Comparing Source of Agricultural Contact Water and the Presence of Fecal Indicator Organisms on the Surface of 'Juliet' Grape Tomatoes

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Executive Summary

Consumption of fresh tomatoes (*Solanum lycopersicum*) has been implicated as the cause of several foodborne illness outbreaks in the United States, most notably in cases of salmonellosis. Potential sources of biological contamination include agricultural workers, feral animals, manure, and water. Although microbial water quality standards exist for agricultural use, little is known about how the levels of fecal indicator organisms in water relate to the counts on the tomato fruit surface. In this study we utilized four types of fecal indicator organisms commonly used in microbial water quality standards (*Enterobacteriaceae*, total coliforms, fecal coliforms, and *E. coli*) to monitor the water quality of two surface ponds and a groundwater source. We also monitored the fruit surface of treated grape tomatoes over the 2009 and 2010 growing seasons. Water source and time of year showed significant differences in the counts of fecal indicator organisms in irrigation water, with groundwater having significantly lower counts of all fecal indicator organisms than the two surface water sources. Considerable variability in bacterial counts was found in the surface water sources over the course of the season, perhaps explained by environmental variables such as water temperature, pH, precipitation, and air temperature. The microbial counts on the surfaces of the tomato fruit did not reflect the counts in water applied to the plants. Only certain indicator organisms had a significant difference among treatments, with results differing between the two sampling seasons. This lack of association between the fecal indicator organisms present in the water samples and on the tomato fruit surface questions the reliability of using indicator organisms for water testing.

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Introduction

The number of foodborne illness outbreaks resulting from fresh fruit and vegetable consumption has increased in recent years, so that fresh produce now accounts for 29% of foodborne outbreaks (*7*). This is due in part to the increase in produce consumption, the source of consumed produce, and year-round availability (*7, 25*). Fresh consumption of tomatoes in particular has increased in the United States, and between 1996 and 2008 tomatoes were responsible for 17.1% of produce outbreaks (*7*). There is no inactivation step to kill microbes on raw fresh-market tomatoes before consumption (*20*).

Several sources are implicated in the contamination of tomatoes with pathogens including manure, feral animals, and agricultural contact water for irrigation and pesticide applications (*14*). Despite knowledge that the use of contaminated surface water for irrigation or pesticide application on food crops has been associated with the transmission of foodborne pathogens, this surface water is still used in agriculture today *(14).* Microbial water standards are published by the World Health Organization (WHO) for wastewater reuse in agriculture, and by the United Fresh Produce Association (UFPA) for agricultural water use on tomatoes (*8, 17*). Water standards such as these are being incorporated into Good Agricultural Practices (GAPs), a series of guidelines implemented in agriculture to help reduce microbial hazards (*14, 15*). Although microbial water standards exist, little scientific data is available on how microbial counts in water affect the microbial counts on the tomato phyllosphere, and thus how effective these standards are in preventing foodborne illnesses.

The WHO and the United States Environmental Protection Agency (EPA) use fecal indicator organisms to monitor the infection risk that water or foods pose for humans (*22*). Fecal

indicator organisms are used instead of actual pathogenic organisms due to their cost effectiveness, rapid results, and ease of monitoring in the field and in laboratories (*8*). In order to be classified as a fecal indicator organism, the organism needs to be consistently and universally present in feces, and must not be able to multiply in natural waters (*8,14,30*).

The fecal indicator organisms used to monitor water quality in this study were *Enterobacteriaceae,* total coliforms, fecal coliforms, and *E. coli. Enterobacteraceae* are a family of gram-negative facultative anaerobes (*24*). Total coliforms are a subset of *Enterobacteriaceae*, and include bacteria from the genus *Escherichia, Citrobacter, Enterobacter, Klebsiella,* and *Salmonella* (*14*). Although total coliforms are used as indicators of microbial quality of water, they are unreliable indicators of fecal contamination because they are capable of growing in the environment and in water systems (*30*). In a study isolating over 1000 coliform strains sampled from several types of water, it was found that 61% of coliforms were of non-fecal origin. Notably, if a water source contains large concentrations of organic matter, coliforms may be present in higher numbers (*14*).

Similar to the aforementioned broad categories of fecal indicator organisms, certain genera of fecal coliforms will reproduce in environmental conditions without a fecal origin (*4*). When using water standards based upon quantitative results, environmental amplification can lead to false conclusions on the microbial water quality.

Of the fecal indicator organisms used, *E. coli* is considered to be the most reliable one (*4, 30*). As an indigenous member of the intestinal flora in warm-blooded animals, *E. coli* is specific to fecal contamination and cannot grow surface water free of fecal contamination (*30*). The microbial water standards used by the UFPA and the EPA are based upon the presence and levels

of *E. coli* in water for agricultural and recreational use (*12, 13, 17*). Water that will not come into contact with tomato fruit, such as in trickle irrigation, must have less than 126 colony-forming units (CFU)/100 ml of water. Any agricultural water that will contact fruit most be potable: *E. coli* levels much be undetectable (less than 0 CFU/100 ml) *(12,13)*.

Petrifilms serve as an alternative method to the use of ISO culture-based methods and are an industry-recognized means of measuring indicator organisms (*24*). Composed of dehydrated MacConkey-based media on disposable foils, the media in Petrifilms contain a cold water-soluble gelling agent, bile salts to select for *Enterobacteriaceae*, and violet red to suppress gram-positive bacteria (*24*). The inclusion of additional indicators in the media (depending on the type of Petrifilm) facilitates the enumeration of various organisms (*1, 2, 3*).

To the best of our knowledge, there is little scientific information addressing the effect of microbial water quality on the presence and quantity of fecal indicator organisms on the tomato fruit surface. This information is essential to determine the validity of microbial standards for water to be used in tomato production. In this study the level of several fecal indicator organisms in three water sources (two ponds and one groundwater source) were compared to the number of indicator organisms on the surfaces of grape tomatoes treated with the above sources. Water samples were taken and tested frequently during the sampling seasons to determine the influence of seasonality and various environmental variables on the counts of fecal indicator organisms. To determine the effect of microbial water quality on the surface contamination of grape tomato fruit, water from the three water sources was used in pesticide applications on the tomato plants.

Materials and Methods

     Field Design. Field studies for 2009 and 2010 were completed at the University of Maryland's Wye Research and Education Center in Queenstown, Maryland (38° 56', 76° 07'). The soil at the site was classified as a Nassawango silt loam (fine-silty, mixed, semi-active, mesic, Typic Hapludult). Plots were arranged in a randomized complete block design with 5 blocks along a moisture gradient; each block was spaced 9.1 m from the next and contained three experimental units located 9.1 m apart. During the 2009 growing season, each of the experimental units was comprised of paired rows located 1.8 m apart. Each row contained a grape tomato cultivar ('Juliet'). In 2010, the experimental units were re-randomized under the same block design and each treatment plot contained only one row of five 'Juliet' grape tomato plants, with an in-row spacing of 0.61 m. In 2009 and 2010 the greenhouse grown transplants were planted on June 10 and June 2, respectively. Plants were grown on black agricultural plastic mulch and trained with a four-string stake system, as per DelMarVa's recommended production practices. When needed, the field plot was trickle irrigated and fertigated using well water.

     The tomato field was placed on a 7 to 14 day spray schedule. Water was sampled from the same three water sources used for the pesticide treatments applied to the field plot: a groundwater (well) source (W-G), a surface pond (W-S), and a pond that was treated with copper sulfate (Cutrine Ultra) (W-CS) as an algaecide on August 25, 2009 and June 4, 2010**.** Water from each of these sources was mixed separately with standard agricultural chemicals (Appendix A) and applied to plots with a CO_2-pressurized boom sprayer. Each treatment was applied with a separate sprayer manifold consisting of nozzles, hoses, and a tank. In 2009, the spray treatments

were applied on July 2, July 14, July 28, August 9, August 20, August 30, and September 10. In 2010, the spray treatments were applied on July 26, August 8, August 22, August 30, and September 7.

Water Sampling. In 2009, 50 ml water samples were collected from the source in sterile centrifuge tubes on a biweekly basis from June 4 to July 20 (Appendix A). From then on sampling occurred weekly until September 21 (with the exception of August 2). At each sampling date in 2009, one sample of surface water was collected aseptically at three locations around each of the ponds**.** On August 17, 2009, repetitions were added for the water samples, one repetition was taken at each of three points around the pond.

Sampling during the 2010 season occurred biweekly from June 2 to July 19, and weekly once tomato sampling began from July 27 to September 15 (Appendix A). Three replications of surface water samples were taken at the site of water collection for agricultural contact water.

Before sampling at each location in the two surface water sources, water was agitated with effort to minimize disruption to the silt. Water temperature and pH were recorded at each sampling site using a handheld pH/ORP meter, model HI98121 (*Hanna Instruments*, Woonsocket, RI). At each sampling date, three well water samples were taken from a faucet located within the chemical preparation room. Air temperature and precipitation were recorded daily at a permanent weather station at WREC. Precipitation measurements were taken with NOAA IV Precipitation Gauge (*ETI Instrument Systems*, Fort Collins, CO). The air temperature measurements were taken with a temperature and relative humidity probe, HMP45C-L (*Campbell Scientific*, North Logan, UT). The maximum air temperature of the previous day and

the total precipitation of the three days prior to sampling were used in the environmental calculations.

During the 2010 sampling season, samples of each water source were taken directly from the sprayer, termed "spray catches". Three samples of each source were taken on July 27, August 10, August 23, and September 8, 2010. After filling the appropriate 3-gallon canister to capacity with treatment water, water was run through the spray manifold before sampling. Spray catch samples were collected directly from one of five nozzles (the other four nozzles were covered), before pesticides were added to the canisters. The canisters and spray manifolds were rinsed out with sample water and stored with lids until the next use.

Sampling. Aseptic sampling of ripe fruit occurred at seven dates in 2009, weekly from July 20 (Julian day 200) to September 14 (Julian day 256), and at six dates in 2010, weekly from July 28 (Julian day 208) to September 8 (Julian day 251). During the 2009 sampling year all tomatoes were harvested ripe (with the exception of July 20, in which tomato samples were picked green). During the 2010 sampling year, all sampling dates consisted of a sample of ripe tomatoes.

A sample consisted of six tomatoes (calyx intact) cut with ethanol-sterilized scissors from various locations on the plants and aseptically placed into a Whirl-pak® bag. Between each sample, scissors were disinfected with ethanol. To prevent contamination, gloves were changed between replicates and the tomatoes were never touched with gloves directly (they were handled only within the bag). Once harvested, the samples were kept at 5°C for a maximum of four hours, until they could be weighed and processed in the laboratory. Grape tomato phyllosphere washes were recovered by adding 100 ml of sterile water (in 2009) or phosphate-buffered saline

(PBS, in 2010) to each tomato sample. Each sample was then carefully massaged until the entire surface of each tomato, including under the calyx, was thoroughly wetted (for approximately one minute).

Enumeration of *Enterobacteriaceae,* Total Coliforms, Fecal Coliforms and *E. coli.* 3M petrifilms®(3M, St. Paul, MN) were used to quantify the number of colony-forming units (CFU) of *Enterobacteriaceae,* total coliforms and fecal coliforms on the tomato fruit surface in 2009 and 2010. A 1.0 ml aliquot from each serial dilution (ranging from 10^0 to 10^{-3}) of fruit surface washwater was plated onto each of the *Enterobacteriaceae* and total coliform petrifilms, which were then incubated for 24 ± 2 hours at $38 \pm 1°C$. Fecal (thermotolerant) coliforms were enumerated by plating 1.0 ml of washwater on total coliform petrifilms, and incubating for 24 ± 2 hours at $44 \pm 1°C$. In the 2010 sampling season, *E. coli* were enumerated by plating 1.0 ml of washwater on *E. coli* petrifilms, and incubating for 48 ± 2 hrs at $38 \pm 1°C$. Serial dilutions of water samples ($10^0 – 10^{-2}$) were plated in the same manner as the fruit surface washes. After incubation, bacterial colonies were counted using a stereomicroscope, per the manufacturer's instructions (*1, 2, 3*). If counts could not be performed immediately after removal from the incubator, petrifilms were placed in a freezer at -20°C until they could be enumerated.

Enrichment and qualification of *Salmonella* spp. RapidChek® *Salmonella* Test Kit (Strategic Diagnostics Inc., Newark, DE) was used to test for *Salmonella* spp. in water and phyllosphere samples. Samples were processed according to manufacturer's instructions (*26*)**.** *Salmonella* tests using the RapidChek *Salmonella* TestKit were completed on water samples on the dates of June 29, August 17, and August 31, 2009, and July 19 and August 23, 2010. The surface of grape tomato fruits were tested on the dates of August 17 and August 31, 2009, and August 23, 2010.

Statistical analysis. *Enterobacteriaceae,* total coliforms, fecal coliforms, and *E. coli* counts from the water samples and tomato phyllosphere were log transformed prior to statistical analysis. An analysis of variance (ANOVA) was performed using PROC MIXED procedure of the SAS version 9.2 (SAS institute, Cary, NC, USA), to determine the effect of sampling date and treatment on bacterial counts. Environmental variables were analyzed using the stepwise regression procedure (PROC REG) to determine the relationships between maximum air temperature, pH, water temperature, date, and precipitation on bacterial levels in water samples.

Results and Discussion

Microbial Water Quality in 2009

In 2009, the counts of *Enterobacteriaceae* fluctuated between 3.11 and 5.11 log CFU/100 ml over the sampling season for W-CS and between 3.27 and 4.29 log CFU/100 ml for W-S (Fig. 1). Counts of total coliforms fluctuated between 2.30 and 4.47 log CFU/100 ml for W-CS and between 2.60 and 4.08 log CFU/100 ml in W-S (Fig. 2). As seen in Figure 3, the counts of fecal coliforms were lower than the total coliform and *Enterobacteriaceae* counts. Although levels of fecal coliforms in W-CS fluctuated between 0 and 3.58 CFU/100 ml, and W-S fluctuated between 2.00 and 3.77 log CFU/100 ml (Fig. 3), the counts in two surface water samples (W-S and W-CS) were not significantly different.

Microbial Water Quality in 2010

As mentioned above, in all of the 2010 sampling dates W-G samples had undetectable levels of the measured fecal indicator organisms (Fig. 4 to 7). Counts of *Enterobacteriaceae* in W-S fluctuated between 3.04 and 4.93 log CFU/100 ml and W-CS samples varied between 3.50 and 6.30 log CFU/100ml in the 2010 sampling season (Fig. 4). The total coliform counts in W-S ranged between 2.00 and 4.54 CFU/100 ml and W-CS fluctuated between 2.00 and 5.42 log CFU/100 ml (Fig. 5). The counts of both

fecal coliforms and *E. coli* fluctuated markedly during the course of the 2010 season, but were generally

lower than the *Enterobacteriaceae* and total coliforms counts. These results can be explained by the

specificity of the fecal indicator organisms: *Enterobacteriaceae* and total coliforms are broadest and

second broadest groups, respectively, therefore they are expected to have the higher counts. Fecal

coliforms in W-CS ranged between 2.00 and 5.16 log CFU/100 ml, and the *E. coli* fluctuated between 0

and 2.60 log CFU/100 ml (Fig. 6 to 7). At days 208 and 228, W-S samples had significantly lower counts

than W-CS samples. In the W-S samples, fecal coliforms ranged from 0 to 4.53 log CFU/100 ml, and the

E. coli counts fluctuated between 0 and 4.1 log CFU/100 ml during 2010 sampling (Fig. 6 to 7). The *E.

coli* counts, measured only in the 2010 season, showed significant variation over both source and date,

with   W-S ranging from 0 log CFU/100 ml to 3.00 log CFU/ 100 ml, and W-CS ranging from 0 log

CFU/100 ml to about 2.5 log CFU/100 ml (Fig. 7).

 Water Quality Discussion

    Samples from the three water sources showed significant differences in counts of

Enterobacteriaceae, total coliforms, and fecal coliforms. These results were caused by a significant effect

of source and date, and an interaction between the two main effects. Despite the significant interaction

between source and date, there were clear similarities in the bacterial counts over the course of the 2009

and 2010 sampling seasons. In both seasons, the log-transformed counts of the fecal indicator organisms

on the groundwater samples (W-G) represented colony counts of 0 CFU/100 ml, or counts under our

detectable limit. Consequently, the W-G colony counts did not vary throughout the season and samples

had significantly lower bacterial counts than the pond water (W-S) and copper-sulfate treated pond water

(W-CS) samples (Fig. 1 to 7). Among both 2009 and 2010, the levels of *Enterobacteriaceae* and total

coliforms did not differ significantly between W-S and W-CS, the two surface water sources (Fig. 1 to 2;

4 to 5).

Bacterial counts from W-G were significantly lower than those from W-CS and W-S in 2009 and 2010, which was expected as groundwater sources often have a lower risk of fecal contamination. Groundwater sources are not open to surface contamination and they benefit from the soil's natural ability to filter out pathogens (*14, 29).* Surface water sources, in contrast, are uncovered and can be inhabited by wildlife and are therefore at higher risk of contamination. These factors might explain the higher levels of *Enterobacteriaceae,* total coliforms, fecal coliforms, and *E. coli* observed in W-S and W-CS samples.

Enterobacteriaceae and total coliforms were variable throughout the season, reaching maximum values of up to 6 log CFU/100 ml in the W-S and W-CS samples. Fecal coliforms varied up to 5 log CFU/100 ml, showing more variability in the 2010 sampling season. Of the four fecal indicator organisms used in this study, *Enterobacteriaceae* and total coliforms are the two most inclusive groups, which likely explains the higher levels of these two organisms in the surface water samples.

Microbial Water Quality: Environmental Effecs

Environmental data from the two sampling seasons were combined into a single stepwise regression model, and run with counts of fecal indicator organisms using date and water source. Since the W-G samples had no detectable colony counts, only samples from the two surface ponds (W-S and W-CS) were included for environmental analyses. Regression data are presented in Table 2. Fluctuations in our counts were dependent upon sampling date and several environmental factors: maximum air temperature from the previous day, water temperature, and pH of the water.

The environmental variables that caused a significant change in bacterial counts differed in the two years of this study and by indicator organism (Table 2). Air temperature, water temperature, and pH had a significant effect on total coliform counts but only pH and water temperature significantly affected *Enterobacteriaceae* and fecal coliform counts. These environmental variables measured only explained up to 27% of the variability in bacterial counts in the two surface water sources used in this study. None

of the environmental variables affected the *E. coli* counts in the surface water samples, possibly due to the infrequent occurrence of *E. coli* in the water samples.

Studies have shown that bacterial inactivation is determined by several environmental factors, including light intensity, temperature, pH, and turbidity (*28*). An *E. coli* survival study in a tropical estuary showed that sunlight was the most important inactivation factor. Ultraviolet radiation (a measure of light intensity) damages microbial DNA, thereby causing inactivation. Predatory organisms such as protozoans and bacteriophages exerted considerable pressure on the *E. coli* populations, although the dissolved organic and inorganic substances in the environment did not affect *E. coli* inactivation (*10*).

Grape Tomato Data in 2009

The enumeration of bacteria from the grape tomato phyllosphere did not clearly correlate with the level of indicator organisms in the water sources used for the pesticide treatments. Whole seasonal averages for all indicator organisms studied are displayed in Table 3. Phyllosphere bacteria counts from the 2009 sampling season showed no significant difference over date, however treatment had a significant effect ($P<0.05$). In 2009, the tomatoes treated with groundwater (Phy-G) had a significantly higher number of total coliforms (3.41 log CFU/100 ml) than tomatoes treated with surface water (Phy-S)(1.29 log CFU/100 ml). Tomatoes treated with copper sulfate water (Phy-CS) had a mean count of 1.89 log CFU/100 ml total coliforms. Phy-S tomatoes had significantly higher fecal coliform counts (4.02 log CFU/100 ml) than both the Phy-G (2.67 log CFU/100 ml) and Phy-CS (2.46 log CFU/100 ml). Tomatoes had no significant differences in counts of *Enterobacteriaceae* between the three treatments in 2009 (Table 3).

Grape Tomato Data in 2010

In the 2010 sampling season date had a significant effect on *Enterobacteriaceae* and fecal coliforms washed from grape tomatoes. Additionally, the water source significantly affected the *Enterobacteriaceae* counts: the Phy-G samples had significantly lower counts of *Enterobacteriaceae* (4.32 log CFU/100 ml) than Phy-CS (5.28 log CFU/100 ml) counts. Levels of *Enterobacteriaceae* on Phy-G and Phy-S were no statistically different. The remaining three indicator organisms showed no significant differences in bacterial counts in response to treatment (Table 3).

Grape Tomato Discussion

Bacterial levels in the water source did not correspond with the bacterial levels on the grape tomato fruit surface. In most instances, the significantly higher mean bacterial counts in the two pond surfaces were not reflected in the phyllosphere. These results indicate that there are other factors affecting bacterial colonization of the tomato phyllosphere, so that the bacterial load of applied pesticide treatments may have had less effect than predicted. A five-year study of reclaimed wastewater use in agriculture reported similar results when applied to several horticultural crops *(9)*. Although the reclaimed wastewater used in that study had significantly higher total coliforms and fecal coliforms, the differences were not reflected in the soil and plant tissue samples analyzed.

Other studies have addressed the complex ecological and physical interactions between phyllosphere-associated bacteria and their host plants, indicating that several factors influence the survival of epiphytic bacteria *(11)*. The tomato phyllosphere environment is affected by environmental stresses such as UV radiation, high winds, heat, lack of moisture, as well as an acidic pH and the presence of the antimicrobials glutamic acid and tomatine *(6, 16, 27)*. These antimicrobials select for the growth for the growth of acid-tolerant microbes such as *Pseudomonas syringae pv.* Tomato, and *Lactobacillus* spp. (*27)*. The smooth texture of the tomato skin may also prevent attachment and enhance bacterial sloughing from the fruit surface *(18)*. Competition and cooperative relationships among colonized

bacteria have a role in the colonization of new bacteria, such as those contained in the contact water used to apply pesticides *(11)*. The combination of environmental factors and stresses from the tomato phyllosphere may have prevented bacteria in the pesticide water from colonizing the tomato fruit surfaces in a measurable fashion.

Pesticides used may also play a role in bacteria's persistence in tomato fruit. Studies showed that different *Salmonella* serovars and *E. coli* 0157:H7 can survive and amplify within water containing chlorothanonil (Bravo), a commonly used fungicide, and one used in the tomato treatments. When a cocktail of *Salmonella* and *E. coli* were applied to tomato plants with this pesticide mixture, the tomato fruit contained 2 log CFU/g less bacteria than the tomato leaf surfaces. Despite their survival, bacterial levels were greatly reduced on the tomato fruit after 45 hours, presumably due to the smooth surface of the tomato skin *(18)*.

Spray Catch Results

Spray catch samples were taken at four sampling dates during the 2010 season, directly from the sprayer. These samples were compared to the source water samples at the same four dates. For the *Enterobacteriaceae,* totally coliforms, and fecal coliforms, there was a significant interaction between water source, date, and sample type (P<0.0001). This indicates that the water sampled from the source and the water used in treatment applications varied significantly for these indicator organisms (Table 1). The only significant difference between spray catch samples and source samples appeared in W-G. For each of the fecal indicator organisms in this water source, measured spray catch levels were higher than the source samples (Table 1). The differences between spray source and pond source were not as large in the W-S and W-CS samples and had no significant difference (Table 1). Across the three water sources, the sample type (source versus spray catch) had no significant effect on the counts of *E. coli.*

While separate spray manifolds were used for each water source, it is possible that some contamination occurred in the storage shed. A floating white precipitate was observed in some of the

groundwater spray catch samples, even though each spray manifold was rinsed and sample water was flushed through the manifolds before each sampling. It is possible that the white precipitate is spray residue from the previous sampling. Suspended solids within a water sample increase the amount of surface area that bacteria can attach to and colonize, thus bacterial colonization is often associated with a higher degree of turbidity and suspended solids (*14, 21*). Studies have shown that turbidity is associated with total coliforms, as the increased surface area allows for attachment and the formation of biofilms that protect from antimicrobials and environmental variables *(21, 23).* Despite the efforts to prevent bacterial contamination, the spray catches for the W-G source still show elevated counts. This shows that disinfection and proper storage of pesticides and equipment could also play a significant role in preventing foodborne illnesses, regardless of the water source used.

Fecal Indicator Organisms

The fecal indicator organisms measured in this study were selected for their compatibility with current industry and water standards. However, three genera of bacteria (*Klebsiella, Enterobacter,* and *Citrobacter)* have been shown to reproduce within the environment and in water systems without requiring a fecal source, which is significant because these genera classify within the fecal indicator organisms *Enterobacteriaceae*, total coliforms and fecal coliforms *(14, 30).* Thus, these indicator organisms cannot serve as completely reliable measures of fecal contamination. In order to obtain a confident measure of microbial quality, it is advisable to use multiple indicator organisms at a time *(21).* Of the fecal indicator organisms used, *E. coli* is considered the most reliable because it is the only coliform measurement specific to fecal contamination. Unlike other organisms measured by fecal coliform tests, *E. coli* is not considered to be an environmental organism *(30).*

The WHO and UFPA have adopted microbial standards for the use of agricultural water to help prevent the contamination of tomatoes and foodborne illness outbreaks resulting from their consumption *(8, 17)*. These guidelines are based upon the EPA standards for freshwater recreational water (for non-

foliar application of agricultural water, such as trickle, furrow, or seep irrigation), allowing for a geometric mean of 126 CFU *E. coli* per 100 ml water, or 2.1 log CFU per 100 ml (*13*). The levels of *E. coli* in W-S and W-CS exceeded this standard on several dates throughout 2010 by as much as 1 log. These results demonstrate the variability in microbial levels over the course of a sampling season and the need for farmers to submit water samples for microbial analysis on multiple dates throughout the season.

The UFPA also published guidelines on the recommended level of *E. coli* allowed for water used in foliar applications of pesticides and irrigation. Since the water may directly contact the tomato fruit these standards are based upon EPA standards for potable water: 0 CFU (undetectable levels) of *E. coli* per 100 ml. Based on this standard in the 2010 season, only the groundwater samples in this study would have been suitable for surface applications during the period comprising the first three sampling dates of the season and W-S on several dates toward the end of the season. Despite that difference in suitability of the sources used for mixing pesticides, we found it difficult to demonstrate a difference in fecal indicator organisms washed from the tomato phyllosphere.

The unexpected results of this study may have been influenced by several factors. As mentioned above, some of the spray catch samples from W-G had significantly higher counts of some bacteria when compared to the source water, which had no detectable levels (Table 1). In this study we took measures to clean and separate the spray manifolds, however that may have not been enough to keep bacteria from colonizing the equipment. The volume of water used for pesticide applications was less than 5 gallons of water per source, which may not have been a large enough volume to apply a significant amount of treatment to the tomato phyllosphere. Since spray rates are regulated and the treatments were applied using standard agricultural practices, the amount of water used could not have been altered to increase output of water.

Additionally, it is possible that environmental variables not viewed in this study should be taken into consideration to predict bacterial counts, such as inactivation due to UV radiation and turbidity of the

water sources. Certain environmental variables such as pH and water temperature also have diurnal patterns which fluctuate in the course of one day *(19).* Such patterns were not measured in this study, and may explain the lack of correlation between our colony counts and measured environmental variables.

Conclusion

Our results demonstrate that the relationship between the microbial quality of agricultural contact water and the surface of grape tomatoes is not as straightforward as previously thought. Despite the significant effect that water source and date had on the counts of *Enterobacteriaceae*, total coliforms, fecal coliforms, and *E. coli* from the water samples, these differences did not correspond to the water eluted from the grape tomato fruits. Only certain indicator organisms showed a significant difference among treatments, and those results differed between the two sampling seasons. These results justify the need for frequent water testing when monitoring microbial water quality, and suggest that monitoring other sources of contamination may be as important in preventing foodborne illnesses on tomatoes as monitoring just water source.

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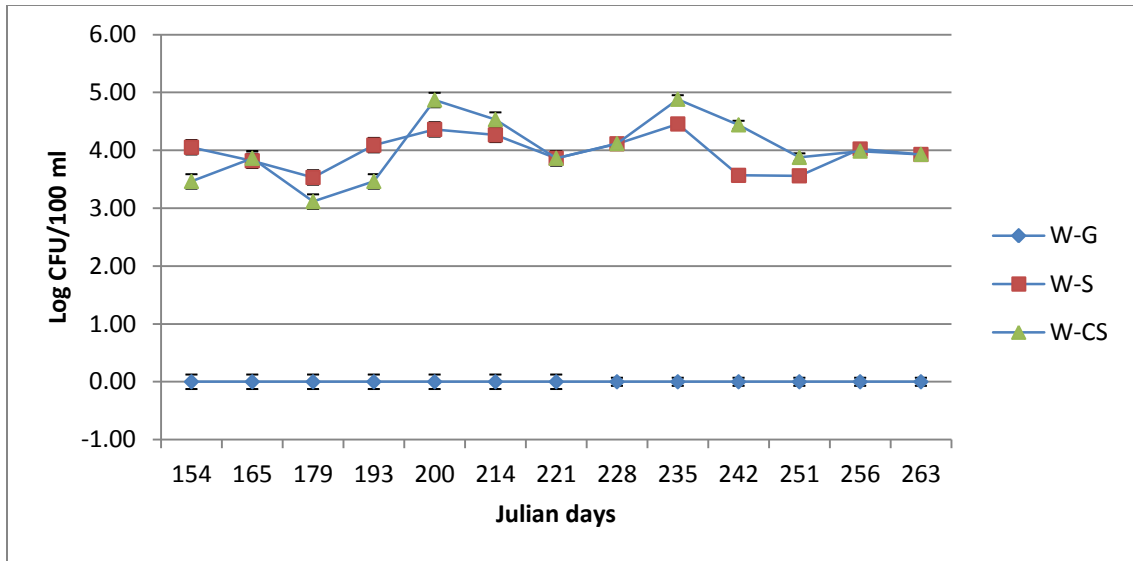
Figure 1. Change of *Enterobacteriaceae* counts over the course of the 2009 sampling season. Organized by water source: groundwater (W-G), surface pond (W-S), and a copper-sulfate treated surface pond (W-CS). Counts are the log-transformed means (per 100 ml) of three replicates.
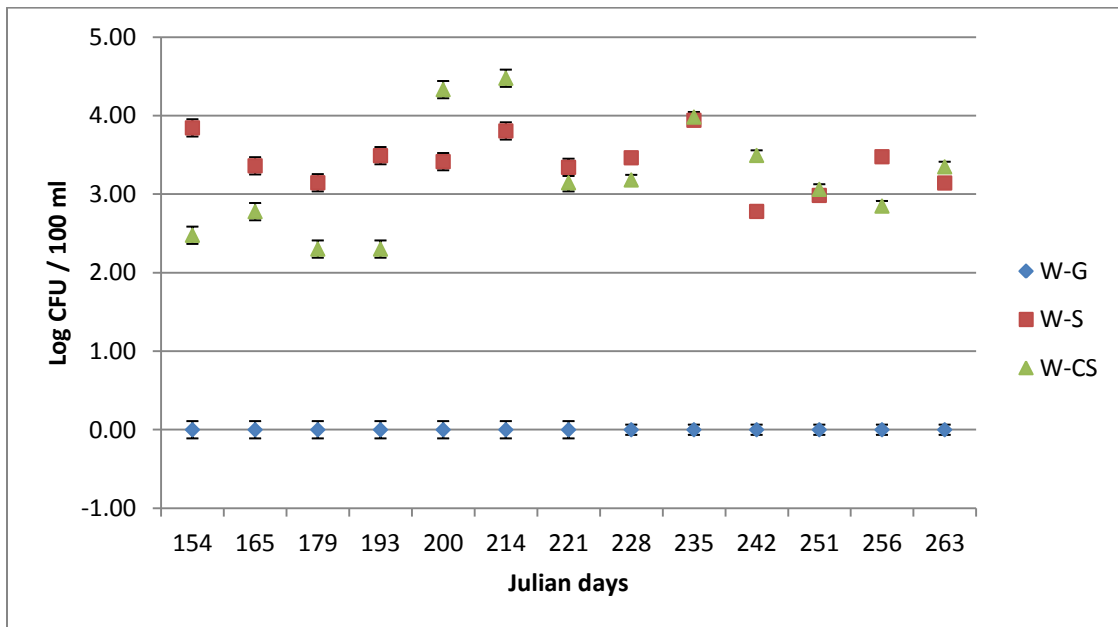

Figure 2. Change of total coliform counts over the course of the 2009 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.
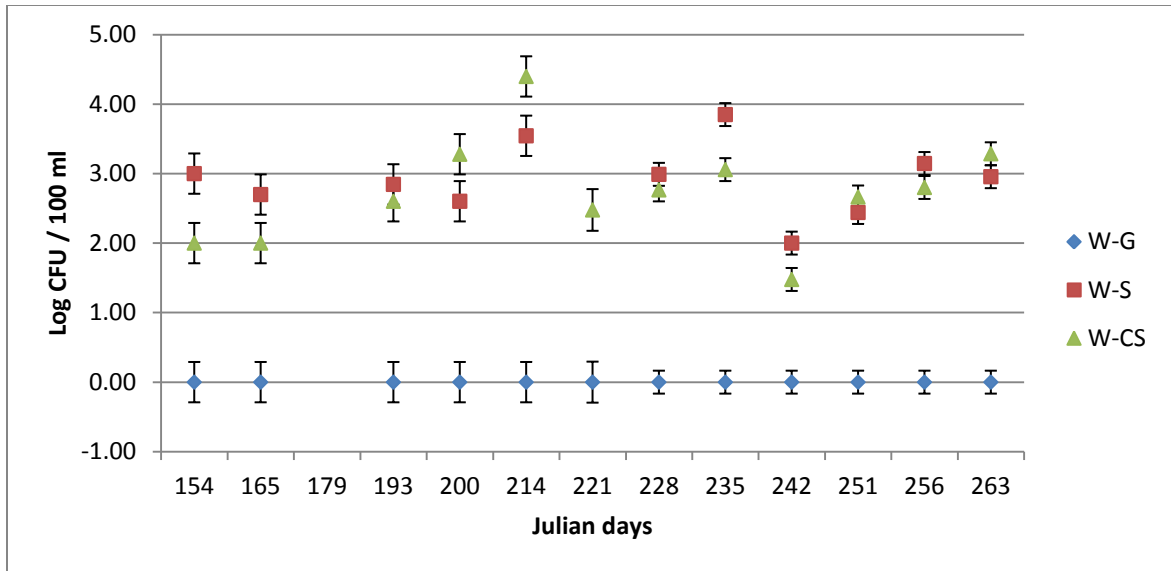
Figure 3. Change of fecal coliform counts over the course of the 2009 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.
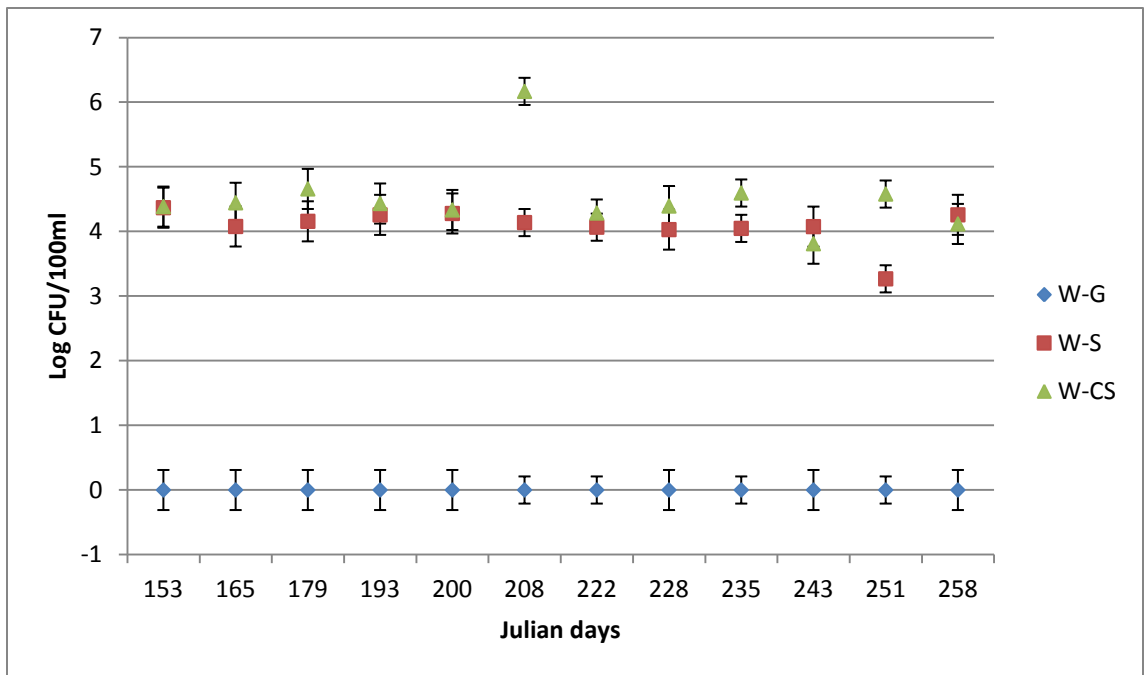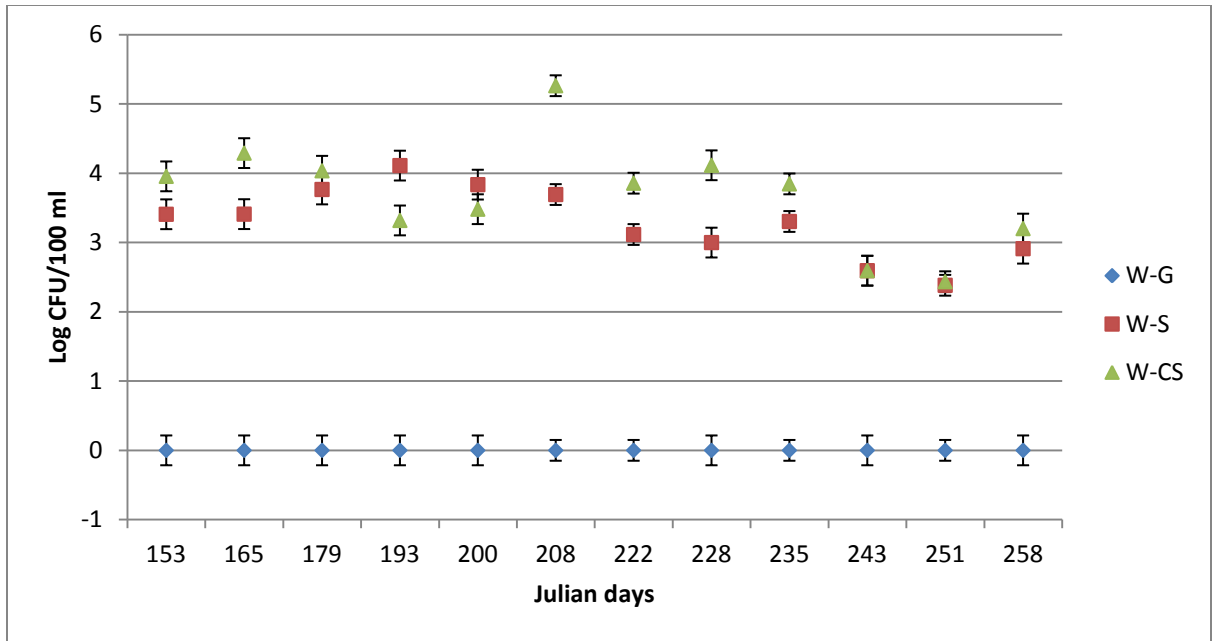

Figure 4. Change of *Enterobacteriaceae* counts over the course of the 2010 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.
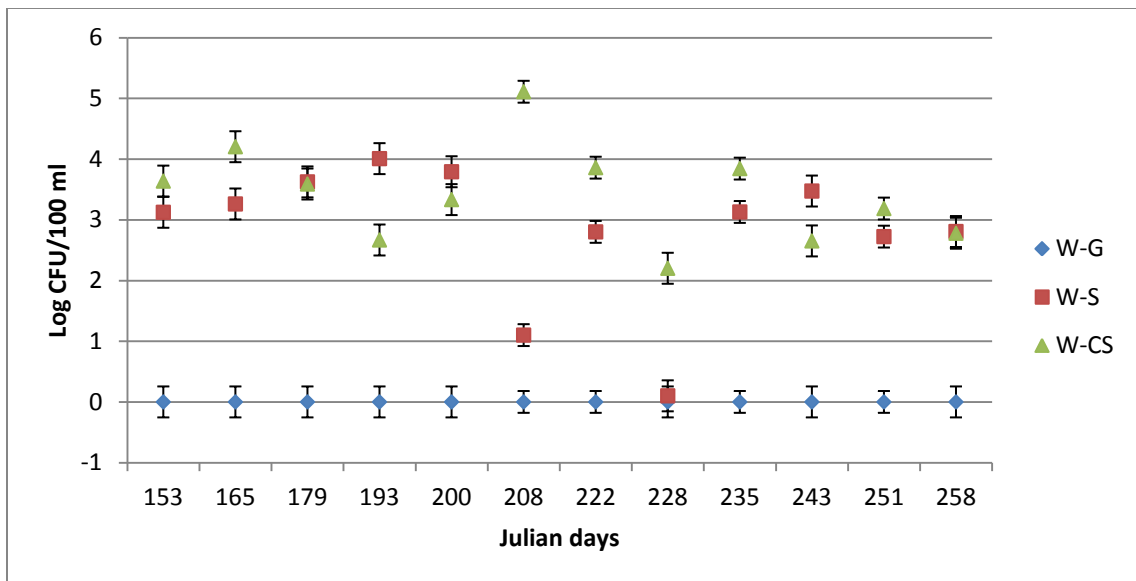
Figure 5. Change of total coliform counts over the course of the 2010 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.

Figure 8. Change of fecal coliform counts over the course of the 2010 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.
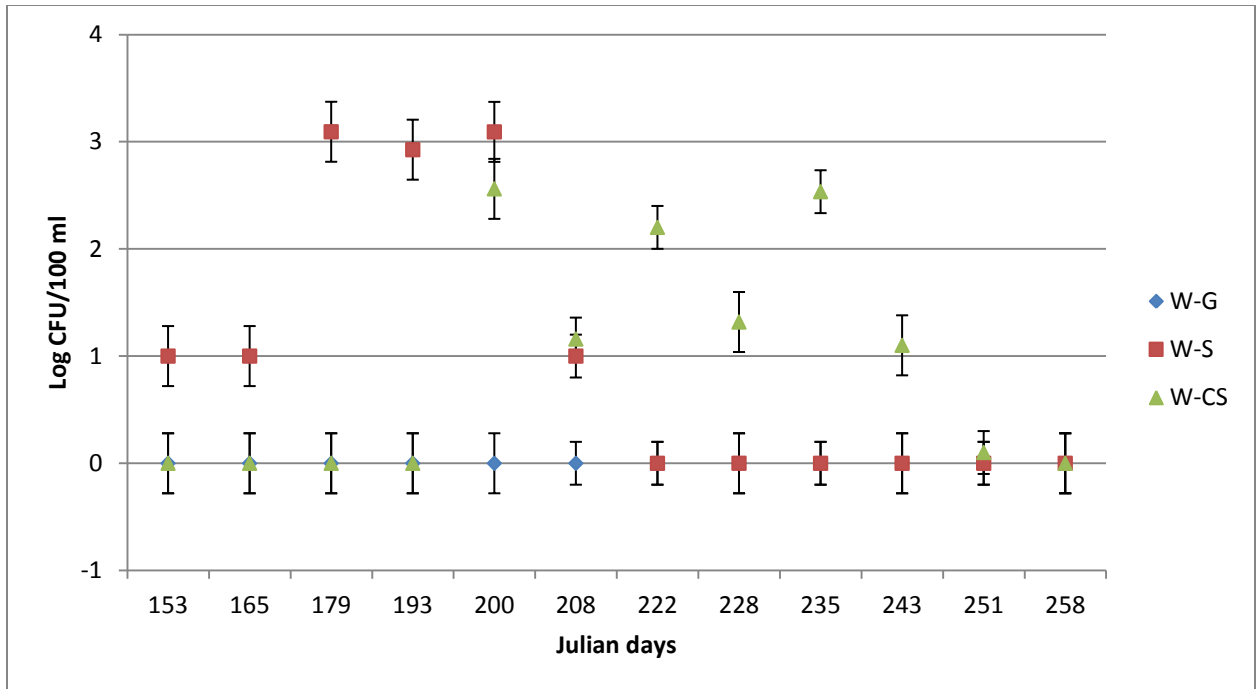
Figure 7. Change of *E. coli* counts over the course of the 2009 sampling season. Organized by water source: W-G, W-S, and W-CS. Counts are the log-transformed means (per 100 ml) of three replicates.

Tomato Treatment	Sample Type	*Enterobacteriaceae**	Total Coliforms*	Fecal coliforms*	*E. coli*
W-G	Source	0.00a	0.00a	0.00a	0.00a
	Spray catch	3.40b	0.78b	1.37b	0.00a
W-S	Source	3.88a	3.12a	2.44a	0.00a
	Spray catch	3.81a	2.95a	2.55a	0.00a
W-CS	Source	4.91a	3.85a	4.00a	1.50a
	Spray catch	5.05a	4.40a	4.52a	1.78a

Different letters within a column indicate significant differences.

Table 1. Level of four indicator organisms in two sample types (source and spray catch) across three water sources. Log-transformed data is reported as log CFU/100ml, and is the mean of three repetitions and four sampling dates in 2010 (Tukey means comparison test, $P<0.05$).

Indicator Organism	Intercept	pH	Water Temp (°C)	Max Air Temp (°C)	Model R^2
Enterobacteriaceae	4.26 (<.0001)	-0.14 (0.03)	0.05 (0.0002)	NS	0.21
Total Coliforms	3.81 (<.0001)	-0.21 (0.01)	0.13 (<0.0001)	-0.06 (0.002)	0.27
Fecal Coliforms	-1.33 (0.20)	0.29 (0.003)	0.07 (0.007)	NS	0.13
E. coli	NS	NS	NS	NS	NS

Note: Numbers in parenthesis are p-values from an Analysis of Variance.

Variables with P>0.05 were marked as Non-significant (NS).

Table 2. Change in *Enterobacteriaceae,* total coliforms, fecal coliforms, and *E. coli* as described by several environmental variables over the 2009 and 2010 sampling seasons. Values are the coefficients of a regression equation (following a stepwise regression).

Year	Tomato Treatment	*Enterobacteriaceae*	Total Coliforms	Fecal Coliforms	*E. coli*
2009	Phy-G	4.97a	3.40a	2.66a	---
	Phy-CS	4.56a	1.89ab	2.45a	---
	Phy-S	4.83a	1.28b	4.01b	---
2010	Phy-G	4.32a	2.40a	2.10a	0.00a
	Phy-CS	5.28b	1.89a	2.44a	0.00a
	Phy-S	4.76ab	2.27a	2.52a	0.00a

Means followed by different letters are significantly different (P<0.05) based upon Tukey analysis.

Table 3. Summary of 2009 and 2010 fecal indicator organism counts on grape tomato fruit surface, organized by water source treatments. Means are presented as Log CFU / 100 ml water.

Appendix A.

2009 Work Log/Spray Treatments

Spray treatments		Water Sampling	Fruit Sampling
		June 4	
June 10: Planted tomato plants			
		June 15	
		June 29	
July 2	Chlorothalonil + Spinosad		
		July 13	
July 14	Chlorothalonil + Spinosad		
		July 20	July 20
July 28	Chlorothalonil + Spinosad + Pyraclostrobin		
		August 3	August 3
August 9	Chlorothalonil + Propamocarb hydrochloride+ Spinosad		
		August 10	August 10
		August 17	August 17
August 20	Chlorothalonil + Spinosad + Abamectin		
		August 24	August 24
August 25: Treat copper sulfate pond (W-CS)			
August 30	Chlorothalonil + Spinosad + Abamectin		
		August 31	August 31
		September 9	September 9
September 10	Chlorothalonil + Spinosad + Abamectin		
		September 14	September 14
		September 21	

2010 Work Log/Spray Treatments

Spray treatments		Water Sampling	Fruit Sampling
		June 2	
June 4: Treat copper sulfate pond (W-CS)			
Planted: June 10			
		June 14	
		June 28	
		July 12	
		July 19	
July 26	Chlorothalonil + Spinosad	July 26	
August 9	Chlorothalonil + Spinosad	August 9	
			August 10
August 15	Water treatments (no pesticides)	August 15	
			August 16
August 22	Chlorothalonil + Spinosad	August 22	
			August 23
August 30	Chlorothalonil + Spinosad	August 30	
			August 31
September 7	Chlorothalonil + Spinosad	September 7	
			September 8
		September 15	