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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES AND TOXIC
SUBSTANCES
WASHINGTON, D.C. 20460

April 22, 2004

MEMORANDUM

SUBJECT: Technical review of Monsanto's submission: "A Final Report on Studies to Assess Production of *Helicoverpa zea* from Alternate Host Plants and from the External Unsprayed Non-Bt Cotton Refuge for Bollgard® Cotton," EPA Reg. Nos. 524-478 and 524-522, Submission dated March 13, 2004, MRID# 46224-01)

TO: Leonard Cole (PM-90)
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FROM: Sharlene R. Matten, Ph.D., Biologist
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**PEER
REVIEW:** Alan H. Reynolds, M.S., Entomologist
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**ACTION
REQUESTED:** Provide a technical review of Monsanto's submission: "A Final Report on Studies to Assess Production of *Helicoverpa zea* from Alternate Host Plants and from the External Unsprayed Non-Bt Cotton Refuge for Cotton" submitted as part of the terms and conditions of Bollgard®¹ cotton (EPA Reg. No. 524-478) and Bollgard® II cotton (EPA Reg. No. 524-522).

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CONCLUSIONS

1. Based on these two-year, five state studies, both C₃ and C₄ alternative hosts serve as unstructured refugia that is much greater than the local 5% external, unsprayed structured non-*Bt* cotton refuge. CBW moths are produced on alternative hosts in sufficient numbers throughout the cotton growing season to mate with any putative resistant CBW moths emerging in Bollgard or Bollgard II cotton fields and dilute resistance. That is, the susceptible CBW moths coming from alternative hosts will reduce the intensity of Cry1Ac and Cry2Ab2 resistance selection in CBW and lower the likelihood of resistance evolution. These findings support the general predictions of the Gustafson et al. (2004) model. Therefore, the results of the alternative host studies support the continuation of the 5% external, unsprayed structured non-*Bt* (*Bacillus thuringiensis*) cotton refuge.
2. The Gustafson et al. (2004) model is limited and cannot appropriately consider the spatial and temporal dynamics of CBW utilization of alternative hosts by generation. See separate review of this model (MRID# 462224-03).
3. Results from these CBW alternative host studies have no bearing on *Bt* resistance management strategies for *Heliothis virescens* (tobacco budworm) and *Pectinophora gossypiella* (pink bollworm) to the *Bt* proteins expressed in Bollgard (Cry1Ac) and Bollgard II (Cry1Ac and Cry2Ab) cotton.

CLASSIFICATION: The alternative host studies are “acceptable.” However, additional CBW resistance modeling work is recommended. See recommendations below.

RECOMMENDATIONS

1. Extend the use of the 5% external, unsprayed structured non-*Bt* refuge until such time the conditional registrations for the Cry1Ac plant-incorporated protectant (PIP) expressed in cotton (Bollgard) and Cry1Ac and Cry2Ac PIPs expressed in cotton (Bollgard II) expire.
2. Refine an appropriate CBW resistance management model with the parameters values obtained from these alternative host studies so that both the spatial and temporal dynamics of CBW utilization of alternative hosts by generation can be considered. Each cotton production system (geography) should be modeled, e.g., North Carolina, The Delta, Georgia.

BACKGROUND

On September 29, 2001, EPA approved an amendment to Bollgard® cotton registration extending the registration until September 30, 2006, except for the 5% external, unsprayed refuge option which expires on September 30, 2004. As a condition of this registration, EPA required that Monsanto Company conduct CBW alternative host research studies that would

provide data that might support these hosts as natural refuge and allow the continuation of the 5% external, unsprayed structured refuge option. As described in EPA's 2001 *Bt* (*Bacillus thuringiensis*) Plant-Incorporated Protectants Biopesticides Registration Action Document (BRAD) (EPA, 2001), research topics must include, but are not limited to the following: "mating and oviposition behavior of *Helicoverpa zea*, fitness of adults and adult population densities coming from the alternative hosts vs. unsprayed and sprayed *Bt* cotton, whether insect pest emergence synchrony with pests emerging from *Bt* cotton, the proximity of alternative hosts to *Bt* cotton, and refinement or construction of new resistance management models that include alternative hosts appropriate for different cotton production regions, e.g., North Carolina vs. Louisiana. Studies must be conducted across the cotton belt where cotton bollworm is an economic pest. The sites must represent a range of conditions that will affect cotton bollworm biology. Conditions must include such factors as irrigation, soil types, and climatic conditions." EPA registered Bollgard II®¹ cotton on December 23, 2002 (EPA Reg. No. 524-522) with the same CBW alternative host data requirements as Bollgard cotton. The draft research protocol was submitted to EPA on December 1, 2001 and reviewed as "acceptable" by EPA on January 28, 2002 (Matten, 2002) and final research protocols were submitted to EPA on March 14, 2002. A two-year regional multi-year project was initiated in 2002 to determine the effectiveness of alternative hosts as unstructured/natural refugia for production of *Helicoverpa zea* (cotton bollworm, CBW). This study was designed to examine the distribution and abundance of CBW alternative hosts through aerial mapping and the productivity of these hosts through larval populations and trapping of CBW adults. Monsanto submitted an interim progress report on the studies described in the protocols March 14, 2003 and reviewed ("acceptable") by EPA on July 22, 2003 (Matten, 2003, **Appendix 1**). EPA required a final report by March 15, 2004. The final report is the subject of this review.

The final report of the results of the two-year study (2002-2003) is divided into five sections:

1. Literature review on CBW biology and behavior that identifies key alternative hosts of CBW in the U.S. and provides the rationale for the design of the field studies.
2. Description of the methods used in the two-year study and the analyses performed.
3. Results of aerial mapping, supplemented by USDA/NASS (National Agricultural Statistics Service) data on cropping patterns, to describe the distribution of common CBW alternative hosts in cotton-growing states. These data are compared to the conclusions of the literature review described in Section 1.
4. Results of larval surveys quantifying the relative productivity of these alternative hosts (corn, peanuts, sorghum, and soybeans) and demonstrating when CBW utilizes them. These results, together with those of Section 3, are used to generate a description of the amount of refuge provided by alternative hosts in different regions of the U.S. at different times.
5. Results of adult trapping, and C3/C4 assays performed on these adults are used to verify which alternative hosts are producing CBW adults at any given time and place. These results are compared to the predictions generated in Section 4.

SUMMARY OF MONSANTO'S FINAL REPORT (Head and Voth, 2004; MRID# 462224-01)

1. Literature on cotton bollworm host use

In this section, Monsanto highlights major points gleaned from the comprehensive literature review of the biology and dispersal of the CBW and tobacco budworm (*Heliothis virescens*, TBW) in North America carried out by Dr. John Benedict, Professor Emeritus, Texas A & M University and Texas Agricultural Experiment Station, Corpus Christi, Texas (Benedict, 2004). EPA recognizes other points of interest from this literature review and has included them in the summary below. Benedict (2004) is an update of an earlier white paper on the biology of TBW and CBW (Caprio and Benedict, 1996) reviewed by EPA in a 1998 White Paper (EPA, 1998). The major aspects of the biology and ecology of CBW are summarized below.

Host plant species for CBW. According to the published literature (Benedict, 2004), there are approximately 130 crop and non-crop hosts of CBW. Crop hosts include both monocotyledonous and dicotyledonous species, and annuals as well as perennials. A total of 30 crop hosts for CBW were identified by Benedict (see Table 5 in Benedict, 2004). CBW larvae are found at high densities in alfalfa (rating = 3), garbanzo bean, hyacinth bean, corn (maize), cotton, grain sorghum, soybean, and tomato and at somewhat lower densities (rating = 2) in chrysanthemum, crimson clover, okra, pigeon pea, peanut, sweet pepper, Irish potato, tobacco, and vetch. These crop hosts belong to five plant families: Fabaceae, Asteraceae, Poaceae, Malvaceae, and Solanaceae. Approximately 100 non-crop hosts (non-cultivated, native and introduced host plants) are known to be hosts for CBW (Appendix Table 1 in Benedict, 2004).

Generations and population age structure. Benedict (2004) points out that the number of generations of CBW varies with the latitude and duration of warm weather in spring and fall. The more northern cotton producing states like North Carolina and Virginia are thought to have only 4 generations per year; while, the warmer southern cotton producing states allow completion of 5 generations per year. In Florida and Texas, it is possible for heliothines to complete 6 generations depending on weather conditions.

Population age structure varies throughout the year, starting with nearly 100% of the individuals is in the adult stage as they emerge from overwintering pupae to start the next generation, then moving into the egg stage, then larval, pupal, and adult in the first spring generation. However, because of the extended emergence of overwintering adults, and their egg laying period of approximately 10 days, larval instars begin to overlap with one another and with recently deposited eggs. The established dates of emergence of overwintering adults in the spring to estimate the timing of the first generation in various locations is summarized in Table 4 in Benedict (2004).

Timing of adult emergence is affected by the crop, host plant nutritional quality, and weather. Females start emerging from pupae 1 to 5 days earlier than males. Migrating males will have an opportunity to mate first with locally emerging females. Females will mate once each night for 3 to 5 consecutive nights, and males will mate repeatedly throughout their life. The adult life span in the field is estimated at 5 to 10 days or more depending on the temperature, humidity, food quality, and amount of time spent in flight. A single female may deposit from 200 to 2,000 eggs. Females typically choose the host plant that has the optimum density of flower buds/fruit for the larval stage to feed upon and to provide nectar for the adults. One study indicated the relative egg laying preference of CBW among key crops when they are all at optimum flowering states as: corn>tobacco>soybeans>cotton. The oviposition preferences correlate with difference in larval performance on different host species. In one study, larvae were found to require 12.5 days to develop corn ears, 16.4 days on soybeans, 17.3 days on alfalfa, and 18.3 days on cotton.

CBW has a variable number of instars ranging from 5 to 7, with 5 to 6 being the most common. CBW larvae can develop to the pupal stage in 12-17 days on cotton in midsummer when daily temperatures reach above 32°C, or in 25 days or longer in the spring or fall when the temperatures are cooler (Table 1 in Benedict, 2004). At 27°C, CBW larvae require 12.5 days to develop on corn ears, 14.7 days on cowpeas, 16.4 days on soybeans, 17.3 days on alfalfa, and 18.3 days on cotton.

Sequential use of hosts by CBW. Each cotton production area has different mixtures of crop and planting dates and non-crop hosts that CBW can utilize in succession during a growing season. The typical sequence begins when overwintering adults emerge and begin laying eggs on the available cultivate and non-cultivated hosts from March to June depending upon the geographic region (Table 4 and Appendix 1 in Benedict, 2004) including alfalfa, Carolina and wild geranium, crimson and Persian clover, hairy vetch, and corn seedlings. Many of the non-cultivated hosts are found in rights-of-ways, drainage ditches, along fences, and in and around cultivated fields. The larvae of the second and third generations usually occur from June through the middle of August in the cotton belt states. Corn is the preferred host at this time. CBW is also found on other crops, such as beans, cotton, peppers, sorghum, tomato, and forages, such as alfalfa, clovers, and vetches. Corn matures in late July and supports a small second generation in the whorl-stage and then a large third generation. The adults of this third generation must seek other hosts, such as cotton, peanuts, soybeans and other crop and non-crop hosts, to produce a fourth generation. The overwintering generation is thought to develop on wild hosts and cotton in the Mississippi Delta and on late planted or regrowth cotton, peanuts, soybeans, and tobacco, and on wild hosts (e.g., deergrass, beggarweed, morning glory, prickly sida) in the Carolinas.

Adult production by host plant species. Benedict (2004) indicates that there are possibly hundreds of studies that have determined the number of heliothine larvae produced throughout the season on various cultivated and uncultivated. However, there are few studies that have determined the numbers of adult heliothines produced per acre by any host plant species, and even fewer that have determined the number of adults produced per acre by all host plant species for a crop production system. The past larval and adult studies are

summarized by Benedict (2004). Results show that densities of heliothine larvae and adults vary with (1) production area, (2) host plant species, (3) heliothine species, (4) time of the season, (5) yearly patterns of weather, (6) agronomic practices, (7) insecticide use including *Bt* cottons, and (8) management of uncultivated hosts. Benedict concludes that at this time there aren't any studies that provide a clear "snapshot" of the sources, and proportion of moths produced by each source, for all the adult heliothines in any cotton production system.

Dispersal and migration. Benedict (2004) indicate that there are three types of heliothine movement: (1) short-range movements over distances of 300 to 3,000 feet that involve feeding, egg-laying, mating, and sheltering; (2) long-range movements above the host plant canopy up to 30 feet over distances of one to ten miles occur less frequently than short-range movement and involve movements, generally downwind, between different hosts, egg laying sites, and between emergence sites and egg laying/feeding areas; and (3) true long-distance wind-born migratory movements, less frequent than either short-range or long-range movements, occur downwind in high speed winds in the upper level air from 300 feet to more than a mile above the plant canopy, and occur over distance of 10 to 300 miles in a night. The proportion of adult heliothine population engaging in short, long-range, and true migratory movement is unknown.

Early spring migratory (true long-distance) movements of CBW northward correlated with weather patterns are very predictable. Some researchers believe the majority of CBW that infest U.S. crops (moving into the southeast and Delta states) are produced from migrants originating in the early spring in the cornfields of northern Mexico and south Texas. The carbon isotope data analyzed by Gould et al. (2002) differentiating CBW use by C_4 and C_3 hosts indicate CBW, over the growing season and 5-6 generations, can make a round-trip from Mexico and south Texas, to the cotton belt states and central U.S., then back in the fall. Marked-release-recapture studies show that CBW adults tend to disperse across the landscape, becoming more concentrated in the areas populated by preferred host plants in the most suitable growth stage for adult and larvae feeding and reproduction.

Genetic diversity and gene flow. Studies summarized by Benedict (2004) indicate that gene flow and interbreeding are high for CBW based on F_{ST} values (Table 8 in Benedict, 2004). F_{ST} values range for from 0.002 (extremely low) to 0.0072 for CBW and from 0.002 to 0.048 for TBW, a 10-fold difference between these two species. Heterozygosity among 23 enzyme loci was 5.5% in CBW populations in the U.S. indicating little genetic variation among CBW populations. Based on these data, individual CBW over large regions (perhaps most of the U.S. and Mexico east of the Rockies) contribute to a common gene pool making the population size very large. The data show that the current level of dispersal results in considerable interbreeding and mixing of genetic traits and maintains genetically homozygous populations over considerable regions of the U.S. and Mexico.

Monsanto conclusions from the literature review. Head and Voth (2004) concluded that CBW utilizes a very large number of crop and non-crop hosts in cotton growing states of the U.S., many in preference to cotton, based on the large body of published literature summarized in Benedict (2004). CBW is capable of extensive movement among hosts, even

those more than 20 miles apart. Alternative hosts should provide a large and effective unstructured refuge for Bollgard cotton in the U.S. They indicate that more detailed studies at a finer spatial scale than are available in the published literature are needed to confirm this conclusion. The studies that are described in subsequent sections carried out in five important cotton-growing states document which alternative crop hosts are available in each region and provide estimates of their relative productivity at different times.

2. Study design and methods

Overall study design and rationale. Monsanto (Head and Voth, 2004) describes it conservative approach to assessing the role of alternative hosts in the life history of CBW. These studies focus on four alternative crop hosts, field corn, peanut, sorghum, and soybeans, that are highly attractive to CBW and are grown on a substantial number of acres (based on USDA/NASS data from 2002) in the relevant cotton-growing states (see Table 2 in Head and Voth, 2004). For the 12 states listed in this table, the four alternative hosts represented at least 71% of the cotton acreage and in many cases, even more. Head and Voth (2004) indicate that the amount of “effective” refuge these hosts provide is very large even though it excludes other crop hosts and many non-crop hosts of CBW.

Study locations. Five states, Arkansas, Georgia, Louisiana, Mississippi, and North Carolina, were selected as representative of the five cotton-growing regions of the U.S. and cotton production practices (see Table 3 in Head and Voth, 2004). Several sampling sites (one in each of four adjacent counties) were selected in each state, except for Arkansas, where two sampling sites were set up within one of the counties.

CBW larval and adult sampling. A detailed description of the methods used for sampling adult and larval CBW are found in Appendix 1 of Head and Voth (2004). These methods, submitted to EPA, March 13, 2002, have been reviewed and accepted by EPA. A brief summary of the main study elements is described below.

CBW larval sampling. To determine which crop hosts supported larval CBW populations at any given time, surveys of larval abundance were conducted in the commercial crop fields adjacent to the crop interfaces throughout the course of the 2002 and 2003 growing seasons. Large CBW larvae (fourth and fifth instars) were counted on a weekly basis in the relevant cotton, corn, peanut, sorghum, and soybean fields. No statistical comparisons were made due to the irregular distribution of larvae, high variability, and missing values. Discussion of these data is found in Section 4.

CBW adult sampling. In both 2002 and 2003, 16-24 sets of adult pheromone traps were established in each of the five states (each location). Each trap set consisted on two to three traps. Each set was placed at a different crop interface that consisted of a Bollgard cotton field and an adjacent alternative host crop, including corn, soybean, sorghum (in Arkansas, Louisiana, Mississippi), peanuts (in Georgia and North Carolina), conventional cotton or Bollgard cotton (see Table 3 in Head and Voth, 2004). All of the crop fields were in

commercial production and crop interfaces were grouped into blocks and replicated four times in each state, except in Arkansas where crop interfaces were arranged in a randomized design. In total, there were over 100 trapping locations monitored on a weekly basis from May or June until September or October. For the number of moths caught in the pheromone traps, a separate ANOVA was run on the log-transformed data for each data in each state in each year. Tukey's HSD multiple comparison procedure was used to compare all treatment means for each state and week within 2002 and 2003. Discussion of these data is found in Section 5.

Crop source of CBW moths. To determine the crop source of the CBW adults caught in the pheromone traps in 2002 and 2003, the wings of representative samples of adults were analyzed using carbon isotope analysis. This method, described by Gould et al. (2002), distinguishes adults that feed on C₃ versus C₄ hosts. That is, moths which feed on C₃ hosts will have a different ratio of ¹³C to ¹²C than those that feed on C₄ based on the carbon assimilation physiology of each type of plants. Typical C₃ hosts are cotton, peanuts, clover, and soybeans; while, corn and sorghum are typical C₄ hosts. Using this method, CBW moths collected can be segregated into those that were from C₄ plants and those that were from C₃ plants. Wings from up to 100 adults from a given trapping location and date were pooled. The percentage of those moths that were from C₄ plants was calculated by comparing the value obtained from a standard curve generated from samples of known composition. A standard curve was generated each year. An ANOVA was run on the percentage of moths coming from C₄ plants and pair-wise comparisons were performed for each state and week within 2002 and 2003. Discussion of these data is found in Section 5.

Assessment of the spatial and temporal distribution of alternative hosts. Satellite imagery (was used to determine the relative acreage of each alternative hosts crop around the crop interfaces (i.e., traps) in 2002 and 2003. Images were done and cover types were analyzed at two scales: within 1 mile of the trap and within 10 miles of the trap. The land use categories used in analyzing the images were: cotton, corn, peanut, sorghum, soybean, rice, sugar cane, trees, unproductive (water, roads), and other (data not available because of clouds or the crop already had been harvested). The satellite imaging analysis was carried out by Southern Illinois University and field validation was done at each sampling location by local cooperators.

Data from the USDA/NASS (2002) database was also used for the same counties and CBW host crops, covering the period from 1995-2002. These data were compared qualitatively with the cropping patterns observed in the satellite images to determine how much the distribution of alternative hosts varied with the spatial scale and among years. Discussion of the satellite imaging and USDA/NASS analyses of alternative host distribution is found in Section 3 as well as Section 5.

3. Alternative host distribution in cotton growing states of the U.S.

Alternative host distribution as determined by satellite imagery. Table 1 summarizes the acreages of corn, peanut, sorghum, and soybean in the satellite images relative to the

acres of cotton for 2002 and 2003. State and county data are from the USDA/NASS (2002) database (same data presented in Table 2 and Table 4 in Head and Voth, 2004). Corn, peanuts, sorghum, and soybeans represent a combined acreage at least 71% of the cotton acreage at the state level in these five cotton-growing states. In the counties in which the traps were located, the combined acreage was at least 52% of the cotton acreages. Based on the satellite imaging, the proportion of alternative hosts was lower in the vicinity of the traps than at the state or county levels, particularly within 1 mile of the trap. Within 1 mile of the traps, alternative hosts represented an average of at least 24% of the cotton acres in 2002 and 59% in 2003. Within 10 miles of the traps, alternative hosts represented an average of at least 31% of the cotton acres in 2002 and 65% in 2003. In Georgia, estimates under represent the total alternative hosts acres because corn acres were not included due to the timing of the satellite photographs relative to harvesting of certain crops. In North Carolina, no satellite imaging data are available for 2002 because of cloud cover. At the 5th percentile level of the alternative host crop distribution for the 1-mile and 10-mile circles, shown in the final column of Table 1 (Table 4 in Head and Voth, 2004), alternative hosts represented at least 9% and 15% (48% if Georgia is excluded because its acres did not include corn) of the cotton acreage in 2002 and 33% and 48% in 2003, respectively. Head and Voth (2004) concluded that the “acreage of alternative hosts of CBW is substantial at all scales in all regions and years, including those with large cotton acreages.” They note that these estimates are conservative because only a limited number of alternative hosts crops are considered.

Alternative host distribution over time. Figures 1a-e (taken from Head and Voth, 2004) represent the state and county data for counties where sampling occurred for the years 1995 to 2002. These figures show that the acreages of cotton and the major alternative crop hosts of CBW are relatively stable over time and that prominent alternative host crops in each state have not changed in status from year to year. For example, the acreage of soybeans in Arkansas; soybeans and corn in Louisiana and Mississippi; soybeans, corn, and peanuts in North Carolina; and corn and peanuts in Georgia were consistently high over this time period. Head and Voth (2004) conclude that the state and county distribution of the prominent alternative hosts in 2002 and 2003 is consistent with the data collected from 1995 to 2002.

Monsanto’s conclusions from analyses of cropping patterns. Monsanto (Head and Voth, 2004) concludes that both the USDA/NASS (2002) data and satellite image analysis support the central findings of the literature review (described in Section 1, Benedict, 2004). While there are many alternative hosts, substantial areas of corn, peanuts, sorghum, and soybeans are present in all major cotton-growing areas at all spatial scales. Cropping patterns of these prominent alternative hosts are stable over time in each state or county. Head and Voth (2004) suggest that this means that these same cropping patterns will be expected in the future.

Head and Voth (2004) indicate that the data presented in Sections 4 and 5 will document that these alternative hosts are truly producing large numbers of CBW adults throughout the season and that these adults move considerable distances from where they emerge. Both of

these factors are important to IRM, the first to determine the size of the refuge (alternative hosts) and the second to determine the proximity of the refuge.

4. Larval CBW populations on alternative hosts at different times in the season.

Larval populations on alternative hosts over time. Tables 5 and 6 in Head and Voth (2004) give the larval distributions over time on cotton and the alternative host crops in each of the five states in 2002 and 2003, respectively. To make these data clearer, Head and Voth (2004) have indexed (index ranging from 0 to 3) the larval abundance on the various host crops to make the patterns clearer (see **Figures 2a-e and 3a-e**). These figures are based on the original data in Table 5 and 6 in Head and Voth (2004). An index of 1 corresponds to a larval density in the appropriate range of 20-200 per acre; a 2 corresponds to 200-2,000 per acre; and a 3 corresponds to >2,000 per acre and more often 10,000-30,000 larvae per acre.

In all five regions and in both years, corn supported the largest and earliest larval populations as was expected based on the literature. The earliest appearance of CBW larvae on corn was in Louisiana (June 3) in 2002 (Table 5 in Head and Voth, 2004) and in Arkansas (June 2) in 2003 (Table 6 in Head and Voth, 2004). CBW larvae remained high in corn from early June through the end of July in Arkansas, Georgia, Louisiana, and Mississippi and through the end of August in North Carolina. Populations routinely exceeded 5,000 (index of 3 in Figure 2a-e and 3a-e). Head and Voth (2004) indicate that the CBW populations were slightly lower in 2003 than in 2002. CBW larvae started appearing in other crops two to four weeks after they began to rise in corn. Populations were seen on the sorghum and cotton fields generally beginning mid-June or early July, and on soybean beginning in mid-July or August in Arkansas, Louisiana, and Mississippi. Sorghum peaks were comparable to corn in some cases, like Mississippi, but were of short duration, about two weeks. Cotton and soybean populations peaked at much lower levels than those in corn (Bollgard cotton had the lowest), one order of magnitude (index of 2) compared to corn (index of 3). In North Carolina, soybean populations peaked in late August and had comparable populations to corn (extended peak in late July until mid-August), both had indices of three. In the southeast, Georgia and North Carolina, larval populations on cotton started to increase in mid to late July, several weeks later than the Delta states, and this population coincided with population appearing on peanuts. These populations persisted for several weeks longer into late August and early to mid- September after populations had dropped in the Delta states.

The phenology and size of the alternative populations relative to cotton is particularly important to IRM. Head and Voth (2004) note that the larval populations in corn overlapped with the earliest larval populations in cotton and were much larger in size than the larval populations on cotton. Similarly, sorghum, peanut, and soybean larval populations overlapped with later CBW generations on cotton and were comparable in size to the populations on cotton.

Predicting adult production by alternative hosts over time. The larval surveys can be used to predict when the relevant crop fields produced CBW adults, as well as their level of

productivity, based on typical development times drawn from the literature (see review in Benedict, 2004). Two assumptions were used to generate qualitative and quantitative predictions concerning the dynamics and composition of the adult populations in different regions and at different times during the growing season: (1) time to adult emergence is approximately 14 days and (2) adult lifespan is approximately 7 days. Head and Voth (2004) extrapolate from the larval survey performed on the crop fields immediately adjacent to each trap to fields of the same crops in a larger area (i.e., within 1 mile or 10 miles of the trap sites). They infer that the adult emergence from these fields should be representative of fields of the same crops at some distance. The quantitative predictions are presented in Section 5 and compared with direct observations of the adult populations drawn from adult trapping. Qualitatively, Head and Voth (2004) predict that adult CBW populations should emerge on corn by mid-June or earlier in all states and adults should be predominant in all states until at least early August. Later season adult populations (mid-August and later) are predicted to be a mixture of adults from cotton, soybean, peanut (in the southeast) and sorghum (in some of the Delta states). The data presented in Section 2 demonstrate that sufficient acreages of the relevant host crops exist in these regions.

Monsanto conclusions from the larval population surveys. Monsanto (Head and Voth, 2004) concludes that “larval populations were high on the identified alternative host crops and generally at least equal to if not much higher than those on cotton. In addition, the phenology of these populations overlapped with the populations found on cotton.”

5. Adult CBW populations produced by different alternative hosts.

Number of adults found near different host crops. The numbers of CBW adults captured in pheromone traps at the interface of Bollgard cotton fields and selective alternative crop host fields in 2002 and 2003 are presented in Tables 7 and 8 in Head and Voth (2004). Comparable numbers of adult CBW were captured at all the different crop interfaces in a given state. No statistical differences were observed except in early season samples from Arkansas and a few sporadic cases. In 2002, there were 621 pairs of means and 21 pairs of means (3.4%) were found to be statistically significant at the 95% confidence level. In 2003, there were 878 pairs of means and 30 pairs of means (3.4%) were found to be statistically significant at the 95% confidence level. The comparable numbers captured at each crop interface suggest that the size of the adult CBW populations captured in the pheromone traps are not a function of the local host crops because the timing and the number of adults captured is greater than that predicted based on the adult emergence numbers from these host crop fields at any given time. Head and Voth (2004) conclude that the CBW adults must be moving broadly across the landscape in search of alternative host crops, corn in the early season and other crop hosts later in the season. This conclusion is consistent with the literature (see Benedict, 2004) that CBW adults are highly mobile and are capable of dispersing soon after emergence as adults and before mating, and will seek attractive hosts throughout the landscape. The relevant spatial scale for studying cropping patterns is 10-miles rather than 1-mile from the trap. Head and Voth (2004) use the carbon isotope analyses to more precisely determine the appropriate spatial scale of moth production.

Sources of adult moths caught in the pheromone traps, as determined by C_3/C_4 analysis. Tables 10 and 11 in Head and Voth (2004) give the percentage of CBW adults caught in pheromone traps in 2002 and 2003, respectively, that fed on C_4 alternative hosts at each crop interface in each of the five states. The values in these tables were produced by comparing the results of C_3/C_4 analyses of composite samples (pooled up to 100 samples from each trap on each date) with the calibration curves generated from moths of known composition (see Appendix 3 in Head and Voth, 2004). In 2002, three pair-wise treatment mean comparisons (<1%) out of a total of 604 total comparisons were statistically significant at the 95% confidence level. In 2003, five pair-wise treatment mean comparisons (<1%) out of a total of 6836 total comparisons were statistically significant at the 95% confidence level. Overall, these data confirm that adult moths disperse rapidly throughout the landscape after emerging because the composition of the moths collected at any given crop interface is not a function of that interface.

Figures 4 and 5 (taken from Head and Voth, 2004) show the percentage of moths derived from C_4 hosts for each state over time, averaged over all crop interfaces (there was no statistical difference in C_4 percent at any given crop interface). Across all five states, the same general pattern was observed. Early in the season, the CBW moths collected come from a mixture of C_3 and C_4 plants (20-80% from C_4 hosts). By late June, the CBW moths collected come nearly all from C_4 sources, and remain constant until early to mid August. At this point, the percentage of CBW moths coming from C_4 hosts declines to around 20-50% by late August. The percentage of moths from C_4 hosts then rose again in the final month of the season to a maximum of 50-80%. These data demonstrate that C_4 alternative hosts are making a significant contribution to the CBW adult population throughout the season. Depending on the state, at least 10-30% of the moths collected are derived from C_4 hosts. These quantitative data agree very well with the literature on CBW host use and the qualitative predictions made based on the larval populations. **Figure 6** graphically summarizes the sources of CBW adults at different times in the growing season based upon the C_3/C_4 analyses, field surveys of larval and adult populations, and published literature.

Comparing observed C_4 percentage with predicted values. **Figures 7a-e** compare the predicted and observed percentage of CBW moths coming from C_4 plants at different spatial scales. Separate prediction curves were generated at three different spatial scales: at the 1-mile scale using the corresponding satellite image data and the observed larval abundances (translated to adult emergence curves); at the 10-mile scale using the corresponding satellite image data and correcting the larval abundance curves for the greater variability in phenology expected at the larger scale; and at the county level using the USDA/NASS data from 2002 and the same emergence curves used at the 10-mile scale. Each of these prediction curves is graphically represented along with the observed curve for Arkansas, Louisiana, Mississippi, and North Carolina. In Georgia, only observed and county level curves were graphically represented because corn was not part of the satellite imaging analysis. All of the predicted curves underestimate the importance of the C_4 contribution. The fit is better for the 10-mile and county level predictions than the 1-mile scale because these scales build in greater variability. These analyses suggest that the scale of adult CBW movement is greater than 10 miles.

Head and Voth (2004) indicate that the early season moth production cannot be explained based upon the local production from corn, cotton, peanuts, sorghum, and soybeans. Based on the literature (see Benedict, 2004), substantial evidence exists for early season northward migrations of CBW from Mexico and southern Texas. Regardless of the precise source, the moths are coming from a combination of alternative host crops, most from weedy hosts, and none from cotton.

Head and Voth (2004) note that the sources of late season CBW moths are less clear than that of early season moths. They cite evidence from Gould et al. (2002) that suggest that moths were migrating southward from northern Corn Belt late in the year. Alternatively, they suggest that some amount of delayed emergence of adults from corn and/or sorghum fields may be occurring. In addition, weedy hosts may be playing some role in producing CBW adults late in the season as well as throughout the season.

6. Monsanto's overall conclusions from the two-year alternative host studies

Head and Voth (2004) conclude that this multi-state, two-year study conclusively demonstrates that the majority of adult CBW in all regions of the U.S. cotton belt are originating from both C₃ and C₄ alternative host plants. A significant portion of the CBW population is derived from alternative hosts throughout the season and in all regions. CBW adults are dispersing long distances in search of suitable oviposition and feeding sites. "Together, this means that the effective refuge for Bollgard cotton from the perspective of CBW is more than 50% for almost any Bollgard field, no matter where or when it is planted, and never is less than 10-20%, even without taking structured refuges into account. Thus, minimal selection for Cry1Ac resistance in CBW will be occurring through the use of Bollgard cotton."

EPA REVIEW

Purpose of the alternative host data

As a condition of this registration, EPA required that Monsanto Company conduct CBW alternative host research studies that would provide data that might support these hosts as natural refugia and provide a final report of these data to EPA by March 15, 2004. EPA stated in the terms and conditions of registration (described in EPA's 2001 *Bt (Bacillus thuringiensis)* Plant-Incorporated Protectants Biopesticides Registration Action Document (BRAD) (EPA, 2001)) research topics must include, but are not limited to the following: "mating and oviposition behavior of *Helicoverpa zea*, fitness of adults and adult population densities coming from the alternative hosts vs. unsprayed and sprayed *Bt* cotton, whether insect pest emergence synchrony with pests emerging from *Bt* cotton, the proximity of alternative hosts to *Bt* cotton, and refinement or construction of new resistance management models that include alternative hosts appropriate for different cotton production regions, e.g., North Carolina vs. Louisiana. Studies must be conducted across the cotton belt where cotton bollworm is an economic pest. The sites must represent a range of conditions that will affect cotton bollworm biology. Conditions must include such factors as irrigation, soil types, and

climatic conditions.” Monsanto Company (Head and Voth, 2004) submitted to EPA on March 13, 2004, the alternative host data collected over the 2002 and 2003 cotton growing season in support of the continuation of the 5% external, unsprayed structured refuge option for both Bollgard and Bollgard II cotton products. The final report of the CBW alternative host studies was divided into five sections: 1) literature review, 2) protocols, 3) cropping pattern analysis, 4) larval productivity on alternative hosts relative to cotton, and 5) adult productivity and carbon isotope analysis. These studies were conducted in five cotton-growing states: Georgia, Louisiana, North Carolina, Arkansas, and Mississippi representative of different cotton production systems in which CBW is a major economic pest. The EPA review that follows evaluates whether Monsanto Company has provided sufficient data to demonstrate that CBW alternative hosts provide a large and effective unstructured refuge for Bollgard and Bollgard II cotton in the U.S to allow continuation of the 5% external, unsprayed structured refuge option.

Literature review

Benedict (2004) is a comprehensive literature review of the major aspects of the biology and ecology of CBW and *Heliothis viresens* (tobacco budworm, TBW). This literature review summarizes the information on CBW hosts, sequential host utilization, larval and adult productivity on each of these hosts, movement and dispersal properties, and gene flow. There are over 130 crop and non-crop hosts of CBW. CBW has the ability to move amongst hosts that are more than 10 miles apart. The basis for the alternative host studies was to prove whether these alternative hosts provide a large and effective unstructured refuge for Bollgard and Bollgard II cotton in the U.S. Each cotton production area has different mixtures of crop and planting dates and non-crop hosts that CBW can utilize in succession during a growing season. Based on the review of the literature, peanuts, corn, sorghum, and soybeans were chosen as alternative crop hosts for CBW. Head and Voth (2004) were able to compare the observed and predicted larval and adult productivity on each of these hosts (C_3 and C_4 alternative hosts more generally) to the literature. Benedict (2004) is an update of an earlier white paper on the biology of TBW and CBW (Caprio and Benedict, 1996) that was reviewed by EPA in a 1998 White Paper (EPA, 1998).

“Effective refuge” size

EPA disagrees with Monsanto’s definition of “effective refuge” size as described in Section 2 of Head and Voth (2004) and in Gustafson et al. (2004). That is, “effective refuge” size is not calculated by simply adding up the total acres per county represented by the four alternative crop hosts (corn, sorghum, peanuts, and soybeans) as a percent of cotton acres, at least 71% according to Head and Voth (2004). “Effective” refuge size may be calculated in a semi-quantitative fashion, as Head and Voth (2004) have done in Section 5 of the study, by examining the observed and predicted percentage of adults derived from C_3 and C_4 hosts in the five states across the landscape (see Figures 7a-e). These values are based upon the C_3 / C_4 analysis (see 5. above), field surveys of larval and adult populations (see 4. and 5. above), satellite imaging and USDA/NASS data base analysis of cropping patterns (see 3. above),

and the published literature (see 1. above). “Effective refuge” size is a weighted average of the proportion of moths coming from each alternative host for each CBW generation (5 to 6 generations) in each cotton production system (geography).

Cropping patterns

Each cotton production area has different mixtures of crop and planting dates and non-crop hosts that CBW can utilize in succession during a growing season. Head and Voth (2004) used both satellite imaging analysis at the 1-mile and 10-mile radius around each trapping location and USDA/NASS (2002) data to analyze the cropping patterns. This analysis indicated that corn, peanuts, sorghum, and soybeans are present over substantial areas in all of the major cotton growing areas at all spatial scales examined although there was variability. The acreages represented by each of these alternative hosts in the counties where sampling occurred remained relatively constant from 1995 to 2002 based on USDA/NASS data. The alternative host studies done in 2002 and 2003 are representative of the cropping patterns in other years in the five representative cotton-growing states.

Larval productivity

Larval productivity was measured over time on cotton and other alternative hosts in five states in 2002 and 2003, respectively. In all five regions and in both years, corn supported the largest and earliest larval populations as was expected based on the literature (Benedict, 2004). CBW larvae remained high in corn from early June through the end of July in Arkansas, Georgia, Louisiana, and Mississippi and through the end of August in North Carolina. CBW larvae started appearing in other crops two to four weeks after they began to rise in corn. Populations were seen on sorghum and cotton in mid-June or early July, and on soybeans beginning in mid-July or early August in Arkansas, Louisiana, and Mississippi and extended until late August. Sorghum peaks were comparable to corn peaks, but were shorter in duration, about two weeks. Cotton and soybean populations peaked at much lower levels than those in corn, about one order of magnitude lower. Bollgard cotton had the lowest CBW populations as one would expect. Larval populations on cotton in Georgia and North Carolina started to increase in mid to later July, several weeks later than the Delta states, coincided with populations appearing in peanuts, and persisted until early September. There were large CBW populations in soybeans late in the season. In North Carolina, the CBW production was generally synchronous across all host crops sampled and represented one generation. Here, there were CBW larvae produced in similar numbers on corn, peanut, and soybean compared to non-Bollgard cotton, but production in all crops was synchronous. From an IRM perspective, larval populations in corn overlapped (were in synchrony) with the earliest larval populations in cotton and peanut, sorghum, and soybean populations overlapped with later CBW generations on cotton. Larval production in the alternative hosts was similar or greater than peak production in cotton (Bollgard or non-Bollgard cotton).

Data from the larval productivity studies were used to qualitatively estimate the adult emergence from the relevant crop fields as well as their relative level of productivity based on the typical development times (Benedict, 2004). Head and Voth (2004) assume that the

larval populations will develop into adults in approximately 14 days and adults will have an average life span of 7 days. This translates into seeing adults in corn by mid-June (or earlier) and these adults should dominate the landscape until early August. The later season adult populations are predicted to dominate in mid-August on sorghum, cotton, soybeans, peanuts, and soybeans (depending on the region). Head and Voth (2004) compare these predicted adult emergence times with the observed adult productivity on C_3 and C_4 alternative hosts to semi-quantitatively determine which alternative hosts are producing CBW moths in what amount during the cotton-growing season (see discussion in “*Adult productivity and origin of moths*” below).

In predicting adult productivity based on the larval productivity numbers, Head and Voth (2004) assume that the proportion of adults emerging from various crops is equivalent to the larval density on the respective crops. However, as Gould et al. (2002) note that is not always possible to accurately predict adult production based only on larval production because development is impacted by predation, parasitism, differential suitability of soils for pupal development, and weather. In 2002, Peters et al. (2004) showed that in larval productivity studies conducted in Louisiana, mean larval survival to adult emergence was 64, 71, 74, 79, and 87% on field corn, grain sorghum, MG 6 soybean, non-*Bt* cotton, and Bollgard cotton, respectively. In 2003, mean larval survival to adult emergence was 66, 58, 100, 82, and 100% on field corn, grain sorghum, MG 6 soybean, non-*Bt* cotton, and Bollgard cotton, respectively. Larval progression peaks were in the order of corn > sorghum > cotton > MG 6 soybean. Temporal occurrence of Bollgard and non-Bollgard cotton was similar in both years. Peters et al. (2004) conclude that the only crop in Louisiana that exhibited similar temporal synchrony with CBW larvae in Bollgard cotton was MG 6 soybean. Data summarized in Peter et al. (2004) in Louisiana show the variability CBW adult production on various hosts from year to year and the difficulty one might have in predicting adult production based on larval productivity numbers. Because of the difficulty in predicting adult productivity based on larval productivity numbers, a separate set of field studies was conducted to assess adult productivity. Adult productivity was determined by a combination of pheromone trapping data and carbon isotope analysis to partition moths into those that came from C_3 and C_4 alternative hosts (Head and Voth, 2004). These studies are reviewed below.

Adult productivity and origin of moths

According to the literature (Benedict, 2004), there weren't any studies that provided a clear “snapshot” of the sources, and proportion of moths produced by each source, for all the adult heliothines in any cotton production system. Head and Voth (2004) summarize the adult productivity data (pheromone trapping) and the C_3/C_4 carbon isotope data collected in 2002 and 2003. These authors compare the observed adult productivity on C_3 and C_4 alternative hosts to the predicted adult emergence patterns based on the larval productivity studies to semi-quantitatively determine which alternative hosts are producing CBW moths in what amount during the cotton-growing season (synchrony).

Pheromone traps collected moths at the interfaces of Bollgard cotton:corn, Bollgard

cotton:soybean, Bollgard cotton:peanut, Bollgard cotton:sorghum, Bollgard cotton: cotton, and Bollgard cotton: Bollgard cotton fields. Fields containing peanuts were only used in Georgia and North Carolina. Fields containing sorghum were only used in Arkansas, Louisiana, and Mississippi. In total, there were 100 trapping locations across the five states. Results showed that comparable numbers were captured at each crop interface in all five states with the exception of a few early season samples from Arkansas and a few other sporadic cases (i.e., no statistical difference). These data indicate that the size of the adult CBW populations captured in the pheromone traps is not a function of the local host crops because the timing and the number of adults captured is greater than that predicted based on the adult emergence numbers from these host crop fields at any given time (using the larval productivity analyses). This means that the CBW adults must be moving broadly across the landscape in search of alternative host crops, corn in the early season and other crop hosts later in the season. This conclusion is the same as that of Head and Voth (2004) and is consistent with the literature (see Benedict, 2004). CBW adults are highly mobile and are capable of dispersing soon after emergence as adults and before mating, and will seek attractive hosts throughout the landscape.

Head and Voth (2004) use the carbon isotope analyses to provide a semi-quantitative determination of the appropriate spatial scale and source of moth production.. Approximately 100 moth wings from each trapping location were pooled for C_3/C_4 carbon analysis. The percentage of those moths that were from C_4 alternative hosts was calculated by comparing the value obtained to a standard curve generated from samples of known composition. Early in the cotton-growing season, the CBW moths collected were from a mixture of C_3 (non-cotton) and C_4 alternative hosts, anywhere from 20 to 80% are coming from C_4 alternative hosts. During the period of late June to early or mid August, the percentage of moths coming from C_4 alternative hosts (predominantly corn) rises to nearly 100%. Following this period, the percentage of CBW moths coming from C_4 alternative hosts declines to 20-50% by late August to early September because of the rise of adult emerging from C_3 host like cotton, soybean, and peanut. At that point, the percentage of moths coming from C_4 alternative hosts increases to 50-80% by the end of the growing season. These same trends were seen in all five states. On a qualitative level, these data agree well with the predicted adult emergence patterns on each alternative host based on the larval productivity studies and the literature on CBW sequential host use (see Benedict, 2004). Based on the C_3/C_4 analysis, the total adult production from C_4 alternative hosts of CBW (averaged across all five states) is at least 20%-30% and peaks in early July through the end of August at 80-100%.

While one can determine the percent of C_3 and C_4 alternative hosts contributing CBW moths at different times in the growing season, the exact source and proximity of these contributing alternative hosts cannot be determined from the data provided in Head and Voth (2004). That is, the C_3/C_4 carbon isotope analysis can only partition the CBW population into those that came from C_3 and C_4 alternative hosts, but cannot distinguish which C_3 alternative host (soybeans, clover, or peanuts etc.) or which C_4 alternative host (corn/maize, sorghum etc.). These data show the CBW moths are being produced by both C_3 and C_4 alternative hosts across the landscape. Head and Voth (2004) overlaid the observed and predicted (based on

the larval productivity data) percentage of CBW moths coming from C_4 alternative hosts at various spatial scales (1-mile and 10-mile scales using the satellite imaging data and the county level using the NASS data from 2002) for each of the five states. They conclude that the scale of adult CBW movement must be greater than 10-miles. EPA agrees with this conclusion. This means that a proportion of the CBW adult population captured into the pheromone traps must not be produced locally. However, one cannot determine the proportion of moths produced locally from those that are produced at greater distances based on these studies. One refinement to the C_3/C_4 analyses would be to do the analyses on wings from single moths rather than pooled moths to more precisely determine the relative proportion of moths coming from C_3 or C_4 alternative hosts at in each trap at each trapping location.

Predictions based only upon local production from corn, cotton, peanuts, sorghum, and soybeans cannot explain where the early season moths come from, nor, why the percentage of C_4 alternative hosts rises again at the end of the season. Based on the literature (reviewed by Benedict, 2004), CBW moths move northward from southern Texas and Mexico early in the Spring. These moths may be produced on corn, on vegetables, and on wild hosts.

The source of late season CBW adults may be southern migrants coming from the Midwest corn belt (Gould et al. 2002). Gould et al. (2002) used stable carbon isotope analysis to assess alternative host use by CBW. They found that non-*Bt* C_4 plants, probably corn, in Mexico and the U.S. Corn Belt appear to serve as important alternative hosts (non-structured refuge) for CBW. Late-season CEW moths captured in Louisiana and Texas maybe migrants whose larvae developed on corn in more northern locations. These authors provide indirect evidence (they did not establish the origin of these moths, i.e., which C_4 or C_3 plants and the distances they are migrating) for migration of CBW from corn-growing areas in the northern U.S. to cotton-growing area in the southern U.S. The authors conclude that when C_4 plants are suitable (e.g., corn), less than 10% of CBW moths are developing on cotton, and that later in the season, 50% of the moths are produced in cotton and soybean. Corn is most likely serving as the predominant C_4 host for CBW. Southern corn is probably an important C_4 host for early season; while, the Corn-Belt corn serves as an important C_4 host for mid-season. The findings of Head and Voth (2004) are the same as those of Gould et al. (2002).

Modeling

Monsanto Company modified Caprio's (1998a) two-patch, deterministic, non-random, population genetics model to create a new model, Gustafson et al. (2004; original report dated September 10, 2001 submitted as a public comment to EPA Docket OPP-00678B) that included insecticidal oversprays of Bollgard cotton fields and the utilization of alternate hosts as natural refugia as parameters. The Gustafson et al. (2004) model is reviewed separately. Gustafson et al. (2004) showed how the model output (i.e., years to resistance) was sensitive to both of these parameters. The model predicted that alternative hosts as "effective" refuge would delay the evolution of CBW resistance to the Cry1Ac toxin expressed in Bollgard cotton. Monsanto (Lahman, 2004) states that the data in Head and Voth (2004) demonstrate the contribution of alternative hosts is at the levels assumed in the model presented in Gustafson et al. (2004). This means that the alternative host data support

the conclusion in Gustafson et al. (2004) that alternative hosts are “effective” refuge and will delay the evolution of CBW resistance to the Cry1Ac toxin expressed in Bollgard cotton. CBW resistance will be delayed even further to the two-toxins (Cry1Ac and Cry2Ab2) expressed in Bollgard II cotton. As Lahman (2004) notes, Bollgard II provides significantly higher levels of control of CBW than Bollgard cotton.

EPA stated in the terms and conditions of registration (see EPA, 2001) that the alternative host data collected in 2002 and 2003 should be used to refine or construct new resistance management models that include alternative hosts appropriate for different cotton production regions. The Gustafson et al. (2004) model or other appropriate resistance management model was not refined with the alternative host data. As noted earlier, EPA disagrees with Monsanto’s method of calculating “effective” refuge in both Gustafson et al. (2004) and in Head and Voth (2004). “Effective refuge” size is a weighted average of the proportion of moths coming from each alternative host for each CBW generation (5 to 6 generations) in each cotton production system (geography). The Gustafson et al. (2004) model is limited in its predictive ability because it cannot segregate the relative contribution of each alternative host on the evolution of CBW resistance by generation. The Gustafson et al. (2004) model has two large patches: 1) Bollgard and 2) alternative hosts (i.e., all alternative hosts *en masse*, no generation segregation). It assumes that all alternative hosts are equally effective and that they all produce moths in complete synchrony with cotton (Bollgard or non-Bollgard) and that it is generation independent. This is not a “stepping stone” model, there is no sequential host utilization by generation. While there is synchrony of CBW moth production in alternative hosts and cotton; this synchrony is not perfect as shown by the data in Head and Voth (2004). While CBW is polyphagous and disperses over great distances to feed on attractive hosts (i.e., it feeds on different hosts sequentially so that different generations will feed on different hosts) as they become attractive over the landscape (see literature review, Benedict, 2004). There is also a huge differential in moth production on these alternative hosts as seen in the data summarized by Head and Voth (2004) and in the literature review (Benedict, 2004). There is no spatial or temporal dynamic in this model. Despite the limitations of the Gustafson et al. (2004) model, one can conclude that, on a very qualitative level, the alternative hosts data support the predictions of this model. That is, inclusion of alternative hosts as unstructured refugia will delay CBW resistance longer than without the inclusion of alternative hosts and only if the 5% external, unsprayed structured refuge was considered.

Because of the limitations of the Gustafson et al. (2004) model, it cannot appropriately consider the spatial and temporal dynamics of CBW utilization of alternative hosts by generation. Monsanto should refine an appropriate CBW resistance management model with the parameters values obtained from these alternative host studies so that both the spatial and temporal dynamics of CBW utilization of alternative hosts by generation can be considered. Each cotton production system (geography) should be modeled, e.g., North Carolina, The Delta, Georgia. Two examples of CBW models that include both spatial and temporal dynamics of CBW by generation are Caprio (1998b) and Storer (2003).

Relevance of CBW alternative host data to other pests

Results from these CBW alternative host studies have no bearing on *Bt* resistance management strategies for *Heliothis virescens* (tobacco budworm) and *Pectinophora gossypiella* (pink bollworm) to the *Bt* proteins expressed in Bollgard (Cry1Ac) and Bollgard II (Cry1Ac and Cry2Ab) cotton.

Summary

Based on these two-year, five state studies, both C₃ and C₄ alternative hosts of CBW serve as unstructured refugia that is much greater than the local 5% external, unsprayed structured non-*Bt* cotton refuge. Data from the carbon isotope analysis indicates that C₄ alternative hosts contribute 20-95% of the CBW moths in and around cotton fields (both Bollgard and non-Bollgard) throughout the season. Corn is the greatest producer of CBW adults in the early season; while, sorghum, peanuts, soybeans, and cotton are producers of CBW adults in later generations. These data are important because Bollgard does not produce a high dose of toxin for CBW and a very large refuge must be maintained to reach the 500:1 ratio of susceptible moths to resistant moths (see EPA, 2001; Matten and Reynolds, 2003). Based on the alternative host data presented in Head and Voth (2004), CBW moths are produced on C₃ and C₄ alternative hosts (unstructured refugia) throughout the landscape (spatial scale is greater than 10-miles) in sufficient numbers throughout the cotton growing season to mate with any putative resistant CBW moths emerging in Bollgard or Bollgard II cotton fields and dilute resistance. At the 10-mile spatial scale surrounding the trapping sites, alternative hosts (corn, soybean, peanut, and sorghum), represented at least 9% of the cotton acreage in 2002 and 48% of the cotton acreage in 2003. Susceptible CBW moths from alternative hosts will reduce the intensity of Cry1Ac and Cry2Ab2 resistance selection in CBW and lower the likelihood of resistance evolution. Therefore, the results of the alternative host studies support the continuation of the 5% external, unsprayed structured non-*Bt* (*Bacillus thuringiensis*) cotton refuge.

Despite the limitations of the Gustafson et al. (2004) model, one can conclude, on a very qualitative level, that the alternative hosts data support the predictions of this model. That is, inclusion of alternative hosts as unstructured refuge will delay CBW resistance longer than without the inclusion of alternative hosts and only if the 5% external, unsprayed structured refuge was considered.

CLASSIFICATION: The alternative host studies are “acceptable.” However, additional CBW resistance modeling work is recommended. Monsanto should refine an appropriate CBW resistance management model with the parameters values obtained from these alternative host studies so that both the spatial and temporal dynamics of CBW utilization of alternative hosts by generation can be considered. Each cotton production system (geography) should be modeled, e.g., North Carolina, The Delta, Georgia. The Gustafson et al. (2004) model is limited and cannot appropriately consider the spatial and temporal dynamics of CBW utilization of alternative hosts by generation.

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Table 1. Acreages of cotton, and relative acreages of other important CBW host crops, at various spatial scales in five states. (Reprinted Table 4 from Head and Voth, 2004, p. 11)

Table 4. Acreages of cotton, and relative acreages of other important CBW host crops, at various spatial scales in five states. State and county data are from NASS (2002). Local data within 10 miles and 1 mile of trapping sites are from satellite images taken in 2002 and 2003, and are averages for all of the images (trap sites) within that state in that year.								
State	Year	Scale ^a	Cotton ^b (Acres)	Corn (%)	Peanut (%)	Sorghum (%)	Soybean (%)	Total ^c (%)
Arkansas	2002	State	920,000	28	0	25	313	366
	2002	County	43,100	17	0	17	168	202
	2002	10 mi	51,800	2	0	4	59	65 (53, 82)
	2002	1 mi	945	5	0	5	22	32 (9, 165)
	2003	10 mi	38044.8	36	0	32	74	142 (119, 180)
2003	1 mi	677.2	23	0	15	44	82 (36, 161)	
Georgia	2002	State	1,360,000	21	37	2	10	71
	2002	County	38,500	12	36	0	4	52
	2002	10 mi	23,800	-	29	0	2	31 (15, 45)*
	2002	1 mi	455	-	19	0	5	24 (10, 45)*
	2003	10 mi	27011.4	19	44	0	2	65 (48, 93)
2003	1 mi	472.6	19	35	0	5	59 (33, 113)	
Louisiana	2002	State	495,000	113	0	33	133	280
	2002	County	46,840	89	0	19	53	161
	2002	10 mi	31,000	98	0	7	75	180 (95, 321)
	2002	1 mi	553	61	0	13	45	119 (24, 268)
	2003	10 mi	28319.6	64	0	12	39	115 (81, 194)
2003	1 mi	596.3	53	0	18	32	103 (35, 642)	
Mississippi	2002	State	1,150,000	46	0	7	119	172
	2002	County	69,375	48	0	13	138	199
	2002	10 mi	55,000	42	0	1	60	103 (74, 126)
	2002	1 mi	778	30	0	4	41	75 (29, 178)
	2003	10 mi	46053.3	40	0	0	70	110 (83, 156)
2003	1 mi	740.9	37	0	4	32	73 (33, 221)	
North Carolina^d	2002	State	920,000	86	11	1	140	238
	2002	County	39,400	28	21	2	43	94
	2002	10 mi	38,600	34	13	2	35	84 (48, 253)
	2002	1 mi	637	25	12	1	27	65 (19, 251)

^a State = total for the state in 2002; county = average of the three or four counties where studies were conducted in 2002; 10 mile and 1 mile = average area of that crop within 10 miles or 1 mile of the traps in that location and year.

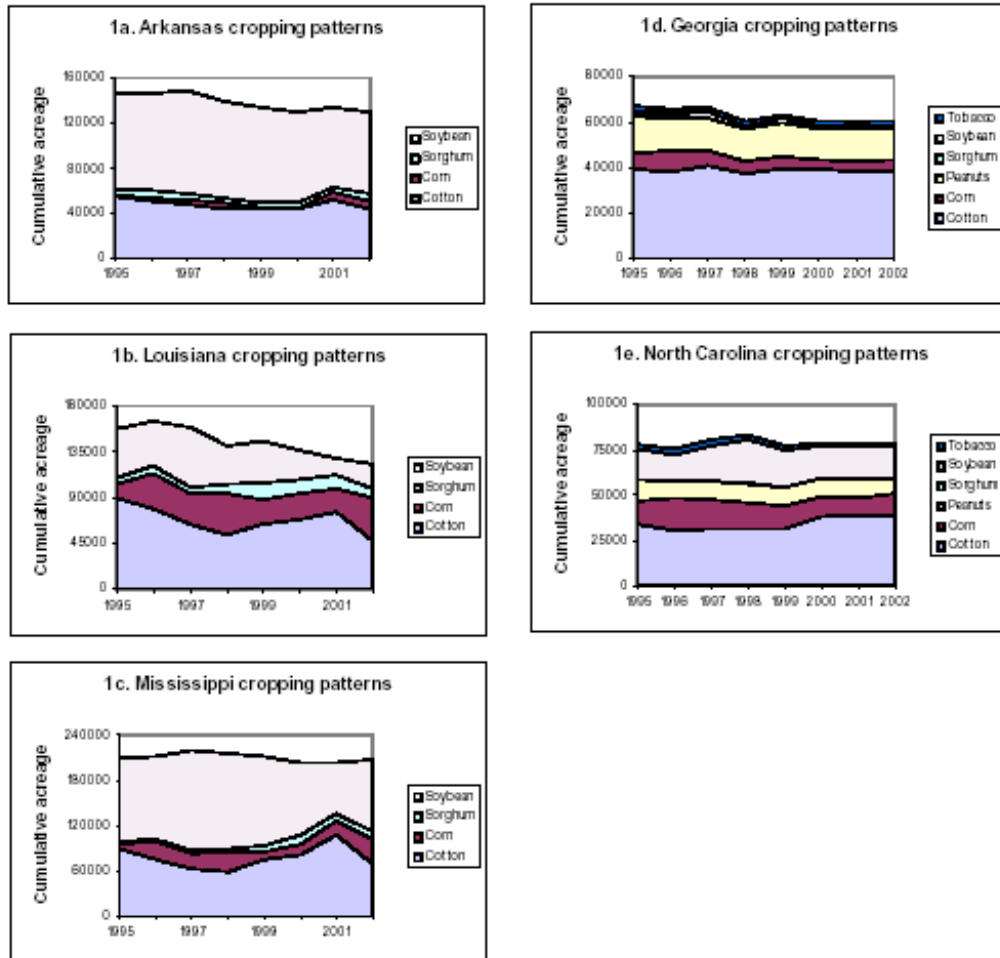
^b Cotton value is in acres; acreages for crops other than cotton are expressed as a percentage of the cotton acres.

^c Total of the four alternative host crops. At the 10-mile and 1-mile scales, the values in parentheses are the 5th and 95th percentiles calculated from the photographs for all of the traps in that state in the relevant year.

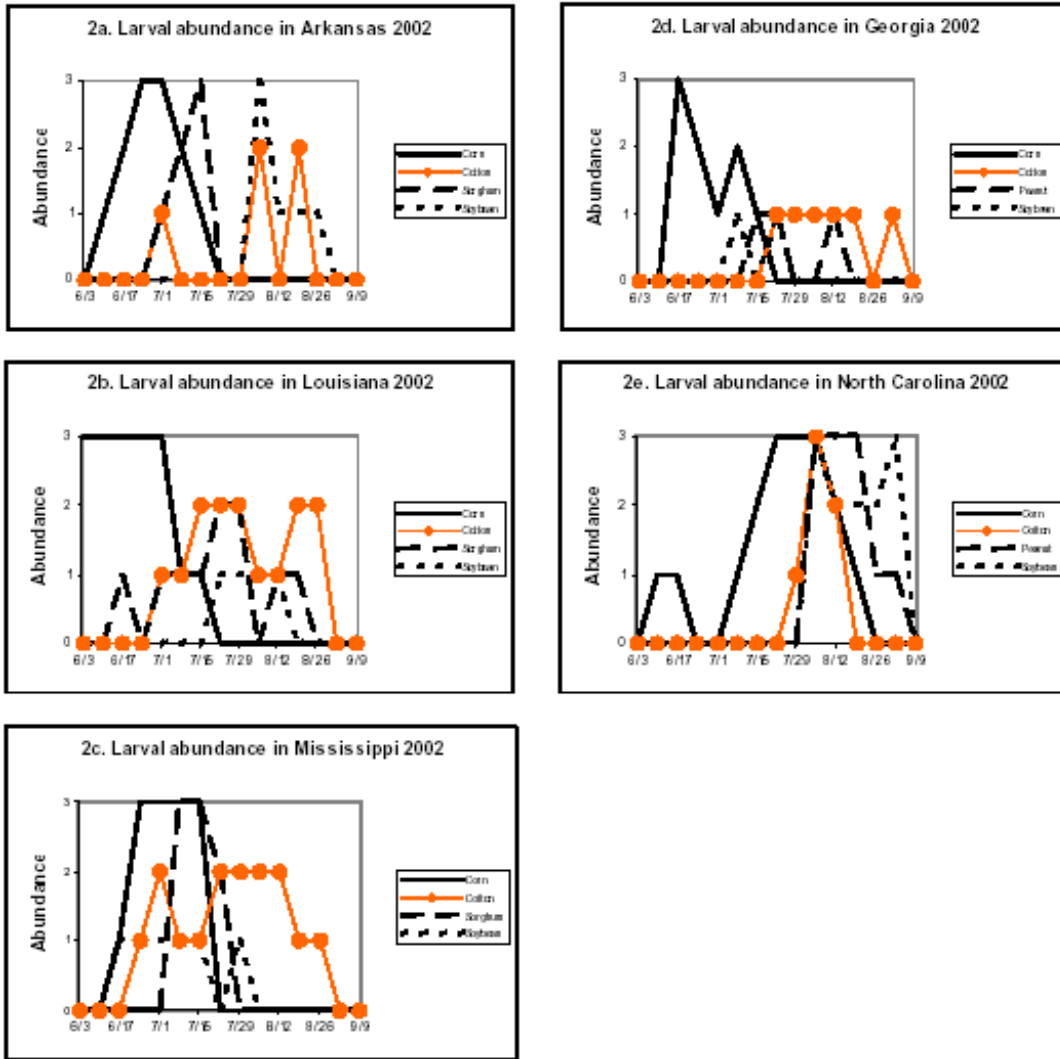
^d Satellite data was not available in North Carolina in 2003 due to persistent cloud cover. The cooperator estimates that there were not significant changes in acreage from 2002.

* Corn acreages around the trap sites could not be calculated in Georgia in 2002 because crops were harvested before aerial photographs were taken. Thus totals for alternative host acres are underestimates in this case.

Figures 1a-e. Acreages of major alternative crop hosts of CBW in five states between 1995 and 2002 (from USDA/NASS, 2002). (Reprinted from Head and Voth, 2004, p. 12)



Figures 2a-e. Larval abundance by crop in five states in 2002. Index: 1 = 20-200 larvae/acre; 2 = 200-2000 larvae/acre; 3 = >2000 larvae/acre (and generally >10,000). (Reprinted from Head and Voth, 2004, p. 16)



Figures 3a-e. Larval abundance by crop in five states in 2003. Index: 1 = 20-200 larvae/acre; 2 = 200-2000 larvae/acre; 3 = >2000 larvae/acre (and generally >10,000). (Reprinted from Head and Voth, 2004, p. 17)

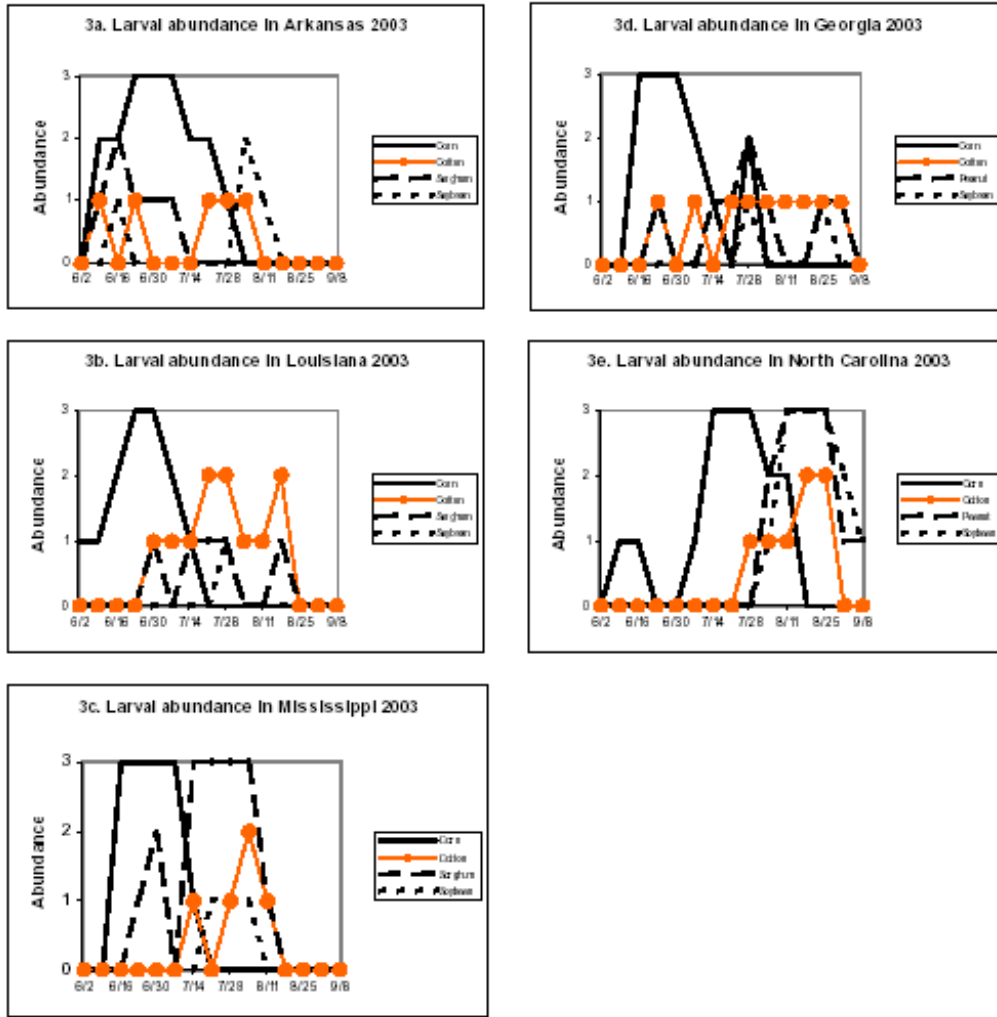


Figure 4. Percentage of C₄ Adults by State in 2002. (Reprinted from Head and Voth, 2004, p. 24)

Figure 5. Percentage of C₄ Adults by State in 2003. (Reprinted from Head and Voth, 2004, p. 24)

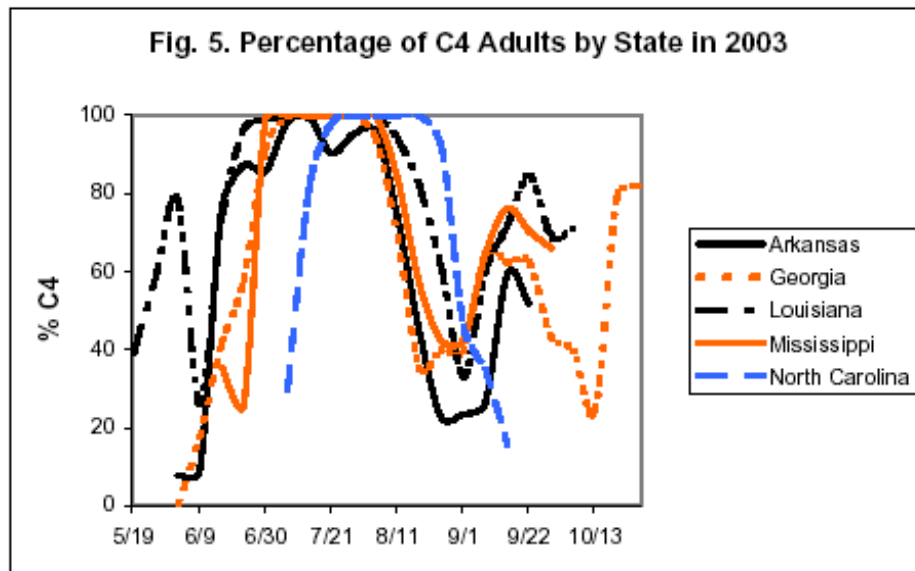
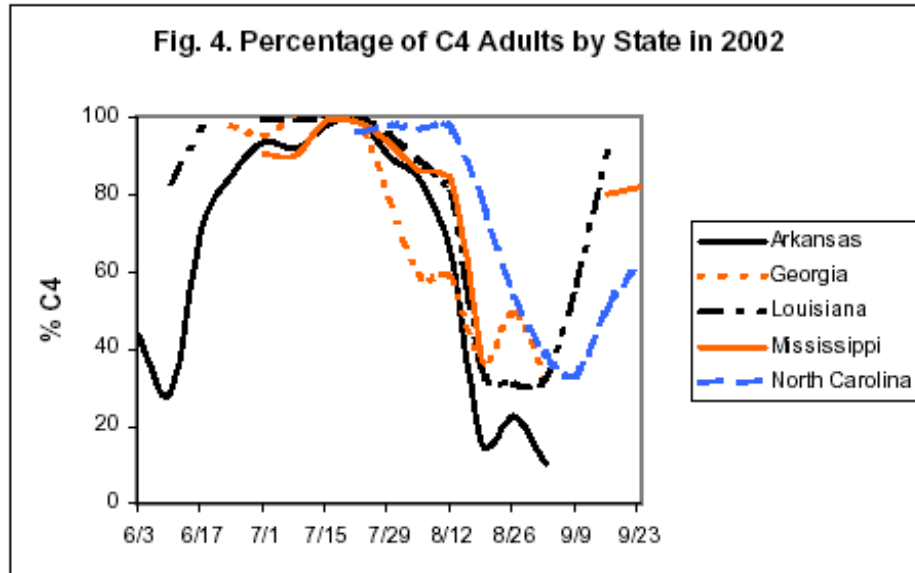


Figure 6. Sources of CBW adults at different times in the growing season based upon C_3/C_4 analyses, field surveys of larval and adult populations, and published literature. The curve is derived from the 2003 data in Figure 5, but averaged across the states. (Reprinted from Head and Voth, 2004, p. 25)

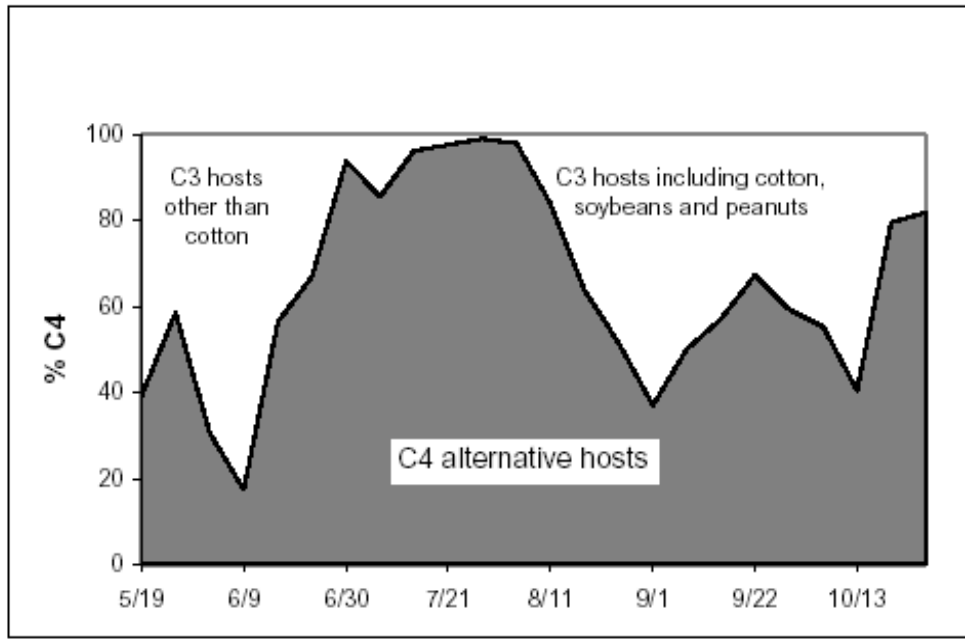
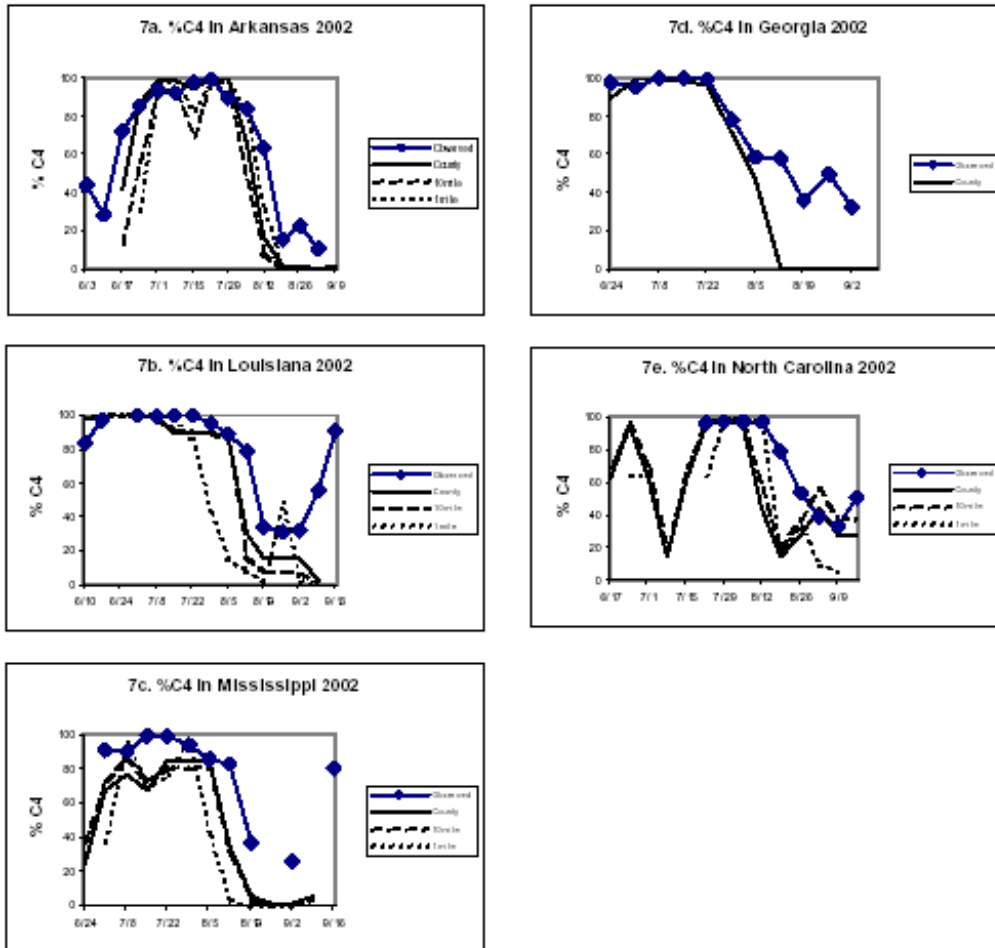


Figure 7a-e. Observed and predicted percentage of CBW moths coming from C₄-type crop hosts in five states in 2002. (Reprinted from Head and Voth, 2004, p. 26)



APPENDIX 1. 2002 Alternate Host Studies – An Interim Report and Review

Summary of 2002 Alternate Host Studies

Landscape level monitoring. Approximately 100 sampling locations were established in commercial crop production fields in North Carolina, Georgia, Mississippi, Louisiana, and Arkansas. Each sampling location consisted of a Bollgard cotton field and an adjacent alternate host crop (corn, soybean, sorghum (mid-South locations), peanuts (Southeast locations), non-Bollgard and Bollgard cotton with an appropriate refuge. The sprayed refuge option was used instead of the unsprayed refuge option in some sampling locations. The final protocol called for the unsprayed refuge option to be used since this is the refuge option that is considered to be weakest. Preliminary results (summarized below) indicate that there is broad scale movement of moths in the landscape indicating the refuge type is immaterial to the conclusions of this work. All commercial crop fields were managed according to standard agronomic practices. Large (L4-L5) cotton bollworm larvae were quantified weekly from the Bollgard cotton field and paired alternate host field at each location starting when the cotton bollworm larvae traditionally start to cause significant feeding damage in the cotton crop and continuing until larval productivity ended in cotton. Adult cotton bollworm moths in the landscape were determined by placing pheromone traps at the Bollgard cotton-alternate host crop field interface at each sampling location. This approach allowed cotton bollworm larvae production to be correlated with subsequent adult moth production in the landscape. To further determine the crop source for cotton bollworm adult moths, representative samples of adult moths were collected from the pheromone traps and the wings were analyzed for the ratio of ^{12}C to ^{13}C to determine whether the moths originated from plants with C_3 (e.g., cotton, soybean, peanuts) or C_4 (e.g., corn, sorghum) type carbon assimilation physiology. In addition, satellite imaging was used to determine the relative proportion of each alternate host crop around each sampling location.

Larval productivity. The average seasonal cotton bollworm larval productivity for the crops sampled in order of greatest larval production to least larval production was: corn >> soybeans > non-Bollgard cotton >> Bollgard cotton. Corn produced between 5,000 to greater than 30,000 larvae per acre during the reproductive stages of corn growth. In the mid-South, larval production on sorghum was almost equal to larval production in corn; however, there was considerable variability, with some location producing greater than 100,000 cotton bollworm larvae per acre while other locations produced no larvae, even between sampling sites within the same state. In the Southeast region, peanuts produced larvae in intermediate numbers between that of corn and soybean. Larval productivity from soybeans was relatively high in North Carolina and Louisiana, but was lower in other states. In Arkansas, late-planted soybean had greater larval productivity than early-planted soybean and non-Bollgard cotton

In Georgia, Mississippi, Louisiana, and Arkansas, cotton bollworm larval productivity in corn and sorghum generally peaked prior to significant cotton bollworm larval infestations in cotton. This would imply that cotton bollworm moths emerging in corn and sorghum most likely migrated to other crops, including Bollgard and non-Bollgard cotton, where the next generation

of cotton bollworm moths were likely produced. In North Carolina, the cotton bollworm production was generally synchronous across all host crops sampled and represented one generation. Here, there were cotton bollworm larvae produced in corn, peanut, and soybean compared to non-Bollgard cotton, but production in all crops was synchronous.

Adult moth production (pheromone traps). In contrast to the large differences in cotton bollworm larval productivity between crops and sampling sites, there were no significant differences between the crops for the number of seasonal moth captures in pheromone traps when evaluated across all sampling locations except for some sampling locations in Mississippi. Sampling at the interface of the Bollgard cotton-Bollgard cotton fields was added to the study when it was inadvertently left off of the final protocol. Moths were collected in large numbers in pheromone traps at sampling sites that had no larval productivity (e.g., Bollgard cotton-Bollgard cotton interfaces) as well as other sampling sites. These results generally indicate that large numbers of adult moths emerge from larvae produced from different host crops. In the mid-South region, pheromone traps continued to attract cotton bollworm adult moths after larval productivity had ended in all host crops at the sampling sites. These results suggest that adult moths are coming from vegetation other than the crop hosts being sampled. In other words, the landscape is saturated with moths and the moths migrate in large numbers to other crops including Bollgard cotton fields. Satellite analysis is being conducted to provide an understanding of the specific relationship between the various host crops, other vegetation, and the sampling locations and long-distance movement of adult cotton bollworm moths. Results from the 2002 season were not available.

Also ongoing is the analysis of adult moth wings for the ratio of ^{12}C to ^{13}C . These analyses will give some indication about whether moths originated from C_3 (soybean, cotton, peanuts) or C_4 (corn, sorghum) plant sources. Preliminary results show that in Georgia, Mississippi, Louisiana and Arkansas, a high percentage of the cotton bollworm adults moths collected during July were from C_4 plant sources than from C_3 plant sources. The subsequent generation of moths produced in mid-August through early September was primarily from C_3 plant sources. Moths in mid-September were primarily from C_4 plant sources. Similar results were reported for North Carolina except all the moth generations were approximately two weeks later than in the other states.

Gossypol analysis of cotton bollworm moths was originally proposed as part of the final protocol, but was dependent on the validation of the protocol. The presence of gossypol would indicate whether the moths were from cotton or non-cotton C_3 hosts. Monsanto indicates that the procedure for the gossypol analysis of cotton bollworm moths could not be validated. Therefore, this portion of the study could not be completed. Monsanto indicates that the larval productivity data will be used to estimate the proportion of moths coming from cotton compared to other C_3 crop hosts.

Specific alternate host evaluations (strip tests). Research trials were established in North Carolina, Georgia, Mississippi, Louisiana, and Arkansas to examine larval productivity. Each trial consisted of replicated 0.25-acre plots of the alternate host crops. Each crop were planted and managed according to standard agronomic practices for each region. Crops included in the

study were Bollgard and non-Bollgard cotton, corn, soybeans, grain sorghum (mid-South), and peanuts (Southeast). Large (L4-L5) cotton bollworm larvae from these treatments were collected and allowed to pupate under laboratory conditions to quantify adult emergence as an estimate of the relative productivity and fitness of adult cotton bollworm coming from each host crop.

Preliminary results generally followed the same trends observed in the landscape monitoring of commercial fields described above. In North Carolina, larval production was synchronous for all crops. Corn, soybean, and cotton were all major contributors of larvae, but larval productivity from peanuts was relatively low. A high percentage of larvae collected from various crops was able to pupate in the laboratory. In the mid-South region, there were very high levels of larvae produced in corn and grain sorghum in late June through mid-July. The subsequent generation was produced in cotton and soybeans. The productivity in grain sorghum was approximately 1-2 weeks later than in corn. Larval productivity in soybean depended on the crop maturity group chosen and location. For example in Louisiana, the productivity of group IV soybeans was essentially zero; whereas, the productivity of group VI soybeans was approximately equal to unsprayed non-Bollgard cotton. In Mississippi, larval productivity in groups IV and V soybeans was very low; whereas in Arkansas, there was moderate productivity in groups V and VI soybeans, but low in group IV soybeans.

EPA Review

Monsanto has fulfilled the terms and conditions of the registration, 524-478 (September 29, 2001) by providing an interim status report of the alternate host studies. In general, larval productivity experiments indicate that larvae were produced in the order of corn >> soybeans > non-Bollgard cotton >> Bollgard cotton. Larval production was variable between sampling sites even within the same state. In North Carolina, larval productivity was synchronous among crops and represented one generation, but was relatively low in peanuts as compared to other crops. In Georgia, Mississippi, Louisiana, and Arkansas, larval productivity in corn and grain sorghum peaked prior to significant larval productivity in cotton. Both the larval productivity data and pheromone trap data indicate that large numbers of moths were collected from different host crops other than cotton. There were large numbers of moths in traps at sampling sites where larval productivity was low. The carbon isotope analysis data supports the conclusion that moths are originating from both C₃ and C₄ hosts and moths migrate across the landscape. For Georgia, Mississippi, Louisiana, and Arkansas, preliminary results indicate that moths collected in July originated from C₄ plant sources, the subsequent generation produced in mid-August through early September was primarily from C₃ plant sources, and then in mid-September the moths originated from C₄ plant sources. North Carolina data show the same trend, although the time is about two weeks later than in the other states.

There are limitations to these studies. These studies are designed to determine the numbers of insects emerging from various crops at the current time. As such, this research will only provide a snap-shot in time. If the landscape changes (as certainly it might in different cotton production areas) or if new insecticides are introduced, the relative contribution of alternate hosts will change. Therefore, the relative value of this kind of work toward understanding the cotton ecosystem will be somewhat limited. It will not be possible to quantify the number of moths

coming from different hosts, nor will it be possible to identify how far moths migrate in the landscape. That is, moths may be moving short distances or long distances. In some cases, depending on the landscape, it is possible that if there are no local hosts then one can make some conclusions that moths are moving in to the landscape from further away.

In addition, the attractiveness of the pheromone traps is rather limited. Pheromone traps are not designed for quantitative analysis. The majority of the moths are likely to be pulled in from <400 meters, although winds will carry moths many kilometers. More extensive life table analysis would provide more certainty in the interpretation of the results and be more useful in understanding the biotic and abiotic factors in operation. One cannot determine from either the larval productivity or the pheromone trapping data the relative value of each alternate host as a refuge. The only conclusion one can make is that larvae are produced by different hosts and that moths are coming from different hosts other than cotton. It is also possible to conclude that corn produces a lot of larvae and a lot of moths relatively early in the season. A second conclusion appears to be that alternate hosts are producing a lot of moths in the landscape, but where they come from or how many are coming from what alternate host cannot be easily determined. The carbon isotope analysis and the satellite imaging might provide useful information as to the relative contribution of C₃ and C₄ hosts, but not really anything about the migratory behavior of cotton bollworm. One cannot determine the effectiveness of these alternate hosts, only that alternate hosts exist, at least in some geographic areas.

The preliminary conclusions regarding cotton bollworm alternate hosts contributions appear to be different in North Carolina than in Georgia, Arkansas, Mississippi, and Louisiana. Larval productivity was synchronous with all alternate hosts in North Carolina, although numbers produced differed among the hosts. The mid-South states do not show the same kind of synchrony. Before any definitive conclusions can be made regarding the relative contributions of alternate hosts as effective refuges for cotton bollworm the second year of these two year studies must be completed and the analyses finished. Monsanto must supply a final report (with actual data) to EPA by March 15, 2004. It should be cautioned that results from these studies will have no bearing on resistance management for *Heliothis virescens* (tobacco budworm) and *Pectinophora gossypiella* (pink bollworm) in Bollgard cotton.