The Relationship Between Formae Speciales, Races, and Vegetative Compatibility Groups in *Fusarium oxysporum*

James C. Correll

Department of Plant Pathology, University of Arkansas, 217 Plant Science Building, Fayetteville 72701.
Accepted for publication 4 February 1991 (submitted for electronic processing).

Fusarium oxysporum is a common soilborne plant pathogen with a worldwide distribution. As a species, it probably causes more economic damage to agricultural crops than any other plant pathogen. Within the species, however, there is a high level of host specificity with over 120 described formae speciales and races capable of causing vascular wilt diseases of many agricultural crops (2). Historically, strains of *F. oxysporum* have been divided into formae speciales on the basis of virulence on a particular host or group of hosts. Further subdivisions of formae speciales into races often are made based on virulence to a particular set of differential host cultivars that vary in disease resistance.

Although virulence has been an extremely useful characteristic for differentiating strains of *F. oxysporum,* there are some inherent problems associated with characterizing strains based solely on pathogenicity. Groupings based on host-pathogen interaction (i.e., virulence) are dictated by the genetic makeup of the host or simply the differential cultivars one has available to distinguish strains. Pathogenicity tests also can be somewhat subjective as they are influenced by many variables, such as temperature (39), host age (15), and method of inoculation (24). For example, up to 11 races of *F. oxysporum* f. sp. *pisi* initially were identified on the basis of subtle differences in disease severity between strains (1). Furthermore, classification of strains based solely on pathogenicity precludes the characterization of nonpathogenic strains of *F. oxysporum.* Nonpathogenic strains represent a major component of the fungal microflora of agricultural soils, and an understanding of diversity in this portion of the population must be developed if we are to fully understand diversity among the virulent strains of this species.

More recently, due to work initiated by Puhalla (24), strains of *F. oxysporum* of various formae speciales have been grouped on the basis of vegetative compatibility. This approach provides a means of characterizing subspecific groups based on the genetics of the fungus rather than on the host-pathogen interaction. In addition, vegetative compatibility allows for the characterization of the nonpathogenic portion of the population. Over the past 5 yr, a considerable amount of research has been conducted on vegetative compatibility in *F. oxysporum.* This work, coupled with the integrated use of powerful molecular tools, has greatly helped in our current understanding of the pathology, population biology, and race relationships of this organism. However, our understanding of this complex fungal pathogen is far from complete.

It is generally assumed that *F. oxysporum* has a set of vegetative incompatibility (*vic*) (or heterokaryon incompatibility [*het*]) loci that are similar to the closely related species, *F. moniliforme* (*Gibberella fujikuroi*). In *F. moniliforme,* a minimum of ten *vic* loci control vegetative compatibility (27,37). Vegetative compatibility in these fungi is considered homogenic, meaning that two fungal strains are only vegetatively compatible if the alleles at each of the corresponding *vic* loci are identical. Theoretically, 1,024 (2^{10}) distinct vegetative compatibility groups (VCGs) could exist (assuming only two alleles occur at each *vic* locus) in a population of *F. moniliforme.*

Strains of *F. oxysporum* readily can be tested for vegetative compatibility by pairing nitrate nonutilizing (*nit*) mutants that are generated on media containing 1.5–4.0% potassium chlorate (6,36). Colony growth on this medium usually is greatly restricted. After 7–14 days, fast growing, chlorate-resistant sectors usually can be observed originating from the initially restricted colony. When these chlorate-resistant sectors are grown on a minimal medium containing nitrate nitrogen as the only nitrogen source, they typically have a thin expansive morphology with no aerial mycelium indicative of a *nit* mutant. *Nit* mutants then can be phenotypically classified by their growth on a basal medium amended with one of several different nitrogen sources. Phenotypically distinct mutants (particularly *nit*1 and NitM mutants), when paired on minimal medium containing nitrate as the sole nitrogen source, will produce a zone of wild-type growth (aerial mycelium) where the two *nit* mutant colonies come in contact. This occurs as a result of hyphal fusion and nutritional complementation in the heterokaryotic cells. These complementary *nit* mutant testers then can be used to test other strains for vegetative compatibility. A heterokaryon can form only between *nit* mutants of vegetatively compatible strains. Thus, isolates that are vegetatively compatible belong to the same VCG.

The limitations of this technique depend on the particular forma specialis or group of strains being examined. Specific limitations include difficulty in recovering *nit* mutants from certain isolates on chlorate-containing media, weak heterokaryon reactions observed between *nit* mutants of some strains (12,14), cross-compatibility reactions observed between certain isolates and different VCGs (33), and the presence of heterokaryon, or vegetatively, self-incompatible isolates (7,8,16).

Several patterns of VCG diversity have been identified in *F. oxysporum.* Initially, Puhalla (36) found that there was a correlation between VCG and forma specialis based on the examination of a limited number of strains. Isolates in the same VCG belonged to the same forma specialis and strains in different formae speciales were in different VCGs. The subsequent examination of numerous formae speciales has supported Puhalla's (33) initial generalization for the most part, but it has revealed that the relationships between formae speciales, race, and VCG in *F. oxysporum* can vary from relatively simple to rather complex, depending on the particular formae speciales.

Within several formae speciales, it has been demonstrated that the relationship between race and VCG is rather simple, where all isolates of a given race, even from a widespread geographical area, belong to the same VCG. For example, with *F. o. apii* race 2 (8), a pathogen of celery, and *F. o. vasinfectum* race 3 (20), a pathogen of cotton, a large collection of isolates from diverse geographical locations each correspond to a single VCG (Table 1). Isolates of *F. oxysporum* pathogenic to crucifers also could be divided into three distinct VCGs, with each VCG containing isolates pathogenic to a given crucifer host (3). Among isolates of *F. o. niveum,* a pathogen of watermelon, three VCGs have been identified (25). All race 2 isolates belong to a single VCG, whereas race 1 isolates from throughout the United States (all areas except Florida), Australia, and Taiwan belong to a second VCG, and the third VCG consists of all watermelon isolates from Florida. With the above formae speciales, it is possible to use vegetative compatibility, with some limitations, as a method for identifying and differentiating formae speciales and races of these pathogens.

Detailed examination of several other formae speciales has

revealed a much more complex relationship between formae speciales, race, and VCG. Within certain formae speciales, there are cases where more than one race may occur within a single VCG and others where isolates of a single race may belong to several different VCGs. For example, four VCGs have been identified among isolates pathogenic to pea (10,26). Isolates of races 1 and 6 of *F. o. pisi* both are in a single VCG, race 5 is in a second VCG, and race 2 isolates occur in at least two additional VCGs (Table 1). *F. o. melonis* and *F. o. cubense,* pathogens of melon and banana, respectively, also have a very complex race-VCG relationship. Eight VCGs have been characterized in *F. o. melonis* with one VCG (0134) containing isolates from four different virulence phenotypes or races (16). Eleven VCGs have been characterized in *F. o. cubense* from a worldwide collection (34,35); multiple races have been found to occur in a single VCG and multiple VCGs exist for a given race. A similar degree of complexity of race and VCG occurs within *F. o. lycopersici;* in one case, the three known races are found within a single VCG and others where a single race is composed of multiple VCGs (11). Where there is a complex race-VCG relationship within a forma specialis, vegetative compatibility cannot be used to identify races. However, even in these instances, vegetative compatibility still can be quite useful in distinguishing pathogens from nonpathogens as well as characterizing genetic diversity within the pathogen population.

F. o. asparagi and race 1 isolates of *F. o. lycopersici,* pathogens of asparagus and tomato, respectively, each have a unique VCG diversity compared with other formae speciales studied thus far. Over 46 distinct VCGs have been identified among a collection of isolates pathogenic to asparagus in greenhouse pathogenicity tests (12). Likewise, race 1 isolates of *F. o. lycopersici* were found to belong to at least 41 different VCGs (11). This very high level of VCG diversity found within *F. o. asparagi* and *F. o. lycopersici* may be similar to the VCG diversity that has been found among the nonpathogenic strains examined colonizing celery roots (9) or from soil (13). The high degree of VCG diversity found among nonpathogenic strains of *F. oxysporum* may be particularly useful in studies where nonpathogenic strains have been used as biological control agents (28,32,38). Specific strains, where VCG could be used as a naturally occurring marker, could be examined in studies with field soil with a typically high background population of *F. oxysporum.*

Kistler and Momol (24) proposed several general models to explain the evolutionary relationship between formae speciales and VCG in *F. oxysporum.* The models incorporate events involving host specialization and genetic isolation to explain the race-VCG diversity that exists within this plant pathogenic fungus. In their models, host specialization is defined as the ability to cause disease on a given host. In a broader sense, host specialization can be defined as the ability of the fungus to parasitize a host without necessarily inciting disease (19,31). It is with this basic premise that I would like to propose several working models to help explain the degree of VCG diversity thus far observed in *F. oxysporum.*

One assumption I have made is that the parasitic, but nonpathogenic, portion of the population may represent some primitive or "basal" population structure of this species (4). From this primitive population, which has a high degree of VCG diversity, mutations to virulence may occur among isolates of the various VCGs. Probably the vast majority of these mutations would never result in disease due to the low probability that an isolate in which the mutation occurred would be in close proximity to a susceptible host. However, if a mutation occurred in an isolate that was in close proximity to a susceptible host (i.e., the roots or possibly the vascular system as a nonpathogenic parasite), then this isolate may proliferate and lead to an epidemic. Further mutations to alter virulence then could occur within this virulent VCG as resistance genes are deployed to combat the existing virulent phenotype. There is some circumstantial evidence that this may have been observed in a population of race 1 of *F. o. lycopersici* (13). Mutations to alter virulence also may occur even in the absence of the selection pressure imposed by introducing resistance genes.

Models I, II, and III (Fig. 1) diagrammatically represent how changes in virulence could result in various patterns of race-VCG

TABLE 1. The relationship between formae speciales, race, vegetative compatibility group, and mitochondrial DNA restriction fragment length polymorphism pattern of *Fusarium oxysporum*

Formae speciales	Race designation	Number of VCGs identified	RFLP patterns identified[a]	Model[b]
apii	2	1	···	I
asparagi	none	>46	···	II?
conglutinans	1	1	A	II
	2			
matthioli	1	1	B	II
	2			
raphani	1	1	C	I
cubense	1	6	···	II
	2	4	···	II
	4	4	···	II,IV
dianthi	2	2	A,B	III
lycopersici	1,2,3	1	···	III
	1,2	2	···	II,III
	1	41	···	II?
	2	14	···	II
	3	2	···	II
melonis	0,1,2,(1,2y)	1	D	III
	1	2	D,F	II
	2	2	A	IV
	2	1	B	I
	(1,2w)	1	C	I
	0	1	E	I
niveum	1	2	···	II
	2	1	···	I
pisi	1,6	1	A	III
	2	2	B,C	II
	5	1	D	I
vasinfectum	3	1	···	I

[a] Patterns are from references (17,21,26,29). Different letters within a forma specialis indicate different RFLP patterns.
[b] The race-VCG models are diagrammatically outlined in Figure 1.

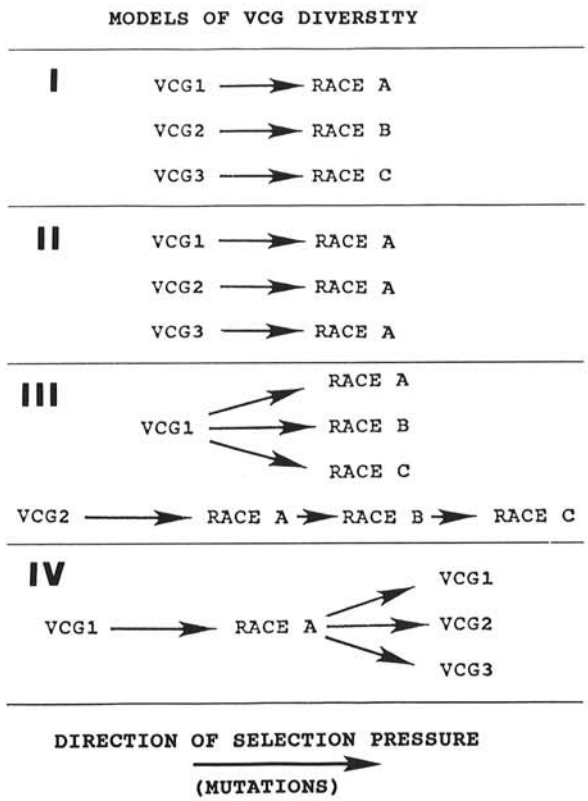

Fig. 1. Models of race-VCG diversity in *Fusarium oxysporum.*

diversity. Indeed, examples of all three patterns of race-VCG diversity have been identified in the various formae speciales examined thus far. In addition, these three models are supported by mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) and genomic DNA RFLP data (5,17,18, 21–23,29,30). In general, mtDNA RFLP patterns are similar or identical within a VCG, but quite different between VCGs, even VCGs of the same formae speciales. For example, in the cases where there are multiple races within a single VCG (*conglutinans* [races 1 and 2], *matthioli* [races 1 and 2], *pisi* [races 1 and 6], and *melonis* [VCG 0134-races 0, 1, 2, and (1,2y)]), each has only a single mtDNA RFLP pattern, indicating that the races within a forma specialis most likely originated from a common genetic background (i.e., the same VCG) (Table 1).

Forma specialis is a very useful grouping of primary importance to plant pathologists. However, in many cases, forma specialis appears to be a grouping of genetically diverse isolates possibly polyphyletic in origin. There are, however, instances where different VCGs within a forma specialis are probably monophyletic in origin, and these are represented in model IV (Fig. 1). There is evidence that this may have occurred within VCGs 0130 and 0131 of *F. o. melonis* (17,18). For example, these two VCGs have the same virulence phenotype and identical mtDNA RFLP patterns. Thus, a change in vegetative compatibility group phenotype, possibly a single gene mutation, could have resulted in vegetatively incompatible isolates within this group that eventually diverged into two distinct VCGs. Race 4 isolates of *F. o. cubense,* which belong to two distinct VCGs (0120 and 0129), also have a similar mtDNA RFLP pattern suggestive of a common origin (33). There also is preliminary evidence of "newer" VCGs originating from populations of "older" VCGs in *F. o. cubense* (33,34). Although it is remotely possible that different VCGs that have polyphyletic origins could have very similar or identical mtDNA patterns, this appears to be less likely than a change occurring in the VCG phenotype.

The pattern of genetic diversity identified within a given forma specialis may have a direct bearing on breeding for resistance. For example, if a particular race of a pathogen belongs to several different VCGs, this race may be genetically very heterogeneous (models II and III). It is not currently known if the mechanism for virulence to a particular host is identical, similar, or different within a genetically diverse forma specialis.

The models presented are simplified, but partially may explain the race-VCG diversity identified thus far. Based on these observations, one would assume that virulence, VCG phenotypes, and mtDNA are all changing independently of one another and at different rates (18,33). Consequently, caution should be used in making any generalizations about race and VCG diversity in *F. oxysporum* based on the examination of a relatively small number of formae speciales. It is hoped that the next five years will be as productive as the past five in advancing our understanding of the biology of this cosmopolitan fungus.

LITERATURE CITED

1. Armstrong, G. M., and Armstrong, J. K. 1974. Races of *Fusarium oxysporum* f. sp. *pisi,* causal agents of wilt of pea. Phytopathology 64:849-857.
2. Armstrong, G. M., and Armstrong, J. K. 1981. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. Pages 391-399 in: *Fusarium*: Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park. 457 pp.
3. Bosland, P. W., and Williams, P. H. 1987. An evaluation of *Fusarium oxysporum* from crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. Can. J. Bot. 65:2067-2073.
4. Brasier, C. M. 1987. The dynamics of fungal speciation. Pages 231-260 in: Evolutionary Biology of the Fungi. A. D. M. Rayner, C. M. Brasier, and D. Moore, eds. Cambridge University Press, Cambridge. 465 pp.
5. Coddington, A., Matthews, P. M., Cullis, C., and Smith, K. H. 1987. Restriction digest patterns of total DNA from different races of *Fusarium oxysporum* f. sp. *pisi*–An improved method for race classification. J. Phytopathol. 118:9-20.
6. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. Phytopathology 77:1640-1646.
7. Correll, J. C., Klittich, C. J. R., and Leslie, J. F. 1989. Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). Mycol. Res. 93:21-27.
8. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Identification of *Fusarium oxysporum* f. sp. *apii* on the basis of colony size, virulence, and vegetative compatibility. Phytopathology 76:396-400.
9. Correll, J. C., Puhalla, J. E., and Schneider, R. W. 1986. Vegetative compatibility groups among nonpathogenic root-colonizing strains of *Fusarium oxysporum.* Can. J. Bot. 64:2358-2361.
10. Correll, J. C., Puhalla, J. E., Schneider, R. W., and Kraft, J. M. 1985. Differentiating races of *Fusarium oxysporum* f. sp. *pisi* based on vegetative compatibility. (Abstr.) Phytopathology 75:1347.
11. Elias, K. S., and Schneider, R. W. 1991. Vegetative compatibility groups (heterokaryons) in *Fusarium oxysporum* f. sp. *lycopersici.* Phytopathology 81:159-162.
12. Elmer, W. H., and Stephens, C. T. 1989. Classification of *Fusarium oxysporum* f. sp. *asparagi* into vegetatively compatible groups. Phytopathology 79:88-93.
13. Gerdemann, J. W., and Finley, A. M. 1951. The pathogenicity of races 1 and 2 of *Fusarium oxysporum* f. *lycopersici.* Phytopathology 41:238-244.
14. Gordon, T. R., and Okamoto, D. 1991. Vegetative compatibility groupings in a local population of *Fusarium oxysporum.* Can. J. Bot. 69:168-172.
15. Hart, L. P., and Endo, R. M. 1981. The effect of time of exposure to inoculum, plant age, root development, and root wounding on Fusarium yellows of celery. Phytopathology 71:77-79.
16. Jacobson, D. J., and Gordon, T. R. 1988. Vegetative compatibility and self-incompatibility within *Fusarium oxysporum* f. sp. *melonis.* Phytopathology 78:668-672.
17. Jacobson, D. J., and Gordon, T. R. 1990. The variability of mitochondrial DNA as an indicator of relationships between populations of *Fusarium oxysporum* f. sp. *melonis.* Mycol. Res. 94:734-744.
18. Jacobson, D. J., and Gordon, T. R. 1991. *Fusarium oxysporum* f. sp. *melonis:* A case study of diversity within a forma specialis. Phytopathology 81:000-000.
19. Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. Phytopathology 61:1213-1217.
20. Katan, T., and Katan, J. 1988. Vegetative-compatibility grouping of *Fusarium oxysporum* f. sp. *vasinfectum* from tissue and the rhizosphere of cotton plants. Phytopathology 78:852-855.
21. Kistler, H. C., Bosland, P. W., Benny, U., Leong, S., and Williams, P. H. 1987. Relatedness of strains of *Fusarium oxysporum* from crucifers measured by examination of mitochondrial and ribosomal DNA. Phytopathology 77:1289-93.
22. Kistler, H. C., and Momol, E. A. 1990. Molecular genetics of plant pathogenic *Fusarium oxysporum.* Pages 49-54 in: Fusarium Wilt of Banana. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN. 140 pp.
23. Kistler, H. C., Momol, E. A., and Benny, U. 1991. Repetitive genomic sequences for determining relatedness among strains of *Fusarium oxysporum.* Phytopathology 81:331-336.
24. Kraft, J. M., and Haglund, W. A. 1978. A reappraisal of the race classification of *Fusarium oxysporum* f. sp. *pisi.* Phytopathology 68:273-275.
25. Larkin, R. P., Hopkins, D. L., and Martin, F. N. 1990. Vegetative compatibility within *Fusarium oxysporum* f. sp. *niveum* and its relationship to virulence, aggressiveness, and race. Can. J. Microbiol. 36:352-358.
26. Leong, S. A., and Holden, D. W. 1989. Molecular genetics of pathogenesis. Annu. Rev. Phytopathol. 27:462-481.
27. Leslie, J. F. 1991. Mating populations in *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 81:1058-1060.
28. Louvet, J., Alabourette, C., and Rouxel, F. 1981. Microbiological suppressiveness of some soils to Fusarium wilts. Pages 261-275 in: *Fusarium:* Diseases, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. Pennsylvania State University Press, University Park. 457 pp.
29. Manicom, B. Q., Bar-Joseph, M., Kotze, J. M., and Becker, M. M. 1990. A restriction fragment length polymorphism probe relating vegetative compatibility groups and pathogenicity in *Fusarium oxysporum* f. sp. *dianthi.* Phytopathology 80:336-339.
30. Manicom, B. Q., Bar-Joseph, M., Rosner, A., Vigodsky-Haas, H., Kotze, J. M. 1987. Potential applications of random DNA probes and restriction fragment length polymorphisms in the taxonomy of

the Fusaria. Phytopathology 77:669-72.
31. Menzies, J. G., Koch, C., and Seywerd, F. 1990. Additions to the host range of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. Plant Dis. 74:569-572.
32. Paulitz, T. C., Park, C.-S., and Baker, R. 1987. Biological control of Fusarium wilt of cucumber with non-pathogenic isolates of *Fusarium oxysporum*. Can. J. Microbiol. 33:349-353.
33. Ploetz, R. C. 1990. Population biology of *Fusarium oxysporum* f. sp. *cubense*. Pages 63-75 in: Fusarium Wilt of Banana. R. C. Ploetz, ed. American Phytopathological Society, St. Paul, MN. 140 pp.
34. Ploetz, R. C. 1990. Variability in *Fusarium oxysporum* f. sp. *cubense*. Can. J. Bot. 68:1357-1363.
35. Ploetz, R. C., and Correll, J. C. 1988. Vegetative compatibility among races of *Fusarium oxysporum* f. sp. *cubense*. Plant Dis. 72:325-328.
36. Puhalla, J. E. 1985. Classification of strains of *Fusarium oxysporum* on the basis of vegetative compatibility. Can. J. Bot. 63:179-183.
37. Puhalla, J. E., and Spieth, P. T. 1985. A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi* (*Fusarium moniliforme*). Exp. Mycol. 9:39-47.
38. Schneider, R. W. 1984. Effect of nonpathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f. sp. *apii* and a novel use of the Lineweaver-Burk double reciprocal plot technique. Phytopathology 74:646-653.
39. Williams, P. H., ed. 1981. Fusarium yellows. Pages 124-129 in: Screening Crucifers for Multiple Disease Resistance. University of Wisconsin, Madison.