Actions and Plans for Cotton Biotechnology at Dow AgroSciences

Discipline: Breeding & Genetics

David M. Anderson

Phytogen Seed Company, LLC

Dow AgroSciences

P.O. Box 787

850 Plymouth Ave.

Corcoran, CA 93212-0787 (USA)

Email: dmanderson1@dow.com

Phone: 1-559-992-3988

FAX: 1-559-992-2581

The author thanks his many colleagues, collaborators and staff at Phytogen and within Dow AgroSciences for their many contributions and tireless efforts in building our very successful cotton program.

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Abstract

Dow AgroSciences' market facing cotton biotechnology business in the US is Phytogen Seed Company, LLC. Phytogen Seed Company, LLC was created by Dow AgroSciences and the J. G. Boswell Company in 1998 by joining their respective cotton technology and germplasm assets to establish a single, stronger entity called Phytogen Seed Company, LLC. Phytogen's mission from the outset has been to improve the overall economics of cotton production for growers by becoming the cotton industry's preferred technology provider in high yielding cultivars prized by the mills for their spinning performance. Phytogen's extensive germplasm library and its access to leading edge technology provide the Company with a solid business foundation upon which it has built its reputation as a respected partner in the cotton industry. Dow AgroSciences continues to evaluate long-term needs of the cotton industry with a view toward increasing the global market for cotton fiber by enabling new end uses while continuing to address growers' needs for higher yields and lower costs of production.

Keywords: transformation; germplasm; global cotton fiber demand.

Phytogen Seed Company, LLC, Dow AgroSciences' market facing cottonseed business, was created in 1998 by combining the cotton research, intellectual property and germplasm efforts of two separate programs that had been developing in parallel into a single entity. These included Phytogen owned by the J.G. Boswell Company and the cotton assets of Mycogen owned by Dow AgroSciences. Phytogen was formed with the intent of developing and applying leading edge technology in the creation and implementation of practical solutions to the many problems facing US cotton growers. From the beginning, our mission has been to improve the overall economics of cotton production for growers by becoming the cotton industry's preferred technology provider in high yielding cultivars prized by the mills for their spinning performance. The Company has come a very long way and our history is briefly reviewed here for the edification of the reader.

Phytogen was founded in 1980 as a plant biotechnology company, with the J.G. Boswell Company as the majority shareholder. Boswell, based in California, is the world's largest single producer of long staple cotton. The Company's laboratory operations commenced in Pasadena, CA early in 1981. The focus was to develop plant transformation capabilities and extend them to the high quality Acala cottons grown in the San Joaquin Valley of California. At the same time, David Padua formed Agrigenetics with similar objectives but a much broader crop focus extending beyond but including cotton. Agrigenetics purchased GroAgri Seed Company in Lubbock, TX giving Agrigenetics a

cottonseed production and retail marketing presence in Texas and entry into cotton germplasm development. In 1982 Phytogen filed for patent protection of its plant vector, the first such filing in the industry, and conducted the first field trials with regenerated cotton plants in 1983. In 1984, GroAgri Seed Company hired Dr. Bobby Phipps as cotton breeder to expand its cotton breeding program and to evaluate a hybrid cotton system. Agrigenetics filed certain Bt patent applications during the same year. In 1987, Phytogen filed patent applications covering cotton regeneration and transformation, and in 1988 hired Dr. H.B. Cooper and initiated its SJV cotton breeding program. Phytogen began construction of its cotton breeding R&D facility in Corcoran, CA in 1989. At the same time, Agrigenetics acquired Sungene, moved scientists from the San Jose, CA facility to Agrigenetics' Madison, WI Laboratory and expanded its cotton transformation activities. A collaborative relationship was established with an Indian company for development of cotton germplasm including both pure lines and hybrids. In 1990 the Agrigenetics cotton breeding program was moved to Arizona and the germplasm development program was refocused on breeding for pure lines and hybrid cultivars for picker markets. In the same year, Phytogen worked with Mr. Jack Hamilton to establish a breeding nursery in Lake Providence, LA with the intent of extending high fiber quality germplasm development into the Midsouth. In 1992, Phytogen initiated international field trials of proprietary cultivars in Greece, Spain, Turkey, Argentina, Colombia, Guatemala, South Africa and Pakistan. The Company’s first international sales took place in Greece in 1992. In the same year, Mycogen Corporation acquired

Agrigenetics and subsequently established a cotton winter nursery in Puerto Rico. In 1993 the first commercial sale of a proprietary Phytogen Acala cultivar in the US, Kings Acala Plus, was booked. In 1994 Mycogen appointed a cotton breeder and the cotton breeding station in Leland, MS was established, and in 1995, Phytogen released its first proprietary Pima Cultivar, Oro Blanco Pima. Mycogen's cotton breeding station in AZ was closed and a transgenic breeding station was established at Woodland, CA. In 1996, Phytogen's cotton breeding activities were expanded in the San Joaquin Valley and Boswell sought a technology partner for Phytogen. In the same year, Dow AgroSciences acquired an equity position in Mycogen Corporation. Mycogen moved the cotton transformation activities from Madison, Wisconsin to Dow AgroSciences' Laboratories in Indianapolis. In 1997, Boswell and Mycogen entered into negotiations concerning the formation of a new cotton joint venture. In the same year, Mycogen licensed the cotton germplasm from Mississippi State that lead to the development of PSC355. In 1998, Boswell and Mycogen announced the formation of Phytogen Seed Company, LLC, with Mycogen as the majority shareholder, thereby joining Mycogen's cotton transformation capabilities, technology base, Bt library, insect resistance patent estate, and Mid-South breeding program with Phytogen's western, Midsouth and international cotton breeding programs, cotton transformation capabilities, cotton-related patent estate and seed production expertise. At the end of the year, Dow AgroSciences acquired Mycogen. Dow AgroSciences is now the majority shareholder in Phytogen Seed Company, LLC in partnership with J.G. Boswell.

In terms of gene transfer, cotton biotechnology and input traits, a number of significant patents have issued to Dow AgroSciences out of its own programs including those in Indianapolis, Mycogen as well as from the Boswell/ Phytogen laboratory formerly in Pasadena, California. A few highlights include the patents on *Bacillus thuringiensis* ("Bt") genes in plants for insect resistance (USP 6,943,282), glyphosate tolerant cotton (USP 6,753,463), transformation capabilities including Acala cottons (USP 6,620,990; USP 6,573,437), cotton traits obtained via somaclonal variation (USP 5,859,321) and patents on cotton expressing lectin genes for insect and pest resistance (USP 7,157,617).

The Bt and glyphosate patents are of course of extreme interest to the industry. The Acala transformation is also of particular note in that it provides a significantly different genetic background from the Coker germplasm used extensively in other programs. Genetic diversity is clearly desirable for long-term crop improvement, and simple sequence repeat analysis presents a picture of a cotton germplasm pool that is relatively narrow (de Magalhaes Bertini et al, 2006; Liu et al, 2000;). Greater than 80% of the US cotton crop is transgenic and prior to 2006, all transgenic cottons shared in part a common Coker genetic background. This was due in large part to the recalcitrant nature of most cotton cultivars except Coker to regeneration with the methods employed (Mishra et al). At Dow AgroSciences we employ protocols that provide efficient transformation and regeneration with a broad range of genotypes. Dow AgroSciences' WideStrike™ Insect Protection, commercialized in 2006 in the cotton cultivar

PHY440W, comes out of an Acala transformation event. Hence, WideStrike™ cotton presents the breeders in our introgression program with a different genetic source (Acala) than that (Coker) available to breeders using other Bts such as Bollgard™ from Monsanto and VipCot™ from Syngenta. Lack of genetic diversity is a recognized threat to sustaining high yields (Walsh, 1981; Van Esbroeck et al, 1998). Beyond the arguments around the desirability of not having all commercial transgenic cotton in the US share a common ancestor, the Acala background is providing our breeding program with fiber quality and disease resistance traits not resident in Coker cottons.

Dow AgroSciences' cotton biotechnology program today

As briefly mentioned above, one of the key developments coming out of our research effort is WideStrike™ Insect Protection. WideStrike™ is a new, stacked insect-protection trait researched and developed for use in the cotton market. Transformation was achieved in an Acala cultivar using gluphosinate herbicide as a selectable marker. The trait expresses two *Bacillus thuringiensis* (Bt) proteins, Cry1F and Cry1Ac, in cotton plants, providing season-long protection from a broad spectrum of destructive cotton pests. Results from multiple years of field trials show that WideStrike provides a high level of activity against worm pests such as cotton bollworm, tobacco budworm, beet armyworm, fall armyworm, soybean loopers, cabbage loopers, and pink bollworm. A moderate level of activity is also seen against black cutworm while beneficial insects are unharmed. Trial results have been consistent throughout the Southeast, Mid-

South, Texas and western growing regions. WideStrike™ provides growers with a powerful choice in the market place in cotton insect protection and as we all know, competition is good for the entire industry. Dow AgroSciences continues to explore new insect technologies as stand alone products and in potential combinations with other insect resistance traits.

Gluphosinate was used as a selectable marker in our WideStrike™ transformation process. Given the emergence of glyphosate tolerant weeds in several key areas of the cotton belt, questions are often asked as to the level of gluphosinate tolerance that may be present in WideStrike™ cotton as a potential means for management of glyphosate resistant weeds in Round-up Ready™ cotton. PHY485 WRF and PHY375 WRF, which are stacks of WideStrike™ plus Round-up Ready™ Flex, carry the gluphosinate selectable marker. DAS has issued the following statement on this point. *"The PAT gene (that confers a level of glufosinate-ammonium tolerance) was used as a selectable marker in the development of WideStrike™ Insect Protection technology. However, WideStrike™ cotton cultivars are not promoted or warranted by Bayer or DAS as tolerant to Ignite. 1) Use of glufosinate-ammonium herbicides over the top of WideStrike™ cotton cultivars may result in injury to the cotton crop; 2) Ignite™ can be used per the Ignite™ label with the use of a hooded sprayer; and 3) Glufosinate-ammonium herbicides should not be used to "burn down" WideStrike™ cotton cultivars in replant or other situations as some of the cotton crop may survive such herbicide treatment."*

Dow AgroSciences, as an output of its ongoing trait discovery program, has recently announced the development of a new family of traits providing innovative herbicide tolerance technology that can improve the performance of industry-leading weed control systems in corn, soybeans and cotton. The convenient, cost-effective solution will enhance and protect today's herbicide tolerant crop technology that the majority of growers have adopted and provide an effective resistance management tool. The technology consists of new herbicide traits that provide tolerance to existing broadleaf and grass herbicides. Additionally, it will be compatible with all popular weed control systems, expanding growers' options for protecting their yields and managing changing weed spectrums in crops while preserving the viability of the current industry-leading technologies.

In addition to transformation per se, we have added a number of additional technical capabilities that are a routine component of our cotton R&D effort today. Genome mapping is used for pedigree verification, recurrent parent genome recapture and in support of trait-associated marker development. Marker assisted selection is used to capture and track traits of interest as they are moved from one breeding line or germplasm stock to another. Mutations derived both as a consequence of somaclonal variation as well as induced via mutagenic agents are used to provide novel variants in breeding populations with otherwise excellent agronomic performance. Triple hybrids are being employed for the capture of traits resident in diploids and unavailable by normal breeding

methods with our commercial allotetraploids. Markers are associated with such traits to assist in the introgression process. Dihaploids are being used to create homozygous stocks to enhance marker selection as well as trait capture. New approaches to *in-situ* gene modification are being explored to alter biochemical pathways for output trait analysis as well as to enhance gene expression for input traits such as herbicide tolerance and pathogen resistance.

Dow AgroSciences, thru Phytogen, has successfully moved its technology out of the laboratory and into the field, developing cultivars that are among the top performers in lint yield and gross margin across the cotton belt. Our cultivars consistently place in the top tier in public and private testing programs across the cotton belt, illustrating the rapid gains that have been made in Phytogen's breeding program in bringing forward herbicide tolerance and insect resistance (WideStrike™) in high yielding cultivars with excellent fiber quality. Furthermore, we have been a good partner to the industry by leveraging our germplasm and testing resources through collaborations and alliances with universities as well as private breeding programs. An excellent example is the commercialization of the cultivar PSC355 and its transgenic counterparts (PHY440W; PHY485WRF; PHY415RF). These PSC355 conversions represent high yielding cotton cultivars developed from germplasm licensed to Phytogen from the MAFES, a collaboration that has benefited both parties.

As our germplasm, trait resources, and breeding capabilities have developed, Phytogen has increased market share year on year. This occurred first in the Acala and Pima growing regions of the West, where we have held a 60% to 70% market share for several years. Now, with the launch of our new proprietary germplasm carrying WideStrike™ and WideStrike™ stacked with Round-up Ready™ Flex, we have captured a significant share in several Midsouth and Southeast market segments. Over the last three years, Phytogen has grown from under 2% of the US market in 2004 to a 6.5% share of the total US market in 2007. Phytogen may be the only major US cottonseed company that experienced both total unit sales and market share growth in 2007 versus the prior year.

Planning and working toward the future

World cotton consumption grew steadily during the 9 year period between 1999 and 2007 (Meyer et al, 2007), and consumption outpaced production in every year except two (2001 and 2004). Global cotton consumption is continuing its strong pace in the present year, with mill use in 2007 expected to top 125 million bales and exceeding world production by some 11 million bales. Within the US, total business revenue stimulated by cotton is annually pegged at $120 billion, arguably the greatest of any US agricultural crop. Even with the estimated 28% drop in planted acres in 2007 versus the prior 5 year average, cotton's total economic impact will still exceed $100 billion. Cotton now represents 84% of the fiber used by the US spinning system (Otte, 2007) and consumer preference for cotton over manmade fibers remains strong.

On the surface, one might reasonably expect these to be good times for the US cotton industry in general and for US cotton growers in particular. Unfortunately, this is not and has not been the case. During the last decade, US cotton mill use has fallen from roughly 2/3 of the US crop to its present level of 25% or less. The situation illustrates the importance of the export market for US cotton producers. The average cotton yield per planted US acre has increased about 2.5% per year over the last decade. This might be nearly sufficient to keep pace with rising operating expenses were it not for the poor prices received for cotton. Consider the following. The average price received per lb in 1975 was $0.60, in 1980 was $0.62, in 1985 was $0.62, in 1990 was $0.62, in 1995 was $0.79, in 2000 was 0.52, and in 2005 was $0.50. Does anyone see a pattern here? The price in 2007 is around $0.62, which means in effect that the cotton grower is working for the same wage today that he was in 1975. At Dow AgroSciences/ Phytogen, we see this as the critical issue to be addressed as we move forward toward the future.

Poor global price performance in the face of consumer demand growth is due in large part to carryover stocks during those years in which production has exceeded demand. In spite of the favorable use to production ratio, carryover stocks are high and diminishing only slowly over time. The situation will not improve on its own. As new technologies are launched in cotton globally, world production could easily outpace demand on a regular basis. As an example, if Bt

cotton continues to deliver yield increases at the current rate in India and China, we could see a 15 million bale increase in production per year develop over the next 5 year period due to increased yields. This increase is achievable even without any new ground being brought into production. It doesn't take much foresight to see that it will take a corresponding 15 million bale increase in cotton used by the world spinning system over the same period of time just to maintain prices within their present range.

Increasing yield per acre and lowering costs of production are critical components of profitability for the US cotton grower, but ultimately we believe that the answer to a healthy US cotton farm economy is to be found in achieving a higher world price for cotton fiber. In truth, since we sell into a global market, the ultimate answer to a healthy US cotton farm economy will be secured in a healthy world cotton farm economy. At Dow AgroSciences/ Phytogen, we believe that goal can only be achieved by enhancing cotton fiber's functionality to enable new end uses that in turn drive higher demand, capture a higher proportion of the world spindles (most likely at the expense of man made fibers including polyester), and thereby stabilize and elevate prices over time. As a Company, we are pursuing this goal in our cotton R&D program along two primary tracks.

The first approach, which we at Dow AgroSciences/ Phytogen consider an essential near term fix, is to enable growers of our cultivars to produce and

present cotton fiber to the world market that is longer, stronger, finer and free of contamination. The world market rewards producers of fiber with these qualities and the highest quality bales get higher prices within the range paid, whatever that price range may be. The end users who produce the highest quality fabrics require these attributes and preferentially buy fiber that delivers these qualities. Dow AgroSciences' commitment is that the growers of our cultivars will not have to sacrifice fiber quality to secure yield performance and quality input traits; we want them to enjoy the complete package. For example, growers of our Acala cultivars in the San Joaquin Valley have attained the highest yields available along with premiums paid for the excellent fiber delivered such as for PHY72 Acala, which has a very long staple length. Because of the attributes of the fiber, our growers are able to further enhance demand for their fiber by roller ginning PHY72 Acala as well as PHY725 RF, our new Round-up Ready Flex™ Acala cultivar. These cultivars, although they are Acala (upland) cottons, lend themselves very well to the roller ginning process used to gin Pima (G. barbadense) cottons. Roller ginning PHY72 and PHY725RF typically moves them from staple 39 - 40 up into the staple 41 and staple 42 range, which results in both a higher demand and a higher price. At Phytogen we are leveraging our significant germplasm resources to elevate the fiber quality of our Midsouth upland cotton cultivars to equal and eventually exceed that of the Acalas. We believe that as our seed customers begin producing cottons with these qualities, they will experience opportunities for securing stronger demand and higher prices in global markets.

The second approach being taken at DAS/ Phytogen, which is longer term but one we consider to be essential, is to explore ways to alter cotton fiber to enable new end uses on a global scale. In the end, the path to higher prices received by our growers for their cotton fiber will take us to the point at which we are capturing a higher share of the world spinning system at the expense of manmade fibers; principally polyester. Clearly this will require elevating fiber attributes and developing alterations in fiber structure in order to enable new end uses. But what are those new end uses, what are the attributes that will enable them, and what alterations in fiber structure might be required? The questions are difficult to answer. Are we even asking the right questions? If polyester is our target, we know that fiber strength is one of the critical components we will need to improve. But how much fiber strength is enough? Polyester can be grouped into Low Tensile Strength ("LTS"), Medium Tensile Strength ("MTS") and High Tensile Strength ("HTS") classes. The strongest of our commercial Pima cottons, and we have the highest strength Pimas on the US market, fall just below the strength of the LTS polyester group. We presently have upland cottons under development that go well into the LTS range and Pima cottons that approach the MTS range. How much higher, if at all, do we need to take fiber strength in order to begin to enable end uses presently only allowed by LTS or MTS polyester fiber? Are there other attributes along with strength, such as elongation, that must be altered in order to achieve new functionality? It is Dow AgroSciences' intention to secure answers to these questions, either internally or

thru alliances with other entities, and use those answers to direct our cotton improvement research.

Consumers consistently identify significant unmet needs in cotton fabrics and articulate desired new functionalities including modified permeability, improved durability, shrink and wrinkle resistance, shape retention and fire retardation. Each of these individual categories has an estimated potential market impact in the US alone of $5 billion or more and if achieved in cotton, could significantly elevate cotton's share of the global spinning system. In our view, these are all improvements that can be enabled in cotton but developing fibers that address these consumer demands will require a proactive, coordinated public and private alliance in order to make them a reality. We would welcome participation in such an alliance.

At Dow AgroSciences/ Phytogen we have established a solid cotton business foundation comprising significant germplasm resources, technology and broad intellectual property assets. We are aggressively exploring ways to deliver fiber improvements in future cotton cultivars available to our customers that will ultimately drive stronger mill demand coupled with higher prices paid to growers, all with a view toward achieving Phytogen's original mission: "To improve the overall economics of cotton production for growers by becoming the cotton industry's preferred technology provider in high yielding cultivars prized by the mills for their spinning performance".

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