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The Present and Future Role of Insect-Resistant Genetically Modified Maize in IPM

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Keywords
host plant resistance, gene flow, refuge

Disciplines
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Chapter 5
The Present and Future Role of Insect-Resistant Genetically Modified Maize in IPM

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Abstract Commercial, genetically-modified (GM) maize was first planted in the United States (USA, 1996) and Canada (1997) but now is grown in 13 countries on a total of over 35 million hectares (>24% of area worldwide). The first GM maize plants produced a Cry protein derived from the soil bacterium *Bacillus thuringiensis* (*Bt*), which made them resistant to European corn borer and other lepidopteran maize pests. New GM maize hybrids not only have resistance to lepidopteran pests but some have resistance to coleopteran pests and tolerance to specific herbicides. Growers are attracted to the *Bt* maize hybrids for their convenience and because of yield protection, reduced need for chemical insecticides, and improved grain quality. Yet, most growers worldwide still rely on traditional integrated pest management (IPM) methods to control maize pests. They must weigh the appeal of buying insect protection "in the bag" against questions regarding economics, environmental safety, and insect resistance management (IRM). Traditional management of maize insects and the opportunities and challenges presented by GM maize are considered as they relate to current and future insect-resistant products. Four countries, two that currently have commercialize *Bt* maize (USA and Spain) and two that do not (China and Kenya), are highlighted. As with other insect management tactics (e.g., insecticide use or tillage), GM maize should not be considered inherently compatible or incompatible with IPM. Rather, the effect of GM insect-resistance on maize IPM likely depends on how the technology is developed and used.

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5.1 Introduction

Maize, *Zea mays* (corn), is second only to rice, *Oryza* spp., as a world crop with over 140 million hectares planted. Annually, nearly 700 million metric tons (MMT) of grain are produced, primarily by the United States (USA; 39.5%), China (19.3%), Brazil (6.0%), Mexico (3.0%), Argentina (2.4%) and India (2.0%). As many as five other countries produce 10 MMT or more annually (FAOSTAT, 2007). Because many important pests of maize are lepidopteran stem borers, the first genetically-modified (GM) maize targeted a stem-boring pest, the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Crambidae). Genetically-modified maize resistant to *O. nubilalis* was first commercially grown in the USA (1996) and Canada (1997); a decade later GM maize is grown in 13 countries on a total of over 35 million hectares, (>24% of maize area worldwide). Available varieties of GM maize now include combinations of traits to suppress lepidopteran and coleopteran pests and to provide herbicide tolerance. Additional traits are being tested to improve the efficacy and spectrum of GM insect-resistant maize.

Most maize growers, however, rely on traditional crop protection practices to manage insects, including cultural, biological or chemical (insecticidal) methods. As use of GM maize continues to spread, growers must weigh the appeal of buying insect protection "in the bag" against questions regarding economics, environmental safety, and insect resistance management (IRM). To agricultural scientists, GM maize provides another valuable option to manage pests, but as GM maize expands to include combined aspects of protection from multiple pests, herbicide tolerance, drought tolerance and nutrient enrichment, costs and benefits become more difficult for growers to evaluate. To place the role of insect-resistant GM maize into a broader context, this chapter will discuss traditional management of maize insects and the opportunities and challenges presented by GM maize as they relate to current and future (potential) insect-resistant products. Information specific to different maize-producing countries, including the United States, Spain, China, and Kenya, will be discussed.

5.2 Maize Integrated Pest Management

As in other crops, management of insect (and weed or pathogen) pests has changed greatly over the last several decades. While growers once relied primarily on cultural methods and (natural) biological control, the efficacy of new synthetic insecticides from the 1940s–1970s increased reliance on chemical pest suppression (Casida and Quistad, 1998). Along with grower dependence on insecticides, insect resistance and concerns that insecticides were harming the environment (and human health) led entomologists to develop integrated pest management (IPM) strategies (Stern et al., 1959; Kogan, 1998; Kennedy, chapter 1). The basic goal of IPM is to achieve effective crop protection through the integration of appropriate control actions in a manner that provides economic benefits to growers and society, and benefits to the environment.

Maize IPM includes both preventative and responsive pest management tactics. Preventative tactics are used prior to the occurrence of the injurious stage of the pest and include host plant resistance (HPR), cultural controls (e.g., modified planting dates, crop rotation, tillage), and natural biological control. Responsive management is used when levels of pests occur that are likely to produce crop losses that exceed the costs of suppression. This requires accurate measures of insect populations and an understanding of the relationship between pest injury and the crop plant damage response (Pedigo et al., 1986). The related IPM concepts of economic injury level (EIL) and economic threshold (ET) are discussed by Stern et al. (1959) and in Kennedy (chapter 1). Primary elements of IPM to integrate with GM maize include host plant resistance, cultural control, biological control and limited use of insecticides.

5.2.1 Host Plant Resistance

Host plant resistance refers to the heritable plant qualities that reduce pest losses, in this case from maize-feeding insects. The HPR in modern maize hybrids is the product of efforts by entomologists and plant breeders to enhance resistance. Resistance traits are generally separated into those that lower plant attractiveness to insects (nonpreference or antixenosis), impair development (antibiosis) or allow a plant to compensate for injury by an insect (tolerance) (Painter, 1968). Insect-resistant GM plants and plants bred for HPR may be considered relatively similar because resistance traits are delivered by the plant and are preventative forms of pest management.

Breeding for HPR in maize has focused on lepidopteran and coleopteran pests. In the USA, such efforts have emphasized resistance to *O. nubilalis*, corn earworm, *Helicoverpa zea* (Lepidoptera: Noctuidae), and western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). Antibiosis from hydroxamic acids and flavonoid glycosides in maize has been key for managing pests. The hydroxamic acid DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) deters *O. nubilalis* leaf-feeding in vegetative-stage maize (Klun et al., 1967). DIMBOA also contributes to maize resistance to *D. v. virgifera*, leading to adults with low emergence, weight, and head-capsule width (Xie et al., 1990). Maysin, C-glycosyl flavone, in maize silks inhibits larval growth of *H. zea* and fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) (Waiss et al., 1979; Wiseman et al., 1992). Because of their efficacy, increased levels of DIMBOA and maysin are common in commercially-available maize germplasm (Barry and Darrah, 1991; Widstrom and Snook, 2001). Some resistance to *O. nubilalis* feeding and tunneling is related to elevated levels of cell-wall fiber and lignin (Coors, 1987; Beeghly et al., 1997) or fortification of the epidermal cell wall (Bergvinson et al., 1995). Epidermal leaf toughness also can be used to identify resistant varieties effective across a wide range of lepidopterans, including tropical pests of maize (Bergvinson et al., 1994). Most research related to maize resistance to *D. v. virgifera*, has focused on tolerance, as plants with large root systems or high compensatory root growth are more tolerant to *D. v. virgifera* feeding (Prischmann et al., 2007).

Because many traits related to maize resistance to insects are multigenic (Frey et al., 1997; Meyer et al., 2007), incorporating them into breeding populations has been difficult. However, the use of marker-assisted selection can facilitate breeding once genes for these traits are identified (McMullen et al., 1998). One option to enhance maize HPR and transgenic insect-resistance includes efforts to combine natural traits with transgenic traits for maximum effectiveness (Warnock et al., 2001).

5.2.2 Cultural Control

Farming practices are used to help manage insect pests. Effectively, insect injury is reduced by physically destroying pests (cultivation), or limiting access to crops over time (planting or harvest dates) and space (crop rotation). For example, prior to synthetic insecticides and maize HPR, *O. nubilalis* management was essentially cultural, with stalk destruction in the fall or moldboard plowing of maize stubble in the spring prior to planting (Caffrey and Worthley, 1927; Umeozor et al., 1985). However, such methods only are effective if conducted over large areas. Late or early maize planting also can be used to reduce *O. nubilalis* injury for the first and second generations, respectively (Mason et al., 1996; Pilcher and Rice, 2001).

Crop rotation of maize with non-host crops, especially soybean, is common practice in the US Corn Belt because (in addition to its agronomic benefits) it has largely controlled *Diabrotica* spp. (Chiang, 1973). However, *Diabrotica* spp. have adapted to crop rotation; in areas of Minnesota, Iowa and South Dakota, northern corn rootworms, *Diabrotica barberi*, have extended their diapause for two or more years (Krysan et al., 1986). In Illinois, Indiana, Ohio, Michigan and Wisconsin, *D. v. virgifera* defeat rotation by ovipositing in non-maize crops such as soybeans (Levine and Oloumi-Sadeghi, 1996). For areas of Europe where western corn rootworm has invaded (from the USA), rotations are mandatory when *D. v. virgifera* are detected as a step towards local eradication (Byrne, 2003). Interestingly, crop rotation can be a responsive tactic if densities of soil insects are known before the crops are planted (as suggested for *Diabrotica* spp. by Gillette, 1912).

5.2.3 Biological Control

Populations of many maize pests are naturally suppressed by beneficial predators, parasitoids and pathogens. Natural enemies may be used in importation (classical), conservation and augmentative biological control to control crop pests. Importation of parasitoids has been used in the USA in response to the accidental introduction of *O. nubilalis* in the early 1900s; the tachinid fly, *Lydella thompsoni* (Diptera: Tachinidae), and the wasps, *Macrocentrus cingulum* (Hymenoptera: Braconidae) and

Eriborus terebrans (Hymenoptera: Ichneumonidae), have become established but do not consistently maintain *O. nubilalis* populations below economic levels (Anonymous, 1990; Mason et al., 1994). Similarly, the parasitoid *Cotesia flavipes* (Hymenoptera: Braconidae) was introduced to Kenya from Pakistan (Omwega et al., 1995) to control the spotted stem borer, *Chilo partellus* (Lepidoptera: Crambidae). This pest was accidentally introduced into Africa before the 1930s and has become one of the most damaging pests of maize (Tams, 1932; Overholt et al., 1997). *Cotesia flavipes* has spread in Kenya and Tanzania, becoming the dominant larval parasitoid of stem borers in southeastern Kenya (Zhou and Overholt, 2001; see section 5.4.4).

Conserving natural enemies of maize pests involves limiting negative factors, such as insecticides, and implementing habitat management to improve factors that support natural enemies such as the provision of alternative food sources (Landis et al., 2000). For example, the parasitoid, *E. terebrans* appears to be influenced by the local landscape, causing greater parasitism of *O. nubilalis* near wooded edges compared to field interiors or non-wooded edges (Landis and Haas, 1992); the wooded edges may provide food resources and a favorable microhabitat for adult wasps (Dyer and Landis, 1997). Many growers use modified field edges such as riparian buffers, filter strips, shelterbelts and living snow fences, which increase landscape diversity and may provide habitat for the natural enemies of maize pests.

In recent decades, augmentative biological control has become more feasible through development of efficient rearing protocols, allowing responsive pest management through inundative or inoculative releases of parasitoids or predators. This strategy may be more useful in high-value maize (grown for seed or fresh consumption) than in maize grown for grain. The egg parasitoids, *Tricogramma* spp. (Hymenoptera. Trichogrammatidae), are used for the inundative control of *O. nubilalis* in Switzerland (Bigler, 1986) and *Ostrinia furnacalis* (Lepidoptera: Crambidae) in China (see section 5.4.3), but this strategy has not been cost-effective in the USA (Andow et al., 1995; Gardner et al., 2007). To control *D. v. virgifera*, entomopathogenic nematodes have been tested with mixed results (Munson and Helms, 1970; Wright et al., 1993; Ellsbury et al., 1996; Jackson, 1996). However, it may be possible to breed maize that is more attractive to entomopathogenic nematodes to help manage *D. v. virgifera* populations (Rasmann et al., 2005).

5.2.4 Insecticides

The basic concept of IPM suggests that insecticide use may be appropriate when other methods cannot adequately suppress pest populations. Further, the decision to apply insecticides should be based on the use of sampling information and economic decision levels (e.g., EIL and ET; Kennedy, chapter 1). In major maize-producing areas like the US Corn Belt, sampling information and decision levels for certain pests are well established (for an overview see Steffey et al., 1999). However, similar guidelines are deficient or unavailable for many key maize pests throughout the world, effectively prohibiting judicious insecticide use.

5.3 Insect-Resistant GM Maize: Opportunities and Challenges

As of 2007, all available insect-resistant GM maize express one or more *cry* genes derived from the soil bacterium *Bacillus thuringiensis* (*Bt*). Consequently, the discussion below pertains specifically to maize varieties including *Bt* traits (*Bt* maize), though once other types and combinations of toxins become available, the opportunities and challenges for IPM are likely to be similar. *Bacillus thuringiensis* crystal (Cry) proteins differ from most conventional insecticides because they are toxic to only a small range of related insects. This is because specific pH levels, enzymes, and gut receptors are required to solubilize, activate and bind a given Cry toxin (Federici, 2002; Ferré et al., chapter 3). This specificity and its label as a "natural insecticide" have contributed to the use of *Bt* as a biologically-based insecticide by many organic growers. Certainly the history of safe grower use of *Bt* treatments has contributed to its commercial success in *Bt* plants.

5.3.1 Current Varieties of Bt Maize

Cry proteins are categorized by their spectrum of activity. For maize pests, primary Cry proteins are Cry1 and Cry2 for Lepidoptera and Cry3 proteins for Coleoptera (Schnepf et al., 1998). Registered types of *Bt* maize, called events, are shown in Tables 5.1 and 5.2 and their relative efficacies against key maize pests are shown in Table 5.3. Prior to 2002, lepidopteran resistance and herbicide tolerance often were combined (stacked); now triple stacks with lepidopteran resistance, coleopteran resistance and herbicide tolerance are available. Although not the focus of this chapter, herbicide tolerance traits increasingly will be stacked with *Bt* maize. This technology allows growers to control weeds by spraying with an herbicide without harming the crop. Growers are attracted to this technology because the companion herbicides replace more persistent herbicides, they are convenient to apply, and they can be used in no-till and minimum tillage systems (USDA-ERS, 2002). In the near future, collaborations between biotechnology companies potentially will produce GM maize with as many as eight different traits, including herbicide tolerance and insect resistance (Dow AgroSciences, 2007). While this may present maize growers with new options, it may also complicate the decision-making process on what to plant, especially if growers cannot pick-and-choose any desirable combination of traits.

5.3.2 Opportunities

Insect-resistant GM maize offers both economic and environmental advantages over using conventional insecticides to manage certain maize pests. Responses of US maize growers indicate an awareness of both types of benefits, as growers cite

Table 5.1 Current and previously registered *Bt* maize products for lepidopteran-resistance (LR) and coleopteran-resistance (CR) commercialized in the USA, field maize unless indicated (http://www.epa.gov/pesticides/biopesticides/pips/pip_list.htm, accessed 2 January 2008)

Events	Insecticidal proteins	Traits	Companies	First registered	Trade names
176[a]	Cry1Ab	LR, PAT[b]	Ciba Seeds Mycogen Seeds	Aug 1995	KnockOut® NatureGuard®
Bt11	Cry1Ab	LR, PAT	Northrup King	Aug 1996	Agrisure™ CB
MON810	Cry1Ab	LR	Monsanto	Dec 1996	YieldGard®
DBT418[a]	Cry1Ac	LR, PAT	DeKalb Genetics	Mar 1997	Bt-Extra™
Bt11 (sweet corn)	Cry1Ab	LR, PAT	Novartis Seeds	Feb 1998	Attribute®
CBH351[a,c]	Cry9C	LR, PAT	Plant Genetic Systems	May 1998	StarLink™
TC1507	Cry1F	LR, PAT	Dow AgroSciences Pioneer Hi-Bred	May 2001	Herculex® I
MON863	Cry3Bb1	CR	Monsanto	Feb 2003	YieldGard® RW
MON863 X MON810[d]	Cry3Bb1, Cry1Ab	CR, LR	Monsanto	Oct 2003	YieldGard® Plus
DAS-59122-7	Cry34/35Ab1	CR, PAT	Dow AgroSciences Pioneer Hi-Bred	Aug 2005	Herculex® RW
TC1507 X DAS-59122-7[d]	Cry1F, Cry34/35Ab1	LR, CR, PAT	Dow AgroSciences Pioneer Hi-Bred	Oct 2005	Herculex® XTRA
MON88017	Cry3Bb1	CR, EPSPS[e]	Monsanto	Dec 2005	YieldGard® VT
MON810 X MON88017[d]	Cry1Ab, Cry3Bb1	LR, CR, EPSPS	Monsanto	Dec 2005	YieldGard® VT Triple
MIR604	modified Cry3A	CR	Syngenta Seeds	Oct 2006	Agrisure™ RW
Bt11 X MIR604[d]	Cry1Ab, modified Cry3A	LR, CR, PAT	Syngenta Seeds	Jan 2007	Agrisure™ CB/RW

[a] No longer registered
[b] PAT, phosphinothricin-N-acetyltransferase, which allows use of herbicide glufosinate ammonium (e.g., Liberty®)
[c] Registered for animal feed and non-food use only
[d] Stacks formed from conventional crosses
[e] EPSPS, 5-enolpyruvylshikimate-3-phosphate synthase, which allows use of herbicide glyphosate (e.g., Roundup®)

Table 5.2 *Bt* maize events for all countries that have commercially planted *Bt* maize with total annual grain maize production (MMT), total hectares (million), percentage *Bt* maize and year first produced for each country[a]

Country	MMT	Total ha	% Bt	1st Prod	Current commercial *Bt* maize events
USA	266.8	29.1	49[b]	1996	MON810, Bt11, TC1507, MON863, DAS-59122-7, MON88017, MIR604
Canada	9.2	1.2	49[c]	1997	MON810, Bt11, TC1507, MON863, DAS-59122-7, MON88017, MIR604
South Africa	9.6	3.1	44[d]	1997	MON810, Bt11
Argentina	15.9	2.5	63[d]	1998	MON810, Bt11, TC1507
Spain	4.2	0.4	21[e]	1998	MON810
France	14.3	1.7	1[e]	1998[f]	MON810
Portugal	0.7	0.1	<1[e]	1999[g]	MON810
Germany	3.7	0.4	<1[e]	2000	MON810
Honduras	0.5	0.3	<1[d]	2001	MON810
Philippines	5.1	2.5	5[d]	2003	MON810, Bt11
Uruguay	0.2	<0.1	<1[d]	2003	MON810, Bt11
Czech Republic	0.6	<0.1	<1[e]	2005	MON810
Slovakia	0.8	0.1	<1[e]	2006	MON810
Brazil	40.8	12.3	0	2008	MON810, Bt11

[a] MMT production, Total ha (million), average 2002–2006 (FAOSTAT, 2007)
[b] USDA-NASS, 2007
[c] Stratus Agri-Marketing Inc., 2006 figure
[d] James, 2007
[e] http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/191.eu_growing_area.html (accessed 16 January 2008), 2007 figures.
[f] no planting 2001–2004
[g] no planting 2000–2004

unique opportunities to protect yield and reduce handling (and use) of insecticides to explain their rapid adoption of *Bt* maize (Pilcher et al., 2002). Economic benefits in the USA from *Bt* maize depend on maize prices and levels of pest populations; in some years, planting *Bt* maize can be an economic disadvantage (Carpenter and Gianessi, 2001), but under typical conditions should provide increased profits to *Bt* maize growers (Sankula, 2006). Research with *Bt* maize in Spain and the Philippines (Demont and Tollens, 2004; Yorobe and Quicoy, 2006; Gómez-Barbero et al., 2008) also suggests growers gain financially from using transgenic insect control. Brookes and Barfoot (2006) estimated that in the USA from 1996 to 2005 the cumulative decrease in insecticide active ingredient (a.i.) use on *Bt* maize was 4% (6,400 MT). Most of the reduction in insecticide a.i. was from lepidopteran-active *Bt* maize. However, coleopteran-active *Bt* maize shows a much greater potential benefit in the near future, as insecticides used against *Diabrotica* spp. comprise 25–30% of the global total in maize (James, 2003; Rice, 2004).

Table 5.3 Important lepidopteran and coleopteran (*Diabrotica* spp.) maize pests in USA, China, Spain, Kenya, Argentina (Arg) and Philippines (Phil). Cry proteins are rated 4 (excellent control), 3 (good control), 2 (some control/suppression) and 1 (no effect).[a] The "x" indicates the pest occurs in that country

Maize pests	Common names	Family	Cry Proteins			USA	China	Spain	Kenya	Arg.	Phil.
Lepidopteran borers			Cry1Ab	Cry1F							
Busseola fusca	African maize stem borer	Noctuidae	2.5	2					x		
Chilo partellus	Spotted stem borer	Crambidae	3	3					x		x
Diatraea grandiosella	Southwestern corn borer	Crambidae	4	4		x					
Diatraea saccharalis	Sugarcane borer	Crambidae	4	4		x				x	
Ostrinia nubilalis	European corn borer	Crambidae	4	4		x		x			
Ostrinia furnacalis	Asian corn borer	Crambidae	4	4			x				x
Sesamia nonagrioides	Mediterranean corn borer	Noctuidae	3.5	3				x			
Other Lepidoptera											
Agrotis spp.	Cutworm	Noctuidae	1[b]	2.5[b]		x	x	x	x	x	x
Helicoverpa armigera	Corn earworm	Noctuidae	2.5	2			x	x	x		x
Helicoverpa zea	Corn earworm	Noctuidae	2.5	2		x				x	
Mythimna separata	Oriental armyworm	Noctuidae	3	i.d.[c]			x				
Pseudaletia unipuncta	Armyworm	Noctuidae	2	2		x	x	x		x	
Spodoptera exigua	Beet armyworm	Noctuidae	2	3.5			x		x		
Spodoptera frugiperda	Fall armyworm	Noctuidae	2	3.5		x				x	
Striacosta albicosta	Western bean cutworm	Noctuidae	2	3		x					
Coleoptera			Cry3Bbl	34/35[d]	mCry3A[e]						
Diabrotica spp.	Corn rootworms	Chrysomelidae	3	3	3	x				x	

[a] Approximate ratings pooled from the authors and industry experts; control might vary depending on event, hybrid and environment
[b] Tests conducted with black cutworm, *A. ipsilon*
[c] insufficient data
[d] binary Cry34Abl+Cry35Abl
[e] modified Cry3A

Another benefit of insect-resistant GM maize is reduced occurrence of ear molds. Because insect damage provides a site for infection by molds, *Bt*-protected maize can have lower levels of the *Fusarium* mycotoxins, fumonisin and deoxynivalenol (Munkvold and Hellmich, 1999; Dowd, 2000). Consequences of contamination with mold may be serious, as fumonisins can cause fatal leucoencephalomalacia in horses, pulmonary edema in swine, and cancer in laboratory rats. Economic analysis suggests that US farmers save $23 million annually through reduced mycotoxins (Wu et al., 2004), though mycotoxin reduction could be a significant health benefit in other parts of the world where maize is a diet staple (Wu, 2006a, b).

One more potential benefit of *Bt* maize is area-wide suppression of pest populations. There is increasing evidence that *O. nubilalis* populations in the US Corn Belt have been suppressed by *Bt* maize (Hellmich, 2006; Storer et al., chapter 10). This phenomenon could have implications for refuge and IRM (see section 5.3.3.3).

5.3.3 Challenges

Detractors of *Bt* maize suggest several challenges, including the potential for effects on non-target organisms and gene flow between *Bt* maize and non-*Bt* maize, to outweigh any benefits. Other issues to consider include whether insect resistance to *Bt* can be managed, and whether the use of insect-resistant GM maize conflicts with the basic principles of IPM.

5.3.3.1 Effects on Non-target Organisms

With regard to non-target organisms, no surprising effects have been observed with *Bt* maize, which confirms the specificity of the *Bt* proteins. Most studies in the USA, Europe and China suggest *Bt* maize has little if any impact on predators and parasitoids and, when compared with maize treated with chemical insecticides, *Bt* maize often results in increased biodiversity (Bourguet et al., 2002; Candolfi et al., 2003; Dutton et al., 2003; Bhatti et al., 2005a, b; Daly and Buntin, 2005; de la Poza et al., 2005; Dively, 2005; Pilcher et al., 2005; Romeis et al., 2006; Fernandes et al., 2007; Marvier et al., 2007; Wang et al., 2007; for general reviews see O'Callaghan et al., 2005; Romeis et al., 2008; chapter 4). Although maize is not a major source of pollen for honey bees, *Apis mellifera* (Hymenoptera: Apidae), the US Environmental Protection Agency (USEPA) requires information on possible effects of *Bt* maize on honey bees. Feeding studies suggest pollen from *Bt* maize has no effect on honey bee larvae or adults (Hanley et al., 2003; Babendreier et al., 2005; Rose et al., 2007; Duan et al., 2008). Specialist insects that depend on target pests are the exception to the generalization that *Bt* maize does not impact non-target organisms. This is particularly true for some parasitoids, which may become less abundant along with their herbivorous hosts (Pilcher et al., 2005; Romeis et al., chapter 4; Storer et al., chapter 10). Also, fewer saprophagous dipterans have been observed in *Bt* maize fields, which has been attributed to the indirect effect of reduced lepidopteran plant injury (Candolfi et al., 2003; Dively, 2005). Studies on possible effects of *Bt* maize on soil microorganisms also suggest little if any impact (Blackwood and Buyer, 2004; Devare et al., 2004; Thies and Devare, 2007).

Two groups of studies raised questions about the possible effects of Cry toxins expressed in *Bt* maize on non-target organisms. First, research on the predatory lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), indicated lacewing larvae were negatively affected when they fed on lepidopteran larvae that consumed *Bt* maize expressing the toxin Cry1Ab (Hilbeck et al., 1998). However, subsequent research showed *C. carnea* was not directly affected by the toxin, but indirectly by feeding on intoxicated, moribund prey (Dutton et al., 2002; Romeis et al., 2004; Rodrigo-Simón et al., 2006; Lawo and Romeis, 2008). Later, studies with larvae of the monarch butterfly, *Danaus plexippus* (Lepidoptera: Danaidae), suggested monarch populations would be reduced from feeding on milkweed leaves coated with *Bt* maize pollen (Losey et al., 1999; Jesse and Obrycki, 2000). Again, more thorough research indicated the likely impact *Bt* maize on monarch was negligible because of limited exposure and low toxicity of *Bt* maize pollen to monarch larvae (Hellmich et al., 2001; Oberhauser et al., 2001; Pleasants et al., 2001; Sears et al., 2001; Stanley-Horn et al., 2001; Zangerl et al., 2001; Wolt et al., 2003; Dively et al., 2004). *Bt* maize event 176, which produces a high level of *Bt* protein in the pollen, had acute effects on monarch larvae fed milkweed foliage containing levels of pollen commonly encountered in maize fields during pollen shed (Hellmich et al., 2001; Stanley-Horn et al., 2001; Zangerl et al., 2001), but this early type of *Bt* maize was an exception, had limited planting and is no longer commercially available. Most recently, a preliminary study suggests that *Bt* maize pollen or detritus might have negative effects on caddisfly larvae (Trichoptera) in streams located in or near *Bt* maize fields (Rosi-Marshall et al., 2007), but the risk was not well established.

5.3.3.2 Gene Flow

The transfer of genetic material between populations (i.e., gene flow) is often considered to be a potential problem between GM crops and their wild relatives. In most areas of the world producing GM maize, however, production is isolated from related species that could hybridize with *Z. mays*. Gene flow as an environmental concern is thus restricted to those areas where wild relatives of maize occur (e.g., Mexico). In addition, in some areas such as the European Union (EU), gene flow issues with GM maize usually involve cross pollination or seed contamination of non-GM maize. Some growers, particularly of organic maize, demand little or no contamination from GM pollen or seed and generally object to production of any GM maize. This has been a particularly controversial issue in Europe. In 2003, the EU stipulated that labeling of food or feed as genetically modified was not required unless GM material exceeded a 0.9% threshold (European Union, 2003a, b). This legislation set the stage for the coexistence of GM and non-GM crops, but isolation distances and other measures to limit mixing or GM and non-GM products needed to be defined. Most research indicates separation of a minimum of 50 m between GM and non-GM maize is adequate to restrict outcrossing to less than the 0.9% threshold (Brookes et al., 2004; Devos et al., 2005; Sanvido et al., 2008), but others suggest as little as 20 m may be adequate, especially if several rows of non-GM maize are used as a buffer around GM maize (Messeguer et al., 2006; Weber et al., 2007).

5.3.3.3 Insect Resistance Management

Insect pests, including maize insects, commonly have developed resistance to conventional insecticides when they are overused (Georghiou, 1986). Larvae of *D. v. virgifera* evolved resistance to soil-applied cyclodiene insecticides by the 1960s (Ball and Weekman, 1962) and adults evolved resistance to methyl parathion in the 1990s (Meinke et al., 1998). Consequently, scientists and growers are concerned that overuse of *Bt* maize could produce pests resistant to *Bt* toxins (Tabashnik, 1994; Gould, 1998; Frutos et al., 1999). Though maize stem borers have not evolved resistance to insecticides (perhaps because insecticide exposure is limited once larvae bore into the plant), several important lepidopteran pests have been selected for resistance to *Bt* toxins in the laboratory (Tabashnik, 1994; Ferré and Van Rie, 2002; Ferré et al., chapter 3), including *O. nubilalis* (Huang et al., 1999; Alves et al., 2006) and *O. furnacalis* (Xu et al., 2006). In the field, only the diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae), has evolved resistance to *Bt* sprays (Tabashnik et al., 1990) and with the possible exception of *S. frugiperda* resistance to Cry1F maize in Puerto Rico (Matten et al., chapter 2), no insects have evolved resistance to *Bt* crops (Tabashnik et al., 2003; Ferré et al., chapter 3).

Various strategies have been proposed for managing insect resistance to *Bt* maize, but currently the high-dose/refuge (HDR) strategy appears to be the most commonly recommend (Bates et al., 2005; Matten et al., chapter 2; Ferré et al., chapter 3). With this strategy, insects that feed on the *Bt* maize are exposed to an extremely high dose of toxin, which makes insect resistance functionally recessive (Gould, 1994). Refuges complement the high dose because they provide a population of susceptible insects that are not exposed to *Bt* toxin. Consequently, rare resistant moths that develop on *Bt* maize, instead of mating with each other, mate with the overwhelming number of susceptible moths from the refuge (Tabashnik and Croft, 1982; Gould, 1998). This process essentiality dilutes resistance genes and maintains a population of susceptible insects. This strategy should be effective as long as plants express a high dose of the toxin, genes conferring resistance are rare, and there are many insects from the refuge available to mate randomly with resistant insects (Gould, 1998). In addition to the biological factors, economic and social aspects of IRM cannot be ignored (Mitchell and Onstad, 2007; Hurley and Mitchell, 2007).

Studies have been conducted to establish baseline *Bt* susceptibility of maize pests in the USA (*O. nubilalis*, Marçon et al., 1999; *H. zea*, Siegfried et al., 2000; *D. v. virgifera*, Siegfried et al., 2005), European Union (*O. nubilalis*, González-Núñez et al., 2000; Saeglitz et al., 2006; *S. nonagrioides*, González-Núñez et al., 2000; Andreadis et al., 2007), and China (*O. furnacalis*, He et al., 2005). In general, these studies have found insect susceptibly to *Bt* varies little among populations.

If extensive planting of *Bt* maize results in an area-wide reduction of the pest population, then the size of *Bt* maize refuges could be adjusted to keep pest populations below EILs. Such an approach, however, would involve addressing IRM refuge requirements first so that insects do not evolve resistance. For example, the hypothetical insect pest in Fig. 5.1 (pre-*Bt* maize, A) is regularly above the EIL and requires annual IPM control measures. The use of *Bt* maize, however, could reduce the annual pest populations below the EIL. The question then becomes what percentages of *Bt* maize and corresponding non-*Bt* maize (or refuge) would keep the pest populations consistently below the EIL? In this example, insect populations resulting from a Refuge 1 strategy (B) are low, but from time to time they exceed the EIL, where the refuge maize might require treatment. On the other hand, insect populations from a Refuge 2 strategy (C) are consistently below the EIL. Obviously, growers would prefer a strategy that reduces or completely eliminates intervention. Such an approach, however, must be coordinated with IRM requirements because too little refuge could lead to pest resistance to *Bt* maize. But, hypothetically, for some pests there could be a balance between IRM requirements and reducing the need for control in refuge maize.

5.3.3.4 Conflicts with IPM Principles

A final challenge to consider for insect-resistant GM maize is the perception that current hybrids are used in ways that directly conflict with the underlying principles of IPM. For example, *Bt* maize varieties generally produce high levels of toxins

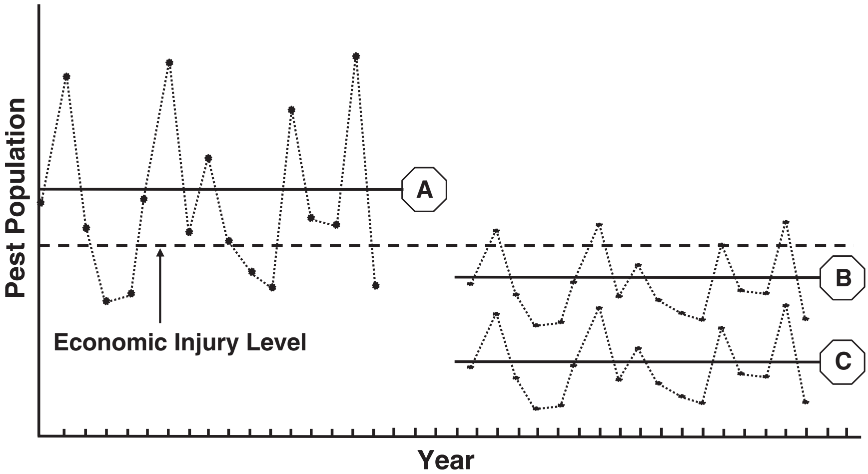

Fig. 5.1 Hypothetical equilibria for pest populations that are influenced by *Bt* maize. Populations vary annually but fluctuate either above the economic injury level (EIL) as in the pre-*Bt* maize pest equilibrium (A), near the EIL as in *Bt* maize with Refuge 1 (B), or well below the EIL as in Refuge 2 (C)

throughout the season whether pests are present or not. Similarly, the appeal of growing *Bt* maize as insurance against pest problems means that some growers will plant insect-resistant GM maize even if pests are not expected to reach damaging levels. Both of these points relate to the IPM concept that insecticides should only be used at times and locations where pests are expected to reach damaging levels. However, sustained high levels of plant resistance or unnecessary planting of resistant varieties have not been considered undesirable for HPR produced through conventional breeding. This suggests that perspectives on this issue are influenced by whether GM insect resistance is thought of as more similar to insecticides or to conventional host plant resistance. If insect-resistant GM maize is perceived as an insecticide, then such objections point out inappropriate uses of the technology. But if GM insect resistance is more accurately a form of host plant resistance, then objections that GM maize conflicts with IPM principles appear to be reflect a double standard for GM-derived plant resistance compared to conventional HPR.

Economic and practical constraints in production of hybrid maize seed also may result in GM maize traits being used when pests are not expected to reach damaging levels. This is a result of the fact that as the number of GM products increases, the number of GM-trait combinations increases geometrically. The high production costs for each hybrid (back-crossing into appropriate germplasm, etc.) and limited inventory space might compel seed providers to sell some stacked traits in areas where their use in pest management is not justified. For example, a grower may want to plant herbicide tolerant maize with *Diabrotica* spp. control, but does not need *O. nubilalis* control. If a stack containing all three is the only option, then the grower may be forced to accept the lepidopteran-active trait in the hybrid, but may or may not have to pay the associated technology fee. Does such a scenario promote the best IPM practice? If stacked maize products potentially lead to high use and compromise refuge requirements then, at least from an IRM perspective, this is not the best practice. On the other hand, is the use of an unneeded trait acceptable if IRM requirements are not compromised? As more products are developed, these situations will become more complex.

5.3.4 Future Types of Insect-Resistant GM Maize

Though commercially available insect-resistant maize varieties use single or multiple *Bt* (Cry) toxins to suppress lepidopteran and coleopteran pests, it seems likely that new technologies will appear continuously for several years. Two main areas of interest for future types of GM maize include developing insect-resistance products for additional pests and improving lepidopteran- and coleopteran-active products to delay the evolution of resistant insects.

To broaden the spectrum of insecticidal activity, maize varieties may include additional *cry* genes, vegetative insecticidal proteins (VIP), lectins, protease inhibitors, chitinases, RNA interference, and others (e.g., Baum et al., 2007; Malone et al., chapter 13). For example, the VIP proteins produced by *B. thuringiensus* show a different mode of action than Cry proteins (Estruch et al., 1996; Lee et al., 2003), and should allow management of black cutworm (*Agrotis ipsilon*; Lepidoptera: Noctuidae) and fall armyworm (*S. frugiperda*) (VIP3A; Lee et al., 2003). Other types of toxins likely will be useful in conferring insecticidal properties to more diverse maize pests; sucking insects like aphids and leafhoppers that are important virus vectors are likely targets.

Improving resistance to more effectively delay evolution of insects resistant to GM maize is a related research area. As in other crops, this may be accomplished by combining or "pyramiding" two or more toxins with different modes of action, including some of the relatively novel toxins noted above. Current commercial cotton varieties include multiple Cry toxins that target the same pest species (Bollgard II or Widestrike; Ferré et al., chapter 3; Naranjo et al., chapter 6). Experimental maize pyramids that target Lepidoptera include hybrids that produce VIP3A and Cry1Ab toxins (Dively, 2005) and hybrids that produce Cry2Ab2 toxin and a chimeric protein Cry1A.105 (USEPA, 2007). Of course, because the use of multiple, complementary toxins may delay resistance, it also may allow changes in the type or size of allowable refuges.

In general, it seems that combinations of multiple, complementary toxins will allow GM maize to protect against several arthropod pests and improve resistance management (Roush, 1998). Other strategies like the use of inducible promoters (which cause expression of traits in response to specific triggers) may be used to transform insect management in GM maize from a preventative strategy to a responsive one (Bates et al., 2005; Christou et al., 2006), perhaps eliminating some of the concerns noted above (see section 5.3.3.4).

5.4 Case Studies from GM and Non-GM Maize Producing Countries

The following case studies on the USA, Spain, China and Kenya provide a cross section of countries that use or are considering the use of *Bt* maize. Other early adopters of the GM technology include Canada, South Africa and Argentina, all of which produce >9 MMT of maize per year (Baute et al., 2002; James, 2003; Gouse et al., 2005, 2006; Trigo and Cap, 2006; FAOSTAT, 2007). Information also is available on the experiences of *Bt* maize growers in the Philippines (Yorobe and Quicoy, 2006).

5.4.1 United States of America Case Study

In 2007, USA growers harvested 34.8 million hectares of maize and produced 338 MMT of grain (USDA-NASS, 2007). Maize production is concentrated in the Corn Belt, especially Iowa, Illinois, Nebraska, Minnesota, and Indiana. In 1996, the first insect-resistant GM maize hybrids were sold, using *Bt* genes to suppress *O. nubilalis* and the southwestern corn borer, *Diatraea grandiosella* (Lepidoptera: Crambidae).

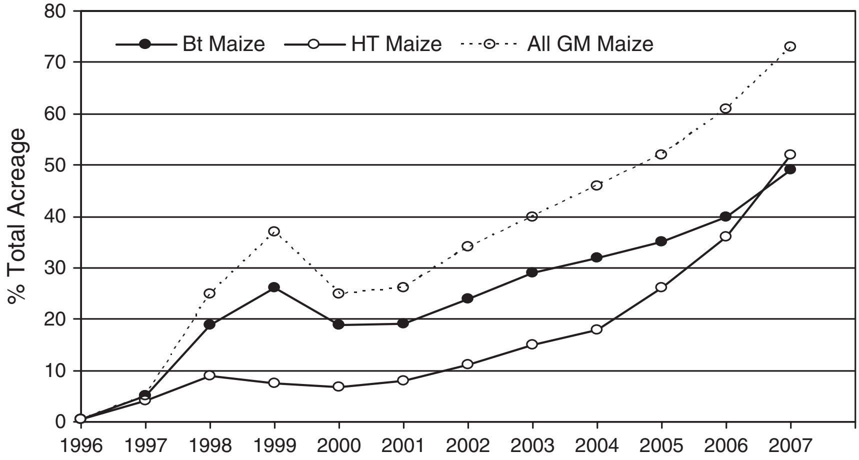

Fig. 5.2 Adoption of GM maize in the United States, 1995–2006. Data for insect-resistance (*Bt*) and herbicide-tolerance (HT) include stacked varieties that combine both types of GM traits (http://www.ers.usda.gov/Data/BiotechCrops, accessed 28 November 2007)

Adoption of transgenic maize in the USA has been rapid, especially after herbicide-resistant maize and *Bt* maize for the control of *Diabrotica* spp. were commercialized in 1997 and 2003, respectively (Fig. 5.2). Prior to *Bt* maize, host plant resistance, crop rotation and insecticides formed the foundation of IPM for key insect pests of maize in the Corn Belt. This case study though focuses on the most important Corn Belt pests, *O. nubilalis* and *Diabrotica* spp.

5.4.1.1 Major Insect Pests and Their Control

The European corn borer, accidentally introduced into the United States in the early 1900s, is the most important maize stem borer in the USA. The current range of *O. nubilalis* in North America covers the Corn Belt as well as southern states from Florida to east Texas, as far west as the Rocky Mountains, and into southern Canada. In the Corn Belt states, *O. nubilalis* is usually bivoltine, but there may be from one to four generations annually depending on latitude.

Prior to the introduction of *Bt* maize, cultural practices (i.e., changes to planting or harvest time, post-harvest stalk destruction) and HPR were major tools to reduce the devastating effects of *O. nubilalis* on maize yields. A combination of in-field monitoring of *O. nubilalis* and insecticide applications based on treatment thresholds could prevent losses of ~10–30% (Linker et al., 1990; Tollefson and Calvin, 1994; Mason et al., 1996). Many growers, however, elect not to use insecticides against *O. nubilalis* because applications must be timed after most eggs hatch but before larvae tunnel into the stalk (where they are protected from insecticides). Furthermore, most modern maize hybrids have some tolerance to *O. nubilalis* injury, so it is likely that without insecticide use, *O. nubilalis* usually represented a modest but chronic problem. In higher value crops, such as seed maize, popcorn and sweet corn (Shelton et al., chapter 9), management is more aggressive and in some cases may use biologically-based insecticides or biological control (e.g., Kuhar et al., 2003; Musser et al., 2006). Another stem borer, *D. grandiosella* can be a more destructive pest of maize than *O. nubilalis*. Southwestern corn borers were first reported as a pest of maize in 1913 and now are found from Arizona to Georgia and north to Missouri, and is an important maize pest in parts of Kansas, Missouri and Kentucky, where it can cause yield losses up to 50%, if not controlled (Chippendale and Sorenson, 1997).

It is debatable whether *O. nubilalis* or *Diabrotica* spp. have caused greater losses for US maize growers, but relative to insecticide use, the complex of western (*D. v. virgifera*), northern (*D. barberi*) and southern (*D. undecimpunctata howardi*) corn rootworms is unchallenged. In the USA estimates of insecticide a.i. applied annually to control this pest complex range from 2,400 to 3,500 MT (Gianessi et al., 2002; James, 2003; Rice, 2004). This represents approximately 60% of the total insecticides used on maize pests in the USA and, as mentioned previously, 25–30% of the insecticides used against maize pests worldwide.

Annual rotation of maize with other crops has been an effective management tool for *Diabrotic*a spp. Yet the high efficacy of chlorinated hydrocarbons against soil-dwelling insects, especially *D. v. virgifera*, has led many growers to plant maize continuously. This was especially the practice in areas of Nebraska and Kansas, where irrigated maize has high-yield potential. However, the development and spread of insecticide-resistant *D. v. virgifera* during the late 1950s made the need for new *Diabrotica* spp. management strategies clear. Subsequently, an understanding of the relationship between adult populations in one year and larval damage the following year allowed producers to assign a risk level to larval injury and use responsive, rather then preventative, tactics (Pruess et al., 1974; Stamm et al., 1985). Either crop rotation or application of an insecticide was recommended if adult populations of more than one per plant were detected the previous year. However, discovery of *Diabrotica* spp. resistance to crop rotation has undermined the crop rotation tactic in many parts of the Corn Belt (see section 5.2.2).

5.4.1.2 Current Use of GM Maize in the USA

Since 1996 the use of GM maize has increased rapidly in the USA. There was a dip in grower use in 2000, but this has been followed by a steady increase to almost 75% adoption in 2007 (Fig. 5.2). Use of lepidopteran-active *Bt* maize approaches or exceeds 50% of the total area of production through much of the Corn Belt, with highest concentrations in northwest Iowa, and southwest Minnesota. High use percentages also occur in parts of Kansas, Oklahoma, Texas, Pennsylvania and Maryland (Fig. 5.3). Commercial, coleopteran-active *Bt* maize, which has demonstrated high consistency in suppressing corn rootworms (Moellenbeck et al., 2001; Vaughn et al., 2005), was first planted in 2003. Since then the adoption of *Bt* maize in eastern Corn Belt states, such as Illinois and Indiana, has greatly increased (USDA-ERS, 2007), at least partially in response to rotation-resistance in *D. v. virgifera*.

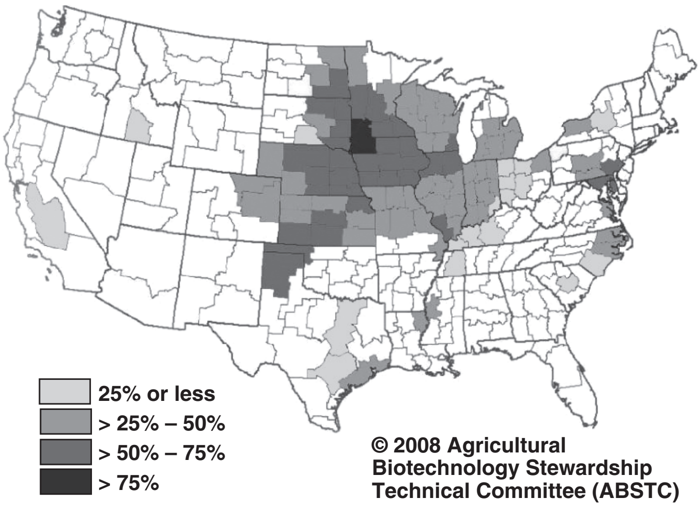

Fig. 5.3 Percentage of total maize hectares planted in 2006 to lepidopteran-active *Bt* maize hybrids in USA crop reporting districts in which > 40,468 hectares (100,000 acres) of maize were planted (Agricultural Biotechnology Stewardship Technical Committee)

The USEPA promotes IRM in *Bt* maize by mandating the use of structured refuges (also see Matten et al., chapter 2; Ferré et al., chapter 3). As of early 2008, in lepidopteran-active maize there is a mandate for a 20% refuge in the Corn Belt and 50% refuge in cotton-growing areas, with the refuges planted within one-half mile (~800 m) of the *Bt* maize (Matten et al., 2004). There is a higher refuge mandate in cotton-growing areas because maize serves as an important refuge source for *H. zea*, which often is a devastating pest of cotton (Naranjo et al., chapter 6). Coleopteran *Bt* maize in the USA also has a 20% refuge mandate, but the refuge maize must be planted adjacent to the *Bt* maize. To promote good IRM stewardship, registrants are required to monitor for resistance, educate growers about the importance of IRM, monitor for grower compliance, and develop remedial action plans in case resistance develops (Matten et al., 2004, chapter 2). Surveys suggest, at least in the USA, most growers understand the importance of planting refuges and most of them follow refuge recommendations (Goldberger et al., 2005; Alexander, 2007); although grower compliance could be lower in the future if Bt maize use percentages continue to increase. Thus far, ten years of resistance monitoring in the US Corn Belt has found no detectable changes in Cry1Ab susceptibility among *O. nubilalis* populations (Siegfried et al., 2007).

5.4.1.3 Effects on Integrated Pest Management

Growing GM insect-resistant maize is likely to impact several aspects of IPM, including the amount of insecticides used, potential problems with secondary pests, and basic decision-making processes used by maize growers with regard to crop production. The effectiveness of biological control (whether natural or through intervention) will almost certainly be increased as insecticide use decreases. The value of lepidopteran-active *Bt* in reducing insecticide use has been modest (Hunt et al., 2007), but novel products with multiple lepidopteran toxins may lead to greater reductions in insecticide use, especially in southern states, if better control of additional maize pests (e.g., *H. zea*, *S. frugiperda*) can be developed. As mentioned previously, the potential for coleopteran-active *Bt* maize to limit insecticide use is considerable; and if resistance to crop rotation for *D. v. virgifera* and *D. barberi* continues to spread, the value of *Bt* maize will become even greater.

Concerns that the use of insect-resistant *Bt* maize could lead to increased problems with secondary pests may stem from experiences with *Bt* cotton, where declining insecticide use against target lepidopteran pests allowed increases of some previously minor pest species (Naranjo et al., chapter 6). However, this seems less problematic for US maize growers with some minor exceptions. For coleopteran-active *Bt* maize, there are anecdotal reports of more problems with minor soil insect pests (e.g., grubs, wireworms). If such problems become widespread or persistent, the most likely result will be increased use of seed treated with systemic insecticide. Additionally, the recent eastward spread of western bean cutworm, *Striacosta albicosta* (Lepidoptera: Noctuidae), through the Corn Belt, which could be related to *Bt* maize or increased use of minimum tillage, poses a potential problem for growers of maize that rely on the *Bt* toxin Cry1Ab (Catangui and Berg, 2006; Storer et al., chapter 10). Although the use of current *Bt* maize with Cry1F or future hybrids with multiple lepidopteran-active toxins should allow this pest to be managed without insecticides.

Lastly, like reliance on insecticides, the use of insect-resistant GM maize could have undesirable impacts on how growers make decisions regarding pest management and crop production. In particular, over-use of *Bt* maize or complete reliance on genetic modifications for insect management could reduce the use of IPM practices that help control secondary pests. For example, since many minor pests are suppressed by crop rotation, recent trends towards more continuous maize production may contribute to new or worsening pest problems. To reduce the likelihood that *Bt* maize is relied upon exclusively and unnecessarily, a model available over the internet, the *Bt* maize Economic Tool (BET; www.btet.psu.edu) provides growers useful information by estimating the likelihood of net benefits from planting *Bt* maize. Growers are allowed to input specific information regarding their production plans and see predicted outcomes based on long-term averages for weather and *O. nubilalis* abundance. The combined information on pest and maize phenology, site-specific weather data and economics generate color-coded maps to help growers determine where *Bt* maize, on average, is economical.

5.4.2 Spain Case Study

After France and Italy, Spain is the third largest producer of grain maize in Western Europe. In recent years the area of maize production has varied from 400,000–500,000 ha.

Because most maize is irrigated with up to 700 mm per year, the production area depends on availability of fresh water. Though maize is planted throughout most of Spain, cultivars, agronomic practices, and yield vary substantially among regions. Most of the production is devoted to livestock feed, with minor amounts for starch, sweet corn and popcorn.

5.4.2.1 Major Insect Pests and Their Control

Three groups of insects are targets of pest management by maize growers in Spain. In addition to two species of stem borers, wireworms (Coleoptera: Elateridae) and cutworms (Lepidoptera: Noctuidae) are primary soil pests. Finally, a group of sucking insects, aphids (Homoptera: Aphidae) and leafhoppers (Homoptera: Cicadellidae), are important because of their role as vectors of maize viruses.

Stem borers, including the Mediterranean corn borer, *Sesamia nonagrioides* (Lepidoptera: Noctuidae) and *O. nubilalis*, are the most damaging maize pests in all parts of the country. *Sesamia nonagrioides* is considered the more damaging species because it is more abundant and produces longer tunnels than *O. nubilalis*. For the stem borers, particularly for *S. nonagrioides*, insecticides are not generally used because larval tunneling limits the efficacy of insecticide-based management. When insecticides are used against stem borers, foliar applications are made (first generation) or insecticides are incorporated into irrigation water (second generation). As a cultural control, modification of planting dates is rarely used because the timing of flights for adults of the two stem borers are distinct, typically separated by four weeks. Most maize cultivars grown in Spain offer a low degree of resistance to stem borers, which is the main tactic used to limit losses due to these insects, and recent efforts have been devoted to looking for new sources of HPR (Butrón et al., 2006). Other control measures include tillage to prevent emergence of adult moths, but this is only effective when it is practiced over large areas. Ideally, tillage takes place after adults of the parasitoid *L. thompsoni* have emerged and exerted their suppressive effect on the stem borer population. In high-value seed maize, inundative biological control with *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) is sometimes used against *O. nubilalis*.

Injury from soil insects, such as wireworms or cutworms, is typically managed using insecticidal seed treatments. Though damage by these pests to maize seed and roots may be overestimated by growers, the absence of reliable economic thresholds and the low visibility of injuries caused by these insects present major obstacles to reducing the use of insecticide treated seed (Piqué et al., 1998). Also, because seeds treated with systemic insecticides appear to delay the development of aphid and leafhopper populations, there is an additional incentive for growers to buy treated seed (Pons and Albajes, 2002). Though the western corn rootworm *D. v. virgifera* has been found in Europe as far west as France, the pest is not yet present in Spain.

5.4.2.2 Current Use of GM Maize in Spain

For many years Spain has been the only country in the EU to grow a significant amount of *Bt* maize. Though *Bt* maize accounted for only about 21% of the total area planted to maize in Spain in 2007, it comprises approximately 50% of the maize grown in some areas. Spanish legislation concerning GM crops follows the general EU framework. Though EU legislation on genetically-modified organisms has been in place since the early 1990s, additional regulations have been developed since then. In the last decade, many experimental authorizations have been granted for GM crops, but few have been approved for cultivation, import and processing for feed and food. The EU Commission first allowed growers to cultivate Cry1Ab maize (Event 176) in 1997, but authorization for 176 was cancelled in 2005. Currently only event MON810 is authorized for cultivation, but other events have been allowed for import and use in processing or for grain (Bt11, 1998; NK603, 2004; MON863 and DAS1507, 2005; and MON863 × MON810, 2006). Periodic updates to the list of authorized GM crops in Europe can be found at http://www.gmo-compass.org/eng/gmo/db/ (accessed 3 January 2008).

The current approval of only MON810 for cultivation in Spain means only lepidopteran-active *Bt* maize is grown. Besides resistance to the two stem borers, the *Bt* varieties also reduce the occasional ear injury produced by *Helicoverpa armigera* (Lepidoptera: Noctuidae). There are no obligatory measures for IRM in Spain but recommendations are based on the high-dose/refuge strategy. Studies conducted by Spanish public institutions suggest that 400 m is a common dispersal distance within which *S. nonagrioides* matings occur at random (Eizaguirre et al., 2006) and that resistance alleles are rare in Spanish populations of *S. nonagrioides* and *O. nubilalis* (Andreadis et al., 2007). Annual monitoring has been conducted with no reported changes in susceptibility of the two stem borer species to the only *Bt* toxin deployed in the field, Cry1Ab (Farinós et al., 2004).

5.4.2.3 Effects on Integrated Pest Management

In spite of the debate on the cultivation of *Bt* maize in Spain, growers have steadily increased their use of *Bt* maize. A survey sponsored by seed companies, revealed that 96% of *Bt* maize growers in Spain were quite satisfied with transgenic varieties to prevent losses due to stem borers. A more in-depth evaluation of the socio-economic impacts of *Bt* maize in Spain used empirical data from on-farm performance for the three-season period 2002–2004 (Gómez-Barbero et al., 2008). In the three main areas growing *Bt* maize growers had 4.7% increases in yield and €85 in gross margins per hectare.

More relevant to IPM practices were the results regarding growers' use of insecticides (Gómez-Barbero et al., 2008). The survey indicated that conventional maize growers were about twice as likely to use insecticides for stem borer suppression (56%, conventional and 30%, *Bt*), and applied on average more than twice as many applications per year as *Bt* maize growers (0.86, conventional and 0.32, *Bt*). The differences between probability of treatment and mean number of applications likely reflects the more common use of multiple applications by conventional growers, among whom 21% used two or more applications compared to 2% for *Bt* maize growers. One likely effect of reduced use of foliar insecticides is conservation of natural enemies, which deter population development of secondary pests, such as aphids and spider mites (Acari: Tetranychidae) (Romeis et al., chapter 4). Other pests that are not currently controlled by *Bt* maize, however, such as *H. armigera* may be more problematic and need to be monitored.

As in the USA, growers may be tempted to use insect-resistant GM maize when it is not justified by economics or IPM principles. Though growing *Bt* maize was profitable across all three main *Bt* maize growing areas, improvement in gross margins ranged from €125/ha in Aragon (northeast) to €7/ha in Castilla La Mancha (central), suggesting that the use of *Bt* maize is not appropriate in all situations and should be used based on the best available economic and ecological data.

5.4.3 China Case Study

China is the second largest producer of maize in the world. In 2004, approximately 24 million hectares of maize were grown, producing a total yield of 125 MMT (average yield ~4.8 t/ha; Wang et al., 2005a). Unlike maize production in the USA, growers in China typically farm relatively small plots with the total production area divided among 100 million maize growers.

5.4.3.1 Major Insect Pests and Their Control

The Asian corn borer, *O. furnacalis*, is the most significant insect pest of maize and occurs in most maize-growing areas from Heilongjiang (northern) to Hainan (southern) provinces. Estimated losses due to this insect range from 6–9 MMT per year (Zhou et al., 1995). Similar to *O. nubilalis* in the USA, direct yield losses come from *O. furnacalis* injury to vegetative stage maize, but the greatest impacts are indirect, from larval feeding on silks and kernels that leads to ear rot, mycotoxin production and reduced grain quality (Zhou et al., 1995; Wang et al., 2005a). Other lepidopteran pests of concern for maize in China include *H. armigera* and *Spodoptera exigua* (Lepidoptera: Noctuidae). In particular, problems with *H. armigera* in maize appear to be increasing as cropping systems from the 1990s have changed (Wang et al., 2001), probably due to the more frequent use of no-till farming and the associated high survival of *H. armigera* pupae in the soil.

Several practical IPM tactics have been developed for *O. furnicalis* including biological, cultural and chemical management. For example, early spring applications of the entomopathogen, *Beauveria bassiana*, over maize stalks can kill ~80% of overwintering larvae, which significantly decreases the number of egg masses in the field and reduces the percentage of infested plants (Wang et al., 2003). Other biological control efforts include mass releases of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) egg parasitoids on an area of 1.0–1.3 million hectares per year in the northeastern provinces. More recently, the scale of the releases of *T. dendrolimi* has been expanded to 2 million hectares, which includes Huang-Huai-Hai summer maize and northwestern maize regions (Wang et al., 2005a). The program produced 60–85% parasitism of *O. furnacalis* and reduced damage to maize by 65–92% (Piao and Yan, 1996), equal to or better than what is achieved by insecticide-based suppression. With one or two releases, costs are estimated at US$4 or 6/ha, respectively. One remarkable management measure has been an extensive network of light (high intensity mercury-vapor lamp) traps over 320,000 ha; traps reduced *O. furnacalis* plant infestations by ~60% with captured moths used to feed chickens on nearby farms (Yang et al., 1998; Wang et al., 2003). Finally, granular insecticide applications and *B. thuringiensis* insecticides have been used for whorl-stage suppression of *O. furnacalis*.

Though IPM plays an important role in controlling *O. furnacalis* in maize, most growers do not manage Asian corn borer populations because of the costs and required skills, safety and environmental concerns, and uncertainty about the benefits of the management (Zhou et al., 1995).

5.4.3.2 Current Use of GM Maize in China

Although *Bt* maize is not grown in China, its commercialization is currently under consideration by the Chinese government (Wu and Guo, 2005). Extensive laboratory and field trials have been conducted to evaluate the efficiency of transgenic maize on target lepidopteran pests and the potential ecological risks to non-target arthropods (Wang et al., 2005b, c, 2007; Li et al., 2007).

Cry1Ab-expressing maize provided excellent control of *O. furnacalis* in laboratory bioassay and field trials (He et al., 2003a, b, 2004). Neonates of *O. furnacalis* did not survive when fed different tissues of *Bt* maize hybrids that produce Cry1Ab toxins (events MON810 and Bt11) (Wang et al., 2004a). Neonates of *H. armigera* did not survive when fed silk, ear and husk tissues in the laboratory; however, there was low survival of *H. armigera* after artificial infestation of silk-stage maize plants in the field (Chang et al., 2006). Perhaps this was due to cannibalism, which provided a way for some *H. armigera* to avoid the *Bt* toxin. Similarly, laboratory tests demonstrated excellent control of *S. exigua*, but some larvae survived on artificially infested plants in the field. Cry1Ab maize had good control for the less serious lepidopteran pest, oriental armyworm, *Mythimna separata* (Noctuidae) (Wang et al., 2004b, 2005b), but the effects of *Bt* maize on other less serious lepidopteran pests, including the yellow peach borer, *Conogethes punctiferalis* (Pyralidae); sugarcane striped borer, *Proceras venosatus* (Crambidae); and millet borer, *Chilo infuscatellus* (Crambidae), are unknown.

Resistance management research related to *Bt* maize also is in progress. Research on resistance mechanisms and biology of resistant individuals is ongoing for a laboratory-selected *O. furnacalis* strain resistant to Cry1Ab (Xu et al., 2006; He et al., 2007). Additionally, research has started to identify sources of non-*Bt* crops and natural plant refuges for *O. furnacalis* in the different maize growing regions of China. If the variety of crops (e.g., maize, millet, sorghum, wheat, vegetables, soybean, peanut, canola) and natural vegetation produce sufficient numbers of the primary maize pests, then refuge plantings of non-*Bt* maize may not be needed in some areas.

5.4.3.3 Effects on Integrated Pest Management

Bt maize could become a major component of IPM in all the maize-growing areas of China. This assumes *Bt* maize would be affordable, effective, easy for growers to use, and environmentally sound. Potentially the main positive impact would be increased control of *O. furnicalis* in areas (>50%) where currently no control tactics are used. Yet even in areas where control with *T. dendrolimi* has been successful, *Bt* maize could offer an economically viable alternative.

The value of *Bt* maize should be considered a long-term issue. Although preserving the efficacy of *Bt* maize in China using IRM may be challenging. *Ostrinia furnacalis*, *H. armigera* and *S. exigua* will be considered target pests. As in the USA, both *Bt* maize and *Bt* cotton are produced in many of the same areas, which complicates matters since maize is considered a refuge for *H. armigera* (Wu and Guo, 2005). Furthermore, if refuges should be required, given the large number of small farms, it is uncertain whether grower compliance could be assured (or even adequately measured).

It is possible that for some Chinese maize growers, GM insect-resistance would provide considerable benefits in reducing reliance on insecticides, which might also reduce illnesses and deaths related to insecticide use (as has been reported for *Bt* cotton, Pray et al., 2002; Hossain et al., 2004; Qaim et al., chapter 12). However, these benefits are difficult to estimate because of limited data. In addition, reduction in insecticide use will increase opportunities for natural biological control (Romeis et al., chapter 4), especially for the control of secondary pests such as mites, corn leaf aphids and thrips, especially *Frankliniella tenuicornis* (Thysanoptera: Thripidae). At present it is unclear whether traditional IPM practices for lepidopteran maize pests would be enhanced or replaced by *Bt* maize.

5.4.4 Kenya Case Study

Africa grows 26 million hectares of maize, accounting for 18% of global area but only 6.6% of the global production. On average, maize yields within industrial countries are around 8.3 t/ha while for sub-Saharan Africa the average is only 1.3 t/ha (FAOSTAT, 2007). Maize production in Kenya fits the pattern in sub-Saharan Africa; with a production area one-third greater than Canada, total yields from

Kenya are less than one-third that of Canadian maize growers, averaging 1.7 t/ha (FAOSTAT, 2007). Kenyan growers are challenged more by poor soil fertility, drought, and limited funds than by insect pests (De Groote et al., 2004a). As a result, growers usually are unable to make adequate investments in fertilizer or improved maize varieties (Freeman and Omiti, 2003). Biotechnology, however, has the potential to improve agricultural production and sustainability in Kenya and other countries in Africa (Thomson, 2008).

5.4.4.1 Major Insect Pests and Their Control

Key insect pests for Kenyan growers include lepidopteran stem borers and coleopteran storage pests. Maize growers estimate losses from stem borers at 13% (De Groote, 2002); the most important species are the spotted stem borer, *C. partellus*, and African stem borer, *Busseola fusca* (Lepidoptera: Noctudidae) (Ong'amo et al., 2006). Other less common species, including coastal stem borer, *Chilo orichalcociliellus* (Lepidoptera: Crambidae), and pink stem borer, *Sesamia calamistis* (Lepidoptera: Noctuidae), and the African sugarcane borer, *Eldana saccharina* (Lepidoptera: Pyralidae), also occur in Kenya and other maize growing countries in sub-Saharan Africa.

The spotted stem borer was introduced from South Asia and first reported in Kenya in the 1950s (Nye, 1960) and now attacks maize and sorghum at elevations below 1,500 m. In contrast, the African stem borer is prevalent in high- and mid-elevation areas causing at least 10% yield loss (Ong'amo et al., 2006). Both species are attacked by the native parasitoid, *Cotesia sesamiae* (Hymenoptera: Braconidae). *Cotesia flavipes*, which was introduced to help suppress *C. partellus* (Overholt et al., 1997), and the tachinid, *Sturmiopsis parasitica* (Diptera: Tachinidae), is a common parasitoid of *B. fusca* (van Rensburg et al., 1988). Another IPM tactic that combines biologically- and culturally-based pest management is the so called "push-pull strategy" developed by the International Center for Insect Physiology and Ecology (ICIPE) (Khan et al., 1997). In this system, maize is intercropped with grasses such as molasses grass (*Melinis minutiflora*) or desmodium (*Desmodium uncinatum*, *Desmodium intortum*) that repel or push the stem borers *C. partellus* and *B. fusca* away from maize. Though the repellent effect is not absolute, molasses grass also produces a volatile that attracts the parasitoid *C. sesamiae*, increasing the rate of parasitism fourfold. Additional plantings of trap crops around maize, Napier grass (*Pennisetum purpureum*) or Sudan grass (*Sorghum vulgare* var. *sudanense*), help attract or *pull* stem borers out of maize. The push-pull strategy can reduce stem borer populations by 75%. It also addresses other problems of maize growers by helping suppress witchweed (*Striga* spp.), improve soil fertility and provide livestock forage (Khan et al., 2001).

After harvest Kenyan growers must contend with the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae), and the larger grain borer, *Prostephanus truncates* (Coleoptera: Bostrichidae). These beetles can cause losses of 10% or more through consumption of grain, reduced grain quality and contamination with insect body parts. Cultural management of these pests involves storing ears over the cooking area where heat and smoke reduce losses. Open-pollinated and hybrid varieties are being developed with conventional host plant resistance by KARI (Kenya Agricultural Research Institute) and CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo) (IRMA, 2002).

5.4.4.2 Current Use of GM Maize in Kenya

Insect-resistant GM maize is not commercially available in Kenya, but pending biosafety legislation may soon allow growers access to GM crops. Comprehensive recommendations for an environmental risk assessment for *Bt* maize in Kenya have been proposed (Hilbeck and Andow, 2004), but many feel they are not appropriate and will delay the adoption. Progress towards approval of GM crops has been facilitated by KARI and CIMMYT through the Insect Resistant Maize for Africa (IRMA) project. The IRMA project holds annual stakeholder meetings to create public awareness on the potential of *Bt* maize and provide policymakers opportunities to visit GM crop improvement research in industrial and developing countries (IRMA, 2000). To date, the IRMA project has conducted the only tests of *Bt* maize in Kenya after material transfer agreements for various *Bt cry* genes (*cry1Ab*, *cry1Ac*, *cry1Ba*, *cry1E*, *cry1Ca*, and *cry2Aa*) were acquired, and new events were developed using a ballistic transformation protocol (Bohorova et al., 2001).

In the laboratory, all events tested produced high mortality of *C. partellus*, but emergence holes noted in field plots raise concerns about the durability of those events (Mugo et al., 2008). The events that provided the best control for *B. fusca* were Event 396 (*Rice Actin* promoter, *cry1Ab*) and Event 127 (*Maize Ubiquitin* promoter, *cry1Ba*), which reduced leaf feeding by 30% in 96-hour bioassays but did not cause a significantly higher mortality than controls (Mugo et al., 2008). In order to release a *Bt* maize variety in Kenya, it is likely that the IRMA project will need to work more closely with the private sector, which has already commercialized *Bt* maize varieties that provide effective control against *B. fusca* and are planted on over 1.2 million hectares in South Africa (James, 2007). KARI and CIMMYT can provide the technical support needed to meet the environmental impact study and testing requirements for a regulatory dossier, and to develop stewardship strategies for smallholder farmers. The commercial event that is most widely used is Monsanto's MON810, which offers good early season control of *B. fusca* in South Africa.

Ongoing research in Kenya also is exploring resistance management for *Bt* maize. Early screening for resistance development in *C. partellus* and *B. fusca* showed no changes in susceptibility to Cry proteins over four generations of selection, increasing hopes that resistance can be effectively managed (Mugo et al., 2005; Tende et al., 2005). However, it is currently unclear how insect resistance management and monitoring for resistance would be conducted. The situation in Kenya shares some features with China, particularly an abundance of very small farms, which may complicate IRM efforts. Consequently, the IRMA project has attempted to determine whether an effective refuge may exist within the existing mixed cropping system. Effective natural refuges appear to exist in some areas and seasons, but alternate hosts such as sorghum may need to be promoted to provide an adequate refuge in arid regions or where maize occupies large areas during the long-rain season from April to June (IRMA, 2005a; Mugo et al., 2005).

5.4.4.3 Effects on Integrated Pest Management

Though insect-resistant GM maize is not commercially grown in Kenya, its potential effects can be examined from two perspectives. The first is to consider whether *Bt* maize resistant to stem borers would improve profitability of maize farming in Kenya. In this case, profitability relates to IPM because of the limited potential for Kenyan farmers to afford expenses associated with improved crop production (e.g., fertilizer) and other pest management efforts (weed suppression and management of other insect pests). The second is the potential for *Bt* maize to impact other IPM practices, particularly insecticide use, which can be estimated.

Information on profitability of *Bt* maize is available from on-farm trials across Kenya. To estimate the potential value of stem borer suppression with *Bt* maize, yields were assessed with and without insecticide use (De Groote et al., 2004b). With overall stem borer losses averaging 13%, current maize production levels equate to about US$80 million. Assuming an effective GM event is found for *B. fusca*, most of these losses (~$10 million) could be preventable. The economic effects of *Bt* maize production elsewhere in sub-Saharan Africa may be informative. Side-by-side plantings of *Bt* and near-isoline maize varieties managed by South African growers showed considerable differences (Gouse et al., 2006). *Bt* maize was perceived to produce greater quality grain and improved yields from 21–62%, depending on location, reinforcing the idea that given affordable seed, *Bt* maize could markedly increase yield and grower profits.

The side-by-side plantings in South Africa also were used to investigate effects on insecticide use (Gouse et al., 2006). No significant differences in insecticide use were found between *Bt* and near-isoline maize, but this may be attributable to a combination of indiscriminate insecticide use and low stem borer numbers. The likelihood of insecticide use by growers varied 20-fold between areas and during two study years; more than half of the growers admitted not observing any stem borers. Other information on the potential impacts of *Bt* maize on IPM include monitoring efforts on non-target species, which indicate that abundance of beneficial non-target arthropods is either unaffected or increased with *Bt* maize (IRMA, 2005a, b).

Collectively, current information suggests that *Bt* or other insect-resistant GM maize could permit greater resources to be committed to pest management and reduce the need for insecticide use. However, this outcome is not assured. The value of *Bt* maize to IPM depends on how maize growers utilize the technology. Ideal use of *Bt* maize would include reduction of insecticide use with maintenance of other traditional IPM practices.

5.5 Summary and Conclusions

Bt maize has revolutionized pest control in a number of countries and may allow growers to expand maize production into regions where high pest populations have made growing maize unprofitable. On balance, benefits of *Bt* maize appear to outweigh possible negative effects. Growers are attracted to convenience of the technology as well as yield protection, reduced use of chemical insecticides and improved grain quality. Some scientists, however, suggest the verdict is still out on *Bt* maize and that more research is needed to sort out issues related to possible non-target effects, gene flow and insect resistance management. With regard to the non-target issue, no surprising negative effects have been found with current *Bt* maize hybrids. Overwhelmingly, experiments have shown toxins produced by *Bt* maize have little if any effects on non-target organisms and, when compared to maize treated with chemical insecticides, *Bt* maize fields usually have higher biodiversity. Gene flow is an important issue, especially related to maize seed producers and organic growers. However, as long as GM material thresholds are reasonable, isolation distances and other measures may effectively limit gene flow. IRM remains a challenge because current high-dose/refuge strategies require growers to plant structured refuges, usually non-*Bt* maize; and often the high-dose criteria for plants are not met for all important pests. However, maize hybrids with genes pyramided against specific lepidopteran and coleopteran pests soon will be available, which should improve resistance management and may allow changes in refuge type and size.

The country-specific case studies indicate there are a variety of ways in which *Bt* maize and future GM maize varieties may affect the practice of IPM by maize growers. Potential benefits, including reduced insecticide use and increased ability to invest in crop production and protection (for growers in developing nations), are considerable. Yet there are challenges, including the possibility that GM maize will displace tools like cultural pest management or conventional host plant resistance. This is most important for resource-limited growers in places such as China and Kenya, who currently rely on a diversity of tactics to manage insect pests. Of course, the worst scenario for the future would include the abandonment of traditional IPM tactics followed by misuse and failure of GM maize due to evolution of pest resistance.

Compared to other IPM practices growing *Bt* maize it is not knowledge intensive because the technology is in the seed. This should be attractive to growers in developing countries where poor infrastructure and inadequate extension services sometimes limit the use of traditional IPM (Shelton, 2007). Growers in developing countries, however, often have other agronomic factors besides pest management to consider before deciding to grow *Bt* maize, as well as social and economic challenges. Nevertheless, *Bt* maize has the potential to reduce extreme yield variability due to lepidopteran pests, which would be an advantage for subsistence growers.

Overall, GM maize should not be considered inherently compatible or incompatible with IPM; rather, like synthetic insecticides developed decades ago, the compatibility of insect-resistant GM crops depends on how they are developed and utilized. As noted, efforts in developing future products may reduce potential problems with secondary pests by broadening activity of GM maize and reducing the chances of resistance evolution by targeted pests. Finally, growers and scientists should understand that GM pest resistance is an important component of maize IPM, but traditional pest management practices must be maintained in order to avoid reliance on a single tactic.

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