

## RANGE OF PATHOGENICITY OF FLORIDA CULTURES OF THE FOOT ROT FUNGUS

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In the search for *Phytophthora*-tolerant rootstocks, many citrus varieties and hybrids were inoculated as bare root seedlings in aerated water with composite inoculum of *Phytophthora parasitica* from 4 or more sources. Sweet orange seedlings, which are very susceptible, were included in each series of plants tested to check the effectiveness of the inoculum. The amount of infection on the sweet orange seedlings, however, varied from series to series, although the same amount of fungus was used each time.

A change in the particular combination of cultures for the composite inoculum seemed to be related to a variation in the amount of infection. For example, the combination of cultures 5, 7, 9 and 11 seemed to give higher infection ratings than the combination of cultures 6, 8, 10 and 12. This apparent relation indicated a possible variation in pathogenicity of the fungus cultures.

Another indication of differences in pathogenicity of cultures was manifested by varying success in infecting sweet orange seedlings with the fungus in soil. Culture P-12, selected arbitrarily to screen soil fungicides, caused only mild infection on sweet orange seedlings, even though the inoculum in the soil was heavy and growing under ideal conditions. Other cultures in previous experiments had produced severe root rot infection in soil.

These indications were the basis for investigating the variability of pathogenicity of individual cultures collected throughout several citrus-producing counties in Florida.

### MATERIALS AND METHODS

Thirty-four cultures of *P. parasitica* from bark, roots, and soil, collected in 7 counties of the citrus area, were tested for their pathogenicity on sweet orange seedlings. The seedlings from a single seed source were grown in the greenhouse in a peat moss-vermiculite medium. Ten plants of uniform size with 10 to 15 leaves

were selected for each replication. The fungus was grown at room temperature in 100 ml. of lima bean broth for 5 to 7 days, washed with demineralized water, and incubated at room temperature in rain water for 24 hours to produce sporangia and zoospores. A modification of Klotz, DeWolfe, and Wong's method of inoculation was used (1). The roots and lower stems of the seedlings were inoculated by immersion for 20 to 24 hours in a tank of aerated water (82-88°F.) containing mycelium, sporangia, and zoospores from 600 ml. of broth. After inoculation, the plants were incubated in beds containing a peat moss-vermiculite mixture (78-84°) and ratings of the degree of infection were made at 3 weeks. Severity of infection by each culture of *P. parasitica* was rated from symptoms in the top from 1 to 5 (5 = wilting and yellow), tap root from 1 to 5 (5 =  $\frac{2}{3}$  or more showing infection), and feeder roots as 1, 3, or 5 (slight, moderate, heavy), giving a maximum rating of 15 for each plant or 150 for each of the 10-plant replications.

### RESULTS

Numerical values proportionate to the amount of infection for 10 plants are given in Table 1 for 15 of the 34 cultures which represent the range of pathogenicity found. Check plants (plants in aerated water without inoculum) have a base figure of 30. Slight feeder root sloughing occurred from the handling and the water which accounts for the 4 and 8 increases over the base

TABLE 1.--Range of pathogenicity on sweet orange seedlings of 15 *Phytophthora parasitica* cultures from various sources.

| Culture No.        | Part from which isolated | County of origin | Rating for 10 plants |       |       |                    |
|--------------------|--------------------------|------------------|----------------------|-------|-------|--------------------|
|                    |                          |                  | Rep 1                | Rep 2 | Rep 3 | (average) Reps 1-3 |
| P-27               | root                     | St. Lucie        | 137                  | 130   | 127   | 131                |
| P-30               | bark <sup>1/</sup>       | Orange           | 130                  | 123   |       | 127                |
| P-14               | root                     | St. Lucie        | 108                  | 135   |       | 122                |
| P-19               | bark                     | Lake             | 91                   | 117   | 103   | 104                |
| P-20               | root                     | Orange           | 96                   | 110   |       | 103                |
| P-5a               | root                     | Orange           | 110                  | 72    | 99    | 94                 |
| P-34               | soil                     | Brevard          | 86                   | 96    | 60    | 81                 |
| P-10               | bark                     | Seminole         | 82                   | 89    |       | 86                 |
| P-21               | bark                     | Polk             | 82                   | 87    |       | 85                 |
| P-36               | root                     | St. Lucie        | 82                   | 74    | 73    | 76                 |
| P-24               | root                     | Hardee           | 65                   | 82    | 76    | 74                 |
| P-12               | bark                     | Seminole         | 63                   | 68    |       | 66                 |
| P-13               | bark                     | St. Lucie        | 46                   | 65    | 60    | 57                 |
| P-22               | root                     | Brevard          | 40                   | 49    | 58    | 49                 |
| P-6                | bark                     | Lake             | 43                   | 34    |       | 39                 |
| None <sup>2/</sup> |                          |                  | 34                   | 38    |       | 36                 |

1/ Trunk bark at bud union.

2/ Plants in aerated water without fungus.

figure of 30 in replications No. 1 and 2, respectively. Cultures with a value of 90 or more caused severe damage to the tap root and almost complete destruction of the feeder roots, whereas cultures with a value of 70 or less caused little or no damage to the tap root and only slight to moderate damage to the feeder roots. Intermediate values correspond to intermediate root damage.

In general, severity of top symptoms did not correspond to severity of root symptoms. Although 3 cultures (P-27, P-30, P-14) with high ratings caused permanent wilting, 5 cultures (P-20, P-21, P-24, P-13, P-22) with high to low ratings caused no top symptoms. The remaining cultures produced top symptoms ranging from yellow veins in the lower leaves to yellowing of the entire foliage.

Although variations in pathogenicity exist, so far there seems to be no relation between the source of the cultures (bark, root, soil, or geographical location) and pathogenicity.

#### DISCUSSION

Further investigations may reveal that variations between cultures collected from a single grove may be as great as variations between cultures from different groves in widely scattered geographical locations. Also, the relative order of pathogenicity of the cultures might be altered if some have slightly different optimum temperatures for maximum disease. However, with the

cultures used under the conditions of this experiment, it was evident that variation in pathogenicity does exist between cultures of *P. parasitica* collected in Florida. This variation in apparently not due to the amount of inoculum since the quantity of sporangia and zoospores was observed to be essentially equal for each culture. If the amount of inoculum had been unequal it is doubtful that this would have influenced the results since the same infection rating was obtained in a separate test with a highly pathogenic culture when using  $\frac{1}{2}$  and  $\frac{1}{4}$  of the amount of inoculum.

These observations warrant further investigations to characterize the foot rot and root rot fungus before extensive screening tests for root-stock tolerance can begin. Screening tests that have been made with citrus varieties and hybrids for *P. parasitica* tolerance may be valid only for the particular culture or cultures used in the tests.

#### SUMMARY

Cultures of *Phytophthora parasitica*, the fungus causing foot rot and root rot on citrus, exhibited a range of pathogenicity on sweet orange seedlings from slight damage to the root system and no foliage symptoms to almost complete destruction of the root system and permanent wilting of the foliage.

#### LITERATURE CITED

1. Klotz, L. J., T. A. DeWolf, and P. P. Wong. 1958. Decay of fibrous roots in citrus. *Phytopath.* 48:616-622.

## ASEPTIC GROWTH OF RADOPHOLUS SIMILIS (COBB) THORNE ON OKRA ROOT CALLUS TISSUE

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Use of tissue cultures in nematological investigations has been explored by a number of workers. Mountain (7) demonstrated that *Pratylenchus minyus* could be grown on excised roots of corn, tobacco, and red clover and that aseptically reared nematodes were pathogenic to tobacco (6). Tiner (9) described a method for

growing *Pratylenchus penetrans* on excised corn roots. Peacock (8) was able to grow *Meloidogyne* spp. on excised tomato roots growing in tissue culture. Widdowson et al. (11) were able to grow cultures of *Heterodera rostochiensis* Woll. on excised tomato root tips and used these cultures to observe the development of *H. rostochiensis* under aseptic conditions. Feder (1) cultured the burrowing nematode, *Radopholus similis*, on excised okra root tissues, and Feder and Feldmesser (2) used *R. similis* individuals reared aseptically in this manner to study the nematode fungal complex attacking citrus roots.

Excised okra roots grow rapidly in tissue culture and must be transferred at frequent intervals (Fig. 1). In addition, the cultures are bulky