

ISB NEWS REPORT

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RISK ASSESSMENT NEWS

Gene Flow from Transgenic Oilseed Rape

Tristan Funk, Peter Westermeier & Gerhard Wenzel

Introduction

Since the introduction of genetically modified (GM) plants for commercial production in 1996, the global area of GM crops has continuously grown to 90 million hectares in 21 countries in 2005.¹ Ninety-six percent of the area grown with GM plants was located in six countries (United States: 55%, Argentina: 19%, Brazil: 10%, Canada: 6%, China: 4%, and Paraguay: 2%). Recently, new EU regulations (Nos. 1829/2003 and 1830/2003) concerning the traceability and labelling of GM food and feed products were passed by the European Parliament and the European Council. Labelling is not obligatory for food and feed products with GM proportions below 0.9% of the ingredients considered individually or products consisting of a single ingredient, provided that this presence is adventitious or technically unavoidable during seed production, cultivation, harvest, transport, or processing. In addition, a recommendation on guidelines for the development of national strategies and best practices was published by the European Commission involving cultivation distances between GM crops and non-GM crops, buffer zones, cropping intervals, the control of volunteer plants, etc., to ensure the co-existence of GM crops with conventional and organic farming. Particularly for oilseed rape, which can be described as a high-risk crop for crop-to-crop gene flow due to cross pollination by the vectors insects and wind, specific rules for cultivation are discussed.

The main objective of this study was the examination of short distance outcrossing of transgenic oilseed rape in the nearest neighborhood. The experimental design allowed the detailed determination of the effects of distance and wind direction on pollination frequencies and distribution. For regulations of co-existence of GM crop cultivation with conventional and organic farming, the relationship between distance and outcrossing is of major interest.



THE ISB NEWS REPORT

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In a three-year field trial, the outcrossing frequencies and distribution from plots with different ratios of transgenic plants (100%, 1.0% and 0.1%) containing the *pat*-gene for resistance towards the broad-range herbicide glufosinate-ammonium were determined in surrounding acceptor plots within a distance of 3–11 m using a biotest.

Materials and methods

Plant material

The oilseed rape (*Brassica napus* L.) varieties used in the field trial were the conventional winter variety 'Falcon' and the isogenic transformation line 'Falcon GS40/90', which is tolerant towards broad-range glufosinate-ammonium herbicides (trade names BASTA® or Liberty®) due to the integration of the synthetic phosphinothricin acetyltransferase (*pat*) gene derived from *Streptomyces viridochromogenes*. The transgenic plants were used in 'contamination plots' as source of transgenic pollen ('donors'). The non-transgenic variety 'Falcon' was used in surrounding plots ('acceptors') to determine the outcrossing frequencies from transgenic oilseed rape caused by transgenic pollen.

Experimental design

The field experiments were carried out on an experimental station near Munich (Germany) in the years 2001 to 2004. Each transgenic donor plot was surrounded by eight acceptor plots with non-transgenic plants. Plot size was 6 m x 6 m with 50 plants/m². The distance between donor and acceptor plots was 1.5 m (Fig. 1).

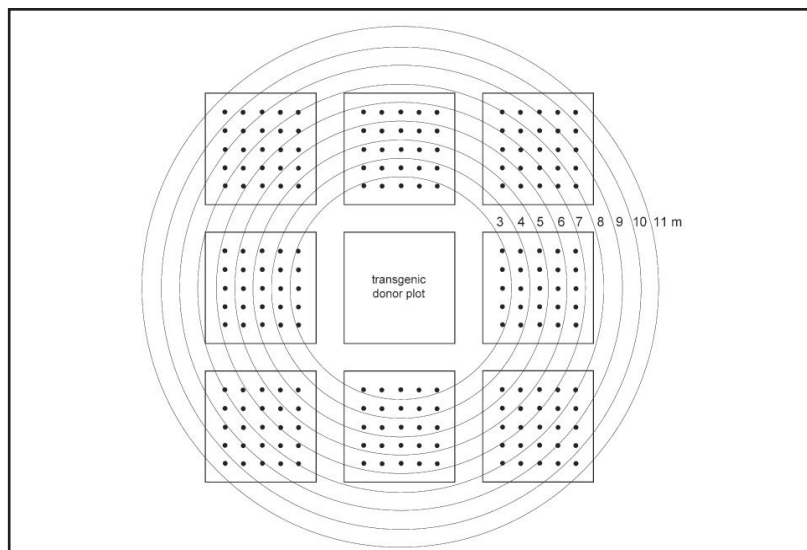


Figure 1: Schematic representation of the experimental design. One transgenic donor plot is surrounded by eight non-transgenic acceptor plots. Plot size is 6 m x 6 m, distance between plots is 1.5 m. Dots within the acceptor plots indicate the sampling points. Circles illustrate the distances to the center of the donor plot.



In 2001/2002, the donor plots containing 100% transgenic plants were grown in two replications. In 2002/2003 and 2003/2004, the donor plots contained different ratios of transgenic plants (100%, 1.0% and 0.1%) in order to simulate transgenic seed contaminations. In the vicinity of the field trial, there were several bee hive colonies: about 10 in the north (distance 1 km); 8 in the south (distance 500 m); and 5 in the west (distance 1 km).

Seed sampling and testing

A grid with 25 sampling points was laid over each of the surrounding non-transgenic acceptor plots (**Fig.1**). Thirty mature pods were collected randomly at each sampling point, resulting in a total of 750 pods per plot. For analyzing the seed samples by means of a biotest, 320 seeds from each sampling point were sown in plastic trays in the greenhouse. Considering a germination capacity of approximately 95%, the technically detectable outcrossing frequency at each sampling point was 0.33% (1 transgenic out of 300 seedlings). After one week, the seedlings were sprayed with a 1% BASTA® solution (200g glufosinate-ammonium/l). Resistant plants could be distinguished after two weeks from non-transgenic plants. In order to exclude false-positives (i.e., non-transgenic survivors), all putative transgenic seedling were retested by qualitative PCR.

For the construct-specific detection of the *pat*-gene, the primer pair *pat_f* 5'-CAC AAT CCC ACT ATC CTT CGC-3' and *pat_r* 5'-TGC TGT AGC TGG CCT AAT CTC A-3' were used to target the 35S-*pat* junction region. PCR control reactions were performed with the primer pair *s_gt-f* 5'-CAA AGA CGA TAA AGG CTA CGG C-3' and *s_gt-r* 5'-TAA TGC TCC GAT CAG AGC TTC C-3' for the *Brassica* specific nucleotide sequence of the S-glucosyltransferase gene.²

Results and Discussion

In 2001/2002, outcrossing frequencies and distribution from 100% transgenic

plots were investigated, whereas in 2002/2003 and 2003/2004, outcrossing was additionally determined from the 1% and 0.1% transgenic plots in order to simulate transgenic seed contamination.

In total, 630,000 seedlings from surrounding acceptor plots were tested in the greenhouse for glufosinate-ammonium resistance. PCR reactions for the exclusion of false-positives were carried out with DNA from all surviving seedlings and resulted in the amplification of a *pat*-specific DNA fragment with the expected size of 141 bp in 87.2% of the seedlings, while an average of 12.8% of the seedlings showed no signals and were therefore classified as false-positives. The combination of biotest and subsequent PCR analysis was very suitable for the detection of outcrossing events because large numbers of seeds could be tested quickly and cost effectively, and therefore a high resolution of the distribution of single outcrossing events could be obtained. The average gene flow within a distance of 3–11 m from the 100% transgenic plots ranged from 0.25% to 0.31% (**Table 1**). Regarding the 1.0% and 0.1% transgenic plots, a marginal and randomly distributed average gene flow amounting to 0.01% and 0.00083% to 0.0065%, respectively, was determined (**Table 1**).

| Transgenic donor plots | 2001/2002 | | 2002/2003 | | 2003/2004 | |
|------------------------|---|-------------------------------|---|-------------------------------|---|-------------------------------|
| | Outcrossing rates from single plots [%] | Average outcrossing rates [%] | Outcrossing rates from single plots [%] | Average outcrossing rates [%] | Outcrossing rates from single plots [%] | Average outcrossing rates [%] |
| 0,1 % | — | — | 0,005 | 0,0065 | 0,0017 | 0,00083 |
| | — | | 0,008 | | | |
| 1 % | — | — | 0,002 | 0,01 | — | — |
| | — | | 0,018 | | | |
| 100 % | 0,29 | 0,29 | 0,25 | 0,25 | 0,31 | 0,31 |
| | 0,29 | | — | | | |

Table 1: Outcrossing rates from transgenic donor plots with different ratios of transgenic plants in 2001/2002, 2002/2003, and 2003/2004.

Figure 2 A–C exemplify the spatial distribution of the outcrossing events around 100%, 1%, and 0.1% transgenic plots. The peaks represent the extent of outcrossing in the surrounding acceptor plots.

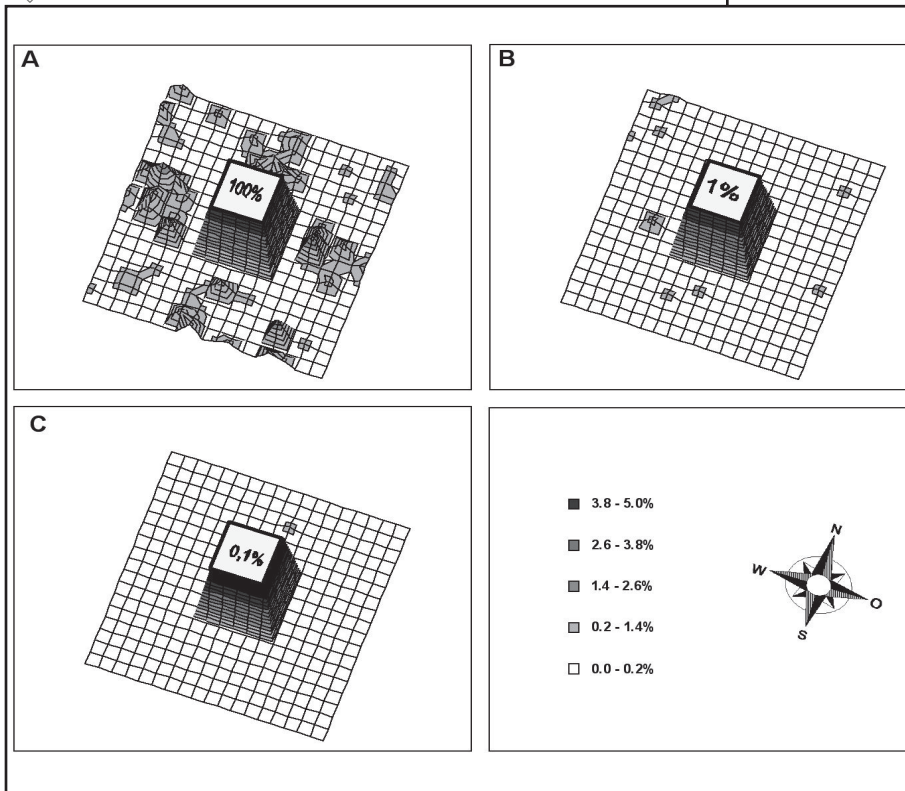


Figure 2: Distribution of the outcrossing events in non transgenic acceptor plots. (A) 100% transgenic donor plot; (B) 1.0% transgenic donor plot; (C) 0.1% transgenic donor plot

For all transgenic plots a random distribution with isolated pollination events became apparent.

The transgenic contamination in neighboring oilseed rape crops therefore was clearly below the EU labelling threshold of 0.9%. The experimental design and the extensive sampling in the acceptor plots allowed a detailed analysis of the outcrossing pattern with respect to the wind and distance parameters.

According to a χ^2 -test, no significant influence of wind on the distribution of transgenic outcrossing was found. Random and undirected gene flow can be explained by insect activity, since honey-bees (*Apis mellifera* L.) and bumble-bees (*Bombus terrestris* L.) play an important role for cross-pollination. Our

observations are supported by results of other outcrossing studies with oilseed rape^{3,4} which observed no directional effects that could be ascribed to wind activity. Several studies showed that pollen will predominantly be deposited by bees on plants close to the pollen source.^{5,6} The overall means of outcrossing events from the 100% transgenic donor plots were plotted against the distance. The pollination declined exponentially with increasing distance (**Fig. 3**). Applying the fitted curve, outcrossing remains even in the nearest vicinity below the actual threshold of 0.9% for GMO contaminations of food and feed, thus making recommendations postulating cultivation distances of 200 - 500 m questionable. The effect of the distance on the number of outcrossing events was found to be highly significant ($P < 0.0001$) by correlation analysis.

Nevertheless, as shown in this study the effects of the pollination vectors, wind and insects, are often interacting, especially at short distances, and cannot be predicted completely.

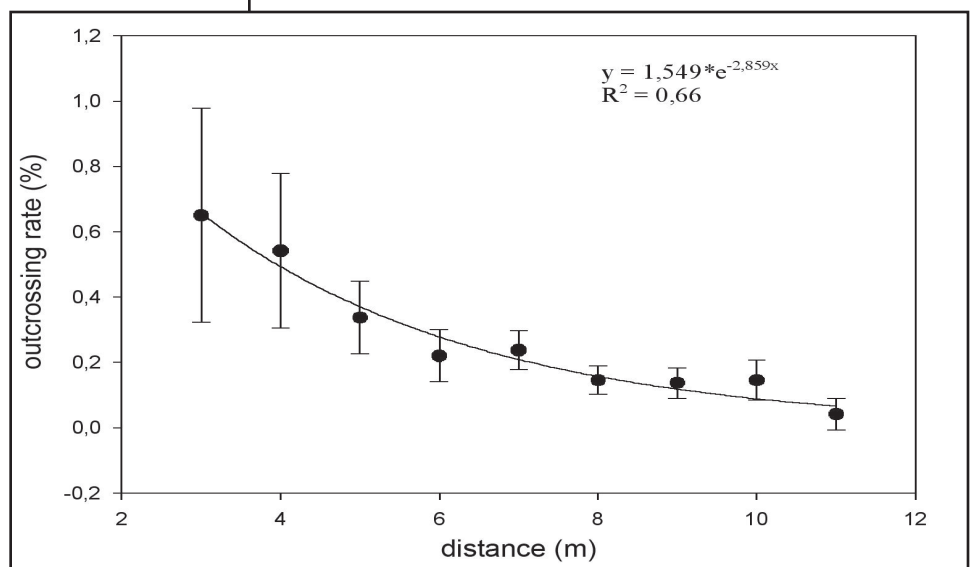


Figure 3: Average outcrossing events as a function of distance from the 100% transgenic plots. The best linear least-squares regression curve fit for the relationship is shown. Standard deviations are represented with error bars.



PLANT RESEARCH NEWS

Protective Plague Vaccine Produced in Tobacco Leaves

Luca Santi and Hugh S. Mason

In the 20 years since the approval of recombinant insulin produced in *Escherichia coli*, biopharmaceuticals have experienced persistent growth, and demand is expected to climb. Classes of biologicals, especially recombinant proteins as antibodies, subunit vaccines, enzymes, hormones, immunomodulatory molecules, receptor agonists, and microbicides, promise to deliver a new wave of therapies. All these molecules are complex polypeptides that must be synthesized by living organisms. Currently most are produced in mammalian cells, which, together with yeast, insect cells, and *E. coli*, are the most traditional biological factories.

Plants as bio-factories

Transgenic plants comprise a convenient alternative system that has extensively demonstrated great potential in studies conducted during the last two decades.¹ The production of subunit vaccines in particular has been widely validated using different plant heterologous gene expression approaches. Numerous candidate vaccines were proven to confer some degree of protection in animal challenge studies against toxins or viral or bacterial pathogens, and to stimulate humoral and mucosal immune responses in human subjects.

Plants are able to express a large variety of proteins and to perform the post-translational modifications required for proper biological function. Plant systems are much less likely to harbor microbes that are pathogenic to animals than are mammalian cells. Lastly, and significantly, plants offer the possibility of an easy scale-up, especially when compared to the previously mentioned expression systems that rely on fermentation technology.

The demand for hundreds of kilograms of recombinant proteins per annum requires utilization of bioreactors with capacities of up

Another objective of this field trial was to measure the persistence of transgenic seeds in the soil. By measurements of 'good agricultural practice' (reduced soil cultivation, application of common herbicides, crop rotation), the number of germinable seeds in the soil seedbank could be reduced by 99.7% to 100% in comparison to the input during harvest within a period of two years.

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to 20,000 liters, with extraordinary associated costs for cleaning, revalidation procedures, and preparation of complex culture media. On the other hand, scaling up transgenic plant production would only involve increasing the acreage in open fields or greenhouse space. Furthermore, the use of edible plant tissues for direct oral delivery would eliminate most of the costs for downstream processing and purification. There are three main methods for production of recombinant proteins in plants: stable transformation of the nuclear or chloroplast genomes, and viral transient infection.

Plant viral expression systems

The use of plant viral vectors offers several advantages.² Recombinant protein expression can reach very high levels in a relatively short time, ranging from 3 to 14 days post-infection, depending on the system used. The small genome size of most plant viruses facilitates molecular engineering, allowing the facile generation of large numbers of different constructs that can be quickly tested. Fully functional and systemic infectious vectors are easily transmissible by mechanical inoculation, making large-scale infections feasible. The major limitation is that the acquired trait is not genetically transmissible and a new infection must be performed on every new plant. Also the environmental containment of the modified virus causes some concerns.

Several expression vectors have been developed using different types of plant viruses; the most common are based on single stranded positive RNA viruses like the tobacco mosaic virus (TMV). The first vectors developed, the so called "full virus" vectors, consisted of the addition of a heterologous open reading frame, encoding for the protein of interest to the viral genome, and driven by an extra subgenomic promoter. The next generation systems were "gene replacement" vectors in which a viral gene, usually encoding the coat protein, was substituted with the gene of interest. The extreme evolution of this concept led to deconstructed viral vectors missing several components of the original virus and usually delivered to the plant by independent constructs.³

Plague

The etiologic agent of plague is the Gram-negative bacteria *Yersinia pestis*. It is generally accepted that in the course of human history plague has been the cause of three pandemic

infections responsible for hundreds of millions of deaths worldwide. Nowadays plague is still endemic in Africa, Asia, regions of the former Soviet Union, and the Americas, where it persists mostly in rodent populations.

There are two major forms of the disease: bubonic and pneumonic. In bubonic plague, *Y. pestis* is transmitted to humans via the bite of infected fleas, often resulting in the formation of "bubos," which are enlarged lymph nodes typically localized in the axillary and femoral areas. Pneumonic plague occurs when the bacteria infect the lungs; it is considered fatal (mortality rates of almost 100%) and can be transmitted by aerosol from infected to naïve hosts.⁴ For these reasons pneumonic plague is of particular concern in light of biological warfare, and in fact, during the cold war the former Soviet Union produced enough aerosolized *Y. pestis* for use as a weapon. The United States, before dismantling its bio-weapon program in the late 1960's, also tested the deadly potential of aerosolized bacteria.

Although antibiotics for plague are available, their effectiveness relies on a prompt diagnosis of the disease, and moreover, strains with acquired resistance to antibiotics have been isolated. Killed whole cells (KWC) vaccines and live attenuated vaccines have been extensively investigated and used in different countries. In the U.S., until 1999, a KWC plague vaccine was used especially for subjects at risk. It is no longer in production due to the poor protection conferred against the pneumonic form, as well as the high incidence of side effects. A live attenuated vaccine was tested in the Soviet Union, but it also showed severe local and systemic side effects. Hence the development of a safe and effective vaccine was needed.

The protective efficacy of subunit vaccines against plague has been demonstrated for many years. Two antigens, F1 and V, and a fusion of both, F1-V, have been selected. The F1 antigen is highly expressed by *Y. pestis* and is exported to form an extra-cellular capsule that surrounds the bacteria. The V antigen is a secreted protein involved in the pathogenic process. In a collaborative effort that included the Bidesign Institute at Arizona State University, Icon Genetics (Halle, Germany), and the U.S. Army Medical Research Institute for Infectious Disease, we demonstrated that sequence-optimized genes and a robust transient expression system generated



high levels of expression of all three antigens in leaves of *N. benthamiana*. The plant-derived antigens, administered subcutaneously in guinea pigs, generated systemic immune responses and provided protection against an aerosol challenge of virulent *Y. pestis*.⁵

Plant-derived subunit vaccine against *Y. pestis*

The first step was the creation of a synthetic version of the open reading frames of the antigens. The coding sequences were optimized for expression in dicotyledonous plants using preferred codons and eliminating spurious mRNA destabilizing signals, potential methylation sites, 5' intron splicing sites, and putative plant polyadenylation signals. The vector system used belongs to the last generation of deconstructed viral vectors. It was developed by Icon Genetics, recently acquired by Bayer Innovation GmbH; it is based on TMV and has been extensively modified to increase performance.³ The vectors are delivered to the plant cell nucleus by *Agrobacterium tumefaciens* (agroinfection) lines carrying two separated proviral cDNA modules: a 5' module containing the viral replicases and movement protein, and a 3' module harboring the gene of interest driven by the coat protein subgenomic promoter. Specific phage-derived recombination sequences are located on each module.

The co-delivery of the two proviral modules together with a third *Agrobacterium* line carrying a construct that directs constitutive expression of the phage PhiC31 integrase leads to the assembly of the complete viral vector in the plant cell nucleus. At this point the vector is transcribed, processed, and exported into the cytosol, where as a positive single stranded viral RNA molecule, it undergoes amplification and translation. This particular system has a deletion of the TMV coat protein, thus limiting its ability to spread systemically throughout the plant. Without the coat protein, the virus is still able to move from cell to cell but loses its ability for systemic infection, allowing stringent containment of the virus.

Distinct localization signals are located on different 5' modules. The pairwise combination of these modules with the 3' module allows the rapid generation of proteins that are targeted to different cellular compartments in order to evaluate the best localization for each target protein. In this specific case, the different 3' modules, each one

containing one of the plant-optimized coding sequences for F1, V, and F1-V, were coupled with 5' modules for cytosolic, chloroplastic, and apoplasmic accumulation. In all cases, the cytosolic accumulation gave the best results. F1 and V were expressed at levels of 2 mg/g of leaf fresh weight and the fusion F1-V at 1 mg/g. These amounts are at least an order of magnitude greater than any antigen expressed in stably nuclear-transformed plants. After purification, the proteins were assayed on SDS/PAGE with Coomassie staining. Antigenicity was evaluated using both ELISA and Western blot analyses.

For vaccine testing in animals, 25 µg of plant-derived antigens, mixed with alum as adjuvant, were administered subcutaneously. Groups of eight guinea pigs were dosed at days 0 (prime), 30 (boost 1) and 60 (boost 2). Serum analysis revealed that all proteins were highly immunogenic. The antibody titers specific for V in particular increased significantly just after the priming dose. Four weeks after the last dose was administered, the animals were challenged with an aerosol dose considered essentially 100% lethal to unvaccinated controls, and in fact, all sham-immunized mice were dead within 6 days after exposure.

Conversely, all antigen-vaccinated groups showed significant rates of survival at 21 days post-exposure. V-vaccinated animals showed the highest survival rate (six of eight), followed by F1-V (five of eight), and F1 (three of eight). Moreover, vaccinated animal mortality was significantly delayed beyond day 6. The majority of the animal challenge experiments reported in literature are carried out by injection of lethal doses of *Y. pestis*. The aerosol exposure used in our study offers a more reliable way to test protection for the pneumonic form of the disease. In addition, to our knowledge this is the first time that F1, V, and F1-V have been individually compared for protection in the same animal study. In conclusion, we have demonstrated that a rapid and robust plant based expression system could be used to produce an effective vaccine against plague.

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REGULATORY NEWS

European Regulators Curdle Plans for Goat Milk Human Antithrombin

Phillip B C Jones

On February 22, 2006, the European Medicines Agency refused marketing authorization for ATryn®, a recombinant form of human antithrombin. The ATryn® application had been the first submitted to a US or European regulatory authority requesting approval of a recombinant therapeutic protein produced in the milk of genetically modified animals.

The strategy of producing therapeutic proteins in transgenic animals' milk arose during the mid-1980s when the fledgling biotech industry faced the challenge of synthesizing properly folded and glycosylated proteins. While vats of mammalian cells offered one tactic, the cultures often produced low yields. To increase efficiency, researchers considered the mammary glands of transgenic cows, goats, and rabbits.

In the early 1990s, Genzyme began a program to produce therapeutic proteins in the milk of transgenic goats. The company spun its operation

off as a separate company, Genzyme Transgenics, which was later named GTC Biotherapeutics. The company's scientists used microinjection to insert a genetic construct into the nucleus of a one-cell embryo. In the case of ATryn®, the construct included a human antithrombin gene under the control of a beta casein promoter.

GTC marketed its technology platform to pharmaceutical companies. Although the company's production method worked, the question remained whether regulatory agencies would approve therapeutics synthesized by transgenic animals. This uncertainty nurtured the 'pharma' industry's hesitancy about producing recombinant drugs in milk. Nobody seemed keen on being the first to try the approval process.

To test the regulatory waters, GTC sought approval for the sale of antithrombin to a small target population. The company aimed at subjects with an inherited antithrombin deficiency, who need the protein to prevent damaging blood clot formation during high-risk situations, such as major surgery.

After testing the anticoagulant in experimental models, GTC investigated the effects of the drug on people with congenital antithrombin deficiency who were undergoing surgery (five subjects) or giving birth (nine subjects). Since either event presents risks of bleeding, these individuals cannot take the anti-clotting drugs that they normally use.

GTC included the results of the clinical investigations in the ATryn® marketing application. The European Medicines Agency rejected the application, because GTC had performed studies with five surgery patients rather than the recommended twelve, and because the agency wants the company to carry out additional studies to assess whether patients developed antibodies in response to ATryn® treatment. GTC announced that it would appeal the decision, emphasizing that the agency had not based its negative ruling on the fact that the drug had been produced in transgenic goats.

The microinjection technique used by GTC in its ATryn® project tends to generate embryos that give rise to transgenic animals at a rate of one to five



percent. To produce its newer drugs in transgenic animals, GTC uses the more efficient somatic cell nuclear transfer method. This technique can also produce clones.

Clone, Clone on the Range

The art of cloning – the production of an animal genetically identical to a single parent – has undergone a revolution in the last decade. During the 1970s, embryo splitting, or blastomere separation, offered a means to clone animals. In this technique, a researcher divides an embryo and implants embryo segments into a surrogate mother. Embryo splitting has several limitations: only a few clones can be produced from an egg, and an embryo gives few clues about the traits of the developed clone.

Cloning came of age in 1996 with the birth of Dolly the sheep, the world's first mammal cloned from an adult cell. Researchers at Scotland's Roslin Institute produced Dolly using somatic cell nuclear transfer. In this process, the nucleus of a somatic cell from the animal to be cloned is injected into an enucleated oocyte and the embryo is transferred to the uterus of a surrogate mother. Theoretically, nuclear transfer can be used to make an unlimited number of copies of one animal.

After Dolly, nuclear transfer enabled the cloning of cattle, goats, and pigs. The era of livestock cloning had arrived. Proponents of livestock cloning claim that it benefits consumers by providing reliably high quality food products, while farmers can select and propagate animals with superior genetics without risking gene reshuffling associated with sexual reproduction. The technology's supporters also claim that cloning may allow the replacement of grain-fed livestock with grass-fed livestock. Since grass does not require the quantities of fertilizers and pesticides required for growing grain, cloning may benefit the environment.

Although some view livestock cloning as an extension of centuries-old selected breeding practices, critics have voiced social, ethical, and religious concerns. For example, animal welfare groups claim that livestock cloning poses unnecessary health risks to farm animals. Opponents of livestock cloning also suggest that

seemingly healthy clones and their progeny may harbor subtle defects that could make their food products unsafe to eat.

The Food and Drug Administration has pondered the safety of food products obtained from the offspring of clones. In 2001, the FDA's Center for Veterinary Medicine requested the National Research Council to identify any safety concerns that cloned animals might present to food, animals, and the environment. In its 2002 report, the NRC concluded that "there is no current evidence that food products derived from adult somatic cell clones or their progeny present a food safety concern." The NRC committee recommended the collection of additional information about the composition of food products to ensure that cloned animal products do not differ from those of normal animals in ways that might affect human health.

Two years later, the NRC published a second report on the safety of cloned animals in the food supply. Again, the organization could find no scientific evidence that cloning produced an unintended compositional change that posed health risks in humans.

The University of Connecticut's Xiangzhong Yang led a team of US and Japanese researchers in an investigation of potential compositional changes induced by cloning. They compared the produce of two beef and four dairy clones generated by nuclear transfer with that from normal animals of similar age and breed. Meat was analyzed against more than 100 quality criteria, while milk was analyzed for protein, fat, lactose, and urea nitrogen.

Published in the May 3, 2005, issue of the *Proceedings of the National Academy of Sciences*, their study indicated that the composition of meat and milk from somatic animal clones did not differ significantly from that of genetically matched comparators or industry breed comparators. Although they found that meat and dairy products met industry standards, Yang and colleagues cautioned that their pilot study should provide guidelines for more conclusive studies with larger numbers of clones with different genetic backgrounds.



The FDA has been cautious as well. In 2003, the Center for Veterinary Medicine published a draft summary of its science-based review of the risks that may arise in livestock cloning. The agency could find no evidence that consumption of edible products from clones of cattle, pigs, sheep, or goats poses a greater risk than consumption of those products from their non-clone counterparts. Nevertheless, the FDA requested producers to abstain from placing edible products from clones into the food supply until the agency has concluded its safety evaluation.

For years, the FDA has promised a final policy and, several times, approval seemed imminent. Most recently, *The Washington Post* reported in October 2005 that the agency was expected to rule soon that milk from cloned animals and meat from their offspring are safe to eat. Yet, in response to written questions, Stephen F. Sundlof, director of the Center for Veterinary Medicine, informed *The Washington Post* that the FDA “really can’t provide a reliable estimate on the time frame” for releasing a policy. With the future of a new food industry at stake, the agency thoughtfully chews on safety issues.

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INDUSTRY NEWS

***Triticum aestivum L (Wheat) —
 An incredibly complex genetic soup***

Tracy Saylor

The irony of wheat (*Triticum aestivum L.*) – which some want to see spared from genetic manipulation through biotechnology – is that the genetic manipulation of this crop over thousands of years makes decoding and thus ‘manipulating’ the genes of bread wheat all the more difficult today.

Wheat started out as a grass, and about 9,000 years ago, wild einkorn wheat was harvested in the Fertile Crescent. Then around 8,000 years ago, a mutation or hybridization occurred within emmer wheat, resulting in a plant with seeds that were larger, but could not sow themselves through the wind. While this plant could not have succeeded in the wild, it produced more food for humans, and within cultivated fields, it outcompeted plants with smaller, self-sowing seeds to become the primary ancestor of modern wheat breeds.

“All of this genetic engineering (hybridizing) was conducted thousands of years ago by ancient farmers completely unaware of modern genetics or the difficulty of hybridizing polyploid plants,” explains Answers.com (<http://www.answers.com/topic/wheat>).

Bread wheat today is referred to as a ‘hexaploid’ species (6x chromosomes), containing three different ancestral genomes, each of which has seven pairs of chromosomes, for a total of 42 chromosomes. The amount of DNA within wheat over thousands of years of transformation makes for an incredibly complex genetic soup. The bread wheat genome is one of the largest and most complex of all crop species, and in fact is even more complex than the human genome. The size of the wheat genome is approximately 13.5 gigabases; the human genome is only about 3 gigabases.

National Association of Wheat Growers Urges Biotech Wheat

Needless to say, constructing a genetic road map of wheat is a long, tedious process because of the sheer amount of material to be mapped. Bikram Gill, Kansas State University, explained efforts now underway to sequence the wheat genome at the



recent Grain Congress in San Antonio, representing the annual meeting of the National Association of Wheat Growers. Gill, recognized as an international expert in wheat genetics research, heads a team responsible for mapping the genome of the wheat plant so breeders can identify important genetic traits and create new varieties of wheat with specific desirable characteristics, like more resistance to disease and insects, and better end-use traits. More information about this effort can be found online at www.wheatgenome.org.

Also at that meeting, the National Association of Wheat Growers and U.S. Wheat Associates approved several amendments and additions to their Biotechnology Position Statement. Among the provisions was a joint resolution of the USW/NAWG to "support continued research and development of Syngenta's fusarium tolerance transgenic trait in wheat and ... work proactively with stakeholders in the food system for the benefit of customers and consumers worldwide, U.S. wheat producers and the whole U.S. wheat industry."

The Scientific Cost of Not Pursuing GM Wheat

Forrest Chumley, associate director for research at Kansas State University, says that in science, success is the best recruiting tool, and failure to develop and commercialize biotech traits may lead to a decline in wheat research investments, reduced student enrollments, and lost research opportunities in the future.

Chumley discussed "the scientific cost of not pursuing genetically-modified wheat" during the research forum at the Grain Congress. In science, there is a certain amount of competition for research talent, he says, and success is the best recruiting tool. Without biotech, Chumley speculated, "can we continue to attract the best and brightest to wheat research and education?"

Chumley also speculates that the wheat research field may stagnate and be bypassed by the Ag research community's "best and brightest" without biotechnology.

Chumley says wheat as a research field has been "moderately" successful in non-biotech research advancements, but has failed at making strides within biotechnology. He says there are several reasons why investment in wheat biotech has been comparatively low compared to other crops:

- Wheat is generally viewed as a "low value" commodity, with low value inputs.
- Wheat is a commodity fragmented by six types or classes, making the tremendous expense of developing a biotech trait more difficult to recover.
- Planted acres have been in steady decline since 1980, even before the arrival of biotechnology.
- Wheat has a complex, challenging molecular genetics background that makes biotech research more tedious than in other crops.
- Transgenic wheat research has a comparatively small community, with very little private research.
- There is a lack of "pull" from the industry.

The continued acceptance of other biotech crops around the world – including more acreage and more biotech crops (rice is around the corner) – will help pave the way for wheat, says Chumley. "Biotechnology represents the most rapid adoption of any agricultural technology ever," he says. "There isn't one credible case of someone becoming sick by this technology."

Chumley says that of 12,173 field test permits granted by USDA-APHIS since 1987 for researching crop biotech traits, 5,535 have been for corn (45.4%) and 396 for wheat (3.2%). "That's about a 15-fold difference, and I don't think corn is 15 times more important than wheat."

He says that the earliest permit filed with APHIS was in 1994 by Monsanto to test glyphosate tolerance. The work was shelved by the company a decade later because of resistance to the technology. Chumley says there were just three public biotech wheat field test permits pending as of early 2006, filed by the University of Minnesota, Kansas State University, and Oklahoma State University. He says public biotech wheat research now is focused on drought and fusarium resistance.

"In biotech we have a trait that promises to take a dangerous toxin out of the food supply, so why not," says Chumley, referring to DON or vomitoxin from scabby wheat. "But with increased biotech acceptance, biotech will be hard to keep out of wheat. It will come."

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