

Genetic Diversity and Pathotypes of U.S. Fov

Jinggao Liu

Al. Bell

Carlos Ortiz

Tanya Wagner

Jose Quintana

Sandria Prom

Lorraine Puckhaber

Elaine Chen

Jinggao Liu

USDA-ARS-SPARC-ICCDRU

College Station, TX

Bob Kemerait

University of Georgia

Bob Nichols

Cotton Incorporated

Kathy Lawrence

Auburn University

James Olvey

O&A Enterprises

Jason Woodward

Texas A&M University

Marin Brewer

University of Georgia

Fov meeting at Cotton Breeders' Tour

Chandler, AZ

September 24, 2017

Acknowledgement:

This research is supported
in part by Cotton Incorporated

Formae Speciales and Races

- Many *Fusarium oxysporum* isolates appear to be host specific
- Isolates that attack cotton were designated as *Fusarium oxysporum* f. sp. *vasinfectum* (Fov): **Polyphyletic**
- Isolates of Fov have been categorized into races based on **differential virulence** to several plant species and cultivars (cotton, soybean, tobacco, okra, alfalfa)
- Races 1 and 2: USA
- Race 3: Egypt
- Race 4: India
- Race 5: Sudan
- Race 6: Brazil
- Races 7 and 8: China

Race Concept in Fov is Invalid and Impractical

- Virulence of isolates was not always stable but varied among experiments (Armstrong et al.)
- Genetic purity of differential host was not established (cotton cultivars are often formed by combining multiple lines to maintain some heterogeneity and **susceptibility differ** in individual member)
- Susceptibility scores were arbitrary (10 to 20% vs 50% wilted plants) and framed to conform to gene-for-gene concept (Ebbels vs Armstrong, Kappelman)
- Available cultivars were not able to differentiate known races of Fov
- **No genetic basis for resistance was established and gene-for-gene relationship as occurs with races is unlikely**

Pathotypes of Fov: Vascular Competent vs. Root Rot

Differentiated Based on Disease Assays:

- **Infested-soil assay:** Germinated seeds (1-2 cm) were transplanted into sandy loam/washed sand mix. One mL of 5×10^6 conidia/mL was injected into each of six locations 1 cm away from the radicle. Incubated at 23° C, 13-hour days and 18° C, 11-hour nights.
- **Interaction with nematode:** Root-knot nematode eggs (6 x 1ml 1000 eggs) were injected into soil one week after Fov treatments in the infested-soil assay.
- **Stem-puncture assay:** Plants grown in a greenhouse mix. At fourth true leaf stage, a drop (20-30 μ L) of 10^7 conidia/mL was placed on the stem surface below the cotyledonary node. A syringe needle was punctured into xylem through the drop. Incubated as above.
- **Root-dip assay:** Plants grown in a sandy loam/washed sand mix (3:1). Roots (two true leaf stage) were dipped in a 10^6 conidia/mL of Fov (5 min) and transplanted (25° C, 13-h days and 20° C, 11-h nights). (**Most stressful to plants**)

VCG 0113, 0114, 01111
(Races 3, 4, 7 and AusBio)

No Nematode

Soil
Inoculated



Stem
Puncture
Inoculated



Root
Dip
Inoculated



Vascular Incompetent
Root Rot

15 VCGs

(includes races 1, 2, 6, and 8)

No Nematode

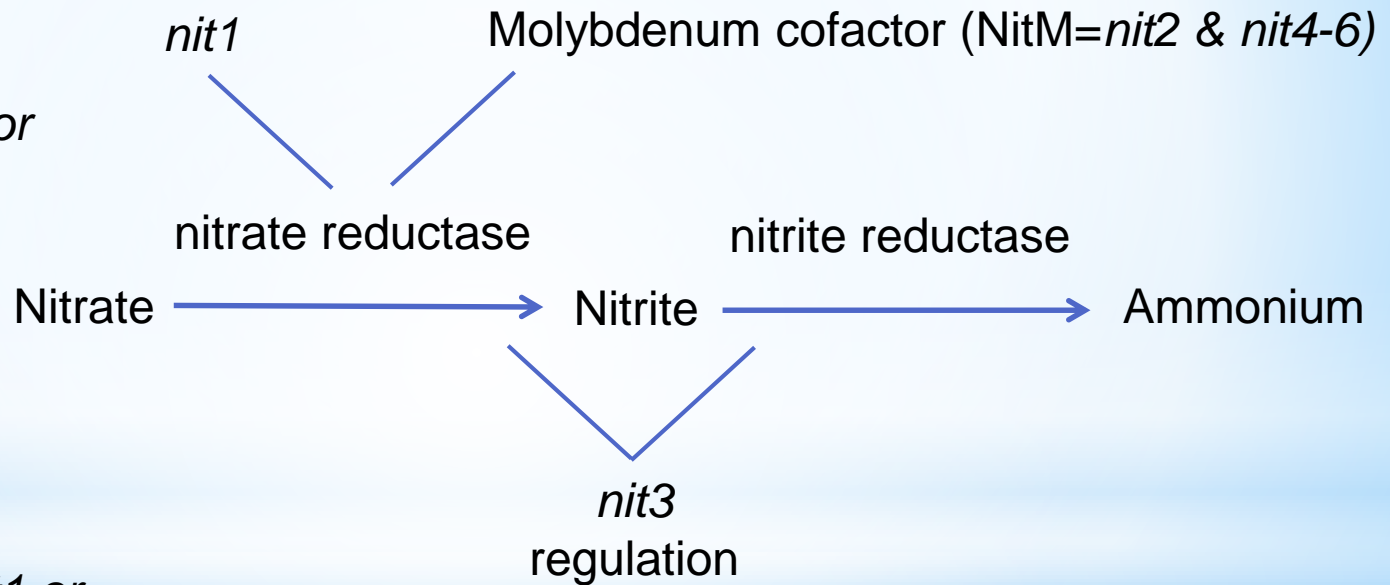
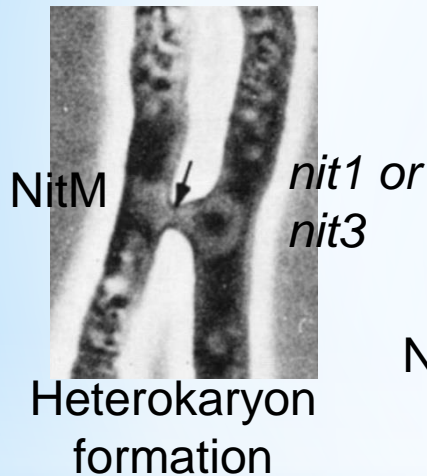
With RKN



Vascular Competent
Vascular Invasion

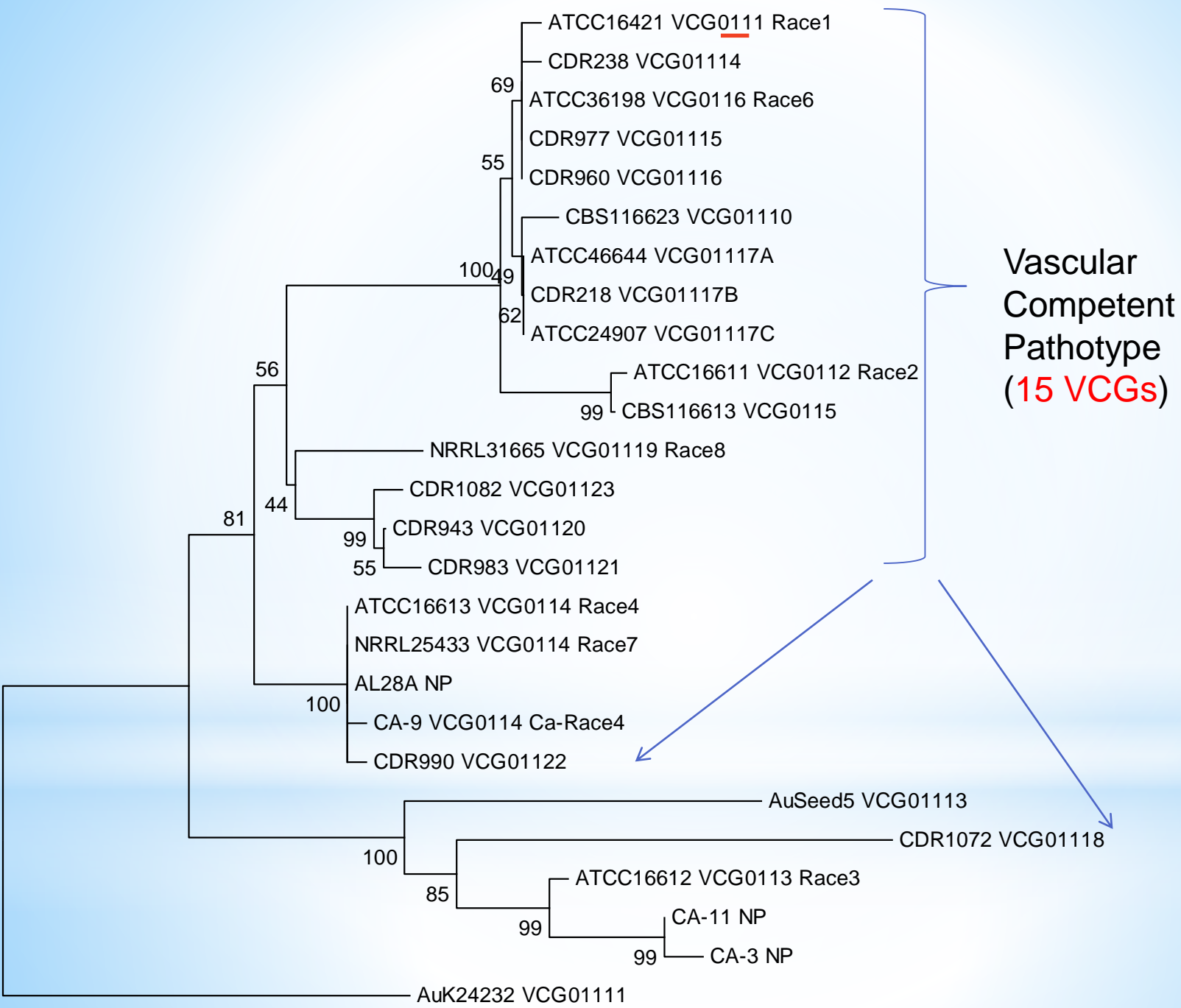
Genetic Diversity of Fov

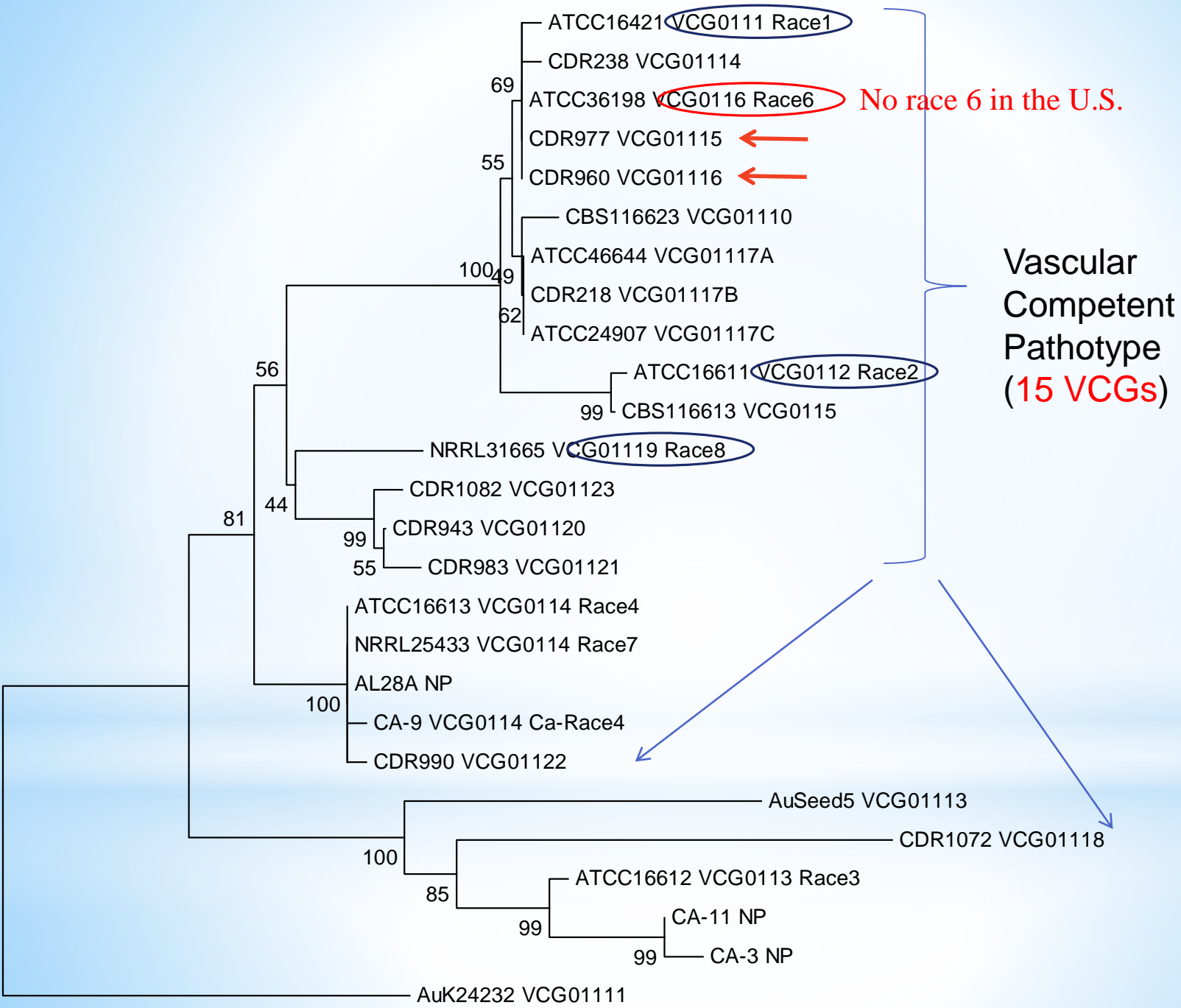
- Vegetative compatibility test using nitrate utilization mutants is used to determine genotypes as groups (VCG). The compatibility is governed by a dozen or so *vic* loci and requires all of them to be the same for the two isolate to be compatible. Thus, compatible isolates have similar genetic makeup (fine fingerprint capability but lack phylogenetic information).



(Puhalla 1974, 1985)

- DNA sequence analysis (EF, PHO and BT: nearly 6kb).





No race 6 in the U.S.

Vascular Competent Pathotype (15 VCGs)

AuK24232 VCG01111

AuSeed5 VCG01113

CDR1072 VCG01118

ATCC16612 VCG0113 Race3

CA-11 NP

CA-3 NP

100

85

99

99

100

ATCC16613 VCG0114 Race4

NRRL25433 VCG0114 Race7

AL28A NP

CA-9 VCG0114 Ca-Race4

CDR990 VCG01122

CDR983 VCG01121

CDR943 VCG01120

CDR1082 VCG01123

NRRL31665 VCG01119 Race8

CBS116613 VCG0115

ATCC16611 VCG0112 Race2

100

ATCC46644 VCG01117A

CDR218 VCG01117B

ATCC24907 VCG01117C

55

CDR960 VCG01116

CBS116623 VCG01110

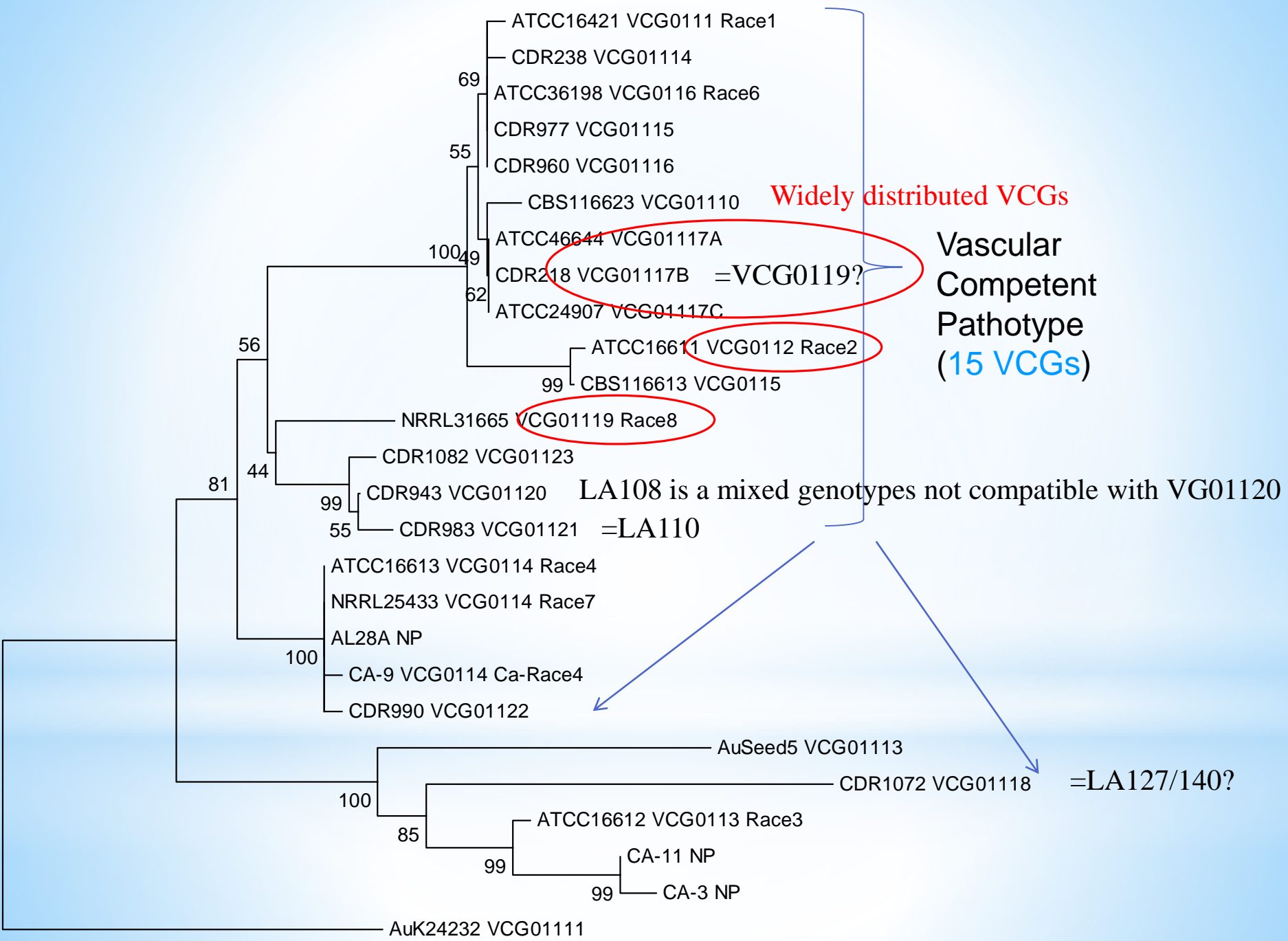
69

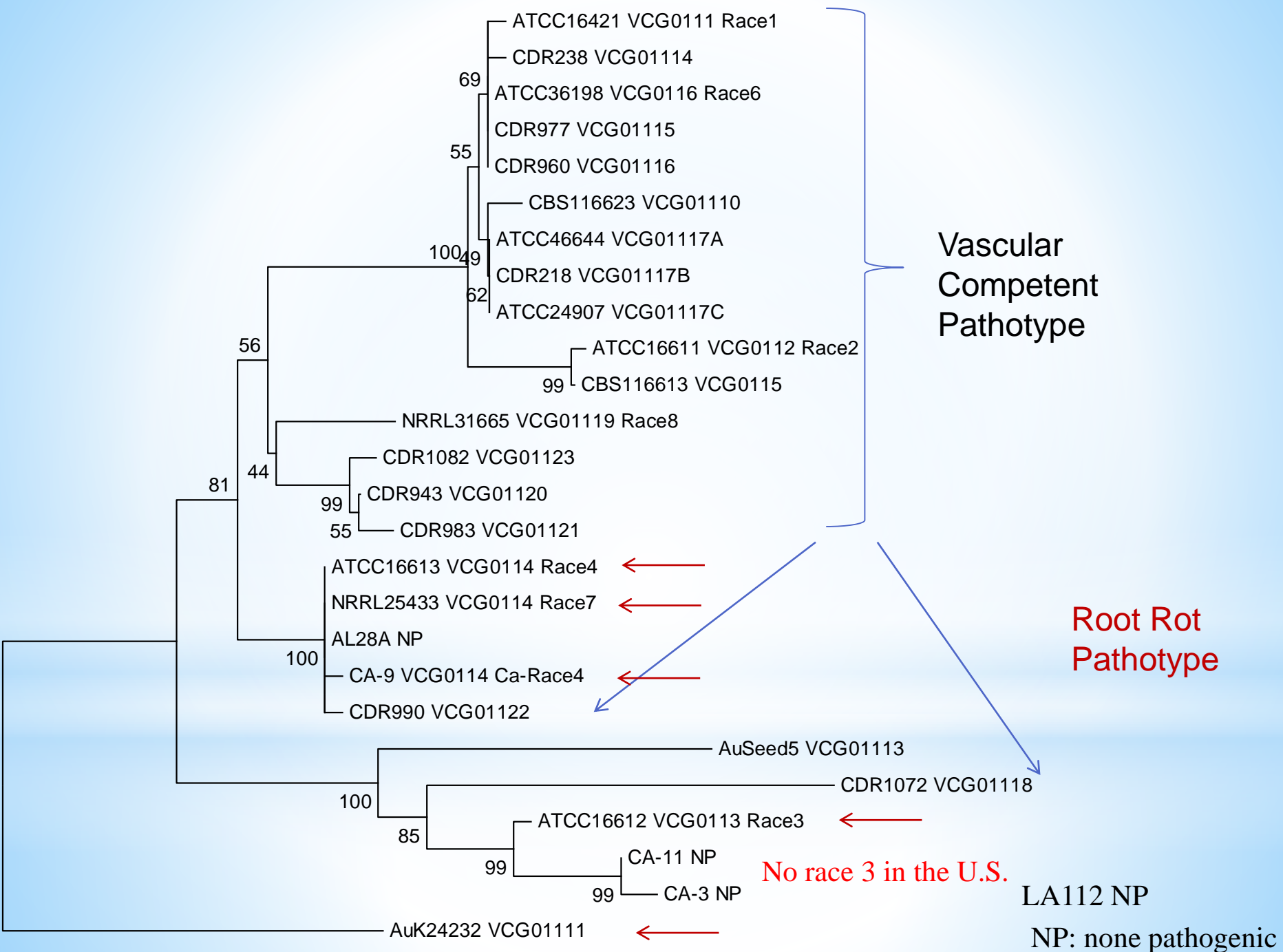
CDR238 VCG01114

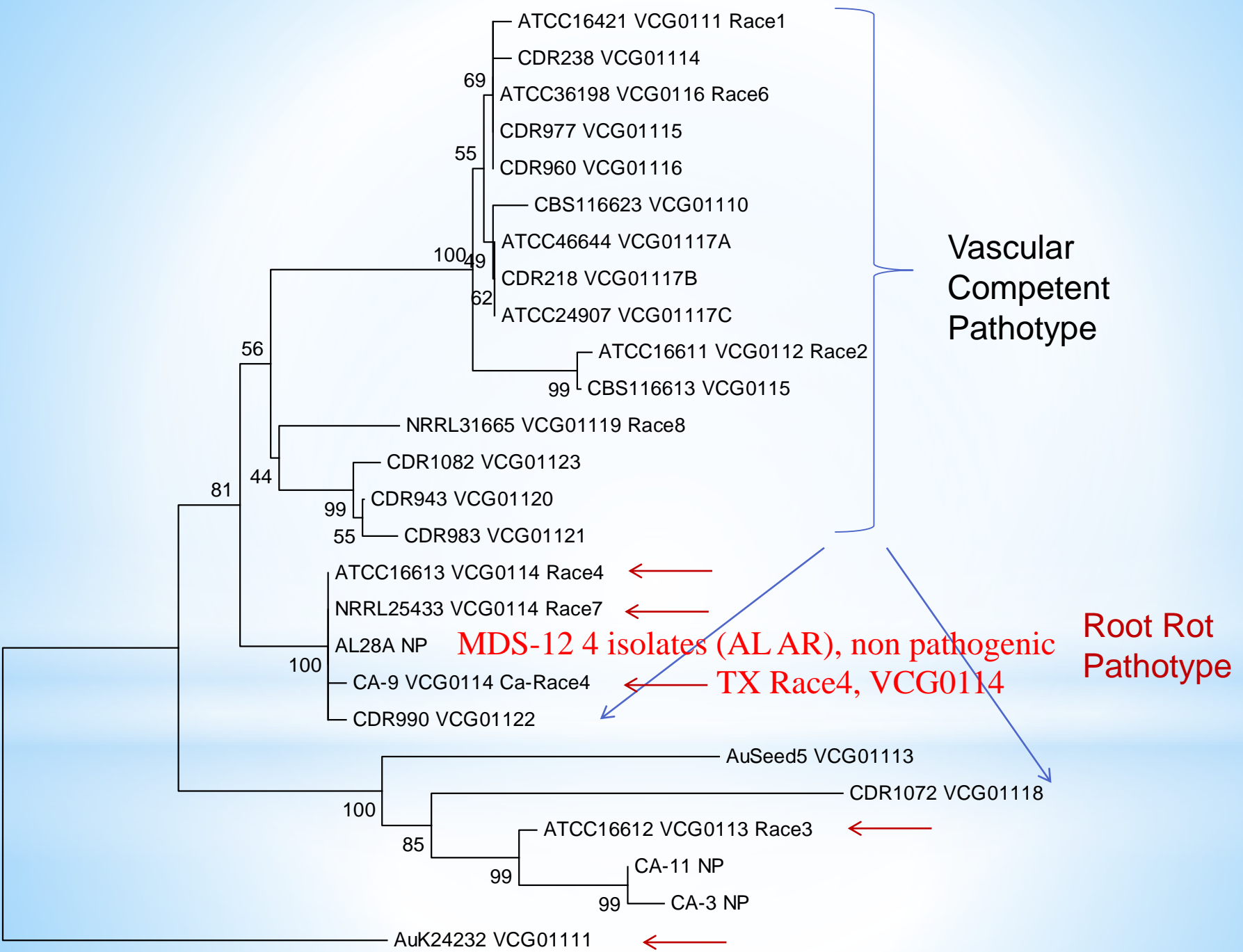
CDR977 VCG01115

ATCC16421 VCG0111 Race1

ATCC36198 VCG0116 Race6



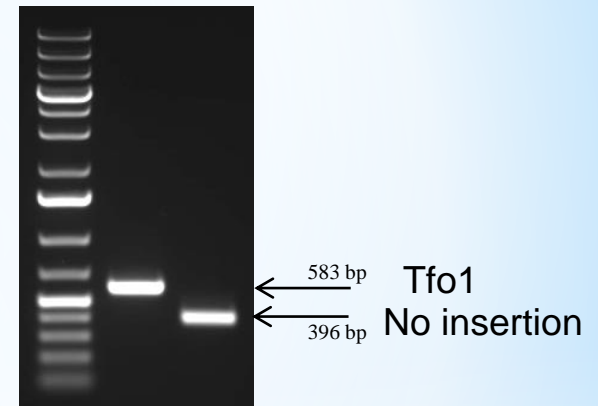
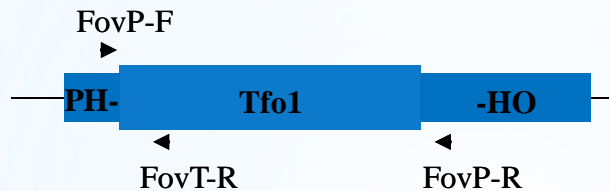




Detection of California and Texas Race 4 Isolates

- Developed a PCR-based system for the identification of California Race 4 Fov isolates based on the unique PHO-Tfo1 insertion event

Most CA isolates
46% of TX isolates



(Ortiz et al., 2017, Plant Disease)

54% of TX isolates
A few China isolates
One CA isolate (MITE
Instead of MuDR)



Soil-Infested Assay

Pima S7

24 DAI

1 of 4 flats



← Control
(inoculated
with water)

Each cup is
inoculated with a
different TX race
4 isolate.

Bell, 2017

Genetic Diversity, Virulence, and Nematode Interactions of Fov Causing Cotton Wilt in Georgia

- Locally severe outbreaks of Fusarium wilt of cotton in Georgia, USA since 2011
- Concern about new genotypes
- Surveyed 7 fields in five counties
- Conducted VCG and DNA analyses
- Pathogenicity assays:
 - Stem puncture assay
 - Soil-infested assay
 - Interaction with root-knot nematode in the soil-infested assay

Frequencies of VCGs among Fov obtained from infected plants in five counties of Georgia in 2014

County ^a , Field, and DP Cultivar	No. of Infected Plants ^b	Number of Infected Plants per VCG							
		0111 ^c race1	0112	01117B	01117C	01118	01119	01120	01121
Evans, 3A, 1050 B2RF	10	0	2	8	1	0	0	0	3
Evans, 3B, 1050 B2RF	16	0	2	10	3	0	0	0	2
Berrien, 2, 1454 NR B2RF	23	3	0	1	5	0	0	0	17
Tift, 1A, 1252 B2RF	13	0	0	1	0	0	0	0	4
Tift, 1B, 1252 B2RF	15	0	0	5	0	0	0	0	5
Cook, 4, 1050 B2RF	17	0	0	6	1	7	0	0	9
Grady, 5, 1454 NR B2RF	13	0	0	6	0	1	1	5	2
Total	107	3	4	37	10	8	1	5	42

^aCounties were arranged from northeast to southwest along the Georgia cotton belt that runs parallel to the geographic fall line. Berrien, Tift and Cook Counties share borders among them.

^bNumber of infected plants analyzed.

^cInternational VCG code. Race 1 reference isolate ATCC16421 belongs to VCG0111, race 2 reference isolate ATCC16611 to VCG0112, and race 8 reference isolate NRRL31665 to VCG01119.

Frequencies of VCGs among Fov obtained from infected plants in five counties of Georgia in 2014

County ^a , Field, and DP Cultivar	No. of Infected Plants ^b	Number of Infected Plants per VCG							
		0111 ^c race1	0112 race2	01117B	01117C	01118	01119 race8	01120	01121
Evans, 3A, 1050 B2RF	10	0	2	8	1	0	0	0	3
Evans, 3B, 1050 B2RF	16	0	2	10	3	0	0	0	2
Berrien, 2, 1454 NR B2RF	23	3	0	1	5	0	0	0	17
Tift, 1A, 1252 B2RF	13	0	0	1	0	0	0	0	4
Tift, 1B, 1252 B2RF	15	0	0	5	0	0	0	0	5
Cook, 4, 1050 B2RF	17	0	0	6	1	7	0	0	9
Grady, 5, 1454 NR B2RF	13	0	0	6	0	1	1	5	2
Total	107	3	4	37	10	8	1	5	42

^aCounties were arranged from northeast to southwest along the Georgia cotton belt that runs parallel to the geographic fall line. Berrien, Tift and Cook Counties share borders among them.

^bNumber of infected plants analyzed.

^cInternational VCG code. Race 1 reference isolate ATCC16421 belongs to VCG0111, race 2 reference isolate ATCC16611 to VCG0112, and race 8 reference isolate NRRL31665 to VCG01119.

All VCGs of Georgia Fov Belong to Vascular Competent Pathotype

- Stem-puncture assay with 'Rowden': Caused 33-60% and 40-67% reductions in Shoot and Leaf Weight compared to control inoculations, and increases in Percent Leaves Wilted from 0.7% (control) to 27-54% (Fov inoculated).
- Soil-infestation assay and interaction with RKN (Rowden):
 - RKN **alone** did **not** cause significant reductions in Shoot Weight, or increases in Percent Leaves Wilted and Vascular Browning Index compared to water control.
 - Fov **alone**, overall, did **not** cause significant reductions in Shoot Weight, or increases in Percent Leaves Wilted and Vascular Browning Index compared to water control.
 - Fov + RKN, overall, caused significant reductions in Shoot Weight, or increases in Percent Leaves Wilted and Vascular Browning Index compared to Fov treatment alone.

Pathogenicity of Root Rot Pathotype of Fov

- Causes disease without nematode in the soil-infested assay
- Does not cause disease in stem puncture assay
- Mainly rot the root and confined to the lower part of the stem before killing the plant
- Prefer neutral or alkaline clay soil
- Often associated with irrigated fields

Symptoms and Vascular Browning (% of brown stain in the stele tissue at ground level)



Asut Biot

Ca Race 4

Race 4

Ca Race 4

<<< Coker 312, 9 weeks after inoculation >>>

R-4



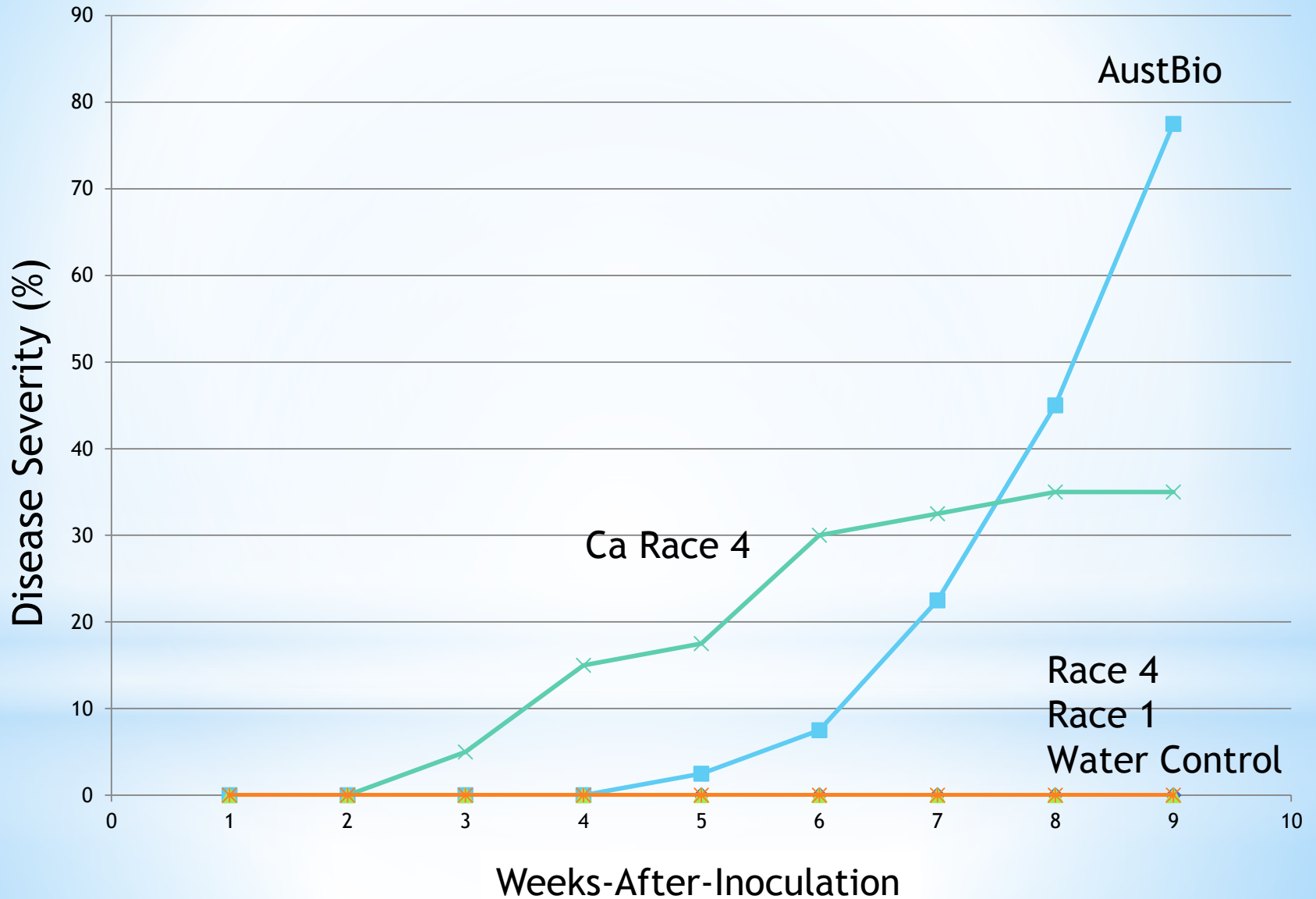
CA 9



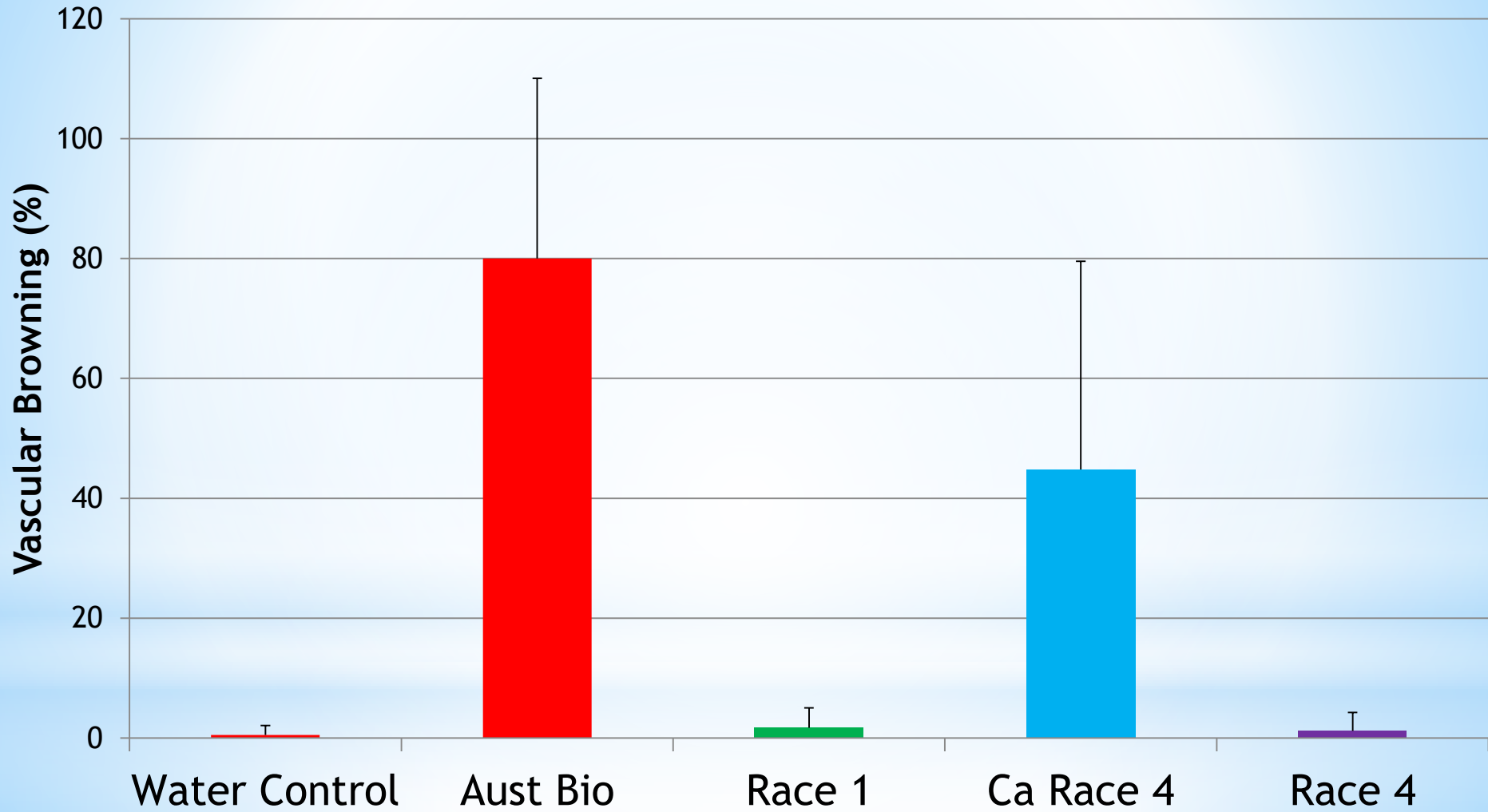
CA 14



Disease Progression of Coker 312 Inoculated with Race1, Race 4, Cal-race 4, and AustBio



Vascular Browning (%) 9 Weeks-After-Inoculation



Implications for Breeding

- Root Rot Pathotype, VCG0113, 0114, and 01111
(races 3, 4, 7, and AusBio):
 - ❖ Breed for resistance to Fov
 - For race 4, resistances from *G. arboreum* Acc. No. 190, Pima S-4, Pima S-6, SBSI 12B2 are introgressed into Upland lines (Bell)
- Vascular Competent Pathotype: 15 VCGs
(including races 1, 2, 6, and 8):
 - ❖ Breed for resistance to nematode: good resistances to RKN and RN are available
 - Mi₁, Mi₂, Ren₂, Ren₃ line (Bell) is being tested in Georgia and Texas

As many Fov fields in Georgia are infested with sting nematode, the test may also provide evaluation of the line's effectiveness against Fov-Sting nematode complex
- Stacking the Fov and nematode resistances to control both pathotypes (Bell)