

GENETIC DIVERSITY ON VCG'S OF *VERTICILLIUM DAHLIAE* ON SUNFLOWER: UPDATE

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OUTLINE

- ▣ Symptoms and Introduction
- ▣ Objectives
- ▣ Methods
 - * *AFLP*
 - * *Traditional Nit Mutant Assay*
 - * *SSR's*
- ▣ Preliminary Results
- ▣ Future





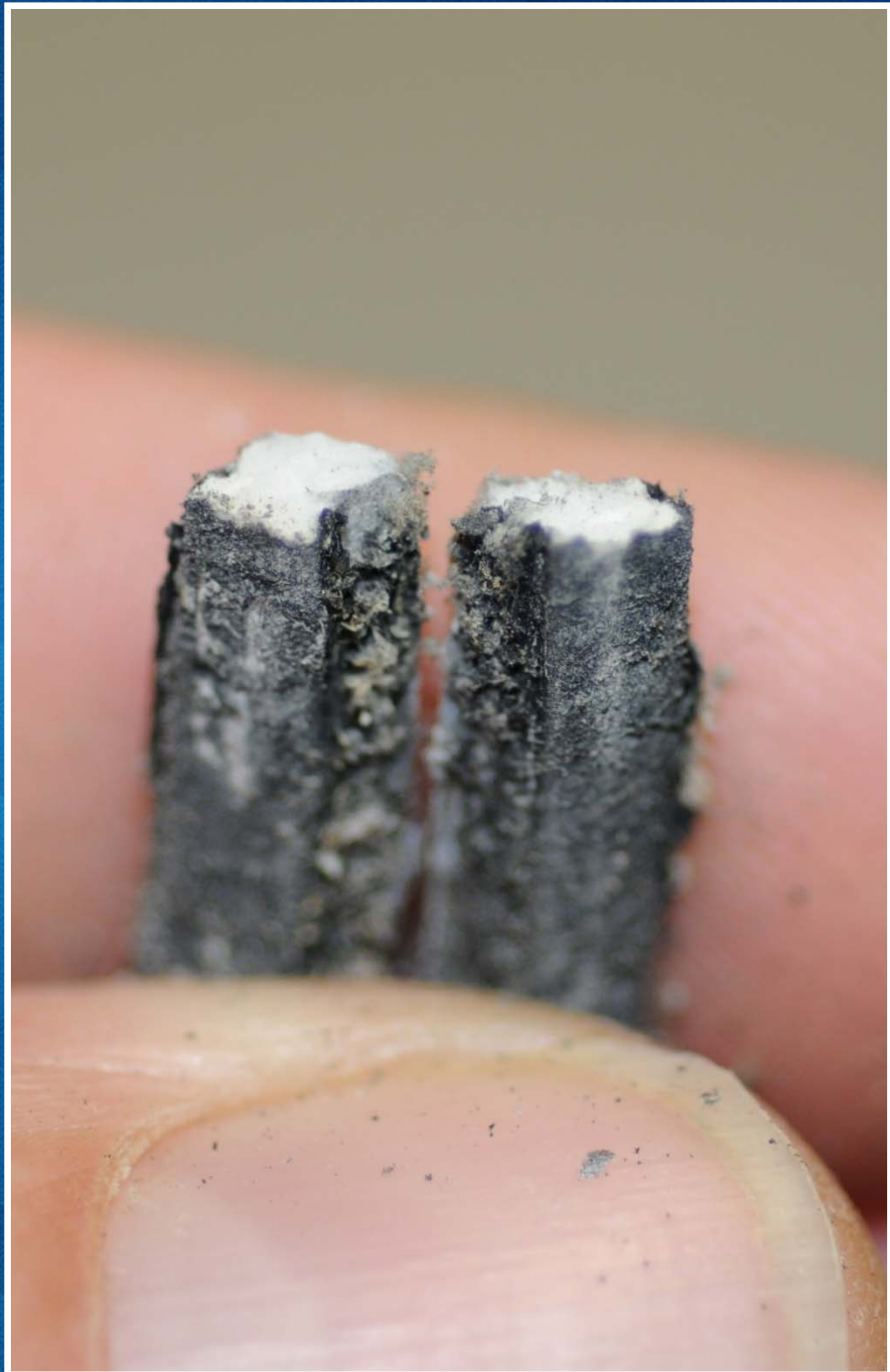












Introduction: Verticillium wilt

- ▣ *V. dahliae*: fungal pathogen – soil borne
- ▣ Microsclerotia persist for years.
- ▣ No sexual stage that allows recombination of genes
- ▣ Vegetative compatibility grouping (VCG)
- ▣ Yield reductions significant

Introduction

- ▣ Effective resistance in North America for 20 yrs
- ▣ Resistance not conferred in Argentina - 1985
- ▣ Resistance overcome in 2002 (MN) & 2004 (ND)

Objectives

- ▣ Determine the VCG(s) **and genetic diversity** of *V. dahliae* on Sunflower
- ▣ Aggressiveness of VCGs on sunflower

Techniques

VCG and Genetic Diversity

- ▣ PCR's – **Correlated to VCG**
- ▣ Amplified Fragment Length Polymorphism – **VCG**
- ▣ Simple sequence repeats (SSR) – **Diversity**
- ▣ Traditional NIT-mutant assay - **VCG**

Aggressiveness

- ▣ Greenhouse screening : Root Dip

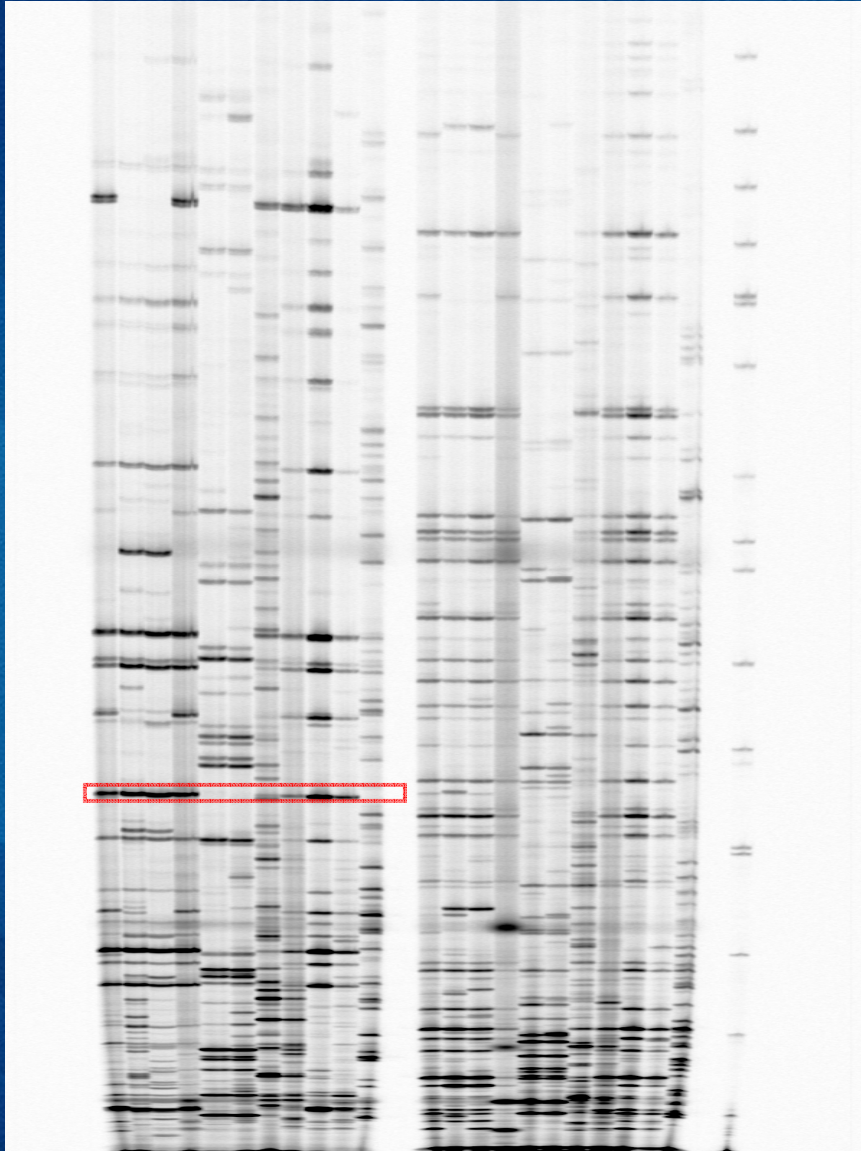
Isolates

- ▣ Tester isolates
- ▣ Isolates obtained in North and South America
 - Other crops
 - Other Regions



Methods and Preliminary Data

Methods: AFLP



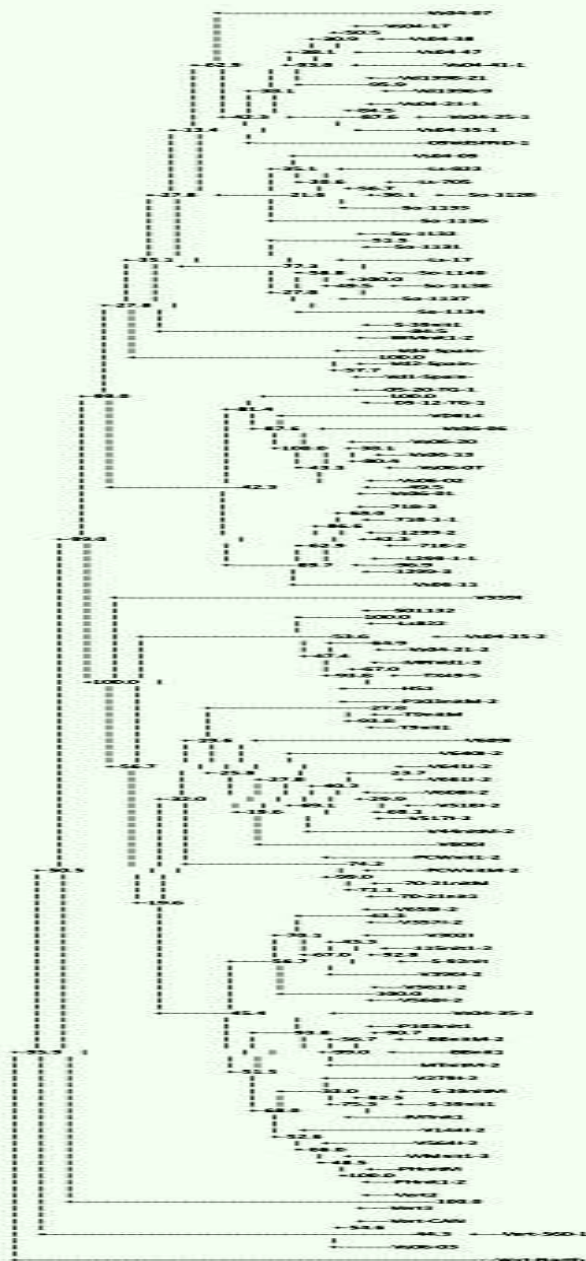
- - ▣ Collado Romero, 2008 correlated AFLP pattern to VCG
 - ▣ DNA extracted, Licor Sequencer, manually scored polymorphisms

Methods: AFLP

- ▣ Collado Romero, 2008 correlated AFLP pattern to VCG
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Sunflower Samples (n=97)



VCG 2A and 4B

VCG 1

VCG 6

NIT Mutant Screening

- ▣ Creation of mutant isolates
- ▣ Use of multiple media
- ▣ Compatibility assessment using 'tester' isolates

VCG – Traditional (n=50)

County	VCG
United States	1B, 2A, 2B, 4A, 4B, 6
Argentina	1B, 2B, 4A, 4B, 6
Australia	1B, 2A, 2B
South Africa	6
Canada	2B
China	1B

Results to date:

- ▣ Six different VCG found in sunflower
- ▣ No obvious geographical differences between continent identified...
- ▣ Scoring and analysis of approximately 400 - 600 isolates will continue (AFLP and SSR) - *winter*
- ▣ Representative isolates will be evaluated for aggressiveness -*spring*

Acknowledgments

- ▣ National Sunflower Association
- ▣ All persons who provided *V. dahliae* isolates

Thank you and Questions

Methods: PCR

Primer pair	Amplicon	Reference
VertBtF/R	115	Atallah et al., 2007
DB19/22	526 or 543	Carder et al., 1994
INTD2F/2R	462	Mercado-Blanco et al., 2003
NDF/R	1410	Perez-Artez et al., 2000
INTND2F/2R	824	Mercado-Blanco et al., 2003
DB19/espdef01	334	Collins et al., 2005
INTND2F/3R	688	Collado-Romero et al., 2008
INTND2F/MCR2B	964	Collado-Romero et al., 2008

Our Study

Collado-Romero et al.(2006)

PCR Pattern	334	824	462
A	+	-	+
B	+	-	-
C	-	+	-
None amplified	-	-	-
D	+	+	-

Collado-Romero et al.(2006)

Our Study