# Managing the Risk of Insect Resistance to Transgenic Insect Control Traits: Practical Approaches in Local Environments

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ABBREVIATIONS: *Bacillus thuringiensis* (Bt), Central Institute of Cotton Research (CICR), Genetic Engineering Approval Committee (GEAC), Insect Resistance Management (IRM), Insect Resistance Action Committee (IRAC), Integrated Pest Management (IPM), Organization for Economic Co-operation and Development (OECD), Quality Control and Assurance (QC/QA), United States Environmental Protection Agency (US-EPA)

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**ABSTRACT**

**BACKGROUND:** Growers have enthusiastically embraced crops genetically improved to express *Bacillus thuringiensis* (Bt) proteins for insect control because they provide excellent protection from key damaging insect pests around the world. Bt crops also offer superior environmental and health benefits while increasing grower income. However, insect resistance development is an important concern for all stakeholders; including growers, technology providers and seed companies that develop these genetically improved crops. Given the marked benefits associated with Bt crops, insect resistance management (IRM) must be a consideration when cultivating these crops.

**RESULTS:** The technical data and practical experience accumulated with Bt crops in many global regions can inform different aspects of resistance management leading to robust, science-based IRM plans. A range of elements should be considered in assembling any IRM strategy, including: pest biology/ecology, product deployment patterns, local cropping systems, insect susceptibility monitoring, stakeholder/grower communications, and a remedial action plan should resistance develop. Each of these elements is described in more detail, with specific examples of how these elements can be combined and tailored to the local/regional environments and grower practices.

**CONCLUSION:** IRM plans need to be suitable for the given production situation. What works for large monoculture production systems in North America is unlikely to be appropriate for the small, more diverse agriculture of Southeast Asia or Africa. Though it is clear that Bt crops impart considerable value to growers, it is also clear that it is in the best interest of all stakeholders to preserve Bt proteins for the long-term benefits they provide.

## 1 INTRODUCTION

Insect control traits introduced into plants using modern biotechnology methods have shown high economic value across the globe. An assortment of crops expressing different *Bacillus thuringiensis* (Bt) proteins has been commercialized for insect control and additional products are under development. Growers have embraced crops (i.e. maize, cotton, potato and rice), genetically improved to express different Bt proteins (i.e. Cry1Ab, Cry1Ac, Cry1F, Cry1A.105, Cry2Ab, mCry3Aa, Cry3Bb, Cry3Aa, Cry34/35, Vip3A), as they provide excellent protection from key damaging insect pests in global regions.[1] There is a history of safe use of these proteins, both for the environment and human health.[2,3,4] Recent evaluations have shown that these traits provide economic value to adopting countries through increased grower income.[5,6] The continuing value of this technology can be enhanced through appropriate stewardship, such as insect resistance management (IRM) plans, that can prolong trait efficacy against the target insect pests.

The evolution of insect resistance is an ongoing concern to all concerned with crop protection, including those who use insecticide applications, cultural practices and host plant resistance. This article was commissioned by the Insect Resistance Action Committee (IRAC), a specialist technical group of the industry association CropLife International, to provide additional guidance for the development of IRM plans that address these stakeholder concerns. Over the past four decades, hundreds of insect species have developed resistance to one or more control measures, severely impacting the economics of crop production. Most cases of insect resistance to date involve synthetic chemical insecticides,[7] but resistance has also developed to microbial agents, such as sprayable formulations of Bt.[8] Bt crops that express Cry proteins throughout the growing season were anticipated to have an even greater risk for resistance development than that for sprays, thus they were introduced into the marketplace with mandatory, proactive IRM plans. To date, with over 10 years of commercial use of transgenic Bt crops in multiple countries, no confirmed cases of widespread field resistance to Bt crops have been documented. This durability has been attributed, at least in part, to proactive IRM plans that have helped to mitigate the resistance risk. Given the marked benefits associated with transgenic Bt crops, IRM must be a key consideration when introducing these crops into agricultural environments.

## 2 RESULTS

### 2.1 How to develop a robust IRM plan

Durable, science-based IRM plans have been based upon an extensive array of research data that has been collected before and since the initial introduction of Bt crops. Many important factors, such as pest biology, pest/crop interactions and computer simulation models for IRM plans have been investigated. However, in the beginning it was understood that no matter how detailed the results, science alone will not result in a robust IRM plan in the absence of practical field experience, information on local/regional environments and grower practices.

IRM plans need to be suitable for the given production situation. While practical experience has accumulated with Bt crops in the US, Canada, Australia, Argentina, Philippines and China,[9-11] and can inform certain elements of an IRM plan, other aspects must take into account the unique pest population spectrums and distinctive agricultural practices found in local and regional environments. Because of uncertainties, there is a regulatory temptation to be overly conservative in setting IRM requirements and a technology-user temptation to be less cautious. Practical IRM needs to strike a balance between these competing perspectives. What works for large monoculture production systems in North America is unlikely to be appropriate for the small, more diverse agriculture of Southeast Asia or Africa. The goal should be to enable growers to have access to the technology while providing stewardship that will provide an acceptable level of protection against resistance.

The introduction of Bt crops brought powerful new pest control agents to growers, which are so effective and safe that the United States Environmental Protection Agency (US-EPA) made the unprecedented demand for resistance management plans prior to market introduction. Specifically, these early IRM plans were based upon: 1) plant tissue expression levels of the insect control protein that were characterized as ‘high dose’, 2) the use of refuges where susceptible insects could thrive, and 3) the assumption that resistance to the Cry proteins in plants would be functionally recessive and rare in insect populations. A refuge of non-Bt plants is planted within or close to the Bt crop so that any rare resistant insects emerging from the Bt fields can easily find and mate with susceptible insects. In this scenario, the genetics of resistance are assumed to be recessive such that heterozygous offspring remain susceptible to the amount of the Bt protein expressed in the crop. In theory, this IRM strategy should delay resistance development as long as the frequency of resistant alleles is low, and the refuge produces sufficient numbers of susceptible insects that mating between resistant and susceptible insects is significantly more likely to occur than mating between insects each carrying resistance genes. The refuge requirements set by the US-EPA for Bt crops are described in Table 1 for maize and Table 2 for cotton. Refuges can be economically practical for growers especially if chemical insecticide treatments used in the refuge can protect the yield while allowing sufficient susceptible insects to survive.

Other elements of these initial IRM plans included: 1) annual monitoring for target pests' susceptibility to the Bt protein expressed in the crop in high market adoption areas, 2) communication and education for growers so that they fully understand and carry out resistance measures, 3) monitoring growers for compliance with the IRM plan (i.e. ensuring that the refuge plots are large enough and at the appropriate distance from the Bt field), 4) enforcement of compliance through removal of technology from growers found repeatedly out of compliance, and 5) a remedial action plan should resistance be detected. IRM plans developed for other countries or regions should be tailored to their specific needs and while the elements outlined above should be considered, some may not be appropriate to the local conditions.

Pyramiding of Bt proteins into the same plant often controls not only a broader insect spectrum, but may also provide superior IRM properties if the insect control proteins act by unique mechanisms of action on the same target pest. Bt proteins act through a receptor-mediated mechanism and receptor alteration has been the most frequently identified resistance mechanism associated with high levels of resistance.[12-14] This improved IRM strategy is based on the concept that resistance in an insect to two different toxins present in the same plant is far less likely than that of resistance to a single toxin.[15-17]

Evaluating the impact of IRM refuge acres on the production of susceptible insect populations can also be used to optimize refuge deployment for extending trait durability. The non-Bt crop refuge provides one source of susceptible insects but it may not be the only source because many insect pests are generalists (e.g. Cotton bollworm, *Helioverpa zea*; old world bollworm, OWB, *Heliothis armigera*; tobacco budworm, *Heliothis virescens*; cabbage looper, *Trichopusia ni*), feeding and developing on a variety of different host plants.[18] They can develop on other cultivated crops as well as non-crop alternative hosts, such as those in uncultivated areas and weedy field borders. Other insects are classified as specialists (i.e. European corn borer, *Ostrinia nubilalis*; western corn rootworm, *Diabrotica virgifera virgifera*) feeding on only one or a couple different crop species. For the generalists, an alternative host refuge may produce equal to or larger quantities of insects than the structured non-Bt crop refuge. The US-EPA acknowledged this when they removed the need for a structured non-Bt cotton refuge for pyramided Bt cotton products, relying instead on the abundance of non-cotton plants to provide a natural refuge for Noctuidae species.[4]

In other areas of the world, a variety of IRM tactics have been utilized for Bt crops taking into account local cropping practices. Australian authorities and growers worked together to establish IRM plans with the introduction of single-gene Bt cotton in 1996 by limiting each grower to a cap of 30% of the total cotton grown per farm, thereby ensuring a large non-Bt cotton refuge. This cap was removed when pyramided Bt cotton was introduced seven years later, reflecting the reduced resistance risk associated with pyramided products. Some other continuing elements of the IRM plan in Australia restrict planting times, limit insecticide use on refuges and require cultivation after harvest ('pupa-busting'), all in an effort to minimize resistance risk. Because farm production in China is typically a mixture of small plots of cotton, maize, soybean, wheat and peanut (<5 ha in total) that serve as natural hosts for the major pest, OWB, there is no requirement for a separate structured non-Bt cotton refuge for Bt cotton.[10] To date, Bt maize has not been approved in China, thereby limiting selection pressure on OWB. In India, each bag of Bt cotton includes a second bag containing an additional 20% of non-Bt cottonseed to plant a refuge. Information is shared on the proper deployment of the non-Bt refuge. The Philippines, where farm size is also small, adopted IRM requirements for Bt maize that base the need for a structured IRM plan on market penetration, similar to the Australia plan but with different metrics. Until growers in a region plant above 80% of their maize crop to hybrids containing a Bt trait, they are not required to plant non-Bt maize as a refuge for the Asian corn borer, *Ostrinia furnacalis*. All the plans described above include either annual insect susceptibility monitoring and/or routine monitoring for insect damage and follow up testing if populations of the primary target insects are found damaging a Bt field.

## 2.2 The importance of stakeholder participation

Adoption of Bt crop IRM plans to date has been successful in part because they were developed with broad stakeholder participation. Experts representing trait providers, academic institutions, regulatory authorities, grower and commodity groups, and other local/regional support groups have each had a role during the development and maintenance of an IRM strategy. Successful resistance management should begin before introduction of Bt crops through the establishment of a local infrastructure of experts who can provide input on key pest biology and pest/crop interactions that can help to tailor IRM recommendations. Scientific experts should evaluate the available data and determine if additional research is needed to support implementation of an initial IRM plan and to further refine the deployment of an IRM strategy as new research data and experience with the technology becomes available.

Grower groups and farm advisor organizations can provide specific crop production practices and assist in the evaluation of how IRM components can be practically integrated and how information on IRM can be effectively disseminated. Commodity groups along with technology developers can provide technical information for the development of educational materials for stakeholders and users. Government authorities can determine if the proposed IRM plan meets society's need for sustainable use of the technology and can ensure compliance. While this is the ideal approach, it must be realized that not all situations will meet this model. For example, in developing countries, the infrastructure might not exist for widely educating growers on IRM. In these cases, it will be necessary to explore options where the trait provider or seed distributor takes on more of the responsibility for implementation.

Regardless of the specific region for the cultivation of the Bt crop, a range of elements should be considered in assembling an IRM strategy. These elements include: pest biology and ecology, expected product deployment patterns, local cropping systems and use of the Bt crop within an Integrated Pest Management (IPM) program, insect susceptibility baseline and monitoring options, stakeholder and grower communications and education, and a remedial action plan in the event of resistance development. Each of these elements is described in more detail in the following sections.

## 2.3 Pest biology and ecology for major pests

The core of the IRM plan is based on the key target pest biology and pest / crop interaction. Most crops have a complex of primary and secondary pests. Many secondary pests can be controlled via other IPM tools if the key target pest is controlled by the trait. The primary target pests of the transformed crop should be identified for the region and the efficacy of the Bt crop characterized for each. The target pest biology information should include the history of control measures, such as classes of insecticides sprayed in the area and the combination of biologically-based approaches adopted, to assess the potential for resistance development. The life cycle of the insect pest, including the number of generations of the pest in different growing regions and seasons, annual migrations and the movement of both larval and adult stages of each target pest on the crop and other host plants, should be investigated. Insect movement has a direct impact on the design of a refuge for producing susceptible insects, if this is an aspect of the IRM plan. The location, timing and distribution of feeding damage on the crop should be understood.

## 2.4 Product deployment strategies

Characterization of the Bt crop as it relates to the IRM plan should include the amount and pattern of Bt protein(s) expression in the different plant parts and across the growing season. This characterization of the Bt crop will help identify possible control gaps where additional control measures might be necessary. There are multiple techniques to reduce insect selection pressure, incorporating methods based on IPM wherever possible. These include, but are not limited to, the use of refuge, scouting and applying insecticides as needed, rotating different modes of action, limiting specific Bt proteins to certain crops, capping sales to limit market penetration, destruction of crop residues, using locally adapted plant varieties with native resistance, and multiple traits targeting the same pests. The best IRM plan will match the available products with the local environment and conditions in an IPM manner.

The ideal level of expression for Bt crops provides: 1) adequate control of the target pest(s) below economic thresholds, 2) consistent expression throughout the growing season to ensure control as pest populations increase, and 3) expected control of partially resistant insects. The level of target pest control will determine the amount of non-Bt plant refuge needed to support sufficient Bt susceptible populations. In some cases the refuge is a structured refuge, i.e. a planting of the non-Bt crop, matched by maturity level and genetics to the Bt crop, planted in close proximity. The refuge can also be unstructured, consisting of alternative host plants, such as other crops and weeds, or a combination of the two so long as sufficient susceptible insects are produced.

A pyramiding strategy for insect control consists of the simultaneous use of two insecticidal agents with different modes-of-action, therefore knowing the potential for cross-resistance patterns between the proteins is useful. Insecticidal proteins, which bind to different receptors in the insect midgut, such as Cry1 and Cry2 Bt proteins in TBW, are examples of such a strategy. Researchers have highlighted the IRM advantages of pyramiding Bt proteins in the same crop variety indicating that the refuge size could be greatly reduced or a natural refuge may be sufficient without increasing the risk of insect resistance,[16,19] as in the US for pyramided Bt cotton.[4]

## 2.5 Local cropping systems

Agricultural cropping systems vary by crops, country and culture. High production agriculture may utilize crop monocultures across very large regions with few wild or uncultivated areas. Other environments may support a wide diversity of crops on small plots, or crops intermixed with wild areas within the landscape. The risk of resistance development is impacted by these patterns, with increased risk associated with monoculture cropping and higher market penetration.

Monitoring the adoption rate of Bt crops on a regular basis is important for identifying the highest risk areas. With knowledge of market penetration, regional market triggers could be useful risk management elements, such as for refuge deployment and insect susceptibility monitoring. Specific market caps can limit the planting of Bt crops or favor Bt in one crop but not another as a method of reducing selection pressure. As an example, in the Philippines, when a specific market penetration level is reached (i.e. 80%), then all single gene Bt maize crops must deploy a structured refuge; otherwise the prevalence of non-Bt plants can serve as the refuge.

The practice of saving a portion of the crop seed harvest for planting in the next growing season can have important negative impacts on resistance management for Bt crops. The practice would undermine IRM efforts by; 1) removing quality control and assurance (QC/QA) efforts in the production of the seed resulting in variable trait expression, 2) limiting the accuracy of market penetration, 3) weakening insect monitoring plans, and 4) preventing effective grower communication and education efforts. Without proper QC/QA by a professional seed company, uniform high dose deployment of the trait and even trait purity can be compromised. If the market penetration numbers are inaccurate, market triggers and caps become impractical and insect monitoring for insect susceptibility will be hampered because the correct sampling locations are erroneous. And if technology providers, distributors and dealers are unable to identify growers than the ability to communicate/educate will be impossible.

## 2.6 Baseline & monitoring insect susceptibility

Insect monitoring encompasses two basic approaches: monitoring the insects for changes in susceptibility to the expressed protein and monitoring fields for signs of unexpected levels of damage due to a key target pest. Routine insect monitoring, if done properly, can detect small shifts in pest susceptibility well before widespread resistance occurs at the field level. This enables implementation of actions to mitigate the crop damage and manage Bt-resistant pest populations. Possible resistant insect populations are indicated when insect damage surpasses economic thresholds causing severe crop damage. It may be possible to contain local or isolated hotspots of resistance with appropriate mitigation measures. Once widespread field failures occur, due to resistance in the key target pest, it may be too late to rescue or sustain the Bt trait efficacy against the resistant species in the affected region;

however, if the crop remains effective against other key pests, the overall utility of the Bt trait may be maintained.

As a first step, measuring the baseline susceptibility of pest populations to the trait across the growing area should be characterized prior to widespread Bt crop planting. Subsequent annual monitoring will compare insect susceptibility to these baseline data focusing on regions of highest anticipated levels of adoption. Insect sampling could cover many different areas in a given region, but should focus on areas of high levels of market penetration and those areas where insecticide treatment of the non-Bt crop is highest. Insect sampling should be done in areas nearby, but not within or adjacent to the Bt crop in order to collect sufficient numbers for testing and to ensure that the sample collected is representative of the local pest population. To represent a location, it is recommended that at least 100 larvae, 100 adults, 50 mated females or 50 egg masses are collected to establish a population, but if populations in the field are small, one half of these numbers will also provide a valid sample. Developing reliable, accurate high throughput bioassays for detecting significant changes in population sensitivity to Cry proteins, such as the discriminating dose technique,[20] is the goal.

Grower monitoring of fields for damage due to the key target pests is an important component of early detection of resistance. Therefore, contact information should be clearly indicated to growers so they can report any findings of damage in the Bt crop to seed company or technology provider representatives. This contact information can be provided to distributors, dealers and customers in the various forms of product literature, i.e. grower guides and product labels. Once a report is made, one-on-one communication with the grower should be initiated to investigate the source of the damage. If it is verified that the damage was in a Bt field and a key pest was involved, a technical representative should visit the grower to investigate the level of crop damage, to assist the grower in mitigating the problem to preserve the growers crop, and to report the results for further follow up, if justified. If further follow up confirms that this is a case of suspected resistance, additional investigations should be conducted to confirm the level of sensitivity to the Bt protein compared with baseline measures. If necessary remedial action measures that are commensurate with the severity of the incident should be initiated, which may include reporting to regulatory authorities.

### 2.7 Communication/education: Grower, distributor and dealer

Resistance management is the responsibility of all stakeholders. The use of Bt crops provides a completely new insect control option for the marketplace. Though it is clear that the availability of Bt crops imparts considerable value to growers, it is also clear that it is in the best interest of all stakeholders to preserve Bt proteins for the long-term benefits they will provide. In fact, the proactive effort for grower education on the responsible use of Bt crops in the US as well as the speed at which these products have been adopted has been greater than for any other single insecticidal product in history.

At commercialization, grower education on the proper use of the crops and associated IRM plans is a key component of how the product is marketed and sold. Seed companies, distributors and growers should be educated about the properties and correct product use, including the consequences of resistance development. For example, attendance at grower education meetings could be a requirement for purchase of the product. Other mechanisms for education could include technical bulletins, product brochures, sales meetings, articles in trade journals, presentations by local experts, and grower guides that accompany the commercial product. All of these can be used in addition to the traditional label that accompanies the Bt crop product and which outlines the contents of the product and standard directions for use. What is important is that growers be trained through familiar venues that are effective for their situation, taking into account the culture, education levels, avenues of access to information and how products are procured. Complex IRM plans that require growers to perform additional tasks beyond their normal planting and cultivation activities could be counter to good stewardship so it is better to keep both the plan and the message simple and direct for the grower.

### 2.8 Remedial action plan should resistance develop

The implementation of a remedial action plan should occur if field resistance to a Bt crop is confirmed in a key target pest. Remedial action plans could have multiple components, such as planting structured refuge, applying insecticides, and/or temporarily halting sales, depending upon the situation. The immediate remedial action goals should be to protect the grower's crop investment, characterize the resistant insects to enable the development of case-specific management options and contain the spread of these insects utilizing the best available control methods.

Furthermore, once it has been determined that the damage is caused by a targeted insect and that the plants contain the Bt protein, the investigation should also move to collection and testing of the insect population(s) for susceptibility to the Bt protein. Testing should be done in the specific area of crop damage along with surrounding regions to delineate the scope of resistance and compare the bioassay results to pre-commercial baseline data. Under a worst-case scenario, where insect resistance is confirmed and appears to be spreading, sales of crops expressing this Bt protein could be restricted in the affected region in future growing seasons. Other options for controlling the key pests should be identified for growers, including cultural control, insecticide applications and use of alternative transgenic insect control traits.

## 3 CASE STUDY: OFFICIAL INDIA REQUIREMENTS FOR Bt COTTON

The Genetic Engineering Approval Committee (GEAC) is the official body in charge of commercial release of transgenic crops in India, including strategies for IRM. This is advocated so as to increase the durability of the technology and reduce chances for resistance development. Accordingly, transgenic Bt cotton has to be planted in the centre of the plot, and non-transgenic cotton has to planted as refuge crop surrounding the central plot such that at least 5 rows or 20% of the total sown area, whichever is more serves as refuge. Mixing of transgenic and non-transgenic seeds for planting is not recommended. The guidelines indicate that each pack of Bt cottonseed, when sold, should also contain required quantities of non-transgenic seeds to meet the requirements of planting the refuge crop. The planting layout has to be indicated in the product literature included in the seed pack. Since the standard pack size of cottonseeds in India is 450 g., each pack of Bt cottonseeds sold contains one pouch of 450 g. of Bt cottonseeds and an additional pouch of 120 g. of non-transgenic cottonseeds. For the few years after the Bt cotton was first released for commercial cultivation in India, the non-Bt counterpart hybrid seeds were used for refuge planting. More recently, the GEAC has allowed the flexibility of including either the non-Bt counterpart hybrid or any other conventional hybrid having a similar phenotype as that of the Bt cotton hybrid to be used in refuge planting.

However, there is no legal basis for the grower to follow the requirement of refuge planting and compliance enforcement has been a challenge. Additionally, it has been suggested by the scientific community that considering the abundant, diverse alternate host availability for the target pest and cropping patterns followed by Indian farmers, the requirement for planting refuge crop may be unnecessary.

The requirement for baseline susceptibility data generation by the applicant prior to commercial release of Bt cotton followed by regular resistance monitoring, post commercial release has been implemented as a part of IRM strategy. Central Institute of Cotton Research (CICR), India's premier public cotton research institute has been entrusted with the responsibility of the regular monitoring for insect susceptibility.

## 4 DISCUSSION & CONCLUSIONS

Protection of Bt crops from insect resistance is not only an important concern for growers, but also for the technology providers and seed companies that develop these genetically improved crops. While hundreds of Bt strains have been identified (http://www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/intro.html), only a few have found commercial success based on successful introgression into target plants and acceptable control levels of important agricultural target pests (i.e. Bt *kurstaki*, Bt *aizawai*, Bt *Berliner*, Bt *tenebrionis*). Intensive screening for new microbial strains and their associated insecticidal proteins has provided a variety of candidates for crop transformation. In addition, trait providers are now developing and commercializing pyramided traits that express two or more different proteins against the same pest to prolong trait durability, and an increased understanding of how Cry proteins work at the molecular level has resulted in customized proteins with improved efficacy and spectrum of activity.[21-23] Despite these scientific advances, Bt proteins remain an important insect control resource that should be preserved for their exceptional efficacy and superior environmental and health benefits.

Should resistance development in Bt crops occur, insect damage would undoubtedly increase, leading to a reduction of crop yield and value of the Bt technology. Confirmed resistance may cause the product to be withdrawn from the market, which would inevitably trigger expanded use of insecticides sometimes accompanied by increased hazards to human health and the environment, losing health and safety benefits that Bt crops provide. However, consequences differ by pest – for key pests, it would lead to reduced yields, but for secondary pests, the major benefit of the technology remains and over-reaction (e.g. withdrawal) could unnecessarily lead to a complete return to synthetic chemistries or put more selection pressure on remaining pest management practices.

IPM is an effective and environmentally friendly strategy that relies on an array of pest control techniques that bring together genetic and biological information with cultural and modern pest management methods for a more sustainable crop production system. As long as crop plants have been grown, farmers have embraced a wide range of tactics to offset insect damage, including low technology techniques where field workers manually remove insect pests from crop plants and sophisticated IPM systems that encompass myriad approaches with Bt crops being only the latest of a multitude of science-inspired tools. IRM employs many of these very same principles as IPM to minimize and manage harmful pest outbreaks using a range of pest control tactics in order to preserve and maintain all possible strategies.

In order to identify the best tactics for a robust IRM strategy, to extend the durability of Bt crops for a given country or region, the following elements should be evaluated: pest biology and ecology, expected product deployment patterns, local cropping systems and use of the Bt crop within an IPM program, insect susceptibility baseline and monitoring options, stakeholder and grower communications and education, and a remedial action plan in the event of resistance development. Each of these elements must be assessed with a specific focus on the local conditions and agricultural practices that should be considered in the development of an IRM plan, because no matter how detailed the scientific information, these local conditions are critical for successful IRM deployment. Effective stakeholder participation, including experts from technology providers, grower and commodity groups, academia and government, are another important factor during the development and implementation of an IRM strategy. As new information is collected from research efforts on pest biology and the genetics of resistance, and as more practical experience accumulates with Bt crops in various regions and countries, IRM plans and tactics should be reviewed and refined to extend the durability of all Bt crops globally.

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**Table 1. Refuge requirement for Bt maize set by the US-EPA[a]**

| Trait target | Refuge size | Deployment | Proximity |
|---|---|---|---|
| Corn borer | 20% (maize regions)<br>50% (cotton regions) | Discrete corn borer refuge | Internal or external blocks within ½ mile (¼ mile preferred) or in-field strips (at least 4 rows wide) |
| Rootworm | 20% | Discrete rootworm refuge | Internal or external blocks adjacent or in-field strips (at least 4 rows wide) |
| Corn borer + Rootworm | 20% (maize regions)<br>50% (cotton regions) | 2 options:<br>1. Common rootworm/corn borer refuge | Internal or external blocks adjacent or in-field strips (at least 4 rows wide) |
| | | 2. Discrete rootworm/corn borer refuges | Separate fields should be used within ½ mile (¼ mile preferred) |

[a] Source: EPA (http://www.epa.gov/oppbppd1/biopesticides/pips/bt_corn_refuge_2006.htm) [Accessed 26 March 2009] Refer to this web page for guidelines on insecticide use on the refuge acres.

**Table 2. Refuge requirement for Bt cotton set by the US-EPA[b]**

| Gene # | Region | Refuge size | Deployment | Proximity |
|---|---|---|---|---|
| Single | All | 1. 5% external unsprayed<br>2. 5% embedded<br>3. 20% external sprayed[b] | 1. At least 50m wide<br>2. At least 50m wide<br>3. N/A | 1. ½ mile (¼ mile preferred)<br>2. Embedded in field<br>3. 1 mile (½ mile preferred) |
| Dual | Arizona, California, New Mexico, west Texas | | | |
| Dual | Southeast US | Natural refuge – no structured refuge requirement | | |
| Single / dual | For PBW only – Arizona and California | N/A | At least one row for every 6 to 10 rows of Bt cotton | Embedded in field |

[b] Source: EPA (http://www.epa.gov/oppbppd1/biopesticides/pips/bt_cotton_refuge_2006.htm) [Accessed 26 March 2009] Refer to this web page for guidelines on insecticide use on the refuge acres.