Decoding plant pathogen strategies: Exploring effector structural insights for enhanced plant resistance

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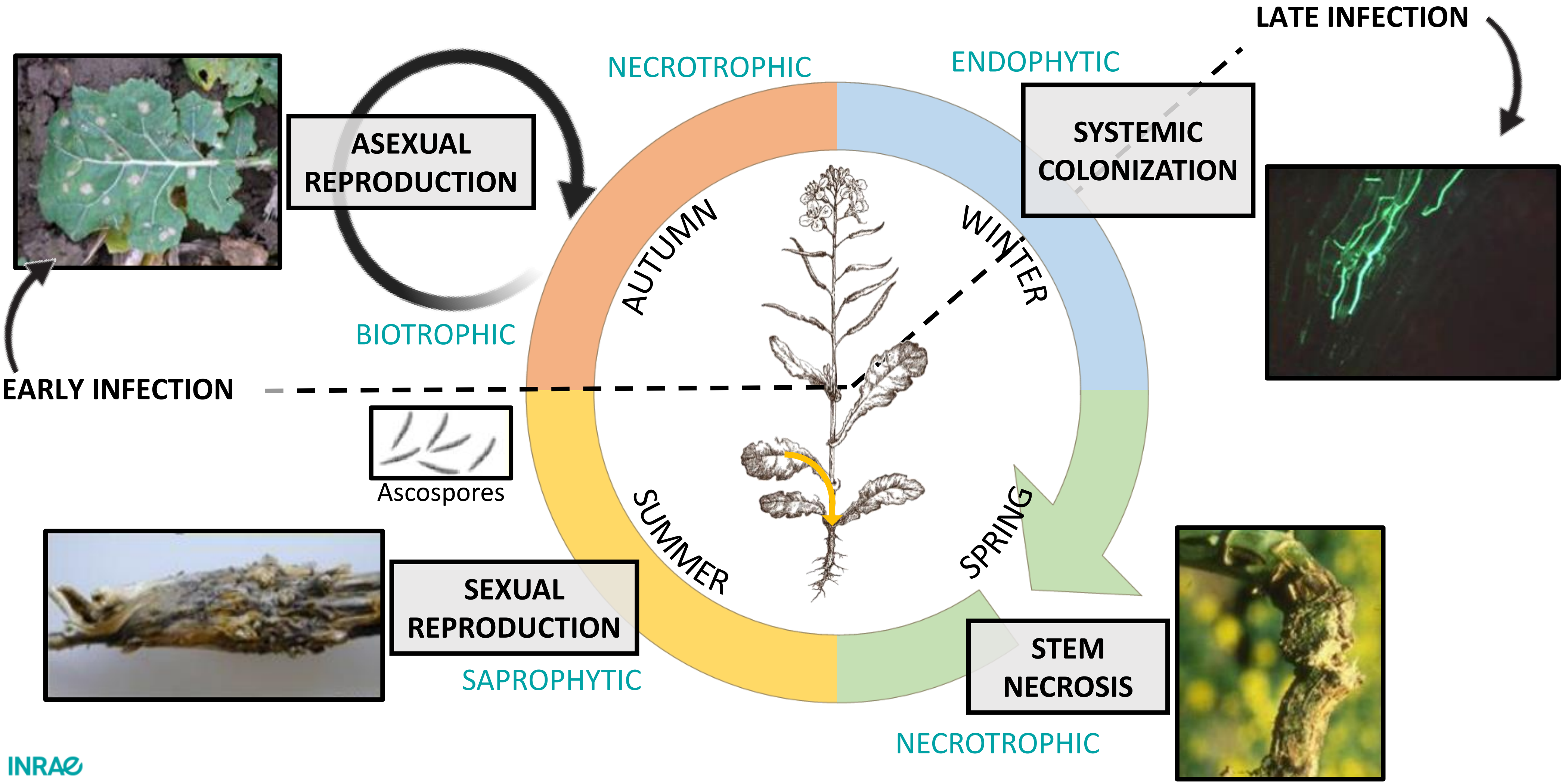
Leptosphaeria maculans has a complex life cycle with alternating trophic stages



LATE INFECTION

NECROTROPHIC

ENDOPHYTIC

ASEXUAL REPRODUCTION

SYSTEMIC COLONIZATION

AUTUMN

WINTER

BIOTROPHIC

EARLY INFECTION

Ascospores

SUMMER

SPRING

SEXUAL REPRODUCTION

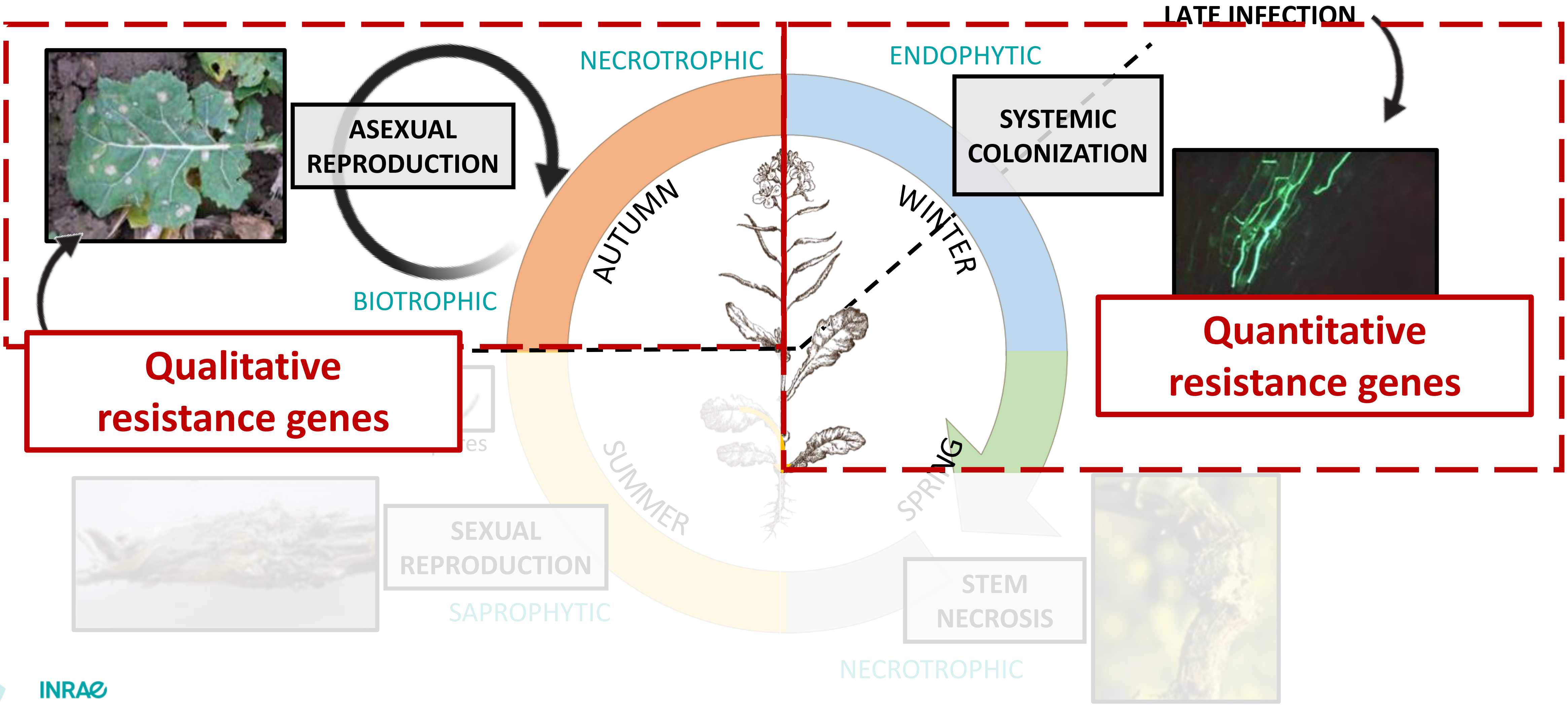
SAPROPHYTIC

STEM NECROSIS

NECROTROPHIC

Rouxel & Balesdent 2005, Mol. Plant Pathol.

The main strategy to control *L. maculans* is based on genetic control

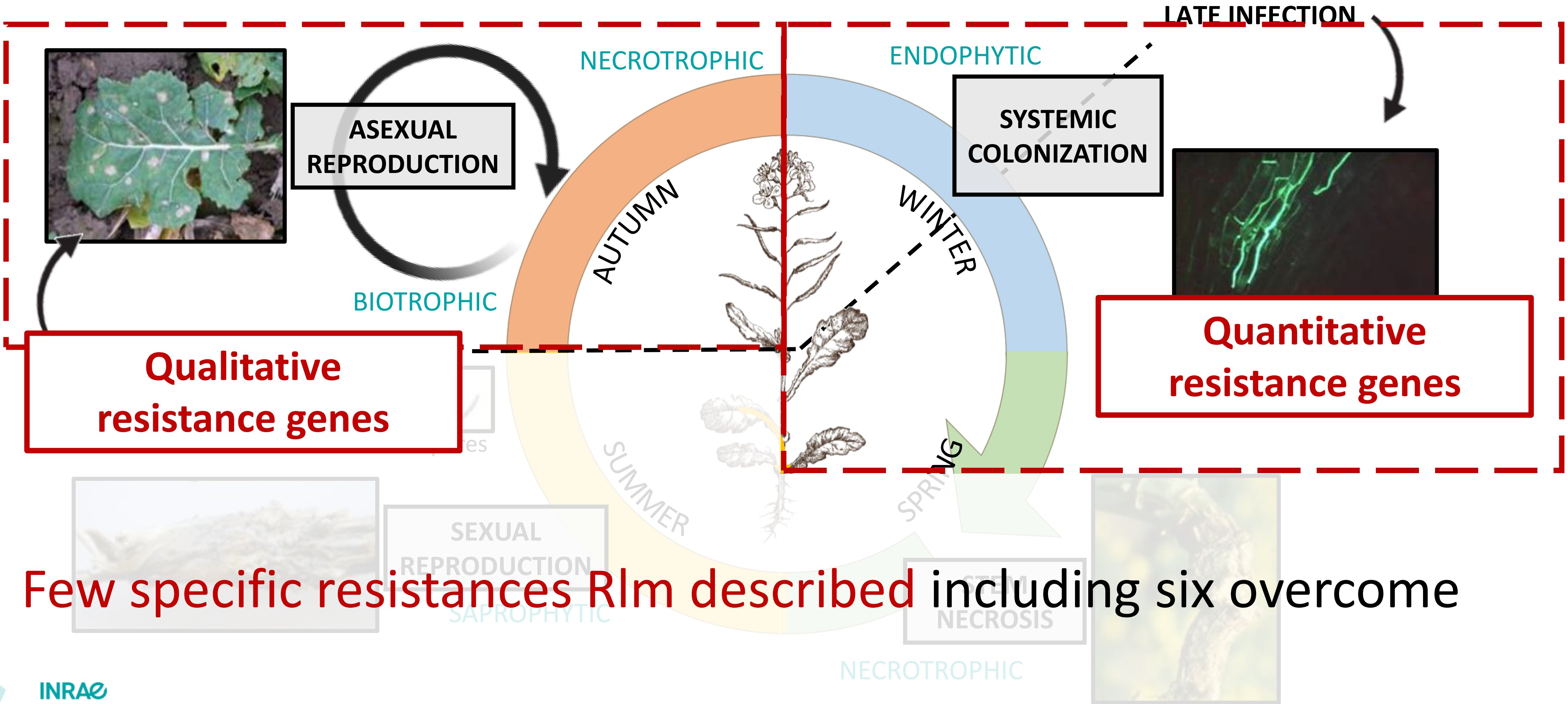


Qualitative resistance genes

Quantitative resistance genes

Rouxel & Balesdent 2005, Mol. Plant Pathol.

The main strategy to control *L. maculans* is based on genetic control



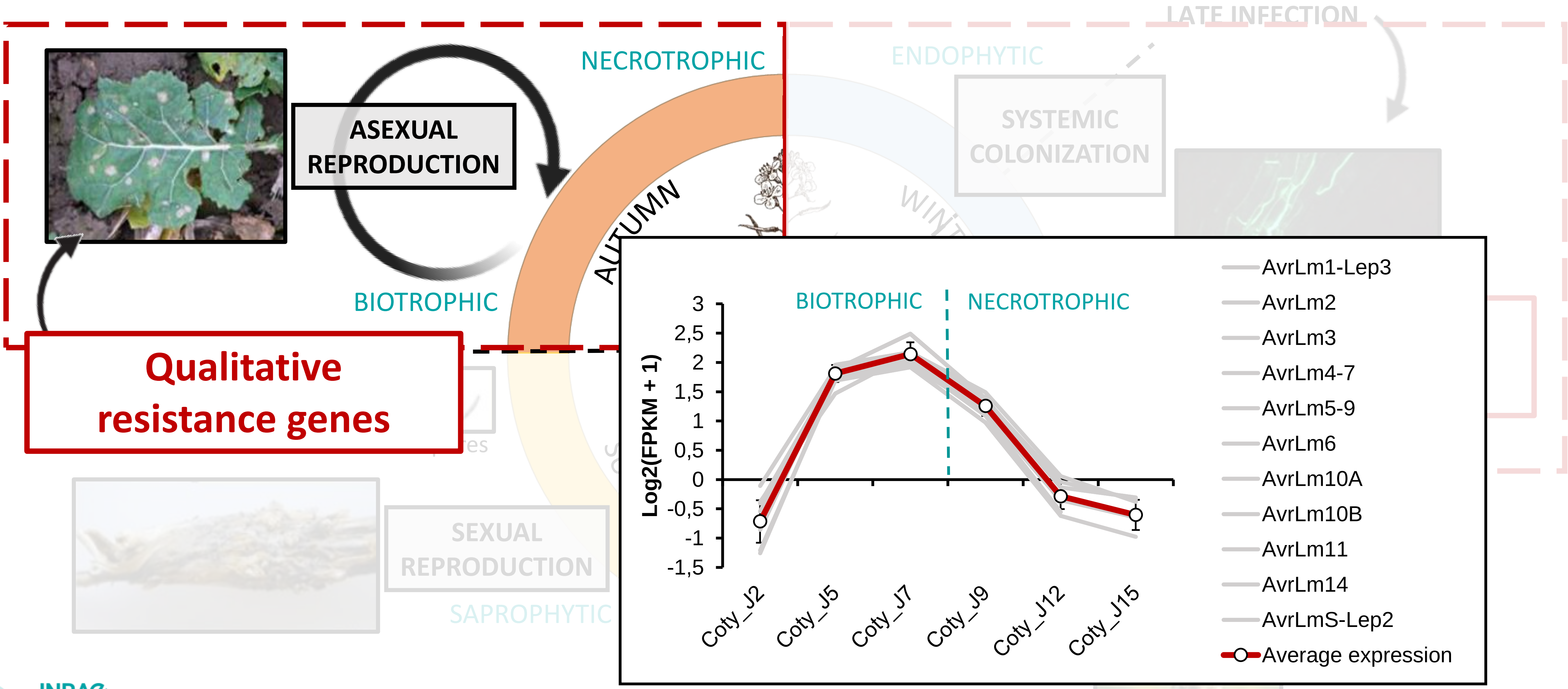
Qualitative resistance genes

Quantitative resistance genes

Few specific resistances Rlm described including six overcome

Rouxel & Balesdent 2005, Mol. Plant Pathol.

11 *AvrLm* genes are co-expressed and up-regulated in early infection …



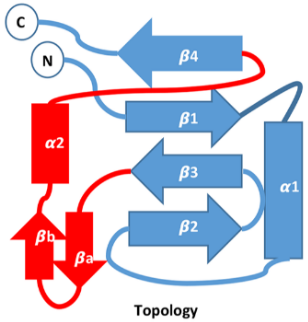
Qualitative resistance genes

Rouxel & Balesdent 2005, Mol. Plant Pathol.
Gay et al. 2021, BMC Biol.

… and four *AvrLm* genes belong to the LARS structural family

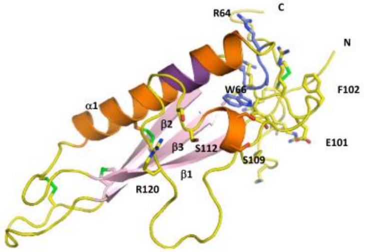
First cristal structure
AvrLm4-7
Blondeau et al. 2015, Plant J.

LARS family
AvrLm4-7, AvrLm5-9,
AvrLmS, AvrLm3
Lazar et al. 2022, PLoS Patho.



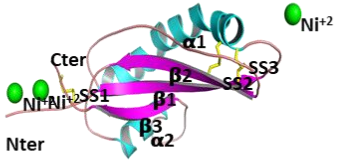
2015

2020

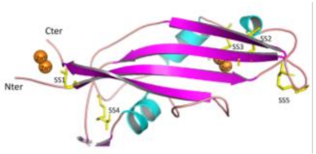


Cristal structures

AvrLm5-9

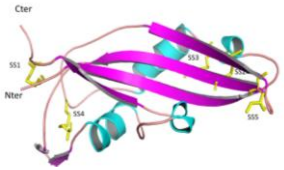


Ecp11-1



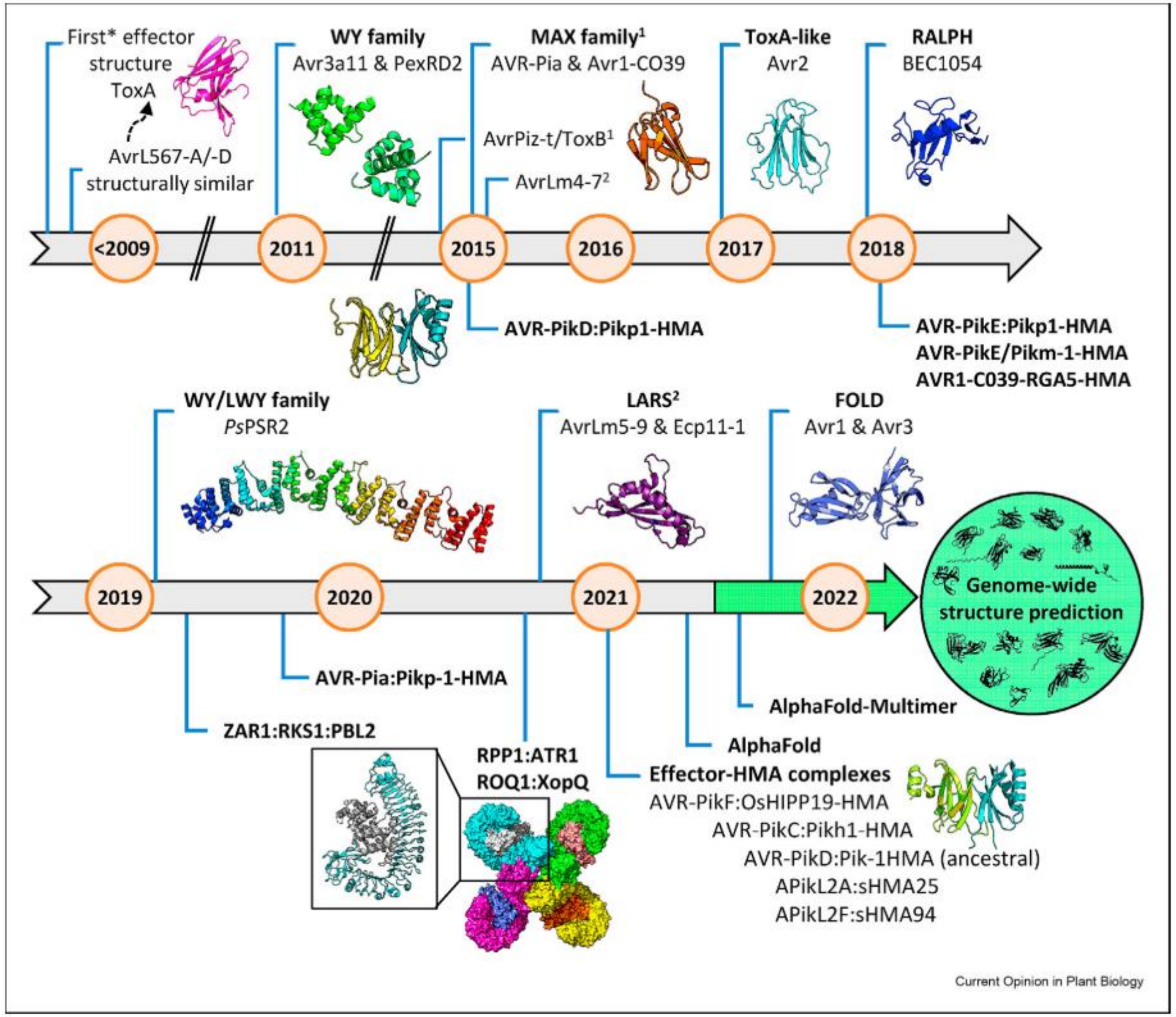
Cladosporium fulvum

AvrLm3/Ecp11-1
sequence identity



Structure prediction
AvrLm3

Different conserved structural families have been described in fungal plant pathogens



Outram et al. 2022, Curr. Opin. Plant Biol.

Many questions can be raised …

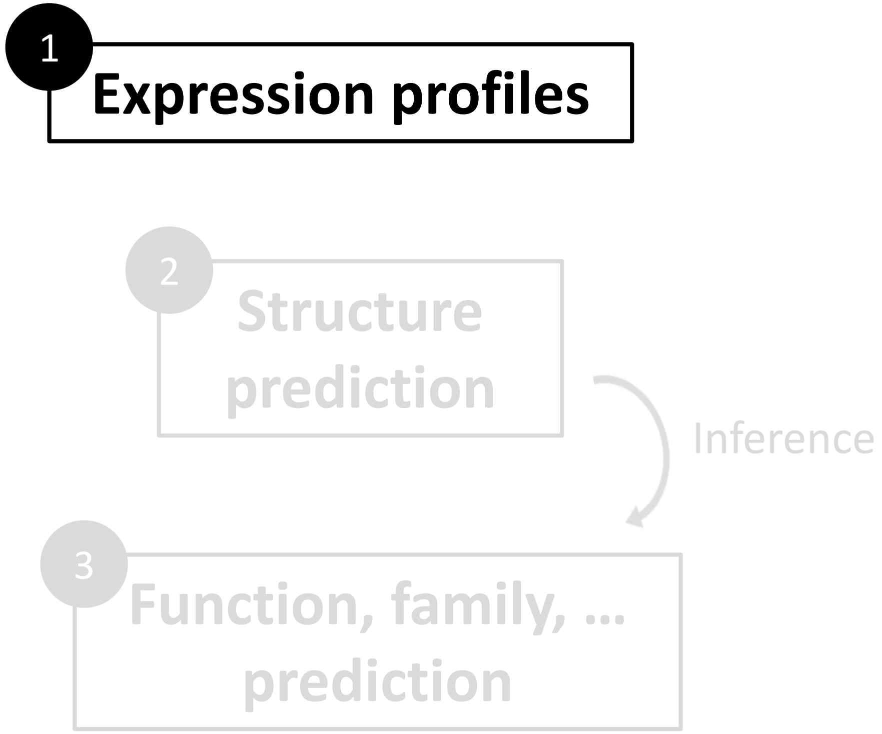
Do *Leptosphaeria maculans* effectors belong to a limited set of structural families?

Is the LARS structural family specific to leaf infection?

OBJECTIVE

Explore structural diversity among *L. maculans* secretome to propose knowledge-driven plant resistance management

Three main strategies are used in this project:



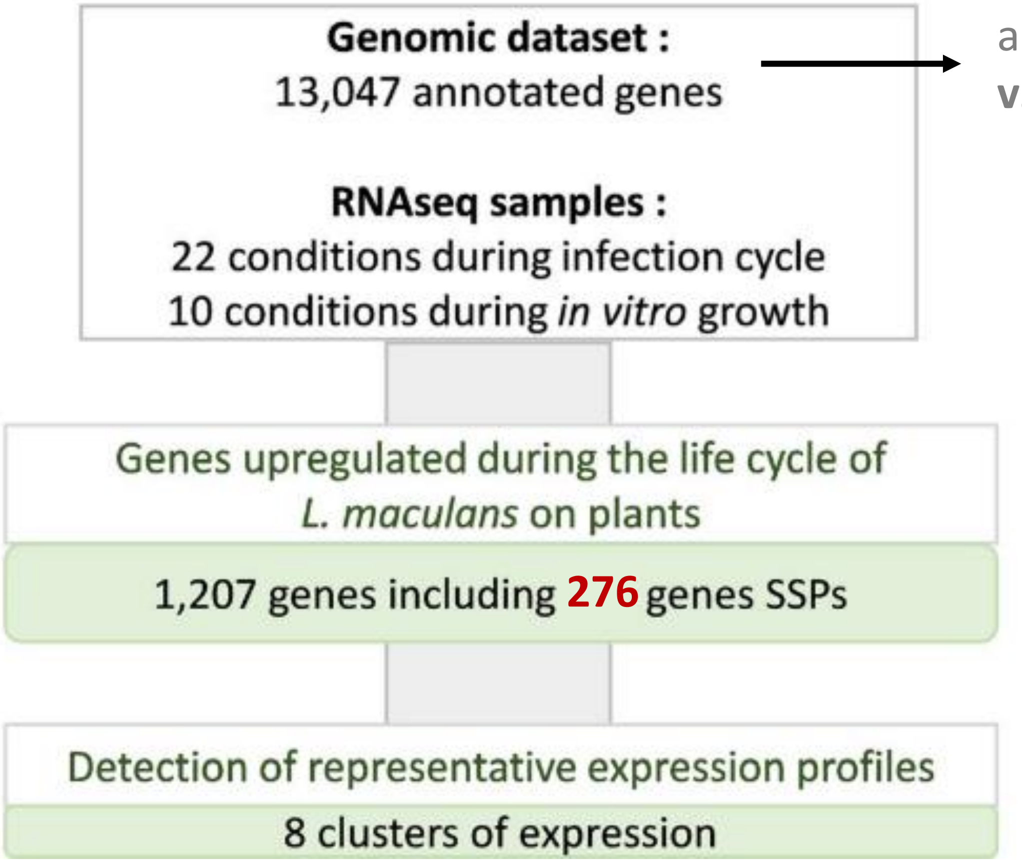
1 **Expression profiles**

2 **Structure prediction**

Inference

3 **Function, family, … prediction**

276 small secreted proteins are upregulated during the life cycle of *L. maculans* on plant



Genomic dataset :
13,047 annotated genes

RNAseq samples :
22 conditions during infection cycle
10 conditions during *in vitro* growth

Genes upregulated during the life cycle of *L. maculans* on plants

1,207 genes including **276** genes SSPs

Detection of representative expression profiles

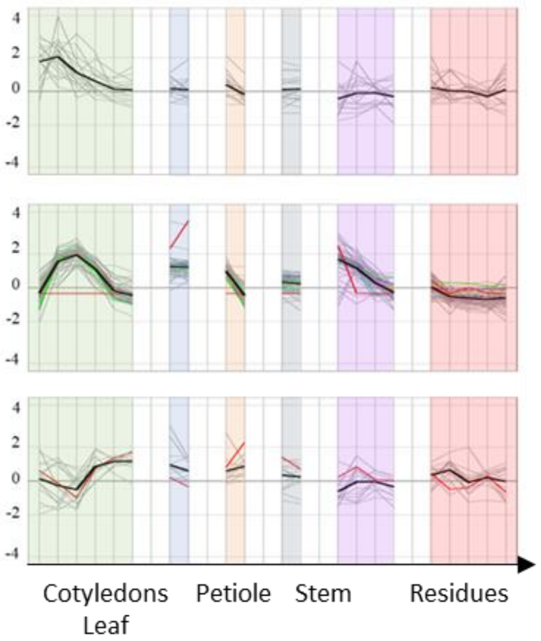
8 clusters of expression

annotation from
v2 of *L. maculans* genome

Gay et al. 2021, BMC Biol.

The *L. maculans* effector repertoire is expressed in eight waves

EARLY INFECTION



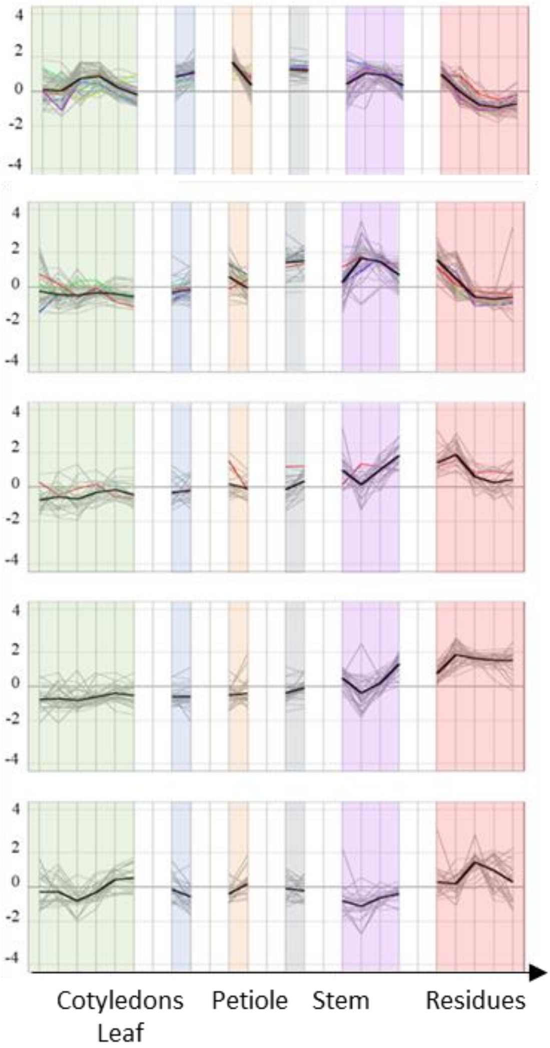
Wave 1:
16 SSPs

Wave 2:
67 SSPs

Wave 3:
14 SSPs

Gay et al. 2021, BMC Biol.

LATE INFECTION



Wave 4:
55 SSPs

Wave 5:
40 SSPs

Wave 6:
22 SSPs

Wave 7:
33 SSPs

Wave 8:
24 SSPs

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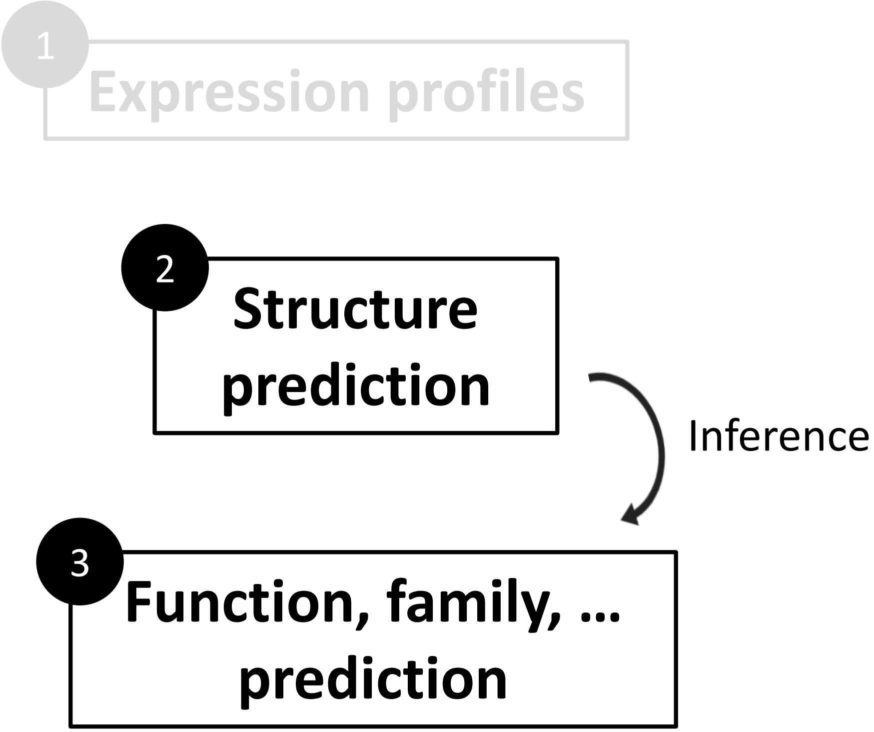

1 **Expression profiles**

2 **Structure prediction**

Inference

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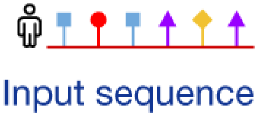
Over half of the effector structures of *L. maculans* are predicted with good confidence

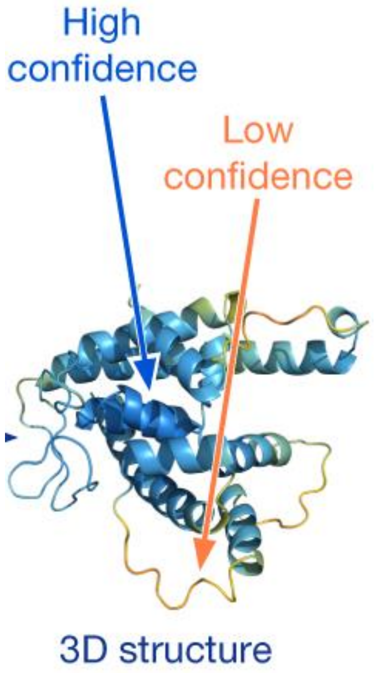
Method: annotation from **v2 of *L. maculans* genome**
Structural prediction: ColabFold v1.5.2-patch:
AlphaFold2 using MMseq2
(Default parameters, all mature sequence, date: 2023)



Alphafold

Model Confidence ⓘ

- 🟦 Very high (pLDDT > 90)
- 🟦 High (90 > pLDDT > 70)
- 🟨 Low (70 > pLDDT > 50)
- 🟧 Very low (pLDDT < 50)



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(Default parameters, all mature sequence, date: 2023)

276 structure predicted

147

High confidence scores (pLDDT>70)

76

Average confidence scores (70<pLDDT>50)

53

Poor confidence scores (pLDDT<50)

Over half of the effector structures of *L. maculans* are predicted with good confidence

Method: annotation from **v2 of *L. maculans* genome**
Structural prediction: ColabFold v1.5.2-patch:
AlphaFold2 using MMseq2
(Default parameters, all mature sequence, date: 2023)

Method: Functional inference based on
the modeled structure: Foldseek
(date: 2023)

276 structure predicted

Inference →

157 structures assigned to structural analog(s)

No difference between waves of expression
Pearson's Chi-squared test
X-squared = 4.8887, df = 7, p-value = 0.6735

147

High confidence scores (pLDDT>70)

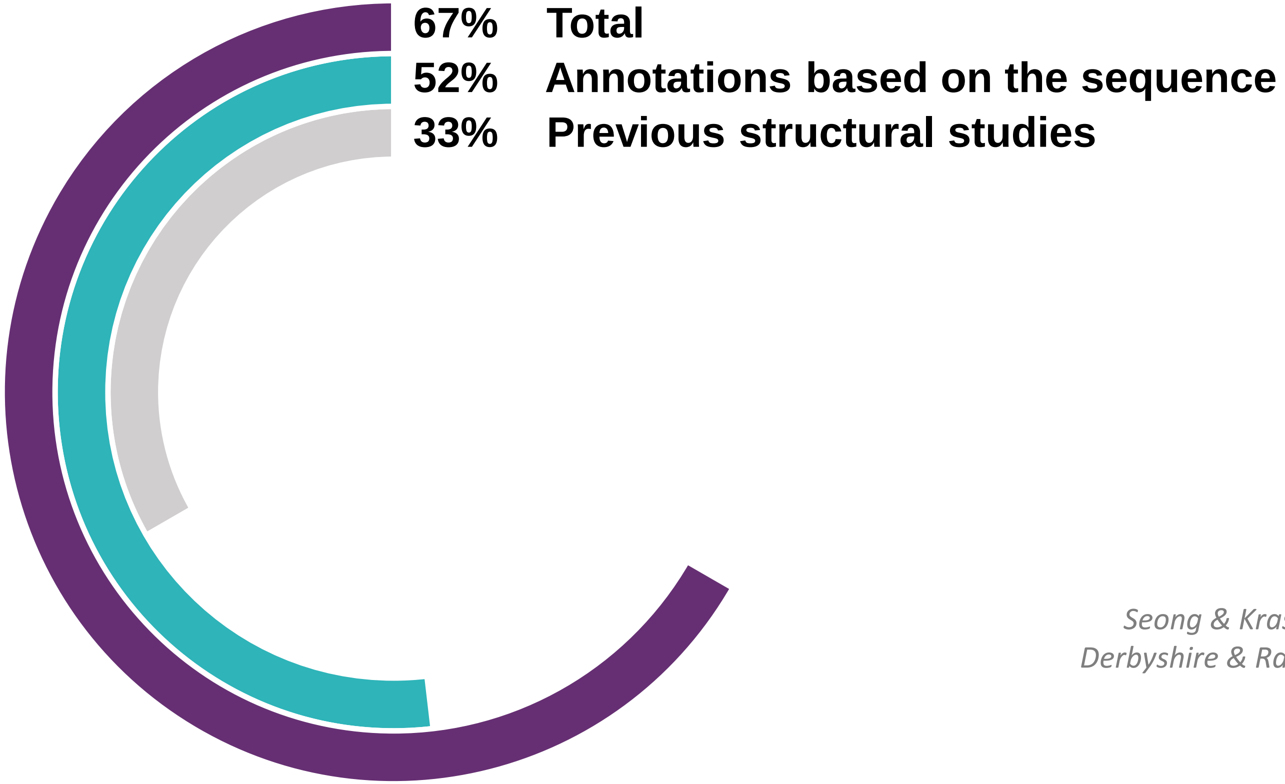
76

Average confidence scores (70<pLDDT>50)

53

Poor confidence scores (pLDDT<50)

The manual and individual investigation of each protein structure provides new information



67% Total
52% Annotations based on the sequence
33% Previous structural studies

Seong & Krasileva, 2023 Nature Microbiol.
Derbyshire & Raffaele, 2023 Nature Commun.

Explanations:

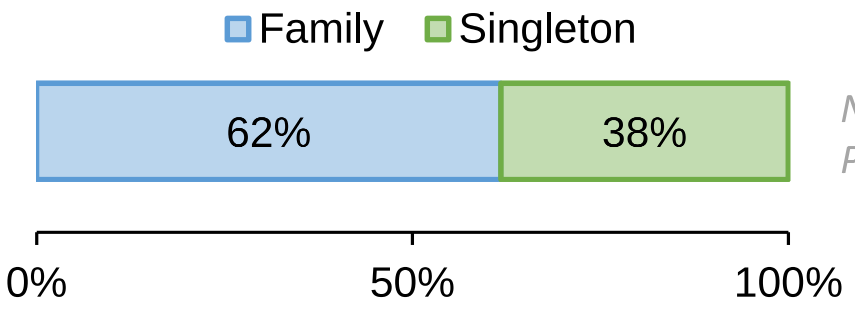
- 20% of effectors missing from v1 genome annotations
- Reduce the analysis set to give more individual consideration to each case
- 3D structures are more informative than protein sequences

Many questions can be raised …

Do *Leptosphaeria maculans* effectors belong to a limited set of structural families?

Is the LARS structural family specific to leaf infection?

The KP6 and LARS families are the most represented in *L. maculans*

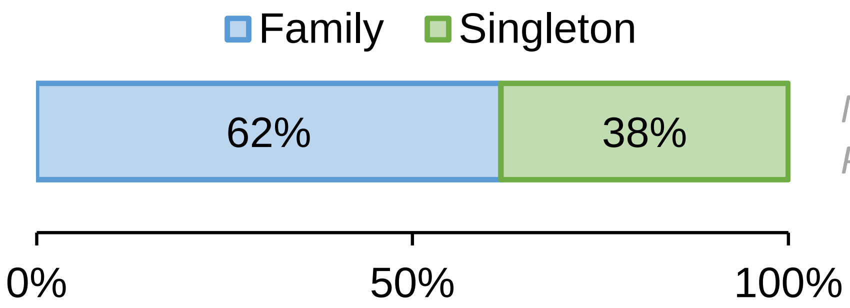


No difference between waves of expression
Pearson's Chi-squared test; ***p-value = 0.3121***

25 structural families (≥2 members)

Sequence identity = 22,42% (min=11%; max=57,8%)

The KP6 and LARS families are the most represented in *L. maculans*



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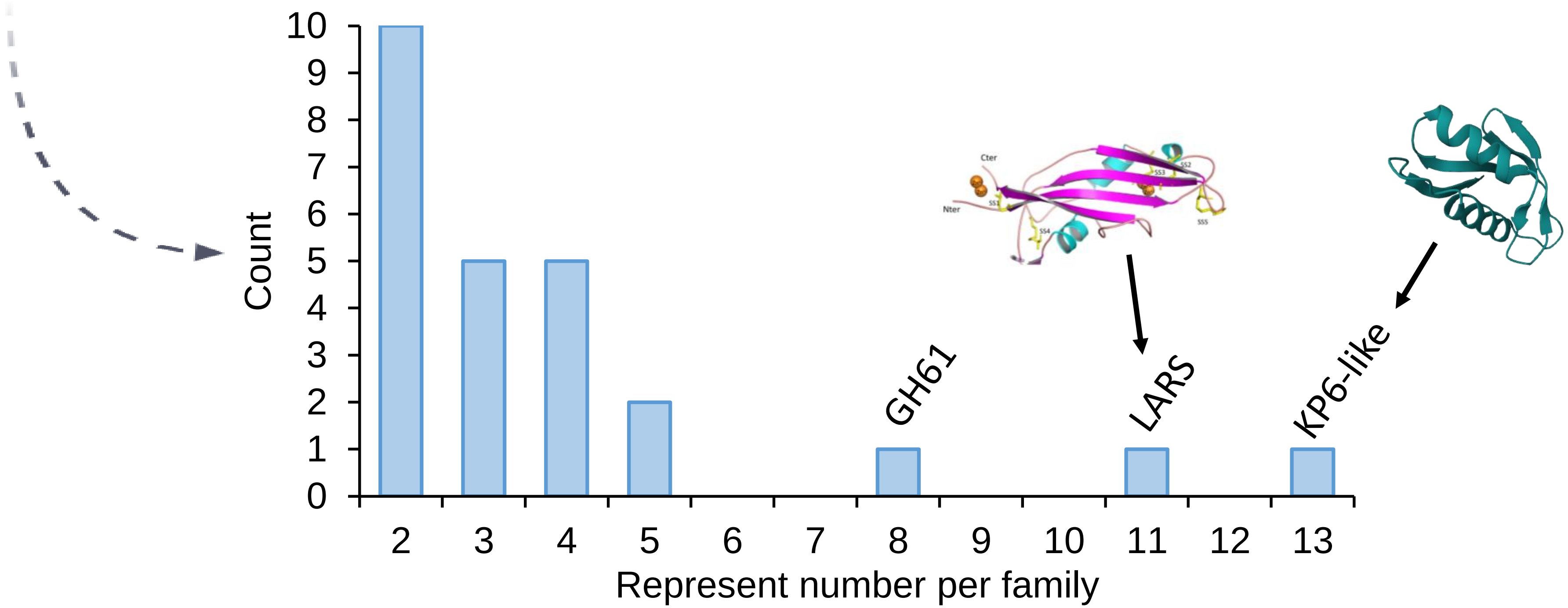
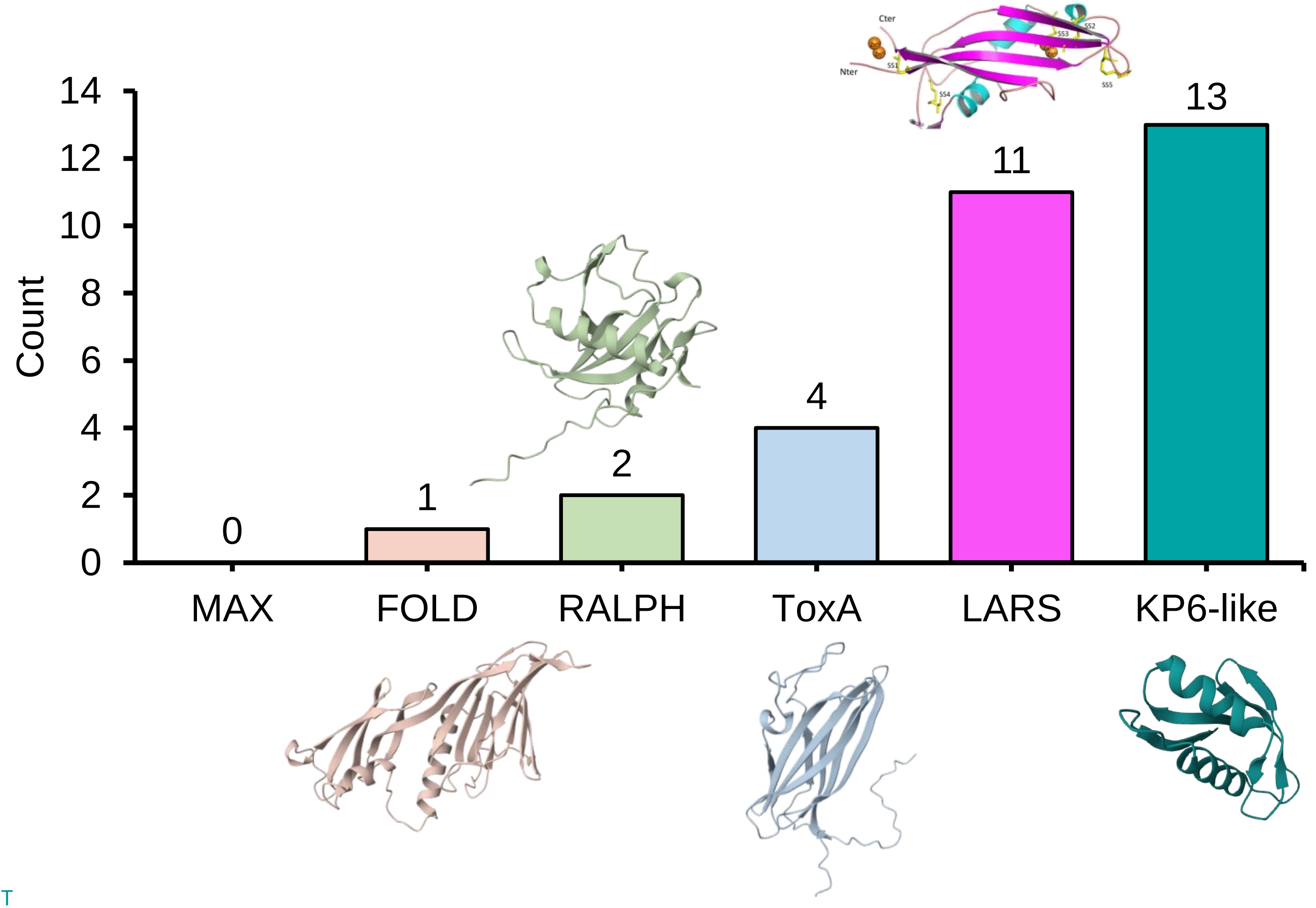


L. maculans secretes a number of structural families common to other plant pathogens



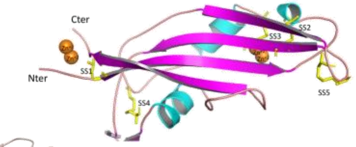
AvrLm proteins belong to a handful of structural families

AvrLm1-Lep3

Tox3

AvrLm2

FOLD



AvrLm3
AvrLm4-7
AvrLm5-9
AvrLmS-Lep2

LARS

AvrLm6
AvrLm10A

KP6-like

AvrLm10B

ToxA

AvrLm11
AvrLm14

Unknown

Rocafort et al. 2022, BMC Biol.
Talbi et al. 2023, Mol. Plant Pathol.
Lazar et al. 2022, PLoS Pathog.

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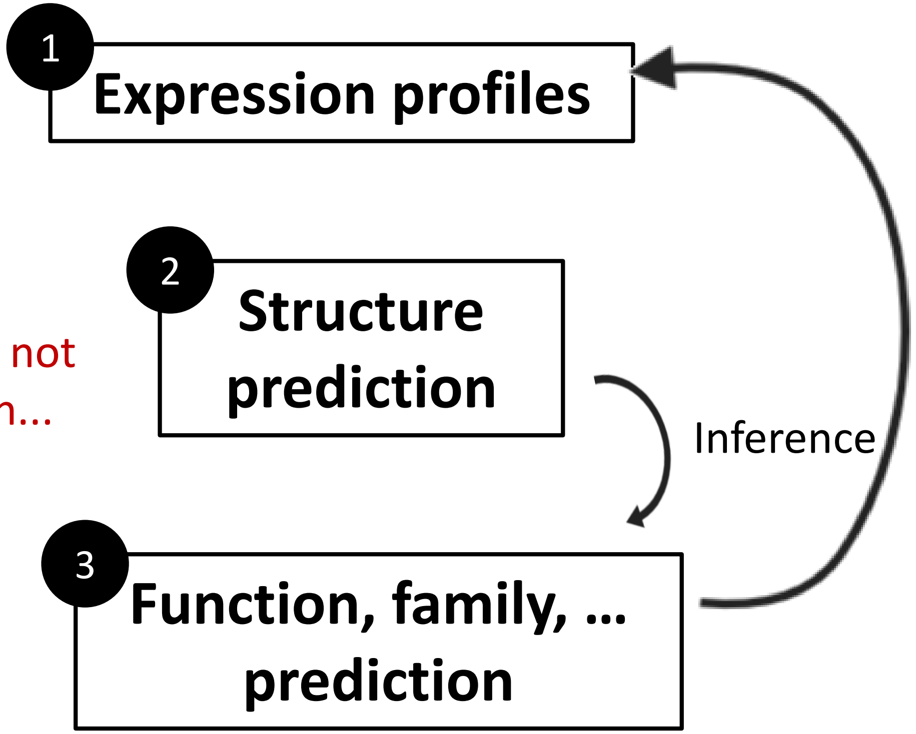
Similarity of structure does not imply similarity of function...



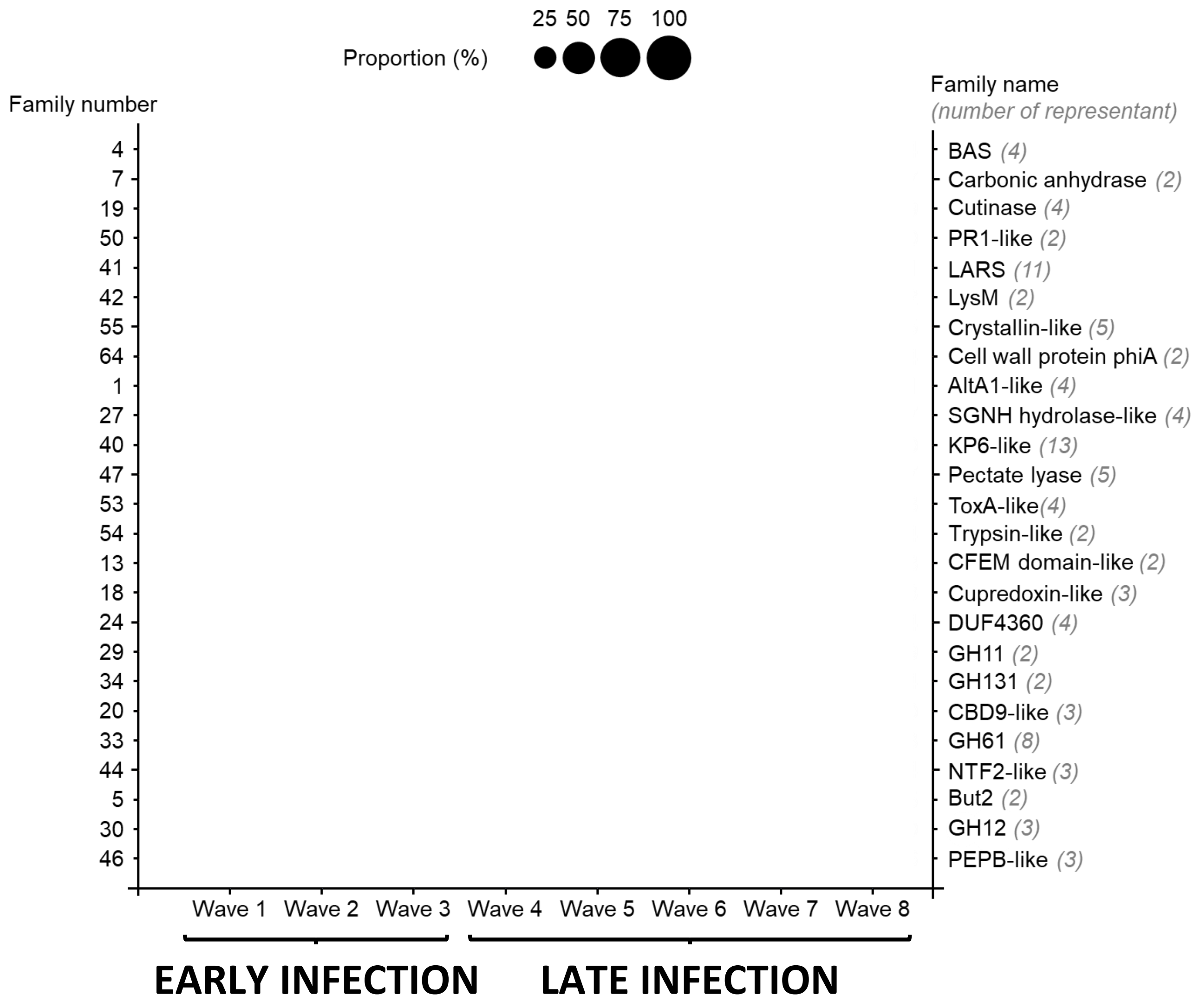
1 **Expression profiles**

2 **Structure prediction**

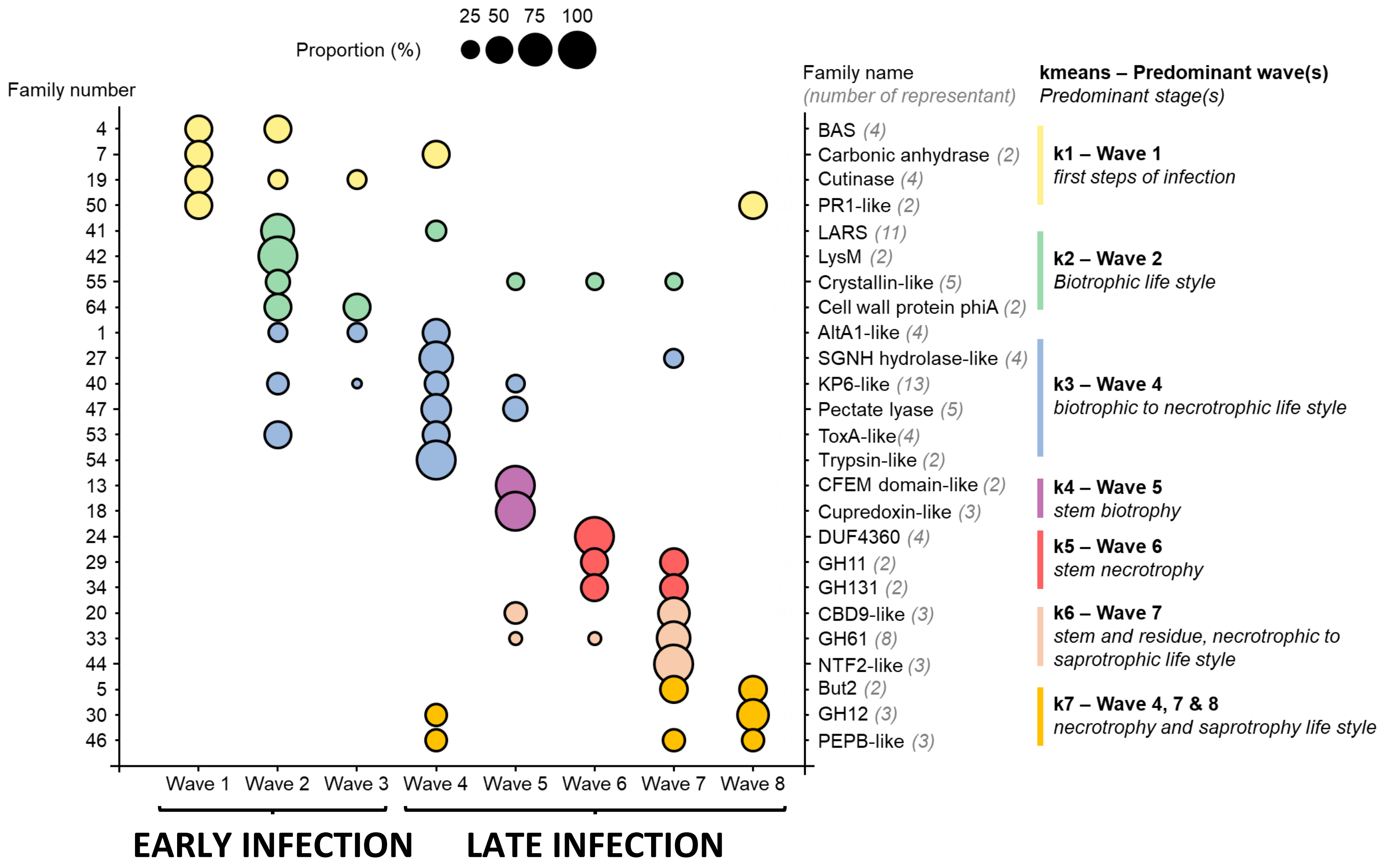
3 **Function, family, … prediction**

Inference

A correlation emerges between putative function and wave of expression



A correlation emerges between putative function and wave of expression



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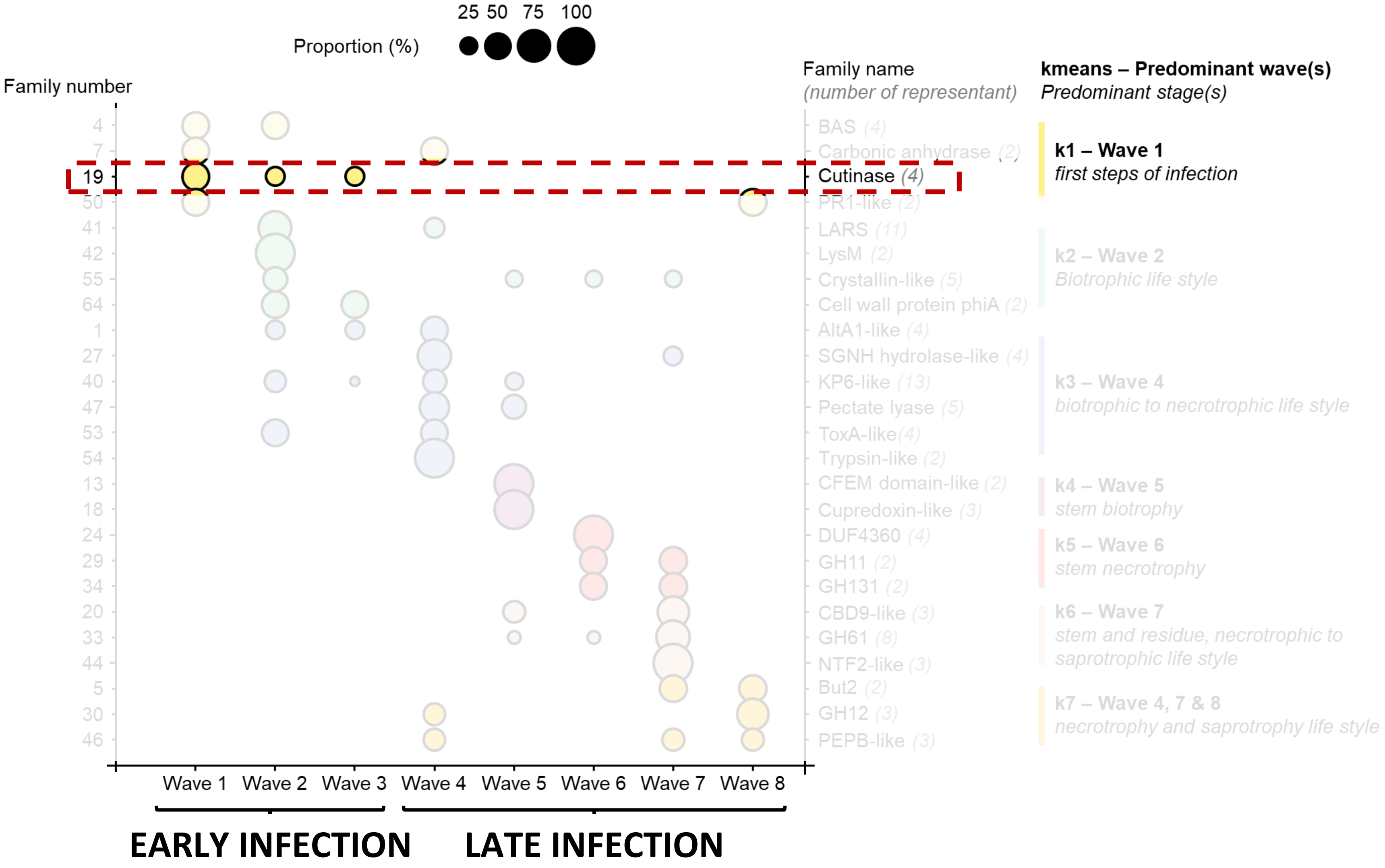

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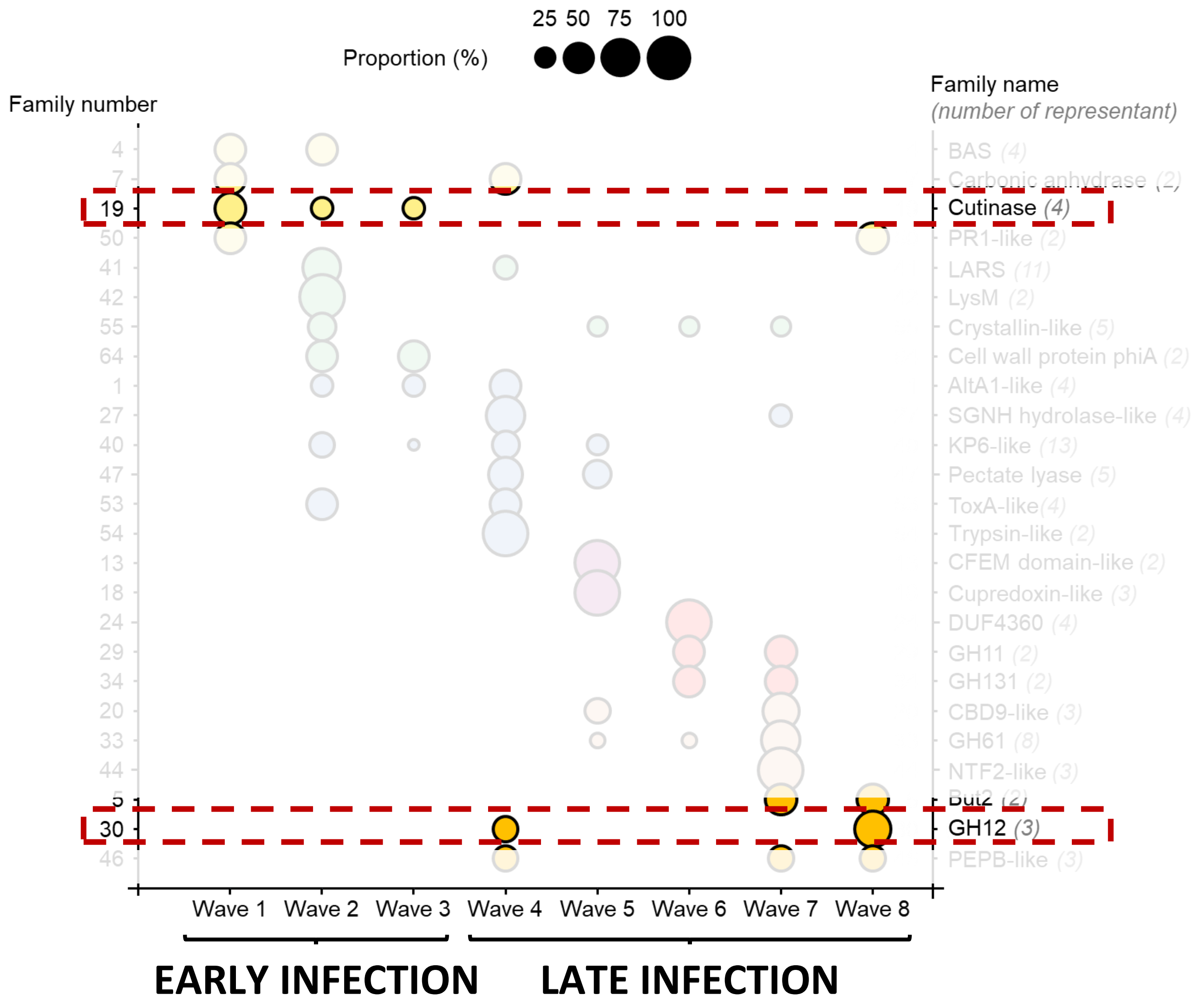


kmeans – Predominant wave(s)
Predominant stage(s)

k1 – Wave 1
first steps of infection

k2 – Wave 2
Biotrophic life style

k3 – Wave 4
biotrophic to necrotrophic life style

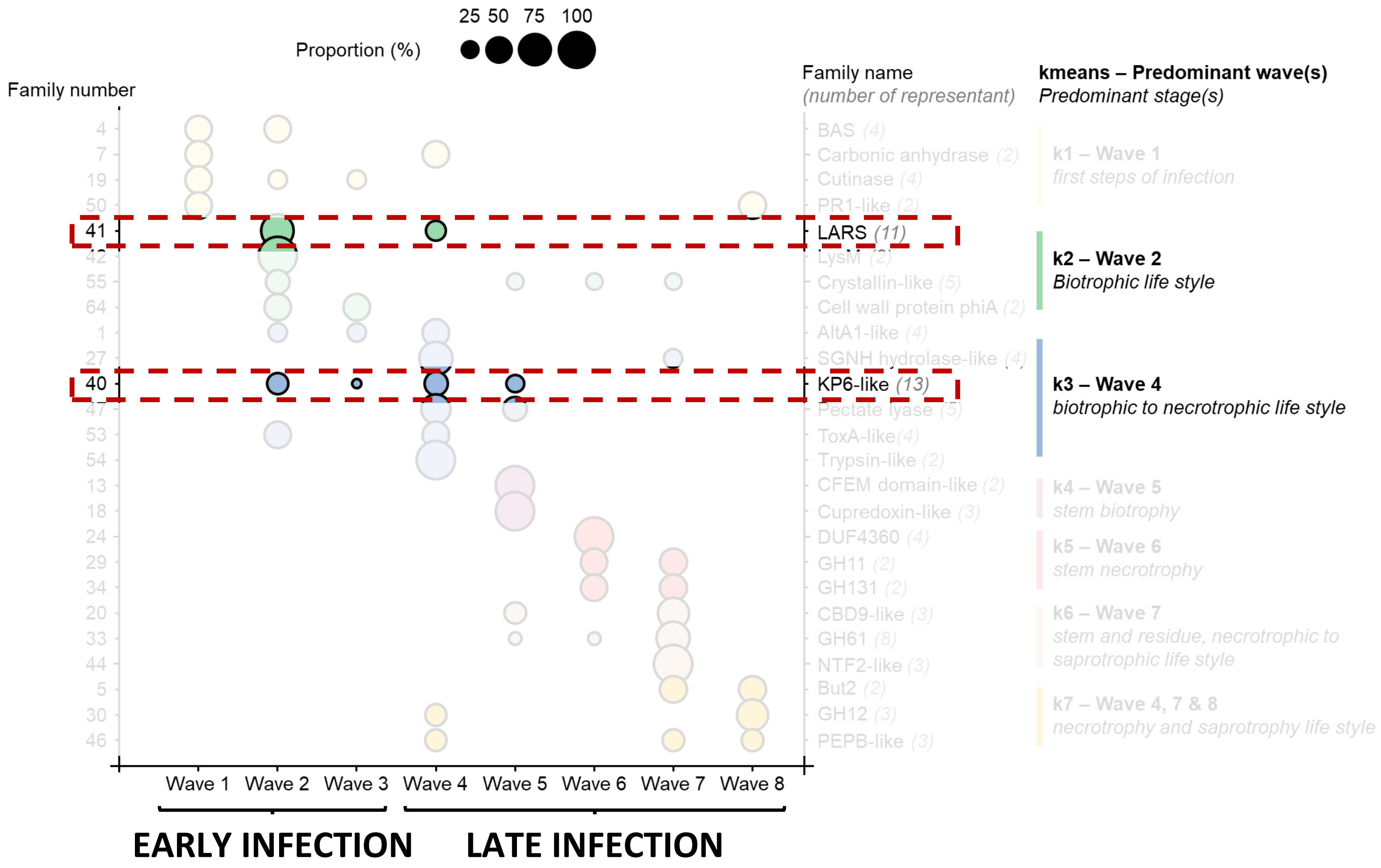
k4 – Wave 5
stem biotrophy

k5 – Wave 6
stem necrotrophy

k6 – Wave 7
stem and residue, necrotrophic to saprotrophic life style

k7 – Wave 4, 7 & 8
necrotrophy and saprotrophy life style

A correlation emerges between putative function and wave of expression



Take-home message

Structural analyses are no substitute for functional analyses

Example:

KP6-like

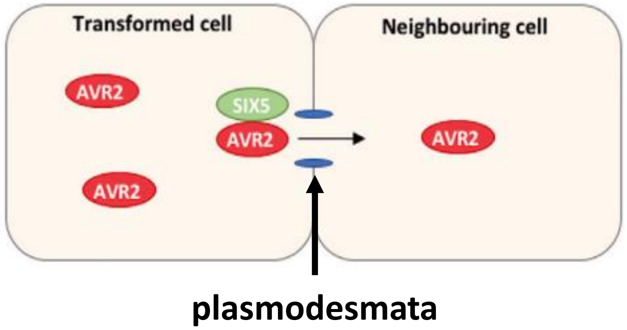
Toxic & antifungal properties

- KP6 protein of the P6 *Ustilago maydis* virus *(Koltin & Day 1975, Appl. Microbiol.)*
- Zt-KP6-1 protein of *Zymoseptoria tritici* *(cf. Lebrun MH & De guillen K communications)*

Cf. Marc-Henri Lebrun
Session 8, friday

Cell-to-cell 'transporters'

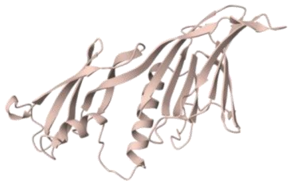
- SIX5-AVR2 pair of *Fusarium oxysporum* f. sp. *lycopersici* *(Ma et al. 2015, New Phytol.; Cao et al. 2018, Mol. Plant)*

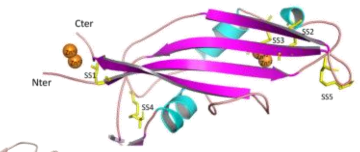


Cf. Isabelle Fudal
Session 8, friday

Take-home message

R proteins recognize a handful of structural families





AvrLm1-Lep3	Tox3	Rlm1-LepR3
AvrLm2	FOLD	Rlm2
AvrLm3 AvrLm4-7 AvrLm5-9 AvrLmS-Lep2	LARS	Rlm3 Rlm4 Rlm7 Rlm5 Rlm9 RlmS
AvrLm6 AvrLm10A	KP6-like	Rlm6 Rlm10
AvrLm10B	ToxA	Rlm10
AvrLm11 AvrLm14	Unknown	Rlm11 Rlm14

Rocafort et al. 2022, BMC Biol.
Talbi et al. 2023, Mol. Plant Pathol.
Lazar et al. 2022, PLoS Pathog.
Borhan et al. 2022, Annu. Rev. Phytopathol.

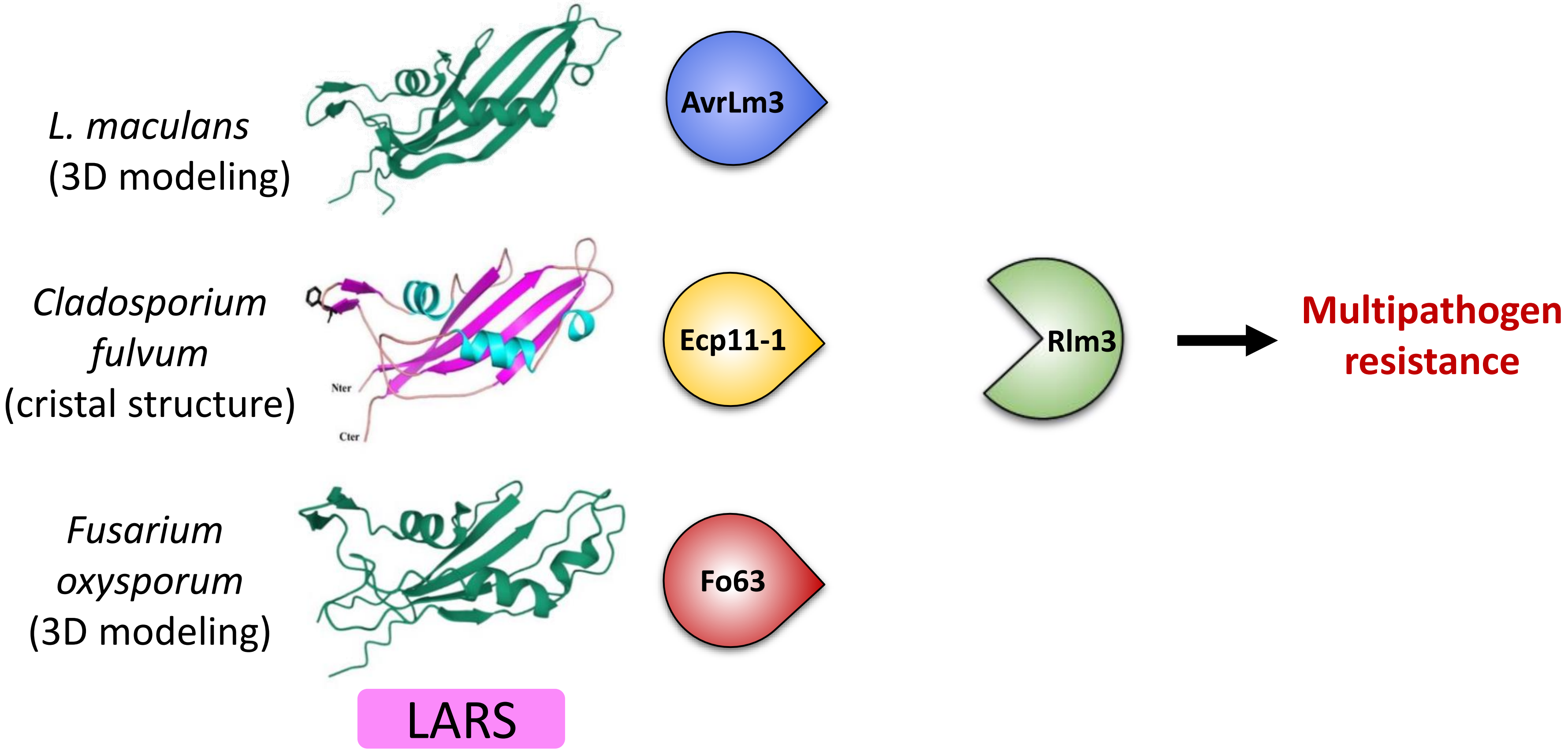
Take-home message

Can broad-spectrum resistances recognizing structural families be identified?

Proof of concept:

- Rlm3 is able to recognize AvrLm3, Ecp11-1 and Fo63 (LARS family)

  (Lazar et al. 2022, PLoS Pathog.; Talbi et al. in prep.)



L. maculans
(3D modeling)

Cladosporium fulvum
(cristal structure)

Fusarium oxysporum
(3D modeling)

Multipathogen resistance

Thanks for your attention

Isabelle Fudal

Herman
van Tilbeurgh

Inès
Li de la Sierra-Gallay