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ARS–154

January 2001

Bt Cotton & Management of the Tobacco Budworm-Bollworm Complex



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Abstract

Hardee, D.D., J.W. Van Duyn, M.B. Layton, and R.D. Bagwell. 2000. *Bt* Cotton & Management of the Tobacco Budworm-Bollworm Complex. U.S. Department of Agriculture, Agricultural Research Service, ARS–154. 40 pp.

Preservation of *Bt* technology is critical for cotton producers across the U.S. Cotton Belt because of increasing insecticide resistance and production costs. Frequent introduction of new transgenic cotton varieties creates a need to continuously evaluate their cost-effectiveness and develop efficient plans for their deployment. This publication explains how *Bt* cotton is developed, how it controls insect pests, and how it can most effectively be used in insect pest management. Restrictions and limitations to the use of *Bt* cotton are discussed, such as insects' development of resistance to it and approaches to preserving the technology for long-term profits.

Audiences for the publication consist of research and extension entomologists in the public and private sectors, consultants, and cotton producers.

Keywords: bollworm, *Bt* cotton, budworm, cotton, *Heliothis virescens*, *Helicoverpa zea*, refuge, resistance monitoring, transgenic cotton

This publication reports research involving pesticides. It does not contain recommendations for their use nor does it imply that uses discussed here have been registered. All uses of pesticides must be registered by appropriate state or Federal agencies or both before they can be recommended.

While supplies last, copies of this publication may be obtained at no cost from D.D. Hardee, USDA–ARS, P.O. Box 346, Stoneville, MS 38776, e-mail: dhardee@ars.usda.gov. Copies also available from F.L. Carter, National Cotton Council, P.O. Box 820285, Memphis, TN 38182.

Copies of this publication may be purchased from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161; telephone (703) 605–6000.

Issued January 2001

Acknowledgments

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The following individuals provided invaluable input during multiple reviews of this publication's content and technical accuracy:

J.J. Adamczyk, Jr., USDA–ARS; C.T. Allen, University of Arkansas; G.L. Andrews, Mississippi State University; J.S. Bacheler, North Carolina State University; J.H. Benedict, Texas A&M University; M.L. Boyd, University of Missouri; J.R. Bradley, Jr., North Carolina State University; E. Burris, Louisiana State University; M.A. Caprio, Mississippi State University; F.L. Carter, National Cotton Council; B.L. Freeman, Auburn University; T.W. Fuchs, Texas A&M University; L.D. Godfrey, University of California at Davis; F.L. Gould, North Carolina State University; M.A. Karner, Oklahoma State University; G.L. Lentz, University of Tennessee; B.R. Leonard, Louisiana State University; S.R. Matten, U.S. Environmental Protection Agency; W.J. Moar, Auburn University; J.W. Mullins, Monsanto Corporation; R.D. Parker, Texas A&M University; P.M. Roberts, University of Georgia; M.E. Roof, Clemson University; C.G. Sansone, Texas A&M University; R.W. Seward, University of Tennessee; J.E. Slosser, Texas A&M University; R.H. Smith, Auburn University; S.D. Stewart, Mississippi State University; N.P. Storer, North Carolina State University; and D.V. Sumerford, USDA–ARS.

The authors also express their appreciation to K.R. Ostlie, W.D. Hutchison, and R.L. Hellmich, authors of "*Bt* Corn & European Corn Borer." 1997. University of Minnesota, St. Paul, NCR Publication 602, for allowing use of ideas and text.

This publication arose from a collaborative multistate and multiagency effort to provide timely guidelines for cotton producers, crop consultants, extension and industry personnel, and agricultural agency administrators on recommended ways to deploy *Bt* cotton technology. Since this technology is changing rapidly, the publication will be updated as needed.

Bt *Cotton* & *Management of the Tobacco Budworm-Bollworm Complex*

Tobacco budworm, *Heliothis virescens* (F.), larva

Bt cotton is one of the first crop protection products from biotechnology.

All *Bt* cotton plants contain one or more foreign genes derived from the soil-dwelling bacterium, *Bacillus thuringiensis*; thus, they are transgenic plants.

The insertion of the genes from *B. thuringiensis* causes cotton plant cells to produce crystal insecticidal proteins, often referred to as Cry-proteins. These insecticidal proteins are effective in killing some of the most injurious caterpillar pests of cotton, such as the larvae of tobacco budworms and bollworms. This new technology for managing insect pests was approved for commercialization in the United States by the U.S. Environmental Protection Agency (EPA) in October 1995 and is now available from several seed companies in this country, as well as in many other cotton-growing countries around the world.

Cotton varieties containing the Cry1Ac *Bt* protein provide protection against three major U.S.

Tobacco budworm, *Heliothis virescens* (F.), moth

cotton pests—tobacco budworms, bollworms, and pink bollworms. *Bt* cotton also reduces survival of other caterpillar pests such as beet armyworms, cabbage loopers, cotton leafperforators, fall armyworms, southern armyworms, and soybean loopers. The protection it provides against tobacco budworms, pink bollworms, and European corn borers is greater than the protection provided by the most effective foliar insecticides. Unfortunately the protection it affords cotton against bollworms is generally less than that provided by registered insecticides.

The preservation of the *Bt* technology is critical because (1) bollworms, tobacco budworms, and pink bollworms continue to develop resistance to foliar-applied insecticides, (2) use of insecticides is tied to ecological concerns, and (3) *Bt* genes are valuable resources. This publication focuses on how *Bt* cotton affects the tobacco budworm and bollworm. It is intended to provide information that will guide producers, research and extension entomologists, consultants, and industry in the proper use and long-term preservation of this valuable technology.

Frequent introduction of new transgenic cotton varieties creates a need to continuously evaluate their cost-effectiveness and develop efficient plans for their deployment. A goal of this publication is to answer questions about the technology by explaining how *Bt* cotton is developed, how it controls insect pests, and how it can be most effectively used in insect pest management. Restrictions on and limitations to the use of *Bt* cotton are discussed, such as insects' development of resistance to it and approaches to preserving the technology for long-term profits.

Tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), cause more damage to cotton than any other insect pest in the U.S. Cotton Belt. The combined cost of controlling these pests and the losses they inflict on cotton production exceeds $300 million a year. Insecticides used against tobacco budworms and bollworms often create other problems, such as higher populations of beet armyworms and cotton aphids and an increased pesticide load in the environment. Frequent exposure of insect pests to insecticides results in the development of insecticide resistance, which reduces the overall effectiveness of available insecticides, increases crop losses, and leads to higher pest control costs and lower farm profits.

The severity of tobacco budworm and bollworm infestations and resistance to synthetic insecticides vary across the Cotton Belt, both between and within the states. Because of this variation and the price of the technology, not all areas of the Cotton Belt are able to economically justify the use of *Bt* cotton. However, where insect infestations are severe, *Bt* cotton offers a new management tool for producers, helps ensure against yield loss in the presence of heavy infestations of insecticide-resistant tobacco budworms, and aids in reducing bollworm damage.

Bt—*What Is It?*

Bollworm larva feeding in boll

The insect-disease-causing organism *Bacillus thuringiensis* (*Bt*) is a naturally occurring soilborne bacterium found worldwide. A unique feature is its production of crystal-like proteins that selectively kill specific groups of insects and other organisms. When the insect eats these Cry-proteins, its own digestive enzymes activate the toxic form of the protein. Cry-proteins bind to specific receptors on the intestinal walls and rupture midgut cells. Susceptible insects stop feeding within a few hours after taking their first bite, and, if they have eaten enough toxin, die within 2 or 3 days.

Different *Bt* strains produce different Cry-proteins, and there are hundreds of known strains. Scientists have identified more than 60 types of Cry-proteins that affect a wide variety of insects. Most Cry-proteins are active against specific groups of insects, such as the larvae of certain kinds of flies, beetles, and moths. For example, Colorado potato beetle larvae are affected by Cry3A proteins; Cry1Ac is used against tobacco budworms; and European corn borers can be killed with Cry1Ab, Cry1F, Cry1Ac, and Cry9c proteins. Other Cry-proteins are active against mosquito larvae, flies, or even nematodes. Some Cry-proteins have been used for more than 30 years in various liquid and granular formulations of natural *Bt* insecticides, mainly to control caterpillars on a variety of crops. The *Bt* cotton varieties presently used against tobacco budworms, bollworms, and certain other caterpillars produce the Cry1Ac protein.

The Why's and How's of Creating Bt Cotton

Bioinsecticides like *Bt* that are sprayed on crops may perform as well as synthetic insecticides in very limited situations, but the performance of *Bt* insecticides has been inconsistent in many instances.

The erratic performance in cotton is attributed to four reasons:

- The toxin is rapidly degraded by ultraviolet light, heat, high leaf pH, or desiccation.
- Caterpillars must eat enough treated plant tissue to get a lethal dose of the toxin, since the toxin has no contact effect.
- The sites where tobacco budworms and bollworms feed are difficult to cover with the foliar-applied sprays.
- *Bt* Cry-proteins are less toxic to older larvae.

A cotton plant modified to produce Cry-protein within the plant tissues that caterpillars eat overcomes most of the aforementioned limitations. The plant-produced *Bt* proteins are protected from rapid environmental degradation since they are not directly exposed to the environment. Incomplete coverage is not usually a problem because the plants produce the proteins in all tissues where larvae feed, thus ensuring that the larvae will eat the Cry-protein. The protein is always present whenever newly hatched larvae feed, eliminating the timing problem associated with foliar application. The result is that *Bt* cotton has a built-in system that efficiently and consistently delivers Cry-toxins to the target pests from the time a newly hatched larva takes its first bite (fig. 1).

Bt cotton offers a vastly improved method for delivering Cry-insecticides to target insects, compared to traditional *Bt* sprays. *Bt* cotton may also be considered a form of host plant resistance, in that the Cry-protein trait is carried in the plant's genes, as is traditional plant resistance to insects.

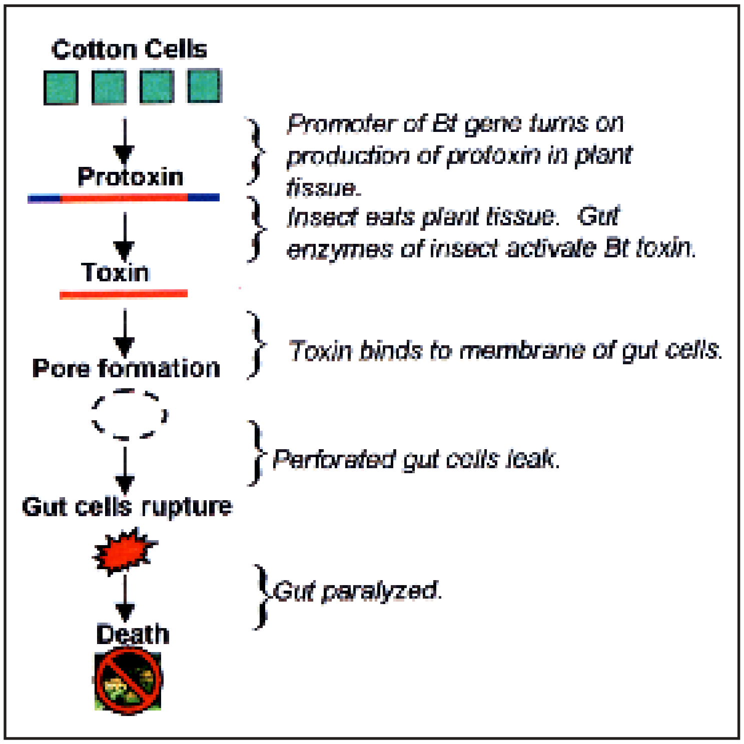


Figure 1. Mode of action for *Bt* toxin after eaten by a tobacco budworm larva. Modified with permission from Ostlie et al. 1997.

Biotechnologists created *Bt* cotton by inserting selected exotic DNA, from a *Bt* bacterium, into the cotton plant's own DNA. DNA is the genetic material that controls expression of a plant's or an animal's traits. Following the insertion of modified *Bt* DNA into the cotton plant's DNA, seed companies moved the Cry-protein trait into high-performance cotton varieties by traditional plant breeding methods. Agronomic qualities for yield, harvestability, fiber quality, and other important characteristics were preserved at the same time the Cry-protein gene was added to commercial varieties.

The three primary components of the genetic package inserted into cotton DNA include:

Protein gene. The *Bt* gene, modified for improved expression in cotton, enables the cotton plant to produce Cry-protein. The first varieties of *Bt* cotton produced in the United States contained one Cry-protein gene—Cry1Ac. Other varieties contain a "stacked" gene complex, for example—one gene for insect control (Cry1Ac) and one gene to protect the cotton from application of the herbicide glyphosate. Future cotton varieties may include these genes, other genes that allow the plant to produce different Cry-proteins, or insecticidal proteins from sources other than *Bt.* There are many possible combinations for crop improvement traits.

Promoter. A promoter is a DNA segment that controls the amount of Cry-protein produced and the plant parts where it is produced. Some promoters limit protein production to specific parts of the plant, such as leaves, green tissue, or pollen. Others, including those used in *Bt* cotton and certain *Bt* corn varieties, cause the plant to produce Cry-protein throughout the plant. Promoters can also be used to turn on and turn off protein production. Current varieties of *Bt* cotton produce some *Bt* protein throughout the growing season.

Genetic marker. A genetic marker allows researchers to identify successful insertion of a gene into the plant's DNA. It also assists plant breeders in identifying and developing new cotton lines with the *Bt* gene. A common marker is an herbicide tolerance gene linked to the *Bt* gene. Following a transformation attempt to place the *Bt* and marker gene into the plant's DNA, plants are treated with herbicide. Plants that were successfully transformed have the *Bt* gene and the herbicide resistance gene and will survive herbicide treatment; plants without the marker gene, and hence without the linked *Bt* gene, will be killed by the herbicide.

This genetic package—a *Bt* gene plus a promoter and marker—can be inserted into cotton plant DNA through a variety of plant transformation techniques. Transformed plants may be affected by the genetic package, as well as the location of the new genes in the plant DNA. The insertion site may affect *Bt* protein production and other plant functions as well. So biotechnology companies carefully scrutinize each transformation to ensure adequate production of *Bt* protein and to limit possible negative effects on agronomic traits.

Following a successful transformation, plants are entered into a traditional backcross breeding program with the variety chosen to receive the foreign *Bt* gene package. The final product, a *Bt* cotton variety, is developed after four or five backcross generations. Even though the new transgenic *Bt* cotton variety may be named after the parent variety, agronomic qualities can be considerably different.

The Safety of Bt *and* Bt *Cotton*

Before registering *Bt* cotton, EPA reviewed data on *Bt* insecticides that had been accumulated for decades. *Bt* Cry-proteins were found to be toxic only to certain insect groups and to have no known negative effects to humans, domestic animals, fish, wildlife, or other organisms. EPA exempted *Bt*-produced Cry-proteins from the requirement of tolerance in food because of their history of safety and because they degrade rapidly in the environment. These Cry-proteins are considered among the safest and most environmentally friendly insecticides known.

Controlling Tobacco Budworms and Bollworms: Does Bt *Cotton Do the Job?*

Bollworm/budworm eggs on a leaf. Courtesy of S. Stewart.

When compared with other insecticide management practices, *Bt* cotton dramatically improves the control of tobacco budworms and, to a lesser extent, the control of bollworms. For example, chemical insecticides, including some new chemical classes, often control from 70 to 95 percent of a susceptible tobacco budworm population. As indicated in table 1, the level of tobacco budworm control achieved with *Bt* cotton can be very dramatic. *Bt* cotton varieties may provide more than 98 percent control of tobacco budworm throughout the growing season. For growers whose cotton is plagued by high densities of insecticide-resistant tobacco budworms, *Bt* cotton is a very welcome technology.

Bt cotton is less effective against bollworms. Still, it can eliminate as many as 60 to 90+ percent of the bollworms infesting a cotton field (table 1). When there are high numbers of bollworms on *Bt* cotton during the bloom stage, growers may need to apply one or more supplemental insecticide treatments to prevent economic damage. This has been well documented through field research. Fortunately, the bollworm can still be managed more effectively and inexpensively with currently available insecticides—except perhaps in South Carolina where resistance to insecticides has been detected.

Bollworm moths lay their eggs on cotton plant terminals, leaves, buds, and flowers. As the eggs hatch, the larvae may move into open blooms where they feed on flower parts, including pollen, that are known to have a lower level of Cry-protein than other plant parts. This feeding on less toxic parts may result in lower mortality. The bollworm in addition is naturally more tolerant of Cry1Ac *Bt* protein than the tobacco budworm. These two factors help explain why more bollworms than budworms survive on *Bt* cotton. Moreover, Cry-protein expression in *Bt* cotton decreases about 80 days postplanting, which may allow higher survival of bollworms. This late decline may also reduce Cry-protein effectiveness against other insect pests that occur later in the growing season and feed on mature leaves.

Table 1. Survival of tobacco budworms, bollworms, and fall armyworms on *Bt* and non-*Bt* cotton genotypes

	Percent survival	
Insect	**1994**	**1995**
Tobacco budworm		
on *Bt* cotton leaf	1	0
on *Bt* cotton square	2	0
on non-*Bt* cotton leaf	86	84
on non-*Bt* cotton square	69	67
Bollworm		
on *Bt* cotton leaf	7	23
on *Bt* cotton square	5	4
on non-*Bt* cotton leaf	80	74
on non-*Bt* cotton square	63	52
Fall armyworm		
on *Bt* cotton leaf	61	76
on *Bt* cotton square	33	25
on non-*Bt* cotton leaf	76	92
on non-*Bt* cotton square	45	42

Source: Modified and reprinted with permission from Jenkins et al. 1997.

Controlling Other Insects

Tarnished plant bug nymph

Southern armyworms. Courtesy of R. Smith.

Tobacco budworms and bollworms are not the only insect pests that attack cotton. Unfortunately, the Cry1Ac protein has essentially no effect on many of them. Pests that *Bt* cotton does not directly affect include boll weevils, cotton aphids, cotton fleahoppers, cutworms, spider mites, stink bugs, tarnished plant bugs, thrips, and whiteflies. In some caterpillar species, *Bt* cotton may provide only 10 to 50 percent control. This partial suppression may be cause for concern in the later years of an insect resistance management program because it does not provide a high-dose strategy (see Glossary) for insects such as beet armyworms, fall armyworms (table 1), southern armyworms, soybean loopers, and yellowstriped armyworms. *Bt* cotton at this time provides good to excellent control of cabbage loopers, cotton leafperforators, European corn borers, salt marsh caterpillars, and cotton square borers. Future varieties of *Bt* cotton may produce different Cry-proteins or other new toxins that will control a wider variety of pests.

The effect of *Bt* cotton on some insect pests may be indirect. For example, *Bt* cotton does not directly affect the cotton aphid, but reductions in insecticide use against tobacco budworms and bollworms allow more of the aphid's natural enemies to survive, and they in turn reduce aphid numbers.

On the other hand, reducing the amount of foliar insecticide may also allow other pests normally controlled by the insecticides to become more abundant. Boll weevils, stink bugs, and plant bugs, for example, have by chance been controlled by foliar sprays applied against tobacco budworms or bollworms. So, reduction in the use of insecticides on *Bt* cotton has allowed these pests to increase in some areas. Offsetting this disadvantage, however, is *Bt* cotton's usefulness where the boll weevil has been eradiated.

Does Bt Cotton Affect Beneficial Insects?

Big-eyed bug

Lacewing larva

Many studies have shown that Cry1Ac in *Bt* cotton is highly selective because it kills only certain caterpillar species. *Bt* cotton has minimal or no effect on beneficial insects, including honey bees, lady beetles, spiders, big-eyed bugs, pirate bugs, and parasitic wasps. However, laboratory research has shown that Cry1Ab protein can indirectly affect green lacewing larvae that eat *Bt*-killed caterpillars. It is not known if Cry1Ac in *Bt* cotton has a similar effect on lacewing larvae. Theoretically, *Bt* cotton may indirectly lower the general abundance of some beneficial insects, since it causes caterpillar populations to decline, resulting in less food for the predators, parasites, and the pathogens that attack them. Offsetting this effect is the positive influence gained from reducing conventional broad spectrum insecticide use in *Bt* cotton. The overall balance of these contrasting influences is currently unknown and is difficult to predict.

The Value of Bt *Cotton to the Cotton Farmer*

As is the case with most new technology, *Bt* cotton offers value to the cotton farmer in specific circumstances (table 2). Information on economic benefits is limited due to the short time the technology has been available and the many new cotton varieties introduced each year. Recently, the technology fee and the seed cost for *Bt* cotton have decreased, which has affected the economics of growing it. Yield data are available from federal and university entomologists and agronomists, as well as seed companies, in nearly every cotton-growing state.

Comparisons of the *Bt* and non-*Bt* cotton varieties generally show that *Bt* cotton offers an economic advantage in instances where effective insecticidal control of certain caterpillar pests is difficult to achieve or is very costly.

Examples of such situations include

- insecticide-resistant tobacco budworms or bollworms
- high populations of susceptible tobacco budworms or bollworms, such as in outbreak seasons or during the initial phases of boll weevil eradication
- situations where a properly timed and applied insecticide management program cannot be achieved, such as in fields that do not allow the proper operation of air or ground sprayers, in remote fields, or in cases where cotton acreage exceeds the amount of equipment or personnel dedicated to insecticidal control
- situations where insecticidal control is excessively costly (for example, more than $40/acre), as may be the case when high infestations are coupled with newer, expensive insecticide products—even though the insecticide program may protect the crop
- situations where eliminating early tobacco budworm sprays allows survival of beneficial insects that reduce the risk of pest infestations

Table 2. Yield comparisons between *Bt* and non-*Bt* cotton

	Yield (lb lint/acre)	
Year	***Bt* cotton (unsprayed)**	**non-*Bt* cotton (sprayed)**
1994	1369	1392
1995	1465	1425

Source: Reprinted with permission from Jenkins et al. 1997.

associated with higher insecticide use (as with beet armyworms or aphids).

These situations suggest that income from reduced insecticide input, along with higher yields as a result of less insect damage, offset the technology fee and favor *Bt* cotton. However, if infestations of tobacco budworms or bollworms are low, or the yield of the *Bt* variety used is low, the technology fee may exceed the value of the *Bt* toxin. Also, a conventional insecticide spray program may allow the farmer to grow certain high-yielding cotton varieties that perform better than available *Bt* varieties. Some economic comparisons show little or no economic advantage to using *Bt* cotton (table 3), whereas the economic returns in other circumstances have been positive (table 4). Even in the areas that economically favor *Bt* cotton, however, there are often situations where high-yielding, non-*Bt* varieties grown under conventional spray programs provide equal or greater economic returns than *Bt* varieties.

Regardless of whether or not a particular variety contains the *Bt* gene, yield potential continues to be the primary consideration when selecting a cotton variety. The Cry1Ac gene in *Bt* cotton is only one of thousands of genes that affect the yield and other characteristics of different cotton varieties. Growers who are selecting cotton varieties should carefully consider the traits of each, including yield performance, relative maturity, fiber quality, ability to withstand adverse weather, and harvestability.

Bt cotton may offer value to the cotton farmer in ways that are hard to measure by short-term economic comparisons. Using insecticides involves complying with certain laws, such as worker protection and pesticide label restrictions. Compliance often makes the grower's job more difficult and increases the risk of consequences arising from noncompliance. Insecticide use in sensitive areas—next to schools, fish ponds, dwellings, medical facilities, roads—can be a concern to the grower and his or her neighbors. If legal and social risks are a concern, *Bt* cotton may have value to the grower by reducing these risks.

Adopting *Bt* cotton may result in a more efficient enterprise, while maintaining a high level of insect pest control. Insect management with insecticides can be time-consuming and involve a significant amount of labor and equipment. If *Bt* cotton reduces the insect control burden and decreases the need for labor and equipment, these resources may be diverted to other farm obligations.

Bt cotton may reduce potential resistance to foliar insecticides in tobacco budworms and bollworms. Since resistance genes selected as a result of one insecticide may be eliminated by a different and unrelated insecticide, the rotation of toxins, including *Bt* Cry-protein, may be able to slow the selection of genes for resistance to any single toxin. Farmers will be obliged to include a non-*Bt* refuge to accompany any *Bt* cotton planted (see refuge section, p. 23). Use of a sprayed refuge provides an excellent opportunity to use newer insecticide classes, as well as effective older chemistries, as aids in reducing development of insecticide resistance in tobacco budworms and bollworms and in adopting resistance management plans for other chemistries. Maintaining effective refuge areas that are close to *Bt* cotton also helps slow resistance to the current Cry1Ac toxin cotton varieties and may decrease resistance to other *Bt* toxins as they are introduced.

Table 3. Cost of *Bt* cotton vs. non-*Bt* cotton in North Carolina, 1999

Expense	*Bt* cotton		Non-*Bt* cotton	
Technology fee*	$19.14		$ 0.00	
Control costs	5.78	@ 0.76 applications/season	19.88	@ 2.65 applications/season
Damage†	0.00	@ 4.41% damage	8.50	@ 5.5% damage
Extra scouting	3.00		0.00	
Total	$27.92		$28.38	

* Projected average cost; varies by seed rate and row spacing.

† Difference in late-season bollworm damage under grower conditions (N=614 fields, 1996–1998).

Source: J. Bacheler, unpublished data.

Table 4. Cost of *Bt* cotton vs. non-*Bt* cotton in Mississippi, 1995–97

Expense	*Bt* cotton	Non-*Bt* cotton
Number of sprays*	6.7	11.7
Control costs	$61.48	$68.15
lb lint/acre	876	789
Economic advantage	$63.22	—

* Average of 1995–1997

Source: Reprinted with permission from Stewart et al. 1998.

Bt *Cotton and Boll Weevil Eradication: Can They Work Together?*

Boll weevil

Both *Bt* cotton and boll weevil eradication have great value for the cotton industry in the United States. Available *Bt* cotton varieties are highly effective against tobacco budworms and provide significant suppression of bollworms and certain other caterpillar species. Consequently, the foliar insecticide treatments required to control these pests in *Bt* varieties are substantially lower than in non-*Bt* varieties (table 5). Many of the treatments used to control tobacco budworms and bollworms also are active against other pests, such as boll weevils and tarnished plant bugs, and lower insecticide use in *Bt* cotton reduces coincidental control of such pests. As a result, *Bt* cotton varieties grown in boll-weevil-infested areas typically require more foliar insecticide treatments (table 5). Eradication of the boll weevil is, therefore, necessary to realize the maximum potential benefit from growing *Bt* cotton.

Eradication of the boll weevil reduces the number of foliar insecticide treatments necessary to control other pests in non-*Bt* cotton. For example, in Georgia, before the boll weevil was eradicated, the amount of insecticide required for control of other pests was notably higher than the amount required following eradication. Data from Georgia show an increase in the need for treatment for other pests during the early years of boll weevil eradication, because the insecticides used destroyed beneficial insects. In the absence of beneficial insects, populations of pests such as tobacco budworms, bollworms, beet armyworms, cotton aphids, and whiteflies often increase.

Bt cotton has proven itself to be a useful tool in minimizing the risk from certain caterpillar pest outbreaks in the early years of a boll weevil eradication program. In recent eradication programs in Alabama, Mississippi, and Louisiana, producers chose to plant more than 80 percent of their acreage to *Bt* varieties, primarily to minimize risks of tobacco budworm outbreaks. However, there has been a negative aspect to this high use of *Bt* cotton. When most acreage is planted to *Bt* varieties, growers apply fewer sprays that provide coincidental control of boll weevil. This makes boll weevil eradication somewhat more difficult and more costly. Greatly overshadowing this negative influence are the positive effects of *Bt* cotton in reducing the risks of secondary pest problems during the initial years of an eradication effort.

Where boll weevils are eradicated, the overall value of *Bt* cotton increases. In areas free of boll weevils, insecticide sprays can be cut back and beneficial organisms more successfully relied on to reduce pest insects. The example from Georgia shows a distinct decline in the need to treat for other pests once the boll weevil was eradicated (fig. 2). An additional reduction in foliar treatments was observed after *Bt* cotton became available in 1996. This example shows that boll weevil eradication and *Bt* cotton are complementary in reducing total insecticide use and lowering insect control costs. Reducing insecticide use and relying more heavily on biological control also benefits efforts to manage insecticide resistance.

Table 5. Average number of annual insecticide sprays for tobacco budworms, bollworms, and boll weevils in Mississippi, 1996–98

Insect	1996	1997	1998
Tobacco budworms and bollworms			
on *Bt* cotton	0.3	0.9	1.2
on non-*Bt* cotton	3.1	3.1	5.2
Boll weevils			
on *Bt* cotton	—	2.6	3.3
on non-*Bt* cotton	—	1.9	1.9

Source: Modified with permission from Layton et al. 1999.

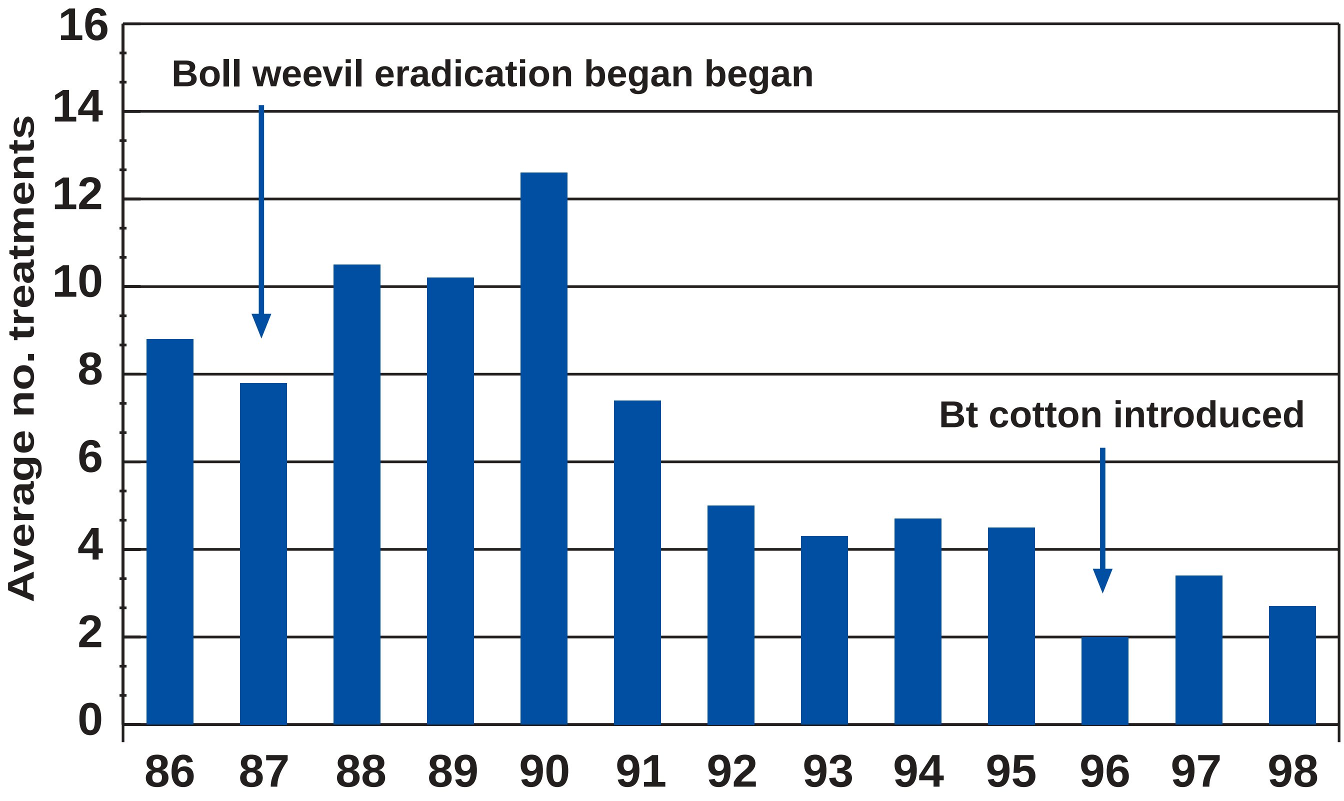


Figure 2. Non-boll weevil treatments in Georgia. Reprinted with permission from Layton et al. 1999.

Resistance to Bt *Cotton in Tobacco Budworms and Bollworms*

It is commonly known that more than 500 species of insects and mites have developed at least some degree of resistance to insecticides (Georghiou and Saito 1983), knowledge clearly showing that many arthropods have the genetic potential for rapid adaptation to chemicals in their environment. Most scientists agree that the tobacco budworm and the bollworm will eventually become resistant to the Cry1Ac protein used in current *Bt* cotton varieties. The tobacco budworm has a well-known reputation for developing resistance to chemical insecticides. Currently it is resistant to most conventional insecticides used on cotton. However, for the time being, it is extremely susceptible to the Cry1Ac protein in *Bt* cotton. The bollworm is inherently more tolerant to this toxin, and it is likely to develop resistance faster than the tobacco budworm.

Field and laboratory studies document the developed resistance of several insects to spray formulations of B.t. toxins. The best-known example is the diamondback moth, a caterpillar pest that attacks cabbage and related plants. It has shown high levels of resistance to *Bt* sprays in Florida, Hawaii, North Carolina, Asia, and other locations (Tabashnik et al. 1990). It has also shown resistance to *Bt* transgenic canola plants. Researchers have already developed laboratory colonies of Colorado potato beetles, European corn borers, tobacco budworms, and bollworms that are resistant to Cry-proteins (fig. 3). The resistant laboratory colonies of tobacco budworms and bollworms demonstrate these insects have the genetic potential to become resistant.

Crop protection with *Bt* cotton is a form of host plant resistance, like resistance of soybean varieties to the soybean cyst nematode. Farmers are familiar with resistant crops losing their protection from pests, like nematodes overcoming soybean resistance and mildew adapting to resistant wheat varieties. While the same fate is predicted for *Bt* cotton, the time necessary to reach economic resistance can be greatly influenced by the way growers and consultants utilize this crop.

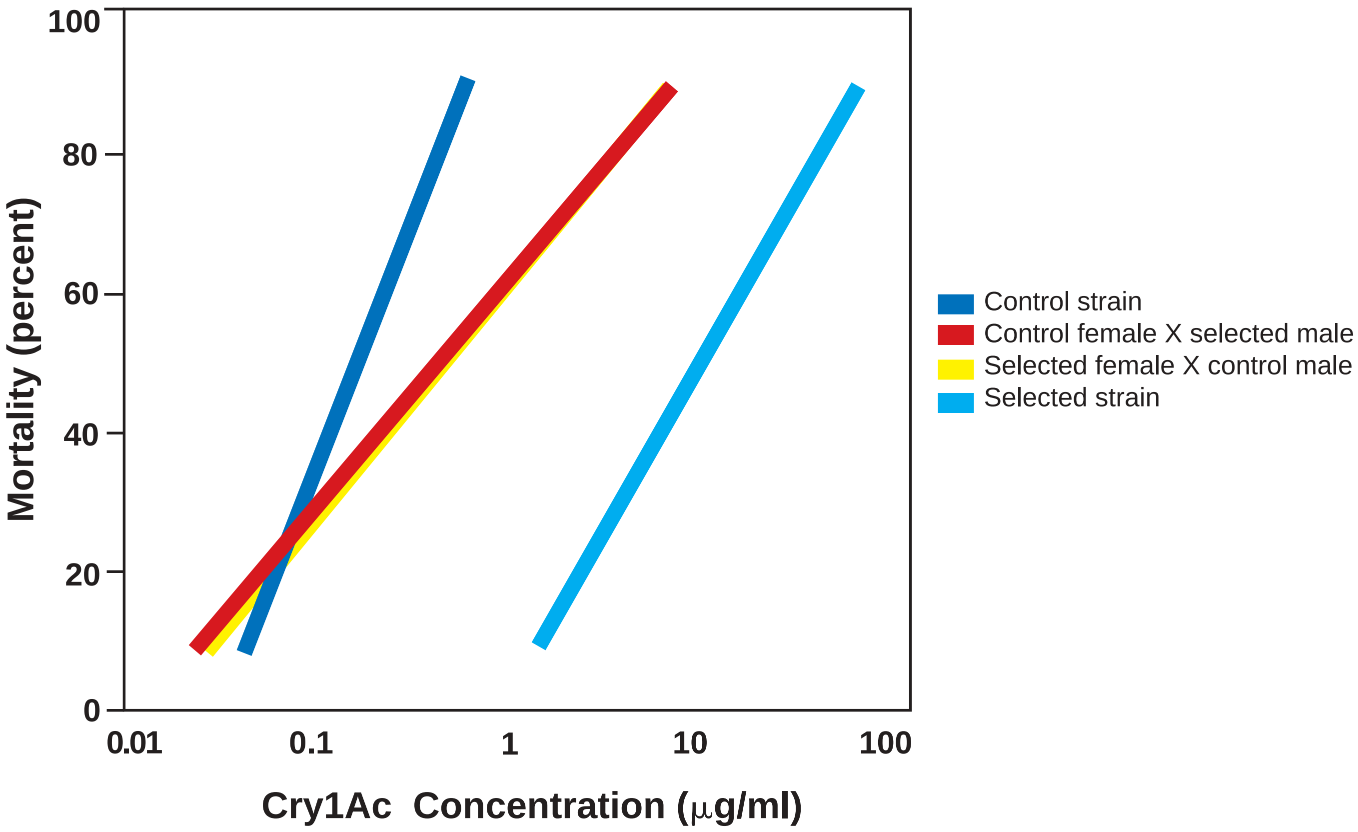


Figure 3. Development of resistance to *Bt* cotton in tobacco budworm in laboratory experiments. Reprinted with permission from Gould et al. 1992.

How Resistance Develops

A variety of factors may influence the rate at which tobacco budworms and bollworms become resistant to Cry-toxin in *Bt* cotton.

These factors include:

- The number of generations of tobacco budworms and bollworms exposed each year to *Bt* plants containing the same or similar toxins
- The percentage of each generation exposed to *Bt* plants containing the same or similar Cry-toxins
- The mortality level that Cry-toxin causes among tobacco budworms or bollworms carrying one copy of a resistance allele and one copy of a susceptible allele. The mortality level is determined by the Cry-toxin concentration in the plant, which in turn may determine the functional dominance of the allele affecting resistance
- The frequency with which Cry-resistance alleles are expressed in the tobacco budworm or bollworm population before exposure to Cry-toxins and the dominant or recessive nature of the resistance alleles
- The migration patterns of tobacco budworm and bollworm moths
- The survival advantage or disadvantage that resistance allele(s) offer tobacco budworms or bollworms both in the presence and absence of Cry-toxins
- The number of susceptible moths available for mating with moths carrying resistance gene(s).

Before exposure to Cry-toxins—by spraying insecticides containing *Bt* or through planting *Bt* crops—very few tobacco budworms and bollworms (perhaps 1 in 100,000 to 1 in 1 million) carry two copies of a resistance allele (RR), meaning they are fully resistant to *Bt* cotton. Some tobacco budworms or bollworms have a single copy of a resistance allele and a susceptible allele (RS); these are called heterozygotes. The overwhelming majority have two copies of a susceptible allele (SS).

Most of the susceptible insects (SS) are killed after feeding on *Bt* cotton, depending on the dose of Cry-toxin in the plant. The heterozygous insects (RS) usually are more difficult to kill than the susceptible insects. Still, heterozygous insects are not considered *Bt* resistant in most instances, because most will die if the toxin dose in the plant is high enough. Resistant insects (RR) are not killed by *Bt* toxin. The difference in survival rates among these three types represents a selective advantage for resistant (RR) tobacco budworms and bollworms feeding on *Bt* cotton, because they will survive while susceptible individuals will die.

As the use of *Bt* cotton increases, a higher percentage of tobacco budworm and bollworm populations will be exposed to Cry-toxins. Relatively more caterpillars carrying resistance alleles will survive to adulthood, while fewer susceptible caterpillars will survive. As a result, more resistant insects will pass on alleles for resistance to new generations.

Because Cry-toxins are expressed in transgenic plants for the entire growing season—compared to insecticides that remain active for short periods—*Bt* cotton further prompts tobacco budworms and bollworms to select for resistance. This exposure can greatly enhance selection for resistant alleles and subsequently accelerate the pace that these insects develop resistance, especially in areas where few alternate hosts are available (fig. 4).

Due to the high, season-long selection for *Bt*-resistant insects, scientists advise that development of field-level resistance could take a relatively short time. However, if *Bt* crops are not used over a high proportion of the total acreage, the rate that insects develop resistance should be slower and *Bt* crops should remain effective for many years (Gould et al. 1992). The rate that resistance develops increases proportionately as the acreage of *Bt* crops expands within a county, state, or region. The presence or absence of alternate non-*Bt* food plants in a particular area may also influence the development of resistance, which will probably occur first in a locale where the use of *Bt* cotton is high and the availability of non-*Bt* hosts, including cotton and other plants, is low.

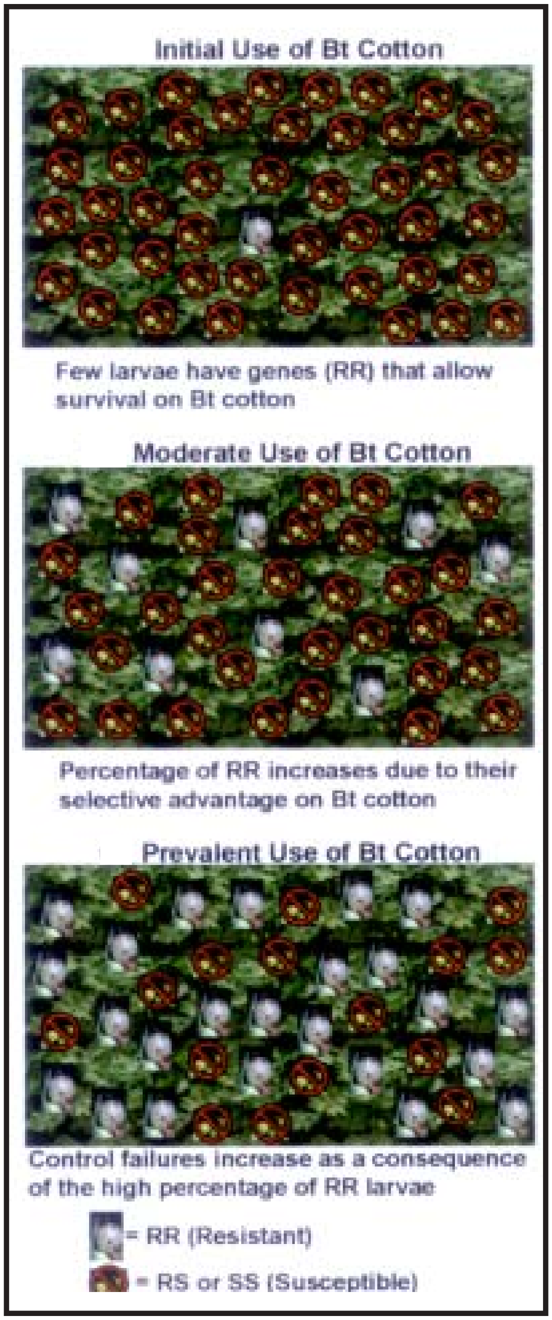


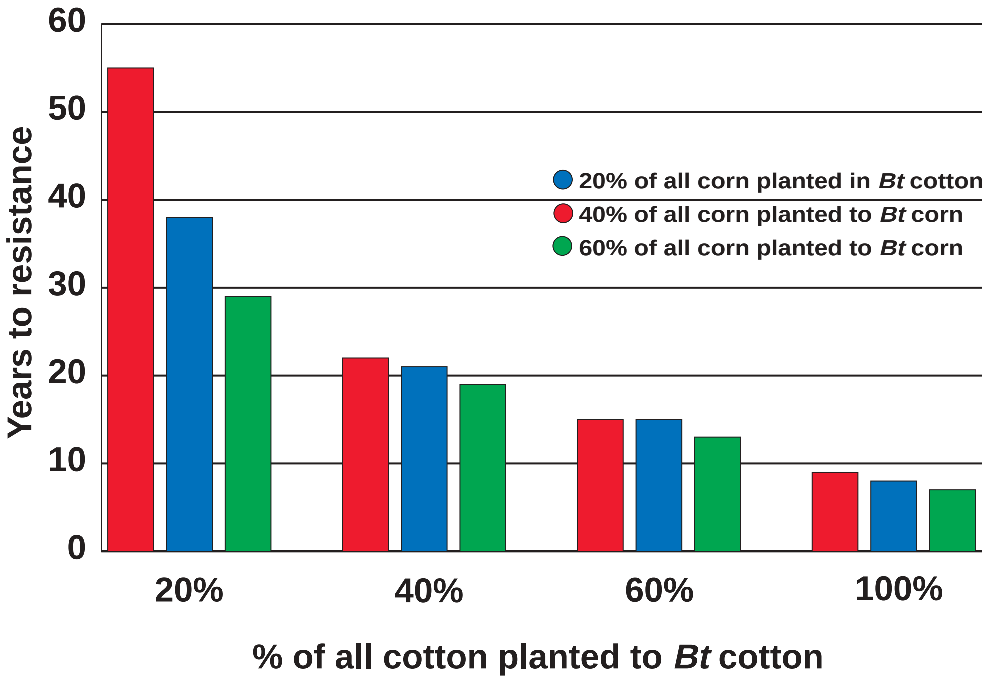
Figure 4. Increase of resistant larvae on *Bt* cotton. Reprinted with permission of D. Sumerford.

Bt *Corn: Does It Hasten Resistance to* Bt *Cotton?*

Several types of *Bt* corn use different *Bt* transformation events and express Cry1Ab, Cry1Ac, Cry1F (not commercially available), or Cry9c toxins. Some brands of Cry1Ab corn express *Bt* toxin in the ears, where bollworm larvae (called corn earworms on corn) may be feeding, but other brands do not have toxin in the ears. Cry9c toxin does not kill bollworms and should not affect development of *Bt* resistance.

Currently, only the Yieldgard corn hybrids express *Bt* protein in ears and may have an effect on *Bt* resistance in bollworms (fig. 5). Tobacco budworms should not be affected by corn since they very seldom infest this crop.

Growing corn in cotton production areas could have a major influence on bollworms' development of resistance to *Bt* cotton. Bollworm cater-



*** Spatially explicit model with 120 patches of corn, 160 patches of cotton, 40 patches of wild hosts, and 80 soybean patches. Five replicates and noncross resistance.**

Figure 5. Computer simulation showing the influence of *Bt* corn and *Bt* cotton on the rate that bollworms develop resistance to *Bt*. Reprinted with permission from ILSI Health and Environmental Sciences Institute 1998.

pillars infest the whorl and ear stages of corn. Moths are strongly attracted to silking corn to lay eggs, and a major portion of the second-generation population may develop in corn ears. Non-*Bt* corn will not select for *Bt*-resistant bollworms but will act as a refuge and delay resistance. The more non-*Bt* corn—other non-*Bt* hosts—grown in a corn and cotton production area, the slower *Bt* resistance develops.

However, growing ear-expressing *Bt* corn can hasten *Bt* resistance in two ways: (1) if non-*Bt* corn is replaced with ear-expressing *Bt* corn, the refuge effect that non-*Bt* corn provides diminishes and bollworm populations are exposed to *Bt* toxin—both from the increase in *Bt* crops and the decrease in non-*Bt* crops; and (2) as mentioned, greater exposure to the *Bt* toxin gives bollworms that are carrying a resistance allele(s) a survival advantage and hastens the pace to resistance. If robust non-*Bt* refuges are maintained for both cotton and corn, the rate that resistance develops is reduced and should allow a prolonged life for both *Bt* products.

Implications of Resistance

Growers know that production costs can increase as insects develop resistance to insecticides. How much of an increase depends on the availability and cost-effectiveness of alternative strategies. If such strategies do not exist or are expensive, production costs and crop losses may be very high. The resistance of tobacco budworms and bollworms to *Bt* cotton is accompanied by costs similar to those encountered with resistance to conventional insecticides.

There are also additional costs to resistance. Cry-toxins in *Bt* plants and Cry-toxin-based insecticides are not easily replaced when insects develop resistance. In the past, growers relied on the availability of new insecticides. There is no guarantee this process will continue. Developing new insecticides and transgenic insecticidal crops is time intensive, difficult, and expensive. Researchers may not be able to develop new insecticides at reasonable costs that conform to environmental and performance requirements as quickly as growers need them. A case in point is the high cost of recently released tobacco budworm insecticides for use on cotton.

The length of time that an insecticide or *Bt* cotton remains effective may depend upon how well growers and pest managers follow resistance management guidelines. Improper usage dramatically decreases the effective life of a product. If *Bt* products are carefully used, their effectiveness may be extended for many years. But if the technology is abused, budworms and bollworms will quickly become resistant. Preserving the effectiveness of *Bt* cotton is one way to keep pest management costs at the lowest level.

Other less obvious risks could also occur. In the past the appearance of resistant tobacco budworm or bollworm infestations increased the frequency of scouting and complicated decision-making by growers and pest managers. If new, selective insecticides are needed to combat Cry-toxin-resistant pests, secondary insects may become more numerous. Also, caterpillars exposed to one Cry-protein (for example, Cry1Ac) may develop resistance to other similar Cry-proteins, even though they have not been exposed to them; this process is known as cross-resistance. When growing *Bt* crops, growers should always use the refuge sizes specified in the licensing agreement in order to slow the development of resistance to the Cry-proteins contained in sprayable *Bt* insecticides and *Bt* crops.

Can Growers Slow Resistance?

A logical, science-based, and proactive resistance management strategy is necessary to prevent tobacco budworms or bollworms from developing resistance to *Bt* cotton in less than 10 years. All members of the cotton industry should practice this strategy to slow development of resistance. EPA has registered products from companies that sell *Bt* cotton seed, and these companies are required to recommend and support insect resistance management (IRM) strategies for *Bt* cotton. IRM is a key element of a good overall integrated pest management (IPM) program.

EPA has accepted a resistance management concept for *Bt* cotton known as the “high dose / refuge strategy.” This approach has two complementary principles: (1) *Bt* plants must produce a high dose of Cry-toxin throughout the season, and (2) effective IRM refuges must be maintained. An IRM refuge consists of a non-*Bt* host crop, and it is intended to produce susceptible tobacco budworms or bollworms or both.

In theory, if *Bt* plants express a high dose of toxin, then all susceptible (SS) pests eating the plant will die, almost all of the heterozygous (RS) insects will die, and resistant (RR) insects will survive. When resistant survivors from the *Bt* crop mate with susceptible insects from a non-*Bt* refuge, the offspring receive one allele—either an S or an R—from each parent. Offspring from the cross-mating will be heterozygotes (RS). If *Bt* plants express a high dose of toxin, almost all heterozygotes will be killed. Eliminating most heterozygotes also eliminates most resistant alleles from the surviving populations and greatly slows the development of resistance.

Without a source for producing susceptible insects—an IRM refuge—the development of resistance is proportional to the dose; that is, the higher the dose, the more rapidly resistance develops. Therefore, the high dose / refuge strategy is a high-risk strategy, depending upon the availability of properly functioning IRM refuges. The high dose, or in other words high effectiveness, is very good for pest control, but it can cause the rapid development of resistance in the absence of effective IRM refuges.

As mentioned, the IRM refuge is acreage planted with a non-*Bt* crop that serves as a host for tobacco budworms or bollworms or both. And the refuge must be close enough to the *Bt* crop to ensure that susceptible moths have an opportunity to mate with resistant ones. This means that moths from the *Bt* cotton and the refuge must emerge at about the same time and be relatively close to each other.

Commercial *Bt* cotton varieties (Bollgard) currently express enough Cry1Ac toxin throughout most of the season to kill all susceptible (SS) and almost all heterozygous (RS) tobacco budworms. Only resistant (RR) tobacco budworms are expected to easily survive. Thus, commercially available *Bt* cotton varieties (Bollgard) should qualify for the “high dose” definition for tobacco budworms.

As mentioned, research shows that bollworms are less sensitive to Cry1Ac toxin than tobacco budworms. Researchers estimate that from 5 to 25 percent of susceptible (SS) bollworm larvae survive on *Bt* cotton varieties now in use (Bollgard), and estimates for heterozygote (RS) survival are significantly higher. So the *Bt* cotton grown now cannot be considered “high dose” against bollworms.

Future IRM Refuge Options

Refuge regulations originally mandated in 1995 for Bollgard varieties will remain in effect through the 2000 growing season. When the registration for Bollgard cotton expires after the 2000 growing season, new refuge requirements may be forthcoming. At the time of publication, no final refuge requirements had been determined for the 2001 growing season and beyond. The issue will be debated before the final decision is made; recommendations will range from complete removal of *Bt* technology from the marketplace, to a minimum of a 50-percent non-*Bt* cotton refuge, to no change from the current 4-percent-unsprayed or 20-percent-sprayed refuge scenarios.

Computer simulation models, along with limited evidence from field and laboratory studies, suggest that the 4-percent-unsprayed refuge or the 20-percent-sprayed refuge required on the original *Bt* cotton label (Bollgard cotton) may not adequately delay resistance in bollworms and tobacco budworms. Studies using computer models (Gould et al. 1992, ILSI 1998) also suggest that bollworm resistance to *Bt* cotton can occur quickly if *Bt* cotton is extensively planted and only small IRM refuges are used. Research indicates that bollworms, and to a lesser extent budworms, have the genetic ability to adapt quickly to *Bt* toxin (Gould et al. 1992, Sumerford et al. 2000, Burd et al. 2000).

In 1999, EPA and the U.S. Department of Agriculture, proposed for discussion the following two structured refuge options to mitigate the resistance of tobacco budworms and bollworms to *Bt* toxins expressed in Bollgard *Bt* cotton:

1 An external refuge of at least 30% non-*Bt* cotton should be implemented. The placement of the structured refuge should be planted within 0.5 miles of the farthest *Bt* cotton in a field to provide *Bt*-susceptible moths. The external refuges of non-*Bt* cotton can be treated with any other registered non-*Bt* insecticide or other insect control measures.

2 In-field refuges of at least 10% non-*Bt* cotton refuge should be implemented. In-field refuges should be planted entirely within the field as blocks, minimum size to be determined based on planter size, to provide *Bt*-susceptible moths. Cotton fields may be treated with any registered non-*Bt* insecticide or other control measures, as long as the entire field is treated in the same manner. This means that *Bt* cotton rows cannot be treated independently from non-*Bt* cotton rows with insecticides or other insect control measures.

In both options, (1) and (2), agronomic practices used for farming the non-*Bt* cotton must ensure adequate production of susceptible [tobacco budworm and cotton bollworm] adults to mate with resistant adults emerging from *Bt* cotton. In particular, termination of growth of non-*Bt* cotton should not be done until termination of growth of *Bt* cotton has begun. In general, agronomic practices for non-*Bt* cotton should be similar, as practical, to those of the *Bt* cotton grown in the same management unit, especially regarding crop nutrition, irrigation, and termination (U.S. Environmental Protection Agency and U.S. Department of Agriculture 1999).

EPA and USDA co-authored this position paper to provide stakeholders with a focal point for discussing future recommendations. The entire document may be reviewed at http://www.epa.gov/oppbppdl/biopesticides/otherdocs/bt_position_paper_618.html. Updated versions of refuge guidelines will be available at this website.

Entomologists in the public sector are concerned with cotton insects' almost 50-year history of developing resistance to sprayable insecticides from almost every chemical class. Sound biological reasons indicate that *Bt* insecticide within plants will be even more vulnerable to the development of insect resistance. Given the reduced pace of developing new replacement insect-control technology, the U.S. cotton industry may face a greater risk of insecticide resistance than ever before.

The refuge plans currently in use are designed for Bollgard cotton varieties that perform like the varieties first marketed (for example, NuCotn 33 and NuCotn 35). These refuges may be inappropriate for new *Bt* genes or stacked gene *Bt* products that may be marketed in the near future. One hopes new *Bt* gene products will deliver a high dose of toxin to the bollworm and secondary lepidopterans and qualify for the high-dose refuge strategy. If these products do qualify, scientific theory should support less restrictive IRM plans for such future products. The development of highly effective transgenic insecticidal cotton plants with multiple toxic genes should be encouraged.

The Economics of Cotton Refuges

The expenses and yield reduction associated with refuges must be viewed as costs of using *Bt* cotton technology. Balancing these are the economic and other benefits realized from *Bt* cotton. In non-*Bt* cotton when insecticides are used for insect management, the chemical costs, application costs, and risks of handling pesticides and possible complaints about them, among other costs, are balanced against the benefit of insect control and higher yields. If, for example, a 90 / 10 in-field refuge plan is used, the 10-percent refuge may be damaged by insects, but the yield and insect control cost over the total acreage may be more profitable than that achieved using other insect management plans.

Preserving *Bt* cotton technology from resistance helps ensure that growers will have effective insect management options in the future. Experience shows that greater crop loss and higher cost insect management typically follow the development of resistance. So, clearly, an economic benefit is often difficult to estimate. There is no guarantee that new and cost-effective biotechnology or insecticidal products will replace existing technology in a timely fashion.

Can the Development of Resistance Be Successfully Monitored?

Monitoring for the development of insect resistance to Cry-proteins is a difficult and imprecise task. It may include surveying the annual use of *Bt* cotton in each county or parish, annual testing of tobacco budworm and bollworm populations for *Bt* sensitivity, and checking *Bt* crops for any changes in the survival rate of tobacco budworms and bollworms. Monitoring tobacco budworm and bollworm populations on *Bt* cotton is important in providing the earliest warning that they are developing resistance; it is also required by EPA. And it improves resistance management efforts.

Companies that register the proteins in *Bt* cotton with EPA are required to keep annual sales records on a county-by-county basis and submit summaries for each state. Surveys of grower use of *Bt* cotton, conducted by Cooperative Extension Service personnel, also may be available and could be used as a guide for monitoring areas where circumstances favor the development of *Bt* resistance by tobacco budworms and bollworms. Researchers predict that resistance is more likely in areas where the use of *Bt* cotton has been high for several years. Other characteristics of a particular region—such as the amount of non-*Bt* cotton acreage and alternative hosts of tobacco budworms or bollworms—may be surveyed to help determine the risk of resistance. Assigning resistance risk categories to unique cotton-growing environments across the Cotton Belt can be helpful in monitoring the efforts at specific sites.

A major difficulty in monitoring is that resistance can develop to an advanced stage before it is easily detected in the field. For example, if 1 of every 100,000 tobacco budworms was resistant when *Bt* cotton was first marketed, resistance levels would advance many-fold—perhaps to 1 per 100 individuals—before field failures could be detected.

Detection of resistance in the field by scouts and growers would likely occur only in outbreak years, unless the resistance level was more than 1 insect per 100. The development of resistance is largely undetectable by measuring field performance of *Bt* cotton. This invisible phase is due, in part, to monitoring techniques that are not sensitive enough to detect early shifts in tobacco budworm and bollworm resistance.

A first step in establishing an effective monitoring program is to document the initial susceptibility level of tobacco budworms and bollworms to the *Bt* toxin. With this information as a baseline, it may be possible to spot small, early changes in susceptibility before field control failure occurs. Laboratory studies are uncovering more information about the frequency with which resistant genes occur and the dominance of these genes. Detecting changes in susceptibility to the Cry-toxins requires precise techniques that provide information from a large number of insects and many locations. The U.S. Department of Agriculture and state universities are cooperating to establish Cry-toxin susceptibility baselines and measure resistance in tobacco budworm and bollworm larvae collected from all across the Cotton Belt, especially from the mid-South and the Southeast (Summerford et al. 2000). Technology companies are also conducting similar tests. If these monitoring studies are sufficiently widespread, changes in the susceptibility of tobacco budworms and bollworms to *Bt* may be successfully detected prior to field control failures. EPA is reevaluating annual monitoring requirements for development of remedial action plans.

Counting the number of surviving caterpillars in fields of *Bt* cotton may be helpful for detecting the development of resistance. Susceptible populations of tobacco budworms are decimated in *Bt* cotton, so scouting for their survival may uncover resistance before economic field failures occur. This method is not highly sensitive, however, and insect resistance to Cry1Ac toxin must reach a relatively high level—for example, one resistant insect in 500 susceptible insects—before detection is possible.

The tolerance of bollworms to Cry1Ac toxin is already too high to allow close measurement of changes in resistance using scouting and larval survival. What's more, less than adequate *Bt* expression in the cotton plants may produce a false positive for resistance. Field scouting will detect only large shifts in the survival rate of bollworms.

Fitting Bt *Cotton Into an Insect Management Program*

As with all conventional insecticides, *Bt* cotton must be managed wisely and used in combination with other insect pest and crop management practices. For more than 20 years, IPM has focused on using insecticides on an as-needed basis only. The decision to use insecticides has generally been based on crop scouting to determine insect pest densities and the use of spray thresholds. Of course, *Bt* crops cannot be used as-needed because the *Bt* toxin is in the plant. For this reason, tobacco budworms and bollworms have greater opportunities for exposure to the toxin, and development of resistance is more likely with *Bt* plants.

Consequently, resistance management must be part of the total pest management package. On the positive side, *Bt* plants, in the absence of insecticide sprays, will help preserve beneficial insects, allowing growers to take advantage of them as a free resource, especially in areas where the boll weevil has been eradicated.

When developing crop production plans, growers should consider all insect management strategies and tactics, with emphasis on the following areas:

Cultural practices. Early crop maturity (which may be optimized with computerized management programs such as COTMAN) should be part of every pest management plan. Most growers realize that early fruit set and early fruit maturity help reduce insect pest infestations. Combined use of optimal planting times and early-maturing cotton varieties reduce crop damage, minimize insecticide use, and diminish the chances of pests' becoming resistant to *Bt* toxin and insecticides. Early fruit set should be protected so maturity is not delayed.

Rotating *Bt* crops with other non-*Bt* crops (temporal refuge) that also are hosts of tobacco budworms and bollworms is an example of resistance management and offers overall pest management benefits. Soybeans, peanuts, and tobacco can harbor tobacco budworms, and these same crops, plus corn and grain sorghum, are all hosts of bollworms. Yieldgard *Bt* corn would not be considered an adequate alternate host, but other available *Bt* corn brands and non-*Bt* corn could serve as alternate hosts. Certain forage crops, such as crimson clover and alfalfa, and many weeds may also serve as hosts to tobacco budworms and bollworms. These alternate hosts can provide an important refuge benefit and help slow the development of resistance. In fact, according to computer models, the rate that bollworms develop *Bt* resistance is directly related to the proportion of non-*Bt* corn (not Yieldgard) in a cotton and corn cropping system.

Beneficial insects. Conventional insecticides often have a significant adverse effect on beneficial insects in cotton. Populations of cotton aphids, beet armyworms, tobacco budworms, bollworms, fall armyworms, and other pests frequently increase following insecticide use due to the destruction of beneficial arthropods. *Bt* toxins do not have this effect. The loss of beneficial insects can also be reduced by using fewer applications of insecticide or by using an insecticide that is less harmful.

Scouting and thresholds. *Bt* cotton is not effective on pests such as boll weevils, plant bugs, and stink bugs. When bollworms, beet armyworms, fall armyworms, and looper moths lay large numbers of eggs, damaging infestations may occur because the level of *Bt* toxin produced is not adequate to fully control them. As a result, *Bt* cotton must be scouted in a fashion similar to that used with conventional cotton but using altered techniques and modified treatment thresholds. Entomologists in each cotton-producing state publish variations for *Bt* cotton scouting and treatment thresholds.

Bt cotton kills almost all newly hatched tobacco budworm larvae and most bollworm larvae, so using eggs as a criterion for insecticide applications may lead to unneeded foliar treatments. When large numbers of bollworm eggs are laid, survival of the larvae may lead to damaging infestations. As a consequence, in a few states where the tobacco budworm and bollworm populations consist mostly of bollworms, extension entomologists have adopted a high egg threshold for *Bt* cotton (table 6). In other states (Arkansas, Mississippi, and Texas, for example) egg thresholds are not recommended as a treatment criterion. Use of the egg threshold is not possible in states where the percentage of tobacco

The beneficial wasp, *Microplitis croceipes*, feeding on bollworm/budworm larva. Courtesy of J. Powell.

budworms is high, since separating tobacco budworms from bollworms without a microscope is difficult and time-consuming in every stage but the adult.

A potential solution to the problem of identifying eggs and small larvae is the development of kits containing species-specific monoclonal antibodies. These kits would allow growers and consultants to make better pest control decisions if surviving larvae longer than 1/8 to 1/4 inch are found in *Bt* cotton. This technology is still being developed.

Scouting *Bt* cotton for tobacco budworms and bollworms must employ somewhat different techniques than scouting non-*Bt* cotton. Newly hatched larvae do not die immediately after feeding on *Bt* cotton. In fact, most states recommend counting caterpillars only of a certain size, such as 1/8 to 1/4 inch long. Caterpillars that reach a minimum size have a greater ability to survive on *Bt* cotton. As the caterpillars become larger, they become more difficult to kill with the *Bt* toxin and can damage the crop unless eliminated quickly. The larvae that survive are most likely to be bollworms. Caterpillars, usually bollworms, often are found in flowers or "bloom tags," so greater emphasis is being placed on examining the blooms and small bolls when scouting.

Tobacco budworm and bollworm damage in *Bt* cotton can also be a target for scouting. Caterpillars that grow beyond a minimum size and feed on terminals, squares, or bolls cause recognizable

Table 6. Bollworm and tobacco budworm thresholds on cotton in South Carolina

	Spray threshold		
Stage	**% Eggs**	**% Square damage**	**% Larvae**
Before bloom			
non-*Bt* cotton	—	20	15
Bt cotton	—	—	—
After bloom			
non-*Bt* cotton	20	3	5
Bt cotton	75	5 (bolls)	30

Source: Roof 1999

Cotton boll damage from budworm/bollworm larvae

damage. The superficial damage of very small caterpillars should not be mistaken for penetrating injury that may indicate a damaging insect population. In some situations, fall armyworms or beet armyworms can cause this damage, so scouts should be careful to properly identify the pest species. Some states have developed damage thresholds for *Bt* cotton.

Insecticide use in *Bt* cotton. In *Bt* cotton, all insect pests not affected by the *Bt* toxin should be managed as they would be in non-*Bt* cotton. It may be necessary to apply insecticide to control thrips, aphids, boll weevils, tarnished plant bugs, stink bugs, and a few tolerant caterpillars such as beet armyworms and fall armyworms. The decision to apply insecticides to *Bt* and non-*Bt* cotton should be based on scouting and treatment thresholds.

Insecticide use depends on several variables, including the abundance of the pest insect and the presence of beneficial insects. Using insecticides against early-season pests, such as boll weevils and tarnished plant bugs, also reduces the number of beneficial insects. As mentioned previously, this reduction may result in higher populations of insect pests and fewer opportunities for reduced insecticide use in *Bt* cotton. In areas where boll weevils have been eradicated and tarnished plant bugs are infrequent, the presence of *Bt* toxin and beneficial insects greatly reduces the amount of insecticide needed on *Bt* cotton.

Bt *Cotton in the Future*

Transgenic crops are one of the most revolutionary developments in agricultural production. As with most new technology, there are exciting possibilities for the economic value of *Bt* cotton and apprehensions about its wise use. In order to preserve *Bt* cotton well into the 21st century, producers, seed companies, scientists, and regulators need to foster strong collaboration to ensure longevity of the technology.

Companies are striving to develop new genes for insertion into cotton plant DNA to provide other possibilities for improving agronomic traits and pest control characteristics. Genes for new insecticidal toxins will be important for managing a wider spectrum of insects and for slowing the pace of resistance. If future varieties express a high dose of toxin and the toxins do not have the same physiological target site, the rate that insects develop resistance could be greatly reduced.

Key Steps—Implementing a Resistance Management Plan

The following summary, based on the principles outlined in this publication, assumes that a voluntary, proactive approach by growers will provide product stewardship for long-term yield benefits and profitability.

1 Use *Bt* cotton in fields where the risk of severe tobacco budworm and bollworm infestations warrants the price premium for seed.

2 Plant *Bt* cotton and non-*Bt* refuge in the required proportions and patterns.

3 Record where *Bt* and non-*Bt* cotton are planted so *Bt* cotton performance can be monitored and non-*Bt* cotton can be scouted and treated as needed.

4 Use all cultural alternatives available for avoiding high late-season tobacco budworm and bollworm populations. These alternatives include early planting, short-season varieties, and protection of early fruit.

5 Continue using an IPM approach for all pests. When designing scouting priorities, keep in mind that other insects—such as beet armyworms, cabbage loopers, cotton square borers, European corn borers, fall armyworms, southern armyworms, soybean loopers, and yellowstriped armyworms—are suppressed at various levels by *Bt* cotton. Still other pests are not affected by *Bt* cotton, including boll weevils, cotton aphids, cotton fleahoppers, cutworms, spider mites, stink bugs, tarnished plant bugs, thrips, and whiteflies.

6 Monitor *Bt* cotton to verify tobacco budworm and bollworm control throughout the season. Since bollworms are not as readily controlled by *Bt* cotton, carefully watch for a heavy egg-lay and scout for surviving larvae lower in the cotton plants, especially in bloom tags. If feeding damage occurs in *Bt* cotton, investigate the cause immediately. If needed, get help in identifying feeding caterpillars. If tobacco budworm or bollworm larvae or excessive damage are discovered, resistance or tolerance to *Bt* cotton is a possibility, and the situation should be investigated.

Verify from field records that *Bt* cotton was planted where excessive damage or larvae are observed. Consult your grower's guide for the seed company's procedure for investigating suspected cases of resistance or tolerance. Immediately notify seed company representatives or extension agents or both if evidence indicates a performance problem.

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Glossary

Allele. An alternative form of a gene. For example, a gene determining the reaction of an insect to *Bt* toxin may occur in the form of an allele for resistance or an allele for susceptibility.

Alternate host. Non-*Bt* plant types other than cotton that support successful reproduction of tobacco budworms or bollworms or both; soybean and corn are examples.

***Bacillus thuringiensis* (*Bt*)**. A naturally occurring soil bacterium that occurs worldwide and produces a toxin specific to certain insects (for example, moths, beetles, blackflies, or mosquitoes).

Beneficial arthropods. Usually refers to insect predators or parasites of pest insects in crops. When abundant, these insects can assist in decreasing certain pests, although they are often seriously reduced by insecticide.

Biotechnology. The science and art of genetically modifying an organism’s DNA, such that the transformed individual can express new traits that enhance the quality of a product (for example, seed oils or fiber quality) or can express resistance to pests.

Boll weevil eradication program. A joint USDA, producer, and state program designed to eliminate the boll weevil as an economic pest from the Cotton Belt.

***Bt* cotton**. Commercial varieties of cotton that contain a gene from *Bacillus thuringiensis* within its DNA. This gene allows the plant to produce Cry-protein within most or all plant tissues. The toxin makes the plant tissue—terminals, squares, or bolls—toxic to some important caterpillar pests.

***Bt* insecticides**. Formulations of *Bt* Cry-proteins manufactured and sold for spraying a wide variety of plants in order to control certain caterpillar or beetle pests. Some formulations are also used for mosquito and fly control.

Cross resistance. Resistance to one or more toxins from exposure to one or more other toxins, such as bollworms becoming resistant to Cry1Ac in cotton by being exposed to CryIAb in corn, or vice versa.

Cry-proteins. Any of several crystalline proteins found in *Bt* spores that are activated by enzymes in the insect’s midgut. These proteins attack the cells lining the gut, cause gut paralysis, and subsequently kill the insect.

Cry1Ac protein (toxin). One of many *Bt* crystalline protein toxins. The Cry-protein used in the first varieties of *Bt* cotton sold commercially to growers.

DNA. Deoxyribonucleic acid, a double-stranded molecule, consisting of paired nucleotide units grouped into genes and associated regulatory sequences. These genes serve as blueprints for protein construction from amino acid building blocks.

Dominance (of an allele). The ability of one allele to determine a characteristic in a heterozygous individual. For example, an allele for resistance to *Bt* toxin may be dominant. As a consequence, an insect heterozygous for resistance, that is, containing an allele for resistance and an allele for susceptibility, is resistant to *Bt* toxin.

Dose of toxin. The amount of toxin eaten per insect. For example, the dose of toxin that a caterpillar receives by eating part of a *Bt* plant can be stated as micrograms of *Bt* toxin eaten per caterpillar or per milligram of the caterpillar's total body weight.

Expression. Production of the desired trait (protein concentration, for example) in a transgenic plant. Expression varies with the gene, its promoter, and its insertion point in the host DNA.

Gene. The basic unit of inheritance; a section of DNA that codes for a specific product (for example, protein) or trait.

Genetic marker. See Marker.

Heterozygote. A diploid organism carrying two different alleles (for example, susceptible and resistant) of a gene.

High dose. A dose of toxin high enough to kill all susceptible target pests and nearly all heterozygotes. Such a dose can be delivered by plants with sufficiently high concentrations of *Bt* toxin.

High-dose refuge strategy. A resistance management approach that uses plants to minimize the rapid selection for resistance to transgenic plants. This strategy relies upon plants that produce Cry-proteins at a concentration sufficient to kill all but the most resistant insects and is used in combination with a non-*Bt* refuge that allows susceptible insects to survive and mate with resistant individuals.

Host plant resistance. Ability of a plant to avoid insect damage, to kill attacking insects, or to tolerate their damage.

Insect resistance management (IRM). A proactive approach to offset and slow insects' resistance to *Bt* crops or insecticides by reducing selection or by counteracting the effects of selection for resistance genes.

Integrated pest management (IPM). A management approach that integrates multiple, complementary control tactics to manage pests in a profitable, environmentally sound manner. Examples of the control tactics include conservation of natural enemies, crop rotation, host plant resistance, and insecticides.

IPM. See Integrated pest management.

IRM. See Insect resistance management.

Larva. Immature stage of certain insect species; examples include caterpillars and grubs.

Lepidoptera. The order of insects that includes moths and butterflies. The plant-eating immature stages are often called worms, caterpillars, or larvae.

Marker. A genetic flag or trait used to verify successful gene transformation and to indirectly measure expression of the inserted genes.

Mode of action. Mechanism by which a toxin kills an insect. For example, the mode of action of *Bt* is ingestion and disruption of cells lining the midgut.

Promoter. A DNA sequence that regulates where, when, and to what degree an associated gene is expressed.

Refuge or insect resistance management (IRM) refuge. A wild host area or an area planted with nontransgenic plants (for example, non-*Bt* cotton or alternative hosts for tobacco budworms or bollworms) where susceptible pests can survive and produce a local population capable of mating with resistant survivors from *Bt* cotton. This mating, by decreasing the likelihood that *Bt*-resistant insects will mate with one another, dilutes resistance in the insect population.

Registration. Legal approval of pesticides and transgenic crops for use in the United States by the U.S. Environmental Protection Agency. Registration is granted after extensive review of toxicology (to mammals, birds, fish, and other nontarget organisms), environmental fate, health and safety issues, and precautions.

Resistance (by pests). The evolved capacity of an organism to survive in response to selection from exposure to a pesticide. The evolution of resistance occurs through a process of genetic accumulation, whereby a population becomes less sensitive to the pesticide following repeated exposure.

Selection. A natural or artificial process that results in survival and better reproductive success of some individuals over others. Selection results in genetic shifts if survivors are more, or less, likely to have particular inherited traits.

Stacked. Describes transgenic plants with more than one introduced gene in a single crop plant variety, such as a “stacked” cotton variety containing a *Bt* gene and a gene for herbicide tolerance.

Transgenic. An organism genetically altered by addition of foreign genetic material (DNA) from another organism into its own DNA.