2002 BELTWIDE COTTON CONFERENCES, ATLANTA, GA – JANUARY 8-12

POLLEN DISSEMINATION FROM ADJACENT FIELDS OF GENETICALLY ENHANCED COTTON IN THE MISSISSIPPI DELTA

Dennis A. Berkey and Bryan R. Savoy
Stoneville Pedigreed Seed Company
Memphis, TN
Steven R. Miller and Paul G. Johnson
Stoneville Pedigreed Seed Company
Stoneville, MS

Abstract

Maintaining varietal and transgenic purity in foundation seed stocks has become more challenging in recent years. The purpose of this study was to evaluate pollen dissemination between two adjacent fields of genetically-enhanced cotton in the Mississippi Delta in 2000 using seed bioassays. Fields located south of Leland, Mississippi were planted with experimental Stoneville varieties containing BXN®/Bollgard® and Roundup Ready®/Bollgard traits. These fields were separated by a 13-foot road. At harvest maturity, 50-boll samples were collected randomly by hand from the bottom, middle, and tops of plants. A BXN® bioassay was used to quantify dissemination of pollen from the BXN field into the RR field by measuring resistance of seedlings to bromoxynil [(3,5-dibromo 4-hydroxybenzonitrile) (BUCTRIL® 4EC)] herbicide. A Roundup Ready® rolled towel bioassay was used to quantify dissemination of pollen from the RR field into the BXN field by measuring resistance of seedlings to glyphosate (ROUNDUP ULTRA®) herbicide. Pollen dissemination from the BXN field to the RR field averaged 1.89% in Row 1 nearest to the road, followed by 0.77% in Row 8, 0.13% in Row 16, and 0.00% in Row 24. Pollen dissemination from the RR field to the BXN field averaged 1.05% in Row 1 nearest to the road, and 0.69% in Row 8. Leaving 8 border rows un-harvested in seed production fields appears reasonable in this production environment to avoid potential contamination from adjacent fields of different varieties, unless a zero tolerance for unintended transgenic events exists.

Introduction

Maintaining varietal and transgenic purity in foundation seed stocks has become more challenging in recent years. Seed producers need to maintain purities for intended events and minimize unintended events in transgenic varieties, while ensuring no adventitious presence of genetically-enhanced seed in conventional varieties. Often, these seed-production challenges occur in close proximity. Fortunately, cotton is largely a self-pollinated crop with natural outcrossing in the 0 to 5.9% range (Meredith and Bridge, 1973). Bees and other insects are the primary vectors of pollen in cotton, with little or no pollen being transported by air currents (McGregor, 1976), although pollen can be a significant field-to-field vector of gene flow in an open-pollinated crop like maize where cross-pollinations occur at distances up to 200 m from source planting (Luna V., 2001).

Isolation distance is a management tool that has been used successfully to control gene flow via pollination in cotton planting seed production systems (Green and Jones, 1953; Kareiva, 1994) and is a requirement of official seed certifying agencies (Mississippi Seed Improvement Assoc., 1999). Frequency of pollen movement decreased to less than 1% by Row 7 in solid-planted cotton in Mississippi (Umbeck et al., 1991). Umbeck et al. (1991) also found no consistent differences in pollen dissemination due to flower position, which indicated no seasonal effects. 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). Seed bioassays have been used to determine the presence of intended and unintended transgenic events (Ashton and Maruschak, 2001; Savoy et al., 2001).

The purpose of this study was to evaluate pollen dissemination between two adjacent fields of genetically-enhanced cotton in the Mississippi Delta in 2000 using cost-effective seed bioassays.

Materials and Methods

Adjacent seed-production fields located south of Leland, Mississippi near the Stoneville Pedigreed Seed Company Mid-South Research Station were identified in 2000 to represent a commercial situation to measure field-to-field pollen dissemination (Figure 1). Fields were planted on 11 May 2000 with experimental Stoneville varieties with BXN®/Bollgard® (BXN) and Roundup Ready®/Bollgard (RR), and these fields were separated by a 13-foot road (Figure 1). Inter-row spacing was 38 inches and final plant populations were approximately 43,000 plants per acre. Major insect pests were plant bugs and white flies, which were effectively controlled with seven timely applications of ORTHENE® (0.5 or 1.0 lb/acre) from 2 June 2000 to 11 August 2000, and one application of BIDRIN® (0.25 lb/acre) on 12 June 2000. These fields were also treated one time per week with ULV malathion as they were in the Boll Weevil Eradication Program.

At harvest maturity on 14-15 September 2000, 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 four locations at about 250-foot intervals. Seed cotton was ginned on a 10-saw table-top gin, and 1200-1500 seeds per replication were obtained. Fuzzy cotton seeds were hand-acid delinted with sulfuric acid, neutralized with calcium carbonate, then treated with a commercial fungicide before being planted for bioassays.

A BXN bioassay was used to quantify dissemination of pollen from BXN field into RR field by measuring resistance of seedlings to bromoxynil [(3,5-dibromo 4-hydroxybenzonitrile) (BUCTRIL® 4EC)] herbicide. Seeds were planted in a moist potting soil/sand mixture in 10 inch x 20 inch nursery flats in a 86°F growth chamber with 16-hour photoperiod and high light intensity (~520 foot-candles). Bromoxynil is a contact herbicide that destroys cell membranes in the presence of light by inhibiting photosystem II. Therefore, plants must be actively growing to have good herbicidal activity. Following a 7-10 day growth period, flats were removed from growth chamber and sprayed with a BUCTRIL 4EC solution (10 ml BUCTRIL 4EC per 1,500 ml water) until runoff. Two days following initial BUCTRIL 4EC application, flats were sprayed again with the same BUCTRIL 4EC solution. Two days after second herbicide application, seedlings were evaluated as BXN tolerant or BXN susceptible. Tolerant seedlings have no damage and are unaffected by BUCTRIL 4EC treatment as bromoxynil is metabolized into non-toxic compounds. Susceptible seedlings appeared as desiccated or burned at two days after herbicide application, followed by complete necrosis. Percent BXN purity was calculated by dividing the total number of tolerant seedlings by the number of seedlings that emerged, then multiplying by 100.

A Roundup Ready rolled towel bioassay was used to quantify movement of pollen from RR field into BXN field by measuring resistance of seedlings to glyphosate (ROUNDUP ULTRA) (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 hard seed. Roundup tolerant seedlings produced seedlings with all essential seedling structures, although growth was inhibited by 25-30% compared to normal seedlings in the standard germination test and minimal secondary root growth occurs (AOSA, 1992). 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.

Eight-hundred seeds per replication were tested in both BXN and RR bioassays. Mean separation was accomplished by calculating 95% confidence intervals around means based on binomial probabilities (Remund et al., 2001). Non-target transgenic purity was determined in source parental seed and progeny of RR by testing 3,200 seeds using the BXN bioassay. Six outside rows were not harvested for seed increase.

Results and Discussion

The highest amount of pollen movement from the BXN field to the RR field averaged 1.89% in Row 1 nearest to the road (Figure 2). The BXN frequency in the RR field decreased to 0.77% by Row 8, was 0.13% at Row 16, and was undetectable at Row 24 (Figure 2). Pollen movement from the RR field to the BXN field averaged 1.05% in Row 1 nearest to the road, with the frequency decreasing to 0.69% in Row 8. These results agree with Umbeck et al. (1991) who reported that the frequency of pollen movement from a transgenic field decreased to less than 1% by Row 7 in solid-planted cotton in Mississippi. These results are reasonably consistent with Llewellyn (1996) who determined that progeny from conventional control plants adjacent to transgenic plants had transgenic progeny of 0.03% at 16 m (approximately 16 rows) into a buffer zone surrounding a transgenic field (Llewellyn, 1996). BXN presence in RR parental seed was 0.031%, which represents the base level for parental seed. Seed harvested from the RR field had a BXN level of 0.250%.

Field to field pollen dissemination in this year and production environment occurred at levels between 0.69% and 0.77% in the eighth row from pollen source. By the sixteenth row, pollen dissemination was undetectable. Isolation and border rows are good management tools to help control pollen movement from field to field. From a practical standpoint, leaving 8 border rows un-harvested in seed production fields appears reasonable in this situation to avoid potential contamination from adjacent fields of different varieties. However, additional border rows and isolation may be necessary if no tolerance exists for unintended events.

References

AOSA. 1992. Seedling Evaluation Handbook #35. Association of Official Seed Analysts, Lincoln, NE.

Ashton, D., and J. Maruschak. 2001. Evaluation of a canola (*Brassica napus* L.) herbicide tolerance bioassay method. AOSA Referee/SCST Research Joint Committee Meeting Project Report, Albuquerque, NM 20-24 May 2001. Association of Official Seed Analysts, Lincoln, NE.

Green, J.M., and M.D. Jones. 1953. Isolation of cotton for seed increase. Agron. J. 45:366-368.

Kareiva, P. 1994. Studying and managing the risk of cross-fertilization between transgenic crops and wild relatives. J. Molec. Ecology 3:15-21.

Llewellyn, D. 1996. Pollen dispersal from two fields of transgenic cotton in the Namoi Valley, Australia. p. 157-166. *In* Molecular breeding: new strategies in plant improvement. International Soc. for Plant Molecular Biology, Dordrecht; Boston: Kluwer Academic Publishers.

Luna V., S., J. Figueroa M., B. Baltazar M., R. Gomez L., R. Townsend, and J.B. Schoper. 2001. Maize pollen longevity and distance isolation requirements for effective pollen control. Crop Sci. 41:1551-1557.

McGregor, S.E. 1976. Insect pollination of cultivated crop plants. P. 171-189. *In* USDA-ARS Handbook No. 496.

Meredith, W.R., and R.R. Bridge. 1973. Natural crossing in cotton (*Gossypium hirsutum* L.) in the delta of Mississippi. Crop Sci. 13:551-553.

Mississippi Seed Improvement Association. 1999. Cottonseed certification standards. p. 26-29. *In* Handbook of seed certification regulations. Mississippi State, MS.

Remund, K.M., D.A. Dixon, D.L. Wright, and L.R. Holden. 2001. Statistical considerations in seed purity testing for transgenic traits. Seed Sci. Res. 11:101-119.

Savoy, B.R., D.A. Berkey, and P.G. Johnson. 2001. Rolled towel bioassay to determine Roundup Ready trait in cotton planting seed. Seed Technology 23:163-166 (*In* press).

Umbeck, P.F., K.A. Barton, E.V. Nordheim, J.C. McCarty, W.L. Parrott, and J.N. Jenkins. Degree of pollen dispersal by insects from a field test of genetically engineered cotton. 1991. J. Econ. Entomol. 84:1943-1950

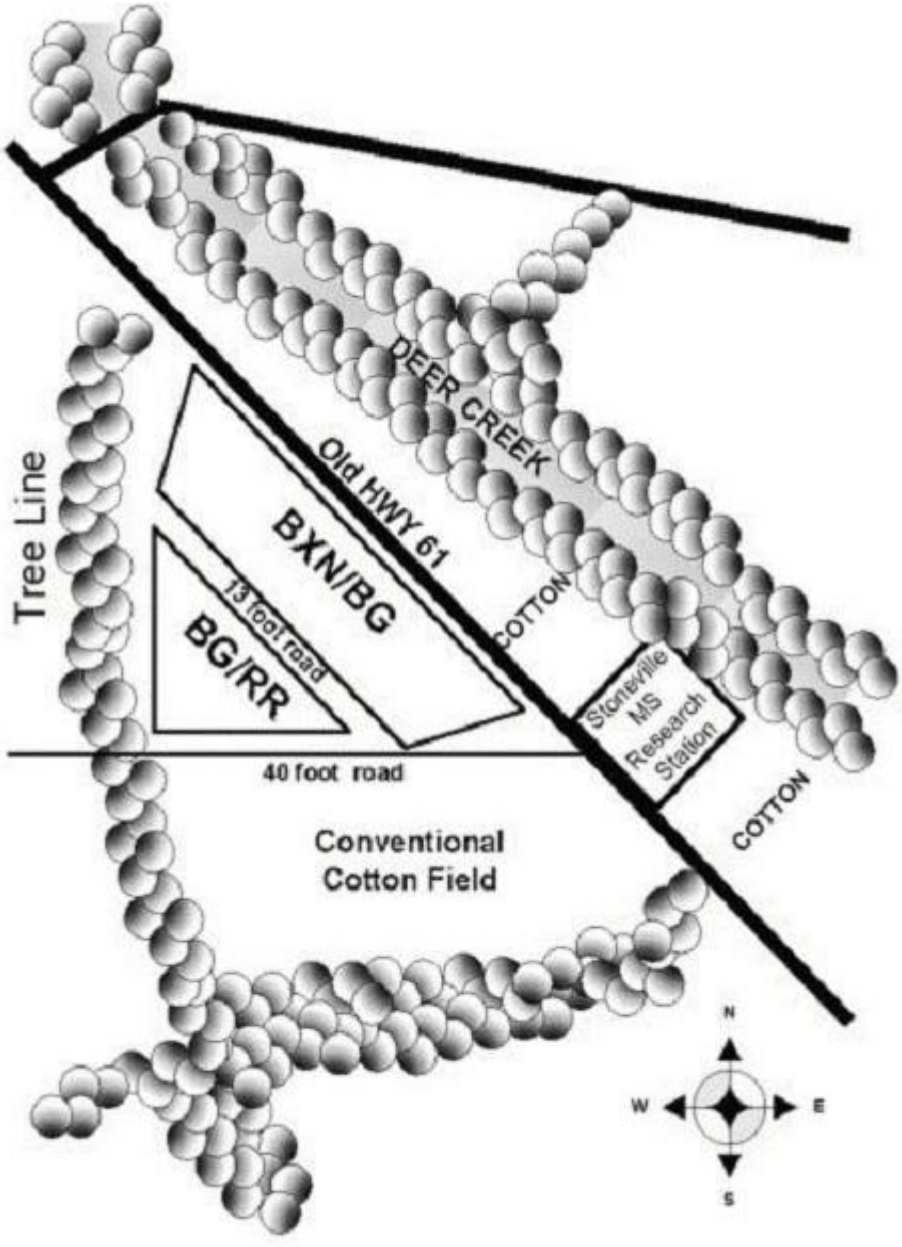

Figure 1. Adjacent BXN and Roundup Ready cotton seed-production fields separated by a 13-foot road, located south of Leland, Mississippi near the Stoneville Pedigreed Seed Company Mid-South Research Station.

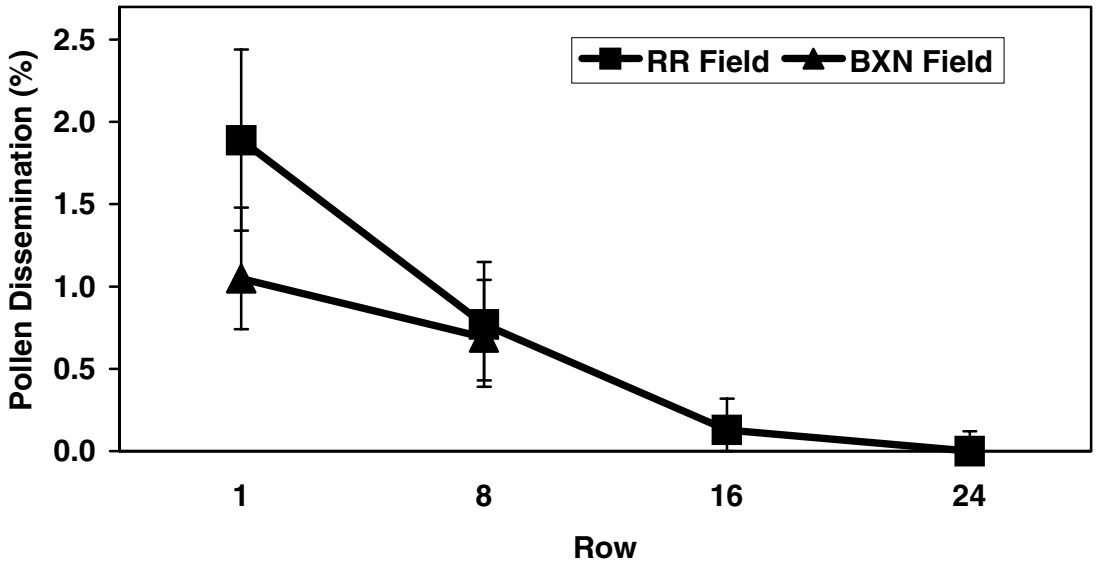

Figure 2. Percent pollen dissemination as a function of number of 38-inch rows from source field in the Mississippi Delta in 2000. Error bars represent 95% confidence intervals around means based on binomial probabilities.