.16	99.44	100.61	101.51	101.11
100.41	101.92	99.72	100.98	102.43	101.88
100.55	102.03	99.75	101.11	103.66	102.50
101.05	103.04	99.89	103.79	103.99	104.12
101.66	103.17	100.87	104.80	104.40	105.10
101.94	104.47	101.08	106.17	105.27	107.24
102.47	107.03	101.60	106.60	105.33	108.03
103.92	107.09	101.68	107.35	107.06	111.28
106.76	110.36	101.85	108.37	107.58	112.41
107.30	112.03	102.82	108.63	110.21	114.35
107.83	112.15	103.01	110.69	110.97	114.44
107.97	114.41	105.54	110.93	113.67	116.63
109.36	114.53	107.83	115.54	114.84	119.03
109.72	118.92	108.68	117.78	117.10	126.97
129.00	118.95	109.15	123.24	122.02	147.39

† Denotes duplicate recovery error was greater than 20 %.

Instrumentation quality control was achieved through use of blank samples, continuing calibration verification (CCV), and lab control sample (LCS) analysis (Klesta and Bartz, 1996). Standard reference materials (SRM) were used to create solutions (Table 5) for initial machine calibration and to create CCV analyte. A second (external)

SRM was used to create LCS analyte. Additionally, instrument software automatically prompted recalibration every 50 samples.

Table 6. Concentration of initial calibration standards, continuing calibration verification, lab control sample, and blank sample analyte.

	Ca	Mg	K	Fe	Mn	Zn
	mg L ⁻¹					
1st	3.00	3.00	100.00	1.00	1.00	1.00
2nd	7.50	7.50	200.00	2.00	2.00	2.00
3rd	15.00	15.00	400.00	5.00	5.00	5.00
4th	30.00	30.00	n.d.†	10.00	10.00	10.00
CCV	7.50	7.50	200.00	2.00	2.00	2.00
LCS	10.00	10.00	100.00	2.00	2.00	2.00
Blank	0.00	0.00	0.00	0.00	0.00	0.00

† Three initial calibration standards were used for potassium

One CCV per 10 samples and one LCS and blank measurement per 15 samples were analyzed. Blank samples were created in the same matrix as sample analyte (5.0 mL 2.0 M HCl, 95.0 mL deionized water). When a CCV had greater than 10 % error, the machine was recalibrated and the samples analyzed after the previous CCV were reanalyzed. All CCV and LCS values were within 10 % error meaning machine measurements are sufficiently accurate (Appendix D) (Klesta and Bartz, 1996).

Carbon and Nitrogen

Instrument quality control was achieved through the use of initial calibration and CCV with a SRM. Initial calibration involved analyzing two blank, two SRM, and two glutamic acid samples as the first six samples per batch. The SRM used for both initial calibration and CCV contained 3.03 % N and 36.2 % C (SRM #391810 1573a Tomato leaves, U.S. Dept. Commerce National Institute of Standards and Technology, Gaithersburg, MD 20899). One CCV per 15 samples was performed. Any data with greater than 10 % CCV error were reanalyzed.

Continuing calibration verification percent recovery was 99.55 to 100.40 % for C and 95.71 to 99.11 % for N (Appendix D). All CCV results were within 5 % error and therefore the numbers are sufficiently accurate (Klesta and Bartz, 1996).

Pathogen isolation

Sanitation

Benchtop surfaces, utensils, and glassware were kept sterilized with 0.6 % NaOCl solution and utensils were sterilized between samples. All work was performed in close proximity to ignited Bunsen burners to minimize airborne contamination (the rising hot air minimizes particles settling onto equipment). Forceps and knives were secondarily sterilized with alcohol and fire between each sample.

Agar solutions were made with clean, autoclaved utensils. Solutions were poured into single use Petri dishes in a sterile room and in close proximity to a large ignited Bunsen burner. All Petri dish lids were kept closed as much as possible when adding plant material to dish. Dishes were sealed with Parafilm[®] wax after plant material was added.

Procedure creation

Ten strawberry plants displaying Verticillium Wilt symptoms were removed from the sample site (July, 2008) and maintained in the Biological Sciences greenhouse (California Polytechnic State University, San Luis Obispo, CA). Laboratory techniques were practiced prior to working with experimental plants.

Potato Dextrose Agar (PDA) with 100 $\mu\text{g mL}^{-1}$ streptomycin-sulfate is recommended media for *Verticillium* spp. isolation from plant tissue (Melouk, H.A. 1992). Hyphae grew rapidly on PDA and dark pigmentation of the media made viewing difficult. Half strength PDA plates (50 % v/v PDA, 50 % v/v water agar) (Appendix F) were made; growth was rapid and media pigmentation made viewing the fungus difficult. Fungal growth on water agar was slow and the media opaque making fungal structures

more visible. Fungal growth was generally visible within two to four days of plating petioles into water agar.

Hyphal tip transfers were made from water agar isolations to both PDA and V8 (Appendix F) juice petri dishes. Dishes were kept in light for approximately 7 days before being put into the dark. A plastic container was covered with aluminum foil to create a dark room. Microsclerotia were produced from both V8 juice agar and PDA agar confirming presence of *V. dahliae*.

Sample preparation

Petiole samples were used for pathogen isolation. Plants displaying Verticillium Wilt symptoms in the middle of the season were petiole sampled (one petiole per plant) prior to death. All surviving plants were petiole sampled as previously described and plated within 24 hours.

Petiole samples were cleaned of the leaf blade, trimmed at the base, and cut into 5-cm sections. Pieces were sterilized in 0.6 % NaOCl for two minutes. Petiole sections were laid onto paper towels and the terminal 3-mm removed and discarded. Remaining sections were cut into 1 to 2-cm pieces for plating (Melouk, 1992).

Plating

Approximately 10 petiole pieces were spaced evenly across a single Petri dish of water agar media. Petiole sections were inserted vertically into agar solution causing the least amount of disruption to agar surface. Plates were labeled and placed on a sterilized and papered benchtop with a single fluorescent tube light hung above the samples. Samples were allowed to grow approximately one week prior to identification.

Identification

Dissecting microscopes were used for viewing of samples. Hyphal growth was radial from the petiole sample. Fungal structures were easily visible on water agar. The production of microsclerotia confirmed *V. dahliae* was the pathogen present. Identification of *Verticillium* was subsequently done using conidiophores and conidia morphology (Melouk, 1992).

Non-experimental techniques

- Conidia transfers (using lab fabricated glass needles) were successfully made from isolates to PDA or V8 juice agar.
- *V. dahliae* was successfully isolated from strawberry petioles, leaf veins, and terminal pieces of leaf blades.

Statistical methods

All parameters were summarized using standard statistical measures of mean, standard deviation, frequency, minimum, and maximum. Logistic regression was used to analyze the effects of soil infestation and treatment on infection status. Soil infestation and treatment were included as main effects however the interaction of soil source and treatment (soil source*treatment) was not included, as sample size did not allow its estimation.

A linear mixed effects model was used to assess the effects of soil infestation and treatment on the measured parameters. Soil source, treatment, and soil source*treatment were included as fixed effects. Row and column location in the greenhouse were included to statistically control for possible systematic spatial differences in plant placement. Random effects were included for blocks and rows nested within blocks. Pairwise comparisons among treatment effects were performed using the Tukey-Kramer adjustment for multiple comparisons. Model fit was assessed using graphical analysis of residuals and a Shapiro-Wilk test for normality. Statistical significance was declared at the 0.05 level. All analyses were performed using SAS for Windows Version 9.3 (SAS Institute, Cary, NC). Statistical analysis was performed by Dr. Jerome Braun of the University of California, Davis.

Significant interactions involving row or column placement were ignored. Treatments were grouped into categories ('fumigant', 'organic', 'ozone') respective to method of treatment.

Data cleaning

Several outliers were identified and removed from statistical calculation (Table 7). Outlier data were removed from the respective data analysis and not any of the other parameters (For example, plant 93 was only removed from Fe analysis). Outlier data were removed from analysis because of suspected human error.

Plants 120, 134, and 150 had single sample metal concentrations approximately double the range of values seen for each respective metal suggesting a dilution mistake (mathematically or in practice) was made. Plant 93 had an iron concentration approximately four times higher the range of iron for all other samples. Additionally, zinc data was transformed using the natural logarithm before analysis.

Table 7. Outliers removed from analysis.

Plant ID	Treatment	Value as mg L ⁻¹
93	NTC	Fe=13.73
120	MB:CP	Mn=4.12
134	Ozone (L)	Ca=146.97
150	NTC	Ca=135.66

Assumptions

Key Factors and Variables

See Appendix A.

Independent key factors and variables

Soil properties

Irrigation drip tape was tied off in the diseased section of the field (The grower did not waste applied water or fertilizer on dead plants). A lack of fertilizer inputs to ‘Infested’ soil may have caused lower sulfate, calcium, and nitrate (Table 1) compared to ‘Clean’ soil. Ammonium sulfate and calcium nitrate are common water soluble fertilizer solutions used in strawberry production potentially explaining increased sulfur, calcium, and nitrate concentrations in ‘Clean’ soil. Innate soil sampling variability could have caused differences in reported chemical data.

Soil physical data was not tested for ‘Clean’ and ‘Infested’ soil samples. Soil physical properties could have been changed during sample removal, transportation, treatment application, and potting (compaction). Soil chemical data suggest soils may not have equal fertility (Table 1). Soil fertility is assumed to be equal.

Treatment applications

Fumigation and ozone applications were outsourced. Soil treated with fumigants and ozone was re-homogenized before potting. Individual plant and pot applications were made with measured quantities and hand applied. Treatment applications are assumed to be equally distributed.

Environment

Plants were spaced on a measured grid creating equal canopy exposure potential between plants. Buffer rows were placed on end rows to reduce outside canopy exposure. Buffer rows were not placed on end columns because the additional amount of required plants would have been burdensome. One half the plants were placed in a position with innately different sunlight and shade exposure than interior column plants could receive. Treatment combinations were randomized to ensure treatments were equally distributed across columns.

Sunlight exposure and shade were not equally distributed within the greenhouse. Blocks were created to account for variability caused by sunlight and shade. Temperature sensors were placed into each block for documentation.

Mid-season death of adjacent plants and variable canopy coverage of individual plants created heterogeneous environmental conditions for surrounding plants.

Treatment randomization, blocking, replication, and buffer rows are assumed to account for heterogeneous environmental exposure experienced by plants.

Cultural practices and limitations to plant growth

Initial planting mortality was assumed to be associated with transplant preparation, transportation, cold storage, and planting damage. Commercially, plant mortality is expected in low percentage and is generally not attributed to soil disease. Plant mortality and subsequent replanting is recognized as a key factor limiting plant growth. Planting depth and associated root damage were assumed to be equal.

Pest pressures tend to develop in clusters spreading outward. Plants were inspected frequently and pest populations quickly treated minimizing damage. All

strawberry plants were treated when applications were made. Pest pressure is recognized as a key factor limiting plant growth.

Bees hive populations and plant pollination need were difficult to match. Individual harvests removed as much as ten aborted fruit per plant. The amount of aborted flowers per plant was not recorded. Pollination is recognized as a major key factor limiting plant growth.

Stolons were removed when present. Removal of stolons is commonly practiced in field settings reducing internal plant competition for photoassimilates between fruit and vegetative structures. Stolon removal and subsequent plant response are recognized as a key factor to plant growth.

Irrigation amount and duration was equal. Applied water is assumed to be uniformly distributed to plants. Fertilizer applications were applied with irrigation water and assumed to be equally distributed to plants.

Plant nutritional health was visually monitored throughout the season. Calcium deficiency was observed and plants were subsequently treated (Ulrich et al., 1992). Calcium deficiency was attributed to high relative humidity (greenhouse humidity not documented) slowing water transpiration thereby reducing mass flow of nutrients into plant roots (Choi et al., 1999). Calcium fertilizer was applied both through irrigation water and by foliar application. Foliar applications were assumed to have uniform distribution and absorption. Plant nutrition is recognized as a key factor limiting plant growth.

Dependent key factors and variables

The quality of data is dependent on the precision and accuracy of data collection (Klesta and Bartz, 1996). Plants, fruit, and samples were treated in a non-biased manner. Consistency and care during measurement and sample taking were practiced. Quality assurance and quality control measures indicate relatively acceptable consistency in laboratory work. Human error throughout the experimental methodology is recognized as the most important key factor to accurate data collection.

Results

Disease presence

Soil was not analyzed for presence of *Verticillium* spp. microsclerotia, thus disease pressure cannot be mathematically represented. Disease presence can be demonstrated by observing non-treated control plants. Every non-treated control plant grown in ‘infested’ soil was infected with *Verticillium* spp. (Table 8). Three of the five non-treated control plants grown in ‘clean’ soil were infected with *Verticillium* spp. All treatments (except 3-scan) had reduced infection in ‘clean’ soil when compared to ‘infested’ soil source.

Table 8. Summary of total infected plants per treatment.

	Clean	Infested
MB:CP	0	3
InLine [®]	1	3
PicClor60	1	1
1-scan	0	2
2-scan	1	4
3-scan	2	1
Ozone (L)	1	2
Ozone (M)	1	4
Ozone (H)	0	3
<i>Bacillus</i> spp.	0	4
Mycormax [®]	2	4
Promote [®]	1	3
NTC	3	5
Sum	13	39

Odds of infection

The probability of *Verticillium* spp. infection was modeled using logistic regression. For each treatment category, plants grown in 'clean' soil had lower probability of infection than did plants grown in 'infested' soil (Tables 9, 10, and 11).

Table 9. Logistic regression analysis of infection status for 'fumigant' treatments.

Effect				Point Estimate	95 % Wald confidence limits	
Soil	C	vs. I		0.124	0.021	0.720
Treatment	InLine®	vs. NTC		0.107	0.011	1.080
Treatment	MB : CP	vs. NTC		0.062	0.006	0.689
Treatment	PicClor60	vs. NTC		0.033	0.003	0.428

Table 10. Logistic regression analysis of infection status for 'organic' treatments.

Effect				Point Estimate	95 % Wald confidence limits	
Soil	C	vs. I		0.051	0.009	0.281
Treatment	<i>Bacillus</i> spp.	vs. NTC		0.069	0.005	0.871
Treatment	MB : CP	vs. NTC		0.036	0.003	0.500
Treatment	Mycormax®	vs. NTC		0.258	0.024	2.730
Treatment	Promote®	vs. NTC		0.069	0.005	0.871

Table 11. Logistic regression analysis of infection status for 'ozone' treatments.

Effect				Point Estimate	95 % Wald confidence limits	
Soil	C	vs. I		0.109	0.033	0.362
Treatment	1-scan	vs. NTC		0.030	0.003	0.360
Treatment	2-scan	vs. NTC		0.174	0.019	1.620
Treatment	3-scan	vs. NTC		0.058	0.006	0.600
Treatment	MB : CP	vs. NTC		0.058	0.006	0.600
Treatment	Ozone (H)	vs. NTC		0.058	0.006	0.600
Treatment	Ozone (L)	vs. NTC		0.058	0.006	0.600
Treatment	Ozone (M)	vs. NTC		0.174	0.019	1.620

Most treatments reduced the odds of infection below the probability of a non-treated control plant (Tables 9, 10 and 11). Plants grown in soil treated with InLine® fumigant had equal odds of infection compared with plants grown in non-treated control

soil (Table 9). Plants inoculated with Mycormax[®] had equal odds of infection compared with plants grown in non-treated control soil (Table 10). Plants grown in ozone treated soil at 2-scan or Ozone (M) levels had equal odds of infection compared with plants grown in non-treated control soil (Table 11).

Yield

Yield refers to the total aggregate weight of harvested fruit from a given plant (Table 12). The average weight of individual berries (Table 15) and the average number of berries produced per plant per treatment (Table 16) describe the characteristics of harvested fruit. Averages are the mathematical mean of select data. In a commercial setting, berry size determines marketability and thereby marketable yield

Pairwise comparisons were made within each treatment category against the industry standard of MB:CP and non-treated control.

Table 12. Strawberry fruit yield (grams).

Treatment	Mean	SE
MB:CP	533.13	101.30
3-scan	520.33	116.24
Ozone (H)	514.96	108.68
PicChlor60	509.17	170.21
1-scan	504.71	64.76
Mycormax [®]	498.33	92.40
InLine [®]	489.74	120.55
2-scan	485.59	172.92
Promote [®]	442.39	158.03
Ozone (M)	438.14	89.86
Ozone (L)	428.49	72.78
<i>Bacillus</i> spp.	410.12	57.57
NTC	362.95	148.42

Though not significant, strawberry plants grown in soil treated with MB:CP tended ($p = 0.0633$, Appendix E) (Fig. 1) to have higher yield than did other fumigants and non-treated control plants.

Figure 3. Strawberry yield for fumigants and non-treated control (error bars represent standard deviation of the mean).

Strawberry plants grown in soil treated with MB:CP tended to have higher average yield than did ‘organic’ treated plants (Table 13). A significant interaction between treatments existed ($p = 0.05$) however, pairwise comparisons were not conclusive (Table 13). Plants grown in soil treated with MB:CP yielded approximately 170 grams (approximately 50 %) more fruit than did non-treated control plants (Table 13). Organic treatments tended to yield more fruit than did non-treated control plants (not statistically significant) (Table 13).

Table 13. Pairwise comparisons of yield using Tukey-Kramer (grams).

Treatment	Estimate	Standard error	Letter Group
MB : CP	533.17	33.11	a
Mycormax [®]	498.58	33.14	ab
Promote [®]	443.71	33.10	ab
<i>Bacillus</i> spp.	411.44	33.10	ab
NTC	362.02	33.27	b

Plants grown in ozone treated soil did not have statistically different yields from plants grown in soil treated with MB:CP or non-treated control. A significant interaction between treatments existed ($p = 0.02$, Appendix E) however pairwise comparisons were not conclusive. Again, plants grown in soil fumigated with MB:CP had higher yield than did non-treated control plants (Table 14).

Table 14. Pairwise comparisons of yield using Tukey-Kramer (grams).

Treatment	Estimate	Standard error	Letter Group
MB : CP	534.95	39.55	a
3-scan	518.64	39.55	ab
Ozone (H)	517.39	39.57	ab
1-scan	505.63	39.54	ab
2-scan	484.04	39.61	ab
Ozone (M)	446.01	41.58	ab
Ozone (L)	431.32	39.64	ab
NTC	358.79	39.65	b

Fruit characteristics

Individual strawberries had a relatively narrow range of average weights among treatments (12.94–14.87 g) suggesting yield was dependent on the number of berries produced (Table 15). MB:CP, 3-scan, and Ozone (H) were the three highest yielding treatments also having the three highest total fruit counts (Tables 9 and 10).

Treatments had a wide range of average number of fruit produced per plant (Table 16). Plants treated with MB:CP (41.86 ± 3.28) had more berries than did non-treated control plants (24.35 ± 3.28) ($p = 0.04$, Appendix E, F). Recall MB:CP plants (533.17 ± 33.11) had higher yield than did non-treated control plants (362.02 ± 33.27) (Tables 12, 13, and 14, Appendix E, F). Plants producing a high number of berries had high yield.

Table 15. Strawberry fruit weight (grams).

Treatment	Mean	SE
Promote [®]	14.87	2.28
Ozone (L)	14.59	1.57
PicChlor60	14.03	1.86
2-scan	13.94	1.67
InLine [®]	13.91	2.16
Mycormax [®]	13.66	1.36
1-scan	13.66	1.89
NTC	13.63	2.66
Ozone (M)	13.55	1.53
3-scan	13.38	1.73
Ozone (H)	13.35	2.43
<i>Bacillus</i> spp.	13.24	2.04
MB:CP	12.94	1.34

Table 16. Strawberry fruit count per plant.

Treatment	Mean	SE
MB:CP	41.60	8.07
Ozone (H)	39.10	7.28
3-scan	38.70	5.72
1-scan	37.90	9.10
Mycormax [®]	36.50	6.55
PicChlor60	36.50	12.44
InLine [®]	35.50	7.96
2-scan	34.50	10.97
Ozone (M)	32.78	7.45
<i>Bacillus</i> spp.	31.60	6.67
Ozone (L)	29.40	4.17
Promote [®]	29.40	8.76
NTC	25.60	10.62

Fruit nutrition

Significant interactions existed for calcium, magnesium, manganese, and zinc concentrations (Appendix E). Table 17 displays a summary of fruit nutrition concentrations for all treatments.

Table 17. Average fruit nutrient concentrations for treatments (fresh weight basis).

	N	C	Ca	Mg	K	Fe	Mn	Zn
	%		mg kg ⁻¹					
MB:CP	1.34	43.13	73.79	111.08	1522.25	4.05	2.23	1.37
InLine®	1.26	42.84	77.02	108.90	1485.70	3.58	1.73	1.70
PicChlor60	1.40	43.61	68.61	105.35	1562.47	3.78	1.98	1.34
1-scan	1.37	43.40	79.89	113.71	1578.23	3.75	2.11	1.24
2-scan	1.42	42.93	75.64	110.92	1584.25	3.86	2.22	1.44
3-scan	1.41	43.53	79.06	111.94	1585.62	3.96	2.27	1.81
Ozone (L)	1.27	42.72	77.33	118.26	1547.79	3.74	1.92	1.33
Ozone (M)	1.30	42.93	82.11	120.10	1546.60	4.16	1.88	1.87
Ozone (H)	1.36	43.30	76.71	118.00	1595.38	3.55	1.74	1.75
<i>Bacillus</i> spp.	1.31	42.97	77.37	110.86	1527.27	3.68	1.74	1.30
Mycormax®	1.32	43.43	80.38	122.20	1562.21	3.72	1.87	1.26
Promote®	1.28	43.03	80.69	120.77	1550.53	3.90	1.94	1.99
NTC	1.38	43.81	73.94	119.60	1590.47	3.66	1.89	2.08
Minimum	1.26	42.72	68.61	105.35	1485.70	3.55	1.73	1.24
Maximum	1.42	43.81	82.11	122.20	1595.38	4.16	2.27	2.08
Fumigant avg.	1.33	43.19	73.14	108.44	1523.47	3.80	1.98	1.47
Ozone avg.	1.35	43.13	78.45	115.49	1572.98	3.84	2.02	1.57
Organic avg.	1.30	43.14	79.48	117.94	1546.67	3.77	1.85	1.52
Avg.	1.34	43.20	77.12	114.74	1556.83	3.80	1.96	1.58

Calcium

A significant soil*treatment interaction existed for fumigant Calcium concentrations ($p = 0.02$; Appendix E). None of the pairwise comparisons were significant (Table 18).

Table 18. Pairwise comparisons of fruit calcium (mg kg^{-1}) using Tukey-Kramer.

Soil	Treatment	Estimate	Standard error	Letter Group
Infested	MB : CP	206.97	17.04	a
Clean	PicClor60	203.25	17.06	a
Infested	PicClor60	203.19	17.01	a
Clean	MB : CP	196.04	17.17	a
Clean	InLine®	170.65	17.03	a
Infested	InLine®	152.14	17.02	a
Infested	NTC	148.42	26.58	a
Clean	NTC	145.23	19.02	a

Magnesium

Plants grown in fumigated ‘clean’ soil (105.1 ± 4.15) had lower fruit magnesium concentration than did plants grown in fumigated ‘infested’ soil (117.36 ± 4.15) ($p = 0.03$; Appendix E).

Manganese

Plants grown in soil fumigated with MB:CP had higher fruit manganese concentrations than did plants grown in soil fumigated with InLine® (Table 19).

Table 19. Pairwise comparisons of fruit manganese (mg kg^{-1}) using Tukey-Kramer.

Treatment	Estimate	Standard error	Letter Group
MB : CP	2.22	0.08	a
PicClor60	1.98	0.07	ab
NTC	1.89	0.07	ab
InLine®	1.73	0.07	b

A significant treatment interaction ($p = 0.0002$; Appendix E) existed for fruit manganese concentration of ‘ozone’ treated plants. A wide range of fruit manganese

concentrations existed; however none of the pairwise comparisons were significant (Table 20).

Table 20. Pairwise comparisons of fruit manganese (mg kg⁻¹) using Tukey-Kramer.

Treatment	Estimate	Standard error	Letter Group
NTC	0.63	0.12	a
Ozone (M)	0.52	0.12	a
Ozone (H)	0.47	0.11	a
3-scan	0.40	0.11	a
2-scan	0.34	0.11	a
MB : CP	0.30	0.11	a
Ozone (L)	0.28	0.12	a
1-scan	0.19	0.11	a

Zinc

Plants grown in ‘clean’ soil (0.29 ± 0.06) had lower fruit zinc concentrations ($p = 0.02$; Appendix F) than did fruit from plants grown in ‘infested’ soil (0.50 ± 0.06). A significant treatment interaction ($p = 0.05$; Appendix E) existed for fruit zinc concentration of ‘organic’ treated plants. A wide range of fruit zinc concentrations existed; however none of the pairwise comparisons were significant (Table 21).

Table 21. Pairwise comparisons of fruit zinc (mg kg⁻¹) using Tukey-Kramer.

Treatment	Estimate	Standard error	Letter Group
NTC	0.63	0.11	a
Promote [®]	0.59	0.11	a
MB : CP	0.31	0.11	a
<i>Bacillus</i> spp.	0.24	0.11	a
Mycormax [®]	0.22	0.11	a

Plant Growth

Treatments with high plant vegetative weight tended to also have high yield (Tables 12 and 22).

Table 22. Strawberry plant weight (grams).

Treatment	Mean	SE
PicChlor60	203.38	43.62
MB:CP	202.66	35.62
2-scan	194.82	43.34
3-scan	192.46	44.59
Mycormax [®]	180.91	36.59
1-scan	180.09	18.66
Promote [®]	170.66	34.93
Ozone (M)	169.34	28.47
<i>Bacillus</i> spp.	167.32	26.16
Ozone (L)	166.05	33.93
Ozone (H)	164.44	26.27
InLine [®]	161.59	46.00
NTC	145.72	22.51

Plants grown with MB:CP (201.50 ± 12.66) had higher vegetative weight than did plants grown with InLine[®] (161.40 ± 12.64) ($p = 0.02$, Appendix E, F). Plants grown with MB : CP (203.06 ± 11.41), 2-scan (194.82 ± 11.44), and 3-scan (193.39 ± 11.42) had significantly higher vegetative weight than did non-treated control plants (143.25 ± 14.31) (p -value 0.003, Appendix E, F).

MB:CP, PicClor 60, and 3-scan were in three of the highest four treatments for both strawberry plant weight and yield suggesting a larger plant produces higher yield than does a smaller plant (Tables 12 and 22).

Discussion

Conditions

A minimum level of disease pressure was needed to evaluate disease control of the treatments. Disease pressure was not as simple as soil with or without *Verticillium* spp. inoculum. Disease pressure was demonstrated in the infection rates of NTC plants (Table 14). Every non-treated control plant grown in 'infested' soil was infected with *V. dahliae* while three of five plants grown in 'clean' soil were infected (Table 14). Plants grown in 'clean' soil had lower probability of being infected than did plants grown in 'infested' soil (Tables 15,16, and 17). Thus, two levels of infectious *Verticillium* spp. pressure were demonstrated to exist.

Many plants were infected with *V. dahliae* yet able to produce fruit. The humid conditions of the greenhouse were hypothesized to keep evapotranspiration rates low thereby relieving potential water stress. Water stress (due to xylem tissue plugged with *V. dahliae*) is the ultimate cause of death for plants infected with *V. dahliae*. The pathogen was present and able to infect the plants; however high humidity did not allow the plants to become stressed and die. *V. dahliae* was isolated from leaf blade material suggesting the entire vascular system of infected plants could be filled with the pathogen. Growing the plants in a greenhouse with more ventilation could have resulted in more plants perishing due to *V. dahliae* infection. The ability of the plants to survive and produce fruit despite being infected with *V. dahliae* suggests strawberries grown in humid coastal regions could tolerate *V. dahliae* infection.

Yield

Yield values from Table 12 and Appendix F are not congruent with yield values from Tables 13 and 14. Table 12 and Appendix F values are the mathematical mean of the treatment samples. The values of Table 13 and 14 are Tukey-Kramer method estimates of the mean for the given sample populations. Estimates are a product of the mathematical mean and standard error for the given sample population. When the sample population is different from one table to another (comparing MB:CP and NTC to either of the three categories of treatment) the standard error for the sample population will change and therefore estimates will not be congruent with mathematical mean of a given treatment value.

In general, yield seemed to be dependent on the number of fruit produced per plant. Individual strawberry weights had a narrow range suggesting a strawberry has a limited size potential regardless of plant health. Plants with the highest yields had the highest fruit counts. Secondly, larger strawberry plants tended to produce more fruit than did smaller plants. Thus, yield was dependent on having a large vigorous plant able to produce more fruit than a smaller plant.

MB:CP treated plants had significantly higher yield than did NTC plants (Table 13). The alternative fumigants PicClor60[®] and InLine[®] tended to have lower yield than MB : CP but the difference was not statistically significant (Appendix E). One could ignore tendencies and declare alternative fumigants were an acceptable form of control because yields were not lower than the industry standard of MB:CP.

Organic treatments tended to increase yield of strawberry plants compared to NTC (Table 13). Though 'organic' treatments did not statistically increase yield compared to NTC one could suggest the treatments have potential value. Organic treatments did not have statistically lower yield than did plants grown with MB:CP

(Table 13). One could ignore the tendencies and declare ‘organic’ treatments had statistically equal yields compared to the industry standard of MB:CP suggesting the treatments are viable alternatives to MB:CP.

Ozone treatments tended to increase yield of strawberry plants compared to NTC (Table 14). Though ‘ozone’ treatments did not statistically increase yield compared to NTC one could suggest the treatments have potential value. ‘Ozone’ treatments did not have statistically lower yield than did plants grown with MB:CP (Table 14). One could ignore the tendencies and declare ‘ozone’ treatments had statistically equal yields compared to the industry standard of MB:CP suggesting the treatments are viable alternatives to MB:CP. The high rates of ozone (both application methods) tended to produce higher yield than did the lower rates (Table 14). Further investigation of rates would be suggested.

Fruit nutrition

Many studies have searched for a nutritional difference between ‘organic’ and ‘conventional’ produce. For this study strawberry fruit did not seem to have a wide range of values suggesting fruit was nutritionally equal (for the measured parameters). The differences in nutrient concentration between treatments can be from a wide array of independent and dependent factors. Instead of focusing on the small differences as caused by treatments, data can be viewed as general strawberry nutritional value. Additionally, the data can be used to estimate the amount of mineral nutrients removed from a field with a strawberry crop. Table 23 provides strawberry nutrient values of previous studies.

Table 23. Mineral concentration of strawberry fruit.

Author	K	Ca	Mg	Fe	Mn	Zn
	g kg ⁻¹			mg kg ⁻¹		
Hakala et al., 2003	2.09	189	161	3.20	3.20	1.20
Tahvonen, 1993	2.00	200	160	4.10	4.60	1.40
Jorhem and Sundström, 1993	n.d.	n.d.	n.d.	n.d.	2.70	0.85
Capar and Cunningham, 2000	1.51	149	123	4.10	3.60	1.00
Kahn et al., 2010	1.63	100	144	10.14	3.39	1.46
Recamales et al., 2007	0.94	250	160	4.58	3.31	2.60
Zhie and Zhao, 2006	1.66	190	150	40.00	78.00	16.30
Hegedus et al., 2008	1.43	222	133	5.40	3.20	1.00
Reganold et al., 2010	15.00	1200	1300	n.d.	n.d.	9.95
Reganold et al., 2010	16.50	1320	1340	n.d.	n.d.	9.96
USDA–ARS, 2010	0.86	130	80	4.90	2.00	0.90

Conclusion

Many factors capable of influencing the measured parameters existed. Sample size and method, planting design, and statistical procedures are assumed to account for innate variability. Laboratory methods were in place to ensure accurate data was collected. All collected data was assumed to be unbiased and accurate. The treatments of this study are representative of a wide range of production practices for strawberry crops and can therefore be viewed as representative of strawberry production in general.

A grower considering the use of pre-plant treatments for disease control would first decide which types of control options are available and what diseases are targeted. The organic grower cannot fumigate soil but a conventional grower can.

Organic strawberry growers are able to use products similar to those used in this experiment. Though no particular treatment increased yield compared to NTC a potential value existed as the treatments did not have statistically lower yield than MB:CP. Combinations of the organic treatments or a different set of growing conditions could have better disease control and yield warranting further investigation.

MB : CP is the best option for widespread disease control. Neither PicClor60[®] nor InLine[®] have been touted to directly compete with MB : CP in widespread efficacy however both were proven as potentially viable in terms of yield.

The amount of nitrogen, carbon, calcium, magnesium, potassium, manganese, iron, and zinc found in strawberry fruit was relatively equal among treatments. Data can be useful to a person estimating crop nutrient removal or to a person calculating the consumptive value of strawberries.

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Appendices

Appendix A. Key factors and variables

Independent	
K. F. Treatment application Var. <ul style="list-style-type: none"> – Form applied – When applied – How applied – Amount applied – Uniform distribution 	K. F. Soil characteristics Var. <ul style="list-style-type: none"> – pH – % organic matter – Element concentrations – Cation Exchange Capacity – Water holding capacity – Drainage – Compaction
K. F. Sampling bias Var. <ul style="list-style-type: none"> – Equipment consistency – Plant identification – Soil sampling – Record keeping 	K. F. Environment Var. <ul style="list-style-type: none"> – Sunlight exposure – Shading – Canopy uniformity – Temperature – Relative humidity
K. F. Cultural practices Var. <ul style="list-style-type: none"> – Plant establishment – Pest damage – Irrigation – Fertilization – Pollination – Harvest – Plant maintenance 	K. F. Plant growth limitations Var. <ul style="list-style-type: none"> – Water needs – Nutrition needs – Respiration needs – Disease control
Dependent	
K. F. Fruit chemical composition Var. <ul style="list-style-type: none"> – Element concentrations 	K. F. Fruit yield Var. <ul style="list-style-type: none"> – Individual berry weight – Harvest date
K. F. Plant data Var. <ul style="list-style-type: none"> – Vegetative mass – Vegetative growth – Stolon production 	K. F. Pathogen control Var. <ul style="list-style-type: none"> – Presence of pathogen – Virulence of pathogen

Appendix A. Independent and dependent key factors and variables. 'K.F.' denotes key factor; 'Var' denotes variable.

Appendix B. Treatment randomization grid.

Row 1	1 buffer row			
Row 2	K2	L2	I2	J2
Row 3	I1	J1	L1	K1
Row 4	O2	P2	M2	N2
Row 5	C1	D1	B1	A1
Row 6	C2	D2	A2	B2
Row 7	M1	N1	P1	O1
Row 8	G2	H2	E2	F2
Row 9	G1	E1	F1	H1
Row 10	N2	O2	P2	M2
Row 11	F1	H1	G1	E1
Row 12	B2	C2	D2	A2
Row 13	B1	D1	A1	C1
Row 14	F2	G2	H2	E2
Row 15	J1	L1	I1	K1
Row 16	J2	K2	L2	I2
Row 17	N1	P1	M1	O1
Row 18	P1	N1	O1	M1
Row 19	D2	A2	B2	C2
Row 20	A1	C1	B1	D1
Row 21	E2	F2	H2	G2
Row 22	J1	K1	I1	L1
Row 23	I2	J2	L2	K2
Row 24	H1	F1	E1	J1
Row 25	M2	O2	P2	N2
Row 26				
Row 27	3 buffer rows b/c plumbing			
Row 28				
Row 29	L2	J2	K2	I2
Row 30	A1	B1	D1	C1
Row 31	H2	F2	G2	E2
Row 32	K1	L1	J1	I1
Row 33	M2	N2	O2	P2
Row 34	E1	F1	H1	G1
Row 35	A2	B2	C2	D2
Row 36	M1	N1	P1	O1
Row 37	P1	M1	O1	N1
Row 38	G2	E2	F2	H2
Row 39	L1	I1	K1	J1
Row 40	K2	I2	J2	L2
Row 41	D1	A1	C1	B1
Row 42	O2	M2	N2	P2
Row 43	H1	G1	E1	F1
Row 44	C2	A2	B2	D2
Row 45	1 buffer row			

Appendix B. Treatment planting map.

Verticillium dahliae expt.			
1 buffer row			
Prom	Bacillus	Ozone (M)	2_scan
Ozone (M)	2_scan	Bacillus	Prom
Filler 1	Filler 2	UTC	Filler 3
1_scan	Mycor	Ozone (H)	CP:Tel
1_scan	Mycor	CP:Tel	Ozone (H)
UTC	Filler 1	Filler 2	Filler 3
MB:CP	3_scan	Ozone (L)	InLine
MB:CP	Ozone (L)	InLine	3_scan
Filler 2	Filler 1	Filler 3	UTC
InLine	3_scan	MB:CP	Ozone (L)
Ozone (H)	1_scan	Mycor	CP:Tel
Ozone (H)	Mycor	CP:Tel	1_scan
InLine	MB:CP	3_scan	Ozone (L)
2_scan	Bacillus	Ozone (M)	Prom
2_scan	Prom	Bacillus	Ozone (M)
Filler 1	Filler 2	UTC	Filler 3
Filler 2	Filler 3	Filler 1	UTC
Mycor	CP:Tel	Ozone (H)	1_scan
CP:Tel	1_scan	Ozone (H)	Mycor
Ozone (L)	InLine	3_scan	MB:CP
2_scan	Prom	Ozone (M)	Bacillus
Ozone (M)	2_scan	Bacillus	Prom
3_scan	InLine	Ozone (L)	MB:CP
UTC	Filler 3	Filler 1	Filler 2
3 buffer rows b/c plumbing			
Bacillus	2_scan	Prom	Ozone (M)
CP:Tel	Ozone (H)	Mycor	1_scan
3_scan	InLine	MB:CP	Ozone (L)
Prom	Bacillus	2_scan	Ozone (M)
UTC	Filler 3	Filler 2	Filler 1
Ozone (L)	InLine	3_scan	MB:CP
CP:Tel	Ozone (H)	1_scan	Mycor
UTC	Filler 3	Filler 2	Filler 1
Filler 1	UTC	Filler 3	Filler 2
MB:CP	Ozone (L)	InLine	3_scan
Bacillus	Ozone (M)	Prom	2_scan
Prom	Ozone (M)	2_scan	Bacillus
Mycor	CP:Tel	1_scan	Ozone (H)
Filler 2	UTC	Filler 1	Filler 3
3_scan	MB:CP	Ozone (L)	InLine
1_scan	CP:Tel	Ozone (H)	Mycor
1 buffer row			

Appendix C. Strawberry plant fertilizer schedule.

Appendix C. Strawberry plant fertilizer schedule

Date	Fertilizer	Form	English rate (gal acre ⁻¹)	Metric rate (L ha ⁻¹)
12/03/09	17-0-0	Liquid	10.0	94.1
12/03/09	Humic Acid	Liquid	5.0	47.0
12/12/09	3-18-18	Liquid	5.0	47.0
12/12/09	Humic Acid	Liquid	2.5	23.5
12/17/09	17-0-0	Liquid	5.0	47.0
12/23/09	17-0-0	Liquid	5.0	47.0
01/07/09	17-0-0	Liquid	5.0	47.0
01/13/09	9-0-0	Liquid	5.0	47.0
01/23/09	3-18-18	Liquid	5.0	47.0
01/31/09	6-30-30	Dry†	8.0	9.0
02/10/09	17-0-0	Liquid	10.0	94.1
02/19/09	3-18-18	Liquid	10.0	94.1
03/04/09	17-0-0	Liquid	5.0	47.0
03/31/09	9-0-0	Liquid	5.0	47.0
03/31/09	Biomin Calcium	Liquid	5.0	47.0
04/10/09	17-0-0	Liquid	2.5	23.5
04/16/09	0-0-25-17(S)	Liquid	7.5	70.5
04/16/09	9-0-0	Liquid	2.5	23.5
04/22/09	3-18-18	Liquid	10.0	94.1
04/29/09	17-0-0	Liquid	5.0	47.0
04/29/09	EDTA-Calcium	Dry	5.0	5.6
05/09/09	9-0-0	Liquid	2.5	23.5
05/09/09	0-0-25-17(S)	Liquid	5.0	47.0
05/15/09	3-18-18	Liquid	10.0	94.1
05/21/09	9-0-0	Liquid	5.0	47.0
05/21/09	0-0-25-17(S)	Liquid	10.0	94.1
05/21/09	EDTA-Calcium	Dry	5.0	5.6
06/15/09	9-0-0	Liquid	7.5	70.5
06/15/09	Biomin Calcium	Liquid	5.0	47.0
06/15/09	Biomin Potassium	Liquid	5.0	47.0
06/23/09	9-0-0	Liquid	7.5	70.5
06/23/09	0-0-25-17(S)	Liquid	7.5	70.5

† Dry units (lbs acre⁻¹ or kg ha⁻¹)

Appendix D: Quality assurance and quality control

Appendix D. Ascending range of continuing calibration verification percentage recovery.

Ca	Mg	K %	Fe	Mn	Zn
93.20	93.33	94.73	97.40	90.65	91.96
94.27	93.33	95.53	97.65	92.25	92.22
94.40	93.73	97.08	98.20	95.30	92.50
94.80	93.86	97.35	98.70	97.60	93.40
95.07	93.87	97.51	99.25	98.65	93.97
95.33	93.87	97.67	99.40	98.70	94.40
95.47	94.00	97.68	99.50	98.75	95.10
95.47	94.00	97.70	99.60	99.00	95.17
95.60	94.13	97.91	99.65	99.05	95.95
95.60	94.27	98.14	99.70	99.05	96.50
95.73	94.40	98.58	99.70	99.15	96.60
95.73	94.40	98.76	99.70	99.15	96.65
95.73	94.53	98.81	99.80	99.20	96.70
95.73	94.53	98.81	99.85	99.35	96.76
95.73	94.53	98.83	100.10	99.35	97.05
95.73	94.67	98.89	100.20	99.40	97.40
95.87	94.80	99.04	100.25	99.50	97.40
96.00	94.93	99.08	100.35	99.55	97.45
96.00	95.06	99.08	100.35	99.60	97.50
96.13	95.30	99.25	100.40	99.80	97.90
96.13	95.33	99.31	100.45	99.95	98.00
96.27	95.60	99.43	100.50	99.99	98.20
96.27	96.00	99.44	100.50	100.15	98.25
96.27	96.00	99.58	100.80	100.20	98.32
96.40	96.13	99.62	100.90	100.25	98.50
96.53	96.80	99.66	101.00	100.35	98.50
96.53	96.80	99.72	101.05	100.35	98.53
96.53	99.20	99.79	101.05	100.50	98.54
96.67	99.20	100.46	101.10	100.55	99.00
96.67	99.20	100.48	101.20	100.65	99.44
96.80	99.33	101.31	101.35	100.65	100.25
96.80	100.40	102.03	101.40	100.70	100.65
96.93	105.47	102.44	102.85	101.00	101.00
96.93	106.40	103.33	103.50	101.20	101.06
97.20	108.27	104.20	103.75	102.05	101.15
97.33	108.67	104.56	105.40	108.25	101.35

Appendix D: Quality assurance and quality control

Appendix D. Ascending range of lab control sample percentage recovery.

Ca	Mg	K	Fe	Mn	Zn
%					
92.80	94.60	92.99	96.50	93.25	93.94
93.00	96.00	93.08	97.50	94.60	94.50
93.10	96.30	93.87	98.40	97.50	94.86
93.40	96.40	94.17	98.65	97.50	95.15
93.40	96.50	95.03	98.80	97.95	95.80
93.60	96.60	95.22	98.85	98.25	96.15
93.90	96.70	95.29	98.85	98.25	96.28
94.70	96.70	95.61	99.10	98.75	96.35
94.90	96.90	95.63	99.10	99.15	96.77
95.20	96.90	95.79	99.50	99.65	98.00
95.20	97.10	95.86	99.75	99.75	98.03
95.30	97.40	95.89	100.35	100.15	98.50
95.50	97.90	96.35	100.65	100.40	98.57
95.70	98.60	96.38	100.85	100.55	98.65
95.70	100.80	97.26	100.95	101.05	98.90
95.80	100.90	97.64	101.30	101.50	99.00
95.90	101.20	97.81	101.30	101.55	101.80
96.00	109.10	99.22	102.00	102.00	102.55
96.67	109.20	100.13	102.60	102.05	102.55
97.00	109.70	101.49	102.80	107.20	*
*†	*	102.71	104.70	108.20	*

†Re – analysis of data created unequal numbers of LCS readings per element analyzed.

Appendix D: Quality assurance and quality control

Appendix D. Percent recovery of standard reference material for carbon and nitrogen analysis.

Batch	N	C
	%	
1	97.00	100.05
2	96.43	100.09
3	96.85	100.19
4	96.79	100.19
5	96.64	100.22
6	97.13	100.17
7	97.44	100.14
8	98.29	100.18
9	98.73	100.17
10	96.44	100.22
11	96.04	100.19
12	96.42	99.78
13	96.37	99.72
14	97.06	100.09
15	96.30	99.55
16	97.34	100.18
17	98.54	100.29
18	99.11	100.40
19	96.50	100.25
20	95.71	100.08
21	95.91	100.06
Min	96.04	100.05
Max	98.73	100.22

Appendix E: P-values for all pairwise comparisons

Appendix E. Fumigant pairwise comparison p-values.

Effect	Berry count	Plant ht (cm)	Yield	Berry wt (grams)	Plant wt	N	C	Ca	Mg	K	Fe	Mn	Zn
						%		(mg kg ⁻¹)					
Soil	0.98	0.51	0.62	0.12	0.94	0.40	0.97	0.62	0.03	0.72	0.09	0.15	0.24
Treatment	0.04	0.36	0.07	0.59	0.02	0.39	0.23	0.19	0.24	0.20	0.20	0.02	0.15
Soil*Treatment	0.15	0.46	0.06	0.46	0.68	0.92	0.72	0.02	0.09	0.64	0.23	0.17	0.53
Row	0.61	0.09	0.88	0.31	0.16	0.66	0.38	0.15	0.91	0.87	0.61	0.36	0.62
Column	0.25	0.52	0.06	0.25	0.09	0.81	0.26	0.62	0.61	0.70	0.88	0.55	0.15

Appendix E. Organic pairwise comparison p-values.

Effect	Berry count	Plant ht (cm)	Yield	Berry wt (grams)	Plant wt	N	C	Ca	Mg	K	Fe	Mn	Zn
						%		(mg kg ⁻¹)					
Soil	0.57	0.26	0.98	0.34	0.90	0.88	0.46	0.77	0.12	0.68	0.25	0.33	0.95
Treatment	0.02	0.39	0.05	0.19	0.13	0.66	0.28	0.39	0.35	0.59	0.64	0.08	0.05
Soil*Treatment	0.08	0.58	0.07	0.38	0.92	0.62	0.60	0.84	0.30	0.94	0.61	0.38	0.58
Row	0.19	0.87	0.07	0.47	0.38	0.77	0.43	0.19	0.50	0.98	0.79	0.31	0.50
Column	0.25	0.49	0.99	0.20	0.74	0.59	0.59	0.36	0.17	0.71	0.77	0.09	0.48

Appendix E. Ozone pairwise comparison p-values.

Effect	Berry count	Plant ht (cm)	Yield	Berry wt (grams)	Plant wt	N	C	Ca	Mg	K	Fe	Mn	Zn
						%		(mg kg ⁻¹)					
Soil	0.85	0.25	0.82	0.75	0.68	1.00	0.97	0.53	0.15	0.72	0.32	0.67	0.02
Treatment	0.002	0.46	0.02	0.57	0.003	0.32	0.06	0.33	0.59	0.62	0.53	0.0002	0.20
Soil*Treatment	0.41	0.90	0.66	0.15	0.93	0.84	0.09	0.09	0.40	0.69	0.44	0.65	0.58
Row	0.63	0.04	0.46	0.04	0.07	0.35	0.02	0.01	0.62	0.82	0.89	0.76	0.93
Column	0.45	0.01	0.20	0.54	0.09	0.53	0.42	0.67	0.69	0.68	0.17	0.94	0.76

Appendix F. Mean and standard deviation for all analyzed data.

Appendix F. Mean and standard deviation for all analyzed data.

Stat	Treatment	Berry count	Yield	Berry wt (grams)	Plant wt	N	C	Ca	Mg	K	Fe	Mn	Zn
							%			(mg kg ⁻¹)			
Mean	MB:CP	41.60	535.13	12.94	202.66	1.34	43.13	73.79	111.08	1522.25	4.05	2.23	1.37
Mean	NTC	25.60	362.95	13.63	145.72	1.38	43.81	73.94	119.60	1590.47	3.66	1.89	2.08
Mean	1-scan	37.90	504.71	13.66	180.09	1.37	43.40	79.89	113.71	1578.23	3.75	2.11	1.24
Mean	2-scan	34.50	485.59	13.94	194.82	1.42	42.93	75.64	110.92	1584.25	3.86	2.22	1.44
Mean	3-scan	38.70	520.33	13.38	192.46	1.41	43.53	79.06	111.94	1585.62	3.96	2.27	1.81
Mean	Ozone (H)	39.10	514.96	13.35	164.44	1.36	43.30	76.71	118.00	1595.38	3.55	1.74	1.75
Mean	Ozone (L)	29.40	428.49	14.59	166.05	1.27	42.72	77.33	118.26	1547.79	3.74	1.92	1.33
Mean	Ozone (M)	32.78	438.14	13.55	169.34	1.30	42.93	82.11	120.10	1546.60	4.16	1.88	1.87
Mean	Bacillus	31.60	410.12	13.24	167.32	1.31	42.97	77.37	110.86	1527.27	3.68	1.74	1.30
Mean	Mycormax	36.50	498.33	13.66	180.91	1.32	43.43	80.38	122.20	1562.21	3.72	1.87	1.26
Mean	Promote	29.40	442.39	14.87	170.66	1.28	43.03	80.69	120.77	1550.53	3.90	1.94	1.99
Mean	InLine	35.50	489.74	13.91	161.59	1.26	42.84	77.02	108.90	1485.70	3.58	1.73	1.70
Mean	PicChlor60	36.50	509.17	14.03	203.38	1.40	43.61	68.61	105.35	1562.47	3.78	1.98	1.34
STD	MB:CP	8.07	101.30	1.34	35.62	0.25	0.90	10.04	15.81	120.26	0.50	0.28	0.21
STD	NTC	10.62	148.42	2.66	22.51	0.13	0.77	7.05	23.49	91.07	0.54	0.24	1.00
STD	1-scan	9.10	64.76	1.89	18.66	0.06	0.53	14.09	12.12	105.14	0.48	0.26	0.25
STD	2-scan	10.97	172.92	1.67	43.34	0.11	1.00	5.92	11.14	104.89	0.47	0.29	0.29
STD	3-scan	5.72	116.24	1.73	44.59	0.14	0.28	14.72	18.94	140.06	0.97	0.40	1.63
STD	Ozone (H)	7.28	108.68	2.43	26.27	0.11	0.59	8.62	16.36	99.89	0.37	0.32	0.89
STD	Ozone (L)	4.17	72.78	1.57	33.93	0.10	0.80	12.44	17.57	92.14	0.67	0.30	0.12
STD	Ozone (M)	7.45	89.86	1.53	28.47	0.13	0.88	16.17	22.62	57.05	1.01	0.26	1.22
STD	Bacillus	6.67	57.57	2.04	26.16	0.12	0.83	8.03	9.70	94.94	0.61	0.33	0.31
STD	Mycormax	6.55	92.40	1.36	36.59	0.06	0.50	12.43	15.84	78.86	0.56	0.42	0.23
STD	Promote	8.76	158.03	2.28	34.93	0.15	1.00	11.90	14.45	70.32	1.03	0.30	0.98
STD	InLine	7.96	120.55	2.16	46.00	0.11	1.30	11.87	17.40	65.09	0.48	0.29	0.74
STD	PicChlor60	12.44	170.21	1.86	43.62	0.11	0.45	7.03	8.70	80.97	0.36	0.19	0.32