Infection of additional hosts of Synchytrium endobioticum, the causal agent of potato wart disease: 3. Tomato as an assay tool in potato wart disease'

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Tomato was investigated as an assay tool for Synchytriurn endobioticurn because of a need for a simple, rapid, consistent and dependable assay. To investigate tomato, tomato seeds or seedlings were grown in Synchytriurn endobioticurn-infested soil or potting mix, and signs of infection were indicated by the presence of sporangia in tomato root tissue. Infection was detected 3 d after inoculation, and increased in an exponential fashion with time. The ED, for tomato was ca. 10 sporangia g^{-1}, but infection occurred at propagule densities as low as one sporangium g^{-1}. Infecrion occurred in seedlings grown in sand to which sporangia were moved by irrigation; downward migration of sporangia was > 10 cm. Tomato infection paralleled potato infection when tomato was grown in soils obtained from field amendment trials. Tomato appears to serve as a useful assay tool for S. endobioticum.

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On a cultive des graines ou des plants de tomate dans un melange dempotage infeste de Synchytriurn endobioticurn et les signes d'infection se sont manifestes par la presence de sporanges dans le tissu radiculaire. L'infection a ete décelée 3 jours apres l'inoculation et s'est accrue de façon exponentielle avec le temps. La DE, de la tomate a ete de 10 sporanges/g de melange, mais l'infection est apparue à des densités de propagules aussi faibles que 1 sporange/g. L'infection s'est manifesté chez les plants cultivés dans le sable où l'irrigation a favorise la migration verticale des sporanges; cette migration vers le bas a depasse 10 cm. L'infection de la tomate a egale celle de la pomme de terre dans les cultures de tomate en sols provenant d'essais d'amendement en plein champ. Il semblerait que la tomate puisse servir d'instrument d'essai biologique pour S. endobioticurn.

Introduction

There are several mechanical methods described in the literature (1, 4, 7, 8, 10) for detecting Synchytriurn *endobioticum* (Schilb.) Perc. in soils. For example, Pratt (10) sieved infested soil through wet-sieves, and retrieved sporangia from the finest mesh sieve: Nelson and Olsen (8) centrifuged infested soil with dibromoethane, or mineral oil plus SAE 10 motor oil, and Glynne (1) used chloroform to float sporangia off: Mygind (7) developed a water flotation technique to retrieve sporangia. These techniques provide answers to quantitative questions, but they have definite floors of propagule extractability, hence detectability (4). The biological method using potato is so fraught with inconsistency and unreliability *(2,* 6) that attention was paid to trials with tomato (*Lycopersicum esculentum* Mill.). Tomato is an additional host of *S. endobioticum* and has been shown to be readily susceptible to infection by this fungus (3). Furthermore, the detection of S. *endobioticum* by tomato involves simple greenhouse culture equipment and standard greenhouse techniques.

This paper is an account of infection experiments with tomato and S. *endobioticum* to determine the first signs of infection and the ED, and to test tomato as an assay tool.

Materials and methods

Tomato cv. Beefmaster (J. Harris Co. Inc.), an extra large fruited hybrid, was selected for experimental work. It was the most susceptible of all cultivars to S. *endobioticum* that we have tested (3).

Both seedlings and seeds were used for infection. Seedlings, at the four leaf stage, were transplanted into units of sporangia-infested soil or potting mix (perlite:peat moss 1:1, v/v), or slurried in sporangia-suspension prior to transplanting. Seeds were planted directly onto infested mix or sand, covered lightly with mix, and germinated under a polyethylene cover. All seedlings were watered by irrigation to field capacity daily, and fertilized weekly with Van den Elft's tomato nutrient solution. Seedlings were raised in a controlled-environment room at 20°C, 80% RH, and 14-h day:

To infest sand or potting mix, sporangia were separated from tumor tissue by homogenization and screening. The separated sporangia were suspended in water, and the densities adjusted to give, when mixed with measured amounts of potting mix or sand, 1, 2, 5, 10, 15, 20, 30, 40 and 50 sporangia g^{-1} potting medium. After mixing by hand to ensure uniform propagule distribution, aliquots of infested media were distributed among "6-inch" plant pots. Five

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seedlings were planted in each pot. To make sporangial slurries, the separated sporangia were suspended in water to give 95,000 sporangia/ml. To assay sporangial movement in soil, 10,000 separated sporangia were deposited on each sand column and then flushed into the sand with water.

Tomato seedlings were harvested at different intervals, depending on the experiment involved, and root areas were cleansed by brushing with a soft brush under running water.

Table 1. Numbers of sporangia of *S. endobioticum* located in root tissue of tomato at different intervals after inoculation*.

Days after inoculation	Numbers of Sporangia range	$\bar{x}$**	Standard Deviation***
0	0	0	0
3	0-3	< 1	1
7	0-6	2	2
9	0-13	4	4
13	0-13	5	4
16	1-21	7	7
20	2-15	7	5
23	3-42	16	12
28	1-65	27	20
30	5-105	41	31
34	12-58	37	17
37	5-227	82	72
41	29-292	124	85
43	42-252	136	79

*Ten samples/treatment, inoculated by slurrying roots in 95,000 sporangia/ml.
**Rounded out to nearest whole number.

Potentially adhering sporangia were removed by ultrasonication, the seedlings were examined microscopically (25 X) (3) and the numbers of sporangia/plant counted.

To assay the downward movement of sporangia, triangular tubes (40 cm L X 3.7 cm W) were constructed from milk cartons and filled with gravel. Another similar tube (20 cm^2) was mounted securely on each gravel tube, and these were 3/4 filled with sieved sand. Resting sporangia were deposited on each sand surface and water was added to the empty 1/4 tube sections in two experiments. The tubes were grouped into six units of four tubes and five units of ten tubes. In the first experiment, the units received 50, 100, 150, 200, 250 or 300 ml water in daily increments of 50 ml; in the second, 5, 10, 20, 40 or 80 ml water in daily increments of 5 ml. The water regimes were based on an ideal of 25 mm water/wk for good potato growth (9). Each sand-filled tube was sectioned into four parts. The sand filled sections, labelled A, B, and C, were embedded separately in potting mix in 10-cm plastic plant pots. Each of the two hundred and forty sections were seeded with at least three Beefmaster seeds. These were culled later to one per section in the first experiment only, and harvested 8 wks after seeding.

To assay the influence of soil amendments on tomato infection, soil samples from amendment plots were planted to tomato transplants. In the field design, microplots each containing nine tubers, set 3 X 3, were amended with either lime, oat/barley straw, dried chopped potato tops, or Ca-, K-, Na- and NH_4- nitrates, singly or in combination. Lime was added to soil at the rate of 1120 kg/ha, straw and potato haulm at the rate of 30 t/ha, and the nitrates at the rate of 168 kg N/ha. Some microplots received no treatment. At harvest, an aliquot of soil was removed at the base of each plant, and aliquots from the same microplots

Table 2. Average number of resting sporangia/tomato seedling 5 wks after inoculation at different levels of sporangial density.

	No. resting sporangia in inoculum (g^{-1} potting mix)											
	1''	2''	5*	5#	10†	10''	10#	15#	20†	30†	40†	50†
No. Seedlings	20	20	20	19	20	20	20	20	20	20	20	20
Median No. Sporangia/ Seedling	0.55	0.6	0.6	26	26	1.2	40	53	27.9	41.8	62.7	71.1
Sporangial Range	0-3	0-6	0-3	7-133	6-99	0-3	11-109	10-140	12-127	9-100	16-151	41-123
Sporangial Age (Wk)	20	20	20	1	8	20	1	1	8	8	8	8
% Infection	30	35	30	100	100	45	100	100	100	100	100	100
Ave. % Infection				65		82						

† First experiment
* Second experiment
# Third experiment

pooled. Numbers of sporangia g^{-1} field soil were estimated by the method of Hampson and Thompson (4). Weights of tumor/treatment were recorded.

Results

Time course of infection

Seedlings were inoculated in slurries and planted. Ten seedlings at a time were removed at 2-5 d intervals after inoculation. From Table 1, it can be seen that sporangia were first evident in tomato tissue by 3 d after inoculation. There were wide ranges of sporangial numbers in the samples at each level of examination. Generally, the average number of sporangia/seedling increased in an exponential fashion; at 1, 3 and 5 wk after inoculation the average sporangial numbers were, respectively, 2, 7 and 137/seedling.

ED_{50}

The number of sporangia to bring about 50% infection of tomato seedlings was determined by transplanting 4-wk old seedlings into potting mix previously infested to different levels of sporangial density. In the first experiment, twenty seedlings at each level of 10, 20, 30, 40 or 50 sporangia/g mix displayed 100% infection; in the second experiment, twenty seedlings each at sporangial densities of 1, 2, 5 or 10 sporangia/g mix displayed 30, 35, 40 and 45% infection, respectively; in the third experiment, forty seedlings each at the 5, 10 and 15 sporangia/g level displayed 100% infection, respectively (Table 2).

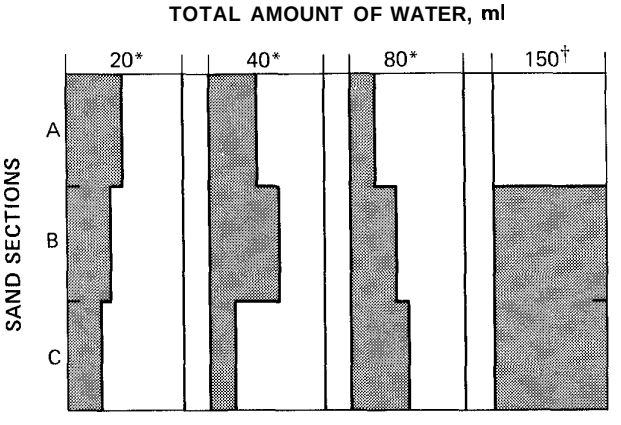

Figure 1. Histogram depicting the depth in sand (particle size, 600-1000 μm) to which sporangia were washed when irrigated. The presence of sporangia was shown by growing tomato seeds or seedlings in a 5-cm top layer of each section. Bar width is proportional to incidence of infection, distance between marks on horizontal axis = 100% infection. A, B and C are top, middle and bottom sand sections, respectively. Irrigation water was applied in multiples of 5(*) or 50(+) - ml units.

Downward migration of sporangia

Figure 1 depicts, in histogram form, the percent infection recorded for tomato at the three levels of sand in the percolation tubes which received 20, 40, 80 and 150 ml water. It may be assumed that the movement (and dispersion) of the propagules within the sand columns is quite complex. The presence of sporangia, however, is shown by tomato. The sporangia were well-moved down the columns. At the 5 and 300-ml levels, respectively, tomato infection in section C was 17 and 100%.

Soil amendment influence on tdmato infection

In order to see whether tomato infection was also influenced by soil treatment, tomato seedlings were grown in the soils which had been amended. Fig. 2 shows the soil treatments grouped (clockwise) in increasing order of disease incidence (percent infection) on potato, viz: I, II, III and IV, which order parallels the infection index and total tomato borne sporangial numbers. The total soil-borne sporangial numbers, however, were I > III > IV > II.

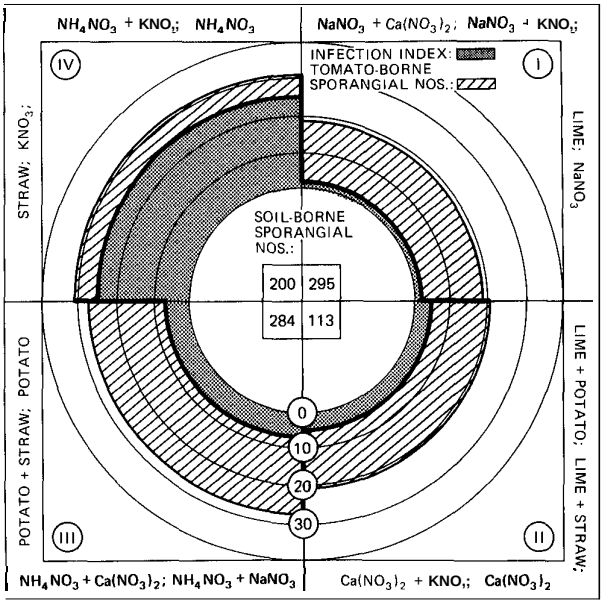

Figure 2. Soil treatments were ranked clockwise in ascending order of disease incidence, and then divided into four groups (I, II, III and IV). The chart shows the total number of soil-borne sporangia (S=85), tomato-borne sporangia (S=6) and infection index value for each quadrant. (Infection Index = weight of tumor mass ÷ number of soil-borne sporangia).

Discussion

The potato has been used as an assay tool by many workers in potato wart disease as the principal host of S. *endobioticum.* It displays, however, erratic and unpredictable infection in field (6), greenhouse and growth room culture (2). Other disadvantages to using potato as an assay plant are that tubers are neither equally available nor in similar physiological condition throughout the year, numbers of potato plants sufficient to be treated statistically consume considerable greenhouse space, under laboratory conditions the potato plant does not appear to be susceptible to low inoculum levels, and laboratory conditions for potato infection have not been clearly defined.

The experiments detailed in this paper demonstrated that tomato (cv. Beefmaster) displayed infection, with S. *endobioticurn,* within a few days of inoculation, and 100% infection at 14 d after inoculation, that one sporangium g^{-1} inoculation media was detectable, and that 50% tomato seedlings became infected at inoculurn densities of ca. 5 sporangia g^{-1}. It was also observed that disease incidence in tomato increased rapidly with time, that numbers of host-located sporangia varied considerably for all tomato samples at any one inoculum level, and that tomato infection appeared to be strongly influenced by the nature of the inoculation medium in much the same way as potato infection responded.

Nevertheless, since tomato appears extremely susceptible to S. *endobioticum,* the fact that seed is readily available, inexpensive, germinates readily, can be used at all times during the year, that seedlings are readily cultivated and transplanted, and that sufficient numbers of tomato plants can be handled to provide statistical treatment of results, tomato gives us a sensitive tool for exploring facets of wart disease that would otherwise be denied us using potato alone.

Acknowledgements

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