



H E X I M A

Development of a disease resistance trait for GM crops

***Dr Robyn Heath
Senior Vice President
Product Development
Hexima Limited
17 October 2011***

- ◆ Australian biotechnology company based in Melbourne
- ◆ Strategy to commercialise GM crop protection technologies with global players
- ◆ Focus on:
 - Major economic targets: fungal disease and insect protection
 - Most valuable GM crops: corn and soy
 - Discovery and Phase I R&D
- ◆ Innovative researchers: strong and growing intellectual property portfolio
- ◆ Agreements with market leaders DuPont/Pioneer and Monsanto

Ag-biotech development phases: a general overview

Hexima
focuses on
Discovery and
Phase I

Hexima's seed
co. partners
focus on Phase
2 onward

Discovery	Phase I	Phase 2	Phase 3	Phase 4
Gene/trait identification	Proof of concept	Early development	Advanced development	Pre-launch
24-48 mths	12-24 mths	12-24 mths	12-24 mths	12-36 mths
High through-put screening Model crop testing	Gene optimisation Crop transformation	Trait development Pre-regulatory data Large scale transformation	Trait integration Field testing Regulatory data generation	Regulatory submission Seed bulk-up Pre-marketing

- ◆ R&D contracts with La Trobe University & The University of Melbourne
- ◆ Three research sites:
 - School of Botany, The University of Melbourne
 - Department of Biochemistry, La Trobe University
 - R&D Park, La Trobe University



New Hexima glasshouse and tissue culture facility, R&D Park, La Trobe University

Hexima programs

◆ Disease resistance

- DuPont/Pioneer joint program for corn and soybean
- Defensins and other antifungal proteins (AFPs)

◆ Insect resistance

- Climate Ready grant
- ARC Linkage with University of Western Australia
- Research licence with Monsanto
- Proteinase inhibitors in combination with other technologies


◆ Multigene expression vehicle (MGEV)

- Enabling technology
- Research licences with DuPont/Pioneer and Monsanto

◆ Pharmaceutical applications

- Private funding

Crop losses due to disease

-  Fungal disease causes ~US\$8.5billion yield loss in corn & soy in the US each year



Corn (US)

Area	32m Ha
Av yield	380 bu / Ha
Price	US\$4 / bu
Av value	US\$48.5b pa
Av disease loss	10-12% pa
Value of loss	US\$5.7b pa

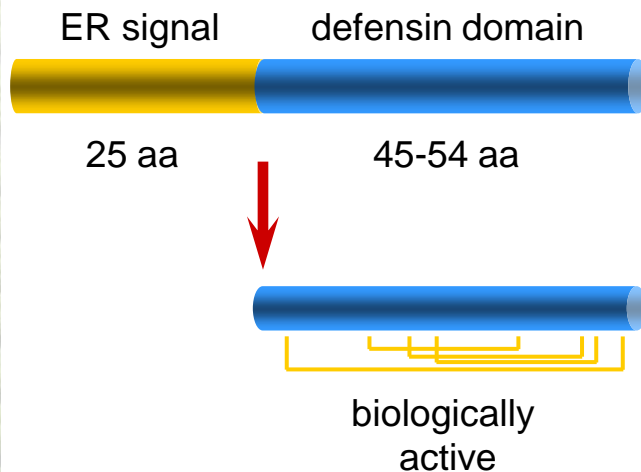
Soy (US)

Area	30m Ha
Av yield	99 bu / Ha
Price	US\$11 / bu
Av value	US\$32.5b pa
Av disease loss	8-10% pa
Value of Loss	US\$2.8b pa



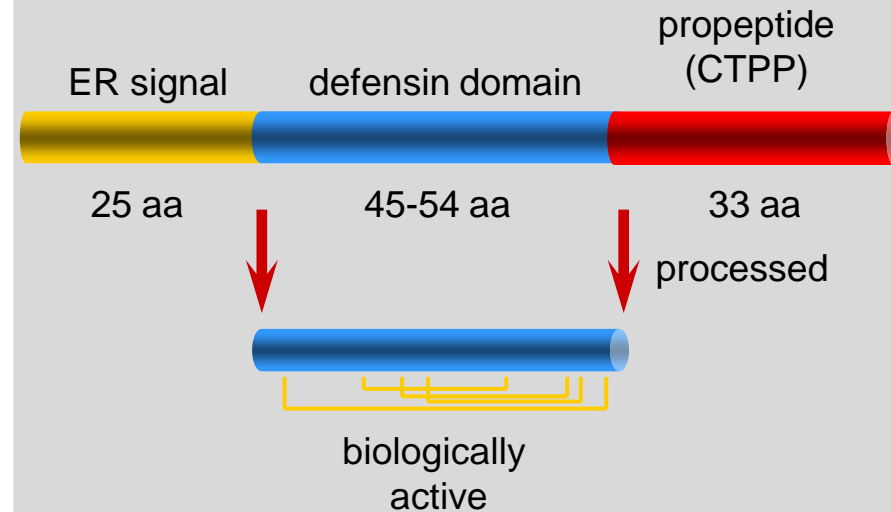
Source: FAPRI, Pioneer, Wrather & Koenning, estimates based on 2008 figures

Class I defensin



- Most defensins in this class
- Most from seeds
- Secreted

Class II defensin



- Most in Solanaceous plants
- C-terminal propeptide (targeting, detoxification)

Nicotiana alata defensin I (NaDI)

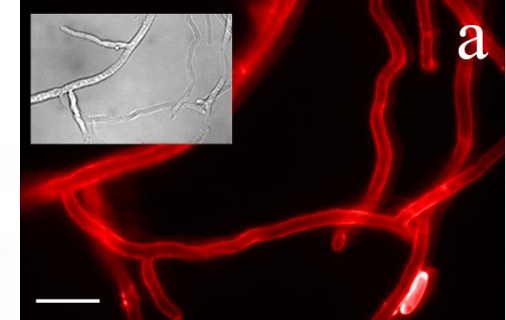
- ◆ Class II defensin
- ◆ Highly expressed in the flowers of *Nicotiana alata* (ornamental tobacco)
- ◆ Inhibits plant and human fungal pathogens
- ◆ Fungicidal

Pathogen	Disease	IC ₅₀ (uM)
<i>Fusarium graminearum</i>	Head blight	0.75
<i>Fusarium solani</i>	Sudden Death Syndrome	0.5
<i>Fusarium oxysporum</i>	Fusarium wilt	1
<i>Leptosphaeria maculans</i>	Blackleg	0.5
<i>Cryptococcus neoformans</i>	Cryptococcosis	1

Mechanism of action of NaDI

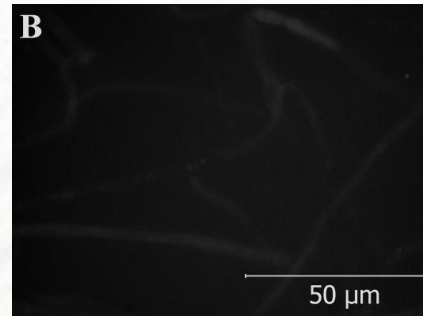
1. NaDI binds to the fungal cell wall

NaDI detected using anti-NaDI antibody

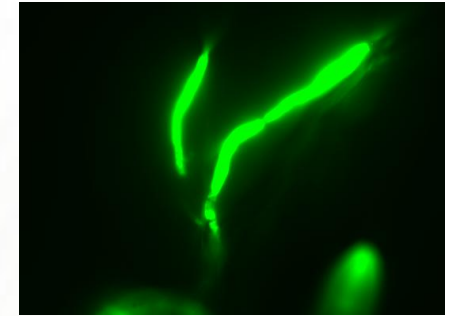


2. NaDI permeabilises the plasma membrane

SYTOX green (DNA stain) - only enters hyphae in the presence of NaDI



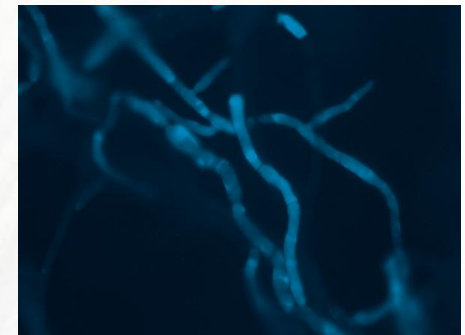
SYTOX green



SYTOX green + NaDI

3. NaDI enters live fungal cells

NaDI tagged with
bimane amine



Synergy between NaDI and PIs

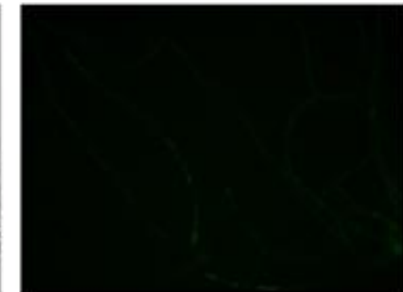
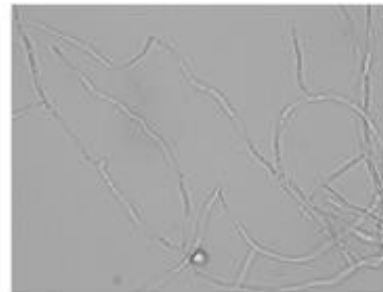
NaDI allows small proteins to enter fungal hyphae and enhance cell death

In vitro assay with NaDI, cysteine proteinase inhibitor (NaCysI) and *Fusarium graminearum* hyphae

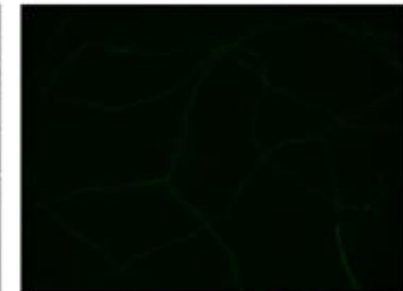
No protein

Bright field

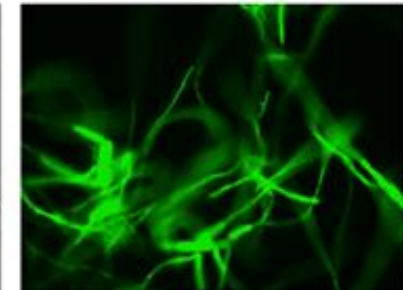
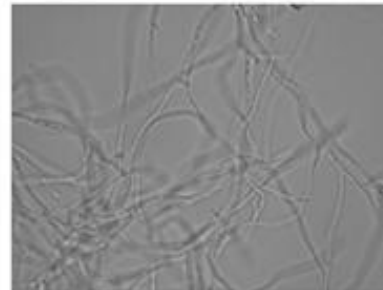
Fluorescence



NaCysI labelled with FITC



NaDI +
NaCysI labelled with FITC



Disease resistance in transgenic crops

Cotton



Canola



Corn

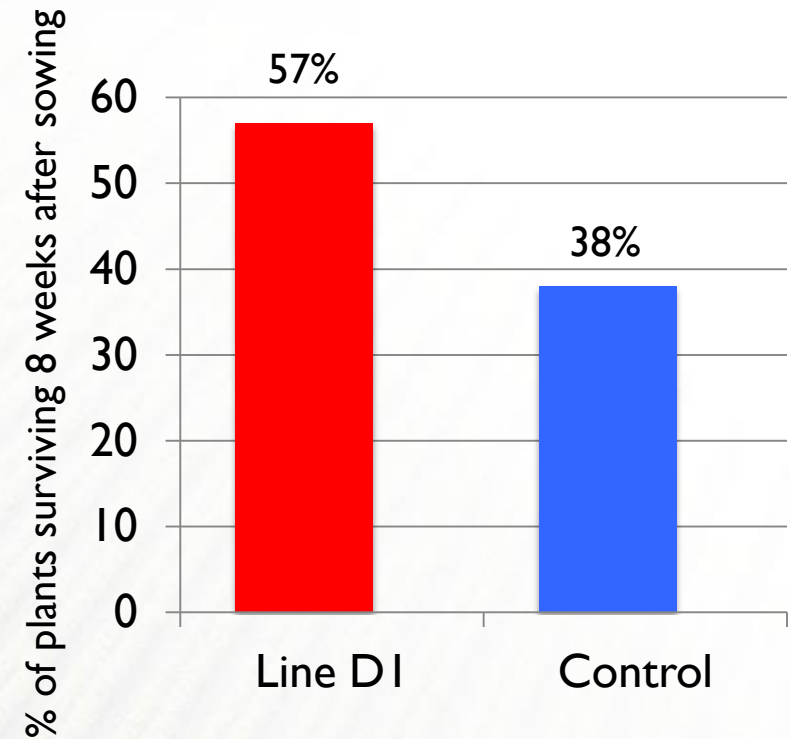


Transgenic cotton expressing *NaDI*

Fusarium oxysporum infected soil bioassay



James McKenna assessing plant survival



- ◆ Transgenic line DI had higher survival than non-transgenic control

Field trials with transgenic cotton

- Assessed transgenic line DI (expressing NaDI)
- 3 years of trials: 06/07, 07/08, 08/09
- Trial sites on private farms in Queensland and NSW
- Target diseases:

Fusarium Wilt (*Fusarium oxysporum*)
- plant death



Verticillium Wilt (*Verticillium dahliae*)
- defoliation



Fusarium wilt field trials



06/07

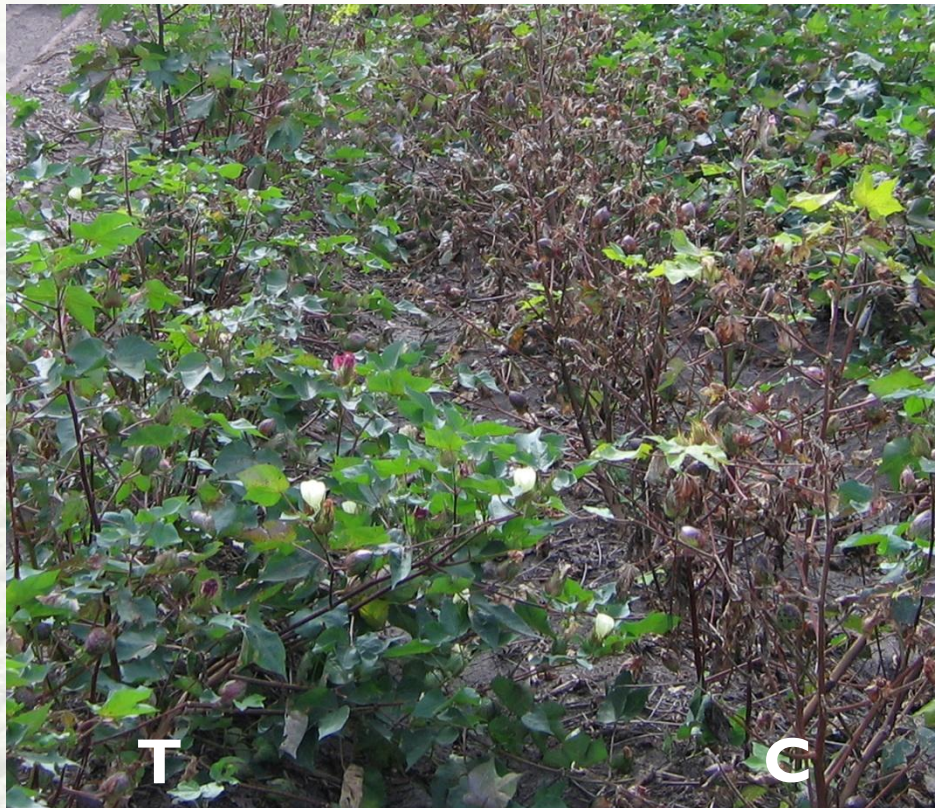
07/08

08/09

- Transgenic cotton line expressing NaDI had
 - Higher tolerance to Fusarium wilt over 3 seasons
 - 2-4 fold increase in lint yields
 - 2-3 times the survival rate
 - No effect on lint quality
- No adverse agronomic differences or yield penalty in the absence of disease

T = transgenic C = non-transgenic control

Verticillium wilt field trial (07/08)



T = transgenic

C = non-transgenic control

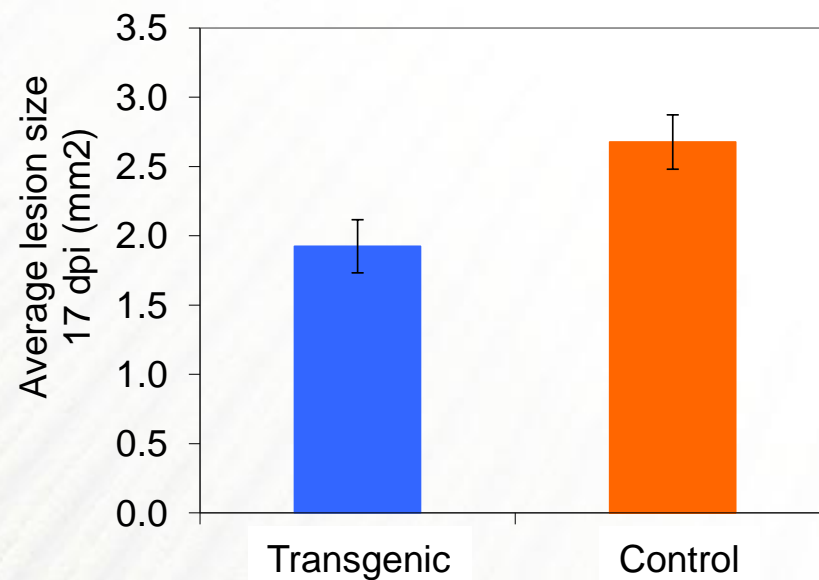
- Transgenic cotton expressing NaDI had:
 - higher tolerance to Verticillium wilt
 - up to a 2 fold increase in lint yield
- No difference in lint quality

Transgenic canola expressing *NaDI*

Bioassays with *Leptosphaeria maculans* (Blackleg)



Inoculated cotyledons



- Transgenic canola expressing *NaDI* had 30% smaller lesions than the non-transgenic control

Corn diseases in the US

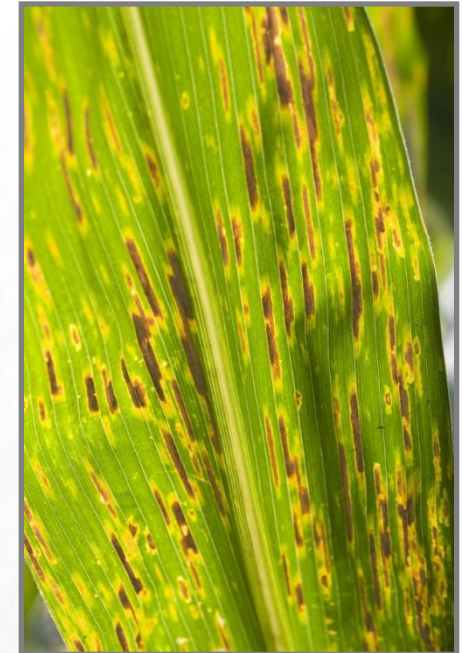
- ◆ Fungal disease affects both yield and quality
- ◆ Fungal toxins are powerful carcinogens



Fusarium ear rot



Anthracnose
stalk rot



Gray leaf spot

Protein/gene discovery

- ◆ Screen for new antifungal molecules from varied sources
- ◆ Robotic platform; laboratory tests against multiple fungal pathogens
- ◆ Selection of multiple leads for testing in plants
- ◆ Select gene combinations for broad spectrum control



Nicole van der Weerden operating the Tecan robot

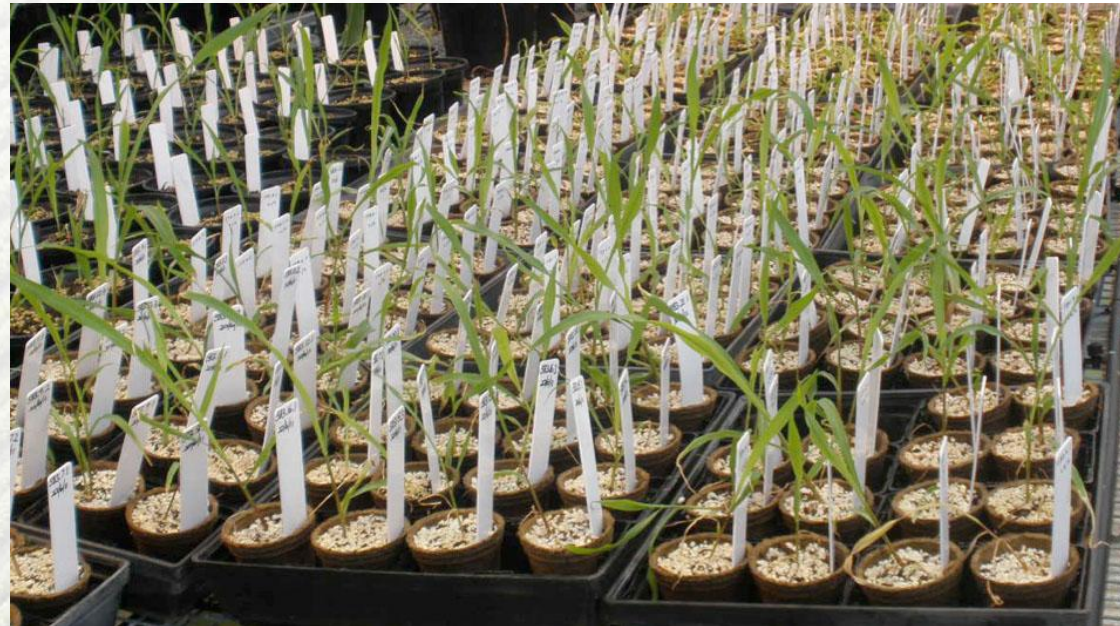
MGEV technology

- ◆ Multiple proteins produced from a single promoter
- ◆ Advantages:
 - co-ordinated expression
 - a single transformation event
 - a single selectable marker



Transgenic corn (DuPont/Pioneer program) H E X I M A

- ◆ *Agrobacterium* transformation of immature embryos
- ◆ Facilities and staff in place to produce and test up to 10,000 transgenic corn plants per year
- ◆ Plants assessed for gene expression, phenotype and resistance to key fungal pathogens



Transgenic corn plants containing AFP genes

Acknowledgements

Professor Marilyn Anderson

Dr Nicole van der Weerden

Melbourne Uni lab

La Trobe Uni lab

Glasshouse & corn transformation lab

