Temperature-Dependent Resistance to Tobacco Ringspot Virus in L8, a Necrosis-Prone Tobacco Cultivar

James W. Hendrix

Department of Plant Pathology, University of Kentucky, Lexington 40506.

ABSTRACT

The burley tobacco breeding line L8, which has black shank resistance derived from *Nicotiana longiflora*, was resistant to tobacco ringspot virus (TRSV) at 24 C. Resistance was expressed as a necrotic reaction on the inoculated leaves and the absence of systemic infection. In a small percentage of plants, a systemic necrotic reaction occurred. At 35 C, L8 plants became systemically infected, and symptoms were considerably different from those of plants incubated at 24 C.

Hybridizing L8 with standard cultivars resulted in loss of resistance, as measured by systemic invasion, but the nature of the systemic symptoms was dependent upon the percentage of L8 germplasm. Resistance to TRSV was not related to resistance to tobacco mosaic virus. L8 tends to have necrotic reactions to stresses, but the factors controlling the various reactions appear to be inherited differently.

Phytopathology 62:1376-1381

Tobacco ringspot virus (TRSV) is prevalent in both flue-cured and burley tobacco-producing regions of the United States (4, 10, 22). Losses have not been considered great enough to require control (22), but ringspot is often quite destructive in individual fields. Little effort has been made to develop TRSV-resistant cultivars of tobacco, although there has been extensive development of resistance to other pathogens (3, 21). Reported here is evidence that the burley tobacco cultivar L8 is resistant to TRSV.

L8, recently registered (7), was developed by Valleau et al. (23) as a source of high resistance to common strains (race 0) of the black shank fungus, *Phytophthora parasitica* var. *nicotianae*. The black

Fig. 1-4. I = inoculated leaf. All plants 39 days old at inoculation. Photographed 14 days later. 1) Inoculated L8 plant incubated at 24 C. Arrows, necrotic systemic symptoms. 2) Inoculated Burley 37 plant incubated at 24 C. 3) Noninoculated L8 plant incubated at 24 C. 4) Inoculated L8 plant maintained at 24 C, with severe systemic necrotic infection.
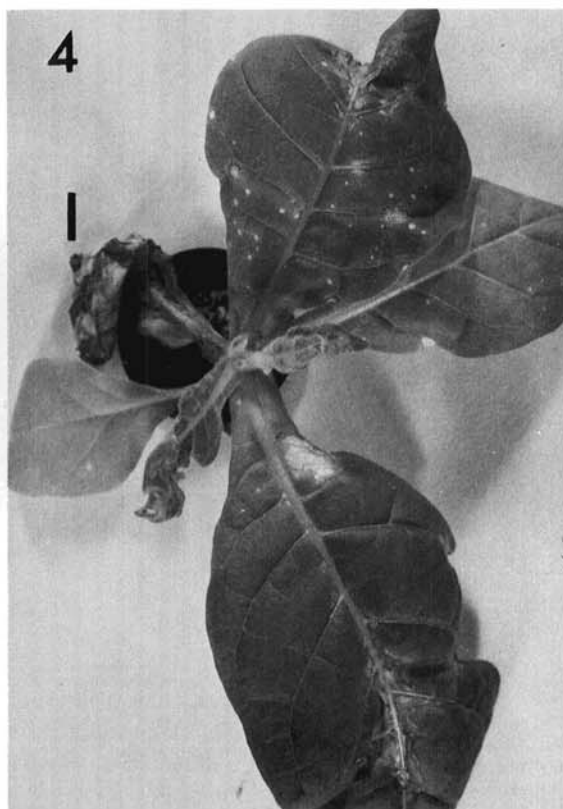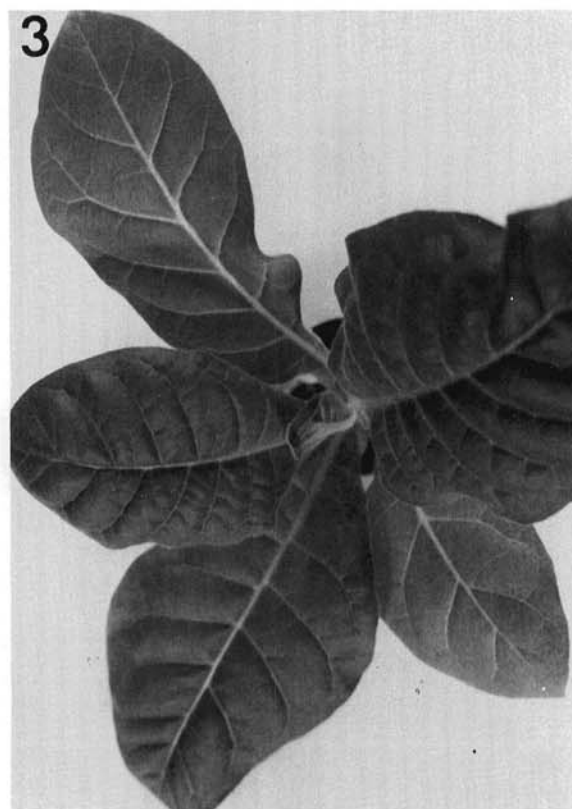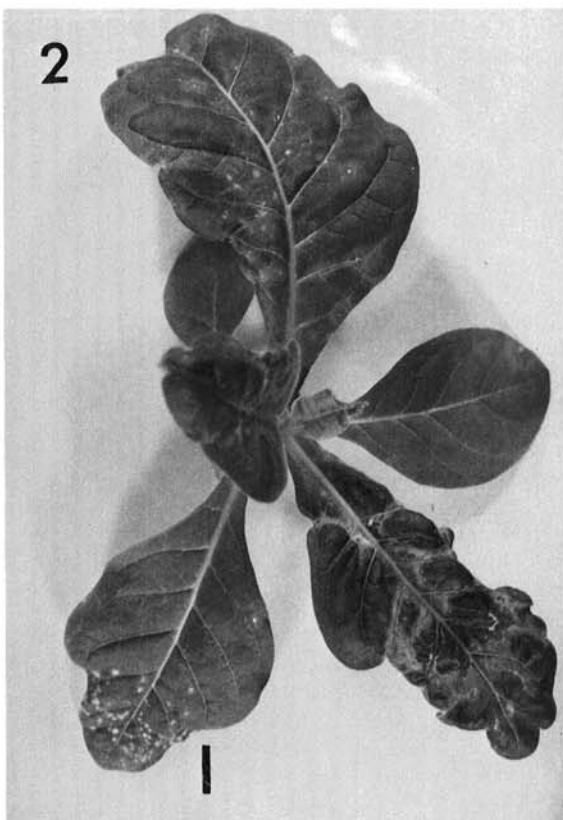
shank resistance was obtained from *Nicotiana longiflora* by chromosomal segmental substitution (5). L8 also has the *N. glutinosa* factor for resistance to tobacco mosaic virus (TMV) and moderate resistance to the black root rot pathogen (7). The utility of L8 has been limited by its tendency toward a physiological leaf spotting reaction (7, 23), and by the frequent development of race 1 of the black shank pathogen, to which it is susceptible, in fields heavily infested with race 0 (14, 19, 23).

MATERIALS AND METHODS.—Plants were grown in the greenhouse in 50-ml black plastic tubes containing 8- to 50-mesh Weblite, an expanded shale product produced by Weblite Corporation, Roanoke, Va. The tubes were watered with Hoagland's nutrient solution from below through holes in the bottoms of tubes.

I prepared inoculum by grinding infected tobacco, cowpea, or cucumber leaf tissue in 0.01 M Na_2SO_3. I added Carborundum to the inoculum before inoculating each plant on a single, well-expanded leaf in the conventional manner by rubbing the leaf with cheesecloth soaked in inoculum.

RESULTS.—Inoculated leaves of L8 plants incubated at 24 C in a growth chamber became severely necrotic and usually died (Fig. 1, 4). Most plants did not become infected systemically. Except for inoculated leaves, they could hardly be distinguished from noninoculated plants (Fig. 3). Some L8 plants became infected systemically to a limited extent (arrows, Fig. 1). A few plants became extensively infected systemically and were severely necrotic (Fig. 4). Such plants were usually, but not always, shaded by adjacent faster-growing plants.

In contrast, inoculated leaves of Burley 37 plants developed spots that were chlorotic first but eventually became necrotic (Fig. 2). Inoculated leaves did not become completely necrotic or chlorotic like those of L8. Burley 37 plants became infected systemically, with severe distortion and extensive chlorosis (Fig. 2).

Indexing to cowpea and cucumber demonstrated that tissues of L8 plants without systemic symptoms were free of the virus, and tissues of L8 and other cultivars with systemic symptoms contained virus. L8 plants, therefore, are not symptomless carriers.

In earlier tests in which greenhouse temperatures were high for several days, systemic invasion occurred in as many as 50% of the L8 plants inoculated. Accordingly, the reactions of L8 and Burley 37 plants incubated at a high temperature were studied.

Local necrotic resistance was not expressed when L8 plants were incubated in a growth chamber at 35 C after inoculation (Fig. 5). Both L8 and Burley 37 plants became systemically infected, although symptoms were mild and consisted primarily of faint oak-leaf patterns (arrow, Fig. 5). Systemic necrosis or extensive leaf distortion did not occur.

Local symptoms were also markedly different at high temperatures from those at low. Chlorotic rings developed on inoculated leaves of L8 plants kept at 35 C (Fig. 5), in contrast to extensive necrosis at 24 C (Fig. 1, 4). Necrotic rings developed on inoculated leaves of Burley 37 plants kept at 35 C (Fig. 6), in contrast to the chlorotic spots developing at 24 C (Fig. 2).

Noninoculated plants grew much better at 24 C than at 35 C. Thus, the severe distortion or necrosis
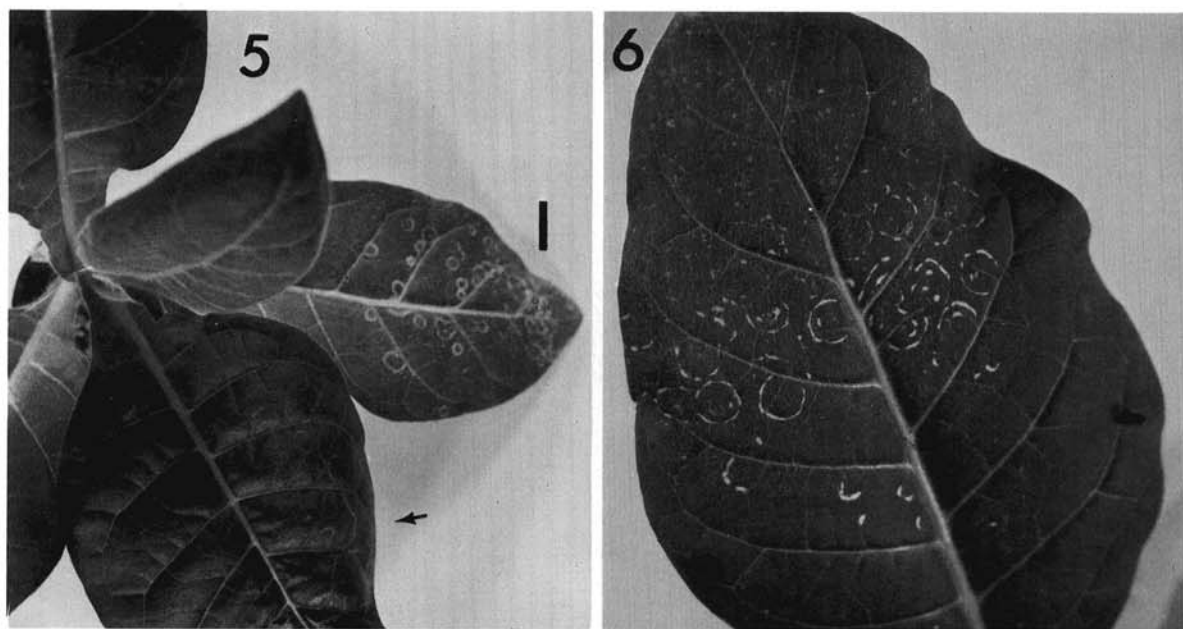

Fig. 5-6. All plants 39 days old at inoculation. Photographed 14 days later. 5) Inoculated L8 plant incubated at 35 C. I = inoculated leaf. Arrow, faint oak leaf and ring patterns. 6) Inoculated leaf of Burley 37 plant incubated at 35 C. Scale 2 times that in Fig. 1-5.

TABLE 1. Systemic infection by tobacco ringspot virus of plants of L8, Burley 37, and crosses between Burley 37 or male sterile Burley 37 and L8[a]

Cultivar or breeding line	No. systemically infected plants/inoculated plants			
	Greenhouse[b] experiment A	Growth chamber[c] experiment A	Greenhouse[d,e] experiment B	Growth chamber[e,f] experiment B
L8	4/20	3/13	0/4	0/6
(MS B37 × L8) × L8	16/20	8/13	4/5	1/6
L8 × B37	18/19	12/13	1/2	1/6
B37 × L8	15/20	13/13	3/6	5/6
MS B37 × L8	20/20	13/13	9/9	5/6
(MS B37 × L8) × B37	20/20	13/13	3/4	3/6
Burley 37	20/20	12/13	7/7	5/6

[a] All plants seeded at same time, inoculated at ages indicated.

[b] Plants inoculated when 28 days old. Data were taken 19 days after inoculation. Temperatures after inoculation: extreme low, 17 C; mean low, 18 C; mean high, 24 C; extreme high, 27 C.

[c] Plants inoculated when 31 days old. Data were taken 16 days after inoculation. Temperatures: 21.5 C, lights on; 19 C, lights off.

[d] Temperatures after inoculation: extreme low, 18 C; mean low, 20 C; mean high, 30 C; extreme high, 34 C.

[e] Plants inoculated when 47 days old. Data taken 14 days after inoculation. Precautions were taken to prevent shading of smaller plants by larger plants.

[f] Temperatures: 21.5 C, lights on; 19 C, lights off.

of systemically infected plants occurred at a temperature suitable for rapid growth. Inoculated L8 or Burley 37 plants were not markedly different in size from noninoculated plants incubated at 35 C.

In studies of the inheritance of resistance, breeding lines with 50 or 75% L8 germplasm sometimes appeared to be more resistant to systemic infection than Burley 37; but over-all, only L8 was clearly resistant (Table 1). In these tests, most of the systemically infected L8 plants became shaded by larger plants; but this was not true for any other line. Reciprocal crosses eliminated the possibility of striking maternal effects on inheritance of resistance.

While the quantitative data suggest that crossing L8 with other cultivars results in the complete or nearly complete loss of resistance, the nature of the systemic symptoms was dependent upon the percentage of L8 germplasm. Systemic infection of L8 resulted in veinal necrosis, with no chlorosis. Burley 37 plants were severely malformed and stunted. Chlorosis, rather than necrosis, predominated. (MS Burley 37 × L8) × L8 had necrotic veins and spots, with little chlorosis, malformation, or stunting. Symptoms were mild, and plants were less severely affected than systemically infected Burley 37 plants. L8 × Burley 37, MS Burley 37 × L8, and Burley 37 × L8 plants had necrotic and slight chlorotic local and systemic symptoms, but plants were not severely malformed or stunted. Some (MS Burley 37 × L8) × Burley 37 plants were severely chlorotic, malformed, and stunted, whereas others seemed considerably more tolerant. In the latter case, the number of plants tested was not sufficient to suggest whether or not these differences in symptoms represent a true segregation.

Burley 21 reacted in a manner similar to Burley 37, and other L8 hybrids (MS Ky 12 × L8 and MS B21 × L8) responded similarly to the Burley 37 and MS Burley 37 × L8 hybrids.

DISCUSSION.—The factor in L8 governing resistance to TRSV apparently is located on the chromosomal segment derived from *N. longiflora*, for the reaction of L8 to the virus is quite similar to that described for *N. longiflora*. Wingard (24) divided plants into groups according to their reactions to TRSV. Wingard's group 4 contained a wide variety of plants, including *N. longiflora*, *N. plumbaginifolia*, *N. glutinosa*, and a number of other species of *Nicotiana*, which seemed to be hypersensitive to the virus. L8, in its inoculated leaf and systemic necrosis symptoms, fits Wingard's description for group 4, except that systemic ring and oak leaf patterns, as illustrated for *N. plumbaginifolia*, and eventual development of symptomless leaves was never observed in L8.

The factor for resistance to TRSV in L8 is not identical with the *N. glutinosa* factor for resistance to TMV, although *N. glutinosa* and *N. longiflora* apparently reacted similarly in Wingard's tests (24). All the cultivars tested in this work except Burley 37 have the *N. glutinosa* factor for TMV resistance, yet all but L8 were susceptible to TRSV.

The responses to temperature of L8 inoculated with TRSV and *N. glutinosa* inoculated with TMV are similar. When inoculated at 21 C, leaves of *N. glutinosa* inoculated with TMV develop small local lesions (18). At 28 C, the local lesions are much larger, and spread quickly. Systemic infection does not occur at 21 or 28 C; but at 35 C there is systemic infection, but no necrosis. The reaction of L8 to TRSV at 24 C is similar to that of *N. glutinosa* to TMV at 28 C.

The effect of high temperature in causing relatively mild systemic symptoms has been noted by others (2, 20). Plants which had produced

symptomless leaves at high temperatures again had symptoms in newly developing leaves when transferred back to low temperatures.

L8 has a strong tendency for necrosis in its responses to a variety of stresses. Race 0 of *Phytophthora parasitica* var. *nicotianae* can grow considerable distances through stems, but the growth is limited to a necrotic zone, in contrast to the complete horizontal invasion of the pith and vascular tissue characteristic of either race of the fungus in stems of the commonly used cultivars (13), and a hypersensitive type of necrotic response occurs in root cells of L8 in advance of race 0 (11). The physiological spotting reaction is a necrotic response to unknown environmental and/or nutritional factors, and the reactions to TRSV and TMV are hypersensitive necrotic responses.

However, all these factors seem to be inherited independently or in dissimilar ways. The resistance to TMV derived from *N. glutinosa* is controlled by a single dominant gene (15, 21), but is not accompanied by resistance to TRSV or black shank. Resistance to race 0 of the black shank fungus also appears to be controlled by a dominant gene, although completely Mendelian ratios beyond the F_1 generation are not obtained (6, 23). The physiological spotting factor, while linked with black shank resistance, is controlled by a recessive factor, for F_1 hybrids of L8 with commercial cultivars are not affected (23). The resistance to TRSV is not controlled by a single dominant factor, for any reduction in L8 germplasm resulted in loss of systemic resistance to the virus (Table 1). However, the nature of the systemic symptoms depended on the proportion of L8 germplasm present, indicating that inheritance may be quantitative or influenced by modifying factors. While the nature of inheritance to TRSV is not known, it is inherited differently than the other necrotic responses.

The utility of L8 as a source of resistance to TRSV is questionable because of the nature of its inheritance. The reactions of L8 hybrids in tests such as those reported here may not be indicative of the degree of control obtainable under field conditions, however. Necrotic resistance to viruses involves virus translocation; and translocation resulting in systemic infection may be influenced by factors such as plant age, plant growth rates, temperature, and mode of transmission, whether to roots by nematodes (8, 12) or to shoots by thrips (17). Resistance of L8 hybrids must therefore be evaluated in the field with natural infection.

If L8 is an inadequate source of resistance to TRSV, Wingard's observations (24) suggest that resistance to TRSV might be obtained from species of *Nicotiana*. The nature of the TRSV-resistance in L8 is not necessarily characteristic of the *Nicotiana* species, including *N. longiflora*. Litton et al. (16) recently found that some sources of *N. longiflora* were resistant to race 1 of the black shank fungus, unlike the parents of L8 (23) and NC 2326 derived from *N. plumbaginifolia* (1, 9). Furthermore, Apple (1) obtained lines with moderate resistance (9, 19) to

race 1 of the black shank fungus, although neither the *N. plumbaginifolia* nor the *N. tabacum* parents were resistant. The physiological leaf-spotting reaction of L8 is not characteristic of its *N. tabacum* parents, and probably not of its *N. longiflora* parent. Probably the best approach would be to make crosses of *N. tabacum* with several resistant species of *Nicotiana* and to make careful selections through the back-crossing and inbreeding generations.

LITERATURE CITED

1. APPLE, J. L. 1962. Physiological specialization within Phytophthora parasitica var. nicotianae. Phytopathology 52:351-354.
2. BENDA, G. T. A., & A. W. NAYLOR. 1958. On the tobacco ringspot disease. III. Heat and recovery. Amer. J. Bot. 45:33-37.
3. BURK, L. G., & H. E. HEGGESTAD. 1966. The genus Nicotiana: a source of resistance to diseases of cultivated tobacco. Econ. Bot. 20:76-88.
4. CLAYTON, E. E., & J. E. MC MURTREY, JR. 1958. Tobacco diseases and their control. U.S. Dep. Agr. Farmers' Bull. 2023. 69 p.
5. COLLINS, G. B., & P. D. LEGG. 1969. Cytogenetics of a burley tobacco breeding line derived from a cross with Nicotiana longiflora Cav. Can. J. Genet. Cytol. 11:382-388.
6. COLLINS, G. B., P. D. LEGG, C. C. LITTON, & M. J. KASPERBAUER. 1971. Inheritance of resistance to black shank in Nicotiana tabacum L. Can. J. Genet. Cytol. 13:422-428.
7. COLLINS, G. B., P. D. LEGG, C. C. LITTON, & J. H. SMILEY. 1971. Registration of L8 burley tobacco germplasm. Crop Sci. 11:606-607.
8. FULTON, J. P. 1962. Transmission of tobacco ringspot virus by Xiphinema americanum. Phytopathology 52:375.
9. GOINS, R. B., & J. L. APPLE. 1970. Inheritance and phenotypic expression of a dominant factor for black shank resistance from Nicotiana plumbaginifolia in a Nicotiana tabacum milieu. Tobacco Sci. 14:7-11.
10. GOODING, G. V., JR., & F. A. TODD. 1966. Virus diseases of flue-cured tobacco in North Carolina. Plant Dis. Reptr. 50:308-309.
11. HANCHEY, PENELOPE, & H. WHEELER. 1971. Pathological changes in ultrastructure:tobacco roots infected with Phytophthora parasitica var. nicotianae. Phytopathology 61:33-39.
12. HENDRIX, J. W. 1961. Soil transmission of tobacco ringspot virus. Phytopathology 51:194.
13. HENDRIX, J. W., & J. L APPLE. 1967. Stem resistance to Phytophthora parasitica var. nicotianae in tobacco derived from Nicotiana longiflora and N. plumbaginifolia. Tobacco Sci. 11:148-150.
14. HENDRIX, J. W., & R. A. FLOWERS. 1968. Development of races of Phytophthora parasitica var. nicotianae in mid-Tennessee in 1966. Tobacco Sci. 12:134-135.
15. HOLMES, F. O. 1954. Inheritance of resistance to viral diseases in plants. Advances Virus Res. 2:1-30.
16. LITTON, C. C., G. B. COLLINS, & P. D. LEGG. 1970. Reaction of Nicotiana tabacum and other N. species to race 0 and 1 of Phytophthora parasitica var. nicotianae. Tobacco Sci. 14:128-130.
17. MESSIEHA, MIMI. 1969. Transmission of tobacco ringspot virus by thrips. Phytopathology 59:943-945.
18. SAMUEL, G. 1931. Some experiments on inoculating

methods with plant viruses, and on local lesions. Ann. Appl. Biol. 18:494-507.

19. STOKES, G. W., & C. C. LITTON. 1966. Source of black shank resistance in tobacco and host reaction to races 0 and 1 of Phytophthora parasitica var. nicotianae. Phytopathology 56:678-680.

20. VALLEAU, W. D. 1941. Experimental production of symptoms in so-called recovered ring-spot tobacco plants and its bearing on acquired immunity. Phytopathology 31:522-533.

21. VALLEAU, W. D. 1952. Breeding tobacco for disease resistance. Econ. Bot. 6:69-102.

22. VALLEAU, W. D., E. M. JOHNSON, & S. DIACHUN. 1963. Tobacco diseases. Univ. Kentucky Coop. Ext. Service Circ. 522-A. 68 p.

23. VALLEAU, W. D., G. W. STOKES, & E. M. JOHNSON. 1960. Nine years' experience with the Nicotiana longiflora factor for resistance to Phytophthora parasitica var. nicotianae in the control of black shank. Tobacco Sci. 4:92-94.

24. WINGARD, S. A. 1928. Hosts and symptoms of ringspot, a virus disease of plants. J. Agr. Res. 37:127-153.