

# Update on Refuge Requirements for Biotech Cotton

*(In this article biotech cotton refers to insect-resistant biotech cotton only)*

Continuing to plant biotech cotton year after year while simply relying on the assumption that the pests targeted by insect-resistant biotech genes will spontaneously crossbreed with a population produced on alternate crops is risky. Such an assumption would need to be tested and verified, and only then could the product be released for commercial purposes. Setting up a monoculture is always bad, since the pests that dine on one crop tend to multiply tremendously, which magnifies crop losses. Rotating crops keeps pests off balance, diversifies the ecology of the fields and helps maintain the fertility of the soil. Refuge requirements were mandatory from the introduction of biotech varieties and their rigorous implementation was recommended, as if they were an integral component of a technology package. The general recommendation was to plant a non-biotech area equivalent to 5% of the total planted area under unsprayed conditions or an area equivalent to 20% of the total under a non-biotech conventional spray application regime. The primary objective of keeping a refuge crop was to extend the usefulness of a particular gene or genes by creating a hybrid population of target insects. It was strongly believed that the target lepidopteran would develop resistance and that the refuge crop would be one way to stave off the development of resistance. However, the resistance problem did not develop as feared and technology users slowly started relaxing the requirement to plant refuge crops.

## Refuge Requirements and Consequences

Originally, two refuge options were available, 5% unsprayed or 20% sprayed. In the case of the 5% unsprayed, five hectares of non-biotech (non-Bollgard) cotton had to be planted for every 95 hectares of insect resistant biotech (Bollgard) cotton. The refuge could not be treated with any insecticide labeled for the control of the targeted bollworms. Secondary pests, mainly sucking insects, could be controlled in the refuge crop area as long as the products used did not affect the activity of target pests. Different requirements were required for different countries. In India, the refuge had to be at least five rows; in the USA, the size of the refuge had to be at least 48 meters wide. Fertilizer application, weed control and other agronomic management operations, including the management of non-target pests, had to be conducted in a manner similar to the one employed with the biotech Bollgard cotton. It was also mandatory to plant the refuge crop within a certain distance of the biotech field/crop.

The 80:20 (20% sprayed) option required that 1/5 of the cotton area be planted using conventional practices. Commercial insecticides could be used to prevent heavy losses, but the crop could not be sprayed with Bt foliar applications. Later,

a community refuge program was also introduced. The community refuge had to meet the requirements of either the 5% unsprayed option or the 20% sprayed option. Farmers could also employ an appropriate combination of the two options. The latest innovation is the Built-in-Refuge/natural refuge/embedded refuge. In this variant, the refuge crop had to be planted at just the right time so that the flowering of the refuge crop would coincide with that of the biotech crop.

Irrespective of the option that may have been followed, losses in non-biotech cotton were an inescapable fact. In the 5% unsprayed refuge, the area that was not sprayed at all against the target insects was highly vulnerable to losses due to bollworms. With the 20:80 option, the 20% sprayed refuge was prone to suffer because of the resistance problem, when it was present, and in any case, it certainly required regular monitoring and spraying. No specific studies are available to ascertain what the cost of strictly following refuge requirements might have been to farmers (in terms of unit area of refuge crop). Apart from the inconvenience of planting and maintaining a refuge crop, farmers had to cope with lower yields and, ultimately, a higher cost of production. These losses varied from year to year depending upon pest pressure and the kind of refuge adopted. The cost of insecticides and any other costs related to pest control (spraying) could also play a role in the income differences between the biotech and non-biotech areas, especially in the case of larger plantations where the non-biotech area might be quite significant.

While no data on losses are available, Piggott and Marra (2007) reported on savings that might be attained by changing the refuge requirement for Bollgard II in favor of a natural refuge. Initially, the authors presented an evaluative model that was appropriate for any cotton-producing state in the United States. They used empirical applications of the model for North Carolina and observed that, based on 2005 data, a cotton grower in North Carolina could be expected to suffer an estimated annual loss of \$56.40/ha. When non-pecuniary benefits were taken into account, the advantage of not planting a refuge crop increased to \$66.50/ha. These amounts would vary from area to area and year to year. The technology and seed companies would also benefit from higher seed sales. Following the very same principle, other countries also promoted the use of a natural refuge for crops other than cotton.

## Refuge Requirements by Country

### Argentina

Argentina commercialized biotech cotton in 1998/99, but the area planted to biotech varieties remained at less than a

quarter of the total for over 15 years. Now it seems that almost all production, especially in the Chaco—the largest cotton-producing province in the country—is biotech. The largest area is planted to Deltapine varieties. Local varieties branded as Guazuncho are planted on about 5% of the area; the rest is all Deltapine, mostly NuOpal, which also has a Roundup Ready gene. The only company supplying biotech seed is Genética Mandiyú.

Genética Mandiyú recommends that 20% of the area be planted to conventional non-biotech cotton varieties. It also recommends that farmers implement the current refuge requirements individually on their farms. Genética Mandiyú further recommends that, whenever necessary, both biotech and conventional cotton could be sprayed with insecticides, but, in no case should Bt-based insecticides be used. Farmers are advised that insects should not be allowed to multiply beyond the expert-recommended thresholds for any insect.

## Australia

In Australia, where biotech cotton was introduced as Ingard, refuge requirements were made mandatory. However, farmers were given the freedom of choosing between pigeon pea and non-biotech cotton as a refuge crop. Two species of bollworm, *Helicoverpa armigera* and *Helicoverpa punctigera*, which had already developed resistance to many insecticides, were targeted as the most prominent and aggressive pests. During the process of deciding whether or not to commercialize biotech cotton, pigeon pea was assessed as a possible refuge crop because it had twice the capacity to produce *Helicoverpa* moths as compared to unsprayed non-biotech cotton. Only half the amount of pigeon pea area was required to produce the same level of population as an equal area of unsprayed non-biotech cotton. A number of factors—large-scale adoption of biotech cotton (over 90% of the cotton area), changes in crop agronomy, changes in the array of insecticides used (due to changes in the pest complex), replacement of Ingard with dual gene Bollgard II—together helped induce many changes in cotton production practices, and improved farmers' profit margins as well. Such changes tempted Australian growers to make even more changes in refuge requirements, which has led to widespread questioning of the continued relevance of refuge crops and their impact on best management practices.

Irrigated Bollgard II Options

Crop	Conditions	% of Bollgard II
Cotton	Irrigated, sprayed conventional cotton	100
	Irrigated, unsprayed conventional cotton	10
Pigeon Pea	Fully irrigated, unsprayed	5

Rainfed Bollgard II Options

Crop	Conditions	% of Bollgard II
Cotton	Dryland or irrigated, sprayed conventional cotton	100
	Dryland or irrigated, unsprayed conventional cotton	10
Pigeon Pea	Fully irrigated, unsprayed	5

Source: Monsanto Australia & New Zealand

In a 2014 paper, Baker and Tann stated that the pigeon pea maintained its substantial superiority over unsprayed, non-biotech cotton as a refuge generator of *Helicoverpa* in all cases involving both the Ingard and the Bollgard II varieties. This claim was supported by field counts of both live pupae and empty pupal cases left behind in the soil by the moths after emergence. There was some evidence that, in time, *Helicoverpa* production had decreased in both pigeon pea and cotton refuges. The incidence of parasitism of pupae increased from the Ingard to the Bollgard II period. The authors did not find any evidence to support any difference between the two refuge crop types regarding the parasitism of *Helicoverpa*.

Pigeon pea could be grown as a refuge crop together with irrigated cotton, but cannot be used as a dryland refuge option due to the poor crop growth of pigeon peas. Furthermore, the timing and duration of flowering may not necessarily coincide with flowering and fruit formation in cotton. The pigeon pea crop should preferably be planted into fallow or rotation fields with no plants growing as ratoons or volunteers. According to Cotton Australia, in 2010/11, around 60% of Bollgard II refuges were made up by pigeon peas. The key issues involved in using the pigeon pea as a refuge crop are: variable seed germination percentage, potential need to replant, and possible weed seed contamination. Initially, in addition to pigeon peas, farmers were allowed to grow 10% unsprayed non-biotech cotton or 15% sorghum or 20% corn. Since sorghum and corn are not preferred hosts for *H. punctigera*, as of 2010/11 both sorghum and corn were removed from the list of eligible refuge crops. Different refuge crops could be grown simultaneously in such a manner that their total area was enough to meet the minimum refuge area requirement. The simultaneous use of sprayed and unsprayed refuges was not allowed. In Australia, there are slight regional differences in refuge crop requirements, but they hinge mainly on whether the cotton is grown under irrigated or rainfed conditions.

The Australian Pesticides and Veterinary Medicines Authority (APVMA) regulates the Resistance Management Plan in the country. The latest resistance management plan comprises several different resistance management strategies that growers can use to protect the effective longevity of the insect resistance technology (Bollgard/WideStrike). Under the current resistance management plan for Bollgard II, the refuge options in Australia are shown at the above table.

## Brazil

In 2006/07, despite the fact that *H. armigera* had not yet become a pest on cotton, Brazil commercialized Bollgard cotton. Average yields were rising and farmers could easily afford the technology. The main thrust was to keep up with technological innovations in cotton production. The adoption rate also showed that although herbicide tolerant cotton was almost a necessity, there was still no urgent need to turn to insect-resistant biotech cotton. Only recently has the

area planted to insect-resistant biotech cotton came to exceed half of the overall planted area.

*H. armigera* is notorious in cotton for being highly reproductive and for its ability to develop resistance to insecticides. The American bollworm, often also referred to as the cotton bollworm or just as bollworm, is geographically widespread. It is a major pest on cotton in Asia, Africa, Europe and the United States. The boll weevil was and is taking a heavy toll on cotton in Brazil, although the country had still not reported any significant *H. armigera* infestation on cotton. The pest was detected attacking cotton and sorghum for the first time in early 2013. Since then, the bollworm population has been increasing. While it is perfectly capable of developing into a major pest, it has not reached an alarming stage yet. With the increase in the area under Bollgard II varieties, the call for refuge cropping is increasing. Seed companies and experts vary in their recommendations on the proportions of the ideal refuge crop. The most commonly favored ratio is 20% sprayed cotton, but the recommendations are not final and much less are they strictly adhered to. In the Cerrado region of Brazil, cotton is grown as a rotation crop alternating with soybeans, but the fact is that the Cerrado, with the highest yields in the world under non-irrigated conditions, has drawn a significant amount of attention and attracted investment. Soybeans also carry the Cry1Ac gene, which makes it more conducive to the development of conditions for the establishment of the cotton bollworm and, hence, more indicative of a greater need for a refuge crop.

## Burkina Faso

Burkina Faso was the second country in Africa and the first country among the West African cotton producing nations to commercialize biotech cotton. Burkina has suffered an insecticide resistance problem and a number of bollworms, such as *Helicoverpa armigera*, *Diparopsis* spp. *Earias* spp., *Sylepta derogata*, *Spodoptera littoralis*, and sometimes even *Anomis flava*, all attack cotton in the country. *Helicoverpa armigera* and *Diparopsis* spp. are the key heliothines that damage cotton. Burkina Faso commercialized biotech cotton through locally acclimatized varieties in 2008/09. The recommended refuge requirement in the country is 20% conventional cotton with six sprays, no more than 1.5 km away from a Bollgard II cotton field when it is collectively implemented by a group of cotton farmers. For an individual farmer, the 20% conventional cotton field should be adjacent to a Bollgard II cotton field. Monsanto is continuing the development of its refuge in the bag product with the capability of being implemented simultaneously with the introduction of new technologies such as Bollard II stacked with Roundup Ready Flex that is currently in the testing phase. Monsanto has been testing its built-in refuge in a bag technology since 2010 and so far the 5% mixture is performing well and suffering minimal losses. At the time this article was written, no hard and fast conclusions had yet been arrived at.

## China

In 1997/98, China became the first country in Asia to commercialize biotech cotton. The move was prompted mainly by the resistance problem and by the yield losses the country had been suffering for almost a decade prior to the adoption of biotech cotton. At the outset, refuge requirements were recommended, but they are no longer compulsory in cotton. The belief is that the refuge is not required because the cotton bollworm, *Helicoverpa armigera*, feeds on other crops growing in the fields at the time that the cotton crop is in the fields. Plot sizes are small in the Yellow and Yangtze River valleys. Alternate crops, mainly corn, peanuts and soybean, compete with cotton. Studies have shown that the “natural refuges” of non-Bt crops other than cotton delay the development by pests of resistance to the Bt toxin. In the Yellow and Yangtze River valleys, fields are so small that people have seen bollworm larvae crawling from a cotton field over to a soybean field that was just adjacent to the cotton field. It is not known if both fields belonged to the same grower.

Farmers in the Yellow and Yangtze River valleys produce cotton on the smallest plots in the world. The average farm size in the two regions is 0.3 hectare/farmer and cotton may be planted on about one third or one quarter of the total farm size. It is not feasible to plant a refuge crop in farming systems on such a small scale. The Northwest region has a minimum need for refuge crop because of harsh winters and low insect pressure.

## Colombia

Colombia commercialized biotech cotton in 2003/04, mainly because growers were under considerable pressure to control a combination of lepidopterans. A number of bollworms, including *H. armigera* and *Spodoptera*, had developed resistance, while the boll weevil remained as the most serious pest requiring attention, from the appearance of the first flower bud and continuing throughout the cotton-growing season. Colombia adopted biotech cotton through Deltapine varieties, since the country imports most of its planting seed from the U.S. Refuge crops had to be planted mainly in the following ratios:

- 80/20 - where 80% of the cotton area was planted with biotech varieties and the remaining 20% with a conventional variety on each farm.
- 96/4 - where 96% of the cotton area on the farm was planted with a biotech variety and the remaining 4% with a conventional variety; lepidopterans are not controlled at all in the 4% area, neither with Bt nor with insecticides.

The issue of managing the refuge areas was difficult, especially in the region of Tolima. Biotech adoption preceded so slowly that in many cases biotech farmers considered that the neighboring non-biotech farmers' cotton was all the refuge crop they needed. Those farmers who did plant a refuge did so on poor soil or under zero attention conditions.

## India

Since its adoption in 2002/03, the area planted to biotech cotton in India increased continuously so that by 2014/15 the biotech cotton area had reached 11.6 million hectares or over 92% of the total cotton area. The concern regarding the development of resistance to biotech cotton by the bollworm was taken seriously from the very beginning. Some studies have shown that the Northern and Central regions require a higher percentage of refuge crops than the Southern region. Results also showed that a sprayed refuge is more profitable than an unsprayed refuge. As a part of the IPM strategy, the Genetic Engineering Approval Committee of the Government had earlier stipulated the planting of a refuge crop of the same non-Bt cotton hybrid on the periphery of the Bt cotton field—at a ratio equivalent to five rows or 20% of the total sown area, whichever was greater—to non-biotech varieties. Conventional insecticides were allowed, as was also mentioned in the case of Argentina. Growers were obliged to implement refuge requirements both in terms of area and configuration. Later it was decided that a non-biotech counterpart of the same species—a similar duration and similar fiber quality variety/hybrid—could be used as a refuge instead of the same isogenic non-biotech counterpart. Subsequently, the Genetic Engineering Approval Committee also approved the cultivation of pigeon peas as a refuge border crop in place of a non-biotech refuge for the country's Central and Southern cotton regions.

The area planted to biotech cotton has increased beyond the refuge threshold of 80% because Indian farmers generally do not comply with the mandated refuge requirements. In 2002, out of 15 fields inspected by the Genetic Engineering Approval Committee of the Government, only 50% of the farmers were found to be in compliance with these requirements. The seed companies provide non-biotech seed along with the biotech seed (450 grams), but farmers may decide not to use the non-biotech seed all the time or use it to fill gaps.

India is evaluating the benefits of built-in refuge technology, which is a proposed new method to be used in planting a non-biotech refuge. The proposed built-in refuge for Bollgard II at the 5% level would consist of 450 grams of Bollgard II hybrid seeds blended with additional non-biotech seeds of the corresponding cotton hybrid as a refuge constituting a minimum of 5% of the final blend. According to the meeting of the All India Coordinated Cotton Improvement Program held at the University of Ludhiana, field tests were conducted in 2011 and 2012 with the aim of assessing the abundance of major cotton lepidopteran pests (*H. armigera*, *S. litura* and *P. gossypiella*) on non-biotech plants in a built-in refuge planting design corresponding to their abundance on a 20% non-biotech structured refuge. The Central Institute for Cotton Research, which led the evaluation, tested a 5% built-in refuge at five field locations (2012); State Agricultural Universities at five locations (2011 & 2012) and Mahyco-Monsanto Biotech Ltd., at 21 field locations (2011 & 2012). The studies demonstrated

that key lepidopteran pests, such as *H. armigera*, *S. litura* and *P. gossypiella*, were able to colonize non-biotech plants (at the 5% and 10% levels) in the built-in refuge format (as measured by fruiting body damage), on a per plant basis, to an extent similar to that of the bollworm colonization on non-biotech plants in the structured refuge format. Data on larval movement was generated by artificial infestation (25-40 second/third instar larvae/plant) of refuge non-biotech plants in the built-in refuge format at three locations. These studies concluded that adjoining Bollgard II plants did not show increased damage over the Bollgard II controls in five out of six data sets. Studies still under way reveal a bias in favor of the built-in refuge technology, but the final recommendations have not yet been released.

## Mexico

Mexico commercialized biotech cotton in 1996/97, the same year as Australia and the USA. The country planted Deltapine varieties and its refuge requirements were the same as in the USA. It seems that the 80/20 and 96/4 options are still viable in Mexico.

## Myanmar

Refuge requirements for biotech cotton in Myanmar are not adhered to properly. This is because the area owned by each farmer is no more than five hectares for 70% of farmers and diverse crops are grown simultaneously with cotton. Most farmers in the rainfed area practice mix cropping (cotton, maize, pigeon pea, sesame, vegetables, etc.). Only some irrigated areas and land owned by large growers who are traditional producers of cotton having high interest in growing long staple cotton grow may require refuge crops. These areas are mainly in Magwe and Mandalay divisions of central Myanmar. In the Sagaing division, farmers are still growing traditional short staple *arboreum* cotton that does not need pesticide.

## Pakistan

Pakistan silently commercialized biotech cotton but it was only years later that it officially declared that it had been growing it on a large scale for many years. Seed is produced locally and distributed without requiring any commitment by the public or private sector to plant a refuge crop. Farmers are not obliged to adhere to any refuge requirements and normally do not plant refuges of their own volition. Maize and sorghum are grown in many parts of the country at the same time that cotton is in the field. According to official statistics, 86% of all growers own less than five hectares, so many alternate host crops are in close proximity to cotton fields. Farm sizes are larger in the southern part of the Punjab province, but farmers are well aware of production technology and efficient enough to report any resistance problem to the agriculture department. It is not known if Bollgard II is also grown in the country, but Cry1Ac seems to have done very well. Farmers were relieved to be able to divert some of their attention away from

the control of the bollworm and devote it to the control of the leaf curl virus disease and to the mealybug, which caused far greater damage than that attributed to the bollworm and to insecticide resistance. Both problems still persist thus relegating bollworm attack to the third rank.

## South Africa

South Africa commercialized biotech cotton in 1997/98, mainly to tackle the problem of *H. armigera* and *Diparopsis castanea*. It was also suspected that both had developed resistance. Large-scale as well as small-scale growers adopted biotech cotton. At that time, success stories at the level of the small-scale farmers of the Makhathini Flats received a great deal of coverage in the media. Adoption of insect-resistant biotech cotton, followed by herbicide tolerance and Bollgard II (in 2010/11) did not help to slow down the declining trend in cotton production in South Africa. The country suffered a decrease in both irrigated and dryland cotton production, especially since 1999. The reduced cultivation of cotton is attributed to the decline in cotton prices and the increasing prices of competing crops such as maize and sunflower. Inability to cover fixed costs forced a number of cotton gins to close down, which had a huge impact on cotton production in the country. It became evident that the cause of this decline did not lie in insect control costs but somewhere else, and has yet to be pinpointed. Cotton was planted on about 11,000 hectares in 2013/14 compared to 111,000 hectares in 1997/98. Most of the area taken out of production was dryland/rainfed. Cotton production is now mostly a business for commercial farmers.

South African farmers are required to plant a refuge crop at a ratio of 20% sprayed or 5% unsprayed area. The resistance management strategy was inclined toward producing higher doses of Bt toxins. Although planting a refuge is compulsory to limit the development of resistance, the level of compliance by farmers is not known. It is also mandatory that the refuge crop should be more than 500 meters away from biotech cotton. Monsanto distributors provide seed to growers, but the purchase is limited to the quantity of seed permitted under the "Monsanto Technology Agreement", which the grower has to sign with Monsanto.

## Sudan

In 2010/11 and 2011/12, after two years of tests, Sudan commercialized biotech cotton using Chinese varieties. The National Variety Release Committee of Sudan released the first biotech varieties in March 2012. In June 2012, those varieties were approved for commercial production by the Biosafety Authority. The approved varieties carry the Cry1A gene and it is believed most of the cotton area is already planted to biotech varieties with no compulsory requirement for a refuge crop.

## United States of America

The original refuge requirements in the United States were either 5% unsprayed area or 20% sprayed area. There was

a strong emphasis on the implementation of the mandatory refuge requirements to be used in connection with the first single-gene insect-resistant cotton. Consequently, refuge requirements were followed more scrupulously during the first few years of commercial use of Bollgard cotton. However, some growers started to violate the requirements as early as the second year after introduction. The introduction of double-gene Bollgard II cotton launched the debate about the need for a refuge crop. Researchers did not challenge the need for susceptible insects, but argued instead that susceptible insects could come from a large number of alternate crops and native host plants. It was already known that bollworms could complete their larval development on a number of plants, including maize, pigeon peas, soybeans and sorghum. In 2003, at the time when Bollgard II was approved, at least two other insect-resistance genes capable of being approved for commercial use were already in advanced stages of development. The view was emerging that insect-resistance genes could be replaced with new genes regularly, such that resistance would not be as serious a threat as was originally believed prior to the commercialization of biotech varieties.

The Environmental Protection Agency (EPA) is responsible for regulating refuge requirements for biotech cotton in the USA. No structured refuge crop is required for stacked insect-resistant biotech cotton (Bollgard II and WideStrike) in the cotton-growing areas from West Texas to the cotton belt in the Southeast. This is because only heliothines are the primary targets. In the area from West Texas out to the West coast, the pink bollworm – a non-heliothine – has a significant presence and requires a different control strategy. Cotton growers in the states of California, Arizona, New Mexico and the western part of Texas are required to plant cotton as a refuge crop. The options included: embedded, 5% unsprayed cotton or 20% sprayed refuge. Some exceptions may still apply due to the pink bollworm eradication programs being implemented in some parts of the above-mentioned areas.

## Factors Responsible for Changes in Refuge Requirements

The reasons for imposing the requirement of a refuge crop were known even before the commercialization of biotech varieties. Preemptive measures were adopted with the primary objective of delaying the development of resistance and that objective is still the fundamental basis for the long-term sustainability of biotech genes. In the mid-1990s, biotechnology applications in the form of insect-resistance genes were new, and the cotton sector had the bitter experience of bollworms developing resistance to insecticides. Some countries managed to recover from the insecticide resistance problem; some major producing countries were at the peak of their resistance issue, and the West African countries were consolidating their thinking to deal with the resistance problem by way of collaborative efforts at a regional level. Insect-resistant biotech cotton leads to lower production costs (thanks to lower insecticide use), higher yields (due to better

insect control) or a mix of both with various degrees of success. If none of the foregoing advantages is obtained, at least there is the benefit of lower environmental pollution. When growers plant a portion of their crop employing conventional practices or under unsprayed conditions, they automatically forgo the benefits of biotech cotton.

It is possible to implement refuge conditions where the land holdings are large and each farmer is planting hundreds of hectares of cotton. But, in countries where each farmer may be planting less than a hectare of cotton, it is practically impossible to maintain a uniform level of refuge crops. Where smallholdings are prevalent, if one out of five farmers do not plant biotech cotton, his conventional cotton automatically serves as a refuge crop for his neighbors.

Cropping systems vary greatly from one country to another. There are countries where, in certain areas, cotton is grown exclusively during a given part of the crop year. Consequently, although many different small farmers may own small tracts of land, the result may be that large contiguous extensions are planted exclusively to cotton. On the other hand, there are cropping systems where other crops that are equally strong hosts for target pests, such as maize, are grown at the same time. This cropping model is commonly practiced in China (Mainland), India, Myanmar, Pakistan and Sudan among other countries that have commercialized biotech cotton.

Aside from the hybrid population of target insects, resistance to a toxin can also be delayed by enhancing its strength. If the toxin is very strong and has a diversified mode of action, there is every chance that the useful life of that toxin may be extended before insects develop resistance to it. The development of Bollgard II has effectively provided the same advantage with regard to resistance. Even before Bollgard II was approved, it was generally believed that stacked-gene cotton, with two or more insect-resistance genes, would have a profound effect on management of resistance in insect-resistant biotech cotton. It was assumed that Bollgard II would extend the useful life of toxins. The third generation of toxins will further minimize the chances of development of resistance.

## Conclusions

Refuge requirements were advised prior to the adoption of biotech cotton. Initially, the most common refuge requirements were those that depended on pest biology, together with many other factors, and they were strictly adhered to during the first few years following the commercialization of insect-resistant biotech cotton. However, in time it became apparent that the resistance problem had not emerged to the degree that was

generally feared in the beginning. Many countries began to consider relaxing the early refuge requirements, mostly due to the natural occurrence of susceptible populations on crops other than cotton. Production in small-scale farming systems involved more than one alternate host crop. Studies showed that there were sufficient populations available in the vicinity of the cotton to interbreed with the population that had survived on the biotech cotton. Many countries have relaxed their refuge requirements, but significantly enough, none has tightened those requirements. In those countries that have not relaxed refuge requirements, the area planted to biotech cotton has exceeded the refuge threshold of 80%. Farmers generally do not comply with mandated refuge requirements due, on the one hand, to the lower income received from the refuge crop and, on the other hand, the complications faced by smallholders in endeavoring to adhere to the recommendations. Small-scale growers planting less than a hectare cannot afford to plant refuge lines for their biotech cotton. In small-scale farming systems, particularly in the biotech cotton producing countries of Asia, comparable areas are planted to legume crops and to corn/maize, which provide an ample natural refuge for biotech cotton. A diversified cropping pattern can serve as an unstructured refuge only if the crops grown are not biotech or, in the event that they are biotech, if they do not carry the same biotech gene(s). Gene pyramiding has also delayed the development of resistance. The next stage in the resistance management strategies that seem to be emerging is unstructured/embedded/built-in refuges.

## References

- Baker, Geoff H. and Collin R. Tann. 2014. Refuge crop performance as part of the Bt resistance management strategy for *Helicoverpa* spp. (Lepidoptera: Noctuidae) in *Australian cotton production systems*. *Australian Journal of Entomology* (Impact Factor: 0.8). 05/2014; DOI: 10.1111/aen.12072, <http://www.researchgate.net/publication/259546274>.
- Piggott, Nicholas E. and Michele C. Marra. 2007. The Net Gain to Cotton Farmers of a Natural Refuge Plan for Bollgard II® Cotton. <http://www.agbioforum.org/v10n1/v10n1a01-piggott.htm>.
- Singla, Rohit., Phillip Johnson and Sukant Misra. 2012. Examination of Regional-level Efficient Refuge Requirements for Bt Cotton in India. *AgBioForum*, The Journal of Agrobiotechnology Management and Economics. Volume 15, Number 3, 2012.
- Technical Information Section. 2007. Refuge requirements and their implications in biotech cotton, *THE ICAC RECORDER*, Vol. XXV, No. 3, 2007.
- Tabashnik, Bruce E., Thierry Brévault and Yves Carrière. 2014. *Insect Resistance to Genetically Engineered Crops: Successes and Failures*. <http://www.isb.vt.edu/news/2014/Jan/TabashnikBrevaultCarriere.pdf>.