Reaction of Nicotiana Species to Alternaria alternata

J. R. Stavely, G. W. Pittarelli, and G. B. Lucas

Research Plant Pathologist and Agricultural Research Technician, Crops Research Division, ARS, USDA, Beltsville, Maryland 20705; and Professor of Plant Pathology, Department of Plant Pathology, North Carolina State University, Raleigh, North Carolina 27607, respectively.

Cooperative investigations of the Crops Research Division, ARS, USDA, Beltsville, Maryland, and the North Carolina Agricultural Experiment Station, Raleigh. Journal Series Paper No. 3279, North Carolina State University Experiment Station, Raleigh.

Accepted for publication 11 December 1970.

ABSTRACT

Nicotiana bonariensis, N. wigandioides, N. debneyi, N. noctiflora, N. repanda, and two accessions each of *N. suaveolens* and *N. longiflora* were consistently among the most resistant of 63 *Nicotiana* species in independent tests of reactions to *Alternaria alternata* conducted at Beltsville, Md., and Raleigh, N. C. *Nicotiana nesophila, N. stocktonii, N. goodspeedii, N. hesperis, N. occidentalis,* and *N. forgetiana* were highly resistant at one of the locations and lightly infected at the other location. Stem lesions developed on 56 species; and typical, restricted lesions developed on the youngest inoculated leaves of 61 species. All species in subgenus Tabacum and all species but *N. thyrsiflora* in subgenus Rustica were moderately or highly susceptible to the disease at one or both test sites. Phytopathology 61:541-545.

Additional key words: Nicotiana subgeneric relationships, breeding for resistance.

The most serious leaf disease of flue-cured tobacco in the United States is brown spot, incited by *Alternaria alternata* (Fr.) Keissl. (*A. tenuis* Nees). All currently grown tobacco cultivars, *Nicotiana tabacum* L., are susceptible to the disease. Certain cultivars show moderate degrees of tolerance (9, 10, 13), but only a Santo Domingo cigar tobacco, Beinhart 1000-1, has a high level of tolerance in the field. The Beinhart 1000-1 tolerance is controlled by a single factor, intermediate in expression (3). A tolerant breeding line, PD121, was derived from a cross with Beinhart 1000-1 (3, 8). However, this tolerance has not yet been transferred to acceptable flue-cured cultivars. Tolerant cultivars, including PD121, have a few spots when inoculum levels are moderate; but with high inoculum levels they develop nearly as much spotting as sensitive cultivars (13, 15).

Through interspecific hybridization, resistance to several important tobacco diseases has been transferred from other *Nicotiana* species to *N. tabacum* cultivars (2, 4, 5, 7). This method has not yet been thoroughly tested for obtaining brown spot resistance in *N. tabacum.*

The reaction to brown spot of only 40 of the 65 *Nicotiana* species has been reported in detail (7, 11, 12). This previous work was complicated by escapes from infection (11) and inconsistent results (7, 11, 12). More recently, greater knowledge of the relationship of temperature and other factors to brown spot initiation has enabled development of improved inoculation methods (15). The objective of our study was to determine, at two independent locations, the brown spot reactions of the available species in the genus *Nicotiana.* A primary purpose for this testing was to discover additional sources and retest reported sources of resistance or immunity. Some of our results have been published (12, 16).

Materials and Methods.—The genus *Nicotiana* contains 65 species according to Goodspeed (6) as amended by Burbidge (1). At Beltsville, Md., 63 species and at Raleigh, N. C., 51 species were tested for their reaction to *A. alternata.* Inoculation methods were the same as those previously reported (12, 17). The incubation temperature was 20 C at Beltsville, 22-28 C at Raleigh. Two highly pathogenic isolates were used at both locations. The isolates used in Maryland and North Carolina were obtained independently (12, 15). The conidial concentration was 1.2×10^5 at Beltsville and 3×10^4 at Raleigh.

All plants were grown in 10.2-cm clay pots in standard Plant Industry Station soil (a 2-year-old, steam-sterilized, composted mixture originally made up with 3 cubic yards of sandy loam, 1.5 cubic yards of cow manure, 16 lb. 5-10-5, and dolomitic limestone to bring the pH to 7.0) or the previously described North Carolina soil (12). Exceptions were *N. spegazzinii* Millán and *N. linearis* Ph., which were grown in 7.6-cm pots and in a 50:50 sand:soil mixture, respectively. Species were given supplemental fertilization if needed. Seeds used at Beltsville were from the USDA *Nicotiana* species collection, maintained by L. G. Burk at the Oxford Tobacco Research Station, Oxford, N. C., and included 1-3 accessions of each of 63 species. Plants of the species were obtained at Raleigh from D. U. Gerstel, Crop Science Department, North Carolina State University. Included in the tests were a tetraploid race of *N. suaveolens* Lehm. (*N. eastii* Kostoff) and an artificial species derived from crossing *N. forgetiana* Hort. ex Hemsley with *N. alata* Link & Otto (*N. sanderae* Hort. ex Watson). Origin and identity of all accessions tested can be obtained from the authors or their institutions.

At Beltsville, all plants were grown in a greenhouse at 24-30 C. Time from seeding to inoculation varied due to the differing growth rates of the various species, but averaged about 13 weeks. Five to 15 species were inoculated/test by spraying all aboveground surfaces of five plants/species, Two check plants of brown spot-sensitive tobacco cultivar Coker 187 Hicks (C187H) were included in each test. Cultivars Hicks, NC95, and a breeding line, PD121, were included in several tests. Two five-plant replicates of each species were tested.

A third replicate was tested in the case of all highly resistant or immune species. When questionable symptoms resulted from the first test, noninoculated plants of species involved were incubated along with inoculated plants in the second test to determine if incubation conditions were responsible for any part of the total symptomatology. The number of lesions per leaf, severity of stem and leaf symptoms, abundance of pinpoint-sized lesions on younger leaves (17), and any unusual symptoms were recorded 16-18 days after inoculation. Data on number and severity of leaf spots were divided into five classes: 0 = immune; 1 = very light, 1-8 spots/leaf; 2 = light, 9-19 spots/leaf; 3 = moderate, 20-49 spots/leaf; 4 = severe, more than 50 spots on at least one leaf. This leaf rating had to be modified in some cases to accommodate variation in leaf size, as certain of the species have leaves less than 5% as large as the leaves on most *Nicotiana* species. Stem lesions were rated in four classes: 0 = immune; 1 = light; 2 = moderate; and 3 = severe.

The age at inoculation, propagation methods, number of plants tested, and rating methods used at Raleigh were the same as previously reported (12), and were similar to those used at Beltsville, except stem infection was rated into five classes at Raleigh.

RESULTS.—Infection was generally less severe at Raleigh than at Beltsville. All of the species in subgenus Tabacum of the genus *Nicotiana* were moderately to highly susceptible to *A. alternata* (Table 1). The most susceptible species in the subgenus was *N. glutinosa* L. (Fig. 1); all accessions had severe leaf, stem and petiole symptoms, and some plants died.

The species in subgenus Rustica were all moderately to severely susceptible to brown spot at Beltsville, except *N. thyrsiflora* Bitt. ex Goodsp., on which leaf infection was light and stem infection was absent. Goodspeed's 55-200 *N. cordifolia* Ph. was less severely infected than the other susceptible species. Leaf lesions on *N. cordifolia* were unique in that they were usually monofacial and visible only on the lower surface (Fig. 2). *Nicotiana knightiana* Goodsp. had light stem infection and numerous, restricted leaf lesions that were usually less than 2 mm in diam. The reactions of *N. knightiana* and *N. glauca* Grah. were unusual in that abscission of many of the older leaves occurred within 16-18 days after inoculation. At Raleigh, leaf symptoms on *N. knightiana* and *N. raimondii* Macbr. were very light, and on all of the rest of the species in the subgenus, except some accessions of *N. rustica* L., symptoms were light or moderate.

Considerable uniformity in response to *A. alternata* was shown in most of those sections of subgenus Petunioides that have a small number of species. The three species in section Repandae and three in section Noctiflorae were lightly infected to highly resistant. In repeated tests at Beltsville, about half the inoculated plants of *N. nesophila* Johnst. (Fig. 3) and *N. stocktonii* Lehm. remained free of leaf symptoms, other than a few pinpoint-sized lesions on the younger leaves; the remainder of the plants of these species had no more than four lesions/mature leaf. Plants of *N. repanda* Willd. were all very lightly infected to a similar degree

TABLE 1. Reactions of *Nicotiana* species to *Alternaria alternata* in independent tests at two locations

Subgenus section species	Disease rating[a]			
	Beltsville		Raleigh	
	Mature leaf	Stem	Leaf	Stem
Tabacum				
Tomentosae				
N. glutinosa	4	3	4[b]	4[b]
N. otophora	4	3	4	4
N. setchellii	4	2		
N. tomentosa	4	2	3[b]	3[b]
N. tomentosiformis	3	2	4	3
Genuinae				
N. tabacum				
cv C187H	4	3		
cv NC95	4	3		
cv PD121	3	2		
cv NC2326			2	2
cv Hicks	4	3	3	2
Rustica				
Paniculatae				
N. benavidesii	4[c]		2[b]	
N. cordifolia	3-4	3	2	0
N. glauca	4	3	2-3[b]	2-3[b]
N. knightiana	4	1	1	0
N. paniculata	4	3	3[b]	3[b]
N. raimondii	4	3	1	1
N. solanifolia	4	3	2	3
Thyrsiflorae				
N. thyrsiflora	2	0	1	0
Rustica				
N. rustica	4	3	2-4[b]	2-4[b]
Petunioides				
Acuminatae				
N. acuminata	4	3	4	4
N. attenuata	2[d]	2		
N. corymbosa	4[c]	3		
N. linearis	2	1		
N. miersii	4	2		
N. pauciflora	2[d]	2	2[b]	3[b]
N. spegazzinii	3[d]	2		
Alatae				
N. alata	3[d]	2	4[b]	2[b]
N. bonariensis	0-1[d]	0	0-1	0
N. forgetiana	2	1	1[b]	0[b]
N. langsdorfii	3[d]	2	3	2
N. longiflora	1-2	1	1	3
N. plumbaginifolia	3	2	3[b]	3[b]
N. sanderae	4	3	2[b]	3[b]
N. sylvestris	4	3	4	3
Bigelovianae				
N. bigelovii	3[d]	3	2[b]	3[b]
N. clevelandii	3	3		
Noctiflorae				
N. acaulis	1-2	1		
N. noctiflora	1	0		
N. petunioides	2[c]	1		
Nudicaules				
N. nudicaulis	3	1	2	1
Repandae				
N. nesophila	0-1	0	2	0
N. repanda	1	0	0-1	0-1
N. stocktonii	0-1	0	2	0
Trigonophyllae				
N. palmeri	3[d]	0	3	2
N. trigonophylla	4	3	4[b]	
Undulatae				
N. arentsii	3[d]	0	1	0

TABLE 1 (*Continued*)

N. undulata	2[d]	1	4[b]	4[b]
N. wigandioides	0[d]	0	0[d]	0-1
Suaveolentes				
N. amplexicaulis	3[d]	3	3[b]	3[b]
N. benthamiana	3[d]	2	3[b]	3[b]
N. cavicola	3[d]	3	1-2	1
N. debneyi	1	0	0-1	0-1
N. eastii	3	1	1	0
N. excelsior	3	2	2[b]	4[b]
N. exigua	4	3	2	0
N. fragrans	4	3		
N. goodspeedii	1	0	2[b]	3[b]
N. gossei	2[d]	3	2	2
N. hesperis	2[d]	2	0-1	0
N. ingulba	2	1		
N. maritima	2	1	3[b]	2[b]
N. megalosiphon	4[c]	2	3	3
N. occidentalis	2	1	**0**[b]	1[b]
N. rosulata	4	2	2	0
N. rotundifolia	3	1	3[b]	2[b]
N. simulans	2[d]	2	2[b]	3[b]
N. suaveolens	0-2	0	1[b]	1[b]
N. umbratica	3[d]	2	2	2
N. velutina	4	3	2	2

[a] Leaf symptoms were rated: 0 = immune; 1 = very light; 2 = light; 3 = moderate; and 4 = severe. Stem symptoms were rated in five similar classes at Raleigh, four at Beltsville.

[b] Data previously published by Ramm & Lucas (12).

[c] Lesions, less than 1 mm in diam, on youngest leaves were less severe.

[d] Small lesions on youngest leaves were more severe.

at both locations, but *N. nesophila* and *N. stocktonii* had more lesions at Raleigh than at Beltsville. Species in sections Bigelovianae, Nudicaules, and Trigonophyllae had light or moderate-to-severe leaf symptoms, with much similarity in each section. Both species in Bigelovianae suffered injury caused by some factor other than *A. alternata* that killed most brown spot-infected leaves.

At Beltsville, *N. wigandioides* Koch & Fint., section Undulatae, was the only species tested that was immune to older leaf and stem infection. However, young leaves of all three species in this section were moderately to severely affected by pinpoint infections. *Nicotiana arentsii* Goodsp. had barely enough infection to be classified as moderate at Beltsville and very light leaf infection at Raleigh; whereas *N. undulata* R. & P. was heavily infected at Raleigh, and lightly at Beltsville.

Species in sections Acuminatae and Alatae ranged from highly resistant to very susceptible. Three species in Acuminatae were lightly infected. One of these three, *N. pauciflora* Remy, suffered considerable damage from some other factor in addition to brown spot. *Nicotiana acuminata* var. *multiflora* (Ph.) Reiche (syn. *N. angustifolia* R. & P.) developed unusually extensive chlorotic halos around leaf lesions, but the plants were not killed as were plants of *N. acuminata* var. *acuminata*. Reactions in Alatae ranged from the high level of resistance of *N. bonariensis* Lehm. (Fig. 4) to the severe reaction of *N. sylvestris* Speg. & Comes. Older leaves of *N. bonariensis* were as immune as those of *N. wigandioides* (Fig. 5), except under nutritional stress, when as many as three lesions developed on a few leaves. Pinpoint lesions on young leaves of *N. bonariensis* were few in contrast to the severe reaction of *N. wigandioides* to this type of infection. Both Gerstel's 28-7 and Kehr's accession of *N. longiflora* Cav. had some plants with older leaf immunity, but other plants with as many as five lesions/mature leaf. Clayton's *N. longiflora*, the source of wildfire resistance in tobacco (4, 5), developed more lesions than the other two accessions.

In Suaveolentes, the most resistant species were *N. suaveolens* and *N. debneyi* Domin. In accessions 00-20/B and D of *N. suaveolens* (received from Gembloux, Belgium, and tested only at Beltsville), some plants remained free of spotting; the remainder had no more than three lesions/leaf. Accession B, synonomous with Raeber et al.'s (11) immune W-90, germinated so poorly that only one plant was obtained, from which the test population was derived. Clausen's accession of *N. suaveolens* had some plants with as little as three lesions/leaf, but others with as many as 15/leaf. All accessions were free of young leaf lesions, and stem infection was absent to very light. *Nicotiana goodspeedii* Wheeler was very lightly infected at Beltsville, and had slightly more infection at Raleigh, while the reverse was true for *N. occidentalis* Wheeler and *N. hesperis* Burbidge. The reaction of *N. cavicola* Burbidge was unusual in that none of the numerous leaf lesions exceeded 2 mm in diam, and most were less than 1 mm. The reactions of *N. fragrans* Hook. (Fig. 6) and, to a lesser degree, of *N. simulans* Burbidge, were unique in that few distinguishable lesions developed on inoculated leaves. Instead, the leaf surfaces rapidly became light brown and necrotic. No such symptoms developed on noninoculated check plants of these species.

DISCUSSION.—Among species for which the reaction to brown spot has not been reported (7, 11, 12) were two of the seven that were consistently very lightly infected to immune, *N. bonariensis* and *N. noctiflora* Hook., as well as *N. nesophila* and *N. stocktonii*, which were highly resistant at Beltsville and lightly infected at Raleigh. Most of the smaller differences in symptom ratings between Beltsville and Raleigh were probably due to lack of opt inoculation and incubation conditions (15) or to slight differences in rating systems. Major differences probably resulted from these factors, from use of different accessions of the pertinent species, and perhaps from use of different isolates of the pathogen. Only *N. debneyi* and *N. repanda*, of all species tested, were found to be more susceptible by other workers than by us (11).

We have given greatest weight to the brown spot reaction of older leaves in assessing the relative resistance or susceptibility of the *Nicotiana* species. Stem lesions are less important in the field than the lesions on mature leaves. The pinpoint lesions, occurring on youngest inoculated leaves, become severe only with artifically high inoculum levels, and are of minor consequence at inoculum levels occurring in the field (17). Thus, the practical value of the consistent immunity in the mature leaves of *N. wigandioides* may be equal to the value of the usual immunity in all leaves of *N. bonariensis* and the high level of resistance in all leaves of *N. longiflora, N. noctiflora, N. nesophila, N. repanda, N. stocktonii, N. debneyi,* and *N. suaveolens.*

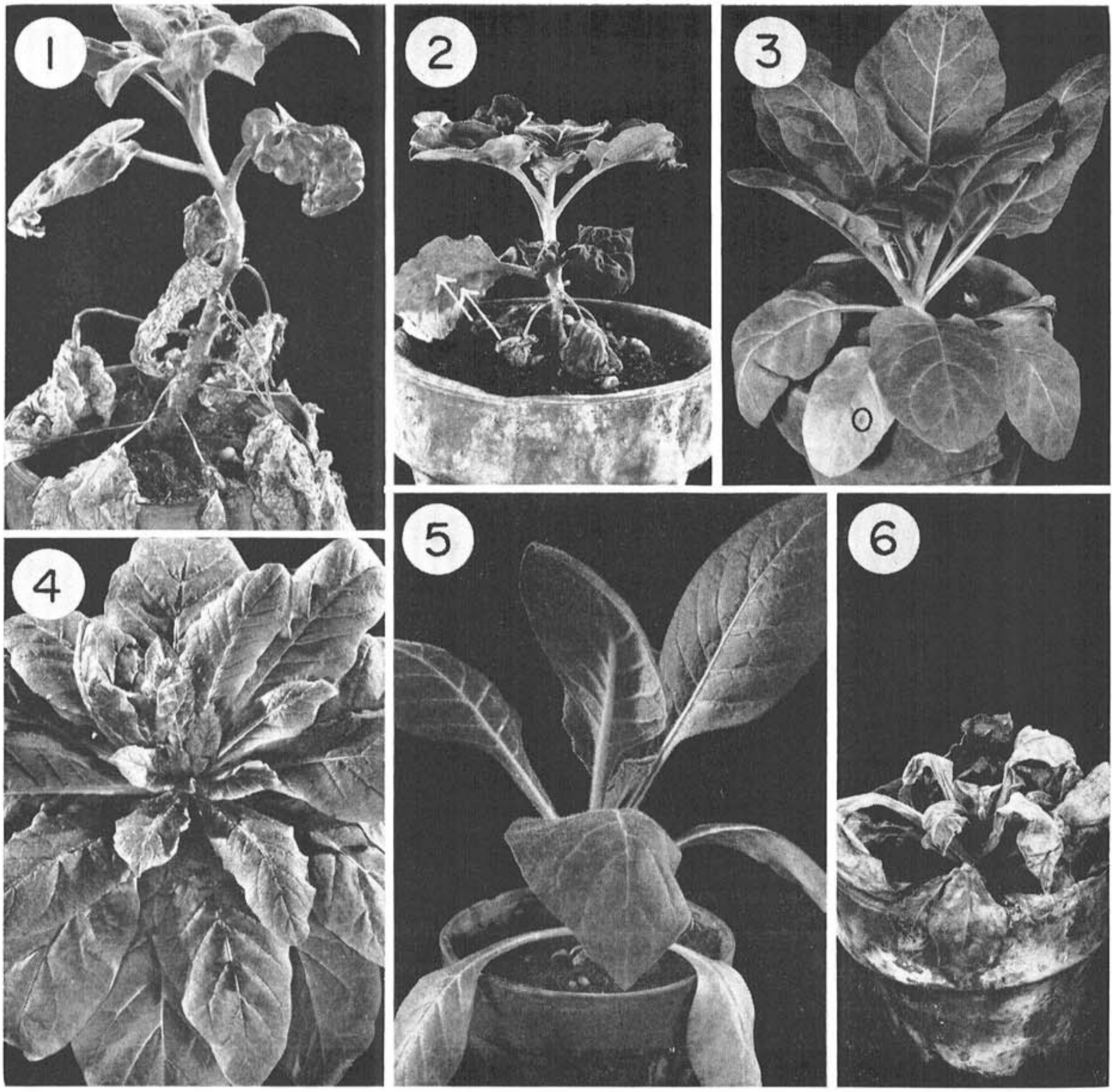

Fig. 1-6. *Nicotiana* species 16-18 days after inoculation at Beltsville with *Alternaria alternata*; **1)** *N. glutinosa* showing severe symptoms; **2)** *N. cordifolia* 55-200. Note lesions visible only from lower leaf surface; **3, 4, 5)** *N. nesophila, N. bonariensis,* and *N. wigandioides,* respectively, showing highly resistant to immune responses; **6)** *N. fragrans* showing extreme reaction to infection.

The brown spot reactions of *Nicotiana* species bore considerable relationship to the taxonomic subdivisions of the genus (6). All of the consistently most resistant species occurred in subgenus Petunioides. Similar disease responses occurred in the species within sections Bigelovianae, Noctiflorae, Repandae, and Trigonophyllae. However, the three species in section Undulatae ranged from mature leaf immunity to moderate susceptibility, and there was a wide range of reactions in the larger sections, Acuminatae, Alatae, and Suaveolentes. The species in subgenera Tabacum and Rustica were all susceptible at Beltsville except *N. thyrsiflora,* which is taxonomically separated at the section level from the other species in subgenus Rustica. All of the species most closely related to *N. tabacum,* which is considered to be an amphiploid originating from a cross between *N. sylvestris* and a member of section Tomentosae (6), were moderately to highly susceptible.

There was no correlation between chromosome number, geographical distribution (6), or the quantities of polyphenols or activity of oxidases (14) of the *Nicotiana* species and their reaction to *A. alternata.*

Clayton concluded, after many years of breeding tobacco for disease resistance, that only a few kinds of resistance justify attempted interspecific transfer. Among these are monogenic-dominant, high-level resistance or immunity; or, if a major disease is involved and adequate resistance cannot be found in *N. tabacum,* high-level polygenic resistance of intermediate dominance (4). Since brown spot is a major disease, and because high-level resistance to brown spot has not been found in *N. tabacum,* we believe that certain *Nicotiana* species may be of value as sources of brown spot resistance. F_1 progeny have been obtained from crosses between *N. tabacum* and certain of the resistant species, including *N. suaveolens, N. longiflora,* and *N. debneyi* (5, 7). Extreme cross sterility between *N. tabacum* and *N. repanda* has been overcome by using a bridge cross, employing *N. sylvestris* as the intermediate, bridging species (2). Hopefully, genes from most of the more resistant species could be transferred to tobacco, with the ultimate development of acceptable cultivars. However, only those species that transmit a high level of resistance to their interspecific F_1 progeny will be useful. The availability of several alternative sources of resistance in the genus is of great potential value.

LITERATURE CITED

1. BURBIDGE, NANCY T. 1960. The Australian species of Nicotiana L. (Solanaceae). Australian J. Bot. 8:342-380.
2. BURK, L. G., & H. E. HEGGESTAD. 1966. The genus Nicotiana: a source of resistance to the diseases of cultivated tobacco. Econ. Bot. 20:76-88.
3. CHAPLIN, J. F., & T. W. GRAHAM. 1963. Brown spot resistance in Nicotiana tabacum. Tobacco Sci. 7:59-62.
4. CLAYTON, E. E. 1954. Identifying disease resistance suited to interspecific transfer. J. Hered. 45:273-277.
5. CLAYTON, E. E. 1958. The genetics and breeding progress in tobacco during the last 50 years. Agron. J. 50:352-356.
6. GOODSPEED, T. H. 1954. The genus Nicotiana. Chronica Botanica Co., Waltham, Mass. 536 p.
7. LUCAS, G. B. 1965. Diseases of tobacco [2nd ed.]. The Scarecrow Press, Inc., N.Y. 778 p.
8. MAIN, C. E., & J. F. CHAPLIN. 1968. Resistance to brown spot in flue-cured tobacco. Phytopathology 58:730 (Abstr.).
9. OKUURA, M., & K. OHBU. 1967. Studies on the control of tobacco brown spot caused by Alternaria longipes (Ell. & Ev.) Mason. IV. The varietal variation of susceptibility to tobacco brown spot. Bull. Utsunomiya Tobacco Exp. Sta. 5:107-134.
10. RAEBER, J. G., & M. A. SCHWEPPENHAUSER. 1966. Susceptibility of tobacco varieties to brown spot, Alternaria longipes (Ell. & Ev.) Mason, p. 599-603. 4th World Tobacco Sci. Congr. Proc. Athens, Greece.
11. RAEBER, J. G., M. A. SCHWEPPENHAUSER, & W. F. T. HARTILL. 1963. Sources of resistance to frogeye and brown spot in the genus Nicotiana, p. 242-247. 3rd World Tobacco Sci. Congr. Proc. Salisbury, Rhodesia.
12. RAMM, C. VON, & G. B. LUCAS. 1963. Variability in susceptibility of Nicotiana species to Alternaria longipes. Plant Dis. Reptr. 47:369-371.
13. RICE, J. C., J. M. KENYON, & E. L. PRICE. 1969. Measured crop performance-tobacco. N.C. State Univ. Res. Rep. 32. 47 p.
14. SHEEN, S. J. 1970. Comparative quantities of polyphenols and oxidases in Nicotiana species. Tobacco Sci. 14:43-46.
15. STAVELY, J. R., & C. E. MAIN. 1970. Influence of temperature and other factors on initiation of tobacco brown spot. Phytopathology 60:1591-1596.
16. STAVELY, J. R., & G. W. PITTARELLI. 1970. Sources of resistance to Alternaria tenuis in the genus Nicotiana. Phytopathology 60:1315 (Abstr.).
17. STAVELY, J. R., & L. J. SLANA. 1971. Relationship of leaf age to the reaction of tobacco to Alternaria alternata. Phytopathology 61:73-78.