Dispersal and Management of *Xanthomonas campestris* pv. *vesicatoria* During Thinning of Direct-Seeded Tomato

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ABSTRACT

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The spread of *Xanthomonas campestris* pv. *vesicatoria* during thinning of direct-seeded tomato was studied in two field experiments at Homestead, Florida. In a warm, humid season (spring 1988), the incidence of bacterial spot was less when plants were thinned in the afternoon when foliage was dry (44%) vs. those thinned in the morning when plants were laden with dew (87%). In a cool, dry season (fall 1988), disease incidence was reduced from 55% to 0% by waiting until afternoon when plants were dry before thinning. Ethanol and 10% povidone-iodine, applied as prophylactic hand washes, reduced disease incidence after thinning by 65% and 81%, respectively, in spring 1988. In fall 1988, no infected plants were detected when povidone-iodine was used; only 3.7% disease incidence was recorded for the ethanol treatment. In most cases, *X. c.* pv. *vesicatoria* could not be detected after hands were washed in either povidone-iodine or ethanol. Based on results of ordinary runs analysis of the distribution of diseased plants, the distribution of infected plants was generally highly clustered in all rows except those where bactericidal hand washes were used. Periodic hand disinfestation may be useful as a component of an integrated program for management of bacterial spot of tomato.

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is the most widespread and destructive disease of tomato (*Lycopersicon esculentum* Mill.) in Florida (22). When epidemics begin early in the crop (before flowering), losses in marketable fruit in excess of 50% can occur (21). Losses are particularly evident among the large-size (premium grade) fruit, compounding the economic effects of the disease.

Chemical control of bacterial spot is often less than satisfactory (9). Despite repeated applications of copper and maneb or mancozeb, growers still report that bacterial spot is their most limiting disease problem (19). Therefore, there is particular interest in development of integrated programs for management of this disease.

For some time, field observations by the authors, pest management scouts, and others have led to the hypothesis that *X. c.* pv. *vesicatoria* is readily spread throughout tomato fields during routine farming operations. Significant manipulations of plants by farm workers still occur in the Florida tomato industry. Approximately one-third of the 17,800 ha of tomato planted in Florida is direct-seeded and subsequently thinned to one plant per hill. Growers prune side shoots on about 70% of the crop. Over 80% of the plants are manually tied to support stakes. In Israel (1), the bacterial spot pathogen has been shown to be dispersed by farm workers and agricultural equipment that have been in contact with diseased plants.

Medical scientists routinely use several topical preparations for dermal antisepsis (8). Several of these agents produce "virtual disinfection," according to Gardner and Seddon (5), when used on the surface of human skin for periods of 20–30 sec. It may be that this technology can be applied to cases of phytopathogens transmitted on the skin.

One objective of this study was to determine the role of the manual farm operation described above (thinning of direct-seeded tomato) in the spread of the bacterial spot pathogen and subsequent disease incidence. In addition, the potential for disease management through the use of bactericidal hand washes during the thinning process was investigated. A preliminary report has been published (20).

MATERIALS AND METHODS

Field plots. Two field experiments were carried out at the University of Florida Tropical Research and Education Center (TREC), at Homestead in Dade County. Experiment 1 was planted 29 March 1988. At the time of thinning and disease assessment, the weather was warm (average daily high = 32 C) and humid. Several convective rain showers occurred beginning 6 days after thinning. These conditions are considered favorable for development of bacterial spot epidemics (11). Experiment 2 was established 28 October 1988. By the time experimental treatments were carried out, weather conditions were less favorable for disease development. Rainfall was negligible, and humidity and temperature were lower (average daily high = 22 C) than expected at that time of the year.

Tomato plants (cv. Duke) were produced in accordance with standard grower practices for Dade County (2). Individual plots consisted of raised plastic-mulched beds spaced 1.8 m apart with 0.3 m between plant holes in the row. Each bed was 15.2 m long, oriented in an east-west direction. In order to minimize splash-dispersal of bacteria, drip irrigation was used (18,26). Foliar fungal diseases were controlled with weekly applications of chlorothalonil (Bravo 720, 1.7 kg a.i./ha). Insect pests were managed as needed, based on periodic scouting of plots (22).

Thinning regimens. Four thinning regimens were studied for their relationship to dispersal of *X. c.* pv. *vesicatoria*. Each plot was machine-seeded in plug mix (a commercial preparation of peat moss and perlite). Hand-thinning of plots was done 26 April 1988 (experiment 1) and 2 December 1988 (experiment 2). Workers began at the east end of rows and worked westward. The intent of the thinning operation was to limit hills to a population of one plant per hill. In some hills, zero or only one seedling emerged. Single seedlings were removed 4 days before treatments were applied so that only hills that required thinning were used; hence the numbers of experimental hills in individual rows varied (Figs. 1 and 2).

One worker was assigned to each block. In one treatment, workers thinned plots without previous exposure of their hands to known sources of *X. c.* pv. *vesicatoria*. In the remaining treatments, they gently handled naturally infected tomato leaves for 10–20 sec. A discrete infestation, using fresh infected tomato foliage, was used before each row of each treatment was thinned. The infected leaves were taken from plants in other

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research plots at TREC on the morning of the experiment. Between treatments, hands were washed with soap and water and allowed to air dry. Some plots were thinned immediately after inoculum exposure. In other plots, 10% povidone-iodine, equivalent to 1% available I_2 (Betadine, Purdue Frederick Co., Norwalk, CT), or 70% freshly prepared ethanol was used for disinfestation of hands between inoculum exposure and thinning. Workers thoroughly washed hands in approximately 10 ml of ethanol or 3 ml of povidone-iodine. After allowing excess fluid on the workers' hands to dry for 2 min, hills were hand-thinned. In most cases, the one remaining seedling in a hill came in contact with the hands of the worker when excess plants were pulled and discarded. Three seconds or less were spent thinning any one hill. The four regimens were conducted in the morning (0730–0930 hours) when plants were naturally wet with dew and in the afternoon (1400–1600 hours) when plants were dry. An additional treatment, in which plants were not thinned, was included in these tests. These plots were planted by hand with two seeds placed in each hill and covered with about 1 g of plug mix that had been moistened just before use. The experiments, consisting of nine treatments, were replicated five times (experiment 1) and four times (experiment 2) in a randomized complete block design.

Collection and analysis of disease incidence data. Plots were rated for incidence of bacterial spot on 9-10 May 1988 (experiment 1) and 20 December 1988 (experiment 2). Without handling plants, all hills were scored for presence (two or more leaves with lesions) or absence of typical bacterial spot lesions on blades and/or petioles. No ratings of disease severity were taken. Records regarding the spatial distribution of diseased plants were maintained. The percentage of hills with diseased plants was calculated for the entire row (26–50 hills) and also for the first 10 hills beginning at the east end of the rows. Calculation of statistics based on these two row lengths allowed for comparison of the effects of the bactericidal hand washes over distance down the row.

Disease incidence data were analyzed by ANOVA, followed by a series of preplanned single-degree-of-freedom orthogonal contrasts (7,24). Data were converted to arcsine square root equivalents before analysis (7).

Ordinary runs analysis (15) was used to assess whether infected plants were randomly distributed or clustered in rows. Runs are defined as successions of like plants (diseased or healthy) in a row. For example, there are 12 runs in the left-hand row in the "thinned" treatment in experiment 2 (p.m.) in Figure 1. If plants are diseased primarily as a result of plant-to-plant spread, as might be expected if mechanical transmission is important, diseased and healthy plants will be clustered. Subsequently, few runs will be recorded. The expected number of runs for a random distribution was compared to the actual number observed. The expected number of runs and Z statistic can be conveniently calculated as described by Madden et al (15). Large negative Z values ($-Z = 1.64$, $P = 0.5$) are considered indicative of nonrandomness or clustering.

Populations of the pathogen on hands of workers. Two experiments were conducted to estimate the actual populations of *X. c.* pv. *vesicatoria* that accumulate

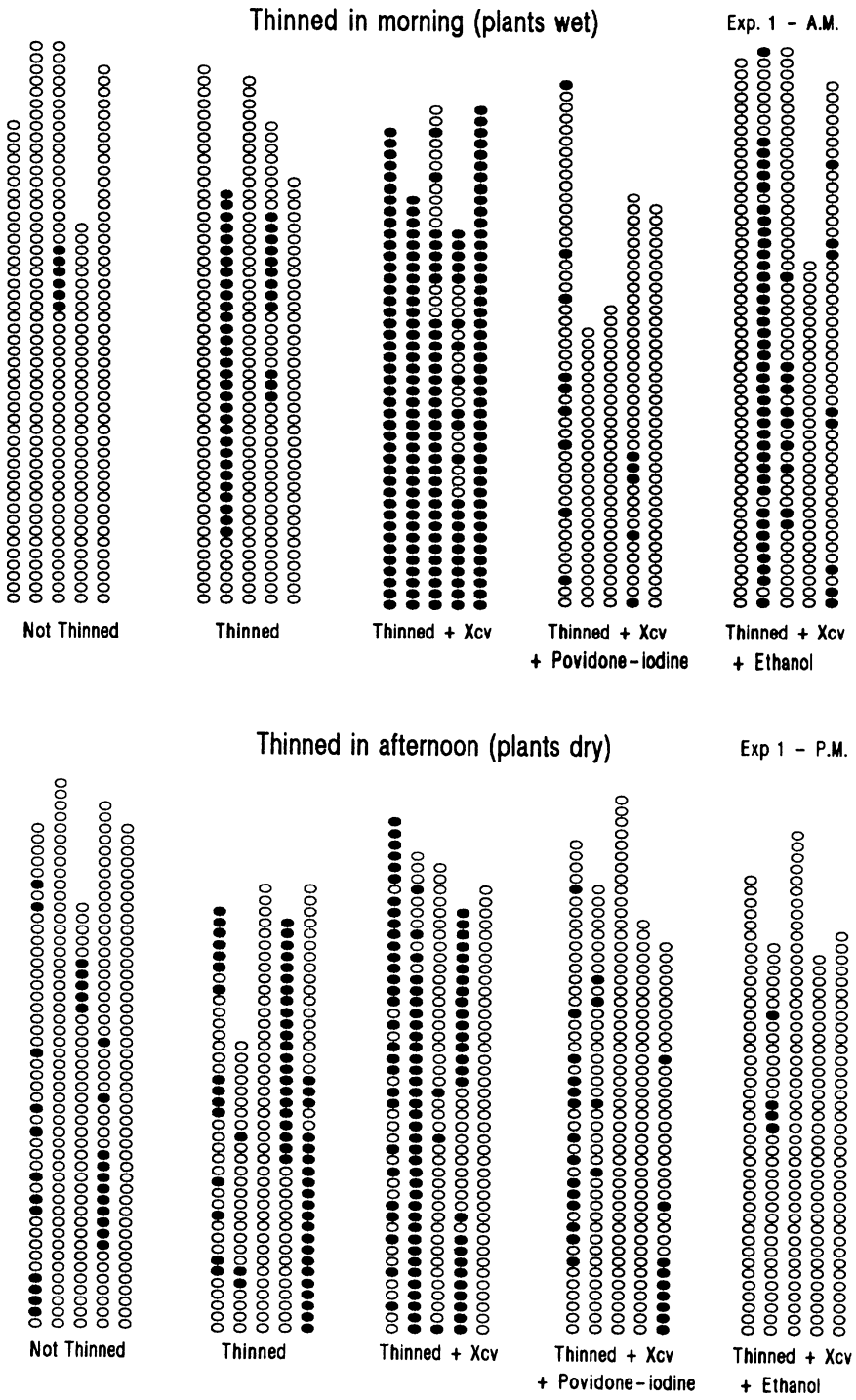

Fig. 1. Distribution of plants with symptoms of bacterial spot 2 wk after application of thinning regimens in spring 1988. From left to right, plots were left undisturbed (not thinned; two plants per hill), thinned without previous exposure to infected foliar tissue, thinned after rubbing tissue with visible symptoms of bacterial spot (*X. c.* pv. *vesicatoria*) between the hands for 10–20 sec, and thinned after interdiction of a prophylactic bactericidal hand wash between inoculum exposure and plant manipulation. The a.m. portion of the figure represents plots thinned 0730–0930 hours when plants were laden with dew. Plants were dry when thinned in p.m. (1400–1600 hours). Solid ovals represent diseased plants; open ovals are symptomless plants. Experiment was planted in a randomized complete block, but treatment replications are grouped for illustrative purposes.

on hands of farm workers. Five workers who had participated in the previously described experiments handled wet plants with visible symptoms of bacterial spot. Foliar tissue was rubbed gently between the hands for 20 sec. Each worker then vigorously rinsed his hands in a 250-ml stream of sterile buffer (14). The rinsate from the washing was collected in a sterile Nalgene tub (26 × 15 × 6.5 cm), transferred to a sterile beaker, and transported to the laboratory in an ice chest. Ten-fold dilutions were made in sterile buffer and 0.1 ml of 10^0, 10^{-1}, and 10^{-2} dilutions was spread with a sterile bent glass rod over the surface of triplicate plates of Tween medium B (17).

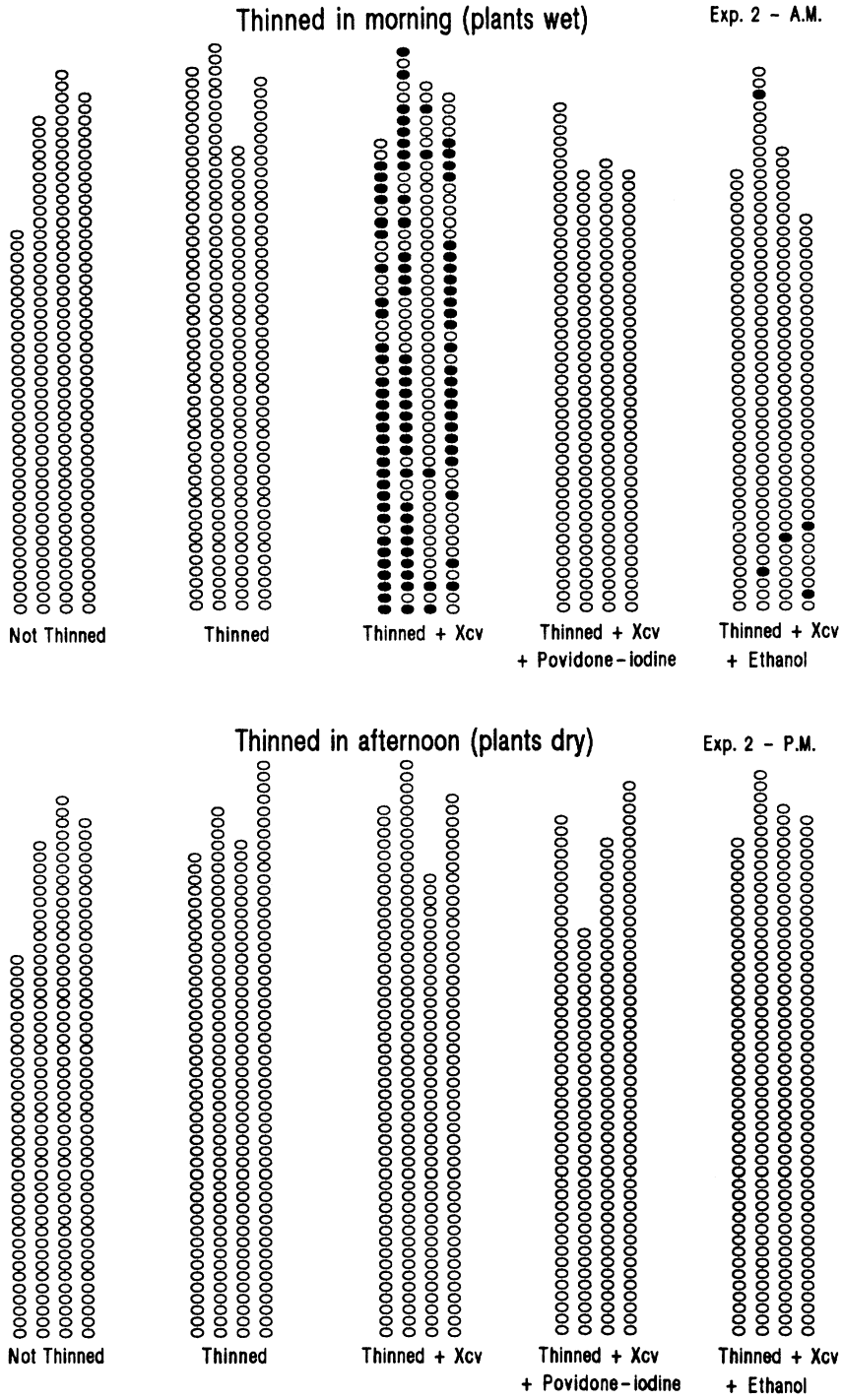

Fig. 2. Distribution of plants with symptoms of bacterial spot 2 wk after application of thinning regimens in fall 1988. From left to right, plots were left undisturbed (not thinned; two plants per hill), thinned without previous exposure to infected foliar tissue, thinned after rubbing tissue with visible symptoms of bacterial spot (*X. c.* pv. *vesicatoria*) between the hands for 10–20 sec, and thinned after interdiction of a prophylactic bactericidal hand wash between inoculum exposure and plant manipulation. The a.m. portion of the figure represents plots thinned 0730–0930 hours when plants were laden with dew. Plants were dry when thinned in p.m. (1400–1600 hours). Solid ovals represent diseased plants; open ovals are symptomless plants. Experiment was planted in a randomized complete block, but treatment replications are grouped for illustrative purposes.

In the same test, the direct bactericidal effects of povidone-iodine and 70% ethanol were measured. After an additional 20 sec of handling infected plants, small amounts of povidone-iodine or 70% ethanol were poured over workers' hands and rubbed until both palms and the back of the hands were thoroughly covered. The film of ethanol was allowed to dry. The excess povidone-iodine was washed off with a gentle stream of distilled water without vigorous rubbing. Preliminary tests showed that the water stream did not remove many *X. c.* pv. *vesicatoria* cells. Hands were then assayed for populations of *X. c.* pv. *vesicatoria* as previously described.

In an attempt to concentrate low populations of the pathogen, remaining rinsate was passed through a 0.45-μm filter. The filter paper was cut up into small pieces which were suspended in 20 ml of sterile buffer in 250-ml flasks. Flasks were agitated on an orbital shaker at 250 rpm for 45 min. Aliquots of buffer were plated on Tween medium B as described earlier.

All plates were incubated at 28 C for 4–5 days. Populations were calculated as colony forming units (cfu) present on the hands of the individual farm worker.

RESULTS

Development of bacterial spot was widespread on tomato plants in rows thinned when wet by workers who had handled diseased foliage for only 10–20 sec (Figs. 1 and 2). When no intervening hand wash was used, 87% (experiment 1) and 44% (experiment 2) of the dew-laden plants thinned in the morning had symptoms about 2 wk later (Table 1).

The incidence of bacterial spot in those rows thinned in the morning was dramatically reduced if a bactericidal hand wash was used (Figs. 1 and 2, Tables 1 and 2). In experiment 1, less than 7% of the plants in rows where povidone-iodine was used were symptomatic. Disease incidence was 22% with the 70% ethanol wash. Reductions in disease incidence were even greater in experiment 2 when environmental conditions were less favorable for disease development. No infected plants were detected when povidone-iodine was used; only 3% of the plants were diseased in rows where 70% ethanol was employed (Fig. 2).

In the absence of bactericidal hand washes, mechanical transmission of spot was reduced by waiting until plants were dry (after 1200 hours) before thinning. Under warm, humid conditions (experiment 1), the number of infected plants was reduced by about half by waiting until afternoon to thin (Table 1, Fig. 1). In cooler, drier weather (experiment 2), we did not observe any symptomatic plants in any experimental plots thinned when plants were dry, even when workers handled diseased plants and no prophy-

lactic hand wash was applied (Fig. 2).

Significantly fewer plants expressing symptoms were found in those plots that were not thinned (Table 2). These results were evident statistically despite the inclusion of data from the bactericidal hand washes in the single-degree-of-freedom contrast comparison. As expected, the canopy was more dense in the unthinned plots, because the plant population was double that of all other treatments. No actual meteorological measurements were made within plant canopies. However, it was noted that plants remained wet longer in the plots that were left unthinned.

The highly efficient transmission of the bacterial spot organism was evident throughout these tests. Ordinary runs analysis supports the conclusion that the distribution of infected plants was for the most part strongly clustered (Table 3). When random distributions were found, they were associated with the low numbers of diseased plants in rows where bactericidal hand washes were used.

Reductions in disease incidence corresponded with reductions in populations of *X. c.* pv. *vesicatoria* on hands of workers. During 20 sec of contact with diseased tomato foliage, workers accumulated about 10^6 cfu of *X. c.* pv. *vesicatoria* on their hands (Table 4). In most cases, *X. c.* pv. *vesicatoria* was not detected after the prescribed hand wash treatment with either povidone-iodine or 70% ethanol. In those cases where the pathogen was detected, bactericidal hand washes still decreased pathogen populations by at least 96%.

DISCUSSION

These studies demonstrate that *X. c.* pv. *vesicatoria* can be transmitted readily from plant to plant in tomato rows during routine thinning operations. Thinning is only one of several manual operations carried out by farm workers. It probably is not as damaging or invasive as the pruning and tying activities. Mechanical transmission may be especially significant when bacterial dissemination by splashing water, as in rainfall or overhead irrigation, is negligible. In our studies, disease spread was limited in the dry season to rows thinned by farm workers after deliberate exposure to *X. c.* pv. *vesicatoria*.

A dramatic reduction in incidence of bacterial spot was recorded when povidone-iodine or 70% ethanol was used as a prophylactic hand wash. While both compounds were efficacious, there are several contraindications to their field use at this time. Farm workers may object to the sticky, uncomfortable texture of povidone-iodine. More importantly, some people are sensitive to iodine or may become sensitized after repeated exposure and develop allergenic dermatitis (4). Applications of ethanol may be associated with excessive skin dryness and possible field consumption. Further experiments are needed to determine just how often the bactericidal hand washes need to be used in the field. Use of either compound may be enhanced by use of rubber gloves. Despite some of these drawbacks, we have shown that bactericidal hand washes hold promise as alternatives to chemical sprays for control of bacterial spot. Several scouting companies in Florida now require their field staff to routinely use ethanol hand washes when inspecting fields for pests.

The potential for dispersal of *X. c.* pv. *vesicatoria* is increased greatly if thinning is done when plants are wet. Therefore, it would seem prudent for farmers to wait until fields are dry before initiating some types of farming operations. However, it is very difficult to persuade field crews and crew leaders that work should be delayed until plants are dry. Crews prefer to work early in the morning in order to avoid the high mid-day temperatures characteristic of Florida. Unfortunately, bacterial spot is usually most severe during those months of the year when temperatures are most oppressive (10,11).

Disease incidence was significantly less if thinning was avoided. A precise method for placing one or two seeds in

Table 1. Mean percentage of plants with visible symptoms of bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), in plots thinned at Homestead, FL, in two experiments[a]

	Diseased tomato plants in a row (%)			
	Entire plot (26–50 hills)		First 10 hills[b]	
Treatments[c]	Plants wet (a.m.)	Plants dry (p.m.)	Plants wet (a.m.)	Plants dry (p.m.)
Experiment 1				
Not thinned	5.2	12.6	0.0	14.2
Thinned, no *Xcv*	19.6	33.4	19.6	28.2
Thinned + *Xcv*	87.2	43.8	100.0	46.6
Thinned + *Xcv* + povidone-iodine[d]	6.6	13.4	8.0	20.1
Thinned + *Xcv* + 70% ethanol[e]	22.4	2.2	26.1	0.0
Experiment 2				
Not thinned	0.0	0.0	0.0	0.0
Thinned, no *Xcv*	0.0	0.0	0.0	0.0
Thinned + *Xcv*	44.0	0.0	55.5	0.0
Thinned + *Xcv* + povidone-iodine	0.0	0.0	0.0	0.0
Thinned + *Xcv* + 70% ethanol	0.0	3.7	10.0	0.0

[a] Experiment 1 was conducted in spring 1988 in warm, humid weather. Experiment 2 was conducted in fall 1988 in cool, dry weather.
[b] First 10 hills starting at east end of row where thinning was initiated after handling infected plants and appropriate application of hand washes.
[c] Workers contaminated hands by gently handling naturally infected tomato foliage for 10–20 sec before thinning of each replication of each appropriate treatment.
[d] Three milliliters of povidone-iodine applied between *Xcv* exposure and thinning of each replication of each treatment.
[e] Ten milliliters of 70% ethanol applied between *Xcv* exposure and thinning of each treatment replication.

Table 2. Preplanned single-degree-of-freedom orthogonal contrasts, contrast sum of squares (CSS), and *F* test values (*F*) for percentage of tomato plants with bacterial spot in plots thinned at Homestead, FL, in spring 1988

	Transformed (arcsine square root) percentage of diseased plants in a row[a]			
	Entire plot (26–50 hills)		First 10 hills[b]	
Preplanned contrasts	CSS	*F*	CSS	*F*
Unthinned vs. thinned	0.514	4.56*[c]	0.987	6.67*
No *Xcv*[d] vs. *Xcv* (inc. bactericides)	0.20	0.175	0.359	2.43
Thinned + *Xcv* vs. thinned + *Xcv* + bactericidal hand washes	3.84	34.08**	5.48	37.09**
Povidone-iodine vs. 70% ethanol	<0.001	<0.001	0.0	0.037
Unthinned wet vs. dry	0.45	0.40	0.152	1.03
Thinned + no *Xcv*, wet vs. dry	0.143	1.27	0.312	2.11
Thinned + *Xcv*, wet vs. dry	1.12	9.95**	1.64	11.12**
Povidone-iodine, wet vs. dry	0.036	0.32	0.04	0.28
Ethanol, wet vs. dry	0.249	2.21	0.44	2.99

[a] Based on visual inspection of all plants for at least two leaves with discrete bacterial spot lesions. Plants rated 2 wk after thinning.
[b] First 10 hills starting at east end of row where thinning was initiated after handling infected plants and appropriate application of hand washes.
[c] * = Significant difference(s) at $P = 0.05$ and ** = significant difference(s) at $P = 0.01$.
[d] *Xcv* = *Xanthomonas campestris* pv. *vesicatoria*.

Table 3. Results of ordinary runs analysis[a] for determination of randomness or clustering of tomato plants with bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), in two experiments at Homestead, FL

Thinning regimen	Morning (plants wet)				Afternoon (plants dry)			
	Expected runs (no.)	Observed runs (no.)	*Z* statistic	Distribution	Expected runs (no.)	Observed runs (no.)	*Z* statistic	Distribution
Experiment 1								
Not thinned[b]	18	7	−694	clustered	49	29	−5.58	clustered
No *Xcv*[c]	70	10	−12.64	clustered	82	24	−9.56	clustered
Xcv[d]	97	24	−10.83	clustered	103	42	−8.62	clustered
Xcv + I_2[e]	27	25	−0.63	random	48	36	−3.50	clustered
Xcv + EtOH[f]	87	34	−9.26	clustered	8	9	>0	random
Experiment 2								
Not thinned	...	...	...	...				
No *Xcv*	...	...	...	...				
Xcv	94	59	−5.01	clustered				
Xcv + I_2	...	...	...	...				
Xcv + EtOH	11	14	>0	random				

[a]Ordinary runs analysis done according to procedures outlined by Madden et al (19).
[b]Control plots seeded by hand to ensure two plants per hill.
[c]Thinned without exposure to bacterial spot inoculum.
[d]Thinned after workers gently rubbed naturally infected leaves between hands for 10–20 sec before each replication of each appropriate treatment.
[e]I_2 = 3 ml of 10% povidone-iodine wash between inoculum exposure and thinning.
[f]EtOH = 10 ml of 70% ethanol wash between inoculum exposure and thinning.

Table 4. Populations in colony-forming units (cfu) of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) on hands of farm workers before and after bactericidal hand washes[a]

Farm worker	*Xcv* cfu on hands before wash	*Xcv* cfu after povidone-iodine wash	*Xcv* cfu after ethanol wash
A	8.8×10^5	NDT[b]	NDT
B	1.1×10^6	NDT	2.0×10^4
C	2.3×10^6	1.4×10^4	NDT
D	7.2×10^6	NDT	NDT
E	1.9×10^6	NDT	7.1×10^4

[a]Leaves of field-grown, diseased plants rubbed gently through hands for 20 sec. Hands rinsed with 250 ml of sterile buffered saline solution and appropriate dilutions of rinsate plated on Tween medium B. Hands were infested before each treatment with an intervening soap and water wash. Two experiments were conducted with similar results; data from one experiment are shown.
[b]NDT = none detected even when concentrated by passage through 0.45-μm filter.

a hill that assures the grower of an acceptable stand would eliminate the need for thinning (23). Such technology might reduce the likelihood of mechanical dispersal of *X. c.* pv. *vesicatoria* early in the cropping season.

Some diseased plants were found where workers thinned plots but did not deliberately contaminate their hands with the bacterial spot pathogen. The contagious nature of the disease in these plots is evident from the distribution of runs in the plots. Epiphytic populations of *X. c.* pv. *vesicatoria* may have been present on plants in the test rows (12,13). These could have been spread during handling of plants. In addition, slight wounds, such as those that result in breakage of trichomes, likely occurred during thinning, forming important portals for pathogen entry (6,25).

The likelihood for substantial improvement in chemical control of bacterial spot seems remote at this time. Growers add maneb or mancozeb to copper bactericides for enhanced control (3), a practice necessitated by the widespread occurrence of copper-tolerant strains in Florida (16). The future availability of ethylene bisdithiocarbamate fungicides is uncertain however. Therefore, it is imperative that growers use all means at their disposal to minimize disease severity or delay its onset. These measures can include clean seed and transplants, drip irrigation (18), and reduction of mechanical spread of the pathogen during thinning and other handling operations.

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