The Influence of Spray Adjuvants on Exacerbation of Citrus Bacterial Spot

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ABSTRACT

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The effect of adjuvants on the spread of *Xanthomonas axonopodis* pv. *citrumelo* applied to nursery plots of citrus (*Citrus* spp.) rootstock trees in simulated wind-blown rain was studied. Commercial adjuvants tested included a penetrant-surfactant, the penetrant or surfactant components of the penetrant-surfactant alone, an antitranspirant, a surfactant, or 1 of 3 formulations of a spreader-binder. Individual rows were treated with the adjuvants or water alone as a control. Bacterial dispersal gradients in all rows were similar and extended the entire 7 m of the nursery rows. Disease incidence, number of lesions per plant, and lesion diameters were determined at selected assay points in each row 28 days after the event. The penetrant-surfactant and its surfactant component significantly increased the total number of lesions per plant and mean lesion diameters compared to the water control. The disease gradient slopes associated with the penetrant-surfactant and its surfactant component were significantly flatter and more extensive than the water control. The penetrant component of the penetrant-surfactant, the antitranspirant, and two spreader-binders adjuvants did not significantly alter the disease gradient compared to the water control. Lesion sizes and numbers were also increased by a surfactant product and the surfactant component of the penetrant-surfactant, but not by the penetrant component of the penetrant-surfactant, the antitranspirant, or the three spreader-binder formulations. These results suggest that surfactants which induce stomatal flooding may enhance infection and exacerbate citrus bacterial epidemics.

Additional keywords: Citrus canker, mesophyll infection, water soaking

Citrus bacterial spot (CBS) caused by *Xanthomonas axonopodis* pv. *citrumelo* (synonym *X. campestris* pv. *citrumelo*), is characterized by flat, spreading, water-soaked lesions that often become necrotic on young foliage of susceptible cultivars and rootstocks of citrus (11,15,16,20). Between 1985 and 1995, over 70 outbreaks of citrus bacterial spot occurred in Florida nurseries. Initially, the confusion with and taxonomic relationship to *X. axonopodis* pv. *citri*, the Asiatic citrus canker organism, resulted in the destruction of an estimated \$250 million worth of nursery and orchard citrus rootstocks and grafted trees (16,36). Serological, genetic, epidemiological, and host range differences between the two organ-

isms (5-9,12,13,22,23,25) were demonstrated, which led the Florida Department of Agriculture and Consumer Services, Division of Plant Industry and the United States Department of Agriculture, Animal and Plant Health Inspection Service to recognize *X. axonopodis* pv. *citrumelo* as a separate organism (7), deregulate it as a pathogen of quarantine concern, and halt further eradication efforts against it. *X. axonopodis* pv. *citrumelo* is now described as an endemic bacterium that causes minor disease problems in citrus nurseries (16,36). Citrus bacterial spot has not been found to occur outside of Florida.

Although the host range of *X. axonopodis* pv. *citri* consists of most citrus species, citrus hybrids, and some citrus relatives, the host range of *X. axonopodis* pv. *citrumelo* is relatively narrow (11,20,22,29,31). More than 75% of the occurrences of citrus bacterial spot have been associated with the rootstock Swingle citrumelo (*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.; 15). Occasionally trifoliolate orange rootstocks (*P. trifoliata*) and grapefruit scions (*C. paradisi*) have been reported as hosts in nurseries (11,15). However, bacterial populations only increase and maintain high numbers in Swingle citrumelo infections and tend to decrease rapidly after an initial increase in grapefruit and other rootstocks, cultivars, and hybrids (4,19-21).

X. axonopodis pv. *citrumelo* is most often spread mechanically by horticultural operations such as budding, hedging, and pruning, which inflict wounds of various types on foliage and young stems, but in some cases it can also be spread by wind-blown rain (9,12,17,34). Simulated wind-blown rain events move the bacteria up to 10 m from source plants and establish infections due to stomatal flooding with bacteria-laden spray water (12,30). Without subsequent wind-blown rain events or commercial spray applications, which generate wind-blown water droplets, epiphytic bacteria populations decline rapidly and associated disease gradients tend to stabilize (12,14,19). Leaf surface populations of *X. axonopodis* pv. *citrumelo* are short-lived, as are infections on nursery trees transplanted to commercial citrus plantations (12,18,37). However, wind-blown rain can cause water congestion of host tissues and force inoculum through foliar stomata (10,21,28,30). When bacteria come in contact with mesophyll tissues, infections can occur even in cultivars with known field resistance (11,21,35).

Very little information exists on the effect of spray adjuvants alone on disease development. The majority of literature relevant to adjuvants deals with the enhancement of the performance of various pesticides, especially fungicides (1-3,26,32,33,38). In 1992, we noted an intensification of CBS symptoms and unexpected spread of the disease within some commercial citrus nurseries (T. R. Gottwald and J. H. Graham, unpublished data). In these cases, a spray adjuvant, i.e. surfactant, was added to spray tank mixes. The purpose of this study was to determine the influence of spray adjuvants, including surfactants, penetrants, and antitranspirants, on bacterial dispersal gradients, the associated establishment of disease gradi-

Fig. 1. Wind speed generated by a mist-blower measured with a thermoanemometer at various distances within citrus nursery test rows.

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ents, and incidence of CBS in citrus nurseries.

MATERIALS AND METHODS

Four field nursery plots were established with greenhouse-grown seedlings of Swingle citrumelo. Each nursery plot consisted of

11 rows, each with 30 uninoculated trees with 0.3 m between the plants within the row and 0.46 m between rows. Trees were about 0.5 to 0.75 m tall, and each had one or more young growth flushes that were susceptible to infection when the experiments began. Four trees previously inocu-

lated with *X. axonopodis* pv. *citrumelo* were placed at the end of each nursery row to serve as an inoculum source. These inoculum source trees were prepared by rubbing the foliage with a suspension of bacteria harvested from petri plate cultures and mixed with Carborundum to facilitate

Fig. 2. Bacterial dispersal gradients and disease gradients established with a mist-blower to simulate blowing-rain events. The mist-blower blew water over inoculum-laden trees and down uninoculated nursery rows treated with various spray adjuvants. Bacterial dispersal gradients all were very similar, indicating no bias among treatments: (A) trial 1; (D) trial 2, (G) trial 3, and (J) trial 4. Disease gradients for each treatment are represented by linear regression: (B and C) trial 1, (E and F) trial 2, (H and I) trial 3, and (K and L) trial 4. Statistical comparisons among treatments within each trial are shown in Table 2.

infections. Source trees were allowed to develop lesions for approximately 21 days prior to being placed in the field and all had severely infected foliage. The experiment was conducted the same day the source trees were placed in the field, after which the source trees were removed. After final data collection from each trial, all trees in the four nurseries were cut back to about 10 cm above the soil surface, and all foliage was removed. As the pruned trees sprouted, new main shoots were selected, trained, and allowed to grow until a uniform stand was again achieved for the next trial.

All trees in individual rows were treated with a single spray adjuvant or water as a control to run-off (50 to 100 ml per plant) via a power sprayer (model PS 12-12, W. W. Grinder Inc., Wichita, KS). Sprayer pressure was maintained at 2.8 kg/cm². The spray nozzle was held approximately 30 cm from the plant surface. Plants were allowed to dry for 24 h prior to initiation of the experiment. Treatments were arranged as a randomized complete block with nurseries as blocks. Each treatment was repeated twice within each nursery plot in randomly selected rows. Thus, there were eight rep-

lications of each treatment. Polyethylene plastic sheeting was used to shield adjacent rows from spray drift. Four such trials were conducted. The names of the commercial adjuvants are not specified at the request of some of the manufacturers. The first two field trial treatments included the following commercial adjuvants mixed with water: penetrant-surfactant (PS; 0.25%, vol/vol); the penetrant portion of the PS (PS-P), i.e., fulvic acid (0.05%, vol/vol); the surfactant portion of the PS (PS-S), i.e., tri-siloxane, an organosilicate (0.20%, vol/vol); and a water control (WC). In the third and fourth trials, other adjuvants tested included an antitranspirant (AT), beta-pinene polymer (1.0%, vol/vol); a spreader-binder (SB1) composed of a volatile methylated seed oil solvent/phthalic-glycerol-alkyl synthetic resin mixture (0.25%, vol/vol); a second formulation of the spreader binder (SB2) with a greater proportion of seed oil (0.25%, vol/vol); a third formulation of the spreader binder (SB3) with a greater proportion of synthetic resin (0.25%, vol/vol); and an additional surfactant (S), a tri-siloxane (0.25%, vol/vol). PS and S products differ in concentration of tri-siloxane (i.e., polyether polydimethyl siloxane copolymer); the PS

contains nearly 100% tri-siloxane, whereas the S contains only 25% tri-siloxane combined with a diluent. The three spreader-binder formulations (SB1, SB2, and SB3) also have some penetrant function due to the presence of the volatile solvent.

After the treatments had dried for 24 h, a tractor-driven mist-blower (Hardi Mini/max model DK2600, Hardi Inc., Davenport, IA) was used to simulate wind-blown rain events. The six nozzles of the sprayer were aligned vertically and adjusted to blow nonchlorinated well water over the inoculum source trees and down individual nursery rows for approximately 60 s per row. The sprayer traversed the entire length of the test rows. Wind speed generated by the speed sprayer was measured at several locations within the row with a thermoanemometer (model 8525, Alnor Instrument Co., Skokie, IL).

Bacterial dispersal gradients were examined immediately after the simulated blowing rain events as soon as the plants dried. Sample plants from each row/treatment were designated at 0, 0.30, 1.22, 2.44, 3.96, 5.79, and 7.92 m from the inoculum source trees. Five leaves were taken from each sample plant, sonicated for 3 min, then

Table 1. *t* test comparison of disease gradient slopes (*b*) resulting from bacterial spread events in experimental plots in which spray adjuvants were used

Trial	Adjuvant ^a	PS-P	PS-S	SB1	AT	SB2	SB3	S	Water control
1	PS	13.25 ^b (412)*** ^c	4.06 (428)***						15.84 (380)***
	PS-P		8.69 (412)***						5.17 (364)***
	PS-S								12.24 (380)***
2	PS	17.58 (358)***	2.23 (358)*						13.12 (287)***
	PS-P		16.68 (428)***						1.13 (357)ns
	PS-S								12.45 (357)***
3	PS			15.47 (380)***	9.75 (316)***	10.20 (348)***	13.25 (356)***		13.47 (348)***
	SB1				3.38 (268)***	0.001 (300)ns	0.78 (308)ns		2.76 (300)**
	AT					3.10 (236)**	2.83 (244)**		1.50 (236)ns
	SB2						0.62 (276)ns		2.27 (268)*
	SB3								1.89 (276)ns
4	PS			20.86 (340)***		12.96 (332)ns	12.96 (332)ns	12.68 (396)***	14.80 (308)***
	SB1					1.00 (244)ns	1.17 (252)ns	11.28 (308)***	2.09 (220)*
	S					6.73 (300)***	8.07 (308)***		9.24 (276)***
	SB2						0.03 (244)ns		2.58 (212)*
	SB3								2.83 (220)**

^a PS = penetrant-surfactant; AT = antitranspirant; S = surfactant; and SB1 = spreader-binder. All are commercial spray adjuvants. Treatments designated PS-S and PS-P are surfactant and penetrant components of the complete PS product, respectively. Treatments designated SB2 and SB3 are different formulations of the parent SB1 product with more seed oil and less resin, and more resin and less seed oil, respectively.

^b *t* test values calculated for the number of degrees of freedom are indicated in parentheses; the number of degrees of freedom is equivalent to the sum of the number of points used in each of the two regressions slopes tested - 2.

^c *, **, ***, and ns indicate differences detected by *t* test for *P* = 0.05, 0.01, 0.001, and not significant, respectively.

shaken for 20 min in 20 ml sterile 0.1 M phosphate buffer. The suspension from each plant was dilution-plated on semi-selective Kasugamycin-Cephalexin-Bravo 720 (KCB) medium (12,20,27), and colonies were counted after 10 days.

Lesions required 3 to 4 weeks after infection to fully develop under field conditions. Therefore, disease gradients were examined 28 days after the blowing-rain event for each trial. Disease incidence for each plant in each row was estimated as the proportion of 10 leaves infected per plant. The average lesion diameter of 20 lesions per plant also was calculated to determine the effect of treatment on lesion size.

Data from the eight individual rows associated with each treatment were pooled. The resulting bacterial dispersal gradients and disease gradients were linearized by linear regression analysis. In those cases

where disease gradients did not extend the full length of the test row, data from the disease gradients were truncated at the distance at which no further disease symptoms were detected. Differences among the slopes of bacteria dispersal gradients and among the slopes of disease gradients were compared by *t* test for all treatment combinations.

At 28 days after the blowing-rain event for the second, third, and fourth trials, the number of lesions per leaf and the diameter of all lesions were measured at 0.30, 1.22, 2.44, 3.96, 5.79 and 7.92 m from the inoculum source in each treatment row. Data from the eight individual rows associated with each treatment were pooled. Differences among the effect of spray adjuvant treatments on the number of lesions per leaf and lesion diameters versus distance were examined by orthogonal polynomial

contrasts analysis, using distance from the source of inoculum as the repeated measure (general linear models procedure, SAS Institute Inc., Cary, NC).

RESULTS

The wind speed produced by the mist-blower decreased rapidly with distance from the exit nozzles (Fig. 1). Wind was not detected at distances greater than 5.5 m due to the lack of sensitivity of the thermoanemometer; however, mist and wind were observed visually to traverse the entire 9.0 m length of the nursery rows.

No significant differences were found among the slopes of the bacterial dispersal gradients associated with different treatments for any of the four trials (Fig. 2A, C, E, and G). Thus, bacterial dispersal events were nonbiased among all spray adjuvant treatments tested.

Fig. 3. The effect of spray adjuvants on the number of lesions per leaf over distance for (A) trial 2, (C) trial 3, and (E) trial 4. The effect of spray adjuvants on lesion diameter over distance for (B) trial 2, (D) trial 3, and (F) trial 4. Differences among trends in number of lesions and lesion diameter are compared in Tables 2 and 3, respectively. For A and B, PS = penetrant-surfactant; PS-S = tri-siloxane (surfactant) component of the PS; PS-P = fulvic Acid (penetrant) component of the PS; and WC = water control. For C through F, PS = penetrant-surfactant; AT = antitranspirant; S = surfactant; SB1 = spreader-binder; SB2 and SB3 are different formulations of the parent spreader-binder product SB1; and WC = water control.

Due to the relative linear nature of CBS disease gradients over the distances examined, disease gradient data were adequately linearized by simple linear regression of nontransformed disease incidence values, rather than by the use of more complex transformations of disease incidence (24). Significant differences existed among disease gradients associated with different spray adjuvant treatments (Table 1). Almost all disease gradients associated with spray adjuvant treatments were significantly different from and more extensive compared to the water control treatment. Exceptions to this were the penetrant component (PS-P) of the PS in trial 2, and the AT and SB2 formulation of the spreader-binder in trial 3 (Table 1). Pre-event application of the commercial spray PS and its surfactant component (PS-S surfactant component tested in trials 1 and 2 only) caused the most extensive CBS disease gradients to form in all four tests (Fig. 3B, D, and F), which were highly significantly different from the disease gradient associated with the water control (Fig. 2). However, the PS-P, S, and SB2 all had some effect on the extent of the resulting CBS disease gradients and were often significantly different from the water control but were not as effective as the PS (Table 1 and Fig. 2).

The results of repeated measures analysis of the number of CBS lesions that developed over distance indicated that the use of the PS resulted in the development of significantly more lesions compared to the water control in trials 2 and 4, in which lesion numbers were measured (Table 2). In trial 1, the use of the PS-S also resulted in the development of significantly more lesions (Table 2 and Fig. 3A, C, and E).

Repeated measures analysis of lesion size over distance indicated that the PS and PS-S significantly increased lesion size compared to the water control in all three trials for which lesion size was measured (Table 3 and Fig. 3B, D, and F). In trial 4, lesion size associated with the S treatment was also significantly larger compared to the water control (Table 2).

DISCUSSION

Bacterial dispersal gradients were established by simulated blowing-rain events, using the mist-blower to blow water over inoculum-laden trees and down uninoculated nursery rows, as previously demonstrated (12). These gradients were consistent within and among nursery plots and treatments with no detectable differences. Therefore, the resulting disease gradients for each row were directly related to the

spray adjuvants tested, without bias due to differences in bacteria dispersal among nursery row locations.

It is apparent from this study that the PS adjuvant and, to a lesser extent, the S and the spreader-binder formulations markedly lengthened disease gradients and increased lesion number and lesion diameter. The greatest increase in disease gradients and increase in lesion size and lesion number were due to the PS and PS-S, a tri-siloxane. However, the PS-P, as well as the SB1 and the S (also a tri-siloxane but at lower concentration) also caused small but significant lengthening of the disease gradients and increases in lesion numbers and sizes. Other adjuvants, such as the AT, SB2, and SB3, had no appreciable effect on CBS disease gradients or lesion size and number.

The reason for exacerbation of CBS by the PS was beyond the scope of this study. However, the manufacturer indicates that the PS lowers the surface tension of water, allowing spray materials to flow over the leaf surface more readily. The PS also causes stomatal flooding, the penetration of surface moisture into stomatal cavities and hydrathodes, thus allowing herbicide and other pesticide materials to make better contact and penetrate foliage (38). One means of defense that plant foliage has

Table 2. Repeated-measures analysis of variance of the trends of total number of lesions per plant over the repeated measure of distance from the inoculum resulting from bacterial spread events in experimental plots in which spray adjuvants were used

Trial	Adjuvant	PS-P	PS-S	SB1	AT	SB2	SB3	S	Water control
2	PS	0.0003 ^a	0.8654						0.0004
	PS-P		0.0004						0.6680
	PS-S								0.0006
3	PS			0.1097	0.3101	0.2091	0.0105		0.2597
	SB1				0.5473	0.7216	0.3019		0.6256
	AT					0.8053	0.1060		0.9089
	SB2						0.1677		0.8949
	SB3								0.1318
4	PS			0.0001		0.0001	0.0001	0.0001	0.0001
	SB1					0.8533	0.3152	0.9001	0.5933
	S					0.7564	0.3784		0.6825
	SB2						0.2359		0.4729
	SB3								0.6349

^a Values represent the probability of a greater *F*. Values of *F* < 0.05 indicate a significant difference among the treatments compared.

Table 3. Repeated-measures analysis of variance of the trends of lesion diameter over the repeated measure of distance from the inoculum resulting from bacterial spread events in experimental plots in which spray adjuvants were used

Trial	Adjuvant	PS-P	PS-S	SB1	AT	SB2	SB3	S	Water control
2	PS	0.0008 ^a	0.0001						0.0001
	PS-P		0.1756						0.4143
	PS-S								0.0287
3	PS			0.0001	0.0078	0.0001	0.0001		0.0070
	SB1				0.1321	0.4747	0.4419		0.5329
	AT					0.0176	0.0148		0.5350
	SB2						0.9542		0.2149
	SB3								0.1989
4	PS			0.0001		0.0001	0.0001	0.0001	0.0001
	SB1					0.8976	0.8976	0.0827	0.2001
	S					0.1096	0.1096		0.0023
	SB2						1.0000		0.1592
	SB3								0.1592

^a Values represent the probability of a greater *F*. Values of *F* < 0.05 indicate a significant difference among the treatments compared.

against invasion of pathogenic organisms and toxic substances is the high surface tensions of water associated with these natural openings (38). The organosilicone adjuvants break this water tension (39). The other tri-siloxane product in the study, S, has similar chemistry; however, the concentration of tri-siloxane was only 25% that of the PS and, therefore, had a similar but reduced effect on disease.

The higher number of lesions is attributed to facilitation of spread of inoculum on foliar surfaces and increased bacterial ingress into stomata cavities. Increased movement of inoculum-laden water from the substomatal chamber into the mesophyll may account for increase in lesion size and number. Other products, which are anti-transpirants, did not have such dramatic effects. A similar exacerbation of a bacterial disease of corn, caused by *Erwinia stewartii*, has recently been noted and attributed to the use of an organosilicone adjuvant, which was used to augment the effectiveness of insecticide treatments against fall armyworm (39).

Spray adjuvants are used in many agricultural situations for a variety of reasons. The results of these tests indicate that caution should be taken when using spray adjuvants, especially those with surfactant characteristics. Both citrus and other crops whose foliage is susceptible to bacterial diseases could experience exacerbation of disease due to the use of surfactants in some situations. These effects of surfactant spray adjuvants also could be further exacerbated or mitigated when adjuvants are combined with other agrochemicals such as herbicides, pesticides, fertilizers, and foliar nutrients, which could have antimicrobial activity or stimulate bacterial growth and/or survival.

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