Recent Advances in *Fusarium* Systematics

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

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*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 microbiota 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 [her]) 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 homogamic, 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 nitrate (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 nitI 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 nitI 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 formae speciales 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 formae speciales based on the examination of a limited number of strains. Isolates in the same VCG belonged to the same formae speciales 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 specific 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. aii* 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 specialia, 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 non-
pathogens 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 specialia 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 specialia
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 in 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 non-
pathogenic, 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 intro-
ducing resistance genes.

Models I, II, and III (Fig. 1) diagrammatically represent how
changes in virulence could result in various patterns of race-VCG
diversity.
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 (1,2)]) each has only a single mtDNA RFLP pattern, indicating that the races within a formae specialis most likely originated from a common genetic background (i.e., the same VCG) (Table 1).

Formae specialis is a very useful grouping of primary importance to plant pathologists. However, in many cases, formae specialis appears to be a grouping of genetically diverse isolates possibly polyphyletic in origin. There are, however, instances where different VCGs within a formae 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 formae 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 formae 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