Institution: University of Leeds

Unit of Assessment: UoA5 Biological Sciences

Title of case study: CS2 Technologies to control plant parasitic nematodes

1. Summary of the impact (indicative maximum 100 words)

Plant resistance provides sustainable control of the $125bn annual world crop losses to nematodes to replace environmentally hazardous pesticides. **Urwin** and **Atkinson** have developed three biosafe resistance technologies that 1) suppress feeding success, 2) reduce root invasion and 3) suppress nematode development by RNA interference. We have developed GM agriculture with leading industry (Sinochem, Monsanto) and in emerging economies through free access to technology, capacity building initiatives, review of collaborative R&D plans (India) and regulatory approval of field trials (Uganda). The work has also influenced policy-makers in the UK and in Switzerland, leading to new security measures for GM field trials in these countries..

2. Underpinning research (indicative maximum 500 words)

Plant parasitic nematodes cause global crop yield losses of $125bn annually **(1)**. Nematicides can cause harm to humans in their production and use but it is their severe environmental harm that is leading to their withdrawal globally, often leaving farmers without adequate nematode control. The partnership of **Urwin** (molecular lead) and **Atkinson** (biological lead) and co-workers have developed three technologies for nematode control from discovery to field evaluation in a continuous on-going collaboration that dates from before 1993. This case study is underpinned by over 50 awards to them with a value in excess of £15,000,000.

Technology 1

Cysteine proteinases are important digestive enzymes for nematodes but not humans that are inhibited by cystatins. Plant cystatins are widely consumed in foods (e.g. maize and rice) and are neither human allergens nor toxins. Transgenic expression of a protein engineered cystatin provides nematode resistance **(2,3)** with efficacy against all damaging nematodes tested in a wide range of crop plants.

The technology has been proven in five UK field trials with potato plants **(4)**. The work at Leeds is internationally distinctive by providing the only published reports of GM field trials for nematode resistance. It can take 15 years between proof of concept and commercialisation of new GM technologies. We have had funding in that phase of the work by companies including Limagrain, Ingleheim Boehringer, Zeneca, Syngenta, Harris Moran Del Monte, Dole and Maui Pine. The cystatin-based technology is currently being commercialised for cotton by China Seeds (Sinochem). Our field trials also established that potato plants expressing Technology 1 do not harm non-target, insect aerial feeders, their natural parasitoid enemies or the soil microbial communities **(5)**.

Technology 2

This invention is based on novel synthetic peptides and a novel uptake pathway we showed along certain nematode chemoreceptive neurons. Peptide uptake leads to the targeted nematodes being unable to sense the proximity of roots but it is not lethal to them and hence harmless to nematodes in the soil community that do not attack roots. Transgenic expression of the peptide protects potato **(6)** and banana plants from nematode invasion and its associated root damage. Faunal analysis using a bar-coding approach establishes no harm to the non-target nematode soil community **(6)**.

We have characterised and utilised promoters that restrict expression of these defences to where in roots nematodes invade or feed. This limits the presence of the novel protein or peptide in the crop, ensuring absence of the expressed GM trait in the yield and adds a large margin of safety to the inherent biosafety of our proteins and peptides. We have also shown any likely levels of protein or peptide ingestion is not harmful to humans.

Technology 3

Atkinson and **Urwin** were the first to show that RNA interference is an effective gene silencing approach for plant parasitic nematodes **(1)**. We have demonstrated that transgenic expression of double-stranded RNA in the host plant interferes with expression of targeted nematode genes so disrupting nematode development. The benefit is a nematode resistant GM crop control without any novel protein production.

Lead academics: Professor P Urwin (Professor of Nematology, co-PI, 1993-present); Professor H Atkinson (Professor of Nematology, co-PI, 1972-present)
Academic collaborators: (all University of Leeds) Prof B.H. Davies (1996-present); Prof P.M. Gilmartin (1991-2007); Prof J.P Knox (1991-present); Prof M.J McPherson (1982-present); Prof P. Meyer (1995-present); Dr S. Kepinski (2006-present).
Postdoctoral Researchers at Leeds: Catherine Lilley (01/2004 - current); Laura Jones (07/2009 – current); Sarah Cowgill (01/1996 – 06/2000): Mirela Coke (08/2010 – current); Kate Warner (08/2009 – current); Hugh Roderick (06/2008 – current); Anil Neelam (07/1999 -11/2000); Manjula Bakhetia (08/2003 – 08/2009); Jayne Green (07/1999 – 01/2009); Elena Zubko (10/1997-09/2000).

3. References to the research (indicative maximum of six references)

1. Urwin PE, Lilley CJ, and Atkinson, HJ. (2002) Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. *Molecular Plant-Microbe Interactions* **15**:747-752 DOI: 10.1094/MPMI.2002.15.8.747 [*Scopus citations (30/08/2013): 147]*
2. Urwin, PE, Atkinson, HJ, Waller, DA, and McPherson, MJ. (1995) Engineered Oryzacystatin-I Expressed in Transgenic Hairy Roots Confers Resistance to *Globodera pallida*. *Plant Journal* **8**:121-131. DOI: 10.1046/j.1365-313X.1995.08010121.x [*Scopus citations (30/08/2013): 153]*
3. Urwin PE, Lilley CJ, McPherson MJ, and Atkinson HJ. (1997) Resistance to both cyst and root-knot nematodes conferred by transgenic Arabidopsis expressing a modified plant cystatin. *Plant Journal* **12**:455-461. DOI: 10.1046/j.1365-313X.1997.12020455.x *[Scopus citations (30/08/2013): 105]*
4. Urwin PE, Green J, and Atkinson HJ. (2003) Expression of a plant cystatin confers partial resistance to *Globodera*, full resistance is achieved by pyramiding a cystatin with natural resistance. *Molecular Breeding* **12**:263-269. DOI: 10.1023/A:1026352620308 [*Scopus citations (30/08/2013): 31]*
5. Celis C, Scurrah M, Cowgill SE, Chumbiauca S, Franco J, Main G, Keizenbrink DT, Green J. and Atkinson HJ. (2004) Environmental biosafety and transgenic potato in a centre of this crop's diversity. *Nature* **432**:222-225. DOI: 10.1038/nature03048 [*Scopus citations (30/08/2013): 27]*
6. Green J, Wang D, Lilley CJ, Urwin PE, and Atkinson, HJ. (2012) Transgenic potatoes for Potato Cyst Nematode control can replace pesticide use without impact on soil quality. *Public Library of Science ONE* **7**:e30973. DOI: 10.1371/journal.pone.0030973 [*Total article views = 3,357 (21/10/2013); Scopus citations (30/08/2013): 3]*

Selected grants awarded: Genome sequence for the potato cyst nematode *Globdera pallida,* BBSRC reference BB/F000642/1, 01/03/2008-31/08/2012, £425k, PI Urwin; Nematode resistant plantain for Africa subsistence growers, BBSRC reference BB/F004001/1, 18/06/2008-17/06/2011, £523k, PI Atkinson; Functional characterisation of novel pathogenicity genes of the parasitic nematode *Globdera pallida*, BBSRC reference BB/H000801/1, 01/07/2010-30/06/2013, £343k, PI Urwin; Development of improved East African Highland Banana, US Agency for International Development, 01/10/2006-31/05/2015, £288k, PI Atkinson.

4. Details of the impact (indicative maximum 750 words)

Strategy for delivering impact.

Urwin, Atkinson & colleagues recognised that their research impacted on many of the critical issues of food security (maximise production, minimise environmental impact, address public concerns). Their patenting and licensing strategy allowed new product development in collaboration with multinational agricultural companies and simultaneous independent collaboration with authorities in developing economies to establish home grown expertise and capability in GM crops. They also engaged with policy-makers and the public to inform and influence the debate

concerning GM safety and use as a solution in food security.

Interactions with Industry:

i) They have been underpinned by patents granted to the University (>12 granted patents, e.g. publication numbers: PL362116; HK1032073; AU6063999; CZ20002005; US2001016954; GR3026133; US2003221209; US5863775; US5824876; EP1231274; AU691020; US5589622).

ii) Opportunities are international given UK agriculture produces only 1% of the world crop harvest. The largest seed company in China (China seeds, Sinochem) is funding us (2012-2015, value £500k) to develop nematode resistant cotton **[A]** (Technology 1) as one of the first technologies in their newly formed plant biotechnology unit. Monsanto bought a licence to key USA crops, under a patent filed by the University of Leeds (Technology 3) and has made several payments totalling £44,000 to the University since 2008 **[A]**. Technology 1 is being put into crops to confer nematode resistance for pineapple, coffee and flowers in USA (Hawaii Agriculture Research Center, (HARC), Beltsville Agricultural Research Center) and aubergine and rice in India (Indian Agricultural Research Institute, (IARI)) **[B].**

Influencing Government policy:

i) Our recommendation to have protected national field-trial sites (Atkinson & Urwin 2008, *Nature* 453, 979) has led to the Swiss government establishing such a scheme **[C]**. We were invited to meet the Minister for the Environment (August, 2008) who subsequently publicly supported our research. The Secretary of State for Environment, Food & Rural Affairs cited our work in the House of Commons as an exemplar of research to address the critical questions concerning GM food (namely, "what is the impact on biodiversity?") **[C]**.

ii) Atkinson gave evidence to the UK Parliamentary and Scientific Committee in 2010 concerning the potential of GM crops to enhance global food security, drawing on Urwin/Atkinson research on GM strategies to combat potato cyst nematode **(**references **1-6) [D]**. He also contributed to, and reviewed, a government publication on GM crops **[E]**.

International impact:

i) The expertise has been applied internationally. Atkinson co-chaired (December 2011) an evaluation of the 3rd phase of the Indo-Swiss Collaboration in Biotechnology (2007-2012, expenditure £6.9m, circa 25 project partners) at the request of the Department of Biotechnology, The Ministry of Science and Technology, India **[F]**. The detailed recommendations shaped the 4th phase (2013-) of this international collaboration.

ii) Drawing on knowledge gained from consents granted by DEFRA to Urwin and Atkinson for their UK field trials (e.g. 09/R31/01, 07/R31/01; references **5 and 6**), Atkinson was also a major contributor to the successful submission by The National Agricultural Research Organisation of Uganda to that country's National Biosafety Committee for the first field trials of GM bananas and plantains for nematode control **[G]**. The trials were subsequently planted in 2012.

iii) Strategic work is currently centred on cotton for deployment in China **[A]** and, in collaboration with other institutes, plantain and cooking bananas in Uganda (Technologies 1 and 2, National Agricultural Research Labs, Uganda) and Kenya (International Institute of Tropical Agriculture). Researchers using Technology 1 are developing nematode resistant pineapple in Hawaii (HARC) **[B]** and rice and aubergine in India (IARI).

iv) The work on cooking bananas is central to our effort to develop public goods (Technologies 1-3). This crop suffers estimated losses to nematodes in Africa of 71 ±16% and is vital food for 100 million, mainly poor, Africans. The crop's sterility and slow improvement by traditional cross-pollination techniques underpin its value for GM based improvement. Transgenic bananas with our nematode resistance are in authorised field trials in Uganda following planting in 2012. Our expertise (funded by BBSRC, RCUK, DFID, EU and USAID **[H]**) has enabled scientists in Uganda and other developing countries to deliver these benefits. We have hosted capacity building programmes for >20 scientists from developing countries funded by DFID, BBSRC (e.g. SARID initiative, BB/F004001/1 and ISIS1873), The Foreign and Commonwealth Office (470529) and USAID (APSPII). We donate technology through DFID, USAID, BBSRC and RCUK to provide public goods for Sub-Saharan Africa and India.

Social impact:

The work has led to numerous radio interviews (e.g. BBC Radio 4 *Farming Today*) and television appearances (e.g. BBC's *Countryfile*, 2009, and the Channel 4 programme *What the green movement got wrong*, 2010). Our work has been widely reported in newspapers, (e.g. a major article in the Sunday Times Magazine **[I]** and The Financial Times **[I]**) and included in an exhibition at the Science Museum in London.

5. Sources to corroborate the impact (indicative maximum of 10 references) *websites accessed 08/07/2013*

[A] Contract between Sinochem and University of Leeds, detailing the work to be carried out to develop nematode resistant cotton, and grant information indicating funding of £500,000 *(copies available on request).* Licence payments from Monsanto of £44,000 in REF period *(copy of contract and invoices available on request).*

[B] Genetic transformation of crop and flower species using Technologies 1-3: Wang, M.L. *et al.* (2009) Production of tr*ansgenic pinea*pple (*Ananas cosmos*) plants via adventitious bud regeneration. *In vitro cellular and developmental biology - plant* **45**(2):112-121 DOI: 10.1007/s11627-009-9208-8; Cabos, R.Y. *et al.* (2007) Plant proteinase inhibitors as a natural and introduced defense mechanism for root-knot nematodes in *Coffea arabica*. *Journal of Nematology* **39**(1):100 ISSN: 0022-300X; Fitch, M.M.M. *et al*. (2011) Improved transformation of Anthurium. *HortScience* **46**(3):358-364 ISSN: 0018-5345.

[C] Romeis, J. *et al*. (2013) Plant biotechnology: Research behind fences.*Trends in Biotechnology*. **31**(4):222-224 DOI: 10.1016/j.tibtech.2013.01.020. Hansard 6th Nov 2008: Column 347: T8 [233477] and reply from The Secretary of State for Environment, Food and Rural Affairs *(copy available on request).*

[D] Meeting took place 15 December 2009 at Portcullis House, London. Published as Atkinson H.J. (2010) Come back GM – All is forgiven. *Science in Parliament* **67**, 27-28 *(copy available on request).*

[E] Houses of Parliament : Parliamentary Office of Science and Technology PostNote #412 GM in Agricultural Development June 2012; http://www.parliament.uk/briefing-papers/POST-PN-412.pdf

Also: email trail evidencing Atkinson's involvement as reviewer of POST note *(copy available on request).*

[F] Reference to ISCB evaluation report in IAUA News (Quarterly Newsletter of Indian Agricultural Universities Association 2011, Volume 11 number 4, (see entry on Indo-Swiss collaboration in biotechnology under Assam Agricultural University, Jorhat). http://www.iauaindia.org/news_universities_oct_dec11.html

[G] African Agriculture blog.com (2012) Uganda: trials of nematode-resistant GM banana to begin in 2012 http://www.africanagricultureblog.com/2012/03/uganda-trials-of-nematode-resistant-gm.html (March 19, 2012).

[H] Agricultural Biotechnology Support Project II, East African Highland (EAH) banana resistant to black Sigatoka and nematodes (http://www.absp2.cornell.edu/projects/intersect.cfm?productid=23&countryid=8).

[I] Published on 27th June 2010. Why genetically modified crops are good for us. Sunday Times Magazine, p48-54 *(copy available on request)***;** Published 4th February 2012. Bananas become ripe GM target, Financial Times Magazine http://www.ft.com/cms/s/2/5fa2963e-4c86-11e1-b1b5-00144feabdc0.html#axzz2YSRe1pKk