Epidemiology and management of bacterial leaf spot on watermelon caused by *Pseudomonas syringae*

E. A. Newberry, L. Ritchie, and B. Babu, North Florida Research & Education Center, University of Florida, Quincy; T. Sanchez, K. A. Beckham, and J. B. Jones, Department of Plant Pathology, University of Florida, Gainesville; J. H. Freeman, North Florida Research & Education Center, University of Florida, Quincy; N. S. Dufault, Department of Plant Pathology, University of Florida, Gainesville; and M. L. Paret, North Florida Research & Education Center, University of Florida, Quincy

Abstract

Bacterial leaf spot of watermelon caused by *Pseudomonas syringae* has been an emerging disease in the southeastern United States in recent years. Disease outbreaks in Florida were widespread from 2013 to 2014 and resulted in foliar blighting at the early stages of the crop and transplant losses. We conducted a series of field trials at two locations over the course of two years to examine the chemical control options that may be effective in management of this disease, and to investigate the environmental conditions conducive for bacterial leaf spot development. Weekly applications of acibenzolar-*S*-methyl (ASM) foliar, ASM drip, or copper hydroxide mixed with ethylene bis-dithiocarbamate were

effective in reducing the standardized area under the disease progress curve ($P < 0.05$). Pearson's correlation test demonstrated a negative relationship between the average weekly temperature and disease severity (-0.77 , $P = 0.0002$). When incorporated into a multiple regression model with the square root transformed average weekly rainfall, these two variables accounted for 71% of the variability observed in the weekly disease severity ($P < 0.0001$). This information should be considered when choosing the planting date for watermelon seedlings as the cool conditions often encountered early in the spring season are conducive for bacterial leaf spot development.

Bacterial leaf spot of watermelon caused by *Pseudomonas syringae* has been an emerging disease in the southeastern United States in recent years. It was first reported in Florida during the spring 2013 season, and was subsequently discovered in Georgia in 2015 and North Carolina in 2016 (Dutta et al. 2016; Newberry et al. 2015; Quesada-Ocampo 2016). The symptoms consist of circular necrotic lesions, sometimes with a white to tan center, surrounded by a chlorotic halo (Supplementary Fig. S1). As the disease progresses, these lesions become irregular in shape and may encompass the entire leaf as a foliar blighting. Disease outbreaks in Florida were widespread in 2013 and 2014, and resulted in severe blighting at the early stages of the crop leading to transplant losses. Additionally, these epidemics were associated with an increase in production costs estimated at \$60 to \$100 per acre due to additional chemical inputs necessary for management of the disease (Newberry et al. 2015).

Foliar diseases of various cucurbits associated with *P. syringae* are generally referred to as angular leaf spot (Williams 1996); however, the disease has also been reported as bacterial leaf spot and bacterial blight depending upon the host and manifestation of symptoms (Lamichhane et al. 2015). Recent characterization of *P. syringae* strains associated with disease outbreaks on watermelon, cantaloupe, squash, and cucumber has shown that the pathogen is genetically diverse (Fatmi et al. 2008; Newberry et al. 2016; Slomnicka et al. 2015), and phylogenetically distinct from the organism originally described as the causal agent of the disease, *P. syringae* pv. *lachrymans* (Bradbury 1986; Williams 1996). Examination of the host range of *P. syringae* strains recovered from symptomatic cucurbit tissue has shown that the pathogen has a broad host range, and can infect most members of the Cucurbitaceae family (Hopkins and Schenk 1972; Morris et al. 2000; Newberry et al. 2016).

In 2015, watermelon was among the three largest crops in total vegetable and melon production in the United States. Each year, Florida and Georgia are consistently among the top watermelon producers nationally, with the crop being grown on 16,600 ha that had a farm gate value of \$170 million in 2015 (USDA 2016). Due to the increasing demand for hybrid varieties, the transplant production industry has become an integral part of the production of watermelon and other vegetable crops in the southeastern United States (McAvoy and Ozores-Hampton 2015). *P. syringae* pv. *lachrymans* is commonly considered to be seed-borne, as seed to seedling transmission of the pathogen to cucurbits in both naturally and artificially infested seed has been documented over the years (Bhat et al. 2010; Leben 1981; Shila et al. 2013). The high plant populations found in transplant production facilities, combined with dense spacing, humid conditions, and overhead irrigation make transplant production an important factor in the dissemination of seed-borne bacterial pathogens (Walcott 2008). *P. syringae* has also been associated with the water cycle, and both rain and irrigation water have been identified as potential sources of inoculum in disease outbreaks (Morris et al. 2013; Riffaud and Morris 2002).

The reported severity of angular leaf spot on numerous cucurbit crops tends to vary depending on the region and climate where the disease occurs (Bhat et al. 2010). The epiphytic population size of *P. syringae* is commonly considered to be favored by cooler temperatures and frequent rain (Hirano and Upper 1990); however, some authors have reported a positive relationship between angular leaf spot severity and temperature with optimal conditions ranging from 24 to 28°C (Hansen 2009; Wiles and Walker 1952; Williams 1996). In the southeastern United States, watermelon is primarily produced from May through July, and planting dates range from mid-December to April where seedlings may encounter cooler conditions (Elwakil and Mossler 2013). A better understanding of the specific environmental conditions favorable for the development of bacterial leaf spot as they relate to commercial watermelon production in the spring season is necessary to develop management recommendations.

Copper bactericides have been successfully used to control angular leaf spot of cucumber, and are commonly recommended as an effective chemical control option for other cucurbits as well (Bhat et al. 2010; Williams 1996). Unfortunately, the efficacy of these compounds is unclear as several authors have reported that copper bactericides did not provide economical control of angular leaf spot on cucumber, and in some cases a complete failure in disease control

Corresponding authors: M. L. Paret; E-mail: paret@ufl.edu; and N. S. Dufault; E-mail: nsdufault@ufl.edu

*The e-Xtra logo stands for "electronic extra" and indicates that one supplementary figure is available online.

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was reported on either cucumber or cantaloupe (Bhat et al. 2010; Riffaud et al. 2003). Copper hydroxide and systemic acquired resistance inducers have been identified as effective management options for bacterial fruit blotch of watermelon in greenhouse or field conditions (Hopkins 1991; Hopkins et al. 2009). As no watermelon varieties are commercially available which are resistant to bacterial leaf spot caused by *P. syringae*, understanding the efficacy of these products in managing this emerging disease will be important in reducing its impact in the southeast United States.

In response to the widespread and severe disease outbreaks occurring in Florida, the primary objective of this study was to assess the effectiveness of copper hydroxide alone or in combination with ethylene bis-dithiocarbamate (commonly called mancozeb), as well as foliar and drip applications of the plant inducer, acibenzolar-S-methyl, for the management of bacterial leaf spot of watermelon in the field. In addition to the chemical control of the disease, the environmental factors associated with the development of bacterial leaf spot were investigated. Four field trials were conducted over the course of two years and at two locations near major watermelon producing regions in Florida (Elwakil and Mossler 2013). In these trials, we simulated two potential scenarios of pathogen introduction relevant to the documented life cycle of the pathogen and cucurbit production practices in the Southeast; i) disease outbreaks are associated with the use of transplants that were infected with *P. syringae* before they reached the field (i.e., seedborne/greenhouse level contamination), and ii) disease outbreaks are the result of a field-level introduction of the pathogen.

Materials and Methods

Bacterial strains and culture. Three bacterial strains (13-139A, 13-C1, and 13-141A) recovered in Florida from diseased watermelon tissue in the epidemic of 2013 and characterized as being highly virulent on watermelon were used in each experiment (Newberry et al. 2015). To prepare the bacterial inoculum, each strain was cultured at 28°C for 24 h on King's medium B agar (KB) (King et al. 1954). Bacterial cells were harvested from the plates using a sterile glass rod and were suspended in sterile $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ solution (0.01M). The resulting cell suspension was adjusted to $\sim 10^8$ CFU/ml spectrophotometrically ($\text{OD}_{600} = 0.3$), and diluted to a final concentration of $\sim 10^7$ CFU/ml. Cell suspensions from the three different bacterial strains were then mixed together in equal proportions to create the final inoculum suspension. All bacterial strains were preserved in 30% glycerol at -80°C for long-term storage.

Experimental design. A total of four field trials were conducted in this study at the University of Florida North Florida Research and Education Center (NFREC; Quincy, FL) and the University of Florida Plant Science Research and Education Unit (PSREU; Citra, FL) during the spring of 2014 and 2015. These field trials were designed to

simulate two potential inoculum scenarios for pathogen introduction. For the first scenario in which watermelon transplants were contaminated before they reached the field, half of the seedlings at each trial were inoculated in the greenhouse 7 to 10 days before transplanting, and approximately 3 weeks before initiation of the test treatments. For the second scenario, half of the seedlings in each trial were inoculated in the field 2 weeks after transplanting, and 3 to 4 days after initiation of the test treatments to mimic a situation in which the plants became infected in the field from an external source of inoculum. For each inoculation, seedlings were sprayed with the bacterial suspension described above until run-off using a handheld sprayer. Seedlings inoculated in the greenhouse were sprayed in the seedling flats, placed in a transparent plastic bag, and incubated for 48 h, whereas seedlings inoculated in the field were sprayed before sunrise.

The effectiveness of foliar and drip applications of acibenzolar-S-methyl (ASM) (Actigard 50WG, Syngenta Crop Protection, Greensboro, NC), and foliar applications of copper hydroxide with or without ethylene bis-dithiocarbamate (EBDC) (ManKocide or Kocide 3000, Certis, Columbia, MD), were assessed for the management of bacterial leaf spot in the field. The minimum label rates were used for ManKocide (2.24 kg/ha) and Kocide 3000 (0.56 kg/ha), while the mid label rate was used for Actigard (0.035 kg/ha). Triploid watermelon variety Troubadour (Harris Moran, Modesto, CA) and diploid pollenizer watermelon variety Pollen Pro (Siegers Seed Co., Holland, MI) were used in all experiments. Seeds were planted in seedling trays consisting of 128 cells with an individual cell dimension of $3.5 \times 3.5 \times 4.5$ cm (length $\times$ width $\times$ height) containing commercial potting media, and maintained under greenhouse conditions at $28 \pm 4^\circ\text{C}$ and relative humidity at 75 to 85% for a period of 4 to 5 weeks before transplanting.

Raised bed plots were prepared with black polyethylene mulch and maintained for weeds, diseases, insects, and nutrient management (Freeman et al. 2015). Bed dimensions for each plot were 13.70×3.05 m in Quincy, FL, and 9.10×3.05 m in Citra, FL. For each trial, seedlings were spaced 91.40 cm apart, and each plot was transplanted with 8 to 9 Troubadour and 2 Pollen Pro seedlings at the beginning of the spring season. The transplant and harvest dates for each trial are listed in Table 1. Treatments were arranged in a randomized complete block design with four replications per treatment/inoculation method. Disease severity was assessed beginning the second week after transplanting, corresponding to the beginning of canopy formation in the greenhouse-inoculated plots, and the week following inoculation in the field-inoculated plots. The disease assessment periods were from 4/7/14 to 5/2/14, 4/16/14 to 5/15/14, 3/31/15 to 5/1/15, and 4/6/15 to 5/7/15 for the Quincy 2014, Citra 2014, Quincy 2015, and Citra 2015 trials, respectively. The total proportion of necrotic/symptomatic leaf tissue was determined for each

Table 1. Data summary of field trial conditions and bacterial leaf spot disease severity among the greenhouse and field inoculated, untreated plots from four field trials conducted in Quincy and Citra, FL, during the spring 2014 and 2015 seasons

	Field trial							
	Quincy 2014		Citra 2014		Quincy 2015		Citra 2015	
Inoculation method ^v	GH	Field	GH	Field	GH	Field	GH	Field
sAUDPC ^{w,x}	76.9 b	99.8 a	21.6 e	28.0 de	42.8 c	39.7 dc	16.9 e	1.8 f
Maximum severity ^w	88.6 ab	90.0 a	32.8 d	43.8 cd	76.8 ab	56.3 bc	32.8 d	2.3 e
Transplant date	March 24		April 2		March 17		March 24	
Number of treatment applications	4		3		3		3	
Harvest date ^y	July 16		June 26		June 17		June 16	
Average temperature ($^\circ\text{C}$) ^z	17.4		21.8		20.0		21.8	
Total rainfall (cm) ^z	38.8		10.1		20.4		8.7	

^v At each field trial, half of the seedlings were inoculated in the greenhouse (GH) approximately 7 to 10 days before transplanting, and the other half were inoculated in the field the second week after transplanting.

^w Standardized area under the disease progress curve (sAUDPC) and average maximum disease severity among the untreated-inoculated controls; treatments labeled with the same letter within a row are not significantly different (Tukey's HSD, $P < 0.05$).

^x The sAUDPC is based on five weekly observations from each trial location and year.

^y Watermelons were harvested once at maturity.

^z The average temperature and total rainfall from the date of transplanting to the last disease severity assessment for each field trial.

plot at weekly intervals (every 6 to 10 days) using the Horsfall-Barrett scale (Horsfall and Barratt 1945), and the midpoint averages of the disease severity scale were used to obtain the average weekly disease severity (Redman et al. 1969). These weekly averages were used to calculate the area under disease progress curve, which was then standardized by the rating period (sAUDPC) (Madden et al. 2007). In each trial, symptomatic leaf tissue representative of the symptoms observed in the field was collected, surface sterilized in 0.6% sodium hypochlorite for 1 min, and rinsed twice in sterile tap water. The leaf tissue was then macerated in sterile tap water, and a loopful of the resulting suspension was streaked onto KB medium. After 48 h, *P. syringae* colonies were confirmed by the presence of fluorescent pigment and oxidase negative reaction. At the end of the experiment, fruits were harvested once at maturity and used to calculate the total kilograms per hectare for each plot.

Data analysis. All statistical analysis was performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC). Analysis of variance of the total sAUDPC and average maximum disease severity among the untreated plots from each field trial was performed using the PROC GLM procedure, and Tukey's honest significant difference (HSD) was used for analysis of mean separation ($\alpha = 0.05$). A mixed linear model analysis of variance was used to determine effects of inoculation method (IM) and treatment on the sAUDPC and yield using the PROC GLIMMIX procedure with the restricted maximum likelihood method. The fixed effects in this model were IM, treatment, and the interaction of IM and treatment. Random effects in this model included block and the residual error term. The least squares means were separated using Tukey's HSD ($\alpha = 0.05$).

Data from the Florida Automatic Weather Network (FAWN) stations at each trial location were used to obtain environmental data. The average weekly temperatures were calculated from the daily average air temperatures recorded at 60 cm above the soil. Similarly, the relative humidity (RH %), dew point ($^{\circ}\text{C}$), solar radiation (watts per square meter), and precipitation (cm) were recorded at 2 m above the soil and used to calculate the weekly averages. The average number of hours between observations with RH $\geq 90\%$, and the number of precipitation events ≥ 0.05 and 0.1 cm were calculated at 2 m above

the soil from the hourly averages. Pearson's correlation test was conducted using the PROC CORR procedure to correlate environmental variables with the average weekly disease severity. Linear regression and multiple regression approaches were used to model the relationship between the dependent variable (average weekly disease severity) and the independent variables correlated with disease severity using the PROC REG procedure.

Results

Disease management. At the date of the first disease rating, average disease incidence was $\geq 95\%$ among each of the greenhouse-inoculated treatments in every field trial. The presence of anthracnose lesions and other fungal diseases on plant tissue became difficult to differentiate from bacterial leaf spot approximately 6 weeks after transplanting in the Citra 2014 and Quincy 2015 field trials; therefore, only observations corresponding to 40 ± 5 days after transplanting were used to calculate the average weekly disease severity and the average total sAUDPC. Due to the low disease pressure among the field-inoculated plots from Citra 2015, these data were removed from the analysis and mean separations were conducted using the greenhouse-inoculated plots alone. As a result, the effect of inoculation method was not tested in this field trial.

Both methods of inoculation and treatment effects were significant with $P < 0.05$ in all field trials. A significant interaction between inoculation method and treatment was not detected at $\alpha = 0.05$ in any trial; however, this interaction was significant at $\alpha = 0.10$ in Citra 2014 and Quincy 2015 and $\alpha = 0.15$ in Quincy 2014 (Table 2). When considering the effect of treatment independent of inoculation method, all test treatments significantly reduced the total sAUDPC in relation to the untreated-inoculated control, with the exception of the copper hydroxide treatment from the Citra 2014 trial (Table 3). Because of the ineffective control observed with this treatment in Citra 2014, the efficacy of copper hydroxide-EBDC was assessed in the Citra 2015 trial. Significant differences between the foliar and drip applications of ASM were only observed in the Quincy and Citra 2015 trials, and copper hydroxide-EBDC produced statistically lower levels of total sAUDPC in relation to all other

Table 2. Type-3 tests of mixed model fixed effects in the analysis of treatment on the standardized area under the disease progress curve (sAUDPC) from four field trials (Quincy 2014, Citra 2014, Quincy 2015, and Citra 2015)

Effect	df _n	df _d	sAUDPC							
			F-value				P-value			
			Quincy 2014	Citra 2014	Quincy 2015	Citra 2015	Quincy 2014	Citra 2014	Quincy 2015	Citra 2015
IM ^y	1	21	49.98	15.17	25.02	-	<0.0001	0.0008	0.0002	-
Treatment	3	21	52.30	13.86	51.86	118.3	<0.0001	<0.0001	<0.0001	<0.0001
IM*Treatment ^z	3	21	1.99	2.40	2.70	-	0.1468	0.0968	0.0697	-

^y IM indicates the main effect of inoculation method that contains two levels: greenhouse inoculated and field inoculated. The effect of inoculation method was not tested in the Citra 2015 trial due to low disease pressure in the field inoculated plots.

^z IM*Treatment refers to the interaction of main effects: inoculation method (IM) and treatment.

Table 3. Effect of acibenzolar-S-methyl (ASM) foliar, ASM drip, and copper hydroxide on the bacterial leaf spot severity and average yield based on a mixed model analysis of variance

Treatment ^x	sAUDPC ^y				Yield (kg/ha) ^z			
	Quincy 2014	Citra 2014	Quincy 2015	Citra 2015	Quincy 2014	Citra 2014	Quincy 2015	Citra 2015
ASM foliar	63.4 b	12.0 b	19.8 c	7.0 c	60,696 ab	55,004 a	66,286 a	50,130 a
ASM drip	68.6 b	15.1 b	28.5 b	10.1 b	65,807 a	49,330 ab	65,857 a	59,633 a
Copper hydroxide	39.9 c	21.3 a	6.6 d	4.6 d	63,734 a	48,721 ab	63,596 a	55,229 a
Untreated	88.3 a	24.8 a	42.1 a	16.9 a	43,645 b	36,355 b	66,643 a	44,778 a

^x Copper hydroxide mixed with EBDC was tested in all field trials except Citra 2014, where the copper hydroxide alone was applied. Treatments were applied at weekly intervals beginning the second week after transplanting; three treatments were applied in all field trials except Quincy 2014, which had four treatment applications. The effect of treatment was considered independent of inoculation method (i.e., greenhouse inoculation or field inoculation); treatments within a column labeled with the same letter are not significantly different (Tukey's HSD, $P < 0.05$).

^y sAUDPC = standardized area under the disease progress curve; data are the means of eight replicates per treatment except Citra 2015, which had four replicates due to low disease severity in the field inoculated plots.

^z Yield data are the means of eight replicates per treatment.

Quincy 2014

■ GH inoculated □ Field inoculated

Citra 2014

■ GH inoculated □ Field inoculated

Quincy 2015

■ GH inoculated □ Field inoculated

Citra 2015

■ GH inoculated □ Field inoculated

Fig. 1. Interaction between inoculation method and treatment based on a mixed model analysis of variance from four field trials. Black bars indicate the average sAUDPC among plots planted with seedlings that were inoculated in the greenhouse approximately 3 weeks before initiation of the test treatments, and gray bars show the average sAUDPC among plots inoculated in the field, 3 to 4 days after application of the first test treatment. The interactions significant at $\alpha = 0.10$ are shown. Error bars indicate the standard error of the least squared means and letters over bars indicate significant differences among the means within each field trial (Tukey's HSD, $P < 0.05$). In Citra 2015, the effect of inoculation method was not tested due to low disease pressure.

treatments from every trial in which it was tested with a $P < 0.05$ (Table 3). Although the ASM drip treatment significantly reduced the sAUDPC in all field trials independent of inoculation method, this treatment did not statistically differ from the greenhouse-inoculated, untreated controls in the Citra 2014 and Quincy 2015 trials (Fig. 1). In Citra 2014, neither the greenhouse nor field-inoculated plots statistically differed from the untreated control, and the field-inoculated plots displayed significantly greater disease severity in relation to all other test treatments (Fig. 1).

Yield response. In all field trials, neither inoculation method nor the interaction of method and treatment were significant (data not shown), so the effect of treatment on the yield was considered independently. Significant treatment effects were observed only in the Quincy and Citra 2014 trials with probabilities of 0.0083 and 0.0229, respectively. In Quincy 2014, the average yield among plots that received disease management ranged from 60,696 to 65,807 kg/ha, while the untreated plots averaged 43,645 kg/ha. There were no significant differences in yield among the ASM foliar, ASM drip, and copper hydroxide-EBDC treatments, but only the yield from ASM drip and copper-EBDC treatments was significantly greater than the untreated control ($\alpha = 0.05$). In the Citra 2014 trial, the average yield among the treated plots ranged from 50,064 to 56,347 kg/ha whereas the untreated plots averaged 37,698 kg/ha (Table 3). In this trial, only the ASM foliar treatment significantly differed from the untreated control ($\alpha = 0.05$).

Environmental conditions and disease severity. The average maximum disease severity from the four field trials ranged from 32.8 to 88.6% among the greenhouse-inoculated plots. In the Citra 2015 trial, the maximum disease severity among the field-inoculated plots reached only superficial levels (2.3%), but these values ranged from 43.8 to 90% in the other three trials (Table 1). Because of this low disease severity observed in Citra 2015, only the severity data collected from the greenhouse-inoculated, untreated plots were used to correlate with environmental variables. In the Quincy 2014 greenhouse-inoculated plots, severity ratings from the initial observation to the threshold of disease severity (86.25%), which occurred 4 weeks after transplanting, were included in the analysis. All observations from the Citra 2014, Citra 2015, and Quincy 2015 trials were used, and a total of 18 observations were included in the analysis.

Pearson's correlation coefficient indicated that all environmental variables investigated were significantly correlated with disease severity, with the exception of the average weekly relative humidity and the average number of relative humidity hours $\geq 90\%$ with $P < 0.05$ (Table 4). The average weekly temperature and rainfall displayed the strongest relationship with disease severity, producing correlation coefficients of -0.77 and 0.60 , respectively ($P < 0.05$) (Table 4). Linear regression of the average weekly temperature with disease severity exhibited a negative relationship and produced a regression coefficient of 0.60 (Fig. 2A). The square root transformed

average weekly rainfall with the intercept constrained to 0 was used to model the relationship between rainfall and disease severity (Fig. 2B). This model was significant with a probability < 0.0001 , and produced a regression coefficient of 0.74 (Fig. 2B). The relationship between temperature, rainfall, and disease severity was defined using the following multiple regression model: $y = 130.56 - 5.24(T) + 21.28(RF)$, where y is disease severity, T is the average weekly temperature, and RF is the square root transformed average weekly rainfall. Both independent variables significantly contributed to the model and a random distribution of residuals was observed. The parameter estimates for the multiple regression model are listed in Table 5.

Discussion

To our knowledge, this is the first study to directly examine the management of bacterial leaf spot on watermelon caused by *P. syringae* pv. *syringae* and the specific environmental conditions important in disease development. Here we showed that weekly applications of ASM foliar, ASM drip, or copper hydroxide-EBDC beginning the second week after transplanting were effective in reducing the disease severity in the field (Table 3). We hypothesized that the potential sources of inoculum contributing to the recent epidemics in Florida originated from either a seed-borne/transplant level introduction of the pathogen, or from an external source of contamination occurring in the field. In this study, we sought to replicate these two scenarios of pathogen introduction by inoculating half of the plants at each trial in the greenhouse, before application of the test treatments, or in the field, 3 to 4 days after the first treatment application.

In three field trials, copper hydroxide-EBDC was the most effective treatment in reducing disease severity (Table 3). Even under the epidemic conditions that were observed in the Quincy 2014 trial, this compound performed equally well as both a curative or protective treatment (Fig. 1). Following copper hydroxide-EBDC, the ASM foliar and drip treatments were also effective in reducing the sAUDPC under high disease pressure. The foliar application produced numerically lower sAUDPC levels when compared with the drip in all field trials, and provided a quantitative improvement in disease management (Table 3). Although the ASM drip treatment was consistently effective in reducing disease severity independent of inoculation method (Table 3), this treatment provided variable results when seedlings were inoculated before reaching the field in Citra 2014 and Quincy 2015 (Fig. 1).

Copper is often recommended to be applied as a protective treatment for angular leaf spot of cucurbits, and reported to be ineffective after an epidemic is underway (Williams 1996). It was therefore interesting to note that overall we did not find stronger evidence for an interaction between inoculation method and treatment in these field trials (Table 2). In the first month after transplanting, canopy formation occurs at a rapid rate and the application of copper hydroxide or ASM may still protect newly developed foliage from secondary dispersal of inoculum when disease develops at the early stages of the crop. The interaction of inoculation method and treatment was also likely to be dependent on both environmental conditions and the residual activity of the chemical treatment. Studies conducted on tomato and kiwifruit have shown that copper residues declined on the leaf surface by over 70% at 5 days after application, or by 50% after 2.5 cm of rainfall (Gaskin et al. 2013; Jones et al. 1991). The Citra 2014 trial received a heavy rain event (1.3 cm in 2 h) after the first treatment application, which may have been sufficient to remove the majority of residual copper from the leaf surface prior to the field inoculation. This may provide one explanation as to the significant differences between inoculation method among the copper hydroxide treated plots; however, both treatments were ineffective in reducing disease severity in this field trial (Fig. 1).

Upon examination of the yield response, there was no evidence that the manner of pathogen introduction to the plots was a significant factor (data not shown). Under epidemic conditions and high disease pressure as was observed in Quincy 2014, the management of bacterial leaf spot was associated with a potential increase in yield of approximately 50% (Table 3). It was unexpected to note a significant

Table 4. Environmental variables correlated using Pearson's correlation coefficient with the average weekly bacterial leaf spot severity among the greenhouse inoculated, untreated plots from four field trials, $N = 18$ observations

Variable ^x	Disease severity	
	Correlation coefficient	P-value
Temperature	-0.77	0.0002
Rainfall	0.60	0.0081
Dew point	-0.51	0.0309
$RF \geq 0.05 \text{ cm}^y$	0.51	0.0293
$RF \geq 0.1 \text{ cm}^y$	0.57	0.0141
Relative humidity	0.31	0.2065
Relative humidity hours ^z	0.21	0.3992
Solar radiation	-0.48	0.0438

^x All environmental variables are the weekly averages except the number of hourly precipitation events.

^y Number of hourly precipitation events $\geq 0.05 \text{ cm}$ or 0.1 cm .

^z Average number of weekly relative humidity hours $\geq 90\%$.

treatment effect in Citra 2014 as more moderate disease pressure was observed in this trial (Table 1). A high amount of variability was seen in the yield data, and only the foliar application of ASM resulted in a significant yield increase (Table 3). ASM is labeled for watermelon to manage various fungal and bacterial diseases. Because anthracnose

and other fungal issues were noted in this trial, we cannot confidently conclude that this treatment effect was primarily influenced by bacterial leaf spot.

In addition to the significant yield losses associated with the disease in Quincy 2014, the harvest date in this trial occurred approximately

Fig. 2. Linear regression analysis of the average weekly temperature and rainfall with bacterial leaf spot severity among the untreated plots from four field trials conducted in Citra and Quincy, FL. The average weekly disease severity among the untreated plots planted with seedlings inoculated in the greenhouse was plotted against: **A**, the average weekly temperature, $P = 0.0002$, $R^2 = 0.60$, root mean square error = 15.45; and **B**, the average weekly rainfall fitted with a square root transformation and the intercept constrained to 0, $P < 0.0001$, $R^2 = 0.74$, root mean square error = 21.5.

1 month later than the other three trials, which were all transplanted at similar times (Table 1). The stunting of severely blighted seedlings and delayed harvest is a significant factor that should be taken into account when considering the impact of this disease. Growers in the north-central region of Florida often have a window of just a couple of weeks to plant early and obtain the maximum return for the crop by reaching the peak of the market, while facing the risk of extended cool weather conditions and frost damage (Mossler et al. 2013). As a clear yield effect was not observed under low to moderate disease pressure (Table 3), understanding the specific environmental conditions that are conducive for disease development may help to reduce unnecessary chemical inputs.

Pearson's correlation test demonstrated a clear negative relationship between the average weekly temperature and disease severity (Table 4). The coefficient of determination produced by a linear regression model using temperature as the independent variable explained 60% of the variability observed in the disease severity ratings, indicating that temperature is a key factor in determining the development of bacterial leaf spot of watermelon (Fig. 2A). When the average weekly temperature exceeded 22.5°C, only moderate to low levels of disease severity were observed from the four field trials conducted in this study (Fig. 2A). This is likely an overestimation of the ideal temperature conditions conducive for disease development

Table 5. Parameter estimates for a multiple regression model incorporating the average weekly temperature and the square root transformed rainfall as independent predictors of disease severity. Severity ratings from the greenhouse inoculated-untreated plots from four field trials were used in the model development, $N = 18$ observations.

Effect	DF	Parameter estimates			
		Estimate	Standard error	t Value	Pr > t
Intercept	1	130.56	26.70	4.89	0.0002
Temperature	1	-5.24	1.19	-4.39	0.0005
Sqrt(Rainfall)	1	21.28	8.97	2.37	0.0314

Fig. 3. Observed versus predicted disease severity values produced from a multiple regression model incorporating the average weekly temperature and square root transformed average weekly rainfall as independent predictors of disease severity, $P < 0.0001$, $R^2 = 0.71$, root mean square error = 13.6.

under natural conditions, and is in contrast to the optimal temperature conditions (24 to 28°C) as determined by Wiles and Walker (1952) and commonly reported for angular leaf spot (Hansen 2009; Williams 1996). It is unclear how these conditions might vary for different cucurbit crops or genetically distinct populations of the pathogen.

The effect of rain in the development of bacterial leaf spot was also clear from these studies, and the average weekly rainfall was found to be significantly correlated with disease severity with probability of 0.0081 (Table 4). Very high disease pressure was observed in the Quincy 2014 trial with maximum severity levels exceeding 88% in both greenhouse and field inoculated plots (Table 1). This trial received a total of 18.4 to 30.1 cm more rainfall than any of the other three trials from the period of transplanting to the last disease assessment date (Table 1). Regression of the average weekly rainfall and disease severity fitted with a square root transformation described this relationship well, producing a regression coefficient of 0.74 ($P < 0.0001$) (Fig. 2B). The resulting curve indicated that the 50% disease severity threshold corresponded to approximately 1 cm average weekly rainfall when inoculum is present in the field (Fig. 2B).

When incorporated into a multiple regression model with the average weekly temperature, these two independent variables accounted for 71% of the variability observed in the disease severity ratings ($P < 0.0001$). This model tended to over predict disease severity when the observed values ranged from 5 to 20%, and under predict disease severity when more moderate to high disease pressure was observed (Fig. 3). Although we show that temperature and rain are critical factors in determining the development bacterial leaf spot, this was by no means a new insight for this disease in general. However, this was the first study to directly examine the specific environmental variables important in the development of the disease on watermelon. Further work is needed to more precisely quantify these environmental variables so that the risk for disease development can be more accurately assessed.

In summary, the results of this study indicated that ASM foliar, ASM drip, or copper-EBDC were effective in reducing disease severity when applied at weekly intervals beginning the second week after transplanting. However, ineffective disease control was observed with copper hydroxide in one field trial, suggesting that copper bactericides may provide unreliable control of bacterial leaf spot. This observation was consistent with other reports from cucumber and cantaloupe over the years (Bhat et al. 2010; Riffaud et al. 2003), and indicates that this product should not be solely relied upon for the management of this disease. A negative relationship between the average weekly temperature and disease severity was observed, and is in contrast with the optimal temperature conditions commonly reported for angular leaf spot of cucurbits. When incorporated into a multiple regression model, the average weekly temperature and square root transformed average weekly rainfall were robust predictors of disease severity. This information should be taken into account when considering the planting date of watermelon seedlings as the extended cool conditions often encountered in the early spring season for watermelon production are conducive for disease development. As transplant production is likely a critical point for secondary dispersal of *P. syringae* cells, an understanding of the efficacy and timing of these treatments in reducing epiphytic bacterial populations in transplant production is needed to reduce the risk for widespread contamination of watermelon transplants before they reach the field.

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