dollars) spent annually on insecticides worldwide, it was estimated that nearly $2.7 billion could be substituted with Bt biotechnology applications (Krattiger 1997). Economic advantage gained during 1999 by Bt cotton alone has been estimated to be $213 million in the USA. Cultivation of transgenic crops has led to a reduction in pesticide use and significant increase in yield (Cannon 2000). Unfortunately, there are also concerns that the benefits of genetically transformed plants will be short-lived (McGaughey & Whalon 1992). Despite the potential advantages of using Bt crops, the possibility of their widespread use has raised some potential problems. Decades of indiscriminate insecticide use have demonstrated that exposing insect populations to high levels of toxins results in evolution of resistance to insecticides (Roush & McKenzie 1987). Recently, several species of insect pests have been selected for resistance to Bt in the laboratory, indicating that biological pesticides can suffer the same fate as the chemical pesticides (Liang et al. 2000, McGaughey et al. 1998a).

DEVELOPMENT of RESISTANCE in INSECTS to Bt GENES

Several studies have shown that insect pests can adapt to Bt toxins under laboratory conditions (Shelton et al. 2002). Certain pests such as *Plodia interpunctella* (McGaughey 1985), *Heliothis virescens* (Stone et al. 1989), *Plutella xylostella* (Tabashnik et al. 1990), *Spodoptera exigua* (Moar et al. 1995), and *Ostrinia nubilalis* (Huang et al. 1997) have been shown to develop some degree of resistance to *B. thuringiensis* under laboratory conditions. Evolution of insect resistance to insecticidal proteins produced by Bt would decrease our ability to control agricultural pests with genetically engineered crops designed to express genes coding for these proteins (Gould et al. 1992). Information on development of resistance in insects to Bt toxins has been summarized below.

Indian meal moth, Plodia interpunctella:

The first studied case of resistance to Bt-strains was *P. interpunctella*, which had developed 100-fold resistance following 15 generations of laboratory selection with Dipel (McGaughey 1985). On further selection, after 36 generations, the resistance levels reached 250-fold (McGaughey & Beeman 1988). *Bacillus thuringiensis* sub sp. *kurstaki* caused a narrow spectrum resistance to Cry1Ab and Cry1Ac toxins, while sub sp. *aizawai* and *entomocidus* strains caused broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1Ac, Cry1B, Cry1C, and Cry2A (McGaughey & Johnson 1994).

Diamondback moth, Plutella xylostella

Although there are no instances of insects developing resistance to Bt transgenic plants in the field, diamondback moth, *P. xylostella*, is the first insect known to have evolved high levels of resistance to Bt as a result of repeated use of formulated Bt insecticide (Tabashnik et al. 1990). A diamondback moth colony derived from field population in the Philippines that was regularly exposed to Dipel showed more than 200-fold resistance to Cry1Ab (Ferre et al. 1991). As much as 1640-fold resistance to Bt has been recorded in localized populations of diamondback moth from Hawaii, Florida, and Asia (Tabashnik et al. 1992). In field populations of *P. xylostella*, resistance to Bt sub sp. *kurstaki*, containing Cry1A(a,b,c), Cry2A, and Cry2B toxins and to a lower extent Bt sub sp. *aizawai*, containing Cry1A (a,b), Cry1C, and Cry1D toxins has been observed in various countries (Tabashnik 1994). Laboratory selection of *P. xylostella* using Cry1Ca protein and in later generation transgenic broccoli expressing Cry1Ca, increased Cry1Ca resistance to 12400-fold (Zhao et al. 2000b). Resistance to Cry1A toxins from Bt sub sp. *kurstaki* caused cross-resistance to Cry1F, but not to Cry1B or Cry1C (Tabashnik et al. 1996). Contrary to the assumption that independent mutations are required to counter each toxin in *P. xylostella*, an autosomal recessive gene conferred extremely high resistance to Cry1Aa, Cry1Ab, Cry1Ac, and Cry1F (Tabashnik et al. 1997). In a *P. xylostella* colony possessing 1500-fold resistance to a commercial formulation, the resistance rapidly fell to 300-fold in the absence of selection, but remained stable at this level in subsequent generations (Tang et al. 1996).

Cotton bollworm/ legume podborer, Heliothis /Helicoverpa

Helicoverpa armigera is capable of developing resistance to Cry1Ac in 7 to 8 generations (Kranthi et al. 2000). Highly mobile polyphagous pests such as *Helicoverpa* may develop resistance to Bt on one transgenic crop and then disperse, nullifying the effectiveness of a wide range of Bt transgenic crops expressing the same or similar Cry proteins. Pests with resistance to CryIA proteins in transgenic plants may also display significant resistance to Bt biopesticides. A laboratory strain of *H. virescens* developed resistance in response to selection with the Bt toxin CryIAc. In contrast to other cases of Bt-toxin resistance, this strain exhibited cross-resistance to Bt toxins that differ significantly in structure and activity (Gould et al. 1992). Over 10000-fold resistance to Cry1Ac was obtained in *H. virescens* colony on selection with Cry1Ac protoxin (Gould et al. 1995). The insecticidal activity of Bt in leaves and squares of transgenic cotton plant was high during the second generation of the insect, but declined in the third and fourth generations of *H. armigera* in North China. The surviving third and fourth generation larvae, after feeding on flowers of Bt cotton, fed on the bolls until pupation, which caused selection in field populations of *H. armigera*. The increase in resistance was 7.1-fold

after 17 generations of selection in the laboratory (Zhao et al.1998). Liang et al. (2000) found that the resistance ratio of *H. armigera* to Bt transgenic cotton, after selection for 16 generations was 43.3, and inheritance of resistance was controlled by a single autosomal incomplete recessive allele.

European corn borer, Ostrinia nubilalis

There has been a significant decrease in susceptibility across generations for selected strains of *O. nubilalis* after chronic exposure to formulated Cry1Ab (Huang et al. 1997, Josette et al. 2001). Similarly, a 162-fold increase in resistance to transgenic Cry1Ac has been observed in European corn borer after 8 generations of laboratory selection (Bolin et al. 1999). Event 176 Bt corn hybrids express high levels of Cry1Ab toxin in green plant tissue and pollen, but extremely low levels in the silk and kernels (Koziel et al. 1993), on which second generation *O. nubilalis* larvae have been shown to survive (Siegfried et al. 2001). Zoerb et al (2003) stated that successfully developed *O. nubilalis* larvae have either survived exposure to sublethal doses of Cry1Ab Bt toxin or exploited plant tissues that do not express the toxin, and they further implicated that Event 176 hybrids do not satisfy requirements for high dose that are recommended for resistance management purposes.

Pink bollworm, Pectinophora gossypiella

Field collected pink bollworm quickly evolved resistance to Cry1Ac under laboratory selection (Patin et al. 1999, Simmons et al. 1998, Tabashnik et al. 2000). *Pectinophora gossypiella* selected with Cry1Ac protoxin developed 300-fold resistance to Cry1Ac protoxin, and high levels of cross-resistance to Cry1Aa and Cry1Ab protoxin, and low levels of resistance for Cry1Bb protoxin (Tabashnik et al. 2000a). Three selections with Cry1Ac in artificial diet increased resistance of pink bollworm to >100-fold relative to a susceptible strain (Liu et al. 2001).

Tobacco caterpillar, Spodoptera spp.

In general, *Spodoptera* spp. larvae are not very susceptible to the Cry toxins (Strizhov et al. 1996). However, Cry1C toxin had been reported to be toxic against *S. exigua* (Visser et al .1988) and *Spodoptera littoralis* (Van Rie et al. 1990a). Selection to Cry1Ca caused 850-fold resistance to Cry1Ca and cross-resistance to Cry1Ab, Cry9C, and Cry2A, as well as to a recombinant Cry1E-Cry1C fusion protein in *S. exigua* (Moar et al. 1995), while in *S. littoralis*, 500-fold resistance to Cry1Ca and partial cross-resistance to Cry1D, Cry1E, and Cry1Ab has been recorded (Muller-Cohn et al. 1996).

BASIS for DEVELOPMENT of RESISTANCE Mutations in insects that cause disruption of any of the steps involved in the mode of action could confer resistance to Bt (Heckel 1994). Decreased solubilization of the Bt crystal, decreased cleavage of the full-length Bt protein into an active fragment, increased proteolytic digestion of the active fragment, decreased binding of the active fragment to the midgut epithelium, and decreased functional pore formation are the major changes in the Bt toxicity pathway responsible for evolution of resistance (Gill et al. 1992). Previous genetic and biochemical analyses of insect strains with resistance to Bt toxins has indicated that: (i) resistance is restricted to single group of related Bt toxins, (ii) decreased toxin sensitivity is associated with changes in Bt-toxin binding to sites in brush-border membrane vesicles of the larval midgut, and (iii) resistance is inherited as a partially or fully recessive trait. If these three characteristics are common to all resistant insects, specific crop-variety deployment strategies could significantly diminish problems associated with resistance in field populations of the target pests (Gould et al. 1992). Recent studies have shown that the genetic basis of resistance to Bt toxins in insects is similar to resistance to chemical insecticides, which is conferred by multiple physiological mechanisms under independent genetic control. In *Heliothis*, the existence of separate, independently assorting resistance genes has already been confirmed by linkage analysis with marker loci (Heckel 1994). Heckel et al. (1997) identified a major Bt- resistant locus in a strain of *H. virescens* exhibiting up to 10000-fold resistance to Cry1Ac toxin. Despite many potential mechanisms of resistance, the best-characterized and most widely observed mechanism of resistance to *B. thuringiensis* is reduced binding of toxin to midgut membranes (Van Rie et al. 1990b). Changes in the binding affinities of toxin receptors on the brush border membranes of the insect midgut have been identified in Bt resistant *P. interpunctella* (Van Rie et al. 1990b), *P. xylostella* (Ferre et al. 1991), *H. virescens* (MacIntosh et al. 1991), and *Trichoplusia ni* (Ballester et al. 1994). Cry1Ab and Cry1Ac have the same receptor in the midgut of *O. nubilalis*, with the receptor having a higher affinity for Cry1Ab than for Cry1Ac (Denolf et al. 1993).

Studies on a field population of *P. xylostella* have also suggested that, apart from reduced binding, other biochemical mechanisms are involved in resistance to Bt (Martinez-Ramirez et al. 1995). Some evidence for reduced conversion of protoxin to toxin and increased degradation of toxin also has been reported (Forcada et al. 1996, Oppert et al. 1994, 1997). In *H. armigera*, the excessive degradation of protoxin in midgut juice triggered by receptor binding of activated toxin was presumed to be responsible for low sensitivity of the insect to Bt (Shao et al. 1998). Toxin binding in resistant *T. ni* selected with Cry1Ab did not correlate with resistance, since there was no cross-resistance to

Cry1Ac (Estada & Ferre 1994), which shares the same binding site of Cry1Ab as demonstrated in *O. nubilalis* midgut membrane (Denolf et al. 1993).

When the midgut proteinases from resistant strain of European corn borer were characterized, there was a 35% decreased hydrolyzing efficiency in activation of Bt protoxin compared with the susceptible strain (Huang et al. 1999). However, in studies by Liu et al. (2000), Cry1C toxin was found to be significantly more toxic than was Cry1C protoxin to resistant strain of diamond back moth, but not to susceptible strain. If reduced conversion of Cry1C protoxin to toxin is the sole mechanism of resistance, both susceptible and resistant larvae should be equally susceptible to Cry1C toxin. Further, they observed similar binding of 125I-Cry1C to brush border membrane vesicles from the Cry1C resistant and susceptible strains and concluded that reduced binding of Cry1C to midgut target sites was not a mechanism of resistance in diamondback moth. Mohan & Gujar (2003) also found no differences in proteolytic patterns of Cry1A protoxins in both susceptible and resistant populations of diamondback moth. They also stated that the differences in susceptibility of two populations to *B. thuringiensis* Cry1Ab were not due to midgut proteolytic activity. McGaughey et al (1998b) indicated that apart from toxin solubility and/or proteinase activation in the insect midgut, postbinding events such as receptor aggregation, pore formation, ionic fluxes, and insect recovery may also be involved in resistance development. Following Cry1Ac ingestion by *H. virescens*, similar histopathological changes were observed in midgut epithelium in both susceptible and resistant colony (Forcada et al. 1999, Martinez-Ramirez et al. 1999), suggesting that resistance is due to a more efficient repair (or replacement) of damaged midgut cells (Ferre & VanRie 2002).

Research conducted over the past 10 years has indicated that it is likely that the increased use of Bt toxins from transgenics will result in a rapid evolution of resistance in insects (Gelernter 1997). However, selection of plants for horizontal resistance is more durable rather than vertical resistance, and the current research on transgenic plants, particularly incorporation of the Bt delta endotoxins into crops for control of insects appears to be proceeding on a vertical resistance model, based on complete resistance conferred by one or a few genes. These varieties, like those produced through conventional resistance breeding, may become susceptible to the target pests. This may undervalue the benefits of Bt in IPM approaches (Waage 1996), as it runs the risk of breakdown of resistance in the long-term.

It may be uneconomic to develop Bt-transformed crops unless we develop strategies to extend their usefulness. Wigley et al. (1994) proposed a plan in which the major elements to be considered for deploying Bt genes among crops are: (i) assess the risk of Bt resistant insects evolving and dispersing out of the crop to infest others; (ii) characterize the diversity of Bt protein binding sites in the guts of key polyphagous pests; and (iii) use the above information to deploy Bt genes among different transgenic crops.

RESISTANCE MANAGEMENT Resistant management strategies require detailed knowledge of the toxins' mode of action and genetic response of resistant insects. Unfortunately, insects show great variability in their genetic responses to Bt toxins. Schnepf et al. (1998) emphasized that laboratory selection experiments may give rise to very different outcomes from field situations. However, several resistance management strategies have been proposed to delay adaptation to Bt-transgenic crops by pest populations (McGaughey & Whalon 1992, Raymond et al. 1991, Tabashnik 1994). The most promising with currently available technology is the use of refuges of non-transgenic crops, augmented wherever possible, with high toxin expression in the plants and avoiding mosaics of different toxins and pesticides (Roush 1997a).

THE REFUGE STRATEGY The primary strategy for delaying insect resistance to transgenic crops under large monocultures is to provide refuges of non-Bt crop plants that serve to maintain Bt-susceptible insects in the population. This potentially delays the development of insect resistance to Bt crops by providing susceptible insects for mating with resistant insects (Liu et al. 1999).

The refuge strategy is expected to work if resistance to Bt is inherited as a recessive trait. The basic goals of the mixture strategy are two fold: (i) reduce the difference in fitness between susceptible and resistant insects, and (ii) reduce the degree to which a resistant insect can pass on its phenotypic trait to its offspring. Refuges can consist of fields planted with non-Bt plants or of non-Bt plants within the Bt plants. The large numbers of susceptible insects that survive on the refuge plants are then available to mate with the small number of resistant insects that survive on the Bt plants. The offspring of susceptible (SS) x resistant (RR) matings will be RS, and therefore, will not survive when they feed on high dose Bt plants.

The US Environmental Protection Agency (EPA), which regulates transgenic pesticidal crops, believes that scientifically sound long-term insect resistance management (IRM) strategies are essential to the protection of Bt microbial pesticides, transgenics, and reduction in the risks from the use of pesticides. The EPA has imposed mandatory IRM requirements for Bt cotton. Two structured refuge requirements have been imposed: 4% unsprayed or 20% sprayed crops (Matten 2000), and the refuge fields must be within 0.8 km of their Bt fields (EPA/ USDA 1999). Obviously,

enforcing a similar system for small holding farmers will not be possible in most parts of Asia. In a typical village in Asia, it is unlikely that all farmers will plant Bt crops on all their land, and farmers grow several diverse crops, which serve as hosts for *H. armigera*. In such a scenario, it may not be necessary to enforce the cultivation of refuge crops (Sharma and Ortiz 2001). Bt genes will be one of many factors that the farmers will consider when choosing which varieties to grow. The governments can promote the maintenance of refuges by restricting the number and diversity of Bt cultivars that can be released. For example, in the Indian state of Punjab, rice farmers grow traditional Basmati varieties and modern semi-dwarf varieties. Stem borer damage is higher in basmati varieties, and thus the government could authorize the release of Bt-transformed basmati varieties, but not Bt-transformed semi-dwarf varieties (Cohen 2000).

Although Bt cotton that produces Cry1Ac toxin has been effective against pink bollworm (Patin et al. 1999, Tabashnik et al. 2000b), the slower development of resistant larvae on Bt cotton as compared to susceptible larvae on non-Bt cotton could reduce the probability of mating between susceptible and resistant insects, and this asynchrony could reduce the expected benefits of the refuge strategy (Liu et al. 1999, Liu et al. 2001, Storer et al. 2001). Though there was slow larval growth, the corn borer larvae were successful in completing development on transgenic corn plants, causing similar amounts of damage as on non-Bt plants (Storer et al. 2001). Each insect/Bt crop system may have unique management requirements because of the biology of the insect, but the studies have validated the need for a refuge (Shelton et al. 2000). Therefore, care must be taken to ensure that refuges, particularly those sprayed with insecticides, produce adequate numbers of susceptible insects. Models and experimental data showed that separate but adjacent refuges might be superior to other strategies for insects that can move between plants in their larval stage (Shelton et al. 2002).

A concern is often raised that insect damage in non-Bt fields will increase after introducing Bt crops. The implication is that farmers will be even less likely to grow non-Bt crops because of the increased damage, and therefore there will be even fewer refuge fields. However, Cohen (2000) suggested that with diamondback moth on Bt collards and the European corn borer on Bt maize, many of the moths that emerge from fields of non-Bt crops would disperse and lay their eggs in Bt fields. In contrast, very few moths will emerge from Bt fields and move from Bt fields to non-Bt fields. As a consequence, insect damage in non-Bt fields may decrease if most fields are planted with Bt crops.

There is also a debate regarding the spatial design of the refuge system (separate/seed-mixture) to be adapted. Roush (1997a) pointed out that seed mixes can actually promote resistance development for insects that move from plant to plant. There is no evidence to show that moths can detect whether or not a plant contains Bt toxin. In some studies, it has been found that after the feeding begins, caterpillars move away from Bt plants faster than from non-Bt plants, but very few larvae crawl far enough to move from one field to another. Ramachandran et al. (1998) found that *P. xylostella* larvae move away from transgenic canola plants within 24 hours. Similarly, *H. virescens* and *H. zea* larvae are known to move between plants, so seed mixtures might not work. For endophytic insects such as *P. gossypiella* and other stem and root-feeding species with limited larval and adult movement, within-field refuge would be best (Gould 1998). Mallet & Porter (1992) pointed out that in seed mixture refuge system, if the pest's feeding stages could move between plants, instead of ingesting a high dose of toxin or no toxin at all, they would often consume intermediate doses nullifying the advantages of high-dose refuge. The same argument can be extended to transgenic plants with tissue specific expression of toxins. Because of the importance of maintaining appropriate refuges, insect biology and behavior should also to be considered for implementing a refuge system that is practical and economic.

Increasing the size of the refuge delays the development of resistance. Some workers have called for refuges as large as 50%, if farmers are allowed to spray them, which may present a dilemma and reduce farm profitability (Gould & Tabashnik 1998). On the other hand, farmers may be reluctant to sacrifice a large number of refuge plants to insects just to maintain susceptible alleles. In China, *H. armigera* naturally possesses a vast refuge as it can feed on corn, soybean, peanut, and many other crops. Studies that have monitored the sensitivity of *H. armigera* field populations to Bt insecticidal protein Cry1Ac from 1998 to 2000 indicated that *H. armigera* is still susceptible to Cry1Ac protein (Wu et al. 2002b). Although development of *H. armigera* on Bt cotton was much slower than on common cotton, there was a high probability of mating between populations from Bt cotton and other sources due to scattered emergence pattern of *H. armigera* adults and overlap of second and third generations. Thus, in a cotton, soybean, and peanut mix system, non-cotton crops provided a natural refuge (Wu et al. 2002a). As indicated earlier in the diverse cropping systems of the tropics (Sharma et al. 2001), where the insects have several alternative and wild hosts, there may not be any need to grow the refuge crops.

FUSION GENE STRATEGY Theoretical models suggest that pyramiding two dissimilar toxin genes in the same plant has the potential to delay the onset of resistance

much more effectively than single-toxin plants released spatially or temporally, and may require smaller refuges (Roush 1997b). Because of diversity among Bt toxins found in nature, one of the most tempting resistance management strategies is to use two or more of these toxins in mixtures, rotations, or sequences. Laboratory as well as field studies have been conducted to evaluate the efficacy of dual protein transgenic crop plants against several lepidopteran pests (Greenplate et al. 2000a, Stewart & Knighten 2000, Stewart et al. 2001). The basis for this strategy is sometimes referred to as "redundant killing" because insects adapted to one toxin may be susceptible to the second toxin. If the plants contain two Bt toxins at a high dose, insects that are able to survive on a plant with one high-dose toxin are rare, and insects that are able to survive on plants with two high-dose toxins will be very rare. If such insects are homozygous for resistance alleles for two different genes, and if the frequency of the allele for resistance to each gene is 10-3, then insects of the genotype R1R1R2R2 will occur at a frequency of only 10^-12, i.e., 1 out of 1 trillion. Because such insects will be very rare, fewer susceptible insects will be needed to ensure that resistant insects do not mate with each other. Therefore, fewer refuge fields will be necessary, although it is still very important to have some refuge fields.

Similar levels of Cry1Ac have been reported in near isogenic lines of cotton expressing either Cry1Ac alone or Cry1Ac and Cry2Ab (Greenplate et al. 2000b). Activity of single and double toxin genotypes remained greater than the conventional cottons against tobacco budworm. However, Bollgard II, with double toxin, may have greater efficacy against lepidoptera that mainly feed on reproductive structures. Increased activity of Bollgard II (Cry1Ac and Cry2Ab) may be due to increased potency of Cry2Ab, increased overall expression level of Cry2Ab, or possibly a synergistic combination of Cry1Ac and Cry2Ab.(Adamczyk et al. 2001). Dual toxin (Cry1Ac and Cry2 Ac) Bt cottons will provide substantially better control of *H. zea*, *S. frugiperda*, and *S. exigua* compared with the existing single toxin (Cry1Ac) Bt cultivars, and may not require supplemental insecticidal applications (Stewart et al. 2001). Hybrid rice plants expressing a fusion gene, Cry1Ab and Cry1Ac, under the influence of rice *actin*1 promoter are highly resistant to the larvae of both leaffolder and yellow stem borer (Tu et al. 2000). The expression level of the fusion gene (20 ng-1mg soluble protein) in the genome was sufficient to control the lepidopteran insects (the LD 50 for yellow stem borer neonate is 7.58 mg-1ml diet, whereas that for striped stemborer is 7.41 mg-1ml diet) (Attotham et al. 1994).

Serine protease inhibitors synergized Bt against four species of moths and *Leptinotarsa decemlineata* (MacIntosh et al. 1990). Lee et al. (1996) found that a combination of Cry1Ac and Cry1Aa exerted a synergistic effect on gypsy moth larvae, whereas a combination of Cry1Aa and Cry1Ab was antagonistic. Hence, while considering a pyramiding approach, an examination of whether co-expression of multiple toxin genes will have a synergistic effect needs to be undertaken. Similarly, if Bt toxin genes are to be integrated with protease inhibitor genes, protease inhibitors that do not affect the protease-mediated cleavage to release activated Bt toxin but that are still capable of inhibiting digestive process of the insect need to be engineered.

The strategy of "pyramiding," i.e., combining two toxins in a single transgenic plant will, at best, substantially reduce the size of the needed refuge and at worst, produce resistance to both toxins in the same amount of time as for a single toxin (Roush 1997b). Cross-resistance among toxins and the ability of insects to develop resistance to multiple toxins will limit the success of this approach (Roush 1998). Studies have shown that there are large differences in the cross-resistance spectrum of the insect species that have been selected for resistance using single toxins or toxin mixtures. Polygenic inheritance and the existence of multiple mechanisms of resistance may be involved in broad-spectrum resistance, and may limit the use of multiple toxin strategies for managing resistance (McGaughey 1994). Although, the independence of Cry1C resistance from Cry1A resistance in diamondback moth suggests that Cry1C and Cry1A toxins might be useful in rotations or mixtures for delaying resistance (Liu & Tabashnik 1997), the dominance of resistance can vary for a given pest from different locations. However, pyramiding of two or more insecticidal genes in the same plant is a promising long-term strategy for delaying resistance, and one which is more forgiving on refuge size. The so-called, high dose strategy, combined with the use of refuges, is widely agreed to be the best technical approach for managing resistance, and evidence is accumulating that 'separate' refuges are more effective at conserving pest susceptibility than 'mixed' refuges (Cannon 2000).

THE HIGH-DOSE APPROACH Doses of toxins that do not make life hard for susceptible individuals, either by killing them or by reducing their reproductive output, do not select for resistance. On the other hand, doses that are sufficient to kill all individuals in a population, including the most resistant genotypes, do not select for resistance either, because no one is favored by discrimination. However, slow decay of toxin residues means that there will almost certainly be a time period where discrimination works strongly in favor of resistant individuals in a population (Tabashnik & Croft 1982). Low-dose insecticide applications have been shown to create high risks of resistance

development (Georghiou & Taylor 1977) and the theoretical potential for spraying crops with extremely high doses of one or more insecticides has been discussed often (Roush 1989, Tabashnik & Croft 1982). The "high-dose refuge" strategy is the most widely used and has been implemented in North America (Alstad & Andow 1995). When an insecticide spray kills 95% of the susceptible (SS) individuals, the survival of RS individuals is likely to be significantly higher, unless the alleles governing resistance happen to be phenotypically recessive (i.e, the RS and SS insects are physiologically identical). Instead of hoping that resistance is phenotypically recessive, the high dose approach attempts to make resistance alleles "effectively recessive" even if they are not phenotypically recessive (Gould 1998). Similarly, dose that is insufficient to kill the insects bearing one copy of a major resistance allele renders resistance functionally partially dominant. Hence, the only commercially available approach to reduce the likelihood of resistance development is the use of a high dose of a single gene, producing 25 times the toxin concentration needed to kill susceptible insects in combination with a refuge.

High concentrations of Cry1Ac in bolls of transgenic cotton are essential for achieving functionally recessive inheritance of resistance (Liu et al. 2001). Further, extensive planting of transgenic corn hybrids having sub-optimal production of the toxin and resulting in only moderate effects on *H. zea* would raise concerns about the rapid evolution of resistance (Storer et al. 2001). If transgenic plants could be made to express enough toxins to overcome all homozygous resistance alleles, the crop in question would become a non-host. The lack of a "high dose" in current Bt cotton cultivars for *H. armigera* and the small scale production systems of cotton indicates that the "high dose/refuge" resistance management strategy is not feasible for Bt cotton in northern China (Zhao et al. 2000a). Under these circumstances, supplemental control of *H. armigera* with insecticides is essential to grow Bt cotton for a longer period (Ru et al. 2002). Resistance in insects to Bt can be dramatically reduced through the genetic engineering of chloroplasts in plants. Several copies of the Bt genes could be expressed per cell via the chloroplast genome as opposed to only two copies via the nuclear genome in a diploid cell. The Cry2Aa2 protoxin levels in chloroplast-transformed tobacco leaves are between 2 to 3% of total soluble protein, and are 20-to-30-fold higher than current commercial transgenic plants (Kota et al. 1999). If a toxin is consistently produced by a plant at a highly toxic concentration without having a negative effect on yield, and the toxin does not affect non-target organisms, then the constraints on high dose strategy would be quite low.

Another serious concern regarding the success of high dose strategy is that the hypothesis of resistance being recessive does not hold in different insect species. Inheritance of resistance showed incomplete dominance in *O. nubilalis* to a commercial preparation of Bt (Huang et al. 1999), and in *H. virescens* to Cry1Ab (Sims & Stone 1991). While, Tabashnik et al.(1998) demonstrated dominant resistance to Cry1Aa in a strain of *P. xylostella* having field-evolved Bt resistance.

CONTROLLED EXPRESSION of TOXINS Mono-cultivation of Bt transgenic crops is likely to select intensely for resistance because pests will be exposed to Bt even when they are not causing economic damage (Mallet & Porter 1992). The degree of yield reduction caused by a pest population is dependent on its density, as well as on when and where insects feed on the plants. Expression of toxin coding genes could be limited to vulnerable plant parts, and at times when toxicity is needed most. If a pest causes no damage when it feeds on mature leaves, but causes severe stunting when it feeds on buds and developing leaves, then toxin production only in buds would be useful. Having Bt expressed in plants so that the insect population is subjected to selection pressure for particular periods of time (e.g., through an inducible promoter) or in particular plant parts (e.g., through tissue-specific promoters) may provide larger refuges for susceptible alleles both within the field and within a region while at the same time minimizing the crop loss (Roush 1997b). This can be achieved by using gene constructs having a tissue specific promoter.

In *P. xylostella*, resistance to Bt declined when exposure to insecticide ceased (mean R = -0.19). In four other pests (*H. virescens*, *L. decemlineata*, *Musca domestica* and *P. interpunctella*), resistance to Bt declined slowly or not at all (mean R = -0.02) in the absence of exposure to Bt (Tabashnik et al. 1994). Similar loss of resistance in *O. nubilalis* was observed in the absence of selection pressure (Bolin et al. 1999). This can be exploited for formulating resistance management strategies by enforcing complete restriction on cultivation of certain Bt cultivars for a specified period.

Solutions to resistance management involve complex strategies. The track record of resistance management for chemical pesticides is not encouraging. The wisdom gained from previous pesticide failures should provide impetus for the proactive development and implementation of management strategies for transgenic crops. Keeping this in view, Cohen (2000) made four practical recommendations for promoting the sustainable use of Bt crops, based on existing knowledge of the principles of resistance management:

- Do not release Bt varieties that do not have a high dose of toxin. Toxin titers of 2 µg/g of leaf fresh weight or 0.2 % of soluble leaf protein have been shown to act as high doses against most insect pests of crops.

- Release only Bt cultivars that have two Bt toxin genes, which are not closely related to each other, and both should be expressed at a high dose.

- Do not release Bt-transformed versions of all popular crop varieties. Some popular non-Bt varieties should remain available to improve chances that some non-Bt fields (refuges) will exist.

- Implement resistance monitoring programs to serve as an early warning system for governments and farmers and provide valuable information for improved deployment of future pest-resistant cultivars.

However, the farm-level implementation of resistance management will face practical and social obstacles. A survey conducted by US maize growers has shown that in the year 2000, almost 30% of the farmers failed to comply with the refuge protocols designed to prevent or delay the emergence of insects resistant to Bt toxins (Dove 2001). Ensuring effective resistance management practices is a challenge that will require coordination from all sectors (public and private) concerned with crop protection, and will require the commitment of growers and advisers that current technology for crop protection is a precious resource vital to profitable production. There is a continuing need for interaction between ecologists, geneticists, and plant breeders in determining system-wide impacts and devising optimal ways of deploying insect-resistant crops. The current state of knowledge is not sufficient to support any single proven resistance management strategy that may be recommended as a general approach to avoid resistance to transgenic Bt plants, and demands thorough examination of the tritrophic interactions that occur between insecticidal proteins, the plant, and the insect.

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Generating Baseline Data for Insecticide Resistance Monitoring in Cotton Aphid, *Aphis gossypii* Glover

ABSTRACT The baseline susceptibility data were generated for the six commonly used insecticides viz., thiamethoxam, imidacloprid, dimethoate, methyl demeton, acephate, and monocrotophos in cotton ecosystems for the field population of *Aphis gossypii*. Populations were collected from the cotton fields of the Department of Cotton, Agricultural College and Research Institute, TNAU, Coimbatore, India. IRAC method No. 8 developed and recommended by Insecticide Resistance Action Committee (IRAC) with a slight modification was used for arriving the lethal concentrations. The base line susceptibility data were created for seven generations. The LC50 values varied from 0.3412 to 1.0414 for thiamethoxam, 0.4583 to 1.8055 for imidacloprid, 3.0096 to 10.6924 for dimethoate, 12.598 to 49.2606 for methyl demeton, 1.4615 to 5.3284 for acephate, and 1.1866 to 3.7057 for monocrotophos. The LC95 values varied from 10.8617 to 35.2153 for thiamethoxam, 17.9171 to 43.4310 for imidacloprid, 49.1667 to 629.6511 for dimethoate, 418.4538 to 1174.6270 for methyl demeton, 36.1800 to 130.4890 for acephate, and 24.9571 to 139.4943 for monocrotophos.

KEY WORDS: Insecticide resistance, *Aphis gossypii*, diagnostic doses

INTRODUCTION The importance of *Aphis gossypii* Glover as a cotton pest is increasing throughout the world (Leclant and Deguine, 1994). High aphid populations may stunt and retard cotton seedling growth and development as a result of its feeding. Late season populations can cause decreased fiber quality as the result of stickiness and the development of sooty mould associated with honeydew dropped onto cotton fibers (Isely, 1946). There has been a general decline in the effectiveness of several insecticides to control *A. gossypii*. The intensity of aphid infestations has increased over the last ten years and the use of insecticides to control aphids is questioned.

The pest problem is aggravated more rapidly due to control failures in many areas. Though control failure may be due to many factors, one of the major factors is the development of resistance to insecticides. The chief objective in resistance monitoring is to exaggerate the differences between susceptible and resistant individuals such that the frequency of misclassification is greatly reduced (Ffrench-constant and Roush, 1990). This is fulfilled by fixing the diagnostic doses.

Resistance to *A. gossypii* is in the initial stages of development and no systematic work has been done so far on monitoring of insecticide resistance in India as it has been done in *Amrasca devastans* (Distant) (Jaya Pradeepa and Regupathy, 2002), *Helicoverpa armigera* (Hub), *Plutella xylostella* (Linn.), and *Spodoptera litura* (Niranjan Kumar and Regupathy, 2001). Given the background, the present study was undertaken to determine the diagnostic doses for the commonly used insecticides in cotton for *A. gossypii*.

MATERIALS and METHODS The test insects were collected from the cotton field, Department of Cotton, Agricultural College and Research Institute, TNAU, Coimbatore, India. The population was maintained for seven continuous generations without exposure to pesticides under the laboratory conditions for generating the baseline data, i.e. fixing diagnostic doses.

The dilutions required were prepared from the commercial formulations of insecticides using distilled water. The dosages were attained after preliminary range finding studies for constructing log-concentration-probit-mortality (lcpm) lines (Regupathy and Dhamu, 2001).

The wingless adults aphids of ca 1.45mm size and weighing ca 0.19mg were taken from the culture maintained for the treatment. Each replication consisted of 10 aphids and there were three replications. Bioassays were conducted following the procedure based on the standard *Bemesia tabaci* Gennadius susceptibility test, IRAC method No.8 developed and recommended by the Insecticide Resistance Action Committee, with slight modification.

The experimental setup consisted of two disposable cups, one as an inner test chamber and the other as an outer water reservoir. The cup that served as the inner test chamber was taken and a hole was pierced in the centre of the bottom side of the cup.

The young green uncontaminated leaves were selected and the petiole was cut to a length of approximately four cm. The leaves were dipped in the concentrations for five seconds holding the leaf by the petiole with fine forceps. Care was taken to avoid the damage to the petiole. Then the leaves were left for