REPORT OF AN EXPERT PANEL ON BIOTECHNOLOGY IN COTTON

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EXPERT PANEL ON BIOTECHNOLOGY IN COTTON

As a next step in the process of providing objective, science-based information on the use of biotechnology in cotton, a proposal to create an expert panel on biotechnology in cotton was initiated on January 28, 2000. An expert panel was charged with preparing a balanced scientific report on cotton biotechnology written in language understandable to most persons. This is intended to help the ICAC in an education effort on genetically engineered (GE) cotton.

In its second report to the 58th Plenary Meeting, the Private Sector Advisory Panel (PSAP) said:

> "The use of cotton in the developed nations faces potential resistance due to increasing and well orchestrated environmental concerns over the use of biotechnology to develop insect resistant seeds…
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> "While the current focus is on food products, this has already impacted the use of cottonseed oil, meal and cakes in the European Union and could affect cotton yarn and finished textile products.
>
> "This issue must be addressed to appropriately educate and inform our governments and assure the consuming public that there are significant benefits to consumers from the adoption of biotechnology, for example, decreased pesticide use. The public must be assured that cotton is an environmentally friendly product…"

This report, prepared by the Expert Panel on Biotechnology in Cotton (Annex 3), addresses the following points to provide objective, sound science-based information on cotton biotechnology:

- Scope of biotechnology and genetic engineering in cotton
- Comparison of conventional breeding and genetic engineering
- What are the benefits of using GE cotton?
- What are the risks and potential impacts on human health of using GE cotton?
- What are the impacts on the environment of using GE cotton?

As part of this report a bibliography (see Annex 2) of studies relevant to the subject of biotechnology in cotton is provided.

Scope of Biotechnology and Genetic Engineering in Cotton

Biotechnology includes a wide variety of experimental techniques used to evaluate and manipulate the genetic material of organisms. In general these include molecular analysis of the genetic material, hybridization among least related parents, organ and cell culture, plant regeneration, microbial biochemistry, and molecular biology and genetics. Most of the techniques are straightforward and are considered as natural extensions of traditional genetic techniques. Only when these techniques are used in combination to develop plants that contain genetic material that they could not obtain by sexual means has concern arisen. Even then the prevailing expressions of concern are selective, and little or no opposition is expressed to breeding based on micropropagation, hybridization among least related parents, genetic mutations induced by various intrusive techniques, or to the creation of novel plants by fusion of plant cells.

The scope of this report is limited to the category of biotechnology involving genetically engineered (GE) plants. The term "genetically modified organisms" (GMOs) is inappropriately used in the popular press when "genetically engineered" is intended. "Genetically modified" in its generic sense is applicable to all economic plant species domesticated from their wild progenitors through selection and breeding. "Genetically engineered" applies to organisms that have received and express genetic material through a variety of molecular techniques known as recombinant DNA (rDNA) technology. Surprisingly, plants with gene deletions created by recombinant DNA technology have also been designated as GE, so the current public debate tends to be driven, in part, by the techniques used rather than the end product. (The same gene deletion created by chemical or radiation would not result in a GE plant, although the end product is the same.)

Cotton is one of the lead crops to be genetically engineered and since its introduction in 1996, production of GE cotton has been one of the most rapidly adopted technologies ever. By 1999/2000, 2.63 million hectares or 12% of world cotton area was planted to transgenic varieties in Argentina, Australia, China (Mainland), Mexico, South Africa, and the USA. Many other countries are evaluating the performance of these cottons and some are preparing to plant them on a commercial scale.

Although a considerable number of genes have been engineered into cotton, only a few are important in commercial production. Primarily the selection of genes exploited by biotech and seed companies, thus far, address categories of management input (pest control) to crop production rather than output (yield potential and quality) of the crop. The major economic costs in crop management are insect and weed control, so GE cotton with genes that lower these costs would impart the highest added-value to the seeds in the near-term (equivalent to the reduction in cost required to chemically control pests). Naturally these were the first to be developed by commercial companies because of the economic incentive. Specific genes that are currently popular in commercial varieties are discussed below, but in general, genes that provide resistance to various destructive moth larvae and genes that impart tolerance to specific herbicides dominant the current GE varieties of commercial cotton. Herbicide-tolerant genes allow cotton crops to survive effective herbicide treatments by the farmer; the weeds are destroyed but the cotton is not harmed. Crops containing a gene derived from the ubiquitous soil bacterium *Bacillus thuringiensis* (Bt) produce a protein that is toxic to Lepidopteran larvae/caterpillars that attack cotton.

Before addressing specific concerns related to production of transgenic cotton, a basic understanding of the techniques involved in genetic engineering compared to conventional breeding will provide a better perspective of the limitations, benefits, and potential negative consequences of biotechnology and GE organisms in the environment.

Comparison of Conventional Breeding and Genetic Engineering

Traditional breeding. Man has genetically modified plants from the day selections were first made from among wild plants and placed under casual cultivation. This was called “automatic selection” by Harlan (1975). Harvesting and repeated sowing of the harvested material was a powerful filter for selecting genetically modified plants (mutants) with traits of agronomic value, such as absence of seed dormancy, flowering at the same time and maturity, large seeds, shatter-proof pods, and better regeneration ability in vegetatively propagated crops. Automatic selection was unconsciously practiced for millennia. This was followed in the domestication process by directed selection in which yield and specific traits of interest to the grower were selected and propagated. Specific examples in cotton include longer fiber length, stronger fibers, and white lint versus the brown color, very short fiber of the wild types. Wild races of our current crops were domesticated and developed by these methods. Thus, over time, the collecting of desirable genes and discarding the undesirable ones has altered the genetic make-up of our modern crops so that many barely resemble the parents from which they arose.

Systematic selection of plants was carried out by amateur and professional agriculturists throughout the world long before Mendel (1866) established the basis of genetics. The scientific basis of plant breeding was established soon after the rediscovery of Mendel’s work in 1900. The basic concept of plant genetics is that traits are controlled by genes, that genes are located on discrete structures called chromosomes located in the nucleus of each cell of an organism, and that mixing (recombination) of parental genes occurs during the formation of sex cells in the first generation progeny. The number of different chromosomes in a cell is specific to the species (52 in the case of *G. hirsutum;* “upland cotton”), but in a normal plant, two copies of each chromosome are present. One set of chromosomes (26 chromosomes) is contributed by the male parent and one set by the female parent. All the chromosomes together make up the “genome” of the plant. Two copies of each chromosome means that each gene is present in at least two copies, although many genes may be present in multiple copies in the genome. A plant trait controlled by one gene pair, such as fiber color, is called a qualitative trait. However, most traits of agronomic or economic importance are quantitative, that is, controlled by the interaction of many genes. Up to 50,000 genes may be present in a plant, and their time of expression and interaction determine the progression of growth and development of the plant in its environment.

For most traits different versions of the controlling genes exist as a result of mutation and natural divergence of isolated plant populations. It is this diversity of gene type that provides the basis of traditional plant breeding. The breeder attempts to introduce a large number of “desirable” genes from a range of different genetic sources into a single superior genotype. By traditional methods this is done by sexual hybridization of a donor parent with the receiving parent. Thus, genetic transfer is limited to plants that are sexually compatible, and in most cases usable genetic resources are limited to some, but not all, members of the same plant genus. In cotton one of the most suitable examples could be producing extra fine quality fiber equivalent to Egyptian/Pima cotton from upland cotton varieties.

In addition to the limited number of genes of interest from a donor source, a single copy of the other 50,000 genes that constitute the species is also transferred to the initial hybrid. While selecting for the trait of interest, the traditional breeder must eliminate the vast majority of genes contributed by the donor parent that are detrimental to the performance of the superior genotype. This process involves several plant generations using various backcross, intercross and self-pollination strategies. Experience has shown that genes that are physically near each other on a chromosome are “linked” and are usually inherited together. Since traditional breeding deals with large blocks of DNA, breaking the linkage between a useful gene and a “detrimental” gene requires large populations and intense scrutiny to identify “recombinant” plants containing only the useful genes. Some linkages may be impossible to break.

Because of the imprecise nature of traditional breeding, genetic diversity tends to be increased when the breeder introduces exotic germplasm into the breeding pool. Most breeders accept that genetic diversity helps buffer crop response to environmental fluctuations and promotes stability of crop performance. However, the difficulty of breeding out unwanted genetic material encourages breeders to use cotton germplasm in their programs that has already been highly selected. In spite of the difficulties inherent in traditional breeding, yield gains of approximately 6.8 kg/ha/yr were achieved by US cotton breeders between 1960 and 1996 (Meredith, 2000). However, cotton yield appears to be on a plateau or slight decline since 1987 (Meredith, 1995). The lack of genetic diversity in commercial varieties is now considered a problem that, in part, compounds the criticism that genetic engineering limits genetic diversity because of backcross breeding rather than forward breeding.

Genetic engineering. Genetic engineering is a breeding strategy that attempts to avoid the problems associated with the transfer of large blocks of genetic material between two parents. The current state of technology allows only a very limited number of foreign genes (from any life source) at a time to be introduced into a plant. However, single gene traits cause the least disruption of the existing plant genome and are much easier to develop in subsequent breeding efforts. Two major components are required to accomplish genetic engineering. The first is a knowledge of plant genomic structure and the structure of a single gene, and the second is the ability to develop a complete plant from a single cell (regeneration). Not all varieties of cotton can be regenerated from a single cell, so direct genetic engineering is usually limited to the few varieties that can be. These are not the most elite lines, so a series of backcrosses (crossing the first initial GE plant and subsequent progeny with elite lines) and selection are required to put the new gene into the best varieties. This, of course, reintroduces the problems associated with combining large blocks of genes between two parents. Consequently, new methods of genetic engineering are being developed where the need to regenerate plants from single cells is avoided and an elite variety can be used directly as the recipient.

Genes are composed of DNA, a linear series of four basic chemical subunits. The linear order (sequence) of these subunits determines the regulation and expression of the genes. Each chromosome consists of one exceptionally long double-stranded DNA molecule, and the genes are arranged linearly along the strand, usually with long stretches of non-functional DNA between them. In its simplest form a gene consists of three main areas of DNA sequence, each with a different function: 1) a sequence at the start of the gene, called the promoter, dictates when, where, and how much of the gene product will be produced; 2) a central region, called the coding region, provides the genetic code for the gene product; and 3) a terminal region tells where the gene ends. Existing cell biochemical components will interact with the integrated genetic material and treat it as a functional gene if these three components are present and in the correct order. With a few notable exceptions concerned with cell function, the final product of a gene is a protein. The function of each gene/protein is specific, but collectively these functions range from nutrition storage and cell structure to metabolic catalysts (enzymes) and plant defensive agents. Proteins with the latter two functions are the ones used in current GE cotton lines.

In reality, individual plant cells, not plants, are "transformed" by insertion of foreign DNA. Various techniques are available to do this, but the most common method relies on a system provided by nature. The bacterium that causes the crown gall disease, *Agrobacterium tumefaciens,* is nature's own genetic engineer. It transfers some of its own DNA to plant cells as a part of the disease process. Scientists have removed the disease- causing DNA from selected strains of the bacterium and discovered that the bacteria, while no longer causing the crown gall disease, retain the ability to transfer to a plant cell any DNA placed at the same location as the disease-causing DNA that was removed. This natural system, then, allows any gene to be transferred to a plant cell through the bacteria. The specific chromosome and site where new DNA is inserted in a cell is more or less random, so it may be inserted into an area where the gene is poorly expressed, it may insert into an existing gene and inhibit it, or it may insert into a genetically favorable site. For this reason it is necessary to do many transformation and regeneration events, then select the transgenic plant that gives the best performance.

Only a limited number of cells incorporate the foreign DNA into a chromosome, so these must be selected from among the vast number of cells that do not incorporate the DNA. At the technical level, selection of transformed cells has relied upon inclusion of a second gene in the foreign DNA in tandem with the gene of interest. In most cases this second gene codes for an enzyme that inactivates an antibiotic that would normally kill the cell. When a tissue that has been infected with *Agrobacterium* carrying the DNA to be inserted is cultured on a medium containing the antibiotic, transformed cells will grow while non-transformed cells will die. The bacterium is killed by a second antibiotic in the medium that has no effect on any plant tissue. Questions have been raised concerning the use of antibiotic resistance genes in GE crops. In the case of genes that confer tolerance to herbicides, the herbicide itself can be used as the selective agent for transformed cells, in which case a second gene for cell selection is not necessary.

In **summary**, traditional breeding methods deal with blocks of chromosomes based on sexual hybridization and recombination. Often genes for entire metabolic pathways are involved that may result in changes of a variety of secondary products. Genetic engineering deals with a very limited number of defined genes (usually two) designed to impart traits to a crop that are not present in the traditional germplasm breeding pool. In every case, thus far, the end product of the inserted genes has been the protein itself rather than any secondary metabolic product that could trigger more actions and interactions.

What Are the Benefits of Using GE Cotton?

The introduction of GE cotton has provided growers with a new tool for managing insects and weeds in cotton. Numerous benefits of this technology accrue to the grower, the global cotton industry, and society on many levels — economic, environmental, and social. These benefits include **direct benefits**, such as reduced pesticide use, improved crop management effectiveness, reduced production costs, improved yield and profitability, reduction in farming risk, and improved opportunity to grow cotton in areas of severe pest infestation. **Indirect significant benefits** of the technology include improved populations of beneficial insects and wildlife in cotton fields, reduced pesticides runoff, air pollution and waste from the use of insecticides, improved farm worker and neighbor safety, reduction in labor costs and time, reduction in fossil fuel use and improved soil quality.

The most significant benefit of biotech cotton to date has been the reduction in insecticide usage for the control of certain bollworms. Numerous studies, conducted across the United States and in Australia, China, Mexico, and Spain, have demonstrated an overall reduction in sprays for Lepidopteran pests (Benedict and Altman 2000, Mullins and Mills 1999, Novillo et al. 1999, Obando-Rodriquex et al. 1999, Xia et al. 1999, Wier et al. 1998, Bacheler et al. 1997, Bryant et al. 1997, ReJesus et al. 1997, Roof and DuRant 1997, Stark 1997, Mitchner 1996, Davis et al. 1995). The number of spray reductions ranges from 1.0 to 7.7 sprays per crop season. Of the research reviewed for this report, an average reduction of 3.6 sprays per crop season is achieved when a grower uses Bt varieties versus non-Bt varieties.

Seven academies of science from around the world (the Royal Society of London, the U.S. National Academy of Sciences, the Brazilian Academy of Sciences, the Chinese Academy of Sciences, the Indian National Science Academy, the Mexican Academy of Sciences and the Third World Academy of Sciences) issued a report, *Transgenic Plants and World Agriculture*, in July 2000 spelling out the promise of agricultural biotechnology to alleviate hunger and poverty in the world. The paper urges governments to base their decisions regarding biotechnology on sound science and indicates that it will be critical to use the best science to make wise choices with respect to these technologies. It was pointed out that public health regulatory systems need to be put in place in every country to identify and monitor any potential adverse human health effects of transgenic plants, as is the case for any new plant variety. Likewise, environmental concerns must be addressed and assessed against the agricultural technologies currently in use that cause environmental problems. Procedures that most nations already have in place to approve the use of new crop plants could serve as the model for a more formal risk-assessment process.

Also the report, *Genetically Modified Pest-Protected Plants: Science and Regulation*, published in April 2000 by the U.S. National Academy of Sciences, found to be valid the principles that “There is no evidence that unique hazards exist either in the use of recombinant DNA techniques or in the movement of genes between unrelated organisms” and “Assessment of the risks of introducing recombinant DNA-engineered organisms into the environment should be based on the nature of the organism and the environment into which it is introduced, not on the method by which it was produced”.

Potential unintended benefits of genetically modified cotton. The initial commercial GE cotton crops are designed primarily to deal with pests. To the extent that they reduce overall pesticide use, they reduce the potential for collateral damage to non-target species, including humans. Even if the effect of the technology is merely to substitute one pesticide for another, the net effect might be to reduce negative environmental consequences. For example, this would occur if the new pesticide affects fewer species or degrades more quickly.

Herbicide-tolerant crops also can contribute to soil-related environmental improvements. With less need for weed control, fewer passes are made through the field. Total soil movement, and hence soil erosion, is reduced. Reduced work in the field results in less soil compaction, which in turn corresponds to better aeration in the soil and better water conditions in the root zone. Finally, the reduced need for machinery reduces fuel consumption.

Genetic changes at a single gene level could also contribute to fiber quality, germplasm evaluation and to genome mapping. All these are discussed by Stewart in ICAC Review Articles on Cotton Production Research No.3, *Biotechnology of Cotton*, published in 1991.

What Are the Risks and Potential Impacts on Human Health of Using GE Cotton?

In the United States the impacts of GE cottons to human health have been investigated and approved prior to their use by the U.S. Food and Drug Administration (FDA). FDA is making this review mandatory prior to use and is establishing guidelines for voluntary labeling. Australia, Office of the Gene Technology Regulator, which coordinates assessments from the relevant health and environment authorities, also has robust regulatory requirements. Other countries and international groups do similar reviews prior to approval.

Safety assessment of GE cotton on human and animal health is science- and risk-based and has focused on the following:

- A detailed understanding of the biology of cotton, including the uses of the products derived from cotton.
- A biochemical characterization of the introduced proteins, estimation of the levels of the protein in the important plant products, and a detailed assessment of the safety of the introduced proteins. The safety assessment includes: 1) a history of safe consumption of the proteins by humans or animals; 2) any prior animal toxicity testing of the proteins; 3) results from the field and lab safety studies to assess the allergic effects, toxicity and digestibility of the expressed proteins; and 4) assessment of the dietary consumption of the proteins by humans and animals of cotton products.
- A determination of any unintended effects on the quality traits of the crop as a result of the insertion of the genetic material or the resulting protein expression. This concept is termed “Substantial Equivalence”. In cotton, testing of this concept included multiple location trials of agronomic characteristics and plant morphology, fiber quality, and nutritional components of the cottonseed, oil and meal. These nutritional composition studies include proximates (protein, fat, carbohydrates, ash moisture and calories), fatty acid spectrum, amino acid spectrum, and gossypol. Additionally, the equivalence of cottonseed oil and meal was also determined.
- Feeding studies with cottonseed or cottonseed meal conducted in rats or other animals to determine any adverse health or behavioral effects.
- Review and testing of cotton products used in medical and personal hygiene products and food.

Changes at the molecular level can be made to produce a particular compound that could trigger actions extremely important for the cotton industry. For instance researchers in the private and public sectors after many years of research developed a system which would cause the plant to produce only infertile seed. This technique was named the Technology Protection System (TPS) and it means that farmers had to buy seed every year. TPS could be used not only with GE cotton but in other varieties too. The plans to commercialize TPS have been withdrawn, in part, because of public objections to the technology. GE could be used to produce genetic characteristics that might be objectionable by some farmers because of their traditional approaches to seed use and/or production practices.

The apparent usefulness of herbicide-tolerant GE cotton is to minimize the use of herbicides. However, critics contend that it will encourage farmers to rely on the technology and use herbicides extensively on GE cotton. Since herbicide usage is already high and often has low efficacy in traditional cotton production, such contention is valid only if herbicide usage increases on GE cotton, which has not been the case for herbicide-tolerant cotton varieties grown in the US. Any assessment must also consider the persistence and environmental load of the traditional herbicides compared to the herbicide to which the crop is engineered to be tolerant.

Even before GE cotton became available, fears were expressed that insects could develop resistance to the toxin produced by the Bt gene. Now it is almost universally accepted that insects will eventually develop resistance to the toxin, thus, measures have already been adopted to delay the development of resistance. The potential for resistance to develop in the target insects also means there is a need to routinely re-engineer cotton with new genes that will produce toxins with different modes of action.

Currently, only two types of GE cottons involving three different genes have been commercialized and neither demonstrates any interaction with other genetic material in the cotton plant to produce deleterious effects. But, such interactions are not impossible.

A review of all safety information indicates that GE cotton does not pose any different risks to human or animal health than conventional cotton. Each of the proteins introduced into GE cotton commercialized to date has been shown to not require a tolerance level by the U.S. Environmental Protection Agency (EPA). This means these proteins are considered safe for human or animal consumption. Tolerances set by the EPA establish allowable, safe limits of pesticides in food (i.e., cottonseed oil) and feed (i.e., cottonseed, cottonseed meal, cottonseed hulls). Additional approvals for the use in food and feed of products derived from GE cotton have been obtained following scientific review in Japan, Australia, Argentina, South Africa, Mexico, Canada and China. Scoured and bleached cotton, as it is used for medical and personal hygiene products as well as for chemical cellulose products, does not contain DNA or protein from a transgenic plant.

What Are the Impacts on the Environment of Using GE Cotton?

In the U.S. the U.S. Department of Agriculture (USDA) is responsible for field testing of all agricultural biotechnology crops. USDA evaluates whether a technology could pose a threat to plant or animal health. The U.S. Environmental Protection Agency (EPA) has regulatory authority for crops such as Bt cotton, which claim pesticidal properties (i.e., pest-protected plants). EPA regulates (40 Code of Federal Regulations part 152.20) environmental exposure to these crops to ensure there are no adverse effects to the environment, non-target insects and other organisms (e.g., microbes, earthworms, and nematodes). EPA has announced that they will amend these regulations on the oversight of biological control agents by the end of 2000 to clarify how they regulate genetically engineered plant pesticides. Other countries and international groups conduct similar reviews prior to approving the use of GE cottons.

The impact of GE cotton on the environment has had science- and risk-based assessments that have focused on the following components:

- Agronomic performance of all new cotton varieties is typically assessed through field observations to determine morphology, yield, lint quality, plant growth characteristics, and susceptibility to diseases and insects. These factors were all unaffected by the insertion of genetic material, except for the targeted differences in the proteins produced and the commensurate yield increases as an indirect consequence.
- An assessment of the biology of GE plants for pest or weediness potential relative to conventional cotton includes the potential for cross-pollination to weedy relatives, dormancy and germination changes, and overwintering potential. The inserted genetic material in these cotton products behaves as any other DNA that is transferred to progeny through Mendelian inheritance. For gene flow to occur via normal sexual transmission, certain conditions must exist: the two parents must be sexually compatible, their periods of fecundity must coincide, a suitable pollen vector must be present and capable of transferring pollen between the two parents and resulting progeny must be fertile and ecologically fit for the environment in which they find themselves. Wild populations of *G. hirsutum* are relatively rare and tend to be widely dispersed. Most grow in non-agricultural areas. Cotton is normally considered a self-pollinating crop, but can be cross-pollinated by certain insects. However, the possibility of cross pollination of the introduced genes from GE cotton to other *Gossypium* species or to other plants of the same family is extremely low to nil for the following reasons and has been confirmed in cross-pollination studies:
    - Upland and Egyptian/Pima cotton has 52 chromosomes and is incompatible with cultivated or wild diploid cotton species having 26 chromosomes and, therefore, cannot cross and produce fertile offspring.
    - Although cross pollination to species having 52 chromosomes can occur, commercial cotton production generally does not occur in the same geographical locations as the wild relatives. For example, cross-pollination to *G. tomentosum* in Hawaii is possible, but no commercial cotton is grown in Hawaii.
    - There are no identified species outside the cotton family that are sexually compatible with cultivated cotton.

- An assessment of impacts on non-target insect species has been conducted. Testing was conducted with the Bt protein due to its insecticidal properties. A large amount of testing has been conducted on the sprayable Bt products with demonstrated safety to non-target organisms. These results were confirmed for Bt cotton. The insects tested represent major insect classes and included adult and larval stages of honeybees, green lacewing, ladybird beetles, and parasitic Hymenoptera, as well as common soil organisms, earthworms and springtails. The absence of toxic effects in the non-target organism studies, even at the protein (Cry1Ac) levels considerably above the maximum predicted environmental exposure, demonstrate that Bt protein would not have adverse impacts on these and related non-target organisms. Additional field observation studies of impacts of Bt cotton on non-target organisms have shown increases in populations due to the reduction in non-specific pesticide use. Research with Bt corn on the persistence of these toxins, and their possible ecological and environmental effects in soil, demonstrated that the protein (Cry1Ab) is released in root exudates from Bt corn grown in the lab and in natural soil in the field. However, no significant difference was observed in the amount of toxin in the soil between Bt corn and non-Bt corn, nor was there an effect on soil microbes, earthworms, and nematodes, which are non-target species. Extensive field study research on the effect of Bt pollen on monarch butterflies show that the concentration of Bt pollen adhering to milkweeds (the main food staple of the larvae) within a few (1-5) meters of cornfields is typically too low to cause mortality of even small monarch caterpillars that might be present during pollen shed. Due to its large particle size (90-100 microns), most corn pollen deposits stay within the cornfield. Cotton pollen is in the same size range as corn pollen, however, cotton pollen is spiny and never transported by wind as corn is. Cotton pollen leaves a flower only when harvested for food by bees, and these are unaffected by Bt proteins. Non-target Lepidopteran larvae are not exposed to Bt toxins from GE cotton away from the plant itself. Any larva that forages on the cotton is, by definition, a target pest. Adult butterflies and moths may visit a cotton field for nectar, but they do not eat pollen and nectar contains no protein.
- An assessment of the environmental fate of the introduced proteins has been conducted. Soil degradation of the protein (Cry1Ac) alone or in cotton tissue was studied under both lab and field conditions, each showing rapid elimination of insecticidal activity in the soil, which was comparable to half-lives reported for microbial products.

Based on the low levels of environmental exposure to the introduced proteins and the data generated in the environmental safety assessments listed above, there are no anticipated adverse effects on the environment nor have any been reported since the introduction of GE cotton in 1996 in any country where it is produced. Indeed, the most significant impact on the environment from the use of GE cotton involves many of the benefits of the technology, such as reduced pesticide use, as described in the benefits section, above.

Conclusion

Currently available genetically engineered cotton is resistant to certain bollworms and tolerant of specific herbicides. But, this is not the only use of genetic engineering technology. Genetic engineering can be used in a variety of ways and can produce results that may not be imagined at this stage. The ability of researchers to bring changes at the molecular level is an additional tool in the hands of breeders who have been limited by the available genetic material with which they could work. The technology has tremendous applications not only in the agronomic field but also in quality and chemistry of the plant itself. Current research involves the addition of genes, but undesirable genes could be deleted too. Changes at the gene level better enable geneticists to use directed breeding and construction of choice genotypes. However, genetic engineering is a new technology and we have much to learn about its use. The technology has tremendous applications and utility but must be watched and used carefully without sacrificing the environmental safety, natural flora and fauna and sustainability.

Annex 1: *Other Information*

Insect Resistant GE Cotton

1. Bt Cotton

The era of transgenic cotton began when Perlak et al. 1990, introduced cry 1A(b) and cry 1A(c) genes into cotton plants and transformed plants showed a high level of resistance to *Heliothis*. The Monsanto Company received authorization to launch transgenic cotton, known as Bollgard cotton, for the 1996 crop season. The transgenic cotton was tested in all the major cotton production regions in the USA and plants were analyzed for insect control efficiency compared with traditional chemical insecticides. The transgenic cotton varieties in the USA have been approved by the US Environmental Protection Agency (EPA), the US Department of Agriculture and the US Food and Drug Administration. During the field and laboratory tests, it was demonstrated that transgenic cotton is highly effective against neonate larvae of *Heliothis virescens* (Tobacco budworm), *Helicoverpa zea* (cotton bollworm) and *Pectinophora gossypiella* (pink bollworm) (Wilson et al. 1992); the toxin gene delivers the Bt protein directly to the neonates immediately after they hatch when they are most susceptible. The Bt gene from the original genetically engineered mother plant was transferred to advanced cotton cultivars through backcrossing. It has facilitated the introduction of the Bt gene to cotton varieties that have a good agronomic base and desirable fiber properties and are otherwise not responsive to regeneration in vitro. The control of insects in GE cotton was reported to be comparable with synthetic pyrethroid insecticides and their yield level was comparable to or higher than the same variety treated with traditional insecticides. The GE cotton requires less number of sprays and reduces the cost of cultivation. The *in planta* delivery of crystal proteins does not have any undesirable effects on predators and parasites, so transgenic plants can be effectively used in an IPM system (Fischhoff, 1996). GE cotton in China has also been developed combining Bt and the Cowpea trypsin inhibitor (CpTI) gene (Zhao et al. 1997).

GE cotton has been one of the most rapidly adopted technologies ever. The transgenic cotton plants engineered for resistance to the lepidoptera group of insects are being commercially cultivated in Australia, China Mexico and the US, and are at field testing stage in number of countries. During its first year of commercial production (1996), 1.8 million acres were planted to BT cotton in the USA, 75000 acres in Australia, 5000 in Mexico and in India GE cotton is at field testing stage but its area declined in 1997. In 1998/1999, 2.63 million hectares or 8% of world cotton area was planted as transgenic varieties in Argentina, Australia, China (Mainland), Mexico, South Africa, and the USA. Many other countries are evaluating the performance of such cottons and some are ready to plant them on a commercial scale.

2. Other Insecticide Proteins for Insect Resistance

Most of the transgenic plants are based on Bt delta-endotoxins. There are other insecticidal proteins, e.g., polyphenol oxidases, proteinase inhibitors (Hilder et al., 1987), and X-amylase inhibitors, which interfere with the nutritional needs of the insect by interfering with digestive enzymes. Proteinase inhibitors are highly specific and act as insect growth retardants.

Trypsin inhibitor genes. Proteinase inhibitors are highly effective on a particular class of digestive enzymes and act as growth retardants (Jongsma et al., 1995). Trypsin inhibitase effect inhibitors affect insects by reducing their capacity to assimilate plant proteins. The insect reduces feeding, which leads to starvation. The insects possessing proteinase inhibitors switch the proteinase composition in their gut to overcome this effect (Jongsma et al., 1995). Cowpea trypsin inhibitor has been engineered into cotton and shown to reduce damage by lepidopterans (Zhao et al., 1997) substantially. Chitinases also have insecticidal properties and are presumed to target chitin-containing structures, such as the peritrophic membrane, which protect midgut cells in the insect gut lumen (Ding, 1995).

Lectin genes. Lectins are carbohydrate-binding glycoproteins found in many plant species that possess a broad range of antimicrobial and insecticidal properties (Cavlieri et al., 1995). The insect toxicity of lectins relates to their ability to bind midgut cell membranes and impair the absorption of nutrients by the insects thereby inhibiting their growth. Rajgura et al. (1998) observed that F2 plants grown from seed of individual regenerated plants of Coker 312 transformed with lectin genes, when fed to neonate larvae of *Heliothis virescence,* inhibited larvae growth.

Herbicide Tolerant GE Cotton

Herbicide tolerant GE cottons have received less notice than Bt cotton, but these were the first commercial GE cottons in the US and are now grown on a larger acreage in the US than the insect resistant cottons. Herbicide tolerant cottons allow the spraying of broad-spectrum herbicides over the plants, eliminating weeds and reducing labor and equipment. Herbicide tolerant genes have been commercially developed by two U.S. Companies, Calgene and Monsanto, and at least two other companies Aventis and Novartis, presently have herbicide tolerant cotton lines under development.

The US company, Calgene, developed cottons tolerant to the herbicide bromoxynil (Buctril®), which have been introduced into the US market as Stoneville BXN varieties. The gene action in BXN varieties is dependent upon a degradation enzyme isolated from the bacterium *Klebsiella sp.* which breaks down the herbicide bromoxynil. BXN cotton tolerance to Buctril® is very high and has been field tested at levels ten times the labeled rates without damage to the plants. The herbicide has superior activity against morning glory species and is a useful product for the weed complex common to the Mid-South area of the US.

The Monsanto herbicide-tolerant cottons were developed with a gene to reduce damage to cotton plants by glyphosate (Roundup®). The glyphosate herbicide blocks the production of aromatic amino acids, and the transgenic plant compensates by overproducing the key enzyme in the amino acid pathway. The utility of the Roundup Ready (RR) cottons are presently limited because they can only be sprayed over the top for the first four leaves of growth without a delay of boll production. This limitation has not stopped adaptation of RR varieties. They are the dominant GE cottons in the U.S. with 39 commercial varieties and 37% of the 1999 U.S. acreage.

Development of Resistance to Bt Cottons and Resistance Management

Biotechnology can help in the management of insecticide resistance because the Bt varieties possess inherent ability to withstand lepidopteran insects. However, experience gained with synthetic chemical insecticides and resistant crop varieties developed through conventional breeding suggests that insects could adapt to transgenic plants within a relatively short span due to continuous exposure of the lepidopteran insect pests to the Bt toxins (Fischhoff, 1992; Federici, 1998; Shen et al., 1998). The risk of development of resistance in Bt cotton crops is probably greater than that for Bt pesticide formulations due to continuous and extensive expression of the delta-endotoxin in the plant tissues. The physiological mechanism of development of insect resistance to Bt delta-endotoxin includes change in gut pH or in enzymes that would affect dissolution and activation of the proteinaceous crystal. Cross-resistance among toxins occurs in some insect species. The selection of a population of *H. virescens* for resistance to one type of toxin of cry IA(c) can lead to resistance to a broad range of other toxins. These insects developed resistance not only to cry IA(c), and closely related cryIA (a) and cryIA(b) toxins, but also to the more distant toxins, cry IIA, cry IB and cry IC. Recently it has been reported that *Helicoverpa armigera* have developed resistance to Bt in Yauggu and Xiuxiang provinces of China (Shen et al., 1998). Due to the development of resistance to Bt toxin the average mortality of newly hatched larvae of *H. armigera* declined significantly (16-29%) as compared to the susceptible strain. It is difficult to predict the mechanisms by which insects develop resistance to various toxins. But in cases where resistance is attributed to modification in the binding sites, resistance seems to be inherited as a major recessive or partially recessive gene. In this case the level of resistance is high, and cross-resistance is limited and involves toxins that share the same binding site. On the other hand, where resistance is due to unknown modifications, it seems to be inherited in additive gene action and level of resistance is moderate. New strategies are needed to maximize the durability and utility of GE cotton.

1. Insecticide Resistance Management Strategies

Insecticide-resistance-management (IRM) strategies include transgenic plants as a central focus in an IPM system (Fischhoff, 1992; Federici, 1998; Shen et al., 1998). The other factors aim at reducing the selection pressure to delay the emergence of insect populations that are resistant to Bt toxin. The enhanced expression of toxin genes, use of tissue specific promoters, use of refusia, a combination of GE cotton with chemical insecticides for effective IPM, gene pyramiding, breeding for broad spectrum host plant resistance in GE cotton, and identification of new proteins with insecticidal activity against lepidopteran species, will be helpful in IRM. (William et al., 1992, 1998).

2. Use of Refugia

Insect resistance to the Bt toxin is related to recessive genes or accumulation of several minor genes. Susceptibility to the toxin is maintained in the broad insect population by management strategies that promote mating of wild-type (susceptible) insects with the few individuals that could have developed resistance (homozygous recessive genes). This is done by allowing a population of unselected toxin-sensitive insects to survive on non-transformed plants (refugia) in the immediate vicinity. The refugia should assure that the number of individuals with dominant genes (susceptible) available for mating are much higher than resistant individuals surviving on the Bt cotton, thus the development of homozygous recessive (resistant) breeding populations are suppressed. GE plants can be organized differently according to the target insect's biology. Seed mixes and intermingled blocks of non-transgenic and Bt transgenic plants can be envisaged for management of resistance to Bt by refusia (Roush et al., 1998; Luttrell and Caprio, 1996). The refugia should be close enough to the toxin expressing plants for the sensitive insects to be able to mate with those having developed resistance.

3. Biotechnology, Plant Breeding and IPM

The traditional options of using a variety bred for broad-based host plant resistance as a component of pest management practices should not be ignored simply because it is low technology or a traditional concept. The resistant, or even a tolerant variety, when cultivated in combination with integrated IPM strategies, like release of natural enemies, proper rates of pesticide application, chemical rotation, application of pheromones for mating disruption, attraction and killing, and timely monitoring has assisted in delaying the development of insecticide resistance in *H. armigera*. The idea that different pests are bound to develop resistance to transgenics should not be dismissed. On the other hand, GE cottons with a particular trait used in combination with a cotton variety bred by traditional plant breeding, which is tolerant to a number of biotic and abiotic stresses, could be used in an effective IPM system. Zhang et al. (1998) stressed the need for cultivation of cotton varieties possessing several insecticidal genes in the rotation or mixture of GE cotton with a single insecticidal gene, coupled with the use of strong tissue specific or chemically induced promoters. Biotechnology provides new tools, which can be applied to different systems to solve problems, just as traditional plant breeding, or organism selection, is a tool to improve crops and microbes. The products of biotechnology are unlikely to completely replace other pest control means but can be effectively integrated into pest management systems and integrated crop management strategies.

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