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POLLEN DISPERSAL FROM TRANSGENIC COTTON FIELDS

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Abstract

Potential pollen dissemination from transgenic cotton fields has implications in breeding, seed production, seed certification, and regulatory disciplines. The purpose of this study was to evaluate pollen dispersal from transgenic fields to conventional fields in commercial seed production environments in Arizona, Mississippi, and Texas. Roundup Ready rolled towel bioassays and enzyme-linked immunosorbent assays were used to quantify the presence of transgenes in seeds harvested from conventional fields. A 55-foot non-planted buffer was effective in maintaining 100% varietal purity in a conventional field in Arizona. In Mississippi, seeds harvested in a conventional field at distances of 12 ft or more from a RR pollen source had similar levels of adventitious presence as the parental source seed. Pollen dispersal was greatest in the Texas environment, plus pollen dispersal varied between locations within a conventional field at 10 ft and 20 ft from the RR pollen source. Transgenic percentage in seed harvested from conventional fields dropped sharply from 3 ft to 50 ft from the transgenic pollen source, and then leveled off at distances greater than 50 ft at a transgenic percentage similar to parental seed across production environments. The results from these studies indicate that pollen dispersal from transgenic cotton fields can be effectively managed using field isolations using a combination of unplanted areas and unharvested border rows. A 50 ft isolation provided adequate control of pollen dispersal across production environments evaluated in these studies.

Introduction

Understanding potential pollen dissemination from transgenic cotton fields has applications in breeding, seed production, seed certification, and regulatory disciplines. Fortunately, cotton is largely a self-pollinated crop with a low natural outcrossing, with bees and other insects as the primary vectors of pollen dispersal (Meredith and Bridge, 1973). Seed producers use isolation as a management tool to minimize the likelihood of adventitious presence (AP) of genetically enhanced seeds in conventional varieties, or the presence of unintended events in transgenic varieties. Leaving eight 38-inch border rows unharvested, in combination with a 15 ft road, in seed production fields in Mississippi avoided potential contamination from adjacent fields of different varieties (Berkey et al., 2002). Frequency of pollen movement decreased to less than 1% by Row 7 in solid-planted cotton in Mississippi (Umbeck et al., 1991). Progeny from conventional control plants adjacent to transgenic plants had transgenic progeny of 0.4% at 1 m to below 0.03% at 16 m into a buffer zone surrounding a transgenic field (Llewellyn, 1996). Preliminary results in California in 2000 and 2001 have shown that pollen flow is less than 1% at 30 ft or more from the pollen source (A. Van Deynze, unpublished data, 2002). This same research showed that a high level of pollinators increased pollen flow up to about 50 to 60 ft compared with a low level of pollinators. A gene flow study in China showed a gene flow frequency at 5, 10, 20, and 50 m of 0.61, 0.16, 0.09, and 0.03%, respectively (Zhang et al., 1997).

Field isolation requirements have been established by regulatory authorities that help ensure the maintenance of varietal purity based on past pollen dissemination studies. For example, for Foundation class seed, USDA states that the "minimum isolation shall be at least of 100 feet if the cotton plants in the contaminating source differ by easily observable morphological characteristics from the field to be inspected, plus at least 1320 feet between upland and Egyptian (Pima) types (USDA-APHIS, 2002)." State seed certifying agencies have individual regulations that govern field isolation requirements that range from 15 to 25 ft for cotton varieties with similar morphological characteristics and greater isolation requirements for varieties that differ by morphological characteristics or species (Arizona Crop Improvement Association, Inc., 2002; California Crop Improvement Association, 2002; Mississippi Seed Improvement Association, 1999; Texas Department of Agriculture, 1997). The purpose of this study was to evaluate pollen dispersal from transgenic fields adjacent to conventional fields in commercial seed production environments in Arizona, Mississippi, and Texas.

Materials and Methods

Arizona

Neighboring seed-production fields located north of Coolidge, Arizona in Pinal County were identified in 2002 to represent a commercial situation to measure field-to-field transgenic pollen dispersal. Fields were planted on 15 April 2002 with a conventional variety next to a commercial Bollgard® (BG) variety. Approximately 60 feet separated these fields (Figure 1). Inter-row spacing was 38 inches and the final plant populations were approximately 40,000 plants per acre. At harvest maturity on 18 September 2002, 50-boll samples were collected by hand from the bottom, middle, and tops of plants in a 10-15 ft row length. Six reps were taken for every distance from the BG pollen source. Distances from pollen source of 58, 67, 79, 91, 103, 127, 175, 223, and 265 feet were sampled.

Mississippi

Adjacent seed-production fields located south of Leland, Mississippi in Washington County near the Stoneville Pedigreed Seed Company Mid-South Research Station were identified in 2002 to represent a commercial situation to measure field-to-field pollen dispersal (Figure 2). Fields were planted on 7 May 2002 with experimental varieties. Conventional varieties were planted in the same field as a Roundup Ready® (RR) variety, with no field row separating the conventional from RR fields (Figure 2). Inter-row spacing was 38 inches and final plant populations were approximately 41,000 plants per acre. Major insect pests were plant bugs and lepidopteran pests, which were aggressively managed with timely insecticide applications. At harvest maturity on 24 September 2002, 50-boll samples were collected randomly by hand from the bottom, middle, and tops of plants in a 10-15ft row length. Each row was sampled at six locations at about 200-foot intervals. Distances from pollen source of 3, 12, 24, 36, 48, 72, 120, 168, and 216 feet were represented.

Texas

A sixty-acre conventional seed production field in Terry county, Texas in the High Plains region was used to measure pollen dispersal in 2002. Neighboring fields east and west were planted to RR and BG/RR commercial varieties respectively (Figure 3). Borders between fields were 10 feet on the west and 8 feet on the east. Inter-row spacing was 40 inches and final stand density was approximately 40,000 plants per acre. On 28 Sep. 2002, at harvest maturity, six-50 boll samples were randomly collected by hand from top, middle and bottom bolls at nine different distances (10, 22, 34, 47, 60, 85, 136, 187, 237 feet) from transgenic pollen sources.

Seed cotton samples from all locations were ginned using a tabletop gin. Samples were hand-acid delinted using concentrated sulfuric acid, neutralized with either calcium carbonate or baking soda, and treated with a commercial fungicide before being analyzed.

Laboratory Assays

An enzyme-linked immunosorbent assay (ELISA) was used to measure potential dispersal of pollen from a BG field into a conventional field in Arizona by determining the presence or absence of Cry1Ac endotoxin protein in cotton seeds. Microplates, antibodies and reagents were obtained from Agdia, Inc. (Elkhart, Indiana; http://www.agdia.com). Eight, one-hundred seed sample pools were analyzed for each of six replications per treatment for a total of 4,800 seeds per treatment. One-hundred seed sample pools were counted, and then ground together in small jars using a commercial Waring laboratory blender and a custom blade assembly. Protein extraction buffer was added to the ground sample, and thorough mixing occurred prior to extracting aliquot for ELISA. Sample pools were evaluated as either positive for Cry1Ac or negative, and an assumption of 1 seed out of 100 was made for positive sample pools.

A RR rolled towel bioassay was used to quantify potential movement of pollen from RR fields into conventional fields by measuring resistance of seedlings to glyphosate (ROUNDUP ULTRA) (Association of Official Seed Analysts, 2003; Savoy et al., 2001). Planting media was prepared by adding 2250 ml of a 0.30% ROUNDUP ULTRA [3 ml ROUNDUP ULTRA (480 g glyphosate L^{-1}) to 1 liter of water] solution to 1000 g of dry germination towels (Anchor Paper Company, 25 cm x 38 cm, 38#, unbleached) in a 20-liter plastic container. After the solution was poured on the towels, the container was covered and the towels and solution were allowed to equilibrate for at least one hour. Fifty delinted, fungicide-treated seeds were planted per towel sub sample, with two towels below the seeds and one towel placed on top of seeds. Towels were rolled and rubber bands were placed around the middle of the roll. Ten rolled towels with samples, plus two control towels, were placed in a plastic container, such as a No. 7 Rubbermaid 3.78 liter container (34 cm x 34 cm x 25 cm) with two 6-mm air vents in the lid. Each container was placed upright in a 30°C germinator for seven days. Known tolerant and susceptible checks were planted to verify that the ROUNDUP ULTRA solution was mixed properly and to provide visual standards for tolerant and susceptible seedlings. At the end of the seven-day test duration, seedlings were evaluated as either: Roundup tolerant, Roundup susceptible, dead, or abnormal. Roundup tolerant seedlings produced seedlings with all essential seedling structures, although growth was inhibited by about 25-30% compared to normal seedlings in the standard germination test. Roundup susceptible seedlings had shortened hypocotyl-radicle lengths with obvious black lesions on hypocotyls. The percent RR purity was calculated by dividing the number of Roundup tolerant seedlings by the number of normal and abnormal seed sprouted, and then multiplying by 100.

Statistical Methods

Eight-hundred seeds for each of six replications were tested in both RR bioassay and BG ELISA. Mean separation was accomplished by calculating 95% confidence intervals around means based on binomial probabilities (Remund et al., 2001). Adventitious presence of transgenes was determined in source parental seed and progeny from conventional fields by testing 4,800 seeds using the appropriate RR bioassay or BG ELISA. All mean data points from Mississippi (2000 and 2002) and Texas (2002) were analyzed using regression analysis.

Results and Discussion

Arizona

There were no significant differences found between the mean percentages of BG in source seed and any of the distances from pollen source. Source seed had a 0.52% level of adventitious BG, which is significantly greater than any other source seed used in these studies. These results indicate that the 55-foot non-planted buffer was effective in maintaining varietal purity in the conventional field in this production environment (Figure 1). As a point of reference, Arizona Crop Improvement Association regulations require a 25-foot minimum between varieties with similar species and morphological characteristics (Arizona Crop Improvement Assoc., Inc., 2002).

Mississippi

There were no significant differences found between the mean percentages of adventitious presence of RR in source seed and distances of 12 ft or greater from RR pollen source (Figure 4). The average RR% (0.22% RR) at 3 ft into the conventional field was the only distance that was significantly greater than source seed AP of 0.00% RR. It is important to note that RR and conventional fields were planted row-to-row with no unplanted space or buffer crop in between fields (Figure 2). Based on these results, it appears that the 15 ft field isolation requirement used in Mississippi is adequate in preserving varietal purity (Mississippi Seed Improvement Assoc., 1999).

Pollen dissemination results in this 2002 study are significantly lower than reported in an earlier study in the same locale near Stoneville, Mississippi in 2000, which indicates year and production environment effects on pollen dispersal. In 2000, pollen dissemination from a BXN® field to a RR field averaged 1.89% at approximately 16 ft from a RR pollen source, followed by 0.77% at 37 ft, 0.13% at 61 ft, and 0.00% at 85 ft. (Berkey et al., 2002). Pollen dissemination from a RR field to a BXN field averaged 1.05% at 16 ft from a BXN pollen source, and 0.69% at 37 ft (Berkey et al., 2002).

Texas

Locations within a field had a significant effect on RR percentage in seed harvested from the conventional field, with the W location having a higher RR% at 10 and 20 ft from the pollen source than the E location (Figures 5). The prevailing winds were from the southwest to the northeast, which could explain the higher level of transgene presence in the W location (Figure 3).

Within the west side location (W), the average RR% at the 10 and 22 ft sampling points was greater than the RR% at the 34 ft sampling point or greater distances (Figure 5). The 34 ft distance was significantly greater than the 60 ft distance and greater distances. There were no statistical differences between RR% in the 84 ft and greater distances compared to the parental seed RR level. Within the east field location (E), average RR% at 9 and 21 ft was significantly greater than at 47 ft or greater distances (Figure 5). There were no statistical differences between the RR% in the 49 ft sampling point and greater distances compared to the RR% in the parent seed. Based on data from this field study, it appears that 34 ft of isolation may be required in the Texas environment measured to keep AP levels below 1% in conventional varieties grown next to transgenic varieties.

Summary and Conclusions

A 55-foot non-planted buffer was effective in maintaining varietal purity in the conventional field in the AZ production environment. In MS, seed harvested in a conventional field at distances of 12 ft or more from a RR pollen source had similar levels of AP to parental source seed. Pollen dispersal was the greatest in the Texas environment, plus pollen dispersal varied between locations within a conventional field at 10 ft and 20 ft from the RR pollen source. In field location E, RR% was similar to parental seed at distances of 49 ft or more; in field location W, RR% was similar to parental seed at distances of 84 ft or more.

A regression analysis was performed on means in fields in Mississippi (2000 and 2002) and Texas (2002) where baseline AP in parental seed was between 0.00 and 0.10% ($\alpha = 0.05$) as this level represents a pure source of conventional seed. Transgenic percentage in seed harvested from conventional fields dropped sharply from 3 ft to 50 ft from the transgenic pollen source, and then leveled off at distances greater than 50 ft at a transgenic percentage similar to parental seed (Figure 6). The results from these studies indicate that pollen dispersal from transgenic cotton fields can be effectively managed using field isolations of 15 to 84 ft depending on the production region. It also appears that the isolation distance used can be accom-

plished using a combination of unplanted areas and unharvested border rows during seed harvesting operations. A 50 ft isolation provided adequate control of pollen dispersal across the years and production environments evaluated in these studies.

More research in commercial environments is needed to more fully understand effects due to year, production environment, and pollinator activity. Baseline level of adventitious presence of genetically-enhanced seed in parental source seed, and error rates of test methods should be determined to accurately interpret effectiveness of isolations.

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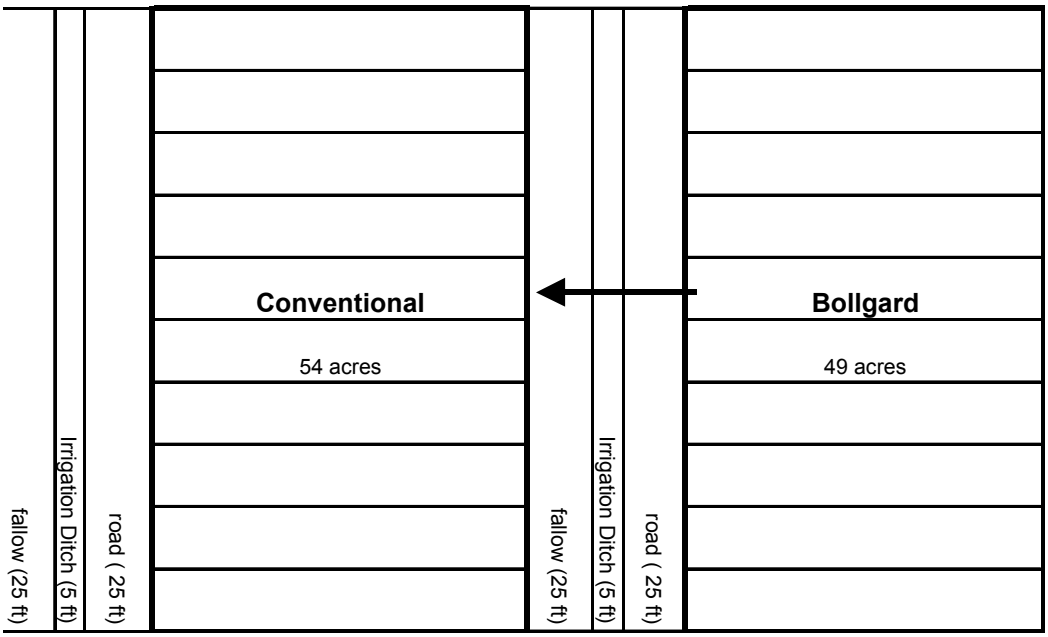

Figure 1. Neighboring conventional and Bollgard cotton seed-production fields separated by a 55-foot unplanted isolation zone, located north of Coolidge, Arizona in 2002. The arrow indicates the direction of potential pollen dissemination from the Bollgard field to the conventional field.

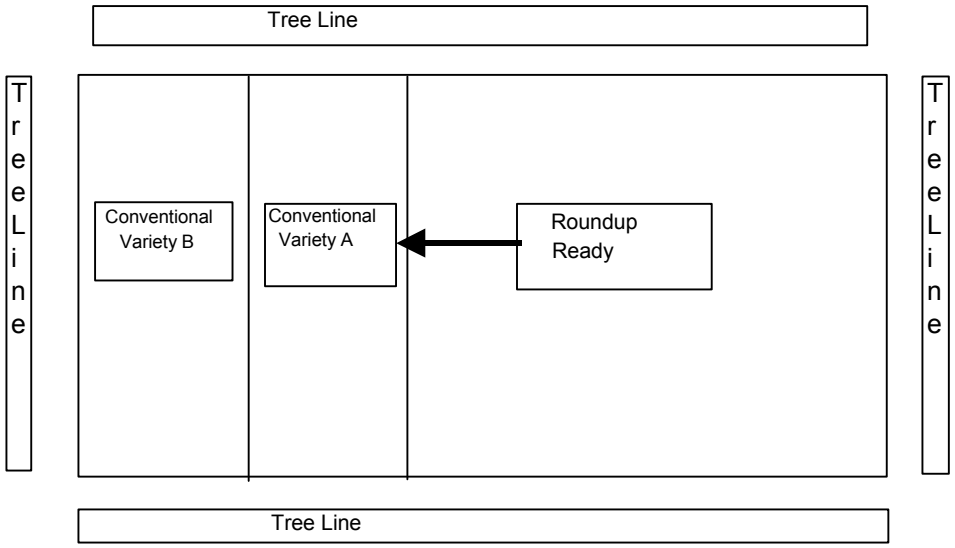

Figure 2. Adjacent conventional and Roundup Ready cotton seed-production fields planted row-to-row, located south of Leland, Mississippi in 2002. The arrow indicates the direction of potential pollen dissemination from the Roundup Ready field to the conventional field.

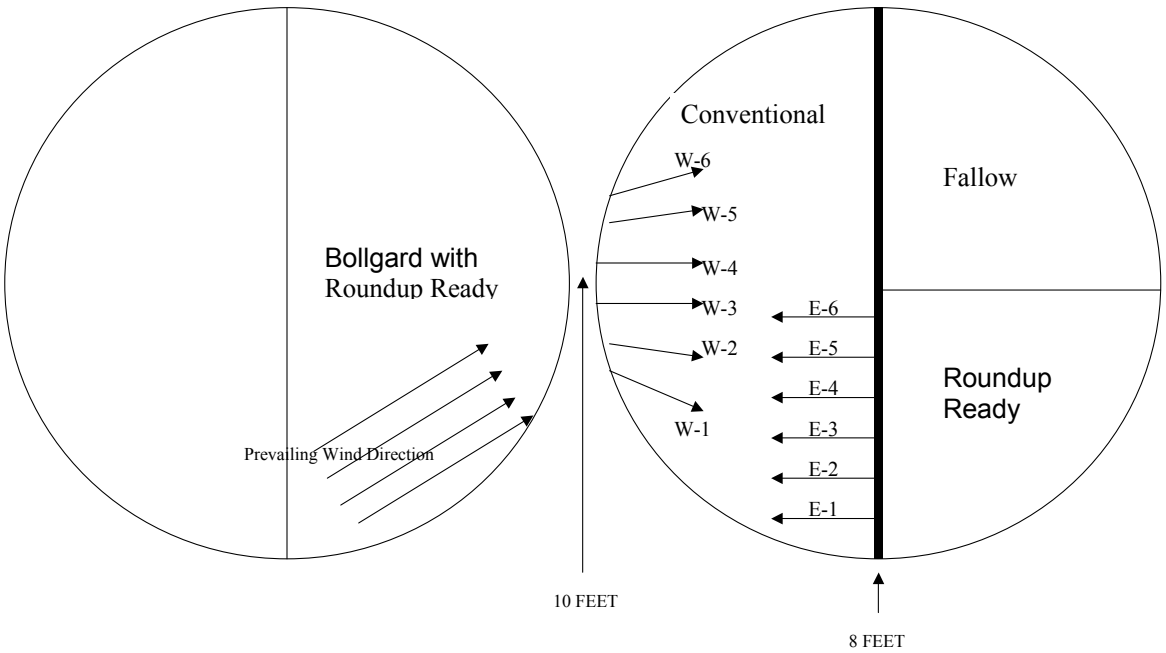

Figure 3. Neighboring conventional, Roundup Ready, and Bollgard with Roundup Ready cotton seed-production fields, located in Terry county, Texas in the High Plains region in 2002. Potential pollen dissemination was measured in the east (E1-E6) side of the conventional field, and in the west (W1-W6) side of the conventional field.

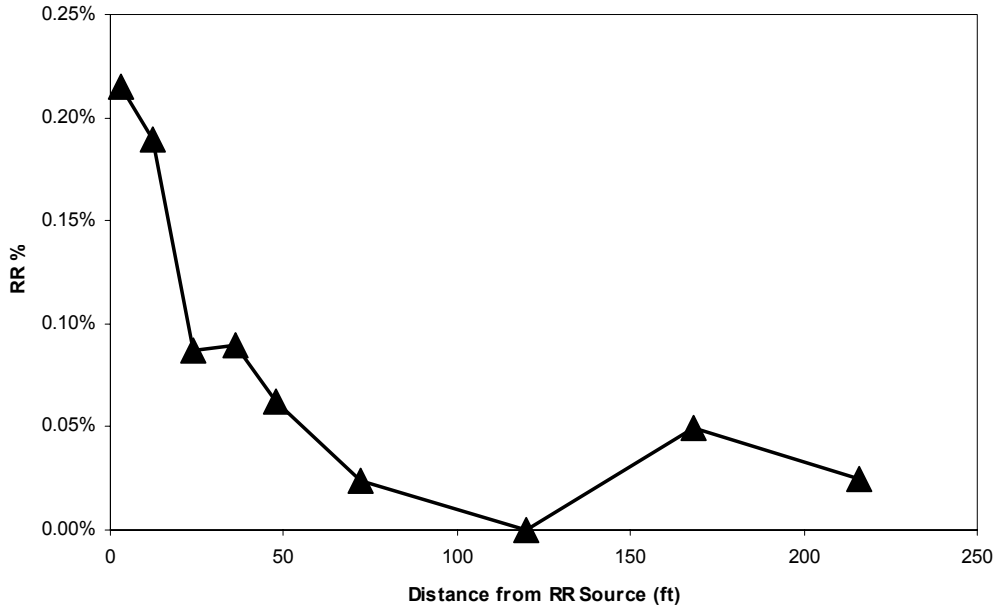

Figure 4. Percent Roundup Ready (RR) seeds in seed harvested from a conventional field as a function of distance (ft) from Roundup Ready pollen source near Stoneville, Mississippi in 2002. Note that adventitious presence in parental (source) seed was between 0.00-0.10% (α = 0.05) based on binomial probabilities.

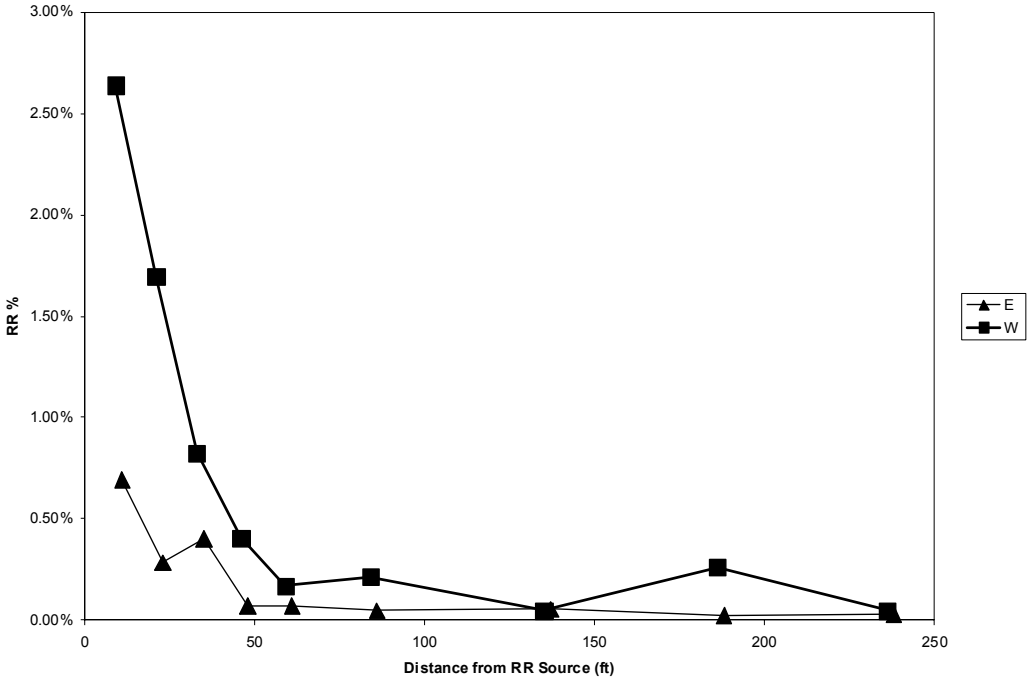

Figure 5. Percent Roundup Ready (RR) seeds harvested from a conventional field as a function of distance (ft) from Roundup Ready pollen source in Terry County, Texas in 2002. Note that adventitious presence in parental (source) seed was between 0.00-0.10% (α = 0.05) based on binomial probabilities.

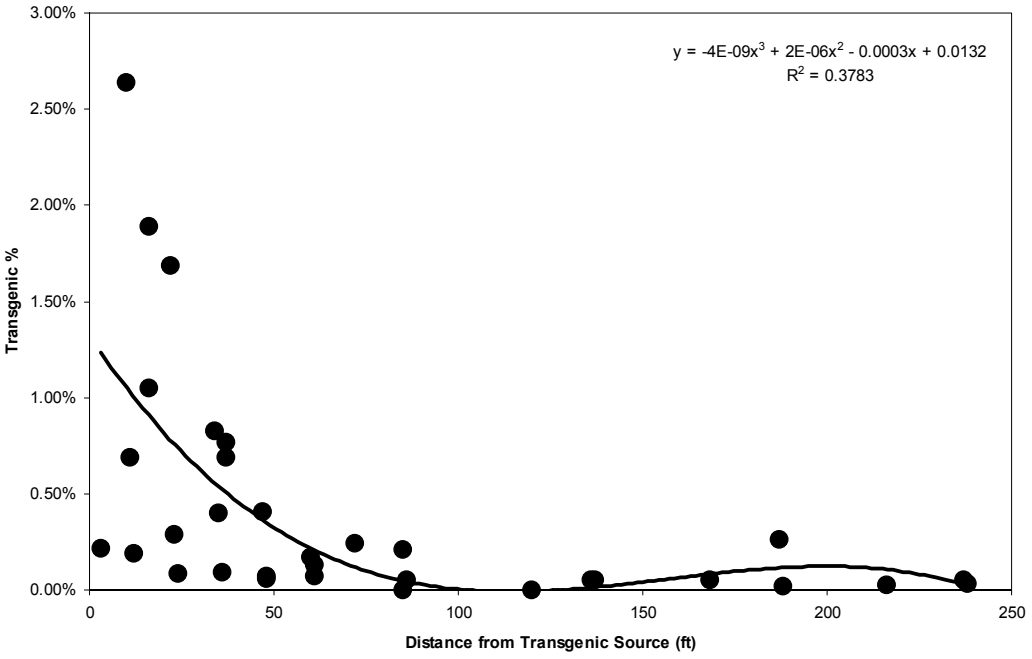

Figure 6. Percent transgenic seeds harvested from conventional fields as a function of distance (ft) from transgenic pollen source across Mississippi and Texas locations in 2000 and 2002. Note that adventitious presence in parental (source) seed was between 0.00-0.10% (α = 0.05) based on binomial probabilities.