

**Resistance to Race 0 of *Phytophthora parasitica* var. *nicotianae* in Tissue Cultures of a Tobacco Breeding Line with Black Shank Resistance Derived from *Nicotiana longiflora***

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We thank Annette Barfield and Janet Finley for assistance.

Paper 77-10-11-99, Kentucky Agricultural Experiment Station.

Accepted for publication 20 July 1977.

ABSTRACT

MARONEK, D. M., and J. W. HENDRIX. 1978. Resistance to race 0 of *Phytophthora parasitica* var. *nicotianae* in tissue cultures of a tobacco breeding line with black shank resistance derived from *Nicotiana longiflora*. *Phytopathology* 68:233-234.

Tissue cultures derived from stems of tobacco (*Nicotiana tabacum*) plants either susceptible to races 0 and 1 of *Phytophthora parasitica* var. *nicotianae* 'Burley 21' or with resistance to race 0 derived from *N. longiflora* 'L8' were inoculated with race 0 or 1 of the pathogen. Tissue cultures derived from Burley 21 plants were susceptible to both races,

whereas cultures derived from L8 plants were resistant to race 0 and susceptible to race 1. These results correlate with the reactions of stems or roots of these cultivars to races 0 and 1 and provide further evidence of the similarity of black shank resistance of *N. longiflora* and *N. plumbaginifolia*.

Resistance to the black shank disease in tobacco is derived from two major sources in the United States (6). Moderate resistance to races 0 and 1 of *Phytophthora parasitica* Dast. var. *nicotianae* (Breda de Haan) Tucker has been transferred from the cigar cultivar, Florida 301, into burley and flue-cured cultivars of *Nicotiana tabacum*. Lines with high resistance to race 0 but high susceptibility to race 1 have been developed from *N. plumbaginifolia* Viv. for flue-cured tobacco (2) and from *N. longiflora* for burley tobacco (8). Cultivars with Florida 301-type resistance are susceptible when stem-inoculated, but those with *N. plumbaginifolia* or *N. longiflora* resistance are resistant when stem-inoculated with race 0 (5, 9). Resistance derived from *N. plumbaginifolia* and *N. longiflora* appears similar in being inherited essentially as a simple dominant factor (1, 2).

Helgeson and coworkers (3, 4) found tissue cultures derived from plants with resistance from *N. plumbaginifolia* were resistant to race 0 and susceptible to race 1 in a manner similar to whole plants. We conducted this study to determine if tissue cultures derived from plants with *N. longiflora* resistance reacted to races of the black shank fungus as do tissue cultures from *N. plumbaginifolia*.

Callus tissue was derived from 2.5-cm-long stem segments of Burley 21, which is susceptible to both races, and L8, which has resistance derived from *N. longiflora* (1, 5, 8). Stem segments were surface sterilized in 1% sodium hypochlorite for 10 min and rinsed twice with sterile water. Pith explants were obtained from the stem segments and cultured on a modified Murashige and Skoog basal medium (7) containing per liter; 6 g agar, 3 mg 2,4-dichlorophenoxyacetic acid, and 0.5 mg kinetin.

Callus tissue produced from the pith explants was removed, divided, and subcultured three successive times to ensure that only callus tissue free from the original stem explant tissue was used. All callus cultures were subcultured monthly and grown in 9-cm diameter petri plates containing 40 ml of basal medium at 27±1 C under a 16-hr photoperiod [5,649-5,918 lux (525-550 ft-c)] before and after inoculation.

Calli (six replicates per treatment) were inoculated with race 0 isolate 1156 or race 1 isolate 1452 (5). Inocula were 4-mm diameter disks taken from the margin of a colony growing on 25 ml of 1.7% agar in 9-cm diameter petri plates. An inoculum disk was placed on top of each callus, with care taken to prevent direct contact between the fungus and the medium. After inoculation, plates were incubated in a plastic bag to maintain high humidity. Reactions of the calli to the fungi were made according to the rating system of Helgeson et al. (3) as follows: 0, no visible mycelium; 1, mycelium visible on medium surrounding the callus but aerial mycelium not present; 2, aerial mycelium present on portions but not completely covering the callus; 3, aerial mycelium completely covering the callus surface.

All calli developed from Burley 21 were rated 3 by 14 days after inoculation with either race. The calli were completely covered by aerial mycelium, and the fungi were growing vigorously over the medium. Fungal growth was much more vigorous in the presence of Burley 21 callus than on the tissue-culture medium without callus. After 14 days, calli derived from L8 all were rated 1 if inoculated with race 0 and 3 if inoculated with race 1. Calli of L8 inoculated with race 1 were indistinguishable from Burley 21 calli inoculated with either race. Four wk after inoculation with race 0, L8 calli were rated 2. Aerial mycelium was present but not uniform over the top of the callus, and limited growth occurred on the medium surrounding the callus. Although our inoculation method and basal medium were different from that of Helgeson et

al. (3), the results were similar. Fourteen days after inoculation, the reactions to race 0 of calli derived from *N. plumbaginifolia* was near 1 (3), and a rating of 1 was found for L8 calli inoculated with race 0 at the same time interval.

Valleau et al. (8) considered the black shank resistance of *N. longiflora* and *N. plumbaginifolia* to be identical, but Wills (9) found leaf tissue of L8 to be susceptible whereas leaf tissue of 1071, a breeding line with black shank resistance derived from *N. plumbaginifolia*, was resistant to race 0 but susceptible to race 1. Our observations and those of Helgeson and coworkers (3, 4) indicate that the reactions of tissue cultures derived from tobacco with black shank resistance derived from *N. longiflora* and *N. plumbaginifolia* are similar to each other and to the stem reactions of the whole plants from which they were derived. This work provides further evidence of the similarity of these sources of resistance.

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