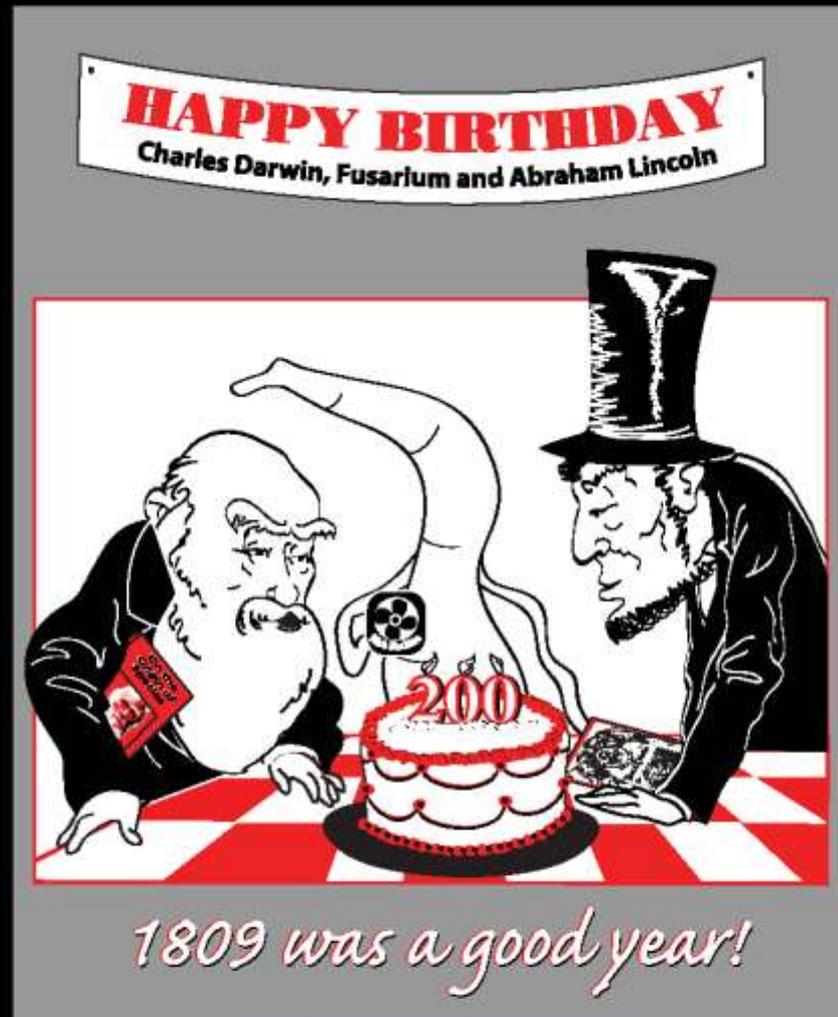# Vegetative Compatibility – A Native Fungal Mechanism for Inducing Death in *G. zeae*

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Outline

- Background on *vic* genes in general
- A multi-step vegetative compatibility model
- Genetic evidence for the multi-step model
- Cloning *vic* loci
- Evolutionary non-conservation of *vic* loci
- Mapping *vic* loci
- Utilization of *vic* loci

Vegetative (Heterokaryon) (In)Compatibility

- Is a self-recognition system in fungi
- Has a multi-genic basis
- Some molecular details are not evolutionarily well conserved
- Different in ascomycetes than in basidiomycetes
- Leads to death of non-identical heterokaryons, via a relatively complex apoptotic process
- Governed by a series of *vic* or *het* loci
- In *Fusarium*, used mostly for population analyses

Some Genera with Vegetative Compatibility Systems

- *Aspergillus*
- *Ceratocystis*
- *Cochliobolus*
- *Colletotrichum*
- *Cryphonectria*
- *Cryptostroma*
- *Diaporthe*
- *Fusarium*
- *Hypoxylon*
- *Leptographium*

- *Leucostoma*
- *Leucocytospora*
- *Morchella*
- *Neurospora*
- *Penicillium*
- *Podospora*
- *Sclerotinia*
- *Septoria*
- *Trichoderma*
- *Verticillium*

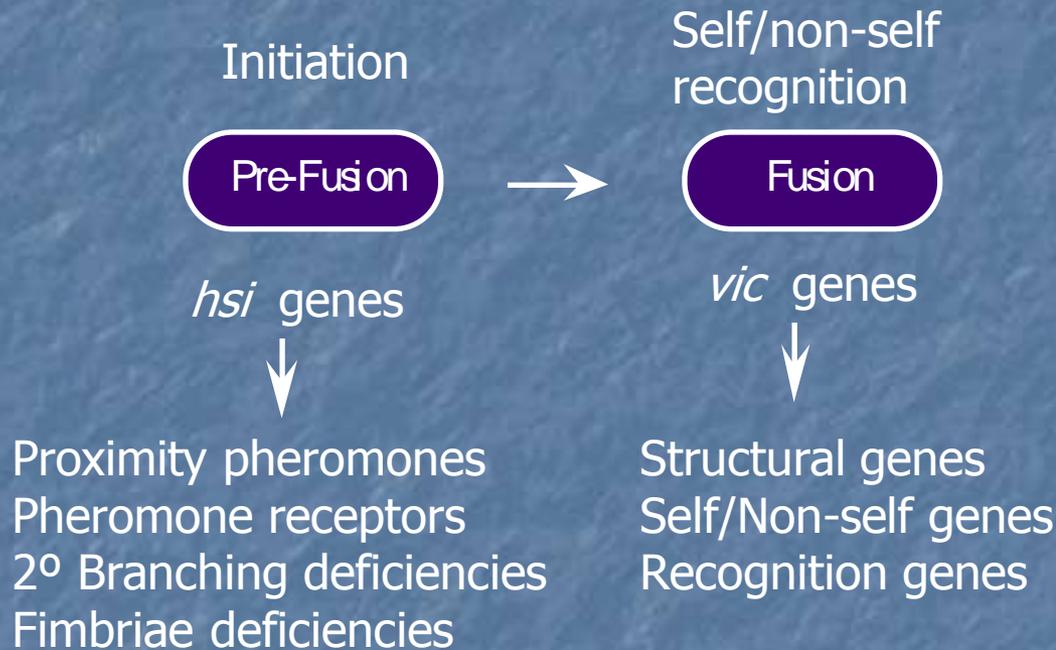# Genetics of Vegetative Compatibility in Ascomycetes

- Stable heterokaryon formed only when all *vic* locus pairs are homozygous
- Unstable heterokaryons may form with one or a few differences
- Two types of interactions – allelic and genic
- Number of loci involved is usually large (10-15)
- Strains that are vegetatively incompatible are sexually cross fertile
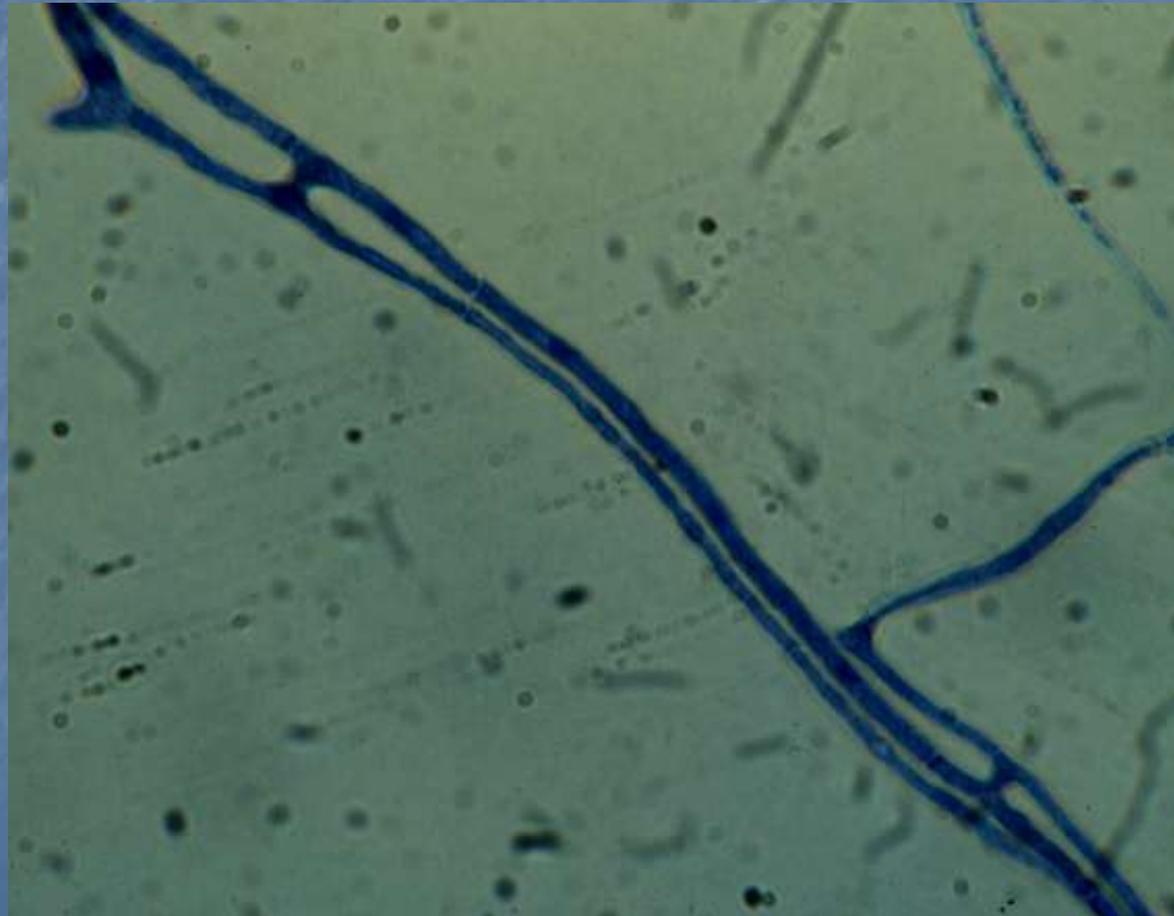
Possible Progeny with Three Segregating *vic* Loci

(*vicA vicB vicC* × *vica vicb vicc*)

- *vicA vicB vicC*
- *vicA vicB* vicc
- *vicA* vicb *vicC*
- *vicA* vicb vicc

- vica *vicB vicC*
- vica *vicB* vicc
- vica vicb *vicC*
- vica vicb vicc

Two-step Vegetative Compatibility Model

Initiation

Self/non-self recognition

Pre-Fusion → Fusion

hsi genes

vic genes

Proximity pheromones
Pheromone receptors
2º Branching deficiencies
Fimbriae deficiencies

Structural genes
Self/Non-self genes
Recognition genes

Heterokaryon Self-Compatible
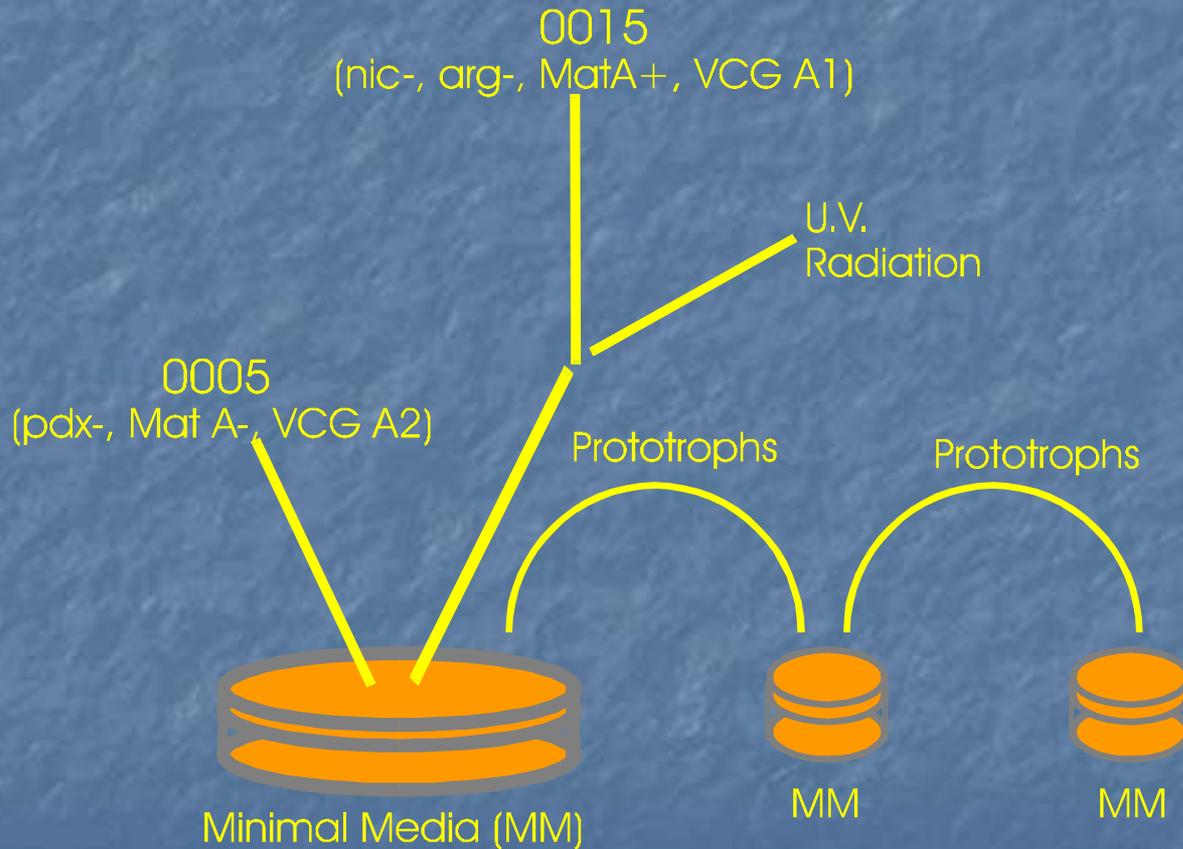
Heterokaryon "Self"-Incompatible

Ideal Interactions

Compatible

Incompatible

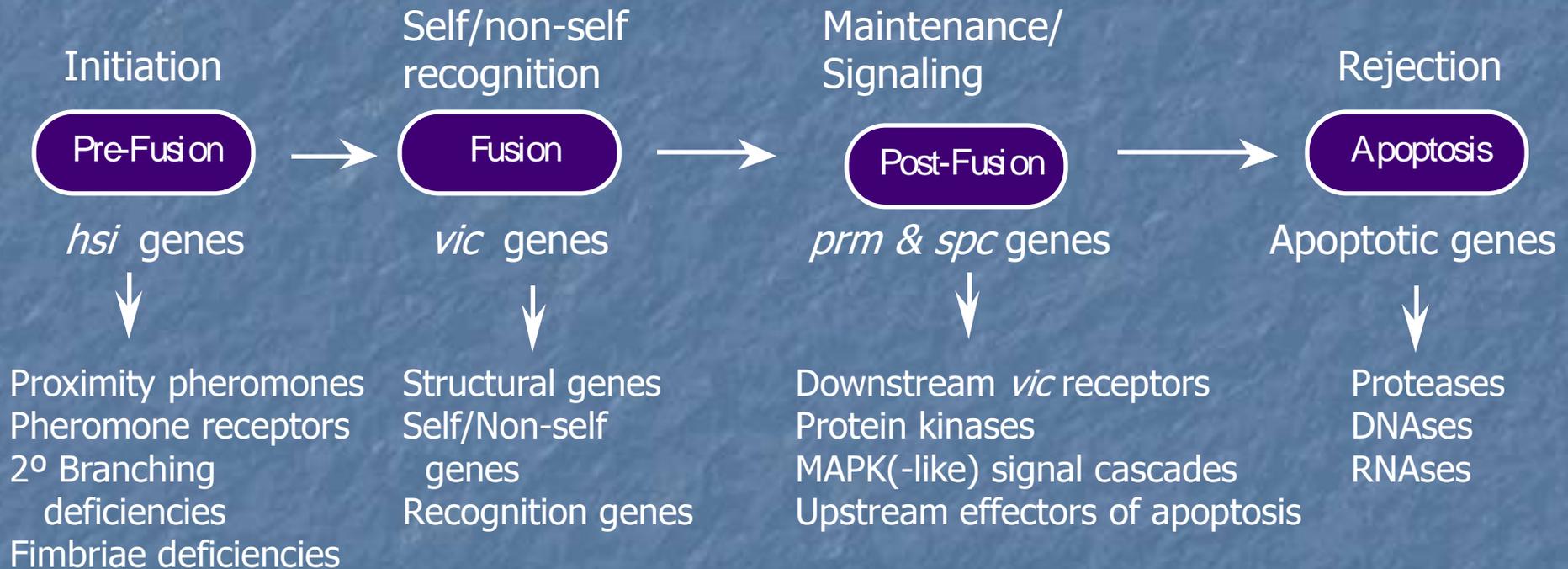# *PRM* & *SPC* Mutants in *F. verticillioides*

- Overcome differences at multiple *vic* loci
- Numerous mutants (38) at multiple loci (at least 12) can block *vic*-death reaction (*prm*)
- One mutant (*spc*) can form interspecies heterokaryons
  - Not allelic with *prm* mutants
  - Wild-type and mutant differ in levels of multiple transcripts when growing vegetatively

Four-step Vegetative Compatibility Model

Initiation

Self/non-self recognition

Maintenance/ Signaling

Rejection

Pre-Fusion → Fusion → Post-Fusion → Apoptosis

hsi genes

vic genes

prm & spc genes

Apoptotic genes

Proximity pheromones
Pheromone receptors
2° Branching
  deficiencies
Fimbriae deficiencies

Structural genes
Self/Non-self
  genes
Recognition genes

Downstream *vic* receptors
Protein kinases
MAPK(-like) signal cascades
Upstream effectors of apoptosis

Proteases
DNAses
RNAses

Cloned *vic* Loci

- *Neurospora* – allelic interactions(?)
  - *mat/tol*
  - *het-c/pin-c* – membrane + HET domain protein
  - *het-6/un-24* – ribonucleotide reductase + HET domain protein
- *Podospora*
  - *het-s* – prion
  - *het-c/het-d* & *het-c/het-e* – non-allelic interactions – HET domain proteins involved
- 55 HET domain proteins in *Neurospora* genome, 129 in *Podospora* genome, >100 in *F. graminearum* genome

Vic Locus Cloning Strategy

- Successful transformation results in cell death
- Sometimes two tightly linked loci – may need both
- Requires chromosome walking or suspect gene(s) identity
- Simultaneously transform strains that differ at the *vic* locus
- When one strain fails to transform, then the DNA fragment carries the *vic* locus

Neurospora's *het-c* Locus

- First *vic/het* gene to be cloned
- Two tightly linked genes
  - *het-c* – membrane protein
  - *pin-c* – HET protein (alleles have 62-76% nucleotide identity!)
- Three alleles – Killing severity depends on the alleles that are interacting
- *het-c* homologs in *Podospora* and *Fusarium* do not have *vic* function
  - *het-c* gene names in *Podospora & Neurospora* are not for the same gene
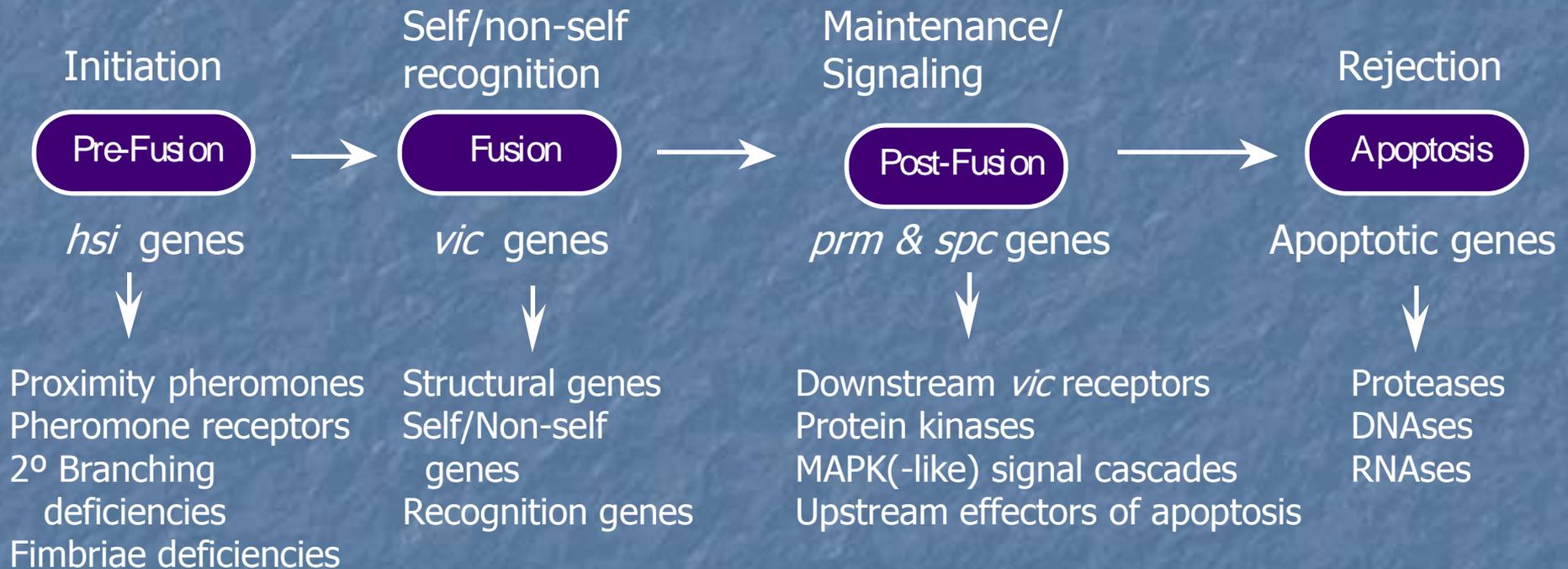- At the molecular level are all "allelic" interactions really multi-gene?

Mapping *vic* Loci in *Fusarium verticillioides*

- A model for what is being done in *F. graminearum*
- Cross strains that differ at multiple (10?) *vic* loci that carry a common nutritional mutant
- Plate progeny on lawn of conidia that can grow only if complementation occurs (selects 1/1024 *vic* types)
- Recover prototrophs and separate partners
- Look for distortion of 1:1 ratio (preferable 100:0) of linked markers
- 10 loci on at least 9/12 chromosomes; only one chromosome with two loci
- Some loci have < 100% penetrance in killing phenotype, *i.e.*, must differ at two loci to be dead
- AFLP flanking markers often separated by only a few map units (avg. 21 kb/map unit)

Mapping *vic* Loci in *Fusarium graminearum*

- ☑Cross strains that differ at multiple (10?) *vic* loci that carry a common nutritional mutant (recreate existing mapping cross with additional markers)
- ☑Plate progeny on lawn of conidia that can grow only if complementation occurs
- Recover prototrophs and separate partners (in progress – have ~100, goal is 500)
- Look for distortion of 1:1 ratio of linked markers (preferable 100:0) – not yet started
- Localize on existing genetic map and physical sequence
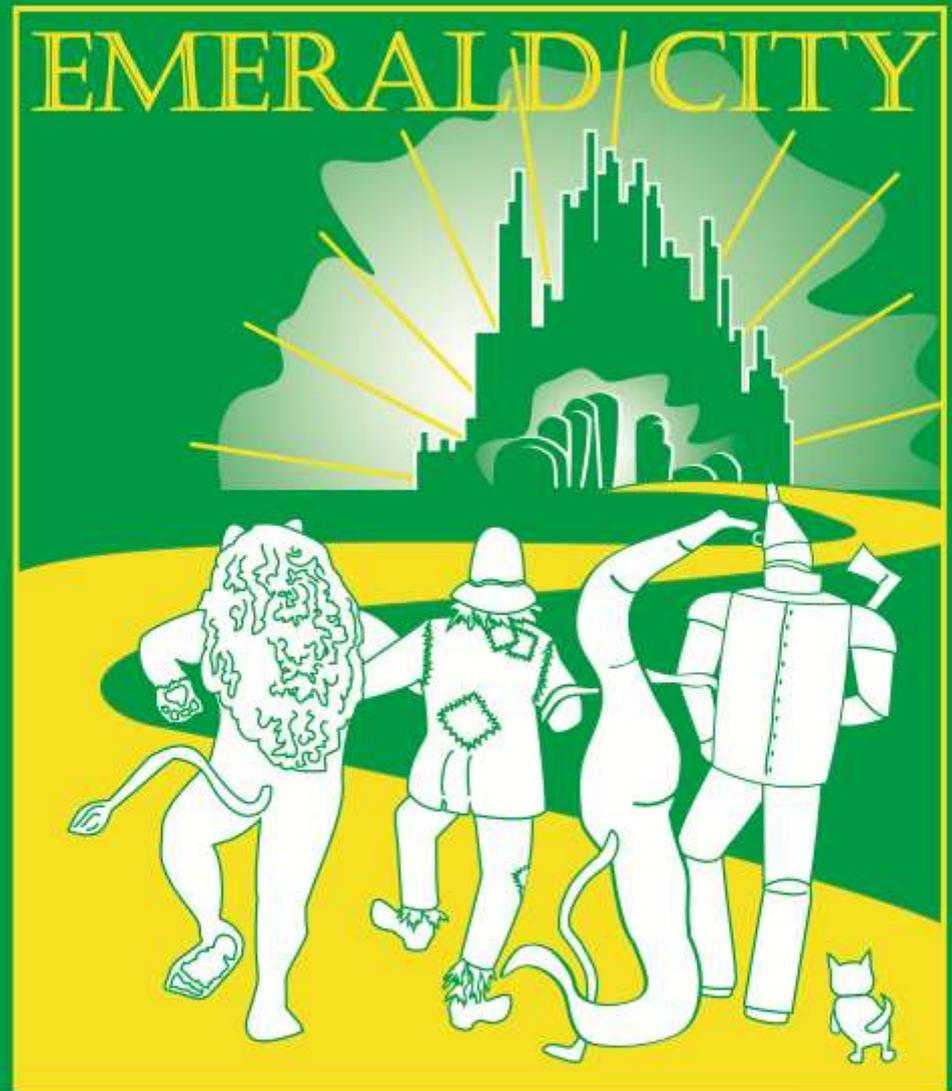- Prove identity by cloning and looking for killing phenotype

Four-step VCG Model

Initiation

Self/non-self recognition

Maintenance/ Signaling

Rejection

Pre-Fusion → **Fusion** → **Post-Fusion** → **Apoptosis**

hsi genes

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RNAses

Utilization?

- Ultimately looking for ways to trigger cell death

- Could use *vic* genes directly as triggers, probably by incorporating them into transformed host plants

- Could identify roles played in the cell and look for within host (resistance) or external (fungicide) ways of triggering pathway

Questions?