A Brushing Method of Inoculation for Screening Tomato Seedlings for Resistance to *Septoria lycopersici*

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ABSTRACT

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Screening of tomato (*Lycopersicon esculentum*) seedlings for resistance to *Septoria lycopersici* was more accurate by brush-inoculation of leaves than by dipping or spraying. An inoculum concentration of 10^6 spores per milliliter or higher was applied to both sides of leaves with a camel's-hair brush, pots containing inoculated seedlings were placed in shallow trays in a thin layer of water, and the inoculated seedlings were covered with a moisture-proof plastic canopy for 48 hr at 24 ± 2 C in a greenhouse. Differences in susceptibility were most evident 8 days after inoculation. Although more time-consuming than dipping or spraying, this method resulted in more uniform disease development and provided reliable differentiation between susceptible and resistant genotypes.

Septoria leaf spot of tomato (*Lycopersicon esculentum* Mill.) has been an important disease in Canada and the eastern United States (2,8). It remains one of the most important diseases in commercial tomato production during periods of rainfall, frequent dew, or frequent overhead irrigation. Resistance to *Septoria lycopersici* Speg. was noted as early as 1935 (1,6,9), but absence of practical levels of resistance within *L. esculentum* impeded the breeding efforts (7). Fungicides such as maneb, captafol, and chlorothalonil have proved effective for disease control, so the breeding effort has been reduced. Currently, seven or more fungicide sprays are used on commercial field tomato crops to control Septoria leaf spot, early blight, and fruit anthracnose. Public concerns about the effects of pesticides on environmental quality has led to a renewed interest in breeding for disease resistance.

A highly resistant *L. pimpinellifolium* (L.) Mill. line, PI 422397, having a single dominant resistance gene, has been identified (4). A breeding program was initiated at Harrow Research Station to incorporate resistance from this source into improved, commercially acceptable tomato cultivars. In order to assay the progenies of crosses, a reliable and precise method of screening is needed. This paper reports a method for screening tomato seedlings for resistance to *S. lycopersici*.

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MATERIALS AND METHODS

Inoculum. Spore suspensions of *S. lycopersici* were prepared from 3-wk-old colonies on PDA; 5 ml of sterile distilled water was added to each plate, and the surface of the culture was scraped to dislodge the spores. The spore suspensions derived from several plates were pooled, and spore concentrations were determined with a hemacytometer. Unless stated otherwise, the inoculum concentration used in this study was 10^6 spores per milliliter.

Inoculation. Seeds of a susceptible tomato cultivar, H2653, were sown in 20 × 40 cm peat trays. Two weeks after germination, when the first true leaf emerged, the seedlings were transplanted to 10-cm peat pots (two plants per pot) filled with an autoclaved soil mixture consisting of sand, peat, and loam (2:1:1). Assays were made in a greenhouse on tomato seedlings at the three-leaf stage (approximately 5 wk after sowing). Spore suspensions were brushed onto both sides of the leaves with a camel's-hair brush. The inoculated seedlings were covered with a moisture-proof plastic canopy for 4 days at 24 C in a growth chamber with 14 hr of light. After removal of the plastic canopy, the plants were kept in the same growth chamber for symptom development. The light source consisted of cool-beam fluorescent lamps supplemented with incandescent lamps with an intensity of 280 $\mu E \cdot m^{-2} \cdot s^{-1}$ at bench level. Disease severity was scored 8 and 15 days after inoculation using a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100% leaf area with symptoms.

The effect of inoculum concentration on disease severity was investigated using concentrations of 10^1 to 10^7 spores per milliliter. The effect of temperature was investigated at 12, 16, 20, 24, 28, and 32 C in a growth chamber maintained at 80% relative humidity (RH) during or after the moist period.

The minimal moisture period needed for *S. lycopersici* to infect tomato was determined by placing inoculated seedlings under a plastic cover at 100% RH for 8, 16, 24, 32, 40, and 48 hr in a growth chamber at 24 C. The effect of photoperiod on disease development was investigated by subjecting inoculated plants to differing periods of light (0–24 hr in 4-hr increments) during or after the moisture period.

Two previously reported methods, spraying (3,4) and dipping (2), were compared with brushing, using optimal conditions for disease development, i.e., inoculum concentration of 10^6 spores per milliliter, temperature at 24 C, moisture period of 48 hr, and a photoperiod of 14 hr. The method of inoculation was compared on three tomato lines: H2653, a susceptible line (R. E. Pitblado, *personal communication*); PI 372364, a

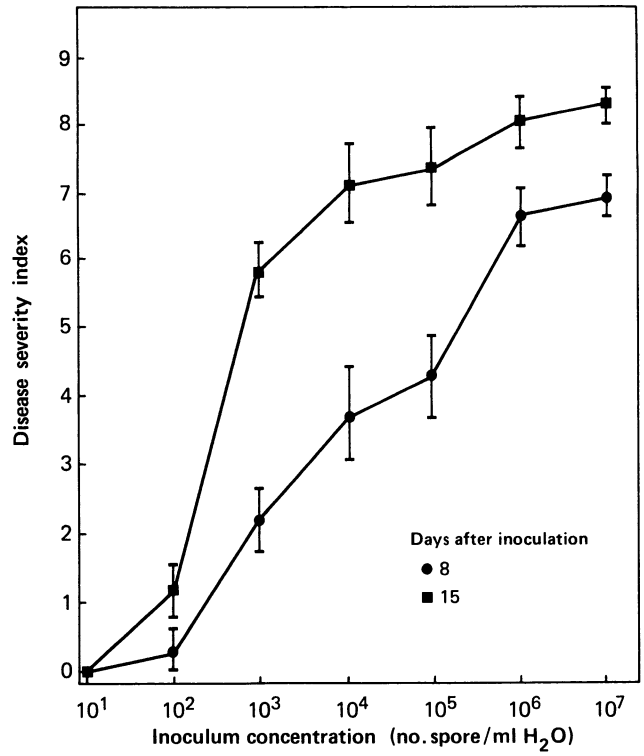

Fig. 1. Effect of inoculum concentration of *Septoria lycopersici* on severity of Septoria leaf spot on a susceptible tomato cultivar, H2653, inoculated by the brush method. The experiment was conducted at 24 C, 14 hr of light, and 48 hr of moisture with 100% RH. Disease severity was scored 8 and 15 days after inoculation using a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100% leaf area with symptoms. Vertical bars represent standard error of the mean ($n = 40$).

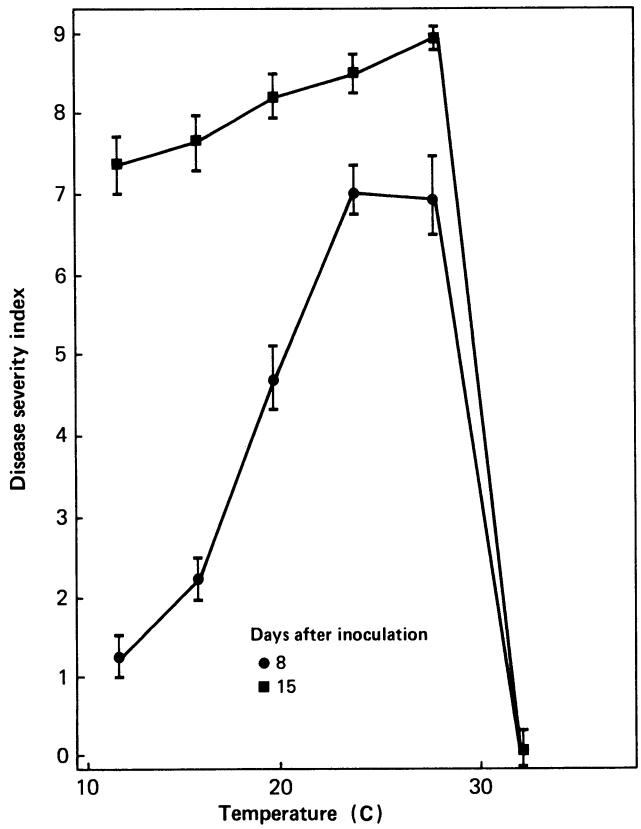

Fig. 2. Effect of temperature on the severity of Septoria leaf spot on a susceptible tomato cultivar, H2653, inoculated by the brush method. Inoculated plants were covered with plastic for 48 hr, then transferred into growth chambers at various temperatures and with 80% RH for disease development. Disease severity was scored 8 and 15 days after inoculation using a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100% leaf area with symptoms. Vertical bars represent standard error of the mean ($n = 40$).

line with horizontal or nonspecific resistance (5); and PI 422397, a highly resistant line (4). All experiments were repeated.

RESULTS

Seedlings inoculated with spore concentrations less than 10^2 per milliliter developed low levels of disease (Fig. 1). Disease severity increased exponentially with increasing inoculum concentrations between 10^2 and 10^6 spores per milliliter but did not differ significantly between 10^6 and 10^7 spores per milliliter. After 15 days of incubation, however, differences in disease severity were reduced at 10^3 to 10^6 spores per milliliter.

Maximum disease development occurred between 24 and 28 C at 8 days after inoculation (Fig. 2). However, increased disease development in plants kept at lower temperatures was observed after 15 days of incubation.

Percent leaf area infected after 8 days of incubation increased from 23 to 73% for periods of moisture of 24 and 48 hr, respectively (Fig. 3). Percent leaf area infected for each moisture period was 10–20% greater after 15 days than after 8 days of incubation (Fig. 3).

Photoperiod (ranging from 0 to 24 hr in 4-hr increments) had little or no effect on disease development. However, plants kept under constant darkness showed a slight general yellowing, and those kept under constant light showed rugosity in young leaves.

On the basis of the preceding experiments, the following inoculation conditions were considered optimal and used in subsequent experiments: inoculum concentration of 10^6 spores per milliliter, temperature at 24 C, moisture period of 48 hr, and photoperiod of 14 hr. Comparison of the three inoculation methods (Table 1) showed that the brushing method gave the greatest disease severity (7.4) and the lowest variations among plants and among tests. The disease severity of plants inoculated with dipping and spraying averaged 3.9 and 3.7, respectively. Variation among plants within a given test was smallest with brushing and greatest with dipping (Table 1).

The brushing method was used in test assays of three tomato genotypes having different susceptibilities: H2653 (highly susceptible), PI 372364 (horizontal or nonspecific resistance) (5), and PI 422397 (resistant gene or specific resistance) (4). H2653 had severe disease, with large (av. 4.7 mm in diameter) lesions (Table 2). PI 372364 had moderately severe disease, with slightly smaller (av. 3.6 mm in diameter) lesions, and defoliated rapidly. Disease severity in PI 422397 was approximately one-half that of H2653 and 30% less than that of PI 372364; lesions were small (av. 1.2 mm in diameter).

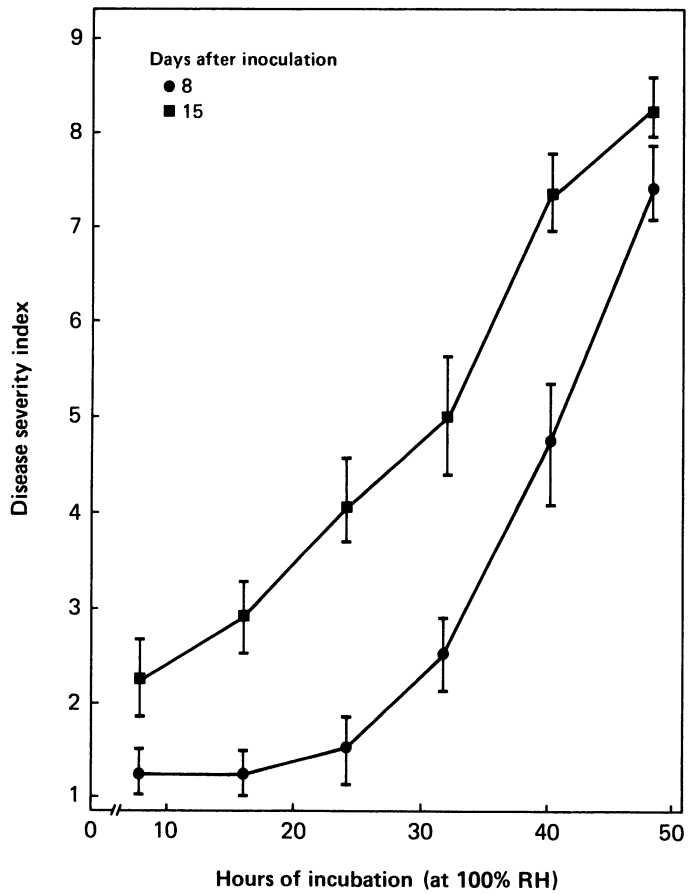

Fig. 3. Effect of incubation period at 100% RH on severity of Septoria leaf spot on a susceptible tomato cultivar, H2653, inoculated by the brush method. The experiment was conducted at 24 C and 14 hr of light with an inoculum concentration of 10^6 spores per milliliter. Disease severity was scored 8 and 15 days after inoculation using a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100% leaf area with symptoms. Vertical bars represent standard error of the mean ($n = 40$).

DISCUSSION

Brushing was clearly superior to spraying and dipping as an inoculation method for screening tomato seedlings against *S. lycopersici*. The large variation in leaf spot development among plants and tests after spray inoculation appears to be associated with the amount of inoculum applied and the uniformity of its delivery. Furthermore, inoculum may bead up and run off if applied in excess, owing to the surface tension of the epicuticular wax. Dipping was initially thought to be a better alternative to spraying (2), although our results showed little difference. Dipping apparently does not improve infection rate and uniformity of infection, as evidenced by the relatively low disease severity index and high variability among plants and tests. Foliar trichomes and epicuticular wax likely repel the spore-containing water droplets. On the other hand, the spore suspension applied with a camel's-hair brush appeared to spread more uniformly on the leaf surfaces. This may be because the friction of the brush hairs breaks the trichomes and reduces the surface tension of the epicuticular wax.

Although applying a spore suspension to leaves with a camel's-hair brush is tedious, the consistency and uniformity of the results make it the most accurate inoculation method for differentiating

Table 1. Comparison of disease severity on tomato cultivar H2653 in four tests on four different days after inoculation by brushing, spraying, and dipping with a spore suspension (10^6/ml) of *Septoria lycopersici*

	Disease severity[z]		
Test	**Brushing**	**Dipping**	**Spraying**
1	7.3 ± 0.4	4.5 ± 2.1	3.0 ± 0.7
2	7.4 ± 0.6	3.2 ± 1.8	1.5 ± 0.5
3	7.1 ± 0.6	4.1 ± 2.5	4.2 ± 1.1
4	6.8 ± 0.7	3.6 ± 1.4	6.1 ± 1.4
Av.	7.2 ± 0.3	3.9 ± 0.6	3.7 ± 1.9

[z] Rated 7 days after inoculation on a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100%. Each value is the mean of four replications of 10 plants each. Analysis of variance showed that brushing differed significantly ($P \leq 0.05$) from dipping and spraying but that dipping and spraying did not differ significantly from each other.

Table 2. Comparison of disease severity on three tomato cultivars of different susceptibility inoculated by the brushing method with *Septoria lycopersici* (10^6 spores per milliliter)

Cultivar	Type	Disease severity[y]	Av. lesion diameter[z] (mm)	Defoliation of inoculated leaf (%)
H2653	Very susceptible	6.7 ± 0.8	4.7 a	0
PI 372364	Horizontal resistance	4.2 ± 1.4	3.6 b	50
PI 422397	Resistant gene	3.0 ± 0.7	1.2 c	0

[y]Rated 8 days after inoculation on a 0–9 scale, where 0 = less than 10% leaf area with symptoms, 1 = 10–20%, 2 = 20–30%, to 9 = 90–100%. Each value is the mean of four replications of 10 plants each.

[z]Each value is the mean of four replications of 50 lesions each. Means followed by a different letter are significantly different ($P \leq 0.05$).

susceptible from resistant genotypes. Over time, the method should reduce the amount of work, facilities, and time required.

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