

# ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

MAY 2003

## *In This Issue:*

FAO E-mail Conference -  
Regulation of GMOs ..... 1

Weighing the Economic Costs  
and Benefits of Bt Cotton in  
South Africa ..... 1

Two Genes for One Protein:  
Application of Ssp DnaE Split  
Intein in Plant Genetic  
Engineering ..... 4

Agrobacterium tumefaciens-  
Mediated Transformation of  
Temperate Forage Grasses ..... 5

Regional Suppression of Insect  
Pests with Bt Crops ..... 7

Risky Genomics: The Roles  
of Risk/Benefit Assessment  
and Rational Decision  
Frameworks ..... 9

### **Stakeholder Workshop**

Future Directions and Research  
Priorities for the USDA  
Biotechnology Risk Assessment  
Research Grants Program  
(BRARGP)

June 9-10, 2003

Westin Embassy Row Hotel,  
Washington, DC

*Register Today!*

<http://www.isb.vt.edu>

## NEWS AND NOTES

### **FAO E-MAIL CONFERENCE — REGULATION OF GMOs**

The FAO Electronic Forum on Biotechnology in Food and Agriculture is devoting its next e-mail conference to the theme of “Regulating GMOs in developing and transition countries.” This moderated conference covers the agro-industry, crop, fisheries, forestry and livestock sectors and begins in late April, lasting for four weeks. It is organized by the FAO Working Group on Biotechnology and is the first conference to be held this year. All messages posted during the conference will also be placed on the Forum website (<http://www.fao.org/biotech/forum.asp>). To join the Forum send an e-mail to [mailserv@mailserv.fao.org](mailto:mailserv@mailserv.fao.org) leaving the subject blank and entering only the following two-line text message:

subscribe BIOTECH-L  
subscribe biotech-room1

Those who are already Forum members should omit the first line of the above message. For more information, contact [biotech-mod1@fao.org](mailto:biotech-mod1@fao.org).

## PRODUCTION NEWS

### **WEIGHING THE ECONOMIC COSTS AND BENEFITS OF BT COTTON IN SOUTH AFRICA**

*Stephen Morse, Richard Bennett and Yousouf Ismael*

It has often been claimed that agricultural biotechnology could provide many benefits for farmers in the developing world. However, until recently there has been a shortage of data obtained under field conditions to either support or contradict this assumption. This article summarises some of our work, funded by the University of Reading, designed to help address this gap in knowledge by focussing on the Republic of South Africa, which in 1997/98 experienced the first commercial release of a GM crop (Bt cotton) in the whole of sub-Saharan Africa. Not only that, but one of the target groups for the release was small-scale farmers in the Makhathini area of KwaZulu Natal near the border with Mozambique.

Throughout the world the cotton crop is susceptible to damage from a range of insect pests such as the bollworm complex, jassids, and aphids. It is estimated that in 1994 approximately 24% of the insecticides sold in the world were used on cotton<sup>1</sup>. Insecticides are expensive, and of course their use poses a serious

### THE ISB NEWS REPORT

The material in this News Report is compiled by Information Systems for Biotechnology, funded as the National Biological Impact Assessment Program by a grant from USDA/CSREES to Virginia Tech. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the view of the US Department of Agriculture or of Virginia Tech. The News Report may be freely photocopied or otherwise distributed with attribution.

Current and past issues of the ISB News Report are available at <http://www.isb.vt.edu>.

**Editor:** Ruth Irwin  
rirwin@vt.edu

#### **To order your free subscription:**

Email: Send to [news@nbiap.biochem.vt.edu](mailto:news@nbiap.biochem.vt.edu)  
Type *subscribe newsreport*  
*[your name]* in the message section. Do not include any additional text.

Phone: Call 540-231-3747

Web: Connect to <http://www.isb.vt.edu>.  
Select "News Report," Subscribe."

ISB welcomes your comments and encourages article submissions. If you have a suitable article relevant to our coverage of the agricultural and environmental applications of genetic engineering, please email it to the Editor for consideration.

#### **Information Systems for Biotechnology**

207 Engel Hall  
Virginia Tech  
Blacksburg, VA 24061  
Tel: 540-231-3747  
Fax: 540-231-9070  
Email: [isb@vt.edu](mailto:isb@vt.edu)



potential threat to the health of farmers and their workers, including children, as well as to the environment. While not removing the need for insecticide application, the insect resistant Bt (*Bacillus thuringiensis*) cotton variety should help alleviate these problems.

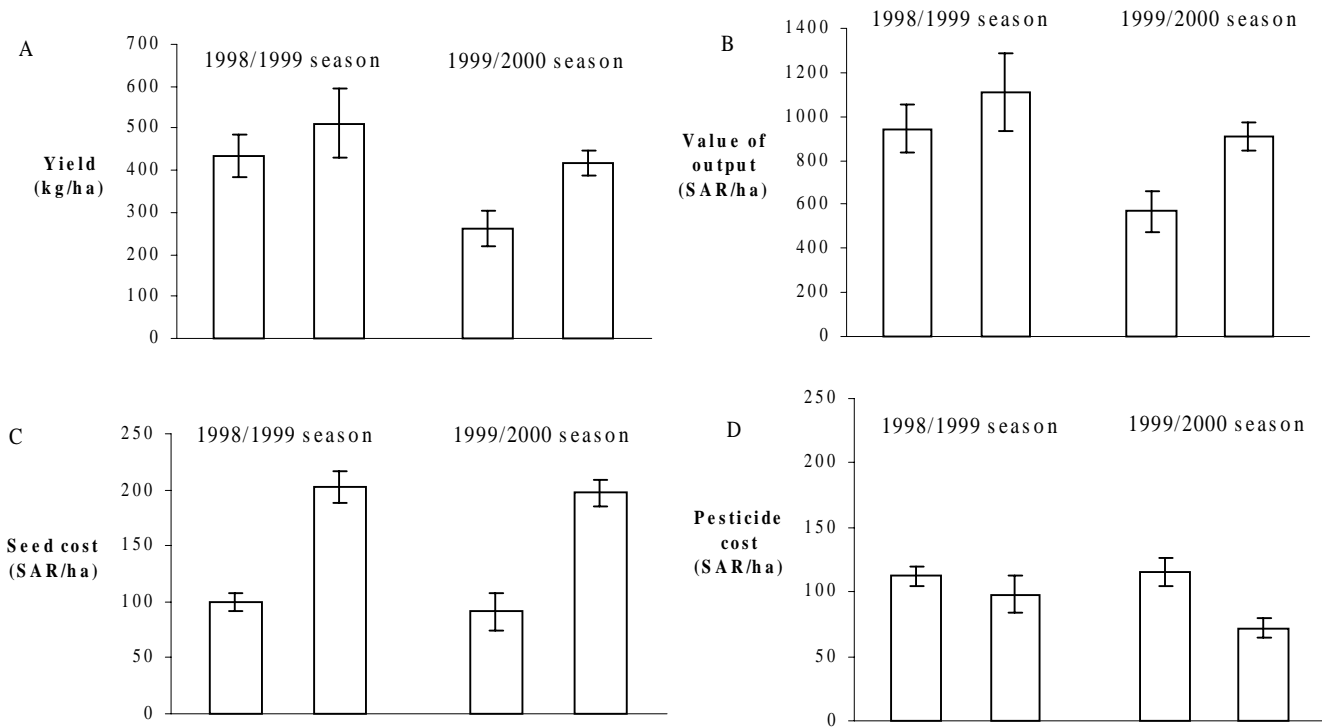
The commercial release of Bt cotton in South Africa was made possible by the Genetic Modified Organism Act of 1997, and, in the 1997/98 season, a few farmers in Makhathini grew the variety as a trial. Adoption expanded rapidly following the first commercial release in 1998/99, and by 2001/02 it was estimated that approximately 90% of farmers were growing Bt cotton. This rate of adoption is not surprising given the importance of agriculture, particularly cotton, in the livelihood of local farmers and the need to limit pest attack. Many of the main insecticides used in Makhathini, particularly the organophosphorus-based insecticides, are highly toxic. In addition, carrying a knapsack sprayer for many hours to spray one hectare of cotton under the heat and humidity of Makhathini is a tiring task.

However, were there economic benefits to growing Bt cotton? Our fieldwork designed to address this question began in early 2000, and the first phase was a pilot study of 100 farmers to look at economic impact for the 1998/99 and 1999/2000 seasons (Figs. 1 and 2). In both growing seasons the Bt cotton gave higher yields per hectare than the non-Bt varieties, although the gap was most marked in the 1999/2000 season, probably because of an unusually heavy rainfall which reduced average cotton yields for all growers. It is noticeable that the proportional fall in yield in 1999/2000 was higher for non-adopters. For the other two components of gross margin, the seed costs per hectare were higher for the Bt adopters, while their pesticide costs were lower. However, for adopters the gains in pesticide saving per hectare were much less than the extra seed cost per hectare, although again there was a seasonal effect. In 1998/99 the reduction in pesticide cost/ha was only 13%, while in 1999/00 it rose to 38%. This was probably due to two factors:

- 1) a lack of familiarity with the technology in the first season; and
- 2) adopters may have been reluctant to take the risk of technology failure and hence applied insecticide close to their 'normal' rate for bollworm control.

The overall result was an increase in gross margin per hectare for adopters (Fig. 2)<sup>2</sup>. In the first season the difference between the gross margins of Bt adopters and non-adopters was only 11%, while in the second season this rose to 77%.

In 2001 a more extensive study was implemented based on a detailed analysis of records held by the major supplier (Vunisa Cotton) of inputs (including credit) to farmers in the area. This same company is also the only purchaser of cotton. Vunisa has kept records of all transactions with the farmers since the first commercial release of Bt, and the authors are highly indebted to



**Figure 1.** Components of the gross margin for Bt cotton adopters and non-adopters in Makhathini. Within each season the bar on the left is for non-adopters of Bt cotton while the bar on the right is for adopters. Error bars are one standard deviation. Value of output is given by yield X price/kg (assuming SAR 2.175/kg).

the company for opening up their files. As a result:

- 1) data for three seasons could be compiled (1998/99 to 2000/01); and
- 2) sample sizes for analysis were much larger (between 254 and 1196) than in the pilot study when it was only possible to interview 100 farmers.

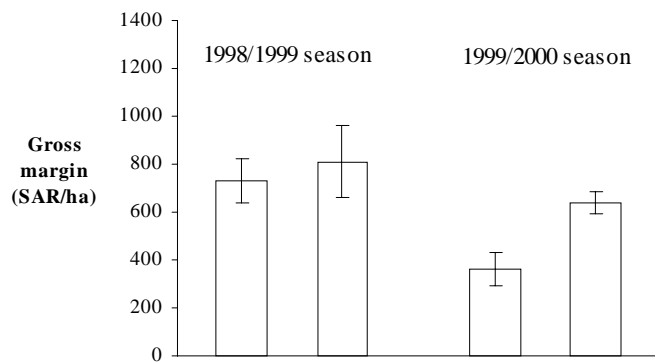
A detailed survey of 32 farmers was also conducted with the particular aim of better understanding issues of ‘spraying behavior’. This allowed the gross margin analysis to go beyond a consideration of just seed and pesticide costs. The results are still being analysed, but broadly confirm the main findings of the pilot study and indeed provide evidence for even greater benefits.

Besides economic impacts of Bt cotton, the research has also investigated other important issues such as patterns of adoption (size of farm, age of farmer, gender), ergonomics (time taken and distance walked during spraying), and incidence of accidental poisoning from insecticide. One concern with the rapid increase in adoption of Bt has been the acceptance of refugia as a means of managing potential ‘resistance to the resistance’. Results suggest that only 50% of the respondents had received some form of training on refugia, and, of these, approximately one half had actually planted a refugia plot. However, one interesting development was that many of the Bt adopters continued to

grow an area of non-Bt cotton, not because of resistance management but as insurance in case the Bt cotton failed.

Our findings suggest that the use of Bt cotton has provided economic and other benefits for the farmers of Makhathini and indeed mirror the findings of others<sup>3</sup>. Better gross margins and less time and energy spent spraying fields as well as the reduced risk of poisoning can only be beneficial, and this does not even take into account an assumed environmental benefit arising out of a reduction in pesticide usage. There are concerns, such as the lack of farmer understanding and implementation of refugia, and clearly there are important issues of sustainability to consider.

One does need to keep reported benefits in perspective. After all, Bt cotton is not a panacea that will solve the complex issue of poverty overnight. The highest gross margin advantage per hectare from our research was approximately SAR 700, which translates to about USD \$70/ha (USD \$1 = approx. SAR 10). The median urban daily wage rate in South Africa is reported to be between USD \$4.00 and \$4.50<sup>4</sup>, although agricultural wage rates in Makhathini are much lower (USD \$1.00/day). Therefore, in terms of options, the gross margin advantage is at best equivalent to about 15 to 18 days paid employment in one of the cities, provided one is able to travel and can find the work, of course. Nevertheless, if Bt cotton is managed



**Figure 2.** Average gross margin per ha for Bt adopters (right bars) and non-adopters (left bars). Error bar is one standard deviation.

properly it can be a positive and potentially sustainable contribution to farmer livelihood in Makhathini.

References and Notes

1. Myers D. *New Internationalist*, May 2000.
2. Gross margin has been calculated simply on the basis of revenue minus seed and pesticide costs (labour and water, for spraying, costs are not included).
3. See for example the following:  
 Pray C, Ma D, Huang J, and Qiao F. (2001) *World Development* 29(5): 813-25.  
  
 Qaim M and Zilberman D. (2003) *Science* 299: 900-902.  
  
 Traxler G *et al.* (2002) *Transgenic cotton in Mexico: Economic and environmental impacts*. Proceedings of the 5th International Conference on Biotechnology. Ravello, Italy.
4. Stryker JD *et al.* (2001) *Increasing demand for labour in South Africa*. Research Report, USAID, Bureau for Africa, Office of Sustainable Development, Washington.

*Stephen Morse, Richard Bennett and Yousouf Ismael*  
 University of Reading, Reading, Berkshire UK  
 s.morse@reading.ac.uk

**PLANT RESEARCH**

**TWO GENES FOR ONE PROTEIN:**

**APPLICATION OF *Ssp* DnaE SPLIT INTEIN IN PLANT GENETIC ENGINEERING**

*Jianjun Yang*

Inteins are internal protein elements mediating post-translational protein splicing. A typical intein element consists of 400 to 500 amino acid residues. It contains four conserved protein splicing motifs A, B, F, and G, as well as

a homing endonuclease sequence embedded between motifs B and F. During classic protein cis-splicing, the intein catalyzes a series of reactions to remove itself from the precursor and ligate the flanking external protein fragments (i.e., exteins) into a mature protein. Three conserved amino acid residues at intein-extein junctions are directly involved in the protein splicing reactions. They include a Ser or Cys at the intein N-terminus (the first amino acid in motif A), an Asn or Gln at the intein C-terminus (the last amino acid in motif G), and a Ser, Thr, or Cys at the beginning of the C-extein. The embedded endonuclease plays a role in mobilizing intein genes. It can be artificially deleted or naturally absent from the intein sequence without compromising protein splicing. According to InBase, the intein database, nearly 140 putative inteins have been identified from prokaryotes and single-cell eukaryotes<sup>1</sup>. But no intein has been found in higher plants. Nor had any known intein been demonstrated to function in plant cells.

Inteins can artificially be split into an N-terminal half (Int-n) that contains the A and B motifs and a C-terminal half (Int-c) that contains the F and G motifs. When the split intein fragments are fused with different protein or peptide precursors, they are able to conduct protein trans-splicing that assembles two separate precursor molecules into a mature hybrid protein. Recently, a pair of functional and naturally split intein coding sequences were identified from the split *DnaE* genes in the genome of *Synechocystis* sp. PCC6803<sup>2</sup>. One of the split genes encodes a fusion protein tandem containing 774 N-terminal amino acid residues of the DnaE protein and 123 amino acid residues of the DnaE Int-n. The other split gene encodes a protein sequence for 36 amino acid residues of the DnaE Int-c, followed by 423 C-terminal amino acids of the DnaE protein. Although the two genes are located 745 kb apart on opposite strands of the *Ssp* PCC6803 genome, the mature protein product is an intact 1197 amino acid catalytic subunit of DNA polymerase III, lacking any intein sequence due to the protein trans-splicing. The *Ssp* DnaE intein can mediate a trans-splicing reaction when fused to foreign proteins, both *in vitro* and *in vivo*, in bacterial and mammalian systems. Compared to the artificially split inteins, the *Ssp* DnaE intein catalyzes protein trans-splicing reactions with higher efficiency and under milder conditions.

For the first time, we have reported the reconstitution of protein trans-splicing in plant cells<sup>3</sup>. In our work, a pair of plant-optimized Int-n and Int-c coding sequences was synthesized based on the amino acid sequences of the *Ssp* DnaE split intein and the rule of plant codon usage. At the same time, beta-glucuronidase (*GUS*) gene was divided



into two parts to serve as reporter. *GUSn* encodes the first 203 amino acid residues of the enzyme (GUS-n), while *GUSc* encodes the C-terminal 415 amino acid residues of the enzyme (GUS-c). GUS-c starts with a native Cys to meet the requirement for an extein sequence to participate in the splicing. These split intein coding sequences were translationally fused to the divided *GUS* reporter genes in appropriate orders to form *GUSn-Intn* and *Intc-GUSc* fusions. They were in turn placed between 35S promoter (5'35S) and NOS terminator (*NOS3'*), resulting in chimeric constructs of 5'35S::*GUSn-Intn*::*NOS3'* and 5'35S::*Intc-GUSc*::*NOS3'*. To test the intein's function in plants, these two chimeric constructs were integrated into the same binary vector and used to co-transform *Arabidopsis* plants. We found that a functional full-length beta-glucuronidase, along with its precursors of GUS-n/Intn and Int-c/GUS-c, was present in the transgenic plants. This result suggests that the *Ssp* DnaE intein catalyzed protein trans-splicing in the plant cells. The chimeric constructs were also used to transform *Arabidopsis* individually. Neither construct was able to produce a functional full-length beta-glucuronidase by itself in transgenic plants. However, when both of them were brought into the same *Arabidopsis* plant by classic genetic crossing, a functional full-length beta-glucuronidase was detected in the hybrid plants, showing a result identical to that observed in the co-transformed *Arabidopsis*. Therefore, our work demonstrated the reassembly of a functional beta-glucuronidase in plants cells through the intein-mediated protein trans-splicing. In addition, our work shows that the *Ssp* DnaE intein is active throughout the entire transgenic plant. The reassembly of beta-glucuronidase has been carried out in many agriculturally important tissues and organs, such as leaves, roots, stems, and seeds.

Because of its capability of seamlessly ligating two separate protein fragments together without leaving any footprint, intein-mediated protein trans-splicing has great potential in plant genetic engineering. One area of application is to use it as a "molecular switch" to turn on a gene expression mechanism or a metabolic pathway through reassembly of gene regulators or metabolic enzymes. Combined with plant breeding techniques or specific promoters, the mechanism and pathway can be activated in a designated generation, tissue, developmental stage, or with special stimulators. Intein-mediated protein trans-splicing may limit the environmental impact of herbicide resistance genes by keeping different parts of the gene in plastid and nuclear genomes while assembling gene products in the cytosol<sup>4</sup>. Another attractive area is to use inteins to produce larger and more complex proteins. For example, a larger heterologous protein or enzyme is usually

more difficult, and sometimes impossible, to synthesize in plants or other organisms. If protein trans-splicing can be integrated into a production platform, however, smaller fragments of the protein could be synthesized and then assembled into a complete molecule. Production of advanced protein polymers and therapeutic proteins often requires assembly of the building blocks, which include different functional domains and peptide backbones. By utilizing the protein trans-splicing mechanism, these building blocks could be stacked in separate transgenic traits and assembled into the protein polymer or therapeutic protein in the hybrid progenies through genetic crossing.

#### References

1. Perler FB. (2002) *Nucleic Acid Res* 30: 383-384.
2. Wu H, Hu Z, and Liu X.-Q. (1998) *Proc Natl Acad Sci USA* 95: 9226-9231.
3. Yang J, Fox GC Jr, and Henry-Smith TC. (2003) *Proc Natl Acad Sci USA* 100: 3513-3518.
4. Sun L, Ghosh I, Paulus H, and Xu M-Q. (2001) *Appl Envir Microbiol* 67: 1025-1029.

Jianjun Yang  
Sr. Research Biochemist  
DuPont Central Research and Development  
jianjun-gene.yang@usa.dupont.com



### AGROBACTERIUM TUMEFACIENS-MEDIATED TRANSFORMATION OF TEMPERATE FORAGE GRASSES

Andrew J. E. Bettany

Our laboratory has been involved in the tissue culture and genetic transformation of forage grasses for many years. In that time, we have developed methods based on protoplast transformation, silicon-carbide "whiskers," and microprojectile bombardment. Recently we have produced the first report of *Agrobacterium tumefaciens*-mediated transformation of the temperate, widely grown forage species Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*)<sup>1</sup>.

The first report of gene transfer in plants mediated by the soil pathogen *Agrobacterium tumefaciens* was made in 1984<sup>2</sup>. For nearly ten years it was believed that this interaction would only work in dicotyledonous plants, because of the distinctive "wound" response. However, as evidence accumulated, it became apparent that with specific experimental alterations gene transfer could also

be made to take place between *A. tumefaciens* and monocotyledonous species.

*Agrobacterium*-mediated gene transfer is considered desirable because of the following advantages:

1. Transgenic DNA is preferentially integrated into transcriptionally active regions of the genome;
2. Transgenes are normally integrated in low copy number;
3. Vector DNA is excluded from the transfer of any T-DNA;
4. Co-transformed T-DNAs may be inserted at different integration sites, often on different chromosomes (allowing segregation of desired transgenes and undesired selectable markers); and
5. Large (>50kb) DNA fragments can be inserted (e.g., BACS).

For these reasons, we have been working on a system to obtain transgenic forage grasses via *A. tumefaciens*-mediated transformation.

### Plant material

Selection of plant and bacterial genotypes is important for success. We have used established plant genotypes, which have proven success in tissue culture and regeneration of plants. Many forage grasses are outbreeders, so that embryo-derived cultures will all be different genotypes. Thus, it is more complex to screen for amenable genotypes, and new cultures must be started from shoot tips or inflorescences. The genotypes we use have been selected after an extensive screen for tissue culture responsiveness. The source material in our experiments was callus-derived from shoot tips. However, the source of the material does not seem to matter so long as highly regenerative, actively dividing cells of a suitable genotype are obtained. We have found that callused tissue, even if only on auxin-containing medium for a few days, is more successfully transformed than explants directly.

### Agrobacterium

We elected to use an *Agrobacterium* strain and T-DNA binary vector that had proven successful in the transformation of another monocot species, rice (*A. tumefaciens* strain LBA4404, binary vector pTOK233<sup>3</sup>). This system had the added advantage of containing the selectable marker *hph*, encoding resistance to the antibiotic hygromycin, which we had extensive experience in using. We have noted that using fresh stocks of *Agrobacterium* from glycerols works better than stocks maintained on plates. Many different media have been described for growth of *Agrobacterium*. Although suspension cultures grow better in richer media (e.g., MGL), pelleting the *Agrobacterium* proved to be difficult in such media, so we

routinely use medium AB<sup>4</sup> containing 50 mg l<sup>-1</sup> hygromycin. Reported conditions for pelleting *Agrobacterium* also vary widely; we determined that 2,700 x g for 10 minutes was adequate. We have found it essential to induce the *vir* gene functions of *Agrobacterium* by a pre-incubation in medium containing 100 mM acetosyringone.

### Infection and regeneration of plants

For maximum infection, active cell division is essential; therefore we use cells 3–4 days after sub-culture. Cells were washed in the induction medium before addition of induced *Agrobacterium*—we have found it helpful to do this at 25–28°C. We then plated immediately onto solid medium and co-cultivated in the dark at 25°C for three days. It is important to remove excess *Agrobacterium* at this stage by blotting, since wet cultures rarely retain embryogenic potential for long. We regenerated plants using stepwise selection in liquid culture (0→50 mg l<sup>-1</sup>→75 mg l<sup>-1</sup> hygromycin) over a period of 25 days, after which cells were plated onto solid medium. All media also contained 250 mg l<sup>-1</sup> cefotaxime to kill the *Agrobacterium*. Embryogenic colonies were transferred to regeneration medium containing 50 mg l<sup>-1</sup> hygromycin but no cefotaxime.

### Analysis of Regenerated Plants

Although we only produced a limited number of plants (eight *Lolium* and two *Fescue*) most of these were shown to be independent transformants by Southern blotting. What we found most surprising was that the majority of the plants (6/7 of those analysed) contained rearranged or truncated T-DNAs; usually this proportion is nearer 1/10. We can only speculate as to the reasons for this, but it is worth mentioning that the design of the Southern blotting experiment can significantly under-estimate the degree of rearrangement. Indeed a recent paper<sup>5</sup> has suggested that T-DNA rearrangements may be more common than has been reported.

Unfortunately, only two of our plants expressed both transgenes (the selectable gene *hph*, encoding resistance to hygromycin, and the non-selected gene *uidA*, encoding the enzyme beta-glucuronidase). We could show that most plants contained at least one intact copy of each transgene. Therefore, we think this may be due to co-suppression effects, since both genes were driven by the same promoter and most plants contained more than one copy of each gene.

By the time the plants flowered, none were still expressing the *uidA* gene. Only three *Lolium* plants produced sufficient seed for analysis. Transgene transmission was mainly mendelian, but we saw less paternal transmission than



expected, and male and female transmission was not always identical. We have seen similar patterns in the past from plants transformed via Silicon-carbide whiskers and microprojectile bombardment so we cannot rule out the possibility that this is a consequence of our selection method or, indeed, our genotype.

We have established that *Agrobacterium tumefaciens* can be used to transform temperate forage grasses, but more work still needs to be done on optimising the system.

#### References

1. Bettany AJE *et al.* (2003) *Plant Cell Reports* 21(5): 437-444.
2. De Block M *et al.* (1984) *EMBO Journal* 3: 1681-1689.
3. Hiei Y *et al.* (1994) *Plant J* 6: 271-282.
4. Chilton MD *et al.* (1974) *Proc Nat Acad Sci USA* 71: 3672-3676.
5. Mason G *et al.* (2002) *BMC Biotechnology* 2(1): 20.

Andrew J. E. Bettany  
Institute of Grassland and Environmental Research  
Aberystwyth, Wales, UK  
andrew.bettany@bbsrc.ac.uk

## INSECT NEWS

### REGIONAL SUPPRESSION OF INSECT PESTS WITH BT CROPS

*Yves Carrière, Timothy J. Dennehy and Bruce E. Tabashnik*

Over-reliance on broad-spectrum insecticides can cause environmental contamination, evolution of pest resistance, and disruption of biological control by natural enemies. In many crops, a few key pests trigger most insecticide applications. Transgenic cultivars producing insecticidal proteins of *Bacillus thuringiensis* (Bt) can kill some of these key pests, yet are much less harmful than most synthetic insecticides to nontarget arthropods, wildlife, and humans. Thus, by replacing broad-spectrum insecticides, Bt crops can help to improve pest management and preserve the environment.

Although Bt crops quickly became a significant component of agriculture, little is known about their long-term consequences. Bt crops do provide short-term control of key pests<sup>1</sup>, but scientists have debated whether they could also cause long-term declines in pest populations. In the Febru-

ary issue of the journal *Proceedings of the National Academy of Sciences*<sup>2</sup>, our team of researchers from Arizona reported that Bt cotton caused long-term suppression of a major pest, the pink bollworm (*Pectinophora gossypiella*).

The ten-year study was conducted in 15 cotton-growing regions (between 412 and 4350 km<sup>2</sup> per region) across the state of Arizona. Population density estimates for each region were based on the average number of male pink bollworm moths captured weekly in traps baited with female sex pheromone. A network of >1000 pheromone traps was used in each year to evaluate regional population density in the spring, which reflects population density later in the season. Abundance of Bt and non-Bt cotton fields in each region in the year preceding trapping was quantified with Geographical Information System (GIS) technology. The association between spring population density and regional use of Bt cotton in the previous year was evaluated with multiple regression, which accounted for effects of weather and regional differences other than Bt cotton use.

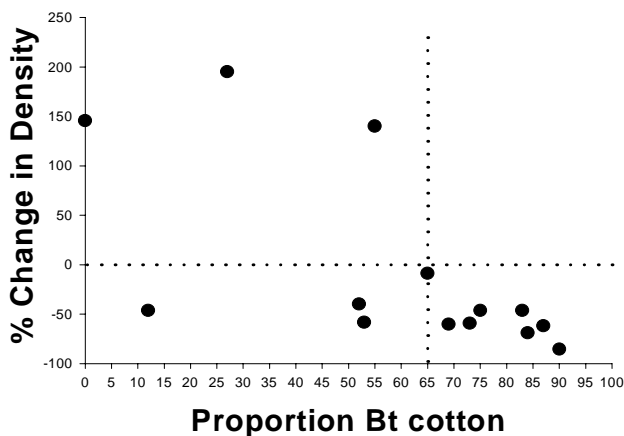
Bt cotton was first used on a large scale in Arizona during 1996, when it accounted for less than 20% of the state's total cotton acreage. From 1997 to 2000, Bt cotton accounted for at least half of Arizona's cotton annually. Pink bollworm population declines started in 1998 and continued through 2001, the last year of the study.

The association between regional abundance of Bt cotton (averaged from 1997 to 2000) and average regional population density for the years before use of Bt cotton (1992-1995) was positive, showing that producers who experienced high pink bollworm pressure before commercialization of Bt cotton became high adopters of the new technology. However, for 1999-2001, after widespread use of Bt cotton, the association between regional population density and abundance of Bt cotton was negative. Thus, intensive use of Bt cotton transformed high infestation regions into low infestation regions.

We compared average regional population density of pink bollworm from 1992 to 1995 (before large scale use of Bt cotton) vs. 1999 to 2001 (after large scale use of Bt cotton). For each region, we analyzed changes in population density—before vs. after large scale use of Bt cotton—as a function of the percentage of total cotton acreage planted to Bt cotton (Fig. 1). Changes in population density were variable in regions where less than 65% Bt cotton was used, with decreases occurring in half of the regions and increases in the other half. In contrast, popula-

tion density declined in all regions where more than 65% Bt cotton was used.

The results described above and other analyses<sup>2</sup> suggest that a threshold value of Bt cotton use of about 65% was required to suppress pink bollworm populations. Among regions with Bt cotton use above this threshold, the percentage change in population density was negatively associated with the percentage of Bt cotton (Fig. 1). The greatest decline in population density was 62%, which occurred in the region where the highest percentage (87%) of Bt cotton was planted.



**Figure 1.** Association between regional proportion of Bt cotton and percent change in pink bollworm population density. Proportion of Bt cotton is the average proportion of Bt cotton from 1997 to 2000 in each region. Change in density (CD) was obtained for each region by subtracting from the average density between 1999 and 2001 the average spring density between 1992 and 1995 (AD). The % change in density was calculated for each region as  $CD / AD \times 100$ . One region was excluded because we had no estimates of its density before 1997. The horizontal dotted line indicates no change in density between 1992-1995 and 1999-2001. The vertical dotted line separates regions with more or less than 65% Bt cotton.

In general, two factors can reduce target pest density in regions where Bt crops are used. First, replacing non-Bt crop fields with Bt crop fields eliminates suitable habitats. Second, most pests do not discriminate for oviposition between Bt and non-Bt crops<sup>3</sup>. Thus, females that move from non-Bt crop fields or patches of wild hosts and lay eggs on Bt plants will waste eggs if most of their progeny die on such plants. This can sufficiently reduce female reproductive rate in non-Bt host habitats to cause a regional decline in pest density.

Simulation and analytical models show that decline of pest populations may occur if use of a Bt crop exceeds a threshold value<sup>2</sup>. Such declines become more likely as abundance of the Bt crop and female movement increase and as the net reproductive rate of the pest in non-Bt host

habitats decreases. Accordingly, suppression of a pest by a Bt crop is less likely to occur in pests that are generalists, sedentary, or have high reproductive rates in non-Bt host habitats.

Throughout its worldwide range, the pink bollworm uses a variety of host plants, but it is a specialist in Arizona where cotton is virtually the only suitable host available. Such ecological specialization would have favored the regional suppressions. Currently, we do not know whether the reductions in pink bollworm density are exclusively due to habitat elimination or a combination of habitat elimination and egg wastage. We plan to acquire more data to determine whether the declines will continue until pink bollworm becomes rare in regions where more than 65% Bt cotton is used.

Control of pink bollworm by Bt cotton has helped to substantially reduce insecticide use in Arizona. The long-term regional suppression of pink bollworm may further reduce insecticide use and enhance implementation of the refuge strategy, which is mandated by the U.S. Environmental Protection Agency for delaying evolution of pest resistance to Bt cotton. This strategy requires that growers plant refuges of non-Bt cotton to provide susceptible insects for mating with resistant insects. By fostering long-term population declines, Bt cotton could reduce the need for pink bollworm control, thereby facilitating deployment of larger refuges and reducing the risk of resistance.

Evolution of resistance would eliminate the benefits of Bt cotton, which has been extremely effective against pink bollworm in Arizona so far. However, the estimated frequency of alleles conferring resistance has been much higher in field populations of pink bollworm than in other pests targeted by Bt crops<sup>1,4</sup>. Therefore, to delay resistance, it is important to maintain an effective refuge near every Bt cotton field<sup>5</sup>. It is becoming increasingly clear that regional abundance of Bt crops and agricultural practices interact with pest behavior and life history to affect refuge efficacy. Accordingly, rigorous field experiments are urgently needed to better understand how refuges should be deployed and managed, especially in regions where Bt crops are abundant.

References

1. Tabashnik BE *et al.* (2000) *Proc Natl Acad Sci USA* 21:12980-12984
2. Carrière Y *et al.* (2003) *Proc Natl Acad Sci USA* 100: 1519-1523
3. Liu Y-B *et al.* (2002) (Lepidoptera: Gelechiidae). *J Econ Entomol* 95:143-148



4. Morin S *et al.* (2003) *Proc Natl Acad Sci USA in press*

5. Carrière Y, Tabashnik BE (2001) *Proc Royal Soc Lond B*  
268:1475-1480

*Yves Carrière*<sup>1</sup>, *Timothy J. Dennehy*<sup>2</sup> & *Bruce E. Tabashnik*<sup>3</sup>  
*Department of Entomology, The University of Arizona*  
<sup>1</sup>*carrier@ag.arizona.edu*  
<sup>2</sup>*tdennehy@ag.arizona.edu*  
<sup>3</sup>*brucet@ag.arizona.edu*

## REGULATORY NEWS

### RISKY GENOMICS: THE ROLES OF RISK/BENEFIT ASSESSMENT AND RATIONAL DECISION FRAMEWORKS

*Robert C. Lee*

#### Background

Risk management is important with regard to new or emerging technologies or programs that have associated risks. Risk is typically defined as the probability and severity of an adverse event<sup>1</sup>, as distinguished from benefit, which can be defined as the probability and magnitude of a positive event. The “precautionary principle” has been advocated as policy or guidance in many risk management scenarios. I focus here on risk management involving genomic technologies (defined here as any application of genomic science, i.e., genetic manipulation of biota). Specifically, genetically modified organisms (GMOs) have been the center of vigorous debate.

Definitions of the precautionary principle seem to be as numerous as the stakeholders involved in these decisions<sup>2</sup>, so for discussion purposes I will assume that, in a broad sense, “precaution” implies managing a risk before an adverse event occurs; e.g., imposing an intervention that prevents or reduces the probability of the event. Precaution could also imply having a mitigation plan in place designed to manage the adverse consequences of an event once the event occurs, although this usage is not as common. Being precautionary is a reasonable and rational course of action and indeed is plain old common sense, *assuming one has good knowledge regarding the risks*. Risk management becomes more difficult, however, when the probability and/or the severity of an adverse event are uncertain, which is the case with many worrisome low-probability, high-consequence risks such as those associated with environmental toxicants, human influenced climate change, and now GMOs.

#### Risk management under a state of uncertainty

In the face of uncertainty, decision-makers may make good decisions, or they may make two classes of risk management judgement errors. They may err in terms of, say, waiting too long to impose controls on a risky technology (thus potentially resulting in the adverse consequences actually taking place). Conversely, they may err in terms of imposing controls too soon or inappropriately without proper evaluation of the risks, benefits, and costs of the technology (thus potentially resulting in inefficient, costly, useless, and/or risky controls). An associated problem arises when a high degree of uncertainty exists (or indeed when unanticipated risks exist), or when psychosocial factors (such as dread of exotic “Frankenstein” type technologies, inappropriate media coverage, etc.) are important. In such cases the perception of risk (as opposed to the “true” nature of risk in terms of probability and severity) and risk attitudes become particularly important<sup>3</sup>. Risk perception and risk attitudes, and more generally, stakeholder values can be highly variable across those stakeholders because of factors such as cultural background, education, employment, and socio-economic status. In cases of technologies that may potentially affect a spectrum of stakeholders both positively and negatively, it is obvious that such stakeholders’ values should be explicitly considered in terms of a decision-making process, although in fact this is rarely done (unless prompted by legal action). The consequence of *not* involving stakeholders is often a decision based on fear and politics, as opposed to reason.

So, how does one go about being “precautious” when one does not know exactly what event will happen, whom the event will affect, and the nature of the consequences? What happens when risks that are perceived to be important by some stakeholders are perceived as trivial by others? What are the tradeoffs involved and implications of controlling some technologies and not others? Typical precautionary principles are at the least simplistic and potentially harmful, because they generally fail to address these and similar considerations.

#### Risk assessment

Risk assessment informs decisions made under a state of uncertainty and is defined as the science and process of characterizing and quantifying risks. Proper risk assessment (often termed “probabilistic” risk assessment) also involves characterizing and quantifying uncertainties. For many years scientists and decision-makers have recognized the value of risk assessment in making risk management decisions regarding industrial technologies such as nuclear power, as well as unintended risks such as environmental contamination<sup>4</sup>. An important distinction must be made:

*Risk assessment is not risk management*; the former is a scientific process that informs the latter. A risk management approach that does not explicitly incorporate risk assessment and uncertainty assessment ignores important information, and thus is inherently flawed.

### **Benefits assessment**

Of course, important decisions regarding technologies are not based on risk alone. Any particular technology worth developing is not just risky, but presumably has some benefits to its proponent/developer and others. These benefits also may be highly uncertain. Essentially, the same approaches and techniques used for risk assessment can be used for “benefits assessment.” Interestingly, even from a business perspective the economic benefits of new technologies are often unclear. For example, biologically produced pharmaceuticals are a “hot item,” but the economic benefits of growing drugs in field plants given the present and likely future regulatory climate are far from clear. When the perspectives of multiple stakeholders (such as consumers, growers, and regulators) are introduced, as they should be, the benefits become much less clear, making components of the genomic technology industry reminiscent of the “dot.com bubble.” There appears to be a prevalent, highly naïve assumption that any new genomic technology, especially those with health care applications, will confer some net benefit to its developers and consumers. Regardless, approval and implementation of technologies are contingent on some sort of balance between associated benefits and risks, whether explicitly stated or not.

### **Tradeoffs**

Consideration of both benefits and risks introduces the additional concept of tradeoffs. Economic costs enter the equation in many ways, but are often viewed as a constraint on resources (e.g., a technology that costs too much may not be worth developing, even though the benefits may be great). Additionally, when a risk management approach is taken, more tradeoffs are introduced. Risk management always costs something. These costs may be economic, either in terms of up-front costs or opportunity losses (i.e., money spent on managing a particular risk could be spent otherwise). Costs may also be associated with foregone benefits or competing risks introduced by the policy, e.g., the foregone health benefits associated with a genomic therapy that is not allowed because of the risks. Uncertainties also introduce tradeoffs. A tradeoff exists between the potential for false negatives (i.e., something is judged “safe” when it is really risky) and the potential for false positives (i.e., something is judged “risky” when it is indeed safe) in risk management decisions.

A risk management approach that does not explicitly address tradeoffs is flawed and can result in inefficient or even harmful decisions. For instance, delaying approval, on the basis of uncertain risks, of a novel life-saving drug that is a result of genomic technology could result in loss of life of patients who may benefit.

### **Being precautionary**

The science of genomic technology is advancing at an extremely rapid rate. Unfortunately, this rate is far outstripping the ability for decision-makers to evaluate the benefits, risks, and costs under present regulatory and advisory systems and risk assessment protocols. This may be a reason why precautionary principles have been proposed as ways to manage these rapidly advancing technologies. To be clear, a precautionary principle is an *ex ante* risk management strategy.

An example of a simple precautionary principle states that a state of uncertainty does not preclude making a decision. This is common sense. However, advocacy-based versions state that risk management action should be taken in the face of a risky technology, and indeed that certainty of safety must be demonstrated by a proponent of a technology<sup>2</sup>. This is simplistic and indeed irrational risk management. Rather than engaging and informing stakeholders, such precautionary risk management takes a rather paternalistic approach, essentially imposing the values of a few experts or advocacy groups on a large spectrum of stakeholders. Such a precautionary principle hamstring true stakeholder involvement and proper risk management by constraining elicitation of stakeholder values and the scientific process of risk/benefit assessment with pre-determined conclusions and the refusal to acknowledge tradeoffs. In its most inappropriate implementation the precautionary principle can indeed impede scientific progress, much of which is targeted toward improvement of the human condition. No drug, therapy, or surgical procedure would ever be approved if it had to be risk-free. Note that rigorous interpretation of precaution potentially also results in a rather nonsensical scenario; i.e., if all actions must be certified as “safe” before implementation, then should not this apply to the precautionary principle itself?

### **Decision frameworks**

An appropriate, rational risk management process will integrate the science of risk/benefit assessment with the policy of risk management, but to my knowledge this is not occurring to date with GMO issues. It is unfortunate that many of the same mistakes that have been made historically in environmental toxicology and other fields are now being made with regard to GMOs; history appears to be



repeating itself. The appropriate and acknowledged way to evaluate highly uncertain risks is to employ risk assessment as a scientific process within an explicit decision framework that directly addresses stakeholder values, tradeoffs, and the impact of uncertainty on decisions.

The term “requisite model” refers to a policy model that contains everything essential for informing the issue at hand<sup>5</sup>. Questions that we may ask to develop such a model include:

- Who are the (legitimate) stakeholders?
- Who bears the consequences of the decision?
- Who is responsible for making/implementing/enforcing the decision?
- What do the stakeholders care about?
- What are their preferences for different outcomes?
- What trade-offs are they willing to make among different consequence dimensions (e.g., cost vs. safety)?
- What are the competing decision options to be evaluated?
- What information do the stakeholders need to make well-informed decisions? What questions are they asking?
- What information is immediately available about the probable consequences of different decisions?
- What data gaps and uncertainties exist, and what means exist to reduce uncertainties?
- What analytic tools and experts are available?
- What are the resource and time constraints on making the decision?

There is a wealth of literature and applications regarding analytic frameworks appropriate for such evaluations, such as multiattribute utility theory (MAUT) and multi-criteria decision-making (MCDM)<sup>4</sup>. It is unclear why such frameworks are not being used to help sort out complex decisions involving genomic technologies, but simple ignorance of the methods may be a reason.

MAUT provides a way of incorporating stakeholder values, preferences, and risk attitudes directly into a transparent, quantitative analytic framework that addresses uncertainties and tradeoffs. Risks, benefits, and costs are balanced in utility functions, which are summary measures of each alternative’s attainment of decision objectives based on stakeholder values.

Another approach for addressing multiple objectives is multi-criteria decision-making (MCDM) in which “inferior” alternatives are eliminated in a systematic fashion, rather than attempting to rank all alternatives. MCDM methods allow for incomplete, ambiguous, and uncertain preferences. They recognize that, while some pairs of alternatives may not be comparable, it may be unnecessary to explicitly compare them to identify the preferred alternative.

While approaches such as MAUT and MCDM do not, by

any stretch of the imagination, provide all the “answers,” they do provide a transparent, balanced basis for informing a decision. These methods have been around for decades, and are routinely applied in many different types of risk management decision-making (such as in engineering and in environmental management). However, genomic technologies pose some special challenges; indeed the potential benefits and risks associated with such technologies may eventually exceed those associated with any other class of technology, and uncertainties are tremendous. There is a pressing need for research that will elucidate the best ways to inform genomic technology decisions. Current risk assessment protocols are inadequate to capture the full range of risks and uncertainties, both short- and long-term. As noted above, there is a great need for efficiency in conducting assessments; the rate of development of the technologies is continually increasing, and risk managers are already overwhelmed. Thus, methodological development and capacity-building for applied assessments are critical. This is not an academic exercise; these are real, very difficult decisions.

It is time to acknowledge that simplistic policy approaches such as the precautionary principle are insufficient to address the complex decision-making that is associated with genomic technologies. It does not take a complex analysis (nor a rocket scientist) to predict that regulation of such technologies without informing decisions via risk/benefit assessment, incorporation of stakeholder values, and rigorous assessment of uncertainties and tradeoffs will result in arbitrary decisions and negative consequences for society.

#### References

1. Haimes YY. (1998) *Risk Modeling, Assessment, and Management*. Wiley.
2. Raffensperger C and Tickner J. (1999) *Protecting Public Health and the Environment: Implementing the Precautionary Principle*. Island Press
3. Slovic P. 2000. *The Perception of Risk*. Earthscan Publications, Ltd.
4. Cox LA. (2001) *Risk Analysis: Foundations, Models, and Methods*. Kluwer Academic Publishers.
5. Phillips LD. (1982) Requisite decision modeling. *J Operat Res Soc* 33: 303-312

Robert C. Lee  
Assistant Professor, Faculty of Medicine, University of Calgary  
Director, Calgary Health Technology Implementation Unit,  
Calgary, Alberta  
rlee@ucalgary.ca

**ISB News Report  
207 Engel Hall  
Virginia Tech  
Blacksburg, VA 24061**

Non-Profit Org.  
U.S. Postage  
PAID  
Blacksburg, VA 24060  
Permit No. 28