HYPOVIRULENCE OF 
CRYPHONECTRIA PARASITICA, 
THE CHESTNUT BLIGHT DISEASE FUNGUS

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Early 20th century range of *C. dentata* (American chestnut). Starting in New Jersey, chestnut blight wiped out ~ 4 billion trees between 1900-1950, encountering virtually no resistance.

Caused by the filamentous ascomycete *Cryphonectria parasitica*
American chestnut *Castanea dentata*, was dominant hardwood tree in northeast US until chestnut blight arrived in late 1800’s

*Tall, straight, fast-growing*
Chestnut blight, caused by *Cryphonectria parasitica* can kill susceptible trees quickly. Chestnut now grows as an understory shrub in North America – continually resprouts.
FIGURE 16.3  Disease diagram of chestnut blight caused by Cryphonectria parasitica. Drawn by Valerie Mortensen.
Conclusion: Chestnut blight fungus invaded North America, first probably in New Jersey, from Asia, probably Japan
What is hypovirulence and how did it come to be used for control of chestnut blight?
Trees seen to be recovering
Fungus in recovering trees phenotypically different
Fungus and aberrant phenotype stable in culture
Aberrant phenotype transmissible in culture and in cankers
Fungus isolated from treated cankers had aberrant phenotype
Hypovirulent, virus-containing isolates of *C. parasitica* grown in culture can be used to transmit virus to virulent isolates in trees, resulting in conversion and healing of those cankers.
**Cryphonectia** viruses and transposons

- **Nucleus**
  - nucDNA

- **Mitochondrion**
  - mtDNA
- **Mitovirus**
- **Reovirus** (2 species)
- **Hypovirus** (4 species)
- **Chrysovirus**
- **Partitivirus**

- **hAT-family transposons** (1 sp)
- **Tc1/Mariner family transposons** (3 spp)
- **Pseudoviridae-family retrotransposons** (4 spp)
- **Metaviridae-family retrotransposons** 1 sp
- **Toti-like***
- **RC1**
Composite diagram of gel containing dsRNAs from different chestnut blight isolates


dsRNA: CHV1, CHV2, CHV3, CHV4

Virulence: ++, +, _
Pigment: ++, +, _
Conidia: ++, +, _
Sequenced: ++, +, _

Hypoviridae
Reoviridae
Narnaviridae
Partitiviridae
Totiviridae

Size (kb): 12.7, 2.8, 1.7, 1.0
Fungal virus families and groups

- **Totiviridae** *(dsRNA)*
- **Chrysoviridae** *(dsRNA)*
- FgV3-like
- **Partitiviridae** *(dsRNA)*
- Megabirnaviridae *(dsRNA)*
- **Mycoreovirus** *(dsRNA)*
- **Narnaviridae** *(ssRNA + sense)*
- **Hypoviridae** *(ssRNA + sense)*
- Endornaviridae *(ssRNA + sense)*
- Barnaviridae *(ssRNA + sense)*
- Mycoflexiviruses *(ssRNA + sense)*
- Botrexviruses *(ssRNA + sense)*
- Sclerodarnaviruses *(ssRNA + sense)*
- **Pseudoviridae** *(RT)*
- **Metaviridae** *(RT)*
- Gemini-like *(ssDNA)*
- Rhizidioviruses *(dsDNA)*

No negative-sense RNA viruses yet identified; no recent dsDNA viruses

Largest genome size: <30 kb

Orange – viruses found in C. parasitica
Hypoviridae, the most important family of viruses for chestnut blight control
Hypoviruses can be seen as lipid vesicles containing dsRNA, but no coat protein, in freeze-substituted mycelium EM pictures.
Hypoviridae was the first named family of viruses whose members lack a coat protein. Structure and contents are poorly understood.

Hillman et al., 1995, 6th Rep. of ICTV, Acad. Press
Cryphonectria hypovirus 1

- First hypovirus identified in Europe, later in Asia, including Japan
- First hypovirus sequenced Shapira et al. 1991)
- Expression of p29 alone results in white phenotype, but not hypovirulence (Choi et al., 1992)
- Released many times into North America, but never found here naturally
- Transgenic isolates released in WV and CT (Nuss, Anagnostakis, MacDonald)
- Subject of many gene expression studies
Hypovirulence viruses in Europe

- Only one species of one family (CHV-1) characterized to date in Europe
- Several independent introductions of CHV-1 to different parts of Europe
- Virus diversity and fungus diversity in Europe lower than that in North America
- Virus recombination events rare
- Found in New Jersey in 1989, then in China 1995

- 2 very small populations

- Most closely related to CHV-1 (55% aa identity)

- The most debilitating hypovirus found to date

- Infectious clones made, but have not been stable

- Chimeras with CHV1 have not been stable
CHV-2 is found in recovering trees in eastern New Jersey.

Tree *NB58* - Five Points, New Jersey, USA

Photo from Dr. Peter Bedker
- Found in Michigan in 1980, then in surrounding area
- Not closely related to CHV-1 or 2
- Stable satellite and defective RNAs may be associated with infection
- Possibly associated with ongoing biological control in Michigan
Cryphonectria hypovirus 4

- First identified in West Virginia (1994)
- Later found throughout North America; not found yet in Europe or Asia
- Very low level dsRNA accumulation - difficult to detect
- May be asymptomatic; only marginally alters virulence and phenotype
- The most common C. parasitica virus in North America
Conclusion: Many viruses can cause hypovirulence in C. parasitica, members of family Hypoviridae most important.
Gaining a clearer perspective on hypovirulence in North American forests - how much sampling is done affects outcome
Chestnut blight mycoreovirus isolations were rare events

- *C. parasitica* strains with these viruses were isolated in West Virginia, USA 35 km from each other.
- More than 100 isolations from cankers from the two trees in which reoviruses were found.
- Only a single isolate from each tree had reovirus.
- MyRV-2: Third canker (Canker C), 18th isolate from that canker contained virus; isolate was called C-18; virus is called MyRV-2/CpC-18.
Virulence tests can be done on living trees, on excised stems, on bark, or on unrelated fruit such as apples.
Using apple fruits to assess virulence of *C. parasitica*

Apple fruit inoculations, 7 day

9Bss1 (V-)
9B21 (V+)
EP155 (V-)
EP155+CHV1
EP155+CHV2
How do Cryphonectria viruses move from one fungal host to another?
Virus is transmitted between compatible, but not incompatible fungal strains. Phenotypic changes follow virus transmission.
Progress on elucidation of details of vegetative incompatibility, hyphal anastomosis, and virus transmission during compatible interaction – Milgroom and now Nuss and coworkers expand on earlier work by Anagnostakis.

From U. Heiniger, D. Rigling
Vegetative incompatibility (vic) genes

- Classical genetic mapping and sequencing of the *C. parasitica* genome allowed for vegetative incompatibility (Vic) genes to be characterized
- Six loci characterized
- Heterologous alleles at different loci had different effects on vegetative incompatibility and on virus transmission
- Gene knockouts allowed for incompatible interactions to become compatible
C. parasitica genome

- Completed for at least three strains, 43.9 mB
- Both mating types completed
- Allowed for mapping of mating type (Mat) loci
- Allowed for mapping of vegetative incompatibility (Vic) loci
- Allowed for mapping of many genes involved with pathogenicity and virulence
- Allowed for mapping of genes involved with virus-fungus interactions
Besides hyphal anastomosis, how are C. parasitica viruses transmitted experimentally to a new host?
Three ways to infect virus-free fungi from cell-free preparations

**Transfection**

- Whole purified virus (Hillman & Suzuki, 2004)
- RNA transcripts from full-length cDNA clone (Chen & Nuss, 1994)

**Transformation**

- Complete cDNA copy of viral genome can integrate into fungal DNA (Choi & Nuss, 1992)
- Complete virus sequence
- Antibiotic resistance

**Introduction to virus-free fungal cells (protoplasts)**

**Virus+ sectors are identified in single large colony resulting from fusion of many cells**

**Extract dsRNA**

- dsRNA + (hypovirulent)
- dsRNA – (virulent)
- dsRNA + (hypovirulent)
- dsRNA – (virulent)

**Virus+ colonies identified from among all antibiotic resistant individual colonies that grow from cells containing cDNA construct in nuclear DNA**
Transfection of EP155 protoplasts with MyRV reovirus

Transfected protoplasts plated here

12 days after plating to regeneration medium

Subculture to PDA

V+

V-

Subculture to PDA
Does hypovirulence work?

- In Asia, it doesn’t matter because trees are largely resistant
- In Europe, where fungus population diversity is low, it provides effective situational control
- In North America, we don’t yet know the long term effect on the forest. It has worked to stabilize populations as a short term control.
- We need to get a better idea of what is going on with large, blighted, surviving trees in North American forests
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