Influence of Developmental Stage on Susceptibility of Tomato Fruit to *Pseudomonas syringae* pv. *tomato*

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ABSTRACT

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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 uninoculated 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 >3 cm in diameter. These results indicate that uninjured tomato fruit are most susceptible to infection by *P. syringae* pv. *tomato* in the period following anthesis and before the fruit reach 3 cm in diameter.

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, however, the epidemiology of the disease must be better understood. Previous studies (2,3,16–18,21) have dealt primarily with identifying the environmental conditions favorable for bacterial speck development. 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 are most susceptible to infection by *P. syringae* pv. *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 plants of cultivar Pik-Red were established at the Michigan State University Botany and Plant Pathology Research Farm at East Lansing. 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. syringae* pv. *tomato* (isolate PtR5) was used as the pathogen throughout this study. Cultures were grown as a lawn for 24 hr at room temperature on a complete medium (11) amended with 100 μg of rifampicin per milliliter (Sigma Chemical Co., St. Louis, MO 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) per milliliter 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: closed calyx, open calyx, open corolla, green fruit $\leq$3 cm in diameter, green fruit >3 cm in diameter, and pink to red fruit. Uninjured 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 greenhouse experiment was conducted in which green fruit were classified into several size categories. Diameters of green fruit were measured (at the widest point), tagged for later identification, and 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 (night) and 27 C (day). Following a 96-hr 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 closed and open calyx stages could not be evaluated at that time because insufficient numbers of flower buds were present. 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 study because the spread of *P. syringae* pv. *tomato* from inoculated plants to control plants could not have been prevented.

Each developmental stage in both greenhouse and field studies was evaluated for the percentage of flower buds, flowers, or fruits that did not develop (nonproductive) and for the percentage of fully

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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 to maturity. The open corolla stage was the only stage in which inoculation significantly increased the percentage of nonproductive flowers compared to the uninoculated control. A higher percentage of fruit inoculated at the green fruit (≤3 cm in diameter) stage developed speck lesions than those inoculated at either the earlier or 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. Uninoculated 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 the field study, a high percentage of the flowers inoculated at the open corolla stage were nonproductive (Table 3). The majority of 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 (≤3 cm in 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 uninoculated 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 produce 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 uninoculated plants compared to those grown under normal greenhouse conditions. The open corolla stage was the only stage in which inoculation with *P. syringae* pv. *tomato* significantly increased nonproductivity compared to the uninoculated 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. syringae* pv. *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 in diameter) uninjured fruit were readily infected while

TABLE 1. Effect of developmental stage of tomato fruit on the incidence of nonproductivity and bacterial speck lesion development following inoculation[a] with *Pseudomonas syringae* pv. *tomato* under greenhouse conditions

Developmental stage at inoculation	Flower buds, flowers, and fruit evaluated (no.)	Flower buds, flowers, and fruit nonproductive[b] (%)	Mature fruit evaluated (no.)	Mature fruit with lesions (%)
Closed calyx	66	80	13	0
Control	101	77	23	0
Open calyx	101	57	43	0
Control	94	51	46	0
Open corolla	116	62[c]	44	5
Control	142	37	89	0
Green fruit ≤3 cm	172	31	118	37
Control	177	35	115	0
Green fruit >3 cm	65	0	65	20
Control	57	0	57	0
Pink to red fruit	8	0	8	0
Control	4	0	4	0

[a] Inoculum concentrations were 10^7 colony-forming units per milliliter.
[b] 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.
[c] Differences between inoculated and control significant at $P = 0.05$ by χ^2 test.

TABLE 2. Effect of tomato fruit diameter on the incidence of nonproductivity and bacterial speck lesion development following inoculation[a] with *Pseudomonas syringae* pv. *tomato* under greenhouse conditions

Green fruit diameter at inoculation (cm)	Fruit evaluated (no.)	Fruit nonproductive[b] (%)	Mature fruit evaluated (no.)	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

[a] Inoculum concentrations were 10^7 colony-forming units per milliliter.
[b] 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[a] with *Pseudomonas syringae* pv. *tomato* under field conditions

Developmental stage at inoculation	Flowers and fruit evaluated (no.)	Flowers and fruit nonproductive[b] (%)	Mature fruit evaluated (no.)	Mature fruit with lesions (%)
Open corolla	150	81	29	21
Green fruit ≤ 3 cm	150	7	139	8
Green fruit > 3 cm	150	0	150	0
Pink to red fruit	150	0	150	0

[a] Inoculum concentrations were 10^7 colony-forming units per milliliter.
[b] 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.

ripening pink to red fruit 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 in diameter) stage was slightly susceptible, while the green fruit (>3 cm in diameter) and pink to red fruit stages were not susceptible. Greenhouse results indicated the open corolla through the green fruit (>3 cm in diameter) stages were susceptible, with the green fruit (≤3 cm in 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 better than size as an indication of maturity. The field open corolla stage may have corresponded to the greenhouse green fruit (≤3 cm in diameter) stage in terms of maturity.

Because there are no stomata on tomato fruit (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. syringae* pv. *tomato* infects uninjured 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. syringae* pv. *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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