Recent Advances in *Fusarium* Systematics

Current Status of *Fusarium* Taxonomy

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All *Fusarium* species have one taxonomic feature in common: production of distinctly shaped macroconidia, usually with a foot-shaped basal cell, when produced in sporodochia (5). This stable feature, when combined with other primary and secondary criteria, composes the basis for the classical approach to *Fusarium* taxonomy. Several taxonomic keys and manuals have been developed to accommodate these criteria (3,11,12,18,19,22,27–30,33).

A fundamental problem inherent in *Fusarium* identification is that members of the genus vary widely in morphological and nonmorphological characteristics, including virulence, and these criteria are used in taxonomic systems. Several taxa within the genus are recognized by the International Code of Botanical Nomenclature (based on morphology) and include section, species, and variety. However, some subdivisions within *Fusarium* species are based on physiology (formae speciales and races) or genetics (vegetative compatibility groups) that are not recognized by the Code according to Article 4.3 (13).

In this discussion, several gaps and problematic areas in the current systems of *Fusarium* taxonomy and identification will be considered. These items include the anamorph-teleomorph connection, section relationships, species delimitation, mutational variants, and subgroup identification. Nomenclatural problems dealing with synanamorphs and generic typification (9,10) and neotypification of species (20) will not be discussed.

Anamorph-teleomorph connection. Teleomorphs of some *Fusarium* species are known and occur in the Hypocreales in the genera *Gibberella* and *Nectria* (10). Both genera produce perithecia that are superficial, with or without a stroma (4). *Gibberella* includes species with ascospores with two or more septations; perithecia are violet or blue to blue-black in color. *Nectria* includes species with ascospores that are one or more septate or muriform (25); perithecia are white, pale orange, or bright red to brown in color (4). In 1983, Rossman (25) reclassified *Calonectria rigidiuscula* (Berk. & Br.) Sacc. (anamorph = *F. decemcellulare* Brick) as *N. rigidiuscula* Berk. & Br.

F. nivale (Fr.) Ces. was transferred to the teleomorph genus *Monographella* as *M. nivalis* (Schaffnit) Müller (4) when it was noted that the fungus produced perithecia and asci with features more closely related to the Amphisphaeriales than to the Hypocreales. The anamorph genus was transferred to *Microdochium* as *M. nivale* (Fries) Samuels & Hallett (26). These decisions ended the two controversies involving whether the fungus was a *Fusarium* species and the naming of the teleomorph.

Teleomorphs are described for 14 of 29 *Fusarium* spp. in nine of 11 sections (Fig. 1) (22). Teleomorphs are unknown for species in the sections *Elegans* or *Arthrosporiella*. Within some sections, the teleomorph has been found for some, but not all, species.

Some *Fusarium* species have populations that are homothallic and heterothallic, either homothallic or heterothallic, and bisexual and self-sterile (4,22).

Two populations of *F. graminearum* Schwabe, designated as groups 1 and 2, cannot be differentiated on the basis of macroconidia but can be distinguished on carnation leaf agar (CLA) (7,22). The group 2 population is homothallic and forms perithecia of *Gibberella zeae* (Schw.) Petch on CLA, while the group 1 population apparently is heterothallic and does not form perithecia on CLA. Isolates of group 2 mistakenly may be classified as group 1 if they have lost the ability to form perithecia by frequent transfer and/or mutation, or if incubated under inappropriate conditions.

Single ascospore cultures have been used to study the range of variation within a species (31), but the taxonomic value of the anamorph-teleomorph connection is of limited usefulness. In many species of *Gibberella* and in heterothallic strains of *N. haematococca* Berk. & Br. (anamorph = *F. solani* [Mart.] Appel & Wollenw. emend. Snyd. & Hans.), perithecial production is rare and compatible mating types often are separated geographically (4). Furthermore, most plant pathologists do not encounter the teleomorph in nature, nor is the teleomorph required to identify *Fusarium* species.

Section relationships. Each section of *Fusarium* contains species that share common morphological characteristics. Yet some taxonomists have placed the same species in different sections. For instance, Booth (3) combined all species with polyblastic conidiogenous cells into the section *Arthrosporiella* (includes *F. semitectum* Berk. & Rav., *F. semitectum* var. *majus* Wollenw., *F. camptoceras* Wollenw. & Reinking, *F. avenaceum* [Fr.] Sacc., *F. sporotrichioides* Sherb., and *F. fusarioides* [Frag. & Cif.] Booth) to demonstrate their close relationship. Nelson et al (22) do not use the presence of polyblastic conidiogenous cells as a section characteristic, and place *F. avenaceum* in the section *Roseum* and *F. sporotrichioides* and *F. chlamydosporum* Wollenw. & Reinking (= *F. fusarioides*) in the section *Sporotrichiella.*

Further complicating the delineation of sections are four recently described species: *F. beomiforme* Nelson, Toussoun & Burgess (21), *F. dlamini* Marasas, Nelson & Toussoun (16), *F. napiforme* Marasas, Nelson & Rabie (17), and *F. nygamai* Burgess & Trimboli (8). All of these species have some characteristics of both the *Elegans* and *Liseola* sections. Table 1 illustrates some

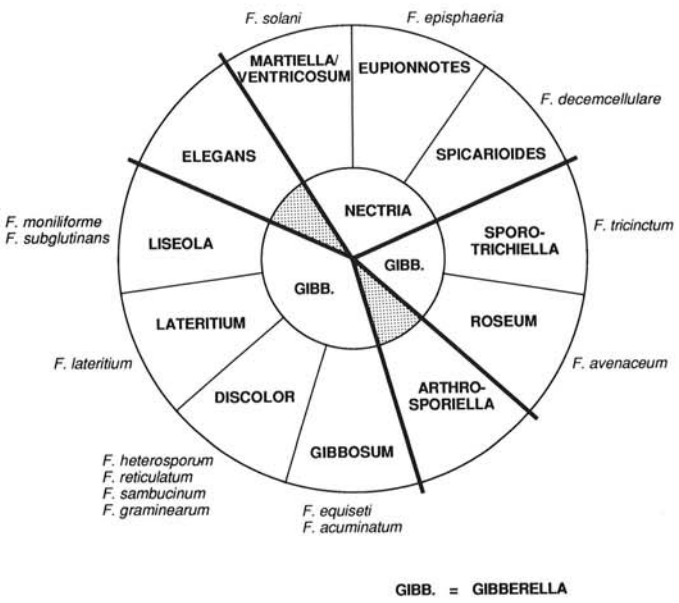

Fig. 1. Relationship of teleomorph (inner circle), where a teleomorph has been identified for at least one *Fusarium* species (named outside circle), to section (outer circle) (33). Placement of species in sections follows Nelson et al (22). Shaded sectors in the inner circle denote that a teleomorph is not known for the corresponding section.

morphological characteristics of *Fusarium* species currently included in the sections *Liseola* and *Elegans.* An example of the controversy is illustrated by *F. nygamai.* This species forms short chains of microconidia, which place it in the section *Liseola;* it also forms chlamydospores, which place it in the section *Elegans.* These incongruities suggest that either a new section should be erected, or the sections *Elegans* and *Liseola* should be combined.

Species delimitation. Three steps are critical in delimitation of *Fusarium* species for a taxonomic system (31): 1) Accumulate a large number of sporodochial-type isolates from many geographic regions if possible (some species may only occur in certain climatic regions). 2) Initiate cultures from single conidia or ascospores and grow them under standardized conditions (22 and C. E. Windels, *unpublished*) to learn the range of variation. 3) Then determine characters useful in species identification. Individual differences in isolates or populations should not necessarily form the basis for designation of a new species (31).

Separation of *Fusarium* species is based on primary and secondary characteristics. Primary characteristics include shape of the macroconidia, presence or absence of microconidia and their shape, whether or not microconidia are borne in chains, and the type of microconidiophore. Secondary characteristics include presence or absence of chlamydospores and their configuration and position, and presence or absence of sclerotia or sporodochia. Colony morphology, pigmentation, and growth rate (colony diameter) can be useful if based on standardized procedures (7). Size and septation of conidia are of limited value. The basis for "lumping" and "splitting" in *Fusarium* taxonomy largely rests on the relative importance ascribed to secondary criteria. However, unless cultures are grown under standardized conditions (22 and C. E. Windels, *unpublished*), both primary and secondary characteristics will not be sufficiently consistent for the development of sound taxonomic keys or for accurate identification.

Both CLA and potato-dextrose agar (PDA), prepared from fresh potatoes, are necessary for identifying *Fusarium* cultures. The microscopic features needed for species identification are based on growth on CLA and culture characteristics are based on growth on PDA. In fact, correct identification may not be possible if other procedures are followed (22). Advantages of CLA over PDA include: sporulation is favored over mycelial growth; conidia and conidiophores are produced in abundance and they are uniform in shape and size; and phenotypic variation is reduced. Conidia produced on PDA often are too variable in size and distorted in shape to provide reliable microscopic features for identification.

Taxonomic systems are not perfect. Occasionally cultures are found that are "intermediates" and intergrade the barriers that separate two species. Also, some isolates of *F. solani* are difficult to separate from members in the genus *Cylindrocarpon.* Occasionally, specimens occur that exclusively contain microconidia, but this problem often can be attributed to mutations or to growth of cultures under nonstandard conditions.

TABLE 1. Examples of morphological characteristics of *Fusarium* species placed in two sections

Morphological character[a]	Section[b]	
	Elegans	*Liseola*
Microconidia		
Chains	−	+
Oval-fusiform shape	+	+
Globose-napiform shape	−	+
Microconidiophore		
Monophialides	+	+
Polyphialides	−	+
Chlamydospores	+	−

[a] + = Characteristic present; − = characteristic absent.

[b] Section *Elegans* includes *F. oxysporum* Schlecht. emend. Snyd. & Hans., and section *Liseola* includes *F. anthophilum* (A. Braun) Wollenw., *F. moniliforme* Sheldon, *F. proliferatum* (Matsushima) Nirenberg, and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas (22).

Within each *Fusarium* species, there is a range of variation in morphological characteristics. With experience it is not unusual to find for a particular species, i.e., *F. oxysporum,* that clones of the fungus isolated from a particular plant species or soil in a geographic area are recognizable. However, if *F. oxysporum* is isolated from another crop, or from another geographic area, other clonal types are found. Experience is the only way to become familiar with the range of variation within each *Fusarium* species. Despite the variation encountered, however, the shape of the macroconidium and other morphological characteristics should still distinguish each species.

Mutational variants. Ideally, all *Fusarium* cultures should represent the sporodochial type. However, in nature and in culture, mutations occur and the sporodochial type is lost. Mutational variants are the nemesis of taxonomists as well as the everyday user of a taxonomic system of *Fusarium.* Starting from the sporodochial type, there are two possible phenotypic expressions of mutations (these appear as sectors or throughout the entire colony). The "mycelial" phenotype usually produces a white, featureless, cottony colony with few or no conidia. The "pionnotal" phenotype produces a slimy, effuse sporodochium of simple conidiophores bearing macroconidia, which may be distorted; the cultures often are more intensely pigmented than the sporodochial type. Neither mutational type has been known to revert to the sporodochial type.

Cultures with mutations often differ significantly from their sporodochial-type parents with respect to morphology and physiology. Because morphology is the basis for identification, standard culturing procedures must be followed to enable cultural variants to be recognized and discarded. Before the importance of initiating cultures from single conidia was recognized, some species were named that likely were mutational variants (22). Mutations in pathogenic isolates also are important, as they often result in a decrease or loss of virulence (22).

Subgroup identification. When the *Elegans* section was revised by Snyder and Hansen, the concept of formae speciales was applied to recognize pathogenic strains that were morphologically indistinguishable from saprophytic strains of the same species, but that differed in their ability to parasitize specific hosts (27). While the concept is most frequently applied in *F. oxysporum,* formae speciales also have been proposed for *F. solani* and *F. lateritium* Nees (3).

Originally it was believed that formae speciales were specific to one host and thus were named according to the Latin name of the host crop (27). Consequently, the form that attacked peas (*Pisum sativum* L.) was designated as *F. oxysporum* f. sp. *pisi* (van Hall) Snyd. & Hans.; the form that attacked beans (*Phaseolus vulgaris* L.) as *F. oxysporum* f. sp. *phaseoli* Kendr. & Snyd., etc.

In reality, host specificity occurs for some, but not all, formae speciales (1,2). Some formae speciales are host specific and cause wilt in a single host based on external symptoms. For some formae speciales, the host range is exceedingly broad. For instance, *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. & Hans., the cause of wilt in cotton (*Gossypium hirsutum* L.), is pathogenic to plants in the families Malvaceae, Solanaceae, and Fabaceae. Another complicating feature is that the early concept of host specificity led to establishment of several formae speciales that later were found to be races of other formae speciales (3).

A difficulty in determining formae speciales and races of wilt fusaria is that uniform conditions in pathogenicity tests are essential. Several factors are critical in making meaningful identifications (C. E. Windels, *unpublished*). These include selection of typical sporodochial-type isolates that are virulent and properly maintained; production, type, and concentration of inoculum; inoculation technique; cultivar selection; plant age and environmental conditions; and disease evaluations.

Identification of races ideally should be based on the use of genetically pure differential hosts (isolines or near-isolines). Armstrong and Armstrong (1) concluded that the number of races of a wilt *Fusarium* depends upon the collection of virulent cultures, availability of differential hosts, criteria for separating races, and diligence of the investigator.

The identification process can be quite involved when an investigator has a population that may be a new forma specialis. In addition to the previously mentioned concerns, a collection of the population in question should be tested against known formae speciales from a wide range of hosts to avoid naming a new forma specialis that already may have been described.

Conclusions. Recently, several analytical techniques involving physiology (6,14,23), genetics (6,15,24), and molecular biology (15,32) have been used to evaluate taxonomic, phylogenetic, or pathogenic relationships between or within *Fusarium* species. These tools should aid in defining and confirming the boundaries and relationships of sections and species that currently are based only on morphology. Whether these techniques will replace microscopic examination of cultures is doubtful. Taxonomic systems must be reliable and reasonably easy to use and should not require sophisticated, expensive equipment operated by highly trained personnel. Advances are anticipated in the development of tests that identify formae speciales and races in vitro rather than in vivo.

Successful application of any analytical procedure will depend on testing a large number of isolates collected from widely separated geographic areas. The range of variation acceptable within a species based on physiology, genetics, or molecular biology will be as important as the range of variation acceptable within a species based on morphology. This common ground will call for cooperation between classical taxonomists and other scientists interested in applying new technology to the genus *Fusarium.*

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