



HEXIMA

# Bioassays for disease resistance in transgenic canola

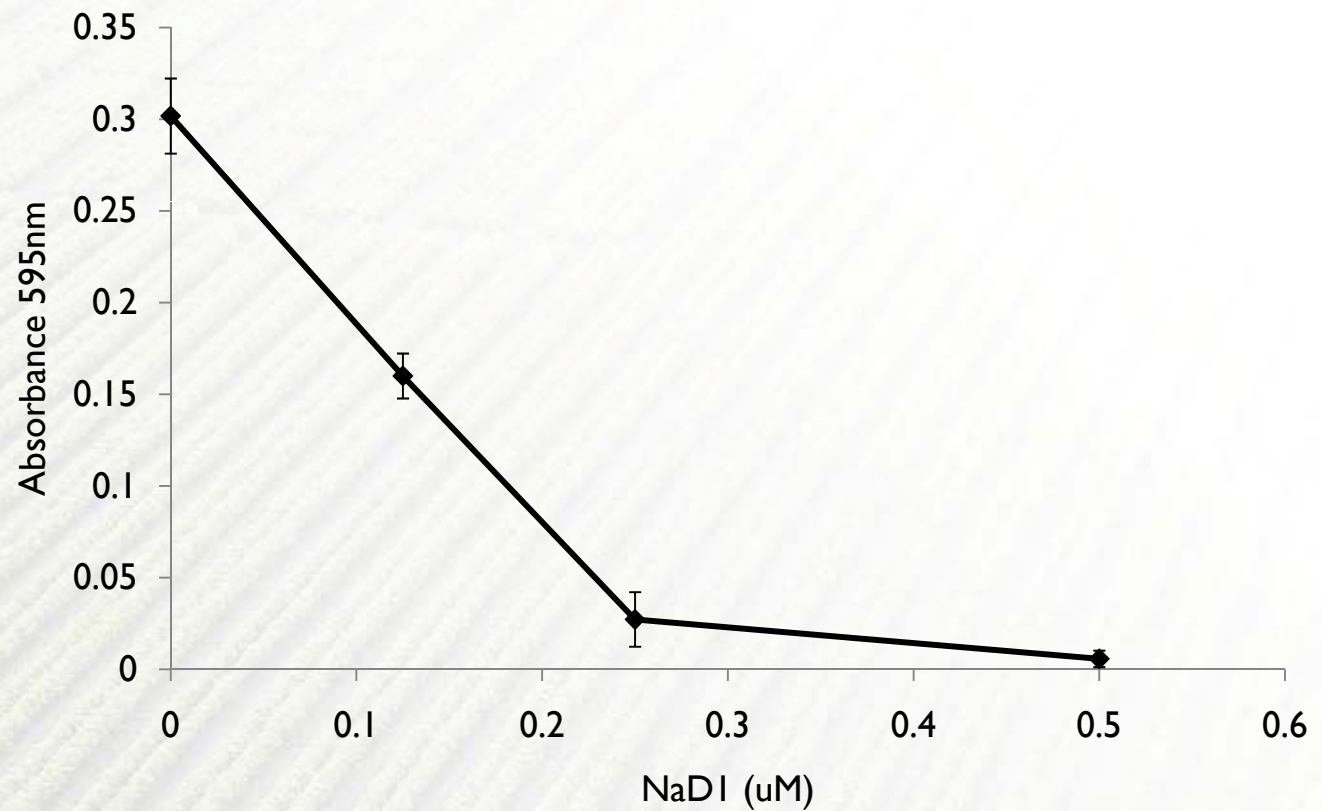
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*23 Feb 2010*

## *In vitro* inhibition of *L. maculans* by NaDI



◆ Fungal growth after 68 hours



## Production of transgenic canola

- ◆ Agrobacterium - mediated transformation
- ◆ RI64 breeding line – not commercialised
- ◆ Initial work in collaboration with Professor Roger Parish's lab
- ◆ Gene construct: 35S promoter driving *N. alata* defensin (NaDI)
- ◆ No agronomic differences observed - transgenic plants fertile



## Blackleg Bioassay: Methods

- Based on method used by the Howlett Lab
  - 10 day old canola seedlings are punctured 4 times with a 26 gage needle
  - Puncture sites are overlaid with 5uL of  $1 \times 10^6$  pycnidiospores (collected from plates)
  - Seedlings are placed in a humid environment for 3 days post inoculation (DPI) and lesions are scored at 10, 14 and 17 DPI



## Blackleg Bioassay: Methods



Germinating seedlings



Wounded seedlings overlaid with pycnidiospores



Developing *L. maculans* lesions



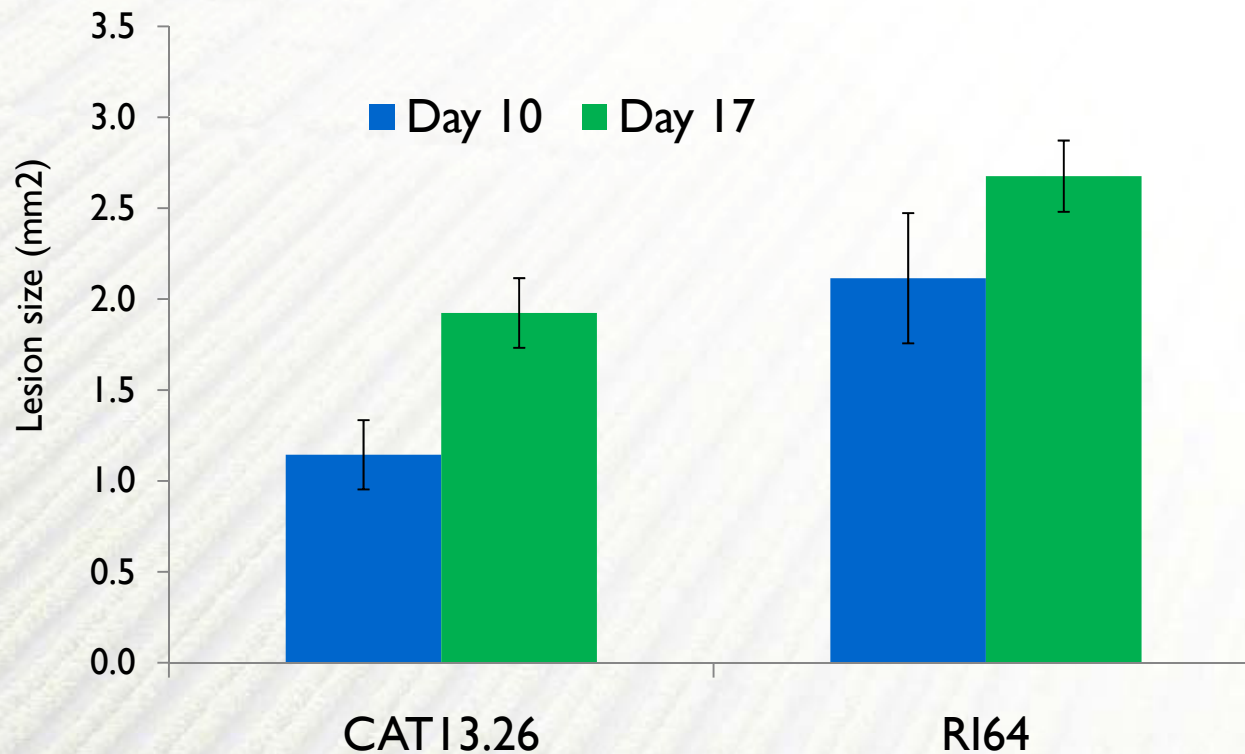
## Lesion analysis: measurement of area

- ◆ Digitize the lesions (take digital photos)
- ◆ Analyze
  - ◆ ImageJ (<http://rsbweb.nih.gov/ij/>) an open source, Java based image analysis software
  - ◆ Identify the lesion area and measure area in  $\text{mm}^2$



## Assessment of transgenic canola expressing NaDI for resistance to *L. maculans*

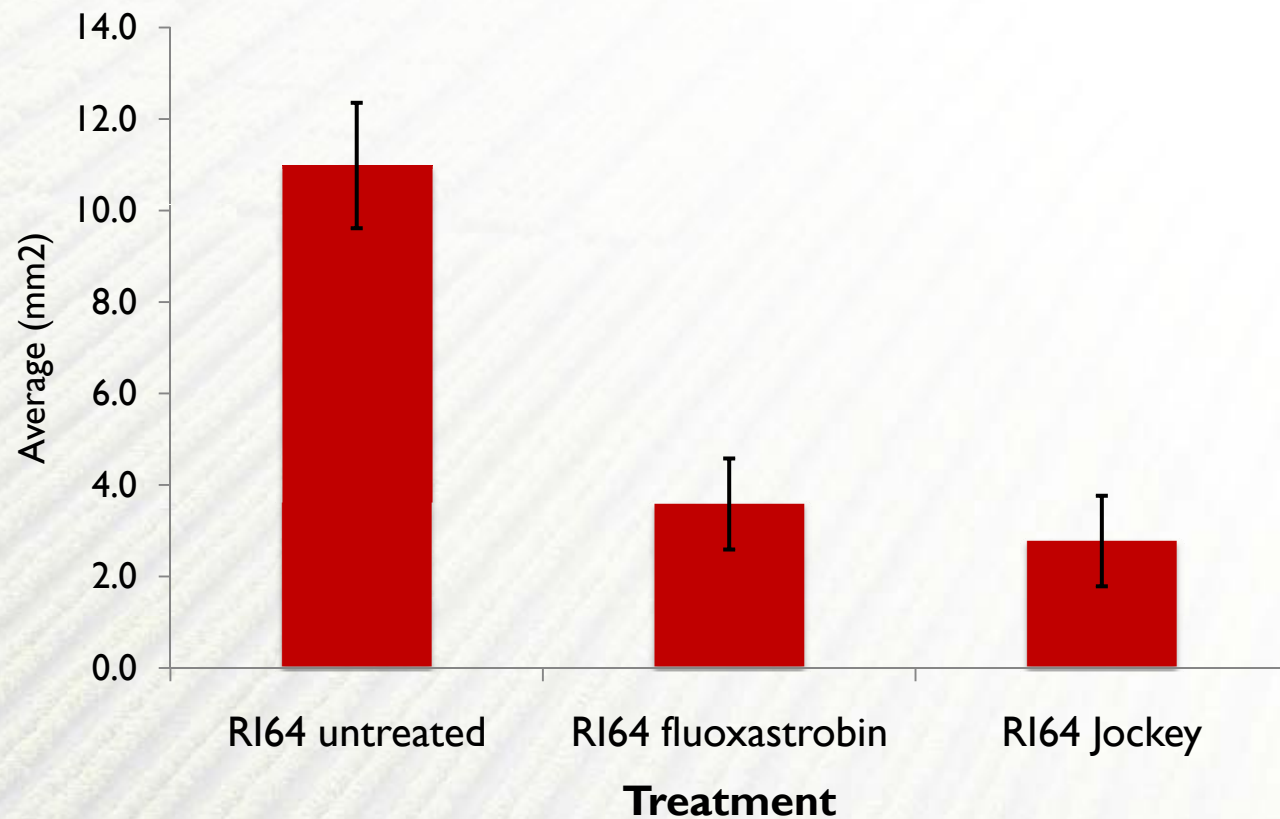
- ◆ Transgenic lines significantly different at 10 and 17 DPI ( $p < 0.05$ )



Error bars represent 95% confidence intervals for mean area



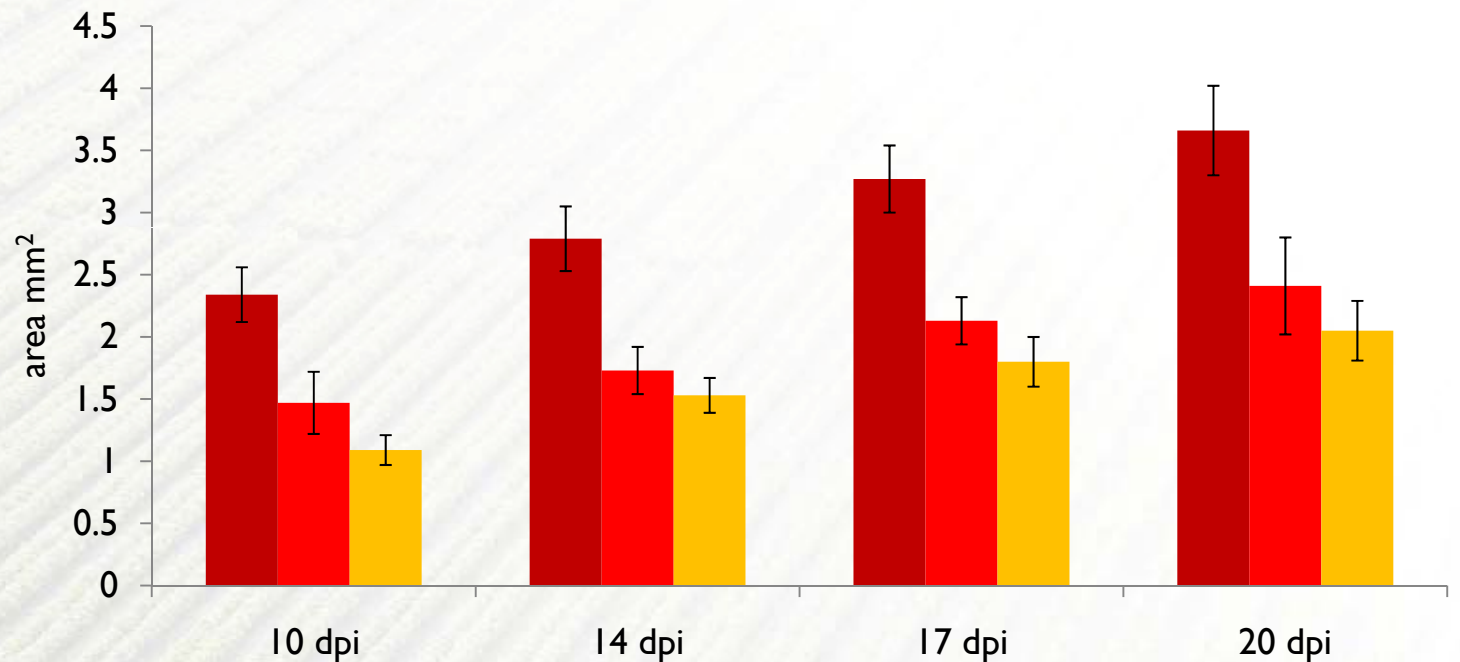
## Assessment of conventional canola seed treated with fungicide prior to sowing



Error bars represent 95% confidence intervals for mean area



## Assessment of fungicide concentration on lesion size



■ RI64 Untreated ■ RI64 Jockey 1/4 strength ■ RI64 Jockey 1/2 strength

Error bars represent 95% confidence intervals for mean area

## Acknowledgements

- Hexima

- Dr Robyn Heath & Prof Marilyn Anderson

- Mr Bruce McGinness

- Parish Lab (La Trobe, Botany)

- Trudi Higginson

- Howlett Lab

- Dr Angela Van de Wouw

- Anton Cozijnsen