D. Insect Resistance Management

1. Introduction

Insect resistance management (IRM) is the term used to describe practices aimed at reducing the potential for insect pests to become resistant to a pesticide. *Bt* IRM is of great importance because of the threat insect resistance poses to the future use of *Bt* plant-pesticides and *Bt* technology as a whole. Specific IRM strategies, such as the high dose/structured refuge strategy, will mitigate insect resistance to specific *Bt* proteins produced in corn, cotton, and potatoes. Academic scientists, public interest groups, organic and other farmers have expressed concern that the widespread planting of these genetically transformed plants will hasten the development of resistance to pesticidal *Bt* endotoxins. Effective insect resistance management can reduce the risk of resistance development. This section provides EPA's scientific assessment of various *Bt* plant-pesticide IRM strategies by reviewing the data and information available to the Agency. The Agency will use this assessment, the report of the FIFRA SAP meeting on October 18, 2000, and all public comments in its development of its risk management decisions for *Bt* plant-pesticides.

The following list will assist the reader with the acronyms for the insect pests discussed in this section.

Acronym	**Common Name**	**Scientific Name**	**Crop**
BCW	Black Cutworm	*Agrotis ipsilon* (Hufnagel)	corn
CBW	Cotton Bollworm	*Helicoverpa zea* (Boddie)	cotton
CEW	Corn Ear Worm	*Helicoverpa zea* (Boddie)	corn
CPB	Colorado Potato Beetle	*Leptinotarsa decemlineata* (Say)	potato
CSB	Common Stalk Borer	*Papaipema nebris* (Guen.)	corn
ECB	European Corn Borer	*Ostrinia nubilalis* (Huebner)	corn
FAW	Fall Armyworm	*Spodoptera frugiperda* (J. E. Smith)	corn
PBW	Pink Bollworm	*Pectinophora gossypiella* (Saunders)	cotton
SCSB	Southern Corn Stalk Borer	*Diatraea crambidoides* (Grote)	corn
SWCB	Southwestern Corn Borer	*Diatraea grandiosella* (Dyar)	corn
TBW	Tobacco Budworm	*Heliothis virescens* (Fabricius)	cotton

The 1998 Science Advisory Panel Subpanel agreed with EPA that an appropriate resistance management strategy is necessary to mitigate the development of insect resistance to *Bt* proteins expressed in transgenic crop plants. The Subpanel recognized that resistance management programs should be based on the use of both a high dose and structured refuges designed to provide sufficient numbers of susceptible adult insects. The 1998 SAP also noted that insect resistance management strategies should be sustainable and to the extent possible, strongly consider grower acceptance and logistical feasibility. The Subpanel defined a high dose as 25 times the amount of *Bt* delta-endotoxin necessary to kill susceptible individuals. The Agency has adopted this definition of high dose. A *Bt* plant-pesticide could be considered to provide a high dose if verified by at least two of the following five approaches: 1) Serial dilution bioassay with artificial diet containing lyophilized tissues of *Bt* plants using tissues from non-*Bt* plants as controls; 2) Bioassays using plant lines with expression levels approximately 25-fold lower than the commercial cultivar determined by quantitative ELISA or some more reliable technique; 3) Survey large numbers of commercial plants in the field to make sure that the cultivar is at the $LD_{99.9}$ or higher to assure that 95% of heterozygotes would be killed (see Andow and Hutchison, 1998); 4) Similar to #3 above, but would use controlled infestation with a laboratory strain of the pest that had an LD_{50} value similar to field strains; and 5) Determine if a later larval instar of the targeted pest could be found with an LD_{50} that was about 25-fold higher than that of the neonate larvae. If so, the stage could be tested on the *Bt* crop plants to determine if 95% or more of the later stage larvae were killed.

Effective IRM is still possible even if the transformed plant does not express the *Bt* protein at a high dose. If the *Bt* plant is non-high dose, the IRM plan could include increased refuge size, increased scouting and monitoring, and/or prohibition of sales of non-high dose products in certain areas.

A structured refuge is a non-*Bt* portion of a grower's field or set of fields that provides for the production of susceptible insects that may randomly mate with resistant insects that may emerge from *Bt* fields and dilute resistance. The size, placement, and management of the refuge is critical to the success of the high dose/structured refuge strategy to mitigate insect resistance to the *Bt* proteins produced in corn, cotton, and potatoes. The 1998 Subpanel defined structured refuges to "include all suitable non-*Bt* host plants for a targeted pest that are planted and managed by people. These refuges could be planted to offer refuges at the same time when the *Bt* crops are available to the pests or at times when the *Bt* crops are not available." The Subpanel suggested that a production of 500 susceptible adults in the refuge that move into the transgenic fields for every adult in the transgenic crop area (assuming a resistance allele frequency of 5×10^{-2}) would be a suitable goal. The placement and size of the structured refuge employed should be based on the current understanding of the pest biology data and the technology. The SAP also recognized that refuges should be based on regional pest control issues.

To address the very real concern of insect resistance to *Bt* proteins, EPA has imposed IRM requirements on registered *Bt* plant-pesticides. Sound IRM will prolong the life of *Bt* pesticides and universal adherence to the plans is to the advantage of growers, producers, researchers, and the American public. EPA's strategy to address insect resistance is two-fold: 1) mitigate any significant potential for pest resistance development in the field by instituting IRM plans, and 2) better understand the mechanisms behind pest resistance.

Beginning with the first *Bt* plant-pesticide registration, the Agency has taken steps to manage insect resistance to *Bt* with IRM plans being an important part of the regulatory decision. The Agency identified (later confirmed by the 1995 SAP) seven elements that should be addressed in a *Bt* plant-pesticide resistance management plan: 1) knowledge of pest biology and ecology, 2) appropriate dose expression strategy, 3) appropriate refuge, 4) resistance monitoring and a remedial action plan should resistance occur, 5) employment of integrated pest management (IPM), 6) communication and education strategies on use of the product, and 7) development of alternative modes of action.

Key to developing an effective IRM plan is an understanding of the pest(s) biology, the dose of the protein expressed in the various plant tissues, and the size and placement of the refuge (a portion of the total acreage using non-*Bt* seed). It is believed that planting a refuge will delay the development of insect resistance by maintaining insect susceptibility. In addition to a structured refuge, IRM plans include additional field research, resistance monitoring for the development of resistance (and increased insect tolerance of the protein), grower education, a remedial action plan in case resistance is identified, annual reporting and communication. IRM plans will change as more scientific data become available. EPA, has in fact, changed IRM plans as new data has become available.

A summary of the Agency's risk assessment of insect resistance development and insect resistance management plans to mitigate resistance is provided below for *Bt* corn, *Bt* cotton, and *Bt* potato products. The detailed Agency risk assessments of insect resistance management are found in the following memoranda: A. Reynolds and R. Rose (OPP/BPPD) to M. Mendelsohn (OPP/BPPD), dated September 11, 2000; S. Matten (OPP/BPPD) to W. Nelson (OPP/BPPD), dated July 10, 2000; S. Matten (OPP/BPPD) to W. Nelson (OPP/BPPD), dated September 11, 2000; and S. Matten (OPP/BPPD) to W. Nelson and L. Hollis (OPP/BPPD), dated July 5, 2000.

2. Corn

The Agency's IRM assessment focuses on two *Bt* plant-pesticides produced in corn: Cry1Ab or Cry9C in either field corn (grown primarily for non-human animal consumption), sweet corn or popcorn (the latter two grown primarily for human consumption). EPA has used the best available scientific information in its IRM assessment and has updated its IRM position as information has become available.

Bt corn IRM plans address corn earworm (CEW) (known as the cotton bollworm in cotton), a pest that is polyphagous, i.e., pests that feed on more than one crop. CEW is a pest of both corn and cotton and early generations may live in corn with subsequent generations in cotton during one growing season. It is possible that as many as six generations of CEW can be exposed to the same or related *Bt* proteins expressed in *Bt* corn and *Bt* cotton, increasing the likelihood of the development of resistance. Because CEW also feeds on other crops (e.g., soybean and tomato), there is also an increased potential for resistant CEW to move to other host crops that may be treated with *Bt* foliar sprays, thus rendering the *Bt* ineffective.

In 1995, at the time of the initial registrations of *Bt* corn, there was no scientific consensus on the details of the IRM plans necessary for prevention of the development of resistance in the two primary target pests, European corn borer (ECB) and CEW. At that time, the putative values for adequate refuge size ranged from 20% to 50% of non-*Bt* corn or other host plants per farm. While the minimum adequate refuge size or structure could not be determined until further research was conducted, it was thought that market penetration of these crops would be sufficiently slow that considerable non-*Bt* corn would remain to act as natural refuges while the additional research was conducted. Thus, the initial *Bt* corn registrants instituted voluntary IRM plans with the requirement that these registrants must submit a refuge strategy by April 1999. From 1995-1997, the registrants agreed to various voluntary refuge requirements in the Corn Belt.

Since 1995, all *Bt* corn registrations have included a resistance monitoring plan for ECB and CEW that contained the following elements: 1) development of baseline susceptibility responses and a discriminating concentration to detect changes in sensitivity, 2) routine surveillance, and 3) remedial action if there is suspected resistance. One of the key purposes of resistance monitoring is to learn whether a field control failure resulted from resistance or other factors that might inhibit expression of the *Bt* Cry delta endotoxin. The extent and distribution of resistant populations can be mapped and alternative control strategies implemented in areas in which resistance has become prevalent. If monitoring techniques are sensitive enough to discriminate between resistant and susceptible individuals, it should be possible to detect field resistance before significant loss of efficacy and eliminate any resistant individuals using other control tactics. In addition, EPA mandated that all registrants must require customers to notify them of incidents of unexpected levels of ECB and CEW damage. Registrants are required to investigate these reports and identify the cause of the damage by local field sampling of the plant tissue and suspect insect populations followed by appropriate in vitro and in planta assays. Any confirmed incidents of resistance are required to be reported to EPA. Based on these investigations, appropriate remedial action is required to mitigate ECB and/or CEW resistance. These remedial actions include: informing customers and extension agents in the affected areas of ECB and/or CEW resistance, increasing monitoring in the affected areas, implementing alternative means to reduce or control ECB or CEW populations in the affected areas, implementing a structured refuge in the affected areas, and cessation of sales in the affected and bordering counties. All registrants have instructed growers to have regular surveillance programs and report any unexpected levels of ECB and CEW damage. Since 1995, there has been no field evidence of ECB, CEW or southwestern corn borer resistance to any of the *Bt* proteins produced in corn. In January 2000, the Agency required that the registrants provide a more detailed resistance monitoring plan that focused on ECB, CEW, and SWCB. The registrants provided the Agency with a revised monitoring plan in March 2000. This monitoring plan is discussed in detail later in this section.

Based on the 1998 SAP Subpanel recommendations, the Agency began to institute mandatory refuge requirements on *Bt* field corn and popcorn products. In 1999, a coalition of *Bt* corn registrants (working with the National Corn Growers Association) approached EPA with a uniform IRM plan for their products. With some modifications to this plan, EPA put in place a consistent set of required refuge strategies for all *Bt* corn products for the 2000 growing season. These requirements greatly strengthened the IRM plan to mitigate ECB, CEW, and SWCB resistance to *Bt* proteins produced in field corn. For the year 2000, EPA required a 20% non-*Bt* corn refuge to planted within ½ mile (1/4 mile in areas where insecticides have been historically used to treat ECB and SWCB) (EPA letter to *Bt* corn registrants, 1/31/00). EPA also required a 50% non-*Bt* corn refuge for *Bt* Cry1Ab corn products in certain southern counties and states where most cotton is grown (EPA letter to *Bt* corn registrants, 1/31/00). The larger refuge was necessary to mitigate the development of resistance to *Bt* proteins in CEW populations feeding on both corn and cotton.

a. Current Insect Resistance Management (IRM) Plans for *Bt* corn

1) MON 810, BT11, and Cry9C

These products are known as "high dose" for ECB based on the 25 X definition described by the 1998 SAP Subpanel (SAP, 1998). Below are EPA's current terms and conditions for IRM for the *Bt* corn plant-pesticide registration for the 2000 growing season:

- "For *Bt* field corn grown outside cotton-growing areas (e.g., the Corn Belt), grower agreements (stewardship agreements) will specify that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower guide/product use guide. Specifically, growers must plant a minimum structured refuge of at least 20% non-*Bt* corn. Insecticide treatments for control of ECB, CEW and/or Southwestern corn borer (SWCB) may be applied only if economic thresholds are reached for one or more of these target pests. Economic thresholds will be determined using methods recommended by local or regional professionals (e.g., Extension Service agents, crop consultants). Instructions to growers will specify that microbial *Bt* insecticides must not be applied to non-*Bt* corn refuges."

- "For the 2000 growing season, grower agreements (stewardship agreements) for Cry1Ab *Bt* field corn grown in cotton-growing areas specified that growers must adhere to the refuge requirements as described in the grower guide/product use guide and/or in supplements to the grower/product use guide. Specifically, growers in these areas must plant a minimum structured refuge of 50% non-*Bt* corn. Cotton-growing areas include the following states: Alabama, Arkansas, Georgia, Florida, Louisiana, North Carolina, Mississippi, South Carolina, Oklahoma (only the counties of Bryan, Caddo, Canadian, Garvin, and Grady), Tennessee (only the counties of Carroll, Chester, Crockett, Fayette, Franklin, Gibson, Hardeman, Hardin, Haywood, Hendersen, Lake, Lauderdale, Lawrence, Lincoln, McNairy, Madison, Obion, Rutherford, Shelby, and Tipton), Texas (except the counties of Carson, Dallam, Hansford, Hartley, Hutchinson, Lipscomb, Moore, Ochiltree, Roberts, and Sherman), Virginia (only the counties of Greensville, Isle of Wight, Northampton, Southampton, Sussex, Suffolk) and Missouri (only the counties of Butler, Dunkin, Mississippi, New Madrid, Pemiscot, Scott, Stoddard)."

- "Requirements for refuge deployment will be described in the Grower Guides/Product Use Guides as described in Section D of the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) IRM Plan submitted to EPA on April 19, 1999. Growers must continue to plant only non-*Bt* corn in the refuge and to plant the refuge within ½ mile of their *Bt* corn acreage. In regions of the corn belt where conventional insecticides have historically been used to control ECB and SWCB, growers wanting the option to treat these pests must plant the refuge within ¼ mile of their *Bt* corn. Refuge planting options include: separate fields, blocks within fields (e.g., along the edges or headlands), and strips across the field. When planting the refuge in strips across the field, growers must be instructed to plant multiple non-*Bt* rows whenever possible."

- "The registrant will monitor for the development of resistance using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. The registrant will proceed with efforts to develop a discriminating concentration assay. The registrant will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB and CEW populations to the Cry1Ab and Cry9C proteins. This resistance monitoring program will be developed to measure increased tolerance to *Bt* corn above the various regional baseline ranges."

- "Populations of ECB and CEW will be collected from representative distribution areas that contain the registrant's *Bt* corn plant-pesticide and monitored/screened for resistance, with particular focus on those areas of highest distribution. The results of monitoring studies will be communicated to the Agency on an annual basis, by January 31 of the year following the population collections for a given growing season."

- "In addition, the registrant will instruct its customers (growers and seed distributors) to contact the registrant (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB and/or CEW damage occur. The registrant will investigate and identify the cause for this damage by local field sampling of plant tissue from corn hybrids that contain the registrant's *Bt* corn plant-pesticide and sampling of ECB and CEW populations, followed by appropriate in vitro and in planta assays. Upon the registrant's confirmation by immunoassay that the plants contain Cry1Ab or Cry9C protein, bioassays will be conducted to determine whether the collected ECB population exhibits a resistant phenotype."

- "Until such time that a discriminating concentration assay is established and validated by the registrant, the registrant will utilize the following to define a confirmed instance of ECB and/or CEW resistance:

  Progeny from the sampled ECB or CEW population will exhibit both of the following characteristics in bioassays initiated with neonates

  1. An LC_{50} in a standard Cry1Ab or Cry9C diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC_{50} for susceptible ECB or CEW populations, as established by the ongoing baseline monitoring program. The source of Cry1Ab crystal protein standard for this bioassay will be *Bacillus thuringiensis* subsp. *kurstaki* strain HD1.

  2. > 30% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1Ab-positive leaf tissue under controlled laboratory conditions.

  Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that the registrant finds it appropriate to alter the criteria specified in the working definition, the registrant must obtain Agency approval in establishing a more suitable definition.

  The current insect monitoring program was expanded to include SWCB and CEW, in addition to ECB. The expanded program must focus monitoring in areas that typically have a high density of *Bt* corn or have historically been prone to high levels of corn borer pressure and where the refuge areas may more likely be treated with insecticides."

- "The current definition of confirmed insect resistance must be used as described above in the ABSTC IRM Plan. Agency approval will be sought prior to implementation of any modified definition of confirmed insect resistance."

- "When resistance has been demonstrated to have occurred, the registrant must stop sale and distribution of *Bt* corn in the counties where the resistance has been shown until an effective local mitigation plan approved by EPA has been implemented. The registrant assumes responsibility for the implementation of resistance mitigation actions undertaken in response to the occurrence of resistance during the growing season. EPA interprets "suspected resistance" to mean, in the case of reported product failure, that the corn in question has been confirmed to be *Bt* corn, that the seed used had the proper percentage of corn expressing *Bt* protein, that the relevant plant tissues are expressing the expected level of *Bt* protein, that it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out. The Agency does not interpret "suspected resistance" to mean grower reports of possible control failures, nor does the Agency intend that extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken."

- "The registrant will maintain a (confidential) database to track sales (units and location) of its *Bt* corn on a county-by-county basis. The registrant will provide annually, on a CBI basis, sales data for each state indicating the number of units of corn hybrids that contain the registrant's *Bt* corn plant-pesticide that were sold. As part of the overall sales report, the registrant will provide a listing of an estimate of the acreage planted with such states and counties with sales limitations. This information will be provided by January 31 of the year following each growing season."

- "The registrant will provide grower education. The registrant will agree to include an active partnership with such parties as: university extension entomologists and agronomists, consultants, and corn grower groups. The registrant will implement a grower education program (in part, as requested by the registrant, through the Grower Agreement setting forth any resistance management requirements) directed at increasing grower awareness of resistance management, in order to promote responsible product use. Insect Resistance Management educational materials for each growing season must be provided to the Agency as they become available for distribution. Survey results and other available information must be used to identify geographic areas of non-compliance with insect resistance management plans. As described in the ABSTC IRM Plan, an intensified grower education program will be conducted in these geographic areas prior to the following growing season. If individual non-compliant growers are identified, they must be restricted from future purchases of *Bt* corn seed."

- "Several aspects of the IRM Plan will operate in synergy to promote grower compliance, however, the cornerstones of the compliance program must be the:

  1. Grower Guides

  These guides must be distributed to each seed customer and updated on an annual basis, as needed. The guides provide complete information for growers regarding routine IRM practices that must be employed, and will be a primary educational and reference tool. Agreed-upon requirements and additional information that was not included in the grower guides for 2000 (e.g., because the requirements were enacted after printing and distribution of the grower guides) is required to be conveyed via supplemental communications to *Bt* field corn seed customers.

  2. Stewardship Agreement (grower agreement).

  Each grower who purchases *Bt* field corn seed must be required to sign a stewardship agreement, which will obligate the grower to follow the required IRM practices as specified in the grower guide/product use guide and/or in supplements thereof.

  3. A Strong and Multi-Pronged Grower Education Program.

  A variety of methods must be employed to promote grower education and to continue to reinforce the need for adherence to all aspects of the IRM program.

  4. Additional mechanisms must also be used to promote grower compliance. For example, training of sales personnel, seed dealers and technical support staff as well as coordination and reinforcement of IRM requirements through other organizations (e.g., NC-205, the Cooperative Extension Service, USDA, National Corn Growers Assn. (NCGA), American Crop Protection Assn., Biotechnology Industry Organization, crop consultants and other crop professionals)."

- "The registrant will confer with the EPA as the registrant develops various aspects of its resistance management research program. The registrant agrees, as a condition of this registration, to submit annually, progress reports on or before January 31st each year on the following areas, as a basis for developing a long-term resistance management strategy which include:

  1. Research data on CEW relative to resistance development and the registrant's plans for producing resistance predictive models to cover regional management zones in the cotton belt based on CEW biology and cotton, corn, soybeans, and other host plants. These models must be field

tested and must be modified based on the field testing performed during the period of the conditional registration. EPA might modify the terms of the conditional registration based upon the field testing validation of the model and might require refuge in the future. EPA notes that there is some scientific work and even some models for CEW on other crops in at least NC and TX that could be used for reference. EPA wants to be in close communication with the registrant as the model development and testing is ongoing. The requirement for development of resistance predictive models may be modified if the registrant provides the results of research that demonstrates resistance to CEW would have no significant impact on the efficacy of foliar *Bt* products and other *Bt* crops. Actual usage data of *Btk* on crops to control specific pests as well as successes and failures and field validated research would be necessary to support such a waiver request. [Satisfied thus far.]

2. ECB pest biology and behavior including adult movement and mating patterns, larval movement, survival on silks, kernels, and stalks, and overwintering survival and fecundity on non-corn hosts. A combination of a comprehensive literature review and research can fulfill this condition. [Satisfied thus far.]

3. The feasibility of “structured” refuge options for ECB including both “block” refuge, “50-50 early/late season patchwork;” research needs to be done in both northern and southern areas on ECB as well as CEW. [Satisfied thus far.]

4. Development of a discriminating concentration (diagnostic concentration) assay for field resistance (field screening) for ECB, CEW and other lepidopteran pests of corn. Specific sampling locations will be established in each state to determine if increases in *Bt* protein tolerance are occurring before crop failures develop. Increased tolerance levels need to be identified before field failure occurs. In monitoring for tunneling damage, the number of trivial tunnels may be less indicative of resistance development than the total extent of tunneling damage (e.g., length of tunnels). The extent of tunneling damage must be monitored as well as the number of tunnels. [Satisfied thus far.]

5. Effects of corn producing the Cry1Ab and Cry9C delta endotoxin on pests other than ECB, including but not limited to CEW, fall armyworm (FAW), and the stalk borer complex. [Satisfied thus far.]

6. The biology of ECB resistance including receptor-mediated resistance

and its potential effect on population fitness, as well as the effects on insect susceptibility to other Cry proteins. More data are needed on protein expression in various parts of the plant at different stages plant development in regard to ECB, CEW and other secondary pests of corn (i.e. stalk borer complex, FAW, and SWCB). [Satisfied thus far.]

7. The registrant must assess the feasibility of using the F_2 screen, sentinel plots, and in-field screening kits to increase the sensitivity of resistance monitoring in 2000. By January 31, 2001, the registrant must provide the Agency with the results from these investigations.

8. The registrant must implement a survey approach similar to the Iowa State University *Bt* Corn Survey (e.g., Pilcher and Rice 1999). A statistically valid sample, as determined by independent market research, of *Bt* corn growers in key states will be surveyed by a third-party. *Bt* corn growers will be included based upon a proportionately stratified random sample designed to balance the survey evenly across seed companies and geographies. In addition to demographic information, the survey will include questions related to insect resistance management such as:

a) What is your primary source of information on *Bt* corn?

b) What percentage of your acres were planted to *Bt* corn this year?

c) Are you following a recommended insect resistance management strategy?

d) If you plant most of your acreage to *Bt* corn, are you likely to scout your non-*Bt* corn for economically damaging populations of corn borers?

e) Did you treat your *Bt* corn acres with an insecticide?

f) What planting pattern did you use for your refuge?

° Planted *Bt* corn as one block in one field.
° Planted *Bt* corn in one block in every field.
° Split seed boxes in the planter and alternated every row or several rows with *Bt* and non-*Bt* corn in every field.
° Planted *Bt* corn in large strips alternated with large strips of a non-*Bt* corn hybrid.
° Planted *Bt* corn in an entire field and planted the border around the field with non-*Bt* corn.

° Planted pivot corners to non-*Bt* corn with the irrigated area of the field planted to *Bt* corn."

Cry9C field corn is not toxic to CEW; therefore, a 20% non-*Bt* corn refuge is appropriate throughout all corn-growing areas including *Bt* corn grown in cotton-growing areas. Insecticide treatments for control of ECB, CEW, and/or SWCB may be applied only if economic thresholds are reached for one or more of these target pests. The rest of the information on the Aventis IRM plan is essentially identical to the description given for MON 810 and BT11.

Table D1. Summary of Current *Bt* Field Corn Refuge Requirements

Active Ingredient	ECB Dosage	Refuge Size in Corn Belt	Refuge Size in Cotton Areas	Grower Agreement	Proximity	Comments/ Other Restrictions
MON 810 & BT 11	High dose	20% sprayed or unsprayed	50% sprayed or unsprayed	yes	½ mile	¼ mile prox. for areas w/ pesticide treat. for ECB, SWCB
Cry 9C	High dose	20% sprayed or unsprayed	20% sprayed or unsprayed	yes	½ mile	¼ mile prox. for areas w/ pesticide treat. for ECB, SWCB

2) BT11 Sweet Corn

A key to understanding the resistance management issues with Attribute BT11 sweet corn is to appreciate the differences in the cultural practices of sweet corn versus field corn. Field corn is frequently grown in large blocks on farms of 500 - 1,000 acres. This results in large areas of field corn monoculture. Conversely, sweet corn is usually grown in blocks of 40 acres or less on farms that produce several crops that are also host plants for ECB and CEW.

In contrast to BT11 field corn, specific refuge requirements were not mandated for this *Bt* sweet corn product because sweet corn harvesting occurs before insects mature and reproduce. Sweet corn is harvested 18-21 days after silking while the plant has active photosynthesis. As a result, in transgenic sweet corn varieties, *Bt* protein production is high at the time of harvest. EPA mandated specific resistance monitoring requirements for ECB, CEW, and FAW, as well as sales reporting requirements. Novartis is required through labeling and technical material to have growers destroy any Cry1Ab (BT11) sweet corn stalks that remain in the fields following harvest in accordance with local production practices. Stalk destruction is intended to reduce the possibility of any insects, including resistant insects, surviving to the next generation. The major aspects of the 1998 insect resistance management plan for Attribute BT11 sweet corn are summarized below.

- "Cry1Ab sweet corn may only be sold for commercial sweet corn production."

- "Novartis Seeds' (Vegetables) must require growers to destroy any Cry1Ab sweet corn stalks that remain in the fields following harvest. This activity must take place either immediately following harvest or a short period of time (a maximum of 1 month) later in accordance with local production practices. Stalk destruction prior to winter will insure that any larvae that happen to be present in the plants after harvest are eliminated. This instruction must appear on all supplemental labeling, technical material, and grower guides."

- "Novartis Seeds' (Vegetables) will perform baseline susceptibility studies and monitor for the development of resistance in ECB, CEW, and FAW populations using baseline susceptibility data and/or a discriminating concentration assay when such an assay is available. Novartis Seeds' (Vegetables) will proceed with efforts to develop discriminating concentration assays for ECB, CEW and FAW, and will ensure that monitoring studies are conducted annually to determine the susceptibility of ECB, CEW, and FAW populations to the Cry1Ab protein. This resistance monitoring program will be developed to measure increased tolerance to the Cry1Ab protein above the various regional baseline susceptibility ranges.

  Novartis Seeds (Vegetables) must participate in baseline susceptibility and monitoring efforts for ECB and CEW currently underway as a condition of registration for Novartis Seeds' (Field Crops) *Bt* field corn registrations (EPA Reg. Nos. 66736-1 and 67979-1). Monitoring locations will be chosen to ensure that representative growing areas of *Bt* sweet corn are included. For *Bt* sweet corn monitoring, adjacent plots of *Bt* field corn may be substituted when practical, provided such plots are within 1500 feet of the *Bt* sweet corn to be monitored. Novartis may summarize both *Bt* field and sweet corn ECB and CEW monitoring in one annual report. However, this yearly monitoring report must provide details as to how and where *Bt* sweet corn was monitored in addition to that information for *Bt* field corn. The insect populations will be monitored for changes in the susceptibility to the Cry1Ab protein. In monitoring for tunnel damage, the number of trivial tunnels may be less indicative of resistance development than the total extent of tunneling damage (e.g., length of tunnels). The extent of tunneling damage should be monitored as well as the number of tunnels.

  Novartis Seeds (Vegetables) must consult with the Agency as well as academic expert(s), on an annual basis, to ensure that this monitoring program is sufficient to measure changes in sensitivity to Cry1Ab in these pests that may result from exposure to the active ingredient in Cry1Ab expressing sweet corn.

  Within one year from the date of this registration [1998], baseline susceptibility studies must be conducted on FAW populations collected from sweet corn growing areas in south Texas and south Florida. Monitoring studies will be conducted on FAW populations collected from sweet corn distribution areas in states in which Novartis Seeds' (Vegetables) Cry1Ab sweet corn plantings exceed 1000 acres. The collected populations of FAW will be monitored for changes in susceptibility to the Cry1Ab protein. [Under review.]

Reports of resistance monitoring will be submitted to the Agency on an annual basis, by January 31 of the year following the ECB, CEW, and FAW population collections for a given growing season and include units sold per state of the Novartis Seeds (Vegetables) Cry1Ab corn. These annual reports will also describe progress towards development of a discriminating dose assay for ECB, CEW, and FAW and any additional research information related to the development of a long-term resistance management strategy. Novartis Seeds' (Vegetables) will confer with the EPA as it develops various aspects of its resistance management research program. [Satisfied thus far.]

In addition, Novartis Seeds (Vegetables) will instruct its customers (growers and seed distributors) to contact Novartis Seeds (Vegetables) (e.g., via a toll-free customer service number) if incidents of unexpected levels of ECB, CEW, or FAW damage occur. Novartis Seeds (Vegetables) will investigate and identify the cause for this damage by local field sampling of plant tissue from its hybrids and sampling of ECB, CEW, and FAW populations, followed by appropriate in vitro and in planta assays. Upon Novartis Seeds (Vegetables)'s confirmation by immunoassay that the plants contain Cry1Ab protein, bioassays will be conducted to determine whether the collected ECB, FAW or CEW population exhibits a resistant phenotype.

Until such time that a discriminating concentration assay is established and validated by Novartis Seeds (Vegetables), Novartis Seeds (Vegetables) will utilize the following to define a confirmed instance of ECB, FAW & CEW resistance:

Progeny from the sampled ECB, FAW or CEW population will exhibit both of the following characteristics in bioassays initiated with neonates:

1. An LC_{50} in a standard Cry1Ab diet bioassay that exceeds the upper limit of the 95% confidence interval of the mean historical LC_{50} for susceptible ECB, FAW or CEW populations, as established by the ongoing baseline monitoring program. The source of Cry1Ab crystal protein standard for this bioassay will be *Bacillus thuringiensis* subsp. *kurstaki* strain HD1.

2. > 30% survival and > 25% leaf area damaged in a 5-day bioassay using Cry1Ab-positive leaf tissue under controlled laboratory conditions.

Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that Novartis Seeds (Vegetables) finds it appropriate to alter the criteria specified in the working definition, Novartis Seeds (Vegetables) must obtain Agency approval in establishing a more suitable definition."

- "Novartis Seeds (Vegetables) will report all instances of confirmed ECB, FAW, and CEW resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of ECB, FAW, or CEW resistance Novartis Seeds (Vegetables) will take the following immediate mitigation measures:

    1. notify growers, extension agents, and university cooperators in the affected area;

    2. recommend to customers and extension agents in the affected area the use of alternative control measures to reduce or control the local ECB, CEW, or FAW population;

    3. require customers and extension agents in the affected area to disc and incorporate crop residues into the soil immediately following harvest, to minimize the possibility of overwintering of ECB, CEW, or FAW;

    4. intensify field surveillance for excessive feeding damage and define boundaries of the affected epicenter.

Within 90 days of a confirmed instance of ECB, FAW and/or CEW resistance, as defined above, Novartis Seeds (Vegetables) will: 1) notify the Agency of the immediate mitigation measures that were implemented, 2) submit to the Agency a proposed long-term resistance management action plan for the affected area, 3) work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented, and 4) implement an action plan that is approved by EPA and that consists of some or all the following elements, as warranted:

1. Informing customers and extension agents in the affected area of ECB, FAW, and/or CEW resistance;

2. Increasing monitoring in the affected area, and ensuring that local ECB, FAW, or CEW populations are sampled on an annual basis;

3. Recommending alternative measures to reduce or control ECB, FAW, or CEW populations in the affected area;

4. Implementing a structured refuge strategy in the affected area based on the

latest research results and coordinated by the Agency with other registrants;

5. If the above elements are not effective in mitigating resistance, Novartis Seeds (Vegetables) will voluntarily cease sale of all of Novartis Seeds (Vegetables)'s Cry1Ab corn in the county experiencing loss of product efficacy and the bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, Novartis Seeds (Vegetables) may sell and distribute in these counties only by obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants.

If EPA agrees that an effective resistance management plan has been implemented which mitigates resistance, Novartis Seeds (Vegetables) can resume sales in the affected county(ies)."

- "Novartis Seeds (Vegetables) will maintain a (confidential) database to track sales (units and location) of its *Bt* corn on a county-by-county basis. Novartis Seeds (Vegetables) will provide annually, on a CBI basis, sales data for each state indicating the number of units of corn hybrids that contain Novartis Seeds (Vegetables)'s *Bt* corn plant-pesticide that were sold. As part of the overall sales report, Novartis Seeds (Vegetables) will provide a listing of an estimate of the acreage planted with such states and counties with sales. This information will be provided by January 31 of the year following each growing season."

- "Novartis Seeds (Vegetables) will provide grower education. Novartis Seeds (Vegetables) has identified primary targets of their education and communication programs. In the processing market, these targets will be field representatives, operations managers, and quality assurance staff. For the commercial fresh market, communication targets will be dealer sales representatives and the growers.

The key communication points will be the:

- importance of insect resistance management (IRM);
- customer/grower roles and responsibilities in IRM;
- cultural techniques that impact IRM;
- importance of scouting for ECB, CEW and FAW damage;
- importance of chemical control for lepidopteran pests as needed; and
- importance of reporting unexpected levels of insect feeding damage to Novartis Seeds (Vegetables).

This material will be delivered to the communication targets through on-site presentations, a

Grower Guide, and an 800 number to which growers can report unexpected damage.

In its Grower Guide, supplemental labeling, and technical material, Novartis Seeds (Vegetables) must specify:

> 1) Growers are required to destroy any Cry1Ab sweet corn stalks that remain in the fields following harvest. This activity could take place either immediately following harvest or a short period of time later in accordance with local production practices. Stalk destruction prior to winter will insure that any larvae that happen to be present in the plants after harvest are eliminated. The statement "Growers are required to destroy any Attribute BT11 (Cry1Ab) sweet corn stalks that are remaining in fields within 1 month following harvest" would suffice.
>
> and
>
> 2) Control for lepidopteran pests, as needed, must not utilize *Bt* microbial products. The statement "No *Bt* microbial pesticides may be used as supplemental insecticide sprays." would suffice;
>
> 3) no *Bt* microbial pesticides may be used as supplemental insecticide sprays;
>
> 4) seed dealers and/or processors may not sell Cry1Ab sweet corn to growers who have been found to not comply with any of the items above."

b. Analysis of the Risks Associated with Current IRM Plans and Alternatives

The risk that insect pests may become resistant to *Bt* plant-pesticides and *Bt* microbial sprays has been acknowledged by many organizations and individuals including EPA's Scientific Advisory Panel (SAP) and Pesticide Program Dialogue Committee (PPDC). SAP meetings and reports in both 1995 and 1998 have confirmed that EPA's approach and elements required in an insect resistance management plan are appropriate. EPA believes that pest biology and the dose of the *Bt* protein expressed in the various plant tissues influence the size and placement needed for an effective refuge. This section is a summary of the key elements of several options for IRM plans for corn and compares the level of risk of resistance development for each scenario. The full risk assessment for *Bt* corn is found in the Agency's memorandum, A. Reynolds and R. Rose (OPP/BPPD) to M. Mendelsohn (OPP/BPPD), dated September 11, 2000.

1) Pest Biology

Knowledge of pest biology is critical for the development of effective IRM strategies and to increase confidence that the IRM plans will be effective at reducing the likelihood that insects will become resistant to *Bt* proteins.

a) ECB (Primary Target Pest)

ECB is a major pest of corn throughout most of the United States. The pest has 1-4 generations per year, with univoltine populations in the far North (i.e., all of North Dakota, northern South Dakota, northern Minnesota, and northern Wisconsin), bivoltine populations throughout most of the Corn Belt, and multivoltine (3-4 generations) populations in the South (Mason et al. 1996). The February, 1998 SAP meeting on IRM identified a number of areas needing additional research including larval movement, adult movement, mating behavior, pre- and post-mating dispersal, ovipositional behavior, fitness, and overwintering habitat (SAP 1998). Since the first registrations of *Bt* corn hybrids in 1995, a significant amount of research has been undertaken in many of these areas, although additional work is still needed. A summary of key aspects of ECB biology that relate to IRM is presented below:

i. Larval Movement

ECB larvae are capable of significant, plant-to-plant movement within corn fields. Research conducted in non-transgenic corn showed that the vast majority of larvae do not move more than two plants within a row (Ross & Ostlie 1990). However, in transgenic corn, unpublished data (used in modeling work) from F. Gould (cited in Onstad & Gould 1998) indicates that approximately 98% of susceptible ECB neonates move away from plants containing *Bt*. Recent multi-year studies by Hellmich (1996, 1997, 1998) have attempted to quantify the extent of plant-to-plant larval movement. It was observed that 4th instar larvae were capable of movement up to six corn plants within a row and six corn plants across rows from a release point. Movement within a row was much more likely than movement across rows (not surprising, due to the fact that plants within are row are more likely to be "touching" as opposed to those across rows). In fact, the vast majority of across row movement was limited to one plant. This type of information has obvious implications for optimal refuge design. Larvae moving across *Bt* and non-*Bt* corn rows may be exposed to sublethal doses of protein, increasing the likelihood of resistance (Mallet & Porter 1992). Given the extent of ECB larval movement between plants, seed mixes have been determined to be an inferior refuge option (Mallet & Porter 1992, SAP 1998, Onstad & Gould 1998).

ii. Adult Movement

Information on movement of adult ECB (post-pupal eclosion) is necessary to determine appropriate proximity guidelines for refuges. Refuges must be established within the flight range of newly emerged adults to help ensure the potential for random mating. An extensive, multi-year project to investigate ECB adult dispersal has been undertaken by the University of Nebraska (Hunt et al. 1997, 1998a). Results from these mark and recapture studies (with newly emerged, pre-mated adults) showed that the majority of ECB adults did not disperse far from their emergence sites. The percentage recaptured was very low (< 1%) and the majority of those that were recaptured were caught within 1500 feet of the release site. Few moths were captured outside of 2000 feet. These results have specifically led to recommendations and guidelines for refuge proximity and deployment.

iii. Mating Behavior

In addition to patterns of adult movement, ECB mating behavior is an important consideration to insure random mating between susceptible and potentially resistant moths. In particular, it is important to determine where newly emerged females mate (i.e., near the site of emergence or after some dispersal).

It is well established that many ECB take advantage of aggregation sites (usually clusters of weeds or grasses) near corn fields for mating. Females typically mate the second night after pupal eclosion (Mason et al. 1996). One recent study suggested that it may be possible to manipulate aggregation sites to increase the likelihood of random mating between susceptible and potentially resistant ECB (Hellmich et al. 1998). Another recent study (mark/recapture studies with newly eclosed ECB) conducted by the University of Nebraska showed that relatively few unmated females moved out of the corn field from which they emerged as adults (Hunt et al. 1998b). This was especially true in irrigated (i.e., attractive) corn fields. In addition, a relatively high proportion of females captured close to the release point (within 10 feet) were mated. This work suggests that females mate very close to the point of emergence and that refuges may need to be placed very close to *Bt* fields (or as in-field refuges) to maximize the probability of random mating.

iv. Ovipositional Behavior

ECB ovipositional (egg-laying) behavior is important for refuge design. For instance, if oviposition within a corn field is not random, certain types of refuge (i.e., in-field strips) may not be effective.

After mating, which occurs primarily in aggregation sites, females move to find suitable corn hosts for oviposition. Most females will oviposit in corn fields near the aggregation sites, provided there are acceptable corn hosts. Oviposition begins after mating and occurs primarily at night. Eggs are laid in clusters of up to sixty eggs (one or more clusters is deposited per night) (Mason et al. 1996).

It is known that females generally prefer taller and more vigorous corn fields for oviposition (Beck 1987). This has implications for refuge design. To avoid potential host discrimination among ovipositing females, the non-*Bt* corn hybrid selected for refuge should similar to the *Bt* hybrid in terms of growth, maturity, yield, and management practices (i.e., planting date, weed management, and irrigation). It should be noted that research has shown no significant difference in ovipositional preferences between *Bt* and non-*Bt* corn (derived from the same inbred line) when phenological and management characteristics are similar (Orr & Landis 1997; Hellmich et al.

1999). Within a corn field suitable for egg laying, oviposition is thought to be random and not restricted to border rows near aggregation sites (Shelton et al. 1986, Calvin 1998).

v. Host Range

ECB is a polyphagous pest known to infest over 200 species of plants. Among the ECB plant hosts are a number of species of common weeds, which has led some to speculate that it may be possible for weeds to serve as an ECB refuge for *Bt* corn. In response to this, a number of recent research projects have investigated the feasibility of weeds as refuge. Studies conducted by Hellmich (1996, 1997, 1998) have shown that weeds are capable of producing ECB, although the numbers were variable and too inconsistent to be a reliable source of ECB refuge. This conclusion was also reached by the 1998 SAP Subpanel on IRM. In addition to weeds, a number of grain crops (e.g., wheat, sorghum, oats) have been investigated for potential as a *Bt* corn ECB refuge (Hellmich 1996, 1997, 1998; Mason et al. 1998). In these studies, small grain crops generally produced less ECB than corn (popcorn or field corn) and are unlikely to produce enough susceptible adult insects.

b) CEW

As was the case with ECB, the 1998 SAP identified a number of research areas that need additional work with CEW. In addition to increased knowledge regarding larval/adult movement, mating behavior, and ovipositional behavior, a better understanding of movement between corn/cotton and long distance migration is also needed (SAP 1998). Additional research regarding CEW biology has occurred since 1998. These data have been submitted as part of the annual research reports required as a condition of registration. The Agency has reviewed these data and has concluded that additional information is needed to improve long-term effective IRM strategies to mitigate CEW resistance.

i. Host Range and Corn to Cotton Movement

CEW is a polyphagous insect (3-4 generations per year), feeding on a number of grain and vegetable crops in addition to weeds and other wild hosts. Typically, it is thought that CEW feeds on wild hosts and/or corn for two generations (first generation on whorl stage corn, second generation on ear stage corn). After corn senescence, CEW moves to other hosts, notably cotton, for 2-3 additional generations. By utilizing multiple hosts within the same growing season, CEW presents a challenge to *Bt* resistance management in that there is the potential for double exposure to *Bt* protein in both *Bt* corn and *Bt* cotton (potentially up to five generations of exposure in some regions).

Given the wide host range of CEW, it has been speculated that wild hosts (weeds) and other non-*Bt* crops (e.g., soybean) may be able to serve as refuge for CEW. However, research into the value of these alternate hosts as reliable producers of CEW is still lacking (1998 SAP).

ii. Overwintering Behavior

CEW are known to overwinter in the pupal stage. Although it is known that CEW migrate northward during the growing season to corn-growing regions (i.e., the U.S. Corn Belt and Canada), CEW typically are not capable of overwintering in these regions. Rather, CEW are known to overwinter in the South, often in cotton fields. Temperature, moisture, and cultivation practices are all thought to play some role in the overwintering survival of CEW (Caprio & Benedict 1996).

Overwintering is an important consideration for IRM--resistant insects must survive the winter to pass their resistance genes on to future generations. In the Corn Belt, for example, CEW incapable of overwintering should not pose a resistance threat. Given that different refuge strategies may be developed based upon where CEW is a resistance threat, accurate sampling data will be needed to accurately predict suitable CEW overwintering areas.

iii. Adult Movement and Migration

CEW is known to be a highly mobile pest, capable of significant long distance movement. Mark/recapture studies have shown that CEW moths are capable of dispersing distances ranging from 0.5 km (0.3 mi.) to 160 km (99 mi.) (some migration up to 750 km (466 mi.) was also noted) (Caprio & Benedict 1996). The general pattern of migration is a northward movement, following prevailing wind patterns, with moths originating in southern overwintering sites moving to corn-growing regions in the northern U.S. and Canada.

It has been assumed that CEW migration proceeds progressively northward through the course of the growing season. However, observations made by Dr. Fred Gould (N.C. State University) indicate that CEW may also move southward from corn-growing regions back to cotton regions in the South (described in remarks made at the 1999 EPA/USDA Workshop on *Bt* Crop Resistance Management in Cotton, Memphis, TN 8/26/99). If this is true (and more investigation is needed for confirmation of this effect), the result may be additional CEW exposure to *Bt* crops. In addition, the assumptions regarding CEW overwintering may need to be revisited--moths that were thought to be incapable of winter survival (and thus not a resistance threat) may indeed be moving south to suitable overwintering sites.

Most CEW flight movement is local, rather than migratory. Heliothine moths move primarily at night, with post-eclosion moths typically flying short distances of less than 200 m (Caprio & Benedict 1996). However, as was indicated by the 1998 SAP, significant research is still needed in this area, particularly as it pertains to CEW and optimal refuge design.

iv. Mating/Ovipositional Behavior

Dr. Michael Caprio (entomologist, Mississippi State University) has indicated that there is significant localized mating among females (i.e., within 600 m (1969 ft.) of pupal eclosion), typically with males that emerged nearby or moved in prior to female eclosion (Caprio 1999). CEW females typically deposit eggs singly on hosts. A recent study (conducted in cotton fields) found that 20% of the eggs found from released CEW females were within 50-100 m (164-328 ft.) of the release point, indicating some localized oviposition. However, males were shown to be able to move over 350 m (1148 ft.) to mate with females (Caprio 2000). These data indicate that, in terms of CEW, refuges may not have to be embedded or immediately adjacent to a *Bt* field to be effective (although the data do not exclude these options). Additional research with mating and ovipositional behavior would provide useful information for CEW IRM.

v. Larval Movement

CEW larvae, particularly later instars, are capable of plant-to-plant movement. At the recommendation of the SAP, EPA eliminated seed mixes as a viable refuge option for CEW (SAP 1998).

c) SWCB and Other Secondary Pests

Some SWCB pest biology data have been provided as part of the annual research reports required as a condition of registration. However, there is still relatively limited information available and more data on SWCB pest biology is needed to tailor IRM strategies for regions in which SWCB and ECB are both pests of economic concern. The 1998 SAP also noted the relative lack of information for SWCB, concluding that "[c]ritical research is needed for SWCB...including: short-term movement, long-distance migration, mating behavior relative to movement (i.e. does mating occur before or after migration)..." Because of this, it is unknown whether IRM strategies designed for ECB (another corn boring pest) will also function optimally for SWCB.

SWCB is an economic pest of corn in some areas (i.e., SW Kansas, SE Colorado, north Texas, west Oklahoma) and can require regular management. Like ECB, SWCB has 2-4 generations and similar feeding behavior. First generation larvae feed on whorl tissue before tunneling into stalks before pupation, while later generations feed on ear tissue before tunneling into stalks. Females typically mate on the night of emergence and can lay 250-350 eggs (Davis 2000).

Research to investigate the movement patterns of SWCB has been initiated (Buschman et al. 1999). In this mark/recapture study, the following observations were made regarding SWCB from the 1999 data: 1) more males than females were captured at greater distances from the release point (similar to ECB); 2) most recaptures of SWCB were within 100 feet of the release site, although some were also noted at 1200 feet; and 3) the moth movement patterns for ECB and SWCB appear to be similar in most regards. Given these results, it is likely that this part of the IRM strategy, refuge proximity guidelines established for ECB, will also be applicable to SWCB. However, the 1999 results were hampered by low SWCB numbers available for testing and the authors have indicated that this work will continue during the 2000 season.

Research for other secondary pests (e.g., BCW, FAW, SCSB, others) is also lacking and may useful for specific regions in which these pests may pose an additional concern. The 1998 SAP indicated that CEW and SWCB should have the highest priority for biology research among the secondary corn pests.

2) High Dose

A high level of *Bt* protein expression (termed "high dose") is considered to be an essential aspect of high dose/structure refuge strategy to mitigate the risk of *Bt* resistance. The lack of a high dose could allow partially resistant (i.e. heterozygous insects with one resistance allele) to survive, thus increasing the frequency of resistance genes in an insect population. For this reason, numerous IRM researchers and expert groups have concurred that non-high dose *Bt* expression presents a substantial resistance risk relative to high dose expression (Roush 1994; Gould 1998; Onstad & Gould 1998; SAP 1998; ILSI 1998; UCS 1998). To mitigate the additional resistance risk of a non-high dose *Bt* corn product, alternate refuge strategies (i.e. larger refuges) may need to be developed.

The 1998 SAP defined high dose as "25 times the protein concentration necessary to kill susceptible larvae" and provided five techniques to verify high dose (defined earlier in this document). As an alternate definition for high dose, Caprio et al. (2000) recommend that a higher, 50-fold value be adopted (rather than 25-fold) because current empirical data suggest that a 25-fold dose may not be consistently high enough to cause high mortality among heterozygotes with known *Bt* resistance alleles. It is also important to consider protein expression over the course of the growing season as some *Bt* corn hybrids may not maintain a steady level of protein expression over the season. The 1998 SAP noted these concerns indicating that the "toxin concentration encountered by the pest" should be the true measure.

Among the currently registered *Bt* corn products, most have been evaluated to determine high dose (via the 1998 SAP verification techniques) for ECB (the primary target pest). It is likely that BT11, MON 810, and Cry9C corn have a high dose for ECB. It is also known that none of the currently registered *Bt* corn products expresses a high dose for CEW (CEW is known to be less susceptible to *Bt* proteins than other targeted lepidopteran pests). However, high dose evaluations for other secondary pests (i.e., SWCB, FAW, etc.) have been sporadic. Ideally, high dose should be evaluated for all susceptible pests, so that appropriate resistance management strategies can be developed. Verification of the high dose using the 1998 SAP Subpanel techniques is recommended for the major target pests of *Bt* corn, ECB, CEW, and SWCB. Below, each registered *Bt* corn product is discussed individually in regard to high dose (as defined by the 1998 SAP) for each of the labeled target pests. It is not expected that label claims of "control" or "suppression" for individual target pests are indicative of high dose.

a) Aventis StarLink Cry9C Corn

Cry9C corn is labeled for "control" of ECB and SWCB and is labeled for "suppression" of BCW and CSB. The Cry9C protein has been shown to be non-toxic to CEW.

For ECB, Aventis (formerly AgrEvo/PGS) submitted the results of two of the high dose verification assays. These tests (techniques #2 and #5 above) confirmed that Cry9C corn does contain a high dose for ECB (MRID # 446796-01). Wang et al. (2000) also have shown that a high dose expression for ECB in Cry9C corn is likely in silk, husk, and kernel tissues in addition to green leafy tissues. Previous data submitted by Aventis (MRID # 442581-16) has shown that the level of protein expression declines slightly over the growing season, but it is doubtful that the decline is enough to challenge the high dose expression for ECB.

High dose verification for SWCB is still needed. Although a high dose is not expected for BCW and CSB, the level of susceptibility to Cry9C will help determine the likelihood of resistance developing with these pests.

b) Novartis BT11 Cry1Ab Corn

According to their grower guides, Novartis BT11 corn is targeted against ECB (claims of "control"), SWCB ("control"), CEW ("control" of 1st generation, "suppression" of 2nd gen.), FAW ("suppression"), and SCSB ("suppression").

Novartis has not submitted any data to the Agency to confirm high dose, via the 1998 SAP guidelines, for any of the targeted pests. However, the Agency is able to conclude that BT11 probably produces a season-long high dose for ECB based on the review of all available data submitted to the Agency. Submitted studies have shown consistent control of ECB from the whorl stage to kernel maturity (VanDuyn et al. 1997; Catangui and Berg 1998). BT11 has also been shown to be effective against late instar ECB as well (Walker et al. 1999).

For CEW, several submitted studies suggest that BT11 does not contain a season-long high dose. These studies revealed excellent control of first generation CEW on whorl stage BT11, but also showed significant survival of second generation CEW on BT11 corn ears (Dively & Horner 1997; VanDuyn et al. 1997). However, in both studies, surviving second generation CEW showed fitness costs (i.e., reduced weight and delayed developmental time). Other research has shown similar results (VanDuyn et al. 1998).

For SWCB, no information on the potential for high dose has been submitted to the Agency. For FAW, one submitted study with BT11 showed good control during whorl stage, but significant infestation during ear stage (Benedict et al. 1998). It is therefore unlikely that BT11 contains a full season high dose for FAW. For SCSB, one study with a limited data set has been submitted, showing good control (VanDuyn 1998). Additional data would be needed to confirm whether

BT11 contains a high dose for SCSB.

c) Monsanto MON 810 Cry1Ab Corn

According to grower guides and product labels, MON 810 is targeted against ECB (claim of "control"), SWCB ("control"), SCSB ("control"), CEW ("suppression"), CSB ("suppression"), and FAW ("suppression").

For ECB, Monsanto has submitted information to verify (with the 1998 SAP guidelines) that MON 810 expresses a high dose (reviewed by EPA, R.Rose/S.Matten memo to M.Mendelsohn, 5/30/99). SAP techniques #2, 3, and 5 were utilized to confirm the high dose expression.

For SCSB, submitted research has shown that MON 810 provides good control versus non-*Bt* corn (VanDuyn et al. 1997, VanDuyn 1998, VanDuyn et al. 1998), although there is not enough information (due to low pest pressure in the tests) to determine if there is a high dose expression. Additional data would be needed to determine whether there is a high dose expression for control of SCSB.

For CEW, submitted studies have shown significant larval survival on MON 810 corn, particularly in ear stage corn (Dively et al. 1997; Dively & Horner 1997; VanDuyn et al. 1997; Benedict et al. 1998; VanDuyn et al. 1998). Therefore, it is unlikely that MON 810 expresses a season-long high dose for CEW. For FAW, MON 810 was found to have good whorl stage control, but significant ear infestation later in the season (Benedict et al. 1998). Given this, and the known lower sensitivity of FAW to Cry1A proteins, it is unlikely that MON 810 has a season-long high dose for FAW. High dose has not been verified for SWCB or CSB with the 1998 SAP techniques. Additional data would be needed to verify whether there is a high dose expression for control of SWCB or CSB.

d) Novartis Attribute Cry1Ab Sweet Corn

Attribute sweet corn is targeted against ECB, CEW, and FAW. Attribute contains the same *Bt* gene as the BT11 hybrid.

For ECB, like BT11, it is probable that Attribute sweet corn expresses a high dose, although it has not been verified with the SAP criteria. Research submitted to EPA specifically for Bt sweet corn has shown virtually no survival of ECB (Dively & Linduska 1998).

For FAW and CEW, it is less likely that *Bt* sweet corn will express a high dose. Several submitted studies have shown (limited) FAW and CEW survival and damage on Attribute *Bt* sweet corn (Dively & Linduska 1998; Whalen & Spellman 1999; Lynch et al. 1999).

The current knowledge base for high dose expression is summarized in the following table.

Table D2. High Dose Summary

HYBRID	SEASON-LONG HIGH DOSE FOR CORN PESTS					
	ECB	CEW	SWCB	FAW	SCSB	CSB
BT11	Probable	NO	Unknown	NO	Unknown	Unknown *
Bt Sweet Corn (BT11)	Probable	NO	Unknown*	NO	Unknown *	Unknown *
MON 810	YES	NO	Unknown	NO	Unknown	Unknown
StarLink Cry9C	YES	NO (no toxicity) *	Unknown	Unknown*	Unknown*	Unknown

YES = high dose verified with 1998 SAP recommended techniques; NO = information indicates that no high dose is likely; Probable = information indicates high dose likely (but not verified by SAP guidelines); Unknown = no or insufficient information available for high dose determination; * = untargeted pest

3) Refuge

The February 1998 FIFRA SAP Subpanel agreed that a high dose/refuge strategy is necessary to mitigate target insect resistance to *Bt* field corn (SAP 1998). A structured refuge should be planted and managed to produce 500 insects susceptible to *Bt* for every one potentially resistant insect. Refuge options should address regional differences and varying levels of the dose of *Bt* in the crop that effect refuge management as well as the need for feasibility and flexibility for the growers. However, if there is not a high dose for the primary target pests, the risk of resistance increases. Larger refuges, increased monitoring, and possible sales restrictions may be used to mitigate some or all of this risk.

a) Deployment of Refuges for all Events

There have been a number of approaches proposed for the optimal design of refuges for *Bt* corn. These include external blocks, in-field strips, seed mixes, temporal refuge strategies, and non-corn hosts. A number of research projects have been undertaken to identify the most appropriate refuge design.

i. Hosts for the Refuge

Non-*Bt* popcorn or field corn provide the best refuge to increase the probability susceptible insects will mate with ECB from the *Bt* corn. Non-*Bt* corn hybrids used as refuges should be selected for growth, maturity, fertility, irrigation, weed management, planting date, and yield traits similar to the *Bt* corn hybrid. Hybrids that are not agronomically similar may result in different developmental times in corn pests that could lead to assortive (non-random) mating between plants in refuge and *Bt* fields.

Recent research has shown that temporal and alternate host, non-corn refuges (e.g. weeds, oats, alfalfa, soybeans) are inadequate strategies (Rice et al. 1997; Ostlie et al. 1997b; Calvin et al. 1997; Mason et al. 1998; Hellmich 1998).

ii. Seed Mixes vs. In-Field Strips vs External Blocks

The NC-205 group has recommended three options for refuge placement relative to *Bt* corn: blocks planted adjacent to fields, blocks planted within fields, or strips planted within fields (Ostlie et al. 1997). In general, refuges may be deployed as external blocks on the edges or headlands of fields or as strips within the *Bt* corn field.

Research has shown that ECB larvae are capable of moving up to six corn plants within or between rows with the majority of movement occurring within a single row. Later instar (4th and 5th) ECB are more likely to move within rows than between rows (Hellmich 1998). This is a cause for concern because heterozygous (partially resistant) ECB larvae may begin feeding on *Bt* plants, then move to non-*Bt* plants (if planted nearby) to complete development, thus defeating the high dose strategy and increasing the risk of resistance. For this reason, seed mixes (refuge created by mixing seed in the hopper) have been eliminated as possible ECB refuges (Mallet & Porter 1992, Buschman et al. 1997).

Buschman et al. (1997) suggested that the within field refuge is the ideal strategy for an IRM program. Since the ECB larvae tend to move within rows, the authors suggest intact corn rows as an acceptable refuge. Narrow (filling one or two planter boxes with non-*Bt* corn seed) or wide strips (filling the entire planter with non-*Bt* seed) may be used as in-field refuges. Data indicate that in-field strips may provide the best opportunity for ECB produced in *Bt* corn to mate with ECB from non-*Bt* corn. Since preliminary data suggests that the refuge should be within 100 rows of the *Bt* corn, Buschman et al. (1997) recommended alternating strips of 96 rows of non-*Bt* corn and 192 rows of *Bt* corn. This would result in a 33% refuge that is within 100 rows of the *Bt* -corn.

In-field strips (planted as complete rows) should extend the full length of the field and include a minimum of six rows planted with non-*Bt* corn alternating with a *Bt* corn hybrid. NC-205 has recommended planting six to 12 rows of non-*Bt* corn when implementing the in-field strip refuge strategy (NC 205 Supplement 1998). In-field strips may offer the greatest potential to ensure random mating between susceptible and resistant adults because they can maximize adult genetic mixing. Modeling indicates that strips of at least six rows wide are as effective for ECB IRM as adjacent blocks when a 20% refuge is used (Onstad & Guse 1999). However, strips that are only two rows wide might be as effective as blocks, but may be more risky than either blocks or wider strips given our incomplete understanding of differences in survival between susceptible borers and heterozygotes (Onstad & Gould 1998).

Given the concerns with larval movement and need for random mating, either external blocks or in-field strips (across the entire field, at least 6 rows wide) are the refuge designs which may provide the most reduction in risk of resistance development. Research indicates that random mating is most likely to occur with in-field strips.

iii. Proximity

The issue of refuge proximity is a critical variable for resistance management. Refuges must be located so that the potential for random mating between susceptible moths (from the refuge) and possible resistant survivors (from the *Bt* field) is maximized. Therefore, pest flight behavior is a critical variable to consider when discussing refuge proximity. Refuges planted as external blocks should be adjacent or in close proximity to the *Bt* corn field (Onstad & Gould 1998, Ostlie et al. 1997b). NC-205 has recommended that refuges be planted within ½ sections (320 acres) (NC-205 Supplement 1998).

Hunt et al. (1997) has completed a study which suggests that the majority of ECB do not disperse far from their pupal emergence sites. According to this mark-recapture study, the majority of ECB may not disperse more than 1500 to 2000 feet. A majority (70-98%) of recaptured ECB were trapped within 1500 feet of the release point. However, in an addendum to the 1997 study, the authors caution that the 1500 foot distance does not necessarily represent the maximum dispersal distance for ECB (Hunt et al. 1998a).

Another mark-recapture ECB project was devoted to within-field movement of emerging ECB (in particular unmated females) (Hunt et al. 1998b). Relatively few unmated females were recaptured (10 over the entire experiment), although the majority of those were found within 85 ft of the release point. This suggests that unmated females may not disperse far from the point of pupal eclosion (this was especially true in the irrigated field). In addition, a relatively high proportion of mated females (31%) in irrigated fields were trapped within 10 feet of the release point, suggesting that mating occurred very close to the point of emergence. Both of these observations indicate that many emerging ECB females may not disperse outside of their field of origin. With respect to resistance management and refuge proximity, these results suggest that refuges should be placed in close proximity to *Bt* corn fields (or as in-field refuge) to increase the chance of random mating (especially for irrigated fields).

While it is clear that ECB dispersal decreases further from pupal emergence points, the quantitative dispersal behavior of ECB has not been fully determined. However, in terms of optimal refuge placement, it is critical that refuge proximity be selected to maximize the potential for random mating. Currently, the proximity requirement for *Bt* corn is ½ mile (1/4 mile in areas where insecticides have been historically used to treat ECB and SWCB) (EPA letter to *Bt* corn registrants, 1/31/00). Based on Hunt et al. data, the closer the refuge is to the *Bt* corn, the lower the risk of resistance. Since the greatest number of ECB were captured within 1500 feet of the field and most females may mate within ten feet of the field, placing refuges as close to the *Bt* fields as possible should increase the chance of random mating and decrease the risk of resistance.

iv. Temporal and Spatial Refuge

The use of temporal and spatial mosaics has received some attention as alternate strategies to structured refuge to delay resistance. A temporal refuge, in theory, would manipulate the life cycle of ECB by having the *Bt* portion of the crop planted at a time in which it would be most attractive to ECB. For example, *Bt* corn fields would be planted several weeks before conventional corn. Because ECB are thought to preferentially oviposit on taller corn plants, the hope is that the *Bt* corn will be infested instead of the shorter, less attractive conventional corn. However, there are indications from experts in the field that temporal refuges are an inferior alternative to structured refuges (SAP 1998). Research has shown that planting date cannot be used to accurately predict and manipulate ECB oviposition rates (Calvin et al. 1997, Rice et al. 1997, Ostlie et al. 1997b, Calvin 1998). Local climatic effects on corn phenology make planting date a difficult variable to manipulate to manage ECB. Additional studies will have to be conducted under a broad range of conditions to fully answer this question. In addition, a temporal mosaic may lead to assortive mating in which resistant moths from the *Bt* crop mate with each other because their developmental time differs from susceptible moths emerging from the refuge (Gould 1994).

Spatial mosaics involve the planting of two separate *Bt* corn events, with different modes of action. The idea is that insect populations will be exposed to multiple proteins, reducing the likelihood of resistance to any one protein. However, currently registered products only express one protein and the primary pests of corn (ECB, CEW, SWCB) generally remain on the same plant throughout the larval feeding stages, individual insects will be exposed to only one of the proteins. In the absence of structured refuges producing susceptible insects, resistance may still have the potential to develop in such a system as it would in a single protein monoculture.

b) Refuge Options

i. High Dose Events; **MON 810, BT11, Cry9C (Field Corn)**

Non-Cotton Growing Regions That Don't Spray Insecticides on a Regular Basis (e.g., Corn Belt)

This region encompasses most of the Corn Belt east of the High Plains. The original USDA NC-205 refuge recommendations included a 20-30% untreated structured refuge or a 40% refuge that could be treated with non-*Bt* insecticides (Ostlie et al. 1997a). In the case of ECB, the primary pest of corn for most of the U.S., it is known that on average less than 10% of growers use insecticide treatment to control this pest (National Center for Food and Agriculture Policy 1999). Due to the fact that many growers do not regularly treat for ECB, NC-205 modified their position in a May 24, 1999 letter to Dr. Janet Andersen (Director, BPPD). In this letter, NC-205 amended their recommendations to include a 20% refuge that may be treated with insecticides. Specific recommendations in the letter were: "1) insecticide treatment of refuges should be based on scouting and accepted economic thresholds, 2) treatment should be with a product that does not contain *Bt* or Cry toxin, 3) records should be kept of treated refuges and shared with the EPA, 4) the potential impact of sprayed refuges should be monitored closely and evaluated annually, and 5) monitoring for resistance should be most intense in higher risk areas, for example where refuges are treated with insecticides."

Since most growers (>90%) do not typically treat field corn with insecticides to control ECB, a refuge of 20% non-*Bt* corn that may be sprayed with non-*Bt* insecticides if ECB densities exceed economic thresholds should be viable for the Corn Belt. Refuges can be treated as needed to control lepidopteran stalk-boring insects with non-*Bt* insecticides or other appropriate IPM practices. Insecticide use should be based on scouting using economic thresholds as part of an IPM program.

Non-Cotton Growing Regions That Spray Insecticides on a Regular Basis (e.g., the High Plains for SWCB)

NC-205 (1998) has noted that there are some areas that regularly require insecticide treatment (e.g., the High Plains for SWCB or spider mites) and that separate refuge strategies may be needed for these regions. The 1997 NC-205 report (Ostlie et al. 1997a) and the 1998 Supplement stated that areas routinely treated with insecticides should expand refuge size from 20% to 40% non-*Bt* corn. This is because highly effective insecticides may significantly reduce the number of susceptible adults emerging from the refuge. In a May 1999 letter sent to Dr. Andersen (BPPD Division Director), NC-205 stated: "A refuge management strategy that is more conservative than the one applied across the greater Corn Belt, yet less restrictive than the one proposed for areas growing both corn and cotton, may be most appropriate in the heavily treated areas jointly infested with SWCB and ECB" (Ortman 1999). The size of the refuge is based on the amount of non-*Bt* corn needed to produce 500 susceptible insects for every resistant insect. When insecticide sprays are used on the refuge, fewer susceptible insects are produced and the refuge area needs to be larger to produce the 500:1 ratio.

Entomologists from Kansas State University (Dr. Randy Higgins, Dr. Lawrence Buschman, and Dr. Phillip Sloderbeck) have indicated that the frequent use of highly effective insecticides in areas that are co-infested with both SWCB and ECB is the issue of concern rather than the mere presence of SWCB. Using highly effective insecticides in these areas will decrease the number of susceptible insects emerging from the refuge and reduce refuge efficacy (Buschman and Sloderbeck 1999; Higgins 1999). As a result of the Agency's new IRM requirements for *Bt* corn products for the year 2000, areas that are routinely treated with insecticides were more specifically identified by the Agricultural Biotechnology Stewardship Technical Committee (ABSTC) in a letter to Dr. Janet Andersen dated March 31, 2000. This area includes counties in southwest Kansas, southeast Colorado, and the Texas/Oklahoma Panhandle.

ii. High Dose (MON 810, BT11, Cry9C) Field Corn Events in All Cotton-Growing Regions

As part of their April 1999 and January 2000 submissions, the NCGA/Industry Coalition requested growers be required to plant a minimum of 20% non-*Bt* corn in the northern portion of the corn/cotton region. The northern corn/cotton region corresponds to northern Arkansas, Missouri Bootheel, northern Texas, and the states of North Carolina, Oklahoma, Tennessee and Virginia. A minimum 50% refuge of non-*Bt* corn was suggested for the southern portion of the corn/cotton-growing region. The southern corn/cotton region corresponds to the entire states of Alabama, Florida, Georgia, Louisiana, Mississippi, South Carolina, as well as southern Texas and southern Arkansas.

Cotton-growing regions represent a higher risk for resistance due to the potential double exposure of CEW to both *Bt* corn (Cry1Ab) and *Bt* cotton (Cry1Ac) during the same growing season. Dr. Mike Caprio (Mississippi State University) developed a corn-cotton ecosystem model for resistance evolution in CEW to *Bt*-endotoxins expressed in plants to examine the movement of CEW between corn, cotton, soybean, and other wild hosts (Caprio 1997). In the model, the presence of *Bt* cotton (160 fields) and the ratio of *Bt* corn/non-*Bt* corn fields (120 total fields) are important factors. As the ratio of non-*Bt* corn decreases relative to *Bt* corn, the time to resistance also decreases; meaning that less non-*Bt* corn planted as a refuge results in quicker resistance. This effect was most pronounced when the percent of *Bt* to non-*Bt* corn exceeded 50%. Caprio's model suggests that even without cross-resistance as a variable, a sizable proportion of non-*Bt* corn (at least 50%) should be planted with *Bt* corn in *Bt* cotton growing regions to avoid the quick evolution of resistance. The years to resistance are also impacted by the percent of Bt cotton relative to Bt corn. Dr. John Van Duyn (North Carolina State University) and Dr. Dick Hardee (USDA) have communicated a recommendation for a 50% non-*Bt* corn refuge in cotton growing areas (Personal communication to S. Matten, 1999).

In terms of the proposed "northern cotton-growing region," a significant increase in *Bt* cotton in these areas has been observed over the past several growing seasons. From 1996 to 1999, the percent Bollgard acreage increased in North Carolina from 3% to 19% (total increase: 250,000 acres), in Oklahoma from 7% to 20% (total increase: 57,773 acres), in Tennessee from 2% to 68% (total increase: 380,000 acres), and in Virginia from 1% to 7% (total increase: 6,214 acres) (MRID # 450294-01). This shows that the *Bt* cotton acreage cannot be predicted accurately and may not be an appropriate justification for reduced refuge.

Dr. Fred Gould (North Carolina State University) has also identified resistance risk issues in southern cotton growing regions (described in remarks made at the 1999 EPA/USDA Workshop on *Bt* Crop Resistance Management in Cotton, Memphis, TN 8/26/99). According to Dr. Gould, CEW are thought to feed on corn in Mexico in the early spring before moving to cotton in the southern U.S. and ultimately corn in more northern areas. If these CEW diapause in the northern areas and all die over the winter, they pose no resistance problem. However, some indirect evidence has indicated that at least some CEW move from northern areas to southern cotton growing regions to overwinter. CEW that move from the north to south to overwinter could be exposed for four generations or more to *Bt* crop hosts.

Drs. Caprio, Van Duyn, and Gould recommend a minimum of a 50% non-*Bt* corn refuge that may be treated only as necessary with non-*Bt* insecticides is needed in all cotton-growing regions to reduce the risk of resistance. Smaller refuges may present a greater risk and may result in a more rapid evolution of resistance. Refuges may be treated to control lepidopteran stalk-boring insects as needed with non-*Bt* insecticides or other appropriate IPM practices. Since cotton is a preferred overwintering site for CEW, post-harvest plowing of *Bt* cotton fields to destroy potentially overwintering CEW pupae may also be an effective tool to decrease the risk of resistance, but further research is necessary.

Cry9C corn is the first *Bt* corn product submitted for registration that does not contain either the Cry1Ab or Cry1Ac proteins. In addition, there are no other currently registered *Bt* pesticide products, either microbial or transgenic, that contain Cry9C. Due to separate binding sites, there should be a relatively low potential for cross-resistance between Cry9C and Cry1Ab or Cry1Ac. In addition, studies have shown that Cry9C is not toxic to CEW and does not cause significant mortality (MRID # 445042-01). Therefore, the increased risk of CEW resistance to *Bt* in cotton-growing regions does not apply to Cry9C.

iii. Non-High Dose Events

Non-Cotton Growing Regions That Do Not Spray Insecticides on a Regular Basis (e.g., Corn Belt)

As indicated earlier, there are no specific non-high dose products for ECB that will be considered in this scientific review. However, an assessment of non-high dose is included here to provide a comprehensive review of all possibilities.

Research regarding refuge size for non-high dose *Bt* events is limited. In general, non-high dose *Bt* corn hybrids pose a higher risk (approximately five times higher) of resistance than high dose events (Onstad & Gould 1998, Gould & Onstad 1998). The International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) has recommended larger refuges (e.g. 40% unsprayed in the North) for non-high dose, or high risk varieties (ILSI 1998). The Union of Concerned Scientists (UCS) has also suggested that a separate resistance management strategy should be developed for varieties that do not meet the high dose refuge strategy. UCS recommended a 50% refuge that should not be sprayed with insecticides for *Bt* corn varieties that do not contain a high dose (UCS 1998).

For non-high dose events, larger refuges may be necessary (Gould 1998, ILSI 1998, UCS 1998). Based on the ILSI and UCS reports, at least a 40% unsprayed refuge in non-cotton growing regions (Corn Belt) is needed to mitigate the threat of resistance. According to the National

Center for Food and Agriculture Policy (1999), the percent insecticide use for ECB control in U.S. field corn is on average < 10%. Since most refuges will not be routinely sprayed and some growers need the option of spraying if pests reach economic injury levels, mandating an unsprayed refuge should not be necessary. The risk of insect resistance to the non-high dose events may also be limited by restricting sales (e.g., a total sales cap or in areas where ECB are univoltine). Since ECB exposure to *Bt* is limited in areas where there is one generation per year, restricting the use of non-high dose events to these areas will likely decrease the risk of resistance.

Non-Cotton Growing Regions That Spray Insecticides on a Regular Basis (e.g., the High Plains for SWCB)

Non-high dose plants have an increased risk of insect resistance which is compounded if the refuge is sprayed with insecticides. The ILSI panel has recommended larger refuges for these non-high dose, or “high” risk *Bt* corn varieties. For areas where the refuge will be sprayed with insecticides, the ILSI recommended an 80% non-*Bt* corn refuge (ILSI 1999). Since there is an increased risk of resistance in areas that are routinely sprayed with insecticides, restricting sales of non-high dose events may reduce the risk. In addition to planting restrictions, the ILSI Panel's recommended 80% (insecticide treatable) refuge is one option that could be implemented to mitigate the risk of resistance.

4) Monitoring

a) Monitoring Strategies

A monitoring program for *Bt* corn is necessary to test the effectiveness of resistance management programs. Detecting shifts in the frequency of resistance genes through resistance monitoring can be an aggressive method to detect the onset of resistance before widespread crop failure occurs.

In general, resistance monitoring plans should include a detailed sampling strategy for all pests susceptible to the expressed *Bt* proteins regardless of whether they are stated on the label. For *Bt* field corn, popcorn, and sweet corn, the susceptible pests would include, but are not limited to: ECB, SWCB, CEW, and FAW. To be effective, the monitoring for resistance should be undertaken in areas where the pests are known to regularly overwinter.

The resistance monitoring plan should not be tied to specific sales thresholds, but be based on sampling areas in which selection pressure for ECB resistance development is the greatest. Samples should be distributed throughout all corn-growing areas, but can be concentrated in higher resistance risk areas (SAP 1998). Certain secondary pests such as BCW, SCSB, and CSB may also need to be monitored, as these pests may be of local or regional significance.

Dr. Blair Siegfried (entomologist, University of Nebraska) has indicated that at least 100 or more insects, with a target of 500-1000 insects, should be collected per location (noted at the June 18, 1999 EPA/USDA *Bt* Crop Insect Resistance Management Workshop in Chicago, IL). Sampling locations should be selected to reflect all crop production practices and should be separated by a sufficient distance to reflect distinct populations. More intensively planted *Bt* corn areas in which selection pressure is expected to be higher should also be targeted.

The utilization of sensitive and effective resistance monitoring techniques is critical to the success of an IRM plan. The following monitoring techniques can be considered as part of a tiered approach to monitoring: 1) Grower reports of unexpected damage; 2) Systematic field surveying of *Bt* corn; 3) Discriminating concentration assay; 4) F_2 screen; 5) Screening against resistant colonies; 6) Sentinel *Bt*-crop field plots.

b) Agricultural Biotechnology Stewardship Technical Committee's (ABSTC) Tiered Approach

In response to requirements detailed in Agency letters to *Bt* corn registrants (12/20/99 and 1/31/00), the ABSTC submitted (March 31, 2000) a refined *Bt* field corn resistance monitoring plan for ECB, SWCB, and CEW for the 2000 growing season. The ABSTC plan concentrates resistance monitoring in areas where *Bt* corn market penetration is highest as well as areas with the highest insecticide use. The plan includes the identification of counties growing more than 50,000 acres of field corn (*Bt* and non-*Bt*) to focus monitoring efforts. ABSTC's proposed plan is designed to detect resistance when it reaches 1 - 5% (a level that allow for detection of resistance before field failures occur). Four corn-growing regions were identified and monitoring for each pest will occur in the regions in which the pests are prevalent. When possible, at least 200 first or second flight adults (100 females), 100 second flight egg masses, or 100 diapausing larvae per site will be collected in each region, though insect population levels may limit the number collected. The Agency concluded that ABSTC’s monitoring program should be adequate, although the program would be improved if sampling locations can be separated by a sufficient distance to reflect discreet pest populations.

c) Monitoring Results

EPA currently mandates that both baseline susceptibility and a discriminating concentration be developed for certain primary target pests including ECB and CEW. Baseline susceptibility data should be collected for each labeled/target pest and consideration should be given for all potentially susceptible pests (e.g., SWCB, BCW, FAW, SCSB) with focus on major economic pests. This information is essential to managing resistance in pest populations, especially in assessing whether a field control failure was due to actual resistance or other factors affecting expression of the *Bt* protein. These baseline data are helpful in documenting the extent and distribution of resistant populations. Continued monitoring efforts are needed to provide the Agency with standardized information to determine whether resistance evolution is occurring.

i. Cry1Ab

Dr. Blair Siegfried (University of Nebraska) has coordinated a standardized monitoring program for ECB (since 1995) and CEW involving LC_{50} susceptibility determinations and diagnostic concentration (LC_{99}) bioassays to determine susceptibility levels to *Bt* corn. In terms of baseline susceptibility (LC_{50}), bioassays have been conducted for ECB (Siegfried et al. 1999a) and CEW (Siegfried et al. 2000a). For 1999, ECB were collected from 14 separate sites and F_1 and/or F_2 generations were bioassayed to determine LC_{50}s. Bioassays utilized dilutions of purified Cry1Ab obtained from *Bt kurstaki* strain HD1-9 (provided by Novartis) spread on artificial diet. Neonate larvae were exposed to the diet less than 24 hours after hatching and mortality and larval weight were recorded seven days later. The results for ECB are displayed in Table 2 and show no significant change in ECB susceptibility (LC_{50} and EC_{50}) to Cry1Ab over the five years of testing. For CEW, LC_{50} values for CEW ranged from 70.3 ng/cm^2 (lab colony) to 221.3 ng/cm^2 (field colony).

Table D3. Mean Susceptibility of ECB to Cry1Ab from 1995 to 1999 (Siegfried et al. 1999a)

Year	LC_{50} (ng Cry1Ab/cm^2) ± SEM	EC_{50} (ng Cry1Ab/cm^2) ± SEM
1995	4.34 ± 0.68	0.37 ± 0.007
1996	6.25 ± 1.25	1.25 ± 0.14
1997	2.12 ± 0.53	0.42 ± 0.007
1998	2.57 ± 0.28	0.43 ± 0.05
1999	4.01 ± 0.49	0.62 ± 0.11

For diagnostic concentration analysis (LC_{99}), baseline susceptibility studies conducted by Marçon et al. (2000) were used to determine the discriminating concentration for ECB. The tests with the discriminating concentrations were conducted in a similar manner to the bioassays to determine LC_{50} values. The results showed nearly 100% mortality for ECB at the discriminating dose (LC_{99}) (Siegfried et al. 1999a). A separate diagnostic concentration analysis (using similar methods) was conducted for CEW (using a dose of 6600 ng/cm^2), which also showed nearly 100% mortality (Siegfried et al. 1999b).

Since none of the populations monitored (ECB and CEW) demonstrated <99% mortality at a diagnostic concentration and the LC_{50} for ECB hasn't significantly changed in five years, it can be concluded that ECB and CEW susceptibility to Cry1Ab has not changed as a result of selective pressure.

An additional study was conducted by Trisyono and Chippendale (1999) to determine SWCB susceptibility to Cry1Ab and establish a diagnostic concentration. A bioassay was conducted that utilized a diagnostic concentration of 110 ng Cry1Ab protein g^{-1} diet. LC_{50}s ranged from 0.15 µg g^{-1} to 1.67 µg g^{-1} seven days after treatment and 0.02 µg g^{-1} to 0.36 µg g^{-1} 14 days post treatment.

LC_{95}s ranged from 2.08 μg g^{-1} to 114.45 μg g^{-1} seven days after treatment and 0.21 μg g^{-1} to 7.63 μg g^{-1} 14 days post treatment. Although LC_{50}s and LC_{95}s were variable and require further refinement, results indicated that the laboratory colonies evaluated were not as susceptible to Cry1Ab as the field collected populations. Furthermore, the results indicated that a bioassay using growth inhibition is more sensitive than one based on larval mortality. Bioassays based on growth inhibition rather than larval mortality may have greater benefits because they require a smaller amount of *Bt* protein, time of observation is flexible because weight gain is being compared to a control, and variation may be minimized.

ii. Cry9C

Dr. Blair Siegfried has coordinated a Cry9C monitoring program for ECB (since 1997) to determine LC_{50} susceptibility and a diagnostic concentration (LC_{99}). For 1999, ECB collected from 14 sites were found to have variable LC_{50} susceptibility, ranging between 11.23 ng/cm^2 and 123.67 ng/cm^2. This variability was likely due to natural causes and not the result of Cry9C selection pressure. In addition, three potential ECB diagnostic concentrations were tested. Two of the three doses, 700 and 1650 ng/cm^2, produced close to the expected 99% mortality. The true diagnostic concentration will probably be between these two doses, although further refinement is needed (Siegfried et al. 1999c).

For SWCB, Aventis has conducted research to determine baseline susceptibility to Cry9C (MRID # 451493-01). Results from three colonies tested in 1998 revealed a mean LC_{50} of 33.8 ng/cm^2, with low variability. Work will be initiated to determine a diagnostic concentration for SWCB and Cry9C.

5) Remedial Action

Remedial action plans are needed to mitigate potential resistance development. A specific remedial action plan would more clearly indicate what actions the registrant will take in cases of "suspected" resistance (i.e., unexpected damage) and "confirmed" resistance. The remedial action plan can also include appropriate adaptations for regional variation and the inclusion of appropriate stakeholders. To fully mitigate resistance, a critical element of any remedial action plan would be that once pest resistance is confirmed, sales of all *Bt* corn hybrids that express a similar protein or a protein in which cross-resistance potential has been demonstrated would be ceased in the affected region.

A remedial action plan has been proposed by ABSTC for *Bt* corn, consisting of two elements: 1) strategies for unexpected damage; and 2) strategies for confirmed resistance. Both components are discussed in the following sections.

a) Actions to be Taken if Unexpected Levels of Insect Damage Occur

ABSTC proposed a strategy for unexpected pest damage in *Bt* corn in the "Industry Insect Resistance Management for Cry1A Plant-Expressed Protectants in Field Corn" (submitted 4/19/99). Aventis submitted a similar plan in 1998 (MRID No. 44504201). The exact language of the ABSTC plan is as follows:

> *"Customers (growers and seed distributors) will be instructed to contact the registrant or authorized distributor if incidents of unexpected levels of target insect damage occur during use of the registrant's Bt corn products. Registrants (or their authorized distributors) will investigate and identify the cause for this damage by local field sampling of plant tissue from corn hybrids that contain the Bt corn plant-expressed protectant and sampling of local pest populations, followed by appropriate in vitro and in planta assays. Upon confirmation by immunoassay that the plants contain the appropriate Cry1A protein, bioassays will be conducted to determine whether the collected insect population exhibits a resistant phenotype.*
>
> *Where available and validated for a target pest species, a discriminating concentration assay will be employed to define a confirmed instance of resistance. For other target pests, until such time that a discriminating concentration assay is established and validated, registrants will utilize the following to define a confirmed instance of insect resistance:*
>
>> *Progeny from the sampled pest population will be considered resistant if they exhibit BOTH of the following characteristics in bioassays initiated with neonates:*
>>
>> *1. An LC_{50} in a standard diet bioassay (incorporating the appropriate Cry1A protein) that exceeds the upper limit of the 95% confidence interval of the mean historical LC_{50} for susceptible pest populations, as established by the ongoing baseline monitoring program.*
>>
>> *2. > 30% survival and > 25% leaf area damaged in a five-day bioassay using the appropriate Cry1A-positive leaf tissue under controlled laboratory conditions.*
>
> *Based upon continued experience and research, this working definition of confirmed resistance may warrant further refinement. In the event that the registrants find it appropriate to alter the criteria specified in the working definition, the registrants will obtain Agency approval in establishing a more suitable definition."*

In the January 31, 2000 letter to *Bt* corn registrants, the Agency agreed with this strategy and the working definition of "confirmed resistance." The letter also clarifies the Agency's interpretation of "suspected" resistance to be:

"...in the case of reported product failure, that corn in question has been confirmed to be *Bt* corn, that the seed used had the proper percentage of corn expressing *Bt* protein, that the relevant plant tissues are expressing the expected level of *Bt* protein, that it has been ruled out that species not susceptible to the protein could be responsible for the damage, that no climatic or cultural reasons could be responsible for the damage, and that other reasonable causes for the observed product failure have been ruled out. The Agency does not interpret 'suspected resistance' to mean grower reports of possible control failures, nor does the Agency intend that extensive field studies and testing to fully scientifically confirm insect resistance be completed before responsive measures are undertaken."

Two other elements which would further mitigate the risk of resistance in the event of unexpected damage (i.e., these measures could be undertaken while the cause of the suspected resistance is investigated) are:

1) The immediate use of alternate control measures to control the pest suspected of resistance to *Bt* corn in the affected region.

2) The destruction of crop residues in the affected region immediately after harvest (i.e. within one month) with a technique appropriate for local production practices to minimize the possibility of resistant insects overwintering and contributing to the next season's pest population.

b) Remedial Measures in Confirmed Cases of Insect Resistance

In cases of "confirmed" resistance (as defined in section A above), ABSTC has proposed the following strategy for Cry1A *Bt* corn hybrids:

"The registrant will report all instances of confirmed pest resistance, as defined above, to the Agency within 30 days. Upon identification of a confirmed instance of resistance, registrants will take the following immediate mitigation measures:

1. Notify customers and extension agents in the affected area,

2. Recommend to customers and extension agents in the affected area the use of alternative control measures to reduce or control the local target pest population, and

3. Where appropriate, recommend to customers and extension agents in the affected area that crop residues be incorporated into the soil following harvest, to minimize the possibility of overwintering insects.

Within 90 days of a confirmed instance of pest resistance, as defined above, registrants *will:*

> *1. Notify the Agency of the immediate mitigation measures that were implemented,*
>
> *2. Submit to the Agency a proposed long-term resistance management action plan for the affected area,*
>
> *3. Work closely with the Agency in assuring that an appropriate long-term resistance management action plan for the affected area is implemented, and*
>
> *4. Implement an action plan that is approved by EPA and that consists of some or all the following elements, as warranted:*
>
> > *a. Informing customers and extension agents in the affected area of pest resistance,*
> >
> > *b. Increasing monitoring in the affected area, and ensuring that local target pest populations are sampled on an annual basis,*
> >
> > *c. Recommending alternative measures to reduce or control target pest populations in the affected area,*
> >
> > *d. Implementing intensified local IRM measures in the affected area based on the latest research results. The implementation of such measures will be coordinated by the Agency with other registrants; and*
> >
> > *e. If the above elements are not effective in mitigating resistance, registrants will voluntarily cease sale of all Bt corn hybrids subject to the Industry IRM Plan in the county experiencing loss of product efficacy and in the bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, registrants may sell and distribute in these counties only after obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants and stakeholders.*

If EPA agrees that an effective local resistance management plan has been implemented which mitigates resistance, the registrants can resume sales in the affected county(ies).”

The Agency has agreed with this strategy for confirmed resistance, with the condition that once resistance has been confirmed, the sale and distribution of *Bt* corn in the affected counties must be halted until an EPA-approved mitigation plan is in place. In addition, because legal constraints prohibit the enaction of remedial action plans by growers, *Bt* corn registrants will assume responsibility for resistance mitigation actions (EPA letter to *Bt* corn registrants, 1/31/00).

In addition to the remedial strategy for confirmed resistance proposed by ABSTC, the following elements could further mitigate the risk of resistance development:

1) Immediate suspension of the sale of *Bt* corn hybrids expressing the same or similar *Bt* protein (i.e. same mode of action, cross-resistant varieties) as the suspected *Bt* corn hybrid harboring the resistant population in the affected region (this was mandated in the 1/31/00 letter).

2) The mandatory use of alternate control measures and post-harvest crop residue destruction in the affected region (the ABSTC plan "recommends" these measures).

3) For mitigation of resistance in the growing season(s) following a confirmed resistance incident(s), use of the following procedures:

a) Maintenance of the sales suspension of all *Bt* corn hybrids (with the same protein or similar *Bt* proteins as the *Bt* corn hybrids with the resistant population) in the affected region, which would remain in place until resistance has been determined to have returned to acceptable levels.

b) The development and use of alternative resistance management strategies for controlling the resistant pest(s) on corn in the affected region.

c) Notification of all relevant personnel (e.g., growers, consultants, extension agents, seed distributors, processors, university cooperators, and state/federal authorities) in the affected region of the resistance situation.

d) Intensified monitoring and surveillance in the affected region(s) for resistance and definition of the boundaries of the affected region. These studies could also include assays to track the decline of resistance in the field and determine the potential for cross-resistance in the resistant population.

6) Cross-Resistance

Cross-resistance is an area of major concern for resistance management and poses risks to both transgenic *Bt* crops and microbial *Bt* insecticides. Cross-resistance occurs when a pest becomes resistant to one *Bt* protein, which then allows the pest to resist other, separate *Bt* proteins. The threat of cross-resistance is particularly acute with *Bt* corn, since there are multiple *Bt* proteins and hybrids currently registered and commercially available (Cry1Ab, Cry9C are presently available, Cry1F is pending, and Cry1Ac was recently discontinued by the registrant). In addition, some pests of corn are also pests of other crops for which *Bt* transgenic varieties are or may soon be available or of crops on which microbial *Bt* insecticides may be used (e.g., CEW on cotton, FAW on tomato). Cross-resistance also poses a risk to pyramid strategies, in which multiple proteins are deployed simultaneously in the same hybrid. However, it should be noted that, to date, the development of cross-resistance has not been shown to be a frequent or likely phenomenon in insect pests exposed in the field to *Bt* crops producing different *Bt* proteins.

In general, it is possible for resistance to *Bt* proteins to occur through a number of different mechanisms, some of which may result in cross-resistance to other proteins. The most well documented mechanism of resistance is reduced (midgut) binding affinity to *Bt* proteins. Different Cry proteins may bind to distinct receptors in an insect. Modifications to these insect crystalline protein receptors have been implicated in resistance to Cry proteins. Other mechanisms that may lead to resistance (and ultimately cross-resistance) include protease inhibition, metabolic adaptations, gut recovery, and behavioral adaptations (Heckel 1994; Tabashnik 1994).

Regarding binding sites, cross-resistance may result if two proteins share the same binding site (receptor) in the insect midgut. Therefore, if exposure to one *Bt* protein results in a modification of the receptor, other proteins sharing this site will be affected as well. An example of a possible shared binding site resulting in cross-resistance was observed with tobacco budworm (TBW). In this case, TBW selected for resistance to Cry1Ac were also found to be resistant to the Cry1Aa, Cry1Ab, and Cry1F proteins (Gould et al. 1995).

Overall, cross-resistance patterns and their underlying physiological mechanisms are very complex and somewhat unpredictable, even within a closely related group of proteins and susceptible insects. To mitigate the risks of cross-resistance to *Bt* corn, additional research will be needed to fully assess the potential for cross-resistance with each *Bt* protein and targeted pest. To date, research has been focused primarily on shared binding site studies with a limited subset of *Bt* protein and corn pests (notably ECB). Further mitigation measures could include the restrictions of certain hybrids determined to be at risk for cross-resistance. This has been done in southern cotton growing regions where CEW, a pest of corn and cotton, may be exposed to multiple *Bt* toxins in both *Bt* corn and *Bt* cotton.

A summary of the cross-resistance knowledge base for each commercially registered *Bt* proteins in corn is presented below.

a) Cry1Ab

Cross-resistance patterns in ECB, the major pest of corn, have proven to be complex. The binding of three *Bt* insecticidal crystal proteins to the midgut epithelium of ECB larvae was characterized by performing binding experiments with both isolated brush border membrane vesicles and gut tissue sections (Denolf et al. 1993). Results demonstrated that two independent insecticidal crystal protein receptors are present in the brush border of ECB gut epithelium. From competition binding experiments, it was concluded that Cry1Ab and Cry1Ac are recognized by the same receptor. Also, the Cry1B protein did not compete for the binding site of Cry1Ab and Cry1Ac and was determined to have a different receptor. Cry1D and Cry1E, two proteins that are not toxic to ECB, were not bound to the gut epithelial cells. Other experiments using laboratory-selected resistant strains to predict survival and cross-resistance in the field on *Bt* corn with ECB have provided different results. A Cry1Ac-resistant ECB strain (produced by Dr. Hutchinson, University of Minnesota) and a Cry1Ab-resistant ECB strain (produced by Dr. Keil, University of Delaware) had a moderate level of resistance, about 30 to 60X. None of the resistant larvae survived on *Bt* corn beyond the second instar. It is interesting to note that the Cry1Ac-resistant ECB were not cross-resistant to Cry1Ab and that Cry1Ab-resistant ECB are not cross-resistant to Cry1Ac (Hutchison, personal communication, reviewed by EPA 1998). Based on receptor binding studies, one would have expected both resistant strains to survive on *Bt* corn. It can be concluded that although two proteins are closely related, there may be different binding mechanisms or binding affinity in ECB relative to other pests, such as DBM or TBW.

Based upon the binding properties of Cry1A and Cry2A proteins in CEW, TBW, and ECB larvae, there appears to be a much lower probability of cross-resistance developing to Cry2A delta endotoxins from resistance to Cry1Ab or Cry1Ac. Because the Cry1A and Cry2A proteins exhibit different binding characteristics and very low amino acid homology, they likely possess different modes of action. However, there is some evidence for the development of broad cross-resistance to Cry1 and Cry2A in at least two laboratory-selected strains: beet armyworm (BAW) (Moar et al. 1995) and TBW (Gould et al. 1992).

Collectively, laboratory-selected strains and isolated field populations indicate that there is a genetic potential for *Bt* cross-resistance to develop to multiple or single Cry delta endotoxins in a number of corn pests from exposure to Cry1Ab. However, cross- resistance patterns and physiological mechanisms are complex and unpredictable, even within related groups of proteins and susceptible pests. Research has suggested that Cry1Ab and Cry1Ac may share binding sites in several tested insect species, although this may not necessarily result in cross-resistance in the field. Other proteins, including Cry2A and Cry1F, may also be at risk for cross-resistance with Cry1Ab, although additional research is clearly needed. Due to the potential cross-resistance between Cry1Ab and Cry1Ac, areas in which *Bt* corn (expressing Cry1Ab) and *Bt* cotton (Cry1Ac) are grown may pose additional risks for resistance in CEW, a pest of both corn and cotton during the same growing season.

Given the unpredictability of cross-resistance among pest species, it would be useful to generate cross-resistance data for SWCB, SCSB, CSB, BCW, and other secondary pests, to gain a more complete understanding of the implications for *Bt* corn.

b) Cry9C

Cry9C corn was the first *Bt* corn hybrid registered that does not contain either the Cry1Ab or Cry1Ac proteins. In addition, there are no other currently registered *Bt* microbial pesticides that contain Cry9C. A study has shown that Cry9C recognizes a different binding site from the one recognized by Cry1Ab or Cry1Ac in ECB and DBM (Lambert et al. 1996). Due to these separate binding sites, there should be less potential for cross-resistance between Cry9C and the Cry1A proteins in ECB, although cross-resistance through other (non-binding site) mechanisms cannot be dismissed. Lambert et al. (1996) also indicated that Cry9C shares a binding site with Cry1C in BAW and DBM, but with a lower binding affinity. While Cry1C alone is not toxic to ECB or cutworms (*Agrotis* sp.), it is toxic to other species which are susceptible to Cry9C (e.g., BAW). Cry1C is also a component of some microbial *Bt* formulations.

Cross-resistance data with other corn pests and *Bt* proteins have not yet been developed for Cry9C. In particular, it would be useful to generate these data for SWCB, BCW, and CSB (other targeted pests of Cry9C corn) with Cry9C and the Cry1A proteins. In addition, studies are needed to investigate the cross-resistance potential between Cry9C, Cry1F and Cry2A (Cry1F and Cry2A are found in microbial *Bt* formulations) for the primary and secondary targeted pests.

7) Compliance

Grower compliance with refuge and IRM guidelines is a critical -- significant non-compliance may increase the risk of resistance for *Bt* corn. To mitigate the threat of resistance, the goal of any IRM strategy should be to establish a level of compliance as close to 100% as possible (many of the refuge models have assumed 100% compliance). To date, surveys of compliance and grower attitudes have indicated that some level of non-compliance must be expected for *Bt* corn -- an expectation of 5 - 15% non-compliance may be reasonable, given the survey results. However, the impact of this level of non-compliance on the threat of resistance is unclear and models should ultimately be updated to reflect some degree of non-compliance.

To achieve a high level of grower compliance, a specific program may need to be developed for *Bt* corn to ensure grower conformity and penalize non-compliance. A number of different compliance mechanisms have been proposed by various stakeholders, but there are uncertainties regarding many of the proposed mechanisms and that further study regarding IRM compliance is needed.

8) Grower Education

Growers are perhaps the most essential element for the implementation and success of any IRM plan as they will ultimately be responsible for ensuring that refuges are planted according to guidelines and that *Bt* fields are monitored for unexpected pest damage. Therefore, a program that educates growers as to the necessity of IRM and provides guidance as to how to deploy IRM should be an integral part of any resistance management strategy. Ideally, the educational messages presented to growers should be consistent (among different registrants) and reflect the most current resistance management guidelines. Specific examples of education tools for growers can include grower guides, technical bulletins, sales materials, training sessions, Internet sites, toll-free numbers for questions or further information, and educational publications.

9) Annual Reports

Written reports on various aspects of IRM, submitted on an annual basis to EPA, are of great aid in the evaluation of the success of resistance management for *Bt* corn. The Agency has received annual reports from *Bt* corn registrants on *Bt* corn sales/market penetration, IRM-related research, grower education, grower compliance and resistance monitoring. It is particularly useful to receive reports from *Bt* corn registrants on grower compliance and resistance monitoring.

10) *Bt* Sweet Corn IRM

Attribute *Bt* sweet corn is a BT11 hybrid and expresses the Cry1Ab protein. It is thought that Attribute, like BT11 field corn, contains a high dose for ECB. The other targeted pests, for which there is not a high dose, are CEW and FAW.

Refuge for *Bt* sweet corn was not recommended for the following reasons: 1) sweet corn is typically harvested earlier than field corn (18-21) days after silking (before most lepidopteran larvae complete development); and 2) all *Bt* sweet corn residues were to be destroyed within one month of harvest (a practice that presumably would destroy any live larvae left in corn stalks).

Regarding crop destruction, it is possible that the crop destruct requirement may not be adequate in itself to mitigate the threat of resistance for ECB. Specifically, there are data (Mason et al. 1983) that show variance among different crop destruct techniques in terms of the number of surviving ECB. The variation in the efficacy of crop destruct techniques may increase the risk for ECB resistance in *Bt* sweet corn. This risk may be mitigated by either: 1) prescribing a specific and effective crop destruct technique, or 2) utilizing structured refuge. Regarding option #1, it should be noted that corn cultivation practices vary (i.e., plow vs. no-till) and certain crop destruct techniques may not be compatible with all practices. In addition, additional research may be needed to verify the most appropriate crop destruct technique.

The threat of resistance for CEW and FAW in sweet corn should be lower than ECB, due to the fact that CEW and FAW typically complete development in corn ears (unlike stalk-boring ECB), which are mostly harvested and removed from the field prior to crop destruction (Lynch et al. 1999).

c. Summary of Risk Analysis for *Bt* Corn IRM

1) Proximity of Refuge:

Refuges need to be placed close enough to the *Bt* field to maximize the likelihood of random mating between resistant survivors from the *Bt* field and susceptible insects from the refuge. Given the knowledge of ECB pest biology (adult movement, mating, and oviposition behavior), risks to resistance will be mitigated if the refuge is placed as close to the *Bt* field as possible. In-field refuge options (such as strips) may provide the best scenario to ensure random mating. For external refuge options (i.e. blocks), it would be advantageous to locate refuges as close to the *Bt* field as possible. Hunt, et al (1997) report most ECB adults disperse within 1500 feet from where they were released. To plant the refuge further than 1/4 mile from the *Bt* corn field may decrease the chance of random mating and increase the risk of resistance.

2) Refuge Options

Refuge Scenario #1: High Dose Events (MON 810, BT11, Cry9C) in Non-Cotton Growing Regions That Don't Spray Insecticides on a Regular Basis (e.g. Corn Belt)

This region encompasses most of the Corn Belt east of the High Plains. The initial USDA NC-205 refuge recommendations (issued in 1997) included a 20-30% untreated structured refuge or a 40% refuge that could be treated with non-*Bt* insecticides. However, due to the fact that many growers do not regularly treat for ECB (< 10%), the USDA NC-205 modified their position to include a 20% refuge that may be treated with insecticides. NC-205 stated that insecticide use should be based on scouting using economic thresholds.

Refuge Scenario #2: High Dose Events (MON 810, BT11, Cry9C) in Non-Cotton Growing Regions That Spray Insecticides on a Regular Basis (e.g., the High Plains for SWCB)

NC-205 has noted that there are some areas that regularly require insecticide treatment (e.g. the High Plains for SWCB or spider mites) and that separate refuge strategies may be needed for these regions. NC-205 recommended that areas routinely treated with insecticides should expand refuge size from 20% to 40% non-*Bt* corn. Insufficient numbers of susceptible moths may be produced in sprayed refuges in this area. The affected region includes counties in Southwest Kansas, Southeast Colorado and the Texas and Oklahoma Panhandles.

Refuge Scenario #3: High Dose (MON 810, BT11, Cry9C) Events in All Cotton-Growing Regions

Cotton-growing regions represent a higher risk for resistance due to the potential double exposure of CEW to both *Bt* corn (Cry1Ab) and *Bt* cotton (Cry1Ac) during the same growing season. Modeling by Dr. Mike Caprio suggests that a sizable proportion of non-*Bt* corn (at least 50%)

must be planted with *Bt* corn in *Bt* cotton growing regions to avoid the quick evolution of resistance. Smaller refuges present a greater risk and may result in a more rapid evolution of resistance. Cotton experts Dr. John Van Duyn and Dr. Dick Hardee have also communicated a recommendation for a 50% non-*Bt* corn refuge in cotton growing areas. For this scenario, the recommendations include refuges which may be treated to control lepidopteran stalk-boring insects as needed with non-*Bt* insecticides or other appropriate IPM practices. Insecticide use should be based on scouting using economic thresholds as part of an IPM program.

Since Cry9C corn has a relatively low potential for cross-resistance between with Cry1Ab or Cry1Ac and Cry9C is not toxic to CEW, the increased risk of CEW resistance to *Bt* in cotton-growing regions does not apply to Cry9C. Therefore, increasing the refuge size from 20% to 50% in cotton-growing regions may not be relevant.

Refuge Scenario #4: Non-High Dose Events in Non-Cotton Growing Regions That Do Not Spray Insecticides on a Regular Basis (e.g., Corn Belt)

Although there are no specific non-high dose products for ECB that have been considered in this reassessment, an assessment of non-high dose is provided for a comprehensive review of all possibilities.

In general, non-high dose *Bt* corn hybrids pose a higher risk of resistance than high dose events. The International Life Sciences Institute/Health and Environmental Sciences Institute (ILSI/HESI) has recommended larger refuges (e.g., 40% unsprayed in the North) for non-high dose, or “high risk” varieties. The Union of Concerned Scientists (UCS) has recommended a 50% refuge that should not be sprayed with insecticides for *Bt* corn varieties that do not contain a high dose (UCS 1998).

As noted for Refuge Scenario #1, because most refuges will not be routinely sprayed and some growers need the option of spraying if pests reach economic injury levels, mandating an unsprayed refuge should not be necessary. ILSI and UCS recommended that a refuge of 40% or more non-*Bt* corn (treatable with non-*Bt* insecticides if ECB densities exceed economic thresholds) is viable for the Corn Belt.

The risk of insect resistance to the non-high dose events may also be limited by restricting sales (e.g., a total sales cap or in areas where ECB are univoltine). Since ECB exposure to *Bt* is limited in areas where there is one generation per year, restricting the use of non-high dose events to these areas will likely decrease the risk of resistance.

Refuge Scenario #5: Non-High Dose Events in Non-Cotton Growing Regions That Spray Insecticides on a Regular Basis (e.g. the High Plains for SWCB)

Non-high dose plants have an increased risk of insect resistance that is compounded if the refuge is sprayed with insecticides. The ILSI panel has recommended larger refuges for these non-high dose, or “high” risk *Bt* corn varieties. For areas where the refuge will be sprayed with insecticides, the ILSI recommended an 80% non-*Bt* corn refuge (ILSI 1999). Restricting sales of non-high dose events can also reduce the risk of resistance development.

Table D4. Summary Table of the Five Potential *Bt* Corn Refuge Scenarios (as described above)

Dose	Region	Recommended refuge by NC-205; ILSI	Proximity	Notes
High Dose	Corn Belt, no regular pesticide treatment for ECB, SWCB	20% sprayable*	< 1/4 mile from *Bt* field	
High Dose	Corn Belt, regular pesticide treatment for ECB, SWCB	40% sprayable	< 1/4 mile from *Bt* field	Region includes counties in SW KS, SE CO and TX, OK Panhandle
High Dose and Non-high Dose	Cotton Region	50% sprayable	< 1/4 mile from *Bt* field	Not applicable to Cry9C corn.
Non-high Dose	Corn Belt, no regular pesticide treatment for ECB, SWCB	40% sprayable*	< 1/4 mile from *Bt* field	Sales restrictions are also an option
Non-high Dose	Corn Belt, regular pesticide treatment for ECB, SWCB	80% sprayable	< 1/4 mile from *Bt* field	Sales restrictions are also an option

*Use of insecticide sprays only recommended by NC-205

3) Information to Improve the Risk Assessment

The insect resistance management strategies can be improved with the collection of additional information. These data are summarized in Table D5 below.

Table D5. Summary of Data Needed to Improve Insect Resistance Management Strategies for *Bt* Corn Products

Data	Pests
Pest Biology: e.g., larval movement, adult movement, mating behavior, pre- and post-mating dispersal, ovipositional behavior, fitness, and overwintering habitat and survival	CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)
North to South Movement	CEW
High Dose Verification (using 1998 SAP techniques)	For Cry9C: SWCB; For BT11: ECB (probable), CEW, SWCB, CSB; For MON810: SWCB, SCSB, CSB
Resistance Allele Frequency	ECB, CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)
Cross-Resistance - Cry1F, Cry2A, Cry9C, Cry1A proteins	ECB, CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)
Evaluation (field studies and models) of Refuge Options (20% external refuge (sprayable) v. 20% in-field) - [Issues to consider: production of susceptible insects (500:1 ratio) in insecticide treated and non-insecticide treated refuges, adequacy of size, structure, and deployment of the refuge, rotation of refuge.]	ECB, CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)
Collection of Baseline Susceptibility Data and Validation of Discriminating/Diagnostic Dose	SWCB, BCW, FAW, SCSB
Evaluation of Resistance Monitoring Techniques, e.g., discriminating v. diagnostic dose, F_2 screen, sentinel plots, gene mapping	ECB, CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)
Grower Compliance - more detailed information on refuge (%, deployment, and management)	ECB, CEW, SWCB and other target pests (e.g, FAW, BCW, SCSB, others)

3. Cotton

a. Current Insect Resistance Management (IRM) Plan

The Agency granted a conditional registration in October 1995 for the Cry1Ac delta endotoxin from *Bacillus thuringiensis* subspecies kurstaki and the genetic material necessary for its production in cotton to control tobacco budworm (TBW), cotton bollworm (CBW), and pink bollworm (PBW).

An IRM plan for cotton has been in place since registration in October 1995. However, an amended plan was accepted in July 2000, predominately to strengthen the refuge requirements. Below are EPA's terms and conditions of the *Bt* cotton plant-pesticide registration for the IRM requirements as of July 2000.

- "Provide literature, information, and research results on target pest biology and ecology such as interfield movement and behavior, the importance of development rate, survival and fecundity on non-cotton hosts of CBW, TBW, and PBW, and the effect of different hosts on the development, survival and fecundity of these pests in order to assess the significance of selected non-cotton hosts as refugia."

- "Data evaluating the potential for cross resistance."

- "Data for baseline susceptibility for PBW, CBW, and TBW. Where the information does not already exist, data must be submitted which provide baseline susceptibility and discriminating doses for these pests."

- "Monitoring for resistance should be in specific locations in selected states which will be monitored annually at a central laboratory location, with duplicate sample collections sent to a second lab for confirmation. Monsanto will also follow up on grower, extension specialist or consultant reports of less than expected results or control failures (such as increases in damaged squares or bolls) for the target lepidopteran pests (PBW, CBW, and TBW) as well as for cabbage looper, soybean looper, saltmarsh caterpillar, cotton leafperforator and European corn borer. Monsanto must articulate in its IRM plan how resistance management strategies would be altered should resistance be detected. A preliminary report on results of this monitoring must be submitted to the Agency annually by November 1 each year and a final report will be submitted to the Agency annually by January 31 each year for the duration of the conditional registration."

- "Annual reports are submitted to EPA on the use of Bollgard® cotton by acreage, locality (state and region, if applicable), and variety."

- "Monsanto will develop and distribute 1) educational materials for growers, 2) the technical bulletin on the use of the product, and 3) materials on how to monitor and report resistance."

- "Monsanto will investigate the influence of *Bt* cotton on secondary lepidopteran pests (cabbage looper, soybean looper, saltmarsh caterpillar cotton leafperforator and European corn borer)."

- "Monsanto must submit data relevant to the expression and degradation of the Cry1Ac endotoxin in various plant parts in correlation with susceptible doses for lepidopteran pests."

- Growers are required to choose and implement one of the following refuge options for the 2000 growing season (Note: These were the refuge requirements for the 1996-2000 growing seasons):

  "1. For every 100 acres of cotton with the Bollgard gene planted, plant 25 acres of cotton without the Bollgard gene that CAN be treated with insecticides (other than foliar *Btk* products) that control the tobacco budworm, cotton bollworm and pink bollworm.

  2. For every 100 acres of cotton with the Bollgard gene planted, plant 4 acres of cotton without the Bollgard gene that CANNOT be treated with acephate, amitraz, endosulfan, methomyl, profenofos, sulprofos, synthetic pyrethroids, and/or *Btk* insecticides labeled for the control of tobacco budworm, cotton bollworm, and pink bollworm. This cotton must be managed (fertility, weed control and management of other pests) in a similar manner as Bollgard cotton.

  NOTE: If cotton with the Bollgard gene exceeds 75% of the total amount of the cotton planted in any single county or Parish in any year, growers in that county or Parish choosing option B the following year will be required to plant the 4% refugia within one mile of the respective Bollgard cotton field. Monsanto will notify growers who are in an affected county or Parish. If EPA grants registration for cotton containing the *Btk* insect control protein with a similar mode of action as the Cry1Ac insect control protein to another company(s), the EPA will determine when the total cotton within a county or Parish exceeds the 75% level. This determination will be made using annual reports or planted acreage submitted by the registrants. Should EPA determine the combined acreage of cotton containing the *Btk* insect control protein exceeds 75%, they will inform the registrants by January 1, that the refuge must be planted within one mile of the respective Bollgard cotton or other *Btk* cotton fields."

  - Growers must chose one of three structural refuge options for the 2001 growing season:

    "1. 95:5 external structured unsprayed refuge
    At least 5 acres of non-*Bt* cotton (refuge cotton) must be planted for every 95 acres of *Bt* cotton. This refuge may not be treated with any insecticide labeled for the control of tobacco budworm,

cotton bollworm, or pink bollworm. The size of the refuge must be at least 150 feet wide. The refuge must be managed (fertility, weed control and management of other pests) similarly to *Bt* cotton. The refuge must be planted within ½ linear mile from the edge of the Bollgard cotton field.

2. 80:20 external sprayed refuge
At least 25 acres of non-*Bt* cotton must be planted for every 100 acres of *Bt* cotton. All cotton may be treated with insecticides (excluding foliar *Bt* products) labeled for control of the tobacco budworm, cotton bollworm, or pink bollworm. Ensure that a refuge is maintained within 1 linear mile (preferably within ½ mile) from the edge of the *Bt* cotton.

3. 95:5 embedded refuge
At least 5 acres of non-*Bt* cotton (refuge cotton) must be planted for every 95 acres of *Bt* cotton. The refuge cotton must be embedded as a contiguous block within the *Bt* cotton field. For very large fields, multiple blocks across the field may be used. For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the *Bt* cotton and the block is at least 150 feet wide. Within the larger field unit, one of the smaller fields planted to non-*Bt* cotton may be utilized as the embedded refuge. This refuge may be treated with any insecticide (excluding foliar *Btk* products labeled for the control of TBW, CBW, or PBW whenever the entire field is treated. The refuge may not be treated independently of the *Bt* cotton field.

For areas affected by PBW only, the refuge cotton may be planted as single rows within the *Bt* cotton field.

In cases where placement of the refuge within one mile of the *Bt* cotton would be in conflict with state seed production regulations, the grower must plant the refuge as close to the *Bt* cotton as allowed."

The chart below provides the status of the conditional IRM data requirements:

(1) to submit literature and information on target pest biology and ecology including the data on the effectiveness of non-cotton hosts as potential refuges (literature review due June 1, 1996 and research data due January 31, 1998) [MRID 44042501, satisfied]

(2) research data concerning target pest biology, including data regarding the effect of different hosts on the development, survival and fecundity of these pests in order to assess the significance of selected non-cotton hosts as potential refuges [MRID 44042501, partially satisfied]
(3) to develop a protocol for determining the likelihood of cross-resistance to other *Bt* endotoxins (due April 1, 1996) and submit data to evaluate the potential for cross-resistance (due January 31, 1998) [Submission, May 22, 1996, no MRID, partially satisfied]
(4) to submit a plan for a workable monitoring program (surveillance, tracking and remediation elements) (due March 1, 1996) [Submission April 2, 1996, no MRID, partially satisfied]
(5) to submit an annual report of monitoring data (annually November 1 each year for preliminary results and January 31 each year for the final report for the duration of the registration) [Satisfied thus far; Submissions: Sept. 16, 1996, Nov. 5, 1996, Jan. 28, 1997(D255743), Feb. 28, 1997, June 25, 1997, July 6, 1997, and Nov. 15, 1997 (D242056); June 23, 1999 (MRID 448633-01, D259355), Jan. 28, 2000 (D263381)
(6) to submit annual use reports (annually November 1 each year for the duration of the registration [Satisfied thus far; Submissions: Nov. 5, 1996, Sept. 25, 1997 (S531144), Nov. 15, 1997 (D242056); Oct. 22, 1998 (D251290); June 23, 1999 (MRID 448633-01, D259355); Jan. 28, 2000 (MRID 450294-01, D263371)]
(7) to continue development and distribution of grower education materials [Satisfied thus far],
(8) to continue to investigate the influence of *Bt* cotton on secondary lepidopteran pests (cabbage looper, soybean looper, saltmarsh caterpillar, cotton leafperforator, and European corn borer) [MRID 450293-01, satisfied]
9) to submit data relevant to the expression and degradation of the Cry1Ac endotoxin in various plant parts in correlation with susceptible doses for lepidopteran pests (due January 1, 1998) [MRID 445166-01, satisfied]

b. Analysis of the Current IRM Plans and Alternatives

The risk of TBW, CBW, and PBW developing resistance to the Cry1Ac delta-endotoxin as expressed in Bollgard® cotton has been recognized by many organizations and individuals including EPA's Scientific Advisory Panel (SAP) and Pesticide Program Dialogue Committee (PPDC), National Cotton Council, Arizona *Bt* Cotton Working Group, and entomologists of the Cotton Insect Pest Management Forum. SAP reports from both 1995 and 1998 have confirmed that EPA's approach and elements for an insect resistance management plan are appropriate, but that modifications may be necessary as new information becomes available. The SAP in 1998 stated that a high dose/refuge strategy should be mandated by the Agency for *Bt* crops, but this strategy should be developed within the current understanding of the technology and be flexible to the growers who have to implement it. The 1998 SAP defined a high dose and the ratio of susceptible to resistant individuals that a refuge should produce as part of a long-term insect resistance management strategy. Understanding the pest biology and the dose of the *Bt* protein are key to determining the necessary size and placement of an effective refuge. In 1999, EPA held a workshop on cotton IRM which included the registrant, academic and USDA researchers, growers, public interest groups, and other stakeholders. The workshop has helped EPA strengthen the IRM program for 2001 and in this reassessment.

The section below summarizes the most current understanding of the effectiveness of current IRM plans and compares the risk of resistance development in alternative IRM strategies. The full risk assessment of the IRM plans for *Bt* cotton is found in the Agency memoranda, S. Matten OPP/BPPD to W. Nelson, OPP/BPPD, dated July 10, 2000 and September 11, 2000, respectively.

1) Pest Biology

Knowledge of pest biology is critical for the development of effective IRM strategies. For example, refuges must be designed with a solid understanding of the target pest to maximize the production of susceptible insects and increase the likelihood of random mating between susceptible and potentially resistant pests.

TBW, CBW, and PBW differ in their impact on cotton on a regionally-specific basis. For example, in the Southeast, CBW, is the predominant pest. In the Midsouth (Mississippi Delta), TBW, is the most important pest; whereas, PBW, is the only lepidopteran pest of importance in Arizona and California. However, there are many parts of the cotton belt in which TBW and CBW are both significant economic pests.

Key literature information (Caprio and Benedict 1996) regarding pest biology, adult movement, mating behavior, gene flow, and alternate hosts for TBW, CBW, and PBW has been reviewed previously by the Agency and is summarized in its 1998 White Paper on *Bt* plant-pesticide resistance management (EPA 1998).

TBW and CBW

Published data indicate that both CBW and TBW are highly mobile insects, capable of significant long distance movement, with CBW being more mobile than TBW. Mark/recapture studies have shown that CBW moths are capable of dispersing distances ranging from 0.5 km (0.3 mi.) to 160 km (99 mi.) (some migration up to 750 km (466 mi.) was also noted) (Caprio and Benedict 1996). The general pattern of migration is a northward movement, following prevailing wind patterns, with moths originating in southern overwintering sites moving to corn-growing regions in the northern U.S. and Canada. Observations made by Dr. Fred Gould (entomologist, North Carolina State University) indicate that CBW may also move southward from corn-growing regions back to cotton regions in the South (Gould's remarks, see EPA/USDA 1999c). If this is true (and more investigation is needed for confirmation of this effect), the result may be additional CBW exposure to *Bt* crops. In addition, the assumptions about CBW overwintering may need to be revisited--moths that were thought to be incapable of winter survival (and thus not a resistance threat) may indeed be moving south to suitable overwintering sites.

The importance of movement at a localized level is important for the design of a refuge because of the need for random mating and oviposition. The 1998 SAP Subpanel noted that research has shown that substantial local population substructure can develop during the summer as a result of restricted movement of TBW and therefore deployment of a refuge is important (SAP 1998). Dr. Michael Caprio (entomologist, Mississippi State University) has indicated that there is significant localized mating among females (i.e., within the same field of pupal eclosion), although males may disperse over great distances and mate. Caprio found that 20% of the eggs following releases were located within a circle ranging from 50 to 100 m (164-328 ft.) from the release point (100-200 m (328-756 ft.) in diameter) (Caprio 2000a).

TBW and CBW are polyphagous insects, feeding on a number of grain and vegetable crops in addition to weeds and other wild hosts (Caprio and Benedict 1996). That is, there are many possible alternate hosts for CBW and TBW during the season. However, the exact utilization patterns vary with climate and cultivation practices. The complexity of movement of CBW and TBW amongst various alternate hosts requires more study before it is possible to determine which alternate hosts may serve as a refuge.

By utilizing multiple hosts within the same growing season, CBW presents a challenge to *Bt* resistance management in that there is the potential for double exposure to *Bt* protein in both *Bt* corn and *Bt* cotton (potentially up to five or more generations of exposure in some regions). Cross-resistance to one or multiple *Bt* proteins in *Bt* corn and *Bt* cotton becomes a concern not only for insects exposed to *Bt* crops, but insects that move to other crops in which *Bt* microbial pesticides are used.

Overwintering is also an important consideration for IRM--resistant insects must survive the winter to pass their resistant genes on to future generations. In the Corn Belt, for example, CBW incapable of overwintering should not pose a resistance threat. Given that different refuge strategies may be developed based upon where CBW is a resistance threat, accurate sampling data will be needed to accurately predict suitable CBW overwintering areas.

PBW

PBW, in contrast to either CBW or TBW, is fairly restricted to cotton in the U.S. and has very limited mobility. In Arizona, only okra and wild cotton act as possible alternative hosts for PBW, but these areas where okra and wild cotton grow are very small and usually isolated from the cotton growing areas.

Understanding pink bollworm dispersal is essential to setting guidelines for the distance between refuges and *Bt* cotton. Studies of PBW in non-*Bt* cotton show that some adults disperse long distances, but most do not (see discussion in Tabashnik et al. 1999). Extensive and intensive recapture studies with PBW indicate that the adults typically move less than one km (0.6 mi.), particularly when suitable cotton is available (Tabashnik et al. 1999).

Based on the published research, additional information is needed to address larval and adult movement, mating behavior, ovipositional preferences, population dynamics, gene flow, survival and fecundity, fitness costs, and the use of alternate cultivated or wild hosts as refuges. Until there is further evidence that other hosts are proved to be suitable, only non-*Bt* cotton should be relied upon as refuge. The varied cropping systems for cotton, including local and regional differences, should also be considered. Additional research will improve the strength and reliability of an IRM plan to effectively reduce the likelihood that TBW, CBW, or PBW will become resistant to the Cry1Ac delta-endotoxin.

2) Secondary Pests

Monsanto [MRID 450293-01, January 28, 2000 submission] has analyzed data involving the influence of *Bt* cotton on secondary lepidopteran pests: cabbage looper (*Trichoplusia ni* Hubner), soybean looper (*Pseudoplusia includens* Walker), saltmarsh caterpillar (*Estigmene acrea* Drury), cotton leafperforator (*Buccalatrix thurberiella* Busk), and European corn borer (*Ostrinia nubilalis* Hubner) (e.g., acres infested, acres treated, and bales lost to these five lepidopteran species by state and state-regions) from the 1996-2000 Cotton Insect Loss Surveys compiled annually by cotton extension entomologists (see Williams, 1996, 1997, 1998, 1999, 2000) to look for any change in the status of these pests. Their analysis indicates no change in the secondary status of these pests either nationally or regionally although levels of infestation may vary widely from year to year. For example, the number of acres treated for secondary lepidopteran pests remained at or below 400,000 acres since 1996 while acres treated for CBW/TBW ranged from 4.4 million to 6.9 million. However, the Cotton Insect Loss Surveys do not allow the parameters surveyed to be specified for Bollgard *Bt* cotton and non-Bollgard (non-*Bt*) acres. Further study of how *Bt* cotton and insect resistance management plans have impacted secondary lepidopteran pests is warranted.

3) High Dose

The 1998 SAP subpanel agreed that Bollgard cotton expressing Cry1Ac produces a high dose to control TBW, PBW, but only a moderate dose to control CBW. With CBW, 20% of more of the individuals may survive exposure to the Cry1Ac delta-endotoxin. An effective insect resistance management strategy for Bollgard® cotton must consider the differential effect of having a high dose for TBW and PBW and not for CBW.

4) Refuge

a) General Issues

i. Influence of *Bt* Corn on Insect Resistance to *Bt* Cotton

Growing corn in cotton production areas could have a major influence of the development of CBW resistance to *Bt* cotton. CBW feeds on corn and then moves into cotton as the corn senesces. CBW may potentially be exposed to the *Bt* protein produced in *Bt* corn and *Bt* cotton over the course of six generations. The more non-*Bt* corn in *Bt* cotton growing areas, the slower *Bt* resistance will develop in CBW (ILSI 1999). Results of a spatially explicit model (ILSI 1999) indicate that at a high market penetration of *Bt* cotton, the risk of resistance will be high. *Bt* corn that expresses the *Bt* protein in the ear will increase the selection pressure for evolution of CBW resistance in cotton-growing areas because several more generations of CBW carrying a resistance allele(s) will be exposed to the *Bt* protein. When *Bt* corn and *Bt* cotton are grown in the same area, multiple exposure to the *Bt* proteins should influence the size of the refuge. The effect of *Bt* corn on the development of CBW resistance to *Bt* cotton should be studied further.

ii. Factors Affecting Refuge

a. Alternate Hosts

Monsanto provided a study by Schneider and Cross (Mississippi State University) entitled Summer Survey of Tobacco Budworm and Cotton Bollworm Populations in the Delta and Hills of Mississippi: 1999 Report (Schneider and Cross 1999) examines the relative importance of non-crop refuges (e.g., weeds and wild host plants) with respect to crop refuges (non-*Bt* cotton and soybean) in one county in each of the "Hills" and "Delta" cropping areas of Mississippi. In both the Delta and the Hills areas, local larval population densities of CBW and TBW were higher on *Abuliton theophrasti* (velvet leaf) than on *Lonicera japonica* (honeysuckle), and TBW densities were higher on these hosts than were densities of CBW. However, the small number of fields involved in this study make it difficult to generalize the results. Therefore, results of Schneider and Cross cannot be extended beyond the localized area in which they did the research in Mississippi. Until there are more data, alternate hosts cannot be relied upon to provide suitable numbers of susceptible TBW or CBW and non-*Bt* cotton may be the only viable refuge.

b. Concerns with Sprayed Refuge

Shelton et al. (2000) used *Bt* broccoli and diamondback moth as a model system to validate the need for a structured refuge through actual field tests. Their results indicated that a seed mix strategy is less effective at conserving susceptible alleles than separate refuge. Shelton et al. (2000) indicated that great care should be used to ensure that refuges sprayed with highly efficacious insecticides produce adequate numbers of susceptible alleles. As Shelton et al. (2000) noted each insect/*Bt* crop system will need to have its own unique insect resistance management requirements.

Gould and Tabashnik (1998) in their evaluation of *Bt* cotton IRM options commented that a 20 percent external refuge that can be treated extensively with insecticidal sprays may result in almost no refuge because all of the susceptible target larvae would be killed. They cited computer simulations and small-scale experiments which indicated that the use of *Bt* cotton and heavily treated insecticides on the non-*Bt* cotton refuge may promote rapid resistance to *Bt* as well as to the insecticides used in the refuge. Further research is needed on the impacts of insecticides on the refuge efficacy.

c. Proximity

Efforts to determine the appropriate size of refuges have relied in part on models, most of which assume that random mating occurs between adults emerging from refuges and *Bt* cotton (Tabashnik 1994 a, b; Gould 1998; Gould and Tabashnik 1998). If refuges are too far from *Bt* cotton, the chance for random mating is reduced which tends to accelerate the evolution of resistance (Caprio 1998). The February 1998 SAP Subpanel recommended that the Agency reexamine the current *Bt* cotton refuge options with regard to the distance between refuges and transgenic crops and the expected production of susceptible insects from different types of refuges. Without appropriate deployment, a refuge's efficiency could be minimized.

TBW/CBW

TBW is a highly mobile moth as demonstrated by mark/recapture studies and from studies of genetic structure (Caprio and Benedict 1996). The importance of movement at a localized level is important for the design of a refuge because of the need for random mating and oviposition. The 1998 SAP Subpanel noted that research has shown that substantial local population substructure can develop during the summer as a result of restricted movement of TBW and therefore, deployment of a refuge is important (SAP 1998). Because of this, Gould and Tabashnik (1998) recommended that the maximum distance between *Bt* cotton fields and the non-*Bt* cotton refuge should be less than or equal to one mile.

Based on ovipositional patterns for CBW, Caprio (2000a) has indicated that untreated embedded refuges should be at least 100 m (328 ft.) wide to minimize the risk of rapid resistance evolution associated with source-sink dynamics (i.e., the refuge must be wide enough so that all females do not lay all of their eggs in the *Bt* portion of a field and close enough to the *Bt* portion of the field so that there can be random mating and random oviposition of adults). Caprio (personal communication, 2000b) indicates a spatial restriction for the refuge of less than one km (or 0.6 miles) may be more appropriate for CBW based on his movement studies.

CBW larvae, particularly later instars, are capable of plant-to-plant movement. For this reason, seed mixes have been eliminated as a viable refuge option for CBW (SAP 1998).

PBW

PBW larvae movement is limited between plants. Gould and Tabashnik (1998) recommended in-field refuges as the best approach to PBW resistance management because "they reduce or eliminate the isolation by distance that could reduce hybrid matings between susceptible adults from refuges and resistant adults from *Bt* cotton." Gould (1998) states that "a within-field refuge (e.g., seed mixture or row by row mixing) would be best because of limited larval and adult movement." Tabashnik et al. (1999) recommended that "to increase the likelihood of the desired random matings between resistant and susceptible pink bollworm adults, the distance between non-*Bt* cotton and *Bt* cotton should be considerably less than one mile under all circumstances." They also suggest that "another way to increase the chances for delaying resistance is to increase the number of susceptible moths emerging from refuges." Therefore, based on PBW adult dispersal information, the refuge should be placed as close to the *Bt* cotton fields as possible, preferably within, or immediately adjacent to the field.

d. Asynchronous Development

Asynchronous emergence of susceptible moths from refuges and resistant moths from *Bt* cotton is another factor that affects random-mating and thus resistance management. If this happened, most of the susceptible moths might mate with each other before the resistant moths emerged which would greatly decrease the ability of the refuge to delay resistance. Laboratory studies have shown that temporal mating can potentially occur among TBW and PBW populations from non-*Bt* and *Bt* cotton, since development is delayed for resistant larvae feeding on *Bt* cotton (Liu et al. 1999; Peck et al. 1999). At the present time, because there are no reports of resistance to *Bt* cotton in the field, there is no way of verifying whether the developmental asynchrony observed in the resistant strain of PBW selected in the laboratory will hasten or slow the evolution of resistance. These results indicate there is uncertainty associated with developmental asynchrony and its effect on the high dose/refuge strategy and further research should be conducted.

Peck et al. (1999) developed a stochastic, spatially explicit, simulation model to examine the factors that may influence the regional development of resistance to *Bt* (Cry1Ac) produced in transgenic cotton. Using this model, Peck et al. (1999) found that the spatial scale and the temporal pattern of refuges can have a strong effect on the development of resistance to *Bt* cotton. Specifically, the time to resistance was significantly longer in regions where the same fields were used as a refuge from year to year when compared to where the refuges were changed randomly from year to year. Neither the spatial scale nor temporal pattern of placement of refuges has been investigated.

To further complicate matters, Adamczyk et al. (2000) found that there were clear differences in the CBW and fall armyworm survival and development when these larvae were fed *Bt* cotton leaves from 17 commercially available varieties. Adamczyk's research demonstrates that current *Bt* cotton varieties express different levels of Cry1Ac endotoxin throughout the plant and that reproductive isolation of populations of intrinsically tolerant Lepidoptera (CBW and fall armyworm) may occur and complicate the refuge strategies even further. This issue needs to be further evaluated.

e. CBW Issues

Bt cotton only produces a moderate dose for control of CBW. The dosage in *Bt* cotton plants is high enough to kill >80% of the CBW larvae (see discussion in EPA's White Paper, EPA 1998). Gould (1998) notes that if a moderate dose is to be sustainable then the refuge size should be significantly increased, but some of the spatial requirements may be less strict then they are for the high dose. Gould and Tabashnik (1998) cited the need for very large, 30 to 50 percent, refuges to manage CBW resistance based on modeling results. With moderate dose *Bt* crops, "larval movement is not expected to reduce recessiveness significantly." However, Gould (1998) also stated that wild hosts and other crops could serve as part of a larger refuge for CBW, but that we lack data on the contribution of these hosts to overall post population size in different geographic areas.

Pyrethroid Oversprays

Because Bollgard controls only about 60% to 90% of the CBW populations, supplementary pyrethroid sprays are a common practice where CBW are prevalent. Monsanto presents the argument that the use of pyrethroids in combination with Bollgard produces 99% or better control of CBW and ensures that the overall selection pressure for *Bt* resistance is weak and countered by other substantial pressures (see January 28, 2000 submission). Brickle et al. (Unpublished 2000 ---- study sent to Monsanto as part of its January 28, 2000 submission) studied the efficacy of different insecticides and rates against CBW in *Bt* cotton and non-*Bt* cotton. Their data indicate that reduced rates of Tracer® (a spinosyn), Larvin® (a carbamate), and Steward® (an oxadiazine) were useful for control of CBW in *Bt* cotton, other insecticides tested were less effective. They hypothesized that the weakened physiological state of surviving CBW on *Bt* cotton may result in a synergistic relationship between the *Bt* protein and certain supplemental insecticides. However, Brickle et al. (Unpublished 2000) do not provide any data to substantiate this synergistic relationship. There is no information from this study on how the CBW resistance rate to *Bt* would be impacted through the use of supplemental insecticides or whether the frequency of *Bt* resistance alleles would be impacted (positively or negatively) by the use of supplemental insecticides such as pyrethroids. Supplemental insecticides may have a disproportionate effect on resistant versus susceptible *Bt* alleles. There is no mention that pyrethroid resistance may actually be accelerated through the use of oversprays on *Bt* cotton. There is also no information on how pyrethroids may affect other non-target insects, e.g., natural enemies. Therefore, this subject should be investigated to help establish the size of the refuge.

North to South Movement

Dr. Fred Gould (North Carolina State University) has indicated that there is a concern for the impact of southward movement of CBW on refuge effectiveness (Gould's comments in EPA/USDA 1999c and June 3, 1999 e-mail from Dr. Fred Gould to Dr. Sharlene Matten). According to Dr. Gould, a number of researchers believe that CBW may be feeding on corn in

Mexico in the early spring and moving to cotton in the southern U.S. before moving to corn in more northern areas. If these CBW diapause in the northern areas, and all die over the winter, they pose no resistance problem. However, some indirect evidence has indicated that at least some CBW move from northern areas to southern cotton growing regions to overwinter. CBW that move from the north to south to overwinter could be exposed to *Bt* crop hosts for four generations or more. Dr. Gould has gathered data that indicated between 48-72% of the CBW in Louisiana and Texas that oviposited in cotton in August and early September developed on corn as larvae. Data collected in Texas indicated that these CBW are migrating from northern areas. While more confirmatory data are needed, preliminary data indicate that there may be additional CBW exposure to *Bt* crops and thus more of a concern for CBW resistance development. This work is critical to establishing refuge size and placement for CBW resistance management.

Resistance Monitoring Studies

Resistance monitoring studies have indicated that there is a statistically-significant increase of CBW "tolerance" (not resistance) to Cry1Ac (about 10-fold) observed from 1996-1998 in South Alabama, Florida Panhandle, South Carolina, and Georgia (Sumerford et al. 1999). These results cause increased concern over the cause of "tolerance" and whether the refuge strategies are adequate for CBW. Resistance monitoring efforts are discussed below.

f. Use of Acephate and Methyl Parathion

The October 31, 1995 registration agreement does not allow the use of acephate, methyl parathion, or a number of lepidopteran control agents to be used on the 4% unsprayed refuge. In 1996, Monsanto requested that the Agency grant approval of the use of 0.5 lb ai/A acephate for control of plant bugs and stink bugs in the refuge. Monsanto supplied data and expert opinion in its January 28, 2000 submission that indicated that neither acephate nor methyl parathion is effective against TBW or CBW at 0.5 lb ai/A. Monsanto states that a rate of 0.5 lb ai/A is sufficient to control plant bugs, but a rate of 1.0 to 1.3 lb ai/A is required for lepidopteran control.

EPA's science review dated October 10, 1996 concluded that use of acephate should not be permitted to preserve the integrity of the refuge (already a small percentage of a grower's cotton acreage) to allow susceptible lepidopteran larvae to develop with minimal selection pressure. Monsanto added the use of 0.5 lb ai/A acephate or methyl parathion on the 4% refuge to their grower guides in 1997 and 1998 for the purposes of controlling plant bugs, and stink bugs in the non-*Bt* cotton refuge and that TBW, CBW, and PBW will be unaffected. The use of these two compounds was discontinued for the 2000 season pending further data from Monsanto indicating the effect of 0.5 lb ai/A acephate and methyl parathion on TBW, CBW, and PBW control in non-*Bt* cotton refuges.

Dr. Mitchell Roof, Clemson University, (expert in Monsanto's submission) indicated that methyl parathion is very effective against stink bugs. "At the rate of 0.5 lb ai/acre, methyl parathion would have little impact on bollworms or budworms, but would be quite effective on stink bugs." He indicates that acephate is largely ineffective against bollworms and budworms at 0.5 lb ai/A, but would be effective against thrips.

The methyl parathion label lists bollworm on the label with a use rate of 2.5 to 6 pints/acre. Budworm does not appear on the label. Additional information is needed to assess whether a 0.5 lb ai/A acephate or methyl parathion affect the production of susceptible adults in the refuge.

g. PBW Issues

Evaluation of a PBW-Resistant Colonies

Pink bollworm from the 1997 collections that survived concentrations of 3.2 and 10 µg Cry1Ac protein/ml were pooled into a composite strain designated AZP-R. This strain was then reared for one generation on diet containing 10 µg Cry1Ac protein/ml and tested for susceptibility to Cry1Ac. In bioassays in which Cry1Ac was added to artificial diet, the resistant strain, AZP-R, was 100 to 460-fold less susceptible than the individual populations from which it was derived, based on LC_{50}s. AZP-R showed a 177-fold reduction in toxicity to Cry1Ac relative to the LC_{50} from the pooled data from all field populations in Arizona. These results with AZP-R show that PBW has the genetic potential for resistance to Cry1Ac. Thus, caution should be used when deploying transgenic cotton producing the Cry1Ac protein in the field.

Preliminary findings discussed in Patin et al. (1999) show that "the resistance of AZP-R conferred the ability for PBW to complete larval development in bolls of greenhouse-grown *Bt* cotton, pupate, and successfully reproduce." These results are the first to show that a laboratory-selected resistant insect (PBW) can survive on a *Bt* crop (*Bt* cotton) that is grown commercially in the U.S.

In another set of greenhouse experiments with *Bt* cotton and non-*Bt* cotton, Liu et al. (1999) looked at the genetics of laboratory-selected resistance and larval development time in a second resistant strain of PBW called APHIS-98R. They found that the laboratory-selected resistance was recessive in inheritance (i.e., the resistant-susceptible heterozygotes died on the transgenic *Bt* cotton plants). Recessive resistance is consistent with one of the assumptions of the refuge strategy. Shelton and Roush (1999) pointed out in their recent commentary in Nature Biotechnology that in other cases in which inheritance of resistance to *Bt* was studied using transgenic crops that recessive inheritance was considered to be the most important factor determining the success of the refuge strategy.

Liu et al. (1999) found that resistant larvae on *Bt* cotton required an average of 5.7 days longer to develop than susceptible larvae on non-*Bt* cotton. They conclude that "this developmental asynchrony favors non-random mating that could reduce the expected benefits of the refuge strategy." This means that because resistant insects developed more slowly than their susceptible counterparts, they may be out of phase for random mating and dilution of resistance in the field, especially late in the season.

Peck et al. (1999) have shown in computer simulations that interactions between developmental asynchrony and season length increase uncertainty because they either hasten or slow the evolution of resistance. However, it is important to remember that there is considerable overlap in generations of this insect occurring in the field, especially late in the season (Shelton and Roush 1999). Asynchronous development may have either negative or positive effects on the effectiveness of the high dose/refuge strategy in the field; therefore, further study is warranted. The laboratory findings of Liu et al. (1999) are worth examining further under typical field conditions in *Bt* cotton fields. Field experiments should be conducted to measure whether susceptible adults would be present at the same time as resistant adults.

Measurements of Initial Resistant Allele Frequency

The ability to rapidly select for resistance in *Bt* cotton in laboratory strains derived recently from field populations of PBW (Patin et al. 1999) implies that the frequency of alleles for resistance to Cry1Ac in 1997 was higher than expected in Arizona field population of PBW. In particular, these results suggest that the allele frequency was higher than 0.001, which is typically assumed in resistance management models and was found to be the case for CBW (Gould et al. 1997). In addition, Tabashnik reported at the January 21, 2000 meeting of the Arizona *Bt* Cotton Working Group that the 1995 estimate of initial resistance allele frequency, 0.001, is not correct. The estimated R allele frequency was significantly greater than 0.001 in 1997 and was greater than 0.001 in 1998 (Patin et al. 1999 and B. Tabashnik's remarks at January 21, 2000 Meeting of the Arizona *Bt* Cotton Workgroup). However, even if the frequencies of resistance alleles are higher than originally estimated in pest populations, there may be considerable overwintering costs associated with resistance so that resistant individuals may not be surviving as well as susceptible individuals. Refuge size and deployment to *Bt* cotton fields may need to be adjusted if resistance allele frequency is higher than originally estimated.

Despite the data on PBW resistant strains and measurements of resistance allele frequency, extensive field data from 1997 and 1998 show that *Bt* cotton remained extremely effective against PBW in Arizona (Simmons et al. 1998; Patin et al. 1999). However, there may be improvements that would reduce the risk of PBW resistance development to the Cry1Ac protein produced in Bollgard cotton.

b) Models

i. Gould's Model for TBW and CBW Resistance Management

Dr. Fred Gould, entomologist, North Carolina State University (personal communication to S. Matten, 2000) modeled the performance of several refuge scenarios (see Table D6 below). The model assumes diploid genetics, random mating, three generations per year, an initial resistance allele frequency of 0.001, does not include density dependence, and is deterministic. Gould varied the degree of mortality of susceptible larvae to account for crops with differing compatibility with the high dose concept. He also varied the degree of recessiveness of the resistance alleles. All scenarios were for external unsprayed refuge options.

Table D6. Gould's Model for TBW and CBW Resistance Management

Fitness of *Bt* plants RR = (homozygous resistant fitness) ; Rr = (heterozygote fitness); rr= (homozygous susceptible fitness)	**Years to Resistance Allele Frequency Reaching 0.50 for Varied Refuge Sizes (Unsprayed)**			
	4% refuge	**5% refuge**	**10% refuge**	**20% refuge**
Case 1: Extremely high efficacy against susceptible insects RR =1.0; Rr =0.01; rr =0.0001	5.3	6.3	11.0	22.7
Case 2: Very high efficacy against susceptible insects RR=1.0; Rr=0.01; rr=0.001	5.7	6.7	11.7	24
Case 3 [Case for TBW]: Extremely high efficacy against susceptible insects RR=1.0; Rr=0.001; rr=0.0001	12	14.7	29	62.3
Case 4 [Case for TBW]: Very high efficacy against susceptible insects RR=1.0; Rr=0.002; rr=0.001	12	14.7	28.3	61
Case 5: Moderate/high efficacy against susceptible insects RR=1.0; Rr=0.02; rr=0.01	6	7	12	23.3
Case 6 [Case appropriate for CBW]: Moderate efficacy against susceptible insects RR=1.0; Rr=0.2; rr=0.1	4	4.3	5.3	7.7

ii. Caprio's Model for CBW Resistance Management

Mike Caprio, entomologist, Mississippi State University (personal communication to S. Matten, 2000b) modeled the effect of different refuge scenarios (see Table D7 below) on CBW resistance. Caprio's model assumes that no corn was in the area, so the results are based on CBW being exposed to cotton through four generations/year. Most areas will have a substantial refuge in corn during the first two generations, so this model might represent a worst case (depending on whether or not *Bt* corn is growing in the area), but not an unlikely one when considering the entire cotton belt. In the model, he assumes 5% survivorship of susceptibles, 2×10^{-3} initial gene frequency, and that resistance is a partially recessive trait (h = 0.1). Overwintering survival was estimated to be 25%. Dispersal associated with overwintering and the first spring generation (from non-crop hosts to cotton) was assumed to be 90%. This estimate was probably low, but was used to overcome scale limitations associated with complex simulations. The daily dispersal rate for the first two generations on crop hosts was assumed to be 80%/day. It is assumed that cotton is not a very good host during this time and CBW moves from field to field. Refuges are assumed to be in the same location each year. However, Caprio notes that this shouldn't be a problem given the high overwintering dispersal and high dispersal during the first two generations. Wild hosts are not simulated. For the last two generations, dispersal is set at 25%/day (i.e., 25% of adults leave a patch per day - -a field may consist of many patches, a patch is 10 acres). Caprio calculated that about 46% of the eggs from females emerging in the refuges are laid in the refuge. With dispersal set to 50% per day, 21% of eggs from females emerging in the refuges are laid in the refuge. This is about what Caprio estimated for refuges that are approximately 300 feet wide (67% dispersal parameter). Larval movement is ignored in this model. The number given by the model is years until 50% of the fields have resistance allele frequencies above 50%.

Table D7. Caprio's Model for *H. zea* (CBW) Resistance Management

Refuge Option	Years to Resistance
Untreated (more like a seed mix or single row)	
4%	3.46 years (+ 2 extinctions)
16%	5.3 years (+ 2 extinctions)
32%	9.5 years
Sprayed external refuges (economic threshold at 4% with 90% efficacy of the larval population)	
0%	2.2
10%	7.25

Refuge Option	Years to Resistance
20%	10.5
30%	14.5
Embedded untreated refuges (50% Dispersal)	
1.25%	8.6
2.5%	10.3
5.0%	19.2
10.0%	24.8
Embedded untreated refuges (67% Dispersal)	
1.25%	7.0
2.5%	8.0
5.0%	12.0
10.0%	22.4

Caprio's simulations for untreated refuges predict that a seed mix or single rows would not be used to effectively manage CBW resistance. To delay resistance more than 10 years, Caprio's model indicates there would have to be greater than 30% non-*Bt* cotton in a (untreated) seed mix. Caprio's simulations predicts that embedded (untreated) options give the greatest benefit for resistance management with the least amount of non-*Bt* cotton planted assuming the refuge is wide enough to create sufficient isolation between the refuges and *Bt*-fields. This isolation ensures that females from refuges lay enough eggs in refuges to maintain a large susceptible population in those areas. At the same time, the refuge should be close enough to the *Bt* portion of the field so that the increased isolation does not lead to an increase in non-random mating that could overcome the effectiveness of the non-random oviposition. Caprio's simulation suggests there must be a balance between isolation limiting source-sink effects (and delaying resistance evolution) and isolation increasing non-random mating (and hastening resistance evolution). These simulations did not consider the influence of alternate hosts, such as corn, on the development of CBW resistance to *Bt*.

iii) Mike Livingston Efficient Refuge Model

Monsanto cites in its January 28, 2000 submission, the results of an economic model developed by Dr. Michael Livingston at Texas Tech University and presented at the January 2000 Beltwide Conferences (Livingston, Carlson, and Fackler 2000a) that predict that use of the current refuge plan will prevent resistance for 20 years using either the 4% or 20% refuge options. Monsanto cites a second Livingston paper that discusses how TBW and CBW resistance to *Bt* and pyrethroids is influenced by assumptions made regarding the use of pyrethroids on refuge acres (Livingston, Carlson, and Fackler 2000b).

Livingston's efficient *Bt* cotton refuge model maximizes the present value of profits attainable by cotton producers over planning horizons of various lengths in the Louisiana cotton production region. Livingston's model has several important biological and genetic assumptions that are different than those used with other modelers and entomologists. Van Duyn (personal communication to S. Matten, 2000) indicated that the following critical parameter assumptions are in question: 1) recessive genetic expression of resistance in CBW and TBW to Cry1Ac (and pyrethroids), 2) high pyrethroid resistance fitness costs that allows resistance to retrograde out of the population in the absence of pyrethroid use, 3) CBW and TBW mortality on *Bt* at 60% and 95%, respectively, 4) lack of CBW and TBW migration in the modeled system, 5) method of estimating initial resistance allele frequency and frequency estimates for CBW, and 6) treatment of all cotton fields with pyrethroids to kill non-lepidopteran pests. All of these biological parameters will affect the number of years to resistance.

All models have limitations and the information gained from the use of models is only a part of the weight of evidence used by EPA in assessing the risks of resistance development. In the case of the Livingston model, the questions raised about the assumptions prevent EPA from considering this model until these critical parameters can be reconciled. Livingston has recommended that EPA not use his model until a sensitivity analysis is completed and corrections are made (personnel communication to S. Matten, 2000a and 2000b).

c) Refuge Scenarios for Evaluation

The table below provides a comparative summary of various *Bt* cotton refuge scenarios which have been proposed or are currently in place.

Table D8. Refuge Scenarios for TBW, CBW, PBW Resistance Management

Refuge Scenarios	**External Unsprayed (Structured)**	**Embedded**	**External Sprayed**
TBW, CBW, and PBW: Required refuge for 2001 growing season * Seed growers must plant the refuge within 1 mile of the Bollgard cotton and as close as possible to *Bt* cotton fields when there is a conflict with seed production regulations	5% external unsprayed (150 ft. wide); planted within ½ mile	5% embedded - at least 150ft. wide (approx. 50 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard cotton and is at least 150 ft. wide. The refuge may treated as long as the whole field(s) (*Bt* and non-*Bt*) is treated. For PBW only, the refuge cotton may be planted as single rows within the Bollgard field.	20% planted within 1 linear mile, ½ mile preferred
TBW and CBW only: Cotton Pest Insect Management Forum	None	10% embedded refuge that is at least 300 ft wide (approx. 80-100 rows); For small or irregularly shaped fields, neighboring fields farmed by the same grower can be grouped into blocks to represent a larger field unit, provided the block exists within one mile squared of the Bollgard cotton and is at least 300 ft. wide. The refuge may treated as long as the whole field(s) (*Bt* and non-*Bt*) is treated.	30% planted within 1 square mile area of the *Bt* cotton (at no point should a *Bt* cotton field be >1 linear mile from a non-*Bt* cotton refuge field)
TBW, CBW, and PBW: Gould and Tabashnik (1998)	None	16.7% embedded refuge (eight rows non-*Bt* cotton for every 48 rows of *Bt* cotton) – The non-*Bt* cotton should be planted in at least sets of two or more adjacent rows. The refuge may treated as long as the whole field(s) (*Bt* and non-*Bt*) is treated.	50% within 1 square mile area of the *Bt* cotton for TBW and CBW or immediately adjacent for PBW
PBW only: Arizona *Bt* Cotton Working Group	None	10% embedded refuge in which at least one row of non-*Bt* cotton must be planted within every six to ten rows of *Bt* cotton. The refuge may treated as long as the whole field(s) (*Bt* and non-*Bt*) is treated.	20% within each square mile of land (one section), non-*Bt* cotton should be no more than one mile from the leading edge of each *Bt* cotton field

Refuge Scenarios	External Unsprayed (Structured)	Embedded	External Sprayed
PBW eradication/ suppression in California: CA Cotton Pest Control Board	0% non-*Bt* cotton:100% *Bt* Cotton - San Joaquin Valley; include Imperial and Palo Verde	None	None

d) Evaluation of Refuge Options

External Unsprayed Refuge Options v. Embedded (Sprayable) Refuge Options for CBW and TBW

Dr. Mike Caprio's (Mississippi State University) simulations for untreated refuges (Table D7) show that a seed mix or single rows cannot be used effectively to manage CBW resistance. To substantially delay resistance more than 10 years, there would have to be greater than 32% non-*Bt* cotton in a (untreated) seed mix. Caprio's simulations predict embedded (untreated) options provide for the longest period to resistance with the least amount of non-*Bt* cotton planted. Dispersal and proximity dramatically affect the years of protection. The refuge must be wide enough so that all females do not lay all of their eggs in the *Bt* portion of a field and close enough to the *Bt* portion of the field so that there can be random mating and random oviposition of adults. These simulations did not consider the influence of alternate hosts, such as corn, on the development of CBW resistance to *Bt*.

EPA's interpretation of Gould and Caprio's models indicate increased years to resistance with the structured 95:5 external unsprayed refuge and the structured 95:5 embedded refuge options over the unstructured 96:4 external unsprayed refuge option which has been in place since 1995. Two improvements are the structure requirement of having a refuge of at least 150 feet and placement requirement of the refuge within ½ linear mile from the edge of the Bollgard cotton field. Structure refers to the dimension (minimum width or number of rows) and proximity of the refuge to the *Bt* cotton fields. These new requirements will seek to balance the advantages of increased isolation between refuges and *Bt*-fields in limiting source-sink effects while at the same time limiting the negative effects of non-random mating. These requirements also address one of the 1998 Science Advisory Panel Subpanel's concerns regarding adequate deployment of the non-*Bt* cotton refuge.

The width (structure) of refuge also affects the years to resistance. Based on oviposition and dispersal data generated by Caprio, a refuge of 100 rows or about 300 feet is more ideal than one that is less than 300 feet wide. Increasing the width of the refuge from 150 feet to 300 feet will increase the likelihood that susceptible adult females will lay at least some of their eggs within the refuge and not within the *Bt* cotton fields (a "source-sink" effect). Thus, dispersal and random oviposition affect refuge size and structure needed to reduce the risk of resistance development. Resistance risk can be decreased if the width is increased from 150 feet to 300 feet, although the uncertainty is very high. Caprio's model indicates that an approximately 300 foot wide embedded (untreated) refuge would be about 35-40% better (or about 5-7 years to resistance using the 50% dispersal scenario) than an approximately 150 foot wide embedded (untreated) refuge (using the 67% dispersal scenario) although there is considerable uncertainty (Caprio, personal communication, 2000).

Table D9. Comparison of Refuge Scenarios Using the Gould and Caprio Models [Table D6 and D7] for CBW Resistance Management

Refuge Scenarios	**Years to Resistance**
External unsprayed (based on Gould model)	
95:5	4.3
90:10	5.3
Embedded (based on Caprio model)	
95:5 (150 ft. blocks) [67% dispersal]	12.0
95:5 (300 ft. blocks) [50% dispersal]	19.2
90:10 (150 ft. blocks) [67% dispersal]	22.4
90:10 (300 ft. blocks) [50% dispersal]	24.8
External Sprayed (based on Caprio model)	
80:20	10.5
70:30	14.5

Source: EPA, based on Gould and Caprio's Models

Gould's simulation for the case representing CBW (Case 6 in Table D6) indicates that the time to resistance would be increased from 4.3 years to 5.3 years, a 20% increase in the IRM benefit. A structured refuge in close proximity to the *Bt* cotton field should increase the years to resistance. The advantage of the 95:5 or 90:10 embedded refuge is that deploying the refuge within the field improves the likelihood of random mating between susceptible and resistant individuals and random oviposition. Refuge distance requirements and minimization of treatment of the refuge will increase the likelihood of success for the high dose/refuge strategy for insect resistance management in *Bt* cotton. However, the size of the embedded refuge may still be too small for the long-term.

Caprio explains (personal communication to Dr. Sharlene Matten, June 16, 2000) that the embedded concept was developed as a compromise between an external sprayed refuge and an external unsprayed refuge to protect the grower from yield losses and the possibility for growers to spray the refuge. Allowances have been made so that growers would be able to spray the embedded refuge when the *Bt* cotton was sprayed while a 95:5 external unsprayed refuge (structured or unstructured) does not. If treatment of the entire field was necessary then both susceptible and resistant individuals should proportionately affected. *Bt* cotton fields (or set of fields) with embedded refuges should be sprayed less than an external refuge. There is little incentive for a grower using an embedded refuge to treat the narrow embedded refuge blocks when an economic threshold hasn't been reached in the *Bt* cotton portion of the field (or a set of small fields within a certain narrowly defined area).

Based on Gould's model (see Table D6) using Case 3 and 4 for TBW, a grower could get a 2- fold resistance management benefit (i.e., more than 2 times as many years until resistance development) of deploying a 10% in-field (or theoretically an unsprayed external) refuge versus deploying a 5% unsprayed external refuge. Based on Gould's simulation, the time to resistance would be increased from approximately 14.7 years to 28-29 years before resistance would be expected to occur. Gould and Tabashnik (1998, Figure 1 on p. 104) indicated that the level of protection of of their 16.7% in-field option would be in the range of about 12 years for CBW (longer for TBW).

The major arguments against an embedded option are that it will be logistically and economically difficult to implement because of design and planting issues, growers and consultants cannot easily distinguish the *Bt* cotton and non-*Bt* cotton rows, and grower non-compliance may be increased (Andrews et al. 2000). The National Cotton Council (NCC 2000) agrees with the concerns raised by Andrews et al. (2000). Both Andrews et al. (2000) and NCC (2000) indicate that more research is needed on the embedded refuge such as the full scale field demonstration of the embedded versus external refuge being conducted this year in Louisiana. Flexibility should govern how refuge is placed and that voluntary compliance is the best method. For seed producers, they argue they cannot comply with an in-field or embedded refuge option because of seed certification distance requirements (see letter from NCC, 2000 and statements made by Tom Kirby, Delta and Land Pine in EPA/USDA 1999c).

Another argument against a 10% embedded refuge is that in areas that have high TBW resistance problems to pyrethroids that it will be too large for growers to afford the expected losses caused by resistant-TBW and will not fit well within the special needs of areas undergoing boll weevil eradication (Andrews et al. 2000). Planting an embedded option may be more labor (cost) intensive than planting an external refuge. Scouting embedded fields would also be an issue and new scouting practices would have to be developed. Field mapping is a necessity to distinguish *Bt* cotton and non-*Bt* cotton blocks within a single field. Unexpected damage (or other performance problems) would have to be investigated very thoroughly to determine the cause.

While there are logistical, economic, and cultural challenges associated with in-field refuge options, these hurdles are not insurmountable and the benefits to resistance management can be considerable. However, consultants and growers had to adjust to the logistical, economic, and attitudinal challenges associated with implementing mandatory refuge options in 1996. Monsanto's grower compliance surveys indicated that greater than 91% of all growers surveyed complied with the refuge requirements even in the first year of mandatory refuge implementation. These data indicated that cotton growers were able to adjust to planting refuge options fairly readily, although there is still some non-compliance. Second, consultants and extension entomologists changed their scouting practices for *Bt* cotton after the 1996 growing season because TBW/CBW were feeding lower in *Bt* cotton plants on blooms (blooms express lower levels of the Cry1Ac protein) than non-*Bt* cotton plants. In this instance, consultants and extension experts adapted scouting practices to *Bt* cotton.

External Unsprayed Refuge Options v. Embedded (Sprayable) Refuge Options for PBW

Gould and Tabashnik (1998) proposed a 16.7% in-field option (i.e., eight rows non-*Bt* cotton within every 48 rows cotton) versus a 10% embedded option proposed by the Arizona *Bt* Cotton Working Group (PBW). Gould and Tabashnik (1998) recommended blocks of eight rows of non-*Bt* cotton for every forty-eight rows of cotton. The non-*Bt* cotton may be planted in sets of two or more adjacent rows. The Arizona *Bt* Cotton Working Group recommended that at least one row of non-*Bt* cotton be planted within every six to ten rows of *Bt* cotton based on planter size. The 95:5 embedded option required by EPA to be implemented for the 2001 growing season allows for the non-*Bt* cotton to be planted as single rows for PBW resistance management. The major disadvantages of this option are related to grower feasibility, both in a logistical and economic sense. That is, growers may not be willing to deploy a 10% versus 5% embedded (or in-field) refuge.

Because PBW does not disperse to a great extent, <1000 m (0.6 mi.) (Tabashnik et al. 1999), the ½ mile linear distance requirement is probably adequate for the 95:5 external unsprayed refuge option. The structure requirement of having the unsprayed refuge be at least 150 ft. wide strengthens the IRM strategy.

Results of a two-year field study described in Patin et al. (1999, also MRID 448633-01) indicate that in-field refuges of one row of non-*Bt* cotton for each five rows of *Bt* cotton showed promise as an alternative effective refuge for managing PBW resistance to the Cry1Ac delta-endotoxin expressed in Bollgard® cotton. The in-field refuges allow susceptible PBW to be generated systematically throughout *Bt* fields and create a better opportunity for resistant individuals to randomly mate with susceptible individuals (a key for the success of any high dose/refuge strategy). The data show that there were adequate number of adult moths produced in the internal in-field plot. In-field treatments had somewhat higher densities of large PBW larvae than did the external refuge non-*Bt* cotton plants. Based on two years of evaluations at Eloy, the yield of the in-field refuge plots was comparable to, or better than, the external plots. An in-field refuge would also simplify grower decisions regarding deployment of the refuge and potentially reduce non-compliance with the current external refuge strategies. Therefore, a single row (or multiple rows) in a 95:5 or 90:10 embedded refuge option may be adequate to mitigate PBW resistance based on its limited dispersal.

80:20 v. 70:30 External Sprayed Refuge Options for TBW, CBW, and PBW

The 80:20 external sprayed refuge with a distance requirement of 1 linear mile (preferable ½ mile) is essentially the same as the previous 80:20 external sprayed option except that a distance requirement has been included. The distance requirement will improve the refuge efficiency of producing susceptible moths in close proximity to putative resistant moths. This distance requirement is adequate based on TBW and CBW dispersal data. However, for PBW, the refuge may need to be placed closer to the Bollgard cotton fields. Based on PBW dispersal data (Tabashnik et al. 1999), a ½ mile or less distance requirement would be better.

Based on computer simulations for CBW (see Caprio's model, Table D7 and Table D9), a 30% external refuge sprayed at a 4% infestation level (90% of the larval populations is controlled) may delay resistance 30% longer relative to a 20% external sprayable refuge or approximately four more years --- 10.5 years to 14.5 years. Caprio comments (personal communication to S. Matten, 2000b) that this same trend is seen for TBW and there would be an even greater number of years until resistance would occur because there is a high dose for TBW and only a moderate dose for CBW.

An external sprayable refuge option can be used by seed producers and non-seed producers alike. In general, an external sprayable refuge option remains a lower risk option than an external unsprayed option because there are greater economic incentives to manage the "sprayed" refuges than "unsprayed" refuges that are placed external to *Bt* cotton fields. There are a number of insecticides, representing several different classes of chemistry, that have been recently introduced that provide effective control of budworm and bollworm. These new materials, along with older materials, can be used in an effective system for management of insect pests in non-*Bt* cotton that are cost competitive with the *Bt* cotton system (comments by Dr. Blake Layton, Mississippi State University, in EPA/USDA 1999c). As a consequence, grower compliance has been reported to be less of an issue with the external "sprayed" refuge than with the external "unsprayed" refuge option.

Gould and Tabashnik (1998) proposed a 50% external sprayed refuge for TBW, CBW, and PBW. These authors recommended that the refuge be deployed within one square mile of the *Bt* cotton fields for TBW and CBW resistance management. This same proximity recommendation was proposed by Van Duyn et al. (2000) for their 70:30 external sprayed refuge for TBW and CBW. Gould and Tabashnik (1998, Figure 1 on p. 104) indicated that the years to resistance of the external sprayed option would be in the range of about 12 years for CBW (longer for TBW).

Gould and Tabashnik (1998) recommended that the non-*Bt* cotton refuge should be planted immediately adjacent to the *Bt* cotton fields (or rows) for PBW resistance management. The Arizona *Bt* Cotton Working Group proposed that the refuge should be placed within one square mile of the *Bt* cotton fields and the non-*Bt* cotton should be no more than one mile from the leading edge of each *Bt* cotton field.

The major disadvantages of this option are related to grower feasibility, both in a logistical and economic sense. That is, growers may not be willing to deploy a 30% or 50% external sprayed refuge versus a 20% external sprayed refuge.

Critics of increasing the external sprayed refuge size have different viewpoints. Gould has indicated (personal communication to S. Matten, 2000) that a 10% unsprayed refuge would be approximately equivalent to a 40 to 50% sprayed refuge based on the efficacy of cotton insecticides used for lepidopteran control. Therefore, Gould's model predicts that a 40 to 50% external refuge that is treatable would approximately double the time to resistance versus a 20% external refuge --- from about 12 years to 28-29 years for TBW. As noted in Caprio's model, a 30% sprayed refuge would increase the predicted time to resistance from about 10.5 years to 14.5 years. On the other hand, Andrews et al. (2000) and the National Cotton Council (2000) express the viewpoint that increasing the size of the refuge will reduce the likelihood for profitable cotton production in the southeastern Cotton Belt where tobacco budworm resistance to pyrethroids is high. An external sprayed refuge may potentially increase the likelihood of cotton bollworm resistance to pyrethroids as well as be less likely to fit within the special needs of areas undergoing boll weevil eradication. They also express the concern that an increase in refuge size will potentially reduce grower compliance because of the impact on grower profitability.

For an external sprayed refuge to be efficacious, a low level of susceptible TBW, CBW, or PBW must survive in non-*Bt* cotton fields to provide a refuge benefit. The rate of resistance development to the *Bt* protein in TBW, CBW, or PBW can be reduced if the refuge fields are managed appropriately. Therefore, it is very important for growers to base all insecticide spray applications to the non-*Bt* cotton fields on scouting results and the use of specific economic thresholds. The Cooperative Extension Service publishes insect scouting guides and thresholds for cotton grown in each state. The danger of aggressive spray programs in the non-*Bt* cotton refuge fields is that they will not be based on proper scouting and economic thresholds; therefore, only a few caterpillars will survive and the refuge benefits would be negligible or non-existent. In addition, aggressive spray programs may increase the likelihood of resistance not only to the *Bt* protein, but also to pyrethroid sprays (and other new chemistries).

Because PBW do not disperse to a great extent, <1000 m (0.6 mi.) (Tabashnik et al. 1999), the linear distance requirement (one mile, a half-mile preferred) for the 80:20 or 70:30 external sprayed refuge options may need to be further evaluated.

Seed Mixtures for PBW

Patin et al. (1999) referred to experiments by Watson (1995) that indicated that seed mixtures of *Bt* (80-90%) and non-*Bt* (10-20%) cotton seed were evaluated by Watson (1995) and judged to be promising. Further research should be conducted on seed mixtures as a strategy for PBW resistance management.

California: Area-Wide Suppression Program for PBW

The California Cotton Pest Control Board (CCPCB) has recommended consideration of a unique PBW suppression plan (see December 1, 1999 letter to EPA). This position was originally stated at the EPA/USDA Workshop on *Bt* Crop Resistance Management in Cotton held in Memphis, Tennessee on August 26, 1999. The CCPCB position focuses on the need for 100% *Bt* cotton in a specific area-wide PBW suppression program for a three-year period followed by a *Bt* cotton free period. This plan would need to be evaluated carefully for its potential impacts on insect resistance because the CCPCB requests that the San Joaquin Valley in California be excluded from any refuge requirements.

Currently, an active regulated PBW suppression program is in place and very minimal acreage of *Bt* cotton is planted. However, CCPCB seeks to have the ability to plant 100 percent *Bt* cotton in this area if there is a serious outbreak of native moths. In this event, 100 percent *Bt* cotton would be accompanied by application of sterile PBW moths, active population monitoring, and other management tools already in place.

The CCPCB also would like 100% *Bt* cotton plantings in conjunction with expansion of PBW Control Areas into the California Southern desert areas of Imperial and Palo Verde Valleys. Currently, 96 percent *Bt* cotton and 4 percent non-*Bt* cotton refuge is planted in the Imperial and Palo Verde Valleys, in conjunction with sterile PBW moths and active populations monitoring conducted by the CCPCB. This program has been underway for three years and has resulted in dramatic PBW population reductions. However, CCPCB would like to use 100% *Bt* cotton in the final year of this area-wide suppression program.

Area-wide suppression/eradication programs using 100% *Bt* cotton may be very effective in reducing/eliminating PBW in very geographically isolated areas over a three-year period. This type of program would need coordination with Arizona because the Palo Verde Valley is partially in Arizona. This type of program would also need the involvement of Mexico because of the Mexicali Valley and the annual spring migration of PBW from Mexico to the U.S. However, an area-wide/eradication program is not a resistance management program. That is, structured refuges are designed to produce susceptible insects to mate with resistant individuals to dilute resistance. An area-wide suppression/eradication program is designed to remove an insect from a particular geographic area, not to maintain susceptible insects using a refuge. One danger of allowing 100% *Bt* cotton is that the only individuals that may survive would be resistant if the area-wide suppression/eradication fails. Thus, the likelihood of PBW resistance would be higher if 100% *Bt* cotton were planted and may occur within a couple of years if the area-wide suppression program fails.

Seed Producer Concerns

In the past, seed producers were exempt from the 96:4 external unsprayed and 80:20 external sprayed refuge requirements. Seed increase acres for 2000 are approximately 250,000 acres out of total of about 13 million acres (see Tom Kerby, Delta and Land Pine, remarks in EPA/USDA 1999c). There are different isolation distances required by states (e.g., Alabama, Arkansas, Arizona, California, Mississippi, Minnesota, Texas) for producing certified seed. Arizona, for example, has about 30% of its approximately 350,000 cotton acres, planted for seed production. In general, fields or portions of fields producing Foundation or Registered seed must be isolated 1,320 feet from any other variety of a similar type or 2,640 feet plus an additional 20 buffer rows from other varieties of widely different types. Fields producing the Certified class of seed must be isolated 660 feet plus an additional 20 buffer rows from other varieties of widely different types or 20 feet from other varieties of similar types. Colored cotton must be isolated from white cotton by a distance of at least three miles. However, colored cotton may be isolated from white cotton by a distance of at least one mile, provided there is an intervening field of cotton of at least 250 feet (100 rows) wide covering the full length of the colored cotton field.

The three refuge requirements to be implemented for the 2001 growing season would now include approximately 250,000 acres of seed production. This represents a significant improvement over the current insect resistant management requirements. In particular, the 95:5 external unsprayed structured refuge and the 80:20 external sprayed refuge with distance requirements can be used by seed producers except where there are specific state limitations on seed certification distances. The 95:5 or 90:10 embedded refuge, cannot be used by seed producers because of seed purity standards.

5) Monitoring

Annual resistance monitoring is a mandatory requirement of registration. Monsanto has provided EPA annual resistance monitoring reports. After four years, there is no evidence of TBW, CBW, or PBW resistance to the Cry1Ac delta endotoxin produced by Bollgard cotton cultivars under field situations. As Caprio et al. (1999) conclude “to effectively monitor the frequency of resistance alleles in wild populations of insects, researchers must balance the concerns of statistical precision at low allelic frequencies, costs of sampling, and the organization and labor required to intensively sample many individuals or families.” To date, centralized testing facilities operated by the USDA/ARS/Southern Insect Management Research Unit for the TBW and CBW programs and by the University of Arizona/Extension Arthropod Resistance Management Laboratory for the PBW program help increase the efficiency and consistency of monitoring for insect susceptibility changes.

The February 1998 SAP Subpanel report recommended a tiered-approach to monitoring. In addition, the Subpanel recognized the need to evaluate large numbers of individuals from as many locations as possible. The focus should be on high risk areas, those areas concentrated, but not limited to annual market penetration. At least 100 or more individuals should be collected per location with a target of at least 500-1000 individuals. Sampling locations should be selected to reflect all crop production practices and be separated to reflect distinct populations. However, there may be instances in which large sample sizes are not realistic. This was also noted by the Subpanel.

The results presented below indicate a reasonably well distributed number of sampling sites throughout the Cotton Belt. However, the optimum amount of sampling effort required during each growing season remains unclear. An influence in the success of a resistance monitoring program is adequate financing of the testing facility and collection of samples.

a) TBW and CBW

Diagnostic doses for CBW and TBW have been developed over several years in insect control labs at Monsanto (Sims et al. 1996). The LC_{99} estimates for the full-length Cry1Ac protein are 6.6 µg/ml for TBW and 13322 µg/ml for CBW. The EC_{99} was 0.058 µg/ml for TBW and 28.8 µg/ml for CBW. Sims et al. (1996) validated the concept of a diagnostic dose in combination with a larval growth inhibition assay to unambiguously separate resistant from susceptible insects using a Cry1Ac protein resistant strain of TBW and F_1 hybrids derived by crossing the resistant strain to a susceptible TBW strain. These data indicate that it may be hard to detect resistance with a simple LC_{50} test or to develop a simple diagnostic mortality dose. A combination of the diagnostic dose and larval growth inhibition assay seems to be the most efficient means of tracking population susceptibility, especially when the assay can used to detect susceptibility changes in resistant heterozygotes.

Field populations of TBW and CBW from the eastern half of the U.S. Cotton Belt have been monitored from 1996 to 1999 for changes in susceptibility to the Cry1Ac proteins by Dr. Doug Sumerford, Dr. Dick Hardee, Dr. L. Adams, and Dr. W. Solomon of the USDA/ARS/SIMRU at Stoneville, Mississippi. The results of the resistance monitoring studies from 1996 to 1998 are summarized in Sumerford et al. (1999) (MRID 448633-01). Dr. Doug Sumerford provided EPA with additional resistance monitoring data for the 1999 growing season. The primary focus is on the diet overlay tests which are more reliable. The results of these bioassays are discussed below.

Monitoring efforts for CBW and TBW resistance were initiated in 1996. Eggs or larvae from CBW and TBW populations were collected from nine states. During 1997 and 1998, all field and laboratory population of TBW and CBW were evaluated for tolerance to Cry1Ac via agar overlays containing a freeze-dried formulation of MVPII powder. The concentrations of Cry1Ac in the agar overlay were 0.05 and 5.0 µg/ml for TBW and CBW, respectively. The concentrations were based on the EC_{98} for the two species (Sims et al. 1996).

Results presented in Sumerford et al. (1999) indicated that there were no significant differences for the percentages of <3rd instar larvae between field colonies of TBW and CBW and their respective laboratory control colonies for tests on non-toxic diet for all the tests from 1997 and 1998. However, when treated with Cry1Ac, significantly more larvae from CBW field colonies reached the 3rd instar than those from the laboratory control strain.

TBW and CBW populations from seven regions sampled in both 1997 and 1998 were pooled for analysis: Alabama, South Alabama/Florida Panhandle, Arkansas, Mississippi Delta, Georgia, South Carolina, and Texas. Results indicate that there were statistically significant regional differences in the percentage of CBW larvae ≥3rd instar (tolerant) after five days of feeding on Cry1Ac in 1998 (9.5%) as compared to 1997 (1.85%). The 1998 populations from southern Alabama and the panhandle of Florida were significantly more tolerant than all other regions: 20% in 1998 and 2% in 1997. Southern Alabama and the panhandle of Florida are areas where very high levels of *Bt* cotton were grown in 1996-1998. CBW populations from the Mississippi Delta, Georgia, South Carolina, and Alabama also showed statistically significant increases in CBW tolerance from 1997 to 1998. Results from data collected in 1999 (Sumerford 1999) indicate these same trends. There were no major changes in the tolerances of TBW larvae in the seven regions sampled, with the exception of Generation 3 from the Mississippi Delta.

Results from the Cry1Ac diet overlay tests presented in Sumerford et al. (1999) and comments by Sumerford and Hardee (2000) indicated that CBW showed a significant decrease in susceptibility from 1997 to 1999. As noted in Sumerford et al. (1999), the measure of "tolerance" is based on a sub-lethal dose of Cry1Ac and should not be interpreted as resistance. Actual resistance is commonly defined as at least a 10-fold difference in LC_{50}'s between susceptible and resistant individuals. Results presented by Sumerford et al. indicate no evidence of field failure due to either TBW or CBW resistance. However, these results do indicate that factors may exist that allow CBW to better "tolerate" Cry1Ac in the field and "tolerance" may be increasing in field populations. The trend in decreased susceptibility for CBW from 1997 to 1999 is an area of concern and should be further investigated. Sumerford et al. (1999) concluded that the genetic basis of the detected small changes in CBW tolerance does not appear to be a major recessive gene, but the tolerance may be due to the quantitative effects of several genes with sub-lethal doses. More research is needed to determine the importance of minor genes in the development of resistance under field conditions.

While there may be entomologists who dispute the significance of the results published by Sumerford et al. (1999), what is clear is that the resistance monitoring should be continued and even increased to determine if the trends observed by Sumerford et al. (1999) will continue. The monitoring results presented by Sumerford et al. may not be conclusive enough to warrant an increase in refuge size at this point in time, but they do raise concerns and should be fully investigated.

There are limitations to the interpretation of the 1997-1999 resistance monitoring data for TBW and CBW based on the sampling strategy. The basic problems are two-fold: sampling was variable and sample size was inadequate. To remedy these two areas of concern, Sumerford and Hardee, USDA/ARS/SIMRU (2000a) indicate that they will conduct a more extensive resistance monitoring program for the year 2000. This program will have a uniform collection protocol, increased sample size per location, and use, in part, a more sensitive monitoring technique, F_2 screen. Implementation of the proposed 2000 monitoring program should improve interpretation, accuracy, and precision of monitoring results. Sumerford and Hardee's resistance monitoring program proposed for the 2000 season does address the 1998 SAP's concerns stated above.

b) PBW

Arizona has conducted a statewide monitoring of PBW susceptibility to Cry1Ac from 1996 to present. The results from 1997 and 1998 were summarized in Patin et al. (1999) (MRID 448633-01). Patin et al. (1999) reported that there were no major decreases in susceptibility of field populations to Cry1Ac in 1996 and 1997. The LC_{50} values differed <5-fold between the seven populations evaluated and ranged from 0.35 to 1.7 µg Cry1Ac/ml. The susceptible reference population, APHIS-S, had an LC_{50} of 0.53 µg Cry1Ac/ml.

Preliminary results from ten populations evaluated from the 1999 growing season indicate that susceptibility levels were similar to 1998 and that there is no evidence of reduced susceptibility of field populations of PBW to Cry1Ac (Dennehy et al. 2000a). However, a 3.3-increase in larvae per boll surviving to ≥third instar in *Bt* cotton in 1999 was observed relative to 1998 (Dennehy et al. 2000a).

Based on the results of extensive field monitoring for resistance in Arizona, the susceptibility of PBW to Cry1Ac in the field remains unchanged. However, there are resistant genes in Arizona PBW populations that confer high levels of resistance to Cry1Ac. In addition, the frequency of alleles for resistance to Cry1Ac in 1997 was higher than expected in Arizona. New PBW refuge options may prove to be more effective reducing the risk of resistance development. Such new options should be tested and, where proven effective, be implemented to reduce the risk of PBW resistance development to the Cry1Ac protein produced in Bollgard cotton. In addition, the PBW resistance monitoring program would be more effective at finding resistance before it became widespread if the entire geographic areas in which PBW is an economic pest (e.g., parts of New Mexico, California, and Texas) was part of the program.

c) Summary

As part of the mandatory terms and conditions of the *Bt* cotton plant-pesticide registration, Monsanto is required to submit monitoring data on the susceptibility of field-collected insect pests to Cry1Ac. No effects, outside the normal ranges of susceptibility to Cry1Ac have been reported for the tobacco budworm or pink bollworm. The cotton bollworm (also known as the corn earworm), however, has a natural tolerance to the Cry1Ac protein. Some degree of increased tolerance (not resistance) to the Cry1Ac protein found in *Bt* cotton in CBW populations from South Alabama, the Mississippi Delta, Georgia, the Florida Panhandle, and South Carolina has been reported based on laboratory bioassays during the three-year period from 1996 to 1998. But, increased tolerance should not be interpreted as resistance. There is no evidence of field failure of *Bt* cotton due to either TBW or CBW resistance. These results, however, do indicate that factors selecting for CBW resistance may already be increasing in the field and that continued monitoring and further analysis is necessary. The Agency will continue its close scrutiny regarding the susceptibility of CBW to the Cry1Ac protein.

6) Remedial Action Plans

EPA required a remedial action plan if there were either suspected or confirmed incidents of insect resistance as part of the terms and conditions of registration. Monsanto is required to instruct customers to contact the company regarding unexpected levels of TBW, CBW, or PBW damage or if resistance is suspected. Monsanto is to investigate and identify the cause of such damage. Based on these investigations, appropriate remedial action is required to mitigate resistance. Resistance monitoring will be intensified in instances of suspected or confirmed resistance. Any confirmed incidents of resistance are required to be reported to the EPA under the terms and conditions of the registration as well as under FIFRA section 6(a)(2). Monsanto has instructed its customers to have regular surveillance programs and report any unexpected levels of TBW, CBW, and PBW damage to them and to their local extension agents. Remedial actions include: informing customers and extension agents in the affected areas of resistance problems, implementing alternative means to reduce or control the resistant populations, increasing monitoring in the affected areas, modifying refuges in the affected areas, and ceasing sales in the affected and bordering counties. Industry cooperation with extension and academic entomologists and consultants is considered important in communicating definitions of “unexpected damage” and appropriate remedial action.

The February 1998 SAP concluded that the 1995 remedial action plans “devised by EPA provide a framework for further refinement. The Subpanel recommended that the current remedial action plans be further defined and refined on a regional and crop-specific basis” (SAP 1998).

To address the concerns of the Subpanel’s recommendations, the Arizona *Bt* Cotton Working Group developed a draft remedial action plan in October 1998 to address PBW resistance and finalized it in April 2000. The Arizona *Bt* Cotton Working Group remedial action plan is quite detailed and addresses the regional specific issues associated with PBW resistance (ABCWG 2000). The remedial action plan includes a definition of putative resistance and verified resistance.

As part of the development of Arizona *Bt* Cotton remedial action plan, the Arizona *Bt* Cotton Rapid Response Team led by the Arizona Cotton Research and Protection Council was formed to investigate field reports of putative resistance and forwards putatively resistant populations to the University of Arizona’s EARML laboratory for testing susceptibility to Cry1Ac. The Rapid

Response Team has documented no "in-field" resistance events since it was instituted. The basic components of the remedial action plan are summarized below:

- Implementation of alternative PBW control measures in the year confirmed resistance is found at the affected site.
- Delineation of a "*Bt* resistance remedial action zone" based on sampling of PBW in the area where resistance was found. The remedial action zone should include all sections of land falling within six miles of the perimeter of the section (s) of land in which verified/reportable resistance occurred.
- Planting of only non-*Bt* cotton in the remedial action zone following a verified/reportable resistance event.
- Use of multiple tactics to suppress the resistant population within the remedial action zone, including: timely crop termination (avoidance of a top-crop) and early cultivation, conventional chemicals, sterile moths, parasitic nematodes [Dennehy's remarks found in EPA, 1999c].

Sumerford and Hardee (2000b) have developed a plan to investigate "problem fields," where growers experience unusual TBW and/or CBW damage in Bollgard fields beginning with the 2000 growing season. Their plan will test progeny from problem fields, use a sublethal diagnostic concentration, and dose-response assay to see if the isolated population fall outside the normal susceptibility parameters determined by baseline data.

The remedial action plan which was implemented in Arizona for PBW in 2000 may serve as a model for what could be done in other regions for TBW and CBW where they are the primary lepidopteran pest concerns. One of the most important parts of a regional remedial action plan is immediate and coordinated action to manage insect resistance in affected areas such as those remedial actions performed by the Arizona Rapid Response Team. Also important would be having a "*Bt* remedial action zone" where no *Bt* cotton is planted in an area where resistance has developed until such time as insect bioassays demonstrate that the frequency of resistance has declined to acceptable levels. EPA expects to work with other parts of the country to enhance the current remedial action plans for areas where TBW and CBW are the major pests.

7) Cross-Resistance

As discussed in Section D2.b.6) above, cross-resistance is an area of major concern for resistance management and poses risks to both transgenic *Bt* crops and microbial *Bt* insecticides. Discussions of cross-resistance are complicated due to the fact that the exact nature and genetics of *Bt* resistance are not fully understood. Resistance may vary substantially from pest to pest, adding to the unpredictability of the system. Cross-resistance occurs when a pest becomes resistant to one *Bt* protein, which then allows the pest to resist other, separate *Bt* proteins. Some pests of cotton are also pests of other crops for which *Bt* transgenic varieties or microbial *Bt* insecticides are available (e.g. CBW on cotton, fall armyworm (*Spodoptera frugiperda*) on tomato). Cross-resistance also poses a risk to pyramid strategies, in which multiple proteins are deployed simultaneously in the same hybrid. However, the development of cross-resistance has not been shown to occur in insect pests exposed in the field to *Bt* crops producing different *Bt* proteins.

Regarding binding sites, cross-resistance may result if two proteins share the same binding site (receptor) in the insect midgut. Therefore, if exposure to one *Bt* protein results in a modification of the receptor, other proteins sharing this site will be affected as well. An example of a possible shared binding site resulting in cross-resistance was observed with TBW. In this case, TBW selected for resistance to Cry1Ac were also found to be resistant to the Cry1Aa, Cry1Ab, and Cry1F proteins (Gould et al. 1995).

The complexity of cross-resistance within a single species or different species is demonstrated by a wealth of experimental evidence. Examples involving TBW are discussed below. Gould *et al.* (1995) selected a tobacco budworm strain (YHD2) for a high level of resistance to Cry1Ac (approximately 2000-fold). The YHD2 laboratory-selected strain was found to be cross-resistant to Cry1Aa, Cry1Ab, and Cry1F and showed limited cross-resistance to Cry1B, Cry1C, and Cry2A. The YHD2 strain was resistant to Cry1A proteins (a, b, and c) as well as Cry1F. Genetic experiments revealed that resistance in the YHD2 strain is partially recessive and is controlled mostly by a single locus or a set of tightly linked loci (Heckel *et al.*, 1997). These results differ from Gould *et al.*'s 1992 published work using his more moderately-resistant laboratory strain of TBW (<50-fold) which showed some broad-spectrum resistance to Cry1Aa, Cry1Ab, Cry1B, Cry1C, and Cry2A (Gould *et al.*, 1992). The resistance levels in this TBW strain were low, and subsequent work showed that resistance was inherited as a nearly additive trait (Heckel *et al.*, 1997). These results show that cross-resistance shows a different pattern to a closely related group of proteins by TBW. It is thus difficult to predict what cross-resistance patterns are likely to be in the field because evolutionary responses will depend on the initial frequencies of each resistance allele, the dominance of the alleles, and how the proteins are used.

Because of the complexity and uncertainty associated with predicting cross-resistance, the Agency has taken unprecedented measures to evaluate the cross-resistance of pest species to the Cry proteins expressed in *Bt* plants. EPA required that registrants submit data evaluating the cross-resistance potential of various insect pests to *Bt* proteins prior to registration.

Based on existing binding site studies with TBW and CBW, there is ample evidence of the cross-resistance potential among Cry1A proteins (EPA, 1998 ; February 28, 1998 Agency Science Review Memorandum S. Matten to W. Nelson; also discussion above). However, these studies do not fully address the cross-resistance potential of TBW, CBW, and PBW to other Cry proteins such as Cry1F and Cry2A. Insects such as the TBW have been shown to have a broad cross-resistance potential to Cry1A, Cry1F, and Cry2A proteins (Gould *et al.*, 1992). Cross-resistance issues are relevant to current *Bt* crops, especially *Bt* corn and *Bt* cotton that deploy Cry1A proteins in which TBW cross-resistance to a number of *Bt* proteins has been demonstrated in laboratory binding studies. Cross-resistance is also important to the livelihood of organic growers who use *Bt* foliar sprays on crops in which CBW is a problem.

Based on the available literature examining the receptor binding properties of Cry1A and Cry2A delta endotoxins in CBW, TBW, and ECB larvae, it is very unlikely that cross-resistance would develop to Cry2A delta endotoxins if resistance develops to Cry1A delta endotoxins in commercially available *Bt* corn and *Bt* cotton. Based on the work of English *et al.* (1994), Cry1A and Cry2A proteins exhibit different binding characteristics and likely possess different modes of action. Because Cry1A and Cry2A proteins exhibit different binding characteristics and very low amino acid homology, they likely possess different modes of action. Therefore, Cry2A may indeed be useful in pyramiding or stacking with other *cry* genes or other non-*Bt* insecticidal genes to combat insect pest resistance. There is, however, some evidence for broad cross-resistance (low levels of resistance) to Cry1A and Cry2A in laboratory-selected strains of beet armyworm (Moar *et al.*, 1995) and TBW (Gould *et al.*, 1992). The Agency will look closely at insect resistance management strategies for *Bt* cotton and *Bt* corn lines that express both Cry1A and Cry2A delta-endotoxins and take appropriate regulatory steps to mitigate resistance development.

Monsanto indicates they are investigating the potential for cross-resistance between Cry1Ac and Cry2Ab for registration of Bollgard II (cotton varieties that express both Cry1Ac and Cry2Ab proteins). Preliminary studies by Bradley *et al.* (2000) and Gould (2000b) provided evidence that highly-resistant strains of TBW (YHD2) and CBW selected on Cry1Ac only showed a low amount of adaptation to the Cry2Ab component in Bollgard II plants. In addition, preliminary bioassays conducted by Dennehy *et al.* (2000b) showed that resistance to Cry1Ac in AZP-R does not confer cross-resistance to Cry2Ab. Insect resistance management strategies will have to account for both Cry1Ac and Cry2Ab being pyramided in *Bt* cotton cultivars. Further study is necessary.

8) Grower Compliance

Grower compliance with refuge requirements is extremely important to the success of any insect resistance management strategy for *Bt* cotton. Lack of grower education and/or poor quality education programs impede successful grower compliance. There are several major grower compliance issues: 1) Is 100% grower compliance achievable, 2) How does lack of grower compliance affect refuge effectiveness, 3) How can the highest level of grower compliance be achieved, and 4) What level of grower compliance has been achieved with current refuge requirements. Annual grower compliance reports submitted by the registrant would help determine how refuge requirements are being implemented.

Monsanto representatives visited Bollgard growers during the summers of 1996, 1997, 1998, and 1999, to discuss their resistance management plans and to review other Integrated Pest Management practices. These representatives looked at field maps, visited fields, and used the gene check kits to confirm the refuge cotton plants were non-Bollgard. The IPM practices discussed included: scouting followed by selective insecticide use to enhance natural enemy populations for additional control; managing for early maturity of varieties; post-harvest stalk destruction to minimize resistance to Bollgard in late-season infestations and soil management practices that encourage destruction of over-wintering pupae. Monsanto presented the results of their Bollgard grower compliance visits from 1996-1999 at the August 26, 1999 EPA/USDA Workshop on *Bt* Crop Insect Resistance Management (see EPA/USDA 1999c; also MRID 448633-01 and MRID 450294-01). The results of Monsanto's grower surveys are shown in Table D10 below.

Table D10. Percent Grower Compliance - Monsanto Study

Year	% Growers Following the Refuge Guidelines
1996	99
1997	98
1998	91
1999	94

Based on Monsanto's grower compliance surveys from 1996-1999, results presented in the Table D10 above indicate that greater than 91% of Bollgard users complied with the refuge requirements. However, the specific questions and the methods in which grower compliance was assessed are not clear.

In 1999, Monsanto offered a refuge incentive program for certain counties in north Alabama and Tennessee to bolster compliance with the 4% unsprayed refuge option. The selected counties had a high percentage of Bollgard cotton. Growers who fulfilled the requirements of managing the 4% unsprayed option properly and who signed a certificate of refuge management compliance in addition to the grower technology agreement received rebate on the technology fee. Of the 117 growers who participated in the program, only two did not meet the requirement and qualify for rebates. Results of the program demonstrate that growers can manage the 4/100 unsprayed refuge option according to the survey results provided by Monsanto. The rebate program shows the positive effect incentives can have on grower compliance.

9) Notification System - 75% Acreage Trigger for 4% Unsprayed Refuge Distance Requirements

Through the end of the 2000 growing season, the Agency has required that Monsanto notify Bollgard retailers and growers in counties/parishes that exceeded that 75% trigger in 1998 that the 4% unsprayed external refuge (if chosen) must be planted within 1 mile of the Bollgard® core acreage. No specific information regarding grower compliance with this distance requirement has been provided to the Agency.

In 1997, there were 33 counties that planted more than 75% of their cotton acreage to Bollgard®

(EPA 1998). In 1998, there were a total of 56 counties/parishes that planted more than 75% of their cotton acreage to Bollgard (MRID 448633-01). Based on the 1999 sales information, Monsanto (MRID 450294-01) reported 115 counties/parishes planted at least 75% of their cotton acres to Bollgard.

Based on Monsanto's farm audits, greater than 91% of the cotton growers have complied with the IRM refuge requirements since 1996. Monsanto's reports to the Agency do not specify whether cotton growers complied with the one mile distance requirement for the 96/4 refuge option in counties/parishes under Notification or whether compliance was strictly measured as a function of refuge size or some other measurement of adequate refuge. In discussions with Monsanto, comments have been made that the Notification system is logistically difficult, i.e., Monsanto must send out thousands of letters to dealers and retailers notifying them of whether they are in counties that have exceeded the 75% trigger. In addition, the Agency is aware that not all growers, and certainly not all University/extension education and researchers, received copies of the Notification letters.

Beginning with the 2001 growing season, the Notification system has been replaced with mandatory structure and deployment requirements for the 95:5 embedded, 95:5 external unsprayed structured, and 80:20 external sprayed refuge options. These three refuge options should help ensure better grower compliance with the refuge requirements as well as improve refuge efficacy. Research shows that refuge deployment is critical to ensure that susceptible moths emerge from refuge fields and can randomly mate with putative resistant moths emerging in *Bt* fields. These changes are in agreement with recommendations by the 1998 SAP Subpanel to reexamine the deployment of the 96:4 external unsprayed and the 80:20 external sprayed refuge options that have been in place for the 1996-2000 growing seasons.

10) Grower Education

Most critical to the success of a resistance management strategy, is communication and education efforts targeting growers to understand and implement the resistance management strategy. *Bt* cotton grower education has been reviewed in EPA's White Paper (EPA 1998). The importance of grower education was emphasized at the EPA/USDA Workshop on *Bt* cotton IRM held in August 1999 (EPA/USDA 1999c).

Based on the review of Monsanto's annual reports submitted for the 1996-1999 growing season, Monsanto has invested in programs and materials to educate growers on the value of incorporating the IRM plan into their farming practices. Monsanto conducts numerous grower and retailer meetings. They also provide financial support to academic and extension researchers. Monsanto also conducts annual grower compliance surveys and field visits. Specific scouting techniques have been developed for *Bt* cotton. A partnership developed between industry, National Cotton Council, State grower organization, universities, extension experts, consultants, and state/federal governmental regulatory agencies would be beneficial to promote insect resistance management.

One example of very good partnership is the Arizona *Bt* Cotton Working Group.

11) Annual Plan Reports

Annual reports are useful to help assess the effectiveness of current *Bt* cotton IRM strategies. The Agency has received annual sales and resistance monitoring reports from Monsanto. However, annual research, grower compliance, and grower education materials would also be pertinent for the Agency's assessment of current and potential IRM strategies.

c. Summary of *Bt* Cotton IRM Risk Assessment

Bollgard cotton expressing Cry1Ac produces a high dose to control TBW, PBW, but only a moderate dose to control CBW. This conclusion was confirmed by the 1998 SAP Subpanel. The 1998 SAP Subpanel concluded that a refuge should produce 500:1 susceptible to one resistant individual in the *Bt* cotton fields. There are data and computer models that suggest that 95:5 external unsprayed and 80:20 external sprayed refuge options may not produce enough susceptible individuals to mate with putatively resistant individuals coming from *Bt* cotton fields. That is, the current refuge options may be too small and not in close enough proximity to *Bt* cotton fields to produce enough susceptible individuals at the right time to mate with putative resistant individuals. Based on TBW and CBW movement and mating information, the threat of resistance is reduced if the refuge is placed within one-half mile or one square mile of the *Bt* cotton fields. Based on PBW movement information, the threat of resistance is reduced if the refuge is placed as close to the *Bt* cotton fields as possible, preferably within the field, or immediately adjacent to the field.

Models by Gould and Caprio predict that the 95:5 external unsprayed refuge option poses a higher risk to resistance than any of the other refuge options under consideration. This is especially true for CBW. Based on Gould's and Caprio's models, a 70:30 external sprayed refuge option or a 90:10 embedded options would appear to mitigate the TBW and CBW resistance risk better than the three refuge options to be implemented in 2001: 95:5 embedded, 95:5 external unsprayed structured, and 80:20 external sprayed. Caprio's model predicts that the time to CBW resistance using the 90:10 embedded untreated (67% dispersal) option will be 22.4 years versus 12.0 years if there was only a 95:5 embedded option. In addition, Caprio's model predicts that the 70:30 external sprayed option would increase the time to resistance from 10.5 years to 14.5 years (about a 30% increase over the 80:20 external sprayed option) for CBW. Gould's model predicts about a two-fold increase in years to resistance for a 90:10 versus 95:5 refuge for TBW resistance management.

In the case of PBW, 100 to 400-fold resistance has been selected in the laboratory from more tolerant field populations. These resistant colonies can survive and reproduce on *Bt* cotton grown in the greenhouse. Further studies on these resistant colonies have shown that resistance was inherited in a recessive fashion, but that there was asynchronous development. Asynchronous development may negatively impact resistance management by impeding random mating, but further study is required to confirm or deny this. Initial resistance allele frequency estimates for PBW were incorrect and, based on data collected in 1997, the resistance allele frequency was significantly higher than the 0.001 estimate in 1995. These data suggest that the concern for PBW resistance in the field may have increased and the margin for error allowed by the current refuge options may be too narrow. Arizona *Bt* Cotton Working Group has indicated that the lowest risk option for PBW developing resistance is the 90:10 in-field refuge option.

Four years of resistance monitoring information for TBW, CBW, and PBW have indicated no significant changes in susceptibility to the Cry1Ac protein. However, a more rigorous resistance monitoring program has been instituted in 2000 to examine potential CBW "tolerance" to the Cry1Ac protein.

Refuge management, choice of land for the refuge, proximity and structure of the refuge to the *Bt* cotton fields, spraying the "unsprayed" refuge, premature termination and grower compliance are issues that have and will affect the efficiency of the refuge. Adding "structure" and mandatory ½ mile distance requirements to the 95:5 external unsprayed refuge option will help reduce certain compliance problems. Increased grower education on the importance of implementing and managing good refuges is still needed. Grower cost incentives may increase compliance. Monsanto had a model program in north Alabama and in one county in western Tennessee that provided incentives to growers to better manage the 96/4 external unsprayed refuge option. The Arizona *Bt* Cotton Working Group have a step-by-step PBW remedial action plan.

Based on all of the scientific data available including computer models, long-term resistance management options should be developed and implemented to reduce the risk of TBW, CBW, and PBW resistance development.

d. Information to Improve the Risk Assessment

The insect resistance management strategies can be improved with the collection of additional information. These data are summarized in Table D11 below.

Table D11. Summary of Data Needed to Improve Insect Resistance Management Strategies for *Bt* Cotton Products

Data	**Pests**
Pest Biology (more information): e.g., larval movement, adult movement, mating behavior, pre- and post-mating dispersal, ovipositional behavior, fitness, and overwintering habitat and survival	TBW, CBW, PBW
North to South Movement	CBW
Resistance Allele Frequency	TBW, CBW, PBW
Cross-Resistance - Cry1A, Cry2A proteins	TBW, CBW, PBW
Evaluation (field studies and models) of Refuge Options - [Issues to consider: production of susceptible insects (500:1 ratio) in insecticide treated and non-insecticide treated refuges, pyrethroid oversprays, adequacy of size, structure, and deployment of the refuge, rotation of refuge.]	TBW, CBW, PBW
Resistance Monitoring Program	Intensify CBW program to investigate "tolerance," Expand and intensify PBW program
Evaluation of Resistance Monitoring Techniques, e.g., discriminating v. diagnostic dose, F_2 screen, gene mapping	TBW, CBW, PBW
Step-by-step Remedial Action Plan [compare to AZ *Bt* Cotton Working Group Plan for PBW]	TBW, CBW
Grower Compliance - more detailed information on percent of refuge acres per farm, deployment, and management	TBW, CBW, PBW

4. Potatoes

The Colorado Potato Beetle (CPB) has demonstrated a distinct ability to develop resistance to a wide variety of conventional insecticides. Based on the analysis of available scientific information, the Agency has determined that there is a potential for resistance to develop to the *Bt* Cry3A delta endotoxin produced in potatoes. The development of resistance could contribute to the loss of effectiveness of this plant-pesticide.

Monsanto developed a resistance management plan for the *Bt* Cry3A delta endotoxin produced in potatoes. The Agency and the March 1, 1995 SAP subpanel reviewed the Monsanto resistance management plan and determined that it is a scientifically-sound and workable resistance management plan to address resistance to the *Bt* Cry3A delta endotoxin produced in potatoes as commercialization began. According to the SAP, the resistance management plan included all of the general elements necessary to reduce the selection pressure on the target pest, CPB, and therefore reduce the probability for resistance to occur. The 1995 SAP recommended that the plan be voluntary and that Monsanto should work with the Agency on refinements to the resistance management plan as more information is gathered during wide-scale commercial use.

a. Current Insect Resistance Management (IRM) Plan

The SAP meeting in 1998 on resistance management recommended that the IRM plan for potatoes be mandatory instead of voluntary. Monsanto has made several modifications to its NewLeaf potato IRM plans over the last five years. In 2000, Monsanto amended their registration to make the refuge mandatory. Growers were already signing contracts which included a refuge requirement. In addition, the current plan focuses on placement of the refuge and encompasses the importance of overwintering sites. The Insect Resistance Management Plan includes:

1) Use NewLeaf potatoes in rotation to reduce CPB.

2) Plant and manage "refuges" to maintain susceptible insect populations. Specific grower recommendations are as follows:

- Do not plant your entire potato acreage to NewLeaf potato varieties, but maintain at least 20% of the total acreage as "refuge".
- Do not use a foliar *Bt* application for CPB control on refuge acres. You may treat CPB in the refuge with insecticides to prevent damage. It is recommended that you use foliar insecticides only when populations reach damaging levels, according to local IPM recommendations.
- Plant every NewLeaf potato field within ½ mile or less of the appropriate current year refuge or Plant every NewLeaf potato field within ½ mile of land that was the designated refuge (non-*Bt* potatoes) last year.

3) Use of every method available to reduce CPB populations such as crop rotation, propane flaming, trench trapping, and overwintering habitat destruction.

4) Monitoring for survival of CPB including a toll free number.

5) Grower education plan.

6) Monitoring for resistance development.

7) Remedial action plan.

b. Analysis of the Risks Associated with Current IRM Plans and Alternatives

The 1998 SAP Subpanel concluded that NewLeaf® and NewLeaf Plus® potato hybrids are maintaining a " high dose" expression of Cry3A throughout the growing season to control Colorado potato beetle (CPB). The dose is at least 50 times that necessary to kill first-instar larvae. Experts meeting in December 1999 agreed that a 20% refuge is sufficient to produce the 500:1 susceptible insects to resistant insects needed for an efficient refuge. They also agree that a one-half mile maximum distance restriction for the refuge is a reasonable recommendation. Monsanto has developed a discriminating dose assay, a surveillance and remedial action plan, and an extensive grower education communication and training program to convey appropriate resistance management tactics. IPM and scouting are discussed in the technical material provided by Monsanto/NatureMark. Based on Monsanto's annual grower surveys, grower compliance with the 20% refuge is >99%. In addition, the recent amendments to make the refuge mandatory and the focus on managing insect overwintering habitat have further decreased the likelihood that resistance of CPB to Cry3A will occur from exposure to *Bt* potatoes. The Agency's full risk assessment of insect resistance development and insect resistance management assessment is found in the Agency's memorandum from S. Matten OPP/BPPD to W. Nelson, OPP/BPPD, dated July 5, 2000.

References

Adamcyzk, JJ., Jr., L.C. Adams, and D.D. Hardee. 2000. Quantification of Cry1Ac-endotoxin in transgenic Bt cotton: Correlating insect survival to different protein levels among plant parts and varieties. 2000 Proceeding of the Beltwide Cotton Conferences. National Cotton Council. In press.

Alyokhin, A.V. and D.N. Ferro. 1999. Relative fitness of Colorado potato beetle (Coleoptera: Chrysomelidae) resistant and susceptible to the *Bacillus thuringiensis* Cry3A toxin. J. Econ. Entomol. 92(3): 510-515.

Alyokhin, A., D.N. Ferro, C.W. Hoy, and G. Head. 1999. Laboratory assessment of flight activity displayed by Colorado potato beetles (Coleoptera: Chrysomelidae) fed on transgenic and Cry3a toxin-treated potato foliage. J. Econ. Entomol. 92(1): 115-120.

Andow, D. A. and D. N. Alstad. 1998. The F_2 screen for rare resistance alleles. J. Econ. Entomol., 91: 572-578.

Andow, D. A. and W. D. Hutchison, 1998. Bt corn resistance management. *In* Now or never: Serious new plans to save a natural pest control. M. Mellon and J. Rissler [eds.]. Union of Concerned Scientists, Two Brattle Square, Cambridge, MA.

Andow, D. A., D. N. Alstad, Y.-H. Pang, P. C. Bolin, and W. D. Hutchison, 1998. Using a F_2 screen to search for resistance alleles to *Bacillus thuringiensis* toxin in European corn borer (Lepidoptera: Crambidae). Journal of Economic Entomology 91: 579-584.

Andrews, G., B. Freeman, A. Harris, G. Herzog, G. Lentz, W. Moar, P. Roberts, M. Roof, R. Smith, M. Sullivan, and S. Turnipseed. 2000. April 4, 2000 letter from Dr. G. Andrews *et al.* to Dr. Janet Andersen, Director/BPPD.

Arizona Bt Cotton Working Group, 2000a. January 31, 2000 Draft Report of the Arizona Bt Cotton Working Group Meeting held on January 21, 2000 (edited by Dr. Tim Dennehy, University of Arizona).

Arizona Bt Cotton Working Group, 2000b. A remedial action plan for responding to pink bollworm resistance to Bt cotton in Arizona. T. J. Dennehy, Chair. April 6, 2000 version.

Ballester, V., B. Escriche, J. Mensua, G. Riethmacher, and J. Ferré, 1994. Lack of cross-resistance to other *Bacillus thuringiensis* crystal proteins in a population of *Plutella xylostella* highly resistant to CryIA(b). Biocontrol Sci. Tech. 4: 437-443.

Bartlett, A. C., 1995. Resistance of the pink bollworm to Bt transgenic cotton. 1995 Proceedings Beltwide Cotton Conferences. National Cotton Council. Pp. 766-768.

Bartlett, A. T., J. Dennehy, and L. Antilla, 1997. An evaluation of resistance to Bt toxins in native populations of the pink bollworm. 1997 Proceedings of the Beltwide Cotton Conferences. Vol. 2. National Cotton Council. Pp.885-888.

Beck, S.D., 1987. Developmental and season biology of *Ostrinia nubilalis*. Agricultural Zoology Review. 2: 59-96.

Benedict, J., D. Fromme, J. Cosper, C. Correa, G. Odvody, and R. Parker, 1998. Efficacy of Bt corn events MON 810, BT11, and Event 176 in controlling corn earworm, fall armyworm, sugarcane borer, and aflatoxin. Unpublished study submitted to EPA (part of Monsanto's 1998 IRM research report). MRID # 447753-01.

Bing, J., 1997. Establishment and survival of second generation European corn borer on Event 176 hybrids. Unpublished study submitted to EPA (part of Mycogen's 1997 IRM research report). MRID # 445436-02.

Boiteau, G., 1988. Sperm utilization and post-copulatory female-guarding in the Colorado potato beetle, *Leptinotarsa decemlineata.* Entomol. Expt. Appl. 47: 183-187.

Bradley, J. R., 1995. Expectations for Transgenic Bt Cotton: Are They Realistic? 1995 Beltwide Cotton Conferences. National Cotton Council. Pp. 763-765.

Bradley, J.R., Jr., J. Van Duyn, and R. Jackson, 2000. Greenhouse evaluation of Bollgard II cotton genotypes for efficacy against feral and Cry1Ac selected strains of *Helicoverpa zea*. Project Code: CT99MON7 and CT99MON8. Study submitted to Monsanto.

Brickle, D.S., S.G. Turnipseed, and M.J. Sullivan, 2000. The efficacy of different insecticides and rates against bollworms (Lepidoptera: Noctuidae) in B.t. and conventional cotton. Unpublished study. [Part of Monsanto's January 28, 2000 submission]

Burd, A. D., J.R. Bradley, J.W. Van Duyn, and F. Gould, 2000. Resistance of bollworm to CryIAc toxin (MVP) 2000 Proceeding Beltwide Cotton Conferences., National Cotton Council, Memphis TN. In Press.

Buschman, L., P. Sloderbeck, and R. Higgins, 1997. Evaluation of refuge planting patterns for corn borer resistance management in Bt-corn. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Buschman, L., P. Sloderbeck, R. Hellmich, S. Ramaswarmy, and R. Higgins, 1999. Dispersal of European and southwestern corn borer moths in Bt cornfields. Unpublished study submitted to EPA (part of Monsanto's 1999 IRM research report). MRID # 450369-02.

Buschman, L. and P. Sloderbeck, 1999. Letter to Dr. Janet L. Andersen (OPP/BPPD), dated 11/30/99.

California Cotton Pest Management Board. 1999. Letter to Dr. Sharlene R. Matten (OPP/BPPD), dated 12/1/99.

Calvin, D., L. Smith, and D. Lehto, 1997. Sequential plantings of field corn: temporal refugia for European corn borer and economic benefits of transgenic hybrids. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID# 444754-01.

Calvin, D., 1998. Tactical implementation of Bt corn hybrids in the Northeastern United States to manage European corn borer resistance. Unpublished study submitted to EPA (part of Monsanto's 1998 IRM research report). MRID # 447753-01.

Cantangui, M., 1997. Early and late planting study. Unpublished research report submitted to EPA (part of Mycogen's 1997 IRM research report). No MRID #.

Catangui, M. and B. Berg, 1998. Performance of Bt-corn exposed to univoltine and bivoltine European corn borers in South Dakota, 1998. Unpublished study submitted to EPA (part of Novartis' 1998 IRM research report). MRID # 447439-01.

Caprio, M., 1997. Corn-cotton ecosystem model for resistance evolution in *Helicoverpa zea* to Bt-endotoxins expressed in plants. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Caprio, M., 1998. Evaluating resistance management strategies for multiple toxins in the presence of external refuges. J. Econ. Entomol. 91: 1021-1031.

Caprio, M., 1999. Personal communication. E-mail correspondence to R. Rose (OPP/BPPD). E-mail dated 2/24/99.

Caprio, M., 2000a. Dispersal and ovipositional patterns of tobacco bollworm (*Helicoverpa zea*) in the corn-cotton agroecosystem. Unpublished study.

Caprio, M., 2000b. Personal communication to Dr. S. Matten, OPP/BPPD. E-mail dated 5/10/2000.

Caprio, M., 2000c. Personal communication to Dr. S Matten, OPP/BPPD. E-mail dated 6/16/2000.

Caprio, M. and J. Benedict, 1996. Biology of the Major Lepidopteran Pests of Cotton (June 24, 1996. Submission by Monsanto, MRID 2204225-01.

Caprio, M.A., and E.J. Grafius, 1990. Effects of light, temperature, and feeding status on flight initiation in postdiapause Colorado potato beetles (Coleoptera: Chrysomelidae). Environ. Entomol. 19: 281-285.

Caprio, M.A., D.V. Sumerford, and S.R. Simms, 2000. Evaluating transgenic plants for suitability in pest and resistance management programs. *In* "Field Manual of Techniques in Invertebrate Pathology" (L. Lacey and H. Kaya, Eds.). Kluwer Academic Publishers (Boston). Pp. 805-828. In Press.

Davis, F.M., 2000. Economic status of southwestern corn borer: December 1998. Unpublished report from USDA (ARS: Mississippi State).

Denholf, P., S. Jansens, M. Peferoen, D. Degheele, and J. Van Rie, 1993. Two different *Bacillus thuringiensis* delta-endotoxin receptors in the midgut brush border membrane of the European Corn Borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae). Applied and Environmental Microbiology. 59: 1828-1837.

Dennehy, T., A. Patin, B. Tabashnik, L. Antilla, Y.-B. Liu, and Y. Carrière, 2000a. Status of pink bollworm susceptibility to Bt cotton in Arizona: 1999 season preliminary update. Unpublished study.

Dennehy, T., M. Sims, B. Tabashnik, Y. Carrière, Y.-B. Liu, L. Antilla, and R. Staten, 2000b. Sustaining efficacy of Bt cotton in Arizona. Unpublished study.

DiCosty, U. R. and M. E. Whalon, 1997. Selection of Colorado potato beetle resistant to CryIIIA on transgenic potato plants. Resistant Pest Management. 9: 33-34.

Dively, G. and T. Horner, 1997. Bt endotoxin-expressing corn and its impact on insect pests and mycotoxin contamination. Unpublished study submitted to EPA by Novartis. MRID # 443474-01.

Dively, G., T. Horner, and D. Ames Herbert, 1997. Impact of Bt endotoxin-expressing transgenic corn on corn earworm and its implications to resistance development and area-wide suppression. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Dively, G. and J. Linduska, 1998. Studies of Bt protein-expressing sweet corn: assessment and management of non-target pests, characterization of blemished kernels, comparative performance of different hybrids, survivorship of target pests after harvest. Unpublished study submitted to EPA (part of Novartis' 1998 IRM research report). MRID # 447439-01.

English, L., H.L. Robbins, M.A. Von Tersch, C.A. Kulesza, D. Ave, D. Coyle, C.S. Jany, S.L. Slatin, 1994. Mode of action of CryIIA: a *Bacillus thuringiensis* delta-endotoxin. Insect Biochem. Molec. Biol. 24: 1025-1035.

Estada, U. and J. Ferré, 1994. Binding of insecticidal crystal proteins of *Bacillus thuringiensis* to the midgut brush border of the cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), and selection for resistance to one of the crystal proteins. Applied Environmental Microbiology. 60: 3840-3846.

Ferré, J., M. D. Read, J. Van Rie, S. Jansens, and M. Peferoen, 1991. Resistance to the *Bacillus thuringiensis* bioinsecticide in a field population of *Plutella xylostella* is due to a change in a midgut membrane receptor. Proceedings of the National Academy of Science USA. 88: 5119-5123.

Ferré, J., B. Escriche, Y. Bel, and J. Van Rie, 1995. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. FEMS Microbiology Letters. 132: 1-7.

Gould, F., 1998a. Evolutionary biology and genetically engineered crops. BioScience 38: 26-33.

Gould, F., 1998b. Sustainability of Transgenic Insecticidal Cultivars: Integrating Pest Genetics and Ecology. Annu. Rev. Entomol. 43: 701-726.

Gould, F., 1999. Personal communication to Dr. Sharlene Matten OPP/BPPD. E-mail dated 6/3/99.

Gould, F., 2000a. Testing *Bt* refuge strategies in the field. Nature Biotechnology 18: 266-267.

Gould, F., 2000b. Final Report: Efficacy of new *Bt*-cotton varieties against resistant strains of

Heliothis virescens. Study submitted to Monsanto.

Gould, F., 2000c. Personal communication to Dr. S. Matten, OPP/BPPD. E-mail dated January 31, 2000.

Gould, F. and B. Tabashnik, 1998. Bt-cotton resistance management. *In* "Now or Never: Serious New Plans to Save a Natural Pest Control. Ed. M. Mellon and J. Rissler. p. 65-105.

Gould, F., A. Martinez-Ramirez, A. Anderson, J. Ferré, F. Silva, and W. Moar, 1992. Broad spectrum Bt resistance. Proc. Natl. Acad. Sci. USA 89: 1545-1559.

Gould, F., A. Anderson, A.Reynolds, L. Bumgarner, and W. Moar, 1995. Selection and genetic analysis of a *Heliothis virescens* (Lepidoptera: Noctuidae) strain with high levels of resistance to *Bacillus thuringiensis* toxins. J. Econ. Entomol. 88: 1545-59.

Gould, F., A. Anderson, A. Jones, D. Sumerford, D. Heckel, J. Lopez, S. Micinski, R. Leonard, and M. Laster, 1997. Initial frequency of alleles for resistance to *Bacillus thuringiensis* toxins in field populations of *Heliothis virescens.* Proc. Natl. Acad. Sci. USA 94: 3519-23.

Granero, F., V. Ballester, and J. Ferré, 1996. *Bacillus thuringiensis* crystal proteins CryIAb and CryIFa share a high affinity binding site in *Plutella xylostella* (L.). Biochem. Biophys. Res. Comm. 224: 779-783.

Greenplate, J.T., 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. J. Econ. Entomol. 92: 1377-1383.

Hardee, D.D., J.W. Van Duyn, M.B. Layton, and R.D. Bagwell, 2000. Bt cotton for management of tobacco budworm and bollworm: Continued effectiveness by managing resistance. U.S. Department of Agriculture, Agriculture Research Service, ARS-154, In Press.

Heckel, D.G., 1994. The complex genetic basis of resistance to *Bacillus thuringiensis* toxin in insects. Biocontrol Sci. and Tech. 4: 405-417.

Heckel, D.G., L.C. Gahan, F. Gould, A. Anderson, 1997. Identification of a linkage group with a major effect on resistance to *Bacillus thuringiensis* Cry1Ac endotoxin in tobacco budworm (Lepidoptera: *Noctuidae*), J. Econ. Entomol. 90: 75-86.

Hellmich, R.L., 1996. 1996 Resistance management related field experiments: Ames, IA. Unpublished study submitted to EPA (part of Monsanto's 1997 IRM research report). No MRID #.

Hellmich, R.L., 1997. 1997 Resistance management related field experiments: Ames, IA.

Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Hellmich, R.L., 1998. 1998 Resistance management related field experiments: Ames, IA. Unpublished study submitted to EPA (part of AgrEvo's 1998 IRM research report). MRID # 447443-01.

Hellmich, R.L., L.S. Higgins, J.F. Witkowski, J.E. Campbell, and L.C. Lewis, 1999. Oviposition by European corn borer (Lepidoptera: Crambidae) in response to various transgenic corn events. Journal of Economic Entomology. 92 (5): 1014-1020.

Higgins, R., 1999. Personal communication to Dr. Sharlene Matten (OPP/BPPD). E-mail dated 7/14/99.

Hunt, T.E., J.F. Witkowski, L.G. Higley, and R.L. Hellmich, 1997. Mark-recapture study examining adult European corn borer dispersal to and among attraction sites–year two of a three year study. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Hunt, T., J. Witowski, and L. Higley, 1998a. Mark-recapture study examining adult European corn borer dispersal to and among attraction sites: addendum to 1997 annual report. Unpublished data submitted to EPA (part of Mycogen and Monsanto's 1998 IRM research report). MRID #s. 448545-01 & 447753-01.

Hunt, T., J. Witowski, and L. Higley, 1998b. Mark-recapture study examining adult European corn borer dispersal to and among attraction sites: year three of a three year study. Unpublished data submitted to EPA (part of Mycogen and Monsanto's 1998 IRM research report). MRID #s. 448545-01 & 447753-01.

International Life Science Institute, 1999. An evaluation of insect resistance management in Bt field corn: a science-based framework for risk assessment and risk management. Report of an expert panel. November 23, 1998.

Lambert, B., L. Buysse, C. Decock, S. Jansens, C. Piens, B. Saey, J. Seurinck, K. Van Audenhove, J. Van Rie, A. Van Vliet, and M. Peferoen, 1996. A *Bacillus thuringiensis* insecticidal crystal protein with a high activity against members of the family Noctuidae. Appl. Environ. Microbiol. 62: 80-86.

Liu, Y-B, B.E. Tabashnik, T. J. Dennehy, A. L. Patin, and A.C. Bartlett, 1999. Development time and resistance to *Bt* crops. Nature. 100: 519.

Livingston, M., 2000a. Personal communication to Dr. S. Matten, OPP/BPPD. E-mail dated

January 27, 2000.

Livingston, M., 2000b. Personal communication to Dr. S. Matten, OPP/BPPD. E-mail dated January 31, 2000.

Livingston, M., G. Carlson, and P. Fackler, 2000a. Efficient Bt refuge policy. 2000 Proceedings Beltwide Cotton Conferences. National Cotton Council. In press.

Livingston, M., G. Carlson, and P. Fackler, 2000b. Bt resistance under conventional insecticide use. 2000 Proceedings Beltwide Cotton Conferences. National Cotton Council. In press.

Luttrell, R.G., L. Wan, and K. Knighten, 1999. Variation in susceptibility of noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. J. Econ. Entomol. 92: 21-32.

Lynch, R.E., B.R. Wiseman, D. Plaisted, and D. Warnick, 1999. Evaluation of transgenic sweet corn hybrids expressing Cry1Ab toxin for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae). Journal of Economic Entomology. 92 (1): 246-252.

Mahaffey, J. S., J. S. Bacheler, J. R. Bradley, Jr., and J. W. Van Duyn, 1994. Performance of Monsanto's transgenic B.t. cotton against high populations of lepidopterous pests in North Carolina, 1994 Proceedings Beltwide Cotton Conferences. National Cotton Council. pp. 1061-1063.

Mallet, J. and P. Porter, 1992. Preventing insect adaptation to insect-resistant crops: are seed mixtures or refugia the best strategy? Proc. R. Soc. Long. B 255: 165-169.

Mason, C.E., M.R. Graustein, and J. Whalen, 1983. Effects of cultivation practices on overwintering European corn borer populations. Published in: Progress Through Research and Extension, Part 1 - Crops Reports. Delaware Agricultural Experiment Station, University of Delaware. pp. 18-19.

Mason, C., and 9 others, 1996. European corn borer: ecology and management. USDA North Central Regional Extension Publication No. 327. Published by Iowa State University, Ames, IA.

Mason, C., J. Whalen, C. Keil, and R. Uniatowski, 1998. Impact of Bt crop protection technology on management of European corn borer in Delaware. Unpublished study submitted to EPA (part of Novartis' 1998 IRM research report). MRID # 447439-01.

Matten, S.R., 1994. December 23, 1994 Memorandum to W. Nelson: Evaluation of Monsanto document (September 1, 1994) [D207200] "Strategies to maximize the utility and durability of Colorado potato beetle resistant potatoes"

Matten, S.R. (For Pesticide Resistance Management Workgroup), 1995. May 2, 1995 Memorandum to W. Nelson: Analysis of SAP and public comments on pesticide resistance management for the Cry3A delta endotoxin expressed in potatoes and the PRMW recommendations.

Matten, S.R., 1999. May 17, 1999 Memorandum to L. Hollis and W. Nelson: Review of Monsanto's proposed amendment and product grower guides to include specific refuge requirements for transgenic potato products, NewLeaf (*Bacillus thuringiensis* (Bt) Cry3A delta endotoxin) and NewLeaf Plus (*Bacillus thuringiensis* (Bt) Cry3A delta endotoxin and the Potato Leaf Roll Virus resistance gene), EPA Reg. Nos. 524-474 and 524-498, Submission letter dated April 26, 1999.

Matten, S. R., P. I. Lewis, G. Tomimatsu, D. W. S. Sutherland, N. Anderson, and T. L. Colvin-Snyder, 1996. The U. S. Environmental Protection Agency's Role in Pesticide Resistance Management. *In* Molecular Genetics and Evolution of Pesticide Resistance. T. M. Brown (ed.) pp. 243-253. American Chemical Society Symposium Series No. 645, American Chemical Society, Washington D. C.

Mellon. M. and J. Rissler, 1998. Now or Never: Serious New Plans to Save a Natural Pest Control. Ed. M. Mellon and J. Rissler. Union of Concerned Scientists. Cambridge, Massachusetts. Pp.149.

Mills, J.M. and J. W. Mullins, 2000. Bollgard Technical Use Guide. Monsanto Company. [submitted as part of the January 28, 2000 submission; Responses to Points in the January 5, 2000 EPA Letter.]

Moar, W., 2000. Comments on Bt Monitoring Results. In: Monsanto's Responses to Points in the January 5, 2000 EPA Letter, Submission dated January 28, 2000. Pp. 15-17.

Moar, W. J., M. Pusztai-Carey, H. Van Faasen, D. Bosch, R. Frutos, C. Rang. K. Luo, and M.J. Adang, 1995. Development of *Bacillus thuringiensis* CryIC resistance by *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Applied and Environmental Microbiology. 61: 2086-2092.

Monsanto Company, 1999. December 16, 1999. Summary of December 9 Research Meeting in Montreal, Canada. [No MRID]

Monsanto Company, 1999. 1998 sales and acreage report, research progress, and resistance monitoring report entitled "Bollgard Cotton 1998 Research Progress and Growing Season Sales Report, June 23, 1999. Monsanto. (MRID 448633-01)

Monsanto Company, 2000. 1999 Annual Bollgard cotton acreage report, January 28, 2000. Monsanto. (MRID 450294-01)

Monsanto Company, 2000. Monsanto proposed IRM plan for 2001; Response to EPA's January 5, 2000 letter; Monsanto request to amend the 524-478 conditional registration to an non-expiring, section 3 (c)5 registration, January 28, 2000 (D263381)

Monsanto Company, 2000. Impact of Bollgard cotton on the status of secondary lepidopteran pests in cotton, January 28, 2000. Monsanto. (MRID 450293-01)

Moyer, D.D., 1993. Trapping Colorado potato beetles with plastic lined trenches. Suffolk County Agricultural News, February 1993, Riverhead, NY.

National Center for Food and Agriculture Policy, 1999. Agriculture biotechnology: insect control benefits. 98 pp.

National Cotton Council. 2000. Letter from National Cotton Council to Dr. Janet Andersen, Director (OPP/BPPD) dated May 3, 2000.
National Resource Council, 2000. Genetically modified pest-protected plants: Science and regulation. National Academy Press. Washington D.C.

NC-205 Regional Research Committee, 1998. Supplemental Report, Supplement to: Bt corn and European corn borer: long-term success through resistance management. October, 1998.

Onstad, D.W. and F. Gould, 1998. Modeling the dynamics of adaptation to transgenic maize by European corn borer (Lepidoptera: Pyralidae). Journal of Economic Entomology. 91 (3): 585-593.

Onstad, D.W. and C.A. Guse, 1999. Economic analysis of transgenic maize and nontrangenic refuges for managing European corn borer (Lepidoptera: Pyralidae). Journal of Economic Entomology. 92 (6): 1256-1265.

Orr, D.B. and D.A. Landis, 1997. Oviposition of European corn borer (Lepidoptera: Pyralidae) and impact of natural enemy populations in transgenic versus isogenic corn. Journal of Economic Entomology. 90 (4): 905-909.

Ortman, E., 1999. Personal communication (on behalf of NC-205) to Janet L. Andersen (OPP/BPPD). E-mail dated 5/24/99.

Ostlie, K. R., W. D. Hutchinson, and R. L. Hellmich, 1997a. Bt-corn & European corn borer: long-term success through resistance management. North Central Regional Extension Publication NCR 602.

Ostlie, K., J. Luedeman, and M. Frenchik, 1997b. Corn planting dates and management strategies for European corn borer. Unpublished study submitted to EPA (part of Novartis' 1997

IRM research report). MRID # 444754-01.

Patin, A.L., T.J. Dennehy, M.A. Sims, B.E. Tabashnik, Y-B. Liu, L. Antilla, D. Gouge, T. J. Henneberry, R. Staten, 1999. Status of pink bollworm susceptibility to Bt in Arizona. 1999 Proceedings of the Beltwide Cotton Conferences. National Cotton Council. Pp. 991-996.

Peck, S., F. Gould, and S. Ellner, 1999. Spread of resistance in spatially extended regions of transgenic cotton: implications for management of *Helitohis virescens* (Lepidoptera: Noctuidae). J. Econ. Entomol. 91(6): 1-16.

Perlak, F.J., T.B. Stone, Y.M. Muskopf, L.J. Peterson, G.B. Parker, S.A. McPherson, J. Wyman, S. Love, G. Reed, and D. Biever, 1993. Genetically improved potatoes: Protection from damage by Colorado potato beetles. Plant Molec. Bio. 22: 313-321.

Pilcher, C.D. and M.E. Rice, 1997. Descriptive statistics of 1996 Bt corn survey. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Rahardja, U., and M. E. Whalon, 1995. Inheritance of resistance to *Bacillus thuringiensis* subsp. *tenebrionis* CryIIIA delta-endotoxin in Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 88: 21-26.

Rice, M.E., J.J. Obrycki, and C. Pilcher, 1997. Adjusting planting dates to manage European corn borer in transgenic Bt corn and non-Bt corn. Unpublished study submitted to EPA (part of Novartis' 1997 IRM research report). MRID # 444754-01.

Rice, M.E. and C.D. Pilcher, 1999. Descriptive statistics of 1998 Bt corn survey. Unpublished study submitted to EPA (part of Novartis' 1999 IRM research report). MRID # 450568-01.

Ross, S.E. and K.R. Ostlie, 1990. Dispersal and survival of early instars of European corn borer (Lepidoptera: Pyralidae) in field corn. Journal of Economic Entomology. 83 (3): 831-836.

Roush, R.T., 1994. Managing pests and their resistance to *Bacillus thuringiensis*: can transgenic crops be better than sprays? Biocontrol Science and Technology. 4: 501-516.

Roush, R.T., 1997a. Managing resistance to transgenic crops. In: *Advances in insect control: the role of transgenic plants.* (Ed. Carozzi, N. And Koziel, M.) Pp. 271-294 (Taylor and Francis, London; 1997).

Roush, R. T., 1997b. *Bt*-transgenic crops: just another pretty insecticide or a chance for a new start in resistance management? Pesticide Sci. 61: 328-334.

Roush, R. T., and G. L. Miller, 1986. Considerations for design of insecticide resistance monitoring programs. Journal of Economic Entomology. 79: 293-298.

Schneider, J.C. and D. Cross, 1999. Summer survey of tobacco budworm and cotton bollworm populations in delta and hills of Mississippi: 1999 Report. In: Monsanto's Responses to Points in the January 5, 2000 EPA Letter, Submission dated January 28, 2000.

Science Advisory Panel, Subpanel on Plant-Pesticides, March 1, 1995, 1995. A set of scientific issues were considered in connection with Monsanto company's application for registration of a plant-pesticide containing the active ingredient *Bacillus thuringiensis* subsp. *tenebrionis* delta-endotoxins. (Docket Number: OPP-00401).

Scientific Advisory Panel, Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides, February 9-10, 1998, 1998. Transmittal of the final report of the FIFRA Scientific Advisory Panel Subpanel on *Bacillus thuringiensis* (Bt) Plant-Pesticides and Resistance Management, Meeting held on February 9-10, 1998. (Docket Number: OPPTS-00231).

Shelton, A.M., J.P. Nyrop, A. Seaman, and R.E. Foster, 1986. Distribution of European corn borer (Lepidoptera: Pyralidae) egg masses and larvae on sweet corn in New York. Environmental Entomology. 15: 501-506.

Shelton, A. M. and R.T. Roush,1999. False reports and the ears of men. Nature Biotechnology. 17 (9): 832.

Shelton, A.M., J.D. Tang, R.T. Roush, T. D. Metz, and E.D. Earle, 2000. Field tests on managing resistance to Bt-engineered plants. Nature Biotechnology. 18: 339-342.

Siegfried, B., T. Spencer, and J. Nearman, 1999a. Monitoring Bt susceptibility of European corn borer and corn earworm to Cry1Ab. Unpublished study submitted to EPA (part of Monsanto's 1999 IRM research report). MRID # 450369-02.

Siegfried, B., T. Spencer, and J. Nearman, 1999b. Susceptibility of corn earworm to the Cry1Ab Bt endotoxin and validation of a diagnostic concentration. Unpublished study submitted to EPA (part of Novartis' 1999 IRM research report). MRID # 450568-01.

Siegfried, B., T. Spencer, and J. Nearman, 1999c. Susceptibility of the European corn borer to the Cry9C Bt endotoxin and validation of a diagnostic concentration. Unpublished study submitted to EPA (part of Aventis' 1999 IRM research report). MRID # 451493-01.

Siegfried, B.D., T. Spencer, and J. Nearman, 2000a. Baseline susceptibility of the corn earworm (Lepidoptera: Noctuidae) to the Cry1Ab toxin from *Bacillus thuringiensis*. Journal of Economic Entomology. Submitted.

Siegfried, B.D., A.C. Zoerb, and T. Spencer, 2000b. Development of European corn borer larvae on Event 176 Bt corn: influence of survival and fitness. Entomologia Experimentalis et Applicata. Submitted.

Simmons, A.L., T. J. Dennehy, B.E. Tabashnik, L. Antilla, A. Bartlett, D. Gouge, R. Staten, 1998. Evaluation of B.t. Cotton deployment strategies and efficacy against pink bollworm in Arizona. 1998 Proceedings of the Beltwide Cotton Conference. National Cotton Council. Pp. 1025-1030.

Sims, S. B., J. T. Greenplate, T. B. Stone, M. A. Caprio, and F. L. Gould, 1996. Monitoring strategies for early detection of Lepidoptera resistance to *Bacillus thuringiensis* insecticidal proteins. *In* Molecular Genetics and Evolution of Pesticide Resistance. T. M. Brown (ed.) Pp. 229-242. American Chemical Society Symposium Series No. 645, American Chemical Society, Washington, D. C.

Sumerford, D., 1999. Abstract for Presentation at Cotton Incorporated Crop Management Seminar: "Cotton Production in the Southeast: New Technology for a New Millennium," November 3-4, 1999, Augusta, GA.

Sumerford, D. And D. Hardee, 2000a. Comments on Bt Resistance Monitoring at Stoneville, MS. In: Monsanto's Responses to Points in the January 5, 2000 EPA Letter, Submission dated January 28, 2000. Pp. 14.

Sumerford, D. and D. Hardee, 2000b. Monitoring studies for cotton: Drs. Sumerford and Hardee USDA-ARS-SIMRU, Stoneville, MS. May 2000.

Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, 1999. Status of Monitoring for Tolerance to Cry1Ac in populations of *Helicoverpa zea* and *Heliothis virescens*: Three-year summary. 1999 Proceeding of the Beltwide Cotton Conferences. Pp. 936-939.

Sumerford, D.V., D.D. Hardee, L.C. Adams, and W.L. Solomon, 2000. Tolerance to Cry1Ac in populations of *Helicoverpa zea* and *Heliothis virescens* (Lepidoptera: Noctuidae): Three-year summary. J. Econ. Entomol. In Press.

Tabashnik, B. E., 1994a. Evolution of resistance to *Bacillus thuringiensis*. Annu. Rev. Entomol. 39: 47-79.

Tabashnik, B.E., 1994b. Delaying insect adaptation to transgenic plants: seed mixtures and refugia reconsidered. Proc. R. Soc. Lond. Ser. B 255: 7-12.

Tabashnik, B. E., N. Finson, M. W. Johnson, and W. J. Moar, 1993. Resistance to toxins from *Bacillus thuringiensis* subsp. *aizawai* in the diamondback moth (Lepidoptera: Plutellidae). Appl. And Environ. Microbiol. 59: 1332-1335.

Tabashnik, B. E., N. Finson, F. R. Groeters, W. J. Moar, M. W. Johnson, K. Luo, and M. J. Adang, 1994. Reversal of resistance to *Bacillus thuringiensis* in Plutella xylostella. *Proc. Natl. Acad. Sci. USA* 91: 4120-2124.

Tabashnik, B. E., Y.-B. Liu, N. Finson, L. Masson, and D. G. Heckel, 1997. One gene in diamondback moth confers resistance to four *Bacillus thuringiensis* toxins. Proceedings of the National Academy of Science USA. 94: 1640-1644.

Tabashnik, B.E., A.L. Patin, T.J. Dennehy, Y.-B. Liu, E. Miller, and R.T. Staten, 1999. Dispersal of pink bollworm (Lepidoptera: Gelechiidae) males in transgenic cotton that produces a *Bacillus thuringiensis* toxin. J. Econ. Entomol. 92(4): 772-780.

Tabashnik, B.E., R.T. Roush, E.D. Earle, A.M. Shelton, 2000. Resistance to *Bt* toxins. Science 287: 42.

Tang, J. D., A. M. Shelton, J. Van Rie, S. De Roeck, W. J. Moar, R. T. Roush, and M. Peferoen, 1997. Toxicity of *Bacillus thuringiensis* spore and crystal protein to resistant diamondback moth (*Plutella xylostella*). Applied Environmental Microbiology. 62: 564-569.

Trisyono, A and M. Chippendale, 1999. Monitoring the susceptibility of the Southwestern corn borer, *Diatraea grandiosella*, to *Bacillus thuringiensis*. Unpublished study submitted to EPA (part of Monsanto's 1999 IRM research report). MRID # 450369-02.

Trisyono, A. and M. E. Whalon, 1997. Fitness costs of resistance to *Bacillus thuringiensis* in Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 90: 267-271.

U.S. Environmental Protection Agency, 1998. The Environmental Protection Agency's White paper on Bt Plant-Pesticide Resistance Management. U.S. EPA, Biopesticides and Pollution Prevention Division (7511W), 401 M Street, SW, Washington, D.C. 14 January 1998. [EPA Publication 739-S-98-001]

U.S. Environmental Protection Agency and U.S. Department of Agriculture, 1999a. EPA/USDA Position Paper on Insect Resistance Management for Bt Crops. http://www.epa.gov/pesticides/biopesticides/otherdocs/bt_position_paper_618.htm (posted June 18, 1999; minor revisions July 12, 1999).

U.S. Environmental Protection Agency and U.S. Department of Agriculture, 1999b. Report of the EPA/USDA workshop on Bt crop resistance management, Rosemont, IL, June 18, 1999. P.A. Stewart, ed. 88 p. American Farmland Trust, Center for Agriculture in the Environment. 80p. (Posted at http://www.epa.gov/pesticides/biopesticides).

U.S. Environmental Protection Agency and U.S. Department of Agriculture, 1999c. Report of

EPA/USDA Workshop on Bt Crop Resistance Management in Cotton. Memphis, Tennessee. August 26, 1999. Ed. Esther Day. American Farmland Trust, Center for Agriculture in the Environment. 80p. (Posted at http://www.epa.gov/pesticides/biopesticides).

U. S. Environmental Protection Agency, 2000. Pesticide Fact Sheet for *Bacillus thuringiensis* subsp. *tenebrionis* CryIII(A) Delta-Endotoxin and the Genetic Material Necessary for Its production in Potato. (Posted at http://www.epa.gov/pesticides/biopesticides).

Van Duyn, J., 1998. Large scale replicated field trials of representative commercial Bt and non-Bt corn hybrids to establish relative insect resistance, yield performance, and commercial value. Unpublished study submitted to EPA (part of Mycogen's 1998 IRM research report). MRID # 448545-01.

Van Duyn, J., 2000. Personal communication to Dr. S. Matten, EPA/BPPD. E-mail dated March 14, 2000.

Van Duyn, J. 1999. Personal communication to Dr. S. Matten, EPA/BPPD. E-mail dated May 7, 1999.

Van Duyn, J., W. Modlin, and N. Storer, 1997. Large scale replicated field trials for representative commercial Bt and non-Bt corn hybrids to establish insect resistance, yield performance, and commercial value. Unpublished study submitted to EPA by Novartis. MRID # 443474-01.

Van Duyn, J., W. Modlin, and A. Summerlin, 1998. Evaluations of Bt corn hybrids against selected corn pests: 1998. Unpublished study submitted to EPA (part of Novartis' 1998 IRM research report). MRID # 447439-01.

Van Duyn, J., B. Layton, and R. Bagwell, 2000. March 22, 2000 Letter from Van Duyn, J., B. Layton, and R. Bagwell to Dr. Janet Andersen, Director/BPPD and attached Position paper entitled "Resistance Management Options for Bollgard Cotton."

Van Rie, J., S. Jansens, H. Hofte, D. Degheele, and H. Van Mellaert, 1990. Receptors on the brush border membrane of the insect midgut as determinants of the specificity of *Bacillus thuringiensis* delta-endotoxins. Applied Environmental Microbiology. 56: 1378-1385.

Venette, R., W. Hutchinson, D. Andow, 1999. Early detection of insect resistance to *Bacillus thuringiensis* in transgenic crops: practical and statistical considerations. Journal of Economic Entomology. Submitted.

Walker, K., R. Hellmich, and L. Lewis, 1999. Injury by late-instar European corn borer (Lepidoptera: Crambidae) on *Bacillus thuringiensis* (Bt) transgenic corn. Journal of Economic

Entomology. Submitted.

Wang, Z., R.L. Hellmich, and L.C. Lewis, 2000. Effects of pollen, husks, silks, and kernels of transgenic corn hybrids on survival of European corn borer (Lepidoptera: Crambidae) larvae. Journal of Economic Entomology. In press.

Watson, T.F. 1995. Impact of transgenic cotton on pink bollworm and other lepidopteran insects. 1995 Proceedings Beltwide Cotton Conferences. National Cotton Council. Pp. 759-760.

Weber, D.C. and D. N. Ferro. 1993. Distribution of overwintering Colorado potato beetle in and near Massachusetts potato fields. Entomol. Exp. Appl. 66: 191-196.

Weisz, R., Z. Smilowitz, and S. Fleischer. 1996. Evaluating risk of Colorado potato beetle (Coleoptera: Chrysomelidae) infestation as a function of migratory distance. J. Econ. Entomol. 89: 435-441.

Whalen, J. and M. Spellman, 1999. Insect control in late planted Bt sweet corn - 1998. Unpublished study submitted to EPA (part of Novartis' 1999 IRM research report). MRID # 450568-01.

Whalon, M.. and D. Ferro. 1998. Bt-potato resistance management. *In:* Now or never: Serious new plans to save a natural pest control. M. Mellon and J. Rissler [eds.]. Union of Concerned Scientists, Two Brattle Square, Cambridge, MA.

Whalon, M. E., D. L. Miller, R. M. Hollingworth, E. J. Grafius, and J. R. Miller. 1993. Selection of Colorado potato beetle (Coleoptera: Chrysomelidae) strain resistant to *Bacillus thuringiensis*. J. Econ. Entomol. 86: 226-233.

Wierenga, J. M., D. L. Norris, M. E. Whalon. 1996. Stage-specific mortality of Colorado potato beetle (Coleoptera: Chrysomelidae) feeding on transgenic potatoes. J. Econ. Entomol. 89: 1047-1052.

Williams, M. R. 1996. Cotton Insect Losses 1995. In. 1996 Proceedings of the Beltwide Cotton Conferences. National Cotton Council, Memphis, TN. Vol. 2: 670-689.

Williams, M. R. 1997. Cotton Insect Losses 1996. In. 1997 Proceedings of the Beltwide Cotton Conferences. National Cotton Council, Memphis, TN. Vol. 2: 834-853.

Williams, M. R. 1998. Cotton Insect Losses 1997. In. 1998 Proceedings of the Beltwide Cotton Conferences. National Cotton Council, Memphis, TN. Vol. 2: 904-925.

Williams, M. R. 1999. Cotton Insect Losses 1998. In. 1999 Proceedings of the Beltwide Cotton

Conferences. National Cotton Council, Memphis, TN. Vol. 2: 785-808.

Williams, M. R. 2000. Cotton Insect Losses 1999. In. 2000 Proceedings of the Beltwide Cotton Conferences. National Cotton Council, Memphis, TN. Vol. 2: In Press.

Additional studies identified by MRID number:

44042501 Caprio, M.; Benedict, J. (1996) Biology of the Major Lepidopteran Pests on Cotton: (Bacillus thuringiensis var. kurstaki CryIA(c) Protein): Lab Project Number: 94-041E1. Unpublished study prepared by Monsanto Co. 82 p.

44258116 MacIntosh, S. (1997) Insect Resistance Management Plan: Bt Cry9C Corn. Unpublished study prepared by Plant Genetic Systems N.V. 454 p.

44347401 Stein, J. (1997) Research Reports Regarding the Interaction Between Corn Earworm (*Helicoverpa zea*) and Novartis Seeds' Insect Resistant Corn. Unpublished study prepared by Novartis Seeds, Inc. 151 p.

44475401 Stein, J. (1998) 1997 Growing Season Report for Event 176-Derived and BT11-Derived Corn. Unpublished study prepared by Novartis Seeds, Inc. 269 p.

44504201 MacIntosh, S. (1998) Updated Insect Resistance Management Plan: Bacillus thuringiensis Cry9C Corn. Unpublished study prepared by Plant Genetic Systems (America) Inc. 16 p.

44516601 Greenplate, J. (1998) Quantification of Bacillus thuringiensis Insect Control Protein (CryIA(c)) in Bollgard Cotton Fruit and Terminals as Affected by Plant Age: Lab Project Number: 17400. Unpublished study prepared by Monsanto Co. 26 p.

44679601 MacIntosh, S.; Jansens, S.; Reed, J. et al. (1998) StarLink Corn: Insect Resistance Management Plan for a Bacillus thuringiensis Cry9C Corn Product. Unpublished study prepared by Plant Genetic Systems N.V. 16 p.

44743901 Stein, J. (1999) 1998 Growing Season Report for Event 176-Derived Field Corn, BT11-Derived Field Corn and BT11-Derived Sweet Corn. Unpublished study prepared by Novartis Seeds, Inc.-Field Crops-NAFTA. 202 p.

44744301 Newhouse, K. (1999) Yearly Reporting on the Acreage Planted to Starlink Corn. Unpublished study prepared by AgrEvo USA Company. 7 p.

44775301 Gustafson, K. (1999) MON 810 1998 Research Progress and Growing Season Sales

	Report: Lab Project Number: 99-088E. Unpublished study prepared by Monsanto Company. 405 p.
44543602	Bing, J. (1997) Establishment and Survival of Second Generation European Corn Borer on Event 176 Corn Hybrids: Lab Project Number: JB-97-02. Unpublished study prepared by Mycogen Seeds. 5 p.
44854501	Bystrak, P. (1999) 1998 Progress Report Activities Relating to Resistance Management in Event 176 Maize: Lab Project Number PB-98-01: PB-98-02: BS-98-01. Unpublished study prepared by Mycogen Seeds and University of Nebraska. 158 p.
44863301	Gustafson, K. (1999) Bollgard Cotton 1998 Research Progress and Growing Season Sales Report: Lab Project Number: 99-531E. Unpublished study prepared by Monsanto Company. 53 p.
45029301	Greenplate, J. (2000) Impact of Bollgard Cotton on the Status of Secondary Lepidopteran Pests in Cotton: Lab Project Number: 94-041E/00-003E. Unpublished study prepared by Monsanto Co. 15 p.
45029401	Reding, H. (2000) 1999 Bollgard Cotton Acreage Report: Lab Project Number: 94-041-E/00-004E. Unpublished study prepared by Monsanto Co. 31 p.
45036902	Stone, T. (2000) 1999 Research Progress and Growing Season Sales Report for MON 810 YieldGard Corn: Research Reports: Lab Project Number: 00-CR-001E. Unpublished study prepared by Monsanto Company. 76 p.
45056801	Stein, J. (2000) 1999 Growing Season Report for Event 176-Derived Field Corn, BT 11-Derived Field Corn and BT 11-Derived Sweet Corn: Lab Project Number. Unpublished study prepared by Novartis Seeds, Inc.-Field Crops-NAFTA. 138 p.
45149301	MacIntosh, S. (2000) Yearly Reporting (1999) On the Insect Resistance Management Plan of Starlink Corn. Unpublished study prepared by Aventis CropScience USA LP. 177 p.