Fungal Pathogenesis in Plants and Animals

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School of Botany,
the University of Melbourne
## Fungal pathogens of plants and animals

<table>
<thead>
<tr>
<th>Feature</th>
<th>Plants</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>Many</td>
<td>Few</td>
</tr>
<tr>
<td><strong>Importance</strong></td>
<td>25-30% crop losses</td>
<td>Immunocompromised hosts</td>
</tr>
<tr>
<td><strong>Life style &amp; environmental niche</strong></td>
<td>Often saprophytes; complex <em>in planta</em> – biotroph; necrotroph</td>
<td>Often soil saprophytes; not obligate</td>
</tr>
<tr>
<td><strong>Experimental systems</strong></td>
<td>Many; often shallow focus; crops</td>
<td>Few; different host cell &amp; immunosuppression regimes</td>
</tr>
<tr>
<td><strong>Host specificity</strong></td>
<td>Often; coevolution</td>
<td>Not often</td>
</tr>
<tr>
<td><strong>Reproduction</strong></td>
<td>Sexual &amp; asexual</td>
<td>Asexual (ascomycetes)</td>
</tr>
<tr>
<td><strong>Dispersal/transmission</strong></td>
<td>Between hosts</td>
<td>Not usually between hosts</td>
</tr>
<tr>
<td><strong>Pathogen requirements</strong></td>
<td>Complex set of physical &amp; molecular barriers to overcome</td>
<td>Inocula must get in, must survive 37°C, avoid or subvert immune responses</td>
</tr>
</tbody>
</table>
## Defence Systems of Plants and Animals

<table>
<thead>
<tr>
<th>Feature</th>
<th>Plants</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical barriers</td>
<td>Cuticle &amp; cell wall; lignin</td>
<td>Skin, respiratory surface</td>
</tr>
<tr>
<td>Basal Innate Immunity</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Circulating Cells</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Other innate defence</td>
<td>Resistance genes</td>
<td>Complement, cells: eg. phagocytes</td>
</tr>
<tr>
<td>Acquired or adaptive immunity</td>
<td>Papillae (callose -β 1,3 glucans); Systemic Acquired Resistance</td>
<td>Antibodies, T and B cells</td>
</tr>
</tbody>
</table>
Disease – damage & environment

Plants: Disease Triangle

Animals: Damage Framework Response (Casadevall)

Pathogen

Host

Environment

WEAK RESPONSE
Immune suppression

DISEASE THRESHOLD

STRONG RESPONSE
Immune activation

DAMPs and RAGE
Ascomycetous fungal pathogens of humans

*Coccidioides immitis*

*Candida sp.*

*Ajellomyces capsulatus (Histoplasma)*

*Aspergillus fumigatus*

Images from [http://fungal.genome.duke.edu/](http://fungal.genome.duke.edu/) John Taylor, Tom Volk
Fungal pathogens of plants

*Puccinia graminis fsp. tritici*
Stem rust of wheat
(R. Park)

*Sclerotinia sclerotiorum*
Stem rot of canola (M Rodriguez)

*Magnaporthe oryzae*
Rice blast

*Leptosphaeria maculans*
Blackleg of canola

Different host specificities & life styles *in planta*
Requirements of a pathogen

- Recognise and attach to host surface
- Germinate (spores)
- Enter host
- Colonise & derive nutrition (survive)
- Avoid or subvert host defence responses
- Reproduce
- Exit and disperse and find new host

Steps are developmentally regulated; mutants arrested at particular stages

Some processes (recognition, pH regulation, oxidative burst, signalling) occur several times during disease

Plant symbionts (eg. mycorrhizae) have similar requirements
Recognise & attach to host surface

Mutations can lead to arrest of invasion

Common traits of pathogenic ascomycetes

Plant

Animals

ascsopore conidium

yeast hypha conidium

dimorphic switching

arthrospores
Pathogenic Fungi & Sites of Entry

Surface Physical Barriers to Invasion

**Plants**

- Cuticle - epoxy fatty acids
- **Cell wall** - Cellulose (β1,4 glucan), callose (β1,3 glucan) & proteins

**Animals**

- **Stratum corneum** - keratin

Plants - more complex physical barriers than animals
Fungal Cell Wall (*A. fumigatus*)

Latge (2010) Cell Micro 12, 863

- Melanin
- Hydrophobins
- Conidia

- Protein
- Galactosaminogalactan
- Chitin
- β(1,3)glucan
- α-(1,3)-glucan
- Galactomannan
- GPI anchor
Recognise
 Attach

ascospore
 conidium

Plant

Common

Cell Wall Integrity

conidium

Animals

yeast
hypha
arthrospores

dimorphic
switching

Basal Innate Immunity

Signalling

Oxidative burst
Basal Innate Immunity: PAMP-Triggered Immunity

- Common to animals, plants & insects
- Interaction between small fungal molecules Pathogen – Associated Molecular Patterns (PAMPs) & transmembrane Pattern Recognition Receptors of host
- Pattern Recognition Receptors: similar domains in animals, plants & insects
- Binding triggers similar signaling pathways and defence responses

Diagram:
- Chitin fragment PAMP
- Pattern Recognition Receptor
- Signaling: outside to inside
- Host Cell
- Defence Responses
Chitin fragments sequestered by fungal effector Ecp6 - inhibiting basal immunity in tomato

- Plant pathogens deliver **effectors**
  - small secreted molecules (often cysteine–rich proteins)
  - interact with host: facilitate infection or induce defence responses
  - often lack homologs in other fungi; often highly polymorphic
- Effectors can overcome basal innate immunity
- Bart Thomma: Wageningen; *Cladosporium fulvum* Ecp6 has 3 LysM (chitin binding domains)

- Ecp6 secreted & induced in planta and essential for virulence

(Bolton *et al.*, Mol Microbiol, 2008)
Chitin fragments sequestered by fungal effector Ecp6 - inhibiting basal immunity

* *C. fulvum* enters plant
* Chitin fragments (PAMPs) released
* Recognition by PRR activates defense (PTI)
* Ecp6 sequesters chitin PAMPs - disease

Bart Thomma
(de Jonge et al., Science 2010)
Recognise, Attach,

Germinate

Plant
ascospore conidium

Common
Cell Wall Integrity

Basal Innate Immunity
Signalling
Oxidative burst

Nutrition: glyoxylate cycle

Light
Siderophores
pH maintenance
Hydrophobins

Animals
yeast hypha
arthrospores conidium

biofilms
Lytic enzymes

Plant

Enter host

Stagonospora nodorum (direct entry)

Leptosphaeria maculans

Magnaporthe oryzae

Rick Howard

Common

Lytic enzymes

Melanin

Stomata

Appressorium

Autophagy

Animals

Receptor & endocytosis

Rick Howard
Oxidative burst

pH maintenance

Host

Avoid
defences &
colonise

Effectors & Resistance
genes

Toxins

Siderophores

Transporters

Host cell death

Common

Nutrition

Oxidative burst

pH maintenance

Toxins

Siderophores

Transporters

Host cell death

Effectors & Resistance
genes

Inhibit immune response

Animals

O₂⁻

Host complement

O₂⁻
Toxins

• Secondary metabolites
  – Usually not crucial virulent determinant - some plant exceptions that are host specific
  – Gliotoxin & aspergillosis; depends on immunosuppression regime of mouse; sirodesmin in *L. maculans* – canola stem
  – Many secondary metabolite gene clusters acquired by horizontal gene transfer
  – Protection for fungus against predators in soil

• Small secreted proteins
  • Important disease determinant: ToxA transferred from wheat pathogens *Stagonospora nodorum* to *Pyrenophora tritici repentis*; requires complementary plant receptor
  • Host specific
Avoid defences & colonise

**Plant**

- Oxidative burst
- pH maintenance
- Toxins
- Siderophores
- Transporters
- Host cell death
- Inhibit immune response

**Common**

- Oxidative burst
- Nutrition
- Toxins
- Siderophores
- Transporters
- Host cell death
- Inhibit immune response

**Animals**

- $O_2^-$
- Host complement

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Effectors & Resistance genes
Effectors and Resistance genes

- Co-evolution of plant and pathogen
- **Avirulence** effector proteins recognised by complementary **Resistance** proteins thus activating defence response (Effector Triggered Immunity)
- Pathogen isolates selected that have mutated Avirulence protein or gained new effectors that suppress **ETI**
- Resistance then ineffective – eg. blackleg of canola
- Plants with Resistance gene with different specificity selected for (in natural environments)
Plant Animals

Colonise

Reproduce

often sexual

Disperse

Plant to plant, infected seed

Not animal to animal
Fusarium oxysporum f.sp. lycopersici 4287

tomato wilt

systemic infection in immunodepressed mice

Root adhesion and penetration

Lung
Heart

Antonio Di Pietro
## Virulence of *Fusarium oxysporum* f.sp. *lycopersici* mutants

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Tomato</th>
<th>Mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>pacC (pH response transcription factor)</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Chitin synthase V (cell wall integrity)</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>White collar 1 (photoreceptor)</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>MAP kinase fmk1 (signalling)</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Rho1 GTPase (signalling)</td>
<td>no</td>
<td>yes</td>
</tr>
</tbody>
</table>

Mutation confers virulence on **either** mouse **or** tomato.
PacC, wc2 important in mice; CHS V, MAP kinase fmk1, Rho1 GTPase important in tomato.
Highlights redundancy in some pathways & complexity of interactions.
Transcriptome analyses of infected plant & animal tissue underway.

Di Pietro & Roncero
<table>
<thead>
<tr>
<th>Group</th>
<th>Target</th>
<th>Plants</th>
<th>Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoles</td>
<td>Ergosterol biosynthesis</td>
<td>Widely used:</td>
<td>Widely used</td>
</tr>
<tr>
<td>Echinocandins</td>
<td>$\beta$ 1,3 glucan synthesis</td>
<td>Not used</td>
<td>New drug</td>
</tr>
<tr>
<td>Nikkomycin Z</td>
<td>Chitin synthesis CHS I</td>
<td>Not used</td>
<td>Trials Coccidioides</td>
</tr>
<tr>
<td>Strobilurins</td>
<td>Mit cytochrome bc complex</td>
<td>Yes; resistance develops; G143A mutations</td>
<td>Not used - toxic</td>
</tr>
<tr>
<td>Bion</td>
<td>Salicylic acid analog: mimics systemic acquired resistance</td>
<td>Expensive (horticulture)</td>
<td>Not relevant</td>
</tr>
</tbody>
</table>
Breakdown of resistance to blackleg of canola

- Example of adaptation of plant pathogen
- *Leptosphaeria maculans* causes blackleg, most important disease; losses 5-10% annually in Australia
- Airborne sexual spores released from fruiting bodies on stubble
- Disease controlled by
  - crop rotation with cereals & legumes, removing stubble (inoculum), sowing crops >500 m from last year’s crop
  - fungicide treatment of seeds
  - breeding for resistance; polygenic, minor gene (quantitative) resistance deployed in Australia until 2000
Blackleg disease in Australia

- **Ascospore inoculum**: April-June
- **Stem cankers**: October-December
- **Growth down stem**: October-December

Photos of disease symptoms and ascospores.
Resistance and Avirulence genes

Plant contains Resistance genes (R genes)
Pathogen contains Avirulence ‘effector’ genes (Avr genes) complementary to R genes

Canola Genotype: \textit{Rlm1}
Fungus Genotype: \textit{AvrRlm1} (Avirulent)

Canola recognises fungus
- Defence responses
- NO INFECTION
Resistance and Avirulence genes

Plant contains Resistance genes (R genes)
Pathogen contains Avirulence genes (Avr genes) complementary to R genes

Canola Genotype: $Rlm1$
Fungus Genotype: $avrRlm1$ (virulent)
Avirulence gene mutated or lost

Canola not recognised by plant
INFECTION & DISEASE
‘Breakdown’ of blackleg resistance

• in 2000 ‘sylvestris’ cultivars of canola released with major resistance gene \((Rlm1)\); no lesions!
Breakdown of resistance to blackleg

Steve Marcroft
Phil Salisbury
‘Sylvestris’ cultivar

2002

Resistance gene *Rlm1*

2003
2003: ‘sylvestris’

$30 million losses in Eyre Peninsula
Seed withdrawn from market

2003: Beacon

Several minor genes

Rlm1
Beer award a long time coming

By PETER HEMPHILL

A SPECIALTY brew from the oldest brewery in the world has scored a win at the Australian International Beer Awards.

The Bavarian State Brewery Wiesn Brauerei, established in 1410 AD in Fussen, Germany, won the champion specialty beer title for its Wiesn Brauerei Kristall Weizenbier.

The awards are run by the Royal Agricultural Society of Victoria and Ballarat University. Nearly 600 entries were received in the competition from 22 countries.

New Zealand brewer Dix de Lux took out the grand champion trophy for its New Wester Pale Ale. The Premier’s trophy for the best Victorian beer went to the Grand Ridge Brewery at Mbitoo North for its Cippeleland Gold Pils.

The Aylett Shawl brewery at Cameydon, NSW, won champion lager with the James Squire Original Pilsner and the University of Ballarat vice-chancellor’s trophy for champion Australian brewery.

Champion international brewery was awarded to the Oriental Brewery of the city of Kungju in South Korea.

Other major Australian winners were the Mitchel Brewing Company of the Sanctuary Cove in Queensland for champion small brewery and the Port Dock Brewery Head in Port Adelaide for its Black Bart Milk Stout.

Big blow: Steve Marcroft says the breakdown in blackleg resistance in some canola varieties is a blow for the oilseed industry.

Black cloud over canola as gene fails

By SIMONE DALTON

CANOLA growers’ bid to avoid blackleg disease may have taken a new turn with reports a gene offering major resistance to the disease may have broken down.

The gene carrying resistance in a wild canola relative, Brassica sylvester, and transferred to some varieties of canola, Brassica napus, by Pacific Seeds. The canola varieties with the B. sylvester resistance are Lepa 80, super 400, super 501T, sur- pass 65XCL, surpass 85XCL, surpass 125XCL and surpass 150XCL.

Other canola varieties generally source resistance to blackleg from several genes.

Breeders have been using the gene carrying B. sylvester resistance in many emerging canola varieties because of its impressive blackleg pro-
closely to see if the resistance spread to more than three sites this season.

“It is a wait-and-see year,” he said. Canola Association president Trent Potter urged people not to panic.

“While that resistance may have broken down, we’ve got other varieties.

Farmers who grow varie-
ties carrying the B. sylvester resistance gene last year should try to destroy as much stubble as possible and try to plant any crops this year at least 500ms away from it.

He also urged growers to use good advice or
2004: trial plots

Several minor genes $Rlm1$
2005: *Sylvestris* cultivars

2005 & 2006: less disease in trial plots than previous years
‘Sylvestris’ resistance is ‘overcome’

- 90% yield losses in Eyre Peninsula; $30 m losses
- Increased frequency of isolates attacking cultivars with sylvestris-resistance, not new strain:
  - Isolates collected before 1988 can cause disease
- Blackleg fungus mates prolifically
  - Billions of individuals in populations of spores
  - Populations rapidly adapt to presence/absence of resistance gene
  - ‘Boom and bust cycle’
- What are the types of mutations in \textit{L.\textit{maculans AvrLm1}} that confer virulence on ‘sylvestris cultivars’
**L. maculans** genome

- **Initiative:** Rouxel & Balesdent, INRA & Howlett; Genoscope (Wincker); annotation URGI (Anselem). Nat. Comm (2011)
- 12,500 genes; 45 Mb - closely related *Stagonospora nodorum* (37 Mb)
  - Repetitive DNA: 36% genome (9% of *S. nodorum*)
  - Degenerated transposable elements: truncated & Class I LTR retrotransposons & Class II TIR DNA transposons
- Abundance of Repeat Induced Point (RIP) mutations:
  - occurs prior to meiosis; mutates multi copy DNA
  - transition from C:G to T:A nucleotides – stop codons
  - genome defence mechanism?
- **Plastic genome** – genes easily lost gained or mutated
Patchwork genome: GC-rich & AT-rich blocks

**GC-rich blocks**
Gene dense (1 gene per 2.4 kb)

**AT-rich blocks**
Gene poor (1 gene/30 kb) RIPed transposons
Enriched for effector genes (avirulence $AvrLm1$, $AvrLm4-7$, $AvrLm6$)
Mutations in *L. maculans AvrLm1* conferring virulence on ‘sylvestris cultivars (*Rlm1*)

- Sequenced *AvrLm1* in 295 isolates:
  - 137 before breakdown of resistance (<2004)
  - 158 after breakdown of resistance (2004 and later)
- Six *AvrLm1* alleles: amino acid substitutions & gene deletions (confer virulence)
- Eight fold increase in deletion alleles after breakdown of resistance – consistent with glasshouse data
- Sequenced other effectors in AT-rich region of genome; deletions, amino acid substitutions and RIP mutations in single copy genes!
- Dynamic fungal genome
Implications for the canola industry

• Each resistance gene costs >$3 million to commercialise
• Maximise durability of resistance genes by sowing canola cultivars with different sources of resistance in rotation
  – reduces selection pressure towards virulent isolates within pathogen populations
  – importance of breeding single gene resistance into lines with quantitative resistance
• Cannot eradicate blackleg disease: stay ahead of it
• Determining frequencies of Avirulence/virulence alleles \((AvrLm1, AvrLm6)\) in populations released from stubble onto tape from spore traps
  – DNA extracted; PCR assay - presence/absence band
  – information sent back to farmers/agronomists to warn of risk of resistance breakdown
  – farmers then grow cultivars with different sources of resistance
Fungal pathogenesis in plants & animals

Many hurdles for fungus to invade plant; fewer to invade animals (survival at 37°C, avoid immune responses)

High degree host specificity for plant pathogens

Effectors key to plant disease: role in animal disease?
   – Few reports of functional, transcriptional or polymorphism analysis of small secreted proteins or in different isolates
   – Evolutionary time within angiosperms much greater than within mammals – may not need a high degree of adaptation of fungi to infect animals

Chitin suppresses defence in Candida; chitin induces defence on tomato

_Fusarium oxysporum fsp. lycopersici_ infects mice & tomatoes: redundancy in signalling pathways
Fungal pathogenesis in plants & animals

• Disease control:
  – Fungicides: efficacy, resistance & cost/benefit
  – Plant breeding: resistance can be broken down by fungi whose populations evolve quickly
• Many plant pathogens can adapt rapidly to selection pressure
  – Reflected in genome structure of *Leptosphaeria maculans*
• Lessons can be learnt from studying fungal pathogens in a range of hosts