PROPOSED GUIDELINES FOR PRE-COMMERCIAL EVALUATION OF TRANSGENIC AND CONVENTIONAL COTTON CULTIVARS

Lloyd May and Steve Brown
University of Georgia
Tifton, GA
Bob Nichols
Cotton Incorporated
Raleigh, NC
Tom Kerby
Delta and Pineland Company
Scott, MS
Jeff Silvertooth
University of Arizona
Tucson, AZ

Abstract

The primary, commercial features of the recently-released, transgenic cotton cultivars are their respective pest management traits, including, tolerance to the herbicides Buctril® (bromoxynil) and Roundup Ultra® (glyphosate), and the capacity to synthesize a bacterial endotoxin *Bacillus thuringiensis* (Bt) for management of lepidopterous insect pests. Many transgenic cultivars have been offered for sale with fewer years of public testing than most growers and their advisors would have liked. Lack of time and resources may have resulted in some having been sold in locations with no previous public testing in the immediate growing area. Despite the lack of public test-information, the collective market share of the transgenic cultivars has increased every year since their introduction, presumably because of high grower interest in their value-added, pest-management features. Obviously, transgenic pest management traits strongly influence the pest management programs that are appropriate for the transgenic cultivars, and the efficacy of the pest management programs may positively affect yields and the costs of production. However, in the Official Cultivar Trials (OCTs), comparison of the transgenic cultivars with non-transgenic (conventional) cultivars has been done using only conventional, and frequently, a high-level of pest management. Concerns, about the lack of public-test data on transgenic cultivars, and about relying solely on OCTs for their evaluation, prompted Cotton Incorporated to convene a working group (Appendix I.). The objective was to seek consensus among public and private sector researchers on how to enable growers to confidently choose the best cultivar and pest-management technology for their situation. The drafting subcommittee of the working group proposed guidelines for cultivar evaluation to a joint meeting of SRIEG-61 (Southern Regional Information Exchange Group 61 - Cotton Breeding), and a new Regional Project in preparation, SRDC-9801, (Southern Regional Development Committee 9801 - Development of Genetic Resources for Cotton). Principal points of the proposal were that a minimum of two years of public test data should be available to growers at the time of first sale, and that the data should include comparison of transgenic cultivars with cultivars generally recognized as having high-yield potential. The proposal also suggested that the testing should provide comprehensive economic evaluation of new cultivars by concurrently evaluating yields, fiber quality, and the efficacy and costs of the respective pest management programs.

Introduction

The released, transgenic cotton cultivars have proprietary, pest-management traits as principal commercial features. These include BXN® cultivars that are resistant to the herbicide Buctril, Roundup Ready® cultivars that are resistant to the herbicide Roundup Ultra, Bollgard® cultivars that constituitively express a bacterial endotoxin of *Bacillus thurigiensis* and cultivars that contain transgenes for both Bt and Roundup Ultra tolerance. Grower adoption of transgenic cotton cultivars has increased every year since their introduction (Tables 1. and 2.). Approximately 60% of the U.S. cotton area was planted to transgenic cultivars in 1999 (USDA-AMS, 1999). Transgenic traits are available in cultivars produced through back-crossing a transformed line with parents from established cultivars or elite lines. As such, they are reselected back-cross cultivars. The agronomic characters of such cultivars may be expected to resemble, but not be identical, to their back-cross parents.

Cotton growers achieve profitable returns by producing high yields, controlling costs of production, and employing marketing strategies that maximize returns. Traditionally, the foundation of a cotton production plan has been cultivar selection. Selection of the cultivar is a factor in determining the anticipated length of the production season, yield and quality goals, disease resistance, and other agronomic traits. With transgenic cultivars that express pest management traits, the pest management program is also largely affected by cultivar selection. Thus, cultivar selection directly influences production costs and returns with the transgenic cultivars to an even greater extent than it does with conventional cultivars.

During the period when transgenic varieties were first being introduced, growers had access to relatively little public-testing data that compared costs and returns of transgenic cultivars that embodied pest management traits with those of high-yielding conventional cultivars. Performance data, including the effects of pest management programs, is needed to effectively evaluate the economic benefits of transgenic technology. Because OCTs do not evaluate the transgenic cultivars within their intended production systems, some

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:503-507 (2000)
National Cotton Council, Memphis TN

individuals have questioned the validity of cultivar performance data from OCTs for the transgenic cultivars. Alternative programs that have been suggested include trials that employ the transgenic cultivar's own pest management program, or comparison of several cultivars using both transgenic and conventional pest management programs.

Objectives for Transgenic Cultivar Evaluation

1. Provide growers with agronomic performance, pest management efficacy, and stress tolerance of transgenic cultivars, including, as applicable, stresses due to pest management chemicals.
2. Design a research program that will generate such information by achieving valid comparisons between conventional and transgenic cultivars. The program will determine cultivar yields and economic returns when grown using the management practices that are appropriate for the respective cultivars.
3. Create industry consensus to efficiently use available public and private research resources to achieve objectives 1. and 2.

Cultivar Development: Testing Genes, Constructs, and Backgrounds

Confidential testing of genes, gene constructs, and genes in transitional germplasm is anticipated as part of the development process for transgenic cultivars. Such confidential testing is analogous to the testing of numbered compounds and formulations as part of the long-standard process for development of plant protection products. Once a transgenic germplasm is fixed in its proposed commercial form, it should be named and offered for public testing. Restrictions on the release of test data to the public would be appropriate if the seed company decides not to commercially release the cultivar, or if poor agronomic performance of a candidate cultivar can be traced to seed quality, such as that attributable to production of seed in a winter nursery. The proposed, two-year testing period would facilitate the transition from development to sales by providing data to the seed companies, hands-on experience with the cultivars for public breeders, pest managers, and agronomists, and a base of information for growers.

Naming a Candidate Transgenic Cultivar

Transgenic cultivars can be derived as unique selections from transformed germplasm, or from germplasm introgressed with the transgene through conventional breeding. In some instances, the naming of transgenic cultivars by appending descriptive letters to the serializations already assigned to the back-cross, parent cultivar may have suggested that the transformed cultivar would perform as did the parent, except for the supplemental effect of the transgenic trait. However, field experience has demonstrated that the transgenic cultivars may differ, in some cases substantially, from their back-cross parents in agronomic performance and management requirements. While the right to name a cultivar resides with its proprietary company, university or breeder, we recommend that in most instances, transgenic cultivars should be assigned unique names, considering the possibility of such differences in performance between back-cross parents and their genetically-modified progeny. Exceptions to this recommendation could be transgenic cultivars that have exhibited performance very similar to that of their recurrent parent. Otherwise, we recommend that yield and performance data be generated using the new germplasm in association with its new name. Technical literature should be made generally available that describes the genetic background of the new cultivar, and provides research-based suggestions for its management. A two-year testing program, involving the public sector, as described herein, would be helpful in better determining recommendations for management.

Years of Pre-Commercial Test Data

Most states that maintain recommended cultivar lists require three years of public testing before a cultivar can be recommended. However, transgenic and non-transgenic cultivars alike generally become commercially available before completion of three years of testing in OCTs. Examples of cultivars that have become widely planted before attaining recommended status include, SureGrow 125, Stoneville 474, Bollgard 33B, Bollgard 35B and Deltapine 458BG/RR (May et al., 1995-1999). The combination of grower desire to acquire certain transgenic traits, and the desire of seed companies to accommodate such demand, has accelerated the introduction of transgenic cultivars. Prudence and the desire to maintain high-quality cotton cultivars suggest that there must be a balance between the need to provide new products for the cotton planting-seed market, to adequately develop and field test new cultivars, and to provide reliable information to growers.

We propose that the cotton industry set a goal to make a minimum of two years of data available to the public when a cultivar is first released for general sales. Such data should include comparisons with cultivars that have produced high yields in OCTs. The data on the new cultivar may come from publicly conducted trials that are published annually or from privately sponsored research, that would be released to the public before general sales. We recognize, however, that even two years of trials may not be an adequate sample of growth environments to characterize all the situations a transgene will face in commercial production.

Information Needed for Pre-Commercial Cultivar Evaluation

Official Cultivar Trials have traditionally evaluated yield potential and fiber quality, and were not designed to demonstrate the value-added characteristics of transgenic traits. Additionally, OCTs are not designed to measure the pest management effects or the chemical tolerance of transgenic cultivars, issues that have been major concerns among growers the first years of sale with the Bt and Roundup Ready cultivars, respectively. Transgenic, pest-controlling cultivars are dual-purpose products providing yields and pest control simultaneously, thus affecting both input costs and gross returns. The evaluation process should estimate net returns for all cultivars, in a manner specific for the production system intended for use with the cultivar, that is by systems testing. **By systems testing, we mean growing the test-cultivars in replicated field trials, using the respective management programs that are appropriate for the individual cultivars, and recording pest management efficacy and costs, cotton lint and seed yields, and fiber quality.** These data would then be used to calculate gross returns, and returns above pest management costs, or other such costs as may be relevant for other types of transgenic varieties. Some systems testing, primarily for pest management effects, is already conducted by seed companies and some public agencies as part of the development process for transgenic cultivars. However, only a few conventional cultivars are included as controls, and not all this data has been made available to the public. Data on systems comparisons with high-yielding conventional cultivars could be released from privately sponsored research or should be generated through a public testing program. To date, a few systems trials have been reported that included cultivar as a variable in the treatment design, in addition to pest management treatments (Bryant et al. 1999, May et al., 2000, Murdock et al., 2000; Wilcut et al., 1998, 1999).

Experimental Designs For Evaluating Cultivars with Pest Management Traits

Trials can investigate the effects of cultivars or pesticide management programs, or both concurrently. In the latter case, the interaction of cultivars and pest management programs are also estimated. The objectives of the test and the complexity of the respective pest management programs mandate the treatments and experimental designs.

Single Factor Designs

A. Independent Cultivar and Pest Management Testing. Cultivars or pest management programs can be field-tested independently using Completely Randomized Designs or Randomized Complete Block Designs (RCBDs). An example of a regional testing program that employs RCBDs is the National Cotton Variety Trials program (Rayburn et al., 1998). In a simple, single-factor test of cultivars, pest management is applied uniformly across all plots. Likewise, in a simple, single-factor pest management test, the same cultivar will be used for all treatments. Thus to evaluate both effects, i.e., cultivar and pest management, two experiments must be done. A limitation of such an approach is that, unless many tests are done, or there is close co-ordination among researchers, the compiled results will be unbalanced. That is, the aggregate database will include the effects of several pest management treatments for a few cultivars, and the yields of many cultivars grown with a few, and sometimes inappropriate, pest management treatments. Data from independent cultivar and pest management experiments may not be averaged to make quantitative estimates of the combined effects of cultivars and pest management on yields or returns. Rank-order assessments among one set of variables, for example, cultivars are valid for the pest management program in which they were derived and should be extrapolated to other pest management programs with caution. In the formal, statistical sense, such extrapolations are limited to circumstances where appropriate two-factor experiments have been done to ascertain that there are no interactions between main variables, e.g., cultivars x pest management programs. Obviously, since no interaction effects are estimated in single-factor experiments, an additional, two-factor experiment must be done to ascertain potential interactions between pest management programs and cultivars. Cultivars and pest management programs may interact because of environmental factors, pest populations, the components of the pest management program, differential cultivar tolerance to pesticides, or other factors.

B. Systems Tests Utilizing Compound Treatments. An alternative approach, employing single-factor designs, is to reduce cultivar and pest management to a single effect by combining, i.e., confounding, them in the parameter treatment. In this approach, treatments are the individual cultivars grown using their respective pest management programs, i.e., the program consistent with their genetic or transformed genetic capacities. We suggest the use of the term systems testing, because the treatments are systems comprising both the choice of cultivar and pest management program. In fact when growers purchase transgenic cultivars, with pest-management traits, they are concurrently choosing both production inputs. Such an experimental approach has the applied objective of making a comprehensive, economic evaluation of the input-package, i.e., the transgenic cultivar expressing the pest-management trait(s). The efficient assembly of treatments for use in such a design can be made only after some prior testing of cultivars and pest management programs. A systems-test approach has been taken using single, high-yielding cultivars as control treatments, and single cultivars to represent types of herbicide-resistant cultivars with single or multiple weed-management programs (Wilcut et al., 1998; Wilcut et al., 1999). The same type of approach also has been done with multiple cultivars with single, representative insect and/or weed management programs appropriate for each of the respective conventional or transgenic cultivars (Bryant et al., 1999).

Two Factor Designs

A. Complete Factorial and Complete Split-Plot Designs. Common two-factor designs such as complete, split-plot and factorial designs, are not efficient for conducting applied experiments intended to contrast the effects of pest management programs on the currently-available transgenic cultivars in comparison with conventional cultivars. In many instances, the effects of pest management treatments, appropriate for the transgenic cultivars, are obvious and very negative when applied to the conventional cultivars. For example, the conventional cotton cultivars do not tolerate the herbicides, Buctril or Roundup Ultra. Likewise, supplemental chemical treatments for insect management that may be sufficient for Bt cultivars, may be grossly insufficient for conventional cultivars. While information about comparative chemical tolerance or absolute levels of pest damage would be necessary in early stages of cultivar development, plots illustrating such gross effects in a test program intended to develop recommendations for candidate commercial cultivars would be superfluous.

However, if working within a single type of transgenic cultivar, split-plot or factorial tests may be used effectively for certain purposes. An example of such an approach is the gene equivalency test required by Monsanto for all candidate Roundup Ready cultivars. In these experiments, cultivars are grown in weed management programs without Roundup Ultra, and with the full, labeled rate of Roundup Ultra. Equivalent yields between plus and minus Roundup Ultra treatments indicate sufficient tolerance. If there is no significant interaction of cultivars with herbicides in the analysis of variance (ANOVA) ($P < 0.05$), this indicates that the group of tested cultivars responded similarly with or without Roundup Ultra.

It has been argued that the OCTs represent a valid means to rank the yields of conventional and Roundup Ready cultivars, because the released Roundup Ready cultivars were not associated with positive ($P < 0.05$) interactions in the gene equivalency test. While such a demonstration of tolerance is clearly a positive finding for gene expression, lack of a significant interaction does not obviate the main effect of pest management nor indicate that the possibility of differential response to pest management has been excluded at the ANOVA test-level. Clearly, when significant interactions are found in two-factor experiments, the main effect of one variable cannot be applied to the individual members of the set of the other variable; however, failure to detect a significant interaction is not equivalent to proving its absence. The **Type I error** (typically $P < 0.05$) estimates the probability of rejecting the null hypothesis when it is true, i.e., that there are **no differences.** Elimination of the possibility of an interaction effect requires the rejecting the hypothesis that there are **real differences**, that is the null hypothesis is false, i.e., the **Type II error**. The probability of a Type II error is not fixed by the experimenter, but by the variability of the data in the experiment. Thus, failure to find a significant interaction at ($P < 0.05$) does not mean that there is 95% chance that, the cultivars, for example, responded in the same manner to the pest management treatments. Since the probability of response depends on the variances, it is generally lower than the selected alpha level. Therefore, using OCT data to rank yields of Roundup Ready and conventional cultivars, when no Roundup has been applied to the Roundup Ready cultivars, is not a conservative assumption and should be done with qualification.

B. Strip-Plot and Nested Split-Plot Designs. The strip-plot and split-plot treatment designs (Gomez and Gomez, 1984) are particularly useful for conducting cultivar x pest management trials, where pest management treatments need to be applied in a systematic manner, while minimizing spray drift to adjacent plots. For example, the response of Roundup Ready cultivars to various herbicide programs can be determined using a strip-plot treatment design. In this plot arrangement, herbicide treatments are the vertical treatment factor (applied in the direction of the row), and cultivars are the horizontal factor (perpendicular to the row) (May et al., 2000).

For certain experimental objectives, the choice of treatments and experimental designs are not simple. As indicated, conventional cultivars cannot tolerate certain pesticides that the transgenic cultivars do tolerate by reason of their genetic transformation, e.g., post emergence applications Buctril or Roundup Ultra herbicide. For efficient use of resources, trials intended to compare the effects of such pesticides across transgenic and conventional cultivars should nest the statistical effect of cultivar within the pest management effect. As an example, Murdock et al. (2000) conducted a trial to compare non-transgenic cultivars grown using a soil-applied herbicide program, with Roundup Ready cultivars produced using the same herbicide program, and with a system using only post emergence applications of Roundup Ultra. In this trial, the main effect of pest management system is found directly from the analysis of variance, but since cultivars were nested within the herbicide programs, comparisons of means between transgenic and non-transgenic cultivars are precluded. An ideal experimental design for comparison of transgenic and conventional cultivars would be one allowing efficient planting, spraying, and harvesting; and the comparison of all treatment means, e.g., pest management, cultivars, and their interaction.

Summary

When a new cultivar is offered for general sales, two years of data should be available to growers from public testing sources, or be released to the public from privately conducted research. All such research information should be supported by published data that meet the standards normally accepted by the scientific community for the substantiation of results. An evaluation system should be established to generate yield performance, pest management efficacy (where applicable), and net return data for all new cotton cultivars in comparison to established, high-yielding cutivars, whether conventional or transgenic. The data should be made available to the public when the decision is made to commercially release the new cultivar. Coordination of evaluation efforts between the public and private sectors, and among plant breeders, crop production specialists, pest management researchers, and agricultural economists in the public sector would facilitate the generation of the data needed for growers to make informed choices about cultivars. Exceptions to such a comprehensive pre-commercial evaluation system obviously could be justified when emergency situations require immediate access to technology, as in 1996, when the Bt cultivars were urgently needed due to failures of synthetic pyrethroid insecticides to control pyrethroid-resistant tobacco budworm (*Heliothis virescens*) in Alabama in 1995.

Currently the Cotton Industry is challenged to define a system for conveying relevant information to cotton growers about the field performance, benefits, and possible disadvantages of transgenic cotton cultivars, when they are first offered for sale. These proposed guidelines represent a consensus, among the majority of representatives of public and private sector agencies serving on the working group, concerned with growers' needs for information on the overall value of transgenic cultivars and identification of one possible means to achieve it. Because transgenic cultivars, with pest-management traits, are dual-purpose products, the bottom-line effects of cultivar and pest management are inseparable. To provide growers with a direct comparison of the economic, agronomic, and pest management performance of transgenic and conventional cultivars, we propose that cultivars should be compared with each other while employing their respective pest management programs, that is in a systems testing program that compound treatments in a single factor design as described in I(B) above.

References

Bryant, K.J., C. T. Allen, F.M. Bourland, and L.D. Earnest. 1999. Cost and return comparison of Roundup Ready® and Bollgard® cotton varieties. P 236-238. *In* Proc. Of the Beltwide Cotton Conf., Orlando, FL. 3-7 Jan. 1999 Natl. Cotton Council of America, Memphis, TN.

Gomez, K.A., and A.A. Gomez. 1984. Statistical procedures for agricultural research. John Wiley & Sons, New York, NY.

May, O.L, M.J. Sullivan, D.K. Barefield, and D. Robinson. Part II. Performance of cotton cultivars and strains in South Carolina. *In* Performance of field crops in South Carolina: Section 3 – Cotton. South Carolina Agric. Expt. Stn. Circ. 184. Revised annually. 1991-1999.

May, O.L., E.C. Murdock, and R. Graham. 2000. Herbicide system affects yield of Roundup Ready® cotton cultivars. *In* Proc. Betwide Cotton Conf., San Antonio, TX. 3-7 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN (In press).

Murdock, E.C., O.L. May, and R. Graham. 2000. Herbicide System by Roundup Ready® and Conventional Cultivar Trial. *In* Proc. Betwide Cotton Conf., San Antonio, TX. 3-7 Jan. 2000. Natl. Cotton Counc. Am., Memphis, TN (In press).

Rayburn, S.T., R. Britton, and E. Keene. 1998. National Cotton Variety Tests. USDA-ARS, Stoneville, MS.

USDA-AMS. Cotton varieties planted. 1995-1999. Revised annually. USDA-AMS, Memphis, TN.

Wilcut, J.W., B.J. Brecke, D.C. Bridges, J.M. Chandler, R. Hayes, R.L. Nichols, and C.E. Snipes. 1998. A beltwide perspective on new weed management technologies in cotton. P. 846. *In* Proc. Beltwide Cotton Conf., Nashville, TN 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Wilcut, J.W., S.D. Askew, B.J. Brecke, D.C. Bridges, S.M. Brown, J.M. Chandler, R.M. Hayes, J.A. Kendig, D.K. Miller, R.L. Nichols, and C.E. Snipes. 1999. A beltwide evaluation of weed management in transgenic and non-transgenic cotton. P. 746. *In* Proc. Beltwide Cotton Conf., Orlando, FL 3-7 Jan. 1999. Natl. Cotton Counc. Am., Memphis, TN.

Acknowledgement and Disclaimer

The authors thank the participants of the Transgenic Evaluation Working Group for their time, efforts, and ideas; and, likewise, thank those who contributed comments to the proposal presented at College Station on July 18, 1999. Not all the participants of the working group agreed with all points in the proposed guidelines, as presented at College Station, nor with all the points presented in this report. The content of this report represents the recommendation of the authors, and is not the official policy of any company or university.

Table 1. Percentage of U.S. Cotton planted with transgenic cotton cultivars since 1995.[†]

Type	1995	1996	1997	1998	1999
	%				
BXN	<0.1	0.1	1.2	5.9	7.8
Bt	0	12.0	17.9	18.0	16.9
Roundup Ready	0	0	3.1	17.1	24.2
Bt/RR	0	0	0.5	3.6	11.4
Total	<0.1	12.1	22.7	44.6	60.3

[†] USDA-AMS (1995-1999).

Table 2. Percentage of U.S. Cotton planted with Bt and Roundup Ready transgenes since 1996.

Transgene	1996	1997	1998	1999
	%			
Bt	12.0	18.4	21.6	28.3
Roundup Ready	0	3.1	20.7	35.6

Percentages include transgenes occurring as solo insertions or in combination. Same source as Table 1.

Appendix I. Transgenic Evaluation Working Group

Participants at the March 30 Working Group Meeting in Little Rock, AR:

Lloyd May – USDA-ARS, Florence, SC - Chair
Daryl Bowman – North Carolina State University
Frank Carter – National Cotton Council
Jane Dever – AgrEvo/Cotton Seed International
Dave Dickerson – Stoneville Pedigreed Seed Company
Bob Hayes – University of Tennessee
Merritt Holman – Louisiana State University
Tom Kerby – Delta & Pineland Company
Roger Leonard – Louisiana State University
Bill Lalor – Cotton Incorporated
Bill Meredith – USDA-ARS, Stoneville, MS
Bob Nichols – Cotton Incorporated
Wayne Smith – Texas A&M University
Richard Voth – Monsanto
Vernon Wenger – Rhone Poulenc Agricultural Company

Drafting Sub-Committee Nominated at the Working Group Meeting:

Lloyd May, Bob Nichols, Tom Kerby, Jane Dever plus
Steve Brown – University of Georgia
Jeff Silvertooth – University of Arizona