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Plant & Food RESEARCH
RANGAHAU AHUMĀRA KAI

Development and Assessment of Biotech Germplasm Resistant to Allium White Rot (AWR)

Eady CC., Kenel F., Glu J. and George, A.

8th February 2010

California Garlic and Onion Symposium. Tulare, Ca. USA

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AIDS of allium crops: Garlic industry develops white rot control program

Apr 19, 2003 12:00 PM, By Dan Bryant

The California garlic industry is developing a four-year routine it hopes will manage white rot, a disease considered the "AIDS of allium crops" throughout the world.

Bob Ehn of the California Onion and Garlic Research Committee (COGRC) talked about the disease and measures to bridle it during the recent Kern County Vegetable Crops Meeting at Bakersfield

AgAlert
THE WEEKLY NEWSPAPER FOR CALIFORNIA AGRICULTURE

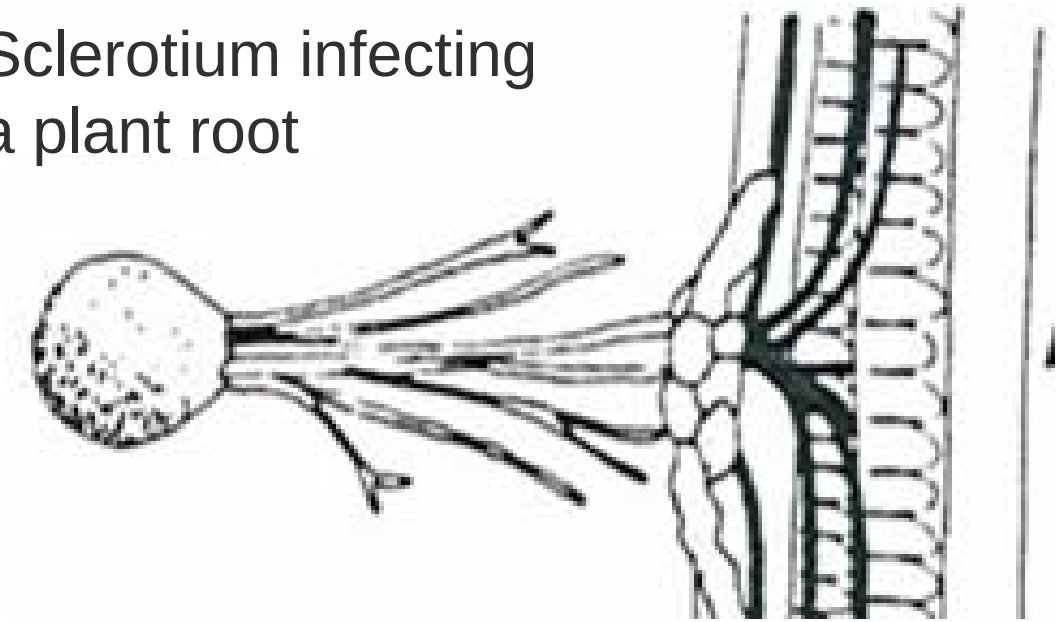
REPRINT

July 22, 2009

White rot in onions and garlic—one of the worst soilborne diseases in California—is becoming a bigger problem in the Central Valley. Over the past three years, infected acreage has increased to 13,000 acres. “It’s a devastating disease and the acreage affected is expanding slowly, but surely,” said Tom Turini,

Strategy: neutralize the production of oxalic acid

Sclerotium infecting a plant root



Natural protection

Wheat root showing oxalate oxidase activity(dark stain)

- Oxalic acid is the **key**
- Mutants that produce no oxalic acid cannot infect plants
- **Biotech approach – Introduce genes that can breakdown oxalic acid**

What have we done so far - Research history

- 2001 – 2005 - PhD project to insert wheat oxalate oxidase gene (*wox)* into onion
  - Two plant lines produced.
  - Data presented to GORAB and funding was obtained:
- 2006-2007 – GORAB funding to create new gene constructs and improve garlic transformation efficiency
  - **Both successfully achieved**

Plant Cell Rep
DOI 10.1007/s00299-009-0814-z

ORIGINAL PAPER

Efficient Agrobacterium tumefaciens-mediated transformation and regeneration of garlic (Allium sativum) immature leaf tissue

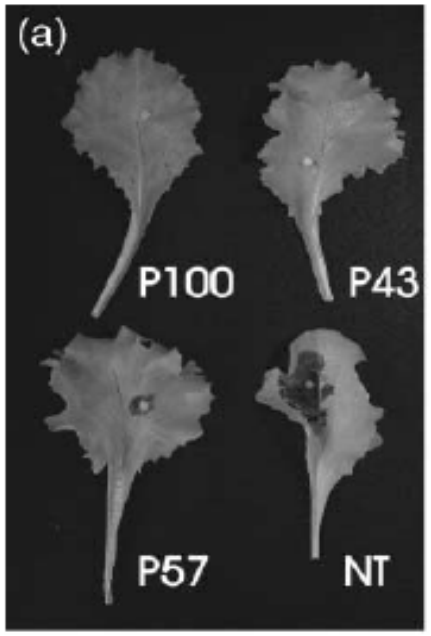
Fernand Kenel · Colin Eady · Sheree Brinch

Acknowledgments California Garlic and Onion Research Advisory Board and New Zealand Foundation for Research Science and Technology (FRST) for funding contributions.

Research history continued

- 2008 – 2010 –GORAB funded assessment of the new gene constructs.
- **Oxalate oxidase from barley *(box*) (Syngenta)**
  - Degrades oxalic acid to hydrogen peroxide and CO_2

- **Oxalate decarboxylase (*oxdc*) *(*Dias et al. 2006)**
  - Degrades oxalic acid to formate and O_2



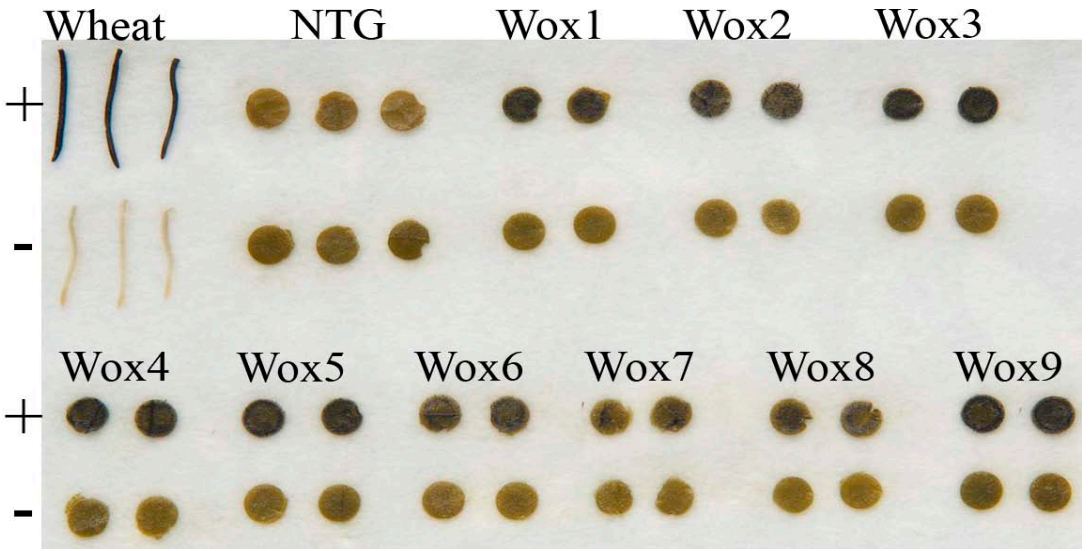
Oxdc lettuce

Assessment plan

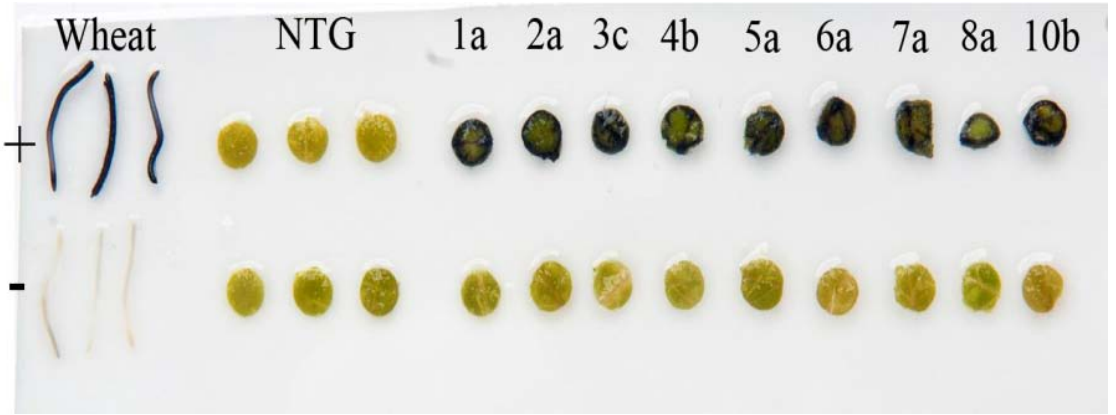
- Transform the constructs into tobacco for rapid comparison against *wox*
  - Enzyme activity assays
  - Fungal challenge using *S. sclerotiorum*
- Transformed into garlic (and onion)
  - Enzyme activity assays
  - Fungal challenge using *S. cepivorum*

Wox and box expression in tobacco

Wox - nine transgenic lines were produced. Staining indicated some activity in all 9 lines

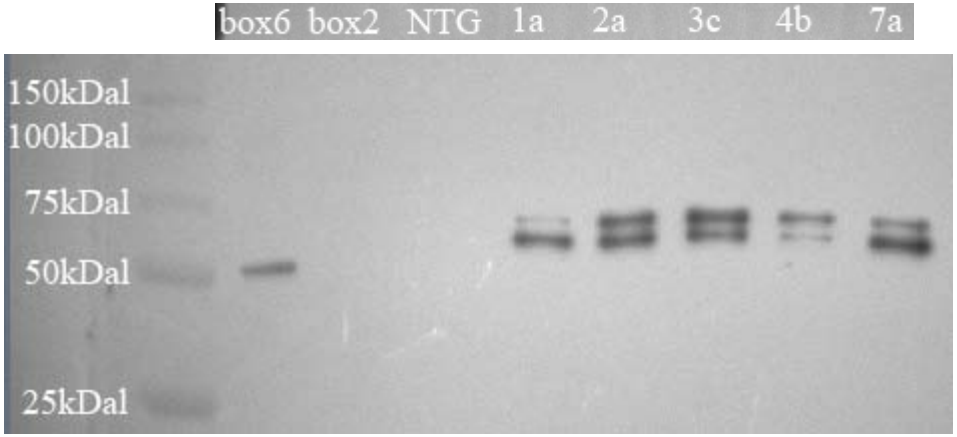


Box - nine transgenic lines were produced. Staining indicated good *box* activity

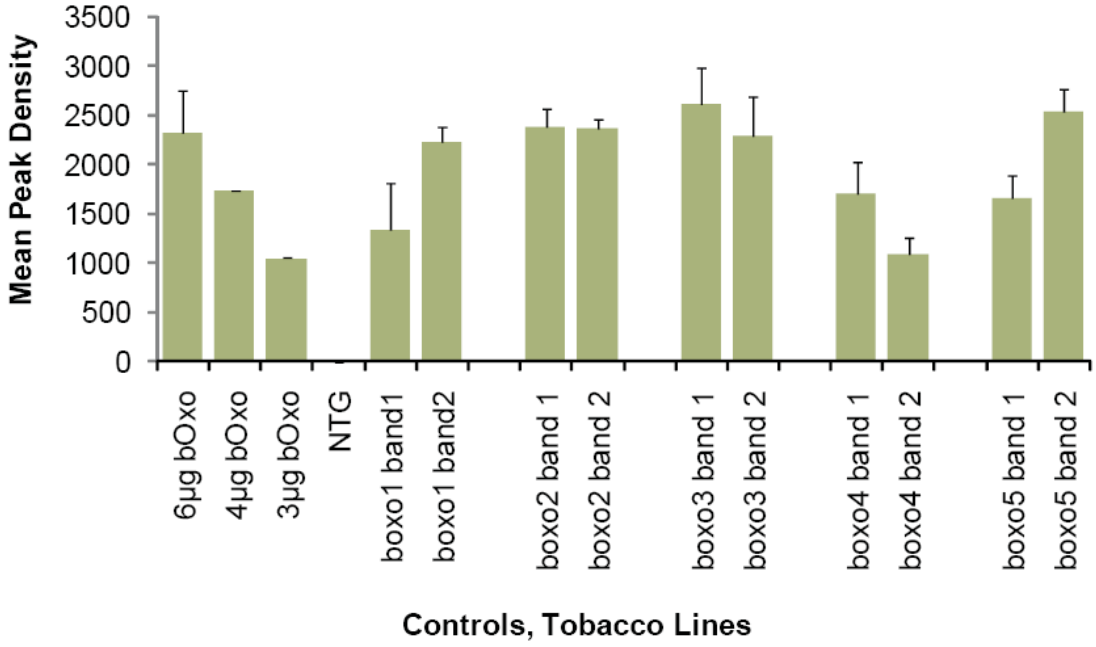


Quantification of activity

Isolate the protein stain for activity and quantify against a standard *box* extract



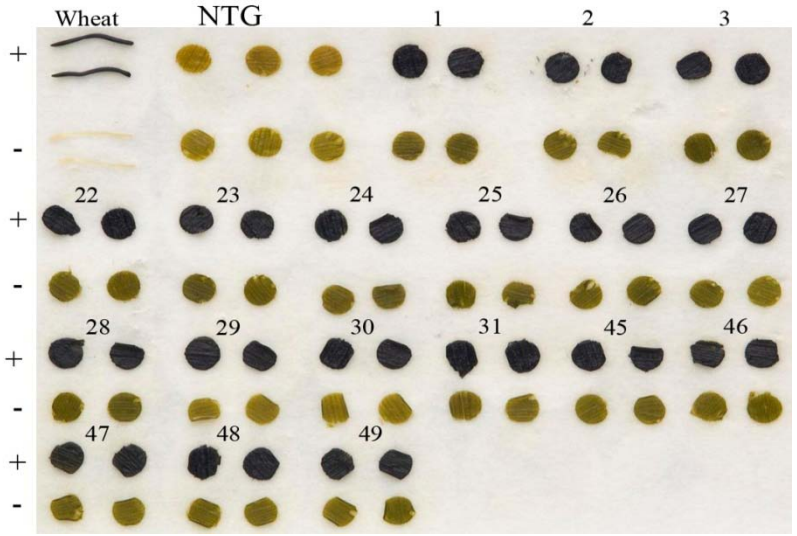
1.5µg of total *box* tobacco leaf protein gave activity ~ equivalent to 6µg of partially purified *box* protein from barley. **Therefore very good activity.**



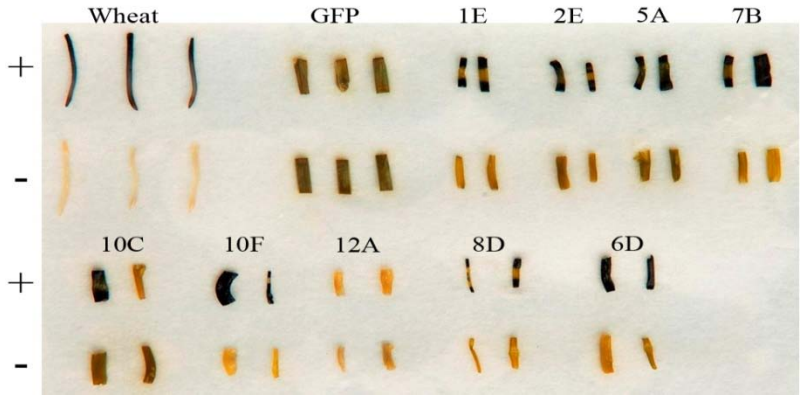
Wox and *box* expression in Allium

***Wox* -** only little activity was observed in the two *wox* onion lines previously produced.

***Box* -**one onion line was produced and clones of this show excellent activity



***Box* -** nine garlic lines were produced. Staining indicated good activity in some lines



Oxalate oxidase gene is active but does it protect against white rot – in tobacco?

Answer – No

Contradicts the hypothesis and several publications which state that oxalate oxidase can protect against white rot pathogens.

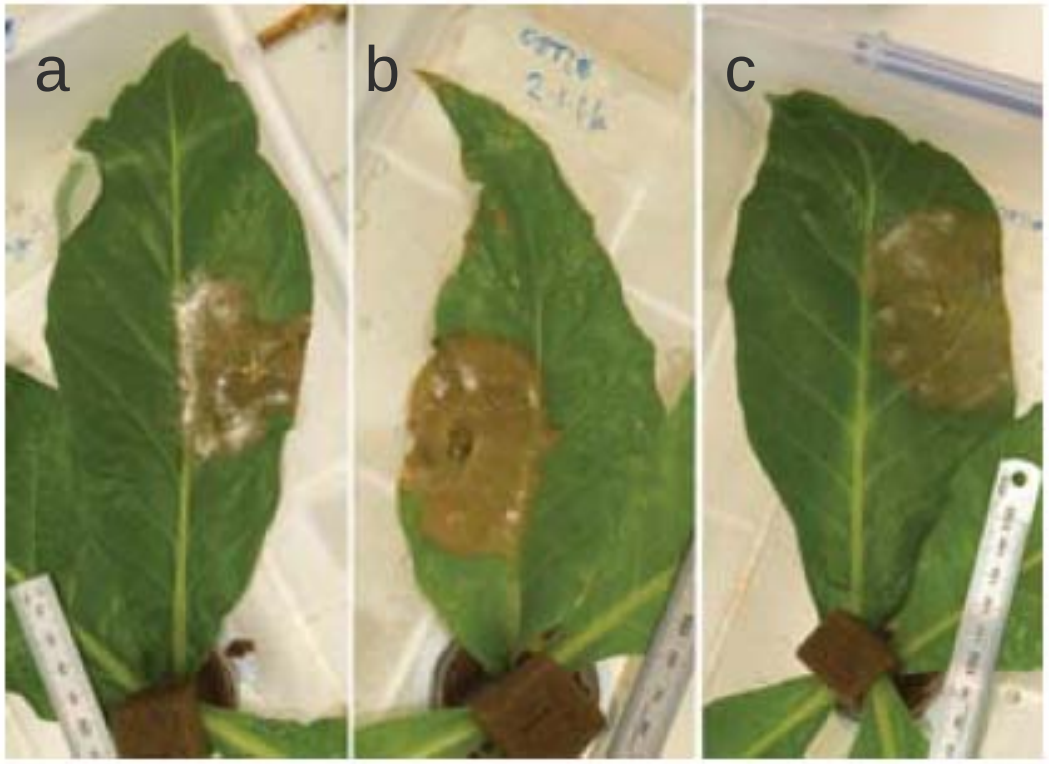


Fig 6. Mechanically wounded tobacco leaves challenged with a *Sclerotinia sclerotiorum* (isolate LU460) infection plug. a) non-transgenic; b) woxo2; c) boxo3.

In onion

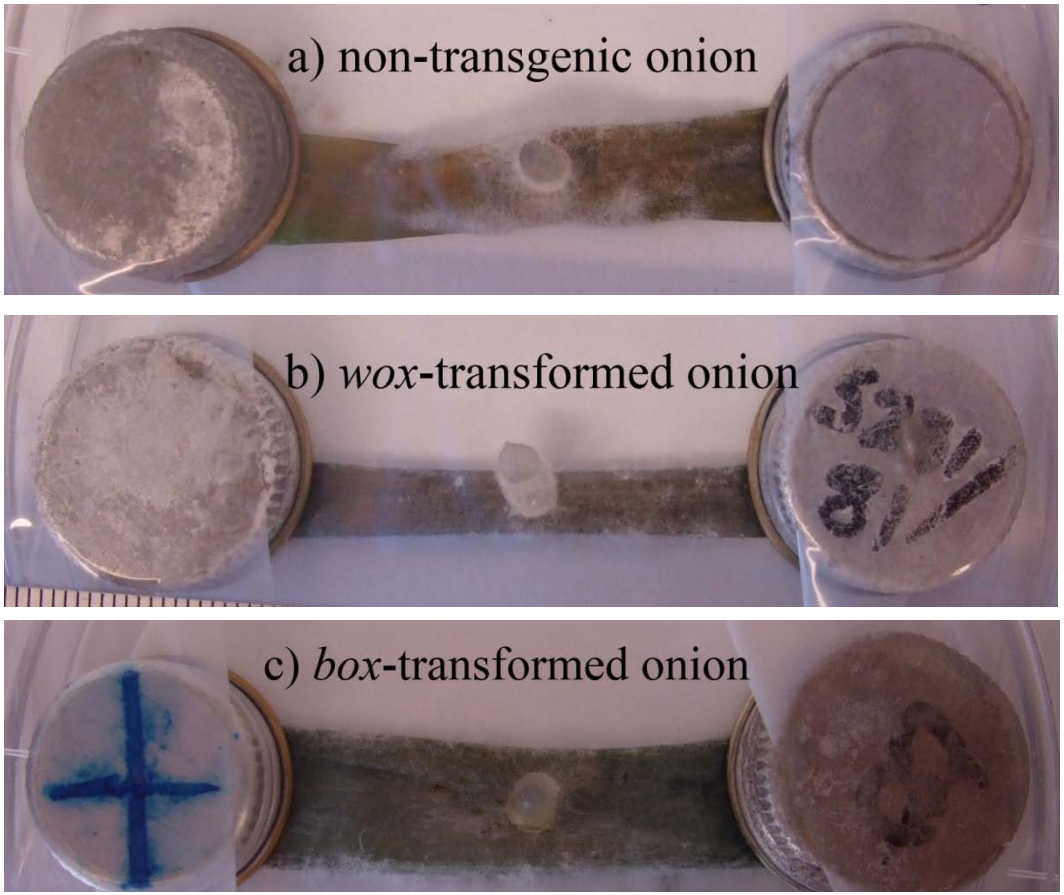
Tested:

Wox onions

Box onions

Box garlic

No resistance was observed



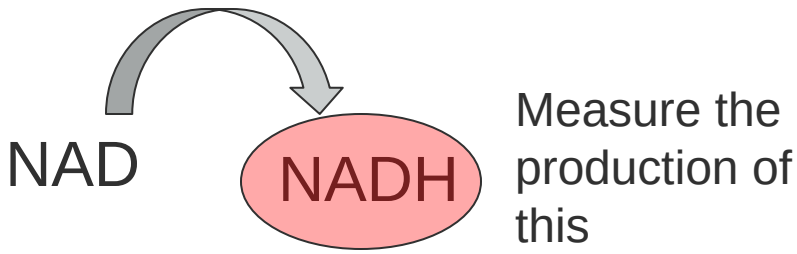
Oxalate decarboxylase

- Ten transgenic tobacco lines were produced.
- *Oxdc* much more difficult to transform into the plants
- No simple stain for activity.

Activity assessed by a complex indirect enzyme-linked assay

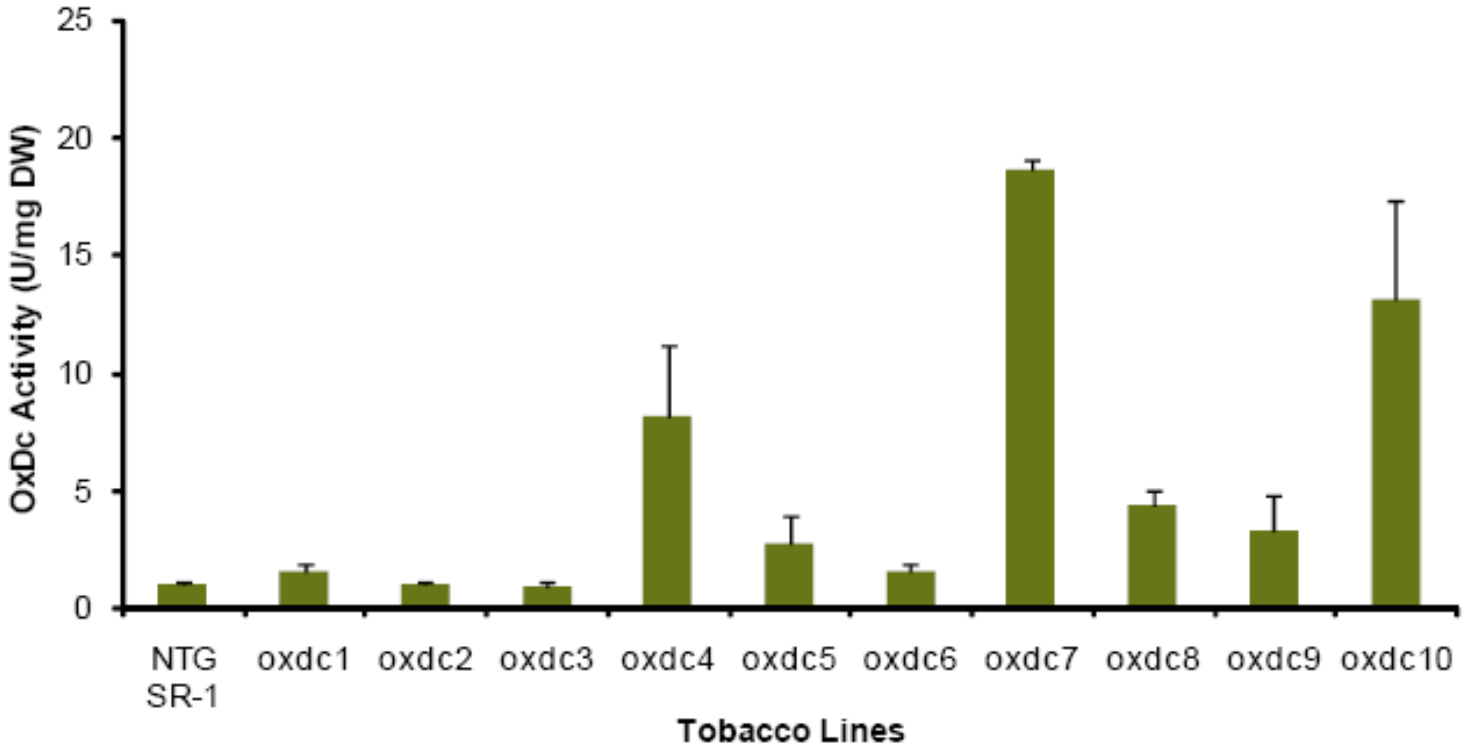
OXDC + oxalic acid → O_2 + formate

Formate + formate dehydrogenase → CO_2



Oxdc activity in transgenic tobacco lines

Ten transgenic lines showed different degrees of activity



* Note only three lines showed significant activity

Oxalate decarboxylase gene is active but does it protect against white rot - in tobacco?

Answer – Yes

Our results correspond with Dias et al (2006) and indicate that when expressed correctly in the plant *oxdc* can protect against white rot pathogens

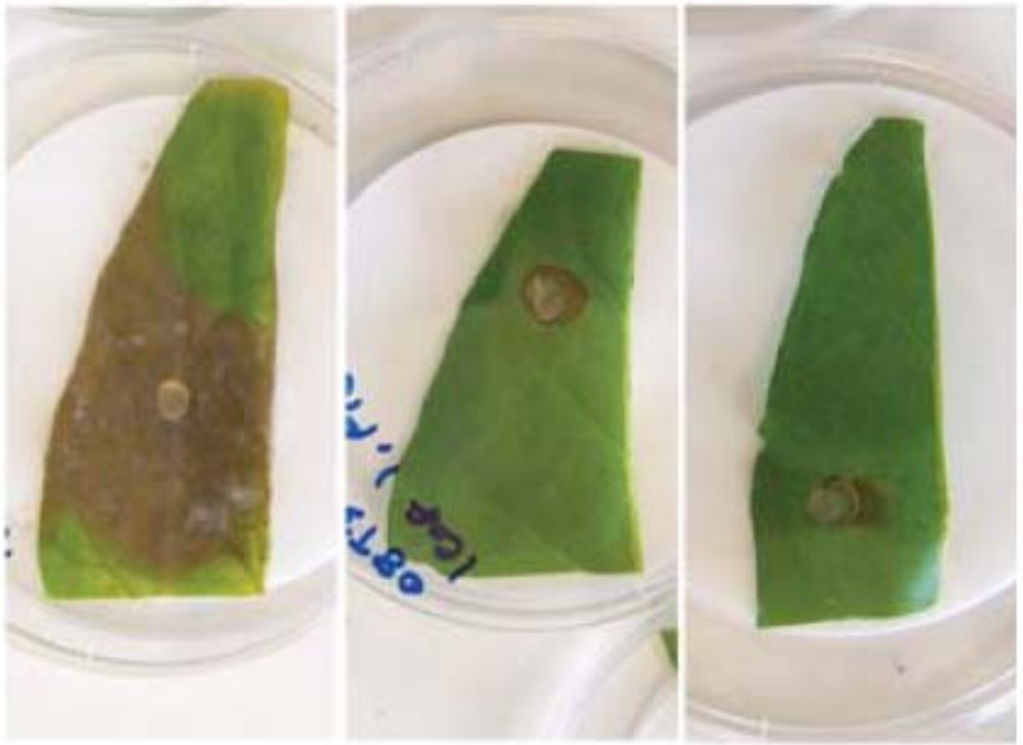
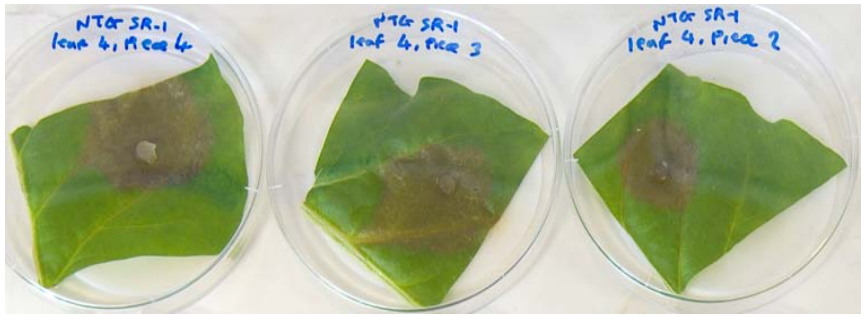


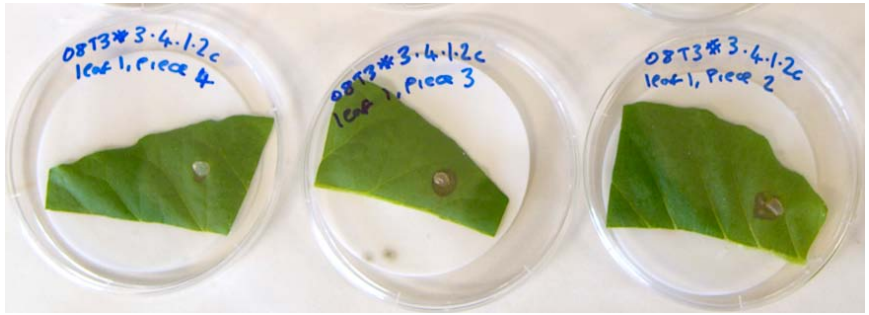
Fig 7. Mechanically wounded tobacco leaf quarters challenged with a *Sclerotinia sclerotiorum* (isolate LU460) infection plug. a) non-transgenic; b) oxdc5; c) oxdc10.

Health/age of leaf tissue was important

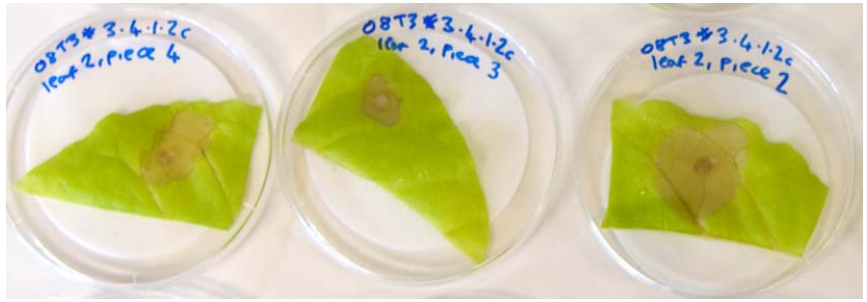
Control | ***Oxdc***

Young





Old



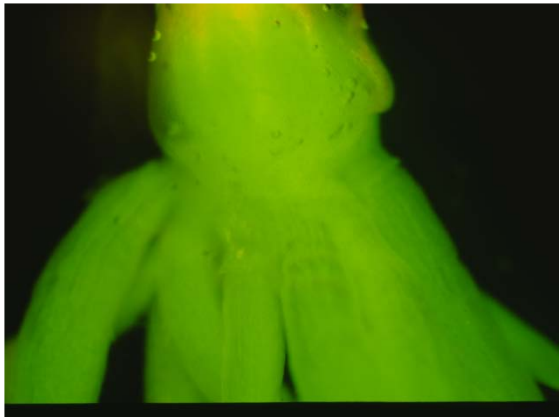
Oxdc in allium species

Initially four transgenic *oxdc* garlic lines were regenerated.

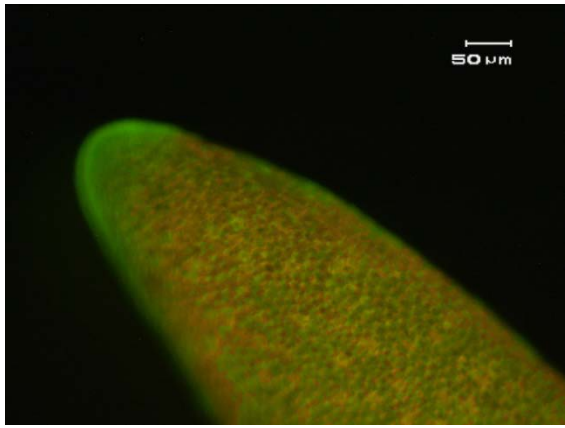
Expression and activity assays reveal that the construct is not being expressed at high level in any of these plants

Plant line	GFP in ex-flasked leaf
0825, 8C	only in stomata
0825, 5D	none
0825, 10B	none
0825, 11F	punctate
gfp line	constitutive

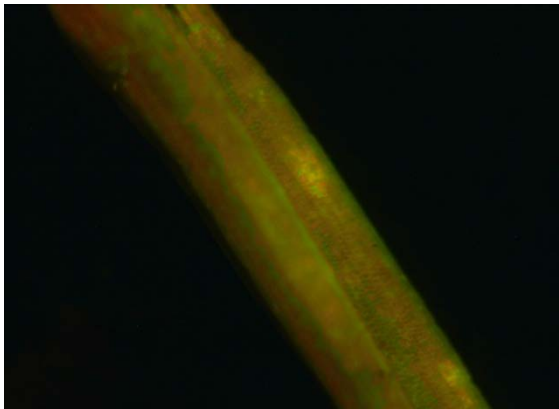
Control GFP

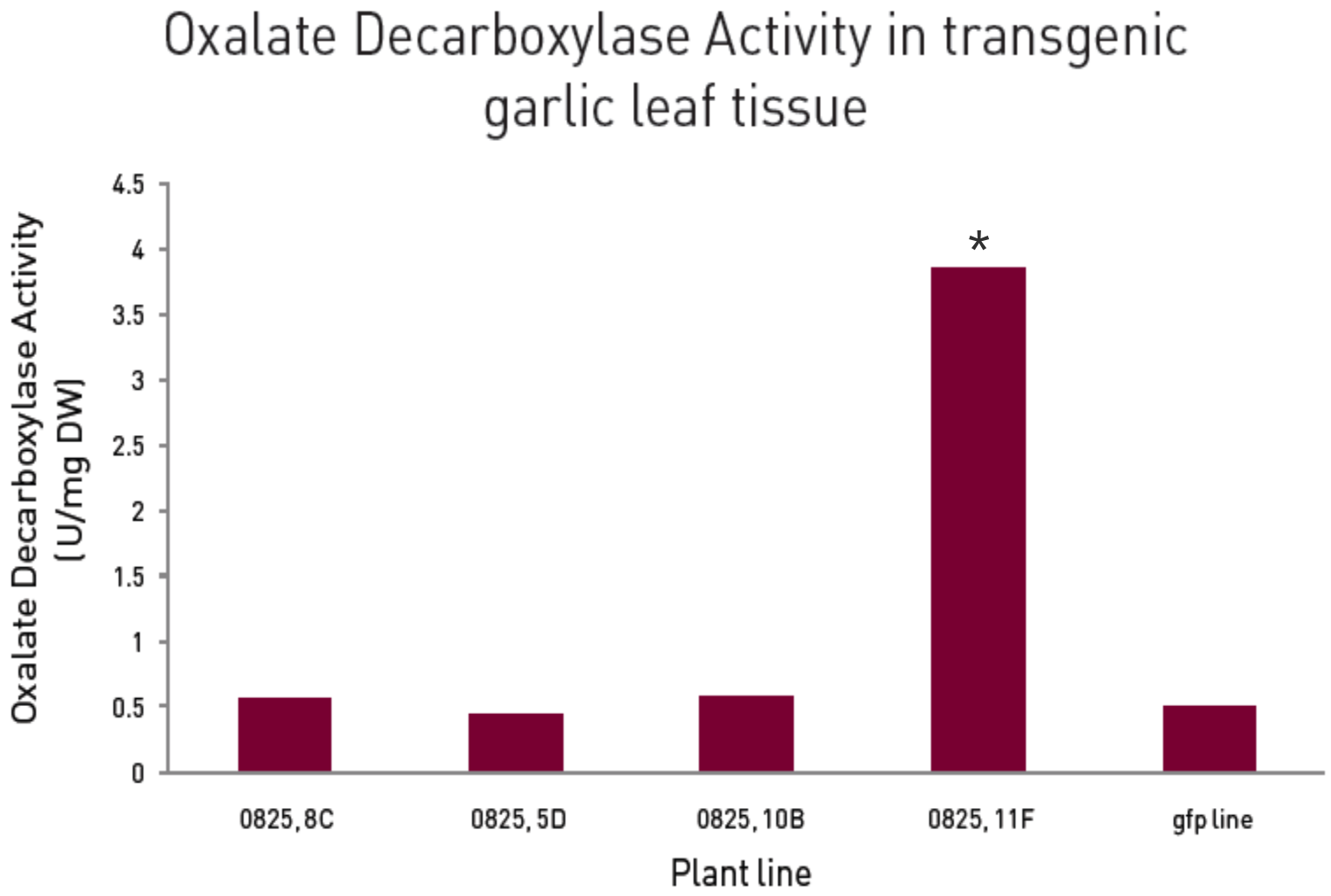


Oxdc root



Oxdc leaf

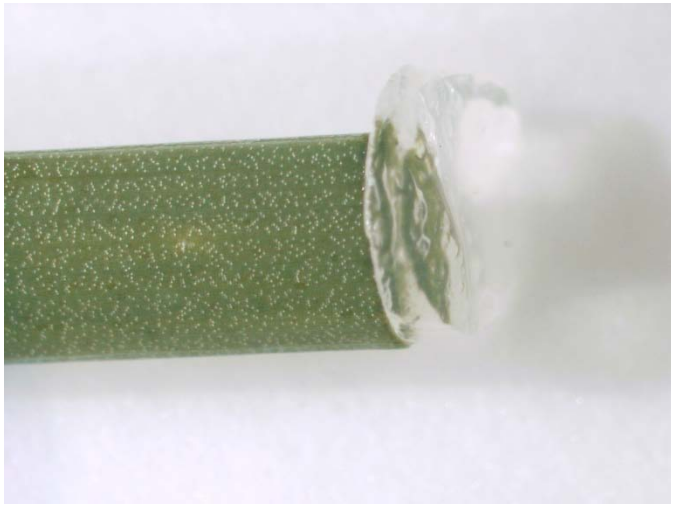


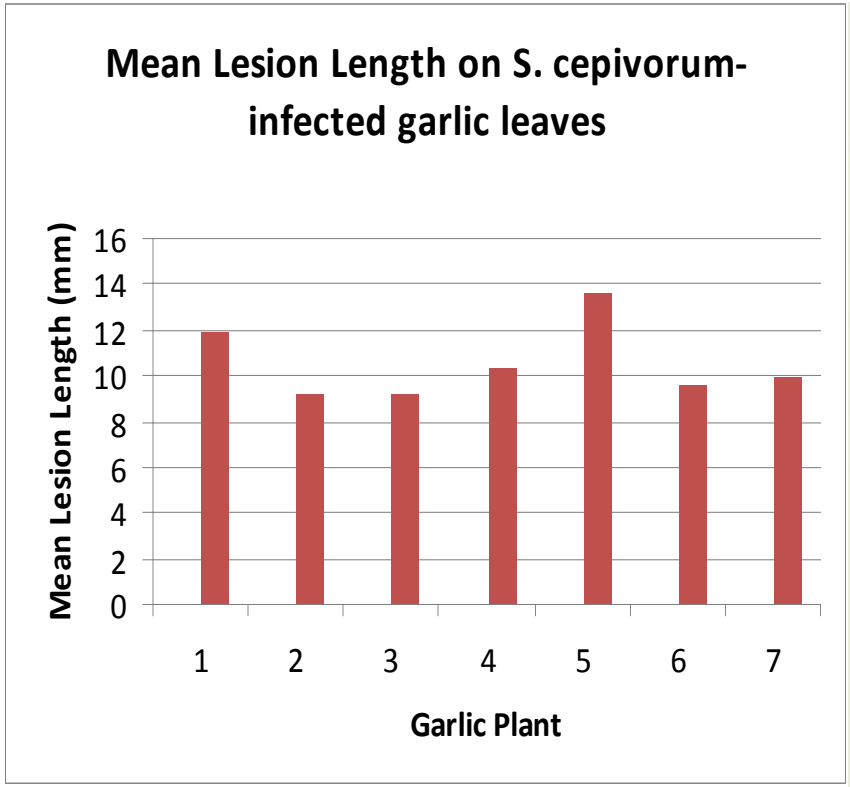


* 0825 11F is only ¼ of OXDC activity in the resistant tobacco lines

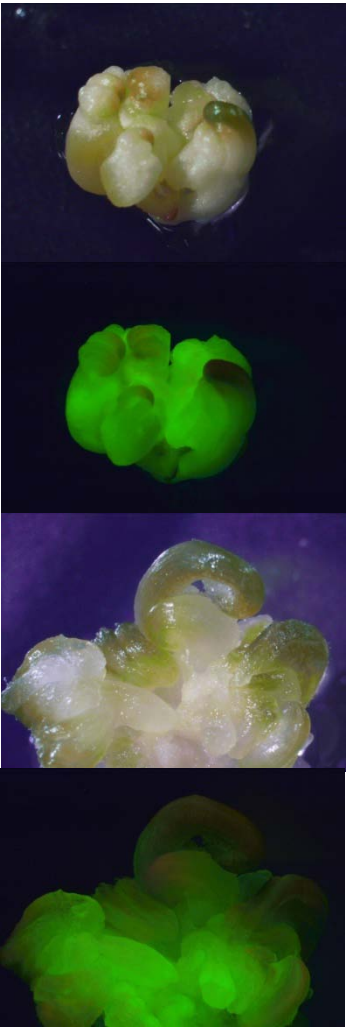
Activity against white rot in garlic (0825 11f)

S. cepivorum hyphae plug on cut leaf surface



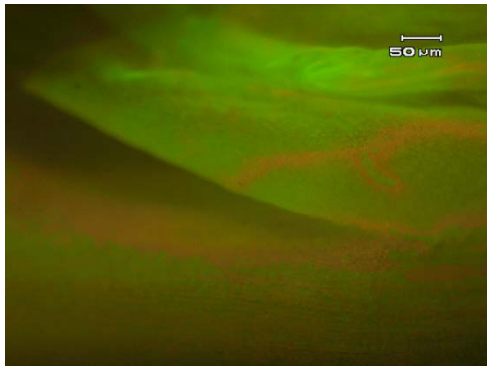


Oxalate decarboxylase in onions



This season we created four *oxdc* onion lines. GFP analysis of these indicate that two have good gene cassette activity.

Bulbs have been produced and *oxdc* analysis will occur later this year.



Summary

- Transgenic plants expressing high levels of oxalate oxidase **do not** show significant tolerance to white rot fungi.
- Transgenic plants expressing high levels of oxalate decarboxylase (Dias et al 2006 plus our tobacco) **do** show tolerance to white rot fungi .
- Our oxalate decarboxylase construct is difficult to transform into plants and expression is often poor.
- Four *oxdc* garlic lines have been produced. These **are not** expressing *oxdc* correctly and **do not** show resistance to white rot
- Four *oxdc* onion plants have been produced. GFP expression indicates **good activity in two lines**. Resistance to white rot is yet to be determined

Summary

Plant	WOX		BOX		OXDC	
	Enzyme activity	WR resistance	Enzyme activity	WR resistance	Enzyme activity	WR resistance
Tobacco	Yes	No	Yes	No	**Yes**	**Yes**
Onion	No	No	Yes	No	**Yes?**	To be tested
Garlic	nt	nt	Yes	No	Low / irregular	Good expression required

New information

Su Kim, K. Min, J-Y. and Dickman, MB*. (2008).Oxalic Acid Is an Elicitor of Plant Programmed Cell Death during *Sclerotinia sclerotiorum* Disease Development. Molecular Plant Microbe interactions 21: 605-612

Institute for Plant Genomics and Biotechnology, Department of Plant Pathology and Microbiology, Texas A&M University, College Station 77843, U.S.A.

- Oxalic acid does not kill by acidification
- Oxalic acid accumulates as oxalate in acidic plant vacuoles,
- Oxalic acid triggers the cells to self destruct

- Does not negate our hypothesis, in fact…

- The OXDC enzyme is active at low pH (Kesarwani et al., 2000),
- Transgenic tobacco and tomato plants with vacuole targeted OXDC showed 5-fold increase in resistance than cytosolic expressed OXDC. (Kesarwani et al., 2000)

- May explain why OXOX has not worked
- And, why low/irregular expression does not work

Future work

- Continue to assessment of current and future *oxdc* Alliums
- Develop refined vacuolar-targetted *oxdc* construct.
- Produce a white rot resistant allium for assessment.
- Develop a business model that will deliver these benefits to the industry.

Acknowledgements

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