

# PRELIMINARY STUDIES ON THE VEGETATIVE COMPATIBILITY GROUPS OF VERTICILLIUM ON SUNFLOWER

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# OUTLINE

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# Introduction

- ▣ *V. dahliae*: cosmopolitan, soil pathogen fungus.
- ▣ > 200 host species worldwide
- ▣ Losses: in US, lettuce (100%), potato (50%), sunflower (30%).
- ▣ Sunflower: reduced head and seed size, oil content and yield.
- ▣ Produce microsclerotia, a survival structures persist for years.

# Intro: symptoms



Slides obtained from Dr. Sam Markell, NDSU.



## Intro: symptoms



Sunflower stalks split open to show the range of *Verticillium* symptoms on pith at the end of the season (late October in south central ND).

Slide obtained from Dr. Gulya, T. 2010 in SD, ND.

## Intro.: resistance to *V. dahliae*

- ▣ V-1 gene ~ effective for 20 yrs (North America)
- ▣ Vd strains not controlled by V-1 in 1985 (Argentina), 2002 (MN), and 2004 (ND)

# Intro.: genetic diversity of *V. dahliae*

- ▣ No sexual stage that allows recombination of genes
- ▣ Vegetative compatibility grouping (VCG)
- ▣ Vd VCGs: VCG1, VCG2 (A,B), VCG3, VCG4 (A,B), and VCG6.
- ▣ On sunflower, VCGs of *V. dahliae* isolates have not been determined on wide scale for populations study (continental & inter-continental)

# Objectives

- ▣ **Determine the VCG(s) of *V. dahliae* on Sunflower**
  - \* **PCR's**
  - \* **AFLP method**
  - \* **Simple sequence repeats (SSR)**
  
- ▣ **Aggressiveness of VCGs on sunflower**
  
- ▣ **SCAR marker for *V. dahliae* to assess a pathogen in plants**



## Methods: isolates

Country	# of isolates	Hosts
Testers	30	Different hosts
Multiple countries	12	?
Canada	23	Potato sunflower
Spain	3	?
Argentina	10	?
Australia	10	?
South Africa	2	?
USA	<b>407</b>	Sunflower, others
ND	350	Sunflower
SD	14	Sunflower
MN	13	Sunflower
TX	13	Sunflower
IN	2	Sunflower
NE	14	Sunflower
WI	1	Potato
<b>Total</b>	<b>497</b>	

# Methods: PCR

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

**Our Study**

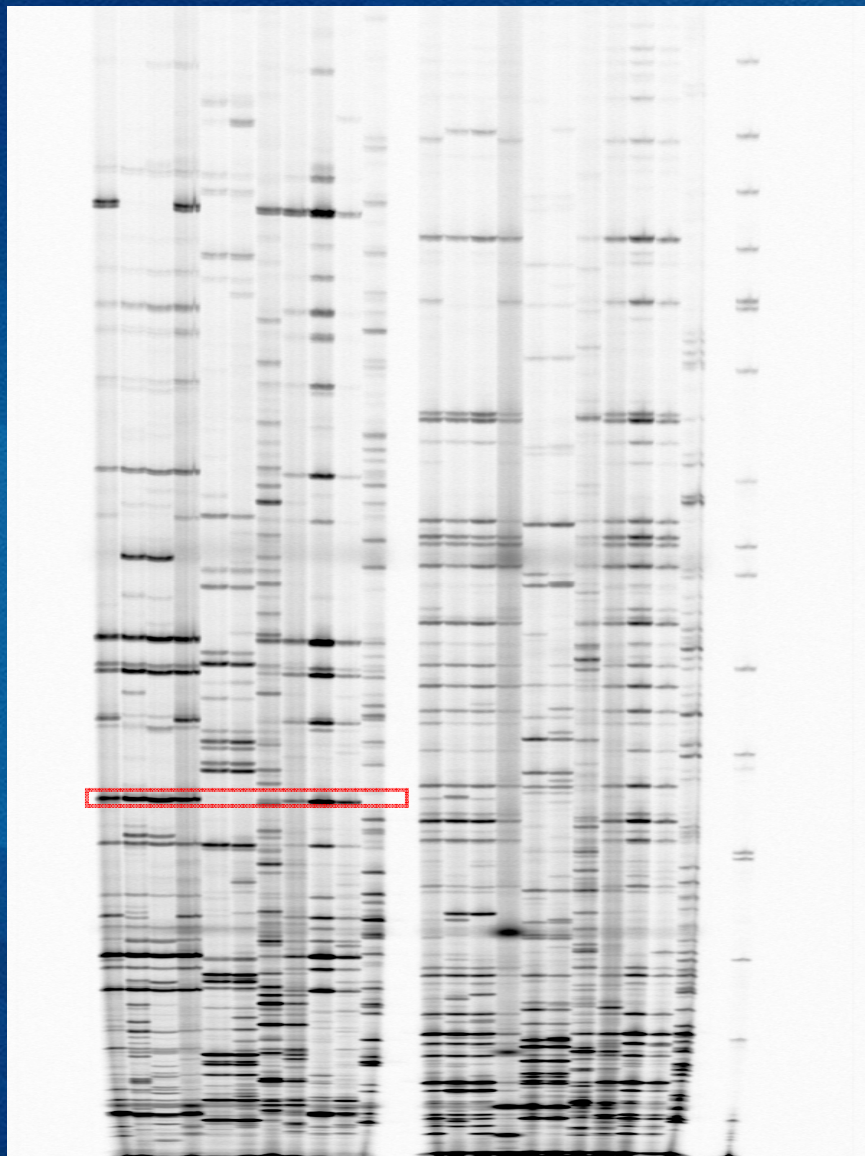
Collado-Romero et al.(2006)

PCR Pattern	334	824	462
A	+	-	+
B	+	-	-
C	-	+	-
None amplified	-	-	-
<b>D</b>	<b>+</b>	<b>+</b>	<b>-</b>

Collado-Romero et al.(2006)

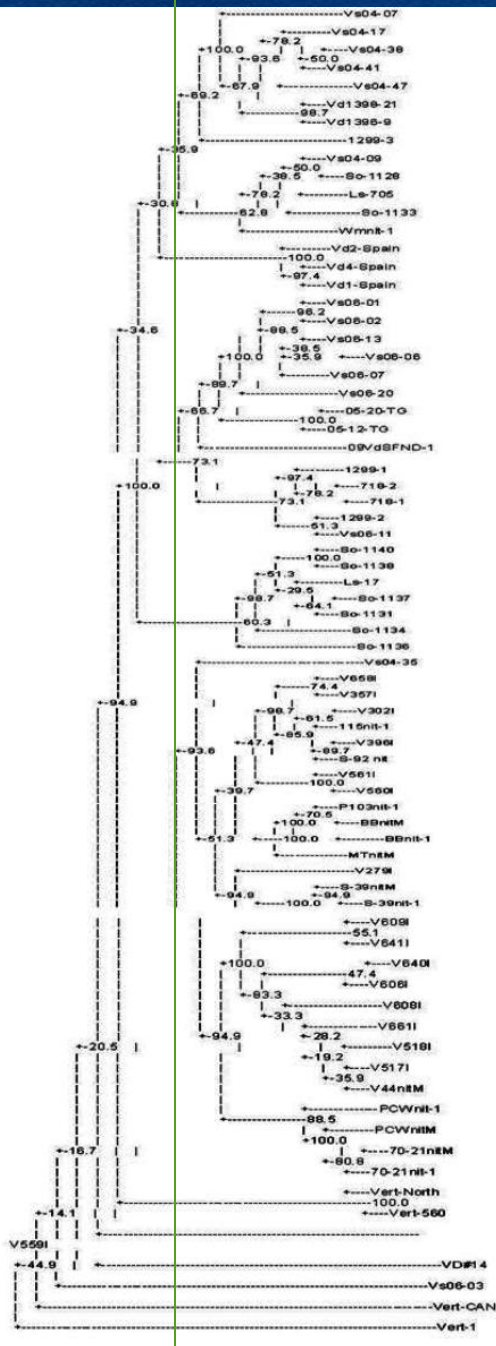
**Our Study**

# Methods: AFLP



- Manually scoring: Present (1) or absent (0).
- Number of bands scored/primer/isolate:
  - EcoRI-AG/MseI-C*: 113
  - EcoRI-AT/MseI-C*: 134
  - EcoRI-GA/MseI-C*: 97
  - EcoRI-GC/MseI-C*: 156
- Collado-Romero et al., 2006
- DNA sequencer: Li-COR
- WINBOOT software was used for clustering analysis.

# Results



Host

PCR pattern

VCG

Potato

D

4A

4B

Lettuce and Spinach

C, D

2A

Sunflower

B

2A

Amur maple

B

2A

Lettuce and spinach

C, D

2A

Testers

Multiple VCGs

Outliers

B

# Conclusions

- Primary results indicate that *Verticillium* on sunflower is belonging to VCG-2A.
  - Host adaptation
  - Aggressiveness: effective breeding
  - International migration of *Verticillium*
- Results are consistent with Collado-Romero et al. (2006) in their isolate clustering and PCR pattern.

## Future work

- Determine VCG of *V. dahliae* isolates (4 month).
- Aggressiveness (4 months)
- Collect isolates from Argentina to compare populations with North America (June 2011).
- Run SSR markers for all *V. dahliae* samples (4 months).
- Design SCAR primers for the different VCG's (3 months).

# Acknowledgments

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

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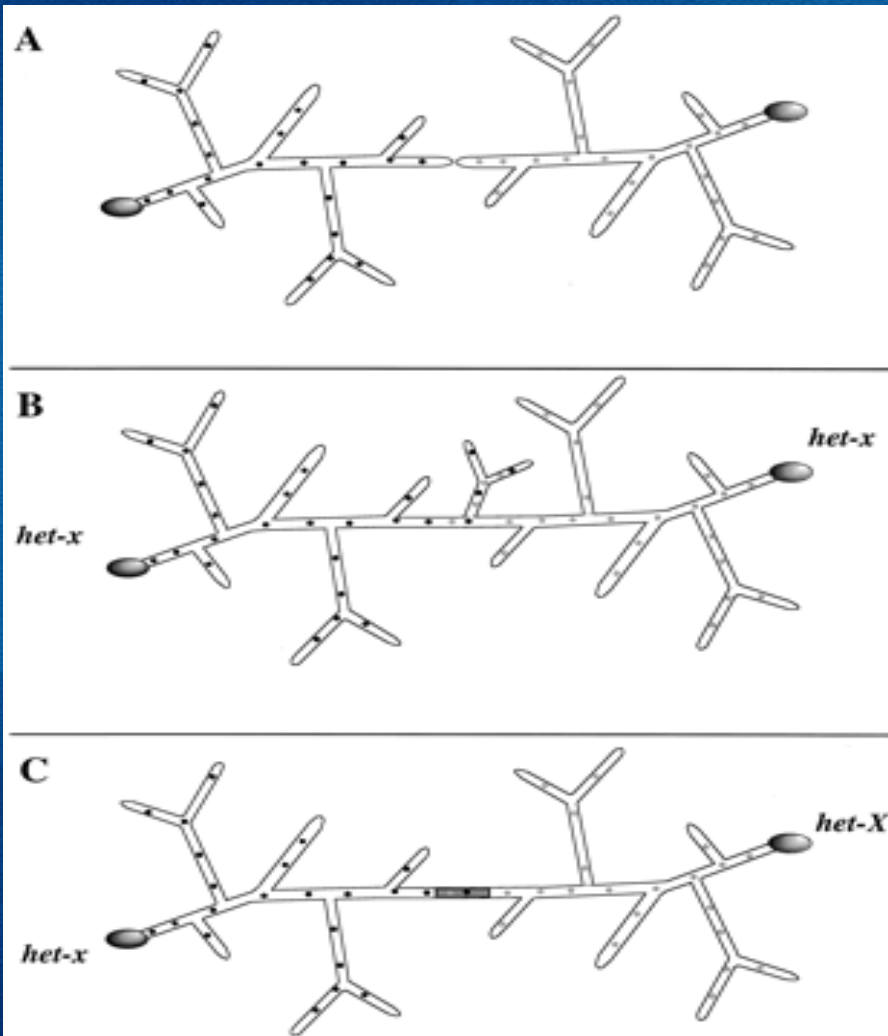


Summary of fungi recovered from culturing sunflower stalks on three different media, PDA, CV8, and PYLA since September to November 2010

Fungus <sup>a</sup>	% Fields	% Stalks
<i>Phomopsis</i>	57	22
<i>Phoma</i>	18	-
Downy Mildew	5	-
Charcol rot	8.4	3.4
<i>V. dahliae</i>	25.8	19.5
<i>Fusarium</i>	62	44.5
Rust	12	-
<i>Rhizopus</i>	0.6	-
<i>Sclerotinia</i>	3.2	-
<i>Colletotrichum</i>	3.2	1.4
Insect	8.4	-
<i>Acremonium</i>	7	3.5
Total fields	155	
Total stalks	1146	

# Vegetative Compatibility Groups

## ▣ What is vegetative compatibility ?



(A) When two different fungal individuals meet, they spontaneously undergo a cell fusion event or anastomosis.

(B) If the two individuals have the same *het* genotype, a heterokaryon is established.

(C) If the two strains differ in *het* genotype, the heterokaryotic cells are destroyed or severely inhibited in their growth

(Saupe et al. 2000. Microbiology and Molecular Biology Reviews 64:489-502)