EPIDEMIOLOGY OF BACTERIAL SPECK OF TOMATO CAUSED BY *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

By

Susan D. Getz

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Botany and Plant Pathology

1982

ABSTRACT

EPIDEMIOLOGY OF BACTERIAL SPECK OF TOMATO CAUSED BY

PSEUDOMONAS SYRINGAE PV. TOMATO

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Bacterial speck of tomato often assumes epidemic proportions and may cause considerable crop damage and serious economic losses under favorable environmental conditions. The pathogen, Pseudomonas syringae pv. tomato, was found to overwinter in Michigan soils in infected tomato leaf tissue which may act as a source of primary inoculum in the spring. Pathogen populations were monitored on susceptible and resistant cultivars in the field and a preliminary predictive model developed. Fruit susceptibility to infection was found to vary at different developmental stages. Green fruit 3 cm or less in diameter were found to be the most susceptible to infection. Scanning electron microscopy revealed numerous natural openings in the fruit surface during the most susceptible developmental stage. These openings appeared to be previous trichome attachment sites and were implicated as possible entry points for the pathogen.

To my parents,

without their love and encouragement

this work would never have been accomplished

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to my major professor, Dr. Christine Stephens, for her guidance, support, and helpful suggestions throughout this research. I am deeply indebted to Dr. Dennis Fulbright for his patience, constant encouragement, and confidence in my research abilities and for his help with many of the experiments. I would also like to thank Dr. Alan Jones for his helpful suggestions, Drs. Melvyn Lacy and Alfred Saettler for critical reviews of the manuscripts, Greg Pontius for countless hours of computerized data analysis and interpretation, Dr. Stanley Flegler for help with the scanning electron microscope work, David Levick for the scanning electron micrograph reproductions, Tom Stebbins for many hours of technical assistance in the field, Marianne LaHaine for typing many drafts of the manuscripts, and Patti Perkins for typing the thesis. Finally, I'd like to thank the people in the Department of Botany and Plant Pathology, especially those in Drs. Stephens' and Fulbright's laboratories, for providing a stimulating environment for learning and research.

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GENERAL INTRODUCTION AND LITERATURE REVIEW

Bacterial speck of tomato (Lycopersicon esculentum Mill.), caused by the bacterium Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato) (18) occurs in many tomato growing areas in the United States (1, 24, 36, 40, 52, 61) and in other countries (5, 6, 12, 14, 30, 31, 37, 38, 62). Within the last decade, this disease has been encountered with increasing frequency on both fresh market and processing tomatoes in Michigan and other tomato production areas (3, 7, 19, 40). There are no published data that adequately explain this recent increase of bacterial speck incidence. The introduction of new, possibly more susceptible, cultivars (59), the establishment of a resident population (40) of the pathogen in field soils via infected seed or transplants, and changes in cultural practices such as the successive cultivation of tomatoes without rotation and the use of overhead irrigation may be partially responsible. A shift from the use of copper compounds to dithiocarbamates for routine spraying of tomatoes to control fungal diseases has been associated with the increased incidence of speck (2, 52). Under favorable environmental conditions, bacterial speck often assumes epidemic proportions and may cause considerable crop damage and serious economic losses (22, 43, 62).

Bacterial speck was first reported in 1933 by Okabe (31) in Formosa and the causal organism given the name Bacterium tomato. The same year, Bryan (9) reported a similar disease of tomatoes in the United States

and proposed the name Bacterium punctulans for the causal organism. In 1943, Dowson (17) listed both Okabe's and Bryan's organisms as synonyms and proposed the new name of Pseudomonas punctulans (Bryan) n. comb., incorrectly giving credit to Bryan for the original description. The name was revised again in 1944 by Altstatt (1) to Pseudomonas tomato. In 1948, Reid (37) reported a bacterial disease of tomatoes occurring in New Zealand and attributed the cause to Pseudomonas punctulans (Bryan) Dowson. In an attempt to determine whether the causal organism reported by Reid was in fact Pseudomonas tomato, Wilke and Dye (56) compared Pseudomonas punctulans and Pseudomonas tomato in several physiological, biochemical, and pathogenicity tests and concluded that the two organisms were identical and the name Pseudomonas tomato (Okabe)Altstatt had priority. In 1978, Young et al. (57) proposed that Pseudomonas tomato be designated as a pathovar of the closely related species Pseudomonas syringae. This nomenclature was accepted in 1980 and the organism became known as Pseudomonas syringae pv. tomato (P. tomato) (18).

P. tomato is a small (0.7 to 1.2 μ by 1.5 to 3.0 μ), gram negative, obligatively aerobic rod (10). The bacterium is motile by means of a tuft of several polar flagella. Fluorescence varies from strain to strain and appears to be related to virulence, the nonfluorescent strains being weaker pathogens (9). Important diagnostic tests which aid in distinguishing P. tomato from fluorescent saprophytic pseudomonads include the presence of arginine dihydrolase (51), oxidase activity (26), and the production of a hypersensitive reaction on tobacco (25). Most strains

of P. tomato utilize D(-)-tartrate, a compound which is rarely utilized by other nomenspecies of the plant pathogenic pseudomonads (12).

Only two host range studies have been reported. Bryan (9) found P. tomato to be pathogenic only on tomato, however Okabe (31) observed symptoms on both tomato and eggplant leaves inoculated with the pathogen. Schneider and Grogan (39, 40) reported that the bacterial speck pathogen is ubiquitous in California and is associated saprophytically with many nonhost plants growing in soils with no known history of tomato culture.

The pathogen produces an extracellular thermostable toxin which is capable of causing chlorotic halos on tomato, bean, and tobacco leaves (45). Optimal temperature for bacterial growth in vitro is 25-30 C and for toxin production is 18 C (32). The chemical composition and mode of action of the toxin are not known. Garber and Shaeffer (20) obtained two exotoxins from culture filtrates of P. tomato but suggested that one may have been a degradation product. Bashan et al. (4) reported that ammonia produced by the bacterium is responsible for the necrotic symptom. In addition, they reported that electrolyte leakage and symptom formation in tomato leaves infected with P. tomato were preceded by the production of toxic quantities of ammonia.

P. tomato enters leaves through natural openings such as stomata or hydathodes and through wounds (3, 5, 9, 32, 37, 41). Free moisture on the surface of the leaves is necessary for infection (16, 32, 40). Symptom expression and disease development are dependent on bacterial strain (9), cultivar (35, 59), temperature (42, 46, 60), and relative humidity (46, 60). Physiological age of the leaves does not appear to

affect disease development (3). Under optimal conditions, small, round, water-soaked areas are visible on leaves approximately 5 days after inoculation (31). Foliar lesions enlarge to approximately 1-3 mm in diameter and are surrounded by distinct yellow-green halos (9, 31, 37). Often several lesions coalesce forming large, irregular necrotic areas on the leaves. Bacterial speck development is inhibited at 22.8 C and above (42), thus the general characterization of bacterial speck as a cool temperature disease. However, Schneider and Grogan (40) showed that a portion of the P. tomato resident population was able to survive under warm, dry conditions in the field for at least 14 days after inoculation, although there were no obvious symptoms. All attempts to obtain disease symptoms by introducing P. tomato directly into stems and leaves, or through wounded roots have failed (3, 32).

It has been reported that infection may cause a decrease in yield (6, 43, 60), however, the most conspicuous and damaging phase of the disease appears on the fruit. P. tomato enters green fruit through wounds, in particular, through broken trichomes (9, 12, 30) and produces small (1-3 mm diameter) superficial black lesions. These small black lesions constitute a severe blemish from the fresh market standpoint and are also objectionable to processors (22). Ripe fruit are not infected (9, 60). Yunis et al. (60) reported that the pH of red skin (5.2) is below the minimum pH required for multiplication of P. tomato.

The bacterium can survive on seed (3, 14, 16, 24, 32), in soil (3, 8, 14, 16, 32, 40), in plant debris (14, 52), and on many nonhost

plants (16, 40). Its ability to overwinter in northern tomato growing areas has never been determined. Currently, there are no commercially acceptable resistant cultivars available, however, promising sources of resistance have been detected in screening tests (33, 35, 59, 62). Bacterial speck control programs are currently built around the concept of prevention. Rotation, the use of disease-free seed and transplants, and the destruction of volunteer hosts are the most practical and effective measures of control, although some control has been achieved by the use of antibiotic and copper sprays (14, 19, 22, 32, 34, 52, 61).

With the recent increase in the occurrence of bacterial speck on Michigan tomatoes, factors affecting initial inoculum and symptom development needed to be investigated. Since a large percentage of Michigan's tomato growers use transplants grown in the southern United States, the speck pathogen was previously thought to be reintroduced into Michigan each growing season on these transplants. However, growers using only Michigan-grown transplants have seen bacterial speck in their fields. In addition, bacterial speck has been particularly severe in the fields that have had a history of bacterial speck incidence (19). These factors suggested that P. tomato has the potential to overwinter in Michigan soils, although this has never been demonstrated. If an epidemiologically significant P. tomato population survives in Michigan from one growing season to the next, infested soil could be a main source of inoculum for the next year's crop.

Isolation of plant pathogenic bacteria from the ubiquitous field microflora has, in general, been difficult and a major obstacle to

epidemiological studies. The introduction of antibiotic markers into plant pathogenic bacteria has improved field recovery and has facilitated basic studies of bacterial epidemiology (13, 15, 21, 23, 48). The use of rifampicin-resistant mutants was tested (54) and effectively used by Weller and Saettler (55) in field studies of the epidemiology of bean common and fuscous bacterial blights. Stadt (47) was able to monitor population levels of rifampicin-resistant Pseudomonas phaseolicola in tolerant and susceptible bean hosts. The use of a similar rifampicin selection system in overwintering and population studies of P. tomato would facilitate reisolation procedures by excluding microflora which often mask the presence of the desired organism.

Several researchers have monitored bacterial population levels and correlated increases with symptom development (27, 50). Perhaps the most sophisticated program of population monitoring is in California where pear orchards are monitored for Erwinia amylovora in order to predict when outbreaks of fire blight might occur (44). The increase in pathogen populations was found to be correlated with weather parameters. These parameters are now monitored and tied to a streptomycin spray program. The result has been a substantial savings for pear growers by reducing the number of sprays necessary for control. However, Sutton and Jones (49) indicated that pathogen population monitoring techniques must be modified for each geographical area. Monitoring techniques used in California did not sufficiently predict disease development in Michigan. Relationships between weather parameters and

bacterial speck development reported in California (42), Georgia (46), and Israel (60) may be significantly different than those encountered in Michigan. Effective and economical spray programs require knowledge of the pathogen-environment relationship in the geographical area in which they are to be used.

Disease prediction based on weather data alone has limited value because other factors such as inoculum potential and host susceptibility are also important in determining disease development. In several other host-pathogen systems, host susceptibility has been shown to vary during plant development (11, 28, 29, 53, 58). Burr and Hurwitz (11) reported that Mutsu apple fruits showed increased susceptibility to Pseudomonas syringae pv. papulans (PSP) beginning 2 to 2.5 weeks after petal fall and continuing for 2 to 4 weeks. They suggested that since PSP enters through stomata, susceptibility may be related to stomatal development and the subsequent development of lenticels. Ripe tomato fruit are immune to bacterial speck infection (9, 60), however, the susceptibility of earlier developmental stages of the fruit has never been examined. The pattern of susceptibility to P. tomato during tomato fruit development may be an important phenomenon to consider when planning a control program for bacterial speck.

The objectives of this research were: (1) to determine whether P. tomato is capable of overwintering in Michigan soils, (2) to determine what environmental parameters can be used to predict P. tomato populations and symptom development on field-grown tomatoes, (3) to

determine the developmental stage(s) at which tomato fruit is most susceptible to infection by P. tomato, and (4) to observe fruit infection sites and follow lesion development with a scanning electron microscope.

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PART I

OVERWINTERING AND INFLUENCE OF WEATHER ON *PSEUDOMONAS SYRINGAE* PV. *TOMATO* POPULATIONS IN MICHIGAN

ABSTRACT

Tomato leaf tissue infected with Pseudomonas syringae pv. tomato (P. tomato) was buried in Michigan field soil in the fall and recovered the following spring to determine if the pathogen was capable of surviving over the winter. P. tomato was reisolated from infected tomato leaf tissue overwintered on the surface and buried up to 18 cm during the winters of 1979-80 and 1980-81. Weather data and foliar P. tomato populations on field-grown susceptible and resistant tomato cultivars were monitored during the 1980 and 1981 growing seasons to identify weather variables that might be used to predict increases in pathogen populations. A predictive model was developed from 1981 temperature and P. tomato population data.

INTRODUCTION

With the recent increase in the incidence of bacterial speck of tomato, caused by Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato) (8), the need to better understand the epidemiology of the disease has become increasingly apparent. Although survival of the pathogen on seed (1, 5, 7, 9, 16) and in soil and plant material (1, 3, 5, 7, 16, 18, 24) has been reported, whether an epidemiologically significant P. tomato population overwinters in northern tomato growing areas has yet to be confirmed.

Because bacterial speck can be a very serious problem one year, but unimportant in others, some method is needed for forecasting disease outbreaks in sufficient time to employ effective, economic, and ecologically sound control measures. Relationships between environmental parameters and bacterial speck severity have been studied in both controlled environments and the field (1, 2, 18, 19, 20, 22, 25), but no direct correlations between field weather and P. tomato populations have been reported.

The objectives of this research were to determine the overwintering potential of P. tomato in Michigan, to identify weather parameters that influence P. tomato populations in the field, and to begin the development of a predictive model that could be used to time protective sprays.

MATERIALS AND METHODS

Overwintering. A naturally occurring rifampicin-resistant isolate of P. tomato (PtR5) was used as the pathogen throughout this study. Inoculum was prepared by incubating a minimal broth (14) culture of PtR5 for 24 hours, then diluting with sterile distilled water (SDW) to a final inoculum concentration of approximately 10^7 colony forming units (cfu)/ml, as determined by standard turbidimetric and dilution plate techniques. Inoculum was applied to leaves of susceptible field-grown tomato plants with a hand-held pneumatic sprayer. Lesions were observed 7 days after inoculation and isolations were made to confirm the presence of the pathogen in the leaf tissue. Noninoculated leaves from susceptible greenhouse-grown tomato plants were used as controls.

In October 1979, infected and noninoculated tomato leaves were placed in separate 18 cm^2 nylon mesh bags (3 mm mesh). The fresh weight of the leaf tissue in each bag was approximately 225 g. The nylong mesh bags containing the leaf material were then buried in field soil at depths of 0, 8, and 18 cm, one infected and one noninoculated leaf bag at each depth. Bags were buried at 2 locations in Michigan. At the Berrien County site (Kalamazoo loamy sand), there were 2 replications at each soil depth and the field was planted with rye. The Ingham County site (Colwood clay loam) consisted of only 1 replication at each soil depth and the field was planted with winter wheat.

Leaf samples were removed from the bags at the Berrien County site in November and December 1979 and isolations made from leaves at each soil depth. Immediately after removing leaf samples, the bags were replaced in their original positions in the ground. The Ingham County site was left undisturbed from the time of burial until the spring. In April 1980, leaf bags were removed from both sites and the remaining leaf tissue evaluated for the presence of the pathogen.

Isolations were made by macerating leaf tissue from each bag in SDW and allowing it to stand for 3 h. From these suspensions, 0.1 ml was spread onto the surface of complete medium (14) amended with 100 μg/ml of rifampicin and 25 μg/ml of cycloheximide (CRC agar). Five plates were prepared from each suspension (bag) and incubated at room temperature for 5 days. Colonies exhibiting phenotypic characteristics similar to isolate PtR5 were selected for further characterization. Isolates that were fluorescent on King's Medium B (KMB) (10), oxidase (12) and arginine dihydrolase (23) negative, and produced a hypersensitive reaction on tobacco (11) were tested for pathogenicity on tomato.

The overwintering experiment was repeated in 1980-81 with the following modifications in procedure. Susceptible greenhouse-grown tomato plants were used as sources of both infected and noninoculated leaf tissue. Leaf bags were buried in November 1980 at the Ingham County site only and were left undisturbed until May 1981.

In addition to determining P. tomato survival on intentionally buried material, an entire plot (23 x 28 m) at the Michigan State

University Botany and Plant Pathology Research Farm, containing plants infected with isolate PtR5, was monitored for overwintering of the pathogen. In October 1980, the plot was divided into 3 sections that were treated in the following manner: (1) plowed, (2) plowed and planted with rye, and (3) tomato plants left standing. In May 1981, each section was plowed and prepared for planting. Care was taken to prevent movement of soil from one section to another in cultural operations. In each section, 3 rows were direct seeded and 3 rows were hand-planted with transplants of the susceptible cultivar Pik-Red (Joseph Harris Co., Inc., Rochester, NY 14624) in an attempt to detect the pathogen in the soil. Tomato plants in each section were checked throughout the growing season for speck development.

Population monitoring. P. tomato populations were monitored on field-grown susceptible fresh market cultivars Pik-Red and Basket Vee (Stokes Seeds Inc., Buffalo, NY 14240) in 1980 and on Pik-Red and the resistant processing cultivar Ontario 7710 (experimental cultivar, R. Pitblado (17)) in 1981. Greenhouse-grown transplants of Pik-Red and Basket Vee were mechanically planted in June 1980 and of Pik-Red and Ontario 7710 in May 1981 at the MSU Botany and Plant Pathology Research Farm. Plots consisted of either 11 rows (1980) or 13 rows (1981) of each cultivar 28 m in length with a row spacing of 1.2 m. Transplants were spaced 0.6 m apart within each row. Carbaryl (1-napthyl-N-methylcarbamate) or methomyl (S-methyl-N-[(methylcarbamoyl) oxy]thioacetimidate) and chlorothalonil (tetrachloroisophthalonitrile)

were used as needed for foliar insect and fungal disease control.

In 1980, inoculum was prepared and applied as described for the overwintering studies. In 1981, inoculum was prepared by washing bacterial cells from CRC agar with SDW after 24 h incubation at room temperature and applied as previously described. Plants were inoculated once in 1980 and twice in 1981.

Isolate PtR5 populations were determined weekly (1980) or 3 times/week (1981) on new and old leaves of each cultivar over a period of 8 (1980) or 13 (1981) weeks. Samples of 60 new leaflets and 20 old leaflets were randomly selected from each cultivar. The fresh weight of 60 new leaflets was approximately equal to that of 20 old leaflets. Leaflets were finely chopped with a sterile scalpel and three 1-g replications were weighed out for each sample. The chopped leaf tissue was ground to a slurry in 5 ml of SDW with a mortar and pestle and then strained through 2 layers of sterile cheesecloth into test tubes. Serial dilutions were prepared with SDW and plated onto CRC agar. Bacterial colonies phenotypically resembling PtR5 were counted after 5 days incubation at room temperature. P. tomato populations were expressed as cfu/g of fresh leaf tissue.

Relative humidity, air temperature, leaf wetness, and irrigation/rain data were collected throughout the growing season. Relative humidity and air temperature were measured with a 7-day recording hygrothermograph (Belfort Instrument Co., Baltimore, MD 21224) placed in a standard weather shelter at ground level. A 7-day recording leaf

wetness meter (M. deWit, Hengelo, The Netherlands), periodically adjusted to remain level with the tomato canopy, was used to measure the duration of leaf wetness. Irrigation/rain was measured with a tipping bucket rain gauge attached to a 7-day event recorder (Weather Measure Corp., Sacramento, CA 95841).

Population model development. Weather data and bacterial populations during 1981 were analyzed using a stepwise multiple regression procedure (15) on the MSU Cyber 750 computer. For the analyses, bacterial populations were transformed to logarithms so that their relationship with the independent (weather) variables was linear and variance was stabilized. Weather variables which did not contribute significantly to the coefficient of multiple determination (R^2) were discarded. Variables which did contribute significantly to R^2 were retained and used to predict bacterial populations. Partial correlation coefficients from multiple regression analyses, and predictions from these analyses, were improved when weather data were analyzed using "days" that ran from 0700 one day to 0659 the next day, rather than from 0001 to 2400 on the same calendar day. Data were plotted using the EPIPLOT software package (Botany and Plant Pathology Department, Michigan State University).

RESULTS

Overwintering. Rifampicin-resistant P. tomato was recovered from infected tomato leaf tissue overwintered on the surface and buried up to 18 cm in both loamy sand and clay loam soils (Table 1). No attempt was made to quantify the results since the degree of leaf decomposition varied between different soil depths and types. Bacterial colonies phenotypically resembling PtR5 were easily distinguished from the soil microflora that were not completely inhibited by the rifampicin and cycloheximide in the selective medium. When tested, suspected PtR5 colonies were always fluorescent on KMB, oxidase and arginine dihydrolase negative, produced a hypersensitive reaction on tobacco, and were pathogenic on tomato. No rifampicin-resistant bacteria were recovered from noninoculated (control) leaf tissue.

Bacterial speck symptoms did not naturally develop on either the direct seeded or transplanted tomato plants in any section of the entire plot that was monitored for overwintering of P. tomato.

Population monitoring. In 1980, P. tomato populations peaked approximately 1 week after inoculation on the susceptible cultivars Pik-Red and Basket Vee (Fig. 1). Populations on new leaves were usually lower than those on old leaves, however, population fluctuations on new and old leaves usually paralleled each other. Temperatures became highly unfavorable (>25 C) for the pathogen during mid-July and there was a noticeable decline in populations. From late-July until the end of the season, populations gradually declined even though

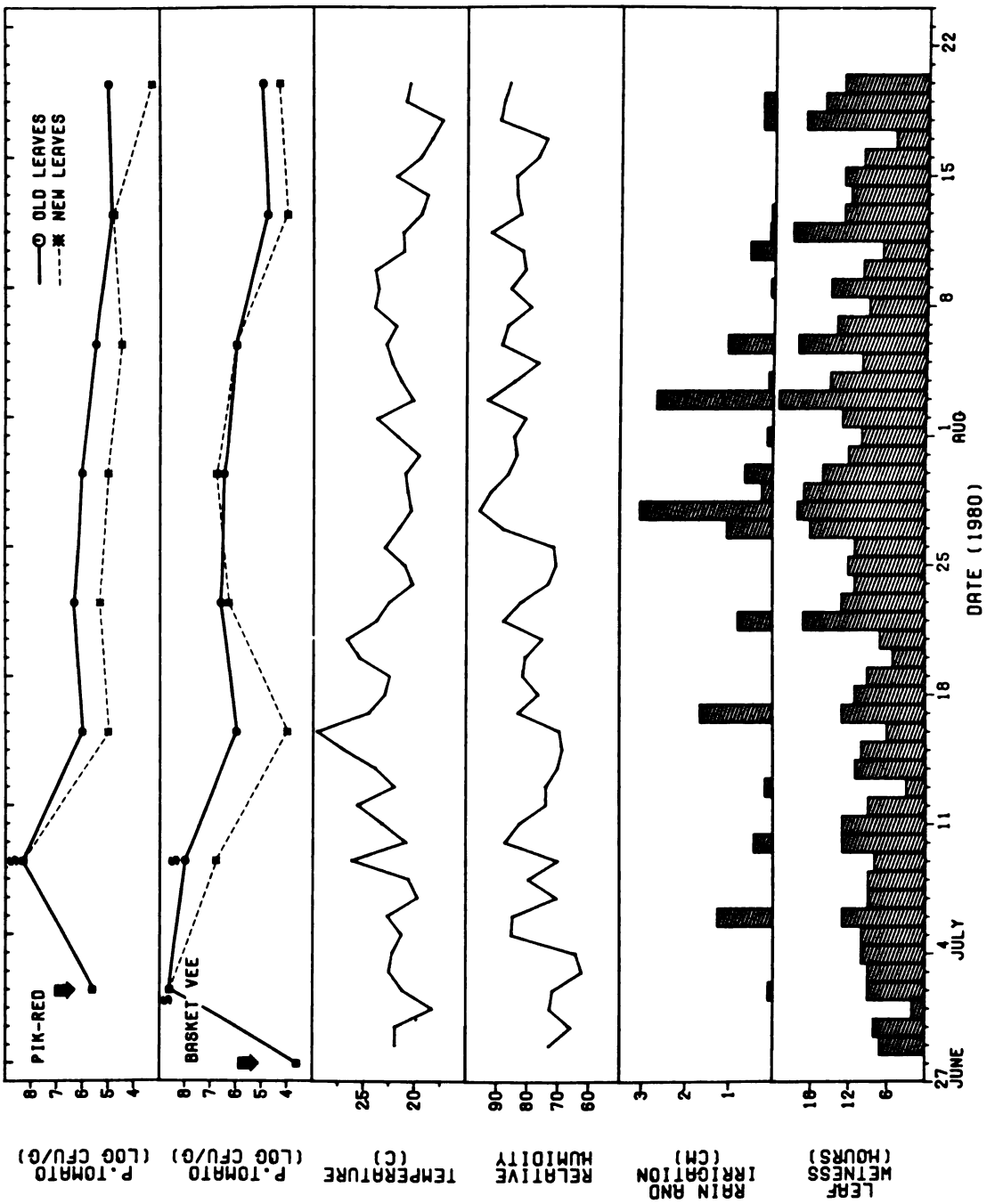
Table 1. Overwintering of Pseudomonas syringae pv. tomato (isolate PtR5) in tomato leaves in Michigan field soils

Year	Soil type, Location	Depth of burial (cm)	Recovery[1] November Infected	November Control	December Infected	December Control	April Infected	April Control	May Infected	May Control
1979-80	Loamy sand,	0 (surface)	+	-	+	-	+	-		
	Berrien County[2]	8	+	-	+	-	+	-		
		18	+	-	+	-	+	-		
	Clay loam,	0					+	-		
	Ingham County	8					+	-		
		18					+	-		
1980-81	Clay loam,	0							+	-
	Ingham County	8							+	-
		18							+	-

[1]Recovery: (+) indicates a rifampicin-resistant, fluorescent, oxidase and arginine dihydrolase negative bacterium that produced a hypersensitive reaction on tobacco and was pathogenic on tomato was recovered from tomato leaf tissue. (-) indicates no rifampicin-resistant bacteria were recovered.

[2]Berrien County results were identical for both replications.

Figure 1. Relationship between climatological changes and the population of Pseudomonas syringae pv. tomato on leaves of field-grown susceptible fresh market tomato cultivars Pik-Red and Basket Vee in 1980. S: symptoms (lesions) present on leaves, +: plants inoculated. Each population point represents the mean of 3 replications, 60 (new) or 20 (old) leaves per replicate.

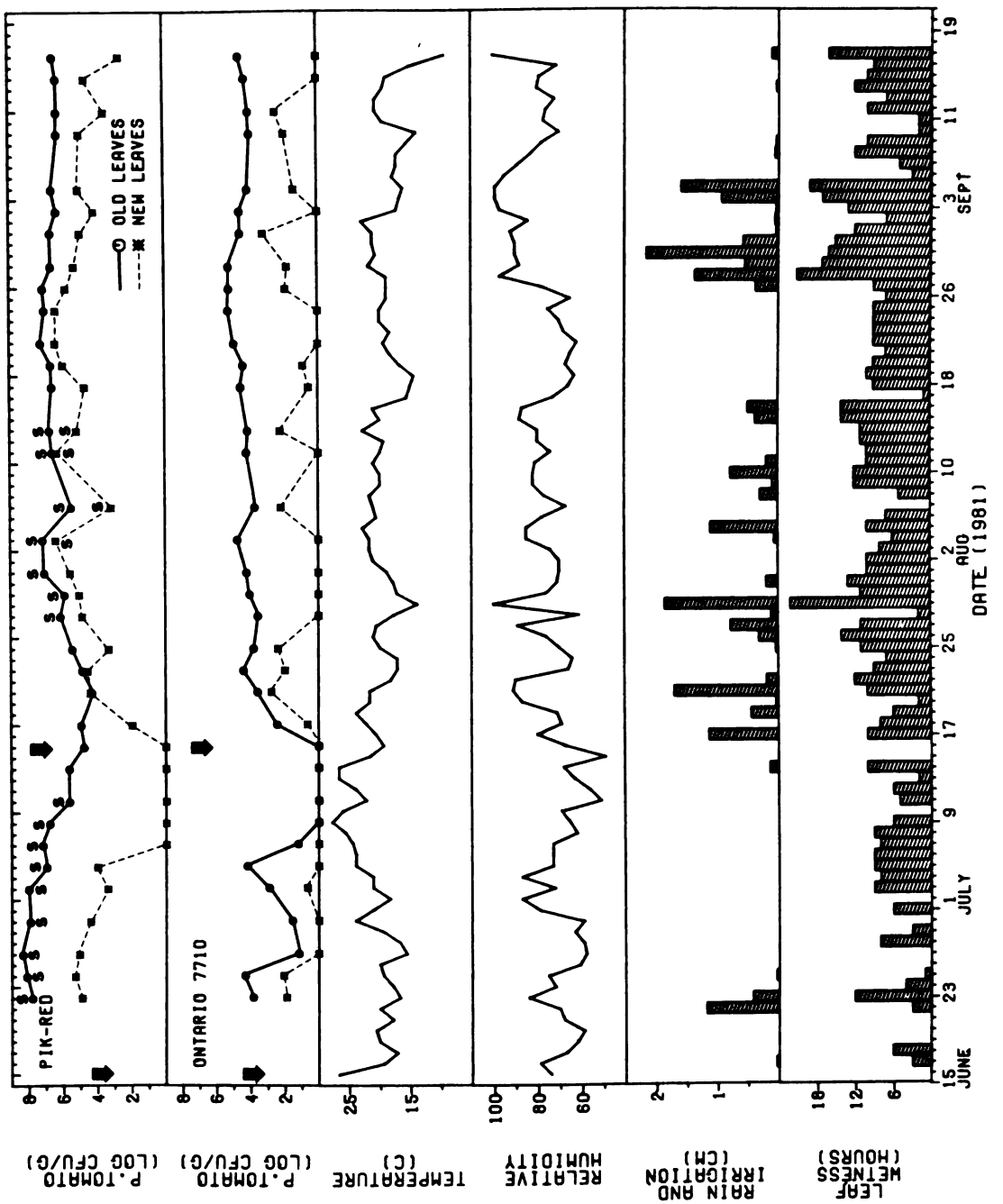

OLD LEAVES
NEW LEAVES
PIK-RED
BASKET VEE
P.TOMATO (LOG CFU/G)
P.TOMATO (LOG CFU/G)
TEMPERATURE (C)
RELATIVE HUMIDITY
RAIN AND IRRIGATION (CM)
LEAF WETNESS (HOURS)
DATE (1980)
27 JUNE
4 JULY
11
18
25
1 AUG
8
15
22

temperature (<25 C) and relative humidity (>80%) became favorable for the pathogen. Speck lesions were present on Pik-Red foliage on 8 July and on Basket Vee foliage on 1 and 8 July. When bacterial populations fell below 10^7 cfu/g fresh leaf tissue (15 July), new lesions did not develop.

In 1981, P. tomato populations peaked 1.5 weeks after the first inoculation on old leaves of the susceptible cultivar Pik-Red (Fig. 2). Populations on Pik-Red new leaves and both new and old leaves of the resistant cultivar Ontario 7710 did not peak until after the second inoculation. As in 1980, temperature (>25 C) and relative humidity (<80%) became highly unfavorable during mid-July and P. tomato populations dropped to undetectable levels on all but susceptible old leaves. Following the second inoculation on 15 July, populations were reestablished on both susceptible new and resistant new and old leaves but quickly disappeared again on resistant new leaves. Populations on susceptible foliage and resistant old leaves remained fairly stable throughout the remainder of the season. As in 1980, population fluctuations on susceptible new and old leaves usually paralleled each other. Symptoms were present on susceptible old leaves 22 June through 10 July and 27 July through 13 August and on susceptible new leaves 3 August through 13 August. Symptoms never developed on resistant foliage.

Population model development. Stepwise multiple regression analysis (15) revealed that during 1981, bacterial populations on susceptible

Figure 2. Relationship between climatological changes and the population of Pseudomonas syringae pv. tomato on leaves of field-grown susceptible fresh market cultivar Pik-Red and resistant processing cultivar Ontario 7710 in 1981. S: symptoms (lesions) present on leaves, ↓: plants inoculated. Each population point represents the mean of 3 replications, 60 (new) or 20 (old) leaves per replicate.


PIK-RED
ONTARIO 7710
OLD LEAVES
NEW LEAVES
P.TOMATO (LOG CFU/G)
P.TOMATO (LOG CFU/G)
TEMPERATURE (C)
RELATIVE HUMIDITY
RAIN AND IRRIGATION (CM)
LEAF WETNESS (HOURS)
JUNE
JULY
AUG
SEPT
DATE (1981)

new leaves at a given time could be predicted with 64% accuracy using average temperature and average temperature squared for the previous 4-day period. A regression equation of the general form:

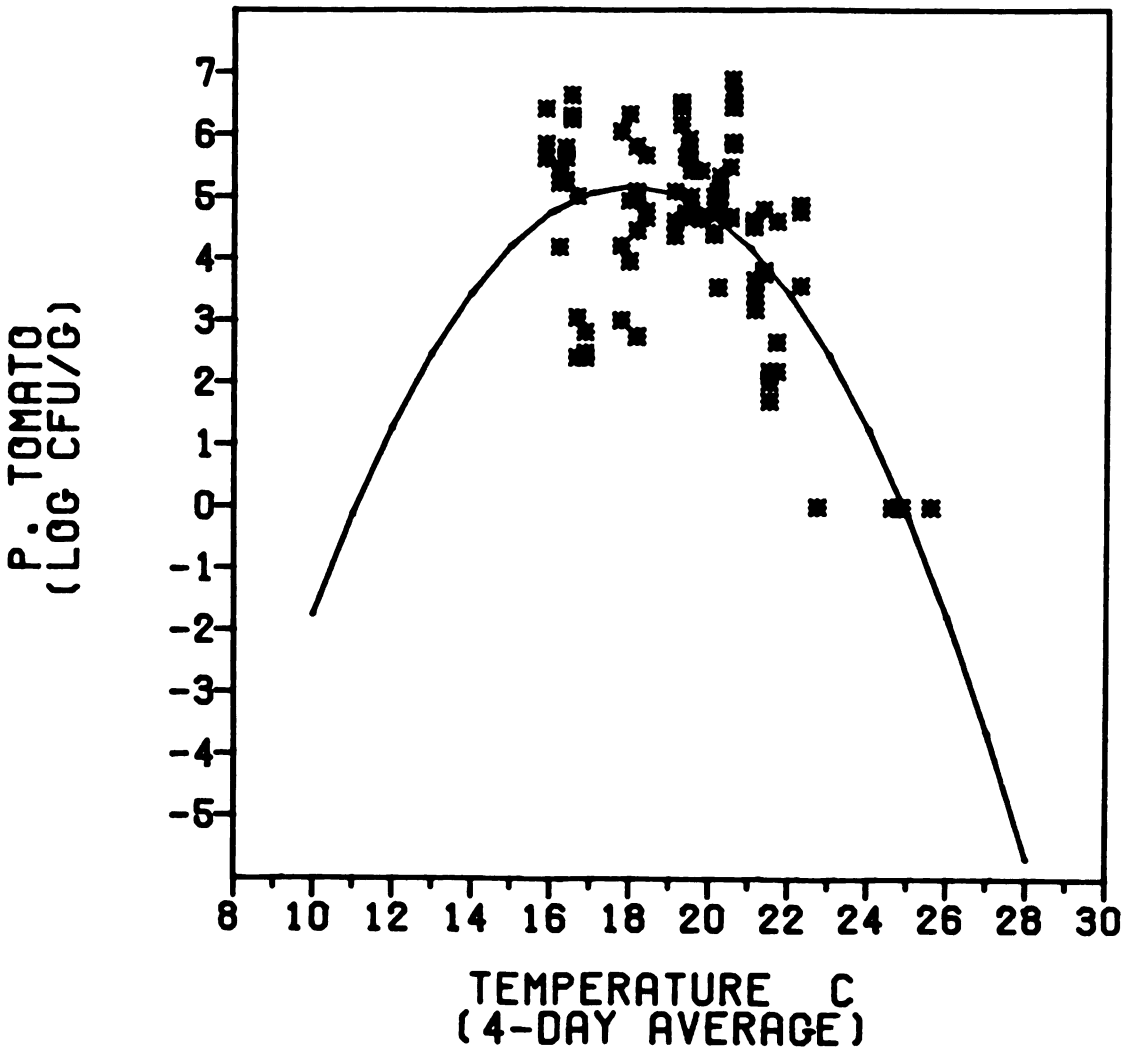
$$\hat{Y} = b_0 + b_1 T + b_{11} T^2$$

was used, where $\hat{Y}$ = estimate of the log of cfu per g of fresh leaf tissue, T = the previous 4-day average temperature (C), b_0 = constant, and b_1 and b_{11} are partial regression coefficients. All coefficients were statistically significant (P=.01). The actual equation is:

$$\text{LOG CFU} = -29.8643 + 3.8926T - 0.108153\ T^2$$

The relationship of temperature to P. tomato populations is shown in a curve generated from the model (Fig. 3). The actual 1981 data points are also included. Examination of residuals, i.e. the difference between the actual data points and those predicted by the regression model, confirmed the assumption that errors were independent and normally distributed.

Figure 3. Relationship between temperature and the population of Pseudomonas syringae pv. tomato on new leaves of field-grown susceptible fresh market cultivar Pik-Red in 1981. The line indicates populations predicted by the equation: LOG CFU = -29.8643 + 3.8926 T - 0.108153 T^2 where T = previous 4-day average temperature. Points (*) indicate actual populations in 1981.


P. TOMATO
(LOG CFU/G)
7
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
8
10
12
14
16
18
20
22
24
26
28
30
TEMPERATURE C
(4-DAY AVERAGE)

DISCUSSION

The use of a rifampicin-resistant P. tomato isolate in combination with our selective medium allowed us to monitor overwintering of the pathogen in the field. Fluctuations in soil temperature, moisture, and microbial activity that normally occur during the overwintering phase are known to influence the rate of plant tissue decomposition (4, 6). This, in turn, would influence the survival ability of P. tomato which is most likely dependent on the presence of host material (4, 21). Artificial situations previously used by researchers to determine the longevity of P. tomato in soil (3, 5, 7, 16) have not simulated the natural situation where temperature, moisture, microbial activity, and other soil parameters are constantly changing.

At the end of the growing season there is ample host plant material providing "protected positions" (13) for the pathogen. As this infected host material senesces, becomes dessicated, and air temperatures decrease, the bacteria probably shift into a hypobiotic state. Bacteria in a hypobiotic state are more likely to survive than are active cells (13). During Michigan winters where ground frost typically reaches a depth of 15-20 cm, plant debris is frozen and microbial activity is substantially reduced. In addition to reducing the rate of host plant decomposition, the low temperatures may directly favor the survival of P. tomato (3).

The greatest reduction in viable P. tomato probably takes place during the warm spring months when soil temperatures increase and

microbial activity resumes. Although not determined, the most favorable site for overwintering was probably in leaf tissue on the soil surface where plant material was wet for shorter periods and where microbial activity would be expected to be less.

Previous studies have shown that soil-borne P. tomato is adequate for initiation of bacterial speck epidemics (7, 18). Our results indicate that the pathogen is capable of surviving over the winter and up to the time at which transplants are set in the field (May). Therefore, overwintered infected plant debris may be a source of primary inoculum in Michigan. Unfortunately, environmental conditions were unfavorable for natural development of the disease in 1981, so naturally infected plants in our experimental plot could not be located to check this point.

Another source of inoculum may be weeds which are known to support epiphytic populations of P. tomato in California (18). Weeds are often present in tomato fields near the end of the season and may also act as survival sites for the pathogen.

Currently, Michigan tomato growers use a 7-day spray schedule for control of bacterial speck. Development of a model to accurately predict the response of P. tomato populations to certain weather conditions would eliminate many of these unnecessary sprays. Our model was specifically developed for predicting P. tomato populations on susceptible new leaves. The model was developed from only one year's data and has not been validated. Additional data are needed to

determine the effectiveness of the model in accurately predicting P. tomato populations in other years. Work is being continued on this model and it will eventually be tested for its effectiveness in timing protective sprays applied after critical population levels of P. tomato have been predicted by the model.

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PART II

INFLUENCE OF DEVELOPMENTAL STAGE ON SUSCEPTIBILITY OF TOMATO FRUIT TO *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

ABSTRACT

Tomato flower buds, flowers, and fruit were inoculated with Pseudomonas syringae pv. tomato (P. tomato) at various developmental stages in greenhouse and field studies. In greenhouse studies, inoculation at the open corolla stage resulted in a significant decrease in marketable yield compared to the noninoculated control. Lesions did not develop on fruit when flower buds were inoculated prior to anthesis. Susceptibility of green fruit decreased as diameter at the time of inoculation increased. Lesions did not develop on inoculated pink or red fruit. In field studies, lesions did not develop on fruit inoculated when they were larger than 3 cm in diameter. These results indicate that noninjured tomato fruit are most susceptible to infection by P. tomato in the period following anthesis and prior to the time fruit reach 3 cm in diameter.

INTRODUCTION

Within the last decade, bacterial speck of tomato (Lycopersicon esculentum Mill.), caused by Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato) (6), has become a serious problem in many tomato production areas (9). Early infection may reduce yield and delay maturity (19, 21), however, the most destructive aspect of the disease is the reduction in fruit quality due to the lesions that form on the fruit surface.

Before effective control strategies can be implemented, the epidemiology of the disease must be better understood. Previous studies have dealt primarily with identifying the environmental conditions favorable for bacterial speck development (2, 3, 16, 17, 18, 21). However, disease prediction based on weather data alone has limited value because other factors such as host susceptibility and inoculum potential are also important in determining disease development. In several other host-pathogen systems, host susceptibility has been shown to vary during plant development (5, 12, 13, 15, 20). The purpose of this investigation was to determine the developmental stage(s) at which tomato fruit is most susceptible to infection by P. tomato.

MATERIALS AND METHODS

In greenhouse studies, tomato plants of the susceptible fresh market cultivar Pik-Red (Joseph Harris Co., Inc., Rochester, NY 14624) were grown in 2-L plastic pots containing VSP Peat Lite Mix (Bay-Houston Towing Co., Houston, TX 77081). A 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18100) was mixed at 5 g/L of water and approximately 0.2 L was applied biweekly. In field studies, several rows of the cultivar Pik-Red were established at the Botany and Plant Pathology Research Farm, Michigan State University. Rows were 17 m in length with a row spacing of 1.2 m. Plants were spaced 0.6 m within each row. Carbaryl (1-napthyl-N-methylcarbamate) or methomyl (S-methyl-N-[(methylcarbamoyl)oxy]thioacetimidate) and chlorothalonil (tetrachloroisophthalonitrile) were used as needed for foliar insect and fungal disease control.

A naturally occurring rifampicin-resistant isolate of P. tomato (isolate PtR5) was used as the pathogen throughout this study. Cultures were grown as a lawn for 24 h at room temperature on a complete medium (11) amended with 100 μg/ml of rifampicin (Sigma Chemical Company, St. Louis, Missouri 63178). Inoculum was prepared by washing cells from the agar surface with sterile distilled water (SDW). Final inoculum concentration was adjusted by dilution with SDW to approximately 10^7 colony forming units (cfu)/ml as determined by standard turbidimetric and dilution plate techniques.

In the initial greenhouse and field studies, tomato fruit development was arbitrarily divided into the following developmental stages: (i) closed calyx, (ii) open calyx, (iii) open corolla, (iv) green fruit 3 cm or less in diameter, (v) green fruit greater than 3 cm in diameter, and (vi) pink to red fruit. Noninjured flower buds, flowers, and fruit of both field and greenhouse grown tomato plants were tagged at different developmental stages and sprayed to runoff from a distance of 30 cm with a fine mist of inoculum. To study relative susceptibility more closely, a second experiment was conducted in the greenhouse in which fruit at developmental stages (iv) and (v) were divided into several size categories. Diameters of green fruit were measured (at the widest point) and the fruit tagged for later identification. Fruit were inoculated as previously described.

In greenhouse studies, both inoculated and control plants were placed in translucent polyethylene-covered chambers with periodic misting (10 sec. every 30 min.) so that plants were continually wet. The temperature within the chambers fluctuated between 20 C (night) and 27 C (day). Following a 96-h incubation period, plants were removed from the chambers and placed on greenhouse benches. Control plants were treated similarly except that SDW was used instead of inoculum.

To maximize both the number of developmental stages present on the field plants and the environmental conditions favorable for bacterial speck infection, the field study was conducted in August and September. This meant that developmental stages (i) and (ii)

could not be evaluated because sufficient quantities of flower buds were not present at that time. To provide a favorable environment for infection (2, 18, 21), field plants were inoculated in the evening when temperatures were cooler and relative humidities higher. Control plants were not included in the field studies because the spread of P. tomato from inoculated plants onto controls could not have been prevented.

Each developmental stage in both greenhouse and field studies was evaluated for the percentage of flower buds, flowers, or fruit that did not develop (nonproductive) and for the percentage of fully developed (mature) fruit that showed typical bacterial speck lesions. Nonproductive flower buds, flowers, and fruit included those producing small fruit that ripened prematurely before attaining a marketable size, those having a persistent calyx but in which the fruit did not develop, and those that abscised (10).

RESULTS

In the initial greenhouse study, a high percentage of flower buds, flowers, and fruit in early developmental stages were nonproductive (Table 1). Fruit larger than 3 cm in diameter at inoculation always developed into mature fruit. The open corolla stage was the only stage where inoculation significantly increased the percentage of nonproductive flowers compared to the noninoculated control. When inoculated at the green fruit ($\leq$ 3 cm diameter) stage, a higher percentage of the fruit developed speck lesions than fruit inoculated at both earlier and later developmental stages. Mature fruit that developed from ovaries and fruit inoculated at closed calyx, open calyx, and pink to red fruit stages never developed speck lesions. Noninoculated controls were symptomless.

In the second greenhouse study, 47% of the fruit between 0.3 and 1.5 cm in diameter at inoculation were nonproductive (Table 2). Fruit 1.6 cm or larger in diameter at inoculation always developed into mature fruit. Of the fruit that reached maturity, 75% between 0.3 and 1.5 cm in diameter, 57% between 1.6 and 2.5 cm in diameter, and 25% between 2.6 and 3.5 cm in diameter at inoculation developed speck lesions. Fruit larger than 3.5 cm in diameter at inoculation never developed speck lesions.

In field studies, a high percentage of the flowers inoculated at the open corolla stage were nonproductive (Table 3). The majority of

Table 1. Effect of developmental stage of tomato fruit on the incidence of nonproductivity and bacterial speck lesion development following inoculation with 10^7 cfu/ml of Pseudomonas syringae pv. tomato under greenhouse conditions

Developmental stage at inoculation		Flower buds, flowers and fruit evaluated (#)	Flower buds, flowers and fruit nonproductive*(%)	Mature fruit evaluated (#)	Mature fruit with lesions (%)
(i)	closed calyx	66	80	13	0
	control	101	77	23	0
(ii)	open calyx	101	57	43	0
	control	94	51	46	0
(iii)	open corolla	116	62[a]	44	5
	control	142	37	89	0
(iv)	green fruit $\leq$ 3 cm	172	31	118	37
	control	177	35	115	0
(v)	green fruit > 3 cm	65	0	65	20
	control	57	0	57	0
(vi)	pink to red fruit	8	0	8	0
	control	4	0	4	0

Table 1. (cont.)

[a]Differences between inoculated and control significant at P=0.05 by X^2 test.

*Nonproductive buds, flowers, and fruit included those producing small fruit that ripened prematurely before attaining a marketable size, those having a persistent calyx but in which the fruit did not develop, and those that abscised.

Table 2. Effect of tomato fruit diameter on the incidence of nonproductivity and bacterial speck lesion development following inoculation with 10^7 cfu/ml of Pseudomonas syringae pv. tomato under greenhouse conditions

Green fruit diameter at inoculation (cm)	Fruit evaluated (#)	Fruit nonproductive* (%)	Mature fruit evaluated (#)	Mature fruit with lesions (%)
0.3 - 1.5	15	47	8	75
1.6 - 2.5	7	0	7	57
2.6 - 3.5	4	0	4	25
3.6 - 4.5	13	0	13	0
4.6 - 5.5	11	0	11	0
5.6 - 6.5	12	0	12	0

*Nonproductive fruit included small fruit that ripened prematurely before attaining a marketable size, those having a persistent calyx but in which the fruit did not develop, and those that abscised.

Table 3. Effect of developmental stage of tomato fruit on the incidence of nonproductivity and bacterial speck lesion development following inoculation with 10^7 cfu/ml of Pseudomonas syringae pv. tomato under field conditions

Developmental stage at inoculation	Flowers and fruit evaluated (#)	Flowers and fruit nonproductive* (%)	Mature fruit evaluated (#)	Mature fruit with lesions (%)
(iii) open corolla	150	81	29	21
(iv) green fruit $\leq$ 3 cm	150	7	139	8
(v) green fruit > 3 cm	150	0	150	0
(vi) pink to red fruit	150	0	150	0

*Nonproductive flowers and fruit included those producing small fruit that ripened prematurely before attaining a marketable size, those having a persistent calyx but in which the fruit did not develop, and those that abscised.

this nonproductivity was due to abscission. As in greenhouse studies, fruit larger than 3 cm in diameter at inoculation always developed into mature fruit. Of the fruit that reached maturity, 21% of those inoculated at the open corolla stage and 8% of those inoculated at the green fruit ($\leq$ 3 cm diameter) stage developed speck lesions. Fruit larger than 3 cm in diameter at inoculation were symptomless.

DISCUSSION

In greenhouse studies, a large percentage of both inoculated and noninoculated early fruit developmental stages did not develop into mature fruit. This high rate of nonproductivity may be attributed to several factors. Our greenhouse-grown tomato plants normally had an average of five to six flowers per cluster of which only two to four flowers produced marketable fruit. The other flowers either abscised or remained attached but did not develop into mature fruit. This may be partially attributed to poor pollination, a common problem in greenhouses (14). Environmental conditions favorable for bacterial speck infection required that, following inoculation, plants be placed under suboptimal conditions for growth and development of the plants. In mist chambers where plants were incubated, factors such as insufficient light and high relative humidity (near 100%) may have been responsible for the increase in the amount of nonproductivity observed on both inoculated and noninoculated plants compared to plants grown under normal greenhouse conditions. The open corolla stage was the only stage where inoculation with P. tomato significantly increased nonproductivity compared to the noninoculated control. At this stage, the majority of nonproductivity was due to flower abscission. Increased ethylene production is often associated with plant pathogens or diseased tissue, and one of the most commonly recognized responses to ethylene is abscission (1). Lesions on the pedicel caused by the pathogen may have stimulated ethylene production in the abscission zone area to the degree that abscission was induced.

In the field study, the majority of nonproductivity observed was due to abscission. Because the study was done near the end of the growing season, field plants already had a heavy fruit set, a condition that is known to result in increased flower abscission (14).

Results from both greenhouse and field studies indicated that susceptibility of tomato fruit to infection by P. tomato varied according to developmental stage of the fruit at inoculation. Similar results have been reported for both bacterial spot (Xanthomonas campestris pv. vesicatoria) (7) and bacterial canker (Corynebacterium michiganense pv. michiganense) (4). Small (≤3 cm diameter) noninjured fruit were readily infected while ripening fruit (pink to red) were never infected. This lack of infection of ripe fruit has been attributed to the natural increase in hydrogen ion concentration as the fruit matures (21), the bacteria being unable to tolerate the more acidic conditions.

Some of the same developmental stages exhibited different susceptibilities depending on whether they were grown under field or greenhouse conditions. In the field, the open corolla stage was the most susceptible to infection, the green fruit (≤3 cm diameter) stage was slightly susceptible while the green fruit (>3 cm diameter) and pink to red fruit stages were not susceptible. Greenhouse results indicated the open corolla through the green fruit (>3 cm diameter) stages were susceptible, with the green fruit (≤3 cm diameter) stage being the most susceptible. These differences may have been due to different rates of fruit development under field and greenhouse

conditions. Rosenbaum and Sando (15) found that fruit age was a better indication of maturity than size. The field open corolla stage may have corresponded to the green fruit ($\leq$3 cm diameter) stage in terms of maturity.

Because there are no stomata on tomato fruits (10), bacterial infection has always been thought to occur through wounds produced as a result of sandblasting, insect punctures, or abrasion with other plant parts (9). However, we obtained substantial infection on young fruit in the greenhouse without wounding prior to inoculation. This suggested that there were natural entry points in the epidermis that allowed invasion by the pathogen. A subsequent scanning electron microscope study provided evidence that P. tomato infects noninjured fruit through openings that remain after trichomes are shed and before the cuticle is fully developed (8).

Our results indicate that the period following anthesis and prior to the time fruit reach 3 cm in diameter is when fruit are most susceptible to infection by P. tomato. In years when fruit development is delayed or advanced by climatic conditions, this period of susceptibility may also vary. Differences in the susceptibility of different fruit developmental stages may warrant consideration in the development of more effective control strategies.

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PART III

SCANNING ELECTRON MICROSCOPY OF INFECTION SITES AND LESION DEVELOPMENT ON TOMATO FRUIT INFECTED WITH *PSEUDOMONAS SYRINGAE* PV. *TOMATO*

ABSTRACT

Tomato ovary and fruit surfaces inoculated with Pseudomonas syringae pv. tomato (P. tomato) were examined with a scanning electron microscope to observe possible infection sites and to follow lesion development. Bacteria were detected on both glandular and non-glandular trichomes present on ovaries during anthesis. Following anthesis, trichomes were gradually lost leaving openings in the young fruit epidermis. Swollen areas of the epidermis that resembled trichome bases were filled with bacteria suggesting that open trichome bases may serve as fruit infection sites. Mature lesions were either sunken or raised and extruded masses of bacteria from cracks in the lesion surface.

INTRODUCTION

Bacterial speck of tomato (Lycopersicon esculentum Mill.), caused by Pseudomonas syringae pv. tomato (Okabe) Young et al. (P. tomato) (4), often assumes epidemic proportions and may cause considerable crop damage and serious economic losses under favorable environmental conditions (7). The most conspicuous and damaging phase of the disease appears on the fruit where small black lesions are formed. These lesions constitute a severe blemish on fruit for fresh market and are also objectionable to processors (7, 9).

Studies of bacterial infection of tomato fruit by Corynebacterium michiganense pv. michiganense and Xanthomonas campestris pv. vesicatoria have been reported (2, 5), but these are relatively old reports published before the advent of scanning electron microscopy. In addition, the cultivars studied have been replaced by commercial hybrids. There have been no studies of fruit infection by P. tomato. Fruit infection sites and early stages of bacterial speck lesion development have been difficult to observe with the light microscope due to problems of resolution and depth of focus. Accordingly, this investigation was conducted to determine possible fruit infection sites and to follow lesion development with a scanning electron microscope (SEM).

MATERIALS AND METHODS

Tomato plants of the susceptible fresh market cultivar Pik-Red (Joseph Harris Co., Inc., Rochester, NY 14624) were greenhouse-grown in 2-L plastic pots containing VSP Peat Lite Mix (Bay-Houston Towing Company, Houston, Texas 77081). A 20-20-20 fertilizer (Peters Fertilizer Products, Allentown, PA 18100) was applied biweekly. Tomato fruit development was arbitrarily divided into the following developmental stages: (i) closed calyx, (ii) open calyx, (iii) open corolla, (iv) green fruit 3 cm or less in diameter, (v) green fruit greater than 3 cm in diameter, and (vi) pink to red fruit.

A naturally occurring rifampicin-resistant isolate of P. tomato (isolate PtR5) was used as the pathogen throughout this study. Inoculum was prepared and applied to each developmental stage as previously described (6).

To observe possible infection sites, ovaries and fruit were sampled at the six developmental stages and prepared for SEM examination (8). Entire ovaries or epidermal blocks (4 x 4 mm) from larger fruit were fixed 2 h in 4% glutaraldehyde followed by 2 h 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. All procedures through dehydration were performed at about 4 C. Following dehydration, tissues were critical-point dried using a Bomar critical-point drier with CO_2 as the carrier gas. Samples were then

mounted on aluminum stubs, sputter-coated with 20-30 nm of gold, and examined in a JEOL JSM - 35C scanning electron microscope.

To follow lesion development, several fruit were inoculated at the green fruit ($\leq$3 cm diameter) stage. Based on initial investigations, greenhouse-grown fruit at this stage was known to be the most susceptible to infection by P. tomato (6). Blocks (4 x 4 mm) were cut from inoculated fruit surfaces 7, 11, or 21 days after inoculation and prepared for SEM observation as previously described.

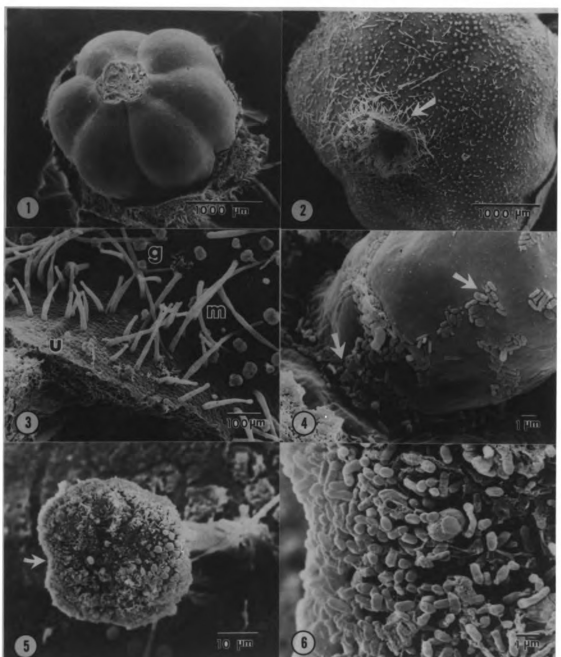
RESULTS

No trichomes were present on tomato ovaries prior to anthesis (closed and open calyx stages) (Fig. 1). The hexamerous nature of the ovary was evident at this early stage of development. Bacteria were distributed over the entire surface with slightly higher populations in depressions between epidermal cells. During anthesis (open corolla stage), the ovary surface was densely covered with unicellular papillary trichomes, long multicellular non-glandular trichomes, and capitate glandular trichomes (Figs. 2, 3); no stomata or natural openings were observed. Bacteria were found primarily on non-glandular (Fig. 4) and glandular (Figs. 5, 6) trichomes.

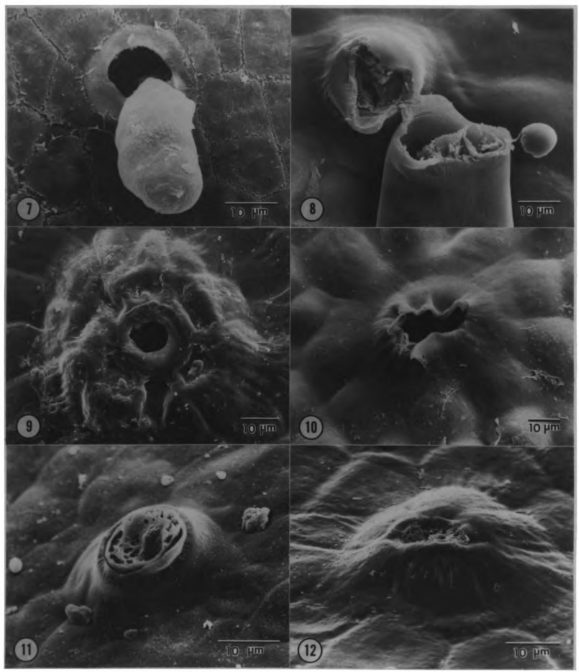
On green fruit 3 cm or less in diameter, few to many trichomes were partially detached (Fig. 7), broken off at their bases (Fig. 8), or completely missing. The actual number of open trichome bases varied according to fruit size, small fruit having more open bases than large fruit. The greatest density of open bases (approximately 12 per mm^2 of epidermis) was observed at the green fruit ($\leq$3 cm diameter) stage which was also the most susceptible stage to P. tomato infection (6). Trichome bases varied in appearance but usually had central openings 10-20 μm in diameter (Figs. 9, 10). Only a few apparently randomly dispersed bacteria were observed on the fruit surface.

On green fruit larger than 3 cm in diameter, many trichomal openings were filled (Figs. 11, 12). As in the green fruit ($\leq$3 cm diameter) stage, very few bacteria were observed on the fruit surface.

Figures 1-6. Scanning electron micrographs of tomato ovaries inoculated with Pseudomonas syringae pv. tomato. 1. Tomato ovary prior to anthesis, X25. 2. Tomato ovary at anthesis, X22. 3. Enlargement from Fig. 2 (arrow) showing unicellular papillary trichomes (u), long multicellular non-glandular trichomes (m), and capitate glandular trichomes (g), X110. 4. Bacteria (arrows) on the surface and at the base of a long non-glandular trichome, X4500. 5. Capitate glandular trichome, X1355. 6. Enlargement from Fig. 5 (arrow) showing bacteria on the head of a glandular trichome, X7505.


1
1000 µm
2
1000 µm
g
m
u
3
100 µm
4
1 µm
5
10 µm
6
1 µm

Figures 7-12. Scanning electron micrographs of natural openings in surfaces of green tomato fruit 3 cm or less in diameter (Figs. 7-10) and greater than 3 cm in diameter (Figs. 11, 12). 7. Non-glandular trichome partially detached, X1715. 8. Broken non-glandular trichome, X1350. 9. Slightly elevated natural opening, X1210. 10. Natural opening, X1035. 11 and 12. Filled natural openings (Fig. 11, X1980; Fig. 12, X1735).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11
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12
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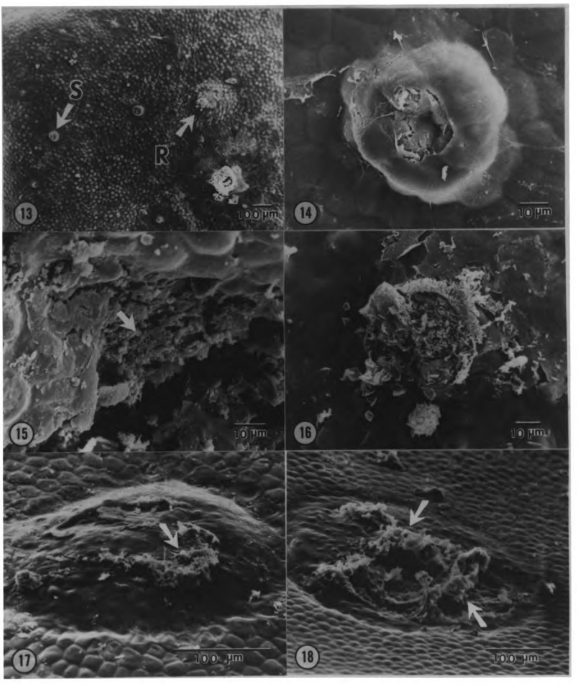
By the time fruit began to turn color, remnants of trichome bases were barely discernible due to a thick waxy cuticle.

The identity of the bacteria observed with the SEM on fruit surfaces was not positively determined; however, isolations from various inoculated developmental stages yielded rifampicin-resistant colonies while isolations from noninoculated developmental stages did not. Bacteria were never observed with the SEM on noninoculated controls.

In the lesion development study, fruit surfaces were examined 7, 11, or 21 days after inoculation. There was no evidence of lesion development and very few bacteria were visible on the fruit surface 7 days after inoculation. Fruit symptoms first appeared to the unaided eye as minute specks 11 days after inoculation. SEM examination of epidermal blocks revealed early stages of lesion development including swollen and ruptured areas (Fig. 13). The similarity between natural openings observed in the epidermis (Figs. 9, 10) and swollen sites (Fig. 14) was apparent. When one of the epidermal swellings (Fig. 14) was removed from the SEM, artificially ruptured with a glass needle, recoated with gold, and placed back in the SEM, large numbers of bacteria were observed inside the tissue (Fig. 15). Naturally ruptured areas of the epidermis exuded copious amounts of bacteria which made it difficult to identify morphological details of the original infection sites (Fig. 16).

Figures 13-18. Scanning electron micrographs of surfaces of green tomato fruit greater than 3 cm in diameter 11 days (Figs. 13-16) or 21 days (Figs. 17, 18) after inoculation with Pseudomonas syringae pv. tomato. 13. Epidermal block showing swollen (S) and ruptured (R) areas, X61. 14. Enlargement from Fig. 13 (S) showing swollen area, X895. 15. Subepidermal view of swollen area in Fig. 14, X250. The swollen area was ruptured with a glass needle and recoated with gold. Masses of bacteria (arrow) were present directly beneath the swollen area. 16. Enlargement from Fig. 13 (R) showing a ruptured area, X975. 17. Raised bacterial speck lesion, X305. Masses of bacteria (arrow) have been extruded from a crack in the lesion surface. 18. Sunken bacterial speck lesion, X205. Bacterial masses (arrows) have erupted from the lesion.


S
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17
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Twenty-one days after inoculation, lesions were clearly visible to the unaided eye and in the SEM appeared either raised (Fig. 17) or sunken (Fig. 18). Bacterial masses extruded through cracks in the lesions.

DISCUSSION

Scanning electron microscopy of tomato ovary and fruit surfaces revealed a developmental phenomenon that resulted in natural openings in the fruit epidermis. Ovaries were densely covered with trichomes at anthesis. When flowers were inoculated, ovarian trichomes were readily colonized by bacteria. During the rapid fruit expansion following fruit set, many of these trichomes were shed or broken off. Open trichome bases were an obvious feature in all epidermal blocks taken from fruit at the green fruit ($\leq$3 cm diameter) stage; hence, the possibility of infection through these natural openings was apparent. P. tomato rods are 0.7-1.2 μm by 1.5-3.0 μm (3). In the presence of free water, P. tomato residing on or near trichome bases could readily invade the fruit through these openings which were usually 10-20 μm in diameter.

Tomato trichomes have been shown to be particularly favorable sites for infection by bacterial pathogens. Trichomal infection by Corynebacterium michiganense pv. michiganense has been observed on both tomato foliage and fruit (2, 10, 11). Gardner and Kendrick (5) postulated that Xanthomonas campestris pv. vesicatoria penetrates the fruit epidermis through broken trichomes or through minute rifts in the cuticle. Previous reports (1, 12) have identified foliar trichomes as habitats and infection sites for P. tomato.

The process of fruit infection by P. tomato is envisaged as follows. Initially, trichomes are colonized by the pathogen. As fruit

enlarges, these trichomes are lost. In the presence of free water, the pathogen invades the open trichome bases and multiplies subepidermally. Eventually, due to internal pressure from expanding masses of bacteria, the epidermis swells and ruptures, resulting in a lesion. The post-symptomatic egress of P. tomato from fruit lesions suggests that, in the presence of free water, lesions may be sources of inoculum for secondary infections.

In our study it was clearly demonstrated that artificial inoculation of young tomato fruit (green fruit $\leq$3 cm diameter) without wounding ultimately produced typical speck lesions. This confirms earlier reports (2, 5) that wounding is not necessary for bacterial infection of tomato fruit. Infection of noninjured fruit probably occurs through open trichome bases that remain after trichomes have been lost.

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