PATHOGENICITY AND VEGETATIVE COMPATIBILITY OF *FUSARIUM OXYSPORUM* ISOLATED FROM TOBACCO

By JAMES A. LaMONDIA[1]

Isolates of *Fusarium oxysporum* pathogenic to Connecticut shade and broadleaf tobaccos were separated into two vegetative compatibility groups (VCG 1 and VCG 2) by demonstrating heterokaryosis based upon complementation tests with nitrate-nonutilizing (nit) mutants (pairing nit M and nit A mutants). The two VCG's did not differ in pathogenicity or virulence to shade or broadleaf tobacco types. Isolates from burley tobacco from Kentucky and flue-cured tobacco from North Carolina were placed in VCG 1. Two isolates of *F.o.* f.sp. *batatas* were placed in VCG 2 and two isolates of *F.o.* f.sp. *vasinfectum* were placed in VCG 1. Neither VCG nor source of *Fusarium* isolate was correlated with ability to cause disease on tobacco type, as all pathogenic isolates caused wilt symptoms on broadleaf and burley, but not on flue-cured tobacco.

Additional key words: *Nicotiana tabacum* L.; Fusarium wilt; heterokaryosis.

INTRODUCTION

Fusarium oxysporum (Schlecht) emend. Synd. & Hans. is the causal agent of the most destructive disease of broadleaf tobacco (*Nicotiana tabacum* L.) in the Connecticut River Valley (13). This fungus is widespread in broadleaf tobacco-growing areas of the valley, and serious losses are common.

Resistance is the principal means of controlling Fusarium wilt in tobacco (14). This resistance appears to be conditioned by several genes with additive effects (10), and recently has been incorporated into broadleaf cultivars (13). The source of this wilt resistance was a Connecticut broadleaf cultivar bred for tobacco mosaic virus resistance from dark fire-cured tobacco (18). All Connecticut shade-grown cigar wrapper cultivars tested were resistant but not immune to Fusarium wilt (J. LaMondia, G.S. Taylor, unpublished data). The source of wilt resistance in shade tobacco is unknown.

Previously, four races of *F. oxysporum* pathogenic to tobacco were distinguished by Armstrong and Armstrong (1). These races were defined by ability to cause disease on flue-cured or burley tobacco types as well as on cotton or sweet potato. *F.o. batatas* race 1 is pathogenic to sweet potato and burley tobacco, but not cotton or flue-cured tobacco. Race 2 is pathogenic to sweet potato, burley, and flue-cured tobacco, but not cotton. *F.o. vasinfectum* race 1 is pathogenic to burley tobacco and cotton, but not sweet potato or flue-cured tobacco. *F.O. vasinfectum* race 2 attacks cotton, burley, and flue-cured tobacco, but not sweet potato. It was not known if this resistance, once incorporated into shade or broadleaf tobaccos, would be effective against isolates pathogenic to flue-cured or burley tobacco types, or even which race(s) of *Fusarium* occurred in Connecticut.

In 1987, severe Fusarium wilt symptoms appeared in a single field of shade tobacco which had been under almost continuous tobacco production since 1945. Research was initiated to investigate the cause of this outbreak and/or to determine if a new race or strain of *Fusarium oxysporum* had developed or had been introduced which could threaten shade tobacco production.

Recently, researchers have used vegetative compatibility, the ability of related isolates to form heterokaryons, to subdivide *F. oxysporum* into vegetative compatibility groups (VCG's) (17). *F. oxysporum* VCG's have been correlated with virulence to host plant cultivars (7, 17), colony size on selective media (7), and isozyme patterns (5). Isolates of *F. oxysporum* pathogenic to melon, banana, and tomato have been classified into VCG's based on host and specific locality (8,11,15). Isolates recovered from diseased shade and broadleaf tobacco in the Connecticut River Valley, and others provided by researchers in other tobacco producing areas, were tested to determine their vegetative compatibility group and for ability to cause disease in shade and broadleaf tobacco.

MATERIALS AND METHODS

Eighteen *F. oxysporum* isolates were collected in 1987 and 1988 from wilted tobacco plants throughout the production areas of the Connecticut River Valley. Other researchers collected eleven isolates from wilted flue-cured tobacco in North Carolina and eight isolates from burley tobacco in Kentucky. Isolates were each tested three times for vegetative compatibility and pathogenicity to tobacco. Isolates of *F.o.* f.sp. *batatas* and *F.o.* f.sp. *vasinfectum* were also included (1). Formae speciales identification of cultures by providers was accepted.

[1]*Associate Plant Pathologist, Dept. Plant Pathology and Ecology, Connecticut Agricultural Experiment Station, Valley Laboratory, Windsor, Conn.*
Contribution received April 30, 1990; Tob. Sci. 34: 105-108, 1990.

Table 1. Vegetative compatibility group (VCG) and pathogenicity of Connecticut *Fusarium oxysporum* isolates to Connecticut tobacco types.

F. oxysporum isolate	Tobacco host	VCG	Pathogenicity[a] bdlf 86-4	bdlf 86-8	shade 86	shade 87	$\bar{X}$
A	Broadleaf	2	6	1	5	3	3.8
B		1	10	3	6	5	6.0
C		1	10	3	5	7	6.3
D		1	2	1	3	2	2.0
E		3	0	0	1	0	0.3
F		2	6	4	2	0	3.0
G		2	9	6	6	8	7.3
H		1	11	6	3	1	5.3
I	Shade	1	12	2	4	6	4.5
J		1	10	4	2	2	4.5
K	Control	-	1	0	0	0	0.3
	F.o. batatas						
L	ATCC 10913	1	5	2	0	0	1.8
M	ATCC 16415	1	0	0	0	0	0.0
		$\bar{X}$	6.3	2.5	2.8	2.6	

linear contrasts	significance level
1. check vs. *F. oxysporum*	0.001
2. VCG 1 vs. VCG 2	NS
3. VCG 1 and 2 vs. VCG 3	0.001
4. 86-4 vs. 86-8 tobacco	0.001
5. 86-8 vs. shade tobaccos	NS
6. shade 86 vs. shade 87	NS

[a] Number wilted plants of 14: 86-4 = *Fusarium* susceptible broadleaf; 86-8 = resistant broadleaf; shade 86 = resistant shade; shade 87 = unknown susceptibility.

Surface-disinfested tissues (0.05% NaOCl for 1 minute) taken from stalks of wilt-symptomatic Connecticut shade or broadleaf tobacco plants were plated onto 20% acidified water agar or Komada's medium (12). Single spores of *F. oxysporum* isolates recovered on Komada's media were cultured on potato carrot agar or potato dextrose agar and identified by spore morphology (20).

All *F. oxysporum* isolates were tested for vegetative compatibility using complementation tests with nitrate-nonutilizing (nit) mutants to verify heterokaryon formation (17). Methods for selecting and characterizing nit mutants have been described (6). Complementation tests were conducted by placing five agar plugs (3 mm^3) colonized by nit A mutants (nit 1 or nit 3) of different isolates equidistantly around a nit M mutant from another isolate on a nitrate minimal medium (6). All nit M mutants were paired with nit A mutants from other isolates in all possible combinations. Not all *F. oxysporum* isolates yielded nit M mutants. Plates were incubated at 18-22° and examined weekly for heterokaryon development. Isolates were considered to be vegetatively compatible if the dense aerial mycelium indicative of a heterokaryon developed within three weeks.

F. oxysporum isolates were tested for pathogenicity against a number of tobacco types. *F. oxysporum* isolates from Connecticut were first tested against *Fusarium*-resistant (86-8) and *Fusarium*-susceptible (86-4) broadleaf cultivars, as well as two shade tobacco cultivars (shade 86 and 87). Shade 87 had a number of plants with Fusarium wilt symptoms under field conditions in 1987. Shade 86 did not have symptomatic plants when grown in the same field the previous year. *F. oxysporum* isolates producing conidia in broth cultures were tested against Fusarium-susceptible broadleaf (86-4), burley (B-21), and flue-cured (C-319) cultivars in later greenhouse pathogenicity tests. Each plant was inoculated with $1.0x10^7$ microconidia applied in suspension to a 1-cm deep 1-cm wide hole in the soil surface approximately 6 wk after seeding. Microcodidia were produced in potato carrot broth shake culture for 36 hr at 20°C. Potato carrot broth was made by autoclaving 10 g potato tissue, 10 g carrot tissue, and 10 g sucrose in 1 L distilled water for 15 minutes, filtering through cheesecloth, and autoclaving again. There were 14 replicate plants per isolate. Uninoculated control plants received water but no fungi in the same manner as inoculated plants. Plants were grown in the greenhouse and rated for wilt symptoms by a subjective rating scale of 0-4 (0 = healthy plants, 1 = stunted or off-color plants, 2 = plants with one symptomatic leaf, 3 = plants with more than one symptomatic leaf, 4 = dead plants) 5 wk after inoculation and the incidence of symptomatic plants recorded.

RESULTS

Isolates of *F. oxysporum* originating from Connecticut broadleaf or shade tobacco were separated into three VCG's and rated for pathogenicity (**Table 1**). Of 10 isolates tested, six were grouped in VCG 1. This group included isolates from both wilted shade and broadleaf plants. Three isolates were grouped in VCG 2, and a single isolate paired with itself but not any VCG 1 or 2 testers and was placed in VCG 3. Pathogenicity, as measured by incidence of wilted plants, was not significantly different between VCG's 1 and 2, but the isolate comprising VCG 3 was nonpathogenic. The pathogenic isolates caused significantly more disease on the *Fusarium*-susceptible broadleaf cultivar 86-4 than on the resistant broadleaf cultivar 86-8 or on the two shade tobacco cultivars tested. There were no effects of *Fusarium* isolate source (host) on the incidence of wilt on shade or broadleaf tobacco types.

Thirty-two *F. oxysporum* isolates from Connecticut, Kentucky, and North Carolina and two isolates each of *F.o.* f.sp. *batatas* and *F.o.* f.sp. *vasinfectum* were tested for vegetative compatibility. Isolates not producing conidia in shake cultures were not tested for pathogenicity. Of 13 Connecticut *F. oxysporum* isolates which paired with testers, six were placed in VCG 1 and seven were placed in VCG 2. Five isolates from Kentucky (62%) and eight from North Carolina (73%) were placed in VCG 1. Both isolates of *F.o.* f.sp. *batatas* (ATCC 10913 and ATCC 16415) were placed in VCG 2, and both isolates of *F.o.* f.sp. *vasinfec-*

Table 2. Incidence and severity of Fusarium wilt caused by *Fusarium oxysporum* isolates from CT, KY and NC on broadleaf, burley and flue-cured tobaccos.

Fusarium oxysporum			Wilt incidence[a]				Wilt severity[b]			
source	VCG	# tested	86-4	B-21	C-319	$\bar{X}$	86-4	B-21	C-319	$\bar{X}$
CT	1	5	5.0	8.0	0.0	4.3	1.2	1.8	0.0	1.0
CT	2	4	6.5	5.0	0.0	3.8	1.7	1.1	0.0	0.9
KY	1	5	4.0	5.0	0.0	3.0	1.1	1.1	0.0	0.7
NC	1	8	4.8	4.0	0.3	3.0	1.3	0.9	0.0	0.7
control	-	-	0.0	1.0	0.0	0.3	0.0	0.0	0.0	0.0
					$LSD_{0.05}$ = 1.5				$LSD_{0.05}$ = 0.4	
		$\bar{X}$ =	4.2	4.5	0.1		1.1	1.0	0.0	
				$LSD_{0.05}$ = 2.2				$LSD_{0.05}$ = 0.5		

[a] Number wilt symptomatic plants of 14, mean of number tested.
[b] Severity scale 0-4: 0 = healthy, 4 = dead,
86-4 = CT broadleaf, B-21 = KY burley, C-319 = NC flue-cured tobacco.

tum paired with VCG 1 testers.

The incidence and severity of Fusarium wilt was not significantly different between *Fusarium* VCG's or between sources of isolates (**Table 2**). Both incidence and severity were greater on broadleaf and burley than on flue-cured tobacco. Vegetative compatibility pairing and pathogenicity of *F. oxysporum* isolates to broadleaf, burley, and flue-cured experiments were repeated additional times with similar results.

DISCUSSION

Severe *Fusarium* wilt of broadleaf tobacco in Connecticut is a disease complex resulting from root infection by *F. oxysporum* and the tobacco cyst nematode, *Globodera tabacum tabacum* (13,16). The incidence of *Fusarium* wilt associated with commercially grown shade tobacco in 1987 appears to have resulted from high *F. oxysporum* densities in soil, infection of roots by *G.t. tabacum*, and suitable environmental conditions, rather than the result of a new race. The shade cultivar that was affected had resistance levels similar to other shade and *Fusarium*-resistant broadleaf cultivars, but like other *Fusarium*-resistant cultivars, it was not immune (9). Pathogenic wilt *Fusaria* can asymptomatically infect resistant plants (2), which could lead to increased inoculum densities under repeated tobacco cultivation.

At least two different VCG's contain isolates of *F. oxysporum* that are pathogenic to tobacco in the Connecticut River Valley. While these fungi may differ genetically in VCG loci, they did not differ in pathogenicity or virulence to Connecticut broadleaf or shade tobacco types.

F. oxysporum VCG 1 contained isolates from Connecticut broadleaf and shade tobacco as well as isolates from burley tobacco in Kentucky and flue-cured tobacco from North Carolina. *F. oxysporum* VCG 2 contained isolates from Connecticut and isolates identified as *F.o.* f.sp. *batatas*. Neither VCG nor host tobacco type of *F. oxysporum* isolate was correlated with ability to cause disease on tobacco differentials, as all pathogenic isolates caused wilt symptoms on both broadleaf and burley tobaccos but not on flue-cured tobacco. Loss of virulence and pathogenicity in culture may be possible for all isolates, but the results of Armstrong and Armstrong(3,4) suggest that factors determining pathogenicity to flue-cured tobacco may be more unstable than for other crops, and may be greatly reduced in relatively short periods of time.

The problems associated with using differential host plants for race identification include difficulties in exactly duplicating experimental conditions, frequent loss of pathogenicity in culture, and differences in virulence of individual isolates. These make the identification of *F. oxysporum* races pathogenic to tobacco difficult to determine. In our experiments, a number of *F. oxysporum* cultures from tobacco and isolates of *F. oxysporum* f.sp. *batatas* and *vasinfectum* lost the ability to cause wilt symptoms on tobacco. Loss of pathogenicity to one or more hosts is a common occurrence for these fungi in culture (1, 19). Further complexity is added by substituting virulence (how much disease occurs) for pathogenicity (ability to cause disease). Everette (9) tested a series of *F. oxysporum* isolates against a number of tobacco cultivars. Certain tobacco cultivars were highly resistant to one isolate but had varying degrees of susceptibility to others. He differentiated at least 17 strains or races of *F. oxysporum* in his collection based on the virulence of individual isolates to different tobacco cultivars.

The importance of race identification may not be readily apparent for burley tobacco as it is susceptible to all four races of *F. oxysporum* attacking tobacco (3). This may also be true for broadleaf tobacco. Rather, race identification may be more important for selection of cover or rotation crops. Because many wilt-*Fusaria* are nonspecific in pathogenicity on several hosts which are not closely related to tobacco, cover crops or crops grown in rotation with tobacco may be susceptible or non-susceptible hosts of these wilt fungi (2,3). The host status of these crops may result in very different inoculum potentials for subsequent tobacco crops.

Connecticut broadleaf tobacco types are susceptible and shade tobacco types are resistant to both VCG's of *F. oxysporum* identified in these experiments. It remains to be seen if the two VCG's identified in these experiments may be correlated with pathogenic races of *F. oxysporum* (1). If so, race determination could be greatly simplified by vegetative compatibility grouping.

ACKNOWLEDGEMENTS

The author is grateful to Dr. H.D. Shew, Eric Tedford, and Dr. W.H. Elmer for providing isolates of *Fusarium oxysporum* and seed of selected tobacco cultivars. Appreciation is also extended to Jane Canepa-Morrison and Stanley Rutkowski for excellent technical assistance.

LITERATURE CITED

1. Armstrong, G.M., and J.K. Armstrong. Formae speciales and races of *Fusarium oxysporum* causing a tracheomycosis in the syndrome of disease. **Phytopathology** 58:1242-1246. 1968.
2. Armstrong, G.M., and J.K. Armstrong. Nonsusceptible hosts as carriers of wilt *Fusarium*. **Phytopathology** 38:808-826. 1948.
3. Armstrong, G.M., and J.K. Armstrong. The *Fusarium* wilt complex as related to the sweet potato. **Pl. Dis. Reptr.** 42:1319-1329. 1958.
4. Armstrong, J.K., and G.M. Armstrong. A race of the cotton-wilt *Fusarium* causing wilt of Yelredo soybean and flue-cured tobacco. **Pl.**

Dis. Reptr. 42:147-151. 1958.

5. Bosland, P.W., and P.H. Williams. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. **Can. J. of Bot.** 65:2067-2073. 1987.

6. Correll, J.C., C.J.R. Klittich, and J.F. Leslie. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetable compatibility tests. **Phytopathology** 77:1640-1646. 1987.

7. Correll, J.C., J.E. Puhalla, and R.V. Schneider. Identification of *Fusarium oxysporum* f.sp. *apii* as the basis of virulence, colony size, and vegetative compatibility. **Phytopathology** 76:396-400. 1986.

8. Elias, K.S., and R.W. Schneider. Genetic diversity within *Fusarium oxysporum* f.sp. *lycopersici* as determined by vegetative compatibility (heterokaryosis). **Phytopathology** 76:1129. (Abstr). 1986.

9. Everette, G.A. Strains of *Fusaria* and their effect on tobacco varieties. **Tob. Sci.** 2:35-40. 1958.

10. Gritton, E.T., G.L. Jones, N.T. Powell, and D.F. Matzinger. Inheritance of resistance to *Fusarium* wilt in flue-cured tobacco. **Crop Sci.** 5:547-550. 1965.

11. Jacobson, D.J., and T.R. Gordon. Vegetative compatibility and self incompatibility within *Fusarium oxysporum* f. sp. *melonis*. **Phytopathology** 78:668-672. 1988.

12. Komada, H. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. **Rev. Plant Prot. Res.** 8:114-124. 1975.

13. LaMondia, J.A., and G.S. Taylor, Influence of the tobacco cyst nematode (*Globodera tabacum*) on *Fusarium* wilt of Connecticut broadleaf tobacco. **Plant Dis.** 71:1129-1132. 1987.

14. Lucas, G.B. *Diseases of Tobacco*. 3rd. Ed. H.E. Parker and Sons, Fuquay-Varina, N.C. 1975.

15. Ploetz, R.E., and J.C. Correll. Vegetative compatibility among races of *Fusarium oxysporum* f.sp. *cubense*. **Plant Dis.** 72:325-328. 1988.

16. Powell, N.T. Interactions between nematodes and fungi in disease complexes. **Ann. Rev. Phytopathol.** 9:253-274. 1971.

17. Puhalla, J.E. Classification of *Fusarium oxysporum* on the basis of vegetative compatibility. **Can. J. Bot.** 63:179-183. 1985.

18. Sand, S.A., and G.S. Taylor. *C2*, A new Mosaic resistant Connecticut broadleaf tobacco. CAES Bulletin 636, 1961.

19. Smith, T.E., and K.J. Shaw. Pathogenicity studies with *Fusaria* isolated from tobacco, sweet potato, and cotton. **Phytopathology** 33:469-483. 1943.

20. Toussoun, T.A., and P.E. Nelson. A *Pictorial Guide to the Identification of Fusarium Species According to the Taxonomic System of Snyder and Hansen*. Pennsylvania State University Press. 43 pp. 1976.