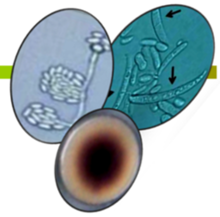
EVOLUTION OF *Fusarium* TAXONOMY: MORPHOLOGICAL, BIOLOGICAL AND PHYLOGENETIC DIAGNOSTIC CONCEPTS



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Regional Workshop on the prevention and diagnostic of *Fusarium* Wilt (Panama disease) of bananas and plantains caused by *Fusarium oxysporum cubensis* – Tropical Race 4 (TR4)
Port Spain, Trinidad and Tobago April 28th-May 9th, 2014

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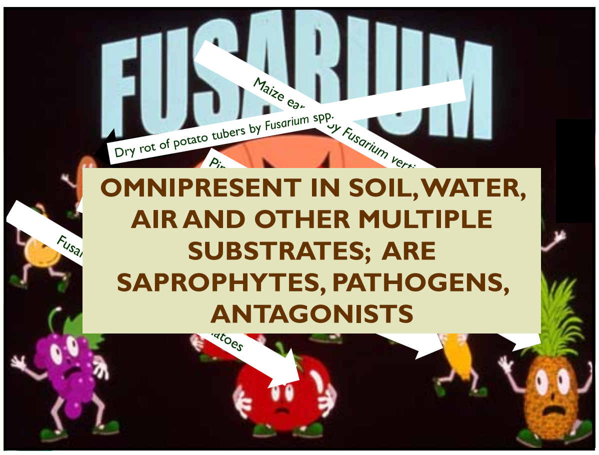


Fusarium species as plant pathogens

- ✓ *Fusarium* species are human, animal and plant pathogens
- ✓ *Fusarium* spp. cause diseases in a wide range of host plants. At least 81 of 101 economically important crop plants of APS list of diseases are caused by *Fusarium*
- ✓ Some of them have a narrow range of hosts; others have a very wide range as *F. oxysporum*
- ✓ They are found in soil, air water and can be transported in vegetable tissues.
- ✓ They can be recovered from the deepest roots in soil until the higher inflorescences of plants.
- ✓ Produce toxins and allergies

Diseases caused by different *Fusarium* species

Sugarcane wilt by *Fusarium sacchari*

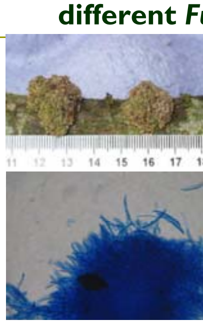
Pokkah Boeng in sugarcane by *Fusarium moniliforme*

Diseases caused by different *Fusarium* species

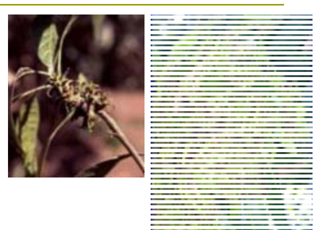
Stem rot and tubers by *Fusarium spp.*

Bakanae in rice by *F. fujikuroi*

Diseases caused by different *Fusarium* species



Green point gall of cacao by *Nectria rigidiuscula (Fusarium decemcellulare)*



Flowering malformation of mango by (*Fusarium manginifera)*

Diseases caused by different *Fusarium* species

Oil palm wilt by
F. oxysporum f. sp. *elaeidis*

F. oxysporum f. sp. *lycopersici*

Diseases caused by different *Fusarium* species

Panama disease by
F. oxysporum f. sp. *cubensis*

Crown rot by
F. pallidoroseum

Diseases caused by different *Fusarium* species

Gibberella zeae
(F. graminearum)

Papaya internal fruit rot by
Fusarium spp.

Fusarium taxonomy

- The taxonomy of *Fusarium* genus has been affected by species concept changes
- In this process in the last 100 years have been recognized by taxonomist, as few as 9 and as much as 1000 species.

Taxonomy of *Fusarium genus:* Historic review

1809. Link create genus *Fusarium*

1910. Appel and Wollenweber (1910) grouped all imperfect fungi Tuberculariacea that have pluricelular macroconidia with croissant shape developed on sporodochia.

1935. Wollenweber y Reinking publish the monography "*Die Fusarien"* and split genera in 16 sections and 65 species.

1940. Snyder & Hansen questioned sections described by Wollenweber & Reinking and reduce all variants to 10 species. Beside propose that all the forms of Elegans section belong to a single specie: ***F. oxysporum***

1955. V. I. Bilai publish in URSS "*The Fusaria (Biology and Systematics)"*

1968. C.M. Messiaen and R. Cassini publish "La systématique des Fusarium"; esencially kept the classification of Snyder & Hansen and propose varieties of some species .

Taxonomy of *Fusarium genus:* Historic review

1971. C. Booth publish The Genus *Fusarium.* CMI. (recognize 14 species)

1981. Nelson Tousson y Manasas publish"*Fusarium Species: An Illustrated* Manual for Identification".

1982. W. Gerlach y H. Niremberg publih"The genus Fusarium – A pictorial Atlas". (recognize 21 species)

1980s. The collaboration among taxomomists of EU, Europe. South Africa, and Australia unify criteria of Gerhlach and Niremberg (Germany), Nelson, Tousson y Marasas in (EU) and Burgess et al. (Australia).

1994. L.W. Burgess *et al*., publish "Laboratory Manual for Fusarium Research"

2006 J. Leslie and B. Summerell publish " The Fusarium Laboratory Manual" recognize 70 species on base of morphological, biological and phylogenetic criteria.

Relationship among the nine species of Snyder & Hansen, the sections of Wollenweber & Reinking and the species described by Leslie and Summerett (2006)

Species of Snyder & Hansen	Sections of Wollenweber & Reinking	Summeret & Leslie (2006)
F. episphaeria	Eupionnotes Macroconia	*F. dimerum, F. merismoides*
F. lateritium	Lateritium	*F. lateritium*
F. moniliforme	Liseola	*F. anthophilum, F. circinatum, F. proliferatum, F. subglutinans, F. thapsinum, F. verticillioides,* and other species in
	Fusarium complex	
		Gibberella fujikuroi
F. nivale	Arachnites	No more consider be Fusarium
F. oxysporum	Elegans	*F. oxysporum*
F. rigidiuscula	Spicarioides	*F. decemcellulare*

Relationship among the nine species of Snyder & Hansen, the sections of Wollenweber & Reinking and the species described by Leslie and Summerett (2006)

Species of Snyder & Hansen	Sections of Wollenweber & Reinking	Summeret & Leslie (2006)
F*. *roseum	Discolor Gibbosum Roseum Arthrosporiella	*F. acuminatum, F. armeniacum F. avenaceum, F. compactum; F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. longipes, F. heterosporum, F. polyphialidicum, F. pseudograminearum, F. semitectum, F. torulosum*
F. solani	Martiella Ventricosum	*F. solani*
F. tricinctum	Sporotrichiella	*F. chlamydosporum, F. poae, F. sporotrichioides, F. tricinctum*

Fusarium oxysporum Schlecht. ex Fr

- ✓ *F. oxysporum* is a complex of anamorphic fungal filamentous species morphologically similars (O'Donnell and Cigelnick, 1998).
- ✓ *F. oxysporum* is omnipresent around the world in different soils.
- ✓ Is the *Fusarium* taxa more economical and agricultural important (Ploetz, 2006)
- ✓ Isolations of *F. oxysporum* are genetically diverse including phytopathogens, saprophytes and biocontrol agents.
- ✓ They include many plant pathogenic representatives. Cause:
  - Vascular wilts,
  - Damping-off problems
  - Root and stalk base rots
- ✓ Have been identified 150 formae speciales with unique pathogenicity or to at an important number of close related hosts

Fusarium oxysporum Schlecht. ex Fr

- Wilts are important in many vegetable, fiber, ornamentals palm trees and banana.
- Pastures and cereals are not affected by *F. oxysporum.*
- Saprophytic populations usually can be aggressive secondary colonizers of diseased plant parts, particularly roots and are morphologically undistinguished from primary colonizers
- Morphology of *F. oxysporum* colonies are highly variable:
  - Produce a floccose sparse or abundant, white to pale violet mycelia.
  - Usually produce a pale violet to dark mangenta pigment in agar media (some isolates do not produce any pigment)

Species definition concepts

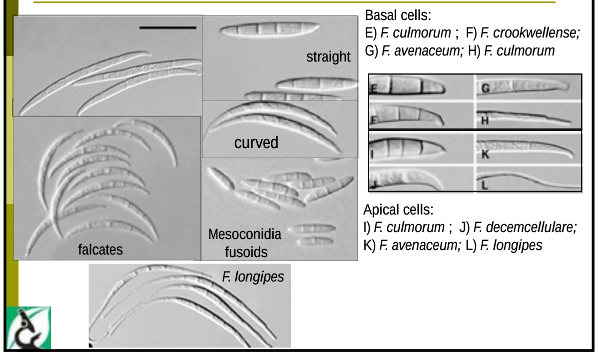
The concepts essentially define criteria to differentiate species. There is three basic concept applied for *Fusarium* :

- → ***Morphologic:*** (constant observable morphological characters among individuals of the same specie and markedly different between different species).
- → ***Biological:*** (crosses among members of the same specie are sexually fertile with viable and fertile progeny).
- → ***Phylogenetic:*** (ADN sequences of conserved genes are cladistically treated to develop phylogenies of those which are grouped in the same group with a common origin, are considered to be of the same specie)

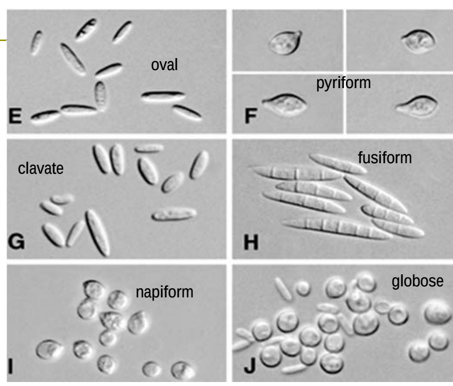
Morphological criteria for specie identification in *Fusarium*

- **Morphological characteristics of structures in carnation leaf agar)**
  - Macro and microconidia (form, abundance, size, how are produced (better from a CLA culture)
  - Chlamydospores (present/absent; single, agglomerates)
  - Conidiophores (mono o poliphyalidics)
- **Cultural characteristics**
  - Growth rate **(in PDA)**
  - Aerial mycelia absent or present and color (PDA)
  - Presence and color of sporodochia in (PDA and CLA)
  - Color of colonies in the superior and inferior part (PDA)
  - Production of volatile aldehydes (odor; rice grain media)

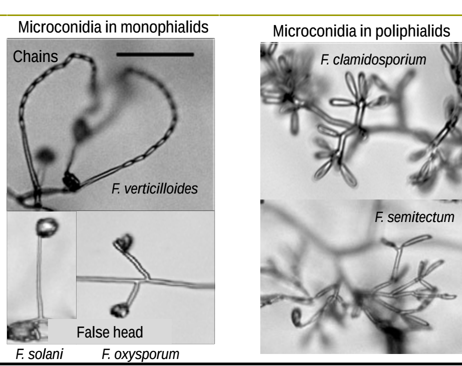
Macroconidia cells (Leslie y Summerell, 2006)



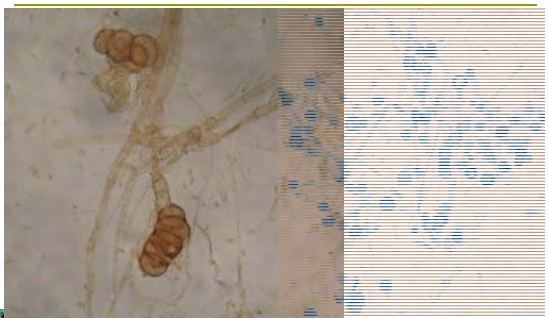
Microconidia shapes (Summerell *et al.* 2003)



Conidiogenic cells of microconidia phialid.



Chlamydospores



F. clamidosporum *F. oxysporum f. sp. cubense*

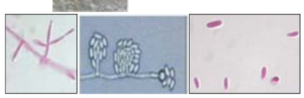
Morphology of *Fusarium oxysporum*

Mycelia floccose sparse or abundant varying from white to purple color.

From pale violet to dark magenta pigment in agar (some isolates do not produce any pigment.

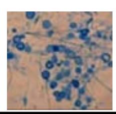
Can produce pionnotal colonies

Microconidia without septa produced in false heads in short monophialides

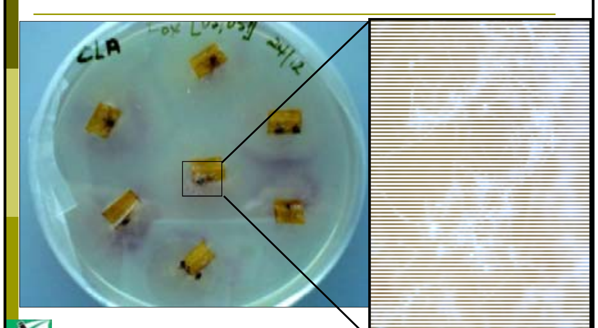


Macroconidia developed in ramified conidiophore on sporodochia

Chlamydospores terminal or intercalated; single or in chains

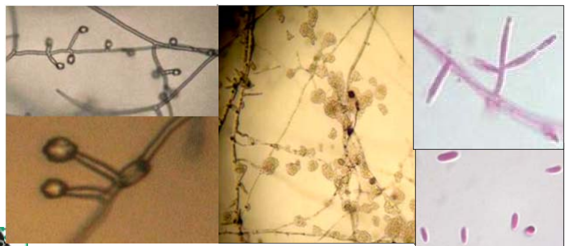


Characteristic of *Fusarium* growth in CLA



Morphology of *F. oxysporum* isolates growth

En CLA and SNA filter paper, produce abundantly microconidia in false heads in short monophyalids developed in hyphae.

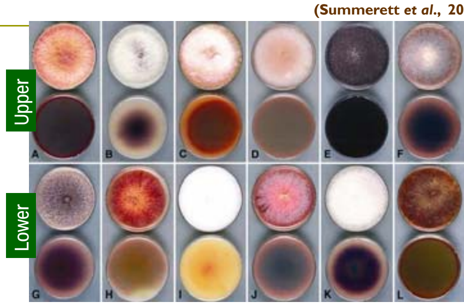


Chlamydospores

- ✓ Can be observed in hyphae on agar surface or inside media.
- ✓ Develop on hypha or conidia in old culture of until a month.
- ✓ True chlamydospores, pseudochlamydospores and swollen cells, can be missed.
  - – Chlamydospores have a gross verrucose like wall and a light color usually from yellow to brown. Can be presents solitary, in chains aggregates, above or below hypha.
  - – Pseudochlamydospores (of thin walls, alone or in short chains are present only in *F. andiyazi*)
  - – Swollen cells are numerous in *Liseola* section

Colonies morphology in potato dextrose agar. A considerable variation among *Fusarium* species

(Summerett *et al.*, 2003)



A) ***F. poae;*** **B)** ***F. oxysporum ;*** **C)** ***F. acuminatum;*** **D)** ***F. nelsonii ;*** **E)** ***F. subglutinans;*** **F)** ***F. nygamai;*** **G)** ***F. pseudonygamai;*** **H)** ***F. lateritium;*** **I)** ***F. thapsinum;*** **J)** ***F. decemcellulare;*** **K)** ***F. verticillioides;*** **L)** ***F. culmorum.***

Biological concept

- ✓ Fertility of sexual crosses (teliomorph development)
- ✓ Host range: formae speciales
- ✓ Vegetative compatibility groups.

Biologic: fertility of sexual crosses

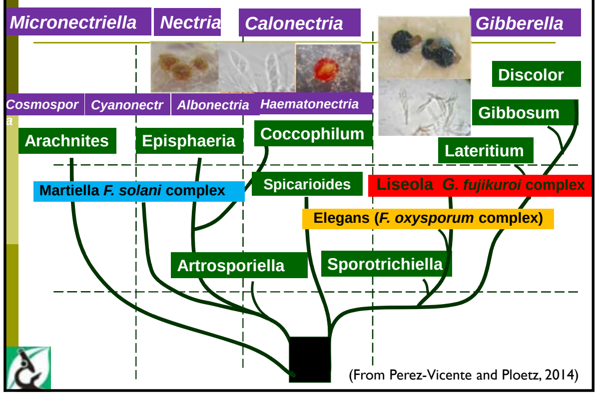
- ✓ Most fertility analysis are realized for of *Gibberella fujikuroi and Haemanectria haematococca* species complex *(Fusarium solani)* differentiation *(Leslie and Summerell 2006).*
- ✓ This approach can not be used to species that do not develop teliomorphe as *F. oxysporum*.
- ✓ There are two idiomorphic MAT (*MAT-1 and MAT-2)* that have to be present for mating occurrence *(Kroonstadt and Staben, 1987).*
  - In *F. oxysporum* has been reported alleles *MAT-1* and *MAT-2* highly similar to those of fertile Fusarium species
  - The not fertility can be consequence of mutation of genes that codify indispensable receptors not codified by *MAT* locus.

Biologic: fertility of sexual crosses

(Protocol described by por Klittich y Leslie,1988):

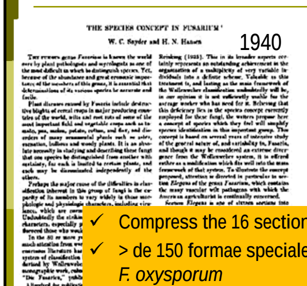
- → Female parent is inoculated in carrot agar and allowed colonize plate by a week.
- → Is inoculated with a spore suspension from the unknown strain (male). Colony is gently shaked with a glass bar
- → Are incubated at < 25°C under light for 4-6 weeks.
- → Avoid seal the plate.
- → If molecular protocols to determine sexual *MAT-1*and *MAT-2* alleles are available, less crosses are required
- → Positive crosses are definitive. Negative ones are not necessarily conclusive.

Fusarium teliomorphs morphologic tree (Booth, 1972)



(From Perez-Vicente and Ploetz, 2014)

Classification in formae speciales by Snyder & Hansen in 1940



THE SPECIES CONCEPT IN FUSARIUM
W. C. Snyder and H. N. Hansen

1940

W.N. Hansen

W.C. Snyder

- ✓ Compress the 16 sections in 9 species
- ✓ > de 150 formae speciales recognized of *F. oxysporum*
- ✓ All Elegans section species grouped in a single *oxysporum* specie

Formae speciales and pathogenic races

- ✓ Most formae speciales attack a single host specie.
- ✓ Some of them attack more than one specie: i.e.; *F. oxysporum* f. sp. *cucumerinum* attack water melon and cucumber **(Cafri et *al.*, 2005).**
- ✓ Genetic bases regarding host specificity are in general unknown (**Baayen et *al.*, 2000**) even when there is recent findings regarding genes involved in pathogenicity in chromosomes LS **(Ma et *al.*, 2010)**
- ✓ It can not be assumed that all individuals in a given single forma special have evolved from a common ancestor. All evidences indicate that monophyletic formae speciales are exceptions **(Leslie y Summerell, 2006).**

Fusarium oxysporum diseases in different plant species

Palm oil wilt by
F. oxysporum f. sp. *elaeidis*

F. oxysporum f. sp. *lycopersici*

Fusarium oxysporum diseases in different plant species

Panama disease by
F. oxysporum f. sp. *cubensis*

F. oxysporum f. sp. *ciceris*

Fusarium oxysporum diseases in different plant species

F. oxysporum f. sp. *cucumeris*

F. oxysporum f. sp. *melonis*

F. oxysporum f. sp. *lentis*

Fusarium oxysporum diseases in different plant species

F. oxysporum f. sp. *asparagi*

F. oxysporum f. sp. *cepae*

Fusarium oxysporum f. sp. niveum en melón

Fusarium spp. cultures growth rate (in mm after three days) in PDA at 25 y 30°C in complete darkness (Burgess *et al*., 1994)

Species	25°C	30°C	Species	25°C	30°C
F. acuminatum	25-35	5-28	*F. decemcellulare*	15-25	11-22
F. andiyazi	27-33	27-40	*F. dimerum*	4-10	5-12
F. anthophilum	25-40	20-45	*F. equiseti*	34-46	28-44
F. armeniacum	44-58	37-54	*F. graminearum*	47-61	5-20
F. avenaceum	28-40	5-25	*F. heterosporum*	28-41	8-30
F. aywerte	40-45	32-42	*F. hostae*	24-29	20-24
F. babinda	25-37	15-22	*F. konzum*	21-34	21-38
F. beomiforme	30-39	36-46	*F. lateritium*	8-20	5-15
F. camptoceras	23-29	20-29	*F. longipes*	37-54	44-61
F. chlamydosporum	34-46	37-55	*F. merismoides*	4-10	5-12
F. compactum	41-54	42-58	*F. musarum*	50-59	44-49
F. crookwellense	54-66	15-25	*F. napiforme*	20-35	20-32
F. culmorum	55-68	15-25	*F. nelsonii*	24-39	26-41

Fusarium spp. cultures growth rate (in mm after three days) in PDA at 25 y 30°C in complete darkness (Burgess *et al*., 1994)

Species	25°C	30°C	Species	25°C	30°C
F. decemcellulare	15-25	11-22	*F. nurragi*	32-40	6-22
F. dimerum	4-10	5-12	*F. nygamai*	25-35	32-42
F. equiseti	34-46	28-44	*F. oxysporum*	25-40	25-40
F. graminearum	47-61	5-20	*F. poae*	42-54	24-39
F. heterosporum	28-41	8-30	*F. polyphialidicum*	23-40	20-38
F. hostae	24-29	20-24	*F. proliferatum*	25-35	25-32
F. konzum	21-34	21-38	*F. pseudograminearum*	39-51	10-25
F. lateritium	8-20	5-15	*F. pseudonygamai*	24-30	24-29
F. longipes	37-54	44-61	*F. redolens*	32-37	33-39
F. merismoides	4-10	5-12	*F. sambucinum*	24-35	11-21
F. musarum	50-59	44-49	*F. semitectum*	35-45	16-33
F. napiforme	20-35	20-32	*F. scirpi*	36-48	36-49
F. nelsonii	24-39	26-41	*F. solani*	21-29	26-36

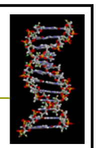
Fusarium spp. cultures growth rate (in mm after three days) in PDA at 25 y 30°C in complete darkness (Burgess et *al*., 1994)

Species	25°C	30°C
F. sporotrichioides	51-61	32-42
F. subglutinans	23-37	11-34
F. thapsinum	19-28	14-27
F. tricinctum	29-39	2-15
F. verticillioides	21-30	22-35

Biological concept: *F. oxysporum* case; vegetative compatibility groups (VCGs).

- *F. oxysporum* is causal agent of an important number of disease with wilt syndrome. The fungus has not known teleomorph.
- Are morphologically identical, and have been differentiated by pathogenicity to specific hosts in formae speciales
- In some species, races are not genetically defined as in *Fusarium oxysporum* f. sp. *cubense*. Vegetative compatibility has allowed to determine population structures.
- In other cases has not been so useful.
- Further more specific informations on the technique will be provide.

The phylogenetic concept: sequences of genome's conserved sectors



In Fusarium phylogenetic analysis, genomic sequences of one or several genes in different analysis types have been used :

- β-tubulin (*tub-2),*
- The translation elongation factor 1-α (*tef-1),*
- Histone H3
- Portions of coding region of nuclear or mitocondrial DNA
  - Intergenic spacers (IGS)
  - ITS spaces

Different techniques has been used:

- RFLP
- AFLP
- PCR
- RAPDs

The phylogenetic concept: sequences of genome's conserved sectors 1-∞*(TEF)*

The translation elongation factor 1-∞*(TEF)* gen codify an essential protein of translation machinery with a high phylogenetic utility due to (Geiser *et al.,* 2006):

(i) Is highly informative at Fusarium species level;

(ii) Have not been detected orthologous copies of Fusarium genera.

(iii) Appears as a single copy and its sequence has a high polymorphism in genome of many closely related Fusarium

(iv) Has been designed universal primers that work thorough the width of all phylogenic genera.

(v) These primers (ef1 and ef2) amplify a ~700 bp of TEF region and kept three introns that complete the half of the amplicon length in all *Fusarium* species.

The phylogenetic concept: sequences of genome's conserved sectors: IGS

Larger intergenic spacers (IGS) has been very widely used in phylogenetic studies in *Fusarium* due to:

(i) Appears as the more rapid evolved spacer region in genome

(ii) Is a very conserved region of nuclear rDNA with sufficient polymorphism to may show considerable differences between close related species and subspecies.

(iii) Appears in multicopies in genome

Fusarium ID database v. 1.0 (Geiser et al., 2006)

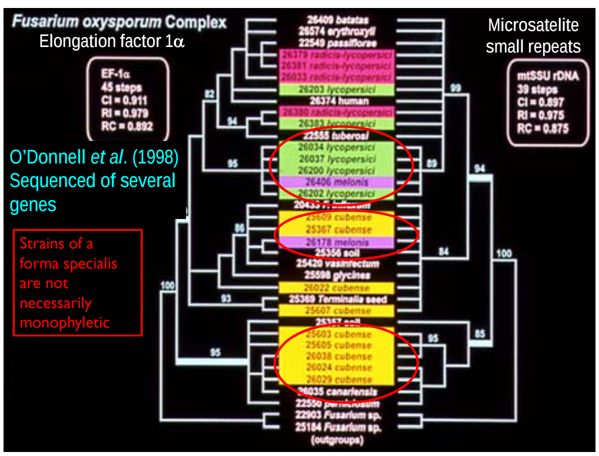
Complex of species	Represented species
Gibberella fujikuroi species complex: Excelent representation	*Fusarium acutatum, F. andiyazi, F. anthophilum, F. bactridioides, F. begoniae, F. brevicatenulatum, F. bulbicola, F. circinatum, F. circinatum, F. sp. cf. concentricum, F. denticulatum, F. dlaminii, F. fractiflexum, F. fujikuroi, F. globosum, F. guttiforme, F. konzum, F. lactis, F. mangiferae, F. napiforme, F. nygamai, F. phyllophilum, F. proliferatum, F. pseudoanthophilum, F. pseudocircinatum, F. pseudonygamai, F. ramigenum, F. sacchari, F. sterilihyphosum, F. subglutinans, F. succisae, F. thapsinum, F. udum, F. verticillioides y* 20 spp.no descritas
Fusarium type A trichotecene producer species and relatives: Weak representation	*Fusarium cerealis (=F. crookwellense), F. culmorum, F. graminearum y 8 spp. relacionadas; F. lunulosporum, F. pseudograminearum*
F. oxysporum and relatives: Good representation.	*Fusarium commune, F. hostae, F. miscanthi,* 45 linajes de cf. *'F. oxysporum', F. redolens.*
F. solani species complex : Good representation.	*Fusarium phaseoli,* 25 linajes de cf. *'F. solani ', F. tucumaniae, F. virguliforme.*
Other linages: Poor representation	

Results influenced by the concept approach

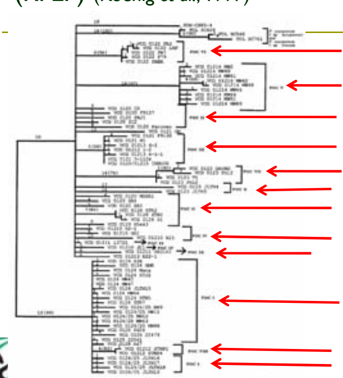
Eg.: ***Fusarium moniliforme***: By decades considered causal agent stalk and ear rot of corn; stem and grain rot of sorghum; bakanae in rice; pokkah boeng in sugarcane.

According the concept:

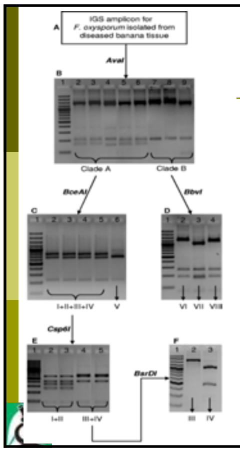
- ✓ **Morphologic:** *Fusarium moniliforme sensu lato* (wide sense)
- ✓ **Biologic:**
  - *F. verticillioides:* stalk and ear corn rot (produce fumonisin)
  - *F. tapsinum:* stalk and grain sorghum rot (produce few fumonisin and high amounts of moniliformin).
- ✓ **Phylogenetic:**
  - *F. andiyazi* (beside *F. verticillioides and F. tapsinum*), few or any aproduction of fumonisin and moniliformin and not toxic to dugs.



Phylogenetic relationship between VCGs in *Foc* (RFLP) (Koenig *et al.*, 1997)



Clados	VCGs
Foc VI	0122
Foc V	01214
Foc II	0120
Foc III	01213, 0120, 0121
Foc VII	0123
Foc X	0123
Foc II	0120, 0126
Foc IV	01210, 0129
Foc IX	01210, 01211
Foc I	0124, 0124/0125
Foc VIII	01212
Foc IV	0124, 0125



PCR-RFLP analysis of rRNA IGS region, and digestion of IGS amplicon with enzymes (AvaI, BceAI, BbvI, Csp6I, BsrDI) to differenciate *Foc* linages

(Foure *et al.*, 2009)

8 linages were differentiated:

- → I (VCG 01219)
- → II (VCGs 1210, 0126)
- → III (VCG 01211)
- → IV (VCGS 0120, 0122, 01215)
- → V (VCGs 0121, 01213)
- → VI (VCGs 0124, 0125, 0128, 01220)
- → VII (VCGs 0123, 01217, 01218)
- → VIII (VCGs (01214)

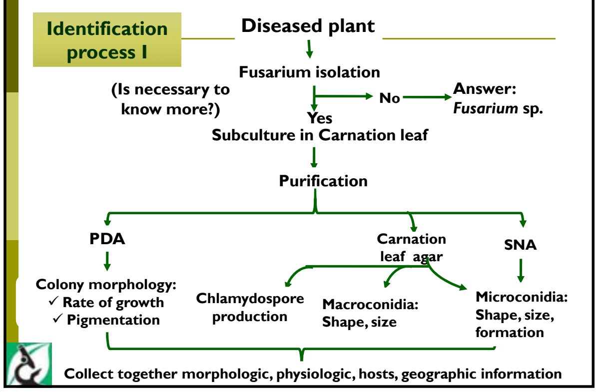
Fusarium taxonomy evolution since the introduction of phylogenetic concept

Specie complex → Complex of species

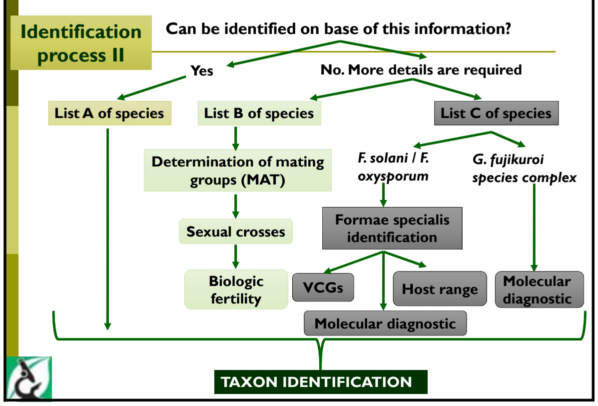
Fusarium oxysporum f. sp. cubense population grouping according different characterization methods

Method	Grouping I	Grouping II
✓ Actual race classification *(Stover, 1962 b; Su et al. 1977)*	Race 4	Races 1 and 2.
✓ Formación de lacinia en K2 *(Sun et al., 1978).*	Present (not always)	Not present
✓ Vegetative compatibility *(Ploetz, 1990 a y b; Brake et al., 1990; Pegg et al., 1993; Moore et al., 1993).*	0120, 0121, 0122, 0126, 0129, 01211, 01213/16, 01215, 01219	0123, 0124, 0125 0128, 01210*, 01212, 01214, 01217, 01218, 01220
✓ Volatile production *(Moore, 1994).*	Odoratum	inodoratum (not always)
✓ RAPD-PCR *(Sorensen, et al. 1993; Sorensen et al., 1994)*	RAPD-PCR group 1	RAPD-PCR group 2
✓ Chromosomic number genome size. *(Boehm et al., 1994)*	Ek I high	Ek II low

Flux graphic for *Fusarium* species identification



Flux graphic for *Fusarium* species identification



Species more commonly isolated that can be identified by different data types

Only morphology SPECIES LIST A	Fertility in sexual crosses SPECIES LIST B	DNA data SPECIES LIST C
F. acuminatum, F. avenaceum, F. chlamidosporum, F. compactum, F. crookwellense, F. culmorum, F. decemcellulare, F. dimerum, F. equiseti, F. gramianearum, F. longipes, F. merismoides, F. oxysporum, F. poae, F. pseudograminearum, F. scirpi, F. semitectum, F. solani, F. sporotrichoides, F. torulosum, F. tricinctum	*F. circinatum, F. fujikuroi, F. proliferatum, F. sacchari, F. sambucinum, F. subglutinans, F. thapsinum, F. verticilloides*	Remanent species of *Gibberella fujikuroi complex* *Many formae specialis* of *F . oxysporum and F. solani.*

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THANKS!