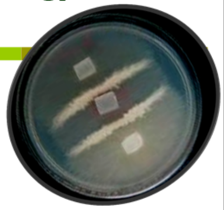
Population biology of Fusarium oxysporum *f. sp. cubense:* VCGs concepts and methodology



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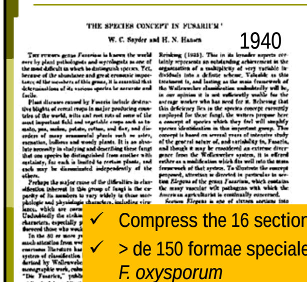
Regional Workshop on the prevention and diagnostic of *Fusarium* Wilt (Panama disease) of bananas and plantains caused by *Fusarium oxysporum cubensis* – Tropical Race 4 (TR4)
Port Spain, Trinidad and Tobago April 28th-May 9th, 2014

Biological concept

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

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

Pathogenic races of *F. oxysporum* f. sp. *cubense*

(Stover and Waite, 1960; Stover, 1962)

Differentials cvs.	Races 1	2	3	4
Gros Mi				+
Apple Si				
Bluggoe				+
Heliconia sp.	-	-	+	+
Cavendish	-	-	-	+

Current classification lack genetic sense; are populations that infect plants of a particular cultivar

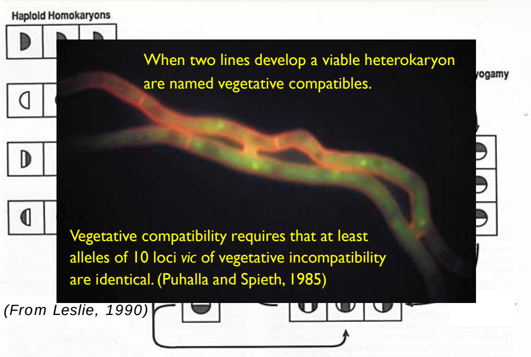
Biological concept: *F. oxysporum* case; vegetative compatibility groups (VCGs).

- *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.

Vegetative incompatibility: vegetative compatibility groups (VCGs)

- To sexual exchage occurs a heterokaryon should develop
- Generally hetero-incopatibility (determined by genes *vic* or *het* prevent heterokaryon formation. Heterokaryotic cells die after interaction of two incompatible cytoplasm.
- These death reaction associated to vegetative incompatibility is not expressed in the sexual phase during fertilization.
- Lethal agents are considered to be labile proteases

Heterokaryon development and vegetative compatibility groups (VCGs)



Characteristics of members of a vegetative compatibility group or VCG

- ✓ Members of a VCG are clonally derived and are genetically related
- ✓ *Vic* loci interact as part of a self recognizing system that allow recognition themselves from others.
- ✓ VCGs can differ in one, various or all vegetative incompatibility loci (*vic*) that are disperse in genome and responsible of the VCG phenotype.
- ✓ Has not been ever reported vegetative compatibility between strains of different *formae speciales* (en *F. oxysporum*).

Characteristics of members of a vegetative compatibility group or VCG.

- ✓ Two vegetative compatible individuals should be also identical for other genes responsible of pathogenicity, ecological adaptation and other characteristics affecting their rol as pathogens.
- ✓ Vic loci can delimitate pathotypes in asexual forms of phytopathogenic fungi as ocurrs in *Fusarium, Verticillium, Rhizoctonia.*
- ✓ Even when a race can be represented in different VCG´s, in a given geographic area prevail a single or a small group of VCG´s

Existing compatibility groups in *F. oxysporum* formae speciales
(Katan y Di Primo, 1999)

VCG number	formae speciales	VCG number	formae speciales
001	*apii* (3)	016	*phaseoli* (5+)
002	*dianthi* (6)	017	*albedinis* (1)
003	*lycopersici* (4+z)	018	*cucumerinum* (6+)
004	*medicaginis* (2)	019	*lilii* (1)
005	*chrysanthemi* (2)	020	*basilici* (1)
006	*tracheiphilum* (1)	021	*matthioli* (1)
007	*pisi* (5?y+)	022	*raphani* (1)
008	*niveum* (3)	023	*tulipae* (1)
009	*radicis-lycopersici* (9+)	024	*canariensis* (1+)
010	*conglutinans* (3?)	025	*papaveris*
011	*vasinfectum* (12+)	026	*radicis-cucumerinum* (2)
012	*cubense* (24+)	027	*betae* (7+)
013	*melonisv* (8+)	028	*ciceris* (1)
014	*elaeidis* (5+)	029	*erythroxyli* (2)
015	*cyclaminis* (3)	030	*lactucum* (1)

Existing compatibility groups in *F. oxysporum* formae speciales
(Katan y Di Primo, 1999)

VCG número	formae speciales
031	*lupini* (2?)
032	*melongenae* (1)
033	*spinaciae* (3)
034	*gladioli* (4)
035	*tuberosi* (6)
036	*batatas* (2+)
037	*nicotianae* (2+)
100	*asparagi* (8)

World *Foc* population structure.

(Ploetz and Pegg, 2000)

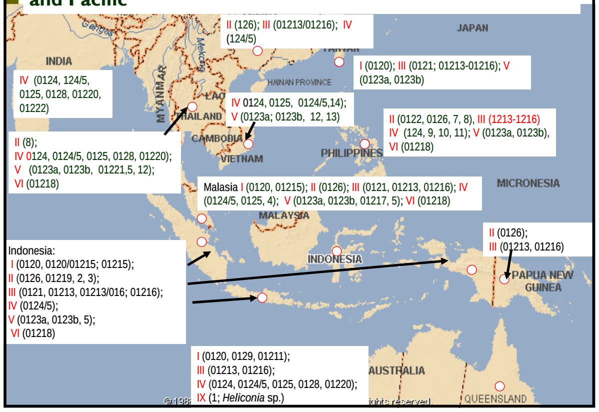
Table 3.1. Vegetative compatibility among strains of *Fusarium oxysporum* f. sp. *cubense*.

VCG[a]	Genomic group/cultivar(s)[b]	Origin(s)
0120–01215	*Musa* sp.; AA: 'SH-3142', 'SH-3362' AAA: 'Gros Michel', 'Highgate', 'Pisang Ambon Putih', 'Pisang Ambon', 'Dwarf Cavendish', 'Williams', 'Mons Mari', 'Grand Nain', 'Lacatan'; AAB: 'Prata', 'Lady Finger', 'Pacovan', 'Huamoa', 'Silk'	Australia, Brazil, Costa Rica, France (Guadeloupe, Guyane), Honduras, Indonesia (Java), Jamaica, Malaysia (Sarawak), Nigeria, Portugal (Madeira), South Africa, Spain (Canary Islands), Taiwan, USA (Florida)
0121	AAA: 'Gros Michel', Cavendish clone	Indonesia (Sumatra), Taiwan
0122	AAA: Cavendish clone ABB: 'Saba'	Philippines
0123	*Musa textilis*; AAA: 'Gros Michel', 'Grand Nain'; AAB: 'Silk', 'Latundan', 'Pisang Keling'; ABB: 'Pisang Awak', 'Kluai Namwa'	Malaysia (West and Sarawak), Philippines, Taiwan, Thailand
0124–0125–0128–01220	AA: 'Niyarma Yik'; AAA: 'Gros Michel', 'Highgate', 'Mons Mari', 'Williams', 'Grand Nain', 'Mbwazirume'; AAAA: 'Jamaica 1242'; AB: 'Ney Poovan', 'Sukali Ndizi', 'Kamaramasenge'; AAB: 'Lady Finger', 'Maçã', 'Manzano', 'Maqueño'; ABB: 'Pisang Awak', 'Ducasse', 'Kayinga', 'Zambia', 'Kluai Namwa', 'Bluggoe', 'Harare', 'Kholobowa', 'Dwarf Bluggoe', 'Mbufu', 'Burro Criolla', 'Pelipita', 'Ice Cream'	Australia, Brazil, Burundi, China, Cuba, Democratic Republic of Congo, Haiti, Honduras, India, Jamaica, Malawi, Malaysia (West), Mexico, Nicaragua, Rwanda, Tanzania, Thailand, Uganda, USA (Florida)
0126	AA: 'Pisang Berlin'; AAA: 'Highgate'; AAB: 'Maqueño'; AAB: 'Pisang Manurung'	Honduras, Indonesia (Irian Jaya, Sulawesi), Papua New Guinea, Philippines
0129	AAA: 'Mons Mari'; AAB: 'Lady Finger'	Australia
01210	AAA: 'Gros Michel'; AAB: 'Manzano'	Cayman Islands, Cuba, USA (Florida)
01211	AA: 'SH-3142'	Australia
01212	AB: 'Ney Poovan'; AAB: 'Silk'; ABB: 'Pisang Awak', 'Kisubi', 'Bluggoe'	Tanzania
01213–01216	AA: 'Pisang Lilin', 'Pisang Mas'; AAA: 'Pisang Ambon', 'Valery', 'Williams', 'Grand Nain', 'Novaria', 'Pisang Nangka', 'Red', 'Pisang Raja Udang', 'Pisang Susu', 'Pisang Barangan'; AAB: 'Pisang Raja Serah', 'Pisang Rastali', 'Pisang Raja', 'Relong'; ABB: 'Pisang Awak', 'Pisang Awak Legor', 'Saba', 'Pisang Kepok', 'Pisang Caputu', 'Pisang Kosta'; Unknown: 'Pisang Batan'	Australia (Northern Territory), Indonesia (Halmahera, Irian Jaya, Java, Sulawesi, Sumatra), Malaysia (West), Taiwan
01214	ABB: 'Harare', 'Mbufu'	Malawi
01217	AAB: 'Pisang Rastali'	Malaysia
01218	AAB: 'Pisang Rastali', 'Pisang Raja Serah'; ABB: 'Pisang Awak', 'Kluai Namwa', 'Pisang Kepok', 'Pisang Siam'	Indonesia (Java, Sumatra), Malaysia (West), Thailand
01219	AAA: 'Pisang Ambon', 'Pisang Ambon Putih'; Unknown: 'Pisang Raja Garing'	Indonesia (Java, Sumatra)
01221	ABB: 'Kluai Namwa'	Thailand

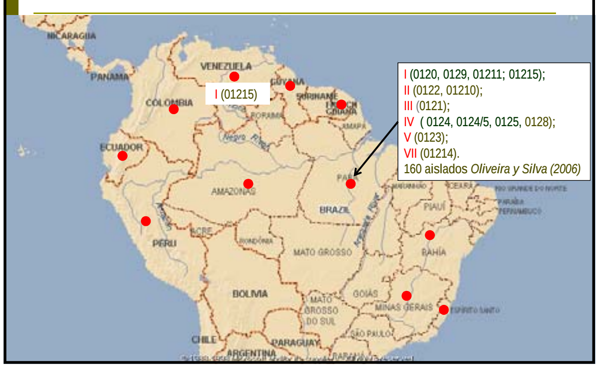
VCG's identified in *F. oxysporum* f. sp. *cubense*

RACES	VCG's
TR4	01213
StR4	0120, 0121, 0122, 0129, 1211
R1 and R 2	All except VCG 01213

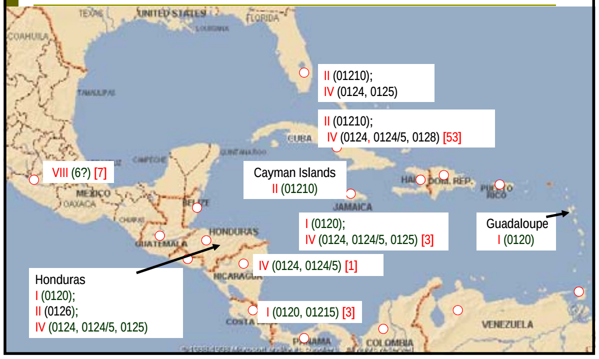
Distribution of Foc lineages and VCGs in South East Asia and Pacific



Distribución de los linajes de *Foc* en América del Sur.

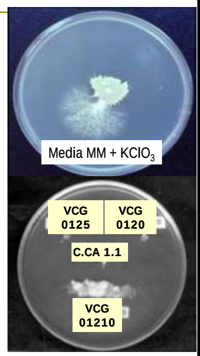


Distribución de los linajes y VCG de *Foc* en la cuenca del Caribe.



Puhalla (1985) and Correll *et al.* (1987) technique based on generation of mutants that do not use N (*nit* mutants)

Technique based in pairing mutants that do not use N allow the visualization of heterokaryon formation



Fluxogram with the general strategy to generate *nit* mutants

Generation of *nit* mutant resistant to $KClO_3$

⬇

***Nit* mutants phenotypes identification in selective media**

⬇ → **Discard** *crn* phenotypes

Pairing of complementary phenotypes (preferently Nit M and *nit 1*) derivated from the same isolate

Robust growth line ⬇ **Self compatible isolate**

Weak growth line ⬇ **Self incompatible isolate**

⬇

Pairing of phenotypic Nit M complementary mutants of reference and *nit 1* derived from isolate in study

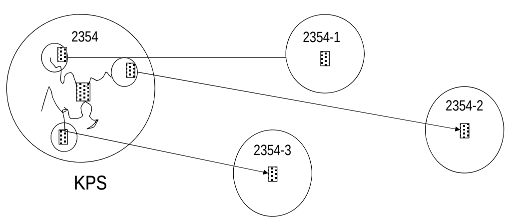
Robust growth line ⬇ Same VCG

Weak growth line ⬇ Different VCG

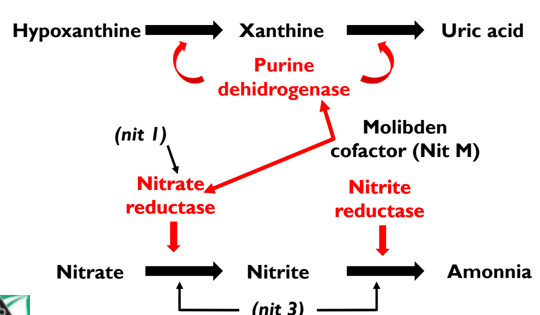
Generation or mutants that do not use nitrate (*nit* mutants)

- Subculture from PDA media or CLA to Minimal Media (MM) + 1.5- 5.0 % de $KClO_3$
- Potassium chlorate is an analogue of nitrate and is take and processed through nitrate reductase metabolic pathway.
- This process produce chloride that is toxic to fungus.
- Colonies of characteristic slow and nudose mycelial growth are produced
- Mycelia from fast growing sectors has suffered mutation for resistance to chlorate (*nit* mutants)
- Mutants of the more advanced border of sector are selected

The different sectors are selected and numbered with isolate number + a consequtive



Metabolic pathway of $N0_3$ and relationship with nit mutant generations for VCGs assays



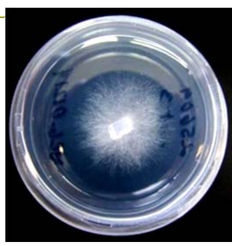
Fisiological assays to determine mutant phenotypes

Nitrate	Minimal media
Nitrite	Basal media + 0.5 g $NaNO_2$/L
Hypoxantine	Basal media + 0.2 g Hypoxantine/L
Amonnia	Basal media + 1.0 g Ammonia tartrate/L.

Phenotype	ClO_3	NO_3	NO_2	Hipoxanthina	NH_4
Wild type	-	+	+	+	+
nit 1	+	-	+	+	+
nit 3	+	-	-	+	+
Nit M	+	-	+	-	+
crn	+	+	+	+	+

(Correl et al., 1987).

Types of mutants that it generates

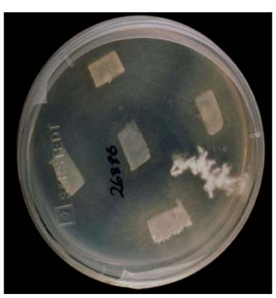


Mutant in minimum media

$KCLO_3$ resistant isolates (*crn*) - an inconvenient

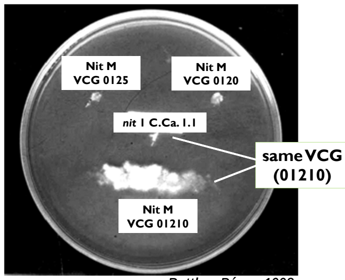
After verify their compatibility, one nit 1 and one Nit M mutant from each isolates was keep it

Compatibility test: matting complementary nit mutants (*nit 1 y Nit M)* derived from the same isolate



Compatible heterocarion

Matting of *nit 1* generated from isolates in study with Nit M from an international collection.



Battle y Pérez, 1998

Precise records

Keep a record of a centralize register of:

- ✓ Origen (place)
- ✓ Cultivar from which was obtained
- ✓ Date of isolations
- ✓ Collector's name
- ✓ VCG that it belong
- ✓ Type of mutant (*nit 1* o Nit M)
- ✓ Time in conservation.