

EFFICACY OF COMPOST TEA ON SEPTORIA LEAF SPOT OF TOMATO IN FIELD AND  
GREENHOUSE STUDIES

by

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## Abstract

With acceptance and utilization of chemical pesticides declining, some vegetable producers are turning to alternative methods to manage plant health issues. Compost tea (CT) has provided control of some foliar pathogens and may provide benefits beyond disease suppression. Despite an increasing body of popular and scientific literature focusing on CT as a biological control option for growers, information on the efficacy of CT is still lacking for many pathosystems. In this study, field trials were conducted to evaluate the efficacy of CT on *Septoria lycopersici*, causal agent of Septoria leaf spot on tomato, in Kansas, in 2006 and 2007. Previous research done at KSU with a similar CT showed adequate control of this pathogen in field and greenhouse studies conducted. Additional work to develop a rapid screening method for efficacy of CT formulations was carried out in the greenhouse at Manhattan, KS.

CT sprayed weekly on tomato plants prior to and after disease onset led to no significant difference in control of the pathogen compared to untreated controls. A contact fungicide (chlorothalonil) provided significant control of the pathogen in 2007, but not in 2006. These results contrast with those obtained in previous K-State research. It is difficult to assess why such striking differences were obtained, but the variation in these results point to the need to identify optimal recipes of CT for this pathosystem.

Preliminary investigations standardized plant age, inoculum concentration, incubation conditions, and incubation interval for measurable Septoria leaf spot disease development on young tomato plants in the greenhouse. Ingredients of the field-tested CT were used to make a variety of CTs to test using the greenhouse-screening assay. Further work on identifying effective CT recipes is needed to substantiate the validity of this screening protocol and to evaluate the correlation of this method with disease suppression in the field.

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## CHAPTER 1 - Introduction

Vegetable growers deal with a challenging array of plant health issues. Among the most common of these issues in areas with hot, humid summer climates is foliar disease. With the acceptance of fungicides declining, attention is turning to alternative approaches for disease control (Scheuerell and Mahaffee, 2002; Schouten, 2002). Compost tea (CT) has been cited as an option for conventional and organic growers to suppress plant pathogens (Diver, 2002; Ingham, 2005a; Kannangara, 2006; Tsrer 1999). CTs are also thought to enhance crop fertility by introducing microorganisms that might aid in soil nutrient retention and extraction, and by adding soluble nutrients, further adding to their potential value as a part of an integrated crop management plan (Diver, 2002; Ingham, 2005a; Kannangara, 2006; Merrill and McKeon, 2001).

CT is an aqueous solution that results from the extraction of microorganisms, fine particulate organic matter, and soluble chemical components of compost, that is intended to maintain or increase the beneficial microorganism population of the source compost (NOSB, 2006). Several methods for producing CT have been reported, with a primary difference among these methods being in the aeration provided during the brew period (Diver, 2002; Ingham, 2005b). Aerated CT (ACT) is CT produced with supplemental air, either through direct injection of air into the brew tank or through mechanical recirculation of the brew tank contents. Non-aerated CT (NCT), also called passively aerated CT, is produced without supplemental air by steeping ingredients in water either undisturbed or stirred occasionally throughout the brew period. Another source of variation in producing CT is in the additives used. Because a primary goal of CT production is to increase the microbial populations in compost, many practitioners include additives to facilitate this increase by providing a nutrient source for microbes. Common additives are molasses, fish hydrolysate, rock dust, soluble kelp, and humic acid (Ingham, 2005b). Each of these additives putatively targets certain groups of microorganisms in the compost. Molasses, fish hydrolysate, and kelp are thought to increase bacterial biomass, while rock dust and humic acids are reputed to increase fungal biomass (Ingham, 2005b).

Several studies have investigated CT efficacy for control of various plant pathogens, and factors that may contribute to efficacy. Al-Dahmani, et al. (2003) investigated the effects of compost source (cow manure, pine bark, yard waste, and organic farm compost), compost



maturity (5, 10, 16 months), aeration (NCT, ACT, and CT produced under anaerobic conditions), compost-to-water ratio (1:1, 1:3, and 1:5), and filtration (0.45 $\mu$ M and 0.20 $\mu$ M) on CT efficacy against the bacterial pathogen *Xanthomonas vesicatoria* on young tomato plants in the greenhouse. In their trials all CT formulations provided significant disease suppression (Al-Dahmani, et al. 2003). Dianeze, et al. (2006) investigated the role of siderophores (compounds produced by microorganisms that supply iron to the cell) in CT efficacy against nine plant pathogens. Reduction of *in vitro* hyphal growth of *Verticillium fungicola*, *V. dahliae*, *Pythium aphanidermatum*, *Phytophthora parasitica*, *Rhizoctonia solani*, and four races of *Fusarium oxysporum* was obtained through the use of ACT, but this suppressive effect was mostly negated when siderophores were deactivated with ferric chloride (FeCl<sub>3</sub>) (Dianeze, et al. 2006).

Elad and Shtienberg (1994) investigated the effects of various production intervals (4 hours, 1 week, and 2 weeks), the addition of a proprietary nutrient broth, pasteurization, and dilution on NCT efficacy against *Botrytis cinerea* on tomato leaves, pepper leaves, and grape berries in growth chamber and greenhouse studies. Producing CT over intervals of ten to fourteen days was found to provide a more suppressive CT than shorter production intervals. The addition of nutrients to increase microorganism populations did not increase the suppressiveness of NCT. Pasteurization to eliminate the microflora of the NCT had no effect on disease suppression, but dilution of NCT did reduce its effectiveness in controlling the pathogen (Elad and Shtienberg, 1996). Welke (2004) investigated the effects of aeration and water-to-compost ratio on the efficacy of CT against *Botrytis cinerea* on strawberry fruit. While both aerated and non-aerated CTs suppressed disease, only aerated CT resulted in greater yields. Interestingly, 8:1 concentrations of water-to-compost resulted in significant differences in disease while 4:1 concentrations did not (Welke, 2004).

Scheuerell and Mahaffee (2004) investigated the effects of aeration, additives, and compost source on CT efficacy against cucumber seedling damping off caused by *Pythium ultimum*. Their investigations reported that ACT and NCT significantly reduced the occurrence of damping off, but only ACT reduced its occurrence consistently. The choice of additives was reported to be more important for suppression of this pathogen than the source of the compost used to make CT (Scheuerell and Mahaffee, 2004). Tsror (1999) investigated NCT production intervals (7 and 14 day) and reported consistently reduced severity of *Alternaria solani* and increased yield of tomato with both formulations. Weltzien and Ketterer (1986) demonstrated

that CT could inhibit colonization of *Plasmopara viticola* on excised grape leaves by dipping or spraying leaves with non-aerated CT. Significant reduction in growth of this pathogen was accomplished with increasing production periods (1-3 days) and increasing intervals between treatment and inoculation (1-24 hours) (Weltzien and Ketterer, 1986).

Each of these studies investigated potential sources of variation in CT efficacy, but only one of them (Scheuerell and Mahaffee, 2004) conducted in-depth investigations into the differences in CT efficacy based on additives. They reported that the additives used in CT production had the largest effect on pathogen suppression in their system of cucumber damping off caused by the oomycete, *Pythium ultimum* (Scheuerell and Mahaffee, 2004). Their results indicated that NCT without additives resulted in no significant reduction of damping-off while NCT produced with either fungal (seaweed powder, humic acids, and rock dust) or bacteria (a proprietary bacterial nutrient solution) promoting additives significantly, but inconsistently, reduced damping-off. Aerated CT produced without additives or with the putative bacteria promoting additive resulted in inconsistent reduction of damping-off, while aerated CT produced with the putative fungal additives consistently gave significant control of the pathogen (Scheuerell and Mahaffee, 2004).

Another variable contributing to CT efficacy may be the level of dissolved oxygen (DO) provided during production. Ingham (2005b) stated that CT production systems that allow DO levels to drop below 6 mg/L can result in the loss of filamentous fungi, protozoa and beneficial nematodes causing the CT to become less suppressive to plant pathogens. However, Scheuerell and Mahaffee (2002) stated that it is not clear that there should be a minimum oxygen level requirement for CT production systems because NCTs suppressed some plant pathogens. In aquatic systems, 5 mg/L DO is generally the lower threshold required for a diverse population of organisms (Davis, 1975). Kannangara, et al. (2006) provided a report of DO levels throughout CT brewing period, but did not consider the efficacy of CT on plant pathogens. The relationship between DO levels in CT during production and CT efficacy remains to be fully understood.

Beneficial microorganisms in CT are thought to suppress plant diseases by occupying spatial niches on the phylloplane, competing with pathogens for leaf/seed exudates, or directly antagonizing pathogens (Diver, 2002; Ingham, 2005a). This would indicate a necessity to optimize microbial communities in CT to maximize antagonistic characteristics and phylloplane establishment, which may lead to greater disease suppression. However, according to Sturtz and

coworkers (2006), microbial communities on the phylloplane of potato after CT application may not resemble the microbial communities in the CT itself. Scheuerell and Mahaffee (2002) suggested that the phylloplane establishment of CT microorganisms might be increased through the addition of spray adjuvants such as commercial spreader-stickers. They also suggested that specific groups of antagonistic microorganisms might increase in abundance on the phylloplane after CT application, so a CT containing those microorganisms could be more suppressive than one lacking them (Scheuerell and Mahafee, 2002).

*Septoria lycopersici* Speg., the causal agent of Septoria leaf spot on tomato (*Lycopersicon esculentum* Mill.), is an important fungal disease of tomato (Parker, et al. 1997). Symptoms are circular lesions up to 1/8 inch in diameter that begin as yellow areas that then turn brown, sometimes with a light or dark border (Sherf and Macnab, 1986). After several days, lesions may begin to produce black pycnidia, which distinguish this disease from others (Sherf and Macnab, 1986). Septoria leaf spot occurs in most U.S. states where tomato is grown and can cause severe defoliation and yield loss (Sherf and Macnab, 1986). All released tomato cultivars are susceptible to this disease (Parker, et al. 1995; Sugha and Kumar, 1998). Control of this pathogen has been achieved through cultural controls and frequent fungicide applications (Blum, 2000; Elmer and Fernandino, 1995; Parker, et al. 1995; Tu, et al. 1998). In Kansas, it is recommended to apply copper-containing fungicides tank mixed with other conventional fungicides in an effort to control common bacterial and fungal foliar diseases (Marr, et al. 1995). A number of fungicides are labeled for preventative use for foliar diseases of tomato (Egel, et al. 2007).

For organic growers, fungicide options are limited. Copper fungicides (Bordeaux mixes, copper hydroxide, copper oxide, copper oxychloride, and copper sulfate) are currently acceptable for control of *S. lycopersici* in organic production, but their use is controversial because they are toxic to many microorganisms at recommended rates (Diver, et al. 1999). Because these fungicides have provided adequate control, historically there has been little work done to develop resistant cultivars (Tu and Poysa, 1990), though breeding programs have recently begun to target this disease (Tu, et al. 1998). In the meantime, CT may provide a means of controlling Septoria leaf spot in tomato production.

Blum (2000) demonstrated that it is possible to reduce the incidence of this disease through the introduction of bacteria and yeast isolates onto the phylloplane. Kashyap (1978)

inhibited leaf necrosis due to *Septoria lycopersici* through the introduction of antagonists *Trichoderma viride* strain 3, *Acremonium charticola*, and *Cladosporium sphaerospermum* strain 3. Silva, et al (2004) reported that when used alone, *Bacillus cereus* moderately lowered area under the disease progress curve (AUDPC) values of *Septoria* leaf spot as well as early and late blight on tomato.

Previous work at KSU demonstrated the ability of CT to reduce the severity of *Septoria* leaf spot. Gangaiah (2005) studied the efficacy of CT against *Septoria* leaf spot and early blight on tomato. He reported greater control and marketable yields with CT and mancozeb than untreated plots, though the treated plots had high (>80%) disease severity. The treated plots were 1.52m (5 ft.) from the untreated plots, exposing them to a large source of inoculum. Fernandino and Elmer (1996) described the relationship between *Septoria* leaf spot severity and distance from fungal population foci. Their results showed that disease severity of plants within two meters of the foci was high, but disease severity decreased as the distance from infected tomato plants increased (Fernandino and Elmer, 1996).

The objectives of this research were to a) re-evaluate the efficacy of the general CT recipe investigated by Gangaiah (2005) in the field, and b) to develop a rapid screening protocol for evaluating CT efficacy in the greenhouse. By increasing plot spacing and introducing physical barriers, thus decreasing the possibility of plot-to-plot disease spread, we hoped to gain a better understanding of the applicability of CT in this pathosystem in the field. In an effort to hasten the process of CT recipe evaluation, studies were conducted to develop an *in vivo* screening investigation in the greenhouse.

## **CHAPTER 2 - Materials and Methods**

### **Field Trials**

Three field trials were conducted to evaluate the efficacy of CT against *Septoria* leaf spot on tomato. Two trials were conducted in the summers of 2006 and 2007 at the Kansas State University Horticulture Research and Extension Center near Olathe, KS. The third trial was an on-farm investigation that took place at Thowe Farms near Manhattan, KS, in 2007. The Olathe trials investigated the effect of CT and chlorothalonil (Bravo Weatherstik, Syngenta Corp., Greensboro, NC, USA) in comparison to an untreated control. No fungicides were used in the Manhattan trial due to farmer preference, leaving only the CT treatment and the untreated control.

### ***Olathe Trial***

#### ***Seedling Production***

Transplants of tomato cultivar ‘Celebrity’ were purchased from a commercial garden center on June 1, 2006. In 2007, seeds of tomato cultivar ‘Rutgers’ were sown in a 200 cell plug tray filled with Metro-Mix 200 (Sun Gro Horticulture, Bellevue, WA, USA) and placed under intermittent mist in the greenhouse, April 21. Seedlings were transplanted into 10.16cm (4 inch) round pots filled with the same media on May 3 and placed on open benching with adequate spacing for unrestricted growth. Pots were watered as needed with 125 ppm 20-10-20 Peters Professional Peat-Lite Special water-soluble fertilizer (Scotts Company, Marysville, OH, USA) until they were taken to the field.

#### ***Field Preparation***

In both years an open field area in the vegetable experiment section at Olathe was tilled to remove weeds. The soil type was Kennebec silt loam, previously cropped to pumpkins in 2005. In 2006, no pre-plant amendments were added prior to bed formation. In 2007, Early Bird Chicken Manure Compost (3-4-2) (CMPP, Inc., High Point, MO, USA) was applied at a rate of 70 pounds of nitrogen per acre and was thoroughly incorporated into the soil prior to bed

formation. Tomatoes were grown on twenty-inch-wide raised beds formed using a Nolt's Compact plastic mulch layer (Nolt's Produce Supplies, Leola, PA, USA). Tomato plants were transplanted into the field on June 3 and May 17 in 2006 and 2007, respectively. Individual plots of tomato were supported through the stake-and-weave method in 2006 and were individually supported with wire cages in 2007.

### ***Experimental Design***

In both years a randomized complete block design with four replications was used (Fig. 1). Each block consisted of one 33.53 m (110 ft) long row oriented east to west, and blocks were spaced 12.2 m (40 ft) apart. Five-plant plots of each treatment were spaced 12.2 m (40 ft) apart in each block. Within each plot, plants were spaced 0.6 m (2 ft) apart. Sorghum sudangrass (*Sorghum x drummondii*) seed was drill-planted between plots (approximately 1.52 m (5 ft) and 5.49 m (18 ft) from rows in 2006 and 2007, respectively) at a rate of 112 kg/hectare (100 lbs/acre) within two weeks after tomato transplant to minimize plot-to-plot interference.

### ***Compost Tea Production***

Forty gallons of CT were brewed weekly using an Alaska Giant tea brewer (Alaska Bounty, Palmer, AK, USA) beginning on June 28 and June 7 in 2006 and 2007, respectively. The components included: 151 L (40 gal) of water, 1.8 kg (4 lbs) of vermicompost (Rising Mist Organic Farm, Belvue, KS, USA), 95 ml (0.4 C) unsulfured molasses, 95 ml (0.4 C) hydrolyzed fish fertilizer (2-4-1) (Neptune's Harvest Fertilizer, Gloucester, MA, USA), 0.4 kg (0.8 lbs) alfalfa-based fertilizer (3-1-5) (Bradfield Industries, Springfield, MO, USA), and 16 ml (0.07 C) (Humisolve TM7 (BioAg Corp., Carson City, NV, USA).

Tap water was added to the brew tank approximately 24 hours prior to use to allow for volatilization of chlorine. All other ingredients were added to a 19 L (5 gal) bucket fitted with multiple perforations, a diaphragm for generation of fine bubbles, and a hook on the base for ensuring submersion. Air was pumped vigorously into the diffusion bucket with a Whitewater LT19 linear air pump (Alaska Bounty, Palmer, AK, USA) for the entire 24-hour brew period. Prior to application, CT was filtered through two layers of nylon stocking to remove particles that could obstruct the spraying apparatus. Dissolved oxygen (DO) measurements were taken every ten minutes during the brew period on July 26, 2007, with a dissolved oxygen probe (Sensorex Corporation, GardenGrove, CA, USA) connected to a model 21X datalogger

(Campbell Scientific, Logan, UT, USA). Output from each record was divided by readings taken from outside of the brewing solution and then multiplied by coefficients from temperature and atmospheric pressure (provided by the manufacturer of the DO probe) to obtain mg/L (ppm) DO.

### ***Treatment Application***

CT was applied weekly with a 4-gallon, piston pump backpack sprayer (SP Systems, LLC, Santa Monica, CA, USA) at rates ranging from 0.5-1.0 gallons (undiluted) per plot to achieve complete coverage of plants, with rates increasing with increasing plant size.

Chlorothalonil was applied with a 4-gallon, piston pump backpack sprayer (Solo Company, Newport News, VA, USA) at a rate of 2 pounds active ingredient per acre. Control plots received no treatment. Initial applications occurred on June 29 and June 8 for a total of 10 and 11 applications in 2006 and 2007, respectively.

### ***Inoculation***

No inoculation was done in 2006. For 2007, lesions of *S. lycopersici* were excised from infected tomato leaves (Manhattan, KS, community garden), surface sterilized in a 10% bleach solution for 30 seconds and incubated on moist filter paper in glass Petri dishes for 48 hours at room temperature to stimulate production of cirrhi from pycnidia. Conidia were stored on one-quarter-strength potato dextrose agar slants at 4.5°C (40° F) (Sundin et al., 1999) until use for field inoculation. Two weeks before field inoculation, slants of *S. lycopersici* were removed from storage and transferred using a sterile loop to 9 cm plates of modified V8 agar (150 ml V8 (Campbell Soup Company, Camden, NJ, USA), 3.0 g CaCO<sub>3</sub>, 15 g agar, 850 ml distilled water). Approximately five days of growth at room temperature in the dark led to significant pycnidia production. 25 ml of sterile distilled water was used to flood the Petri dish; a flamed loop was used to agitate cirrhal masses. The concentration of the spore suspension was determined with a hemacytometer and was adjusted to 1x10<sup>5</sup> spores ml<sup>-1</sup>. Two V8 agar plates received 1 ml of the final solution each. Inoculated plates were stored unsealed, in a dark drawer at room temperature until pycnidia production (approximately 5 days).

On the day of inoculation, 25 ml sterile, distilled water was used to flood each of the two V8 agar plates. Cirrhal masses were agitated with a flamed loop to bring spores into suspension. Spore concentration was adjusted to 1x10<sup>5</sup> spores ml<sup>-1</sup>. This concentration is similar to that used by Blum (2000). A total of 500 ml of solution was reserved for the field application. Prior to

use, 2.06 g of Knox gelatin (NBTY, Inc., Bohemia, NY) was dissolved under heat in 25 ml of sterile distilled water, cooled, and added to the spore suspension to replace the natural adhesive characteristics of the pycnidia that are lost through the addition of water to the spores. On July 5, 2007, each plot received approximately 40 ml of the spore suspension applied with a trigger bottle sprayer to the bottom 1/3 of the plants. No inoculation was done in 2006.

### ***Data Collection***

In 2006 and 2007, disease severity was assessed weekly after the onset of symptoms in the field (approximately 9 weeks after transplanting). One leaf approximately 38 cm (15 in) above ground was randomly chosen on each plant and was marked for repeated measurement. In 2006, visual measurements were taken to reflect the percent lesion coverage per leaf. In 2007, visual measurements were taken to reflect the number of lesions per leaf and the percent lesion coverage per leaf.

In 2006 and 2007, yield data were collected weekly at the same time that disease severity was assessed. Yields of plots were categorized by marketability of fruit. Counts and weights of U.S. #1, U.S. #2, and cull fruits were recorded per plant. Yield values for 2006 were markedly low, thus they are not presented in this report.

### ***Statistical Analysis***

The area under disease progress curve (AUDPC) was determined by the trapezoidal method (Madden, et al., 2007). The general linearized model (GLM) procedure of SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to complete analysis of variance of overall disease severity and weekly and total yield. Individual rating dates were analyzed with the GLM procedure of SAS and mean separation was by Tukey's studentized range (HSD) tests ( $\alpha=0.05$ ).

## ***Manhattan Trial***

### ***Seedling Production***

On April 20, 2007, seeds of tomato cultivar 'Mountain Spring' were sown in a 200 cell plug tray filled with vermiculite and placed in a heated greenhouse at Thowe Farms near Manhattan, KS. After germination, seedlings were transplanted into 6-pack trays filled with Metro-Mix 360 (Sun Gro Horticulture, Bellevue, WA, USA) and then into 10.2 cm (4 inch) round pots with the same media. Seedlings were watered as needed until taken to the field.



### ***Field Preparation***

A small section of farmland was cleared of weeds and a pre-plant amendment of 18-46-0 fertilizer (acquired from the local farmer cooperative) was incorporated into the soil. Raised beds were covered with black plastic mulch and plastic drip tape was laid under the plastic mulch. Tomato plants were transplanted into the field on May 12. Plants were supported through the stake-and-weave system.

### ***Experimental Design***

One row of 'Mountain Spring' tomatoes was planted (Fig. 2). This row was segmented into three blocks, each with two five plant plots. Six untreated plants separated each five-plant plot, and the ends of the row were bordered with five untreated plants. All plants were spaced at two feet.

### ***Treatment Application***

CT was taken from the production site at Olathe each week. Applications were similar to those done at Olathe, with a 4-gallon, piston pump backpack sprayer (Solo Company, Newport News, VA, USA). The initial application occurred on June 9 for a total of 9 applications.

### ***Inoculation***

*S. lycopersici* was not introduced into the field in an effort to avoid unnecessary economic injury to the farmer. However, this pathogen was naturally present due to several consecutive years of tomato grown in the same location.

### ***Data Collection***

Weekly assessments of disease severity were recorded in the same fashion as in the Olathe experiments. No yield data were recorded due to ongoing harvesting by the farmer.

### ***Statistical Analysis***

The AUDPC was determined by the trapezoidal method (Madden, et al. 2007). The GLM procedure of SAS version 9.1 was used to complete analysis of variance of overall disease severity and weekly and total yield. Individual rating dates were analyzed with the GLM procedure of SAS and mean separation was by Tukey's studentized range (HSD) tests ( $\alpha=0.05$ ).

## **Greenhouse Study**

Two sets of trials investigating several CT formulations were carried out over time in departmental greenhouses at KSU, Manhattan.

### ***Seedling Production***

Seeds of tomato cultivar ‘Rutgers’ were sown in 200 cell plug trays filled with Metro-Mix 200 (Sun Gro Horticulture, Bellevue, WA, USA), placed under intermittent mist in the greenhouse, transplanted into 10.2cm (4 inch) round pots filled with the same media approximately 10-14 days later, and placed on expanded metal benching at a spacing of 15.2 cm (6 inches). Plants were produced every two weeks beginning March 27 and ending June 5, 2007. Pots were watered as needed with 125 ppm 20-10-20 Peters Professional Peat-Lite Special water-soluble fertilizer (Scotts Company, Marysville, OH, USA).

### ***Experimental Design***

After 2-3 weeks of growth (4-6 leaf stage), treatments were randomly assigned to the pots. Spacing was increased to 30.5 cm (12 inches) and organized in a completely randomized design.

### ***Treatments***

Each trial included two replications of three CT formulations, three fungicide treatments, and one untreated control. Each treatment consisted of four plants. A total of six CT formulations (CTA-CTF) were tested (Table 1) in two separate sets of trials over the course of the experiment. All formulations included ingredients used in the field trials, but were produced in smaller quantities. CT was produced by filling six 1 L, wide-mouth glass bottles with 900 ml distilled water, and adding ingredients for each tea. Aeration was through a dual output Whisper 2000 air pump (Carolina Biological Supply Company, Burlington, NC, USA) with six-way gang valves. CTs brewed for 24 hours and were filtered through two layers of cheesecloth prior to application.

Formulations of CT evaluated in these trials consisted of compost and water (CTA), individual components (Humisolve TM7, unsulfured molasses, alfalfa-based fertilizer, and hydrolyzed fish fertilizer) of the field trial CT with compost and water (CTB-CTE) and the full recipe used in the field trial (CTF). All ratios of ingredients were held consistent to that of the 40

gallon CT recipe brewed at Olathe. A commercially available, general use form of chlorothalonil (Ferti-lome Landscape and Garden Fungicide) (Voluntary Purchasing Groups, Inc., Bonham, TX, USA) was applied for comparison to CT. Three solution concentrations of chlorothalonil (Table 1) were used because of phytotoxicity that was seen in preliminary investigations. Two weekly applications of CT and chlorothalonil were initiated at the 4-to-6-leaf stage. An untreated control was also included in each investigation. Trials of CTs A, B, and C were carried out four times, and trials of CTs D, E, and F were carried out twice. All treatments were sprayed onto the abaxial and adaxial sides of all leaves with trigger bottle sprayers to achieve complete coverage to the drip point.

### ***Inoculation***

Cultures of *S. lycopersici* were produced in the same manner described for the field inoculation. Sub-cultures were produced bi-weekly to ensure an adequate and consistent supply of inoculum for the study. Spore suspensions were produced in the same manner as described for the field inoculation, except that the total volume was reduced to 100ml, and 0.412g of gelatin was added to the solution.

Plants were inoculated four or five days after the second and final treatment application. All plants were inoculated on the abaxial and adaxial sides of all leaves with a trigger-bottle sprayer and allowed to air dry before being placed in a mist chamber in the greenhouse in a randomized order. Plants were sprayed with tap water to create free water on the leaf surface. The chamber provided 60 seconds of mist every ten minutes. Plants were maintained in the chamber for 48 hours and then returned to the open benching system where they were again placed in a completely randomized design.

### ***Data Collection***

After approximately 10-15 days, lesion development was evaluated by counting the total number of lesions per plant. This method was similar to that used by Blum (2000).

### ***Statistical Analysis***

Since the effects of time were not significant, results of sets of trials were combined for analysis as one experiment. The GLM procedure of SAS version 9.1 was used to evaluate

differences in number of lesions per plant, and means were separated using Tukey's studentized range (HSD) test ( $\alpha=0.05$ ).

## CHAPTER 3 - Results

### Field Trials

#### *Olathe 2006*

Of the five weeks evaluated, only one week showed a significant difference among treatments (Fig. 3). During the tenth week after planting, the fungicide treated plots had significantly lower disease severity than the control plots or CT treated plots. There were no significant differences between the CT treated plots and the control plots at any point throughout the investigation. By the end of the experiment, all treatments had greater than 80% coverage by the pathogen on the selected leaves. The fungicide treated plots tended to be less affected by the disease than the CT treated plots and the control plots, but this difference was not significant.

There were no significant differences in AUDPC values among treatments (Fig. 6).

#### *Olathe 2007*

Results from the five weekly assessments of disease severity (percent selected leaf affected) are presented in Fig. 4. Data collected as number of lesions per leaf gave comparable results, but are not presented herein. Over the first three weeks of the investigation, disease development remained similar among treatments. However, at 13 and 14 weeks after planting, fungicide treated plots exhibited significantly lower disease severity than the control plots and CT treated plots. There were no significant differences between the CT treated plots and the control plots at any point throughout the investigation. By the end of the experiment, infection of selected leaves in both the CT treated plots and the control plots was close to 100%.

The AUDPC values are presented in Fig. 7. The fungicide treated plots resulted in significantly less area under the disease progress curve than the CT treated plots and the control plots. There was no significant difference between CT treated plots and the control plots.

Few significant differences were found for yield data (Table 2). Season-long totals of tomato counts were significantly higher for control plants, but season-long totals of tomato weights were not different among treatments. Among grades (U.S. No.1, U.S. No.2, and cull),

no significant differences were found for number of fruit or weight of fruit between the treatments.

Levels of DO in the CT over the duration of the brew period on July 26 are presented in Fig. 9. Significant drops in the levels of DO indicate that organisms were utilizing oxygen faster than the pump could replace it. On this brewing date, DO levels were less than 6ppm for approximately 7.5 hours.

### ***Manhattan 2007***

Results from the three weekly assessments of disease severity (percent selected leaf affected) are presented in Fig. 5. There were no significant differences between CT treated plots and control plots at any point throughout the investigation. By the end of the three weeks (week 11), levels of disease severity were similar to those found at week 11 during the 2006 Olathe investigation.

The AUDPC values are presented in Fig. 8. There were no significant differences between CT treated plots and control plots.

## **Greenhouse Study**

Few significant differences were found in the results from the developed screening protocol (Fig. 10). Fungicide treated plants exhibited the lowest levels of disease severity, but none of these treatments were significantly lower than the control plants. All CT treated plants, except CT-E, exhibited similar levels of disease severity to fungicide treated plants and control plants. CT-E treated plants gave the highest level of disease severity, but this difference was not significantly higher than CT-F treated plants. High levels of variation among treated plants and control plants did not allow for the detection of significant differences in severity of infection among treatments.

## CHAPTER 4 - Discussion

### *Field Trials*

Yield data for the 2006 field trial in Olathe was not evaluated because yield was markedly low for all treatments. Possible causes for this may have included the late transplant date or the microclimate created by the proximity of the sorghum sudangrass buffers. In 2006, plant buffers were placed approximately 1.52 m (5 ft) from the treatment plots. The fruit set of the tomato plants may have been affected by the limited wind within the plots, thus compromising yield data. For this reason, at Olathe in 2007, plant buffers were placed at a distance of 5.49 m (18 ft) from all plots.

Yield observations from the Olathe, 2007 field trial showed no significant differences in U.S. No. 1 grade fruits among treatments. Total fruit counts, regardless of grade, were significantly higher on untreated control plants than the CT and fungicide treated plants. However, total fruit weights were not significantly different, indicating a smaller average fruit size on control plants. The limited data collected for yield analysis in 2007 is not indicative of expected, whole-season yields.

Compost tea demonstrated no apparent control of *Septoria lycopersici* on tomato in any of the field studies. These results were in contrast to those of Gangaiah (2005), who found consistent inhibition of *S. lycopersici* advancement on tomato plants with several variations of this CT recipe. His results showed control comparable to that obtained by the application of mancozeb. Our results showed no significant inhibition of the pathogen over untreated control plants. Chlorothalonil significantly reduced our AUDPC ratings in comparison to the CT treated plants and the untreated control plants in 2007, but not in 2006. Dillard, et al. (1997) found that chlorothalonil controlled *Septoria* leaf spot better than mancozeb in New York. This may help to account for the difference in CT performance in our trials and those done by Gangaiah in relation to fungicide performance. But differences found in CT efficacy between our trials and those done by Gangaiah, in relation to control plants, were striking.

Though the recipe used in our trials was quite similar to those investigated by Gangaiah, some distinct differences in formulations and sources of ingredients may have contributed to the

differences in results. For instance, we obtained vermicompost from a different source, and used an alfalfa-based fertilizer as opposed to alfalfa pellets. Because microbial populations are considered to be the most significant factor in CT suppressiveness (Scheuerell and Mahaffee, 2002), constituents of a CT recipe that alter the availability of microbial populations (e.g. compost) no doubt have an impact on efficacy. Our investigation into the field performance of CT against *S. lycopersici* on tomato further adds to the body of evidence reporting variability in disease suppression by CT that has become a hallmark of research in this area (Scheuerell and Mahaffee, 2002).

The apparent failure of our CT to control this pathogen could be the result of a number of variables, including the aeration process. Our measurements of dissolved oxygen during the brew period showed a significant dip below the threshold level of DO for aerobic organisms. By most accounts, this drop in DO is an indicator of microorganism feeding and growth (Kannangara et al., 2006; Ingham, 2005b). Filamentous fungi, protozoa, and beneficial nematodes can be lost in a CT if the DO falls below 6mg/L (Ingham, 2005b). Teas that are subjected to these low DO levels tend to be less suppressive to foliar disease because they do not contain beneficial fungi (Ingham, 2005b). However, it has also been reported that about 85% of the suppressiveness of a CT comes from bacterial (not fungal) coverage of the leaf surface (Ingham, 2005a). Further complicating the clarity of the effect of DO on CT suppressiveness, the apparatus used to brew the CT in our experiments was the same one used by Gangaiah. DO levels present in the brewing apparatus were not measured in Gangaiah's work. However, because we used similar recipes, we can speculate that the levels of DO in his teas were comparable to or lower than ours. This is an acceptable assumption when you consider that Gangaiah split the air stream from the air pump during one of his investigations to make two batches (30 gallons each) of CT simultaneously, which further reduced the input capacity of the air pump to the brewing apparatus (Gangaiah, 2005).

The DO levels recorded in our trials only represent one brewing date. The trend observed during this production period could be unrepresentative of DO trends across all of our brewing dates. We could expect to see variations in DO trends among brewing dates because our CT was brewed outside and subjected to environmental conditions that could alter atmospheric variables considered in DO determination. Further investigation is required to substantiate the trends seen in the data presented here with respect to DO levels. As it is, there is very little information on



the trends of DO levels throughout the CT brewing process. No report has related DO levels present throughout a brewing cycle or throughout entire investigations to disease suppression, so it is premature to assert that DO levels had an effect on the disease suppressiveness of our CT. While DO levels in our CT on July 26, 2007 may or may not be representative of DO levels on other brewing dates, we know that DO did drop to dangerously low levels for the maintenance of aerobic organisms and that our CT was not suppressive to *S. lycopersici*.

A number of other variables may account for the lack of efficacy of CT in our trials. Variables such as microbial populations, attachment and survival of microbes, compost source, compost age, brewing time, temperature, pH, and application timing have been cited as possible sources of the variable success reported in disease suppression with CT (Scheuerell and Mahaffee, 2002). There has been no study to date that has considered all sources of variation in CT efficacy. However, with the expanding body of evidence surrounding CT, it is likely that most of these variables will be better controlled in the future.

Results of this study are similar to those found by Olanya and Larkin (2006), who found that CT generally had no effect on inhibiting foliar disease severity caused by *Phytophthora infestans* on potato. They offered several potential explanations for the inefficacy of their CT, including a lack of microbial persistence/retention on the leaf surface and low establishment of potential biological control agents (Olanya and Larkin, 2006). These reasons for the inefficacy of CT are in line with the results of work done by Sturz, et al. (2006), who found that establishment of microbial communities in CT might not provide characteristics necessary for microbial occupancy on the phylloplane of potato. To further identify potential sources of their CT inefficacy, Olanya and Larkin (2006) stated that the microbial constituents of their CT might not have been effective suppressors of *P. infestans*. Elad and Shtienberg (1994) found that individual strains of bacteria found in CT, when applied independently, controlled *Botrytis cinerea* on tomato as well as CT did, indicating that the suppressive activity of CT may be due to relatively few organisms present in the CT.

This illustrates a need to identify microorganisms that compete, antagonize, induce resistance, or inhibit specific pathogens. Identification of effective biological control agents and research leading to CT production that allows for higher concentrations of these agents may lead to greater CT efficacy. Because investigations into the efficacy of CT in suppressing plant

disease through field studies can deplete resources and may not provide usable results, steps should be taken to identify these biological control agents through a more feasible means.

### ***Greenhouse Study***

A protocol for *in vivo* screening of candidate CT recipes under controlled conditions was developed. Several studies of CT efficacy have revealed various time periods between treatment and inoculation. Elad and Shtienberg (1994) only treated plants in their experiments after symptoms of disease appeared. Olanya and Parkin (2006) and Al-Dahmani, et al. (2003) waited 24 hours after treatment to inoculate with their pathogens. Sturz, et al. (2006) inoculated three days after treatment, while Tsrer (1999) waited ten days after treatment to inoculate. Because most application procedures for CT and fungicides suggest weekly applications, a ten-day period between treatment and inoculation may not be indicative of real world results. Weltzien and Ketterer (1986) found that CT efficacy on inhibition of downy mildew (*Plasmopara viticola*) on grape leaves increased when the period between treatment and inoculation increased from 1 to 24 hours, indicating a lag in suppressive action by CT. For this reason, a maintenance period of four-to-five days was used between treatment and inoculation in our screening trials.

Preliminary investigations into the inoculation of tomato plants revealed that four-week-old tomato transplants developed considerably more lesions per plant than 6-, 12-, or 14-week-old plants at a concentration of  $1 \times 10^5$  spores  $\text{ml}^{-1}$  (data not presented). Also, disease development was all but absent when inoculated plants were placed in the mist chamber without water present on the leaves, even under near 100% humidity. Elmer and Fernandino (1995) also noted this phenomenon on the same pathosystem.

Few significant differences were noted among treatments in the greenhouse study. Fungicide treatments did consistently provide moderately low levels of disease severity despite the low concentrations at which they were applied in order to avoid phytotoxicity. The fact that there were no significant differences between CTs A-D is interesting. The addition of humic acid (CTB), molasses (CTC), or alfalfa-based fertilizer (CTD) should have resulted in fewer lesions per plant than CTA due to their supposed role in increasing microorganism biomass (Ingham, 2005b). However, Elad and Shtienberg (1994) reported that the addition of nutrients to CT did not generally improve disease control.

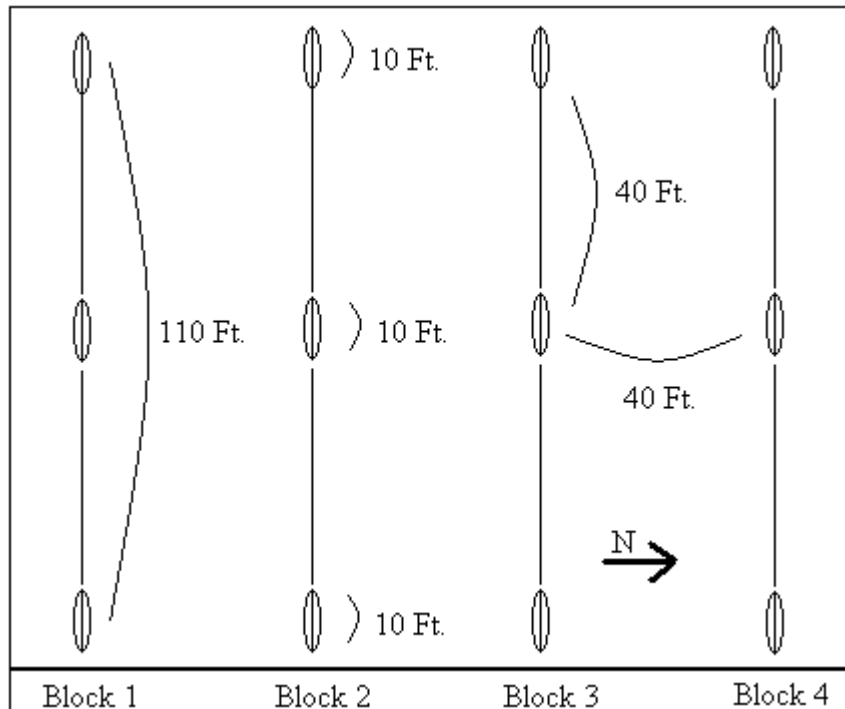
The high lesion development that resulted on CTE and CTF is difficult to explain. The addition of hydrolyzed fish fertilizer to compost and water seemed to have a negative impact on

the suppressiveness of this CT formulation, which may have increased the susceptibility of plants treated with CTF, the complete formulation evaluated in the field. Sturz, et al. (2006) also described this phenomenon by noting that bacteria recovered from the phylloplane of potato after treatment with CT were generally less efficacious against *Phytophthora infestans* than bacteria recovered from the pretreatment phylloplane. It seems intuitive to expect that CTF would develop *S. lycopersici* lesions in a manner similar to that of control plants, because that is what was seen in field experiments. However, we know that *in vitro* studies are often poor predictors of field performance of biological control agents, so *in vivo* studies conducted in the greenhouse should not be expected to relate directly to field performance either. Even with this shortcoming, an *in vivo* screening protocol could provide a framework for identifying potentially effective CT recipes for field evaluation.

In order to assess the utility of a screening method, there should be data that correlates screening results to disease suppression in the field (Scheuerell and Mahaffee, 2002). The results obtained in this screening study do not necessarily correlate to field performance, but perhaps through manipulation of some of the methods of this protocol, better correlation could be observed. For instance, the relatively long period of time over which this study was conducted could have had an impact on the variability that was observed. Had these experiments been conducted in growth chambers, under more controlled conditions, seasonal changes would not have affected the rate of growth and development of tomato plants and *S. lycopersici* as it did in the greenhouse. Furthermore, variation among treatments might be reduced through the use of more appropriate data collection methods or increased replication. It is premature to say that the screening protocol developed in the current study is useful because of the discernible variability that was observed and the lack of correlation to field results.

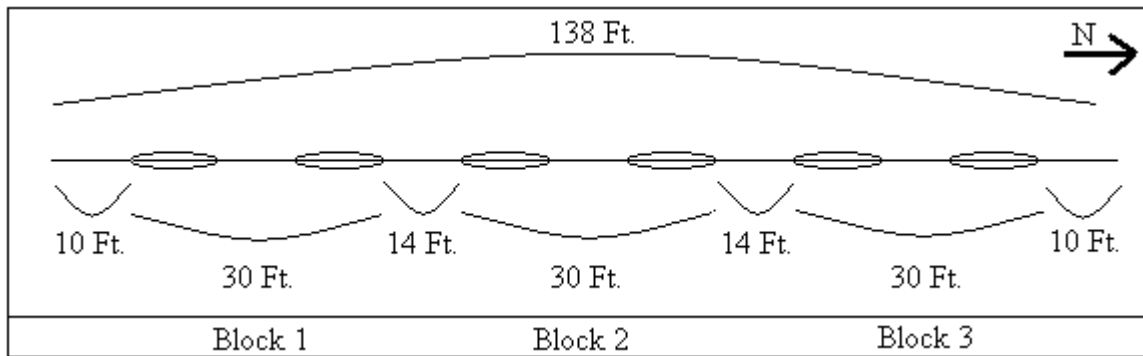
Further screenings are needed to establish the protocol developed in the current study as effective. Much of the general body of research regarding biological control of this disease has identified effective antagonists. By merging the evolving science of CT and the already-present information on biological control, optimal recipes can be formulated. CT formulations effective against *S. lycopersici* as well as other common foliar pathogens of tomato need to be identified before CT utilization can apply to this crop.

## Figures and Tables



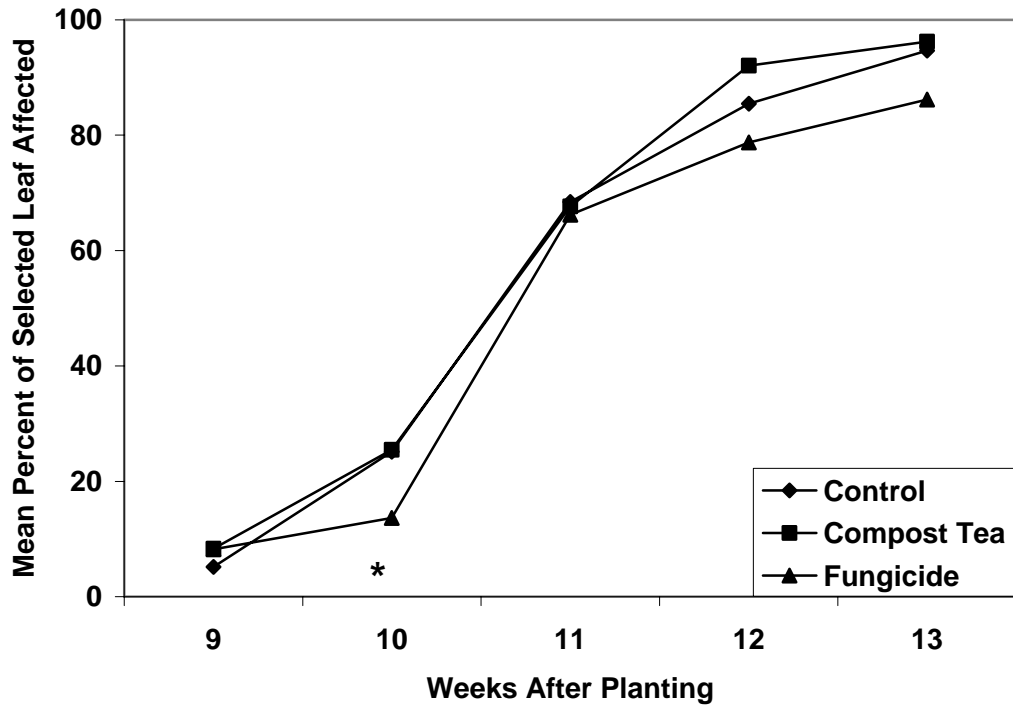
**Figure 1 Experimental Design for Olathe Field Experiments (Summer 2006 and 2007)**

Physical layout of tomato plots in Olathe in 2006 and 2007. Each plot consisted of five plants spaced two feet apart. Rows (blocks) and plots within rows were separated by 40 feet of field space.



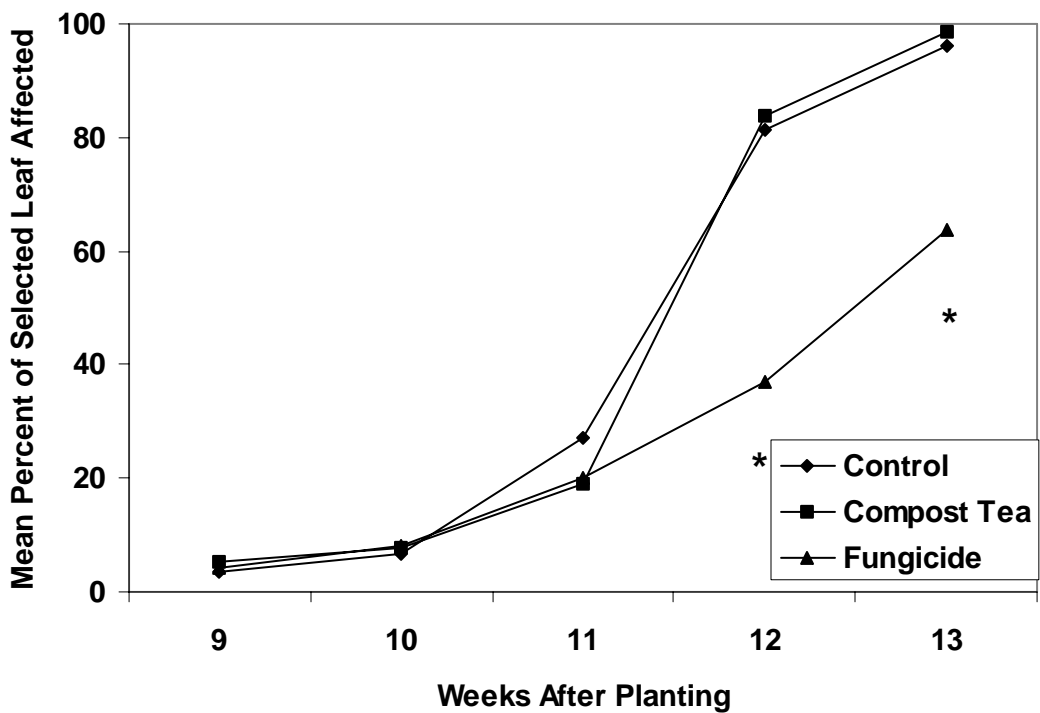
**Figure 2 Experimental Design for Manhattan Field Experiment**

Physical layout of tomato plots in Manhattan in 2007. One row was divided into three blocks of 16 plants, with six plant buffers between blocks. Each block consisted of two plots (CT and control) separated by six plants. Each plot consisted of five plants spaced two feet apart. Each end of the row was buffered with five plants.



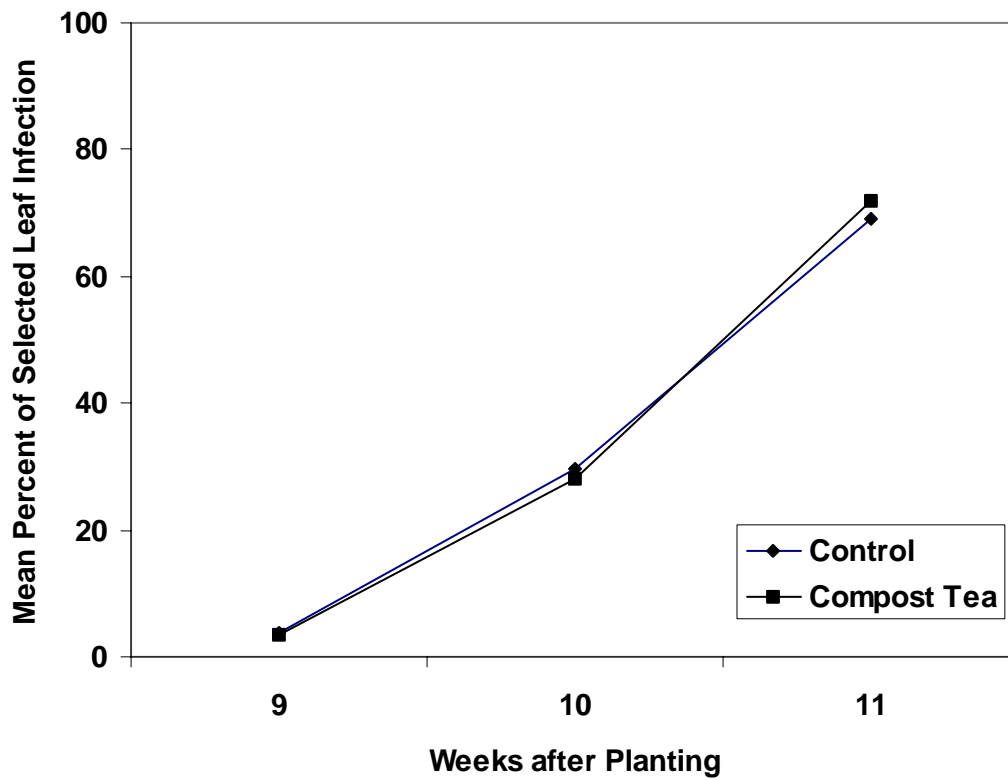
**Figure 3 Disease Severity (Olathe 2006)**

Severity (mean % of selected leaves affected) of Septoria leaf spot on compost tea and chlorothalonil treated plots and untreated control plots at Olathe in 2007. Mean separation was by Tukey's studentized range (HSD) tests,  $\alpha=0.05$ . Significantly different values are noted with (\*).



**Figure 4 Disease Severity (Olathe 2007)**

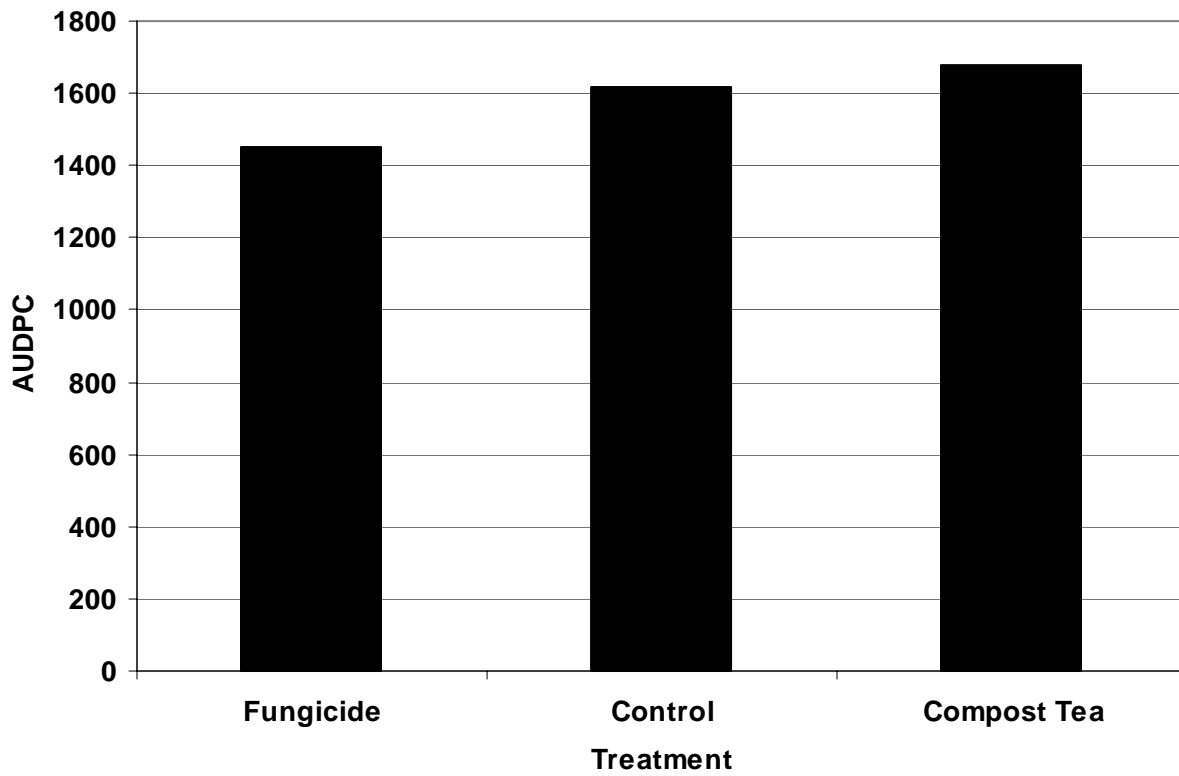
Severity (mean % of selected leaves affected) of Septoria leaf spot on compost tea and chlorothalonil treated plots and untreated control plots at Olathe in 2007. Mean separation was by Tukey's studentized range (HSD) tests,  $\alpha=0.05$ . Significantly different values are noted with (\*).



**Figure 5 Disease Severity (Manhattan 2007)**

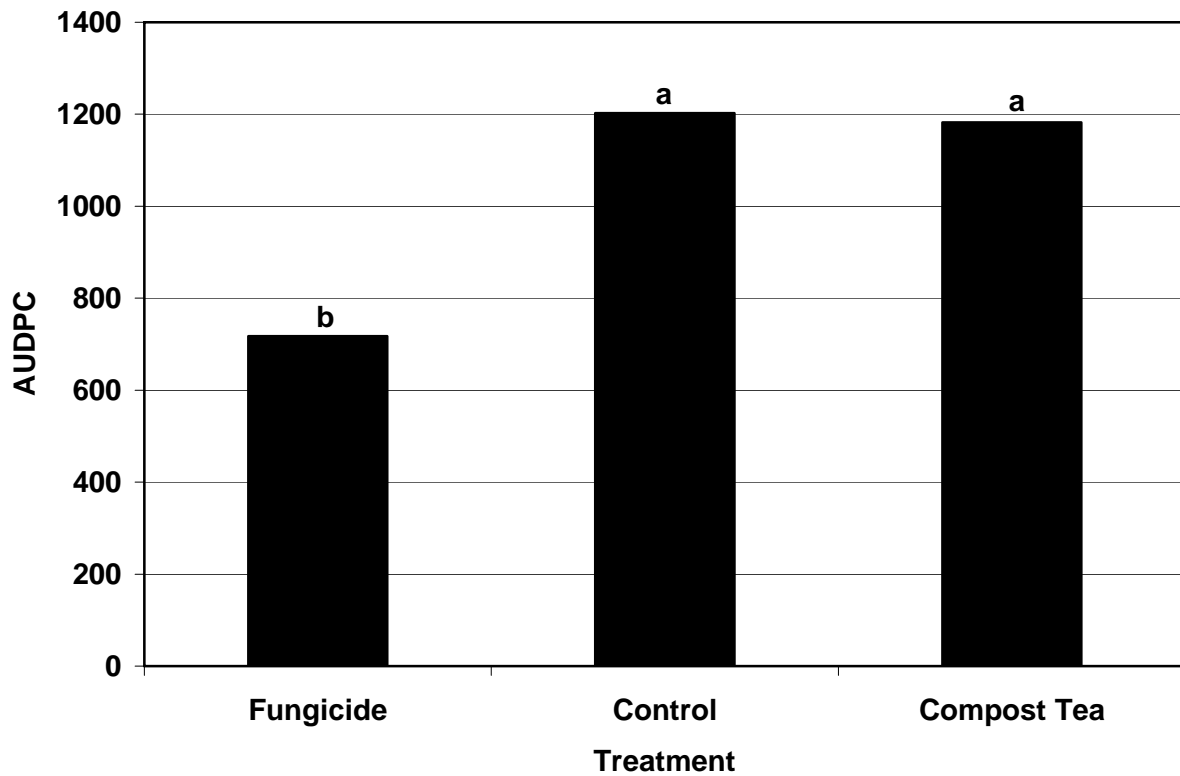
Severity (mean percent of selected leaves affected) of Septoria leaf spot on compost tea treated plots and untreated control plots at Manhattan in 2007. Mean separation was by Tukey's studentized range (HSD) tests,  $\alpha=0.05$ . There were no significant differences among treatments.





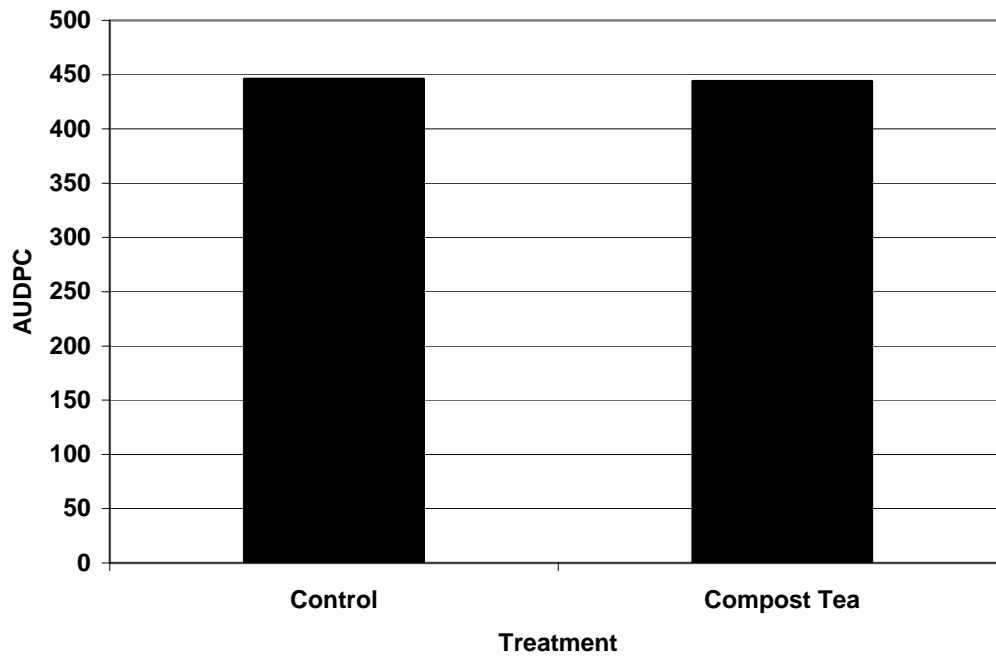
**Figure 6 Area Under Disease Progress Curve (Olathe 2006)**

AUDPC over a five-week period at Olathe in 2006. Figures were calculated using the trapezoidal method from data collected as percent leaf infection. There were no significant differences among treatments according to Tukey's studentized range (HSD) tests,  $\alpha=0.05$ .



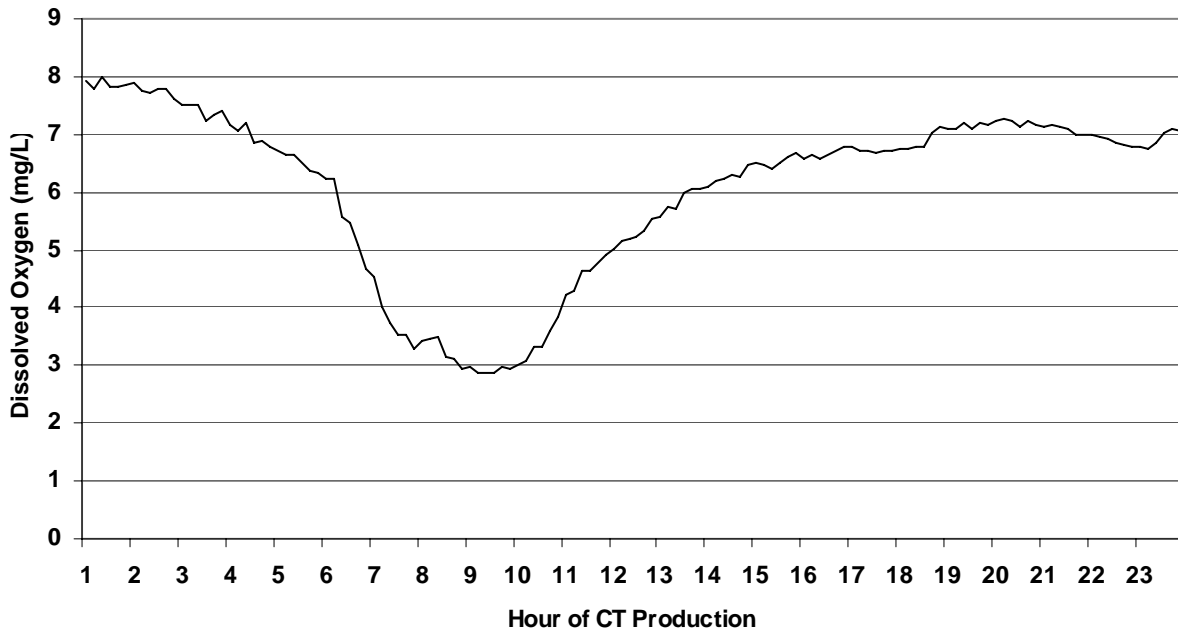
**Figure 7 AUDPC (Olathe 2007)**

Area under disease progress curve over a five-week period at Olathe in 2007. Figures were calculated using the trapezoidal method from data collected as percent leaf infection. Columns with the same letter do not differ significantly according to Tukey's studentized range (HSD) tests,  $\alpha=0.05$ .



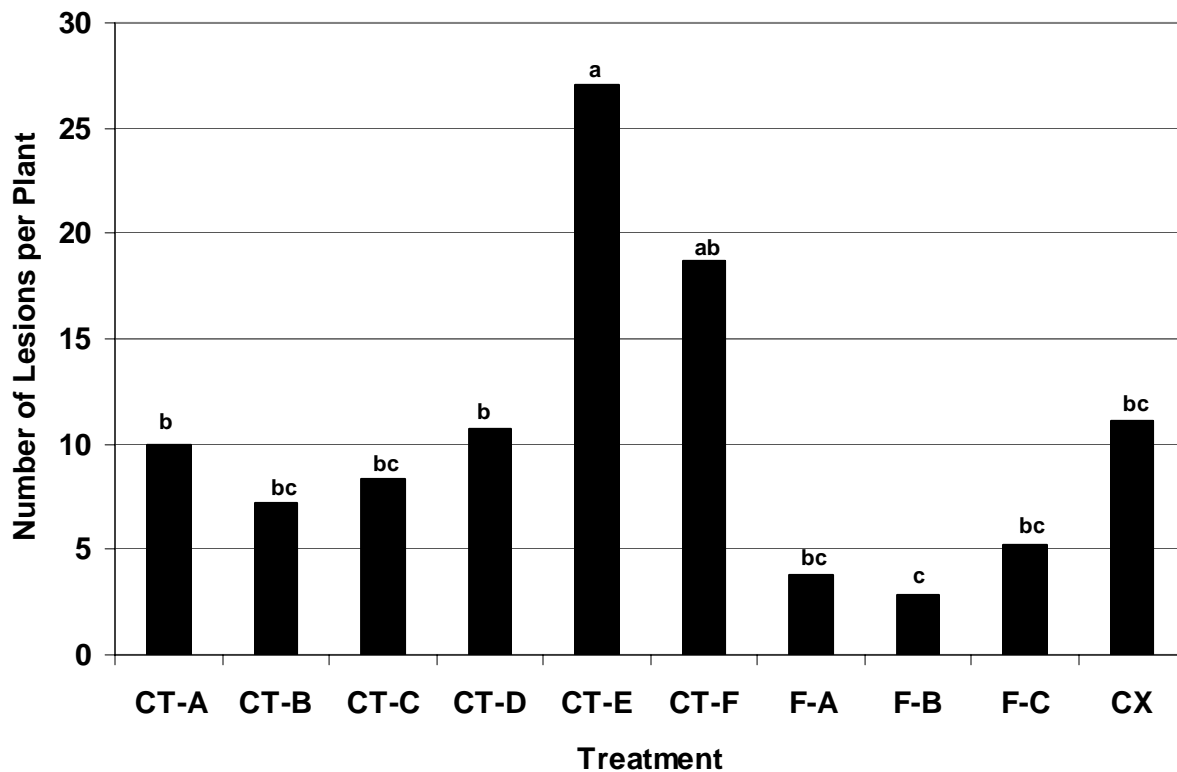
**Figure 8 AUDPC (Manhattan 2007)**

Area under disease progress curve over a three-week period at Manhattan in 2007. Figures were calculated using the trapezoidal method from data collected as percent leaf infection. There were no significant differences among treatments according to Tukey's studentized range (HSD) tests,  $\alpha=0.05$ .



**Figure 9 Dissolved Oxygen Levels During Compost Tea Production**

Levels of dissolved oxygen from CT production on July 26, 2007. DO was calculated by dividing solution readings by atmospheric readings from DO probe (Sensorex Corporation), then multiplying by coefficients from temperature and pressure.



**Figure 10 Disease Severity (Screening Trial)**

Number of lesions on five-to-six week old tomato plants treated with various compost teas (CTA-CTF), fungicide concentrations (FA-FC), or untreated (CX), ten-to-fifteen days after inoculation with *S. lycopersici*. Compost tea recipes and fungicide concentrations can be found in Table 1. Columns with the same letter do not differ significantly according to Tukey’s studentized range (HSD) tests,  $\alpha=0.05$ .

**Table 1 Treatments for Screening Trial**

CT designations and ingredients (900ml CT) and fungicide concentrations

Treatment	CT Ingredients/Fungicide Concentrations
CT-A	10.77 g vermicompost
CT-B	10.77g vermicompost + 0.107g Humisolve TM7
CT-C	10.77g vermicompost + 0.57ml unsulfured molasses
CT-D	10.77g vermicompost + 2.15 g alfalfa-based fertilizer
CT-E	10.77g vermicompost + 0.57 ml hydrolyzed fish fertilizer
CT-F	10.77 g vermicompost + 0.107 g Humisolve TM7 + 0.57 ml unsulfured molasses + 2.15g alfalfa-based fertilizer + 0.57 ml hydrolyzed fish fertilizer
F-A	0.0036% active ingredient
F-B	0.0018% active ingredient
F-C	0.00012% active ingredient

**Table 2 Yield Data (Olathe 2007)**

Counts and weights (lbs.) per plant from compost tea and fungicide treated plots and untreated control plots at Olathe in 2007, by grade. Figures in the total columns are the sums of the grades. Figures with the same letter are not significantly different according to Tukey's studentized range (HSD) tests,  $\alpha=0.05$ .

Treatment	U.S.No.1 Count	U.S.No.1 Weight	U.S.No.2 Count	U.S.No.2 Weight	Cull Count	Cull Weight	Total Count	Total Weight
Control	4.25	1.84	1.65	0.44	0.05	0.02	5.95 <b>(a)</b>	2.30
Compost Tea	3.04	1.50	0.96	0.35	0.06	0.03	4.06 <b>(b)</b>	1.87
Fungicide	2.87	1.43	0.96	0.37	0	0	3.83 <b>(b)</b>	1.80

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