Elicitors: What is available and can we optimise their potential benefits?

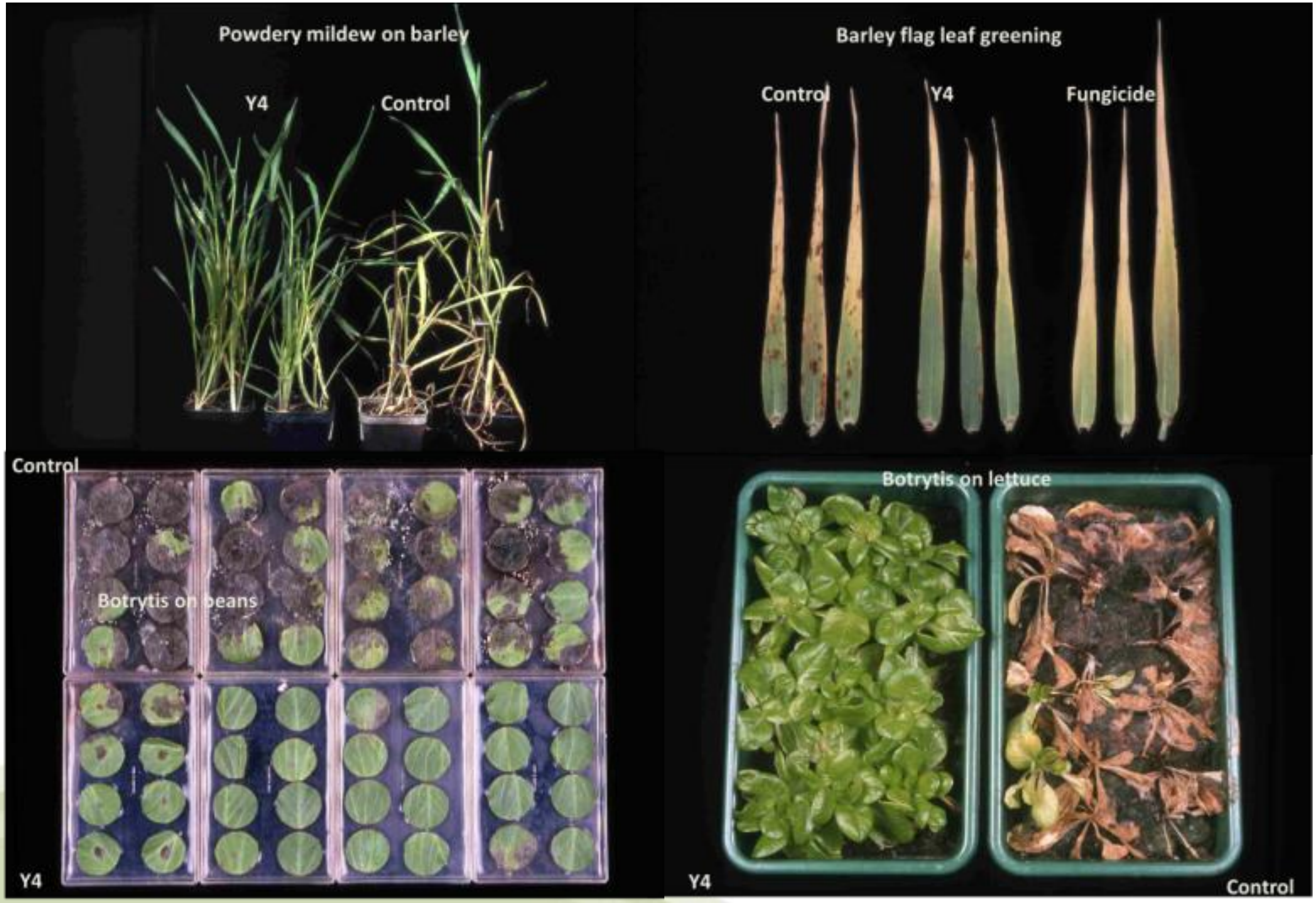
Adrian C Newton[1,2], Tony Reglinski[3], Daniel De Vega Perez[1], Nicola H Holden[1], Lea Weisel[1], Clement Gravouil[2], Ruairidh Bain[2], Neil Havis[2], Dale R Walters[2]

[1]The James Hutton Institute, Dundee, Scotland UK; [2]SRUC, Edinburgh, Scotland UK; [3]Plant & Food Research, Canterbury, New Zealand

SRUC

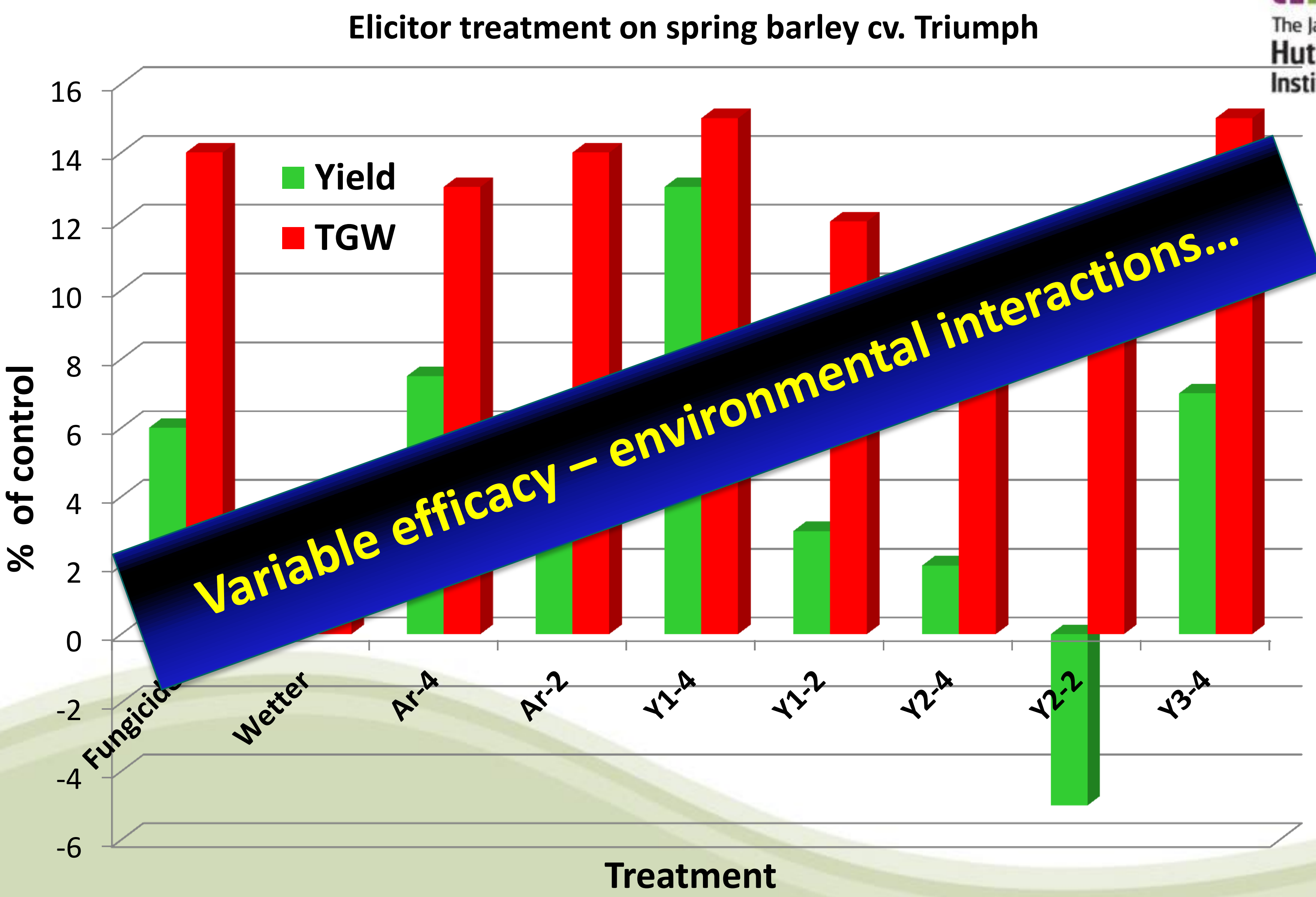
The James Hutton Institute

Yeast-derived elicitor: efficacy

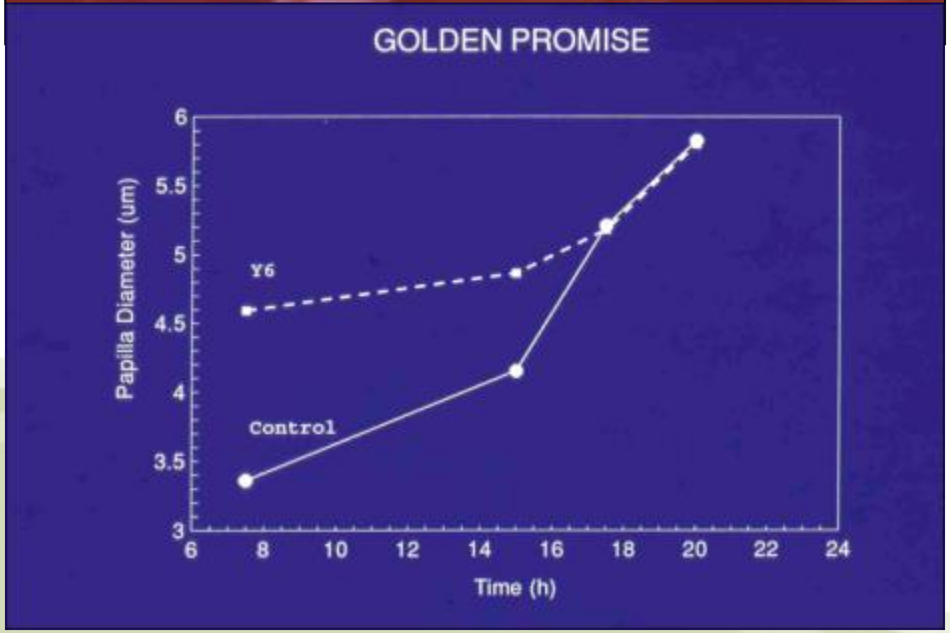


Recent AHDB Projects:

1) Botrytis on tomato – mechanisms
2) Commercial products on field vegetables



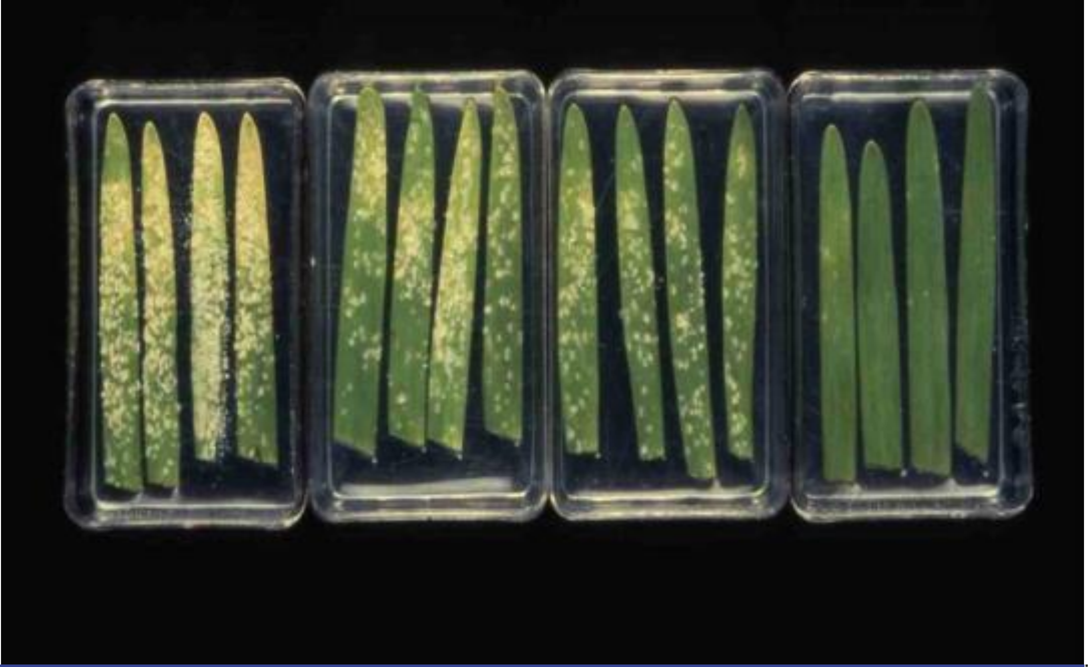
The James Hutton Institute



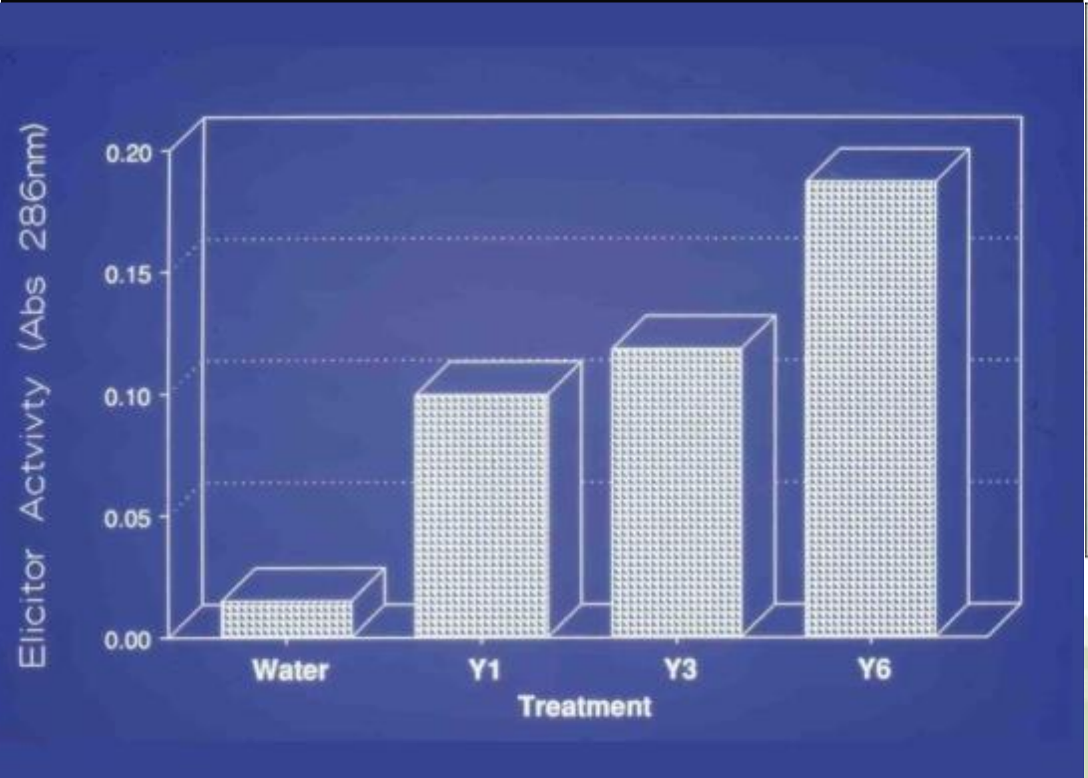
The James Hutton Institute

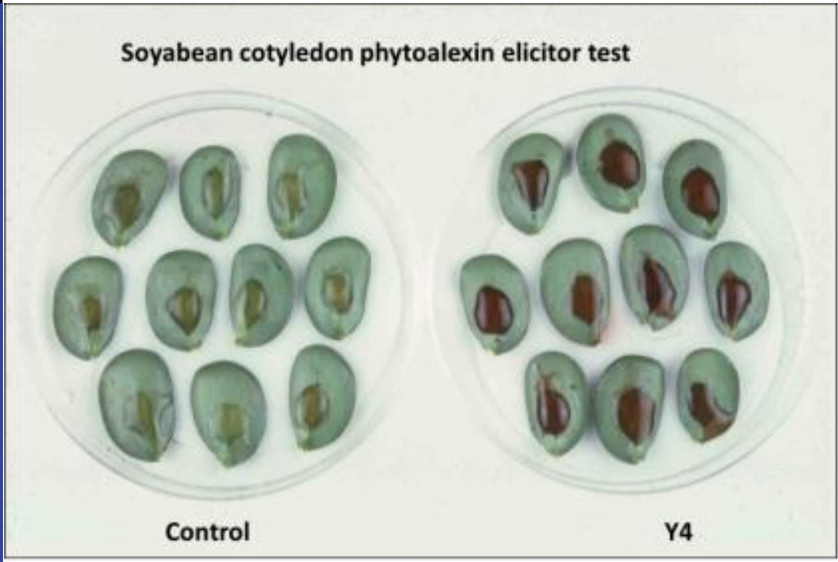
All about
response speed

Tony Reglinski



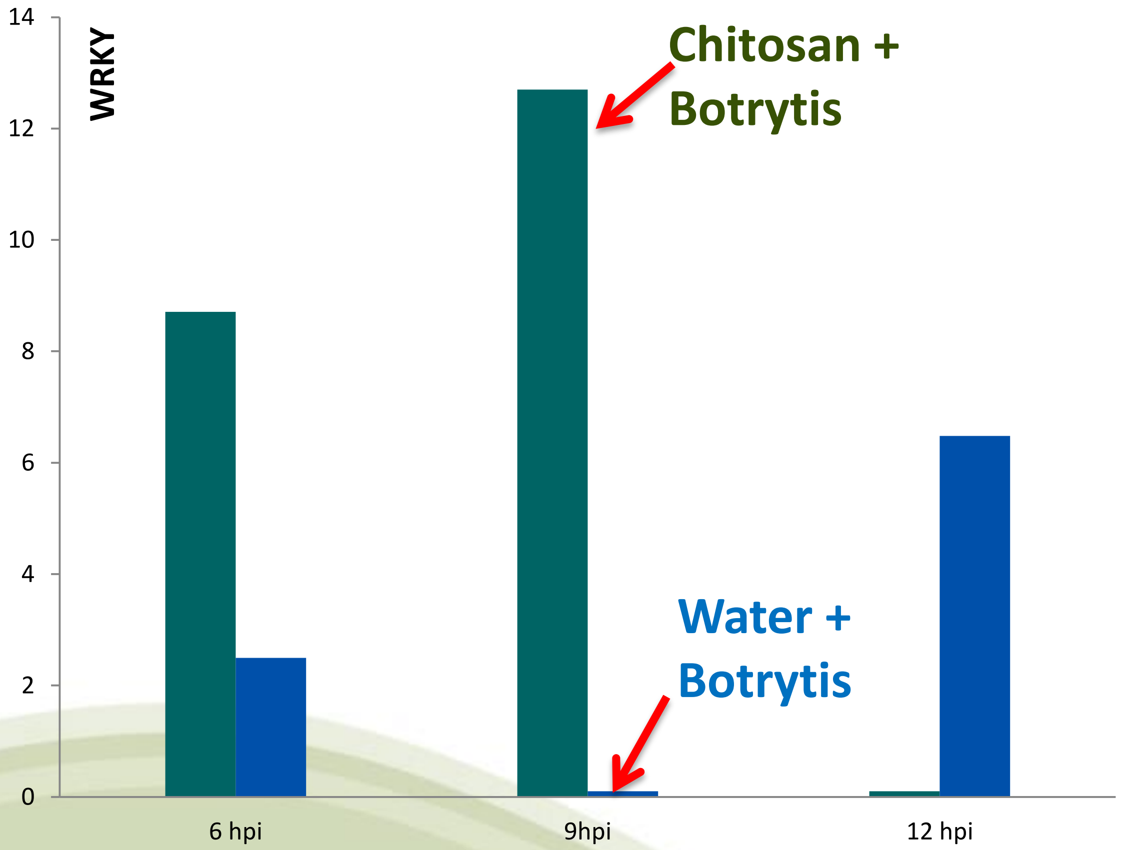
Resistance corresponds with ability to induce phytoalexins





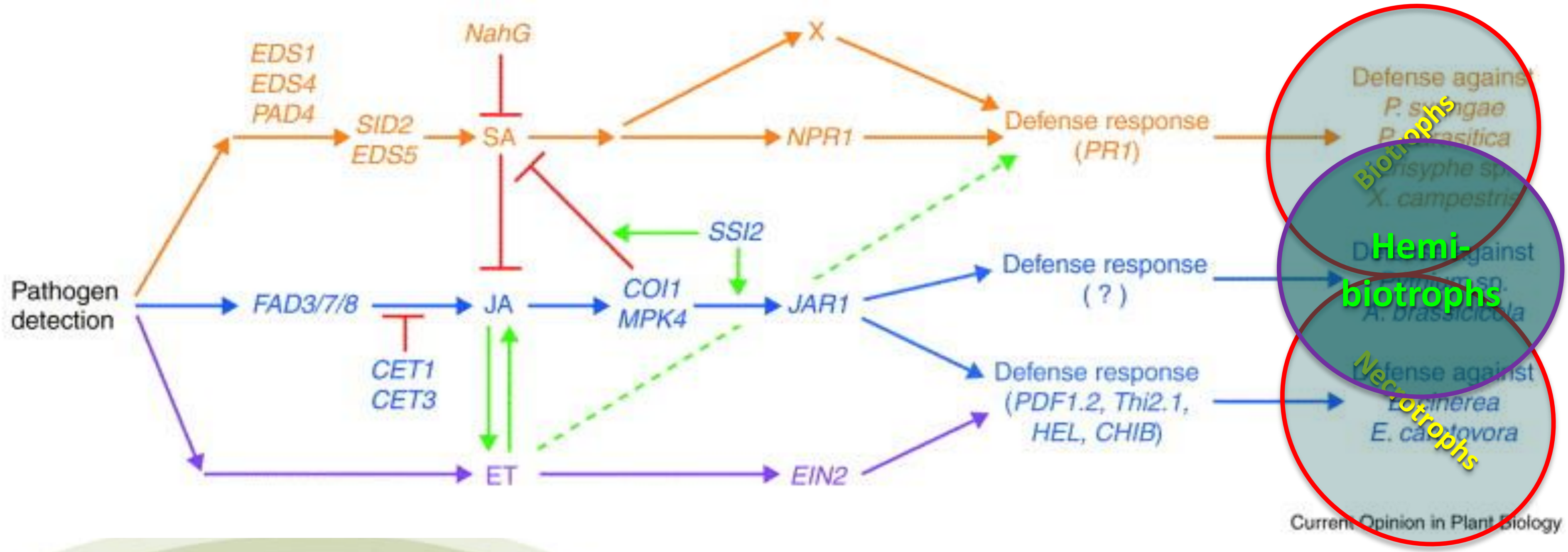
Differential gene expression of regulatory (priming)

(from microarray)



Which pathway(s) to induce for practical use?

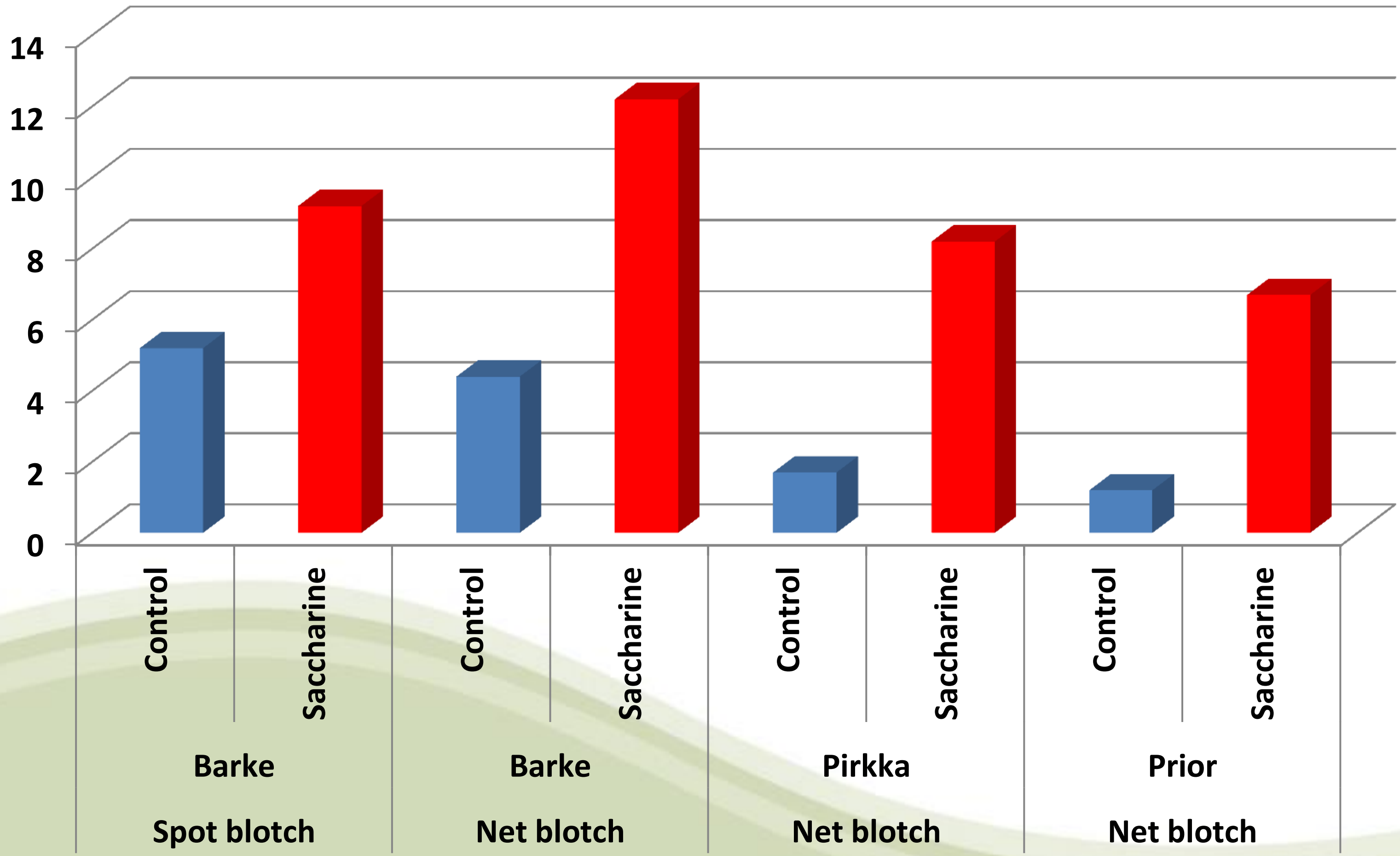
The James Hutton Institute



Biotrophs and necrotrophs respond differently

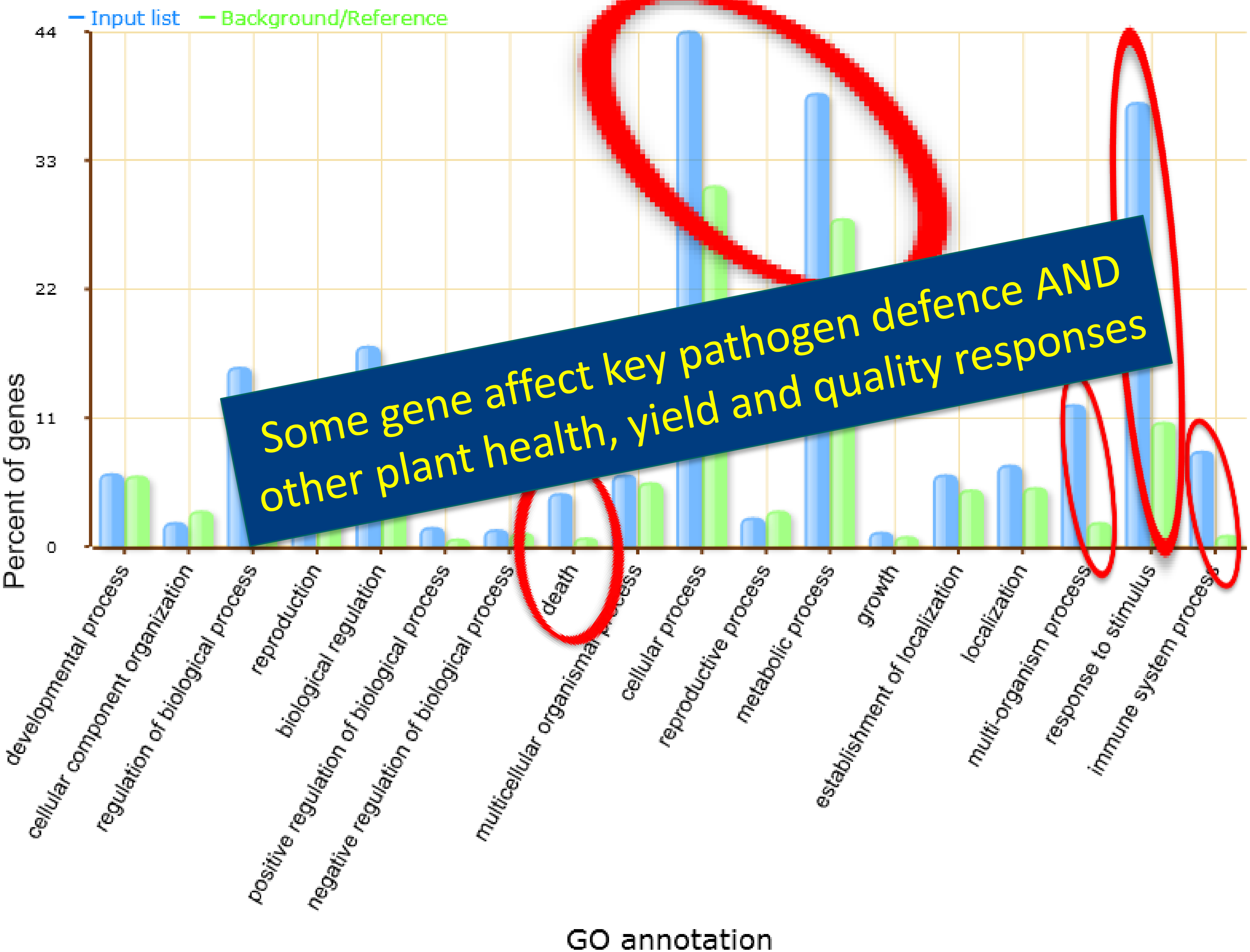
Saccharin induce resistance to *Blumeria graminis* f.sp. *hordei* and *Rhynchosporium commune* in barley (Boyle & Walters, 2006; Walters et al., 2009),

but induces susceptibility to the host-specific necrotrophic fungi *Pyrenophora teres* and *Cochliobolus sativus.*



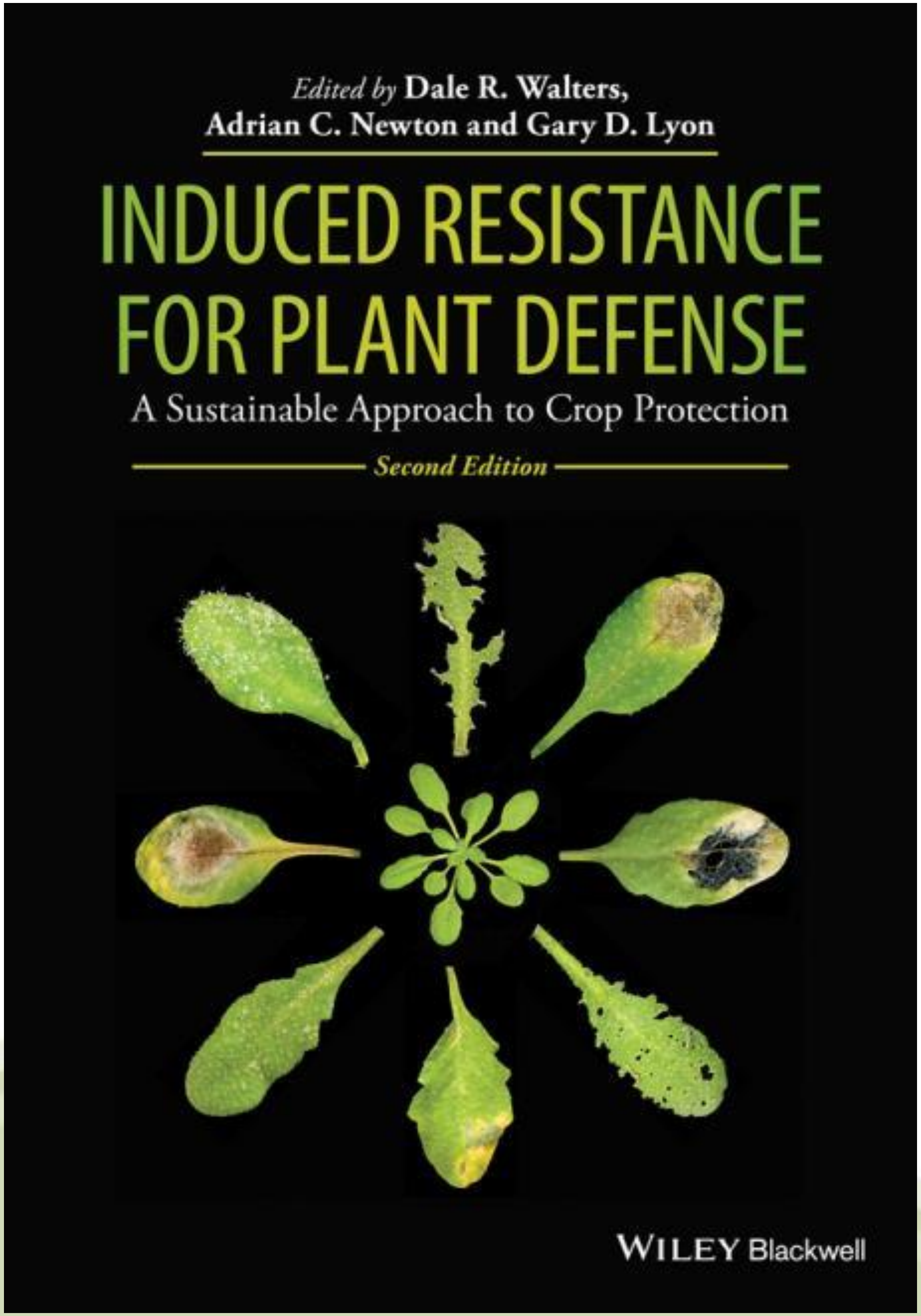
Inger Åhman, 2013. Breeding for inducible resistance. Proceedings of the meeting at Avignon, France, 10-13 June 2013, IOBC-WPRS Bulletin 89, 311-317.

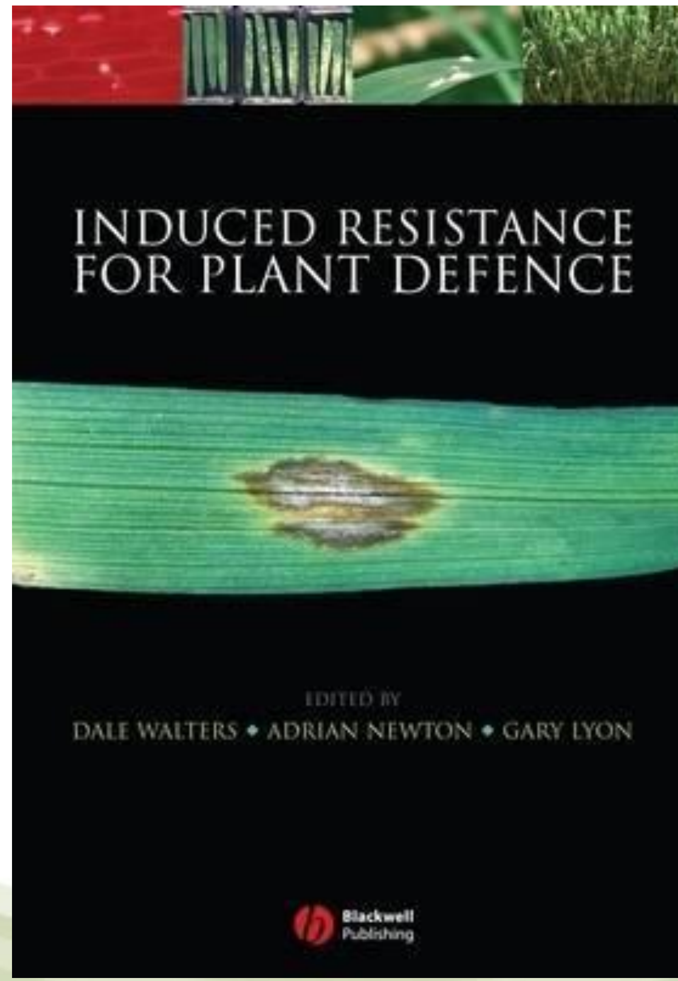
Effects of elicitors on gene expression



Some gene affect key pathogen defence AND other plant health, yield and quality responses

The James Hutton Institute





The James Hutton Institute

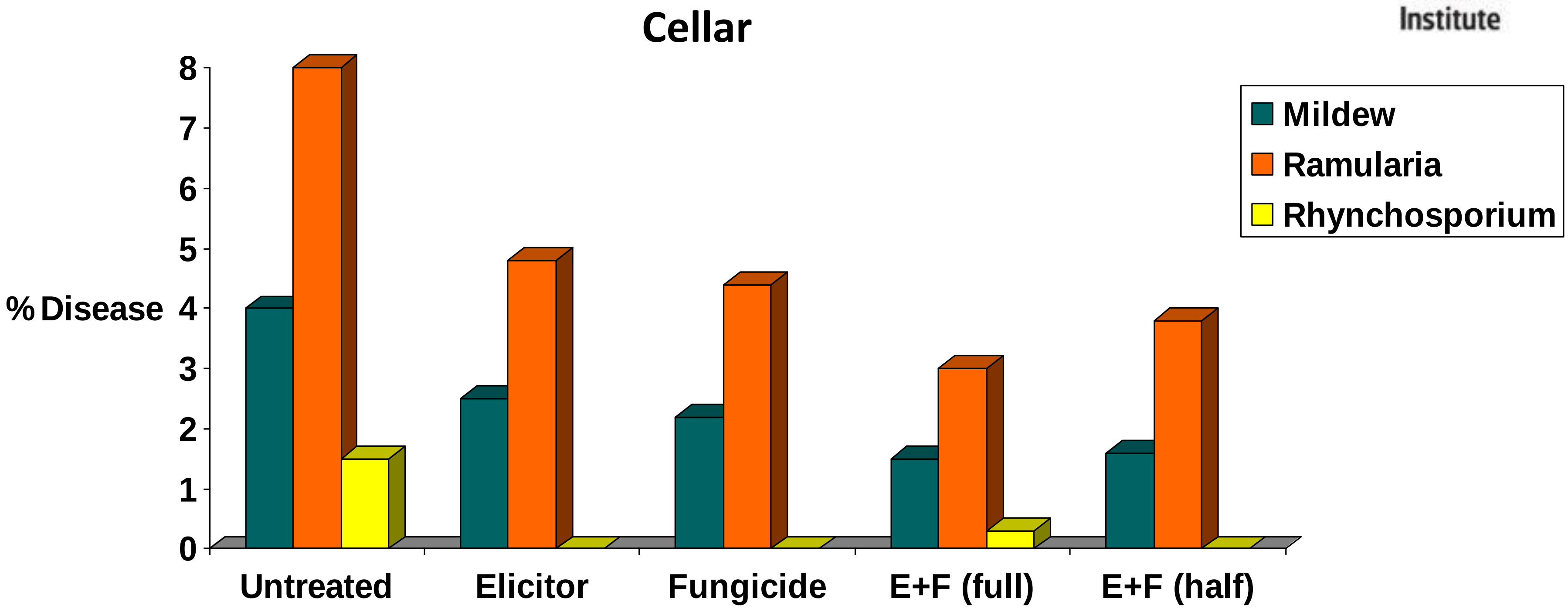
Resistance elicitors and their spectrum of efficiency

Elicitor class	Elicitor	Crop class	Crop species	Type of pathogen	Pathogen species	Reference
Signalling mimic	INA	Dicots	*Brassica oleracea*	Biotrophic oomycete	*Peronospora parasitica*	(van der Wolf et al., 2012)
	BTH	Dicots	*Vitis vinifera*	Biotrophic oomycete	*Plasmopara viticola*	(Perazzolli et a.,. 2008)
		Dicots	*Capsicum annuum*	Biotrophic bacterium	*Xanthomonas axonopodis*	(Yi et al., 2012)
		Dicots	*Capsicum annuum*	Virus	*Cucumber mosaic virus*	(Yi et al., 2012)
		Dicots	*Cicer arietinum*	Necrotrophic fungus	*Didymella rabiei*	(Sharma et al., 2011)
	BABA	Dicots	*Solanum lycopersicon*	Biotrophic fungus	*Oidium neolycopersici*	(Worrall et al., 2012)
	JA	Dicots	*Solanum lycopersicon*	Necrotrophic fungus	*Botrytis cinerea*	(Worrall et al., 2012)
PAMP mimic	Chitosan	Dicots	*Vitis vinifera*	Necrotrophic fungus	*Botryti… …ea*	(Trotel-Aziz et al., 2006)
	Bacterial extract	Dicots	*Brassica oleracea*	Bi…	*…arasitica*	(van der Wolf et al., 2012)
VOC	Nonanal				*…ringae pv.* …gae	(Yi et al., 2009)
	Limonene, Linalool Nonanal			…emibiotrophic fungus	*Colletotrichum lindemuthianum*	(Quintana-Rodríguez & Heil 2013)
Plant extract	of *Datura metel*	Monocots	*Pennisetum glaucum*	Biotrophic oomycete	*Sclerospora graminicola*	(Devaiah et al., 2009)
	of *Ascophyllum nodosum*	Dicots	*Cucumis sativus*		*Alternaria cucumerinum, Fusarium oxysporumg*	(Jayaraman et al., 2011)
	of *Vitex negundo*	Monocots	*Oryza sativa*		*Xanthomonas oryzae*	(Nisha et al., 2012)
	of 'Zimmu' (Allium sp.)	Monocots	Banana fruits		*Lasiodiploida theobromae, Colletotrichum musae &*	(Nisha et al., 2012)

e.g. Bion

Elicitors and fungicides:

differential effects on barley diseases

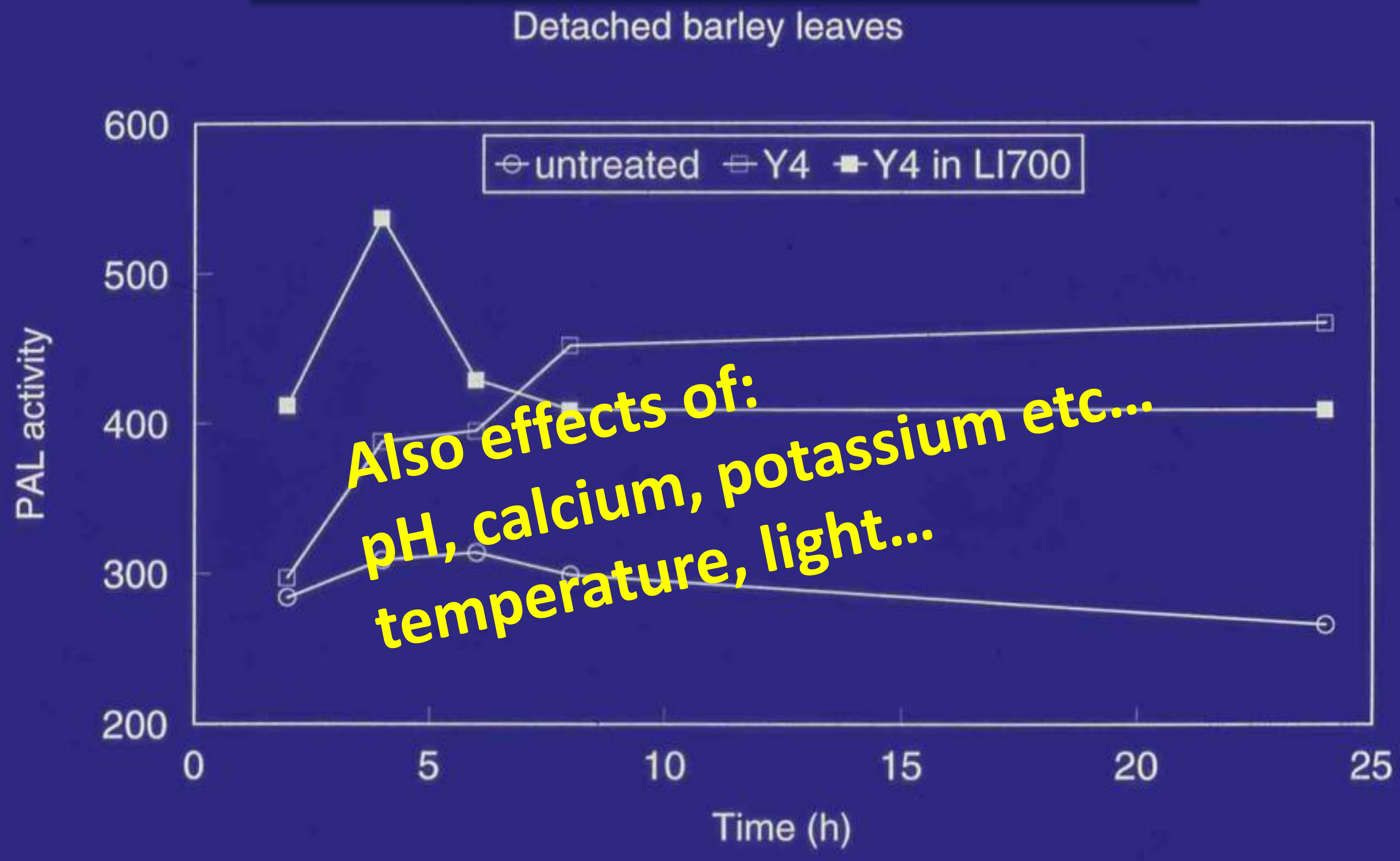


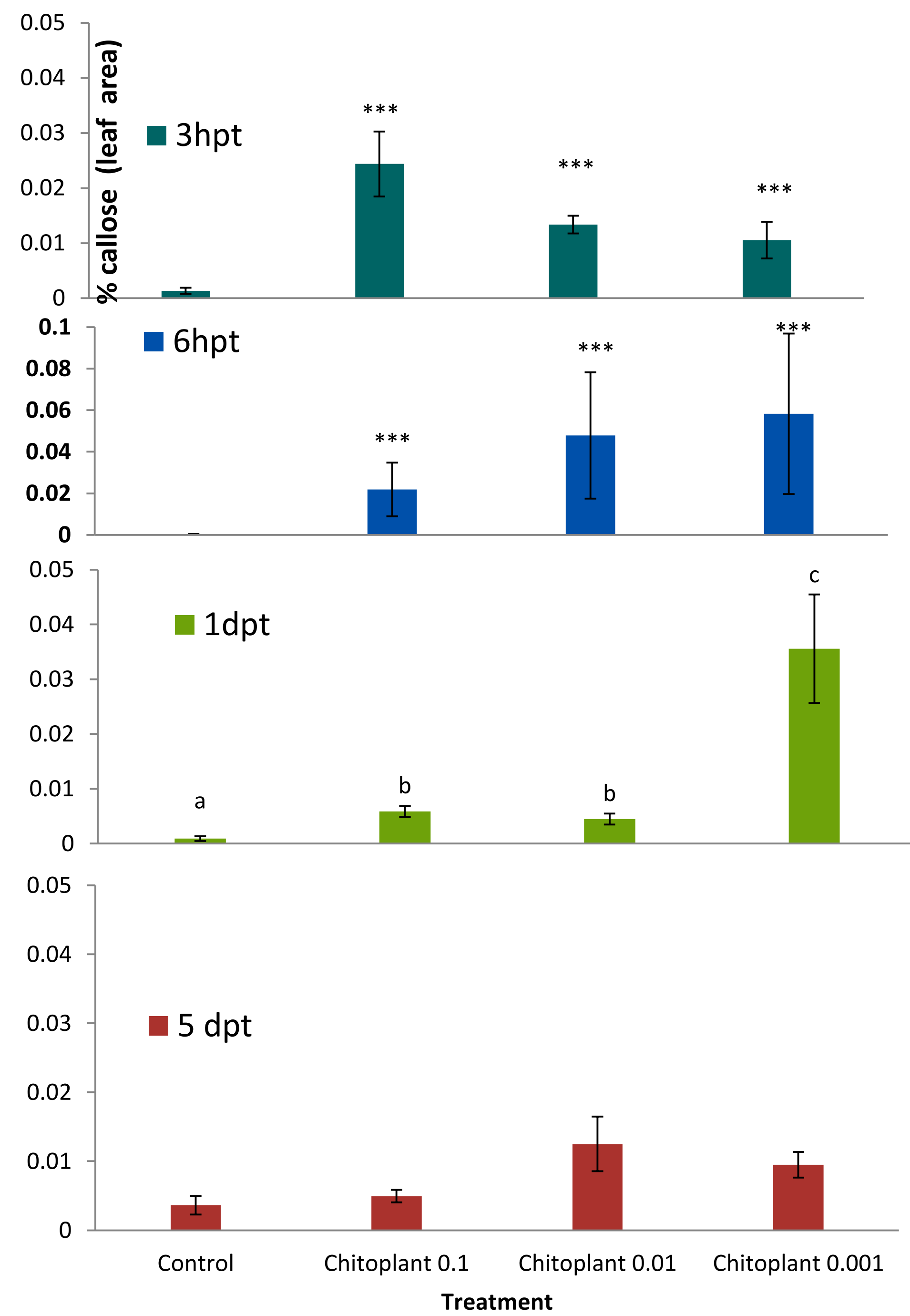
Elicitor = *cis*-jasmone + BABA; associated with > activities of defence enzymes

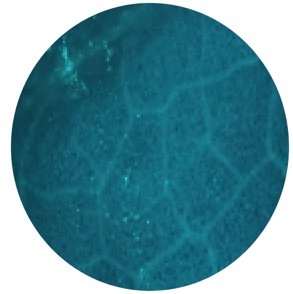
Mildew – E applied GS24; F at GS31+39; E+F together at GS24; E+F (0.5) together at GS39

Dale Walters, SRUC, Edinburgh

Delivery of the effect *in planta*





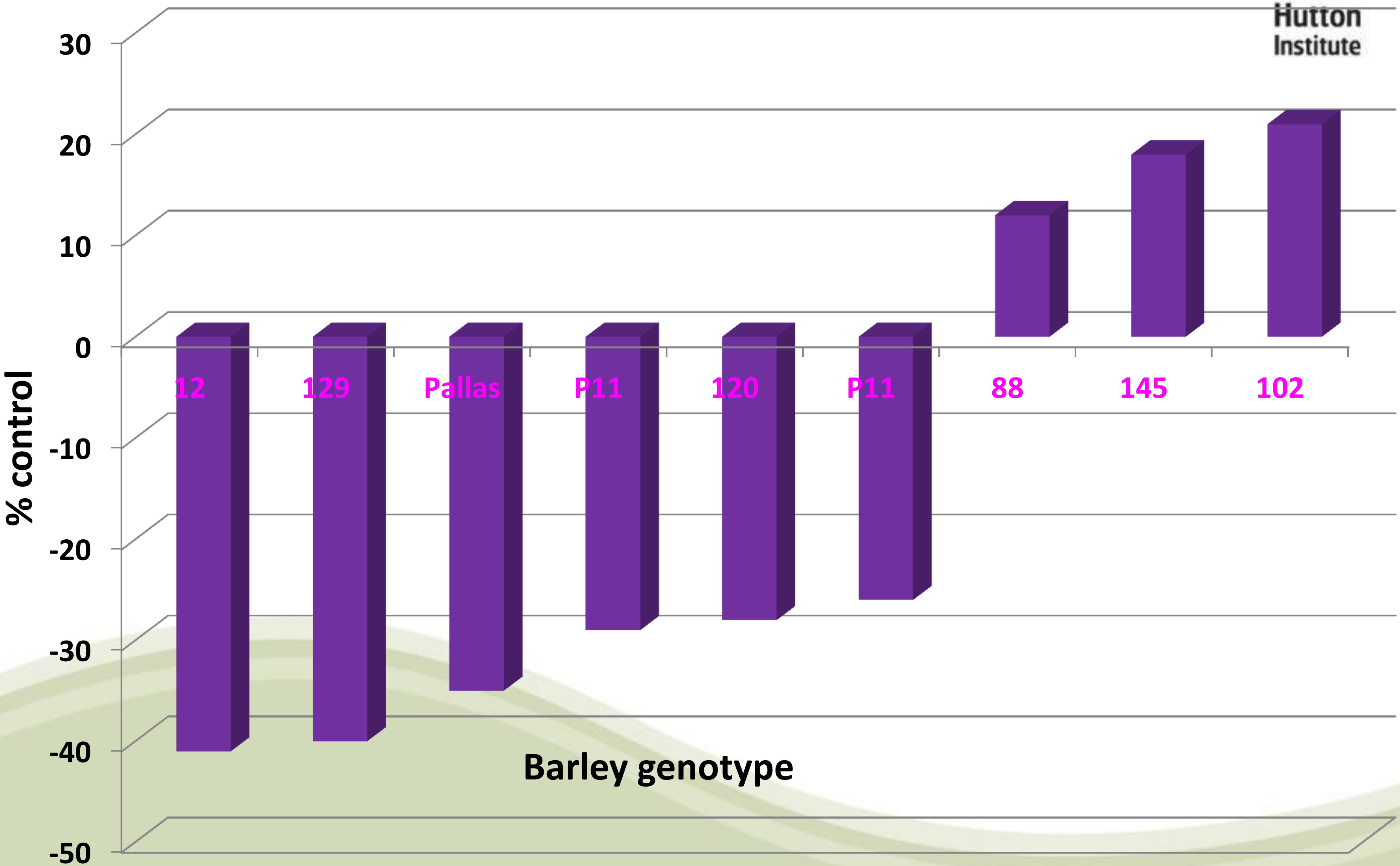


Basal callose deposition induced by Chitosan-IR in tomato cv. Money-Maker

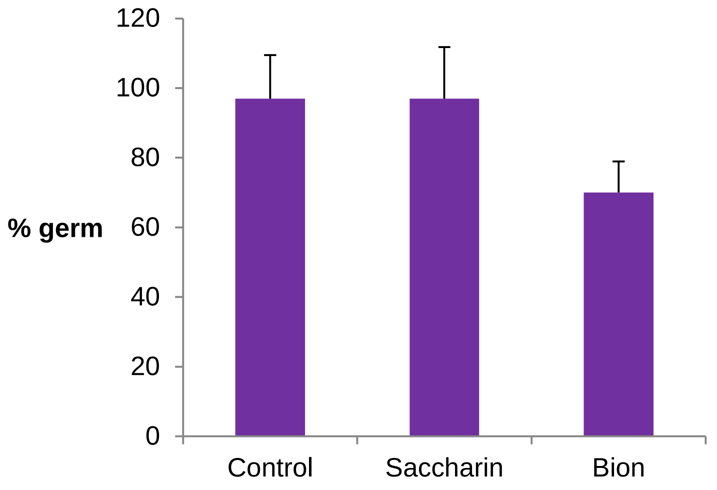
No clear dose-response relationship (Y4 also)

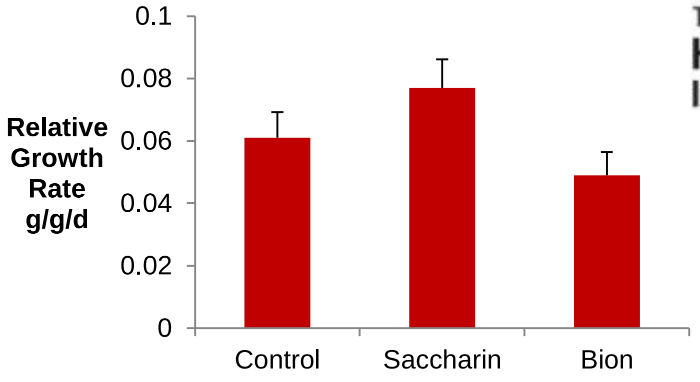
Daniel De Vega Perez

Barley genotype response to elicitors – mildew



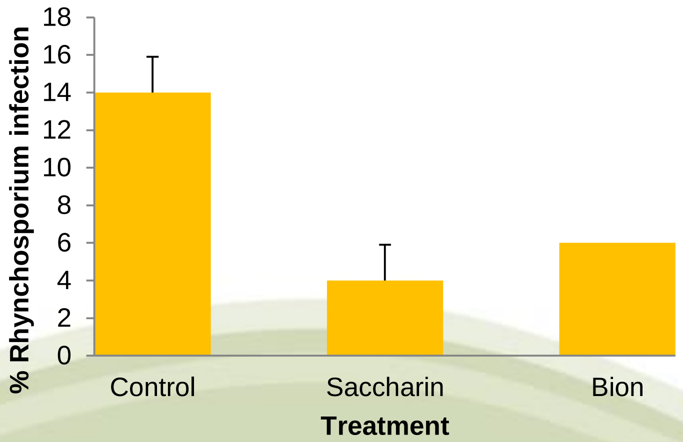
Trans-generational effects of elicitors





- First generation plants treated with saccharin or Bion

- Saccharin had no effect on seed germination or seedling growth rate of progeny (but Bion reduced both)



- **Treatment of parents with saccharin or Bion led to reduced Rhynchosporium infection in progeny**

Epigenetic mechanism: DNA methylation (Jurriaan Ton *et al*. with BABA induction)

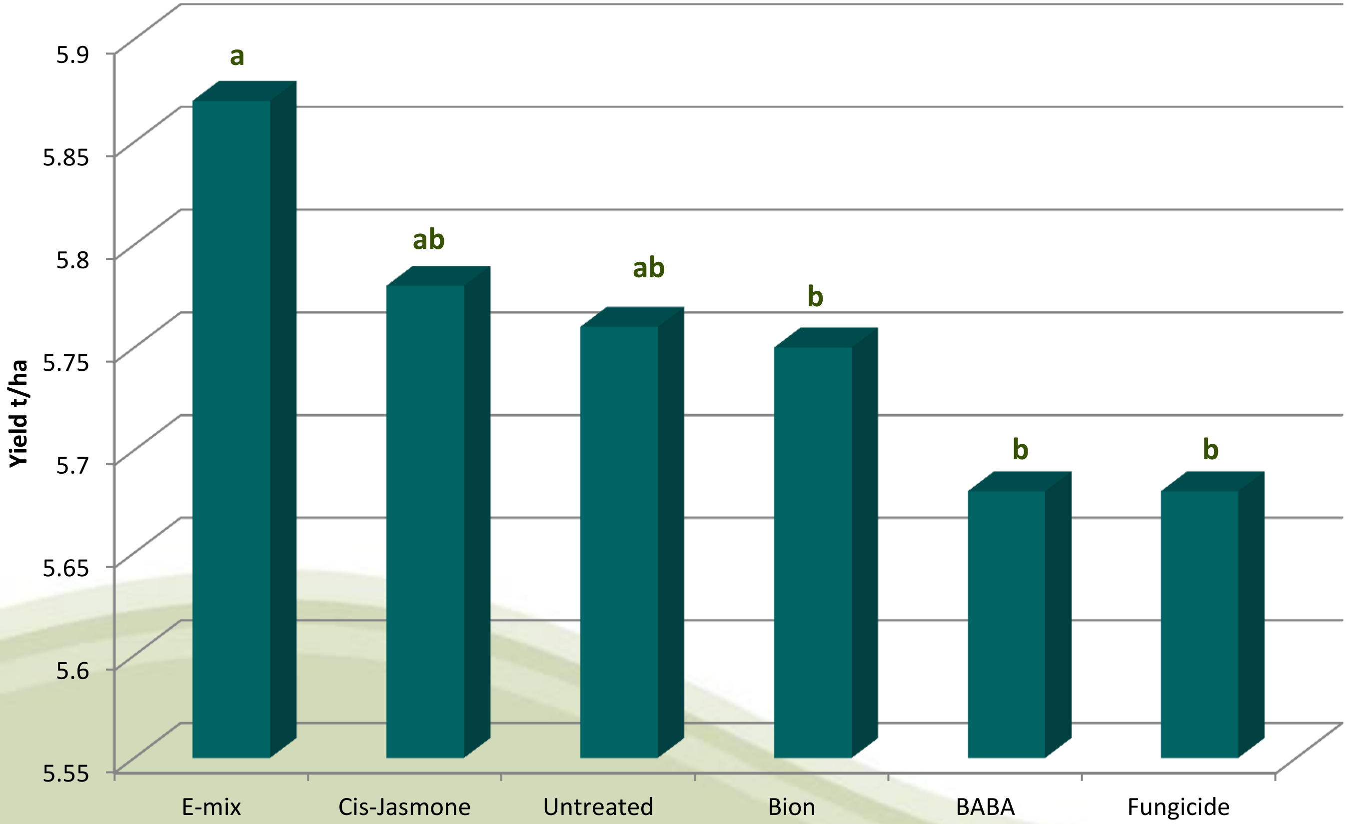
Dale Walters, SRUC, Edinburgh

Trans-generational effects of elicitors

The James Hutton Institute

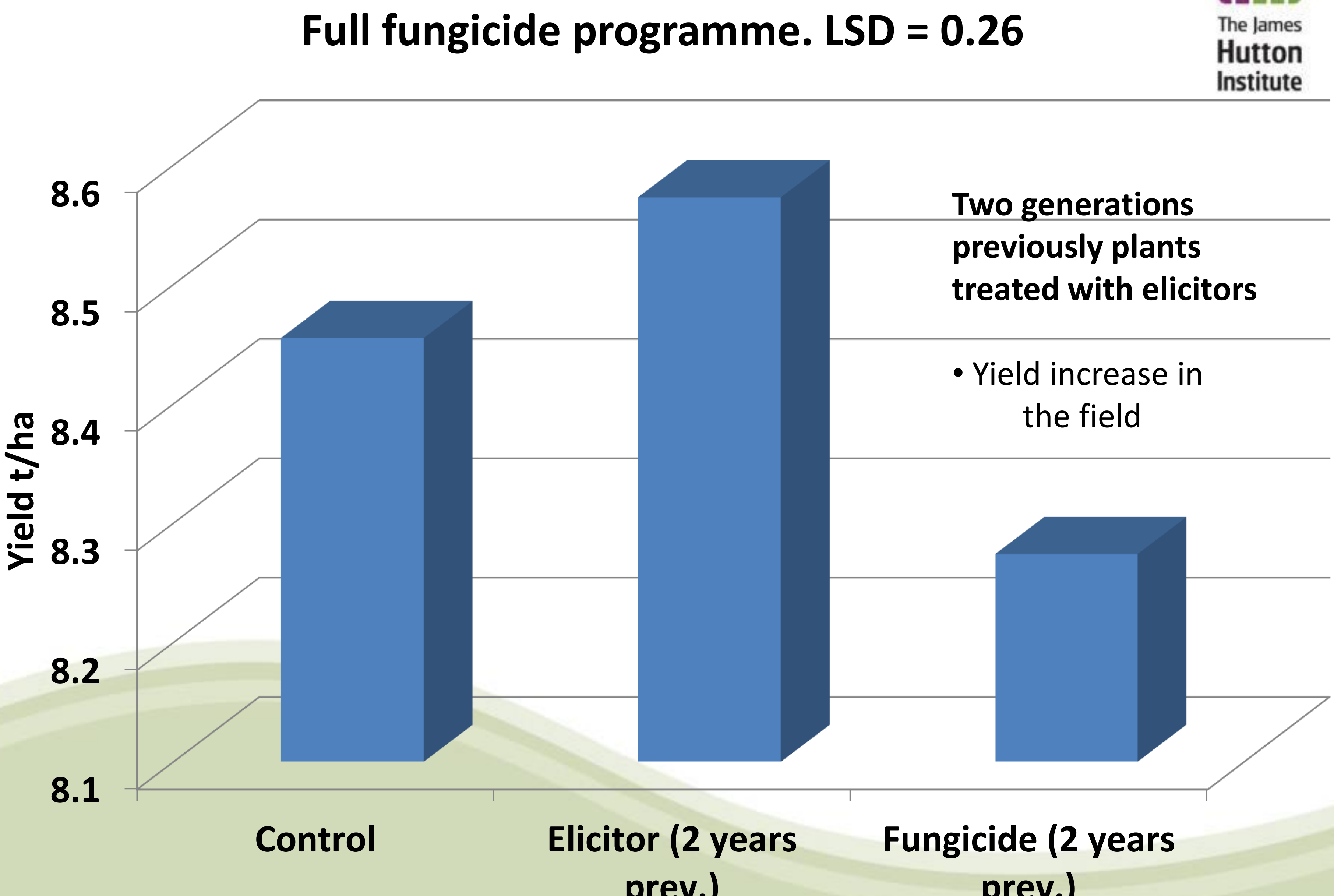
First generation plants treated with elicitors

- Yield increase of **winter barley** in the field



Gravouil & Newton, JHI

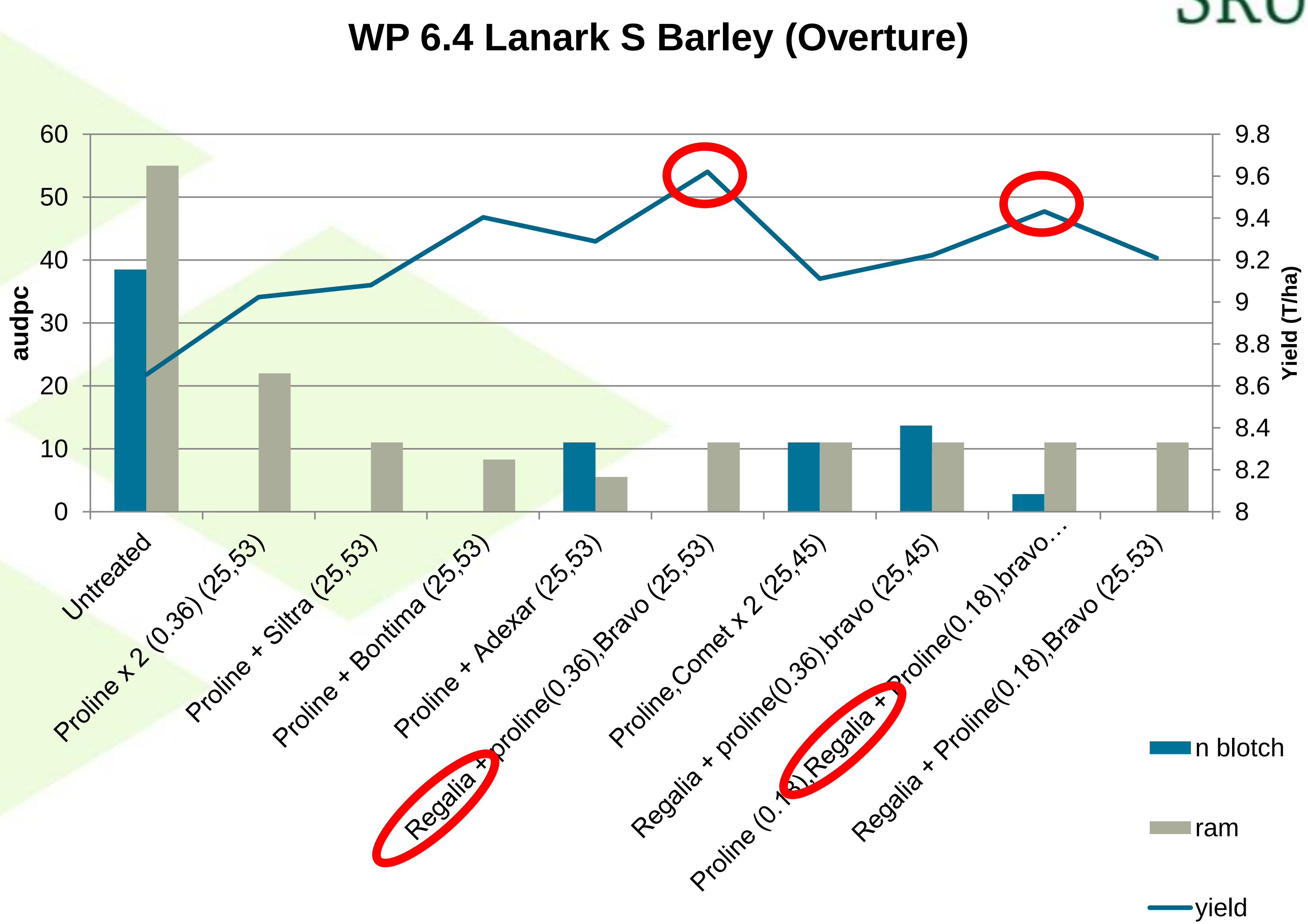
Trans-generational effects of elicitors: **wheat** 2015



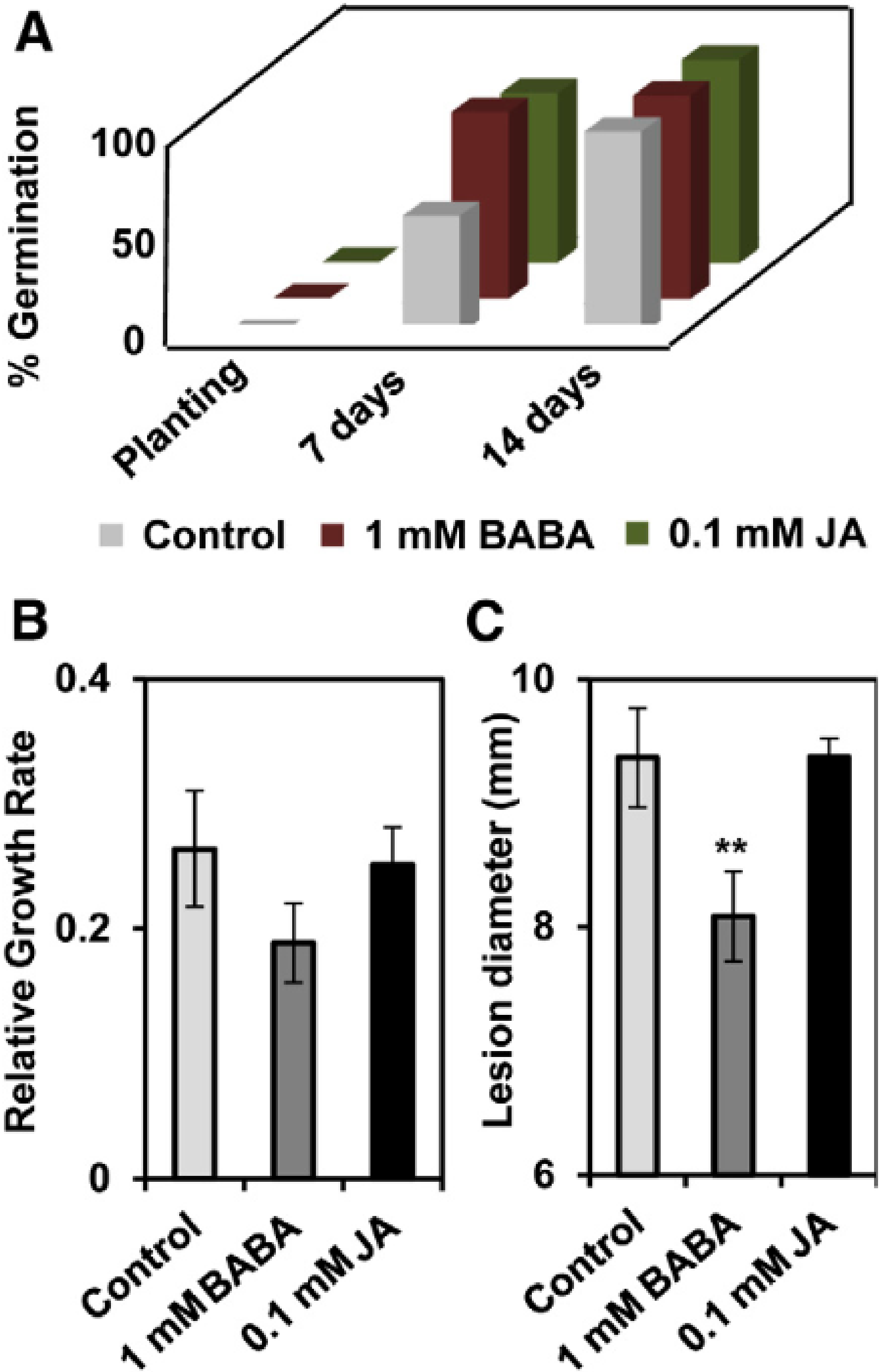
Two generations previously plants treated with elicitors

- Yield increase in the field

Spring barley trials 2014

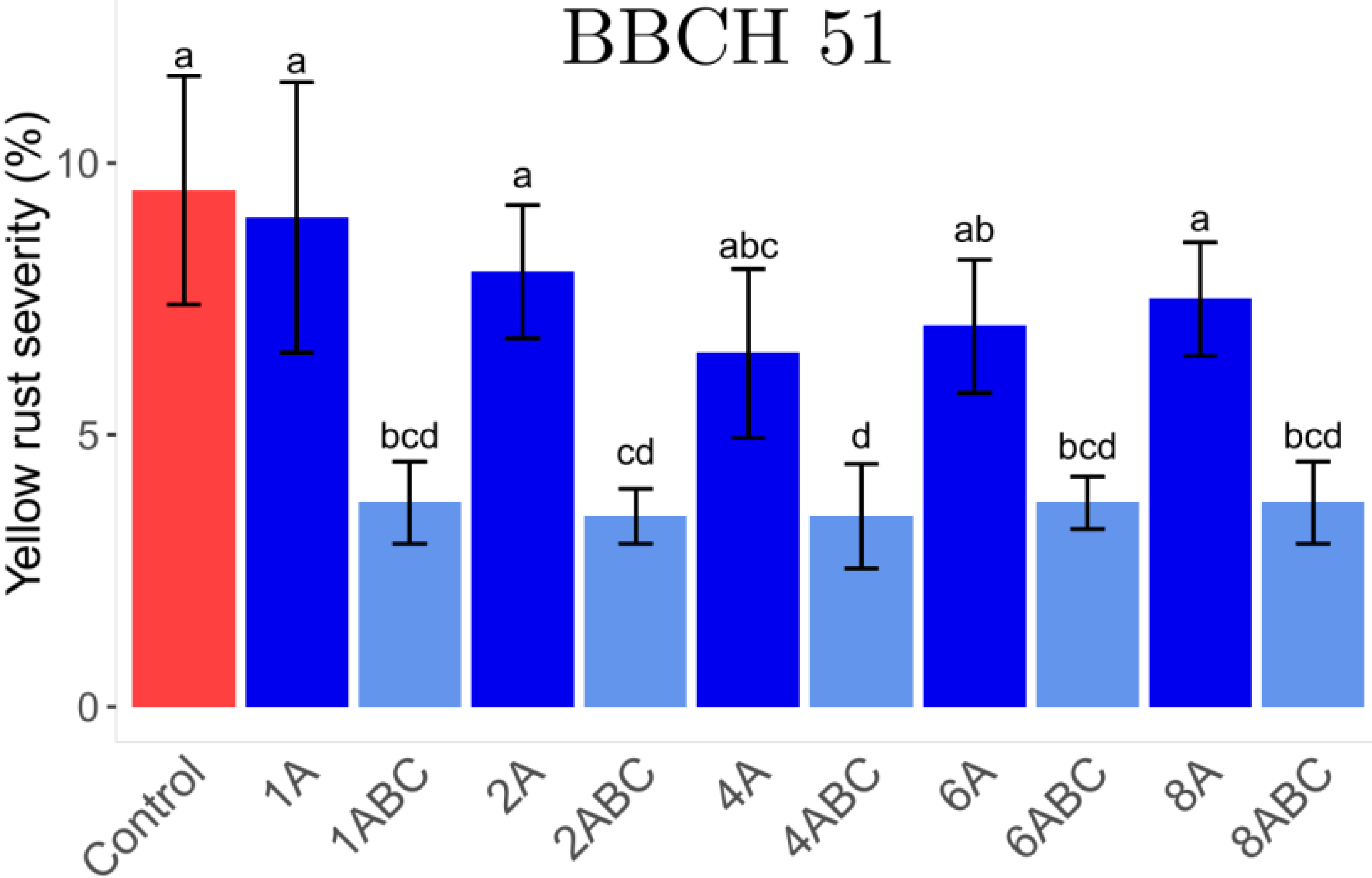


Seed coating?



Estrella Luna and Emily Beardon, 2016. Optimizing chemically induced resistance in tomato against *Botrytis cinerea*. Plant Disease 100, 704-710.

Fig. 3. Effects of seed coating with β-aminobutyric acid (BABA) and jasmonic acid (JA) on soil-grown tomato. Seed were coated in 2% carboxymethyl cellulose (CMC) solution containing water (control), 1 mM BABA, or 0.1 mM JA. Two days later, seeds were planted in soil and plants were grown for 3.5 weeks until challenge inoculation with *Botrytis cinerea*. **A,** Percentages of germinated seeds at 0, 7, and 14 days after planting. No statistically significant differences in germination efficiency were detected between treatments (Fisher's exact tests, $\alpha = 0.05$; $n = 12$). **B,** Average relative growth rates (in centimeters per centimeter per week; ± standard error of the mean [SEM]) between 2 and 3 weeks after planting of coated seed. No statistically significant differences were detected between treatments (t tests, $\alpha = 0.05$; $n = 8$). **C,** Average lesion diameters (in millimeters; ± SEM) at 2 days after inoculation of 4-week-old plants with *B. cinerea*. Seeds were coated with CMC containing water (control), 1 mM BABA, or 0.1 mM JA. Double asterisk indicates a statistically significant difference compared with control-treated plants (t tests, $P < 0.01$; $n = 8$).



The James Hutton Institute

Severity (%) of yellow rust on winter wheat during the 2014 growing season, observed at growth stages 51. X axis labelling: numbers indicate the amount of biofungicide (Bayer: Serenade) applied (l/ha); capital letters from A to D indicate application time point. Error bars represent standard errors and different letters represent significant differences between treatments (P ¼ 0.05). Log transformation was applied prior to statistical analysis.

Antje Reiss and Lise N Jørgensen, 2017. Biological control of yellow rust of wheat (*Puccinia striiformis*) with Serenade ® ASO (*Bacillus subtilis* strain QST713). Crop Protection 93, 1-8.

(Bayer: "Serenade Max is a bio-fungicide/bactericide to aid in the control and suppression of powdery mildew and botrytis and sour rot in grapes, fireblight in pipfruit, botrytis on berryfruit, kiwifruit, persimmons and onions, and various diseases on avocados, citrus, lettuce and turf.")

Inhibitory and cultivar effects of some elicitors

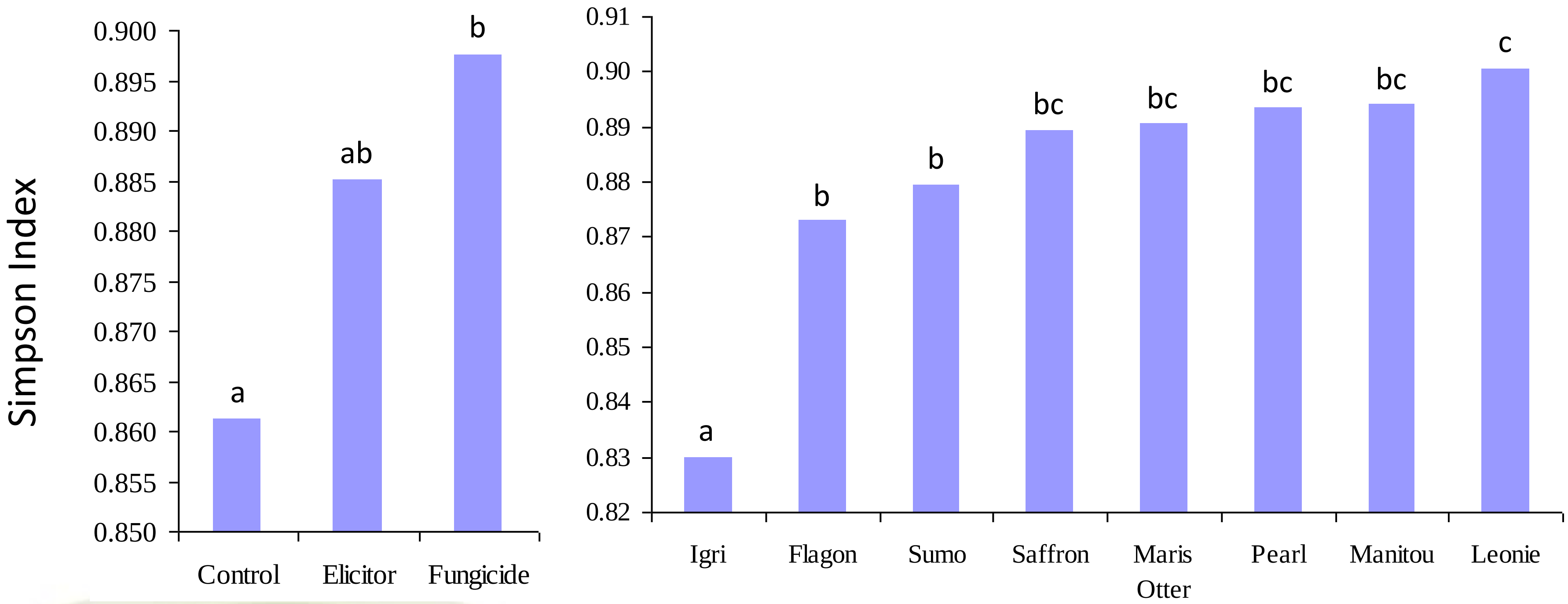
2-week-old tomato cv. Motelle and Money-maker seedlings 1 week after elicitor treatment. BABA-treated plants were smaller than the rest of the treatments and Motelle-BABA treated plants were even smaller than Money-maker BABA-treated seedlings.

Recent reports on:
Costs of resistance induction with elicitors

Elicitor class	Elicitor	Crop class	Crop species	Environment	Type of cost reported	Reference
Signalling mimic	BTH	Monocots	Zea mays	Field	Reduced ear filling and yield	(Small et al., 2012)
		Monocots	Hordeum vulgare	Field	Reduced germination rates in seeds from treated plants	(Walters & Paterson, 2012)
		Dicots	Cicer arietinum	Field	Yield reductions after multiple BTH application	(Sharma et al.,. 2011)
			Arabidopsis	Laboratory	Reduced growth rate and yield	(van Hulten et al., 2006)
	SA	Dicots	Vicia faba	Field	Reduced number of pods and yield	(El-Hendawy et al.,. 2010)
	BABA	Dicots	Arabidopsis	Laboratory	Reduced growth rate and yield	(van Hulten et al., 2006)
	JA	Monocots	Zea mays	Pots in laboratory	Transient decrease in root growth	(Feng et al.,,2012)
		Dicots	Glycine max	Greenhouse	Reduced seed production and germination rates	(Accamando & Cronin, 2012)
	MeJA	Dicots	Hamelia patens	Field	Reduced fruit palatability and removal rate	(Whitehead & Poveda, 2011)
		Dicots	Solanum lycopersicon	Greenhouse	Phytotoxicity and delayed flowering	(Boughton et al., 2006)
PAMP	Fungal	Dicots	Pinus sylvestris	Laboratory	Reduced rooting and survival of seedlings	(Lu et al., 2011)

Microbial Diversity: the Simpson Index

The Simpson Index ranges from 0 (population dominated by one OTU) to 1 (all OTUs are equally present)



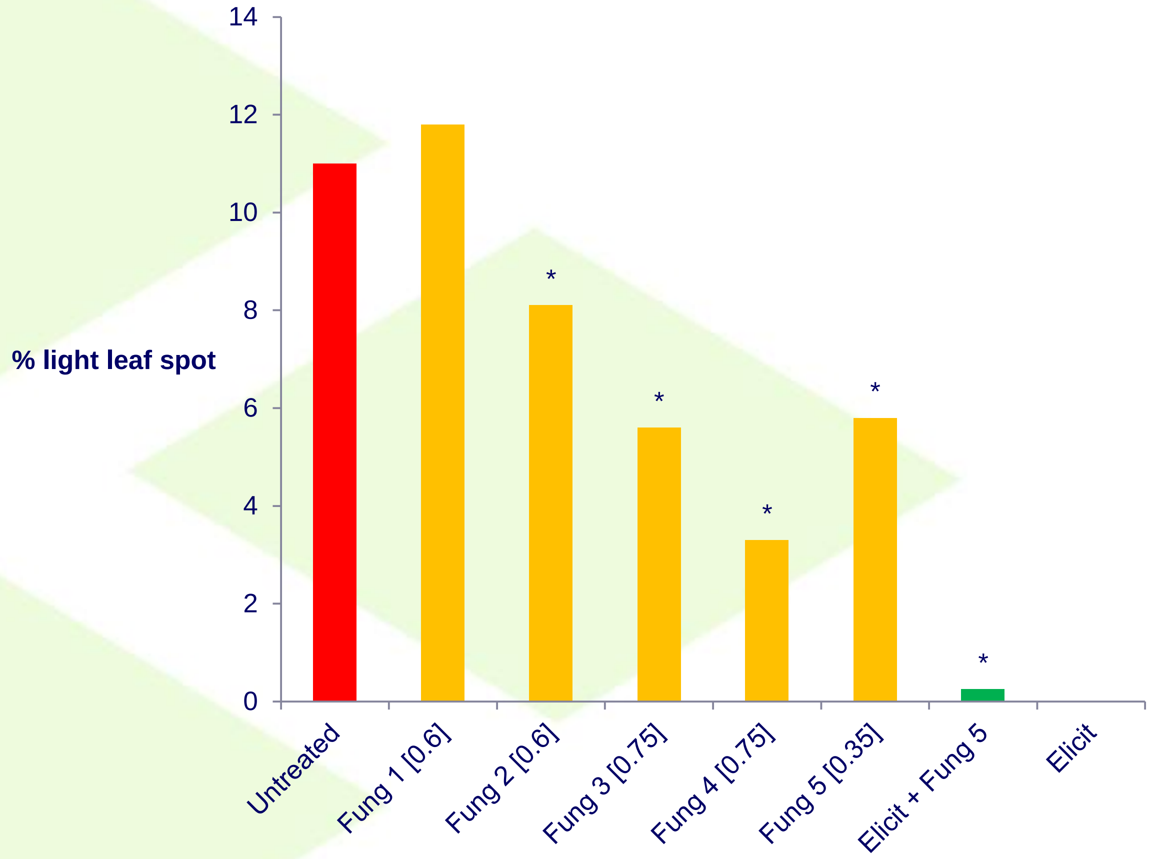
- Treatments significantly (P=0.047, LSD=0.027) affect the microbial diversity by levelling the relative abundance of present OTUs.
- Similarly, there is a significant (P<0.001, LSD=0.021) cultivar effect on the microbial diversity

Gravouil & Newton, JHI

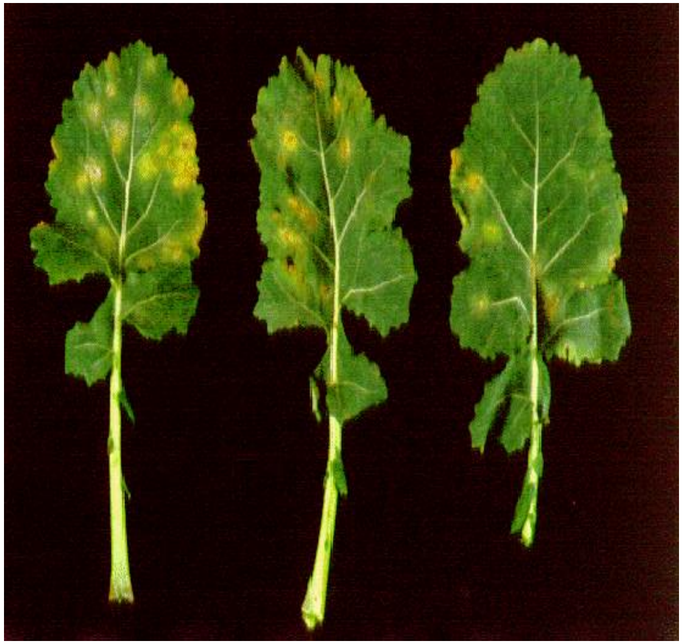
Quick examples…

Using elicitor combinations

SRUC



Elicitor combination controls light leaf spot on winter oilseed rape



Elicitors applied in autumn and early spring

Oxley & Walters (2012) Crop Protection **40,** 59-62

Light leaf spot control - OSR

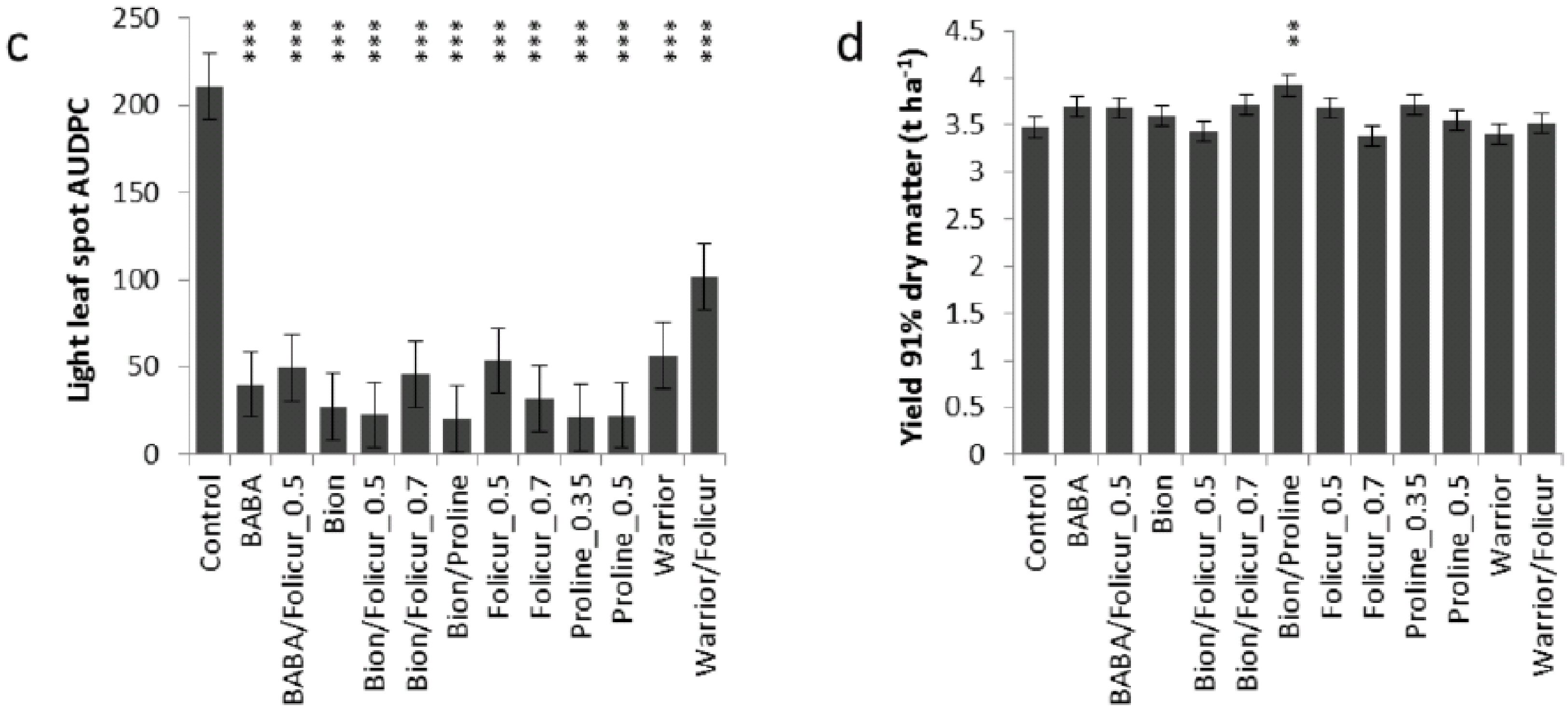
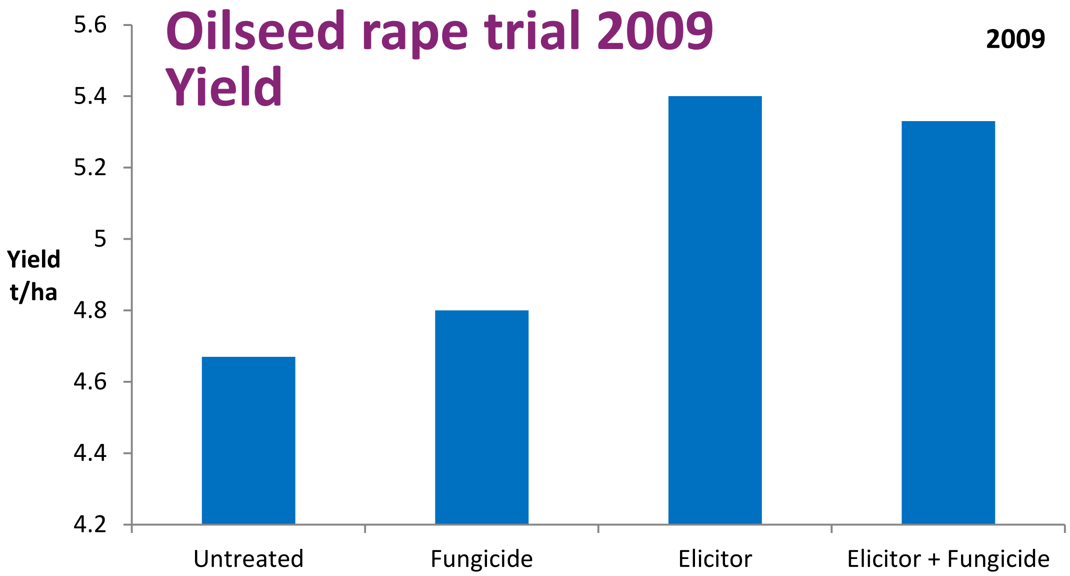


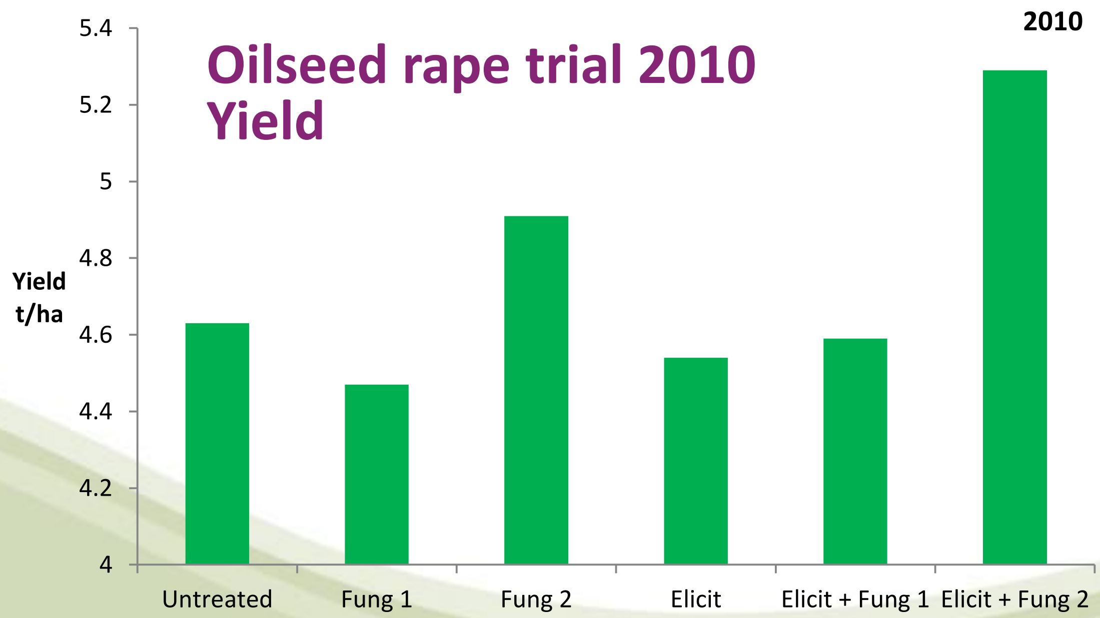
Fig. 1 Field performance of elicitor treatments on winter oilseed rape (WOSR) crops. Effects of elicitors on light leaf spot development measured as the area under the disease progress curve (AUDPC) in 2012-13 (a), 2013-14 (c), 2014-15 (e) and WOSR yield at 91% dry matter in 2012-13 (b), 2013-14 (d), 2014-15 (f). Bars indicate standard error. *** = *P* <0.001; ** = *P* <0.01; * = *P* <0.05.

A



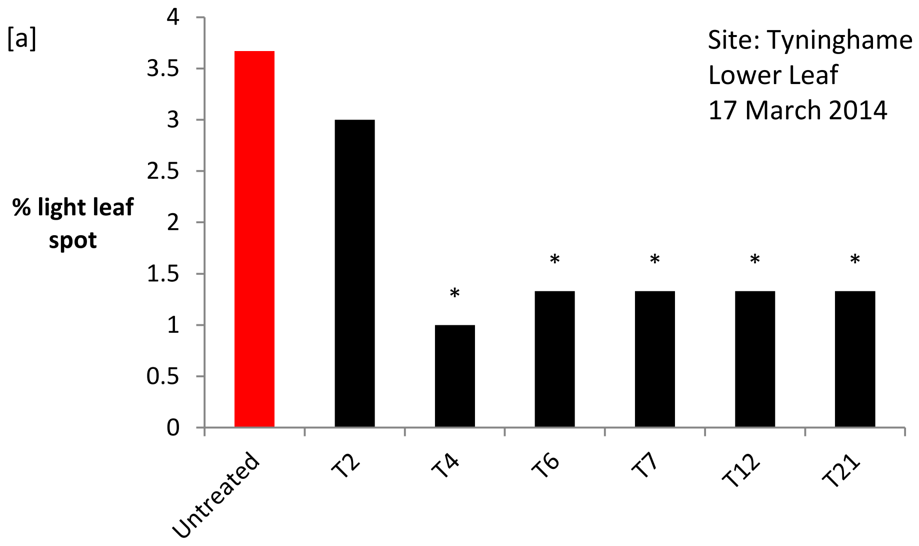
The James Hutton Institute

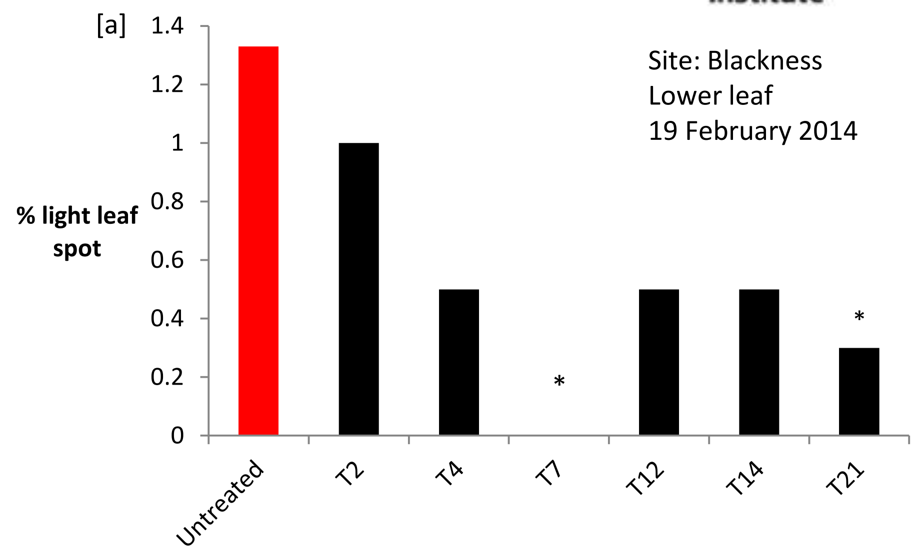
B



Dale Walters, SRUC, Edinburgh

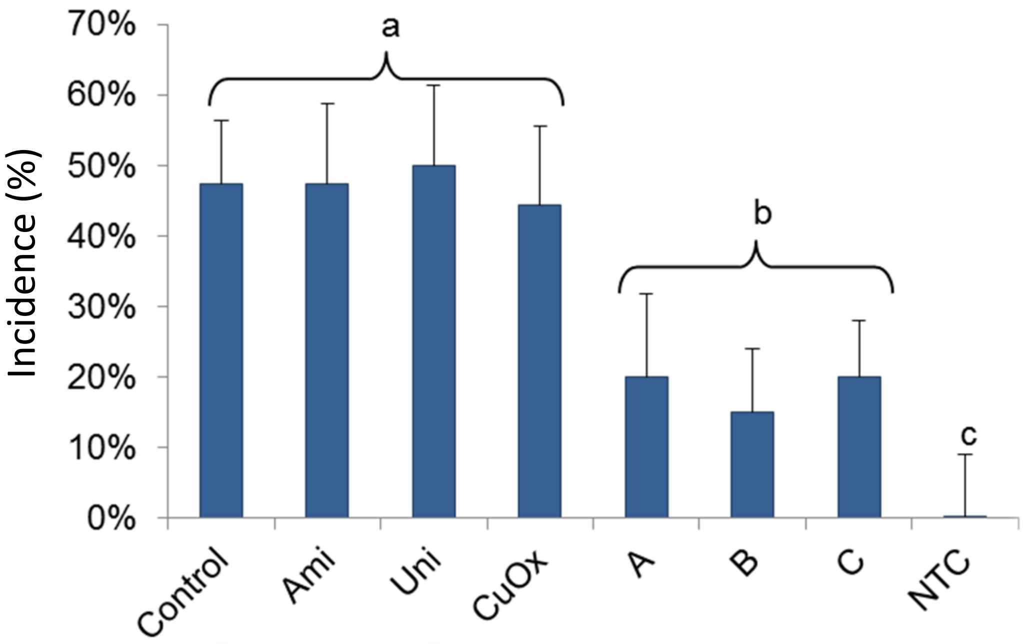
Brussel-Sprouts var. Aurelius





T2 = fungicide programme
T4 = alternate Bion + fungicides
T6 = alternate SiTKO-SA + fungicides
T7 = Bion only – 6 applications
T12 = Bion only – 3 applications
T19 = Bion + Companion – 3 applications
T21 = Bion + Regalia only – 3 applications

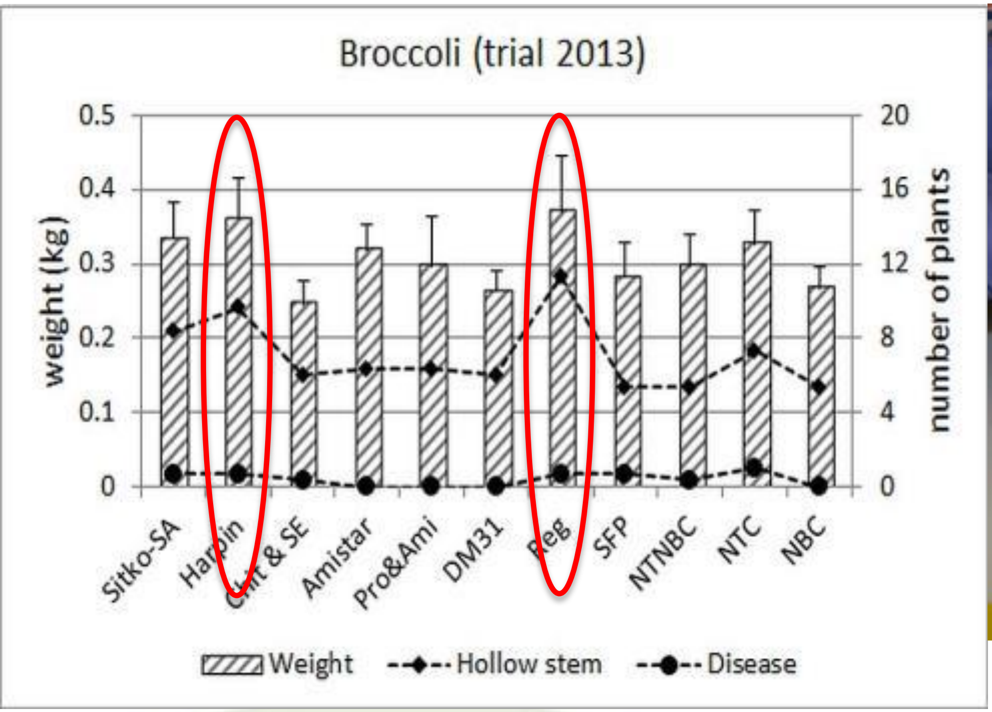
Control of *Bga* in red onion bulbs with fungicides and elicitors



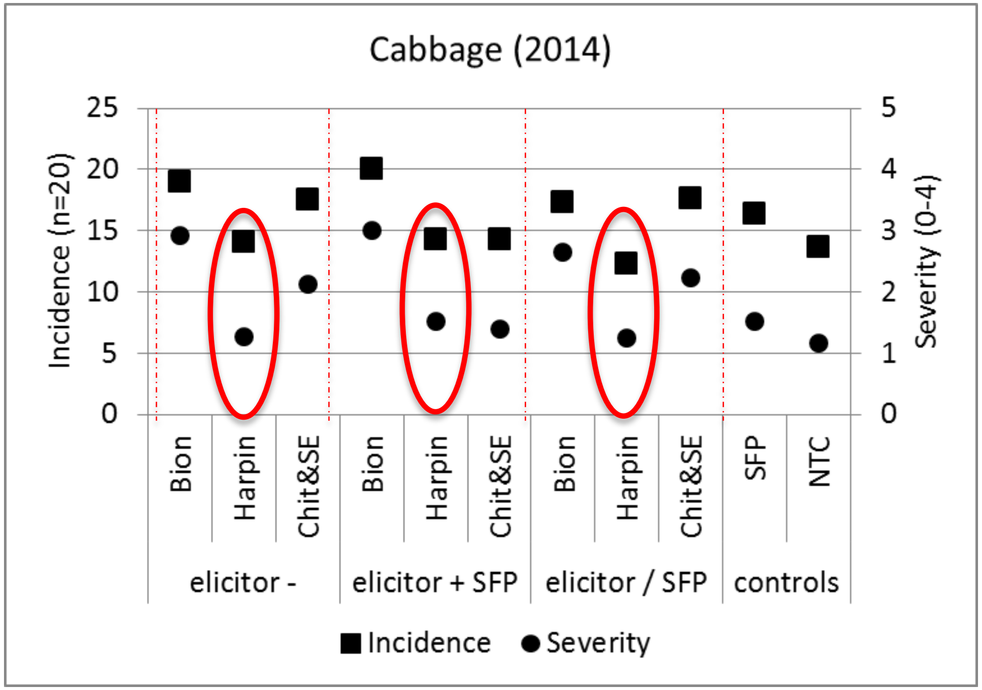
Significant decrease in the incidence and the extent of *Bga*-infected onion bulbs for elicitor treatments compared to fungicides

Hollow stem & stem rot in broccoli

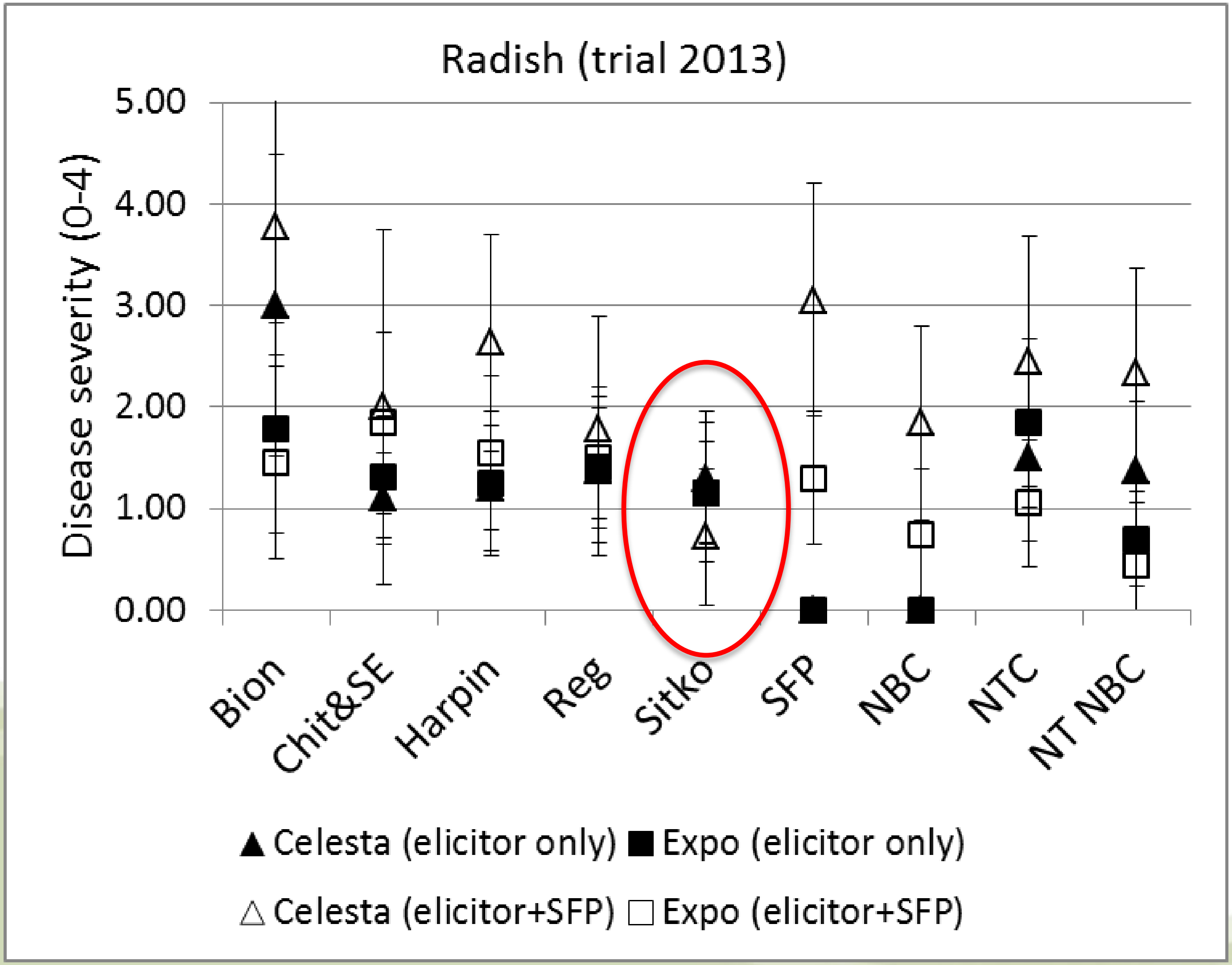
The James Hutton Institute



Cabbage – bacterial rot

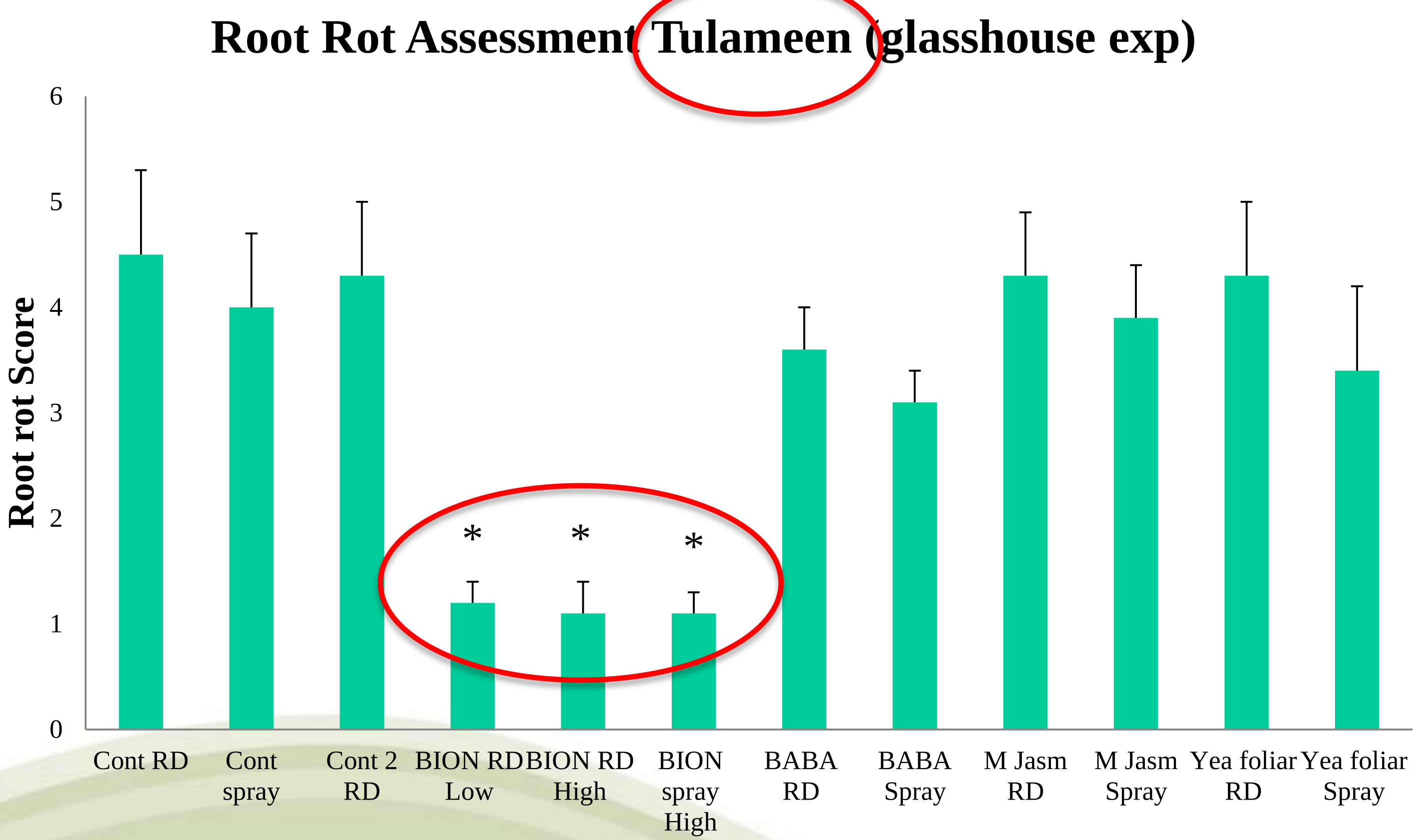


Radish



Radish (trial 2013)
Disease severity (0-4)
5.00
4.00
3.00
2.00
1.00
0.00
Bion
Chit&SE
Harpin
Reg
Sitko
SFP
NBC
NTC
NT NBC
Celesta (elicitor only)
Expo (elicitor only)
Celesta (elicitor+SFP)
Expo (elicitor+SFP)

Phytophthora root rot



Root Rot Score

0 = no symptoms
1 = 0 -3 % roots rotted
2 = 4 – 25 % roots rotted
3 = 26 – 50 % roots rotted
4 = 51 – 75 % roots rotted
5 = 76 – 100 % roots rotted

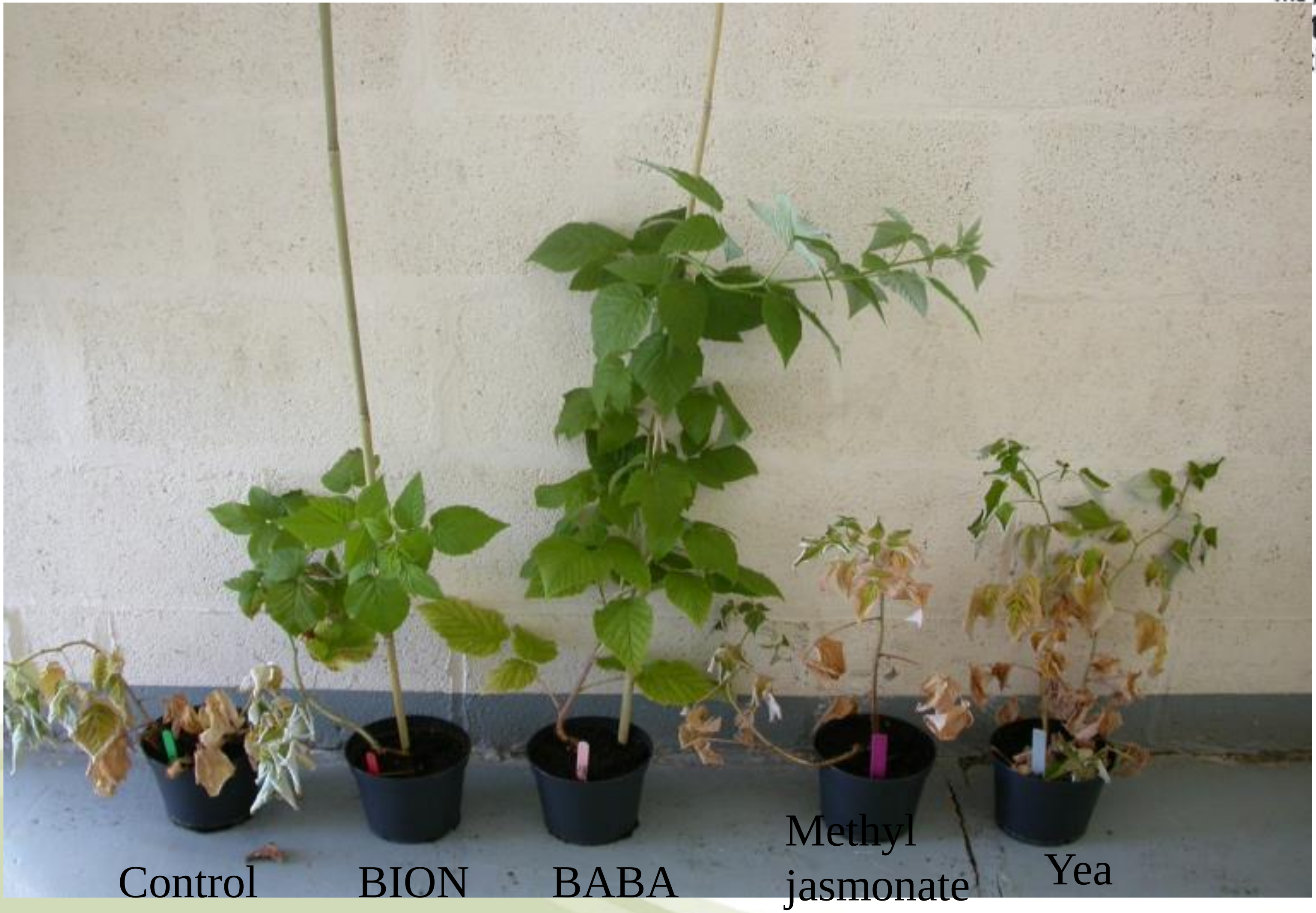
Plants assessed 3 weeks after inoculation

Dale Walters, SRUC

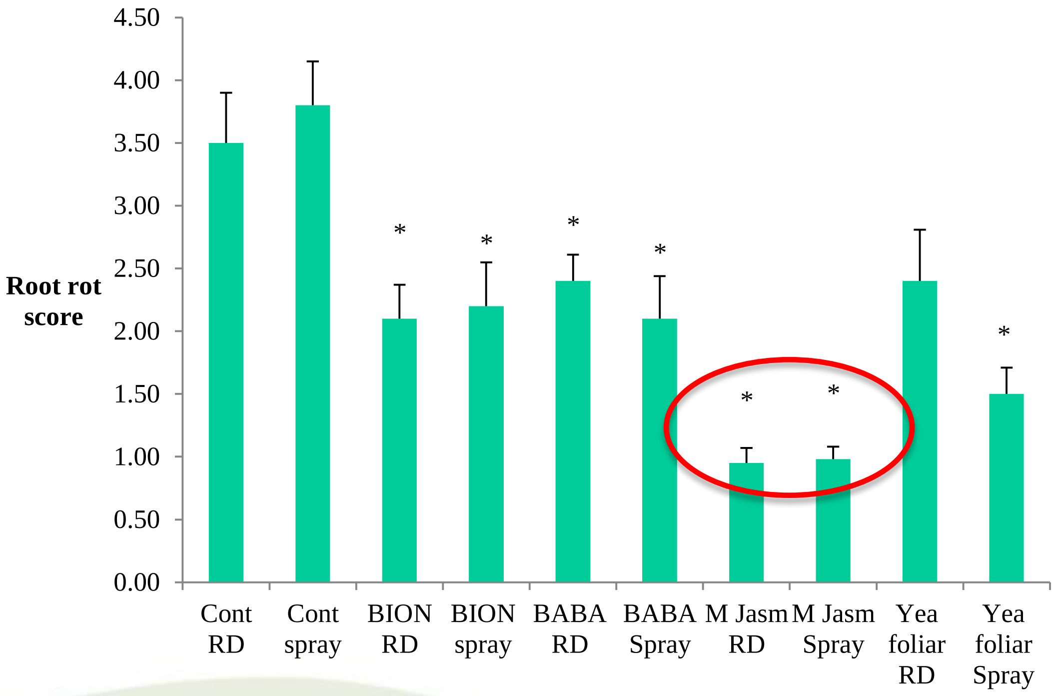

Treated by spraying elicitors
Cont
BION
BABA
Methyl
Jasmonate
Yea


Treated by root drench
Cont
BION
BABA
Methyl
jasmonate
Yea

Elicitor Treatment by Root Drench



Effect of elicitors on Root Rot in Meeker



Root Rot Score

0 = no symptoms
1 = 0 -3 % roots rotted
2 = 4 – 25 % roots rotted
3 = 26 – 50 % roots rotted
4 = 51 – 75 % roots rotted
5 = 76 – 100 % roots rotted

Plants assessed 3 weeks after inoculation

Potato blight control - 2014 Lesion size (cm^2) King Edward

The James Hutton Institute

- Two treatments with significantly smaller lesions than Dithane with C50:
  - Bion + Dithane (p<0.001)
  - BABA + Warrior + Dithane (p=0.019)

  Lesion size for these two treatments was also significantly smaller than for Electis (p<0.001 and 0.047 respectively)

- Bion + Dithane resulted in significantly smaller lesions than Regalia + Bion + Dithane (p<0.021)

Treatment x 6	cymoxanil x 4	25 Sep
Dithane	C50 x 4	1.19
Bion + Dithane	C50 x 4	0.84
BABA + Warrior + Dithane	C50 x 4	1.01
Regalia + Bion + Dithane	C50 x 4	1.07
Electis	C50 x 4	1.10
Regalia + Dithane	C50 x 4	1.43

Ruairidh Bain, SRUC

37

Research Review No. 89

A review of the function, efficacy and value of biostimulant products available for UK cereals and oilseeds

Kate Storer, Sarah Kendall, Charlotte White, Susie Roques and Pete Berry

The James Hutton

Table 2a. Non-microbial products containing a single active ingredient.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Atonik	Arysta	OSR	Synthetic nitrophenols - sodium 5-nitroguaiacolate, sodium o-nitrophenolate, sodium p-nitrophenolate	Higher yields, improved quality, reduce pod shatter, frost tolerance	Other (nitrophenols)	Foliar spray
ALGAFlex	Biotechnica	Cereals & OSR	Concentrated seaweed extract, principally derived from *Ascophyllum nodosum*	Increase yield, strengthen root system development. Improve tolerance to environmental stresses and diseases and increase activity of beneficial microbes	Seaweed extract	Soil drench or foliar spray
BlaminoAM3	Biotechnica	Cereals & OSR	L-amino acids	Strong and sustainable vegetative growth, increase crop yield and quality, improve resistance to environmental stresses, enhanced disease resistance.	Amino acids	Foliar spray
SAPONite	Biotechnica	Cereals & OSR	Plant extract containing active plant saponins	Improve water and nutrient intake, and speed and success of germination.	Other (plant extract)	Seed dressing
BioSilicate	Biotechnica	Cereals	Biologically available silicon	Stronger stalks and stems, reducing lodging, better photosynthesis from extended leaves and extra chlorophyll, reduced heat and drought stress, better resistance to fungal pathogens and sucking insects such as aphids, improved resistance to high salts or toxins	Non-essential chemical elements	Foliar spray

Table 2b. Microbial products.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
Mycortex	Biotechnica	Cereals	Mycorrhizal fungi, *Trichoderma* fungi, beneficial bacteria, humates, saponins	Improve root growth, plant nutrition, N fixation, disease resistance, stress resistance, increase soil microbes, improve soil quality and structure	AMF, PGPB, humic acids, fulvic acids	Granular or liquid - apply at sowing
Symbio liquid endo mycorrhizal inoculant	Symbio	Cereals	Arbuscular mycorrhizal fungal inoculant	Increases plant growth in poor soils, increases yield, healthy plants are more resistant to stress and disease, reduces need for fertiliser and water	Arbuscular mycorrhizal fungi	Seed coat, soil drench or mixed with compost teas

Table 2c. Non-microbial products containing multiple active ingredients.

Product	Company	Target Crop	Product Contents	Product aim (as described on product label)	Product type category	Application type
C Weed 50	Micromix	Cereals & OSR	50% w/v seaweed concentrate produced at lower temperatures utilising only *Ascophyllum nodosum* - formulated with Humic acids and harvested only during selected periods of growth	Earlier establishment, increases early rooting, photosynthetic area, leaf and shoot growth and plant carbohydrate production, improves sugar content in treated crops resistance to disease and pests, storability of treated crops, improves shelf-life of plants and flowers	Seaweed extract	
Matrix/Radical	Micromix	Cereals & OSR	Undisclosed, 'synthhormone', mix of components that have a syngergistic effect when put together	Supports growth and root development, drought amelioration strategy	Other - Growth hormone pre-cursors and analogues	
Optiphite GP	Micromix	Cereals & OSR	N, Phosphite, K, Phosphate, amino acids, humate-lignate active-uptake formulation technology	Reinforces plant disease defence and enhances root development	Phosphite, amino acids, humic substances	
Patron Z	Micromix	Cereals & OSR	N, Zn, Ammonium Acetates, Amino acids (wide range) with alkyl polyglucoside surfactant (with humic acids)	Enhances root and seedling development, increases root mass and length, improves seedling disease resistance, improves nutrient uptake efficiency	Amino acids, humic acids	
VitAmix	Micromix	Cereals & OSR	K, phosphite, humic and fulvic acids, chelated Cu, Mn, Zn, Fe, + Bo, Mo	Improves seedling establishment, promotes root development, reduces disease, corrects deficiencies and prevents physiological disorders	Phosphite, humic substances	
C-Weed AAA	Micromix	Cereals & OSR	*A. nodosum* concentrate produced from a cool extraction process, plus a wide range of L-amino acids from fermentation of plant extracts.	Earlier establishment, increases early rooting, photosynthetic area, leaf and shoot growth, plant carbohydrate production, improves sugar content in treated crops, improves resistance to disease and pests, storability of treated crops	Seaweed extract with L-amino acids	

The James Hutton Institute

Table 20. A summary of the evidence for positive biostimulant effects on plant nutrition, growth and stress tolerance, based on published and unpublished information analysed by this review.

***Low level of evidence: principally laboratory experiments, including little or no data on cereals or oilseed rape;**

****Moderate evidence: greater number of experiments including some that were field-based and/or on cereals or oilseed rape;**

*****Good evidence: wide evidence base including multiple field-based experiments on cereals or oilseed rape.**

Effect Category	Nutrient uptake or access			Plant function & Growth						Abiotic stress tolerance				Biotic stress tolerance	
Product type	N	P	Other	Hormonal	Growth[†]	Yield	Reduced Transpiration	Delay senescence	Improved photosynthesis	Salt	Alkaline	Drought	Cold	Pathogen[††]	Pest[††]
Seaweed extracts	*	*	*	**	**	**			*	*		*	*	*	*
Humic substances	**	*	*	*	**	**			*	*		*			
Phosphite & inorganic salts				*	**	**								**	
Chitin & chitosan derivatives					**	**	*			*		*	*	***	*
Anti-transpirants				***		**a	***		*			**			
Protein hydrolysates & amino acids	*		*		*	*				*		*	*		
Non-essential chemical elements	*	*			*	*		*	*	*	*	*		**	**
Plant growth promoting bacteria	**	**	*	*	***	***				*		*		**	*
Non-pathogenic fungi	*	*	*	*	**	**				*		*	*	**	
Arbuscular mycorrhizal fungi	*	**	*		**	**						*		*	*
Protozoa & nematodes	*			*	*	*									

[†] Above and/or below-ground growth

[††] Resistance or tolerance of pathogen/pest, induced or physical

[a]Yield and other benefits depend on severity of drought conditions; yield penalties may occur when water is plentiful.

Conclusions

- **Potential**: Products and priming
- **Constraints**: Mechanisms of pathogenicity
- **Potential**: Combinations of resistance elicitors
- **Constraints**: Environment, nutrients and formulation
- **Constraints**: Plant genotype interactions
- **Potential**: Trans-generational effects
- **Constraints**: Trade-offs
- **Potential** and **constraint**: Phyllosphere and rhizosphere organisms
- **Potential** and **constraint**: Meeting practical demand

Thanks to:

Scottish Government (RESAS)

Scottish Enterprise Tayside, Scotia Pharmceuticals, Norsk Hydro, AHDB, EU…