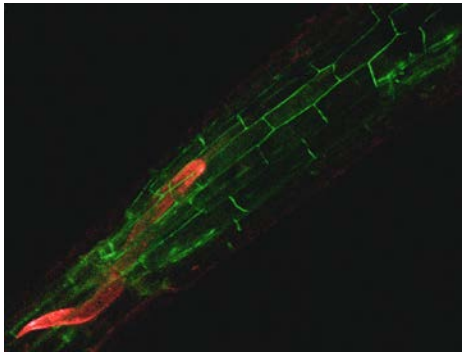
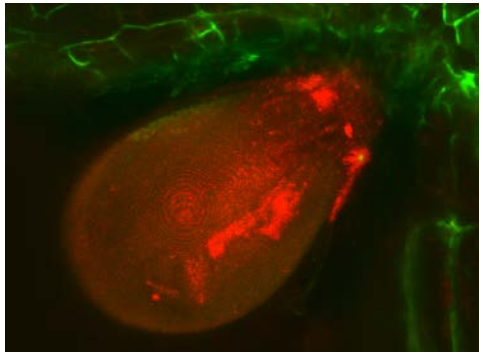
Nematode effectors – function and resistance

John Jones *et al.*


john.jones@hutton.ac.uk

The James Hutton Institute

University of St Andrews

Plant parasitic nematodes are damaging pathogens

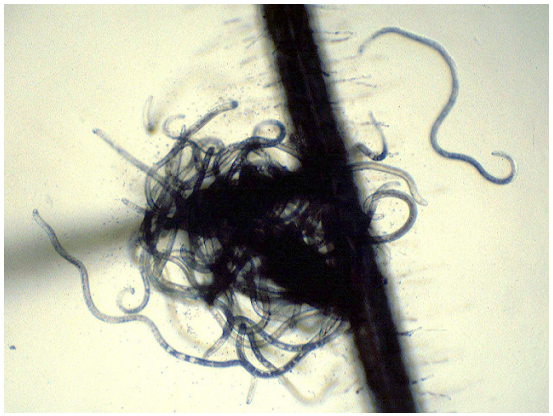
Damage valued at $100 billion pa

- PPNs eat 10% of the world's agricultural output
- 50% of crop losses in developing nations are due to pests and diseases
- Major threat to global food security

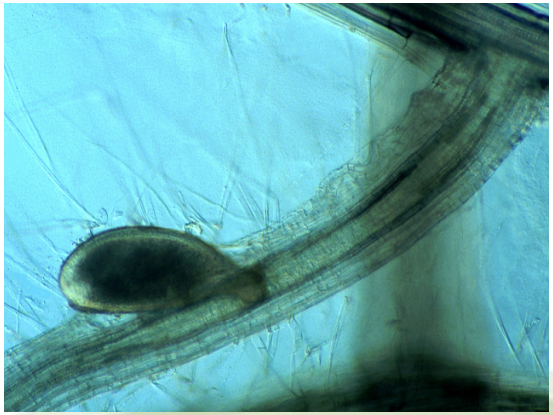
The most damaging species are root-knot nematodes and cyst nematodes

- Potato cyst nematodes are a major problem in the UK
- Effective nematicides being withdrawn

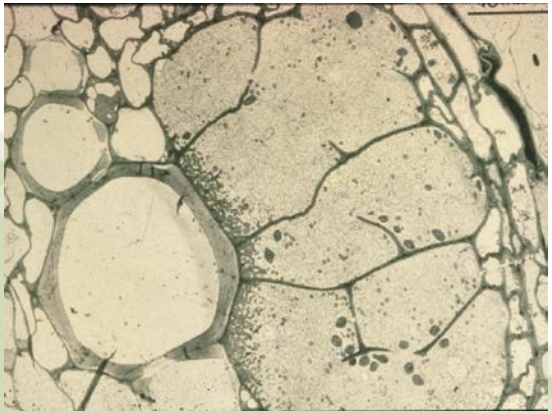
Plant parasitic nematodes can be biotrophic pathogens

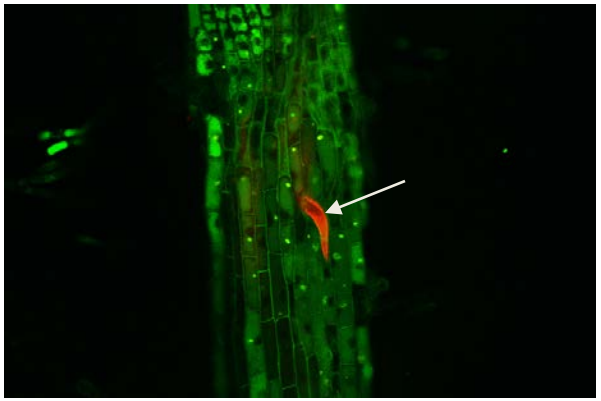


***G. pallida* life cycle**



Syncytium: kept alive for up to 6 weeks





G. pallida genome and transcriptome resources

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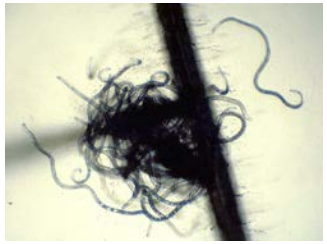
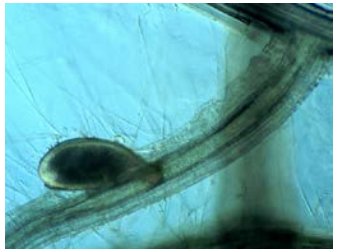


Full genome sequence



parasitic

parasitic

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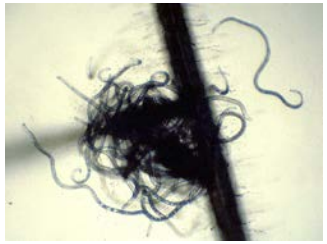
Eggs

Males

J2



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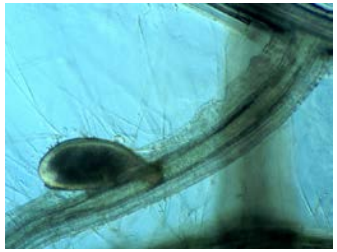

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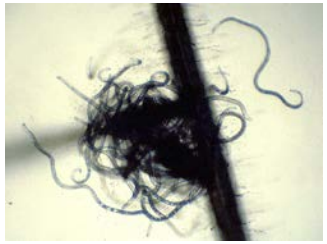

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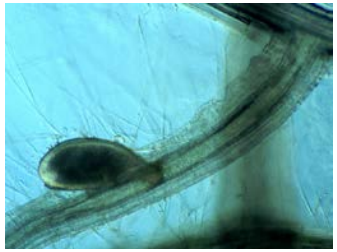

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Powerful tools for understanding plant nematode biology

Effectors of plant parasitic nematodes

Effectors

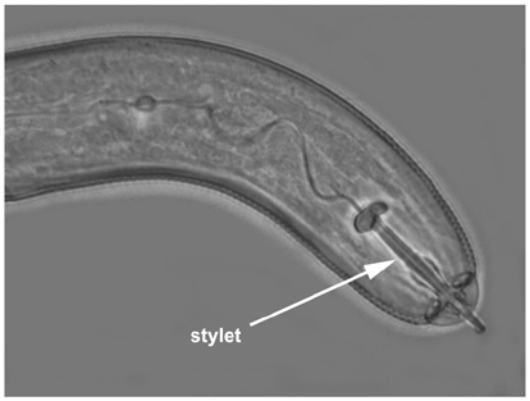
Proteins secreted by the nematode into the host that manipulate the plant to the benefit of the nematode

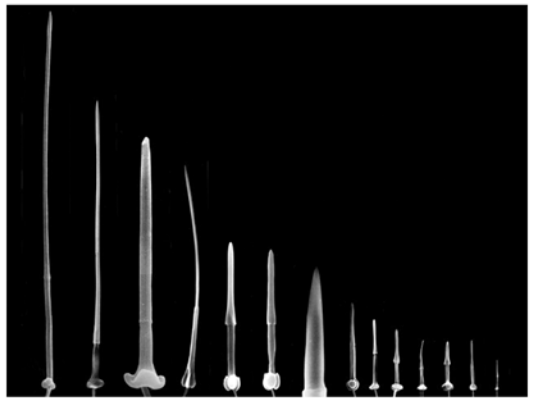
Effectors play roles in:

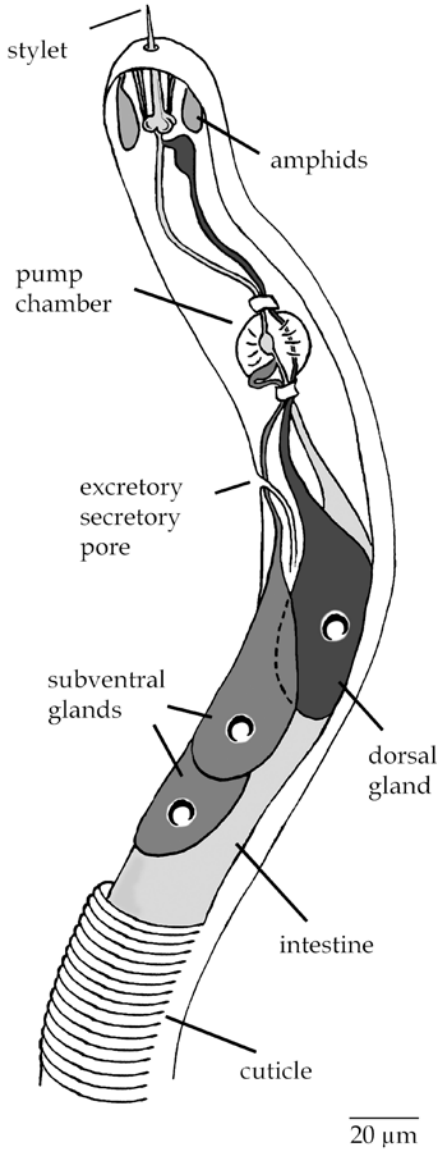
- Invasion & migration
- Induction of the feeding site
- Suppression of host defence responses

Nematode effectors (mainly) from 2 sets of gland cells

- Dorsal gland cell
- Subventral gland cells





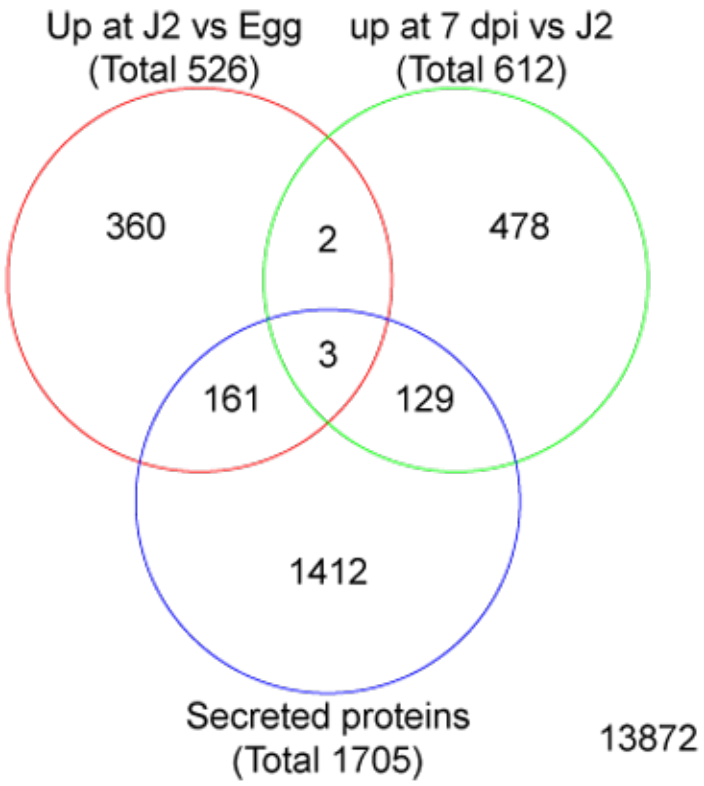


Identifying effectors from nematodes

BLAST search *G. pallida* genome with effectors from other nematodes

Bioinformatic identification of "novel" effector candidates

- Signal peptide, no transmembrane domain
- Upregulated in J2 vs egg or parasitic vs J2



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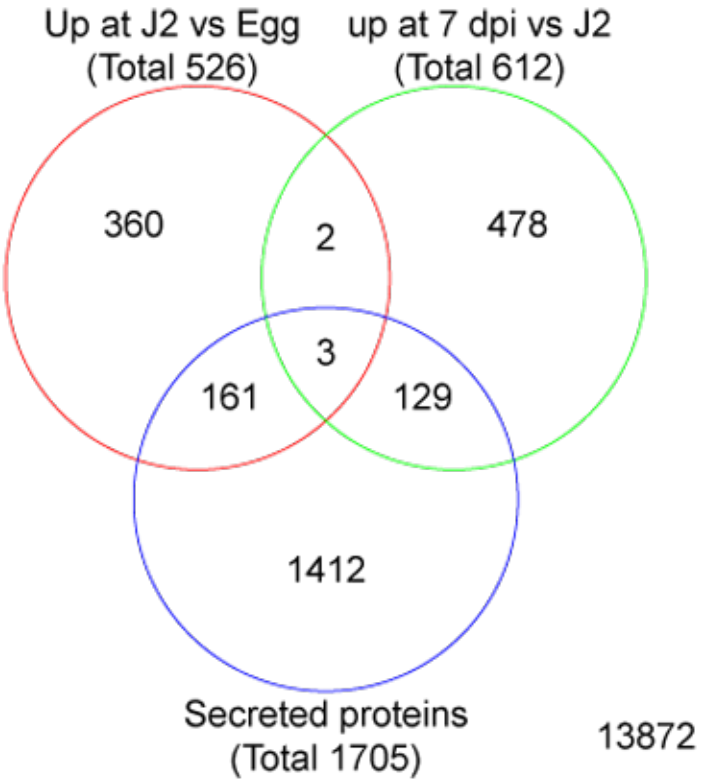

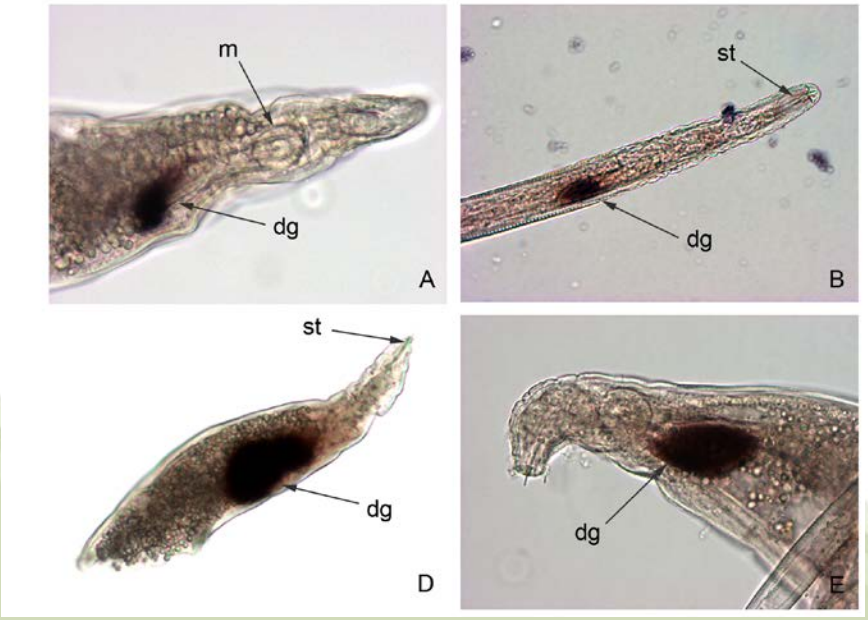

Confirm by looking at where candidates are expressed in the nematode

Categorising/prioritising effectors

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~ 500 potential effectors identified

Need to prioritise

- Presence in a range of populations
- Timing of expression
- RNAi effects
- Subcellular localisation in plants

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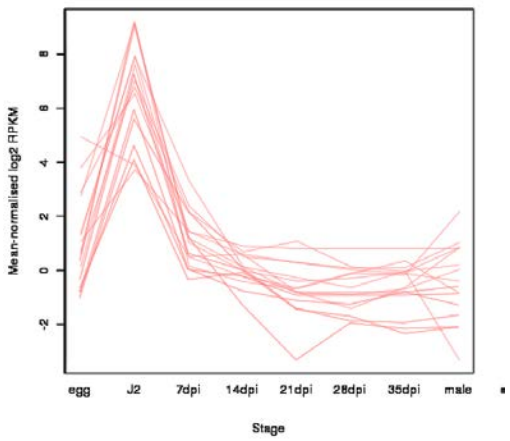
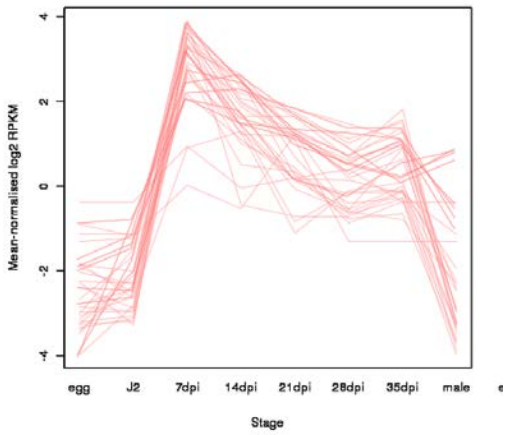


J2 specific



Parasitic stages

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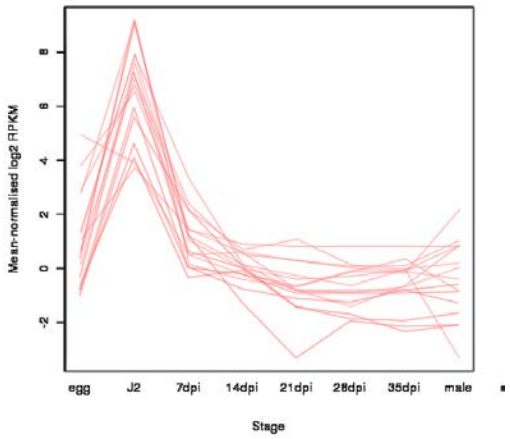

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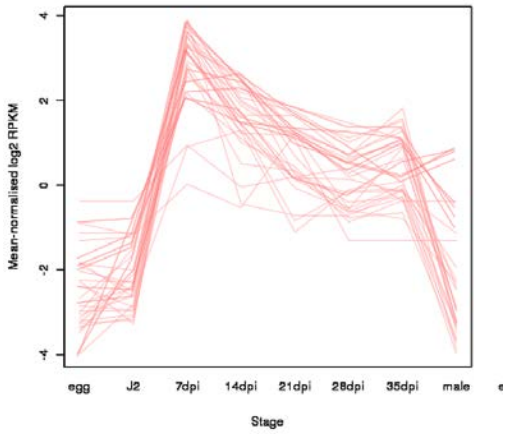
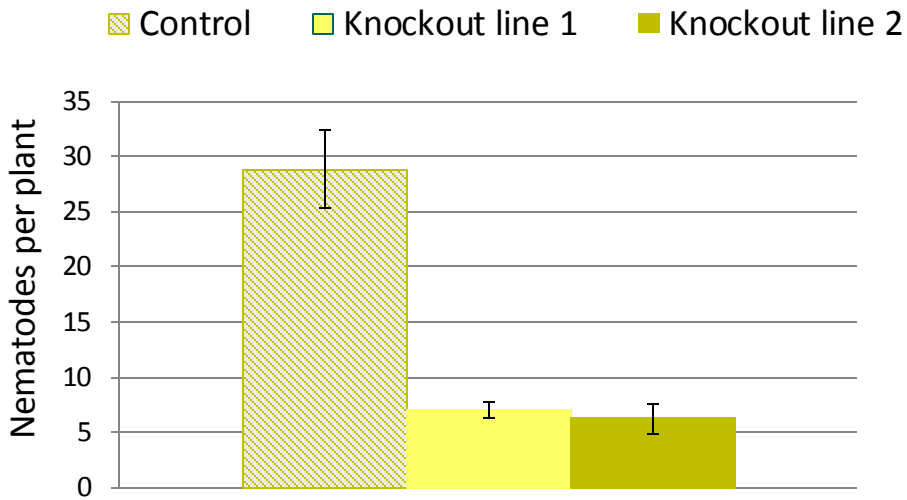


Parasitic stages



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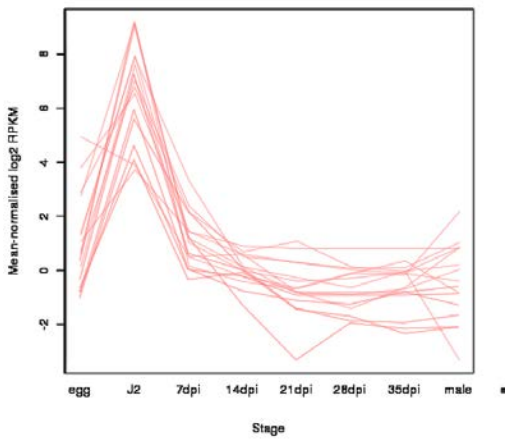

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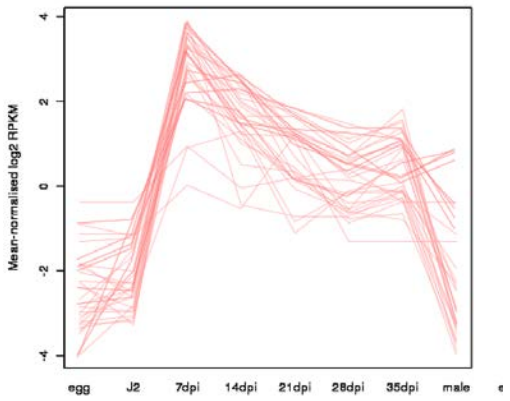

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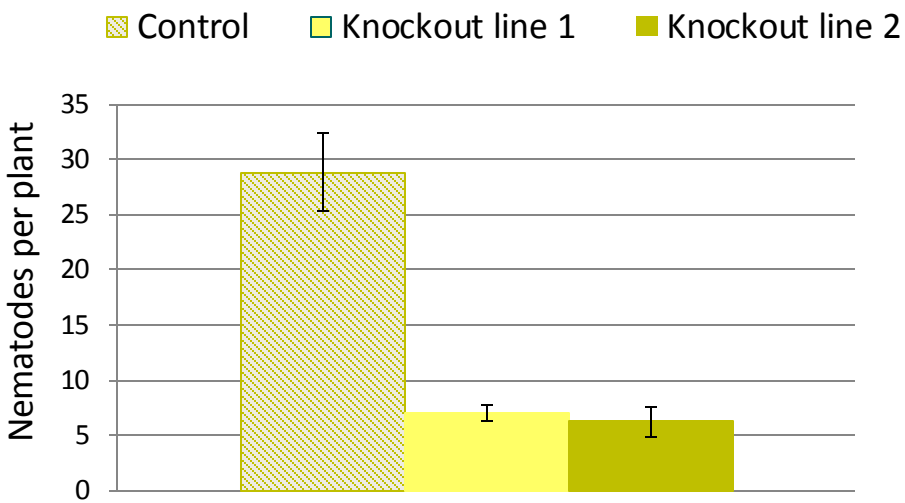

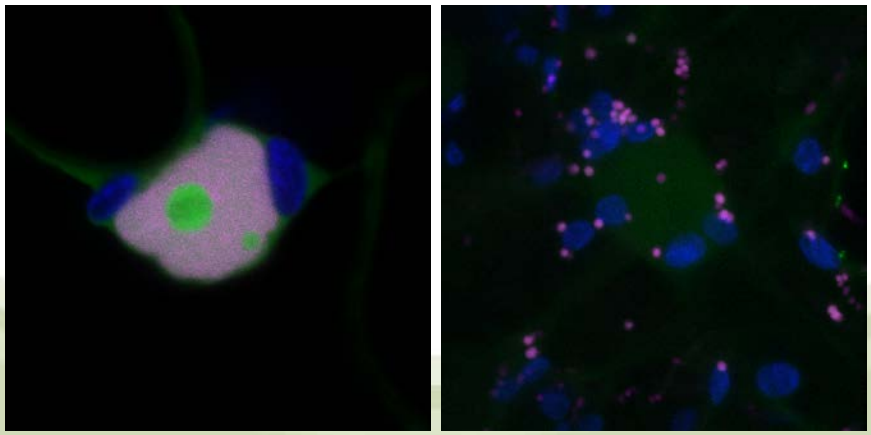

Some *G. pallida* effectors suppress host defences

***e.g* SPRYSEC effector SPRY12**

- Expressed in dorsal gland cell
- Suppresses cell death induced by presence of Gpa2/AvrGpa2

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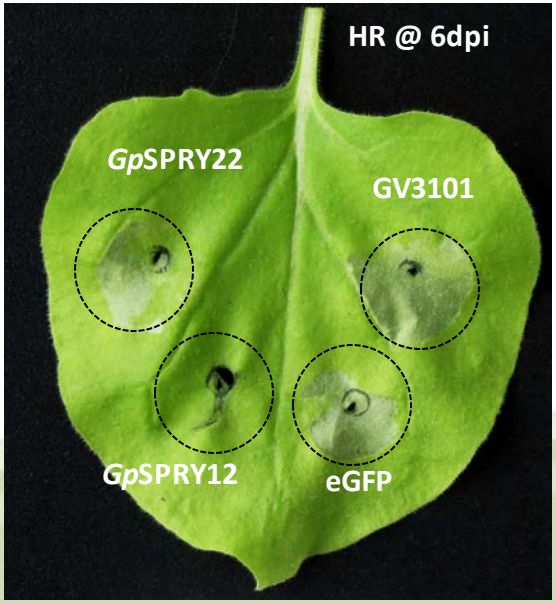

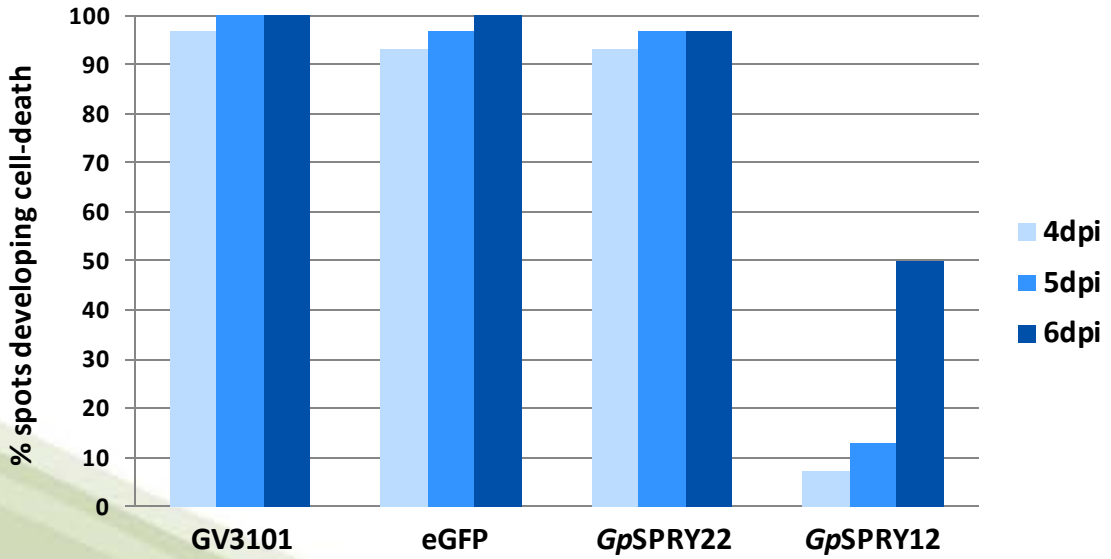

An ability shared with other plant pathogens

Some *G. pallida* effectors may manipulate the host as a food source

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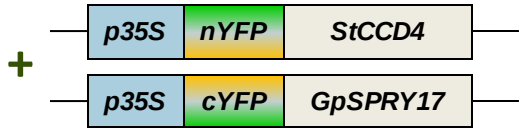
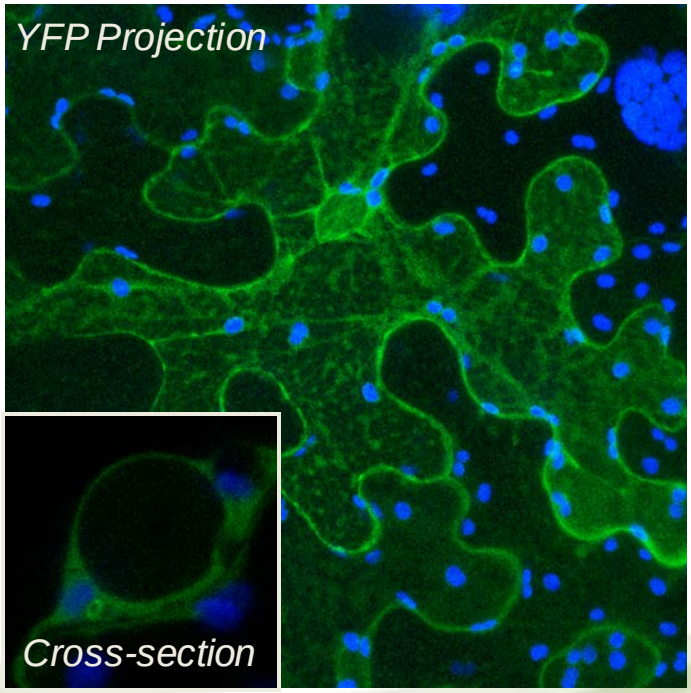
SPRYSEC effector SPRY17 interacts with potato CCD4

- Strong interaction in yeast
- BiFC shows that the interaction occurs in the cytoplasm of plant cells

Reporter *LacZ*

GpSPRY17 + Empty prey vector	[-]
StCCD4 + Empty bait vector	[-]
GpSPRY17 + StCCD4	[++]



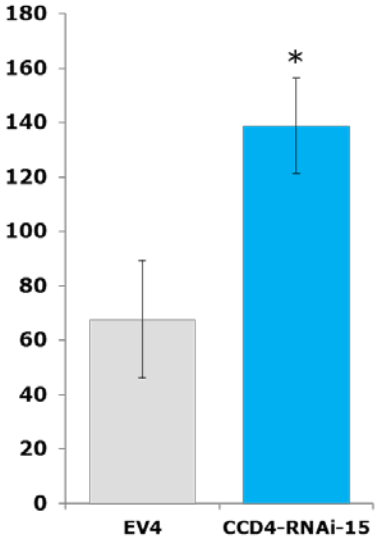


BiFC signal

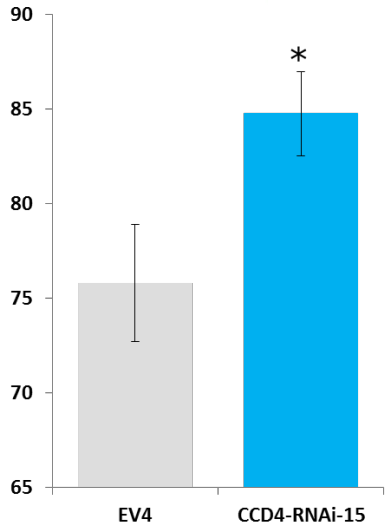
Silencing CCD4 makes plants more susceptible to *G. pallida*

CCD4 is a carotenoid cleavage dioxygenase

- Silencing of CCD 4 leads to increased carotenoid levels in plants
- May be a requirement in the *G. pallida* diet



Nematodes /g fresh root



% of Females

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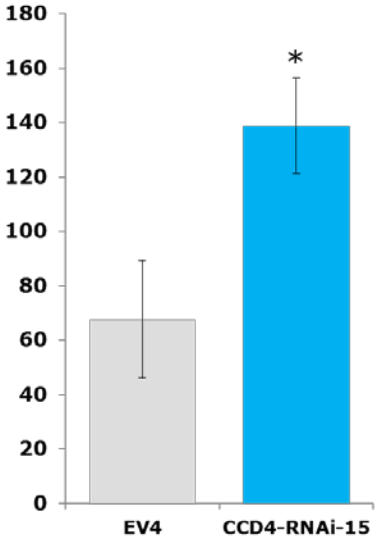

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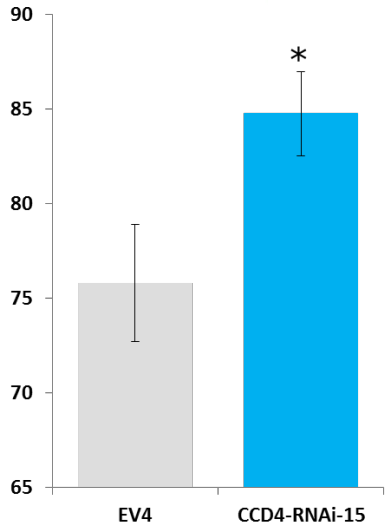

% of Females

A similar pattern is seen in *Solanum phureja*

- Naturally low levels of CCD4
- High levels of carotenoids
- **Highly susceptible to PCN**

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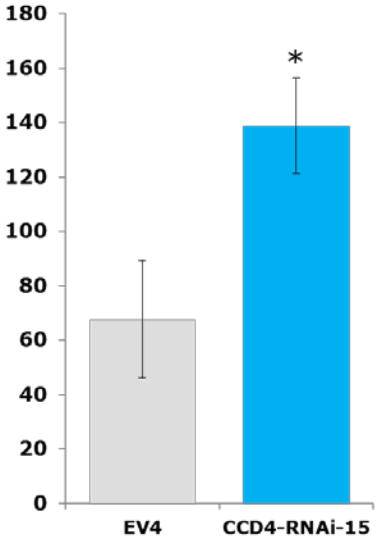

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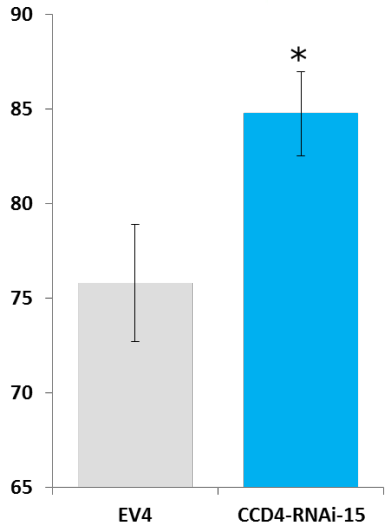

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- Naturally low levels of CCD4
- High levels of carotenoids
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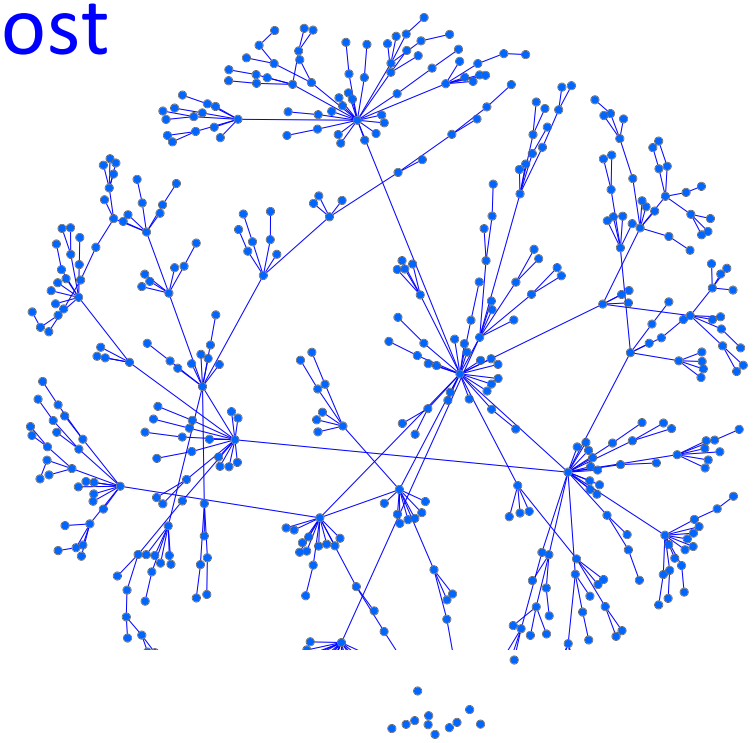
***G. pallida* may inactivate CCD4 in order to improve the nutritional quality of its host**

Common host targets of effectors

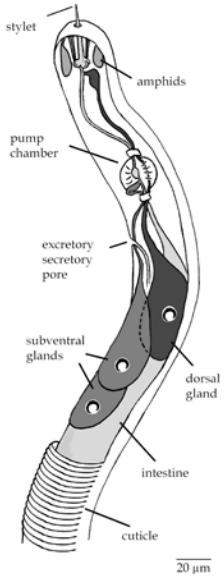
Large scale interaction studies show that some plant proteins interact with many other proteins

- "Hubs"
- Key roles in controlling biochemical networks
- These hubs are frequently targeted by pathogens – control targets

Host



Pathogen



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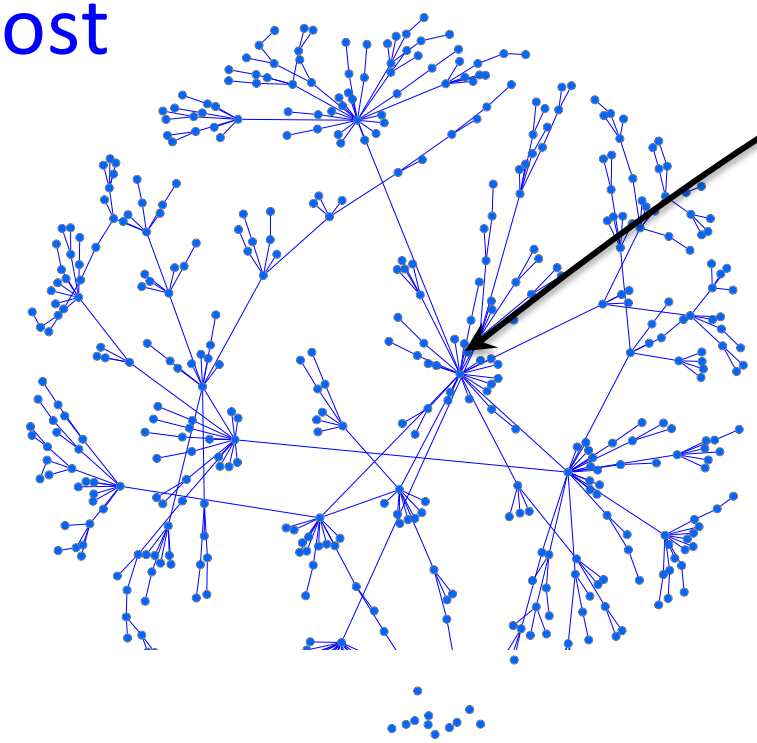

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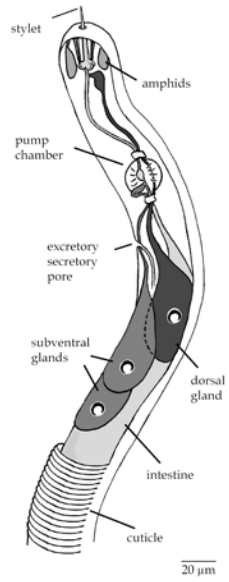

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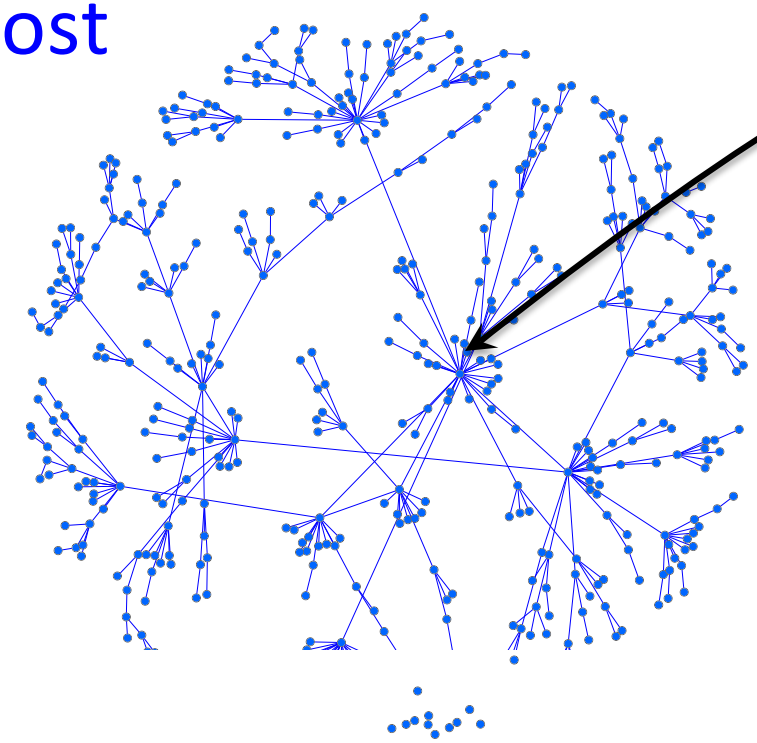

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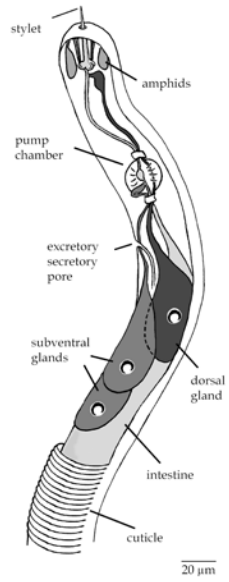
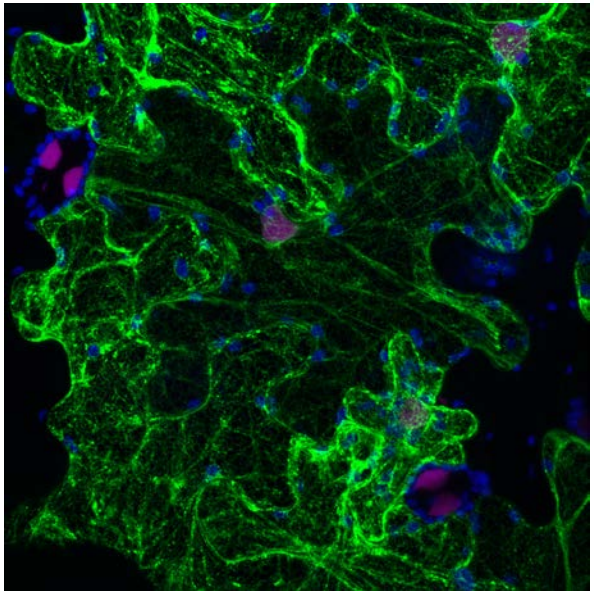


Do *G. pallida* effectors target similar proteins to other pathogens?

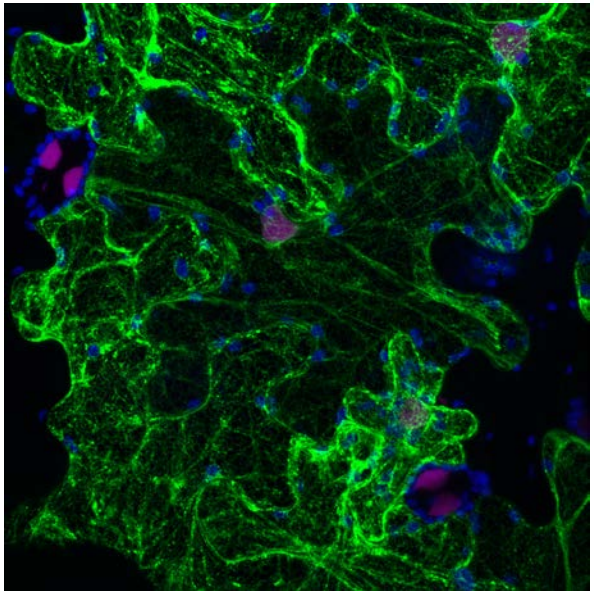
G. pallida effector G16H02



Localises to endoplasmic reticulum in host

- Similar localisation seen for one *Phytophthora infestans* effector
- Interacts with a NAC transcription factor

G. pallida effector G16H02



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***G. pallida* G16H02 effector also interacts with a NAC transcription factor**

G. pallida effector G16H02

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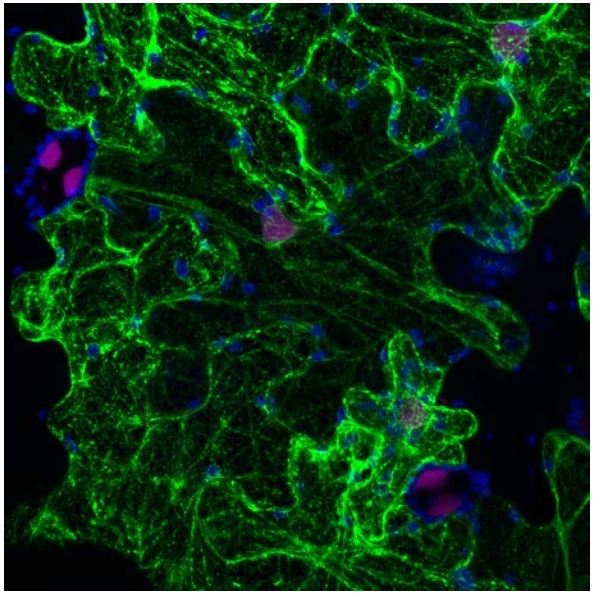
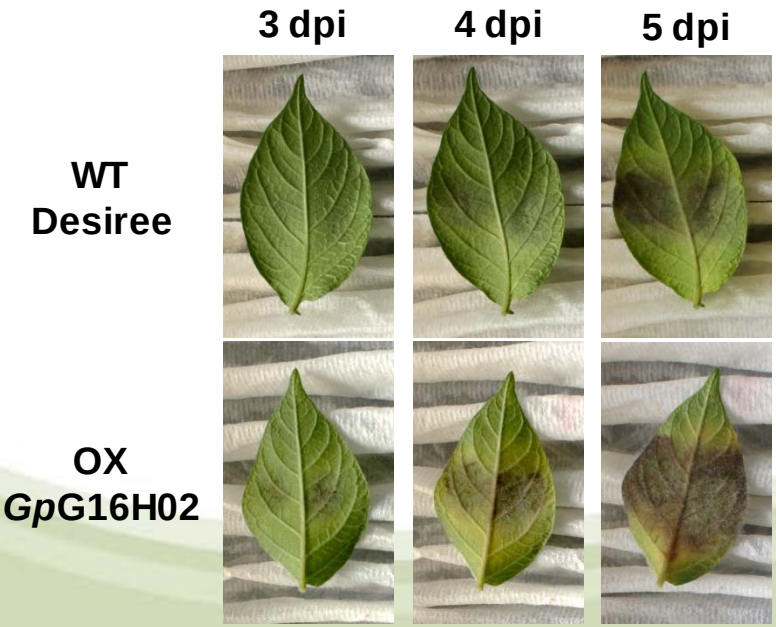


***G. pallida* G16H02 effector also interacts with a NAC transcription factor**

Over expression allows increased infection by *P. infestans*

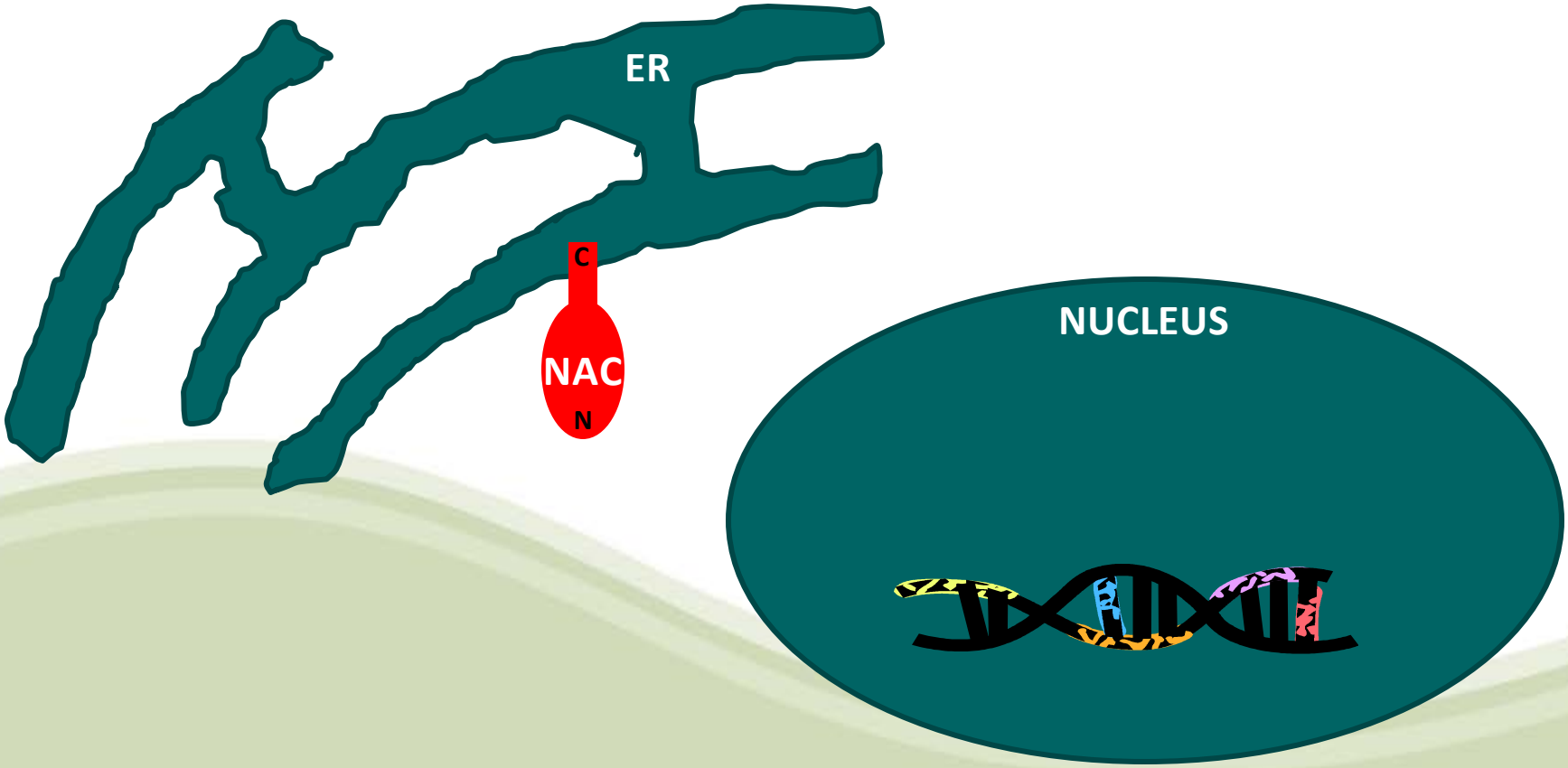


What are NAC Transcription Factors?

One of largest families of plant-specific TFs

Some are membrane bound TFs (MTFs)

MTFs associated with environmental stress responses

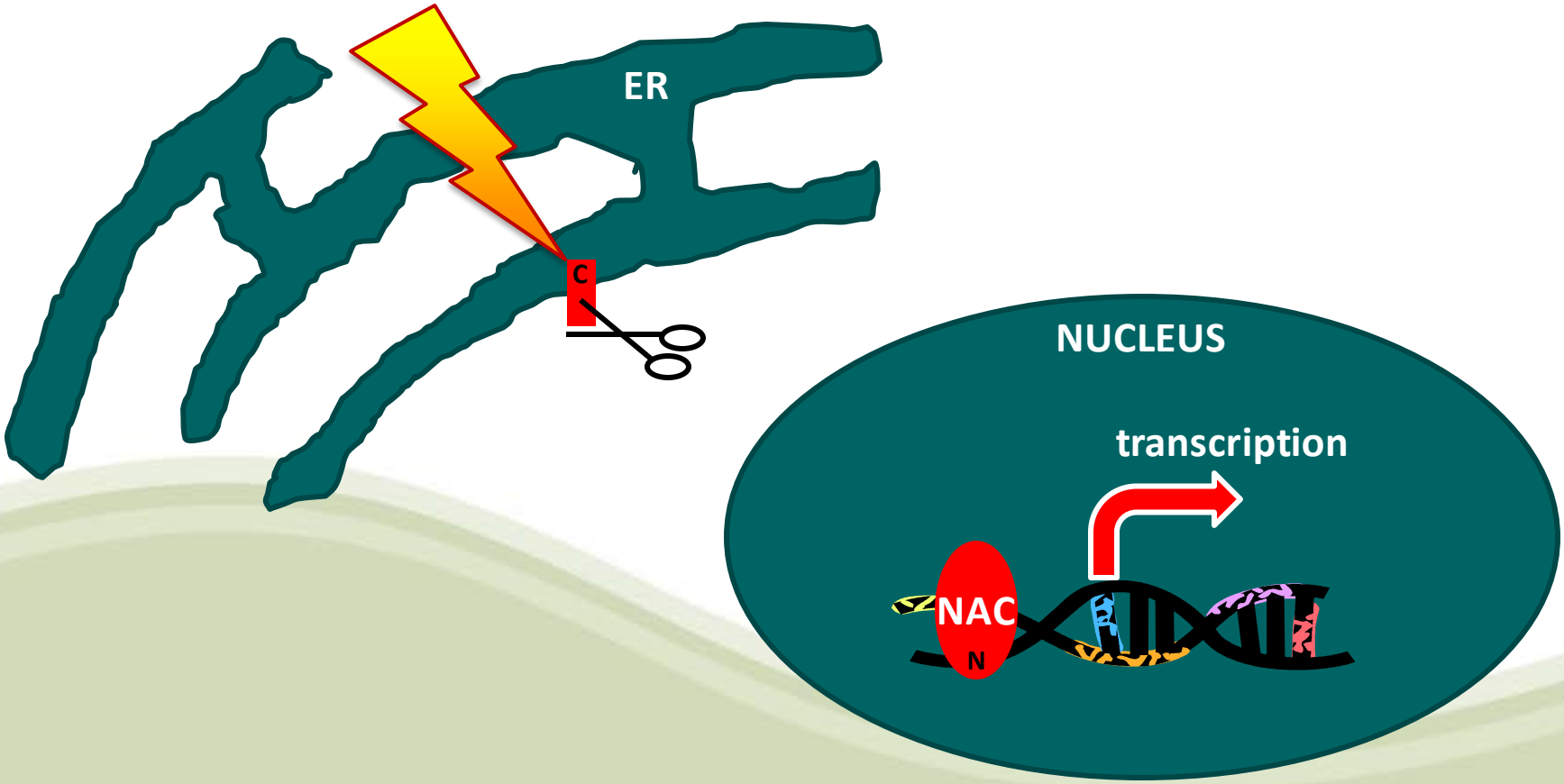


What are NAC Transcription Factors?

One of largest families of plant-specific TFs

Some are membrane bound TFs (MTFs)

MTFs associated with environmental stress responses



The *G. pallida* G16H02 effector causes retention of the NAC TF in the ER

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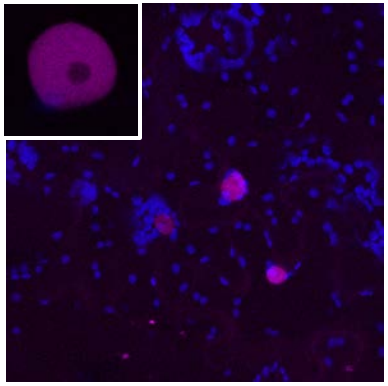
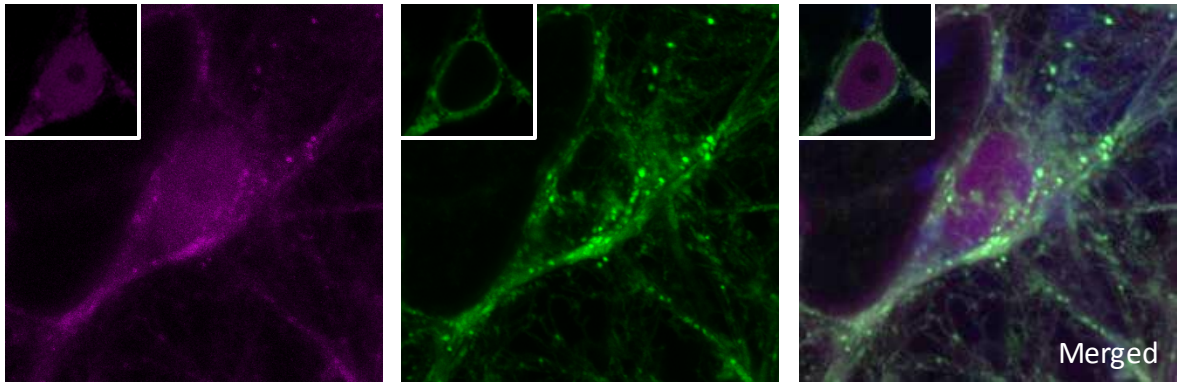
RFP-*St*NAM9



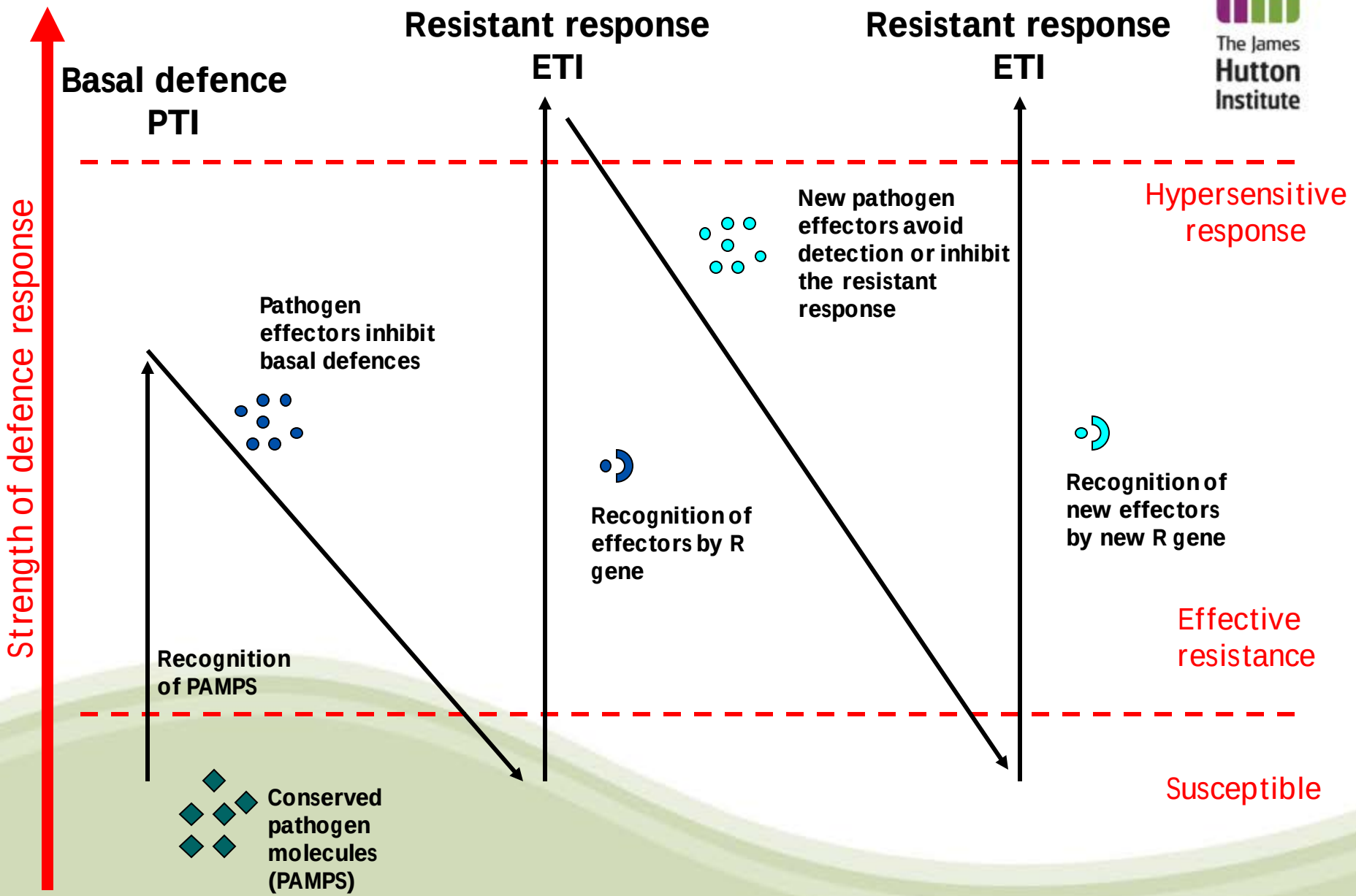
RFP-*St*NAM9
+
GFP-GpG16H02



Similar mode of action to the *P. infestans* effector

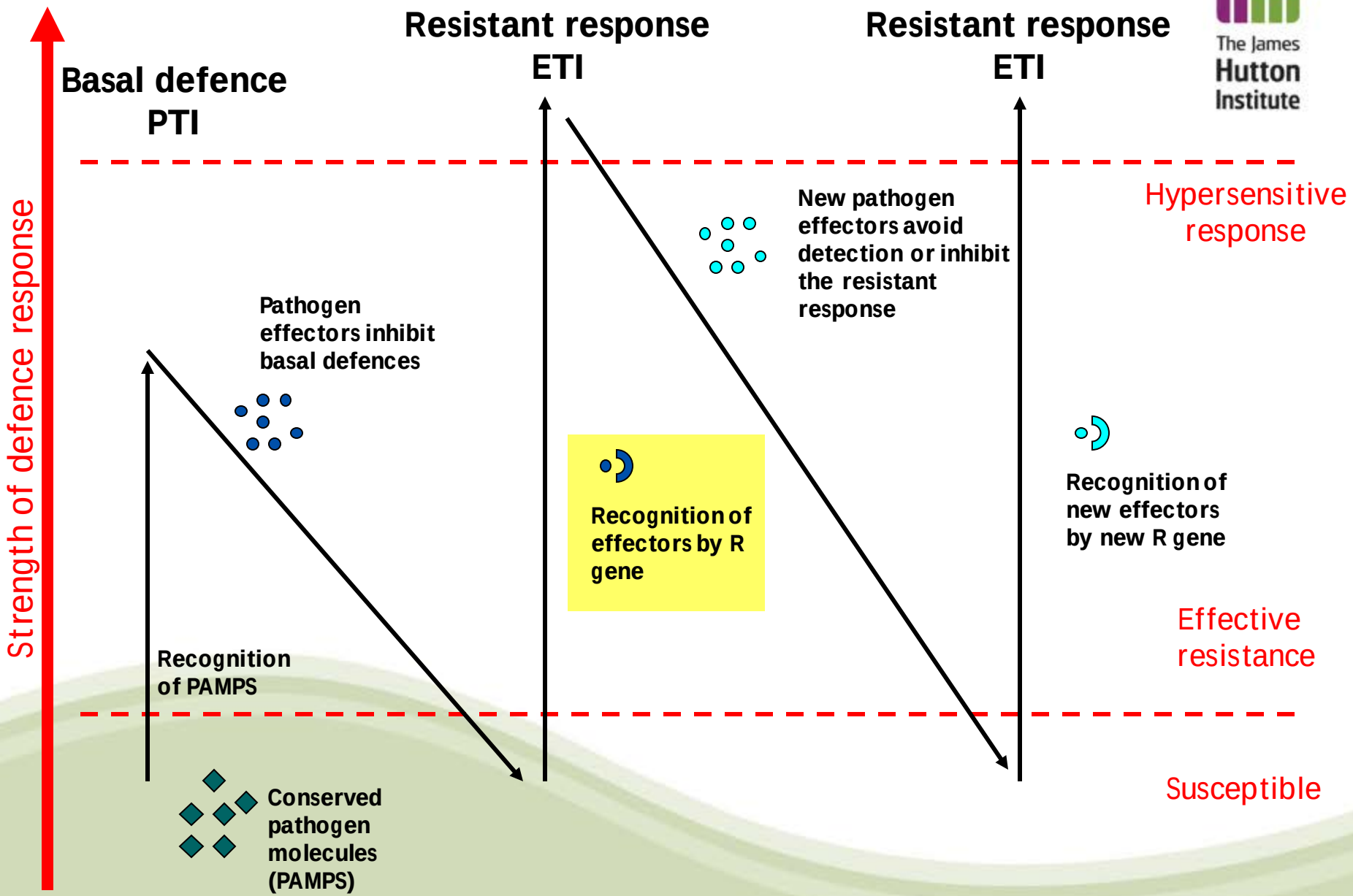
- **Conserved targets of effectors from diverse pathogens**

Identification of effectors: what's the point?



Jones & Dangl (2006), Nature 444, 323-329

Identification of effectors: what's the point?



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Recognised effectors can be used to screen for resistance

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Some effectors are recognised by resistance genes

- Avirulence genes
- SPRYSEC protein – RBP1 = AvrGpa2

Recognised effectors can be used to screen for resistance

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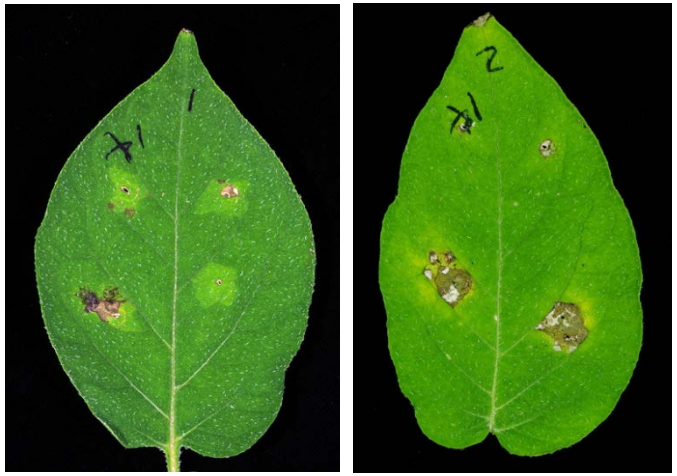
- Avirulence genes
- SPRYSEC protein – RBP1 = AvrGpa2

Avr genes can be used to screen for resistance in breeding lines

- Assays on leaves
- Transient expression of RBP1



Desiree (Gpa2-) **Cara (Gpa2+)**

Identifying Avr genes for new R sources

Additional *G. pallida* sequence resources generated

"Population"	Potential uses	gDNA	RNAseq
Bedale (UK *G. pallida*)	Different virulence compared to sequenced strain	☺	☺
Newton (UK *G. pallida)*	Starting population for selection	☺	☺
Selected lines	Selected for virulence against *verneii* and *andigena*	☺	☺
Luffness	Virulent against *verneii*	☺	☺
Pa1	Avirulent against H2	☺	☺
P4A (S. American *G. pallida)*		☺	☺
P5A (S. American *G. pallida)*	Virulent against *verneii* and *andigena* sources	☺	☺

Analyse variation in effector sequences associated with differences in virulence

New tools for resistance breeding

Resistance that recognises conserved and/or essential effectors is likely to be durable

Summary

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Effectors from *G. pallida* and their host targets have been identified

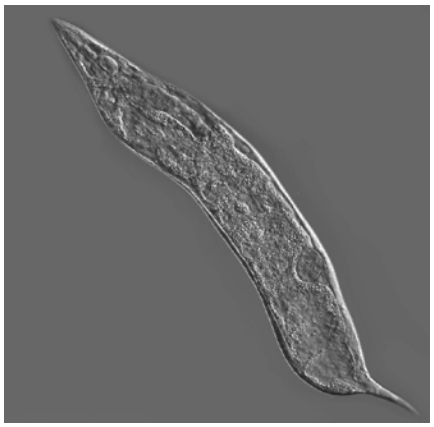
Effectors localise to a range of host structures

***G. pallida* effectors suppress host defence responses**

A *G. pallida* effector may manipulate host biochemistry to improve the nutritional qualities of its host

Effectors from diverse pathogens may have similar host targets

Effectors can be used as tools for identifying resistance



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