Influence of Spore Density, Leaf Age, Temperature, and Dew Periods on Septoria Leaf Spot of Tomato

WADE H. ELMER and FRANCIS J. FERRANDINO, The Connecticut Agricultural Experiment Station, Box 1106, New Haven 06504

ABSTRACT

Elmer, W. H., and Ferrandino, F. J. 1995. Influence of spore density, leaf age, temperature, and dew periods on Septoria leaf spot of tomato. Plant Dis. 79:287-290.

In order to determine the effects of spore density, leaf age, dew period, and temperature on the susceptibility of tomato to *Septoria lycopersici,* growth chamber studies were conducted. Depending on the leaf area, 3–5 ml of water containing 10^2, 10^3, 10^4, 10^5, and 10^6 spores per milliliter were brushed onto the third true leaf up to the seventh true leaf. The log of the disease severity (lesions per square centimeter of leaf) was linearly correlated with the log of the inoculum density (spores per square centimeter of leaf). Older leaves were more susceptible than younger leaves to Septoria leaf spot when inoculum densities were high (10^4 spores per square centimeter of leaf), but younger and older leaves were uniformly susceptible when inoculum densities were low (10^1 to 10^2 spores per square centimeter of leaf). When older leaves were brush inoculated (10^4 spores per square centimeter of leaf), the number of lesions that developed was highest at temperatures between 20 and 25 C, but lesions still developed at 10 C. When brush-inoculated leaves were incubated for 10 days at 50–60% relative humidity without any dew period, the number of lesions that developed did not significantly differ from inoculated leaves that had received 16 hr of dew. Disease severity increased when the dew period extended beyond 20 hr. When leaves were spray inoculated, no lesions developed when dew was absent, but lesions did develop on leaves that were given 24 hr of dew. Our findings suggested that a low incidence of Septoria leaf spot may develop under unfavorable conditions and that leaf abrasion may allow spores to infect in the absence of dew.

Additional keywords: *Lycopersicon esculentum,* foliar diseases, leaf wetness

Septoria leaf spot, caused by *Septoria lycopersici* Speg. is a common late-season foliar disease of tomato (*Lycopersicon esculentum* Mill.) in the eastern United States (1–3,10–12). Symptoms are first noted on older leaves as dark gray, water-soaked spots (1–2 mm in diameter) that develop into small elliptical necrotic lesions (2–5 mm in diameter) often surrounded by a chlorotic zone (3,10–12). As lesions enlarge and coalesce, pycnidia form in the centers and the leaves succumb. Lesions rarely form on young leaves (10).

Septoria leaf spot is routinely controlled by fungicide application (4). However, efforts to limit fungicide sprays have encouraged researchers to study more closely the epidemiology and etiology of the disease. A quadratic relationship between defoliation due to disease and yield was previously demonstrated (7). This model implied that yield was not adversely affected until the plant sustained 50–75% defoliation.

The relationship between conidial inoculum and damage to tomato leaves under different environments is not well defined. Although conidia of *S. lycopersici* are reported as splash-dispersed, experiments using potted tomato plants as trap plants demonstrated that conidia were carried over 30 meters (5,6), and the lesions on the trap plants were low in number and uniformly distributed. However, tomatoes grown in the field commonly have more lesions on their older leaves than on younger ones, suggesting that older leaves may be more susceptible when inoculum densities are high. We found no quantitative information on how leaves of different ages were affected by Septoria leaf spot.

The effect of humidity and temperature on Septoria leaf spot has been reported (1,3,10–12,17). Several studies reported that the maximum lesion number occurred when plants were exposed to 24–48 hr of dew (1,3,10) and that 20–24 C was the most favorable temperature range for lesion development (10,17). However, it was not clear from these studies how disease would develop under less than optimal conditions. Our field observations suggested that severe Septoria leaf spot could develop under conditions of low ambient humidity and cool temperatures. Since epidemiological models are designed to forecast disease under a range of environmental conditions, information on how disease might develop under unfavorable conditions would be useful to predict potentially low levels of disease that may be possible within a field situation. The objectives of this work were to determine the effects of spore density, leaf age, dew period, and air temperature on the development of Septoria leaf spot of tomato.

The authors thank E. O'Dowd for technical assistance.

Accepted for publication 2 December 1994.

MATERIALS AND METHODS

Production of plants. Tomato (cv. Better Boy) seeds were sown into 36-cell plastic trays filled with potting mix (Promix BX, Premier Brand, New Rochelle, NY) and germinated in the greenhouse (18–30 C). After 2 wk, seedlings that had one true leaf were thinned to one plant per cell and fertilized with 40 ml of Peter's 20-8-16 soluble N-P-K (5 g/L). Four weeks later, seedlings were transplanted into 1-L plastic pots filled with Promix BX and were grown for 3–4 wk. Plants in the larger pots received approximately 100 ml of Peter's 20-8-16 soluble N-P-K (10 g/L) solution weekly. Plants possessing 8–9 fully expanded leaves were selected for study.

Inoculum production. Preliminary experiments were conducted to compare the virulence of inocula from 3-wk-old cultures grown on potato-dextrose agar with inocula obtained from re-hydrated infected tomato leaves. Spores from dried infected tissue were superior to the spores from potato-dextrose agar cultures in producing Septoria leaf spot (*data not presented*). A modification of a procedure by Sheridan (14) was used to prepare inocula of *S. lycopersici.* Leaves of Better Boy tomatoes exhibiting actively expanding lesions of Septoria leaf spot were collected the previous season, air dried, and kept in glass jars at 4 C. Approximately 100 g of hand-crushed tissue was placed in 500 ml of sterile distilled water, agitated with a magnetic stir bar for 5–10 min, and filtered through 8–12 layers of cheesecloth. The filtrate was centrifuged at 1,000 *g* for 10 min, and the pelleted spores in each centrifuge tube were bulked. Spores were counted with a hemacytometer and used immediately. Spores of other fungi, such as *Alternaria solani* Sorauer, *A. alternata* (Fr.:Fr.) Keissl., or *Botrytis cinerea* Pers.:Fr were rarely observed.

Effect of inoculum density and leaf age on lesion development. To gain a more accurate estimate of the actual number of spores being applied per leaf, the leaf area (LA) of each leaf was estimated before inoculation by measuring the length (L) and width (W) of the leaf (excluding the petiole), measurements used in the equation LA = L × W ×

0.45 ($r^2 = 0.97$) to predict leaf area. This equation was generated by regressing the products of the lengths and widths from 113 tomato leaves against their leaf areas, which were determined on a leaf area meter (Delta-T Devices, Pullman, WA). A spore suspension containing 0, 10^1, 10^2, 10^3, 10^4, 10^5, and 10^6 spores per milliliter was applied to the adaxial and abaxial side of each leaf with a camel hair paintbrush as described by Tu and Poysa (17). The first and second true leaves from the bottom and all leaves smaller than 5 cm in length were not treated. This resulted in 5–6 leaves being inoculated. All future mention of leaf number refers to the position of the treated leaf. For example, leaf 1 was the oldest treated bottom leaf and leaf 6 was the youngest treated leaf. In the first experiment, 15 ml of the spore suspension was applied per plant, whereas in the second experiment only enough volume was applied to thoroughly wet the leaves. The total volume of remaining spore suspension plus the 1.3 ml absorbed by the paintbrush was subtracted from the original total amount to estimate the amount of inoculum applied per square centimeter of leaf tissue. After the leaf surfaces had dried, plants were placed in a dew chamber (Percival Manufacturer, Model I-35 D, Boone, IA) at 20 C for 24 hr and then placed in a growth chamber equipped with 10 1,500-watt cool-white fluorescent bulbs and 10 60-watt incandescent bulbs set for 12 hr light and dark cycles. Growth chambers were equipped with circulating fans; temperatures varied ±2 C. The relative humidity in the growth chambers was monitored continuously with a hygrothermograph and periodically checked before and after dark cycles with a Bendix Psychron model 566 wet and dry bulb psychrometer (Belfort Instruments, Baltimore, MD). After 10 days incubation, the number of distinct lesions on each leaf was counted. Both experiments contained three replicate plants per treatment. The relationship between the inoculum density (spores per square centimeter of leaf) and disease severity (lesions per square centimeter of leaf) was examined by linear regression analyses on log-log scales.

Influence of dew period and temperature on lesion development. The areas of leaves 1 and 2 were measured and then brush inoculated with a spore suspension containing 10^6 spores per milliliter. Plants were placed in dew chambers at 10, 15, 20, 25, and 30 C, whereupon three plants were removed after 0, 2, 4, 8, 12, 16, 20, or 24 hr and held in growth chambers at the same temperature as the dew chambers. Control plants were brush inoculated with distilled water and placed in the dew chamber for 24 hr and then placed in their respective growth chamber. The relative humidities in these growth chambers averaged 50–60% during experiments conducted in the winter months and 65–75% for studies conducted in the summer. The maximum humidity recorded was 78% and occurred following an irrigation. After 10 days, the lesions were counted. Since only three dew chambers were available, experiments were run using different temperatures in each run. Each temperature/dew period experiment was repeated twice.

Effect of inoculation method and dew period on Septoria leaf spot. To determine whether the abrasive brush-inoculation method affected lesion development in the absence of dew, 5 ml of water containing 10^6 spores per milliliter was either sprayed onto leaves 1 and 2 with an aspirator (17) or brushed on as described before. Once the leaves had dried the plants were placed in a growth chamber set at 20 C that had been adjusted so the 12 hr light cycle had just begun. Relative humidity was closely monitored before and after dark cycles began to ensure no dew was possible. An equal number of plants were also inoculated by both methods and exposed to a 24-hr dew period prior to being placed in the same growth chamber to serve as controls. The number of lesions was counted after 10 days. There were three replicate plants per treatment, and the experiment was repeated.

Influence of temperature on sporulation. To compare the effect of low temperature (10 C) with that of a more optimal temperature (20 C) on number of lesions, pycnidia, and spores produced, leaves 1 and 2 were brush inoculated as described above. Plants were exposed to 24-hr dew periods at 10 or 20 C, and then incubated in a growth chamber at the same temperature. After the number of lesions per square centimeter of leaf was counted, approximately 25 representative lesions were cut out with a scalpel and mounted on a dissecting microscope. The area of the lesion (mm^2) excluding the chlorotic halo was estimated by the equation $((L \times W)/4) \times \pi$ where L = the length and W = the width of each lesion. The number of visible pycnidia was counted. Each excised lesion was air dried on absorbent paper for 18 hr and then placed in a test tube containing 1 ml of sterile distilled water. Tubes were agitated for 1 min, and the number of conidia per lesion was counted using a hemacytometer. Lesion number per square centimeter leaf, number of pycnidia per lesion, number of pycnidia per lesion area, and the number of spores per pycnidium were analyzed using a Student's t test with unequal variances (16).

RESULTS

Effect of spore density on lesion development. Brushing leaves with 10^1, 10^2, 10^3, 10^4, 10^5, or 10^6 spores per milliliter yielded an estimated spore density of 0.1, 1.6, 14.8, 186.4, 1,722.7, and 18,062.4 spores per square centimeter of leaf, respectively, in the first trial and 0.1, 0.5, 3.8, 45.7, 486.4, and 4,587.8 spores per square centimeter of leaf, respectively, in the second trial. When data were plotted on a log-log plot, the resulting total number of lesions per square centimeter of inoculated leaf surface increased linearly as spore density increased (Fig. 1). The slope obtained from experiment 1 was much steeper than the one obtained from experiment 2.

Lesion development on leaves of differing ages. When the age of the leaves was considered, the older leaves were more susceptible than the youngest leaf (Fig. 2), but this effect was only significant at the highest inoculum densities.

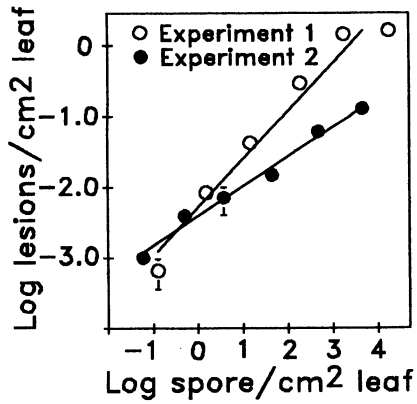

Fig. 1. Effect of spore density of *Septoria lycopersici* on lesion development of tomato. The equation for open circles (experiment 1) was log disease severity (log lesions per square centimeter of leaf) = $-2.8 \times$ log inoculum density (log spores per square centimeter of leaf)$^{0.6}$ ($r^2 = 0.92$); The equation for the filled circles (experiment 2) was log disease severity (log lesions per square centimeter of leaf) = $-2.2 \times$ log inoculum density (log spores per square centimeter of leaf)$^{0.4}$ ($r^2 = 0.96$). I-bars represent standard errors of selected means.

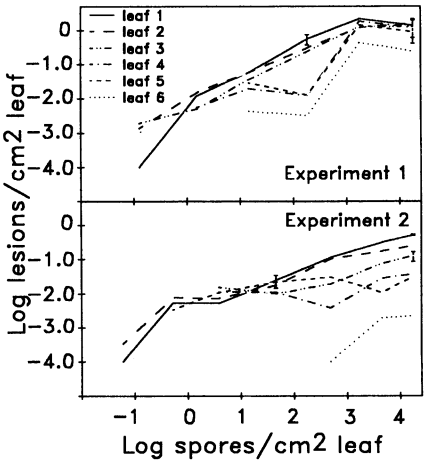

Fig. 2. Response of tomato leaves of differing ages (leaf 1 = oldest through leaf 6 = youngest) for susceptibility to increasing log spore densities of *Septoria lycopersici* per square centimeter of leaf; I-bars represent standard errors of selected means.

During the course of this experiment we noted that younger leaves expanded more rapidly than older leaves. We calculated leaf growth rate during the experiment and found that the younger leaves 4, 5, and 6 expanded about 20% while the older leaves 1 and 2 increased by only 5%. Mean lesion area decreased linearly as leaf position increased. The mean areas of the lesions (LSA) were 14.5, 9.6, 4.9, 2.5, 2.2, and 1.0 mm^2 on leaves (Leaf) 1, 2, 3, 4, 5, and 6, respectively. The equation, $LSA = 19.9 - 4.1$ (Leaf), ($r^2 = 0.60$, $P < 0.001$), best fit this relationship.

Influence of temperature and dew period on lesion development. When temperature was compared, brush-inoculated plants developed the most lesions when they were incubated at 20 C and 25 C, whereas the number of lesions declined at 15 C (Fig. 3). The plants that were grown at 10 C had only a few lesions, and the leaves were stunted and had turned dark green (*data not shown*). Plants that were held at 30 C developed only a few lesions on leaves that had received a 0- or 2-hr dew period, and no lesions developed when the dew period was longer than 2 hr. Exposing brush-inoculated plants to dew periods between 0 and 16 hr did not affect the number of lesions that developed when incubation temperatures were 15, 20, or 25 C. However, dew periods longer than 20 hr increased the number of lesions per square centimeter of leaf at 15, 20, and 25 C (Fig. 3). Length of dew period did not affect the number of lesions per square centimeter of leaf on plants held at 10 C; it averaged 0.063±0.012 lesions per square centimeter (data not shown). Pycnidia were observed within all lesions except those incubated at 30 C.

Effect of inoculation method and dew period on Septoria leaf spot. When dew was absent, brush-inoculated leaves had 0.03 lesions per square centimeter of leaf, whereas spray-inoculated leaves did not develop any detectable lesions. When leaves that were similarly treated were exposed to 24 hr of dew, lesions developed on plants that were inoculated by both methods, but significantly more lesions developed on brush-inoculated leaves than on spray-inoculated leaves (0.12 lesions per square centimeter of leaf compared with 0.04 lesion per square centimeter of leaf, $P = 0.05$).

Influence of temperature on sporulation. The lesion area and number of pycnidia per lesion were 119 and 66% larger, respectively, on plants held at 10 C than on those held at 20 C (Table 1). However, the total number of spores per pycnidium was significantly greater on plants that were incubated at 20 C than on those that were held at 10 C.

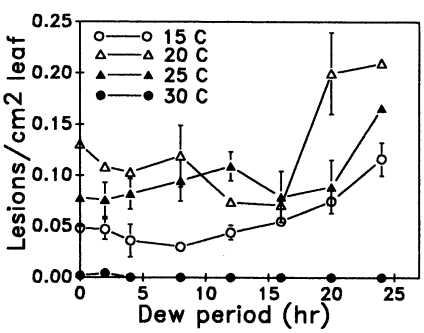

Fig. 3. Influence of dew period and temperature on Septoria leaf spot of tomato. I-bars represent standard errors of selected means.

DISCUSSION

Septoria leaf spot was readily produced on tomato leaves that were brush inoculated with 10^3 spores per milliliter. However, knowing the leaf area, and the amount of inoculum applied to each leaf, provided a more realistic description of the inoculum/damage relationship. The efficiency with which the spores caused lesions decreased as the inoculum concentrations increased in both experiments. Competition for available infection sites may explain this decrease in efficiency. The large differences between the two experiments are unexplained. It is possible that the inocula from the dried leaves was less viable in the second experiment since it had been stored longer, but we have successfully used spores that were recovered from dried tomato leaves up to 8 mo after collection without any noticeable loss in virulence. Similarly, Sheridan (14) reported no loss of virulence when dried celery leaves were used as a source of inoculum for *Septoria apiicola* Speg.

The observation that older tomato leaves were more susceptible than younger leaves to Septoria leaf spot had been mentioned in earlier publications (3,10), but had not been quantified. The increased susceptibility of the oldest leaf suggests that a loss in defense-related mechanisms may occur in these tissues, or these leaves may have been exposed to environments that were more favorable for disease to develop. Several scientists have documented that *S. lycopersici* is inhibited under conditions of high light, which may account for some increased infection in the lower canopy, where radiation is reduced (3,10,11). However, in these studies radiation was fairly constant and shading minimal so this would not completely explain the greater resistance of young leaves. Likewise, the possibility that the lower canopy maintains higher humidity and leaf wetness, and thus develops more infections on these leaves, was not likely in these studies because the growth chambers had circulating fans that maintained a fairly constant air flow over the leaves. Horsfall (9) suggested that older tomato leaves contained less substrates than younger tissue, and fungi such as *S. lycopersici* in "search of sugar" would destroy older tissue more than younger leaves. This hypothesis is currently being examined in our laboratory.

Temperature was more important in the development of Septoria leaf spot than dew period. Optimal temperatures for disease were between 20 and 25 C, but significant disease still developed at 15 C. Tu and Poysa (17) also found that 20–24 C was optimal for lesion production. In the current study, plants held at a temperature of 20 C had significantly smaller lesions and fewer pycnidia per lesion, but more spores per pycnidium than plants held at 10 C. The mean number of spores produced in each lesion, however, did not significantly differ between the two temperatures, which suggested that at cool temperatures lesions may still be capable of producing an appreciable amount of inoculum. The finding that *S. lycopersici* could sporulate at 10 C was surprising and not previously reported.

An unexpected discovery was that Septoria leaf spot developed in the absence of any dew period and that intervals of up to 16 hr did not increase the lesion density. This finding is in contrast to other reports of Septoria diseases on celery (15) or wheat (8), in which periods of 12–15 hr of dew were reported as necessary to incite infection. There can be little argument that some amount of water is necessary for spore germination and infection, and it is possible that a minute quantity may have been tightly held onto the leaf surfaces, but this is difficult to prove. Interestingly, the current study supports the early results of E. A. Bessey (3) who reported in 1916 that tomato leaves that were

Table 1. Effect of temperature on lesion area, number of pycnidia per lesion, and number of spores produced per pycnidium on tomato leaves inoculated with *Septoria lycopersici*[a]

Temperature (C)	Lesion area[b] (mm^2)	No. pycnidia/lesion	No. spores/pycnidium	No. spores/lesion
10	13.8[c]	20.6	15.5	320
20	6.3*	12.4*	161.3**	2,000

[a]Tomato leaves brushed with 10^6 spores per milliliter, held in a dew chamber for 24 hr, then incubated in a growth chamber at same temperature for 7 days.
[b]Lesion area estimated by measuring length (L) and width (W) of lesion, using equation $((L \times W)/4) \times \pi$.
[c]Values followed by *, ** are significantly different using Student's t test for unequal variances (16) at $P = 0.05$, 0.01, respectively.

inoculated with *S. lycopersici* and placed under low ambient humidities were as damaged as those that received constant leaf wetness. Although Bessey (3) did not state what the relative humidity was during his experiments, he reported that the atmosphere in the greenhouse during incubation was very dry and that plants were in a condition of incipient wilt. Therefore, it is reasonable to assume that the relative humidity was less than 90% and that no dew could have formed due to slight changes in the temperature as cautioned by Schein (13). However, we recognized that in the current study the brush-inoculation method may have damaged leaf surfaces and removed subcuticular waxes as mentioned by Tu and Poysa (17), thus creating crevices where spores could be exposed to high humidity. Since the spray-inoculated leaves did not develop lesions when dew was absent, this abrasive treatment may have influenced the infection process. Bessey (3) reported that conditions of low relative humidity were equal to continuous dew for lesion production regardless of whether a water suspension containing the inoculum was directly placed on the leaf with a transfer needle or whether the inoculum consisting of a freshly exuded spore mass was gently placed on the leaf. Our findings suggested that leaf abrasions may be important to spore infection under low humidities. Inasmuch as tomatoes leaves in the field are constantly exposed to physical contact with other leaves, this may explain, in part, why lesions appeared in the field when ambient humidities were deemed unfavorable.

LITERATURE CITED

1. Andrus, C. F., and Reynard, G. B. 1945. Resistance to Septoria leaf spot and its inheritance in tomatoes. Phytopathology 35:16-24.
2. Barksdale, T. H. 1982. Control of an epidemic of Septoria leaf spot of tomato by resistance. Plant Dis. 66:239-240.
3. Bessey, E. A. 1916. The leaf spot disease of tomato. Mich. Agric. Exp. Stn. Tech. Bull. 25.
4. Elmer, W. H., and Ferrandino, F. J. 1991. Evaluation of fungicides for control of Septoria leaf spot and anthracnose of fresh market tomatoes. Fungic. Nematicide Tests 46:177.
5. Ferrandino, F. J. 1993. Dispersive epidemic waves: I. Focus expansion within a linear planting. Phytopathology 83:795-802.
6. Ferrandino, F. J., and Elmer, W. H. 1993. Septoria leaf epidemics within linear plantings of tomatoes. (Abstr.) Phytopathology 83:243.
7. Ferrandino, F. J., and Elmer, W. H. 1992. Reduction in tomato yield due to Septoria leaf spot. Plant Dis. 76:208-211.
8. Hess, D. E., and Shaner, G. 1987. Effect of moisture on Septoria tritici blotch development on wheat in the field. Phytopathology 77:220-226.
9. Horsfall, J. G. 1986. The fungus searches for sugar: an essay. Pages 1-11 in: Vistas in Plant Pathology. A. Varma and J. P. Verma, eds. Malhotra Publishing House, New Delhi, India.
10. MacNeil, B. H. 1950. Studies in *Septoria lycopersici* Speg. Can. J. Res. 28:645-672.
11. Martin, W. H. 1919. Studies on tomato leaf-spot control. N.J. Agric. Exp. Stn. Bull. 345.
12. Muncie, J. H. 1922. Tomato leaf spot and experiments with its control. Pa. State Coll. Agric. Exp. Stn. Bull. 177.
13. Schein, R. D. 1964. Comments on the moisture requirements of fungus germination. Phytopathology 54:1427.
14. Sheridan, J. E. 1968. Conditions for germination of pycniospores of *Septoria apiicola*. N.Z. J. Bot. 6:315-322.
15. Sheridan, J. E. 1968. Conditions for infection of celery by *Septoria apiicola* Plant Dis. Rep. 52:142-145.
16. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics, 2nd ed. McGraw Hill Book Co., NY.
17. Tu, J. C., and Poysa, V. 1990. A brushing method of inoculation for screening tomato seedlings for resistance to *Septoria lycopersici*. Plant Dis. 74:294-297.