A Predictive System for Timing Chemical Applications to Control *Pseudomonas syringae pv. tomato*, Causal Agent of Bacterial Speck

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ABSTRACT


Stage-wise multiple linear regression techniques were used to identify those meteorological and biological variables useful in predicting bacterial speck symptom development caused by *Pseudomonas syringae pv. tomato*. An initial regression model relating temperature, rainfall, and previous population level was developed from 2 yr of data. The model was $BP = -2.99 - 0.14(T) + 1.34(R) + 0.81(P)$, where $BP$ = the predicted bacterial population, $T$ = the average temperature (C) on the previous day, $R$ = the square root of the sum of the daily rainfall + 0.5 for each of the previous 7 days), and $P$ = the population at the previous sampling time. The model accounted for 85% of the observed variation in the population for the years used in model development. In 1984, the equation correctly predicted values above or below the preselected threshold for spray application 12 of 12 times and resulted in three fewer sprays than used in a calendar spray schedule. There were no significant differences in amount of fruit infection between spray schedules. The data base for the regression model was expanded by combining 1984 data with that from the previous 2 yr. The model was $BP = -2.99 + 0.72(R) - 0.11(T) + 0.01(H) + 0.51(P)$ where $BP$ = the predicted bacterial population, $T$ = the average temperature on the previous day, $R$ = the square root of (the sum of the daily rainfall + 0.5 for the previous 6 days), $P$ = the population level at the previous sampling time, and $H$ = the arcsin-square root of the average relative humidity for the previous day. This equation accounted for 46% of the variation in the population for the years used in model development. The model derived from 3-yr data was tested for its applicability to data from 1980 and 1981. Values above or below the threshold were correctly predicted 83% and 86% of the time, respectively. The potential for use of this model in commercial tomato production is discussed.

Additional key words: epidemiology, forecasting, *Lycopersicon esculentum*.

**MATERIALS AND METHODS**

Pathogen. A naturally occurring rifampicin-resistant isolate of *P. s. pv. tomato* was used. Inoculum was prepared as previously described (9). Six-week-old transplants of the bacterial speck-susceptible cultivar, Pik Red, grown in 72-cell flats filled with synthetic soil medium, were obtained from a commercial greenhouse operator in southwestern Michigan. Inoculum was applied to runoff with a hand-held pneumatic sprayer from a height of 25–30 cm. Plants were held in a mist chamber until symptoms developed and then hand-transplanted in the field.

Field plots. Field studies were conducted at the Sodus Horticultural Experiment Station, Sodus, MI, in 1982 and at the Botany and Plant Pathology Research Center in East Lansing, MI, in 1983 and 1984. In each year, the plot used for monitoring populations was $24 \times 6$ m (16 rows, each 6 m long) with 1.5 m between rows and 0.6 m between plants in the row. Plots were cared for according to the standard commercial practices of the area. Carbaryl 80% a.i., 1.4 kg/ha formulated, and chlorothalonil, 1.6 L/ha formulated, were used as needed for foliar insect and fungal disease control. Chlorothalonil has previously been shown to have no effect on populations of *P. s. pv. tomato* (4).

Weather monitoring. Air temperature and relative humidity were measured with a 7-day recording hygrothermograph (Belfort Instrument Co., Baltimore, MD) placed in a standard weather shelter at ground level. Leaf wetness was recorded in 1982 with a deWit 7-day recording leaf wetness meter (Valley Stream Farms, Orono, Ontario, Canada), which was periodically adjusted during the season to remain level with the canopy. Rainfall was measured with a tipping bucket rain gauge and 7-day recorder (Weather Measure Corp, Sacramento, CA). Solar radiation was measured using a 7-day recording mechanical pyranograph (Weather Measure Corp).
Population estimation. Leaf samples were collected two to three times per week starting about 2 wk after transplanting. At each sampling time, 20 symptomless leaflets were randomly selected. Leaflets were bulked and finely chopped with a sterile razor and three 1-g subsamples were weighed out. The samples were homogenized in a blender for 15 sec in 15 ml of distilled water, and the homogenate was strained through two layers of sterile cheesecloth into a test tube. The homogenate was serially diluted 1:10 five times and 0.1 ml of each dilution was spread onto the surface of a complete medium (yeast extract, casamino acids, and monobasic and dibasic potassium phosphate) amended with 100 μg of rifampicin per milliliter and 25 μg of cycloheximide per milliliter. After incubation for 3 days at room temperature (23 C), colony counts were made in petri dishes with 30-300 colony-forming units (cfu) per dish. Final populations were tabulated as log 10 cfu per gram fresh weight.

Population model development. Temperature and relative humidity values each day were the means of 12 readings taken at 2-hr intervals. Precipitation, solar radiation, and leaf wetness values were the sum for each 24-hr period. Temperature and relative humidity means, precipitation, solar radiation, and leaf wetness sums for periods of 1-7 days before a population sampling date were evaluated for correlations with the population levels. Periods beyond 7 days were not considered because symptoms develop in 5-6 days (2,16). The time period for each parameter yielding the highest significant correlation (P ≤ 0.05) was then selected for multiple regression analysis. Stage-wise multiple regression analysis was done using the Minitab (15) statistical package with log 10 bacterial population as the dependent variable and the various weather parameters as the independent variables. To meet the requirement for multiple regression (6) that residuals be normally distributed about zero, transformations were done on several of independent variables. Relative humidity was transformed with arcsin-square root transformation. Rainfall and solar radiation were transformed by adding 0.5 and taking the square root.

The various equations generated were evaluated based on their coefficient of multiple determination (R2) and the significance (P = 0.05) of the partial regression coefficients (3).

Model testing. In 1982, tomato plants (cultivar Pik Red) were planted in two-row plots 6 m long with 1.5 m between the rows and an in-row spacing of 0.6 m. In 1984, single-row plots, 9 m long, were 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 four replications was used. Main plot treatments were a calendar spray schedule (7-day spray interval in 1982 and a 4-day interval in 1984) vs. sprays based on model predictions of population levels (4-min 4-day interval between sprays). Subplot treatments were various types of chemical controls. In 1982, an experimental cupric hydroxide (Kocide 101, 360 g/L a.i.) and mancozeb (Dithane M-45, 360 g/L a.i.) combination (KCC-FMX, 4.7 L/ha) was compared with an unsprayed control. In 1984, streptomycin (Agri-mycin 17, 200 ppm), oxytetracycline (Mycoshield, 200 ppm), cupric hydroxide (Kocide 101, 2.25 kg/ha), and a cupric hydroxide and mancozeb combination (KCC-FMX, 4.7 L/ha) were compared with an unsprayed control. At the end of the season, fruit were harvested and evaluated for the presence of bacterial speck.

The models were evaluated based on their ability to correctly predict population levels above or below the threshold level, as well as on the potential number of times unnecessary sprays might be applied or needed sprays missed. Phi coefficients (5) were calculated as a measure of dependence between actual and predicted population levels.

Threshold determination. Basu (2) estimated that an inoculum concentration of at least 1 x 10^4 cfu of P. s. pv. tomato per milliliter was required for infection. Bashan et al (1), working with tomato plants wounded with Carborundum powder, found that speck symptoms did not develop when inoculated with bacterial suspensions containing fewer than 10^5 cfu per milliliter. Based on these reports and our own greenhouse and field tests, a value of 10^5 (log 5) cfu per gram fresh weight of tissue was chosen as the threshold at which spray applications would be made. The threshold level was chosen to be slightly less than that which actually causes symptom development so that there would be adequate time to make the chemical control applications.

RESULTS

Population model development. In 1982, a previously developed regression model (8) was tested. The equation used was:

\[ BP = -29.86 + 3.89(T) - 0.11(P^2) \]

where \( BP \) = the bacterial population predicted (log 10 cfu per gram fresh weight), and \( T \) = the average temperature (C) for the four previous days. This model accounted for 64% of the observed variation in bacterial population for the year used in model development. For a model to be practical, it must correctly predict bacterial populations above or below the threshold for symptom development. Comparisons between actual and predicted populations were made in contingency tables with the four cells being: 1) both values above the threshold (spray needed), 2) both values below the threshold (no spray needed), 3) the predicted value below and the actual value above the threshold (missed spray), and 4) the predicted value above and the actual value below the threshold (unneeded spray). In our studies, it was meaningful to distinguish between a positive association (correct prediction) and a negative association (incorrect prediction). The phi coefficient selected for use is one measure of association that allows for the distinction to be made. Using the weather and population data from 1982, equation 1 correctly predicted populations above or below the threshold for spraying 9% of the time (Table 1), and there was generally a poor fit between the levels of actual population observed in the field and those predicted (Fig. 1). In every case, the incorrect prediction was below the threshold when actual values were above and would have resulted in missed sprays. The severe underestimation of actual values by the model was an indication that one or more important variables must be missing from the equation (3).

In screening for potential variables, data from 1982 and 1983 were combined to broaden the range of data values used to

<table>
<thead>
<tr>
<th>Equation</th>
<th>Year</th>
<th>A ≤ 5</th>
<th>A &gt; 5</th>
<th>A ≤ 5</th>
<th>A &gt; 5</th>
<th>Phi coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>1981</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>0.61**</td>
</tr>
<tr>
<td>(1)</td>
<td>1982</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>undefined†</td>
</tr>
<tr>
<td>(2)</td>
<td>1983</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>0.67**</td>
</tr>
<tr>
<td>(3)</td>
<td>1984</td>
<td>2</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1.0**</td>
</tr>
<tr>
<td>(3)</td>
<td>1980</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>undefined†</td>
</tr>
<tr>
<td>(3)</td>
<td>1981</td>
<td>2</td>
<td>17</td>
<td>2</td>
<td>2</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*BP = -29.86 + 3.89(T) - 0.11(P^2)*

†Phi coefficient was undefined due to division by zero.

‡BP = -2.99 - 0.14(T) + 1.34(R) + 0.81(P) where BP = the bacterial population predicted (log 10 cfu/g fresh wt) and T = average temperature (C) on the previous day; R = the square root of (the sum of the daily rainfall + 0.5 for each of the previous 7 days); and P = the population level (log 10) at the previous sampling time.

§BP = 0.98 + 0.72(R) - 0.11(T) + 0.01(H) + 0.51(P) where BP = the bacterial population predicted (log 10); R = the square root of (the sum of the daily rainfall + 0.5 for the previous 6 days); T = the average temperature for the previous day; H = the arcsin-square root of the average relative humidity for the previous day; and P = the population level (log 10) at the previous sampling time.

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Construct the equation. The correlation coefficient between the measured bacterial population and each independent variable for the 2-year period is shown in Table 2. Stagewise regression was used to select variables for use in the equation. The equation was:

$$BP = -2.99 - 0.14(T) + 1.34(R) + 0.81(P)$$  

where $BP$ = the bacterial population predicted (log$_{10}$); $T$ = average temperature (°C) on the previous day; $R$ = the square root of (the sum of the daily rainfall + 0.5 for each of the previous 7 days); and $P$ = the population level (log$_{10}$) at the previous sampling time. Equation 2 accounted for 85% of the observed variations in the population for the years used in the model and was significant at the $P = 0.05$ level (Table 3). When field-tested in 1984, there was good agreement between the measured and estimated population levels (Fig. 2). The predictions of population levels above or below the threshold level for symptom development were correct in 12 of 12 samplings (Table 1).

To further expand the range of values for each variable, weather data for the 1984 season were combined with those from the previous 2 years. There were five variables with significant correlations (Table 2). These variables generated the equation:

$$BP = 0.98 + 0.72(R) - 0.11(T) + 0.01(H) + 0.51(P)$$  

where $BP$ = the bacterial population predicted (log$_{10}$); $R$ = the square root of (the sum of the daily rainfall + 0.5 for the previous 6 days); $T$ = the average temperature for the previous day; $H$ = the average relative humidity on the day before sampling. Intercept $0.98$ and **significant at $P = 0.05$.

**DISCUSSION**

The proposed model is based on mean epiphytic bacterial populations reaching a threshold level for symptom development. Lindemann et al. (13) have recently developed a model to predict incidence and severity of brown spot (P. s. pv. syringae) on bean using an apparent threshold level. Their prediction of disease incidence is based on population levels on individual leaflets rather than mean populations. Where they have attempted to predict disease incidence and severity based on the attainment of a threshold population level, this model attempts to predict when that threshold will be reached so that preventive measures may be taken to reduce the population level before the threshold is reached. Because epiphytic bacterial populations are lognormally distributed.

**TABLE 3. ANOVA table for the regression equations used to predict epiphytic Pseudomonas syringae pv. tomato populations**

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Regression coefficient</th>
<th>Mean square</th>
<th>Partial F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation 2: Temperature</td>
<td>-0.14</td>
<td>7.34</td>
<td>18.8***</td>
</tr>
<tr>
<td>Rainfall</td>
<td>1.34</td>
<td>4.12</td>
<td>10.6**</td>
</tr>
<tr>
<td>Previous population</td>
<td>0.81</td>
<td>30.69</td>
<td>78.7**</td>
</tr>
<tr>
<td>Intercept</td>
<td>-2.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual standard error</td>
<td>0.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** and *** indicate statistically significant at $P = 0.05$ and $P = 0.01$, respectively.
distributed (10), the use of bulked samples to obtain a mean population estimate will result in an overestimation of the actual population present. Despite its deficiency, the bulk sampling method 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. To have practical application, the method of sampling must be simple, rapid, and inexpensive. Ideally, it would be done by private consultants, which many growers now use. Because mean estimates are used in generating the multiple regression equations, predicted values will also reflect the overestimation of the populations. If these populations are looked on 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 the threshold for infection is not a constant and may be related, for instance, to changes in host susceptibility (13). 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 to be used for *P. s. pv. tomato* needs to be done.

To avoid problems with extrapolation, weather and population data for 1982 and 1983 were combined to allow a wider range of values. The combining of data from several years 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. One new weather variable, rainfall (*R*), was selected for inclusion in the new equation (equation 2). An important aspect of equation 2 is that a nonweather 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.

At the end of the 1984 season, weather data for 3 yr were combined and resulted in equation 3. A variable accounting for humidity (*H*) was added to this equation. Examination of the partial sum of squares in the analysis of variance showed that the contributions due to *H* and *R* were dependent on the order in which they were entered. This effect indicates a strong relationship between the two variables. The correlation coefficient (*r*) between

\[ H = 0.11(T) + 0.01(H) + \sqrt{R} \]

where *BP* = the bacterial population predicted (logio); *T* = average temperature (C) on the previous day; *R* = the square root of (the sum of daily rainfall + 0.5 for the previous 6 days); and *P* = the population level (logio) at the previous sampling time, which was generated from combined 1982 and 1983 weather data.

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Table 4. The effect of spray timing on fruit infection of susceptible Pik Red tomatoes by *Pseudomonas syringae pv. tomato* at Sodus, MI, in 1982 and at East Lansing, MI, in 1984

<table>
<thead>
<tr>
<th>Spray schedule</th>
<th>Percent fruit infection</th>
<th>Number of sprays</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-day schedule</td>
<td>2.8</td>
<td>7</td>
</tr>
<tr>
<td>7-day schedule</td>
<td>10.7</td>
<td>12</td>
</tr>
<tr>
<td>As predicted</td>
<td>74.9</td>
<td>8</td>
</tr>
</tbody>
</table>

*a* Experiment was set up as a split-plot design. The numbers represent main plot treatment means.

*b* Plants were sprayed based on forecast model predictions of bacterial populations above the threshold required for infection with a minimum 4-day interval between applications.

NS indicates no significant difference between means at \( P = 0.05 \).
predictions with six missed spray periods and two unnecessary sprays. Although the equations with $H$ or $R$ deleted satisfied the defined requirements, the equation containing both variables had the fewest cases of incorrect predictions with two missed spray periods and five unnecessary ones.

Use of equation 2 during the 1984 season reduced the number of spray covers required by three with no significant difference in the percentage of infected fruit (Table 4). This is an important point because chemical application is one of the few variable costs in tomato production. At an application cost of $18.50 per hectare, the saving of three spray covers would have resulted in an increase in gross income of $55.50 per hectare.

Equation 3 provided reasonable estimates of bacterial populations for two different data sets outside of those used to generate the equation. This success suggests that this equation may have application for use in commercial production areas where bacterial speck is a significant problem. Further testing with different cultivars and in other locations will be needed to determine how it might best be used in a practical disease control program.

LITERATURE CITED