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Tomato Anthracnose: A Hypodermic Inoculation Technique for Determining Genetic Reaction[1,2]

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Abstract. A technique developed for inoculating tomato fruits with anthracnose pathogen consists of placing a spore suspension droplet onto a fruit with a hypodermic syringe and then pricking the epidermis under the droplet with the hypodermic needle. Disease symptoms occurred at room temperature and humidity, thus eliminating the need for controlled temperature and humidity. Detectable lesions developed at 95% and 96% of inoculated sites on 'Heinz 1350' and 'Roma' fruits, respectively.

BREEDING for anthracnose resistance in tomatoes has been unsuccessful, possibly because of inadequate inoculation procedures and variation among isolates of the pathogen. There are conflicting reports on the ability of *Colletotrichum coccodes* (Wall.) Hughes to infect uninjured tomato fruits (3, 4, 5, 6, 8, 9, 12). Both pathogenic and nonpathogenic isolates of the fungus have been reported (3, 12).

Tomato lines selected for anthracnose resistance using nonwounding inoculation techniques (4, 5, 7) were susceptible when field-grown in Maryland (4). The reason for discrepancies between results with natural and artificial inoculation is unknown, but it is possible that nonapparent injuries in the field provide an entry for the fungus. If this is true, plant breeders need to select for resistance on the basis of internal fruit environment unfavorable for growth of the pathogen.

Inoculation methods previously reported are not suitable for a genetic study or breeding program because a low percentage of inoculated fruits develop disease. The objective of the research reported herein was to develop a more effective inoculation technique for determining genetic control of anthracnose resistance. The results of the genetic study are reported elsewhere (13).

MATERIALS AND METHODS

Cultivars 'Roma' and 'Heinz 1350' (H-1350), highly susceptible to anthracnose in the field, were used as susceptible genotypes. Plant Introductions (P.I.) 127833 and 129027, previously reported resistant to anthracnose, were used as resistant genotypes. P.I. 127833 is a *Lycopersicon pimpinellifolium,* Mill.; P.I. 129027 is a species cross, *L. esculentum,* Mill. × *L. pimpinellifolium* (1). Two individual plant selections from P.I. 127833 and one from P.I. 129027 were used. These selections and their selfed progenies are referred to as 641-9, 641-4, and 640-1, respectively. The fruit size of each selection is approximately 1 oz.

Tomato fruits with anthracnose lesions were collected from tomato disease plots at the University of Maryland Vegetable Research Farm, Salisbury. A single spore isolate of *C. coccodes* obtained from the diseased fruits was highly pathogenic on wounded and nonwounded 'Roma' and 'H-1350' fruits. The organism was grown on V-8 agar (200 ml of unfiltered V-8 juice and 20 g of powdered agar to 800 ml of distilled water) under continuous light at 23° C (3, 11).

The possibility of determining disease reaction in the seedling stage was investigated in preliminary studies. Tomato seedlings were placed in a humidity chamber 24 hr before inoculation with a spore suspension and then kept in the chamber 48 hr after inoculation. No macroscopically visible lesions developed on inoculated seedlings.

Fruit inoculation techniques were also evaluated in preliminary tests. Methods investigated were: 1) atomizing a spore suspension onto fruit, 2) placing a spore suspension droplet onto unwounded fruit surface, 3) placing fruits onto inoculated soil, 4) spraying paper towels with a spore suspension and placing fruits onto the inoculated towels, 5) placing a spore suspension droplet onto the fruit surface from a hypodermic syringe and then pricking the fruit skin underneath the droplet with the hypodermic needle. Ripe 'Roma' and 'H-1350' fruits were used for each method. With methods 1–4 relative humidity was at or near 100% for 48 hours after inoculation, but with the hypodermic method humidity was not regulated. Spore concentration was about 5,000,000/ml.

Homozygous *L. esculentum* tomato lines 17R8-1, 18R3-1, 19R7-1, 20R7-1, and 38-1-1B, selected for anthracnose resistance using nonwounding inoculation techniques, were field-grown at Beltsville, Maryland, and all were highly susceptible (4, 7). Fruits of these lines were inoculated with the hypodermic technique to determine its effectiveness in indicating susceptibility of the lines.

RESULTS

Lesions developed on fruits with all inoculation methods evaluated, but fewer than 10% of the fruits in methods 1–4 developed symptoms. The remainder of

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Table 1. Mean diameter of anthracnose lesions on ripe and green mature tomato fruits 9 days after hypodermic inoculation with a spore suspension and with distilled water.[a,b]

Cultivar or Line	Spore suspension		Distilled water	
	Ripe	Green mature	Ripe	Green mature
	mm	mm	mm	mm
'Roma'	32.0	9.6	x[c]	x
'H-1350'	27.1	11.7	x	x
641–4	16.6	16.3	x	x
640–1	2.7	5.2	x	x
641–9	2.4	2.4	x	x

[a]One site inoculated on each fruit.
[b]Five fruits or more were inoculated in each category.
[c]No detectable lesion.

this paper reports results obtained with the hypodermic inoculation technique only.

Ripe and green mature check fruits inoculated with distilled water did not develop anthracnose lesions, nor did other organisms enter via the wound and cause rot (Table 1). Lesion size on 'Roma' and 'H-1350' fruits inoculated when ripe was larger than on fruits inoculated when green mature; however, lesion size for 641-4, 640-1, and 641-9 was about the same on ripe and green mature fruits (Table 1). The fruits of the P.I. lines did not appear to become more susceptible as they ripened. The lines 640-1 and 641-9 were more resistant than 'Roma', 'H-1350', and 641-4 as indicated by mean diameter of lesions.

Anthracnose lesions developed on 95% of 'H-1350' and 96% of 'Roma' fruits inoculated (Table 2). Lines 641-9 and 640-1 were again more resistant since only 41% and 63% of their fruits developed detectable lesions, respectively, and the mean diameters of their lesions were considerably less than those of 'Roma', 'H-1350', and 641-4.

The hypodermic technique verified the susceptibility of the tomato lines selected for anthracnose resistance by other researchers (4, 7) using nonwounding inoculation techniques, but which were susceptible when field-grown in Maryland (Table 3).

Discussion

Tomato lines selected for anthracnose resistance but found susceptible when field-grown were also susceptible when inoculated with the hypodermic technique. This technique appears to be more effective in detecting susceptible lines than are other inoculation methods. If the fruits of a line do not develop anthracnose lesions when inoculated with the hypodermic technique, the fruits apparently have an internal environment unfavorable for growth of the pathogen. Such a line should be resistant to natural infection. It is also possible that the hypodermic technique might indicate a line to be susceptible when it would actually be resistant to natural infection.

Table 2. Percentage of tomato fruits infected and mean diameter of anthracnose lesions 9 days after hypodermic inoculation.[a,b]

Cultivar or Line	Fruits inoculated	Fruits infected	Mean lesion diam.
	no.	%	mm
'Roma'	178	96	27.7
'H-1350'	84	95	30.9
641–4	115	84	24.7
640–1	121	63	13.8
641–9	69	41	10.9

[a]One site inoculated on each fruit.
[b]Fruits were ripe when inoculated.

Table 3. Mean diameter of anthracnose lesions 7 days after hypodermic inoculation of ripe fruits of tomato lines previously selected as resistant.[a]

Cultivar or Line	Number fruits	Mean lesion diameter
	no.	mm
641–9	15	0.9
640–1	10	6.1
19R7–1	6	12.0
'Roma'[b]	6	18.3
'H-1350'[b]	3	20.0
38-1-1-B	11	20.3
20R7–1	6	21.2
17R8–1	6	21.5
641–4	5	22.4
18R3–1	4	28.0

[a]One site inoculated on each fruit.
[b]Susceptible genotypes included as checks.

Studies comparing results from natural infection and artificial inoculation are being conducted.

Fruit size differences among tomato lines might be reflected in lesion size several days after inoculation. However, in this study fruit size did not appear to affect lesion development and size. The lines 640-1, 641-4, and 641-9 are small-fruited, yet lesion diameter was consistently larger for 641-4 than for the other 2 lines. Also, mean lesion diameters for 641-4 and 'Roma' were about the same in one experiment.

A good feature of the hypodermic technique is that controlled temperature and humidity conditions are not required for infection and disease development. Room temperature and humidity are suitable for all phases of a test.

Lack of infection of a high percentage of susceptible fruits has been a common occurrence in studies involving artificial inoculation with the tomato anthracnose pathogen (3, 5, 6, 7, 9, 12). The results of this study indicate the effectiveness of the hypodermic technique and its potential usefulness in genetic and breeding programs concerned with anthracnose resistance in tomato.

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