Determination of vegetative compatibility groups using molecular markers, and their aggressiveness of *Verticillium dahliae* occurring on sunflower

Kholoud M. Alananbeh
Samuel Markell
Thomas Gulya
and Neil Gudmestad

January 13th , 2009

OUTLINE

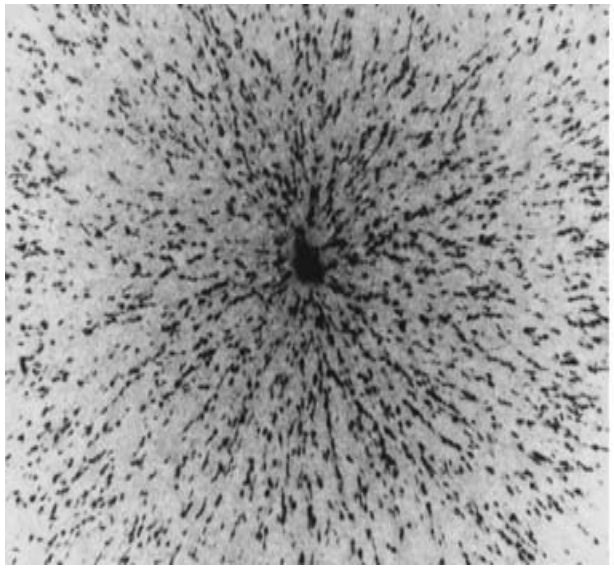
- Introduction
- Symptoms of *V. dahliae*
- Losses
- Genetic diversity
- Vegetative compatibility groups
- Objectives
- Methods

Major diseases of sunflower

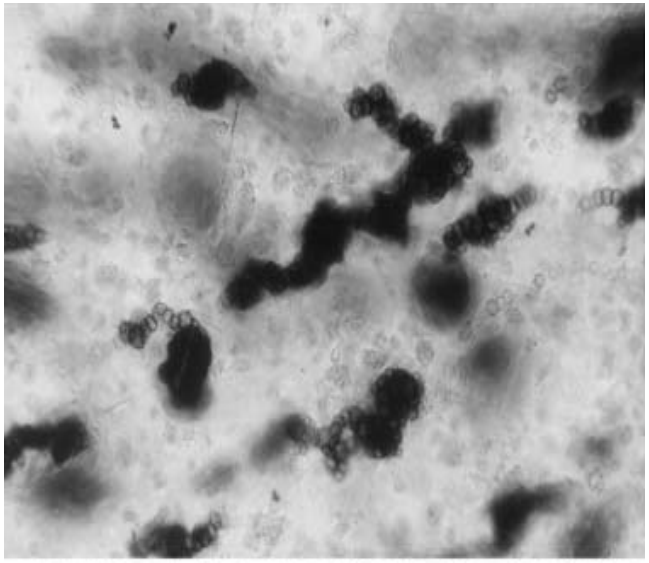
- Sclerotinia stalk and head rot
- Rust
- Verticillium wilt
- Downy mildew
- Phomopsis
- Phoma black stem and leaf spot


Slide obtained from Dr. Sam Markell, NDSU.

Culture: microsclerotia



The microsclerotia are formed in a radial pattern



Globose to elongate V. dahliae microsclerotia

Large, globose to irregularly shaped *V. dahliae* microsclerotia with connected dark hyphae

Goud et al., 2003

Losses & genetic resistance

- The disease has economic implications annually.
- Reduced head size (with severe and mild infection 42% and 18% respectively)
- Seed size, oil content, and yield (up to 66%).
- Managed by genetic resistance

  * V-1 gene ~ effective for 20 yrs

  * Vd strains not controlled by V-1 in 1985 (Argentina), 2002 (MN), 2004 (ND)

Genetic diversity of *V. dahliae*

- No sexual stage that allows recombination of genes
- Vegetative compatibility grouping (VCG)
- Vd has 4 VCGs: VCG1, VCG2 (A,B), VCG3, VCG4 (A,B)
- On sunflower, VCGs of *V. dahliae* isolates have not been determined on wide scale for populations study (Continental & inter-continental)

Importance of studying VCGs

- VCGs vary in their aggressiveness, physiology, and geographic distribution.

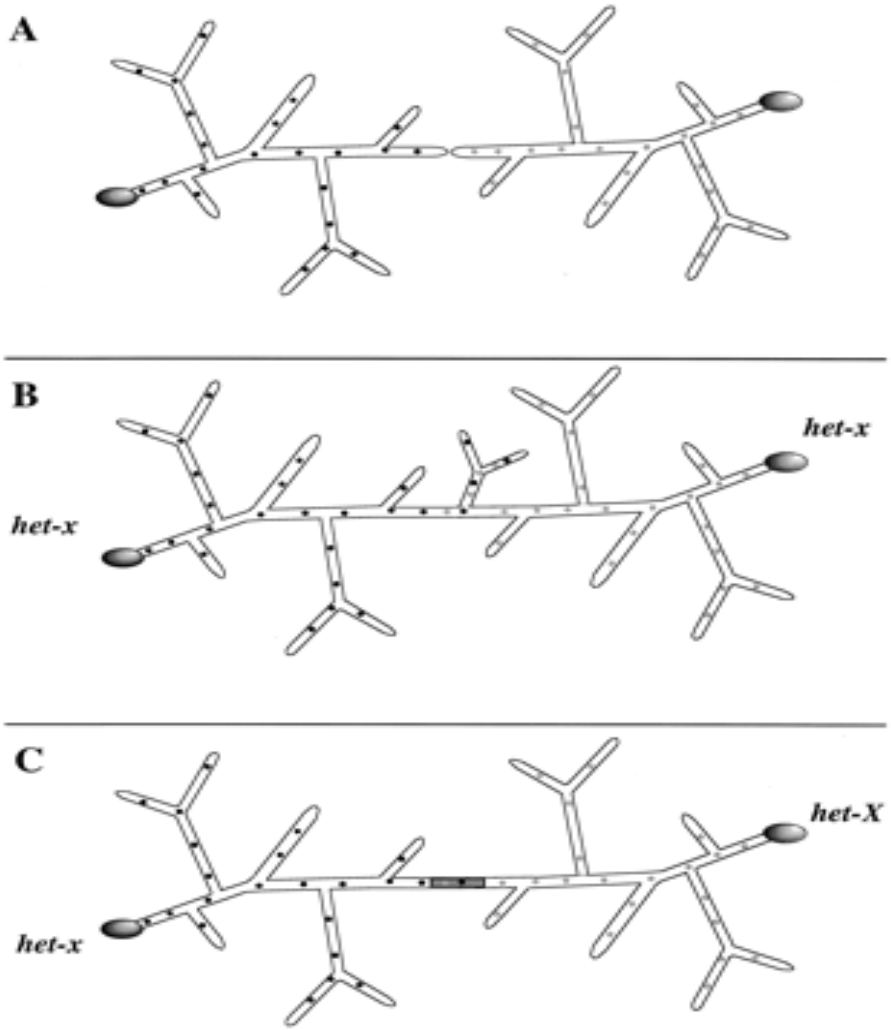
- Breeding programs

- Management (crop rotation)

  *Example: potato Vs mint (Omer et al., 2008)

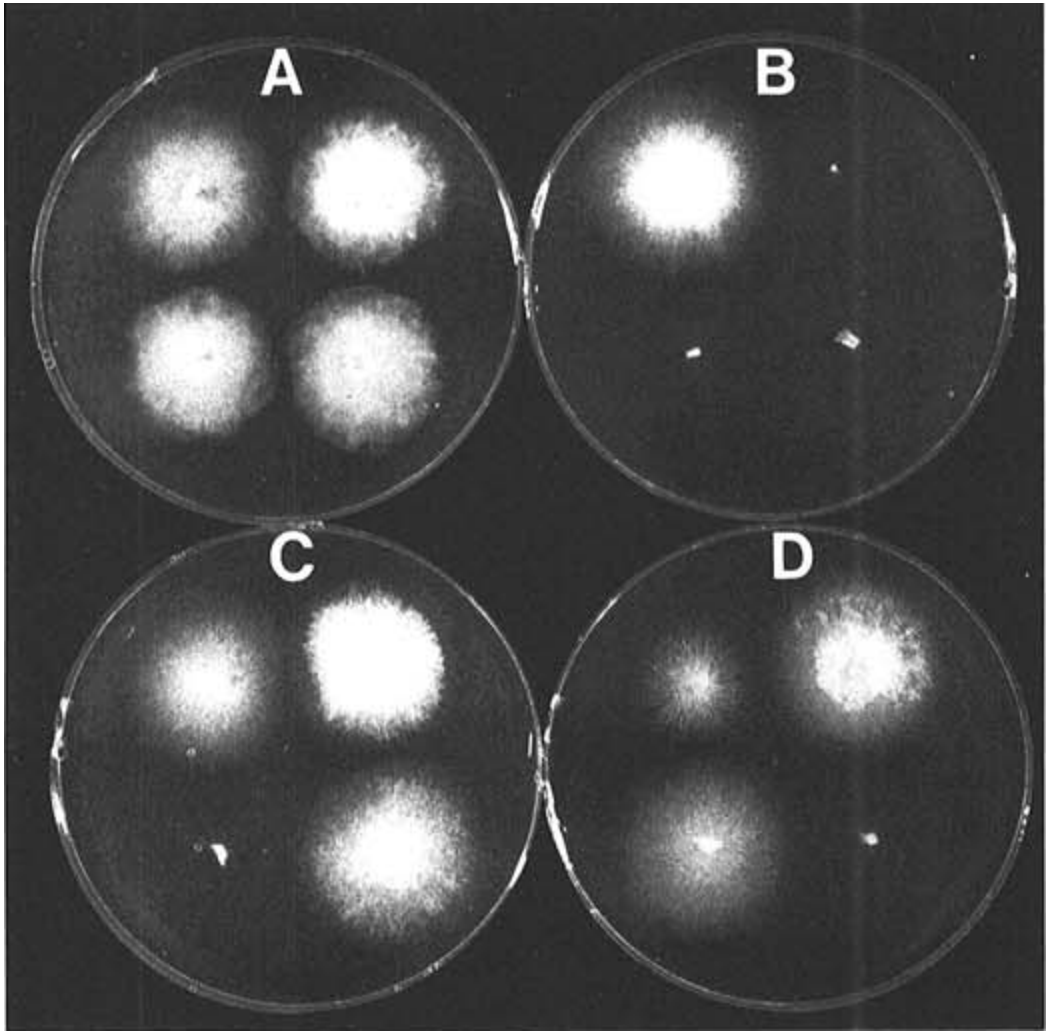
Vegetative Compatibility Groups

- 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)



Nitrate nonutilizing (*nit*) mutants phenotypes of *Fusarium oxysporum* on media with four different nitrogen sources

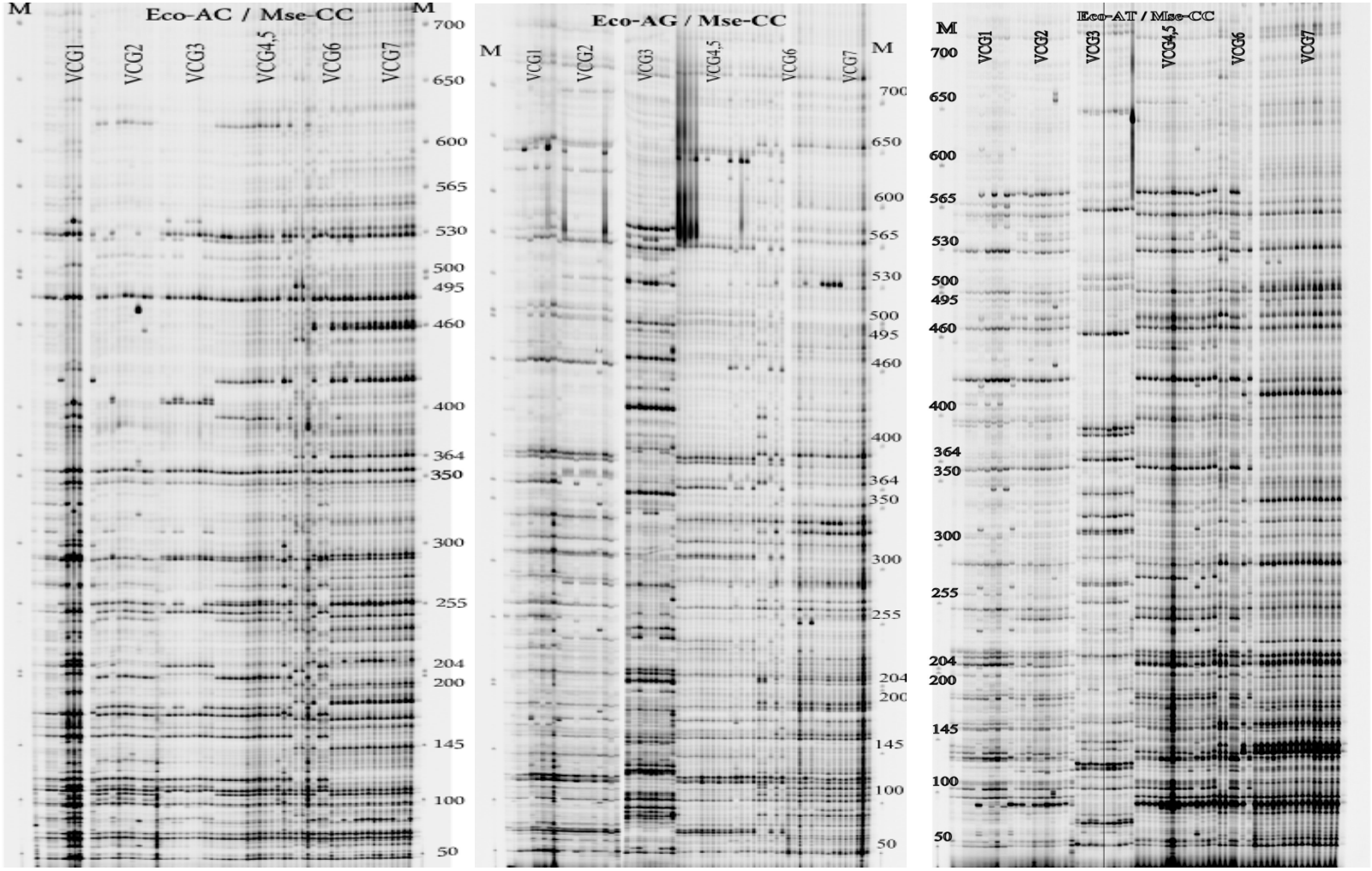
- Stable
- (*nit*) mutants
- Simple
- Many fungal plant pathogens

Limitations

- laborious and time consuming
- mutant are not always possible
- *Vic* or *het* alleles

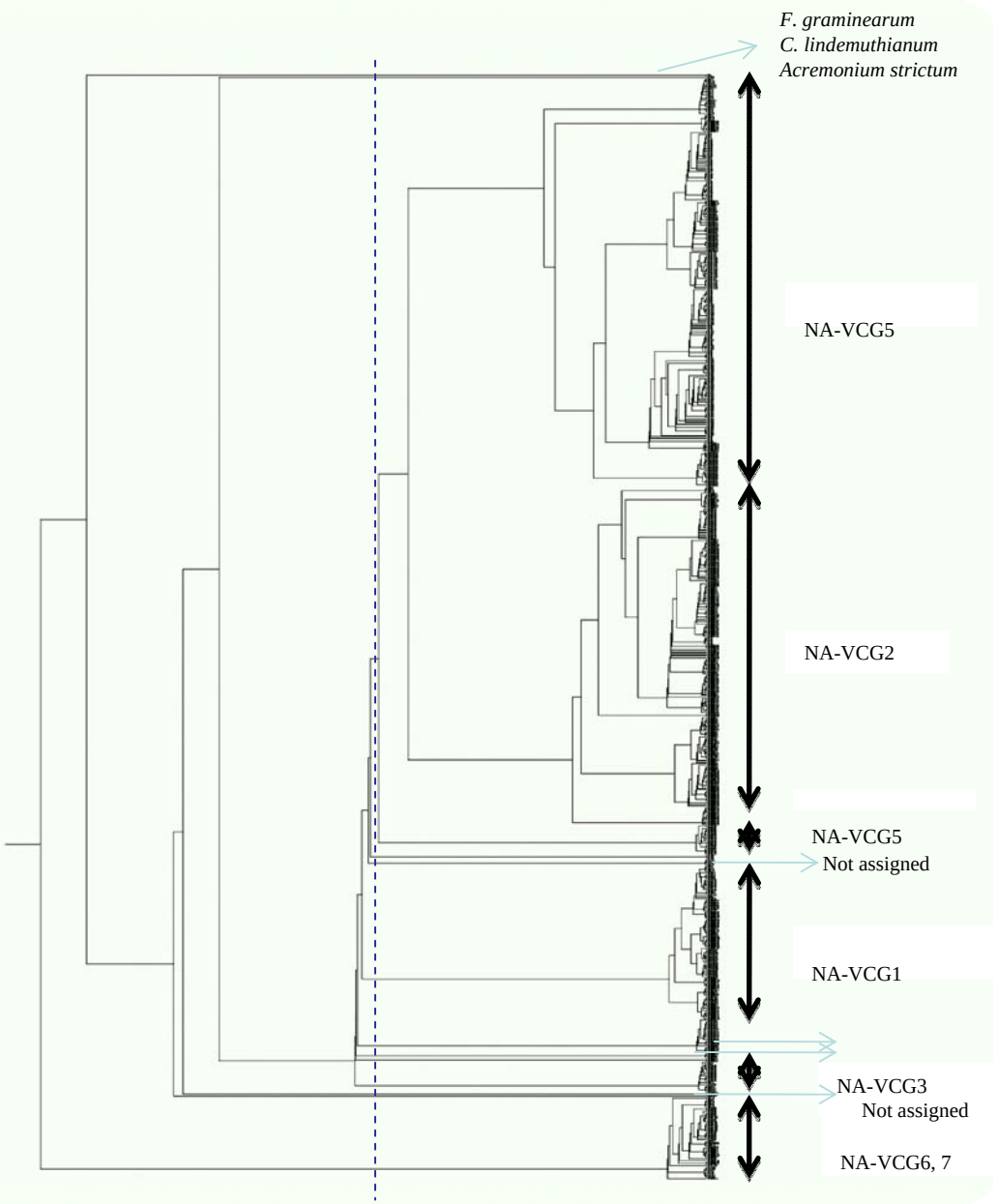
Correll et al., 1987

Amplified fragment length polymorphism (AFLP)



Amplified fragment length polymorphism electrophoresis of *C. coccodes* isolates for the seven NA-VCGs. The three primer sets, Eco-AC/Mse-CC, Eco-AG/Mse-CC, and Eco-AT/Mse-CC were used for selective polymerase chain (PCR) amplification reaction. PCR products were seperated on 6% polyacrylamide/7 urea gel using LI-COR 4300 model. The specific AFLP bands for NA-VCGs can be detected. M: is a standardized, IRdye-700 labeled size marker of 50-bp.

Dendogram generated by 1000 bootstrap reiterations using PHYLIP for AFLP data of 855 isolates of *C. coccodes, and 3* outgroups inclucing *C. lindemuthianum, F. graminearum,* and *A. strictum.*



Project objectives

- Determine the VCG(s) of *V. dahliae* on Sunflower
    * AFLP method
    * Simple sequence repeats (SSR)

- Aggressiveness of VCGs on sunflower

- SCAR marker for *V. dahliae* to assess a pathogen in plants

Isolates

- Collect *V. dahliae* isolates from US
- Dr. Gulya (USDA) and Dr. Rashid's isolates (MB)
- Other parts of the world (Argentina and Europe)
- Tester isolates from Dr. Randy Rowe (PS)

Methods

- Isolates will be cultured, purified, and DNA extracted
- AFLP protocol, will follow Collado-Romero et al., 2008
- Fluorescent DNA sequencer (Li-COR)
- SSR markers (Atallah et al., 2009)
- Aggressiveness: root dip method, hypodermic needle inoculation

Acknowledgements

- **National Sunflower Association**
- **Dr. Samuell Markell**

QUESTIONS ????

SUGGESTIONS !!!