

**Methods for investigating quantitative resistance to
Leptosphaeria maculans (phoma stem canker) in *Brassica
napus* (oilseed rape) in controlled conditions**

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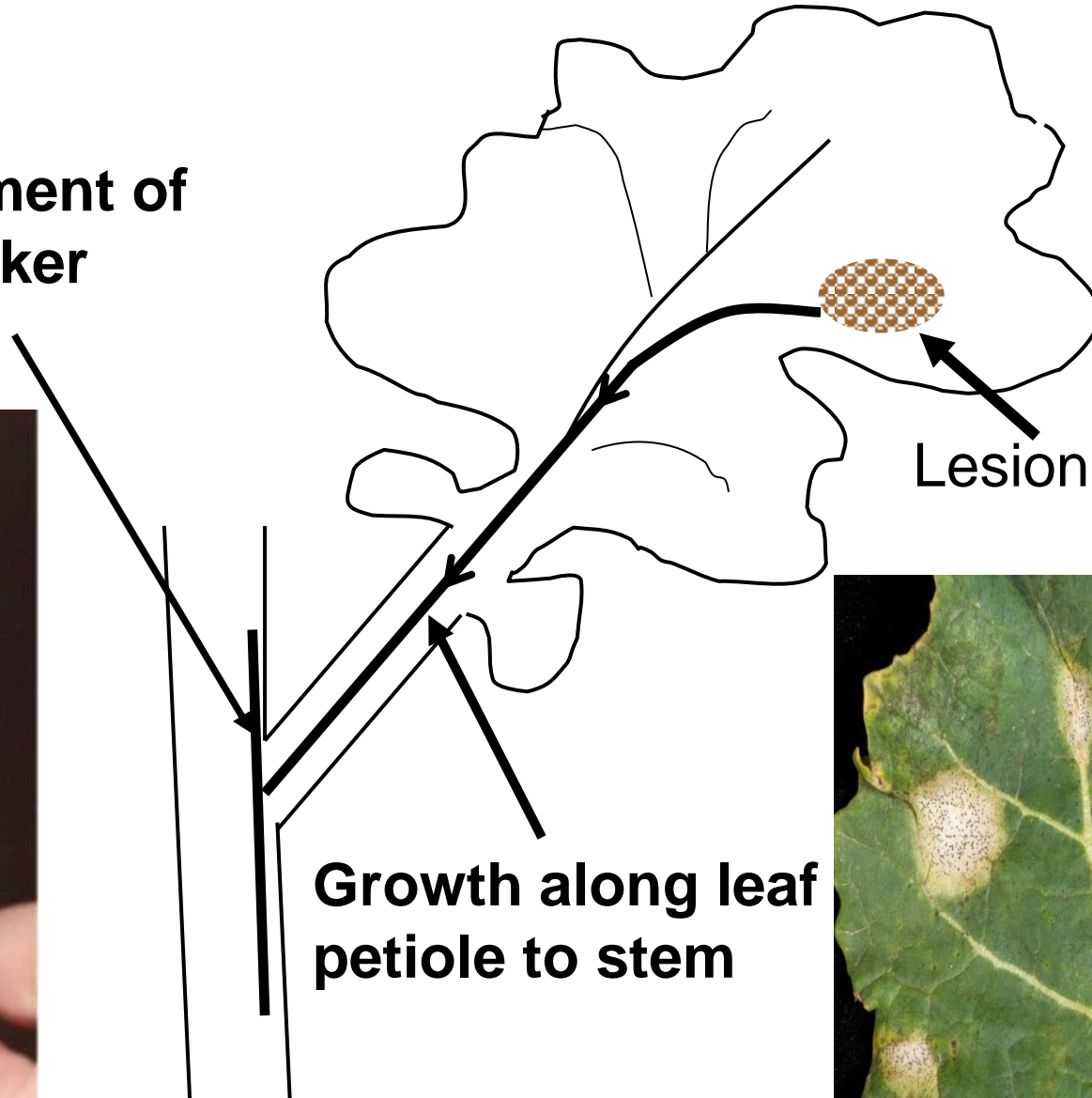
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Importance of the disease

- Stem canker is a major disease on winter oilseed rape in the UK, causing £80-160 M losses annually (£450/t)
- Global (Europe, Canada, Australia) crop losses >£1000M p.a.
- Main UK pathogen
Leptosphaeria maculans

Symptoms of phoma stem canker

Development of stem canker



Severe phoma leaf spot

can kill seedlings of susceptible cultivars



Severe phoma stem canker

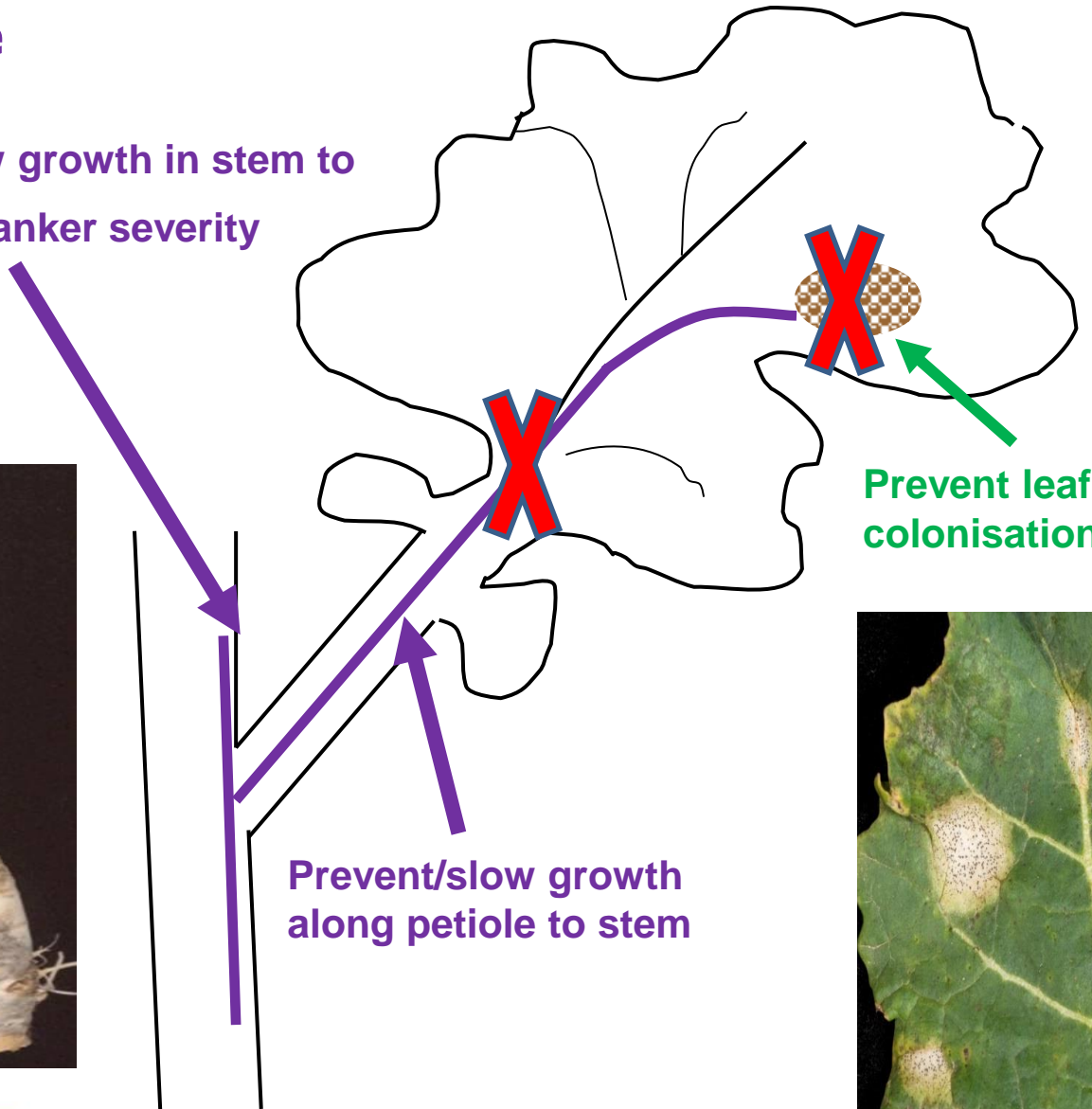
can cause up to 50% yield loss on susceptible cultivars



How to control the disease?

Quantitative resistance

Prevent/slow growth in stem to **reduce** canker severity



R gene-mediated resistance



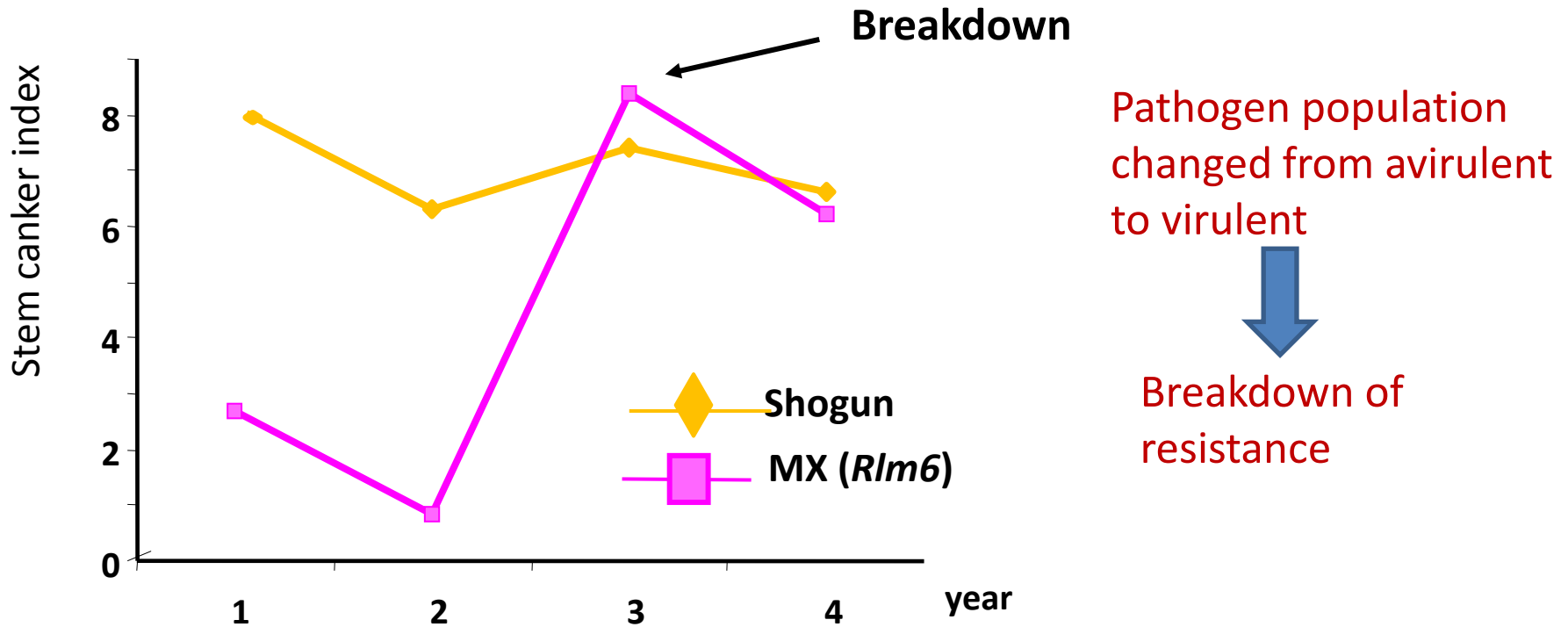
R gene resistance

- Complete resistance
- Easy to assess at seedling stage
- Race specific
- Resistance can be easily breakdown



<i>L. maculans</i> <i>B. napus</i>	<i>AvrLm1</i>	<i>avrLm1</i>
<i>Rlm1</i>	Resistant	Susceptible
<i>rlm1</i>	Susceptible	Susceptible

Example of breakdown of *R* gene-mediated resistance: field experiments in France



Temperature affects *R* gene resistance

Darmor

DarmorMX (*Rlm6*)

15°C



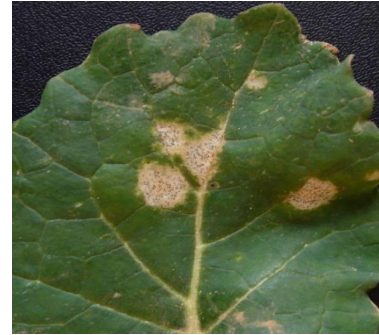
25°C



Quantitative resistance

- **Not race specific**
- **Partial resistance**
- **More durable**

With QR

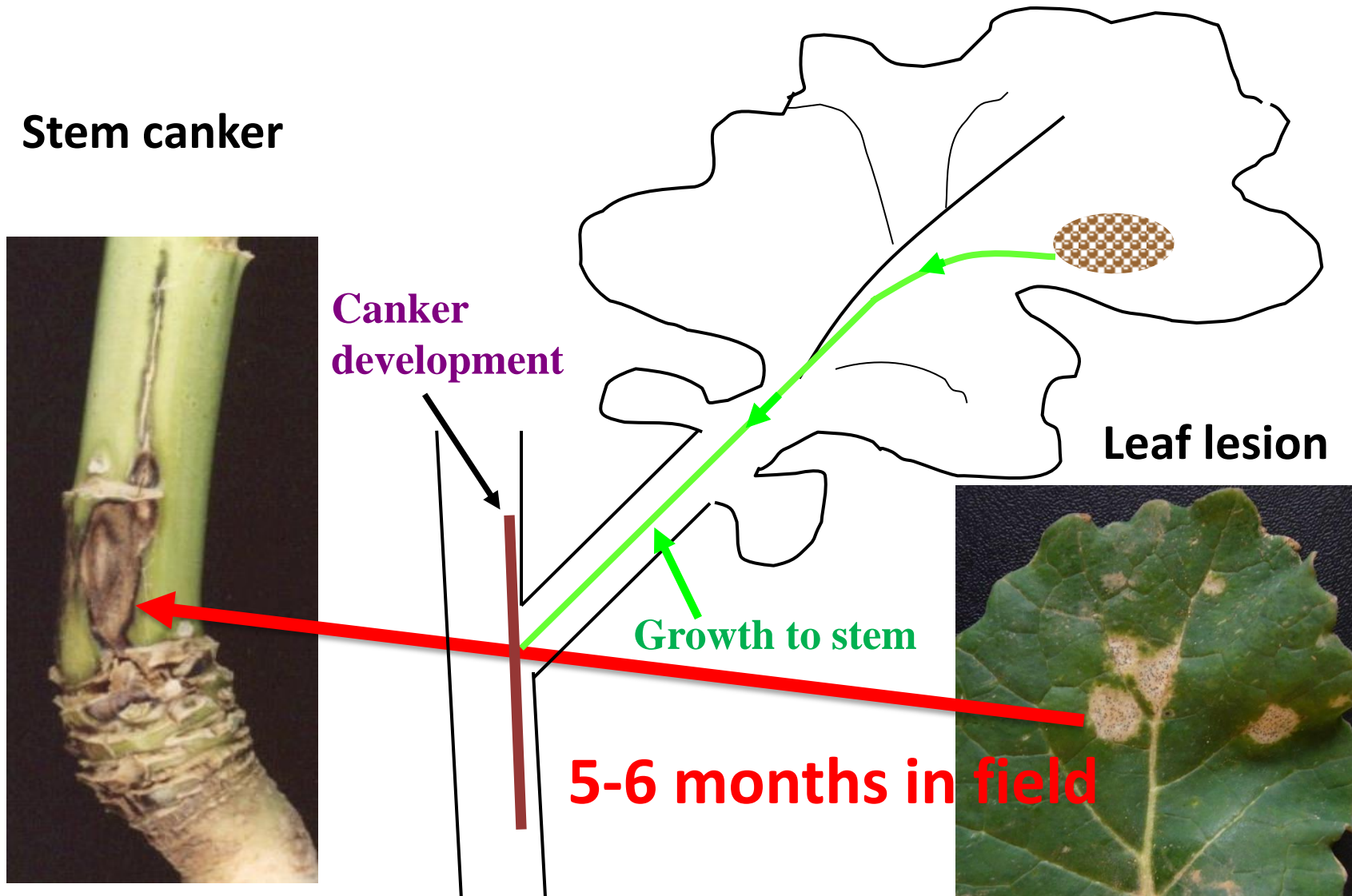


No QR



Difficult to assess quantitative resistance due to long period of symptomless growth

Stem canker



Canker development

Leaf lesion

Growth to stem

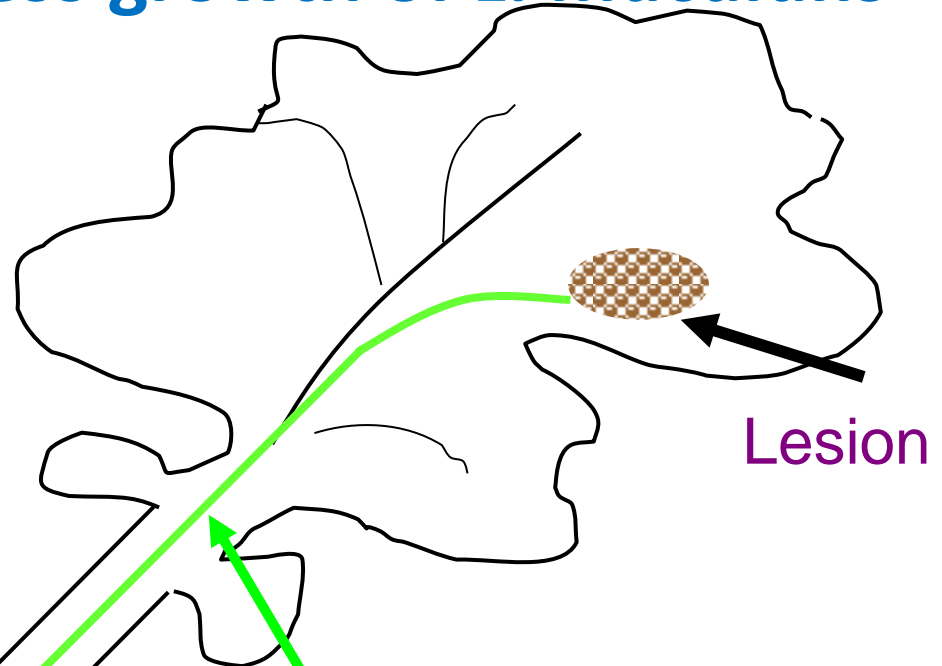
5-6 months in field

Question?

Is it possible to assess quantitative resistance in young plants in controlled environment?

Investigate symptomless growth of *L. maculans*

Stage 2: growth in stem to form stem canker



Stage 1: growth along leaf petiole to stem

Materials

DH lines: A30 (susceptible), C119 (good quantitative resistance)

DH lines from Darmor-*bzh* × Yudal (INRA, Rennes)

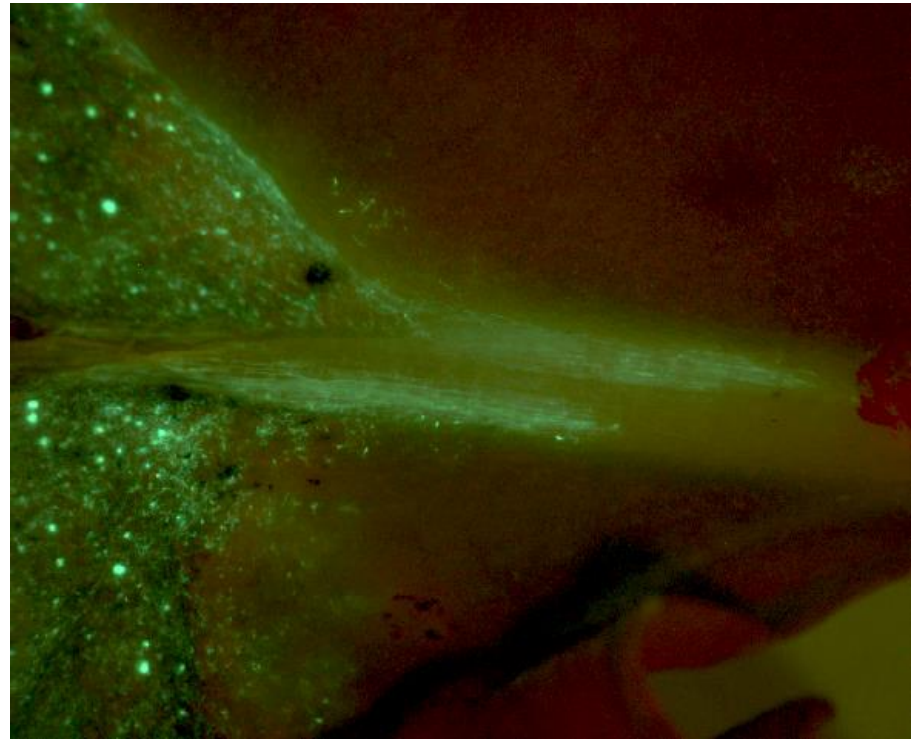
Growth stage 1: growth along leaf petiole to stem



First two leaves were inoculated with ascospores or conidia

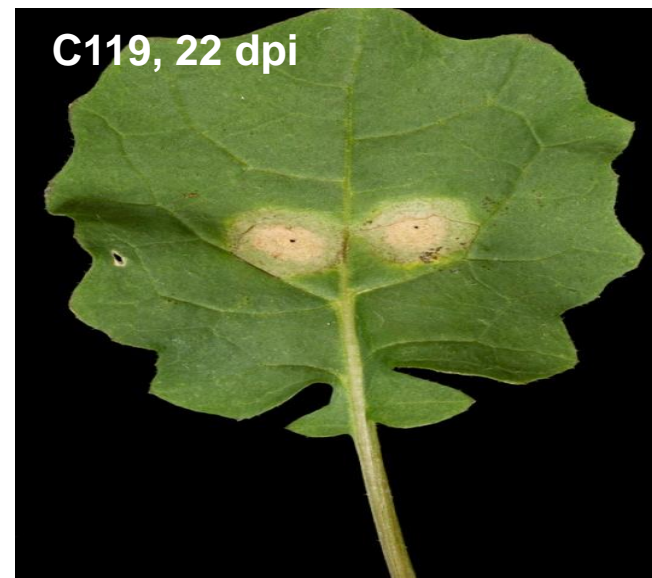
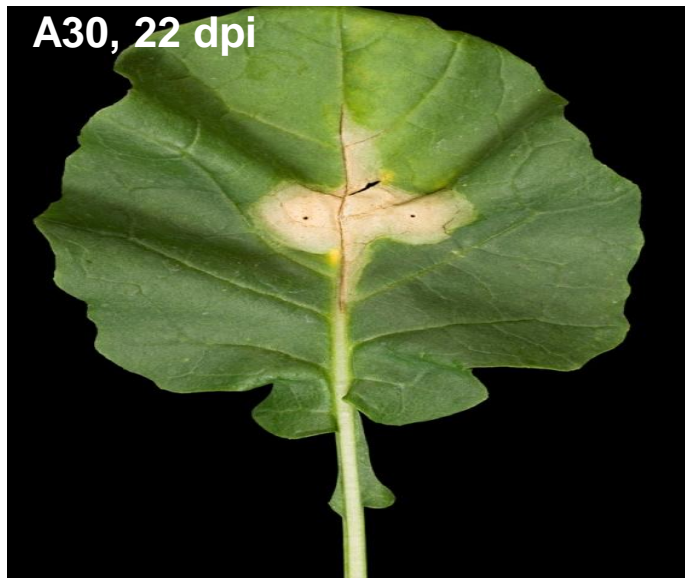
**Growth of *L. maculans* measured by
(1) Green fluorescent protein (GFP)
(2) DNA of *L. maculans* using qPCR**

Symptomless growth along leaf petiole



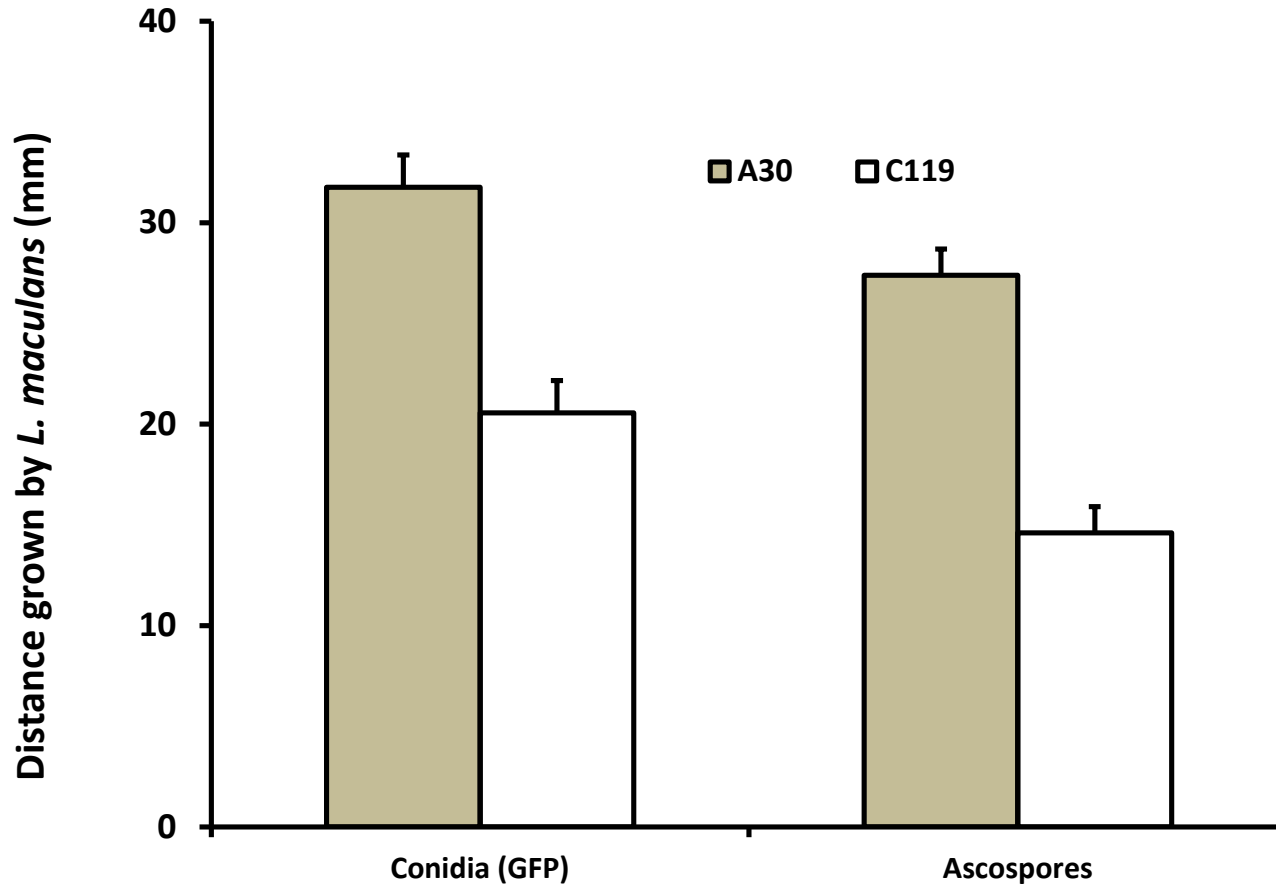
Huang *et al.*, 2014, *PLOS ONE*

Leaf lesion development in controlled environment



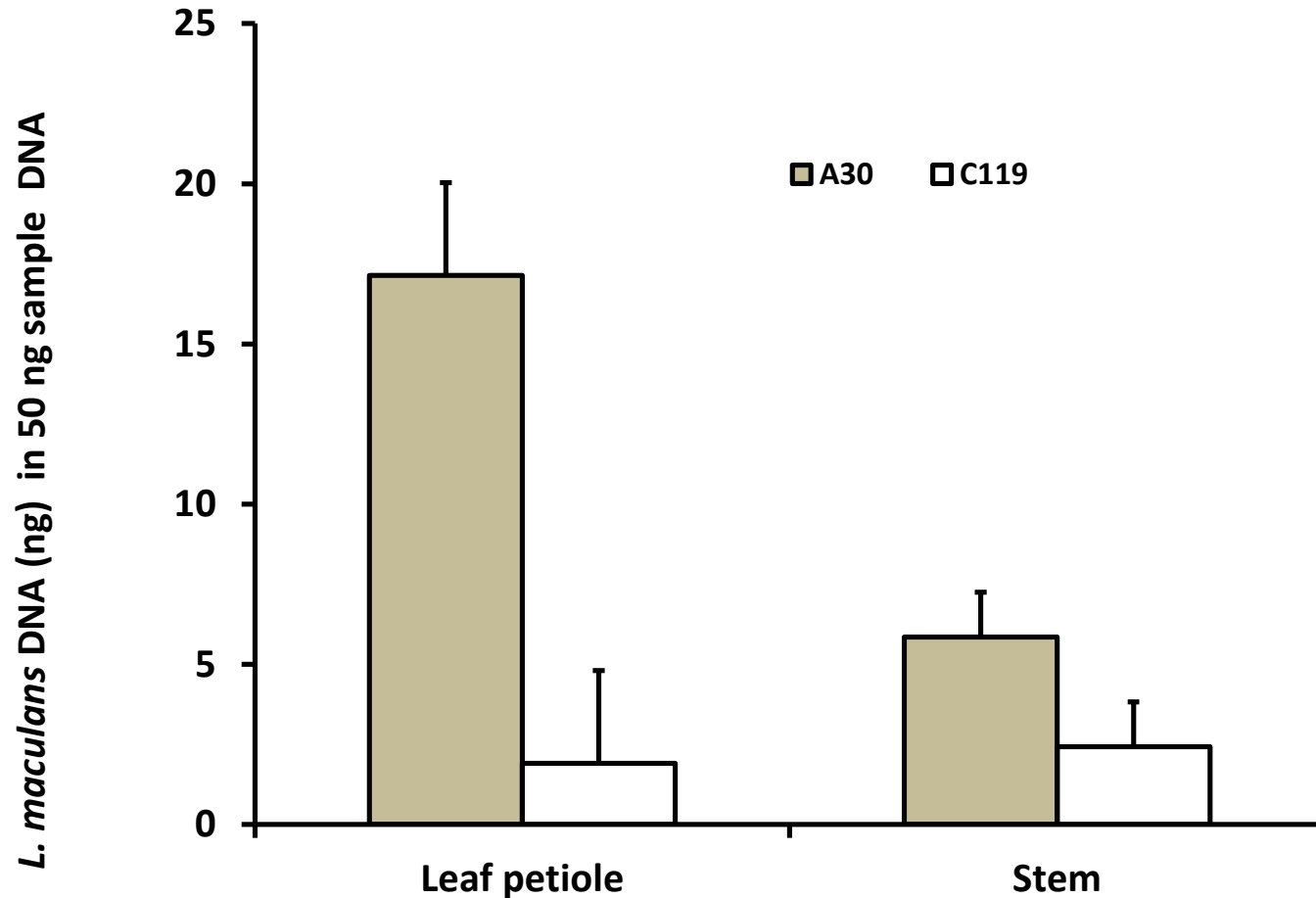
DH lines: A30 (susceptible), C119 (good quantitative resistance)

Growth of *L. maculans* along leaf petiole



DH lines: A30 (susceptible), C119 (good quantitative resistance)

L. maculans DNA in leaf petiole



DH lines: A30 (susceptible), C119 (good quantitative resistance)

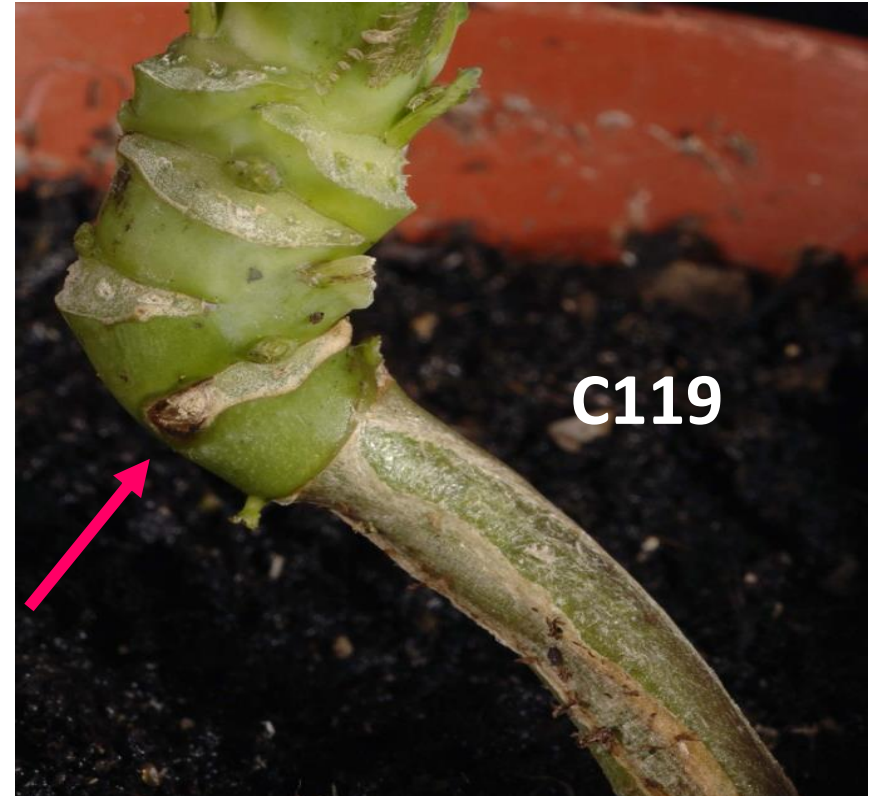
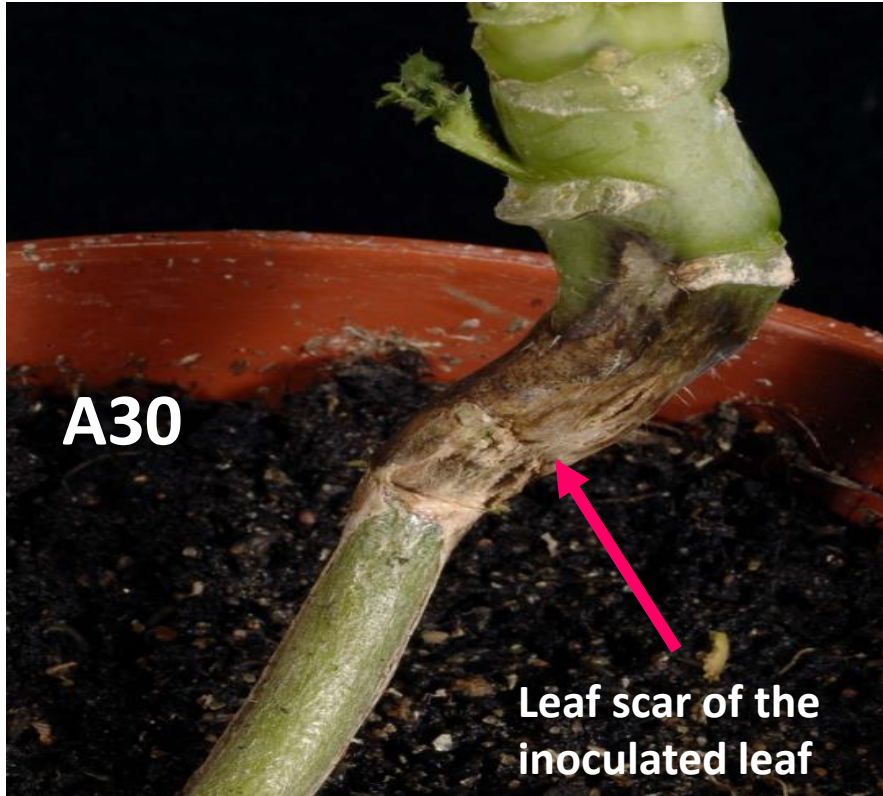
Growth stage 2: growth in stem to form stem canker

Petioles were wounded and inoculated with ascospores or GFP conidia



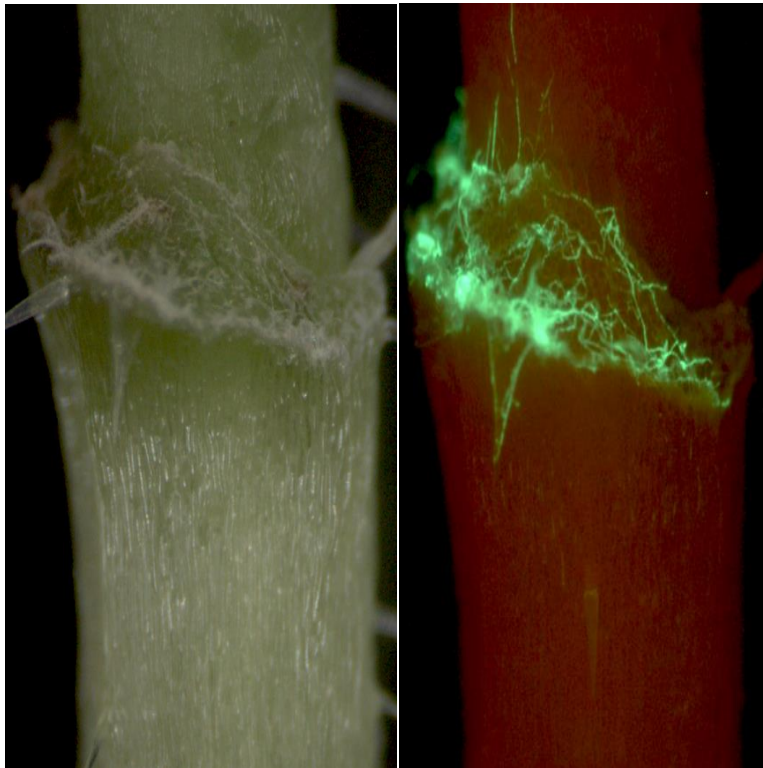
- Growth of *L. maculans* measured by
- (1) GFP (visualise distribution in stem)
 - (2) Stem canker severity score
 - (3) DNA of *L. maculans* using qPCR

Stem canker developed after ascospore inoculation

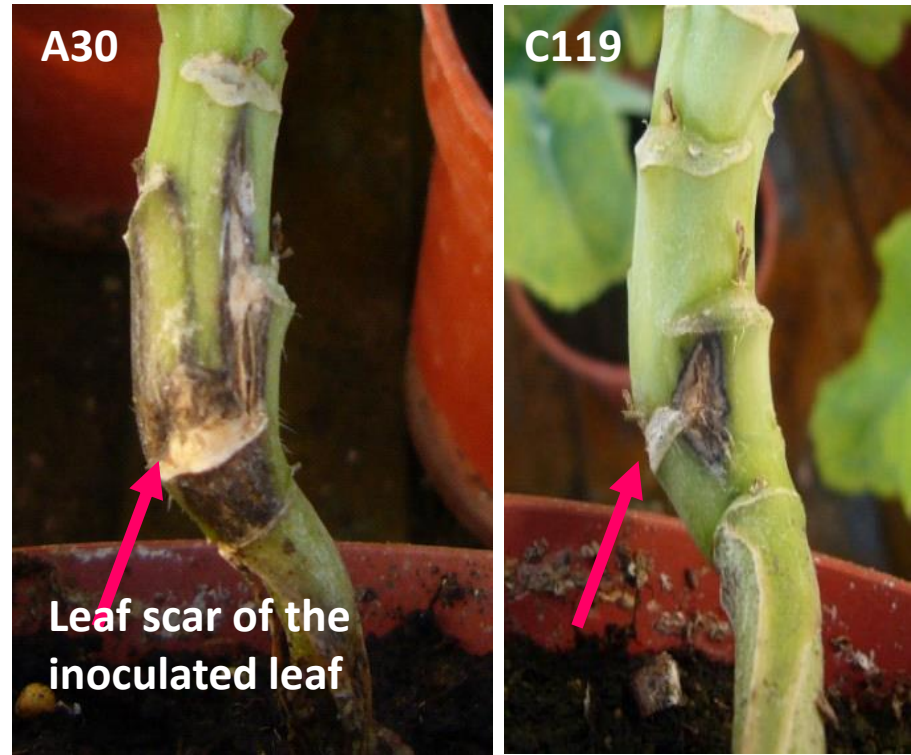


DH lines: A30 (susceptible), C119 (good quantitative resistance)

Stem canker developed with conidial inoculation

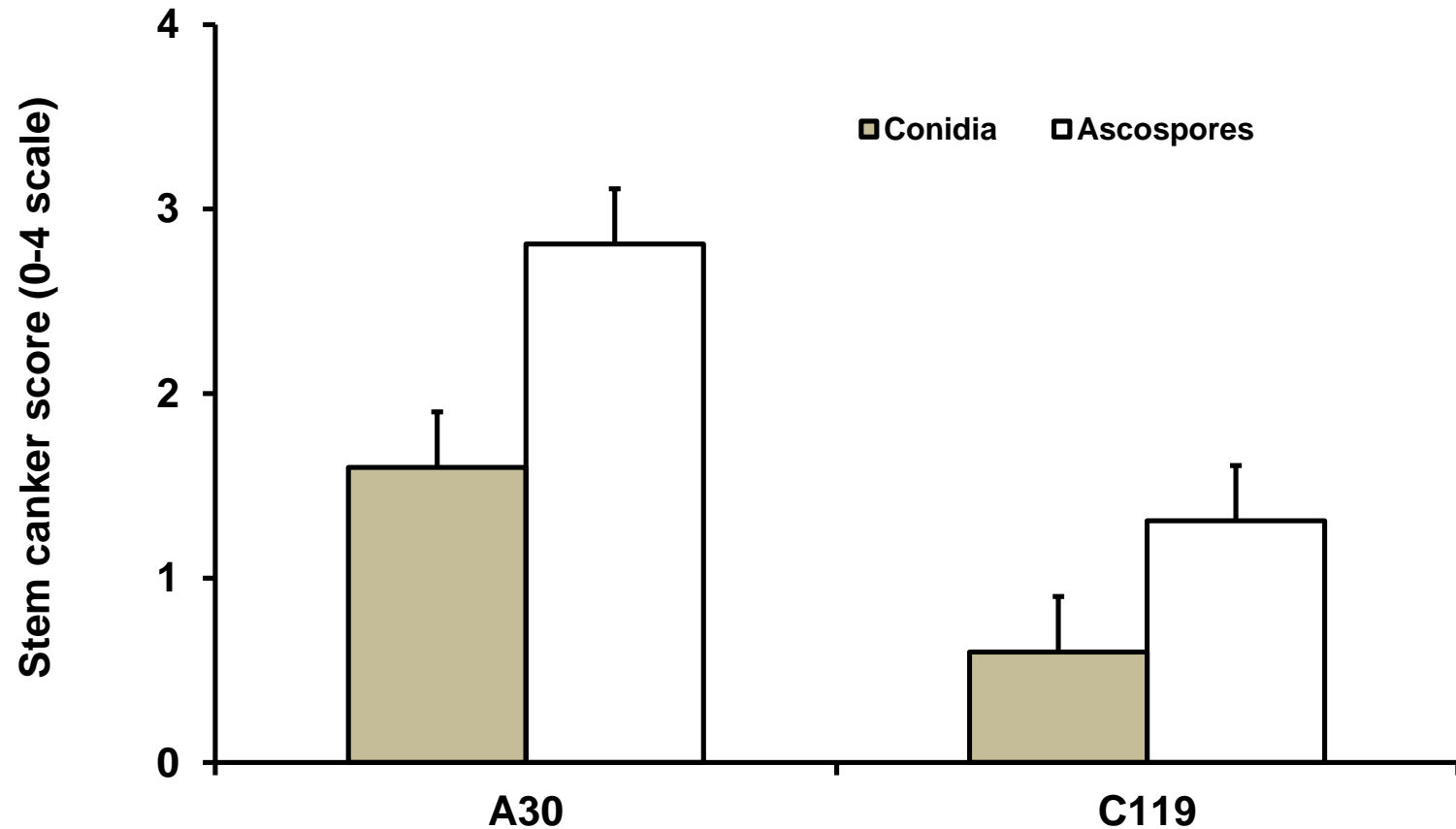


25 dpi



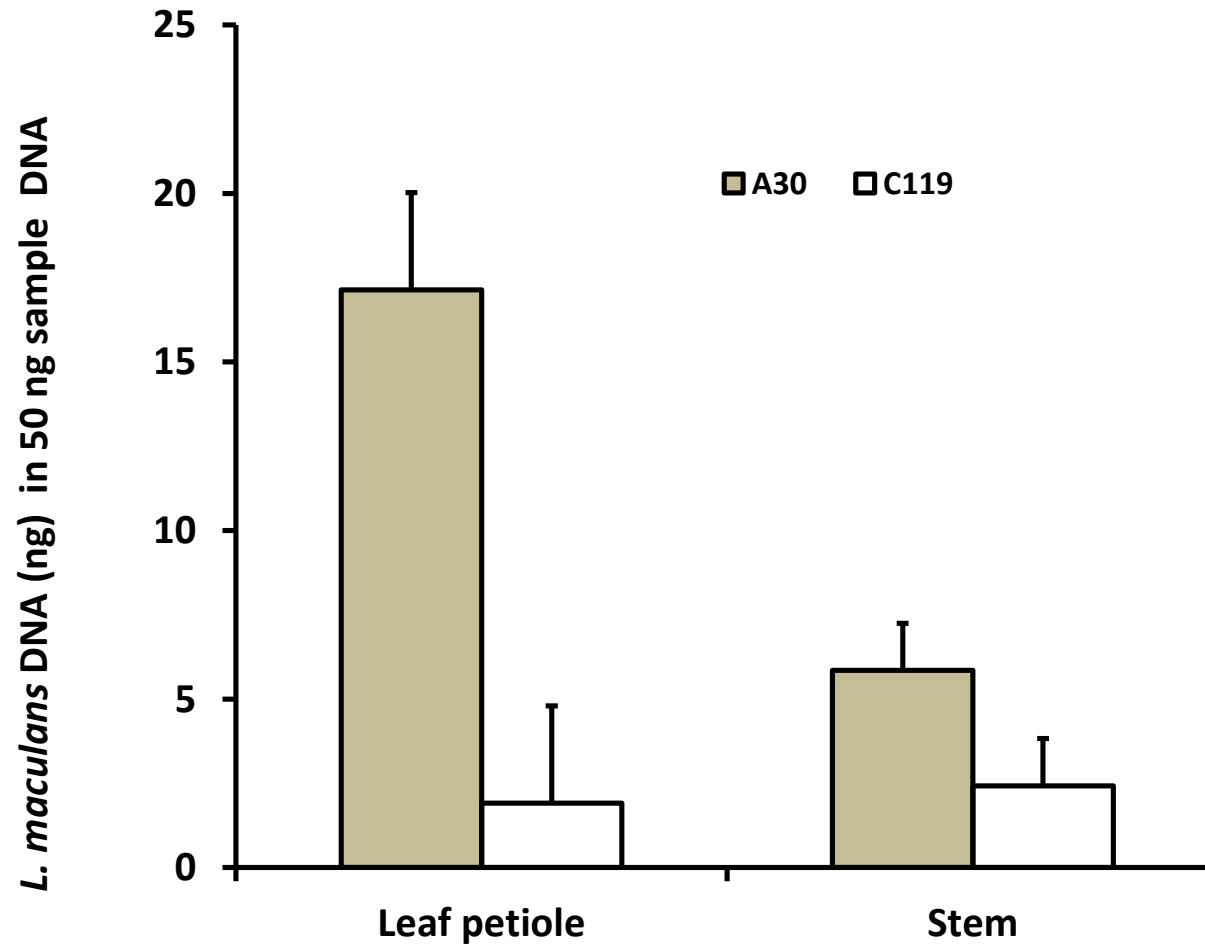
87 dpi

Severity of stem canker



DH lines: A30 (susceptible), C119 (good quantitative resistance)

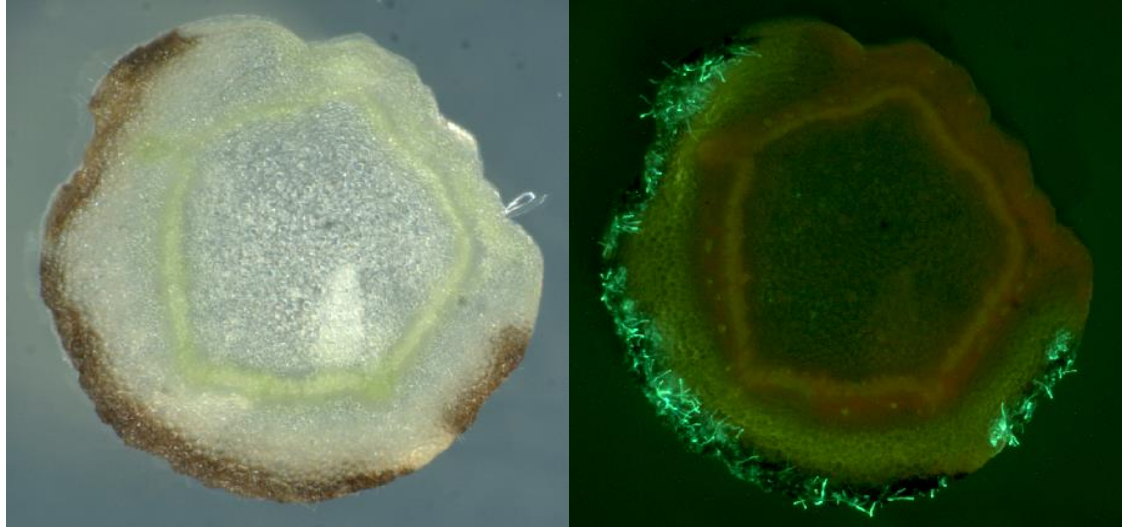
L. maculans DNA in leaf petiole



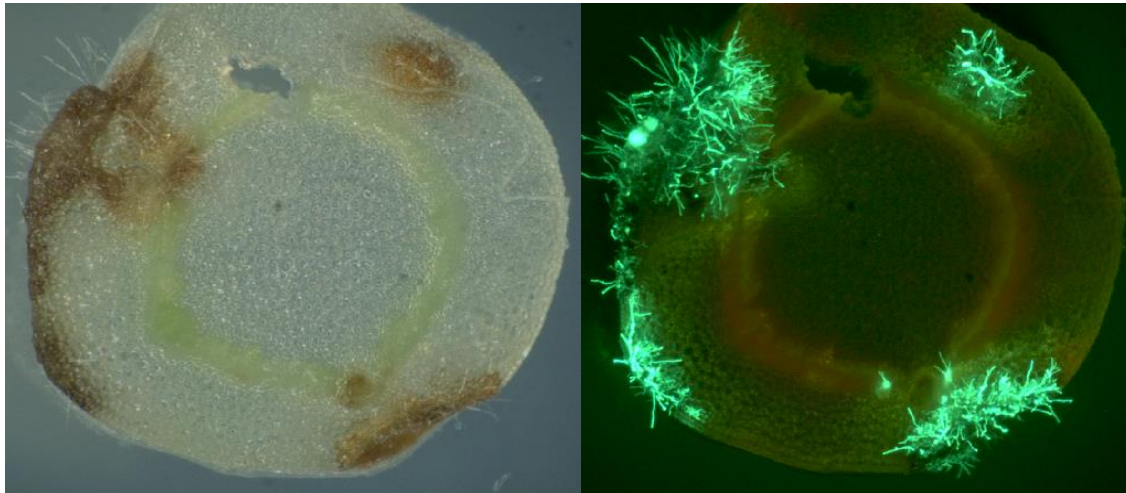
DH lines: A30 (susceptible), C119 (good quantitative resistance)

Quantitative resistance reduced growth of *L. maculans* from stem cortex to pith

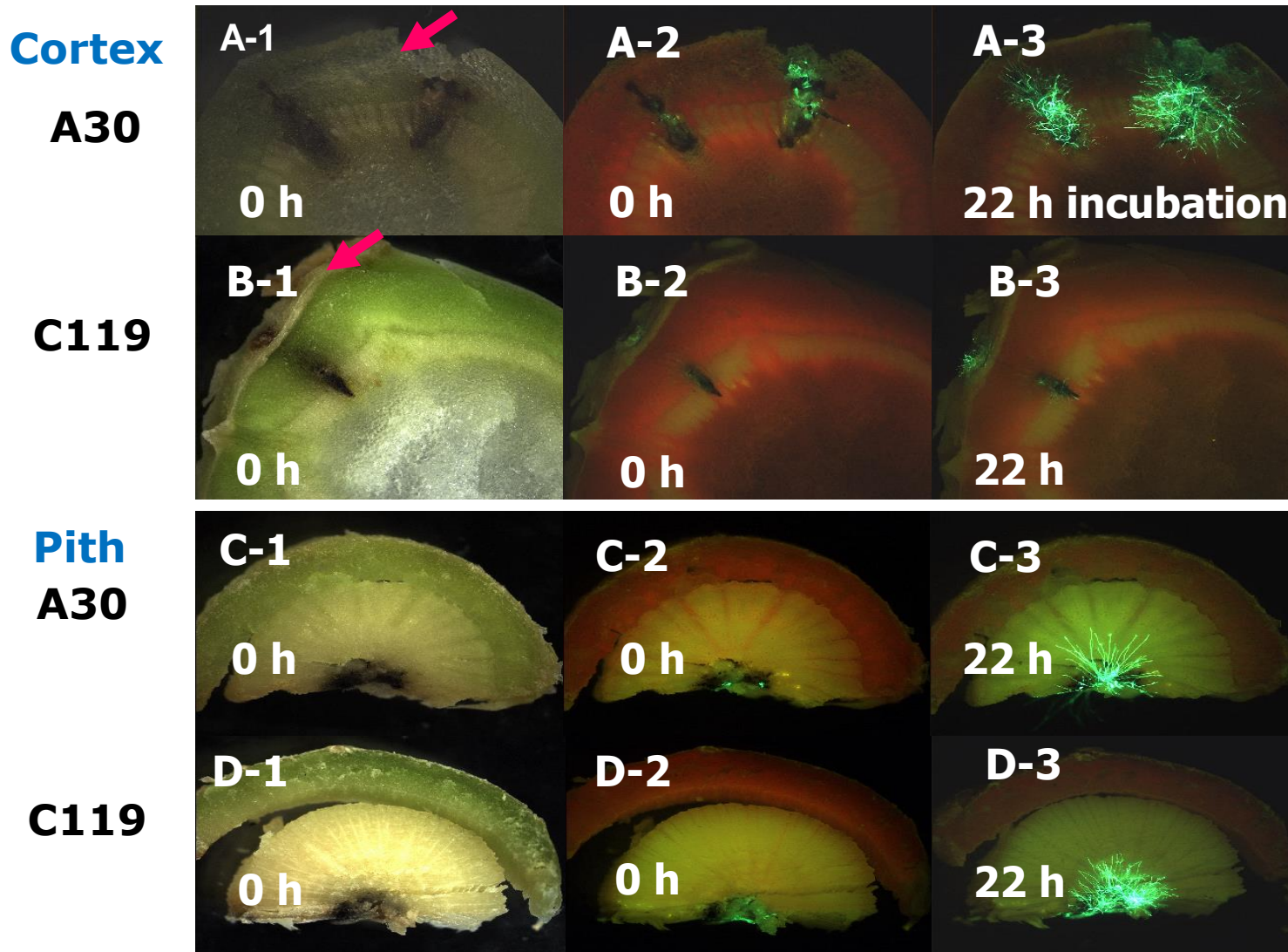
**Darmor
(with QR)**



**Eurol
(no QR)**



Growth of *L. maculans* in stem cortex and pith



Summary

Quantitative resistance to *L. maculans* can be assessed in young plants by leaf inoculation and petiole inoculation

- Leaf inoculation : leaf lesion size, growth distance and qPCR
- Petiole inoculation: stem canker severity score and qPCR
- QR reduces stem canker severity by impeding the growth of *L. maculans* from stem cortex to pith

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- **INRA-Rennes: Regine Delourme, Hortense Brun, Anne-Marie Chèvre, Michel Renard**

A wide-angle photograph of a vast field of bright yellow flowers, likely rapeseed, stretching to the horizon. The sky is a clear, vibrant blue, dotted with numerous fluffy white cumulus clouds. In the distance, a small cluster of buildings and trees is visible on the horizon line. The overall scene is bright and cheerful.

Thank you