
12 Resistance Risks of Bt Cotton and their Management in Brazil

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Introduction

This chapter addresses the risk that insect pests associated with *Bacillus thuringiensis* (Bt) cotton may evolve resistance to Bt proteins in Brazil. Insecticide resistance is a common response among insects to the selection pressure imposed by insecticides. The framework and concepts developed here are designed to be applicable to diploid arthropods evolving resistance to insecticidal transgenes expressed in transgenic plants. With some modification, they should also be relevant to assessing and managing resistance risk in haplodiploid and parthenogenetic arthropods, nematodes, virus, fungi or bacteria to nematicidal, viral, fungicidal or bactericidal transgenes, as well as to herbicide resistance. This framework follows that in Fitt *et al.* (2004), which was developed for the case of Bt maize in Kenya. In this chapter, we more formally integrate the framework into the risk-analysis process, considering briefly how the analysis should be staged to correspond with the development of the transgenic crop (see Andow *et al.*, Chapter 1, this volume).

We have established a series of informational needs that are essential to completing an assessment of risk and the development of a practical risk-management plan. In Fitt *et al.* (2004), we established a series of questions that should be addressed in this risk analysis. Here we integrate these questions under the informational needs to indicate clearly how each contributes to the risk analysis. This chapter focuses primarily on risk analysis prior to any field release of the transgenic crop plant. It concentrates on a comprehensive assessment of the pest/plant system and ecological attributes of the pests that help to define the risk of resistance and indicate possible resistance-management approaches. Additional research during field testing should be used to

address key assumptions and develop an effective, workable and acceptable resistance-management plan and to establish details of the monitoring and response system.

It should be assumed that resistance is a real risk. Experience with insecticide use and basic consideration of evolutionary theory implies that if the Bt crop is used extensively without any resistance-management intervention, resistance should be considered inevitable. The real issue is how to delay its onset. For any given crop there are usually multiple pest species that require control, and any given pest control tactic usually affects multiple pest species. In cotton there are many insect pest species, so it is important to assess which species are at risk of resistance and which is most at risk. In this chapter, we use risk assessment to identify the species most at risk of resistance and then devise risk-management practices that could delay the onset of resistance in this species and all of the others.

To assess the relative resistance risk of a Bt crop, the following issues should be addressed prior to field release:

- Identification of the pest species that are at risk of evolving resistance to the transgene (species, geographic distribution, history of resistance).
- Determination of potential exposure of each species to selection.
- Determination of the likely 'dose' of the transgene toxin to which each species is likely to be exposed.

Dose is a simple measure of 'hazard' projecting the likely effect of exposure in relation to the time to resistance failures. Risk assessment then involves combining information on dose with an estimate of potential exposure. With this information, we can assess the relative resistance risk of the various pest species and identify the species that is most likely to evolve resistance before the others – which might be called the weak link.

Resistance management first focuses around the biological attributes of this weak-link species. We then assess whether the resistance-management strategy constructed around the weak link would also delay the evolution of resistance in other species at risk. While doing this, it is essential that the resistance-management plan be practicable, that is, growers can actually implement it. The resistance-management plan builds on the information from the previous risk assessment, using the following three steps:

- Determination of the likely requirements for resistance management, including refuges.
- Development of the components of a potentially workable resistance-management plan.
- Specification of monitoring needs and development of potential contingency responses.

We then discuss some issues to be considered after field release, but before commercial release. Field and laboratory experiments will be needed to develop an effective, feasible, acceptable resistance-management plan and monitoring and response system. The information presented in this chapter reflects the complexity and dynamic nature of Brazilian cropping systems where

numerous uncertainties exist about the potential deployment of Bt crops and there is limited quantitative understanding of pest ecology. Despite these uncertainties, it is possible to assess resistance risk to various Bt transgenic cottons and to develop a reasonable management plan to delay resistance evolution.

Resistance Risk Assessment

Operational definition of resistance

Resistance is caused by genes that reduce susceptibility to a toxin, and is a trait of an individual. However, it will often happen that resistance is not yet known in a target species at the prerelease stage of development of the transgenic crop. Thus, it is important to define *resistance* operationally, so that resistance can be looked for in advance. This definition will, by necessity, be modified as information becomes available about the expression and inheritance of resistance. This definition is discussed in the section on 'potential exposure of target pests to *Bacillus thuringiensis* (Bt) cotton' below.

In addition, resistance occurs in a field population when there are enough resistant individuals to cause economic damage to the target crop. Hence, it is also necessary that we have an operational definition of *control failure from resistance*; this will be a characteristic of a population and should be easily and unambiguously implemented. An operational definition of control failure from resistance is necessary so that we know what we want to avoid during resistance management and know when we should admit failure and move on. Operationally, a control failure from resistance occurs when the pest causes significant economic damage to the crop. There are several alternative ways to implement this concept. For example, a control failure could be defined as occurring when the pest causes detectable economic damage to the crop, when the pest causes economic damage that is similar to that caused by susceptible insects on a non-resistant crop variety or when the economic damage is considered unacceptable to the grower.

Identification of pest species at risk

Identification of key pest species that could evolve resistance to Bt cotton involves identifying the key target pests first in each of the major geographic regions and then evaluating the resistance history of each species. In some cases, identification of the key target pest species can be difficult because the transgenic crop has not been tested against all relevant species. For Bt cotton in Brazil, there is considerable information about the target species but almost no information about the ability of Bt cotton to control them. The history of resistance is also important for determining resistance risk. This can be clearly illustrated by two Australian pest species, *Helicoverpa armigera* and *Helicoverpa punctigera*, which are significant pests of cotton and often con-

trolled by a range of insecticides. *H. armigera* has historically evolved resistance to all major classes of insecticides deployed against it (Forrester *et al.*, 1993), while *H. punctigera* has not developed field resistance to any insecticide despite being exposed to the same selection in cotton as *H. armigera*. This difference reflects the differing host range and mobility of the two species (Fitt, 1989; Fitt and Daly, 1990; Gregg *et al.*, 1995), and results in a substantial proportion of the *H. punctigera* population avoiding selection in unsprayed crops and non-crop plants. When Bt cotton was introduced into Australia, this history of insecticide resistance clearly identified *H. armigera* as a resistance risk and hence the target for a pre-emptive resistance-management strategy (Fitt, 1997).

We have identified four key target pests for deployment of Bt cotton in Brazil. These are cotton budworm, *Heliothis virescens* (Fabricius); pink bollworm, *Pectinophora gossypiella* (Saunders); cotton leafworm, *Alabama argillacea* (Hübner) and fall armyworm, *Spodoptera frugiperda* (J.E. Smith). These species are all regarded as significant pests of cotton and, historically, *H. virescens* and *A. argillacea* have been regarded as key pests. *P. gossypiella* and *A. argillacea* are specialists, largely restricted to cotton or closely related Malvaceae. *H. virescens* and *S. frugiperda*, in contrast, are polyphagous species with a wide range of unrelated host plants. *H. virescens* appears to be somewhat more specialized in Brazil than in the USA (Gallo *et al.*, 2002). However, with the expansion of agriculture in the Midwest region in Brazil, *H. virescens* also started infesting soybean fields in this region.

Since the introduction of boll weevil in 1983 and of virus-susceptible US varieties in the last 10 years, both the pest spectrum and the distribution of cotton in Brazil have changed dramatically (Ramalho, 1994; Fontes *et al.*, Chapter 2, this volume). About 70% of cotton area is now grown in the Midwest where large-scale developments have occurred only in the last 5–10 years. The infestation of boll weevil is not very critical in the Midwest yet; however, viral diseases (e.g. blue disease) transmitted by the cotton aphid have become extremely important. Maize production has also changed with a diversification of cropping to include extensive areas of autumn and irrigated winter production. As a consequence, *S. frugiperda* has emerged as one of the most significant threats to cotton production. It is regarded as an induced pest in the system. We are thus dealing with relatively new production systems and pest dynamics in environments where relatively little historical data are available.

A number of other Lepidoptera may occur on cotton but all are regarded as too minor to be considered here. Surprisingly, maize earworm, *Helicoverpa zea* (Boddie), a regular pest on US cotton, appears not to infest cotton in Brazil (Degrande, 1998; Gallo *et al.*, 2002). Given that, it could not be considered a resistance risk, although its host range in North America and Central America does include cotton. Research on the ecological genetics of host use in Brazilian *H. zea* may well generate interesting comparisons with its behaviour elsewhere in the Americas.

The geographic distribution of these species varies somewhat among the three major cotton agroecological regions in Brazil (Table 12.1). In the major production area, the Midwest, all of the Lepidoptera are important pests, although boll weevil is a significant and increasing pest and, together with

S. frugiperda, represents the main threat to production in the Midwest. In the Meridian region, *S. frugiperda* is a minor pest of cotton. In the North-east region, where the poorest cotton farmers are found, boll weevil and the specialized Lepidoptera, *A. argillacea* and *P. gossypiella*, are the most important pests. With the expansion of upland cotton in the North-east, *S. frugiperda* is also becoming an important cotton pest in this region. Some characteristics of the farming systems are given in Table 12.2 (see also Fontes *et al.*, Chapter 2, this volume). These data will be important to design a practical resistance-management strategy.

History of resistance

Species with a history of resistance should be prioritized because their *a priori* risk of evolving resistance to Bt cotton is high.

An extensive range of pesticides is registered for use in cotton and maize crops. The organophosphates (OPs), synthetic pyrethroids (SPs) and insect growth regulators (IGRs) are used predominantly (see Table 2.3 in Fontes *et al.*, Chapter 2, this volume). In extensive cotton production of varieties largely derived from US germplasm, the key targets for pesticides are now early-season aphids, which are vectors for viral diseases, and boll weevils that occur in most regions of Brazil. Spraying for these pests suppresses many of the Lepidopteran pests, although three to four insecticide applications are likely to target Lepidoptera specifically. The average frequency of insecticide applications in the three main cotton-production regions varies from 4 to 20 applications per crop (Table 12.2). The Midwest, which produces 86% of Brazilian cotton fibre, is an intensive production system with the highest pesticide input with an average of 16–20 applications per growing season.

Table 12.1. Regional differences in severity of arthropod pests of Brazilian cotton.

Region		Midwest	Meridian	North-east	Resistance history ^a	
	% total cotton area ^b	76.5	12	11		
Target pests	<i>Heliothis virescens</i>	***	***	*	OCI, OPs	
	<i>Alabama argillacea</i>	***	***	***	SPs, OPs	
	<i>Pectinophora gossypiella</i>	***	***	***	None	
	<i>Spodoptera frugiperda</i>	***	*	–	Widespread	
	Other key pests	<i>Anthonomus grandis</i>	**	***	***	None
		<i>Aphis gossypii</i>	***	**	*	OPs

*Severity: *** = most severe.

^aInsecticide class: OCI = organochlorine; OP = organophosphate; SP = synthetic pyrethroid.

^bRemaining area not included here is in the northern region (State of Tocantins).

Table 12.2. Regional differences in farm size, inputs and anticipated impact of Bt cotton on insecticide use in Brazilian cotton.

Region	Midwest	Meridian	North-east
% of cotton production ^a	87	10	2.5
Farm size	Large	Intermediate to large	Small
Inputs	Very high	Intermediate to high	Very low
Average yield (kg/ha) ^b	3357	2518	1770
Insecticide applications	12–20	6–12	4–8
Anticipated reduction in insecticide use from Bt cotton	2–4 applications	2 applications	2 applications

^aRemaining production not included here is in the northern region (State of Tocantins).

^bSource: CONAB (2003).

Most of the applications are targeted to control aphids in the virus-susceptible cotton varieties (six to eight applications), followed by Lepidopteran pests (three to four), boll weevil (two to four) and stink bugs (one to two).

Limited information is available to assess the past history of resistance in Lepidoptera pests of cotton in Brazil (Table 12.1) because there has been no regular monitoring programme in place. Recent baseline assessments and monitoring have demonstrated high frequency of resistance in *S. frugiperda* to OPs and SPs in cotton-producing regions (Diez-Rodriguez and Omoto, 2001). There is also evidence of incipient resistance to IGRs in this species (C. Omoto, Piracicaba, 2004, personal communication). For *A. argillacea*, there is a high probability of resistance to OPs and SPs based on frequent field failures for controlling this pest; however, there is no evidence of pesticide resistance in *P. gossypiella* in Brazil, although measurable background frequency of Bt resistance has been found in field populations in the USA (Tabashnik *et al.*, 2000a). Despite the widespread deployment of Bt cotton in that environment, Bt resistance has not increased in frequency due to other factors (Carrière *et al.*, 2002).

During the 1970s, there are accounts of field failures of organochlorines (OCIs) and OPs against *H. virescens* to the extent that cotton production declined in Brazil. Whether this was due to resistance was not documented at that time. During the early 1980s, prior to introduction of the boll weevil, significant natural enemy populations had developed following reduced use of pesticides. These included high populations of egg parasitoids as one important component of a successful integrated pest management (IPM) approach. In 1983, with the introduction of the boll weevil, significantly increased use of insecticide sprays reduced beneficial organisms, complicating management of

H. virescens (Ramalho, 1994). Subsequently, during the mid 1990s, some field failures of insecticides were reported, but as before, there were no efforts to document that the failures were caused by resistance. These episodes none the less indicate that the Brazilian cotton-production systems are probably quite effective at selecting for resistance in *H. virescens*. We urge that appropriate baseline information on resistance frequencies be collected for the identified key pests prior to deployment of Bt cottons.

Based on the history of resistance to insecticides, we conclude that of the four target species, *H. virescens*, *S. frugiperda* and *A. argillacea* have the highest risk of resistance in Brazil. In addition, based on recent reports of resistance to Bt cotton in the USA (Tabashnik *et al.*, 2000a), *P. gossypiella* also poses a significant resistance risk.

Potential exposure of target pests to Bt cotton

Association with Bt cotton

The association of the target species with Bt cotton is the *maximum period of overlap* of the species on the target crop, in terms of area, spatial distribution and seasonal availability of the crop. Overlap can be evaluated on the basis of presence and absence and general knowledge about the species. More precise, quantitative evaluations will become necessary to develop realistic resistance-management plans (see next section).

The four target species differ markedly in host range and association with cotton (Table 12.3). *P. gossypiella* is a specialist on Malvaceae, particularly on *Gossypium* species. It is thought to feed only on cotton in Brazil. *A. argillacea* is also restricted to cotton. No alternative hosts are known. *H. virescens* has a wide range of recorded hosts, including crops and wild hosts, although in Brazil it is thought to be closely associated with cotton and rarely occurs on other crops (Gallo *et al.*, 2002). Likewise, *S. frugiperda* has an extremely broad range of potential hosts (Table 12.4), though actual usage may be much more constrained.

Table 12.3. Association and fecundity of target Lepidopteran species in Brazilian cotton.

Species	Number of generations in cotton	Number of generations per year	Adult dispersiveness	Cannibalism	Fecundity
<i>Alabama argillacea</i>	3	3	Very high	Low	500–800
<i>Pectinophora gossypiella</i>	3–5	3–5	Very low	Very low	250–500
<i>Heliothis virescens</i>	2–3	2–3	Low	Very high	800–1000
<i>Spodoptera frugiperda</i>	2	6	High	Very high	750–1250

Figure 12.1 illustrates the main periods of cotton production in the three main cotton agroclimatic zones of Brazil. Planting of cotton occurs progressively later from south to north, again reflecting that most cotton is rainfed and rains commence earlier in the south. With the expansion of the second cotton season (planting in January, Fig. 12.1) in the Midwest region, the pest pressure and consequently the pest exposure to cotton will increase in this region. All the four target species have at least two complete generations in cotton. For *P. gossypiella*, *A. argillacea* and *H. virescens*, all generations are associated with cotton (Table 12.3).

S. frugiperda displays a very wide host range (Table 12.4), but clearly prefers grasses. This species is comprised of two genetically different, but

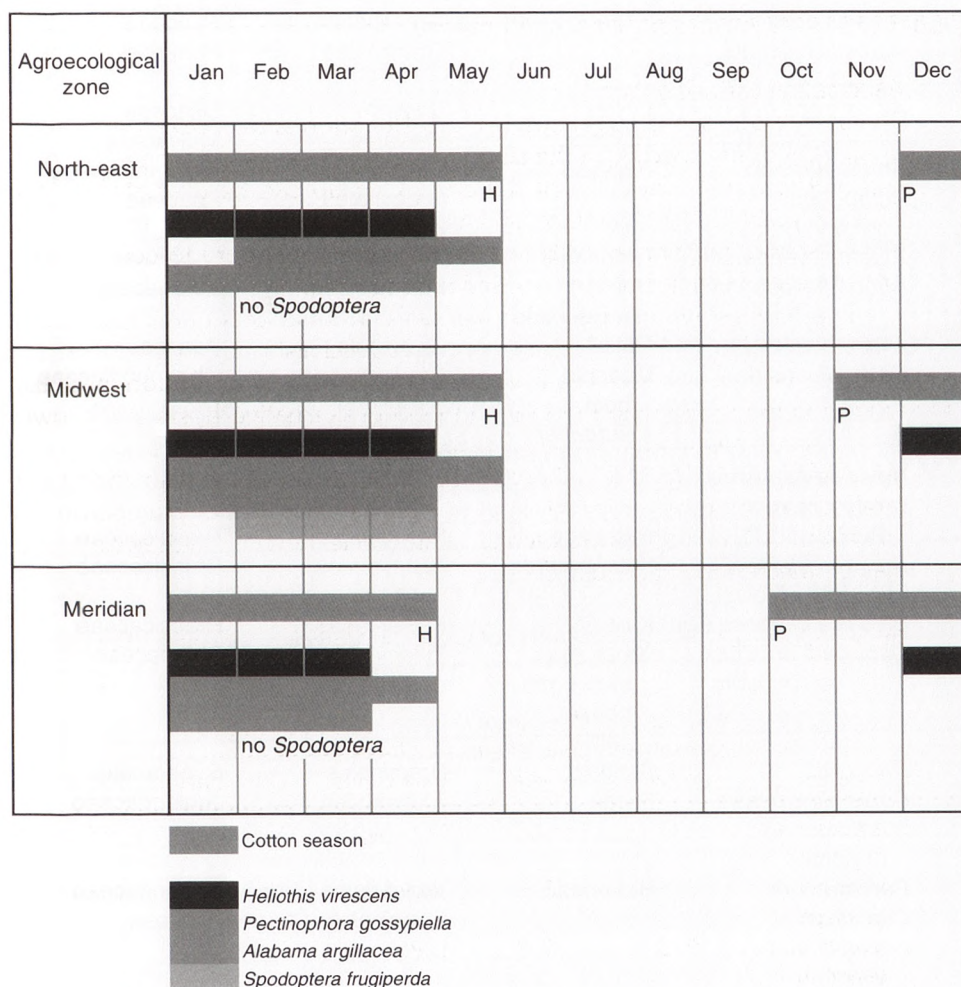


Fig. 12.1. Main cotton-production season and occurrence of key Lepidopteran pests across three main regions. 'P' indicates the typical planting date and 'H' indicates the typical harvest date.

morphologically identical host strains (Pashley, 1986), a 'maize' strain and a 'rice' strain. The 'maize' strain feeds principally on maize, sorghum and cotton, and will feed on a few other hosts when they grow near the primary hosts. In Brazil, maize and cotton are important hosts, as are a number of other crops (Cruz, 1995; Degrande, 1998). Maize appears to be preferred over other hosts. The extent to which populations occur in wild host plants in Brazil is unknown. Non-crop hosts are abundant only during the summer

Table 12.4. List of host plants for fall armyworm, *Spodoptera frugiperda* (Smith), adapted from the *Spodoptera* database (Lepidoptera: Noctuidae) (Pogue, 1995).

Scientific name	Common name	Botanical order	Botanical family
<i>Agrostis alba</i> (L.)		Graminales	Poaceae
<i>Agrostis hyemalis</i> (Walt.)		Graminales	Poaceae
<i>Allium cepa</i> (L.)	Onion	Liliales	Liliaceae
<i>Althaea rosea</i> (Cav.)	Hollyhock	Malvales	Malvaceae
<i>Amaranthus</i> sp.	Pigweed	Caryophyllales	Amaranthaceae
<i>Andropogon virginicus</i> (L.)		Graminales	Poaceae
<i>Arachis hypogaea</i> (L.)	Groundnut	Rosales	Fabaceae
<i>Atropa belladonna</i> (L.)	Deadly nightshade	Polemoniales	Solanaceae
<i>Avena sativa</i> (L.)	Oats	Graminales	Poaceae
<i>Beta vulgaris</i> (L.)	Mangold, beet, sugarbeet	Caryophyllales	Chenopodiaceae
<i>Brassica napus</i> var. <i>napobrassica</i> (L.) Rchb.	Rutabaga	Papaverales	Brassicaceae
<i>Brassica oleracea</i> (L.) var. <i>viridis</i> (L.)	Kale, collards	Papaverales	Brassicaceae
<i>Brassica oleracea</i> (L.)	Kale	Papaverales	Brassicaceae
<i>Capsicum annuum</i> (L.) var. <i>annuum</i>	Green, bell, sweet, red pepper, chilli	Polemoniales	Solanaceae
<i>Carex</i> sp.	Sedges	Graminales	Cyperaceae
<i>Carya illinoensis</i> (Wangenh.) K. Koch	Pecan	Juglandales	Juglandaceae
<i>Carya</i> sp.	Hickories	Juglandales	Juglandaceae
<i>Cenchrus pauciflorus</i> (Benth.)		Graminales	Poaceae
<i>Chenopodium quinoa</i> (Willd.)	Quinoa	Caryophyllales	Chenopodiaceae
<i>Chloris gayana</i> (Kunth)	Rhodes grass	Graminales	Poaceae

Table 12.4. List of host plants for fall armyworm, *Spodoptera frugiperda* (Smith), adapted from the *Spodoptera* database (Lepidoptera: Noctuidae) (Pogue, 1995) – cont'd.

Scientific name	Common name	Botanical order	Botanical family
<i>Chrysanthemum</i> sp.	Chrysanthemum	Asterales	Asteraceae
<i>Cicer arietinum</i> (L.)	Garbanzo, chick-pea	Rosales	Fabaceae
<i>Citrullus lanatus</i> var. <i>lanatus</i> (Thumb.) Matsum. & Nakai	Watermelon	Loasales	Cucurbitaceae
<i>Citrus unshiu</i> (Marcow)	Satsuma orange	Sapindales	Rutaceae
<i>Convolvulus</i> sp.		Polemoniales	Convolvulaceae
<i>Corchorus</i> <i>capsularis</i> (L.)	White jute	Malvales	Tiliaceae
<i>Corchorus</i> <i>olitorius</i> (L.)	Jute	Malvales	Tiliaceae
<i>Croton capitatus</i> (Michx.)	Woolly croton	Euphorbiales	Euphorbiaceae
<i>Cucumis sativus</i> (L.)	Cucumber	Loasales	Cucurbitaceae
<i>Cynodon</i> <i>dactylon</i> (L.) Pers.	Bermuda grass	Graminales	Poaceae
<i>Cyperus</i> <i>rotundus</i> (L.)	Nut sedge	Graminales	Cyperaceae
<i>Dactyloctenium</i> <i>aegyptium</i> (L.) Willd.	Crowfoot grass	Graminales	Poaceae
<i>Dahlia</i> hybrid	Dahlia	Asterales	Asteraceae
<i>Digitaria eriantha</i> (Steud.)	Pangola grass	Graminales	Poaceae
<i>Digitaria ischaemum</i> (Schreb.) (Schreb. ex. Muhl.)	Smooth crabgrass	Graminales	Poaceae
<i>Digitaria sanguinalis</i> (L.) Scop.	Large crabgrass	Graminales	Poaceae
<i>Echinochloa colona</i> (L.) Link	Jungle rice	Graminales	Poaceae
<i>Eleusine indica</i> (L.) Gaertn.	Goose grass	Graminales	Poaceae
<i>Eriochloa polystachya</i> (Kunth.)	Carib grass	Graminales	Poaceae
<i>Fragaria chiloensis</i> (L.) Duchesne	Beach strawberry, Chilean strawberry	Rosales	Rosaceae
<i>Geranium</i> sp.	Geranium	Graminales	Poaceae
<i>Gladiolus</i> <i>glandavensis</i>	Gladiolus	Liliales	Iridaceae
<i>Glycine max</i> (L.) Merrill	Soybean	Rosales	Fabaceae
<i>Gossypium</i> <i>herbaceum</i> (L.)	Tree cotton	Malvales	Malvaceae

Continued

Table 12.4. List of host plants for fall armyworm, *Spodoptera frugiperda* (Smith), adapted from the *Spodoptera* database (Lepidoptera: Noctuidae) (Pogue, 1995) – cont'd.

Scientific name	Common name	Botanical order	Botanical family
<i>Gossypium hirsutum</i>	Upland cotton	Malvales	Malvaceae
<i>Hevea brasiliensis</i> (Muell. -Arg.)	Pokok getah, rubber tree	Euphorbiales	Euphorbiaceae
<i>Hordeum vulgare</i> (L.)	Barley	Graminales	Poaceae
<i>Ipomoea batatas</i> (L.) Lam.	Sweet potato	Polemoniales	Convolvulaceae
<i>Ipomoea purpurea</i> (L.) Roth	Common morn- ing-glory	Polemoniales	Convolvulaceae
<i>Linum usitatissi- mum</i> (L.)	Flax, linseed	Geraniales	Linaceae
<i>Lolium perenne</i> (L.)	Ryegrass	Graminales	Poaceae
<i>Lycopersicon esculentum</i>	Tomato	Polemoniales	Solanaceae
<i>Malus domestica</i> (Borkh.)	Apple	Rosales	Rosaceae
<i>Manihot esculenta</i> (Crantz)	Ubi kayu, tapioca plant, Cassava	Euphorbiales	Euphorbiaceae
<i>Medicago sativa</i> (L.)	Lucerne	Rosales	Fabaceae
<i>Mucuna pruriens</i> (Wall. ex Wight) Baker ex Burck	Velvet bean	Rosales	Fabaceae
<i>Musa</i> x (L.)	Banana	Musales	Musaceae
<i>Nicotiana tabacum</i> (L.)	Tobacco	Polemoniales	Solanaceae
<i>Onosmodium virginianum</i> (L.)		Polemoniales	Boraginaceae
<i>Oryza sativa</i> (L.)	Rice	Graminales	Poaceae
<i>Panicum maximum</i> (Jacq.)	Guinea grass	Graminales	Poaceae
<i>Panicum miliaceum</i> (L.)	Common millet	Graminales	Poaceae
<i>Panicum purpur- ascens</i> (Raddi)		Graminales	Poaceae
<i>Panicum texanum</i> (Buckley)		Graminales	Poaceae
<i>Paspalum conjug- atum</i> (P.J. Bergius)	Sour paspalum	Graminales	Poaceae
<i>Pennisetum clandestinum</i> (Hochst. ex Chiov.)	Kikuyu grass	Graminales	Poaceae
<i>Pennisetum glaucum</i> (L.) R. Br.	Pearl millet, bulrush millet	Graminales	Poaceae
<i>Phleum pratense</i> (L.)	Pearl millet, bulrush millet	Graminales	Poaceae

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Scientific name	Common name	Botanical order	Botanical family
<i>Phytolacca americana</i> (L.)	Pokeweed, poke, pigeonberry	Caryophyllales	Phytolaccaceae
<i>Pisum sativum</i> (L.)	Pea	Rosales	Fabaceae
<i>Platanus occidentalis</i> (L.)	Sycamore	Hamamelidales	Platanaceae
<i>Plumeria rubra</i> (L.)	Frangipani	Gentianales	Apocynaceae
<i>Poa annua</i> (L.)	Annual bluegrass, annual meadow grass	Graminales	Poaceae
<i>Poa pratensis</i> (L.)	Kentucky bluegrass	Graminales	Poaceae
<i>Poa</i> spp.	Pasture grass	Graminales	Poaceae
<i>Polytrias praemorsa</i> (Nees) Hack.		Graminales	Poaceae
<i>Portulaca oleracea</i> (L.)	Purslane	Caryophyllales	Portulacaceae
<i>Prunus persica</i> (L.) Batsch	Peach	Rosales	Rosaceae
<i>Saccharum officinarum</i> (L.)	Sugarcane	Graminales	Poaceae
<i>Secale cereale</i> (L.)	Rye	Graminales	Poaceae
<i>Setaria italica</i> (L.) P. Beauv.	Foxtail millet, Italian millet, German millet, Hungarian millet	Graminales	Poaceae
<i>Solanum dulcamara</i> (L.)	Bittersweet, bitter nightshade	Polemoniales	Solanaceae
<i>Solanum melongena</i> (L.)	Aubergine	Polemoniales	Solanaceae
<i>Solanum tuberosum</i> (L.)	Potato	Polemoniales	Solanaceae
<i>Sorghum bicolor</i> (L.) Moench	Grain sorghum, sorghum, sweet sorghum, milo	Graminales	Poaceae
<i>Sorghum halpense</i> (L.) Pers.	Johnsongrass	Graminales	Poaceae
<i>Spinacia aleracea</i> (L.)	Spinach	Caryophyllales	Chenopodiaceae
<i>Trifolium pratense</i> (L.)	Red clover, purple clover, peavine clover	Rosales	Fabaceae
<i>Triticum aestivum</i> (L.), nom. cons.	Bread wheat, wheat	Graminales	Poaceae

Continued

Table 12.4. List of host plants for fall armyworm, *Spodoptera frugiperda* (Smith), adapted from the *Spodoptera* database (Lepidoptera: Noctuidae) (Pogue, 1995) – cont'd.

Scientific name	Common name	Botanical order	Botanical family
<i>Urochloa decumbens</i> (Stapf) R. D. Webster	Signal grass	Graminales	Poaceae
<i>Urochloa mutica</i> (Forssk.) T.Q. Nguyen	Mauritius grass, paragrass	Graminales	Poaceae
<i>Vaccinium macrocarpon</i> (Aiton)	Cranberry, large cranberry, American cranberry	Ericales	Ericaceae
<i>Vigna unguiculata</i> ssp. <i>unguiculata</i> (L.) Walp.	Southern-pea, cowpea, black- eyed pea, crowder-pea	Rosales	Fabaceae
<i>Viola</i> sp.	Violets	Violales	Violaceae
<i>Vitus</i> sp.	Grapes	Rhamnales	Vitaceae
<i>Vitus vinifera</i> (L.)	Grape vine	Rhamnales	Vitaceae
<i>Wisteria hispida</i> (Maxium)	—	Rosale	Fabaceae
<i>Xanthium</i> <i>strumarium</i> (L.)	Common cocklebur, California-bur	Asterales	Asteraceae
<i>Zea mays</i> (L.)	Maize	Graminales	Poaceae

rainy period and it may be unlikely that much of the population occurs outside maize and cotton during the autumn and winter periods. The 'rice' strain feeds principally on rice, Bermuda grass and Johnson grass, and will also feed on several other species of grass when they are available. When the larvae are very numerous they defoliate the preferred plants and disperse in large numbers, consuming nearly all vegetation in their path. Many host records of this species reflect such periods of abundance, and are not truly indicative of oviposition and feeding behaviour under normal conditions. Meagher and Nagoshi (2004) discuss techniques to monitor the spatial abundance of the host races in Florida and identify differences in distribution of the races in relation to agroecosystem features, which may also apply in Brazil. Nagoshi and Meagher (2003) provide molecular techniques to distinguish the host races, which may be useful in Brazil to untangle the interaction between wild hosts and maize.

Based on their association with cotton, *P. gossypiella*, *A. argillacea* and *H. virescens* are likely to be exposed to more intense selection in Bt cotton than *S. frugiperda*.

Association on other plants with Bt toxin

S. frugiperda, and to a lesser extent *H. virescens*, are the only species that are likely to occur outside of cotton in the field. *H. virescens* will occasionally occur in crops such as soybean, where it may be exposed to Bt sprays, but we regard this exposure as insignificant.

In relation to *S. frugiperda*, some similar Bt transgenes are proposed for use in maize crops. Maize production has diversified in recent years in Brazil, with the main crop grown during summer and a smaller crop of hybrid varieties in autumn and an even smaller winter crop of hybrid varieties in some regions. It is anticipated that 50–100% of the autumn and winter hybrids may eventually be planted to Bt maize that could express Cry1Ab or Cry1F, which provide the best control of *S. frugiperda* (Waquil *et al.*, 2002). By contrast, Cry9C has no effect on this species (Waquil *et al.*, 2002). Under laboratory selection, increased resistance (fivefold based on median lethal concentration (LC_{50})) of *S. frugiperda* to Cry1Ab was observed after four generations (Vilela *et al.*, 2002).

S. frugiperda represents a great risk of resistance evolution should both Cry1Ab maize and Cry1Ac/Cry 2Ab cotton be deployed. This would represent a mosaic of Bt-protein exposure and selection in the two main host plants of this species, hosts which overlap extensively in time and space. Considerable thought will be required to balance the possible introduction of Bt maize into this system, alongside Bt cotton. Below, in the section on 'resistance-risk management', we discuss some ways to balance these needs.

Exposure affected by farming system

The key pests of cotton in the main production regions are currently aphids and boll weevil. Insecticides applied for these pests provide considerable control of the Lepidopteran species and appears to mask the damaging potential of those species. The strength of selection of Bt cotton on the four target Lepidoptera varies directly with the amount of control that Bt cotton will exert above and beyond the control exerted by the insecticides. While there is some disagreement as to how much additional control Bt cotton may exert, it is possible that it will provide little additional control, although the selection pressure for resistance may still be significant (M. Caprio, Mississippi, 2004, personal communication).

Scale of adult movement

The scale of adult movement determines how much mixing and mating can occur between individuals emerging from different fields. For the purposes of relative resistance-risk assessment of the target species, it is not necessary to have precise quantitative data on the species. In general, the less dispersive a species is the greater the risk for resistance evolution (Carrière *et al.*, 2004a). This occurs because sedentary species will be more likely to mate with individuals from the field in which they emerged, and to oviposit in the same fields, which is likely to lead to greater selection pressure on that local part of the population. Hence in assessing the resistance risk, it can suffice to rank the dispersiveness of the target species.

There is little information specific to Brazil on adult movement of the four target species, although *H. virescens*, *P. gossypiella* and *S. frugiperda* have been studied extensively elsewhere. *H. virescens* has been found to be relatively sedentary, although capable of extensive local movement (Schneider, 1999, 2004). *S. frugiperda* is capable of extensive local and interregional movement (Pashley *et al.*, 1985), while *P. gossypiella* is probably the most sedentary of them all (Tabashnik *et al.*, 1999; Carrière *et al.*, 2001, 2004a,b). For *P. gossypiella* in Arizona, it was determined that refuges should not be further away than 0.75 km from Bt-cotton fields (Carrière *et al.*, 2004a,b). *A. argillacea* is believed to undertake extensive long-range movements from Argentina and Central South America through southern and central Brazil to north-eastern Brazil (Medeiros *et al.*, 2003). This movement largely follows the seasonal front of onset of the rainy season. Resident populations undoubtedly remain in all regions. There is no clear evidence of return movements from north to south.

Given this and knowledge from elsewhere, it seems reasonable to rank the dispersiveness as: *P. gossypiella* < *H. virescens* < *S. frugiperda* < *A. argillacea* (Table 12.3).

Likely dose of Cry toxins in Bt cotton

The dose of insecticidal toxin in Bt cotton will be a major factor determining the level of resistance risk. Dose is a measure of 'hazard', which is one of the two components that enter into risk assessment. Dose is defined by both the concentration of the Cry toxin in the Bt plant and the genetic characteristics of the target pest. Is the Bt crop a 'high-dose' or a 'low-dose' plant? A 'high-dose' is defined as one that kills a high proportion (> 95%) of heterozygous resistance genotypes similar to homozygous susceptible genotypes (Tabashnik, 1994a; Roush, 1997; Andow and Hutchison, 1998; Gould, 1998). A 'low-dose' is anything that is not a high-dose.

Resistance management will differ for high-dose versus low-dose plants. Simulation models clearly show that a high dose can delay the evolution of resistance more effectively than a low dose (Roush, 1994; Alstad and Andow, 1995; Gould, 1998; Tabashnik *et al.*, 2003a). A high dose may also allow greater options for resistance management with less restrictions on how non-transgenic refuges are managed (Carrière and Tabashnik, 2001; Ives and Andow, 2002; Onstad *et al.*, 2002; Storer *et al.*, 2003), and so may be more readily implemented compared to low-dose events. Low-dose events will require larger non-transgenic refuges and/or restrictions on the management of these refuges (Ives and Andow, 2002). Indeed, in Australia, growers agreed to cap the area of single gene Bt cotton (low dose for *H. armigera*) to 30% of the total crop in addition to the requirement for refuges (50% sprayed cotton refuge or 10% unsprayed cotton refuge) (Fitt, 2004). In the US, it has been argued that a 50% refuge may be needed for low-dose plants (Gould and Tabashnik, 1998). Simulations conducted during the Brazil workshop also indicated that a 50% refuge was needed for low-dose plants (Fig. 12.2).

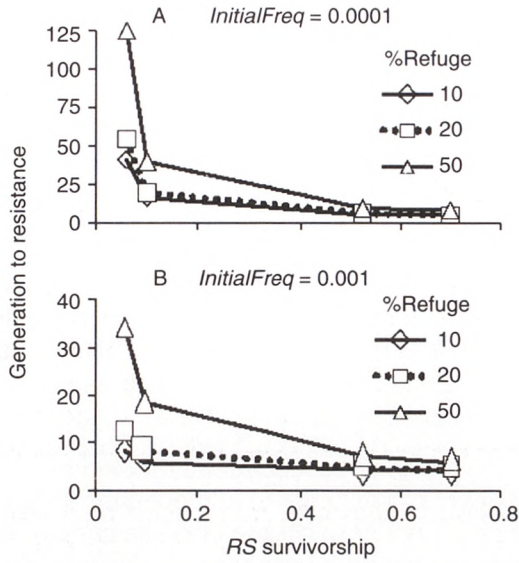


Fig. 12.2. Relationship between Bt cotton efficacy and time to resistance (details in the text under resistance-management section).

To evaluate the 'dose' it is essential to have insects resistant to the Bt crop that can be crossed to create heterozygous individuals that can be challenged with the Bt plant. However, in most cases prior to field release, resistant insects will not have been discovered. When resistance in a target species is not yet known it is not possible to evaluate heterozygous genotypes, so it is impossible to determine if a transgenic plant is high-dose or not. When this occurs, a temporary, provisional, operational definition of 'high-dose' must be used. One such definition for a provisionally 'high-dose' is: a plant that expresses toxin at a concentration that is 25 times the lethal concentration (LC_{99}) of the target pest (Gould and Tabashnik, 1998). This operational definition has been accepted for use by the US-Environmental Protection Agency (US-EPA). One alternative definition is a high dose produces at least 99.99% mortality of homozygote susceptibles relative to a non-Bt control (ILSI, 1999).

As discussed in more detail in Grossi *et al.* (Chapter 4, this volume), several Bt toxins have been incorporated into cotton that have been commercialized outside of Brazil, and many more toxins are under commercial development. Cry1Ac was the first to be commercialized as Mon531 (Bollgard[®], INGARD[®] in Australia). Cry2Ab has been recently commercialized together with Mon531 (Bollgard II[®]). Cry1F has been combined with Cry1Ac in varieties that are under development (Widestrike[®]), and Vip3A may be combined with a Cry1A toxin.

The four target pests differ in tolerance to Bt insecticidal proteins (Table 12.5). These evaluations are based on preliminary bioassay and field-performance data, and could be revised as rigorous evaluations are completed. Cry1Ac and Cry2Ab are present at high enough concentrations in the present

Table 12.5. Estimated effective 'dose' provided by different Bt toxins for target Lepidopteran pests of Brazilian cotton.

Insect	Cry1Ac	Cry2Ab	Cry1Ab	Cry1F	Vip3A
<i>Heliothis virescens</i>	High	High	High	Low	High
<i>Alabama argillacea</i>	High	High	High	Low	?
<i>Pectinophora gossypiella</i>	High	High	High	Low	?
<i>Spodoptera frugiperda</i>	Low	Low	Low, 689.81 ng/cm ^{2a}	High, 36.46 ng/cm ²	High

^aSurface treatment of artificial diet with pure toxin using laboratory colony of *S. frugiperda* in USA. Source: Waquil *et al.* (2004).

Bt cottons to express a high dose for *H. virescens*, *A. argillacea* and *P. gossypiella*. However, toxin expression in these same cotton varieties is a low dose for *S. frugiperda*. Cry1F exhibits the opposite pattern, showing the characteristics of a high-dose event for *S. frugiperda* and low dose for the other three species. The toxicity of Vip3A against the target pests in Brazil is not known to us, but evidence of its high efficacy against *H. armigera* (G. Fitt, Brisbane, 2004, personal communication; Llewellyn *et al.*, 2005, in preparation) suggest it may be high dose for *H. virescens* and possibly for *S. frugiperda*.

Comprehensive laboratory or field information is not available for any combination of pests and specific Bt varieties in Brazil. Based on experience of some of these pests elsewhere, and some preliminary work for some species, we believe that Cry1Ac/Cry2Ab cotton expresses a high dose for *H. virescens*, *P. gossypiella* and *A. argillacea*. For *S. frugiperda*, we consider Cry1Ac/Cry2Ab cotton as a low-dose event. Cry1F/Cry1Ac cotton may be a high dose for all four species because Cry1F is high dose for *S. frugiperda*, while Cry1Ac is high dose for the other three species. We had insufficient information about Vip3A or any Vip3A/Cry1A cottons to evaluate their dose.

Bioassays for estimating LC₉₉

Bioassays estimating LC₅₀ or LC₉₉ or sublethal effects that have been previously correlated with LC₅₀ or LC₉₉ are recommended (Sims *et al.*, 1996). It will be most convenient to conduct the bioassays with purified toxin equivalent to that produced by the transgenic plant. The use of purified toxin allows experiments to evaluate the effects of toxin concentrations many times higher than that present in the transgenic plant.

There are many ways to conduct bioassays. First, the carrier of the toxin should be selected. This can be a natural food source (plant tissue) or artificial diet. Generally, the plant tissue is treated with the toxin by surface application with a series of toxin dilutions. Using artificial diet, the toxin can be provided as a mixture (Gould *et al.*, 1997; Hilbeck *et al.*, 1998) or surface treatment. The surface treatment of artificial diet can be done by applying each dilution to the diet surface in a 128-well bioassay tray (Marçon *et al.*, 1999; Waquil *et al.*, 2004). This method conserves toxin, and is acceptable only when small amounts of toxin are available. This method underexposes larvae that bore

into the diet (Bolin *et al.*, 1999). In all cases, neonate larvae from the F_2 or F_3 generation can be used. In some cases, older larvae can be used. Normally, the trays are incubated for at least 7 days at 27°C, 80% RH and 24 h scotophase or photophase, and mortality and larval biomass are measured to estimate the LC and the growth inhibition (GI) (Marçon *et al.*, 1999).

Transgenic plants can also be used to create a series of toxin concentrations by diluting the tissue into an artificial diet (e.g. Olsen and Daly, 2000). This is advantageous because the toxins are in the same form as expressed in the plant, but is disadvantageous because the maximum toxin concentration that can be evaluated is less than what actually occurs in the plant (Andow and Hilbeck, 2004). When using plant material the specific testing should include:

1. Plant tissues that express the highest concentrations of toxins because they will allow a greater range of toxin concentrations to be tested. However, if the plant produces other secondary plant compounds that adversely affect the target insect pests, tissues that express lower concentrations of these chemicals would be favourable to avoid potential confounding mortality in the assays.
2. Quantification of the actual amount of transgene expression in the tissue that is used.

Plant tissue used without dilution does not allow estimation of an LC_{99} . However, this tissue can, in some circumstances, be used as a discriminating concentration to separate resistant and susceptible phenotypes. This method has been questioned recently by Zhao *et al.* (2002), who showed that the transgenic plant may be less accurate as a discriminating concentration than toxin incorporated into an artificial diet. Thus, the transgenic plant should not be used as a discriminating concentration unless it has been experimentally demonstrated to be an accurate method.

Need to find resistance

Because the actual dose expressed by the Bt plant cannot be determined until resistance genes are recovered in natural populations, assessments of risk without this information should be regarded as preliminary. We have used the limited information available to estimate dose, and we made the precautionary assumption that unless there is evidence that the Bt plant expresses a high dose indicated by consistently high efficacy against a range of field colonies of the pest, then the plant expresses a low dose.

Hence, it is of considerable importance to identify resistance genes in field populations and test their inheritance in the laboratory on Bt plants. Such tests should provide definitive evidence that the Bt plant is a high- or low-dose plant.

For potentially low-dose species, mass selection on laboratory colonies derived from recently collected individuals from the field should be initiated (Akhurst *et al.*, 2003). For potential high-dose target species, mass selection may be less likely to recover resistance (e.g. Bolin *et al.*, 1999; Huang *et al.*, 1999), but in some cases it can be successful (Gould *et al.*, 1997; Tabashnik *et al.*, 2000a, 2002, 2003b; Morin *et al.*, 2003). For high-dose species, additional methods include F_2 screens (Andow and Alstad, 1998; Genissel *et al.*, 2003), in-field screens (Tabashnik *et al.*, 2000a; Venette *et al.*, 2000) and any other approach that can maximize the probability of finding resistant individuals.

If relevant resistance genes have already been recovered in one of the target species in another region or country, a collaboration may be advisable both to use previous data as well as to access the resistant colony for future research. Note, however, that the genetic composition of insect populations varies geographically. Thus, the genetic basis of resistance could differ from one region to the next.

Risk assessment

Potential adverse consequences of resistance

The main potentially adverse consequences of resistance are: control failures; yield loss and economic hardship, when the pest is otherwise difficult to control; increased use of pest-management tactics, such as pesticides, that may be a significant human health or environmental risk; and reduced management options for growers that can increase production costs.

Key pests in Brazil are boll weevil and aphids (as virus vectors) in addition to the four target Lepidopteran species. Insecticides are the primary management tool used presently, with significant numbers of applications in all regions (Table 12.2). IPM systems to reduce insecticide applications have been developed and demonstrated but are not widely adopted (J.M. Waquil, Sete Lagoas, 2004, personal communication). As the four target Lepidoptera are not the key pests, we consider that two to four pesticide applications are the maximum that could be saved by Bt cotton (Table 12.2). Bt cotton is unlikely to reduce insecticide use for boll weevil, but may assist with aphid management if the pesticides currently applied for Lepidoptera control induce outbreaks of aphids. Applications to control boll weevil and aphids also suppress the Lepidoptera. Hence, under the present management practices, control failures and increased pesticide use are unlikely to occur if resistance arises.

Bt cotton may provide more impetus to adopt IPM approaches and so increase pesticide savings and consequent environmental benefits (Fitt, 2002). Resistance evolution would jeopardize these advances, limiting management options for growers. It is difficult to conclude that resistance evolution to the present Bt-cotton varieties will of itself cause substantial harm, because the major problems for farmers are currently boll weevil and aphids. None the less, any reduction in pesticide use will bring environmental benefits. Outside the Midwest region a reduction of two sprays could represent a 30–50% reduction in pesticide use, which is significant and would be threatened if field resistance were to occur. However, this assessment would change substantially if new, environmentally friendly control tactics for boll weevil and aphids become available.

Ranked resistance risk

A history of resistance to pesticides, low mobility of adults, high expected exposure of the population to Bt cotton and a low dose together imply a high resistance risk. Because resistance management usually relies on changing

the exposure rate, the greatest risk will usually be a low-dose species and secondarily species with low dispersal distances. Of course, a history of resistance is a good indicator that resistance to Bt cotton might also occur readily. All of the four target species in cotton have a history of resistance evolution in Brazil or elsewhere, so none can be inferred to have a low resistance risk.

In the Midwest region, all four target species are important pests. The weakest link is *S. frugiperda*, except in locations where considerable areas of non-Bt maize is grown. If Bt maize is allowed or little maize is grown, the resistance risk in *S. frugiperda* is very high (Table 12.6). A low-dose strategy for *S. frugiperda* will be necessary for Cry1Ac/Cry2Ab cottons in this region.

In the Meridian region, *S. frugiperda* is not a major pest in cotton, and all of the other three species are weak links (Table 12.6). Although *P. gossypiella* has the lowest adult mobility of the three species, both *H. virescens* and *A. argillacea* have a great potential for resistance in Brazil and should be taken seriously. A high-dose strategy can be used in this region.

In the North-east region, the key Lepidopteran pests are *A. argillacea* and *P. gossypiella*. Both are weak links for resistance management for reasons similar to the Meridian region (Table 12.6). A high-dose strategy can be used in this region. However, with the expansion of upland cotton in part of this region, *S. frugiperda* is becoming an important pest. In this situation, the same considerations from the Midwest region should be followed.

Resistance Risk Management

Resistance-management requirements

The requirements for resistance management focus firstly around the weak link for each of the three cotton-producing regions of Brazil, then consider

Table 12.6. Summary assessment of the relative resistance risk of the four target Lepidopteran pests of Brazilian cotton. 1 indicates a very high risk and 4 indicates a low risk. All species are likely to be at risk of resistance evolution.

Insect	Region	History of resistance	Movement and risk	Exposure	Hazard (dose)	Risk
<i>Heliothis virescens</i>	Midwest,	1	1/2	1	2/3	2
	Meridian					
<i>Alabama argillacea</i>	Midwest,	1	3	1	2/3	2
	Meridian,					
	North-east					
<i>Pectinophora gossypiella</i>	Midwest,	2	1	1	2/3	2
	Meridian,					
	North-east					
<i>Spodoptera frugiperda</i>	Midwest	1	2	1/2	1/3	1/3

requirements for the other species at risk. In this way the minimum essential requirements for resistance management can be developed.

Seed mixtures should not be used

A seed mixture is often considered as a possible resistance-management tactic. It involves mixing the seeds of Bt and non-Bt cotton in the seed bags or planters so that a mixture of Bt and non-Bt plants occurs in each field. The idea is that the non-Bt plants provide an effective refuge from selection on the Bt plants. While it is true that seed mixtures delay resistance evolution compared to having no refuge at all (Tabashnik, 1994a), they can be seriously compromised by the movement of larvae between plants (Mallet and Porter, 1992). The worst case occurs when Bt resistant heterozygotes can survive the Bt plant long enough to move to a neighbouring non-Bt plant, where they can complete development and vice versa, where susceptible larvae and resistant heterozygotes feeding on non-Bt plants move to Bt plants where susceptibles are killed and heterozygotes survive, so reducing the real value of the refuge.

Our understanding of the behaviour of larvae of *S. frugiperda*, *H. virescens* and *A. argillacea* suggest that interplant movement of larvae is significant and would compromise the effectiveness of seed mixtures as refuges. *S. frugiperda* deposits egg masses from which larvae feed communally, and larvae may move considerable distances from fields or patches of host plants when plant quality declines or the crop is destroyed (Degrande, 1998). *A. argillacea* and *H. virescens* lay eggs singly on certain plant parts. The larvae of these three species move from plant to plant. Larvae of *P. gossypiella*, on the other hand, are very sedentary and rarely move between bolls on a plant. If this species were the only pest of cotton, seed mixtures might be a feasible tactic. However, there is no region in Brazil where this species is the only Lepidopteran pest of cotton. Consequently, we conclude that seed mixtures should not be used in Brazilian cotton (Table 12.8).

Kinds of refuges

A refuge is a habitat in which the target pest can maintain a viable population in the presence of Bt-cotton fields, where there is no additional selection for resistance to Bt toxins and insects occur at the same time as in the Bt fields (Ives and Andow, 2002). The refuge can be managed to control pest damage as long as the control methods do not reduce the population to such low levels that susceptible populations are driven to extinction (Ives and Andow, 2002). Because current cotton pest-management practices in Brazil have not come close to eliminating Lepidopteran pest populations (Ramalho, 1994), it is possible that continuing normal pest management on refuges would not jeopardize resistance management. However, this possibility should be investigated experimentally. Sprayed refuges are likely to be required to be larger in extent than unsprayed ones.

Preliminary data suggest that few wild hosts exist for the four target species during the cotton-growing season. Should the presence of such hosts be proposed, such as for *S. frugiperda*, it will be necessary to provide

scientific data to prove the suitability of such hosts. Specific data requirements are the net population replacement rate, the area of the refuge, the density of adults produced, the relative fitness of these adults (relative to non-Bt cotton or non-Bt maize), temporal synchrony in moth production between the crops and wild hosts and consistency (production year after year).

For *P. gossypiella*, *A. argillacea* and *H. virescens*, which are found mainly on cotton, the only refuge habitat possible at this time is cotton. Cotton is also a suitable refuge for *S. frugiperda*. This means that in the North-east and Meridian regions cotton is the only suitable refuge for resistance management (Table 12.8).

When the same or similar Bt toxins are not used in maize, maize can be a suitable refuge for *S. frugiperda*. *S. frugiperda* is an important pest in the Midwest region, and can be found on cotton from February to April (Fig. 12.1). Maize is produced year round in the Midwest, with significant areas grown at the same time as cotton (Table 12.7). Sorghum is not a suitable refuge for *S. frugiperda* because it is not available at the same time as the *S. frugiperda* is infesting cotton (Table 12.7). Figure 12.3 indicates when during the year each planting of maize is available to *S. frugiperda* in each of the cotton-growing regions of Brazil. The autumn maize crop overlaps with the time during the cotton growth season when *S. frugiperda* is attacking cotton. Thus, the rainfed autumn maize crop can act as a refuge for Bt cotton in those areas of the Midwest where there is significant autumn maize. In addition, millet, which is commonly used to produce biomass in no-tillage systems, may be a suitable refuge for *S. frugiperda*.

Agroecological zone	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
North-east												
Summer (rainfed)	H									P		
Autumn (rainfed)												
Winter (irrigated)				P							H	
Midwest												
Summer (rainfed)	H									P		
Autumn (rainfed)	P					H						
Winter (irrigated)				P							H	
Meridian												
Summer (rainfed)	H									P		
Autumn (rainfed)	P					H						
Winter (irrigated)				P							H	

Fig. 12.3. Phenology of maize production in the three cotton-production regions of Brazil. H = harvesting, P = planting

Table 12.7. Cultivated area (10^3 ha^a) of *Spodoptera frugiperda*-susceptible crops for each season in the Midwest region of Brazil. Figures in parentheses are % of total susceptible crop area.

Crop	Summer	Autumn	Winter ^d	Total
Cotton ^b	545.6 (8.6)	60.0 ^c (0.9)	0	605.6 (9.6)
Maize	777.1 (12.3)	1165.3 (18.4)	370.0 (5.8)	2312.4 (36.5)
Sorghum	0	517.6 (8.2)	0	517.6 (8.2)
Millet ^b	768 (12.1)	664 (10.5)	0	1432 (22.6)
Rice ^b	848.5 (13.4)	0	0	848.5 (13.4)
Wheat	0	122.0 (1.9)	0	122.0 (1.9)
Sugarcane	245.0 (3.9)	245.0 (3.9)	0	490.0 (7.7)
Total	2969 (47.0)	2773.5 (43.8)	370.0 (5.8)	6328.1 (100)

^aSource: CONAB (2003), average of the previous 2 years, except for cotton and rice (just the last season) which had more than 38% increase last year.

^bCultivated area in 2003/2004 season.

^cSource: ORO Consultoria, Rio Verde, GO and Barreiras, BH, Brazil.

^dIrrigated area.

When the same or similar Bt toxins are used in both cotton and maize, then the value of maize refuges could be compromised depending on the area of Bt maize grown. In the section on 'workable resistance-management plans' below, we address this possibility in detail.

Distance to refuge

ADULT MOVEMENT. The necessary maximum distance from Bt cotton to the refuge depends on the frequency and distance that adults disperse. Although detailed dispersal data do not exist for any of the target Lepidopteran species in Brazil, based on observations on these species in Brazil and elsewhere in the world, it may be reasonable to assume that all species undertake sufficient movement at a local (farm) scale of 1–2 km area from their emergence site. For the four target species, it will be necessary to structure a resistance-management strategy to take account of the least mobile of the four (*P. gossypiella*) where adult movements would likely be < 1 km (Table 12.8; Tabashnik *et al.*, 1999).

Table 12.8. Necessary characteristics for resistance management for Bt cotton in Brazil.

Region	Seed mixture	Refuge types	Maximum distance to refuge
Meridian	No	Cotton and maize ^a	2 km
Midwest	No	Cotton and maize ^a	2 km
North-east	No	Cotton	2 km

^aMaize is a suitable refuge provided Bt maize is not planted in the region and the maize is available to the pest Lepidoptera at the same time as cotton.

Methodologies for study of adult movement could involve mark-recapture techniques (Fitt *et al.*, 1995; Tabashnik *et al.*, 1999; Carrière *et al.*, 2001; Cameron *et al.*, 2002; Kfir *et al.*, 2002) or quantification of various polymorphisms within and among populations to estimate gene flow (Han and Caprio, 2002). Flight mill or wind tunnel methods may indicate the propensity for flight but do not indicate the likelihood of long distance movement, and are not recommended. Methods based on genetic polymorphism could be appropriate to estimate gene flow in any of the four target species in Brazil, because they have been long-standing components of the Brazilian environment. It is possible, however, that recent changes in cropping systems, from conventional to no-tillage, during the last 10 years may have dramatically influenced population structure and invalidate this method. Mark-recapture methods would be more suitable than methods based on genetic polymorphism if there is significant variation in dispersal among generations.

It is questionable, however, that such studies would dramatically change the spatial scale of movement assumed in the first paragraph of this section or change the relative rankings among the species. Hence, we do not recommend considerable research on this problem.

MATING BEHAVIOUR. An understanding of adult mating behaviour in relation to the movement of each sex can be crucial to the design of a high dose-management strategy. It is less clear that these details are important for low-dose strategies. Identifying when mating occurs in relation to the site and time of emergence can be critical for high-dose strategies. If all mating occurs soon after emergence and in the natal patch, then intermating among refuge and Bt crop populations may be compromised. Key questions include: When do moths emerge? When and where do they mate after emergence? How far do they move before first mating and subsequently?

In Brazil, these questions will be significant only for the North-east and the Meridian regions, because these are regions where the high-dose strategy can be implemented (at locations where *S. frugiperda* is not a problem in cotton). Very little information is available on mating dynamics of any of the species in Brazil. Despite this lack of knowledge, we believe that additional studies of mating behaviour in relation to movement of each sex are not necessary during the prerelease period. Simulation models can address the various mating scenarios and allow uncertainty to be managed in the initial design of the resistance-management strategy. More specific experimental information will become necessary as the response systems are designed and verified. This should occur when Bt varieties are being tested in the field prior to commercial release.

Workable resistance-management plans

In this section, we propose workable resistance-management plans that incorporate the known scientific information to delay resistance evolution for at

least 20 years. These plans are necessarily preliminary, because several critical pieces of information are missing. Specifically, we consider a high-dose plan for the North-east and Meridian regions (Scenario 1) and two low-dose plans for the Midwest region, one where there is no Bt maize (Scenario 2) and one where Bt maize occurs (Scenario 3). In addition, we outline a high-dose plan for the Midwest region if, sometime in the future, a Bt cotton that expresses a high dose against all four target Lepidopteran pests becomes available (Scenario 4).

Key assumptions underlying the development of these resistance-management plans are given in Box 12.1. We strongly recommend that Bt cotton be integrated into a comprehensive IPM approach that manages aphids and boll weevil effectively and may maximize efficacy of Lepidopteran control by Bt cotton. Research results from Brazil indicate that an IPM approach could have significant value in reducing pesticide applications from the current average of about 15 applications (F.S. Ramalho, Campina Grande, 2003, personal communication). It is entirely feasible that Bt cotton could be included as part of an IPM approach to further reduce pesticide applications in the Midwest region. Unfortunately, cotton growers have not yet adopted IPM approaches, despite extensive efforts to demonstrate on-farm benefits. It appears that many cotton producers no longer fully bury or destroy cotton residues. This undoubtedly exacerbates boll weevil problems. Cultural control of both pink bollworm and boll weevil will be enhanced substantially if the present low-tillage practices are accompanied by adequate stalk destruction (Ramalho, 1994; Degrande, 1998). More extensive field trials are essential to fully demonstrate the value of IPM to growers. For both resistance management of Bt cotton and IPM more broadly, there are distinct advantages in having a contained planting window for cotton, hence we suggest that the second planting season should be avoided even with non-Bt cotton.

Box 12.1. Key assumptions about IPM with resistance management.

Resistance management should be implemented within the framework of a multi-tactic IPM system. For cotton in Brazil, this should include the following elements:

- Appropriate emphasis on monitoring and thresholds for deciding when to make pesticide application, and on conservation of natural enemies.
- Strong emphasis on cultural control of boll weevil. This may include planting windows, crop-residue destruction and available control tactics that conserve natural enemies.
- Development and use of cotton varieties with resistance to aphid-vectorred viruses.
- Establishment of regional working groups for providing on-going oversight of resistance-management recommendations, refuge requirements and remedial action.
- Funding of laboratories to conduct baseline susceptibility studies, sustain on-going resistance monitoring and respond to putative resistance events.

Four approaches can be used to delay resistance evolution. The approach most widely used is to reduce the exposure of the pests to Bt cotton by planting refuges. Specific issues include size, placement, timing of planting and management of refuges. A second approach is to reduce the selective differential between resistant and susceptible insects. The selective differential is the fitness advantage of resistant phenotypes over susceptible phenotypes when both are exposed to the transgenic plant. This can be accomplished by suppressing pests emerging from the transgenic crop with insecticides, physical controls, biological control, etc. A third approach is to reduce heterozygote fitness. A heterozygote has one susceptible allele and one resistant allele. Heterozygotes may have a susceptible or a resistant phenotype. If they are phenotypically susceptible, then they have low fitness on the Bt plant (resistance is recessive), and the rate of resistance evolution is slow. It is possible that natural enemies can alter heterozygote fitness. Little is known about potential selective feeding by natural enemies. If IPM for aphids and boll weevil can be implemented, there would be considerable potential for egg parasitoids and egg predators to reduce exposure of larvae to Bt proteins. The fourth approach can be used only with high-dose strategies. For some species it may be possible to manage the sex-specific movement and mating frequencies to delay resistance evolution (Andow and Ives, 2002). By using chemical and environmental attractants, it may be possible to enhance the movement of males and simultaneously reduce the movement of females from refuges to transgenic fields.

In what follows, we rely on refuges to reduce the exposure of pests to selection. None of the other approaches have been developed sufficiently to incorporate into a scientifically justified resistance-management plan. Refuges should be provided in a structured way with specific areas of non-Bt cotton or non-Bt maize. Such refuges need to be planted in association with Bt cotton in an appropriate spatial arrangement and to provide insects that overlap temporally with those from the Bt-cotton crop.

Given this general outline and what is known of the biology and ecology of the target species in Brazil, we distinguish three scenarios: Scenario 1, where high-dose Bt cotton is introduced and no alternative hosts are present; Scenario 2, where low-dose Bt cotton is introduced with non-Bt maize as an alternative crop; and Scenario 3, where low-dose Bt cotton is introduced and Bt and non-Bt maize co-occur. Scenarios 2 and 3 change the risks and management options for *S. frugiperda*. The other species are not affected, because they are not associated with maize. In addition, we describe briefly a case where high-dose Bt cotton is introduced with Bt maize. This may correspond to future Bt cottons that have yet to be commercialized anywhere.

Refuge options will be needed to cover the spectrum of target pests. In the case of *S. frugiperda*, non-Bt maize (Scenario 2) could be a significant refuge host. Few wild hosts for *S. frugiperda* are known to occur during this period in the growing season. In the case where Bt-maize is introduced (Scenario 3), it is recommended to limit the exposure of *S. frugiperda* by limiting the entire Bt-crop area to 50% of the total area (Fig. 12.2). It is necessary to limit

exposure because Bt-cotton does not express a high-dose relative to *S. frugiperda*, and capping the entire Bt-crop area at 50% is the only way to meaningfully delay resistance evolution and increase the probability that the event will last more than 20 years.

A deterministic simulation model (Caprio, 1998a) was used to assess the requirements for refuge area for Bt cotton in Brazil. The simulations assume monogenic inheritance of a recessive trait and assumed that *H. virescens*, *P. gossypiella* and *A. argillacea* experience a high-dose Bt cotton, whilst *S. frugiperda* experiences a low-dose Bt cotton. The simulations were performed using the deterministic module of the software RRiskBt (Maia and Dourado-Neto, 2003), based on the deterministic version of Caprio's model (Caprio, 1998a). Two simulation-modelling approaches are available in RRiskBt:

- Deterministic approach – prediction of the number of pest generations until the *R* allele frequency (*RFreq*) exceeds a critical value using a deterministic model (Caprio, 1998b).
- Probabilistic approach – the probability of *RFreq* exceeding a critical frequency is predicted using a probabilistic risk-assessment model (Maia and Dourado Neto, 2003).

RRiskBt was developed using the Visual Basic language. A sensitivity analysis module allows us to investigate the influence of *R* allele initial frequency and/or the functional dominance of resistance on the model outputs. Uncertainty analysis tools were incorporated to obtain resistance-risk estimates.

Deterministic approach

The deterministic version of Caprio's model is a simplified version of the stochastic model developed by the same author (Caprio, 1998a). The events of interest are modelled at the time scale of a pest generation instead of a daily scale. Aspects related to refuge layout are not explicitly considered, but incorporated into pre- and postmating pest-dispersal indexes. It is a biological model in which the economics of the refuge are not taken into account. Using this model, the resistance-allele frequency in the target pest population can be projected as a function of pest generation. Such estimates are based on genetic and biological parameters of the target pest and operational factors related to the transgenic crop (Table 12.9). A description of both the deterministic and stochastic versions of the 'Caprio' model can be found in Caprio (2001).

We adopted the deterministic approach here because the studies required for characterization of parameter uncertainty are still in a preliminary stage. We chose sets of parameter values for simulations taking into account the characteristics of the three broad scenarios outlined above for Bt-cotton systems in Brazil (Table 12.10). The simulations were set by varying initial *R* allele frequency, proportion of refuge and survivorship of pest subpopulations corresponding to the genotypes *SS*, *RS* and *RR* in the transgenic cotton (Table 12.11). Low- (LD) or high-dose (HD) scenarios were represented by changing survival rates of susceptible (*SS*) and/or heterozygote (*RS*) genotypes. The *CriticalFreq* was set to 0.50, the *EndCoef* was assumed to be zero and

Table 12.9. Input parameters of the deterministic Caprio model (Caprio, 1998b).

Parameter	Description ^a
<i>InitialFreq</i>	<i>R</i> allele initial frequency in the target pest population
<i>CriticalFreq</i>	<i>R</i> allele critical frequency
<i>RefSurv</i>	Target pest immature-stage survivorship rate from an insecticide applied in the refuge
<i>EndCoef</i>	Endogamy coefficient in the target pest population
<i>SurvSS</i>	Immature stage survivorship of the target pest <i>SS</i> (homozygous susceptible) subpopulation in the refuge
<i>SurvRR</i>	Immature stage survivorship of the target pest <i>RR</i> (homozygous resistant) subpopulation in the refuge
<i>SurvRS</i>	Immature stage survivorship of the target pest <i>RS</i> (heterozygous) subpopulation in the refuge
<i>PreDisp</i>	Premating dispersal index
<i>PosDisp</i>	Postmating dispersal index

^aThe range for all the parameters is the interval [0,1].

Table 12.10. Characterization of the three broad scenarios for potential Bt cotton-growing in regions of Brazil.

Scenario characterization	Scenario 1	Scenario 2	Scenario 3
Crops	Bt and non-Bt cotton (only)	Bt and non-Bt cotton, non-Bt maize (only)	Bt and non-Bt cotton, Bt and non-Bt maize
Bt genes in cotton	<i>cry1Ac</i> + <i>cry2Ab</i>	<i>cry1Ac</i> + <i>cry2Ab</i>	<i>cry1Ac</i> + <i>cry2Ab</i>
Bt genes in maize	None	None	<i>cry1Ab</i> ^a , <i>cry1F</i> ^b or <i>vip3A</i> ^c
Bt-cotton toxin(s) potency	HD against <i>H. virescens</i> , <i>P. gossypiella</i> and <i>A. argillacea</i> ; LD against <i>S. frugiperda</i>	HD against <i>H. virescens</i> , <i>P. gossypiella</i> and <i>A. argillacea</i> ; LD against <i>S. frugiperda</i>	HD against <i>H. virescens</i> , <i>P. gossypiella</i> and <i>A. argillacea</i> ; LD for <i>S. frugiperda</i>
Bt-maize toxin(s) potency	None	None	^a LD against <i>S. frugiperda</i> ^b HD against <i>S. frugiperda</i> ^c Unknown potency against <i>S. frugiperda</i>

HD = high dose, LD = low dose.

^aPotency of *cry1Ab*.

^bPotency of *cry1F*.

^cPotency of *vip3A*.

Table 12.11. Number of pest generations until resistance (N^*) for several high-dose (HD) scenarios (> 95% mortality of heterozygotes relative to susceptibles) resulting from combination of partial recessiveness levels (expressed by SS and SR survival indexes), refuge size and R allele initial frequency ($InitialFreq$). Number of years until resistance for each pest species was calculated by dividing N^* by the number of generations in cotton (Table 12.3).

Scenario	$InitialFreq$	Survivorship			Refuge area (%)	N^*	Number of years		
		SS	RS	RR			<i>A. argillacea</i>	<i>P. gossypiella</i>	<i>H. virescens</i>
HD1	0.0001	0.01	0.02	1	10	22	7.3	4.4–7.3	7.3–11
HD2	0.0001	0.01	0.02	1	20	35	11.7	7–11.7	11.7–17.5
HD3	0.0001	0.01	0.02	1	50	106	35.3	21.2–35.3	35.5–53
HD4	0.0001	0.01	0.06	1	10	6	2.0	1.2–2	2.0–3
HD5	0.0001	0.01	0.06	1	20	9	3.0	1.8–3	3–4.5
HD6	0.0001	0.01	0.06	1	50	25	8.3	5–8.3	8.3–12.5
HD7	0.0001	0.001	0.002	1	10	63	21.0	12.6–21	21–31.5
HD8	0.0001	0.001	0.002	1	20	131	43.7	26.2–43.7	43.7–65.5
HD9	0.0001	0.001	0.002	1	50	492	164.0	98.4–164	164–246
HD10	0.0001	0.001	0.006	1	10	8	2.7	1.6–2.7	2.7–4
HD11	0.0001	0.001	0.006	1	20	18	6.0	3.6–6	6.0–9
HD12	0.0001	0.001	0.006	1	50	98	32.7	19.6–32.7	32.7–49
HD1	0.001	0.01	0.02	1	10	4	1.3	0.8–1.3	1.3–2
HD2	0.001	0.01	0.02	1	20	7	2.3	1.4–2.3	2.3–3.5
HD3	0.001	0.01	0.02	1	50	27	9.0	5.4–9	9–13.5
HD4	0.001	0.01	0.06	1	10	4	1.3	0.8–1.3	1.3–2
HD5	0.001	0.01	0.06	1	20	5	1.7	1–1.7	1.7–2.5
HD6	0.001	0.01	0.06	1	50	15	5.0	3.0–5	5–7.5
HD7	0.001	0.001	0.002	1	10	4	1.3	0.8–1.3	1.3–2
HD8	0.001	0.001	0.002	1	20	6	2.0	1.2–2	2.0–3
HD9	0.001	0.001	0.002	1	50	38	12.7	7.6–12.7	12.7–19
HD10	0.001	0.001	0.006	1	10	4	1.3	0.8–1.3	1.3–2
HD11	0.001	0.001	0.006	1	20	6	2.0	1.2–2	2.0–3
HD12	0.001	0.001	0.006	1	50	31	10.3	6.2–10.3	10.3–15.5

the dispersal indexes, *PreDisp*, *PosDisp* were set to their maximum values (1.0), which assume that the refuge layout allows complete mixing of adults coming from refuge and Bt-crop areas before mating and complete postmating dispersal of females for oviposition. We assumed that the refuge would be sprayed with non-Bt insecticides with efficacy of 80% ($RefSurv = 0.20$). The alternative refuge sizes considered for simulation were 10%, 20% or 50%, based on refuge recommendations adopted in the USA and Australia. The number of pest generations until resistance for high- (Table 12.11) and low-dose scenarios (Table 12.12) was estimated. In some of the high-dose simulations (Table 12.11), resistance evolved in less than 20 years. For these cases, the genetics of resistance of these hypothetical toxins suggests that they would be poorly suited for use in Bt cotton, because it would be difficult to manage resistance.

The correspondence between these simulations and the broad scenarios above (1,2) is determined by the potency of Bt toxin(s) expressed in the cotton/maize system, the target pests that are at resistance risk and the number of generations of these pests in cotton and/or maize. For example, for the high-dose species (*A. argillacea*, *P. gossypiella* and *H. virescens*), scenarios 1, 2 and 3 are equivalent and a 20% refuge is projected to give > 20 years of durability under HD8 conditions (Table 12.11), namely the initial frequency of resistance is < 0.0001, *SS* survival is < 0.001 and *RS* survival is < 0.002 (nearly completely recessive). These survival rates might be expected for effective high-dose events. A 50% refuge gives > 20 years of durability under wider conditions, *SS* survival < 0.01 and *RS* survival < 0.02. Both require that the initial frequency of resistance be low (< 0.0001).

The low-dose cases correspond to *S. frugiperda* (Table 12.12). For those scenarios, *SurvSR* was set to values corresponding to different functional-dominance levels ranging from partial recessiveness to partial dominance (0.06, 0.10, 0.525 or 0.70), keeping *SurvSS* = 0.05 and *SurvRR* = 1.00. The model gives similar results for Scenarios 1 and 2 (Table 12.12) because they differ only in that maize can be a refuge under Scenario 2, while only cotton is the refuge under Scenario 1. Thus, larger refuges will be more readily obtained under Scenario 2 compared with Scenario 1. Under these simulations, the 50% refuge would likely provide about 20 years of durability when initial resistance frequency < 0.0001, for *SS* survival < 0.05 and *RS* survival < 0.10. These might be reasonable survival levels for an efficacious low-dose event. Scenario 3 is more complicated. If Bt maize is produced with a Cry1Ab event, it will represent a low dose for *S. frugiperda* and there will likely be cross-resistance to Cry1Ac (Tabashnik *et al.*, 2000b). In this case, selection for similar resistance alleles will occur in both cotton and maize, and the time to resistance will be reduced according to the number of generations of selection in maize. If Bt maize is produced with a Cry1F event, it will likely be high dose for *S. frugiperda* and there may be no cross-resistance. In this case, the maize refuge for Cry1F and the cotton refuge for Cry1Ac could function together as a refuge for both toxins, and selection for resistance to Cry1Ac would only occur during the two generations in cotton. If Bt maize is a Cry1F event and there is cross-resistance between Cry1Ac and Cry1F, then the evolutionary dynamics will be more complex but faster than the case where

Table 12.12. Number of pest generations until resistance (N^*) for several low-dose (LD) scenarios resulting from combination of R allele initial frequency (*InitialFreq*), refuge size and functional dominance (*DFRes*) levels (PR, partial recessiveness; CD, codominance; PD, partial dominance) expressed by SS and SR survival indexes. Number of years until resistance is calculated for *Spodoptera frugiperda* by dividing N^* by the number of generations on cotton (Table 12.3; no Bt maize and high-dose (HD) Bt maize) or cotton and maize (LD Bt maize).

Scenario	<i>Initial Freq</i>	<i>DFRes</i> level	Survivorship			Refuge area (%)	N^*	Number of years			
			SS	RS	RR			No maize	No Bt maize	LD Bt maize ^a	HD Bt maize ^b
LD1	0.0001	PR	0.05	0.06	1	10	41	20.5	20.5	10.3	20.5
LD2	0.0001	PR	0.05	0.06	1	20	54	27.0	27.0	13.5	27.0
LD3	0.0001	PR	0.05	0.06	1	50	125	62.5	62.5	31.3	62.5
LD4	0.0001	PR	0.05	0.10	1	10	16	8.0	8.0	4.0	8.0
LD5	0.0001	PR	0.05	0.10	1	20	19	9.5	9.5	4.8	9.5
LD6	0.0001	PR	0.05	0.10	1	50	39	19.5	19.5	9.8	19.5
LD7	0.0001	CD	0.05	0.525	1	10	5	2.5	2.5	1.3	2.5
LD8	0.0001	CD	0.05	0.525	1	20	6	3.0	3.0	1.5	3.0
LD9	0.0001	CD	0.05	0.525	1	50	9	4.5	4.5	2.3	4.5
LD10	0.0001	PD	0.05	0.700	1	10	5	2.5	2.5	1.3	2.5
LD11	0.0001	PD	0.05	0.700	1	20	5	2.5	2.5	1.3	2.5
LD12	0.0001	PD	0.05	0.700	1	50	8	4.0	4.0	2.0	4.0
LD1	0.001	PR	0.05	0.06	1	10	8	4.0	4.0	2.0	4.0
LD2	0.001	PR	0.05	0.06	1	20	12	6.0	6.0	3.0	6.0
LD3	0.001	PR	0.05	0.06	1	50	34	17.0	17.0	8.5	17.0
LD4	0.001	PR	0.05	0.10	1	10	6	3.0	3.0	1.5	3.0
LD5	0.001	PR	0.05	0.10	1	20	8	4.0	4.0	2.0	4.0
LD6	0.001	PR	0.05	0.10	1	50	18	9.0	9.0	4.5	9.0
LD7	0.001	CD	0.05	0.525	1	10	4	2.0	2.0	1.0	2.0
LD8	0.001	CD	0.05	0.525	1	20	5	2.5	2.5	1.3	2.5
LD9	0.001	CD	0.05	0.525	1	50	7	3.5	3.5	1.8	3.5
LD10	0.001	PD	0.05	0.700	1	10	4	2.0	2.0	1.0	2.0
LD11	0.001	PD	0.05	0.700	1	20	4	2.0	2.0	1.0	2.0
LD12	0.001	PD	0.05	0.700	1	50	6	3.0	3.0	1.5	3.0

^aAssumes cross-resistance between Cry1Ac and Cry1Ab, and two generations per year in maize.

^bAssumes no cross-resistance between Cry1Ac and Cry1F. If there is cross-resistance, then resistance evolution will be faster and determined by evolution in

there is no cross-resistance. In this case, it will be essential to consider resistance both to Cry1Ac and Cry1F.

For both the high-dose and the low-dose simulations (Tables 12.11 and 12.12), we have assumed that all adults disperse maximally. As discussed below and indicated in previous modelling efforts (Comins, 1977; Caprio, 2001; Ives and Andow, 2002), reduced movement can significantly extend the time to resistance. Several of the target pests are unlikely to disperse maximally during all generations, so these simulation results are likely to represent the worst possible case.

Probabilistic approach

The probabilistic model developed by Maia and Dourado Neto (2003) was based on Caprio's deterministic model. Uncertainty associated with the initial frequency of resistance (*InitialFreq*) was incorporated using Monte Carlo methods (Hoffman and Kaplan, 1999; Hayse, 2000; Abrahamsson, 2002). Initial resistance frequency can be characterized by a probability-distribution function (e.g. truncated normal, uniform, triangular), referred to as the input-parameter distribution. Using Monte Carlo methods, a sample of *InitialFreq* values is taken from the input distribution. We will then run the deterministic model for each *InitialFreq* sampled value, producing in this way an output distribution for *RFreq* at the end of each pest generation. Such a probabilistic approach allows prediction of resistance risk (probability of *FreqR* exceeding a critical value) over time. For details, see Maia (2003) and Maia and Dourado-Neto (2004).

Scenario 1. High-dose Bt cotton, no alternative hosts (Box 12.2)

This strategy is appropriate for the North-east and Meridian regions, where *S. frugiperda* is not an important pest (Table 12.1), and the presently available

Box 12.2. Scenario 1. High-dose Bt cotton, no alternative hosts for target pests.

Suitable for North-east and Meridian regions.

Non-Bt cotton is the only refuge.

Provisional requirements:

- 20% of total cotton area to be planted to non-Bt cotton varieties.
- Bt cotton fields must be located within 1.5 km of their corresponding non-Bt cotton refuge fields.
- Refuge fields must be at least 60 rows wide.
- Refuges can be sprayed with insecticides (cannot be sprayed with Bt products).

Convene regional working groups to formulate appropriate new refuge requirements based on:

1. *G. barbadense* and *G. hirsutum* var. *marie-galante* as potential refuges, including cultivated, feral, volunteer, dooryard and landrace populations. In addition, they should consider how gene flow would affect these refuges (see Johnston *et al.*, Chapter 11, this volume).
2. Geography and extent of use of Bt cotton.

Bt cottons express a high dose for the remaining target Lepidopteran pests (Table 12.5). In these regions, we consider non-Bt cotton as the only suitable refuge.

There are four key provisional requirements for this strategy. First, 20% of the total cotton area should be planted to non-Bt cotton varieties (Table 12.11). This 20% refuge recommendation is consistent with other model results (Andow and Hutchison, 1998; Onstad and Gould, 1998; Roush, 1998; Caprio, 2001; Ives and Andow, 2002; Tabashnik *et al.*, 2003b).

Second, every Bt-cotton field should be located within 2 km of a non-Bt cotton refuge field, which was discussed in the section on 'resistance-management practices' above. Third, the refuge fields should be at least 60 rows wide. The purpose of this recommendation is to reduce movement of females from the refuge to the Bt fields, so that females are more likely to oviposit in the refuge. While this increases the likelihood that adults emerging from the Bt field will mate with each other, this is more than offset by the reduction in selection caused by the non-random oviposition (Ives and Andow, 2002). For example, for the high-dose example HD2 (Table 12.11), resistance occurred in 35 generations. If we assume that 80% of the adults that emerged from the refuges mated globally at random, and of the 20% that mated locally at random, 50% of their eggs were laid in the refuge; then 56 generations were required before resistance occurred. When the non-random oviposition parameter was increased to 80%, then the time to resistance was slowed to 77 generations. Increasing the minimal width of the refuges increases non-random oviposition, which is predicted to decrease the rate of resistance evolution. Fourth, refuges can be sprayed with insecticides but cannot be sprayed with Bt products, as was described in the section on 'resistance-management practices' above.

It will be important to convene working groups in the North-east and Meridian regions to formulate new refuge requirements based on *Gossypium hirsutum* var. *latifolium*, *Gossypium barbadense* and *G. hirsutum* var. *marie-galante*. Especially in the North-east, *G. barbadense* and *G. hirsutum* var. *marie-galante* occur as cultivated, feral, volunteer, dooryard and landrace populations (see Johnston *et al.*, Chapter 11, this volume). The potential effect of gene flow to the feral, volunteer, dooryard and landrace populations should also be considered. In addition, it may be possible to use information on the geography and extent of use of Bt and non-Bt cotton to modify requirements. For example, if the technology is not used very much by growers and refuges occur by happenstance, then it might become possible to relax some refuge requirements.

Scenario 2. High/low-dose Bt cotton, only non-Bt maize (Box 12.3)

This strategy is appropriate for the Midwest region, where *S. frugiperda* is an important pest (Table 12.1). The presently available Bt cottons express a low dose for *S. frugiperda* and a high dose for the remaining target Lepidopteran pests (*H. virescens*, *P. gossypiella* and *A. argillacea*; Table 12.5). Here, non-Bt cotton is the only suitable refuge for the other species, but maize could be an effective refuge for *S. frugiperda*.

Box 12.3. Scenario 2. Bt and non-Bt cotton, non-Bt maize (only).

Potentially suitable for Midwest region.

Non-Bt cotton and non-Bt maize can be used as refuges.

Assumptions

Maize contributes substantially as a refuge for susceptible *S. frugiperda*.

Provisional requirements:

- Two Bt toxins in cotton: Cry1Ac/Cry2Ab.
- 20% of total cotton area must be planted to non-Bt cotton varieties.
- No Bt plants should be more than 1.5 km from their refuge area.
- Refuge areas must be at least 60 rows wide.
- Refuges can be sprayed with insecticides (cannot be sprayed with Bt products).

There are five key provisional requirements for this strategy. First, only two-gene Bt cotton with both Cry1Ac and Cry2Ab should be used. The one-gene Cry1Ac cotton should not be used in Brazil. The two-gene Bt cotton ensures that Bt cotton will act as a high dose against *H. virescens*, *P. gossypiella* and *A. argillacea*, and provides added resilience to the resistance-management strategy (Roush, 1998; Zhao *et al.*, 2003). Unfortunately, this two-gene cotton still expresses a low dose for *S. frugiperda*, so resistance management must be aimed at this weak link.

Second, 20% of the total cotton area should be planted to non-Bt cotton varieties. This is appropriate for the three high-dose species (*H. virescens*, *P. gossypiella* and *A. argillacea*) as discussed in Scenario 1 above (Table 12.11). For low-dose situations, such as *S. frugiperda*, a larger refuge is required. Specifically, our model results suggest that a 50% refuge is needed to ensure a sufficient delay in the time to resistance in this species (Table 12.12). Because autumn maize is an effective refuge for *S. frugiperda*, and we have assumed that none of it is Bt maize, the entire autumn maize crop supplements the 20% non-Bt cotton refuge. It is expected that maize will make up the difference in the needed refuge for *S. frugiperda*.

The remaining conditions are similar to those for Scenario 1. Every Bt-cotton field should be located within 2 km of a non-Bt cotton refuge field; the cotton refuge fields should be at least 60 rows wide; and cotton and maize refuges can be sprayed with insecticides, but cannot be sprayed with Bt products. In addition, we suggest that refuges should preferentially be within Bt-cotton fields. By using embedded refuges, growers may be more likely to manage the refuge in a similar way to the rest of the cotton crop. They may be less likely to spray it unless they are also treating the Bt-crop, and irrigation, crop scouting, etc., are all likely to be similar. By treating them the same, it is more likely that the refuge will function effectively.

Scenario 3. High/low-dose Bt cotton with Bt and non-Bt maize (Box 12.4)

This strategy is appropriate for the Midwest region, where *S. frugiperda* is an important pest (Table 12.1). This is similar to the previous scenario, except that some of the maize refuge for *S. frugiperda* is likely to be ineffective

Box 12.4. Scenario 3. High/low-dose Bt cotton with Bt and non-Bt maize.

Potentially suitable for Midwest region.

Non-Bt cotton and non-Bt maize can be used as refuges.

Assumptions

We are unable to predict the impact of Bt maize on resistance evolution of *S. frugiperda* at this time. Thus, a trigger has been established for refining refuge requirements in cotton on a regional basis in cases where Bt crops gain a 50% market share.

Provisional requirements

- Two Bt genes in cotton – Cry1Ac + Cry2Ab.
- 20% of total cotton area must be planted to non-Bt cotton varieties – halt expansion of Bt crops and refine refuge requirements for cotton if the total percentage of Bt crops in any state equals or exceeds 50% of the planted crops during any month.
- Bt fields must be located within 1.5 km of their corresponding refuge fields.
- Refuge fields must be at least 60 rows wide.
- Refuges can be sprayed with insecticides (cannot be sprayed with Bt products).

Convene regional working group to formulate appropriate new refuge requirements based on:

1. New information on production of susceptible *S. frugiperda* in refuges and wild hosts.
2. New information regarding survival of *S. frugiperda* in Bt crops.
3. Geography and extent of use of Bt cotton.

because it will be Bt maize and hence will not produce unselected moths. This means that additional requirements on refuge size must be considered.

At this time we are unable to predict the impact of Bt maize on resistance evolution of *S. frugiperda*. There are three Bt genes which may be deployed in maize and that vary in their dose against *S. frugiperda*: Cry1Ab – low dose; Cry1F – high dose (Waquil *et al.*, 2004); Vip3A – high dose. Resistance-management requirements in maize will depend on the relative use of these Bt genes. For example, if the low-dose maize becomes prevalent and cross-resistance is a possibility, it may be necessary to impose an area cap on the use of all Bt crops of say 50% of the crop area. If high-dose maize becomes prevalent, then the relative survival of *S. frugiperda* on all possible hosts may play an important role in determining refuge requirements. Thus, a trigger has been established for refining refuge requirements in cotton on a regional basis in cases where Bt maize gains greater than 50% market share.

The provisional requirements for this scenario are the same five requirements as for scenario 2, except that the refuge requirement is altered to take into account the use of Bt maize. The refuge should remain as conventional cotton at 20% of the total cotton area, which is appropriate as a high-dose strategy for the three species (*H. virescens*, *P. gossypiella* and *A. argillacea*) as discussed in Scenario 1 above. However, if the total area of Bt cotton and Bt maize in any state equals or exceeds 50% of the planted cotton and maize

during any month, the expansion of Bt crops should be halted and the refuge requirements should be refined on the basis of the new information. This will ensure at least a 50% refuge for *S. frugiperda*.

It will be important to convene a representative working group for the Midwest region to formulate appropriate new refuge requirements based on research data on the production of *S. frugiperda* from refuges, wild hosts and Bt crops. In addition, it may be possible to use information on the geography and extent of use of Bt and non-Bt cotton and Bt and non-Bt maize to modify requirements.

New kinds of Bt cotton with Bt and non-Bt maize

In addition to the Cry1Ac and Cry1Ac/Cry2Ab Bt cottons, which are now commercially produced in some countries outside of Brazil, several new kinds of Bt cotton are under development. One is based on combining Cry1F and Cry1Ac (Widestrike™, from Dow AgroSciences), and another is based on combining Vip3A with a Cry1A toxin (Syngenta). If Cry1F and Vip3A are expressed at high enough concentrations that they work as high-dose toxins against *S. frugiperda*, then these new combinations would act as high-dose toxins against all four target Lepidopteran pests of Brazilian cotton. Resistance management for these events could follow Scenario 1 described above.

Methods to involve stakeholders, especially growers

It is vitally important that stakeholders, particularly growers, are intimately involved in the implementation of resistance-management strategies. The initial reaction of Brazilian cotton producers is often 'resistance management might work fine in the USA or Australia, but it can't be done here'. This position is understandable given the highly technical basis for the strategies discussed here, the uncertainties that surround them and the added costs in terms of time and money that they impose on growers. None the less, growers need to be convinced of the importance of rigorous management strategies if Bt cotton is to be used sustainably. To this end it will be crucial to involve growers in the regional working groups to develop alternative refuge approaches, and that significant investment is provided for effective extension and educational programmes to support implementation of a proactive resistance management for Bt cotton. All sectors of industry need to provide committed support to sustainable cotton-production systems that minimize environmental impact and optimize grower returns. Ongoing research and extension should support an IPM approach for cotton in which Bt cottons are one important component.

Design of transgenic plants for improved resistance management

We strongly recommend that Bt cotton be deployed as a pyramided two-gene product with both genes expressing a high dose. Pyramiding of additional transgenes can make heterozygotes phenotypically more like susceptible homozygotes and a pyramided plant provides additional safeguards, provided it is used with a refuge (Roush, 1998; Zhao *et al.*, 2003). In the absence of a refuge or when there is considerable cross-resistance, pyramiding is not of itself an effective resistance-management tool, but pyramided plants will still retain efficacy longer than single-gene Bt plants. The combination of Cry1Ac and

Cry2Ab is one example, providing a double high-dose against *H. virescens*, *P. gossypiella* and *A. argillacea*. The combination of Cry1Ac and Cry1F would not represent an acceptable pyramided variety because only one of the toxins provides a high-dose against each target pest. Cry1F appears to be highly active for *S. frugiperda*, but has little activity against the other species (Table 12.5).

Monitoring and response plans

The ultimate goal of monitoring is to obtain timely information that can be used to avoid or lessen the ramifications that pest resistance will have on the economics of agricultural production, and pesticide exposure of humans, wildlife and the environment. In the case of Bt cotton, monitoring information may be used to change the way that Bt cotton is deployed prior to widespread control failures due to resistance, or to justifying continuation of the current use strategy. Necessary steps in achieving this goal with Bt cotton in Brazil will include: (i) establishment of baseline susceptibility of target pests; (ii) detection and isolation of resistant phenotypes; (iii) investigation of putative field control failures; and (iv) documenting the use of Bt cotton and compliance with the resistance-management plan. These objectives necessitate the funding of centralized laboratory facilities in the major cotton-producing regions for the four target pests of Bt cotton. Additionally, Regional Bt-Cotton Resistance Working Groups should be convened annually in each region in order to evaluate the resistance-management strategy in light of new findings, to disseminate new research information and to identify the most critical regional research and education needs.

Monitoring for Bt resistance requires comparing the susceptibility of field-collected individuals with baseline susceptibility data and/or a susceptible laboratory colony. Centralized rearing and bioassay facilities should be identified, and appropriately funded and staffed for this purpose. All methodologies for testing should be standardized between laboratories and strictly adhered to. Monitoring should also involve some level of evaluation of compliance with refuge requirements in each state or region. Baseline susceptibility should be evaluated before the Bt crop is commercialized. Techniques for establishing baseline susceptibility to pesticides of various types are well established (Stone and Sims, 1993; Tabashnik, 1994; Robertson *et al.*, 1995; Sims *et al.*, 1996; Andow and Alstad, 1998; Gould, 1998; Marcon *et al.*, 1999).

Possible monitoring methods

Three kinds of monitoring are essential: crop-damage monitoring, resistance monitoring and compliance monitoring. Crop-damage monitoring involves observation by all parties involved (growers, consultants, extension staff, researchers) of increased damage to crops or numbers of insects. Larvae surviving in Bt-cotton fields should be collected and transported to a facility for bioassay by local farmers, NGOs or agricultural extension or research personnel. Resistance monitoring could involve one of several approaches. A signifi-

cant question is whether the monitoring programme can be sufficiently widespread and intensive to provide a realistic early warning of change in gene frequency for resistance.

All resistance monitoring requires collection of appropriate life stages from the field. In the case of Bt cotton, eggs, egg masses, larvae or adults (using light traps) could be collected and sent to a regional facility for bioassay using a phenotypic screen (Tabashnik *et al.*, 2000a) or for use in a more complicated, but more informative, F_2 screen (Andow *et al.*, 2000). Both methods require a discriminating dose methodology (Tabashnik *et al.*, 2000a) to distinguish resistant and susceptible phenotypes. If a resistant laboratory colony can be developed, then field-collected adults can be crossed with resistant individuals of known genotype and the progeny bioassayed using a discriminating dose to provide information on the genotype of the field-collected adults (Gould *et al.*, 1997). A field-based method could involve sentinel plots or samples could be taken from both refuge and Bt-cotton plots to compare larval densities as an indicator of emerging resistance (Venette *et al.*, 2000). All of these methods establish the frequency of resistance alleles. When conducted over successive years, they allow detection of regional change in resistance frequency. Andow and Ives (2002) compare the cost efficiency of these various methods for Bt maize; a similar comparison could be conducted for Bt cotton.

For refuge compliance (primarily large farms), producers should be required to keep detailed maps of the placement of Bt cotton and refuge crops. Compliance could be estimated by statistical sub-sampling of cotton fields to evaluate the degree of concordance between maps provided by the producer and the observed size and location of Bt and non-Bt fields. Collection of bolls could be made in Bt and non-Bt fields to corroborate designations or antibody tests could be conducted on plant tissue for the same purpose.

Possible responses

Provided it was possible to detect changes in resistance frequency early enough there could be three responses (see Andow and Ives, 2002):

1. Use of other control strategies that result in absolute or high mortality of a putative resistant population (e.g. pesticide overspray, inundative releases of parasitoids, destruction of crop).
2. Increase in size of structured refuge.
3. Eliminate planting of Bt crops in the affected area until susceptibility has returned. A decision on the area affected by resistance should be based on field surveys of damage in Bt crops coupled with knowledge of pest-dispersal propensity (Carrière *et al.*, 2001).

The capacity to respond will be highly dependent on rapid and accurate communication with all producers. It may be very difficult to withdraw Bt cotton from the market once released, particularly when in the hands of small-holders not subject to restrictions on seed availability. Mitigation plans should be formulated with and disseminated to growers through education channels as soon as possible, to illustrate the importance of following recommended resistance-management strategies.

Issues Addressed during Field Testing

Issues to initiate and complete prior to field testing:

- Determine dose of all Bt-cotton types against all four target Lepidoptera.

Issues to initiate prior to field release and continue during field testing:

- Search for resistance in natural populations.
- Conduct resistance monitoring.
- Develop and implement IPM methods (Box 12.1).
- Develop goals and strategies for stakeholder involvement.

Issues to initiate during field testing:

- Evaluate the utility of alternative refuges to *G. hirsutum* var. *latifolium* in the North-east region for *P. gossypiella* and *A. argillacea*, and in the Midwest for *S. frugiperda*, especially maize, sorghum and millet, and also wild hosts.
- Evaluate the distance requirement for *P. gossypiella* and examine pre- and postmating sex-specific movement for all high-dose species.
- Estimate the production of *S. frugiperda* from refuges, wild hosts and Bt crops in Midwest.
- Implement education programmes.
- Develop the monitoring programme.

1. Specify the monitoring/auditing methods and reporting procedures for: (i) establishment of baseline tolerance to the target crop; (ii) early detection of resistance; (iii) control failures due to resistance; and (iv) compliance to resistance-management plan.

2. How can these methods be integrated with other monitoring programmes used on the landscape?

3. Have any discussions occurred with representatives of the farm industry? Has there been any discussion with other stakeholder groups, including the technology industries, and community and environmental groups?

4. How have growers been integrated into monitoring?

5. Quality control. The quality of the data generated in the monitoring efforts may degrade with time. Methods for ensuring that data quality is maintained need to be specified for: (i) early detection of resistance; (ii) control failures due to resistance; and (iii) compliance to resistance-management plan.

6. Using monitoring information. Processing and reporting the monitoring results, and the linkage with the response strategies, need to be evaluated periodically for: (i) early detection of resistance; (ii) control failures due to resistance; and (iii) compliance to resistance-management plan.

- Develop the response plan.

1. Specify the elements of a response plan that is triggered by some monitoring threshold associated with the methods specified above (I.D.1).

2. How will these responses be integrated with the original proactive resistance-management plan?

3. Have any discussions occurred with farmers or other representatives of the farm industry?
4. Has there been any discussion with other stakeholder groups, including the technology industries and community and environmental groups?

Conclusions

There is no question that resistance is a potential risk to be considered with the deployment of Bt cotton. At the same time there is accumulating evidence to show that resistance risks can be managed (Tabashnik *et al.*, 2003b). Due to the complexity and dynamic nature of Brazilian cropping systems, appropriate methods for evaluating resistance risks and their management are necessary. For that reason we evaluated a range of different scenarios to accommodate the possible deployment of Bt cotton and Bt maize. The suite of pest Lepidoptera to consider and their differing sensitivities to Bt proteins further complicates the issues to be considered.

Our overview of the Brazilian agricultural system, the interactions of crops and pests and the conclusions reached here for the resistance-management needs of Bt-cotton deployment were the result of discussions among scientists from different countries, including Brazil. While we identified many gaps and research needs, we conclude that it is possible to formulate reasonable recommendations to minimize the resistance risk for Bt cotton in Brazil. These recommendations should serve as a starting point for a comprehensive research and extension effort to fill the many gaps and commence the critical task of educating growers about the serious need for resistance management, if the potential benefits of Bt cotton are to be realized and sustained. We reiterate that Bt cotton can provide an important component for IPM approaches to cotton production and, despite the added complexity this might impose on growers, the environmental and economic benefits will justify such investment.

For Brazil, the critical first steps are to critically evaluate the efficacy of all Bt-cotton types against all four target Lepidoptera through regulated small-scale trials. At the same time, research should establish the baseline tolerance of field populations to relevant Bt proteins using well-established techniques. This information will serve as the basis for future monitoring programmes and may also uncover resistant individuals that can form the basis for selection experiments to establish resistant strains in the laboratory.

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