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SELECTED STUDIES ON THE EPIDEMIOLOGY, ECOLOGY AND CONTROL OF BACTERIAL SPECK OF TOMATO CAUSED BY PSEUDOMONAS SYRINGAE PV. TOMATO

By

Douglas Joseph Jardine

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To my loving wife and parents;
their patience, understanding, and support
helped to make my dream come true

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ABSTRACT

SELECTED STUDIES ON THE EPIDEMIOLOGY, ECOLOGY AND CONTROL OF BACTERIAL SPECK OF TOMATO CAUSED BY PSEUDOMONAS SYRINGAE PV. TOMATO

By

Douglas Joseph Jardine

A regression model relating temperature, humidity, rainfall and initial population levels to epiphytic populations of Pseudomonas syringae pv. tomato on tomato leaves was developed and tested against field data. The model tested in 1983 was Log_{10} cfu/g fresh wt = -5.49 + 0.1T + 1.35R + 0.81P - 0.01T^2, where T = average temperature (C) on the previous day, R = the sum of the previous 7 days of rainfall, and P = the P. syringae pv. tomato population on the previous sampling date. The model accounted for 85% of the observed variation in observed populations. In 1984, plots which were sprayed based on model predictions received 3 fewer sprays than plots sprayed on a calendar basis, with no significant difference in amount of infection.

Effects of timimg of application and efficacy of selected chemicals were examined. In greenhouse studies, streptomycin provided the highest level of control but was only effective when applied within 24-48 hours of inoculation. In field studies, the efficacy of all chemicals appeared to be related to disease pressure with protection provided only when disease pressure was at

relatively low levels. Antibiotics generally provided better control than did copper compounds.

A no-till management system was evaluated as a potential cultural control practice for bacterial speck. In 1982, population levels of the organism were fewer in no-till plots than in conventionally tilled plots. In 1984, the use of no-till significantly reduced fruit infection to 0.2% compared to 5.5% in conventionally tilled plots.

A field survey was conducted in the spring of 1984 to determine possible overwintering sites of P. syringae pv. tomato. The pathogen was consistently found to be present on leaves and stems of overwintered surface debris. It was never found in association with the roots or rhizosphere of overwintered tomato plants nor with various perennial and winter annual weeds present in the field.

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GENERAL INTRODUCTION AND LITERATURE REVIEW

Bacterial speck of tomato caused by Pseudomonas syringae pv. tomato (P. tomato) has been a recurring problem in Michigan tomato fields since 1978 (11). The disease, while reducing yields (34, 44), has its most devastating effect on fruit quality. The organism enters green fruit and produces small, slightly raised, superficial necrotic lesions (1-2 mm diameter) which do not extend much deeper than the epidermis. Foliar lesions similar in size to those on fruit appear on leaves and occasionally stems and flowers. Often these lesions are surrounded by a chlorotic halo. Yield reductions have been reported (34, 44). The history and physiological characteristics of the pathogen have been well documented by several researchers (12, 26).

Field control to date has depended mostly on crop rotation, the use of disease-free seed, and transplants and fixed copper sprays. Currently, no horticulturally acceptable resistant varieties have been released. Even when resistant varieties become available, their usefulness as a long term control measure may be limited since resistance is controlled by a single dominant gene common to several accessions and named cultivars (29) and the

possibility for resistance breakdown will be high. Pitblado (28) has already reported the development of P. tomato strains cabable of infecting the resistant breeding line Ontario 7710.

Control measures based on prevention have had limited success. Getz (13) demonstrated that the bacterium can overwinter in northern growing areas although this has not yet been shown to be a source of primary infection. Resident populations of P. tomato have also been isolated from roots and foliage of diverse weed and crop plants in soils with and without a history of tomato culture (24, 32). In California, pathogenic isolates of P. tomato were found in fields that had not been planted to tomatoes in over forty years (32).

The production of disease-free transplants has been hindered by the fact that the organism is capable of existing as an epiphyte on the surface of the leaf without causing symptom expression (32, 33, 35). Plants considered to be disease free are shipped from southern production areas. When planted in northern production areas, the bacteria multiply under favorable environmentals conditions to levels capable of producing symptoms. The use of disease-free seed offers protection against the disease, but growers are often reluctant to have hybrid seed treated with hot water because of the decrease in percent germination. P. tomato is known to have survived on dried seeds for 20 years (1).

Control efforts with fixed copper materials on a 7 day spray schedule have yielded mixed results, with copper compounds being effective in some tests (6, 44), but not others (23, 30). Antibiotics have shown promise in some tests (6) but are currently only registered for greenhouse use. The ineffectiveness of copper sprays may be in part due to too infrequent sprays or poor timing. Resistance to normally bactericidal levels of copper has been reported in some Xanthomonas vesicatoria pv. vesicatoria strains (36) and, if present in P. tomato strains, may account for the varying amounts of control.

Disease forecasting could be used to alert growers to the potential onset of disease and allow the application of chemicals or other appropriate controls. Bourke (3) has suggested that in order for a disease to be amenable to predictive forecasting, it must meet four basic requirements: 1) The disease is one which causes economically significant damage in terms of quantity or quality, 2) the disease is variable in time of onset and this variation can be attributed to weather factors, 3) control measures are available and cost effective and 4) laboratory data are available on the nature of the weather dependence of the disease.

In developing a useful disease forecast system, ideally one should look at various parameters of the host, pathogen and environment (3). Although cultivars of a host are generally considered to be either resistant or susceptible,

a particular host's susceptibility may change with age (4, 22, 43). For instance, tomato fruit are no longer capable of being infected by X. vesicatoria or P. tomato once they begin to ripen (44). Indeed, it has demonstrated that green tomato fruit no longer become infected by P. tomato once they reach 3 cm in diameter (14). Plant population density and monoculture of one or a few cultivars may also affect disease progress. Changes in cultivation practices such as sowing time, tillage method, larger fields and irrigation practices may alter a host's susceptibility (3). Wounding from sources such as wind, hail, frost (16), wind-blown sand (5, 40) or insect and mechanical damage can facilitate the entry of pathogens.

Many disease forecast systems assume that an adequate amount of inoculum is present from season to season (7, 8, 41). These systems are difficult to evaluate for if the predicted disease does not occur, it may be either that sufficient inoculum was not available, or the predictive system did not measure and/or interpret the infection periods correctly (18). Forecast systems should take into account conditions both favorable and unfavorable to the pathogen. For example, the occurrence of high temperatures (>29 C) soon after a favorable infection period for downy mildew of lima bean completely negates the effect of the favorable conditions (15). Antagonistic microorganisms can affect the pathogens ability to survive. Erwinia herbicola is known to affect populations of the pathogen E. amylovora

(31, 38) as well as many ice nucleation-active Pseudomonads (21).

Often, attempts are made to simply correlate weather conditions with symptom development (18). More ideally, an attempt should be made to study under controlled conditions, the effect of various environmental factors on the pathogen. Symptom expression and development in P. tomato, for instance, is dependent on the availability of free moisture on leaf surfaces (27, 32), temperature (32, 35, 44) and relative humidity (35, 44).

To date, most disease forecasting systems have been developed for fungal plant pathogens. Predictive systems developed by studying and comparing historical records of disease occurrence and concurrent weather conditions are described as inductive or empirical systems (18). Several empirically derived models have found success over the years. One of the earliest was Mill's tables for the forecast of apple scab (Venturia inaequalis) based on the duration of leaf wetness periods and temperature (25). Several late blight forecast systems based on synoptic weather maps are empirical systems suited to large, but limited, geographical areas (2, 42).

Predictive systems developed from data obtained experimentally in the laboratory or field regarding the relationships of biological and environmental conditions governing host-pathogen interactions are referred to as fundamental or inductive systems. An example of a

fundamental system is that for leaf and stem rust of wheat (Puccinia recondita Rob. ex Desm. f. sp. tritici and P. graminis Pers. f. sp. tritici, respectively) developed by Eversmeyer and Burleigh (9, 10). Using stepwise multiple regression equations, they defined 15 biological and meteorlogical parameters that relate to disease increase after the date of disease prediction. Krause and Massie (18) state that "the classification of disease prediction systems as fundamental or empirical is often an arbitrary process. The accuracy of a predictive system does not depend upon its method of derivation but rather on how well the system has interpreted the biological and meteorlogical relationships that precede infection or disease development".

Recent advances in microcomputer technology have allowed greater use of forecast systems. Blitecast (19), Apple Scab Predictor (17) and the Onion Leaf Blight Predictor (20) are three examples of predictive systems which take advantage of this technology. They are gaining popularity both because of their accuracy and ease of use and economic return.

There are only two bacterial diseases for which forecast systems have been developed. A predictive model has been developed for fireblight (Erwinia amylovora) of apple and pear in the western United States (39), and for Stewart's wilt of corn (37) (Erwinia stewartii). The latter

system is based on the biology of the vector rather than the pathogen or host.

Because current bacterial speck control practices have proven ineffective, new management practices need to be developed. The objectives of this research were: 1) to develop a predictive forecast system for application of preventative chemicals, 2) to determine application intervals for maximum effectiveness, 3) to evaluate the control potential of antibiotics vs. fixed coppers in a predictive system, 4) to investigate the no-till culture of tomatoes as a component of the bacterial speck control management system, and 5) to determine the source of overwintering populations of P. tomato.

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PART I

DEVELOPMENT OF A MODEL TO PREDICT EPIPHYTIC POPULATIONS OF PSEUDOMONAS SYRINGAE PV. TOMATO

ABSTRACT

A preliminary regression model relating temperature to epiphytic populations of Pseudomonas syringae pv. tomato on tomato (Lycopersicon esculentum Mill. Pik Red) was tested. The model failed to accurately predict changes in epiphytic populations. In 1983, a regression model relating temperature rainfall, and previous population level was developed from 2 years pooled data and validated. The model tested was Log_{10} cfu/ g fresh wt = $-5.49 + 0.1T + 1.35R + 0.81P - 0.01T^2$, where T = the average temperature (C) on the previous day, R = the previous 7 day sum of rainfall, and P = the population level at the previous sampling time. The regression model accounted for 85% of observed variations in the population. In 1984, plots sprayed according to model predictions received 3 less sprays than the calendar schedule with no significant difference in amount of infection. The correlation between predicted and observed populations was 0.43. A new regression model was developed by pooling 3 years of data. The equation was Log cfu/g fresh wt = 1.29 - 0.05T + 0.05H - 0.22R + 0.66P, where T = the average temperature (C) for the previous 4 days, H = the average humidity for the previous day, R = the previous 7 day sum of rainfall, and

P = the population level at the previous sampling time. This equation accounts for 64% of the variation in the population.

INTRODUCTION

Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato), the cause of bacterial speck of tomato (Lycopersicon esculentum Mill), exists as a dynamic, epiphytic population on tomato leaf surfaces (16, 17, 19). Research on other epiphytic plant pathogenic bacteria has indicated that symptom development is associated with the attainment of some threshold population level (12, 21). Chemical sprays aimed at eliminating or keeping these bacterial populations below threshold levels to date have provided mixed results. Copper compounds have been effective in some tests (4, 22) but not others (6, 13). Inadequate control may be due to poor timing of chemical application or due to too lengthy intervals between sprays.

If a forecast system capable of predicting the threshold level for symptom expression could be developed, it could aid in the timing of chemical control measures. Relationships between environmental parameters and bacterial speck severity have been studied both in controlled environments and in the field (1, 18, 19, 22). Currently there are very few predictive models for bacterial plant pathogens (15, 20). A disease forecast system which could be utilized similar to the Apple Scab Predictor system (10)

would be useful in controlling bacterial speck chemically. In initial studies (7), a preliminary predictive model for P. tomato relating the 4-day temperature average prior to sampling to the log of the bacterial population on leaves was developed. The objective of this study was to develop a model which would predict the build-up of epiphytic populations of P. tomato to the threshold level which triggers symptom development.

MATERIALS AND METHODS

Field plots. In 1982, field studies were conducted at the Sodus Horticultural Experiment Station, Sodus, MI. In 1983 and 1984, field plots were established at the Botany and Plant Pathology research farm in East Lansing, MI. The bacterial speck-susceptible tomato cultivar, Pik Red, was used each year. In each year, the plot used for monitoring was 24 X 6 m (16-6 m rows) with 1.5 m between rows and an in-row spacing of 0.6 m. Transplants were grown by a commercial greenhouse operator in southwestern Michigan.

Pathogen. A naturally occurring, rifampicin-resistant isolate of P. tomato (PtFr) was used. Cultures were grown as a lawn for 24 h at room temperature on a complete agar medium (11). Inoculum was prepared by washing cells from the agar surface with 5 ml sterile distilled water (SDW). Final inoculum concentration was adjusted by dilution with SDW to approximately 5×10^7 colony forming units (cfu)/ml as determined by standard turbidimetric and dilution plate techniques. Six-week-old tomato plants were sprayed with bacterial inoculum to runoff with a hand-held pneumatic sprayer from a height of 25-30 cm. Plants were placed in a mist chamber until symptoms developed and then taken to the field where all transplanting was done by hand.

Weather Monitoring. Air temperature and relative humidity were measured with a 7-day recording hygrothermagraph (Belfort Instrument Co., Baltimore, MD 21224) placed in a standard weather shelter at ground level. Leaf wetness was recorded in 1982 with a deWitt 7-day recording leaf wetness meter (Valley Stream Farms, Orono, Ontario, Canada) periodically adjusted upward to remain level with the tomato canopy. Rainfall was measured with a tipping bucket rain guage connected to a 7-day recorder (Weather Measure Corp., Sacamento, CA 95841). Solar radiation was measured using a 7-day recording mechanical pyranograph (Weather Measure Corp.).

Population Estimation. Leaf samples were collected 2-3 times per week starting about 2 weeks after transplanting. Samples of 20 symptomless leaflets were randomly selected. Leaflets were finely chopped with a sterile razor and three 1 g replicate samples were weighed out from each sample. The samples were homogenized in a blender for 15 sec in 15 ml distilled water and the homogenate was strained through 2 layers of sterile cheesecloth into a test tube. The homogenate was serially diluted 1:10 and 0.1 ml of each dilution was spread onto the surface of complete medium amended with 100 ug/ml of rifampicin and 25 ug/ml of cycloheximide to control fungal contaminants. After incubation for 3 days at room temperature (23 C), colonies were counted in plates with between 30 and 300 cfu/plate.

Final populations were expressed as $\log_{10}$ cfu/g fresh weight since bacteria are lognormally distributed in the field (9).

Threshold Determination. The threshold population level at which spray applications should be made was determined by greenhouse and field studies comparing inoculum level with lesion development. In greenhouse studies, tomato plants at the five true-leaf stage were sprayed to runoff with suspensions of P. tomato ranging from 10^2 to 10^5 cfu/ml plus a SDW control. After inoculation they were immediately placed in a mist chamber. On succeeding days leaf samples were collected to determine population levels on the leaves. Plants were also observed daily for the development of speck lesions.

In the field study, 16 plants from the population monitoring plot were selected. On July 6, those leaves containing no visible speck lesions were marked on each plant. Populations were monitored and observations for lesion development were made until July 18 when visible lesions became apparent.

Population Model Development. The means or sums of the various weather parameters for periods of 1-7 days prior to a population sampling date were correlated with the population levels. Periods beyond 7 days were not considered since symptoms develop in 5-6 days. The time period for each parameter yielding the highest simple correlation was then selected for multiple regression analysis. Multiple regression analysis was performed using

the Minitab (14) statistical package with $\log_{10}$ bacterial population as the dependent variable and the various weather parameters as the independent variables. In order to meet the requirement for multiple regression (5) that residuals be normally distributed about zero, transformations were performed on several of the independent variables. Relative humidity was transformed with the arcsin-square root transformation. Rainfall and solar radiation were transformed by adding 0.5 and taking the square root.

The various models generated were evaluated based on their coefficient of multiple determination (R^2). Variables were added or deleted to equations based on their contributions to R^2.

Model Validation. In 1982 and 1983, tomato plants (cv.Pik Red) were planted in two row plots 6 m long with 1.5 m between rows and an in-row spacing of 0.6 m. In 1984, single row plots 9 m long were was planted with a between-row spacing of 1.5 m and an in-row spacing of 0.6 m. A guard row was placed between each treatment row. A split plot design with 4 replications was used. Main plot treatments were a calendar spray schedule (7-day spray interval in 1982 and 1983 and 4 days in 1984) vs. sprays based on model predictions of population levels (a minimum 4-day interval between sprays). Subplot treatments consisted of various chemical controls. In 1982, cupric hydroxide + mancozeb at 4.7 L/ha was compared to an unsprayed control. In 1983, streptomycin (200 ppm) and

oxytetracycline (200 ppm) were added to the test. In 1984, cupric hydroxide (2.2 kg/ha) alone was also included along with the 1983 treatments. Multiple regression equations generated in a current year were used to predict population levels in the following year.

Goodness of fit was determined by the correlation between the predicted values using the equation and the actual populations determined by sampling. At the end of the season, fruit were harvested and evaluated for the presence of bacterial speck.

RESULTS

Threshold Determination. The minimum level of detection of epiphytic bacteria using the dilution plating technique is about 10^3 to 10^4 cfu/ml (8). Bacteria could not be detected on leaf surfaces until day 4 (Table 1) and then only on plants which had received initial inoculum levels of 2×10^4 to 2×10^5 cfu/ml. Symptoms first developed on day 6 on plants which had received an initial concentration of 2×10^5 cfu/ml. No other treatments developed symptoms.

On July 6, 16 plants were selected and the highest leaf containing speck lesions on each plant was marked. The mean population on symptomless leaflets from these plants was log 2.85 cfu/g fresh wt (Table 2). On July 9, 11 and 13, no new lesions were observed on the plants even though the population on symptomless leaves had increased to 5.51, 6.83 and 7.60 log cfu/g fresh wt respectively. On July 18, large numbers of new lesions appeared on leaves that had previously been symptomless. Based on greenhouse tests where bacteria developed under ideal infection conditions, and field tests where conditions for infection were less favorable, a value of log 5 cfu/g fresh wt of tissue was chosen as the threshold level at which spray applications would be made.

Table 1. Population levels and symptom development on leaves of greenhouse grown plants inoculated with varying concentrations of Pseudomonas syringae pv. tomato.

Initial concentration (CFU/ml)	Log$_{10}$ (CFU/g fresh wt) Day 0	Day 2	Day 4	Day 6	Day 8
0	0	0	0	0	0
2×10^2	0	0	0	0	0
2×10^3	0	0	0	0	0
2×10^4	0	0	5.0	4.8	3.9
2×10^5	0	0	4.6	7.1**	-

** Lesions appeared on foliage

Table 2. Symptom development and population levels on field grown tomato plants inoculated with Pseudomonas syringae pv. tomato.

Date	Leaf number[z]	Mean population[y]
7/6	4.1	2.85
7/9	4.1	5.51
7/11	4.1	6.83
7/13	4.1	7.60
7/18	11.1	6.00

[z] Numbers in the column represent the higest leaf position from the soil containing visible speck symptoms. These numbers represent the mean of 16 plants

[y] Log_{10} (CFU/g fresh wt)

Population Model Development and Validation. Getz (7) previously developed a second-order temperature-driven regression model of the form:

$$\text{Log cfu/g fresh wt} = b_0 + b_1 T + b_{11} T^2 + E \qquad (1)$$

where T = the previous 4-day average temperature (C). The b values were least squares estimates of the partial regression coefficients and E was a normally distributed random variable with mean zero and variance σ^2. This model accounted for 64% of the observed variation in population. The actual equation derived was:

$$\text{Log cfu/g fresh wt} = -29.8643 + 3.8926T - 0.108153T^2 \qquad (2)$$

There was a reasonably close fit between the actual population levels observed in 1981 and those generated by the model (Figure 1). The correlation coefficient (r) between $\log_{10}$ cfu and temperatures was 0.87. This predictive model was tested in 1982 against a calendar 7-day spray program. The populations predicted in 1982 using the equation generated in 1981 did not coincide well (r = 0.19) with actual populations measured (Figure 2). Ten sprays were applied to plots on a weekly spray schedule while plots sprayed according to the model received 8 sprays. The amount of fruit infection was determined at harvest (Table 3). There was no significant difference in amount of infection between the two spray schedules.

The severe underestimation of 1982 populations by equation 2 suggested that one or more important factors were missing. Relationships between the dependent and

Figure 1. Prediction of epiphytic bacterial populations based on an equation generated using 1981 weather data vs. measured 1981 populations.

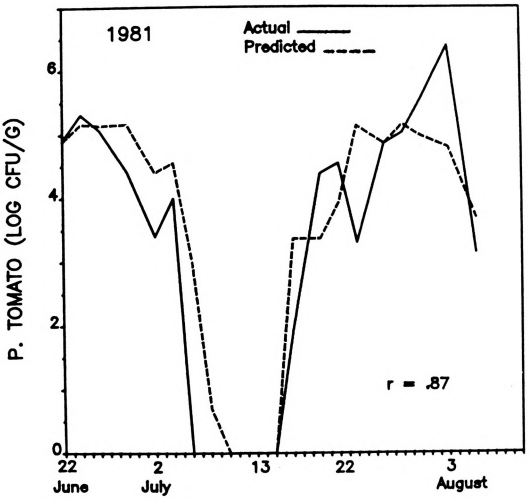

1981
Actual
Predicted
P. TOMATO (LOG CFU/G)
6
4
2
0
r = .87
22
June
2
July
13
22
3
August

Figure 2. Prediction of epiphytic bacterial populations based on an equation generated using 1981 weather data vs. measured 1982 populations.

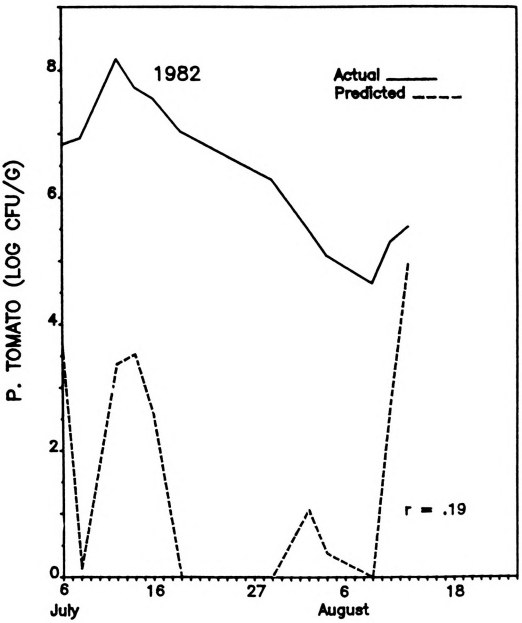

1982
Actual
Predicted
P. TOMATO (LOG CFU/G)
8
6
4
2
0
r = .19
6
16
27
6
18
July
August

Table 3. The effect of two spray schedules on fruit infection by Pseudomonas syringae pv. tomato.

Spray schedule	Percent fruit infection		
	Year		
	1982	1983	1984
4 Days	-	-	2.8
7 Days	67.5	8.5	-
As predicted	74.9	11.0	2.0
	n.s.[z]	n.s.	n.s.

[z] no significant difference

independent variables were visualized by plotting the parameters against each other (Figure 3A-C) and simple correlations were determined. A new equation was generated which took the form:

$$\text{Log cfu/g fresh wt} = -3.97 + 0.07H + 0.64S - 0.13RT + 0.45SR \quad (3)$$

where H = the average relative humidity for the previous 6 days, S = the average solar radiation for the previous 7 days, R = the sum of rainfall for the previous 7 days, and T = the average temperature (C) for the previous 2 days. The new model accounted for 90% of the observed variation in the population. Correlation with the 1982 actual values was high (r = 0.95) (Figure 4).

In 1983, the experiment was repeated using equation 3. Predictions in 1983 tended to overestimate population levels (Figure 5) compared to 1982 when they were greatly underestimated (Figure 2). Ten sprays were applied to plots on a weekly spray schedule while plots sprayed according to the model received 7 sprays. There was no significant difference in amount of infection between the two spray schedules at harvest (Table 3).

Multiple regression equations are not reliable if extrapolated outside the range of values used to construct them (3). Instead of generating new equations each year using a limited range of weather values, data from 1982 and 1983 were pooled and a single equation was constructed which could be used over a wider range of environmental

Figure 3. Relationship between climatological changes and the epiphytic population of Pseudomonas syringae pv. tomato on leaves of the field grown susceptible fresh market cultivar Pik Red, A) 1982, B) 1983, C) 1984.

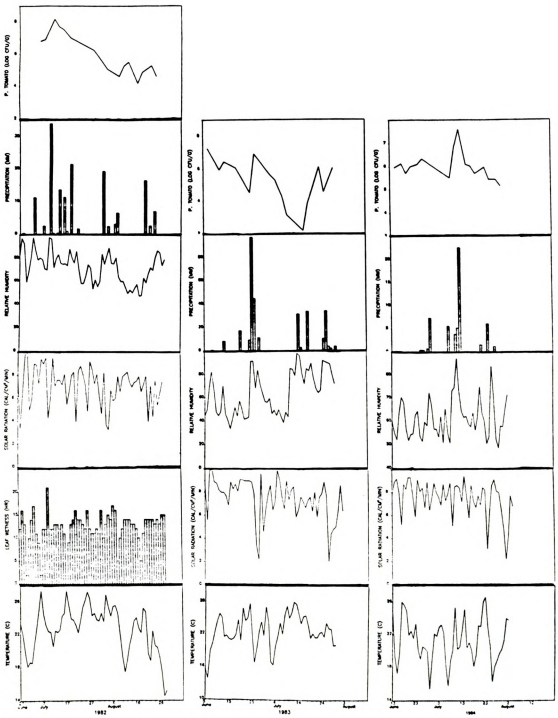

P. TOMATO (LOG CFU/G)
PRECIPITATION (MM)
RELATIVE HUMIDITY
SOLAR RADIATION (CAL/CM²/MIN)
LEAF WETNESS (HR)
TEMPERATURE (C)
1982
1983
1984

Figure 4. Prediction of epiphytic bacterial populations based on an equation generated using 1982 weather data vs. measured 1982 populations.

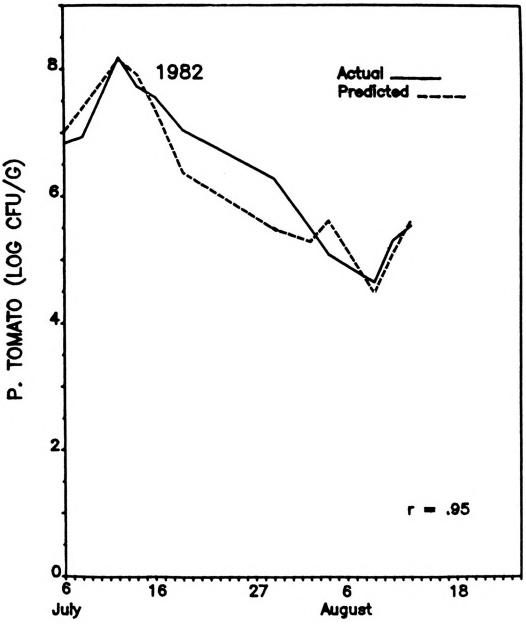
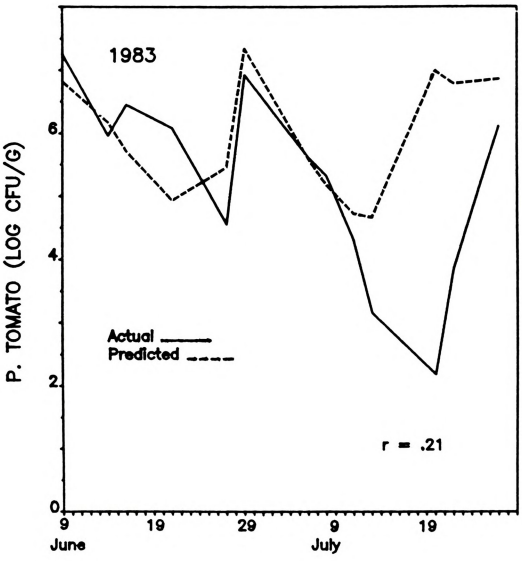

1982
Actual
Predicted
P. TOMATO (LOG CFU/G)
8
6
4
2
0
r = .95
6
July
16
27
6
August
18

Figure 5. Prediction of epiphytic bacterial populations based on an equation generated using 1982 weather data vs. measured 1983 populations.


1983
P. TOMATO (LOG CFU/G)
6
4
2
0
Actual
Predicted
r = .21
9
19
29
9
19
June
July

conditions. The results of this pooling was:

Log cfu/g fr wt = $-5.49 + 0.1T + 1.35R + 0.81P - 0.01T^2$ (4)

where T = average temperature (C) on the previous day, R = the sum of rainfall for the previous 7 days and P = the leaf population level at the previous sampling time. This equation from pooled data accounted for 85% of the observed variations in the population. When predicted values generated from equation 4 were correlated with actual values from 1983, the correlation coefficient (r) was 0.87 (Figure 6). In 1984 tests, the correlation between values generated by equation 4 and actual values was r = 0.43 (Figure 7). The calendar spray schedule was shortened from 7 days to 4 days in 1984 based on greenhouse efficacy experiments. Plots on a regular 4-day spray schedule received 12 sprays while those timed with model predictions of population build-up received 9 sprays. There were no significant differences in yield or infection level between the two treatments (Table 3).

At the end of the 1984 season, weather data were pooled with those from the two previous years. The equation took the form:

Log cfu/g fr wt = $1.29 - 0.05T + 0.05H - 0.22R + 0.66P$ (5)

where T = the average temperature (C) for the previous 4 days, H = the average relative humidity for the previous day, R = the sum of rainfall for the previous 7 days, and P = the leaf population level at the previous sampling time. The equation accounted for 64% of the variations in the

Figure 6. Prediction of epiphytic bacterial populations based on an equation generated using pooled 1982 and 1983 weather data vs. measured 1983 populations.

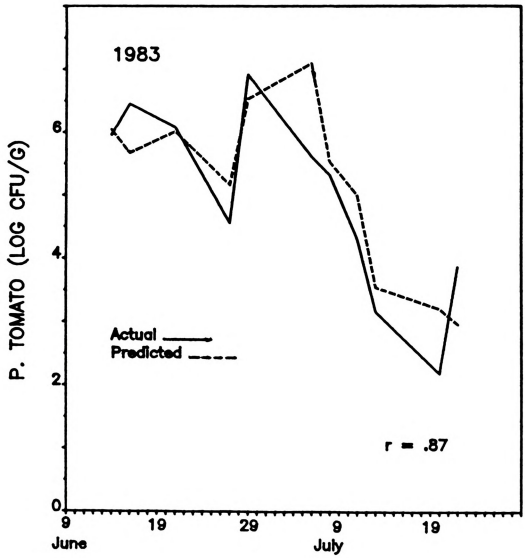
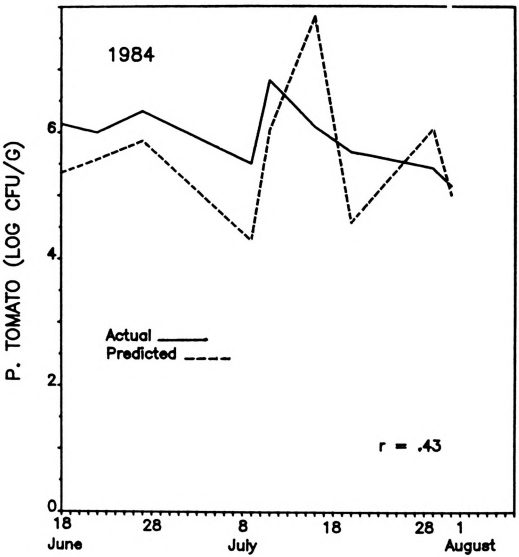
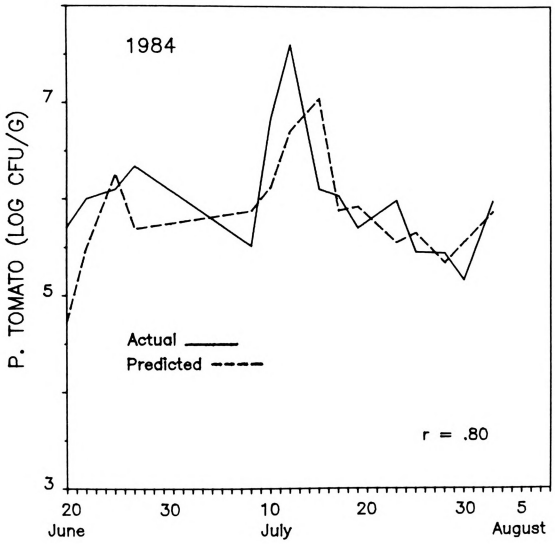

1983
P. TOMATO (LOG CFU/G)
6
4
2
0
Actual
Predicted
r = .87
9
19
29
9
19
June
July

Figure 7. Prediction of epiphytic bacterial populations based on an equation generated using pooled 1982 and 1983 weather data vs. measured 1984 populations.


1984
P. TOMATO (LOG CFU/G)
6
4
2
0
Actual
Predicted
r = .43
18
June
28
8
July
18
28
1
August

population. The predicted values for 1984 generated from this equation were plotted against the actual values (Figure 8). The correlation between the values was r = 0.80.

Figure 8. Prediction of epiphytic bacterial populations based on an equation generated using pooled 1982, 1983, and 1984 weather data vs. measured 1984 populations.


1984
P. TOMATO (LOG CFU/G)
7
5
3
Actual
Predicted
r = .80
20
June
30
10
July
20
30
5
August

DISCUSSION

A multiple regression model was developed for identifying periods when epiphytic bacterial populations attained the threshold level necessary for symptom development. Lindemann et al. (12) recently developed a model to predict incidence and severity of brown spot (P. syringae pv. syringae) of bean using an apparent threshold level. Their prediction of disease incidence was based on population levels on individual leaflets rather than mean populations. They argued that "infections occur on individual leaflets and not on some hypothetical average leaf". Where they have attempted to predict disease incidence and severity based upon population levels, this model attempts to anticipate when a threshold population will be reached so that preventive measures may be taken to reduce the population level before the threshold is reached.

Basu (2) estimated that P. tomato required at least 1×10^6 cfu/ml to produce successful infection. Bashan et al. (1), using tomato plants wounded with carborundum powder, found that speck symptoms did not develop when inoculated with bacterial suspensions containing fewer than 10^4 cfu/ml. In both cases, symptom development was related to some initial inoculum level with no regard given to the

change in populations which might occur on the leaf surface following inoculation. In determining an infection threshold to use with this model, an attempt was made to correlate population levels present on the leaf with the time that symptoms occured. In greenhouse experiments, populations on the leaf surface were between log 4.6 and log 7.1 cfu/g fresh wt before symptoms developed (Table 1). Symptom expression occurred 6 days after inoculation. In field observations, symptoms occurred 5 days after leaf populations reached a high of log 7.6 cfu/g fresh wt (Table 2). Based on the literature and results in greenhouse and field tests, a value of log 5 cfu/g fresh wt of tissue was chosen as the threshold at which spray applications would be made.

This model is based on mean population levels. Because epipiphytic bacterial populations are lognormally distributed (9), the use of bulked samples to obtain a mean population estimate will result in an overestimation of the actual population present. Although I realized the deficiency of the bulk sampling method, it was chosen for its speed and ease of use. The model being proposed requires at least an initial estimate of the population present in the field. In order to have practical application, the method of sampling must be simple, rapid, and inexpensive. Ideally, it will be done by private consultants many growers now use. Since mean population estimates are used in generating the multiple regression

equations, predicted values will also reflect the overestimation of the populations. If these populations are looked upon as relative values, the fact that they overestimate actual values should make no difference as long as the threshold value is chosen accordingly. It has been suggested that this threshold is not a constant and may be related to other factors, for instance, changes that may occur in host susceptibility (12). Environment surely plays a role in determining what the actual threshold will be. Infection of wounded plants when conditions are favorable to the pathogen will likely require a lower threshold than infection of healthy plants when environmental conditions are unfavorable to the pathogen. More work in evaluating the threshold level for P. tomato needs to be done.

Evaluation of Getz's equation in 1982 showed that it significantly underestimated actual populations. According to Butt and Royle (3), underestimation is an indication that one or more important variables is missing. The variables added in 1982 were humidity, solar radiation, and interactions between rainfall and temperature, and solar radiation and rainfall. In addition, the variable T^2 was removed. Leaf wetness did not appear to play an important role in population estimation. It has been demonstrated for P. tomato that leaf wetness periods as short as 6 hrs are enough to induce symptom development on inoculated plants (19). Data from 1981 (7) and 1982 (Figure 3A) indicated that leaf wetness was probably not an important factor under

Michigan growing conditions since dew periods are normally longer than 6 hrs during the summer growing season. Although this equation had a very high R^2 value, the predictions were off by a factor as much as 10^5 late in the season. One of the dangers of multiple regression analysis is that if enough variables are added, regardless of their relationship to the dependent variable, R^2 values near unity can often be attained (3). A second is that often a variable contributes significantly to R^2 because it is correlated with an undetected independent variable and not the dependent variable in question (3). The equation developed in 1982 probably fell victim to both of these dangers.

Data from 1983 were pooled with data from 1982 before deriving an equation. This allowed a wider range of values and limited extrapolation. It was also an attempt to develop an "average best equation" which could be used from year to year rather than developing a new one every year. The 1984 predicted values had a closer fit to actual values than in the previous two years but they still varied by a factor of 10^2 at times. An important aspect of the equation developed in 1983 is that a non-weather variable was added, namely the population level at the previous sampling date (P). The epiphytic bacterial population levels at successive sampling dates represent a time series. The population level measured at any point in this time series would be dependent on what the level was at the previous

point. The variable P thus was included to reflect this relationship. In order to avoid continuous sampling throughout the season, the predicted value generated by the equation on any date could be used as the P value in the next prediction. When this method was used to predict values using 1984 weather data, the correlation with actual 1984 populations was 0.77.

In each of the 3 seasons, use of the model reduced the number of sprays required by 2, 3 and 3 respectively with no significant difference in the percentage of infected fruit (Table 3). This is an important point since chemical application is one of the few variable costs in tomato production. The only way growers can increase net profit is to reduce their variable costs. The total amount of fruit damage in these tests is a reflection of both disease pressure and chemicals used and will be discussed elsewhere (Chapter 2).

The current equation has yet to be tested but with the wider data base used to develop it, predictions are likely to be more accurate. More data needs to be accumulated before the model can be used with confidence.

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PART II

INFLUENCE OF TIMING OF APPLICATION AND CHEMICAL ON CONTROL OF BACTERIAL SPECK OF TOMATO

ABSTRACT

Greenhouse experiments were conducted to determine the effect of timing of application of selected chemicals on control of bacterial speck on artificially inoculated plants. Streptomycin sulfate, oxytetracycline and a copper-mancozeb complex were applied at various times prior to or after inoculation with P. tomato. Only streptomycin provided significant control and then only if applied within 24-48 hr of inoculation.

In field studies, various antibiotic and copper compounds were applied, either using a 4- or 7-day calendar spray schedule or based on a predictive spray model. The efficacy of all chemicals appeared to be related to disease pressure with significant protection provided only when disease pressure was low. Streptomycin generally provided the highest amount of control. The activity of oxytetracycline was increased by the addition of an adjuvant.

INTRODUCTION

Bacterial speck of tomato (Lycopersicon esculentum Mill), caused by the bacterium Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato) continues to be a serious problem in Michigan, Ohio, and other tomato production areas of the United States and Canada (5). Infected plants are characterized by a reduction in quality caused by lesions on the fruit surface and by a reduction in yield (11, 14). Previous chemical control studies have yielded mixed results. Some reports (2, 14) have suggested that copper compounds can be used effectively in a preventative control program while others (7, 10) have shown chemical control to be ineffective, at least under cool temperate growing conditions. Coppers have traditionally been applied on a 7-day spray schedule. It is well known that the selection of material and timing of application are critical for effective disease control (3). The ineffectiveness of copper sprays in some tests may be due in part to the lack of good preventative spray materials or too lengthy intervals between sprays.

Streptomycin sulfate has shown promise in some tests (2) but is currently only registered for greenhouse use. Information on the effectiveness of other antibiotic control

agents is limited (9). The purpose of this study was to 1) evaluate the timing of application of selected chemical control agents and 2) evaluate selected antibiotics and fixed copper compounds for control of bacterial speck in a predictive forecast system.

MATERIALS AND METHODS

Inoculum. A naturally occurring rifampicin-resistant isolate of P. tomato (PtFr) was used as the pathogen throughout this study. Cultures were grown as a lawn on a complete agar medium (6) for 24 h at room temperature. Inoculum was prepared by washing cells from the agar surface with 5 ml sterile distilled water (SDW). Final inoculum concentration was adjusted by dilution with SDW to approximately 5×10^7 colony forming units (cfu)/ml as determined by standard turbidimetric and dilution plate techniques.

Greenhouse Tests. Inoculum was applied to plants at the 5-7 true leaf stage with a hand-held pneumatic sprayer from a height of 25-35 cm. Plants were sprayed until runoff. The pre-inoculation and post-inoculation experiments were each repeated 5 times. In the first 3 experiments all plants were placed in an air-conditioned mist chamber and held at 20 C nights and 24 C days until symptoms developed. Mist was applied for 20 sec every 30 minutes so leaves stayed continually wet. In the last 2 tests, inoculated plants were placed in closed plastic bags on a laboratory bench for 4 days and then removed to a greenhouse bench until symptoms developed.

Tomato plants of the susceptible fresh market cultivar Pik Red (Joseph Harris Co. Inc., Rochester, N.Y. 14624) were grown in the greenhouse in 10 cm clay pots containing Sunshine Mix #1 (J. Mollema and Son, Inc., Grand Rapids, MI 49507). A 20-20-20 soluble fertilizer (Peters Fertilizer Products, Allentown, PA 18100) was applied biweekly. Chemicals were applied with a hand-held pneumatic sprayer at a height of 25-35 cm. A volume of approximately 9 ml was applied to each plant to simulate field spraying (100 gal/A). Four treatments including a SDW control, streptomycin sulfate (Agrimycin 17, 200 ppm), oxytetracycline (Mycoshield, 200 ppm) and an experimental cupric hydroxide (Kocide 101, 360 g/L a.i.) + mancozeb (Dithane M-45, 360 g/L a.i.) combination (KCC-FMX, 5 ml/L) were used. Chemicals were applied 6, 5, 4, 3, 2 or 1 day before inoculation, just prior to inoculation, immediately following innoculation, and at 1, 2 or 4 days after inoculation. Pre-inoculation chemical treatments were applied to the plants and allowed to dry. Plants were then placed into the mist chamber until inoculation. Post-inoculation sprays were made by removing the plants from the mist chamber and applying the chemical. The plants were allowed to dry and then placed back into the mist chamber. In the final two tests, plants were sprayed at the prescribed times and left on the greenhouse bench until inoculation. Following inoculation, plants were placed into plastic bags. Post-inoculation sprays were made by removing

the plants from their bags, applying the chemical and then returning the plant to the bag. The experiments were set up in completely randomized design. There were 3 replications per treatment.

Disease incidence was calculated by counting the number of infected leaflets on each plant and expressing it as a percentage of the total number of leaflets per plant. An arcsin-square root transformation was performed on all data to stabilize the variances.

Field Tests. In 1982, tests were conducted at the Sodus Horticultural Experiment Farm in southwestern Michigan. The 1983 and 1984 tests were conducted at the Michigan State University Botany and Plant Pathology Research Farm near East Lansing. Plants were grown by a commercial greenhouse operator in southwestern Michigan. Six-week-old tomato plants (cv. Pik Red) were inoculated in their flats using the methods described for the greenhouse studies. Following inoculation, the flats were placed into mist chambers for 7 days until symptoms developed. In each of the 3 years, chemical efficacy tests were conducted as part of the predictive forecast model validation (Chapter 1). In 1982 and 1983 plants were transplanted in rows 4.9 m long with 1.5 m between rows and an in-row spacing of 0.6 m. There were two rows per plot. In 1984, a single row 6.0 m long was used with a guard row placed between each plot. The experiment was set up as a split-plot design with 4 replications. Main plot treatments in 1982 and 1983 were a

routine 7-day spray schedule vs. sprays based on model predictions of population levels (Chapter 1). In 1984, a 4-day spray schedule was used vs. model predictions. Subplot treatments consisted of various chemical controls. In 1982, cupric hydroxide + mancozeb at 4.7 L/ha was compared to an unsprayed control. In 1983, streptomycin (200 ppm) and oxytetracycline (200 ppm) were added to the test. In 1984, cupric hydroxide (2.2 kg/ha) alone was also included with the 1983 treatments.

In 1984, a second experiment was conducted to test the efficacy of various fixed copper spray treatments as well as several commonly used antibiotics. The bactericides and rates per liter were, cupric hydroxide (Kocide 101, 2.4 g), cupric hydroxide + mancozeb (KCC-FMX 5 ml), copper salts of fatty and rosin acids (Citcop 5E, 3.7 ml), AR 153845 (composition unknown, 3.7 ml), AR 153845 (3.7 ml) + mancozeb (Dithane M-45, 3.6 g), copper sulfate (Super Cu, 5 ml), copper oxychloride sulfate (COCS, 7.2 g), hexachlorophene (Hexide, 0.6ml), streptomycin sulfate (Agrimycin 17, 1.2 g), oxytetracycline (Mycoshield, 0.9 and 1.8 g), and oxytetracycline (0.9 and 1.8 g) + adjuvant TS 188-30 (composition unknown, 1.2 g). A single row per plot, 9 m long, was planted with a between-row spacing of 1.5 m and an in-row spacing of 0.6 m. A guard row was placed between each treatment row. The experiment was set up in a randomized complete block design with 4 replications.

Transplanting in each year were done by hand. Plots were cared for according to the standard commercial practices of the area. Carbaryl and chlorothalonil were used as needed for foliar insect and fungal disease control. Chlorathalonil has previously been shown to have no effect on P. tomato (7). Disease incidence in all experiments was determined by counting the numbers of speck infected fruit expressed as a percentage of the total number of fruits. In 1984, yields were also determined.

RESULTS

Greenhouse tests. The average amount of infection in each test varied according to the method of incubation. In general, higher levels of infection occurred when plants were placed in plastic bags following inoculation. Though mean levels of infection varied from test to test, the relationship between the various treatments remained constant. Therefore, only one set of data each for the pre- and post-inoculation experiments will be presented.

The effectiveness of all chemical treatments were directly related to time of application. Although the interaction between chemical and time of application (Figure 1) was not significant ($P = .1$) there was a significant decrease in efficacy as the time of application prior to inoculation was increased (Figure 2). Streptomycin provided the best control when compared to the nontreated control but only when it was applied within 1 day of inoculation. Post-inoculation experiments provided similar results (Figure 3). Streptomycin reduced bacterial speck by 39% compared to the control but only if applied within 24 hr of inoculation. The 25% reduction in infection by oxytetracycline was significant when compared to the control but overall levels of infection were still high. The cupric

Figure 1. Effects of chemical and timing of application prior to inoculation on severity of bacterial speck of tomato on greenhous grown plants.

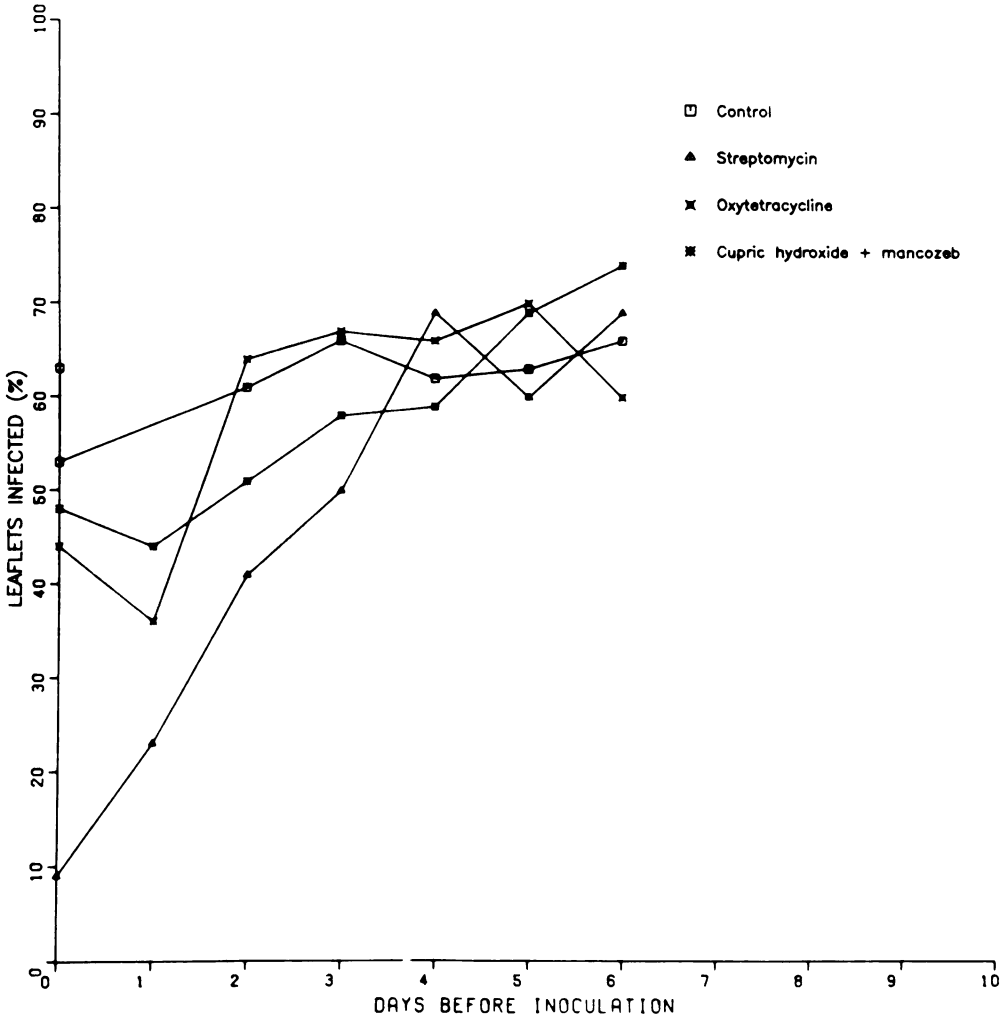

LEAFLETS INFECTED (%)
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
7
8
9
10
DAYS BEFORE INOCULATION
Control
Streptomycin
Oxytetracycline
Cupric hydroxide + mancozeb

Figure 2. Effect of timing of application on severity of bacterial speck of tomato on greenhouse grown plants.

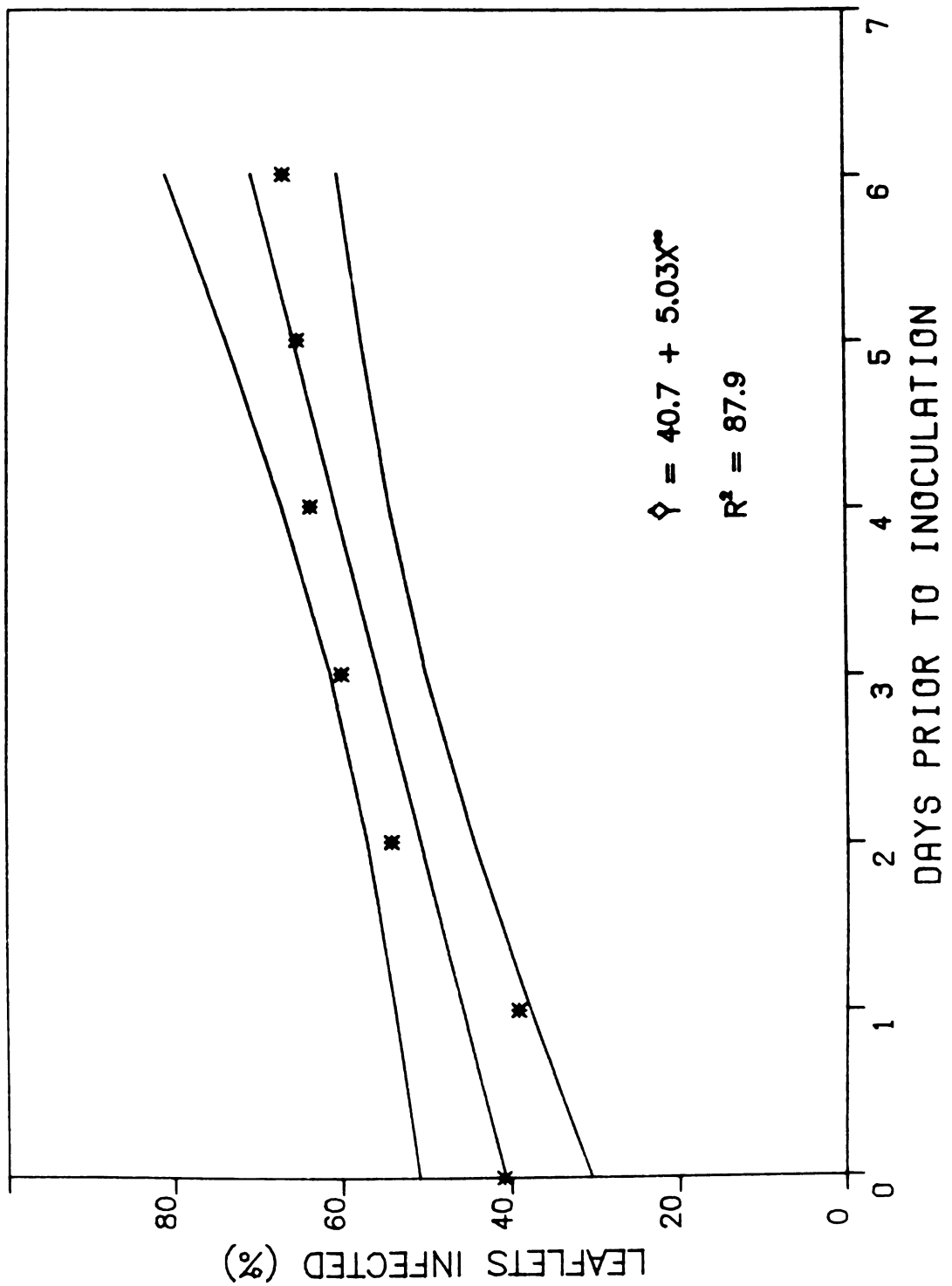
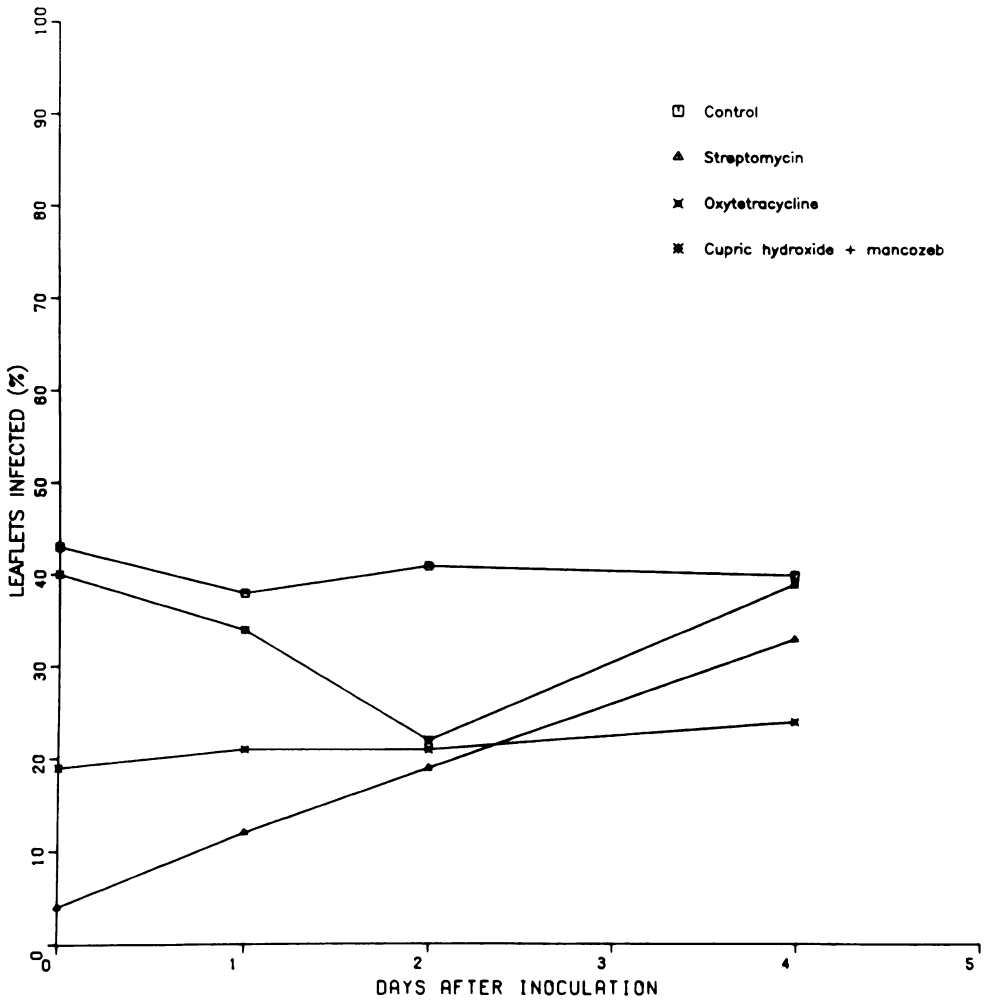

LEAFLETS INFECTED (%)
0
20
40
60
80
0
1
2
3
4
5
6
7
DAYS PRIOR TO INOCULATION
$\hat{Y} = 40.7 + 5.03X^{**}$
$R^2 = 87.9$

Figure 3. Effects of chemical and timing of application following inoculation on severity of bacterial speck of tomato on greenhouse grown plants.


Control
Streptomycin
Oxytetracycline
Cupric hydroxide + mancozeb
LEAFLETS INFECTED (%)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
DAYS AFTER INOCULATION

hydroxide + mancozeb treatment had levels of infection not significantly different from the control. Chemical applications were not made beyond 4 days since symptoms were already beginning to develop.

Field Tests. Field chemical efficacy experiments were done in conjunction with the validation of a predictive forecast system for bacterial speck control (Table 1). In 1982, only the cupric hydroxide + mancozeb combination was used. Disease pressure was high due to cool, wet conditions throughout the summer. There were no significant differences in amounts of fruit infection between control and treatment plots. In 1983, disease pressure was less severe and all 3 chemical treatments significantly decreased speck severity when compared to the control. The 1984 growing season was generally hot and dry and disease pressure was extremely low. All treatments significantly reduced disease severity when compared to the untreated control. There was significantly more speck in the oxytetracycline treatment compared to cupric hydroxide alone but not when compared to the other two chemical treatments.

In 1984, a second, more comprehensive examination of available bactericides was also made (Table 2). All materials except hexachlorophene significantly reduced fruit infection when compared to the control. Of the copper containing compounds, the copper-oxychloride sulfate and cupric hydroxide treatments provided the greatest reduction in disease severity reducing fruit infection to 1.7 and

Table 1. The effect of selected antibiotics and fixed copper compounds for control of bacterial speck in a predictive forecast system.

	Percent fruit infection		
Chemical	1982	1983	1984
cupric hydroxide + mancozeb	68.3[z]	5.6b	0.7ab[y]
streptomycin	-	2.8a	1.0ab
oxytetracycline	-	10.3c	2.5b
cupric hydroxide	-	-	0.5a
control	63.3	20.9d	7.5c

[z] no significant difference

[y] Means within columns followed by the same letter are not significantly different (DMRT P = .05)

Table 2. Effect of chemical treatment on tomato fruit infection by Pseudomonas syringae pv. tomato.

Chemical	Rate/ha	Yield (MT/ha)	Fruit infection (%)
AR 153845	3.5 L	27.6	4.3
AR 153845 + Dithane M-45	3.5 L 3.4 kg	33.5	1.7
Kocide-FMX	4.7 L	27.4	3.3
Citcop 5E	3.5 L	31.9	8.3
Super Cu	4.7 L	29.8	4.7
Hexide	0.3 L	32.8	12.7
COCS	6.8 kg	25.1	1.7
Kocide 101	2.2 kg	32.5	2.7
Agrimycin 17	200 ppm	25.5	0.3
Mycoshield	150 ppm	28.7	9.0
Mycoshield + Adjuvant TS 188-30	150 ppm 1200 ppm	27.7	6.3
Mycoshield	300 ppm	32.8	6.3
Mycoshield + Adjuvant TS 188-30	300 ppm 1200 ppm	32.4	1.7
Control		29.1	15.0
		n.s.	LSD.05 = 5.7

2.2% respectively compared to 15% in the control. The combination of AR 153845 and mancozeb was equally effective reducing infection to 1.7%. Streptomycin was the most effective antibiotic and the best material overall reducing infection to 0.3%. Oxytetracycline at 300 ppm reduced infection to 6.3% compared to only 9.0% for the 150 ppm rate. Oxytetracycline reduced infection to 1.7 and 6.3% respectively by the addition of an adjuvant. There was no significant difference in yields among treatments.

DISCUSSION

Cox (3) has pointed out that selection of chemical and timing of application are critical in control of disease. Many conflicting reports on the efficacy of bacterial speck control chemicals have appeared in the literature (2, 7, 10, 14). In most instances, sprays were applied on a traditional 7-day schedule. The greenhouse studies were undertaken to determine if 7-day schedules were adequate for speck control. Under the ideal infection conditions present in the greenhouse, only streptomycin provided what could be considered adequate speck control, and then only when applied within 24-48 hr of inoculation (Figures 1,3). Efficacy of all the chemicals decreased significantly as the time of application prior to infection was increased (Figure 2). With the exception of streptomycin, application of chemicals following inoculation seemed to have little effect on the progress of disease. Symptoms appeared 4 days following inoculation; thus it would appear that even a chemical with good eradicant properties would have little use more than 2-3 days following infection.

When experiments were conducted in the field, the results were quite different. In 1982, when disease pressure was extremely high due to favorable environmental

conditions, a 7-day spray schedule with cupric hydroxide + mancozeb provided no control of bacterial speck (Table 1). The following year, when environmental conditions were less favorable and disease pressure was lower, a 7-day spray schedule seemed to provide much more effective control. However, only streptomycin and possibly the cupric hydroxide + mancozeb reduced speck severity to levels which would be considered economically acceptable to commercial growers. Based on the greenhouse efficacy results, and the fact that symptoms developed within 4 days of inoculation, the spray schedule in 1984 was shortened to 4 days. Anything less than this would probably not be economically feasible for growers, although a cost-benefit study should be done to confirm this. Although environmental conditions again kept disease pressure low during most of the 1984 growing season, chemical control using a 4-day schedule was excellent (Table 1). In evaluating the 3 years of field data, it appeared that the most important factor in determining the degree of control of bacterial speck was disease pressure. When disease pressure was high such as in greenhouse experiments and in the field during the 1982 growing season, no chemical provided effective control. When disease pressure was low, coppers as well as antibiotics were effective in reducing fruit infection.

The comprehensive bactericide evaluation conducted in 1984 also suggested that coppers and antibiotics were effective when disease pressure was low (Table 2). These

plots were sprayed on a 7-day schedule according to manufacturer's recommendations on the labels. Several of the coppers and antibiotics provided good control when compared to the unsprayed check plots. Only hexachlorophene did not significantly reduce the amount of disease compared to the control. The addition of an adjuvant greatly enhanced the efficacy of oxytetracycline, while the additon of mancozeb to cupric hydroxide and AR 153845 had mixed results. The percent fruit infection increased slightly when cupric hydroxide + mancozeb was compared to cupric hyroxide alone, although the increase was not significant. The addition of mancozeb to AR 153845 improved its performance slightly. Marco and Stall (8) demonstrated that the addition of mancozeb to copper-containing compounds resulted in an increase in the amount of available copper in solution, and this may explain the increased activity of the combined materials which have been reported.

The results of these experiments would indicate that growers in north temperate climates cannot depend on chemical control to consistently reduce the level of bacterial speck infection. Available copper compounds apparently only provide protection when disease pressure is at relatively low levels. Antibiotics, which offer an increased level of protection, are currently not registered for field use. If registration occurs, greenhouse tests suggest that the timing of application is critical. Streptomycin required application within 24-48 hours of

infection for maximum effectiveness. Streptomycin or oxytetracycline plus an adjuvant in combination with a predictive forecast system may provide the type of control which growers need. However, registration in the near future is not likely because of human health concerns associated with antibiotic use and because of the potential for antibiotic resistance. The development of streptomycin resistance in Xanthomonas vesicatoria pv. vesicatoria is well documented (13). It is likely that similar resistance would occur in P. tomato if antibiotics were used on a regular basis. Of greater concern to growers may be the reports of copper tolerance in X. vesicatoria reported from Florida and Mexico (1, 8). Getz et al. (4) has shown that tomato fruit are susceptible to infection only until they reach 3 cm in diameter, therefore, control efforts should be greatest during the early part of the growing season. Growers would be wise to develop a good preventative control program including buying disease free seed and transplants. Even these measures have their drawbacks since hot water treatment of expensive hybrid seed reduces germination and the organism can survive undetected as an epiphyte on leaf surfaces of the plant (12) ready to develop into an epiphytotic when environmental conditions are favorable. Growers should look at alternative cultural practices such as the use of no-till management system to reduce the spread of bacterial speck in a field (Chapter 3).

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PART III

NO-TILL: A POTENTIAL CULTURAL CONTROL PRACTICE FOR REDUCING SPREAD OF BACTERIAL SPECK OF TOMATO IN THE FIELD

ABSTRACT

A no-till management system was evaluated as a potential control practice for bacterial speck of tomatoes. In 1982, population levels of the organism were less in no-till plots than in conventionally tilled plots. In 1984, the use of no-till significantly reduced fruit infection to 0.2% compared to 5.5% in conventionally tilled plots.

INTRODUCTION

Bacterial speck (Pseudomonas syringae pv. tomato) of tomato (Lycopersicon esculentum Mill) occurs in many tomato growing regions in the United States, (9, 12, 13) Canada (3), and other countries (6). The organism contributes to yield decreases (14, 16); however, its most devastating affect is on fruit quality. The organism enters green fruit and produces small, slightly raised, superficial black specks (1-2 mm diameter). Speck lesions are often deep enough to reduce quality even after mechanical removal of skins (9). Attempts to control speck chemically in northern temperate growing regions to date have generally been unsuccessful (10, 11).

Work with other bacterial plant pathogens (4, 15) has indicated that wounding by sand particles plays an important role in development of disease. The addition of carborundum to suspensions of P. tomato prior to inoculation increased infection in greenhouse studies (1). This work suggests that if cultural practices aimed at reducing wounding could be implemented, the severity of speck infections might be reduced. Beste (2) has reported that rye stubble-mulch used in a no-tillage system significantly reduced sand injury due to wind erosion on seedling tomatoes. In this study, the

effect of using a no-till system of tillage for reducing bacterial speck severity is reported.

MATERIALS AND METHODS

A preliminary study was conducted in 1982 to compare the movement of P. tomato in a conventionally tilled and no-till cultural system for growing tomatoes. The plot was established at the Sodus Horticultural Experiment Station in southwest Michigan on a Spinks loamy sand soil. The plot received 56 kg/ha N on the cover crop and an additional 84 kg/ha N prior to planting. The experimental site was seeded to rye (Secale cerealis cv. Wheeler) with a grain drill in October of the previous year. The conventionally tilled plots were prepared by spring plowing with a moldboard plow when the rye was 30 cm in height followed by a disc and a cultimulcher for final seedbed preparation. The rye in the no-till plots was allowed to grow to approximately 75-100 cm in height and was then killed by a single application of paraquat (0.58 kg/Ha) + 0.5% surfactant 3 weeks prior to planting. Tomato plants (cv. `UC 82'), grown in 72-cell flats filled with synthetic soil medium were planted with a single-row Mechanical Transplanter fitted with a double disc coulter to aid in the opening of a planting furrow in the debris. Thirteen rows, 7.5 m long, were planted in a north-to-south direction perpendicular to the prevailing winds with a between-row spacing of 1.5 m and an in-row

spacing of 0.6 m. The western-most row was inoculated 2 weeks after planting by applying a rifampicin resistant strain of P. tomato at a concentration of 5×10^7 cells/ml with a hand-held pneumatic sprayer from a height of 25-30 cm. Plants were sprayed until runoff. The remaining 12 rows were divided into 4 groups of 3 moving from west to east. They were designated west, midwest, mideast and east, respectively.

Leaf samples were collected at approximately two-week intervals beginning one week after inoculation. Samples of 20 symptomless leaflets were randomly selected from each of the four sections. Leaflets were finely chopped with a sterile razor blade and three 1 g replicate samples were weighed out for each sample. The samples were ground in 5 ml sterile distilled water using a mortar and pestle. The tissue was then strained through two layers of sterile cheesecloth into a test tube. A ten-fold serial dilution was done and 0.1 ml of each dilution was spread onto the surface of a complete medium amended with 100 ug/ml of rifampicin and 25 ug/ml of cycloheximide. After incubation for 3 days at room temperature (25 C), colony counts were made in plates with between 30 and 300 colony forming units (cfu)/plate. Bacterial leaf populations have been shown to be lognormally distributed (7), hence all leaf population counts have been transformed to $\log_{10}$ values.

In 1984, six-week-old tomato transplants (cv. Pik Red) grown in 72 cell flats were inoculated with P. tomato prior

to being planted. A bacterial suspension containing approximately 5 X 10^7 cells/ml was applied to the plants while still in the flat with a hand-held pneumatic sprayer from a height of 25-30 cm. Plants were sprayed until runoff. Plants were placed into a mist chamber and held 7 days until symptoms developed and then taken to the field for planting.

The conventionally tilled and no-till plots were prepared as in 1982. Each plot consisted of 18 rows 9 m long with a between-row spacing of 1.5 m and an in-row spacing of 0.6 m. The inoculated plants were planted into the 3 outside rows on the west side of the plots since winds in this area are generally from the west. The remaining 15 rows were planted with disease free plants.

The experiment was set up in a randomized complete block design with 3 replications. Plots were maintained according to standard commercial practices for the area.

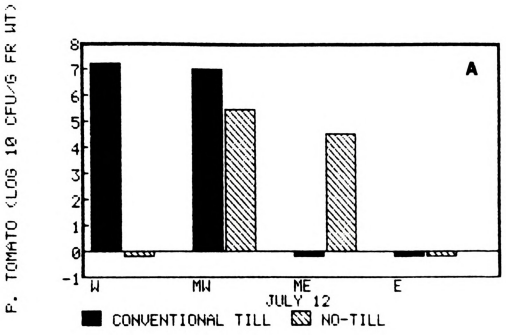
The treatments were evaluated by harvesting fruit from each plot and observing them for speck lesions. Disease incidence was estimated by counting the harvested fruit with speck, and expressing this as percentage infected fruit. An arcsin squareroot transformation of the data was done prior to statistical analysis in order to stabilize the variance.

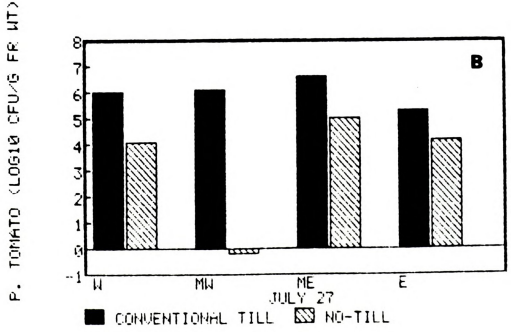
RESULTS AND DISCUSSION

The minimum detection level for the sampling method used in 1982 was approximately 10^2 (cfu)/g fresh weight of leaf tissue. There were no detectable levels of bacteria on leaf samples collected on June 8 and 19. On July 12 (Figure 1A), high levels of the bacteria were found in the 2 conventionally tilled sections immediately adjacent to the inoculated row, and lesions typical of bacterial speck were found on the leaves. Lower levels of P. tomato were found in the center two sections of the no-till plot, however, no speck lesions were detected in these plots. On the July 27 sampling date, bacteria levels in the conventionally tilled plots were higher than in the no-till plots in each of the four sections (Figure 1B). Speck lesions were found in all plots but no attempt was made to quantify them. No samples were collected after this date since fruit had reached the size at which they are no longer capable of becoming infected (5).

The spread of speck into the mideast and east sections from July 12 to July 27 can be attributed to the heavy rainfall during that period. Between July 3 and July 22, 8.5 cm of rain was recorded. Epidemics are thought to occur when inoculum is dispersed by rainsplash during wind-driven

Figure 1. The effect of tillage system on epiphytic populations of *Pseudomonas syringae* pv. *tomato* on susceptible 'UC 82' tomatoes sampled on A) July 12, or B) July 27. The west section was closest to the inoculated row.


P. TOMATO (LOG 10 CFU/G FR WT)
8
7
6
5
4
3
2
1
0
-1
A
W
MW
ME
E
JULY 12
CONVENTIONAL TILL
NO-TILL


P. TOMATO (LOG10 CFU/G FR WT)
8
7
6
5
4
3
2
1
0
-1
B
W
MW
ME
E
JULY 27
CONVENTIONAL TILL
NO-TILL

rainstorms from leaves with lesions to healthy leaves of nearby susceptible plants (8). In tests with bacterial spot (Xanthomonas vesicatoria pv. vesicatoria) on tomatoes, Vakili (15) has demonstrated that the highest levels of infection occured when sandblasting immediately preceded inoculation with the bacteria. The fact that lower levels of bacteria were generally found in the no-till plots compared to the conventionally tilled plots may be an indication that the reduction in soil movement by the mulch in the no-till plots reduces wounding and thereby limits infection by rain-splashed bacteria.

In 1984, an attempt was made to correlate fruit infection with the type of tillage system used. There was a low level of infection in this year which probably can be attributed to extremely dry conditions. For instance, in June and July of 1984 when speck is most likely to develop, only 8.5 cm of rain occurred. This compares to 14.9 cm of rain which fell during the same period in 1982. However, there was significantly less fruit infection in the no-till than in the conventionally tilled plots (Table 1). Presumably, if no-till significantly reduced infection under conditions unfavorable to the pathogen, it could possibly provide even greater benefits during those times when disease conditions are favorable.

Table 1. Effect of tillage system on disease incidence of *Pseudomonas syringae* pv. *tomato* on tomatoes.

Tillage system	Fruit infection (%)[z]
Conventional till	5.5
No-till	0.2

[z] Coefficient of correlation (r) = 0.83*

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PART IV

OVERWINTERING OF PSEUDOMONAS SYRINGAE PV. TOMATO POPULATIONS IN MICHIGAN

ABSTRACT

A field survey was conducted in the spring of 1984 to determine possible overwintering sites of Pseudomonas syringae pv. tomato. The pathogen was consistently found to be present on leaves and stems of overwintered surface debris. It was never found in association with the roots, fruits or rhizosphere of overwintered tomato plants. Likewise, it could not be found in association with the leaves or rhizosphere of various perennial and winter annual weeds present in the field nor in non-rhizosphere soil.

INTRODUCTION

Pseudomonas syringae pv. *tomato* (Okabe) Young *et al.* (*P. tomato*), the cause of bacterial speck of tomato, continues to be a major concern to growers in Michigan, Ohio and southwestern Ontario, Canada. Attempts to prevent introduction of the organism on southern grown transplants are difficult since the organism can survive epiphytically on the leaf surface of plants for long periods of time without symptom development (7, 8). Growers have begun to raise their own transplants in greenhouses in an attempt to avoid this problem but outbreaks of the disease continue to occur. There have been reports that *P. tomato* can survive on tomato seeds for as long as 20 years (1) and this may be a source of infection for greenhouse grown plants. Reports from California (7), Georgia (6) and Israel (3) indicate that *P. tomato* is capable of surviving in the soil and in association with the rhizospere and leaves of non-host plants. Getz (4) has shown that in Michigan, *P. tomato* could be reisolated from artificially infected tomato leaf tissue overwintered on the surface and buried up to 18 cm. The purpose of this research was to determine if there are other potential sources of primary inoculum in Michigan.

MATERIALS AND METHODS

Field Survey. A survey was conducted during the spring of 1984 for the presence of the bacterial speck organism in a field at the Michigan State University Botany and Plant Pathology Research Farm in East Lansing. The field had been planted to tomatoes inoculated with a rifampicin resistant strain of P. tomato in 4 of the preceding 5 years. Samples of weed species present and overwintered tomato debris, including rhizosphere soil, were collected, placed in plastic bags, and stored over ice for transport to the laboratory. Two-2 g samples were taken from each of the various plant parts including the rhizosphere soil and placed in flasks containing 100 ml of sterile distilled water (SDW) and shaken on a wrist-action shaker at 180 rpm for 30 minutes. The wash water was diluted in a log $10^{}$ series with SDW and 0.1 ml aliquots were spread on the surface of complete agar (5) plates amended with 100 ug/ml of rifampicin and 25 ug/ml of cycloheximide (CRC agar). After 72 hr, plates were examined for characteristic colonies. Since some contaminants were also present, plates were also examined under near-ultraviolet (UV) light and flourescent colonies were marked. Flourescent colonies were

subcultured on CRC agar and tested for pathogenicity in the greenhouse.

Inoculum was prepared by placing single colonies into 50 ml of complete broth and allowing them to grow up overnight on a wrist-action shaker. The broth was then placed into a 1000 ml beaker and diluted with 500 ml of SDW to give a final concentration of approximately 5×10^7 colony forming units (cfu)/ml as determined by absorbance readings made with a spectrophotometer. Plants were immersed in the beaker for 1 minute and then placed in a mist chamber for 96 hr. Plants were then placed on a greenhouse bench for 7 days to allow adequate time for symptom development.

Field Baiting. On May 10, speck-susceptible tomato transplants (cv. `Pik Red') were planted by hand into the field from which the samples had been collected following land preparation according to standard commercial practices. Five replicates of 5 plants each were planted at various locations in the field. The plants were placed in single rows with 0.6 m between plants. Two replicates were planted in an east-west direction and 3 in a north-south direction. Plants were allowed to remain in the field for 3 weeks and then sampled for the presence of P. tomato.

Ten leaflets were selected from each plant. Leaflets were finely chopped with a sterile razor and three 1 g replications were weighed out for each plant. The samples were homogenized in a blender for 15 sec in 15 ml distilled

water, and the homogenate was strained through two layers of sterile cheesecloth into a test tube. The homogenate was serially diluted 1:10 and 0.1 ml of each dilution was spread onto the surface of CRC agar. After incubation for 3 days, plates were checked for the presence of P. tomato.

RESULTS

The bacterial speck organism could not be detected in association with any of the various non-host plants tested (Table 1) nor their rhizospheres. Flourescent colonies were observed only in those samples taken from dried tomato fruits, stems and leaves. P. tomato was never detected on tomato roots or rhizosphere soil nor other non-rhizosphere soil samples. Of those colonies growing on CRC agar and testing positive for flourescence, only colonies from the tomato leaf and stem segments were pathogenic. None of the colonies from the fruit caused typical speck symptoms following inoculation.

After 3 weeks in the field, P. tomato could not be detected on any of the plants.

Table 1. Overwintering of *Pseudomonas syringae* pv. *tomato* on tomato debris, and roots and leaves of various weeds in a field with a history of bacterial speck.

Associated plant and common name	Plant part	Flourescent colonies	Pathogenic
Lycopersicon esculentum Mill. (tomato)	fruit	+	-
	leaves	+	+
	stem	+	+
	roots	-	-
	rhiz.	-	-
Capsella bursa-pastoris L. (sheperd's purse)	leaves	-	-
	roots	-	-
Taraxacum officinale Weber (dandelion)	leaves	-	-
	roots	-	-
Plantago lanceolata L. (buckhorn plantain)	leaves	-	-
	roots	-	-
Trifolium repens L. (white clover)	leaves	-	-
	roots	-	-
Agropyron repens L. (quackgrass)	leaves	-	-
	roots	-	-
Poa pratensis L. (bluegrass)	leaves	-	-
	roots	-	-
Non-rhizosphere soil		-	-

DISCUSSION

Use of a rifampicin resistant mutant made it possible to easily determine the identity of the bacteria recovered. These results differ somewhat from those previously reported. Schneider and Grogan (7) found P. tomato to be ubiquitous in soils with no known history of tomato production, especially in the cooler coastal areas of California, as well as on a number of symptomless crop and weed hosts. McCarter et al. (6) were able to find the organism on symptomless hosts in Georgia but only when a vacuum infiltration technique was used. They suggested that P. tomato is disseminated to spring-seeded tomatoes after overwintering on native weeds. In this study, P. tomato was never found in association with symptomless weed hosts nor soil. Vacuum infiltration was not used and the possibility exists that the organism was present but at levels too low to be detected. However, Schneider and Grogan (7) did not need this technique to find the organism on symptomless hosts. Only weed species present in the early spring were sampled since it was felt that these would be the most likely candidates for serving as an overwintering host. When tomato debris was sampled, P. tomato could only be found on leaf and stem samples. This would agree with

Chambers and Merriman (2) who suggested crop debris as the primary source of inoculum after finding that P. tomato survived 25-30 wk in soil with naturally infected tomato debris.

To date, overwintered inoculum has not yet been shown to be a primary source of infection for Michigan grown tomatoes. Disease free tomato plants were placed into a field which had been planted with speck-infected tomato plants in 4 of the previous 5 years. After 3 weeks, during which several periods of speck-conducive weather occurred, no traces of the organism could be found. The experiment was terminated after 3 weeks since the field was to be used for efficacy experiments involving the introduction of speck inoculated plants. The possibility exists that plants simply did not remain in the field long enough for an epiphytic population to become established. Plans are underway to repeat this portion of the study. Disease-free plants will be planted into plots prepared according to customary practices as well as directly into overwintered debris. The plants will be allowed to grow to maturity and will be observed and sampled weekly for the presence of speck.

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APPENDIX

MICROSCOPIC SURFACE COMPARISONS OF SUSCEPTIBLE AND RESISTANT TOMATO FRUIT INOCULATED WITH PSEUDOMONAS SYRINGAE PV. TOMATO

INTRODUCTION

Bacterial speck of tomato (Lycopersicon esculentum Mill.), caused by Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato), causes serious losses to growers in tomato producing regions each year (3, 13). Although the organism can cause considerable yield losses (9, 12), its most devastating effect is on the reduction of fruit quality caused by small necrotic lesions on the surface of the fruit.

Getz et al (2), in scanning electron microscope studies using a speck susceptible cultivar of tomato, suggested that trichomes are gradually lost from fruit surfaces leaving openings in the young fruit epidermis which may then serve as sites of infection for the organism. Research on other epiphytic plant pathogenic bacteria has indicated that symptom development is associated with the attainment of some threshold population of bacteria (5, 10). Currently, there are several known sources of speck resistance (4, 6, 7, 11). It is possible that this resistance may be manifested by morphological changes of the fruit surface which would support smaller populations (i.e. below the threshold) of the bacteria. Schneider and Grogan (8) have reported that tomato mutants, deficient in leaf hairs,

supported smaller resident populations of the bacteria. The purpose of this study was to observe the developing fruit of both a susceptible and resistant cultivar to determine whether there are any morphological differences which may be responsible for the ability of the speck organism to infect the fruit.

MATERIALS AND METHODS

Tomato plants of the susceptible fresh market cultivar Pik Red (Joseph Harris Co., Inc., Rochester, NY 14624) and the resistant breeding line 83-3008-2 (Dr. S. Honma, Department of Horticulture, Michigan State University, East Lansing, MI 48824) were greenhouse-grown in 25 cm clay pots containing a standard greenhouse soil mix (Sunshine #1, J. Mollema and Son, Inc., Grand Rapids, MI 49507). Based on the studies by Getz et al. (2), tomato fruit development was arbitrarily divided into the following developmental stages: (i) open calyx, (ii) open corolla, (iii) green fruit 1 cm or less in diameter, and (iv) green fruit 1-3 cm in diameter.

A naturally occuring rifampicin-resistant isolate of P. tomato (isolate PtFr) was used as the pathogen in this study. Inoculum was prepared and applied to each developmental stage as previously described (2).

To observe possible morphological differences and infection sites, ovaries and fruit were sampled 2 hr and 4 days after inoculation and prepared for scanning electron microscope (SEM) examination. Uninoculated control samples taken at the same times were also prepared. Entire ovaries or epidermal blocks (10 x 10 mm) from larger fruit were fixed 2 hr in 4% glutaraldehyde and then post-fixed

overnight in 1% osmium tetroxide. Both solutions were buffered at pH 7.2 with 0.1 M sodium cacodylate. Fixed tissues were dehydrated in a graded ethanol series (25, 50, 75, 90, 100%) at 4 C. Following dehydration, tissues were critical-point dried using a Sorvall critical-point drier with CO_2 as the carrier gas. Samples were mounted on aluminum stubs, sputter-coated with approximately 30 nm of gold, and examined in a JEOL JSM - 35C scanning electron microscope.

In an effort to avoid trichome damage during the fixation process, an alternate method of fixation was used. Ovaries and epidermal blocks were placed in a petri plate along with a small dish containing 1% osmium tetroxide. The sections were left overnight to allow infiltration of osmium vapors. The sections were then allowed to air dry for 3 days, mounted, sputter-coated with gold and observed.

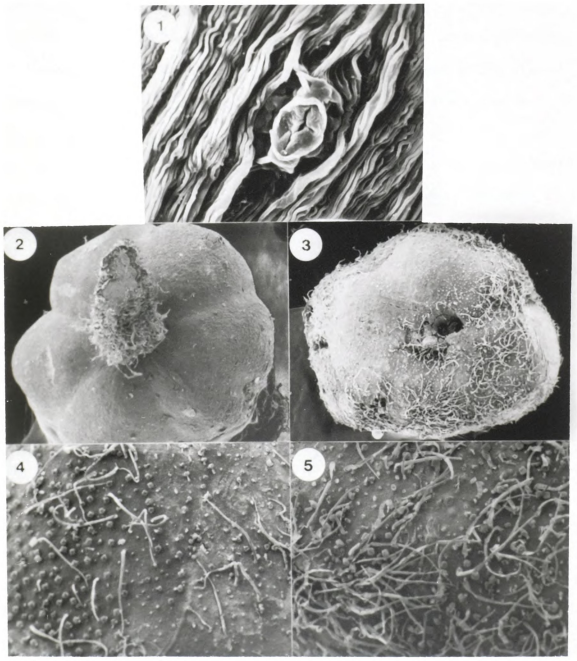
RESULTS AND DISCUSSION

Fixation with osmium tetroxide vapors proved unsuitable. There was extensive collapse of the epidermal tissue (Figure 1) making them unsuitable for further observation.

Getz (2) has reported that no trichomes were present on tomato ovaries of a susceptible cultivar prior to anthesis (open calyx stage) but that during anthesis (open corolla stage) the ovary surface became densely covered with unicellular papillary trichomes, long multicellular non-glandular trichomes and capitate glandular trichomes. Similarly in this study, no trichomes could be found on resistant tomato ovaries prior to anthesis (Figure 2) but they were readily observable during anthesis (Figure 3). Closer views of the surface showed that the susceptible and resistant cultivars both had similar characteristics (Figures 4, 5).

Bacteria could not be observed on the surfaces of the susceptible or resistant fruit 2 hr after inoculation. Two hours may not have been enough time for the bacteria to become attached to the surface and they may have subsequently been washed off during the fixation process. When samples were taken 4 days after inoculation, bacteria

Figure 1-5. Scanning electron micrographs of tomato ovaries. 1. Epidermis of tomato following overnight osmium tetroxide vapor fixation and air drying for 3 days (1400X). 2. Tomato ovary of the resistant cultivar 83-3008-2 prior to anthesis (60X). 3. Tomato ovary of the resistant cultivar 83-3008-2 at anthesis (20X). 4. Surface of the susceptible cultivar Pik Red showing long, multicellular, nonglandular trichomes and capitate, glandular trichomes (45X). 5. Surface of the resistant cultivar 83-3008-2 showing long, multicellular, nonglandular, trichomes and capitate, glandular trichomes (70X).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were readily visible on the surface and trichomes of the susceptible fruit (Figures 6, 7) but not the resistant fruit (Figure 8). Resistant cultivars are known to support reduced populations of epiphytic bacteria (1) and this may explain the absence of P. tomato cells on the fruit surface.

Swollen areas were observed on susceptible green fruit between 1 and 3 cm in diameter 4 days after inoculation (Figures 9, 10). These appear to be similar to the swellings described by Getz et al. (2) and may be an earlier stage of development. Small numbers of bacteria assumed to be P. tomato were found on the surface of these swellings (Figure 11). Swellings could not be found on ovaries less than 1 cm in diameter. When samples of the resistant fruit taken 4 days after inoculation were examined, the same types of swellings were also observed (Figures 12, 13), but no bacteria could be found on the surface. This raises an interesting question. If these swellings are a stage in the development of speck lesions as proposed by Getz et al. (2), how does one account for the similar swellings observed on the resistant fruit since it is known that lesions do not occur on these fruit following inoculation? One possibility may be that the observed swelling is a result of the deposition of some type of protective cuticular material in response to the "wound" created by the loss of the trichome. One could then hypothesize that on the susceptible fruit there may be two types of swellings, one created by the multiplication of bacteria which results in an upward

Figure 6-8. 6. Bacteria present on the surface of the susceptible cultivar Pik Red 4 days after inoculation (3010X). 7. Bacteria present on a capitate, glandular trichome of the susceptible cultivar Pik Red 4 days after inoculation (1840X). 8. Bacteria free surface of the resistant cultivar 83-3008-2 4 days after inoculation (810X).

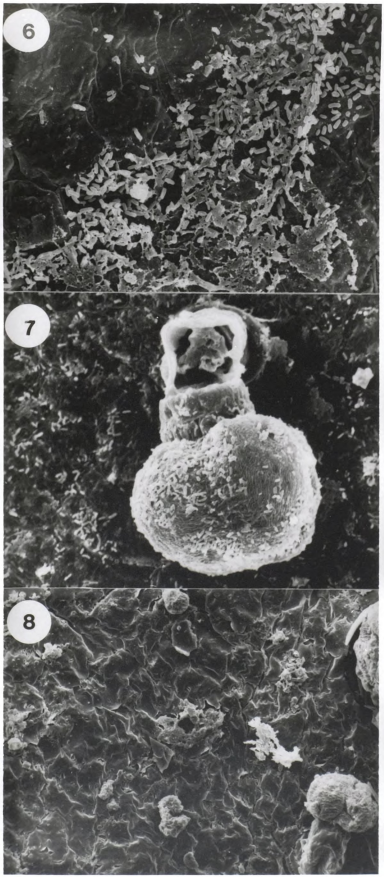
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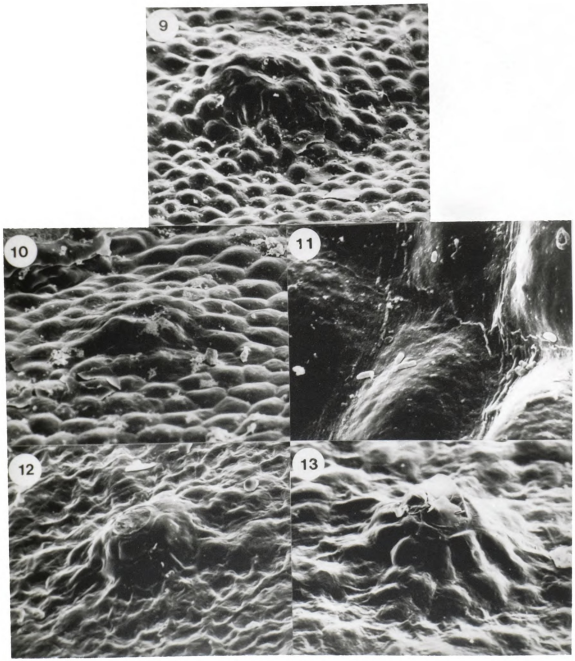
Figure 9-13. 9. Epidermal swelling on the susceptible cultivar Pik Red 4 days after inoculation (515X). 10. Epidermal swelling on the susceptible cultivar Pik Red 4 days after inoculation (815X). 11. Enlargement of Figure 9 showing bacterial in association with an epidermal swelling (5600X). 12. Epidermal swelling on the resistant cultivar 83-3008-2 4 days after inoculation (940X). 13. Epidermal swelling on the resistant cultivar 83-3008-2 4 days after inoculation (1100X).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pressure on the epidermis, and a second caused by the deposition of materials as a wound response. On resistant fruit, only this second type would occur.

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