Herbicide Resistant Biotech Cotton

Weeds must be controlled because they harbor insect pests and compete with the cotton plant for inputs. Experiments have shown that of the three most important operations in cotton, i.e. weed control, application of fertilizers and pest control, weed control is the most important if a cotton grower has limited resources and has to make a choice. Weeds are a bigger problem in intensive farming systems. There may be fields and farms in many production systems where weeds are really not a serious threat. Such situations, however, are rare and do not persist. Weeds may be thought of as a kind of pest and, as such, require an integrated approach for sustainable control.

Weeds may be narrow leafed or broad leafed. It is always comparatively easier to control broad leaf weeds and, fortunately, they are the most prominent in most cotton fields throughout the world. To plan an integrated approach for a sustainable weed control system, it is convenient and useful to be familiar with the biology of weeds.

Area Treated With Herbicides

Country	Area (%)
Argentina	90
Australia	100
Brazil	
Central West	100
Northeast	2
Cameroon	72
China (Mainland)	18
Colombia	
Sinu Valley	100
Côte d'Ivoire	40
India	10
Iran	30-40
Israel	100
Madagascar	1
Mali	37
Pakistan	25-30
South Africa	80
Togo	5
Turkey	80
Uganda	1
USA	91
Vietnam	20
Zambia	5
Zimbabwe	10

Weed Control in the World

Weeds in cotton may be controlled manually, mechanically or chemically by applying herbicides. Crop rotation is another means of minimizing weed intensity. In small farms where the land is worked using animal traction, the fields may be cleared manually. When cotton is not planted in rows, as was the case in many countries not very long ago, hand weeding is the only way to get rid of weeds. Planting in rows allows growers to use mechanical methods of control, but mechanical weeding damages the crop and weeding equipment may be employed in cotton fields only while the cotton plant is below a certain height. Another significant constraint affecting the mechanical elimination of weeds is the inability to remove weeds growing between the plants in a row. Earthing up rows before irrigation can partially control weeds by burying them, but it is only a partial solution. Fields must be free of weeds until the cotton leaf canopy closes the soil to sunlight. The lack of sunlight reduces the weeds' ability to flourish normally and continue consuming inputs provided for the cotton plant. Weed intensity and type will determine the actual number of operations to be carried out. Normally, at least two manual and mechanical passes may have to be performed. Large-scale farming and the high cost of labor dictate exploring other means to control weeds that might also help avoid the shortcomings of manual and mechanical weeding. Chemical weed control is one alternative, but it has its own consequences.

Plant Protection Chemical Use in the World (Sale in Million US$)

Chemical Group	2000	2001	2002	2003	2004
All Crops					
Herbicides	13,796	13,386	12,475	13,348	14,849
Insecticides	8,206	7,744	7,314	7,738	8,635
Fungicides	5,818	5,467	5,450	6,055	7,296
Others	1,364	1,347	1,322	1,374	1,569
Total:	29,184	27,944	26,561	28,515	32,349
Cotton					
Herbicides	675	740	685	673	777
Insecticides	1,548	1,467	1,351	1,423	1,618
Fungicides	57	58	57	60	70
Others	282	266	254	252	280
Total:	2,562	2,531	2,347	2,408	2,745

Source: Cropnosis, Limited, Edinburgh, UK.

Consequences of Herbicide Use

Herbicides are not always the best way of getting rid of weeds. Herbicide applications are clearly the most effective method, but of the three possible options (manual, mechanical and chemical), it is also the most destructive. The consequences of herbicide use, in particular, its effects on the microbial community, are not well understood. Furthermore, the consequences of herbicide use are seldom thoroughly considered prior to the adoption of the chemical option. Unfortunately, the need for efficient weed control drives growers to rely on the use of herbicides. Soil organisms metabolize different herbicides, but it is not known what parts of the microbial community these materials eliminate. They do not seem to do much good against pathogens such as root rot, and there is a need to know what impact herbicides have on beneficial soil-born microorganisms. Herbicide chemicals are assessed by their ability to kill weeds, not by their effects on the soil microflora.

Herbicide use is on the increase in the world, and herbicides have become, or are becoming, an integral part of many weed management systems. Repeated use of the same herbicides that act in a similar manner within the target weed results in the selection of resistant weed biotypes. Herbicide resistance is defined as the inherited ability of a weed to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. Herbicide resistance is currently on the increase along with the use of herbicides. Literature shows that over 250 weed plants have already developed resistance to herbicides. The fundamental reason for this increase in herbicide resistance is that growers continue to use a successful herbicide program until it fails, instead of proactively implementing herbicide resistance management strategies. Loss of herbicide performance brings not only economic losses to growers but also shifts in weed populations because not all the weeds controlled by the chemical will develop resistance at the same time. As natural selection acts and some weeds develop resistance, biotypes of the resistant weeds begin to overwhelm the non-resistant populations. Using mixtures of herbicides, as is done with insecticides, is a resistance management strategy but information is needed to identify which herbicides have similar mode of action.

Herbicides are frequently categorized into families according to various similarities. Examples of herbicide classification categories include: mode of action, application timing, and chemical structure. Herbicide mode of action describes the metabolic or physiological plant process impaired or inhibited by the herbicide. Essentially, mode of action refers to how the herbicide acts to inhibit plant growth. Herbicide site of action describes the specific location(s) within the plant where the herbicide binds. Site of action identifies the herbicide target site within the plant. The most common herbicide classification schemes utilize mode of action; however, in cotton, time of application (pre- or post-) is more commonly used. Herbicide resistance in plants is often due to an alteration of the binding site in the target plant. Rotating herbicides based on different binding sites may provide a more informative classification system.

Classification systems based on mode of action include anywhere from 7 to 13 categories. Some of these systems describe mode of action categories as “cell membrane disruptors,” “seedling growth inhibitors,” and “amino acid synthesis inhibitors.” Rotating herbicides based on these categories could cause confusion among growers. For example, the mode of action category “amino acid synthesis inhibitors” would place the herbicides Pursuit (imazethapyr) and Roundup (glyphosate) in the same family, whereas classification by site of action would place these two herbicides into two distinct families, allowing growers to more accurately rotate herbicides for resistance management.

Herbicides that are applied to one crop may harm a following crop. *Pursuit* is registered for application on peanuts for controlling some of the most commonly occurring weeds, like yellow and purple nutsedge and morning glory. Karnei *et al.* (2002) concluded that Pursuit, if applied to a peanut crop, can significantly injure cotton in the following growing season. Karnei *et al.* (2002) correlated soil herbicide concentration of Cadre and Pursuit to cotton injury and lint yield. Both chemicals were applied in six doses, and treatments showed injury 42 days after planting. The level of injury varied according to the dose and the product, but lower stands resulted in huge yield losses.

Herbicide-resistant Biotech Cotton

The US Environmental Protection Agency (EPA) awarded conditional approval for the sale of the herbicide bromoxynil for use on transgenic herbicide-resistant biotech cotton in May 1995. This was the first commercialization of genetically engineered herbicide-resistant cotton. The bromoxynil-tolerant cotton was traded under the name BXN cotton, and those varieties are still commercially produced. Sold under the trade name Buctril, bromoxynil had long been registered for use on corn, wheat, oats and several other crops. However, it was not previously registered for use on cotton because it killed cotton plants. A few days after its decision on bromoxynil, the EPA also approved commercialization of a second transgenic herbicide-resistant crop. Monsanto received unconditional registration for its glyphosate herbicide (trade name Roundup) on transgenic glyphosate-resistant soybeans. Roundup Ready biotech cotton was commercialized in 1997/98.

During the first year after adoption of glyphosate-resistant Roundup Ready biotech cotton, there appeared an unexpected problem. At the peak boll formation stage, some growers found that their Roundup Ready biotech cotton showed deformed bolls and small bolls that dropped off the plant. The problem was limited to a few isolated fields scattered over thousands of hectares, particularly in the state of Mississippi. It was suggested rather quickly that the problem was associated with the Roundup Ready gene, but only 20% of the Roundup Ready cotton was affected. Researchers looked at other factors such as soil type, weather conditions and herbicide applications, which may have interacted with the engineered gene to cause the abnormalities. Extensive studies were undertaken in the following years, and it was concluded that

Biotech Cotton Area in the USA

Year	All Biotech Area (% of Total Area)	Herbicide Resistant Biotech Varieties (Actual in %)	Share (%)
1996/97	13	< 1	< 1
1997/98	23	4	17
1998/99	45	26	58
1999/00	60	44	73
2000/01	72	61	85
2001/02	78	74	95
2002/03	77	72	94
2003/04	76	74	97
2004/05	80	79	99
2005/06	83	81	98

the problem was not related to the Roundup Ready gene. The problem was correlated with abnormal weather conditions and with production practices followed in the affected area. Early season cold temperatures and multiple applications of Roundup Ready Ultra in a slow growth period caused excessive shedding and changes in boll shape. Since then, no additional complaints have been reported.

Herbicide resistance is the most popular characteristic among the characteristics developed using biotechnology applications. According to the International Service for the Acquisition of Agri-Biotech Applications (James, 2005) biotech crops were grown on 90 million hectares in 2005, and 71% of this area was under herbicide-resistant biotech crops. Herbicide-resistant biotech varieties are available in single and also in stacked form with the insect-resistant trait in cotton and other crops. The herbicide-resistant biotech trait in cotton is extremely popular in the USA. The Agricultural Marketing Service of the US Department of Agriculture estimates the area planted to cotton varieties annually, and a report is published in August/September of every year. Herbicide-resistant biotech varieties were planted on about 95% of the biotech cotton area in recent years. Almost 40% of this area was under pure herbicide- resistant varieties and 60% under stacked gene varieties. Less than 1% of the biotech area was planted to pure insect-resistant varieties. The herbicide-resistant trait in cotton is gaining ground in Australia, but it is not popular in other countries.

Roundup Ready Flex Technology

Herbicide-resistant biotech cotton has become extremely popular in the USA. Since the adoption of biotech cotton in 1996/97, the area planted to herbicide-resistant biotech varieties has continuously increased, as shown in the table above. However, the herbicide-resistant biotech cotton that was resistant to Roundup had a limitation: it could be sprayed up to the four-leaf stage only; beyond that, it could damage the cotton. Consequently, beyond the four-leaf stage, farmers had to resort to other weed control measures. Roundup Ready Flex cotton provided a solution to this problem by creating a more flexible window for over-the-top application of Roundup, one that extended until close to picking time. Roundup Ready Flex was approved for commercial production in 2006/07. According to Croon *et al.* (2005) Roundup Ready Flex is based upon a transformation event identified as MON 88913. Roundup Ready Flex cotton utilizes a *cp4 epsps* gene sequence that encodes for the *CP4 EPSPS* protein. The CP4 EPSPS protein expressed in Roundup Ready Flex cotton is the same protein currently used in Roundup Ready cotton that provides the tolerance to glyphosate. The increased level of glyphosate tolerance in Roundup Ready Flex cotton has been achieved through the use of improved promoter sequences that regulate the expression of the *cp4 epsps* coding sequence.

Roundup Ready Flex technology has been extensively tested in the USA since 2001/02. Trials were conducted by Monsanto, universities and others, who studied its agronomic characteristics, including seed germination, emergence, plant growth and development, harvest quality and compositional elements of the seed. The findings indicated that multiple over-the-top applications of Roundup Ready did not have any negative effects on the agronomical and qualitative characteristics of seed and lint. According to Monsanto, owner of the technology, Roundup Ready products, like Roundup WeatherMAX® and Roundup Original MAX™, may be sprayed over-the-top from emergence through to seven days prior to harvest or 60% open bolls (Murdock and Mullins, 2006).

It is recommended that a maximum rate of up to 79 ounces/ha be applied in each pass when using ground application equipment and up to 55 ounces/ha if applied by air from emergence through 60% open bolls. If the need should arise to apply the herbicide even after 60% of the bolls have opened, the quantity should not exceed 100 ounces/ha. The benefits of extended over-the-top application and the ability to tailor herbicide applications to the weed problem itself instead of to the stage of growth are yet to be verified in the form of in-field commercial performance of the Roundup Ready Flex technology. The only disadvantage Monsanto noted was a potential for leaf injury when combinations of components of glyphosate formulations were applied to Roundup Ready Flex cotton. Therefore, Monsanto has arranged to formulate Roundup WeatherMAX® and Roundup Original MAX™ for use on Roundup Ready Flex cotton to reduce the potential for leaf injury and, consequently, only Roundup WeatherMAX® and Roundup OriginalMAX™ are recommended for over-the-top application on Roundup Ready Flex cotton.

Liberty Link Cotton

Liberty®Link cotton from Bayer CropScience is a transgenic herbicide-resistant cotton similar to Roundup Ready. However, Liberty Link is used with the herbicide gluphosinate rather than glyphosate associated with Roundup Ready cotton. Roundup Ready cotton can tolerate herbicides only up to the four-leaf stage but gluphosinate-containing herbicides like Ignite or Liberty (also marketed under different commercial names) can be sprayed over-the-top of Liberty Link cotton up to the ten-leaf stage. Aside from cost differences, each technology has its own advantages and disadvantages, almost always related to the weed species present in the field. The ultimate objective is to keep the fields clean at a minimum cost and with the least long-term consequences.

Studies conducted in the USA (Barker *et al*., 2005) have shown that repeated emergence of weeds required multiple herbicide applications in order to ensure season-long weed control and achieve high yields. They compared the performance of Liberty Link, Roundup Ready and hand weeding where herbicides were applied early post-emergence, post-emergence and layby applications. Their findings indicated that a one-pass system of early post-emergence or post-emergence herbicide yielded significantly less than the two- and three-pass system (early post-emergence+post-emergence+layby). The two-pass system provided good control of some weeds, but not all weeds, thus affecting yield.

Liberty is a broad-spectrum herbicide. Its killing speed is a little slower than paraquat and a little faster than glyphosate. Liberty causes ammonia accumulation within susceptible plants and, as might be imagined, that causes a quick burn. Liberty also marketed as Basta, Ignite, Rely, Finale and Challenge, can kill a wide variety of plants.

Weed Resistance to Herbicides in Biotech Cotton

Weeds can acquire an inherited ability to survive and reproduce after a dose of herbicide that would normally kill. This means that a normal dose of a chemical that once controlled that particular weed would no longer be effective against that same weed. The development of resistance to herbicides that are used frequently on biotech cotton is an eventual certainty. The threat of weeds developing resistance is no less than the threat of insects developing resistance or morphing into new biotypes or even changing the pest complex. Unfortunately, however, herbicide resistance in biotech cotton has not received the same attention as insect or toxin resistance. Numerous reports in the USA show that weeds have already developed resistance to the most commonly used herbicide -- glyphosate.

Horseweed is a particularly dangerous weed that, if allowed to receive fertilizer and other inputs along with the normal crop, can grow taller than the cotton plant. Horseweed may have different names in different countries, and it is botanically known as *Conyza Canadensi*. It grows straight upright on a central stem surrounded by long, thin leaves. Horseweed was an occasional weed in California, USA, but now it is a common weed in irrigation canal banks, vacant lots, orchard and vineyard floors, roadsides and gardens. According to the University of California, biotypes of horseweed have evolved that are unaffected by the most commonly used herbicides containing glyphosate, which is the active ingredient in over 55 brand-name products approved in California. (http://www.chemicalhouse.com:8080/nl_new/jsp/viewnewsletter.jsp?id=361). The main problem with certain weeds such as horseweed is that they can produce as many as two hundred thousand seeds per plant and any breeze can spread them over hundreds of meters. This is why every effort must be made to ensure that such weeds do not reach the seed formation stage.

Glyphosate-resistant weeds can become a problem when farmers grow Roundup Ready resistant biotech crops in the same field year after year. Rotation of non-biotech crops in a cotton farming system is a valuable tool for avoiding greater problems. Production systems that do not allow for growing other crops in rotation with cotton must consider the weed resistance issue more seriously than systems that allow planting non-biotech crops other than cotton in their cotton fields. Cotton-wheat-cotton is a popular rotation, particularly in many Asian countries, and it is perfectly suitable for avoiding weed resistance problems in biotech herbicide-resistant cotton.

Reports from Australia show that ryegrass has developed resistance to glyphosate. Spurred by the need to minimize the development of resistance to glyphosate herbicides, the Grains Research and Development Corporation (GRDC) launched the Glyphosate Sustainability Working Group initiative. The Glyphosate Sustainability Working Group reported that, although still proportionally low, the incidence of glyphosate resistance in annual ryegrass had doubled in a single year. The practices recommended to grain growers to reduce the risk of resistance development include: use of the double-knock strategy, effective in-crop weed control, alternative herbicides, crop topping and non-herbicide weed control techniques, such as hay and weed seed collection.

Managing Herbicide Resistance in Biotech Cotton

Sustained exposure of weed plants to the same herbicide chemical, as a result of repeated use during a single season against the same weed species, increases the probability that the weed plant will develop resistance to that particular chemical.

Herbicides may be classified in several different ways: site of uptake into the plant (root vs. shoot), means of translocation within the plant (systemic vs. contact), time of application (pre-planting incorporated, pre-emergence, post-emergence), chemical structure similarity (phenoxy vs. triazine) and mode of action. There are many modes of action by which herbicides get dissolved into the weed tissues, above and below ground, and ultimately kill the weed. One such mode of action is through acetolactate synthase (ALS) inhibitors. According to York (2006) there are many herbicides within the four chemical classes (Imidazolinone, Sulfonylurea, Pyrimidinyl benzoate and Triazolopyrimidine) that kill weeds by inhibiting ALS. The herbicides known as Staple and Envoke, which are frequently used on cotton, are ALS inhibitors, and resistance to this group can develop in as few as 3 to 4 years. The other mode of action where resistance is of great concern is the 5-Enol-pyruvylshikimate-3-phosphate synthase (EPSPS) inhibitors. Glyphosate belongs to this group and in the USA a number of weeds affecting cotton have already developed resistance to these groups.

Like insects, weeds of any species may take many years to develop resistance to a particular herbicide, or just a few years. The time it will take a particular species to develop resistance depends on a number of factors. There is no doubt that the frequency with which the same chemical is used to get rid of the same weed species is very important. In the long run, it is much easier and always more economical to avoid development of resistance instead of having to deal with it once resistant weed plants start appearing in the crop. Any economically viable practice that will help minimize the use of herbicides may be recommended to help delay the development of resistance. Many agronomic practices including pre-planting cultivation, early and healthy establishment of the crop, control of seedling diseases, avoidance of abiotic stress, and physiological stress can help the crop compete better against weeds on its own. Rotating crops that do not share the same weeds can also have a long-term impact. In-crop cultivation should be utilized as much as possible to minimize reliance on herbicide control. Early detection is also an important component in any herbicide resistance management strategy.

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