

# **Update on GM wheat field Trials**

**Huw D Jones**Plant Science Dept,
Rothamsted Research. UK



Aphids are significant pests of cereals in the UK.

# Damage grains and also act as vectors for the barley yellow dwarf virus





Barley yellow dwarf virus transmitted by cereal aphids





When aphids are attacked, either by predators or parasitiods, they produce an alarm pheromone

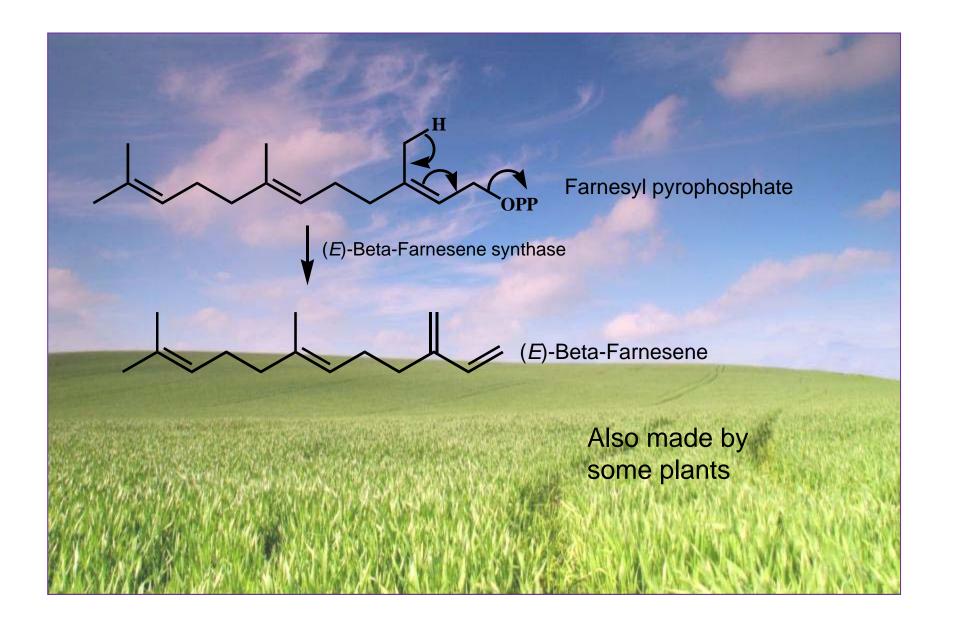
Volatile sesquiterpene: (E)-Beta-farnesene



Lacewing larva (Chrysoperla carnea) attacking Aphis fabae







Proc. Natl. Acad. Sci. USA Vol. 94, pp. 12833–12838, November 1997 Biochemistry

# Isolation and bacterial expression of a sesquiterpene synthase cDNA clone from peppermint (Mentha x piperita, L.) that produces the aphid alarm pheromone (E)- $\beta$ -farnesene

(insect pheromone/plant-insect chemical communication/synomone)

JOHN CROCK, MARK WILDUNG, AND RODNEY CROTEAU\*

Institute of Biological Chemistry, and Department of Biochemistry and Biophysics, Washington State University, Pullman, WA 99164-6340

Contributed by Rodney Croteau, September 25, 1997

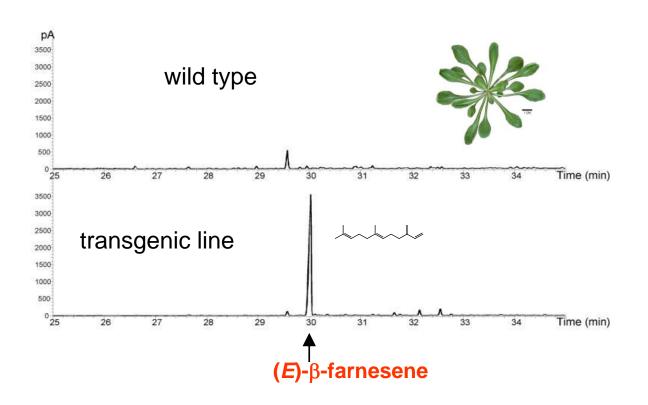
ABSTRACT (E)-β-Farnesene is a sesquiterpene semiochemical that is used extensively by both plants and insects for communication. This acyclic olefin is found in the essential oil of peppermint (Mentha x piperita) and can be synthesized from farnesyl diphosphate by a cell-free extract of peppermint secretory gland cells. A cDNA from peppermint encoding (E)-β-farnesene synthase was cloned by random sequencing of an oil gland library and was expressed in Escherichia coli. The corresponding synthase has a deduced size of 63.8 kDa and requires a divalent cation for catalysis ( $K_{\rm m}$  for  $Mg^{2+} \approx 150 \,\mu{\rm M}$ ;  $K_{\rm m}$  for  $Mn^{2+} \approx 7 \,\mu{\rm M}$ ). The sesquiterpenoids produced by the recombinant enzyme, as determined by radio-GC and GC-MS, are (E)-β-farnesene (85%), (Z)-β-farnesene (8%), and δ-cadinene (5%) with the native C15 substrate farnesyl diphos-

kairomonal oviposition stimulant to the European corn borer (Ostrinia) (18). (E)- $\beta$ -farnesene is the major component of pollen odor in Lupinus and stimulates pollination behavior in bumblebees (19). Feeding by larval lepidopterans, such as Heliothis or Spodoptera (Noctuidae), increases the amount of (E)- $\beta$ -farnesene released by corn; the volatile olefin then is detected as a synomone by the parasitic wasp Cotesia marginiventris (Hymenoptera/Braconidae) for locating the lepidopteran hosts (8). Circumstantial evidence also suggests the lepidopteran induced production and emission of (E)- $\beta$ -farnesene from corn serves as a synomone for Cotesia kariyai (20) and from cotton leaves as a synomone for C. marginiventris (21, 22).

Perhans of greatest significance in plant-insect interactions

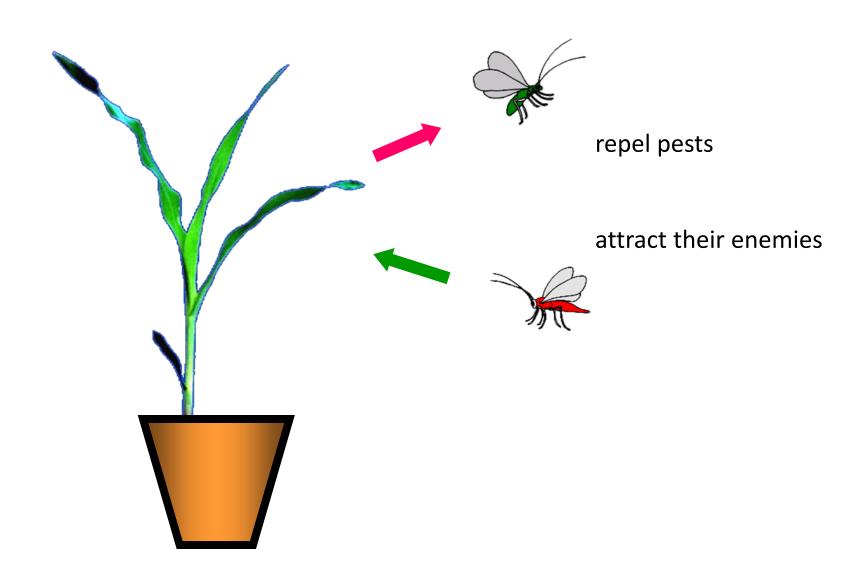
# Transgenic Arabidopsis expressing *CaMV35S:: E*ßf synthase cloned from peppermint [mentha x piperita].

Gas chromatography analysis of volatile chemicals collected from head-space of transgenic Arabidopsis and controls



Beale, MH, Birkett, MA, Bruce, TJA, Chamberlain, K, Field, LM, Huttly, AK, Martin, JL, Parker, R, Phillips, AL, Pickett, JA, Prosser, IM, Shewry, PR, Smart, LE, Wadhams, LJ, Woodcock, CM & Zhang, Y (2006) Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behaviour. PNAS 103: 10509-13

# Crop protection tool? Pest Management



# A New Generation of Insect-resistant GM Crops: Transgenic Wheat synthesising the Aphid Alarm Signal.

BBSRC AgriFood £750,000 @80% FEC 5 yrs. 01.09.2008 – 31.8.2013

PI: John Pickett

Researchers: Johnathan Napier, Huw D Jones, Dr Toby Bruce, Lesley Smart, Janet Martin, Angela Doherty

## **Objectives of project:**

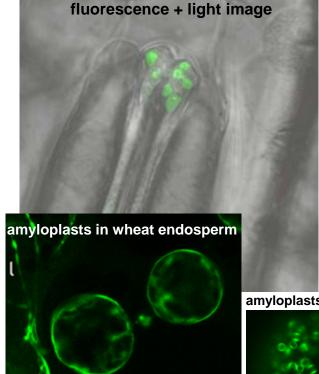
- Express Eßf synthase in transgenic wheat and analyse EBF levels
- Express *E*ßf synthase + *FPP* synthase and analyse EBF levels
- Test whether chloroplast targeting of Eßf synthase + FPP synthase affects overall EBF levels
- Conduct lab expts. to compare behaviour of aphids / predators / parasitoids on transgenic and control wheat
- Conduct field trials of two best GM lines

# Evidence that targeting transgenic terpene synthases to plastids results in enhanced yields of volatile terpenoids

Genetic Engineering of Terpenoid Metabolism Attracts Bodyguards to Arabidopsis Kappers *et. al.* (2005) Science 309:2070-2072

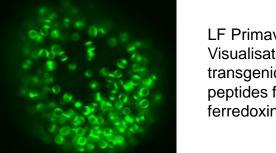
Terpenoid Metabolism in Wild-Type and Transgenic Arabidopsis Plants Aharoni *et. al.* (2003) Plant Cell 15:2866-2884

We have already demonstrated that it is possible to target GFP to wheat plastids using transit peptides:



**GFP** guard cell chloroplasts

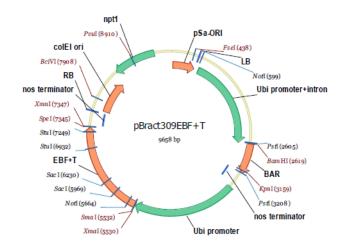
amyloplasts in wheat pollen

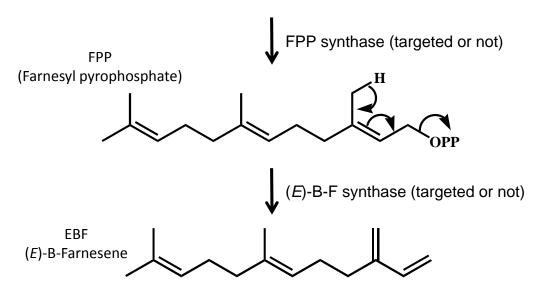


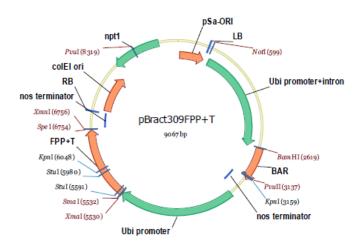
LF Primavesi, H Wu, EA Mudd, A Day and HD Jones (2008) Visualisation of plastids in endosperm, pollen and roots of transgenic wheat expressing modified GFP fused to transit peptides from wheat SSU RubisCO, rice FtsZ and maize ferredoxin III proteins. *Transgenic Research* 17(4): 529-543.

# Approx. 150 transgenic plants made with 4 combinations of gene constructs in var. Cadenza

EBFS + T	EBFS - T	EBFS + T & FPPS + T	EBFS - T & FPPS - T
31	52	33	28

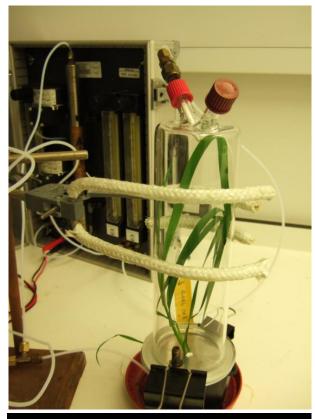






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## Collection of volatiles from transgenic wheat lines





A headspace sample of air surrounding the plant is collected on a Porapak Q filter

The sample is then analysed for (*E*)-ß-farnesene by GC-MS









# GM Field Trial Application submitted to ACRE /Defra 20th June 2011

#### **Legal Notices**

#### ROTHAMSTED RESEARCH Harpenden, AL5 2JQ

Field trial of aphid-resistant wheat plants

Rothamsted Research, AL5 2JQ gives notice that it has applied to the Secretary of State for Environment, Food and Rural Affairs for consent to conduct a field trial with genetically modified wheat under section 111 of the Environmental Protection Act 1990.

Scientists at Rothamsted Research have developed wheat plants that possess a novel, non-toxic aphid resistance trait and the purpose of the proposed trial is to evaluate in the field the efficacy of this approach.

The trial will take place at the Rothamsted Research farm map reference TL 1213 between March 2012 and October 2013.

More information may be obtained by email from field.trial@rothamsted.ac.uk

The Secretary of State will consider any representations made to her relating to any risks of damage to the environment posed by the release of the genetically modified organisms within a period that she shall specify in accordance with the Genetically Modified Organisms (Deliberate Release) Regulations 2002.

The Secretary of State will place information on this proposed GMO release on a public register within 12 days of her receipt of

the application. The public register can be inspected by contacting the Defra Information Resource Centre, Ergon House, Horseferry Rd, London SW1P 2AL (e-mail

defra.library@defra.gsi.gov.uk). This information will also be placed on the Defra website at

http://www.defra.gov.uk/environment/quality/gm/regulation/ registers/applications/index.htm.

The website will include details of how and when representations may be made in respect of the application. Enquiries on this public consultation should be directed to the address below.

GM Team
Defra
Area 8A, 9 Millbank,
c/o 17 Smith Square,
London SW1P 3JR
(email: qm@defra.gsi.gov.uk)

# GM wheat crop trial awaits government green light

#### **Hal Hodson**

A field trial of an experimental GM wheat will begin in March next year if government officials give the go-ahead for the crop to be planted.

One of Britain's leading plant research centres has applied for permission from the government to begin the trial of the GM crop, which is modified to resist attack by aphids. If approved, it would be only the third GM field trial running in Britain; the others, at the John Innes Centre in Norfolk and at Leeds University, are testing different varieties of GM potato. The project will require 24-hour security to prevent anti-GM protesters destroying the crop.

No GM crops are grown commercially in Britain, although GM varieties are grown extensively in other parts of the world notably the US, South America, China and India. The proposed trial is scheduled to run from March 2012 to October 2013. Anyone can submit an objection to the proposals up to 19 August this year.

Prof Maurice Moloney, the director of Rothamsted Research, which has applied for permission to conduct the trial, said the institute's chemical ecologists - who study the natural link between plants and pests - had discovered a way to prevent aphids destroying wheat.

"When aphids are under stress, they release a pheromone that is a signal to other aphids to get out of the locality," Moloney explained. "It turns out that pathway exists in plants, for example in mint. When this pathway is activated, the aphids don't land on that plant."

The chemical, known as (E)-beta-farnesene (EBF), is also found in beer because it occurs naturally in hops. Documentation submitted to the advisory committee on releases to the environment (Acre), the

advisory body for the secretary of state for environment, food and rural affairs, lists more than 300 varieties of plants in which EBF is known to occur naturally.

The new genes are similar to the versions that appear in peppermint, but have been chemically synthesised to function like wheat genes. The GM variety also contains two other genes that originate in bacteria. Dr Shawn Mcguire, a food security scientist at University of East Anglia who was not involved in the work, called GM wheat a "fairly new development". He said that because wheat is self-pollinating, the risk of cross-pollination was much smaller than in other GM crops.

"Wheat is far less promiscuous than oilseed rape and canola, so the issues of



If the trial to modify wheat to resist aphids attacks, gets the go-ahead, it would be the third GM field trial running in the UK

pollen flow and gene flow are less pronounced," Mcguire said.

According to Mcguire, as the pheromone only affects aphids the risks are very different from those represented by more brutish GM crops such as Bt wheat.

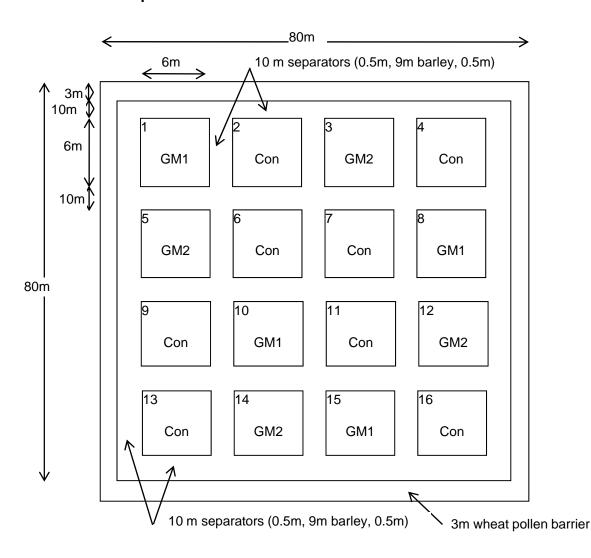
Rothamsted Research emphasised that the project was at the proposal stage and subject to approval. "A public notice appeared in today's Times and this initiates Acre's deliberations," it said.

Claire Oxborrow, a foods campaigner at Friends of the Earth, questioned the value of the research. "Given the fact that wheat is a staple crop, the development of GM varieties is particularly controversial. We're concerned that public money is being spent on research where there is no public acceptance or market," she said.

### **Proposed GM wheat field trial design**

(not draw to scale)

## 4x4 Latin square 6m x 6m plots 2 GM lines plus control



There will be two GM events in the field trial.

Event 2803R6P1 contains one copy of each of the EBFS+T plasmid and the FPPS+T plasmid per haploid genome and emits pure EBF at high levels

Event 2812R9P1 contains 4 copies per haploid genome of plasmid EBFS+T only and emits pure EBF at low levels

#### Department for Environment, Food and Rural Affairs

GM Team Area 8A, 9 Millbank 17 Smith Square London SW1P 3JR



Telephone 08459335577 Website www.defra.gov.uk

Professor Huw Jones Rothamsted Research West Common Harpenden Hertfordshire AL5 2JQ

Our ref: 11/R8/01

16th September 2011

Dear Prof Jones,

PART VI ENVIRONMENTAL PROTECTION ACT 1990 AND THE GENETICALLY MODIFIED ORGANISMS (DELIBERATE RELEASE) REGULATIONS 2002

#### APPLICATION FOR CONSENT REF: 11/R8/01

- I am pleased to enclose a consent for your application to release genetically modified organisms in accordance with the limitations and conditions set out in the Schedule to the consent.
- 2. The conditions attached to the consent require the submission of reports on the outcome of the release. The GM Inspectorate will be in touch regarding arrangements for inspection of the sites and submission of monitoring reports.
- You will receive an invoice in January of each year for the subsistence charge in respect of each release performed under this consent in accordance with Article 5 of the Fees and Charges Scheme 2001.

Please do not hesitate to contact me if I can be of further assistance.

Yours sincerely

David Sherlock

Email david.sherlock@defra.gsi.gov.uk







Trial sown on Rothamsted farm for 2 seasons:

March - Sept 2012 March - Sept 2013



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#### Watch again: GM wheat debate

29 September 2011 | By Alistair Driver

GENETICALLY modified (GM) wheat will be a 'non-starter' unless public concerns over the technology are addressed, according to people on both sides of the GM debate.

Kicking off a Farmers Guardian internet discussion on GM crops, Professor Huw, Jones, one of the scientists leading Rothamsted's GM wheat trial, said the research could generate benefits for farmers and the environment.

"Wheat is the most important UK crop and aphids are a major pest which not only suck sugar reserves from crops but spread virus diseases," he said.

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### Green light for farm trials of genetically modified wheat which is resistant to insects

By DAILY MAIL REPORTER

Last updated at 2:00 AM on 17th September 2011

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A trial of genetically modified wheat was given the green light yesterday.

The crop, which has been modified to make it resistant to insects, will be planted on a three-acre site in spring 2012 and 2013.

It is hoped the wheat will release a chemical that will both deter aphids and attract their predators and parasites.



The Government has backed the trials and say the genetically modified wheat will not damage the environment or human health

19 Sep 2011

Defra has granted consent for Rothamsted Research to conduct a trial of GM wheat in 2012 and 2013.

The Rothamsted application has been evaluated by the independent expert group, the Advisory Committee of Releases to the Environment. It is satisfied that the proposal will not result in any adverse effect on human health or the environment.

In line with ACRE's advice, precautionary conditions have been attached to the consent for the trial. These aim to ensure that no GM material from the trial will enter the food and feed chain. Further information can be found here.



#### Other Wheat Field Trials?



## Deliberate releases and placing on the EU market of Genetically Modified Organisms - GMO Register

П	D /CD /44 /DO /04	United	05/10/2011	Rothamsted	Study of aphid, predator and parasitoid behaviour in wheat producing	
	B/GB/11/R8/01	Kingdom	05/10/2011	Research	aphid alarm pheromone	
	B/DE/10/209	Germany	20/04/2011	University of Rostock	Evaluation of the resistance of KP4 bearing GM spring wheat plants against loose smut in the field with special consideration of effects on resistance against other fungal pathogens	
	<u>B/HU/08/3</u>	Hungary	23/06/2008	Department of Biotechnology, Cereal Research	Field testing of genetically modified wheat lines for improved red rust resistance	
	B/DE/07/195	Germany	23/06/2008	University of Rostock	Field evaluation of loose smut resistance in GM spring wheat expressing KP4 protein with focus on resistance against other fungal pathogens.	
	B/DE/06/178	Germany	09/08/2006	Leibniz-Institut of Plant Genetics	Increase of grain protein content in winter wheat	
	B/IT/04/02	Italy	27/05/2004	Metapontum Agrobios s.c.a r.l.	Study of the stability of the transgene and his hereditability of genetically modified wheat under of open field conditions.	
2	B/ES/04/08- CON	Spain	05/02/2004	Instituto de Agricol. Sostenibile Consejo	Evaluation in field conditions of Fungal resistant wheat.	
	B/DE/03/151	Germany	26/01/2004	Syngenta Seeds	Fungal resistant wheat Germany 2004 (I) & (II)	
	B/DE/02/143	Germany	19/05/2003	Syngenta Seeds	Fungal resistant wheat Germany 2003	
	B/ES/02/17	Spain	06/02/2003	Instituto Nacional de Investigaciones INIA	Safety assessment of the release of transgenic crops spread of herbicide resistance genes from wheat.	
	B/02/R34/4	United Kingdom	16/01/2003	Syngenta Seeds Ltd	To compare the pathogen infestation level and mycotoxin level of wheat expressing an enhanced resistance to Fusarium pathogens with existing non-modified varieties, grown under standard agronomic conditions.	
	02/R36/1	United Kingdom	16/04/2002	Long Ashton RS	Small scale trial of spring wheat to assess control of sucrose synthase activity and starch accumulation in the endosperm.	
	01/R8/4	United Kingdom	16/04/2002	Rothamsted Research	Improved bread making performance of commercial UK wheat	
	97/R8/3	United Kingdom	06/01/1998	Rothamsted Research	Improved bread making performance of spring wheat	









#### Welcome to the Office of the Gene Technology Regulator Website

Ψ.

Licence & Organisat	ion Modified Trait	Organism	Issue Date/ Status
DIR 102 University of Adelaide	Abiotic stress tolerance and enhanced nutrient utilisation	Wheat and Barley	29.6.10 Current
DIR 100 CSIRO	Enhanced carbon assimilation, grain weight, heat tolerance and/or water use efficiency, herbicide tolerance	Wheat	23.6.10 Current
DIR 099 CSIRO	Altered grain composition, Nutrient utilisation efficiency	Wheat and Barley	11.6.10 Current
DIR 094 CSIRO	Enhanced nutrient utilisation efficiency	Wheat and Barley	10.7.09 Current
DIR 093 CSIRO	Altered grain starch composition	Wheat and Barley	5.6.09 Current
DIR 092 CSIRO	Altered grain composition	Wheat	28.5.09 Current
DIR 080/ Vict Uni. Dept of Primary Industries	Drought tolerance	Wheat	30.6.08 Current



### **Greenpeace destroys GM wheat**

Jessica Nairn

Updated July 14, 2011 15:56:01



## Acknowledgements

#### **GM** Wheat

- Angela Doherty
- Caroline Sparks
- Johnathan Napier

### Chemical Ecology

- Toby Bruce
- Lesley Smart
- Gudbjorg Aradottir
- Janet Martin
- John Pickett

### Field Trial

Stephen Goward

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