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Reducing *Fusarium* spp. Inoculum in Irrigation Systems: A Sanitation Case Study in Greenhouse-grown Tomatoes

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***Fusarium* spp. fungi cause several serious diseases in tomato in Florida, notably Fusarium wilt and crown rot. The fungus persists in soil and crop debris and requires intensive efforts to eradicate propagules on field and greenhouse equipment and implements. Samples of greenhouse-grown tomatoes from Suwannee County were diagnosed with a systemic Fusarium disease by the University of Florida–IFAS (UF–IFAS) Plant Disease Clinic in 2009. The plants were grown in coconut fiber-filled lay-flat plastic bags and irrigated by drip emitters at the top of the media. Although the system does not utilize recycled irrigation water, all other components of the irrigation system were reused with each new crop and thus were suspected of contributing to the perennial Fusarium disease occurrence. *Fusarium* spp. were isolated from inside the plastic tubing and the planting media trapped in the ridged shape of the irrigation stakes. Subsequent samples of irrigation stakes were collected and subjected to six sanitization regimens including pressure-washing with and without soaking in sanitizing solutions. We found that washing the stakes to remove planting media and plant debris prior to treatment with any sanitizing solution reduces the inoculum to below detectable levels. Although preliminary, this testing indicates that incorporation of this simple and low-cost step into sanitation protocols may reduce carry-over of Fusarium disease inoculum from one crop to subsequent crops.**

Background

Fresh-market cluster tomatoes are among the most valuable vegetable crops produced in Florida (Pernezny and Roberts, 2008). Fusarium fungi can cause persistent, common fungal diseases of greenhouse tomatoes. Fusarium crown and root rot, caused by the fungus *Fusarium oxysporum* f. sp. radicis-lycopersici Link was first detected in Florida in 1974. The disease has been reported in all major tomato growing areas of the state, but is particularly serious in the acidic, sandy soils of Florida's southern production regions. It is a serious problem for seedling and greenhouse fruit production, and can cause significant yield decreases in field-grown, staked tomatoes in Florida (Zhang et al., 2011). Fusarium crown rot first appears when fruit are setting or sizing. Symptoms include lower leaf marginal yellowing and a slow-to-rapid wilt syndrome that kills the plant (Fig. 1). The lower stem at soil line exhibits vascular discoloration and pith necrosis for a variable distance upward in the stem (Momol and Pernezny, 2006). Fusarium diseases are difficult to manage due to the persistence of the fungi and the fact that few resistant tomato varieties are

Fig. 1.

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available to greenhouse producers. Prevention through sanitation is a critical management step.

This study was conducted in cooperation with Beli Farms in Wellborn, FL. Beli Farms, a greenhouse operation, has been in business for the last 23 years and grows 4 acres of cluster tomatoes and an additional acre of mini cucumbers annually. Most of the greenhouse structures on the farm are covered with polyethylene and are passively ventilated with screened sidewalls and peak ridge vents. The heating system is a hot water system re-circulating through pipes at ground level. Fertilizer is delivered through the irrigation system, which is comprised of polyethylene lines with an individual emitter tube at each plant. The emitter tubing is attached to a plastic stake anchored into the growing media (Fig. 2). When harvest of plants is complete, the stake end is inserted into the emitter tubing to plug the end and cease irrigation at that emitter (Fig. 3).

Extension agents were asked to help identify the cause of a severe disease outbreak in the spring of 2009 that had led to significant loss of revenue, estimated at $150,000. Samples of 'Success' and 'Levanzo' cluster tomatoes submitted to the UF–IFAS Extension Plant Disease Clinic in Gainesville in spring 2009 resulted in isolates of *Fusarium* spp. from plant material and irrigation stakes and tubing. Once the season finished in the summer of 2009, Beli Farms implemented strict sanitation measures to reduce the inoculum of *Fusarium* spp., such as replacing floor plastic and coconut fiber slabs, and using a sanitizing solution to wash all growing areas. However, when the next growing season started in Nov. 2009, it was evident that *Fusarium* spp. were present in the crop. A team of UF–IFAS Extension specialists and the grower met to determine the source of the inoculum. The team discussed the potential role the irrigation system played in serving as a host to the inoculum from one growing season to the next.

Fig. 2.

Fig. 3.

Purpose

This experiment was conducted to evaluate sanitizing regimes for control of *Fusarium* spp. associated with irrigation stakes in a greenhouse tomato production facility.

Materials and Methods

Irrigation stakes representing nine areas of the greenhouse were tested for the presence of *Fusarium* spp. during the spring of 2010. Stakes were washed with a high-pressure nozzle on a household garden-type hose to remove soil debris and then the stakes were sampled for the presence of the pathogen. In order to determine how to disinfect the stakes, household bleach and a quaternary ammonium compound were evaluated for their ability to control *Fusarium* spp. Additional stakes from the same treatments were exposed to the test products and water control overnight (12 h) and over two nights (36 h). Stakes were sampled for the presence of *Fusarium* spp. in two ways: 1) Plant debris was scraped from the stakes with a sterile swab, plated with four replicates per plate onto two petri dishes of general fungal isolation media, and incubated for 5 to 7 d at 25 °C; 2) The debris adhering to the stakes was suspended in water and the solution was cultured on APDA and a semi-selective medium for *Fusarium* spp., Komada's medium. Five replicate plates were cultured from each sample, and were incubated at 25 °C for 5 to 7 d. Colony counts and additional observations were recorded.

Stakes were separated into subsamples of five stakes from each of nine samples. The stakes for treatments 1 through 3 were washed with a high-pressure nozzle attached to a hose. The treatments were: T1, washed then soaked in water; T2, washed then soaked in 6% sodium hypochlorite; T3,washed then soaked in 0.3% alkyl dimethyl benzyl ammonium chloride; T4, unwashed, soaked in water; T5, unwashed, soaked in 6% sodium hypochlorite; T6, unwashed, soaked in 0.3% alkyl dimethyl benzyl ammonium chloride. Stakes were exposed to 24 and 48 h soaking in the test solutions. After the soak period, 0.01 g debris was recovered from the stakes. The debris was suspended into 1 mL of water, vortexed for 20 s, and then 100 µL of the debris suspension was plated onto PDA medium. The inoculated plates were incubated at 25 °C and examined for the presence of typical *Fusarium* spp. colonies.

Results and Discussion

Fusarium spp. colonies were recovered from stakes in five of the nine areas tested (Table 1). It was observed that washing the stakes with a high pressure nozzle attached to a hose reduced the colony recovery to <200 colonies per gram of debris. However, even low inoculum levels such as those found can be enough to incite disease. When the washed stakes were treated with either bleach or the quaternary ammonium compound, the recovery of *Fusarium* spp. colonies dropped to zero (Table 2). When the

Table 1. Number of *Fusarium* spp. colonies recovered from irrigation stakes prior to treatment (% out of 8 possible).

Sample no.[z]	Cultured using PDA	Cultured using Komada's medium	%[y]
S1 (ES, High Probability)	0	0	0
S2 (DS2, High Probability)	2	3	62.5
S3 (DS1, High Probability)	0	2	25
S4 (DS3, High Probability)	0	1	12.5
S5 (CC, High Probability)	2	4	75
S6 (DN Random, High Probability)	4	1	62.5
S7 (DS Random)	0	0	0
S8 (EN Random)	0	0	0
S9 (CC Random)	0	0	0

[z]Represents areas of the greenhouse facility and probability of finding *Fusarium* spp. based on previous samples.
[y]% indicates number out of a total of 8 possible.

Table 2. Total number of *Fusarium* spp. colonies recovered from irrigation stakes after being exposed to various treatments.

	Sample no.								
Treatment[z]	S1	S2	S3	S4	S5	S6	S7	S8	S9
12-hour exposure									
T1	0	0	0	0	0	0	0	2	0
T2	0	0	0	0	0	0	0	0	0
T3	0	0	0	0	0	0	0	0	0
T4	5	1	2	8	4	2	5	1	7
T5	0	0	0	0	0	0	0	0	0
T6	0	0	0	0	0	0	0	0	0
36-hour exposure									
T1	0	0	0	2	0	0	0	0	0
T2	0	0	0	0	0	0	0	0	0
T3	0	0	0	0	0	0	0	0	0
T4	0	0	11	3	11	9	7	1	3
T5	0	0	0	0	0	0	0	0	0
T6	0	0	0	0	0	0	0	0	0

[z]Treatments: T1, washed then soaked in water; T2, washed then soaked in 6% sodium hypochlorite; T3, washed then soaked in 0.3% alkyl dimethyl benzyl ammonium chloride; T4, unwashed, soaked in water; T5, unwashed, soaked in 6% sodium hypochlorite; T6, unwashed, soaked in 0.3% alkyl dimethyl benzyl ammonium chloride.

stakes were not washed prior to treatment, the colony recovery was comparatively high per gram of debris. But when unwashed stakes were treated with either disinfecting compound, *Fusarium* spp. colony recovery was zero. Thus, the use of a sanitizing solution is recommended to control *Fusarium* spp. The length of time (12 h vs. 36 h) the stakes were soaked in the sanitizing solution did not make a difference in controlling Fusarium in this study.

Conclusion

The team was able to identify a previously-unknown source of inoculum of *Fusarium* spp. on the irrigation stakes and in the irrigation tubing. The use of household bleach and a quaternary ammonium compound proved effective in controlling *Fusarium* spp. when used at the recommended rates. The grower took the stakes out and dipped them in bleach and ran bleach through the irrigation system to sanitize the inside of the tubing between seasons when no crop was present. The use of any disinfectant to eliminate the presence of fungal pathogens is dependent upon the amount of active compound available after the organic material in the debris has interacted with the disinfectant. If sodium hypochlorite is used in conjunction with high pressure washing to eliminate the *Fusarium* colonies, the free (available) chlorine should exceed 5 ppm. Results from this trial allowed the farmer to implement sanitation practices after the 2010 season that now effectively control *Fusarium* spp. and reduce infection in the following season.

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