Institution: Durham University

Unit of Assessment: UoA5

Title of case study: Using Biotechnology to Protect Plants against Invertebrate Pests

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

Durham has a long-standing record of research into improving the resistance of crop plants towards pests, which includes pioneering work on genetic engineering of plants for insect resistance. The *CpTI* gene developed in Durham for enhancing insect resistance in transgenic crops has had a major impact on Chinese agriculture, due to the widespread deployment of GM cotton containing genes encoding *Bacillus thuringiensis* (*Bt*) toxin and CpTI. The SGK 321 transgenic cotton line was approved for commercial growing in China in 1999, and by the current REF period *Bt/CpTI* cotton was grown on approximately 0.5 million hectares of land, representing approximately 15% of the total transgenic cotton grown (which in turn represented 67% of total cotton production). The economic value of *Bt/CpTI* cotton is estimated as approx. £600 million per year.

2. Underpinning research (indicative maximum 500 words)

The initial research underpinning the Durham approach to crop protection came from an understanding of the molecular bases of interactions between organisms; specifically, the compounds that plants produce to defend themselves against invertebrate pests[1].

The tools of molecular biology have allowed these compounds to be exploited to produce novel plant defences. Proteinase inhibitors (PIs) which act on the digestive enzymes of insect herbivores are a basic mechanism of plant defence, and engineering plants to increase endogenous PI levels was identified as an early target for genetic engineering experiments, with the aim of protecting crops against major insect pests. The first experiments with PI-encoding transgenes were carried out in Durham with a seed-expressed Bowman-Birk type serine proteinase inhibitor from cowpea (CpTI). Transgenic tobacco plants expressing this "foreign" PI constitutively were significantly protected against attack by lepidopteran larvae (caterpillars)[1,5]. Subsequent work looked at other plant defensive proteins, such as lectins; these defensive proteins bind to the insect gut surface (causing systemic antimetabolic effects in some cases), and a gene encoding the lectin from snowdrop was used in Durham to engineer several plant species, including rice, for partial resistance to sucking insect pests[3-6].

Whereas the initial aims of the research were to exploit plant defensive compounds directly, further research has shown that insect pests are adapted to allow them to tolerate plant defences, and are only partially susceptible to plant defensive compounds. In order to produce more effective pesticides, a new research strategy based on plant defensive proteins has been developed. Studies on snowdrop lectin showed that this protein was able to cross the insect gut wall after binding to the gut surface. This observation suggested that the lectin could be used as a "carrier" to deliver insecticidal toxins to their targets. Spiders, for example, produce peptide toxins in their venom that interact with the ion channels in insect nervous tissue. These molecules are normally injected into the "blood" by the spider sting, and have little or no oral toxicity to insects as they are unable to cross the gut wall to access their sites of action. Conjugation of a spider venom toxin to a lectin "carrier" gave a fusion protein that possessed an oral insecticidal activity towards lepidopteran larvae, which was shown by neither of its components. This result has formed the basis for development of new biopesticides[4].

The exploitation of fusion proteins as biopesticides has required optimisation of recombinant expression systems, allowing production on a kg scale. Concurrently, results showing that these proteins are active if produced in transgenic plants have been obtained; fusion proteins can thus be used for exogenous application, or for endogenous crop protection in GM crops[6].

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

1. Gatehouse, J. A. (2002). Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist 156, 145-169. (193 citations). DOI: 10.1046/j.1469-8137.2002.00519.x

2. Nagadhara, D., Ramesh, S., Pasalu, I. C., Rao, Y. K., Krishnaiah, N. V., Sarma, N. P., Bown, D. P., Gatehouse, J. A., Reddy, V. D. and Rao, K. V. (2003). Transgenic indica rice resistant to sap-sucking insects. Plant Biotechnology Journal 1, 231-240. (54 citations). DOI: 10.1046/j.1467-7652.2003.00022.x

3. Fitches, E., Edwards, M. G., Mee, C., Grishin, E., Gatehouse, A. M. R., Edwards, J. P. and Gatehouse, J. A. (2004). Fusion proteins containing insect-specific toxins as pest control agents: snowdrop lectin delivers fused insecticidal spider venom toxin to insect haemolymph following oral ingestion. Journal of Insect Physiology 50, 61-71. (62 citations). http://dx.doi.org/10.1016/j.jinsphys.2003.09.010

4. Trung, N. P., Fitches, E. and Gatehouse, J. A. (2006). A fusion protein containing a lepidopteran-specific toxin from the South Indian red scorpion (*Mesobuthus tamulus*) and snowdrop lectin shows oral toxicity to target insects. Bmc Biotechnology 6, 18 (32 citations). DOI:10.1186/1472-6750-6-18

5. Gatehouse, J. A. (2008). Biotechnological prospects for engineering insect-resistant plants. Plant Physiol 146, 881-7. (56 citations). http://dx.doi.org/10.1104/pp.107.111096

6. Fitches, E.C., Pyati, P., King, G.F. and Gatehouse, J.A. (2012) Fusion to snowdrop lectin dramatically enhances the oral activity of the insecticidal peptide ω-hexatoxin-Hv1a by mediating its delivery across the insect gut to sites of action in the central nervous system. PLoS One 6: e39389. DOI: 10.1371/journal.pone.0039389

Total grant income to Durham for work on biotechnological methods of protection of plants against insect pests has totalled approx. £2.3 million, with funding from Research Councils (AFRC/BBSRC), Government agencies (DEFRA, SERAD, TSB), industrial companies (AGC, Japan Tobacco) and charitable foundations (Rockefeller Foundation).

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

The strategy of engineering crops for insect resistance by expression of proteinase inhibitors and lectins has been actively pursued by researchers in Asia in particular, representing a multibillion-dollar market (see below). Lectin genes are being actively developed as products for crop protection in India, while the *CpTI* gene is used in commercial transgenic crops in China[2]. China is the world's largest cotton producer, at 7.2 m tonnes in 2011, with the textile sector there employing over 23 million people with fixed asset investment in 2011 of $56.4 billion, up 30.9 percent over 2010 (USDA, http://www.thebioenergysite.com/reports/?id=465).

The *CpTI* gene was developed and published in Durham[1,2] with the aim of enhancing insect resistance in transgenic crops, and has had a major impact on Chinese agriculture, due to the widespread deployment of GM cotton containing genes encoding *Bacillus thuringiensis* (*Bt*) toxin and CpTI. The best known GM cotton variety using these genes is designated SGK 321[4]. The combination of the two gene products in *Bt/CpTI* cotton is stated to show superior protection against cotton bollworm (*Helicoverpa armigera*) when compared to *Bt* cotton, particularly in the late growing stage. Field assays with Asian corn borer, (*Ostrinia furnacalis*) also showed that *Bt/CpTI* cotton consistently showed higher resistance than *Bt*-only cotton[5]. As well as enhanced resistance, *Bt/CpTi* cotton is claimed to show a lower rate of selection of resistant genotypes of cotton bollworm than *Bt* cotton.

The SGK 321 transgenic cotton line was approved for commercial growing in China in 1999, and by 2005 *Bt/CpTI* cotton was grown on approximately 0.5 million hectares of land, representing approximately 15% of the total transgenic cotton grown (which in turn represented 67% of total cotton production). The area over which *Bt/CpTI* cotton was grown in 2005 exceeded that of transgenic cotton varieties imported from outside China (produced by Monsanto)[6]. Figures obtained from the Chinese Academy of Agricultural Sciences (CAAS; personal communication) in 2009 showed that 3.80 million ha were planted with transgenic cotton (77% of total cotton acreage); 95% of this transgenic cotton was locally developed varieties. In 2009, total cotton production was 6.38 million tonnes, with a total value of approx. £6,000 million; this indicates a total economic value of transgenic cotton of approx. £4.4 billion in 2009. An improved *Bt/CpTI* cotton variety developed by CAAS, Zhongmian 41, was estimated to have generated an economic return of more than 6.5 billion RMB, or £710 million, in 2012[7].

The impact of *Bt/CpTI* cotton varieties on ecosystems has been assessed through extensive testing of potential negative effects on non-target organisms and the environment. No negative effects on parasitoids (biological control agents) of cotton bollworm were observed, nor on bees. No negative effects of *Bt/CpTI* cotton on the rhizosphere were detected over a five-year timescale, and *Bt/CpTI* cotton has no acute toxicity to earthworms. The absence of negative effects on the environment, and positive effects in the form of reduced input costs through lower pesticide usage (by up to 40%) have led to the conclusion that *Bt/CpTI* cotton has made a positive contribution to sustainability in Chinese agriculture[8]. The *Bt/CpTI* gene combination for insect resistance has also been introduced into rice in Chinese research, and transgenic rice varieties have been extensively field-trialled on scales up to hectare plots[9]. Protection against a target pest, striped stem borer (*Chilo suppressalis*) was highly efficacious in the field, and superior to insecticide treatment. *Bt/CpTI* rice also shows resistance in the field to a secondary lepidopteran pest, rice leaf folder (*Cnaphalocrocis medinalis*). However, although *Bt/CpTI* rice varieties have been readied for commercial growing, this has yet to take place.

Impact has also been generated through this work as a result of the development of recombinant fusion protein biopesticides[3], funded through DEFRA LINK programmes (starting 2004) in collaboration with the Food and Environment Research Agency, York (Fera) and an Industrial partner, the agrochemical company Isagro Ricerca. As a result of these programmes, a candidate insecticidal fusion protein for commercial development, "FP5", was identified[10]. As part of an ongoing TSB programme (2011-2014), Isagro Ricerca is investing ca. 1 m Euros at Durham to support glasshouse and field trials with a recombinant insecticidal fusion protein, and the protection of potato plants against Colorado Potato Beetle larvae observed in trials has significantly influenced the commercial objectives of this and other companies. Colorado potato beetle costs US growers approx. $150 million annually in insecticide costs (USDA), and development of new methods for control of this pest have potential economic value up to this order, depending on how much of the market is captured. Isagro has invested of the order of 2.5 million euros in the fusion protein programme (ca. 80% during the current REF period), with the expectation of returns an order of magnitude greater than this figure. Funding to develop "FP5" as a commercial biopesticide with the additional partners CPI, for large-scale production, and BTL, for downstream processing, was secured from TSB (2011). This has resulted in employment for 6 scientists at the industrial partners, who have invested a further £0.5 million towards bringing the product to market.

5. Sources to corroborate the impact (indicative maximum of 10 references)

Preparation and publication of intellectual property:

1. Bowman-Birk trypsin inhibitor isolated from *Vigna unguiculata*. US Patent 5,218,104 (1993) V.A. Hilder, A.M.R. Gatehouse, J.A. Gatehouse and D. Boulter.
2. Transformed plant which expresses an insecticidally effective amount of a Bowman-Birk trypsin inhibitor form *Vigna unguiculata* in leaves, stems or roots, and a method for the production thereof. US Patent 5,306,863 (1994). V.A. Hilder, A.M.R. Gatehouse, J.A. Gatehouse, D. Boulter, R.F. Barker and M. Bevan
3. Fusion proteins for insect control. US Patent 7,196,052 (2007). J.A. Gatehouse, E.C. Fitches and J.C. Edwards

Chinese work on Bt/CpTI cotton and rice:

4. Shirong, J.I.A., Sandui, G.U.O., Daochang, A.N., Guixan, X.I.A. (2004) Eds. “Transgenic Cotton” pp. 172-183. Science Press, Beijing, China. ISBN: 9780080449715
5. He, K., Wang, Z., Bai, S., Zheng, L., Wang, Y. (2004) Field efficacy of transgenic cotton containing single and double toxin genes against the Asian corn borer (Lep., Pyralidae). J. Appl. Entomol. 128, 710-715. DOI:10.1111/j.1439-0418.2004.00919.x
6. He, K.L., Wang, Z.Y., Zhang, J.Y. (2009) Monitoring Bt resistance in the field: China as a case study. In “Environmental Impact of Genetically Modified Crops” (eds N. Ferry and A.M.R. Gatehouse) pp. 342-357. CAB International, Wallingford, UK. ISBN: 9781845934095
7. Chinese Academy of Agricultural Sciences. (2012) http://english.caas.net.cn/docs/20120516164318203176.pdf.
8. Russell, D., Deguine, J.P. (2006) Sustainability of Bt cotton in China and India. Cahiers Agricultures 15, 54-59.
9. Qiu, J. (2008) Agriculture: Is China ready for GM rice? Nature 455, 850-852. DOI: 10.1038/455850a

Fusion protein biopesticides:

10. Fitches, E.C. Pyati, P., King, G.F., Gatehouse, J.A. (2012) Fusion to Snowdrop Lectin Magnifies the Oral Activity of Insecticidal omega-Hexatoxin-Hv1a Peptide by Enabling Its Delivery to the Central Nervous System. PLoS ONE, 7(6). DOI:10.1371/journal.pone.0039389