Plant Pathology 602
Plant-Microbe Interactions

Lecture
Fungal and oomycete effectors
What types of resistance can R genes mediate?

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Plant Pathology 602
Plant-Microbe Interactions

- Fungal/oomycete effectors
- What types of resistance can R genes mediate?

Definitions - Kamoun Annu Rev Phytopath 2006

- Effectors are pathogen molecules that manipulate host cell structure and function thereby facilitating infection and/or triggering defense responses
- Unlike the terms "avirulence", "elicitor", "toxin", and "virulence", the term effector is neutral and does not imply a negative or positive impact on the outcome of the disease interaction
- Many effectors are only known by their avirulence function, it is assumed that these effectors have a virulence function of an unknown nature

Fungal and oomycete effectors

Features

- Effectors from diverse pathogens are not necessarily structurally related but share some features
- Must be accessible to plant cells (expressed during infection, extracellular etc…)
- How do they vary between races of the pathogen? Race evolution?
- For a while, relatively few fungal and oomycete Avr proteins known but many discovered recently

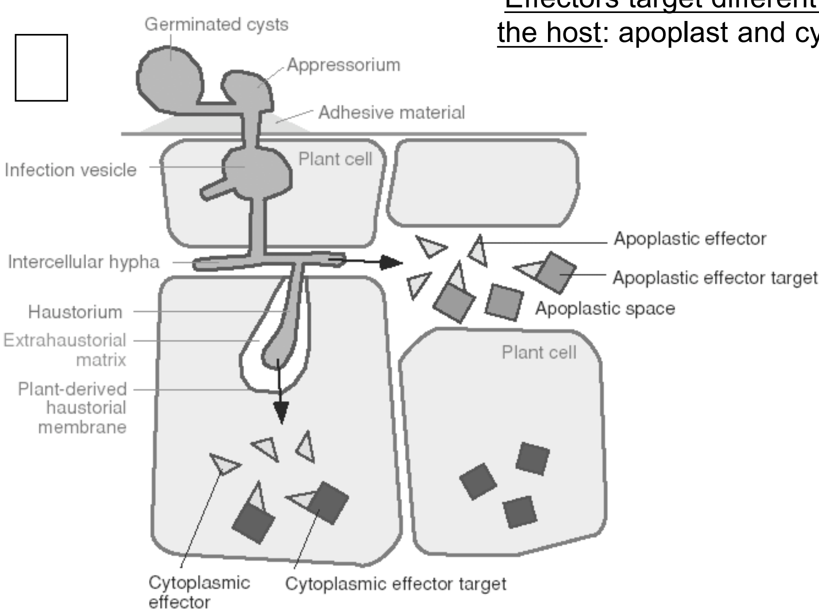
Fungal effectors with Avr function

Pathogen	Avr	Host/R gene	description, virulence function
C. fulvum	Avr9	Tomato Cf9	small cysteine rich, unknown
C. fulvum	Avr4	Tomato Cf4	small cysteine rich, chitin binding
C. fulvum	Avr2	Tomato Cf2	small cysteine rich, protease inhibitor
M. grisea	AvrPita	Rice Pi-ta	metalloprotease
M. grisea	ACE1	Rice Pi33	polyketide synthase
Flax rust	AvrL567	Flax L5-L7	unknown
Flax rust	AvrP4	Flax P4	small cysteine rich
Flax rust	AvrP123	Flax P1-3	small cysteine rich, protease inhibitor

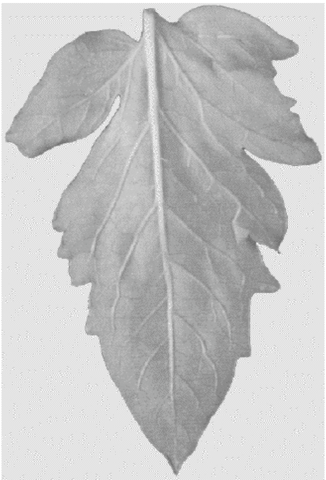
Oomycete effectors with Avr function

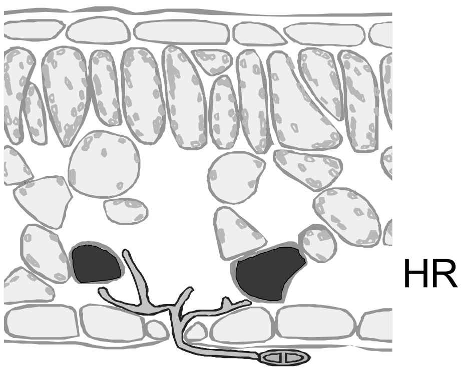
Pathogen	Avr	Host/R gene	description
H. parasitica	ATR1	Arabidopsis RPP1	RXLR effector
H. parasitica	ATR13	Arabidopsis RPP13	RXLR effector
P. sojae	Avr1b-1	Soybean Rps1b	RXLR effector, unknown
P. infestans	Avr3a	Potato Avr3a	RXLR effector, cell death suppressor

<u>Effectors target different sites of the host</u>: apoplast and cytoplasm



***Cladosporium fulvum*-tomato interaction**



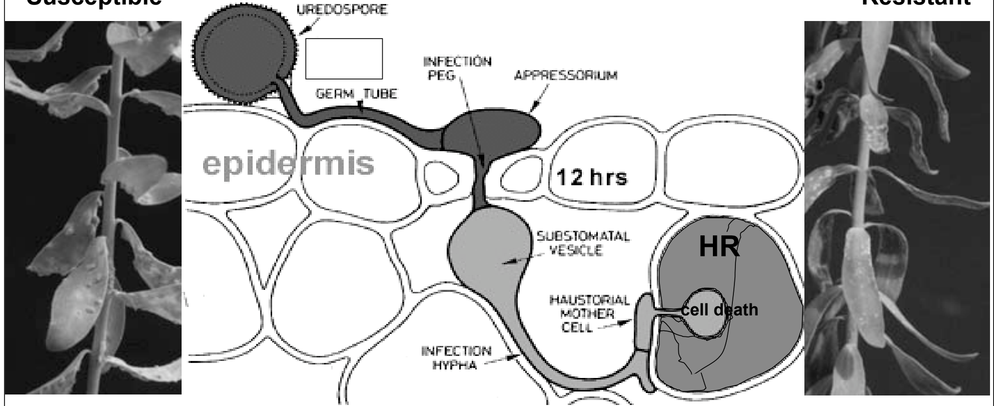


Pathogen: avirulent, plant: resistant

Haustoria: effector delivery into plant cells

Avirulence protein recognition by Resistance protein - Flax-rust interaction

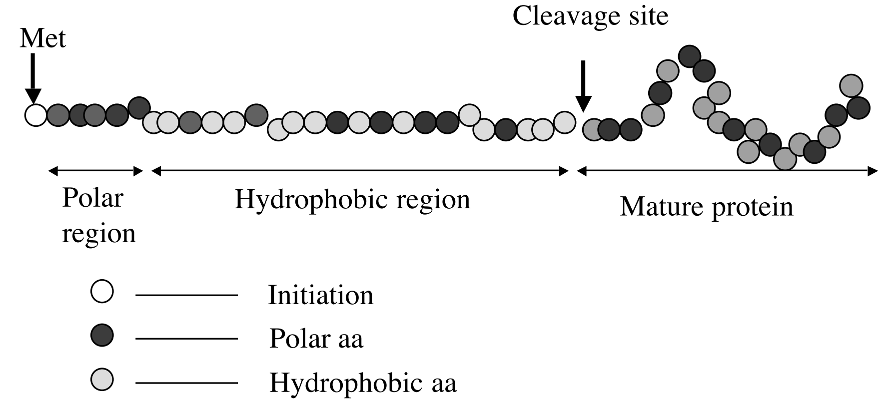
Susceptible **Resistant**



A.M. Catanzariti, P. Doods, and J. Ellis

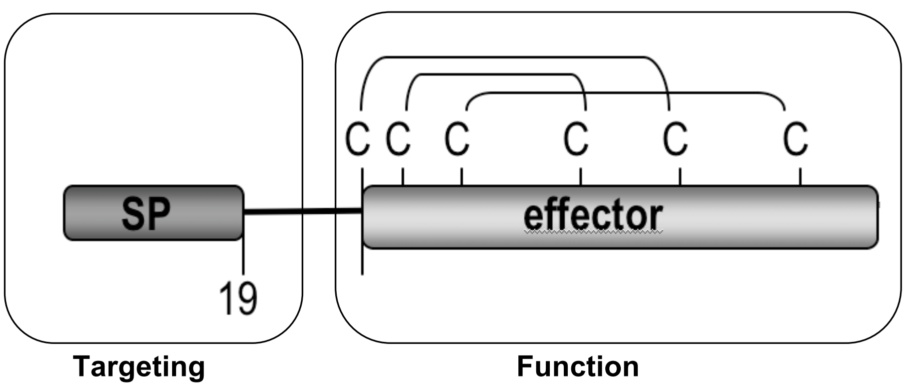
Structure of fungal/oomycete effectors

Structure of signal peptides of secreted proteins

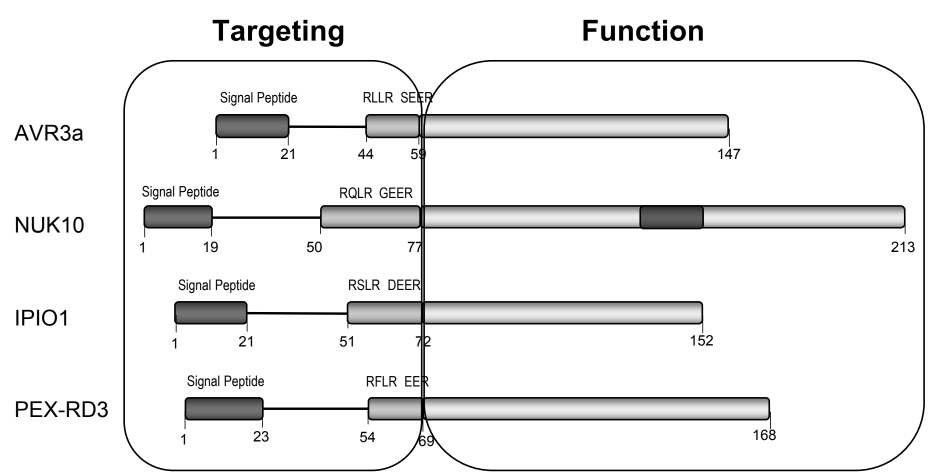


Apoplastic effectors are typically small cysteine-rich secreted proteins

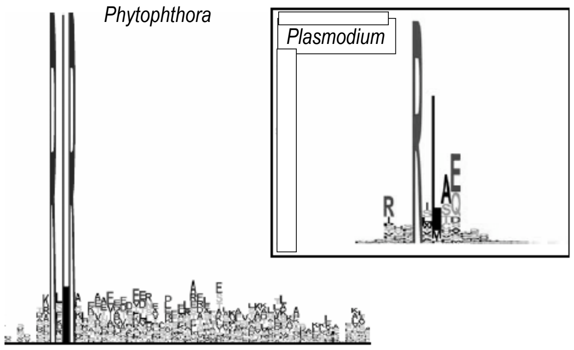
- often get processed upon secretion
- disulfide bridges provide stability in apoplast



Cytoplasmic effectors of oomycetes have a conserved motif (RXLR)

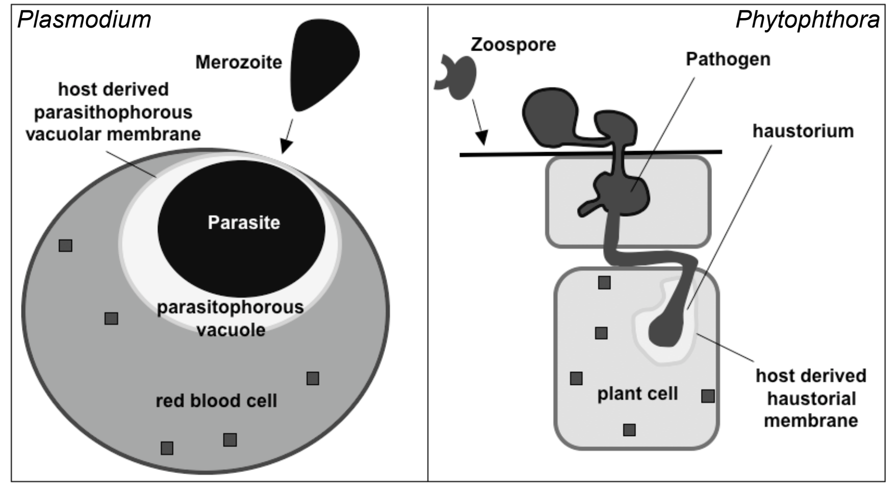


Oomycete RXLR motif is similar to a host translocation motif in the malaria pathogen *Plasmodium*



Marti et al. Science 306:1930 (2004)
Hiller et al. Science 306:1934 (2004)
Rehmany et al Plant Cell (2005)

Conserved effector delivery mechanisms between *Plasmodium* and *Phytophthora*?



How to identify effectors?

- Biochemical approach: effector is purified from pathogen and tested for induction activity in a plant bioassay; gene is then isolated by reverse genetics
- Genetic approach: effector is isolated by map based cloning, complementation experiments or reverse genetic; elicitor activity of gene product determined using a gene expression assay (*E. coli*, virus, *Agrobacterium*)
- Genomics approach: candidate effectors are first identified from sequence database and then assayed for activity using various functional assays

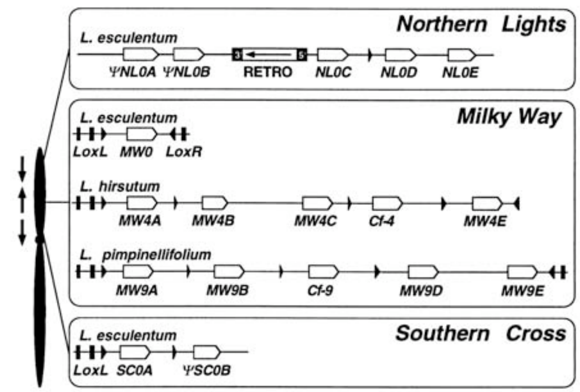
Traditionally, effectors have been identified using biochemical or genetic approaches

- Biochemical purification of elicitor proteins
- Map-based cloning of pathogen avirulence genes
- Slow and low-throughput
- Not always successful

Example: *Cladosporium fulvum* Avr4 and Avr9

- <u>Elicitor activity identified in intercellular fluid</u> (apoplastic fluid) of infected tomato leaves
- <u>Proteins associated with activity purified</u>
- Corresponding <u>gene(s) cloned</u>
- Various gene expression systems were used to test activity of gene products
- <u>Gene knockout and transformation experiments further supported avirulence function</u>
- <u>Avr4/Avr9 sequences in virulent races examined</u>

The Cf-4/ Cf-9 R gene clusters



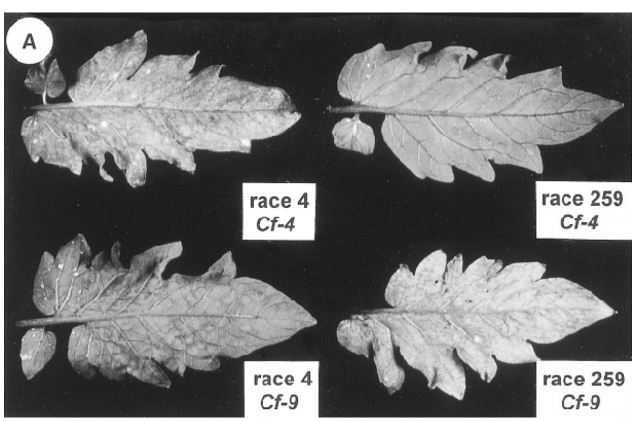
Parniske and Jones 1999 PNAS 96:5850

Example: *Cladosporium fulvum* Avr4/Avr9

Race 4 Race 2 5 9

Cf-4

Cf-9



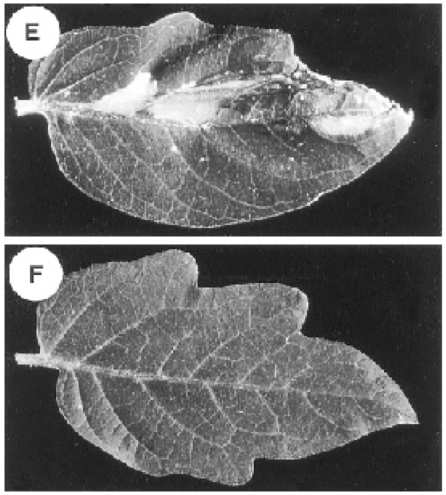
HR inducing activity of *C. fulvum* races on various tomato genotypes

C. fulvum strains	Elicitor(s) produced	Tomato genotypes			
		Cf-0	*Cf-4*	*Cf-9*	*Cf-ECP2*[a]
Wild-type races					
Race 5	AVR4, AVR9, ECP2	—[b]	HR	HR	HR
Race 4	AVR9, ECP2	—	—	HR	HR
Race 2.4.5.9	ECP2	—	—	—	HR
Mutant races					
Race 5-Δ*Avr9*[c]	AVR4, ECP2	—	HR	—	HR
Race 5-Δ*Ecp2*[d]	AVR4, AVR9	—	HR	HR	—

Example: *Cladosporium fulvum* Avr9

Avr9 peptide injected into Cf-9 leaf

Avr9 peptide injected into Cf-0 leaf



Example: *Cladosporium fulvum* Avr4/Avr9

Structure of Avr9 gene

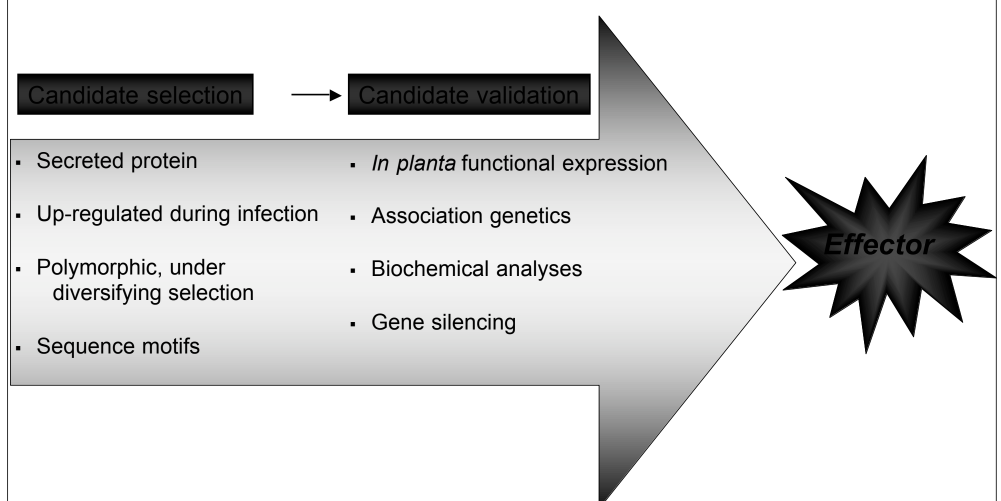
- Single copy gene
- Secreted via signal peptide (type II secretion)
- Active peptide is 28 amino acids; first expressed as a 63 aa protein, secreted as a 40 aa, and then processed by plant/fungal proteases to 28 aa
- Virulent races lack Avr9 gene (no virulent allele! Avr9 deleted)
- Transfer of Avr9 to virulent races is sufficient to make them avirulent on Cf-9 tomato

Example: *Cladosporium fulvum* Avr4/Avr9

Structure of Avr4 gene

- Single copy gene
- Secreted via signal peptide (type II secretion)
- Active peptide is 86 amino acids; first expressed as a 135 aa protein, secreted and then processed by plant/fungal proteases to 86 aa
- Virulent races contain nonfunctional mutated avr4 alleles (mainly single amino acid mutations that make AVR4 unstable in the apoplast, most mutations affect cysteines)
- Transfer of Avr4 to virulent races is sufficient to make them avirulent on Cf-4 tomato

Genomics approach:
Functional genomics pipeline



Cloning of *Phytophthora infestans* Avr3a

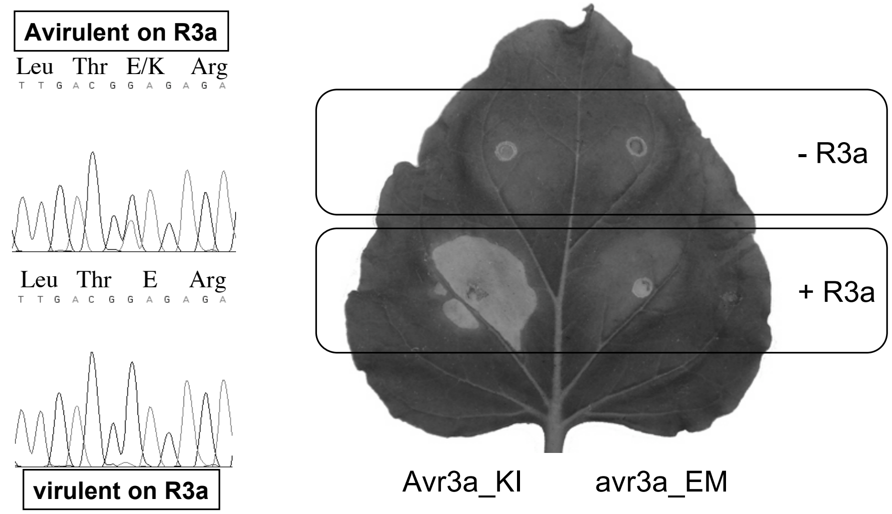
Candidate selection → **Candidate validation**

- Secreted protein
- Polymorphic
- Up-regulated during infection

- Association with avirulence on *R3a* potato (55 isolates)
- Functional expression in *R3a* potato
- Co-expression with *R3a* in *Nicotiana benthamiana*

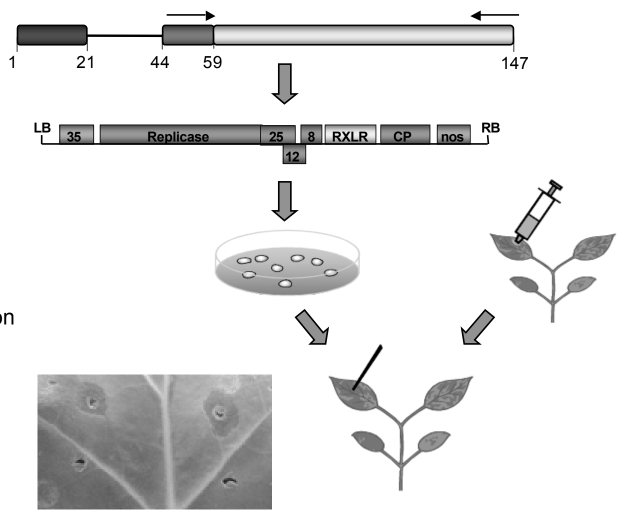
→ ***Avr3a***

Reconstruction of Avr3a-R3a gene-for-gene interaction in tobacco

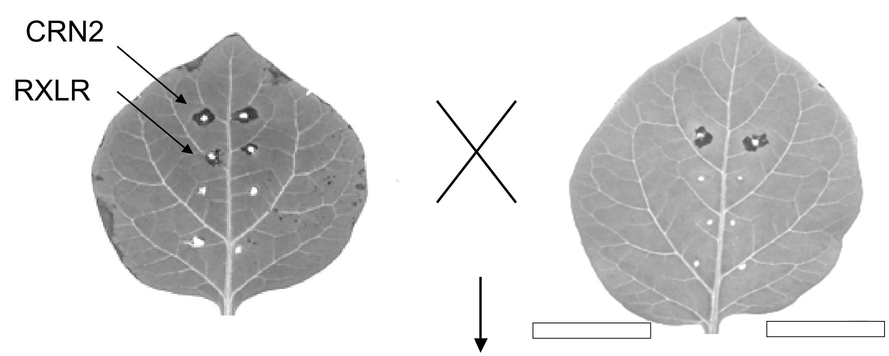


Effectoromics- screening for of Avr-R interactions: (1) in tobacco using cloned R genes

- Amplify RXLR/Avr candidate (allele mining)
- Clone in binary PVX vector pGR106
- Transform/pick up PVX clones in *Agrobacterium*
- Express *R* gene in *N. benthamiana* by agroinfiltration
- Wound inoculate PVX clone in *R* gene expressing leaves
- Confirm by agroinfiltration



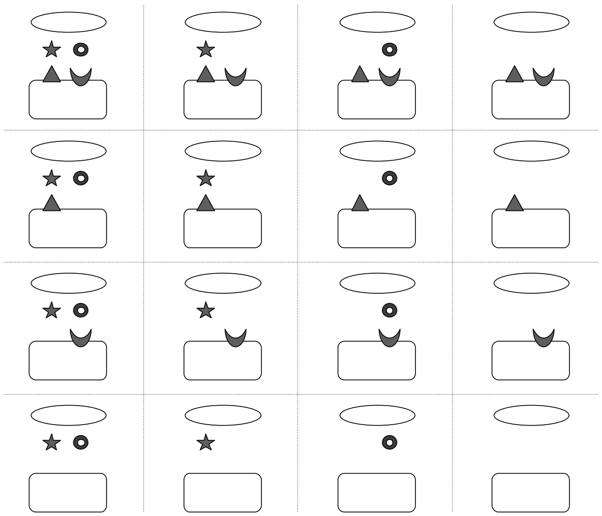
Effectoromics- screening for of Avr-R interactions: (2) screening potato breeding material



Co-segeregation of late blight resistance and effector response

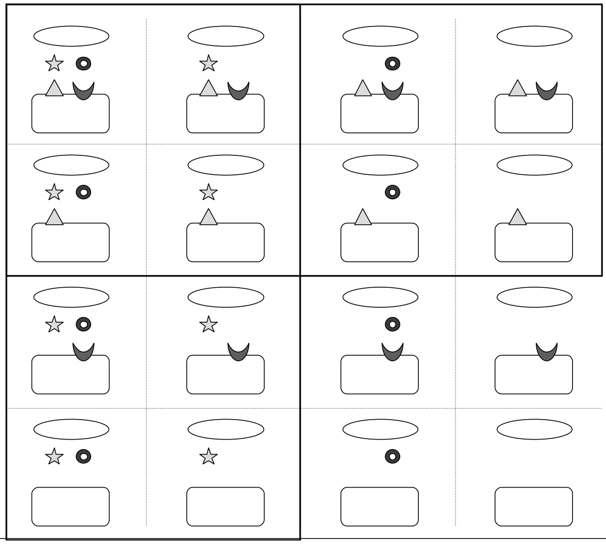
Avr genes help define and dissect R genes

Plant cultivars
(Rs and Rd)



AvrS defines and dissects Rs from other R genes

Plant cultivars
(Rs and Rd)



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- Fungal/oomycete elicitors/Avr proteins
- What types of resistance can R genes mediate?

What types of resistance can R genes mediate?

Traditional negative view

Plant R genes (major genes) only determine:

- Race-specific resistance
- Intraspecific (host) resistance
- Non-durable resistance
- Full resistance
- HR-mediated resistance

What types of resistance can R genes mediate?

Traditional negative view results in rejecting introduction of R genes into some crop plants (example: potato)

- Concept of "R gene-free" germplasm is nonsense
- All plants contain R genes

What types of resistance can R genes mediate?

Traditional negative suggest that R genes cannot mediate complex resistance phenotypes

- Phenotype of a plant does not provide indication about the nature of the genes it carries
- One phenotype can be determined by several genes and one gene can determine several phenotypes
- Effect of a gene depends on the genome context (epistatic effects)

All R genes are not equal!

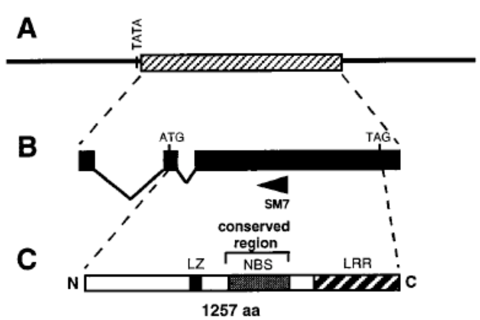
Modern view stemming from recent knowledge about R gene/Avr gene structure and function

Plant R genes can/may determine:

- Broad spectrum resistance
- Nonhost resistance
- Durable resistance
- Partial resistance

Can R genes determine broad-spectrum resistance?

Mi gene (*Rossi et al. 1998 PNAS 95:9750*)



Can R genes determine resistance against all strains of a pathogen?

Mi gene (*Rossi et al. 1998 PNAS 95:9750*)

- Functions in tomato
- Typical CC-NBS-LRR
- Confers resistance to root-knot nematode and potato aphid
- Avr/elicitor unknown
- Common signal/perturbation in nematode and insect?
- A single gene can confer multiple phenotypes

Can R genes be involved in nonhost resistance?

Definition of nonhost resistance:

- Resistance that is determined at the specific or genus level
- In contrast to race- or cultivar specific resistance and partial resistance

Can R genes be involved in nonhost resistance?

Population genetics issue:

- An interaction in which the allelic distributions of the R gene in the plant and the Avr gene in the pathogen are 100% would qualify as a nonhost interaction
- Difficult to study due to the lack of variation and sexual incompatibility between host and nonhost (classical genetics/breeding not possible)

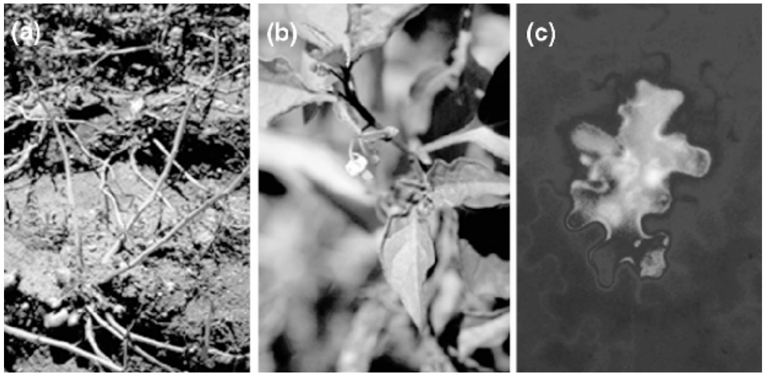
Can R genes be involved in nonhost resistance?

HR is often associated with nonhost resistance:

- Bacteria: HR on nonhosts common; requires hrp (type III secretion) system
- Oomycetes: *Phytophthora* can penetrate all plants and induce an HR on nonhosts
- Fungi: sometimes but saponins and other preformed barriers appear to be more important

Can R genes be involved in nonhost resistance?

HR is often associated with nonhost resistance- example: the weed *Solanum nigrum* and *P. infestans*



Can R genes be involved in nonhost resistance?

Nonhost R genes:

- Example: RPW8 broad spectrum *Arabidopsis* resistance gene against powdery mildew
- Yield durable resistance?
- Likely to include “typical” R genes (NBS-LRR, Cf-like etc…) since HR is involved
- But probably involve other layers of resistance

Can R genes mediate durable resistance?

Durable resistance:

- Resistance that can last in the field over long time periods
- Pathogen is unable to evolve fully virulent races
- Epidemiological concept!!!
- Not necessarily related to mechanism of resistance
- Some R genes are durable

Can R genes mediate durable resistance?

Durable resistance is an epidemiological concept:

- In soybean, single R genes targeted against the soil pathogen Phytophthora sojae tend to last for several years
- In potato, single R genes targeted against the aerial pathogen Phytophthora infestans are quickly overcome by virulent races
- Disease epidemiology and pathogen dispersal are different

Achilles' heel hypothesis-
Dual virulence/avirulence function

- If an *R* gene targets an essential virulence factor of the pathogen then the pathogen is less likely to mutate the dual *Avr/vir* gene to evolve a new virulent race
- Objective is to identify dual *Avr/vir* factors in pathogens

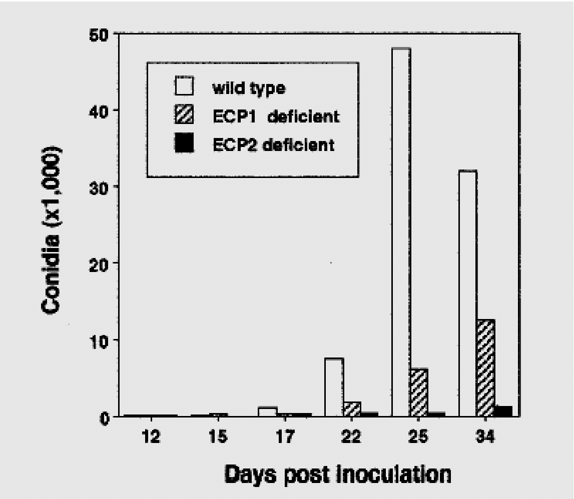
Cladosporium fulvum ECP2

ECP2 is a virulence factor (*Lauge et al. 1997 MPMI 10:725*)

- ECP2 is abundantly secreted by *C. fulvum* in intercellular space of susceptible tomato leaves
- ECP2 knockout mutants of *C. fulvum* show poor colonization and conidation
- ECP2 also function as an avirulence factor since it is recognized by a tomato gene Cf-ECP2

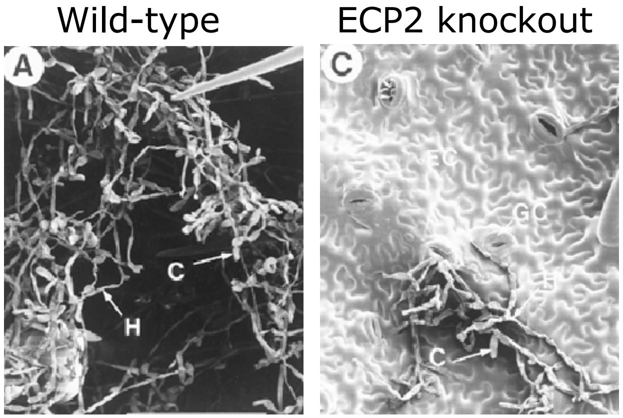
ECP2 is a virulence factor

Quantification of conidia in infected leaves



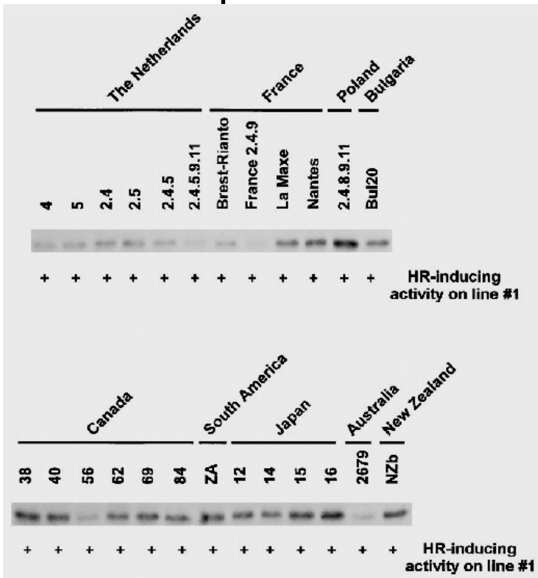
ECP2 is a virulence factor

Scanning EM of lower side of infected leaves



All *Cladosporium fulvum* strains produce ECP2

- ECP2 detected using Western blots of apoplastic fluids
- HR activity on Cf-ECP2 lines also detected with all strains



Cladosporium fulvum ECP2

Summary:

- ECP2 is both a virulence and an avirulence factor
- On most tomato lines, ECP2 functions as a virulence factor
- On Cf-ECP2 lines, ECP2 confers avirulence
- Cf-ECP2 recognizes an essential target of *C. fulvum* (Achilles' heel)
- Does Cf-ECP2 confer durable resistance?
- Cf-ECP2 cloned: typical Cf gene

Can R genes mediate partial resistance?

- Partial resistance: does not provide full resistance to pathogen
- May be more durable than full resistance (well documented in some cases such as potato late blight caused by *P. infestans*)
- Partial resistance may not impose sufficient selective pressure to allow novel virulent races to dominate pathogen populations (*James and Fry 1983 Phytopath. 73:984*)
- If true, then partial resistance should prove durable independently of the mechanism of resistance

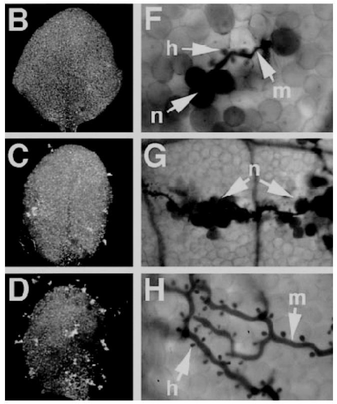
Can R genes mediate partial resistance?

Partial resistance mediated by R genes:

- Traditionally known in many heterozygote plants containing one copy of the R gene (co-dominance)
- Several examples with cloned R genes
- HR can be associated with partial resistance (trailing HR or pathogen escape from HR lesions)

Arabidopsis RPP1-WsB confers partial resistance to the oomycete *Peronospora parasitica*

- *P. parasitica* can sporulate on *RPP1-WsB* plants
- This phenotype is associated with a trailing hypersensitive response
- A similar phenotype was noted on potato cultivars with partial resistance to *Phytophthora infestans*



RPP1-WsA

RPP1-WsB

RPP0

Botella et al. 1998 Plant Cell 10:1847

Can R genes mediate partial resistance?

Cf-9 cluster of genes (*Parniske et al. 1997 Cell 91:821*)

- At least 3 active *C. fulvum* R genes are present in introgressed Cf-9 segment
- One of these genes is Cf-9 (full resistance to Avr9 containing strains); two other genes (Hcr9s) confer partial resistance
- Corresponding Avr genes are being cloned
- Avr9 mutant races are rare and are not fully pathogenic: Cf-9 tomato shows useful resistance

Residual effect of Cf-9 cluster is explained by additional R genes *Lauge et al. 1998 MPMI 11:301*

C. fulvum strains	MM-Cf0[a]	MM-Cf9[b]	MM-Cf9/ *Cf-9* mutant[c]
Avr9^{+} wild types			
Race 4	S[d]	R[e]	WR[f]
Race 5	S	R	WR
Avr9 mutants (transgenic)			
A43Δ*Avr9*	S	WR	WR
B51Δ*Avr9*	S	WR	WR
Avr9 mutants (natural)			
NZb	S	WR	ND[g]
2679	S	S	ND

[a] Genotype lacking the *Cf-9* introgression segment.
[b] Genotype containing the *Cf-9* introgression segment.
[c] Genotype containing the *Cf-9* introgression segment, without a functional *Cf-9* resistance gene.
[d] Susceptible.
[e] Highly resistant.
[f] Weakly resistant.
[g] Not determined.

Summary

- Various types of resistance mechanisms in plants
- Some involve specific recognition of effectors by R genes, others are more general
- Both types of mechanisms can result in a diversity of phenotypic expression of resistance
- R genes may function in different types of resistance
- There is a revival in the use of R genes in biotechnology