Criteria for Quantal Host Response of Tomato Plants to Infection by *Verticillium dahliae*

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ABSTRACT

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Symptoms of Verticillium wilt of tomato were compared as criteria of quantal host response to infection by *Verticillium dahliae*. Vascular discoloration was a reliable criterion but required the destruction of the host. Wilting was the most reliable criterion that could be repeatedly evaluated on the same plant.

Additional key words: dose-response relationship, epidemiology, *Lycopersicon esculentum*

The severity of Verticillium wilt of tomatoes (*Lycopersicon esculentum* Mill.), caused by *Verticillium dahliae* Kleb., is often described by index ratings of external symptoms (2,8,13) or on the degree of vascular browning (2). The indices, though well defined, are subjective measurements. The degree of objectiveness can be raised by recording a quantal host response to inoculation. A further advantage of this method is that the average number of successful infections per plant can be calculated (10). The method requires classification of individual plants as diseased or healthy.

In the case of Verticillium wilt of tomatoes, there is no agreement on the criteria for denoting a diseased plant, although stunting (1,7), wilting (5), partial yellowing of leaves (6), vascular discoloration (6), and reisolation of the pathogen (3) have been considered. Ben-Yephet and Pilowsky (1) compared some of these criteria for evaluating tomato plants for resistance. In the present investigation, we compared the suitability of a different set of criteria for recording quantal host responses of large plant populations.

MATERIALS AND METHODS

A virulent isolate (V_{17}) of *V. dahliae* from diseased tomato plants (11) was used as inoculum. The fungus was grown on potato dextrose agar for 5 days in 1-L Erlenmeyer flasks at 23 C. The conidia were collected by gentle agitation after approximately 50 ml of sterile distilled water had been added to each flask. Mycelium was removed from the suspension by filtering through a double layer of cheesecloth. The conidia were washed six times with sterile distilled water before the suspension was diluted to the required concentration as determined by means of a Fuchs-Rosenthal hemocytometer.

The tomato cultivar Roodeplaat Asbesto, which is highly susceptible to Verticillium wilt, was used. Seedlings were grown in the greenhouse in asbestos trays disinfected with 1% formaldehyde solution and filled with a soil mixture consisting, by volume, of 26 parts steam-sterilized sandy loam soil, 4 parts steam-sterilized coarse sand, 5 parts vermiculite, and 2.5 parts peat moss. To 1 m^3 of this mixture were added 2.5 kg of calmafos (59 g P/kg), 0.5 kg of limestone ammonium nitrate (280 g N/kg), and 0.5 kg of potassium chloride (500 g K/kg). The pH of the soil mixture was 6.05.

Three-hundred tomato seedlings at the second true leaf stage were selected for uniformity and randomly divided into six groups of 50 seedlings. Each group was inoculated by means of a root dip technique (11) with one of the inoculum concentrations listed in Table 1. The seedlings were then transplanted singly into 15-cm clay pots filled with the soil mixture.

The greenhouse temperature varied between 15 and 30 C. To prevent deficiency symptoms, a Hoagland solution was applied 8, 14, and 21 days after inoculation at a rate of 50 ml per pot per application.

The plants were randomized with respect to their position in the greenhouse immediately after being transplanted into the pots. At regular intervals the percentages in each group of plants showing wilting, chlorosis, stunting, or any of these symptoms were recorded. Cotyledons were ignored because of their early abscission. Stunting was determined visually and inoculated as well as uninoculated plants were individually evaluated.

After the final recordings, all plants were cut off 2 cm above soil level and examined for discoloration of the vascular tissue. Segments of stem tissue were also plated on potato-dextrose agar (12).

RESULTS AND DISCUSSION

Wilting and vascular discoloration were the symptoms most easily recognized; they did not occur in any of the control plants (Table 1). These symptoms were always associated with the presence of the pathogen as indicated by positive reisolations. Both symptoms can therefore be ascribed to infection by *V. dahliae* and are considered reliable criteria for host response. Vascular discoloration can be applied only on termination of an experiment, but wilting can be repeatedly recorded on the same plants.

Conroy and Green (3) preferred infection incidence, indicated by positive reisolation of the pathogen, as a criterion of disease severity. The suitability of this criterion is questionable because it is not a host response and because the fungus can be isolated from latently infected plants, although some plants with symptoms failed to yield the pathogen. This is in agreement with results obtained by Fletcher et al (4). Reisolations of the pathogen, however, confirmed its association with observed symptoms. The pathogen could not be isolated from any control plants and symptoms observed on these plants were not considered responses to infection.

Difficulties were experienced in recording chlorosis and stunting, as these symptoms developed erratically. Chlorosis recorded among the control plants during the first two assessments (Table 1) indicated that initially this symptom could not always be ascribed to infection by *V. dahliae*. Toward the end of the experiment, chlorosis due to Verticillium wilt became more pronounced and healthy tissue assumed a darker green color. Consequently chlorosis was considered a reliable criterion only at a later stage of development. Stunting was even more difficult to detect than chlorosis and is not recommended as a reliable criterion for host response to infection. Although 21 days were allowed for this symptom to develop, it was still

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Table 1. Effect of inoculum concentration of *Verticillium dahliae* on tomato plants (cultivar Roodeplaat Asbesto)

Criteria	Days after inoculation	Percentage of plants responding or from which pathogen was isolated[a] at concentration of 1.45× 10^7	10^6	10^5	10^4	10^3	0
Wilting	7	27	16	6	4	0	0
	14	86	52	56	24	12	0
	21	94	74	70	42	6	0
	28	100	90	92	62	24	0
Chlorosis	7	25	18	6	14	12	6
	14	86	60	54	28	12	8
	21	90	76	78	50	6	0
	28	100	94	94	68	24	0
Stunting[b]	21	100	98	86	50	30	8
	28	100	98	80	58	28	8
Vascular discoloration	28	100	96	98	76	24	0
Any symptom of Verticillium wilt	7	33	30	12	18	12	6
	14	90	66	66	32	18	8
	21	100	100	94	68	32	8
	28	100	100	98	86	42	8
Pathogen reisolation	28	100	98	100	82	32	0

[a]Of 49 or 50 plants per inoculum concentration.
[b]Recordings postponed until 21 days after inoculation because of slow symptom development.

recorded on uninoculated plants (Table 1). This can be ascribed to the natural variation in growth rate of plants and to the partial recovery of some infected plants. According to Ben-Yephet and Pilowsky's definition (1), stunting is a relative measurement based on the average height of uninoculated plants. For recording quantal responses in epidemiological studies, however, an absolute measurement is required. Furthermore, Thanassoulopoulos and Kitsos (9) showed that shortening of the stem is not a permanent character and should not be used as an evaluation.

"Any symptom of Verticillium wilt" was also evaluated as a criterion of host response since all the symptoms mentioned previously did not always appear simultaneously on the same plant. Throughout the entire period of observation, uninoculated plants were recorded as responding positively (Table 1). This collective criterion is therefore unreliable and is not recommended.

When the entire experiment was repeated later, similar results, exhibiting the same trends, were obtained. We thus concluded that wilting is the most reliable, sensitive, and practical criterion for a quantal host response of tomato plants to infection by *V. dahliae*. We recommend wilting as the only criterion for use in further experiments on the epidemiology of the disease.

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