

How the Terminator Terminates

An explanation for the non-scientist of a remarkable patent for killing second generation seeds of crop plants

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INTRODUCTION

Genetically modified organisms (GMOs) have become a commercial reality in agriculture. For example, it is estimated that in 1998 over 18 million acres in the United States will be planted in Roundup Ready® soybeans, which were first introduced in 1996 (Horstmeier 1998). These soybeans are engineered by Monsanto Corporation to contain a bacterial gene that confers tolerance to the herbicide glyphosate, or Roundup®, also made by Monsanto. Only two years after the introduction of Roundup Ready® soybeans, over 30% of the corn and soybeans planted in the United States, and close to 50% of the canola planted in Canada, have been genetically engineered to be either herbicide or pesticide resistant.

Monsanto and the other companies that have invested heavily in biotechnology in the last two decades are starting to make some money after years of promises without products, and they are aggressively protecting their patented seeds. In a recent issue of the Farm Journal (Monsanto 1997), Monsanto ran a full page advertisement asking farmers to respect the company's property rights:

“It takes millions of dollars and years of research to develop the biotech crops that deliver superior value to growers. And future investment in biotech research depends on companies' ability to share in the added value created by these crops. Consider what happens if growers save and replant patented seed. First, there is less incentive for all companies to invest in future technology, such as the development of seeds with traits that produce higher-yielding, higher-value and drought-tolerant crops ... In short, these few growers who save and replant patented seed jeopardize the future availability of innovative biotechnology for all growers. And that's not fair to anyone.”

In the future, companies and government breeders who genetically engineer crops may not have to ask for such compliance. If the procedure outlined in a recent patent comes to fruition and is widely used, plant variety protection will be biologically built into the plants themselves.

In March of 1998, a seed company later to be purchased by Monsanto, Delta and Pine Land Company, in collaboration with the United States Department of Agriculture, was awarded U.S. Patent Number 5,723,765: Control of Plant Gene Expression. Although the patent is broad and covers many applications, one application favored by the patent's authors is a scheme to engineer crops to kill their own seeds in the second generation, thus making it impossible for farmers to save and replant seeds.

This 'invention' has been dubbed the 'Terminator Technology' by Rural Advancement Foundation International (RAFI), and that group of researchers have analyzed some of the technology's serious social, economic and environmental implications (RAFI 1998). However, many of the consequences of Terminator cannot be fully appreciated without an understanding of the science behind the invention. In this paper, I outline the steps involved in engineering Terminator Technology into a specific crop. After explaining the process, I then discuss which details might have the devil in them.

OVERVIEW

To help describe the Terminator procedure, I have confined the explanation to only one of the many possibilities covered by the patent. The example I have chosen is cotton seed, which previously has been genetically-engineered with a unique trait, herbicide tolerance. In my discussion, I have assumed that to ensure that the descendants of the herbicide tolerant seeds are not used without compensation to the seed company, the company has additionally genetically engineered the cotton with Terminator. Although this is a hypothetical case -- Terminator cotton is not yet on the market, after all -- all the components of the procedure have been shown to function, at least in the text of the patent for Terminator.

Cotton is not often sold as a hybrid seed, and is thus a likely candidate for Terminator protection. By way of contrast, corn is usually planted as a hybrid, and thus has some measure of variety protection already. This is because the first generation of a hybrid is genetically fairly uniform, and has been bred to have desired characteristics that are not present in either parent alone. When these hybrids make seeds, however, the second generation is quite variable because of the shuffling of genes that occurs during sexual reproduction. Industrial agriculture requires uniformity, because the plants must dovetail with mechanization. Therefore, industrial farmers who grow corn usually buy new seed every year.

There are several major crops which usually are not grown from hybrid seeds. These include wheat, rice, soybeans, and cotton. Farmers often save the seeds from these crops, and may not go back to the seed company for several years--or longer, in some parts of the world--to purchase a new variety.

It would be a big boost to seed company profits if people who now grow non-hybrid crops would have to buy new seed every year. This may have been the major incentive for developing the Terminator Technology.

There likely were other reasons for developing Terminator. One reason may relate to the way in which Terminator's effect differs from hybridization. When Terminator is used, the second generation is killed. With hybridization, the second generation is variable, but alive; and any genes present in the hybrid will be present in the second generation, although in unpredictable combinations. Therefore, a plant breeder who wanted to use the genetic material from the hybrid in his or her own breeding program could retrieve

it from these plants. With Terminator, the special genes, such as the herbicide tolerance of my example, would not be easily available for use by competitors.

Another reason sometimes cited for using Terminator in combination with a genetically-engineered variety is to keep the GMOs from 'escaping' into the environment. Many critics of biotechnology cite problems with releasing GMOs into the wild, noting that their effects on ecosystems and their members would be difficult to predict (Rissler and Mellon 1996). Having all of the second generation seeds die would circumvent this problem altogether.

ROUGH SKETCH AND REVIEW

Terminator is a complicated process to understand and it helps to review beforehand some of the basic information about how genes function during the life-cycle of a plant. Readers with a good grasp of molecular biology may want to skip the review section (A simplified version of basic biological processes) following the general description and proceed directly to the details of the Terminator Technology.

GENERAL DESCRIPTION OF TERMINATOR IN COTTON

In the cotton example, the goal is to develop a variety of cotton that will grow normally until the crop is almost mature. Then, and only then, a toxin will be produced in the (seed) embryos, specifically killing the entire next generation of seeds.

The system has three key components: 1. A gene for a toxin that will kill the seed late in development, but that will not kill any other part of the plant. 2. A method for allowing a plant breeder to grow several generations of cotton plants, already genetically-engineered to contain the seed-specific toxin gene, without any seeds dying. This is required to produce enough seeds to sell for farmers to plant. 3. A method for activating the engineered seed-specific toxin gene after the farmer plants the seeds, so that the farmer's second generation will be killed.

These three tasks are accomplished by engineering a series of genes, which are all transferred permanently to the plant, so that they are passed on via the normal reproduction of the plant.

A SIMPLIFIED VERSION OF BASIC BIOLOGICAL PROCESSES

A plant starts life as a single cell, an egg that has been fertilized by sperm which has been delivered to the egg by the pollen. This first cell divides many times to form the tissues and organs characteristic of the species. The process of going from a single cell to an adult is called development. As development proceeds, cells become different from each other and change. Cells in the leaf become distinct from cells in the root, for example. Most of the differences can be attributed to changes in the kinds and amounts of proteins made in the cells, because many of the structures in cells are made of proteins, and most of the

processes that occur are influenced by enzymes, which are also proteins. Thus, scientists who study development spend a lot of effort describing protein patterns.

By studying which proteins are present in different tissues and organs, biologists have learned that each cell has several thousand different proteins, but most of the proteins are very rare in the cell. A few hundred proteins may be moderately abundant, and a few may be quite abundant. Also, some proteins are found in all kinds of cells and at all times in development, whereas other proteins are only present in a particular tissue, or at a specific time. For example, the gluten proteins responsible for the elasticity of bread dough are found only in the seed, and are present there in very large amounts. In contrast, the enzyme that splits glucose as a first step in releasing energy is found in all living cells, but in fairly small amounts.

Some proteins are made in response to environmental changes, such as increases in temperature, and thus may or may not be present during the life of a particular plant.

The most common way for a cell to control how much of which kinds of proteins are present is to control which genes are functioning (Rosenfeld et al. 1983). Proteins are chains of different amino acids, and the order of amino acids and the length of the chain are unique for each kind of protein. Each unique amino acid sequence is specified by a code on a chromosome in the cell's nucleus. The code is made of DNA. For the purposes of this discussion, a gene is a piece of DNA that contains the code for a specific protein. Genes are present in specific places along the length of the chromosomes.

It turns out that just about every cell has two full sets of genes (one set of chromosomes from the sperm and one from the egg), which code for the proteins made in all of the tissues and organs that an individual plant will need during its life cycle. However, only those genes whose proteins are needed in a particular cell will be used by that cell. These are the active genes. The other genes just sit there on the chromosomes, inactive in that cell, but active somewhere else in the plant.

Whether a gene is active or not depends on complex interactions between the DNA and other molecules in the cell. Specifically, a typical gene can be divided into parts. The first part is a stretch of DNA responsible for interacting with the cell or the environment, and is called the promoter. The second part actually contains the code for the order of amino acids in the protein, and is called the coding sequence. When the gene is active, the promoter is interacting with other molecules in a way that allows the coding sequence to direct the synthesis of a specific protein (through a complex set of steps).

Genetic engineering can be defined as the process of manipulating the pattern of proteins in an organism by altering genes. Either new genes are added, or existing genes are changed so that they are made at different times or in different amounts.

Because the genetic code is similar in all species, genes taken from a mouse can function in a corn plant; and so on. Also, promoters from one coding sequence can be removed and placed in front of another coding sequence to change when or where the protein is made. For example, when the promoter for casein, the major protein in milk, is removed and put in front of the coding sequence for human growth hormone, it causes human growth hormone to be made in cow's milk instead of casein. Of course, in order to make human growth hormone in cow's milk, the engineered gene has to be incorporated into the genetic material of the cow. There are many ways to do this. I will not go into the details here.

The general process of moving genes between species is called transformation, and the result is a transgenic organism. Lately, transgenic organisms are being called genetically modified organisms, or GMOs.

DETAILS OF THE TERMINATOR TECHNOLOGY

The key to Terminator is the ability to make a lot of a toxin that will kill cells, and to confine that toxin to seeds.

To accomplish this, in the case of our cotton example, the plan is to take the promoter from a gene normally activated late in seed development in cotton, and to fuse that promoter to the coding sequence for a protein that will kill an embryo going through the last stages of development.

In the Terminator patent, the authors use a promoter from a cotton LEA (Late Embryogenesis Abundant) gene. This gene is one of the last to be activated. Its protein is not made until the seed is full-sized, has accumulated most of its storage oil and protein and is drying down in preparation for the dormant period in between leaving the parent plant and germinating in the soil. If the engineered gene has the same pattern of expression, LEA-promoter-directed proteins should be made in high quantities, only in seeds, and late in development. It is important for the cotton seeds to go through most of their growth before the toxin acts, because the cotton fiber is an outgrowth of the seed coat and is made as the cotton develops. Further, after the cotton fibers are removed (for human use), the seed is then crushed for oil and protein, both of which are eaten by people and livestock. The cotton crop would be of little use to a farmer if the seeds did not mature normally before dying.

As for a toxin, there are several possibilities discussed in the patent, but the patent authors recommend a ribosome inhibitor protein (RIP) from the plant *Saponaria officinalis*. This protein works in small quantities to stop the synthesis of all proteins. Since cells need proteins for almost everything, they die fairly quickly when they can't make proteins. According to the patent, the RIP is non-toxic to organisms other than plants.

The manipulations of DNA required to engineer a seed-specific promoter/toxin coding sequence gene are done in test-tubes and bacteria, and then the altered gene is put into a cotton plant, using one of several possible well-established methods.

However, this is not all there is to it. If this were all, then as soon as the transgenic plant went through its life cycle and came around to seed development, that would be the end of the project. There would soon be no viable seeds to sell to farmers.

The Terminator patent offers an ingenious method for keeping the toxin gene from being active until long after the farmers plant their crops. The trick is accomplished by inserting a piece of DNA in between the seed-specific promoter and the toxin coding sequence that blocks it from being used to make protein.

At either end of the blocking DNA are put special DNA pieces that can be recognized by a particular enzyme, such as the enzyme called recombinase. Whenever the recombinase encounters these DNA pieces, the DNA is cut precisely at the outside of each piece, and the cut ends of the DNA fuse together, with the result that the blocking DNA is removed. When this happens, the seed-specific promoter is right next to the toxin coding sequence, and is able to function in making the toxin. But this does not happen

immediately. Toxin will not be produced until the end of the next round of seed development, because that is when the LEA promoter is active.

Thus, after the recombinase enzyme does its work, the plant grows normally from germination, through growth of stems, leaves, roots, and all the way through flower formation, pollination and most of seed development. Then, on cue, the seeds die.

All this accomplished, there remains one more problem: How to grow several generations of the genetically-engineered variety so that its seed can be multiplied to sell to farmers?

The Terminator patent solves the dilemma by preventing recombinase from acting until just before the farmers plant their seeds. The patent holders give several possible ways to do this, but concentrate on the following procedure: They propose putting a recombinase coding sequence next to a promoter that is always active -- in all cells, at all times -- but that is repressed. The promoter can be made active again -- derepressed -- by a chemical treatment. Therefore, the seed sellers can treat the seeds right before planting, thus allowing the recombinase to be made then, but not before.

One of the repressible promoter systems they discuss in detail is controlled by the antibiotic tetracycline. A gene that makes a repressor protein all of the time would be put into the cotton plant, along with a recombinase gene that has a promoter engineered to be inactivated by the repressor protein. Under most conditions, then, the repressor would interact with the recombinase gene; no recombinase would be made; the toxin gene would be blocked; and no toxin would be made, even during seed development when the LEA promoter normally would be active.

To activate the toxin gene, seeds just starting to germinate would be treated with tetracycline, right before they are sold to farmers. The tetracycline would interact with the repressor protein, keeping it from interfering with production of recombinase. Recombinase would be made, cutting out the blocking DNA from the toxin gene. The toxin gene would now be capable of making toxin, but would not actually do so until the end of seed development. The next generation would thus be killed.

To accomplish the Terminator effect in cotton, then, three engineered components must all be transferred into a cotton plant's DNA:

1. a toxin gene controlled by a seed-specific promoter, but blocked by a piece of DNA in between the promoter and the coding sequence;
2. a repressor protein coding sequence with a promoter that is active all of the time; and
3. a recombinase coding sequence, controlled by a promoter that would be active at all times, except that it is also regulated by repressor protein, which can be overridden with tetracycline.

The actual transfer of genes into the plant is not a very precise operation. Any one of a variety of methods can be used: the genetically-engineered DNA can be injected into the nucleus of a cotton cell with a tiny needle, or plants cells can be soaked in the DNA and electrically shocked, or the DNA can be attached to small metal particles and shot into the cells with a gun, or viruses and bacteria can be engineered to infect cells with the DNA. In all cases, the genetically-engineered DNA has to find its way to the nucleus, and become incorporated into the plant chromosomes. The number of copies of the inserted genes and their locations on the plant chromosomes are unpredictable, and how well the new genes will function hangs in the balance.

It takes a lot of effort to locate cells that have incorporated DNA in significant amounts and in locations that work. Basically, whole plants have to be regenerated from the cells or tissues that were transformed with the foreign DNA, and then each plant has to be tested for the presence and function of the new genes.

After plants with well-functioning new genes are identified, they are then mated in combinations that result in a line of cotton where both sets of chromosomes (in all of the offspring) have all the components necessary for Terminator to function. These plants are mated together to make a large quantity of seed for sale.

In effect, Terminator Technology gives the seed producer the ability to determine when to set Terminator in motion. Until the recombinase is made, the cotton plants grow normally. After recombinase is made, the second generation of seeds is killed, protecting the patented variety.

SOME PROBLEMS THAT MAY CROP UP WITH THE USE OF TERMINATOR

The patent on this technology is complex. I have described only one of many possible applications of the procedure. Clearly, one cannot determine ahead of time all possible biological ramifications of implementing the patent. However, potential problems have already been noted (Ho 1998). I deal with some of them below.

Will the Terminator spread to other plants?

It is likely that Terminator will kill the seeds of neighboring plants of the same species, under certain conditions. However, the effects will be confined to the first generation, and will not be able to spread to other generations. The scenario might go like this: when farmers plant the Terminator seeds, the seeds already will have been treated with tetracycline, and thus the recombinase will have acted, and the toxin coding sequence will be next to the seed-specific promoter, and will be ready to act when the end of seed development comes around. The seeds will grow into plants, and make pollen. Every pollen grain will carry a ready-to-act toxin gene. If the Terminator crop is next to a field planted in a normal variety, and pollen is taken by insects or the wind to that field, any eggs fertilized by the Terminator pollen will now have one toxin gene. It will be activated late in that seed's development, and the seed will die. However, it is unlikely that the person growing the normal variety will be able to tell, because the seed will probably look normal. Only when that seed is planted, and doesn't germinate, will the change become apparent.

In most cases, the toxin gene will not be passed on any further, because dead plants don't reproduce. However, under certain conditions I will discuss later, it is possible for the toxin gene to be inherited.

In any case, dead seeds, where they occur, would be a serious problem for the farmer whose fields are close to the Terminator crop. How many seeds die will depend on the degree of cross-pollination, and that is influenced by the species of plant, the variety of crop, weather conditions, how close the fields are to each other, and so on. If many seeds die, it will make saving seed untenable for the adjacent farmer. Even if only a few seeds die, they will contain the toxin and any other proteins engineered into the Terminator-protected variety. These new 'components' may make the seed unusable for certain purposes.

WILL SEEDS CONTAINING THE TOXIN MADE BY TERMINATOR BE SAFE TO EAT?

In fact, the effects of the toxin on the uses of the seed are a serious question. This issue is discussed in the patent at the end of page 8. There the authors say that ‘[i]n cotton that would be grown commercially only selected lethal genes could be used since these proteins could impact the final quality of seeds. ... If the seed is not a factor in the commercial value of a crop (e.g., in forage crops, ornamentals or plants grown for the floral industry) any lethal gene should be acceptable.’

This is dangerously reductionist thinking, because people are not the only organisms that interact with seeds. In forage crops, for example, not all of the forage is always harvested before seeds are mature, depending on conditions. How will a particular toxin affect birds, insects, fungi and bacteria that eat or infect the seeds? If a forage crop with toxin-laden seeds is left in the field, and the seeds come in contact with the soil, how will that affect the ecology of soil organisms? These are important questions because a variety of specific organisms are necessary for the healthy growth of plants. Further, a floral or ornamental crop with Terminator may happen to grow near a related crop where the seeds are used, and if pollination occurs, the seeds will contain toxin without that farmer knowing. The toxin could end up in products without anyone’s knowledge. For example, an ornamental sunflower could spread Terminator to an oilseed variety, and then the toxin could end up in edible oil or in sunflower seed meal.

Other potential problems with making novel toxins in edible seeds have to do with allergenicity. The RIP toxin described earlier may not be directly poisonous to animals, but may cause allergic reactions. If the seeds are being mixed with the general food supply, it will be difficult to trace this sort of effect.

WILL DEAD SEEDS HAVE DIFFERENT PROPERTIES THAN LIVING SEEDS?

Although Terminator is supposed to kill seeds very late in development, it is not known what other effects, if any, Terminator may have. Will the dead seeds be more or less easy to store? Perhaps they will respond differently to changes in humidity, or to infection with bacteria and fungi. If dead seeds do behave differently, even a few ‘bad apples may spoil the barrel’, and the problem of partial killing of neighbors’ crops may be even more of an issue.

There also may be nutritional changes in seeds that are killed late in development. Although most of their oils and proteins are present, it is possible that seeds will start to deteriorate or will lack some minor component that is important. The functional properties of specific molecules in foods, for example, are just beginning to be appreciated and are likely to play important roles in preventing diseases. These possibilities require further study.

WILL THE USE OF AN ANTIBIOTIC TO TREAT SEEDS BEFORE PLANTING BE A PROBLEM?

If seed companies do indeed use tetracycline to set the cascade of toxin-gene activation in motion, then they will have to soak a very large amount of seed in the antibiotic. Basically, every seed planted by the

farmer will have to be so treated. How many pounds of cotton seed or wheat seed are needed to plant an acre, and how many acres will be put in? In fact, I am having trouble visualizing exactly how this will work, because the seeds must be treated with tetracycline after they have matured completely (so that the toxin won't be made in the first generation), but before planting (otherwise, the farmer would have to apply antibiotic to the plants). Handling seed that has been soaked seems like a tricky process to me, but perhaps there are viable methods. At any rate, even at low concentrations there will be a lot of tetracycline to handle and dispose of, and large-scale agricultural uses of antibiotics are already seen as a threat to their medical uses. Further, the increased tolerance of bacteria, residual or waste antibiotics may also have a harmful effect on soil ecology.

Again, I am dismayed by the reductionist tone of the discussion of these issues in the patent. On page 7, line 30, the authors state that 'since tetracycline has no harmful effects on plants or animals, its presence would not otherwise impede normal development of the plant, and residual amounts left on the seed or plant after treatment would have no significant environmental impact.' Although it is true that tetracycline has no direct effect on animals, such as humans, the indirect effects can be severe. This is because we depend on a myriad of interactions with microorganisms for our daily functioning, from proper digestion to protection from pathogens. The patient information sheet that comes with any prescription for tetracycline is convincing evidence that tetracycline is not harmless to use.

Plants too depend on microorganisms. They do not function normally without a web of interactions, and indirect effects from substances like tetracycline may prove to be important.

WILL TERMINATOR PREVENT GENETICALLY MODIFIED ORGANISMS FROM ESCAPING?

Clearly, farmers would not want plants genetically modified with Terminator to spread into surrounding areas or to grow from seed as unexpected 'volunteers' in another season. They also would not want the Terminator plants to exchange genes with other varieties or related species. Interestingly, Terminator has been proposed as a method to prevent just such escapes of GMOs and their genes. However, Terminator is not likely to function well for such purposes.

First, it is unlikely that any tetracycline treatment will be 100% effective. For various reasons, some seeds may not respond, or take up enough tetracycline to activate recombinase. In such cases, the plants growing from the unaffected seeds would look just like all the others, but would grow up to make pollen carrying a non-functional toxin gene. The pollen would also carry the genetically-engineered protein supposedly being protected by Terminator, such as herbicide-tolerance. If this pollen fertilized a normal plant, the seed would not die, because no toxin would be made, but the seed would now have the herbicide-tolerance gene and could pass that on. Thus a trait from the GMO would have escaped through the pollen.

Of course, self-fertilized seeds of the Terminator line would also survive in the second generation, if the tetracycline treatment failed, and could be carried off by birds, or grow as 'volunteers' the next season.

Another possibility is that even successfully activated Terminator genes may fail to make toxin because of a phenomenon called gene silencing. In experiments with other GMOs, it was discovered - quite

unexpectedly - that in some cases, previously active (introduced) genes can suddenly stop working. If this phenomenon occurred with seeds containing the Terminator gene, plants containing the silenced toxin gene could grow and reproduce, perhaps for several generations. Thus, Terminator and other engineered genes could be carried into the future, to be expressed -- perhaps still unexpectedly -- at some later time.

Depending on Terminator to prevent GMOs or their traits from spreading unintentionally is unrealistic. 'Escapes' are even more likely to occur in some of the other patent applications, where the genetic components of Terminator will reshuffle during sexual reproduction, and a portion of the seeds will lack the toxin altogether, and thus be viable.

ORGANISMS ARE ALWAYS CHANGING; WILL TERMINATOR MUTATE AND CHANGE CHARACTERISTICS IN SOME DANGEROUS WAY?

If plants were to carry silenced toxin genes, as described above, those genes might suddenly be activated again, causing seeds to die unpredictably in subsequent generations. By the time the phenomenon occurred, however, it might be difficult to ascribe the cause to Terminator.

Another possibility is that the Terminator may be activated at a different time or place in the plant. Fortunately, such events will be self-limiting, because the plants will die. However, for farmers, the instability and unpredictability of GMOs has already been an economic problem. Genes have an ecology--a complex way of interacting with themselves and the environment--that can interfere with the simple linear logic of genetic engineering. A recent article in the Ecologist discussed this problem in detail (Ho et al. 1998).

FINAL THOUGHTS

These are a few of the potential snags that I see in the use of Terminator Technology. My analysis was based on the details of only one of the applications described in the Terminator patent. I am confident that some of the particular problems I have discussed will be addressed by the seed industry before they implement the technology. However, I am also sure that there will be other problems no one yet foresees or imagines. There will be surprises. But whatever the potential biological problems presented by Terminator, in my view they are small in comparison to Terminator's economic, social, and political ramifications (See RAFI 1998).

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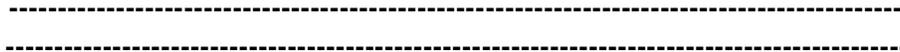
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