Developing "Organic-Ready" Maize Populations with Gametophytic Incompatibility:

2016 Final Project Report

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1. Project Summary:

The purpose of this five year breeding project was to reduce transgenic contamination of organic maize grown in the USA by maintaining the integrity of organic maize seed. Sources of gametophytic incompatibility (Ga1s from tropical corn or popcorn, and Ga2s from teosinte), a trait that strongly interferes with successful fertilization by pollen from plants without the trait, were back crossed with a number of corn populations and inbred lines. Along with other practices to maintain genetic purity of corn varieties, these traits should provide additional insurance against transgene contamination once the seed is deployed into farmers' fields. These improved lines and populations have been increased for public release of corn that will not readily cross with most field corn in the USA. Along with the seeds, videos, presentations, and interviews provided public outreach to highlight the traits, the importance of organic corn, and the work to help protect it.

2. Introduction to Topic:

Maize is an incredibly productive crop that works well in organic crop rotations in many parts of North America. Since the lax release of transgenic varieties of maize in the mid-1990s it has become increasingly difficult to grow uncontaminated organic maize or to find maize seed that is free of transgene contamination (ie. http://non-gmoreport.com/nongeneticallymodifiedfoodorganic/gmocontaminationorganicfoods.php). Maize is an outcrossing species of grass that releases millions of pollen grains from its male flowers to land on the hundreds of silks of the female flowers or ears. Transgenic varieties have become very common and it is now difficult to avoid transgenic pollen that blows on the wind anywhere maize is grown today. This is the critical issue upon which this research and development has focused.

Organic farmers are not required to produce transgene-free crops, but they must plant seed that is free of transgenes. However, buyers may reject maize grain that is contaminated and this has caused some concern among organic farmers. Some have begun planting late to try and avoid the major plume of transgenic pollen and some are spending considerable sums to test seed and crops for transgenic contamination. This is not sustainable. The naturally occurring gametophytic cross incompatibility traits found in maize and teosinte, its close relative, make it hard for pollen grains to join with the eggs unless the pollen is from a plant also carrying the same allele for strong gametophytic incompatibility. This opens up a new opportunity for organic farmers since most maize grown in North America does not carry this trait. Additionally, lines with purple aleurone layers in the seeds are normally used to help in the testing of the success of the gametophytic incompatibility breeding. If the colored layers are over hard, white endosperm, these lines could be used for the production of specialty blue corn hybrids for food corn markets. Therefore, it seems only reasonable to develop colored lines that can be used to increase the value of organic corn both by reducing outcrossing in normal yellow corn and for new, blue varieties.

However, as this is a new approach to maize isolation for most, a campaign is needed to inform farmers and seed producers how to make the most effective use of it. This genetic system is not fool proof, although it greatly reduces outcrossing, and if it breaks down we will immediately be in the same position we are now relative to transgene contamination. Along with this, organic farmers and certifiers will need to learn that synthetic varieties (modern open pollinated varieties) are not transgenic. Public outreach of seeds and information for making use of this trait are needed to successfully employ these seeds to reduce transgene contamination.

Gametophytic cross incompatibility was first noticed decades ago among some lines of popcorn that would set little or no seed if pollinated by dent corn pollen (Thomas 1955). This incompatibility trait is controlled by a single allele, $Ga1^s$, and fully incompatible lines are homozygous or fixed for this trait. Most dent corn is homozygous for the allele ga and cannot pollinate $Ga1^s$ lines, although there are some breeding populations of dent corn with $Ga1^s$ (Kutka 2009) and a patent was granted for yellow field corn lines with $Ga1^s$. Zeigler and Ashman (1994) reported that the trait is widely used in popcorn production to allow planting relatively closely to dent corn fields with much reduced risk of cross contamination.

$Ga1^s$ works because plants with this trait have silks that do not support normal pollen tube growth (and thereby fertilization) for pollen carrying the ga allele. Lausser et al. (2010) reported 0-5% of ga pollen tubes growing 8 cm into silks of homozygous $Ga1^s$ plants with most growing no more than 2 cm. Marcus Zuber, who released white dent corn inbreds with $Ga1^s$ in the early 1990s (Poneleit 2000; www.ars-grin.gov), reported outcrossing rates of 2-5% in homozygous $Ga1^s$ white dent varieties planted adjacent to normal yellow corn. However, dent outcrosses are usually even lower in popcorn fields due to cross incompatibility and the abundance of more competitive $Ga1^s$ pollen in the popcorn fields (Zeigler and Ashman, 1994).

$Tcb1^s$ and $Ga2^s$ are gametophytic cross incompatibility alleles from teosinte that have been crossed into dent corn lines by Jerry Kermicle at the University of Wisconsin (Kermicle and Evans 2010; Evans and Kermicle 2001). These genes appear to work in a similar fashion to $Ga1^s$ and dent corns of the USA are ga2 ga2 and tcb1 tcb1 in genotype. They open up further opportunities to reduce undesirable outcrossing in organic corn in the USA and they won't cross contaminate popcorn either. Use of $Tcb1^s$ has been patented and $Ga2^s$ has not; neither is being used in popcorn so their use in dent corn varieties should not interfere with popcorn production.

Although they are powerful tools, these genes are not a guarantee of long term genetic purity. Populations with these traits will need to be checked for outcrossing from time to time and normal measures to reduce outcrossing must still be put into place to receive the full benefit. Of special note would be reducing the number of volunteer plants in adjacent transgenic fields that might be heterozygous for both transgenes and a gametophytic cross incompatibility trait. Such plants would produce some pollen that carried both an incompatibility gene and a transgene, and these could contaminate plants homozygous for the same incompatibility gene. Also, corn that carries Ga1s should not be grown next to popcorn as these will readily cross contaminate one another, reducing the value of both.

A back cross breeding plan for introducing the $Ga1^s$ trait and fixing homozygous carriers was described, with final homozygous lines identified with a test crossing procedure using lines with purple/blue seeds (Thomas, 1955). This procedure was put into place by the popcorn breeding programs at Iowa State and University of Missouri. Lines of dent corn with purple seeds were developed at Iowa State to use for this testing (Zeigler and Ashman, 1994) and new, early maturing blue lines with hard, white endosperm are currently under development.

Thomas (1955) also reported that homozygous $Ga1^s$ lines used as females can be crossed by lines that are heterozygous because half of their pollen carries the $Ga1^s$ allele. The trait works by setting up a strong competition between pollen carrying and not carrying the allele with non-carriers nearly always failing to grow pollen tubes long enough to fertilize the egg cells. The seeds of a cross with homozygous carriers as females and heterozygous carriers as males results in pure, homozygous seeds (Thomas, 1955). Because of this, homozygous $Ga1^s$ lines can be used as female testers for plants carrying the allele and also as filters to remove the ga allele from a population under development or in hybrid variety formation. This procedure is currently being used in this project for several OP populations that are in development.

Conversations with farmers and researchers suggest there is interest in continuing with organic corn production because it is very profitable and useful in rotation (D. Christensen, pers. comm.; H. Darby, pers. comm.; W. Goldstein, pers. comm.; J. Lazor, pers. comm.; K. Martens, pers. comm.). There is some concern over transgene contamination and the expense of testing for contamination is a new cost required for some sales. I have had several populations contaminated while being grown with participating organic farmers with reasonable isolation in New York and North Dakota. Major Goodman at North Carolina State University and Walter Goldstein at the Manaamin Institute in Wisconsin (pers. comm.) are also working to develop more high value lines with gametophytic incompatibility for their regions.

The patents awarded to Thomas C. Hoegemeyer for his “Puramaize” have greatly cooled interest in the $Ga1^s$ trait by breeders interested in organic corn. Those patents are for using the Thomas method described in 1955 for yellow flint and dent corns that do not carry the waxy gene and they are quite controversial. However, the patents are for making hybrids from elite inbreds carrying the trait in homozygous or heterozygous form and not for population hybrids (from crosses of populations) or for open-pollinated (OP) populations. Work with $Ga2^s$ for hybrids is not patented and I am making every effort to make sure it stays in the public domain.

Can population hybrids and OP populations be of any use today with or without this trait? Kutka and Smith (2007) suggest from an empirical modeling study that synthetic varieties could easily be bred that yield 80% of the best single cross hybrids. A synthetic variety is one bred by intermating a number of inbred lines and then maintaining it as an OP population (Jones, 1918, p. 59). Hayes et al. (1944) reported on the performance of a synthetic OP population that was competitive with several University of Minnesota hybrid varieties at that time. M. Smith (pers. comm.) has developed new synthetics for organic production that yield as well as the best OP varieties available but they stand much better. With more research, better synthetics could be developed that yield up to 90% of the best hybrids, however, to date most organic farmers I have spoken with across the country are very worried by the term and unfortunately confuse synthetic varieties with transgenic technology.

Population hybrids can more quickly provide competitive yields as long as the base lines used to form them show good hybrid vigor in crosses. K. Martens (pers. comm.) reported to me that a double cross hybrid developed at Cornell by M. Smith was a good yielder and easy to produce in his organic fields because each parent was already a tall, vigorous cross. These two line parents could be maintained as OP populations and used for crosses that show the same amount of hybrid vigor (Hayes, et al., 1955). These should also be outside the Hoegemeyer patents given the claims made in the patent applications concerning inbreds and inbred hybrids.

3. Objectives Statement:

First Objective: the development and release of "Organic-Ready" maize populations and lines for organic farmers by crossing the $Ga1^s$ and $Ga2^s$ alleles for gametophytic incompatibility into a number of modern maize populations and lines with maturities from 75-125 days. Thirty seven populations and lines were started in the development pipeline.

Second Objective: the development and release of maize lines with purple seeds ("blue corn") for use in testing for $Ga1^s$ and $Ga2^s$ and for production of organic blue corn.

Third Objective: the education of seed producers and organic farmers on how to use these "Organic-Ready" varieties for reducing the incidence of transgenic contamination.

4. Materials and Methods:

The Thomas (1955) backcrossing methods were used to finish the development of several $Ga1^s$ OP and hybrid parent populations that Seed We Need has been working on since 2001. Similar methods are also being applied to bring the $Ga2^s$ trait into populations and inbred lines for use as OP varieties and single cross hybrids (see results below). White dent lines carrying $Ga1^s$ from Marcus Zuber in Missouri (Mo501w, Mo506w, Mo508w; www.ars-grin.gov), both white and yellow dent carriers from the Maize Genetic Stocks Center in Illinois (401D, 401E; www.maizegdb.org), and the Ga2s lines released by Jerry Kermicle to the Maize Genetic Stocks Center (511L, 511M) have been crossed to populations and inbreds from Seed We Need, Iowa State University and the National Plant Introduction Station (Table 1) in order to generate these new populations and lines. An early version of the Late SS and Late NS populations was released to the USDA (Kutka, 2009) and others have been released to small commercial operations (see results below).

In order to finish this development, new lines with purple kernels are needed. In the final test of successful gametophytic incompatibility plants that should be homozygous are pollinated with pollen from plants with purple/blue colored seed. The following day they are selfed. Those that have the trait working properly will set no blue seeds but the extra day head start will result in some blue seeds in plants where the gametophytic incompatibility system is not working properly. These were then removed from the population. Seed We Need worked to develop new purple/blue lines with white endosperms for the blue corn market. These lines were selfed until homozygous for the colored kernels and new lines were developed by first crossing very good yellow lines with white corn, followed by three or more seasons of back crossing, crossing to colored lines, three or more seasons of back crossing, and then selfing for two seasons. This process continued throughout and is only partly finished due to weather and technique related setbacks. An entire generation had to be rejected because of poor hand pollination technique in the nursery in Chile in 2014-2015, which resulted in mixed yellow, white, and blue kernels in what should have been inbred lines.

The North Dakota nursery (transitional organic until 2015) was used for the 75-85 day populations, whereas the later maturities were worked on in conventional nurseries in New York at Cornell University, Ohio at Miami University, and at North Carolina State University. An organic winter nursery in Puerto Rico headed up by Margaret Smith, and conventional nurseries in Florida (operated by 27 Farms) and in Chile (operated by Vivero Del Sur) were used to speed up progress by adding a second season annually.

Several populations were tested for presence of transgenes prior to further use or release. PCR tests for the two commonly used promoter sequences (CaMV 35S and NOS) were carried out by a commercial lab. One hundred seed samples (ten kernels each from 10 ears) were taken to keep testing costs low. As the ears were hand pollinated negative results were considered to be relatively indicative of a lack of contamination, but absolute certainty cannot be assured.

5. Project Results:
Objective 1:
Painted Mountain (Composite OP) Development and Conversion: The first crosses of Dave Christensen's Painted Mountain with Ga1s carrying lines took place in 2003. Dave then made some further crosses and selections in Montana before sending me seeds of better ears to plant in North Dakota. I had also made crosses of Painted Mountain with earlier and unrelated Ga1s carriers to provide a broader genetic base for the new population. With this grant intensive selection of Painted Mountain and related lines got under way. The basic approach was to select Ga1s lines from the crosses that were homozygous, then to cross various Painted Mountain crosses and improved selections carrying Ga1s onto these mother lines. There were some serious setbacks, the worst being the spray drift incident in 2014 that wiped out all but five plants homozygous for Ga1s, and the drought of 2015 (9" of rain by early September) that killed or badly injured most of the corn. As of 2016 there is a mixed population based on Painted Mountain that is homozygous for Ga1s. Because of the narrow genetic base, an additional year of crossing and back crossing is needed to fully finish this population, but it looks very promising and perhaps improved over the original Painted Mountain. This will be a multicolored flint/flour population for the North. It will be released as **Independence (Cycle 0)** when it is ready.

Badlander (Synthetic OP) and Pete Seeger (Synthetic OP) Development and Conversion: These populations began in the late 1990s as crosses of New England Flints with Painted Mountain and later crosses with early flints from the North (Badlander), or as inbred lines from a number of classic New England Flint varieties and other eight row flint varieties (Pete Seeger). Additional lines were developed by crossing early 8 row flints onto later but much improved lines, followed by inbreeding and reselection. These lines were intermated to form two separate flint populations which were both crossed onto the Ga2s version of the Euro Flint lines described below. Because of the drought of 2015, which greatly reduced the numbers of plants available to work with, the two populations were merged to move forward with back crossing in the winter of 2015-2016. The homozygous version of this Ga2s population will be crossed onto the new version of Pete Seeger and released in 2017 or 2018 depending on weather. This will be a multicolored flint population for the North, and will be released as **Defiance (Cycle 0)**.

Early Riser (Synthetic OP), Falconer (Synthetic OP), and Red River (Synthetic OP) Development and Conversion: Early Riser began as a composite of early populations bred by the University of Guelph. This version has had some popularity among OP corn growers in upstate New York and New England. Falconer is an old variety from North Dakota. Inbred lines were selected and intermated, and also crossed with similar types of lines from early flint by dent crosses. Red River is a synthetic variety bred by intermating 16 inbred lines from North Dakota and New York. Each of these populations has been formulated and intermated in North Dakota and in winter nurseries, then crossed with Ga2s carriers of later maturity, and backcrossed for several generations. The drought of 2015 so depopulated the nursery that in 2015-2016 I intermated all of the remaining plants and backcrossed them onto homozygous plants. The population is a bit diverse now, but basically ready for growth and selection on farm this year (there are about 4 pounds of seed currently). I will almost certainly cross some of this onto improved lines from all three populations again and back cross one more time to beef it up for commercial grain production in the North. The seed as it exists now will be grown out under organic conditions in 2016 in Vermont and selected for maturity, productivity, and good standing ability. This new mixed population is yellow dent and will be released as **Uprising (Cycle 0).**

Dairyland (Composite OP) Development and Conversion: Development of this population continued as part of this project. The original population was formed from a high quality silage composite from the University of Wisconsin and the early stiff stalk synthetic from the University of Guelph in 2004. It has since been grown out in North Dakota in 2006, but it had to be rescued after transgene contamination at the organic farm. As part of this project the cleaned up seed was crossed to a Midwestern population that carries brown midrib and to a Great Plains flint composite with known high quality grain and forage. There were a few setbacks in getting it crossed to the Ga2s carriers, including very untimely flowering in 2014 and some difficulty in reselecting the brown midrib trait. With a successful year of controlled pollinations in New York in 2015, it has again progressed very well. This project currently exists as three populations that need to be intermated and back-crossed onto the Ga2s carrier, followed by reselection for brown midrib. This will take another two to three years. When finished this forage population will be released as **Insurgent (Cycle 0)**.

Hayes Yellow Dent (Synthetic OP) Development and Conversion: Work on this population began in 2003 and the choice of parents was largely driven by results from hybrid variety trials in New York. Inbred parents were intermated to form an 8 line synthetic. Hayes was released to farmers via Albert Lea Seed House a few years ago, but a version was crossed with Ga1s carriers in North Dakota and several back crossings and seed increases were carried out as part of this project. Seed of the final population with gametophytic incompatibility, named **Revolt (Cycle 0)**, was released to Sand Hill Preservation Center and to Green Haven Open Pollinated Seed Group in 2015. Some seed was lost in the mail, but Green Haven Group said they would continue to increase the variety.

Henry Wallace (Synthetic OP) Development and Conversion: This population began as some preliminary crosses among commercial and university corn belt dent inbred lines in 2003. Additional lines were later included and these were crossed with some of my earlier Stiff Stalk and Non-Stiff Stalk crosses with the Ga1s carriers from Missouri as part of this project. These have been through crossing and back crossing to move Ga1s into the new synthetic, the population was pollinated with blue corn pollen and selfed in New York to prove homozygosity, and then it was increased for release in a winter nursery. However, the winter increase limited the ears to just 30, a very narrow population base. Additional back-crosses were carried out in Ohio, in winter nurseries, and in North Carolina. These ears were tested for adventitious presence and came back clean. The population has been released to two Green Haven Open Pollinated Seed Group and to Sand Hill Preservation

Center as **Rebellion (Cycle 0)** in 2015, and five bushels were sent to a cooperative corn breeding project among organic farmers and the University of Illinois in February 2016. It is considered an experimental variety for now, but most of the seed has sold and I look forward to hearing how well it did or did not do for grain production and staying free of contamination. It will be increased under organic management in Illinois this summer.

1776 (Synthetic OP) Development and Conversion: Formation of this eight-line synthetic, based on lines from Iowa and Pennsylvania, began in New York in 2003. An Amish farming group has grown it and passed it around since 2012, and it is carried by Sand Hill Preservation Center. It was crossed with Ga1s carriers from the Midwest (Henry Wallace breeding population) as part of this project and then back crossed onto the Ga1s carriers. It could use another season or two of back crossing and seed increase, with this work shifting from New York and North Carolina to Illinois. It will be released as **1776! (Cycle 0)** when it is ready. As a back up, a version of 1776 that carried Ga2s was bred in New York and currently exists as probably homozygous for Ga2s and 50% 1776 by pedigree. Expansion of the genetic base of this population would be beneficial before release as a viable OP population.

Major Goodman (Synthetic OP) Development and Conversion: As part of this project North Carolina lines carrying Ga1s were intermated and then crossed with other lines with southern adaptation (Argentina, Mexico, North Carolina, Tennessee). Things got off to a slow start with the failure of timely flowering the first year. This original intermating has been followed by several seasons of backcrossing to expand the genetic base away from the original tropical corn parents that carried Ga1s. The final version, called **Liberty (Cycle 0)**, is a white semi-dent for the food grade market. It is being increased in South Carolina in 2016 for public release in 2017.

Zdrowie (Composite OP) Selection and Conversion: This population became part of the project as its values became clear. The parents are populations from Uruguay, Argentina, and Chile, each with excellent agronomic characteristics and stunning, orange seeds. Zdrowie (zdroh-vyeh) is Polish for health, and these may well have higher levels of carotenoids in the grain. These parents were intermated in my backyard and increased for release to organic farmers, starting in Illinois in 2016. However, the population was brought into this project once white cobbed parents in the population were identified in Ohio and North Carolina. These were crossed with Ga1s carriers with white cobs from the other work. The population is being further backcrossed and the organic ready version will be released in a few more years. As it exists right now it is 50% Zdrowie by pedigree and homozygous for Ga1s. Two or three more backcrosses are necessary for public release of a finished population. Seeds will be made public via the USDA after the next backcrossing is complete later in 2016 or early in 2017 as a breeding population.

Euro Flint Conversion: Beginning in 2009 I evaluated some European flint and North Dakota flint inbreds and intermated several that were very early. Selection for maturity and further increase was carried out as part of this project before crossing with a Ga2s carrier. It has since then been through several generations of crossing and back crossing. During the summer of 2015 much of the plants failed due to drought, but a couple of nice, flinty, white cobbed ears that should be homozygous for Ga2s did result. Seed supply is very low after summer of 2015, but I aim to increase it and release to the USDA in fall of 2016. This will be released to USDA as **Euro Flint (Ga2s)** as a gene source, as a tester, and as a parent for future inbred line development.

Stiff Stalk (SS) and Non-stiff Stalk (NS) Population Development and Conversion: Prior to this project I had developed an SS and NS version of these populations that carried Ga1s. I have released full season versions of these (crossed with central Corn Belt lines) to USDA, but they have not released these populations to the public. Work continued with these to prove they had functional Ga1s genes present and to increase the seed. However, some problems in Puerto Rico and New York resulted in some steps having to be repeated. These broad populations are finished and ready for release. I intermated the SS and NS versions for southern Minnesota and crossed these with the Hayes Yellow Dent. I also intermated the SS and NS version for the central Corn Belt and bred this into the Henry Wallace population to expand its genetic base. A test cross of the earlier version of these proved not to be very impressive in a yield trial. That was disappointing, so, I crossed these onto hybrids formed from inbreds of known heterotic groups in order to form improved populations for inbred development, and perhaps also improved heterozygous parents for hybrid seed production. A bit more work is needed to finish these, but thanks to the folks in New York and Florida and some lucky breaks in North Dakota we have 1) an earlier general Stiff Stalk population with Ga1s, 2) an earlier Stiff Stalk population that is 50% early B14 lines, 3) an earlier Stiff Stalk population that is 50% early B73 lines, 4) an earlier Non-stiff Stalk population that is 50% early Iodent lines, and 5) an earlier Non-stiff Stalk population that is 50% early Mo17 lines. These will be released to USDA and any interested breeders in 2016.

Inbred Line Conversions: The number of setbacks for this work was remarkable, even when compared with the population work. Inbreds are by nature weak, slow, and susceptible to many troubles. The biggest problem was inability to get flowering to time out correctly in order to make crosses, but even getting a good number of plants was often a problem in the winter nurseries or when spring weather was bad. Repeat work and the planting extra rows were needed to make much progress. Here are the inbred lines with which we worked and the status of each conversion.

PHK05 Conversion: As an improved CM7 type, this line was crossed with our stiff stalk Ga2s carrier, but setbacks were many – it is a very short inbred and prone to problems in winter and summer nurseries. We currently have heterozygous seed that is 5/8 PHK05 by pedigree and homozygous seed that is about ¼ PHK05. These need to be intermated, and inbred line or continued backcrossing could continue afterwards.

RS710 Conversion: The process of converting this early B14 type to Ga2s has resulted in heterozygous seed that is 7/8 RS710 and homozygous seed that is 1/2 RS710 by pedigree. These lines need to be intermated for further inbred line development or for continued back crossing.

PHM81 Conversion: This early Iodent line from Pioneer got started late and had flower timing issues. We currently have seed that is heterozygous for Ga2s and is ¾ PHM81 by pedigree as well as what should be homozygous Ga2s seed that is ¼ PHM81 by pedigree. These need further back crossing to finish, although they could be used for inbred development as they are right now.

LH160 Conversion: This early Mo17 has been useful in the project. A line selected from its cross with our Ga2s carrier has been used for all further Non-stiff Stalk line work. We had complete failures of the crosses in 2015, but still have a small amount of seed that is 50% LH160 and homozygous for Ga2s by pedigree. Also have a very small amount of seed that is heterozygous for Ga2s and is ¾ LH160. Further work is required, but the homozygous line will be released to USDA for other breeding advances after a seed increase.

LH149 Conversion: Troubles were many, but we made a good deal of progress with this line even if we had complete failures again in 2015. The seed that exists is 7/8 LH149 by pedigree and should be homozygous for Ga2s. It will be released as is to USDA although additional backcrossing or inbred line development would be beneficial.

73-118 Conversion: The LH149 (another B73 type) Ga2s line was used in this development. We have heterozygous seed that is about 29/32 B73 type by pedigree and homozygous seed that is about 27/32 B73 type by pedigree. Once crossed the resulting seed will be 7/8 B73 type by pedigree and homozygous for Ga2s. This would be fine for further inbred development, or continued back crossing with 73-118.

32-311 C-A Conversion: Another difficult inbred, but a very beneficial Non-stiff stalk line for hybrids in the Northeast. We currently have seed that is ¾ 32-311 C-A and a mix of homozygous and heterozygous Ga2s seeds by pedigree. There is also a very small amount of low quality seed that is 7/8 32-311 C-A by pedigree. Some of these should carry Ga2s.

PHJ89 Conversion: So many setbacks with this line! We currently have F2 seed that is 50% PHJ89 and a mix of homozygous and heterozygous Ga2s seeds given the pedigree. It will be made available to USDA for inbred line development and for continued back crossing.

W552 Conversion: This line also got off to a slow start, but we do have seed with some heterozygous Ga2s carriers that is 7/8 W552 and seed that should be homozygous for Ga2s and would be ½ W552 by pedigree. The line that is likely to be homozygous for Ga2s will be released to USDA as a gene source and for inbred line development, but the other seed really needs ongoing backcrossing work to finish it up.

LH54 Conversion: Several setbacks with this Mo17 type line occurred, so the seed we have right now is ¼ LH54 and homozygous for Ga2s by pedigree and also heterozygous seed that is 5/8 LH54. Additional seasons of back crossing would be helpful, although the seed as is could be used for inbred development.

MBST Conversion: This Oh43 type line was brought into the work late. We currently have F2 seed of a cross with our early Ga2s carrier. This seed is ½ MBST and a mix of homozygous and heterozygous Ga2s carriers. It will be released as is to USDA, but could benefit from a few seasons of further back crossing.

LH39 Conversion: Several seasons of failed establishment or pollinations prevented much progress with this conversion. May have some Ga2s carriers from previous years, but not many.

NK778 Conversion: This B37 type line proved to be somewhat immune to blue corn pollen, so that sort of testing would not work. So we switched to backcrossing onto 93-187, a Cornell B37 type line of good utility. The seed we have on hand now includes seed that is probably homozygous for Ga2s by pedigree and is about ½ B37 by pedigree, as well as a mix of Ga2s carriers and non carriers that is about 15/16 B37 by pedigree. It would be best to cross these one more time before public release for use in inbred development or further back crossing.

NK794 Conversion: This is a nice B73 for southern Minnesota and northern Iowa. We had another establishment failure in New York in 2015, but still have very small amounts of seed that is homozygous for Ga2s (5/8 NK794) and a mix of heterozygous seed that is about 15/16 NK794. Additional back crossing is needed to finish this work before viable public release given the small seed supply.

Q381 Conversion: Conversion of this line to Ga2s made some progress after the initial LH149 Ga2s carrier line was identified. We have very small amounts of seed of a homozygous Ga2s version of this that is ¾ Q381 and a mix of heterozygous Ga2s that is 7/8 Q381. I will probably release the homozygous seed to the USDA if there are more than 100 seeds, but the line really deserves some additional back crossing before release for actual use.

B116 Conversion: There were numerous problems with back crossing this line with Ga2s. Little progress was made. Any seeds that carry Ga2s and are less than 50% B116 will be released to USDA to use as breeding lines.

B119 Conversion: Progress was made to the point of having homozygous Ga2s lines that are 7/8 B119. However, lines that are greater than 50% B119 cannot be publicly released. This line can be used for further back crossing until the pedigree of B119 drops to 50% or less. Then the seeds can and will be released to the public.

LH205 Conversion: This conversion project fell behind due to pollination timing and establishment troubles. Progress was not made in 2015 but we did maintain the line that is ¼ LH205 and probably homozygous for Ga2s. The line is, however, is still 27/32 Stiff Stalk in type. Additional back crossing is needed before public release.

LH128 Conversion: This conversion project fell behind due to numerous pollination and establishment troubles. There may be a small amount of seeds that are Ga2s carriers and worthy of release as a breeding line. If so, these will be released to USDA in 2016.

Cornell NS ECB (73-157) Conversion: Attempts at further back crossing failed in 2015, but we do have small amounts of seed that should be homozygous (7/8 73-157) and heterozygous carriers (15/16 73-157). These should be backcrossed and then released to the public as carriers of not only Ga2s but also strong European Corn Borer resistance from natural gene sources.

Cornell SS ECB (NYA665EB/GEMS001 S6) Conversion: This line has been developed to the point of having a very small quantity of seed that is just under 50% this line by pedigree and probably homozygous for Ga2s. We also have a few hundred kernels of seed that is 15/16 this line by pedigree and is a mix of heterozygous Ga2s seeds and non carriers. An additional year of crossing is in order before public release for breeding or use. This line carries strong natural European Corn Borer resistance.

Others: One surprise during the work was contact from Major Goodman at North Carolina State University. He had become familiar with the project via news reports and was working on his own, similar work with tropical corn. Although the NCSU program has had lines with Ga1s for many years, he started backcrossing a new source that seems to include Teosinte Crossing Barrier 1 into lines adapted to North Carolina, and offered to make crosses of his developing lines with early lines from North Dakota. These crosses just barely worked out and seed was moved along for initial selfing in New York in 2015. The seeds that are available should carry the gametophytic incompatibility and some may well flower in the North. This new addition to the project has only just gotten started, but public release of some of the seeds is planned for 2016 via the USDA and other breeder contacts.

Objective 2 Results: Several blue lines were selfed out of crosses of blue corn with corn belt lines. However, these are very short and not all of them are hard enough for food grade use. Additional crosses of some identified white lines with these blue carriers will be made for blue hybrid development, but the blue inbreds themselves will be released to USDA in 2016. They will still be useful for providing blue corn pollen for testing and as sources of blue kernels for other breeders. In 2014-2015 we had an extensive blue corn nursery in Chile, but there were some hand pollination issues and many of the ears came back crossed with yellow field corn. Disappointing!

Objective 3 Results: See section 7 below.

6. Conclusions and Discussion: Clearly, making backcrosses with inbred lines is much more difficult than one might ordinarily think. Also, breeding plans do not always go so easily when the crossing procedure is at all complicated or establishment of lines is poor in some seasons. Setbacks can be many, but progress can also go very smoothly with extra rows, including extra rows that are delay planted to try and guarantee some well-timed flowering. One should plan on larger numbers of plots for each cross as insurance in order to overcome establishment and flowering problems. Some populations did result that appear to be homozygous for one of these gametophytic incompatibility traits. Additional testing is needed, but they are making their way out to the public and it will be interesting to see how well they are received and how well the trait works. Inbred lines and breeding populations were also developed that carry the traits, but they are not all finished to the extent originally proposed. Releases for now will be largely as breeding lines for others to use in their own, private work.

Education about this trait did get attention that was beneficial, like the partnership with Major Goodman at North Carolina University. More will be needed before we all come to see the true value of these traits. Also, more discussions with seed producers are needed. Although many know of the work, most did not express much interest in it. The only seed company to get the Ga2s inbreds to date is in Poland, and only small seed companies actually handle open-pollinated seed corn these days. It will be interesting to see how the new varieties do for them, and whether having this trait increases the acceptance of OP corn varieties.

I would like very much to express my thanks to Margaret Smith at Cornell University, Major Goodman at North Carolina State University, Alfredo Huerta at Miami University, the folks at the University of Puerto Rico who sponsored the organic winter corn nursery, and the teams of technicians at 27 Farms in Florida and at Vivero Del Sur in Chile. They did the bulk of the work for the corn belt and for more southerly regions. Thanks also to my family for allowing me so many hours in the nursery each summer and for allowing so many ears of corn coming through our living space each fall and winter.

7. Outreach (Objective 3):

In 2011 I produced and posted a short video about the project, the importance of this trait, and issues of using the trait in organic corn. This was moved to the NPSAS Farm Breeding Club Channel in 2014.

In 2012, although I ran into bad weather and was unable to attend the conference, I did submit a paper entitled "Using Gametophtyic Cross Incompatibility for Organic Corn Purity" to the conference proceedings for the 6th Organic Seed Growers Conference. This article was reprinted in the MOSES Organic Broadcaster in 2014.

In 2013 I gave a presentation about plant breeding, including an explanation of the value and breeding for this trait in corn, to the then Botany Department at Miami University in Ohio.

In 2014 I gave a presentation about corn breeding at the Northern Grain Growers conference in Vermont. One of the topics was the use of this set of protection traits. I also gave a number of interviews over the phone.

In 2015 MOSES invited me to speak at the Organic Farming Conference. That presentation,"Protecting Organic Corn," is available online (see addenda below). I also gave a version of that presentation at the Indigenous Farming Conference on the White Earth Reservation in March. That same month I also gave that presentation to a group of beginning farmers in North Dakota via a weblink with FARRMS. By December I had learned of a new on-farm corn breeding project in Illinois via my Corn Culture Facebook page. I sent them copies of this same presentation and others about corn breeding, and in 2016 I sent them seeds of my Rebellion (Cycle 0) to increase and use on organic farms there.

8. Financial Accounting:

OFRF Payments:

2011 - $11,500 x 90% = $10,350
2012 - $12,000 x 90% = $10,800
2013 - $11,900 x 90% = $10,710
2014 - $11,800 x 90% = $10,620

Total funds provided to date - $42,480

Expenditures:

2011 – North Dakota 196 rows ($4900), Cornell 88 rows ($2200), Puerto Rico 82 rows ($2050), Chile 68 rows ($1700), Florida 44 rows ($1100). Total – $11,950.

2012 – North Dakota 364 rows ($9100), Cornell 84 rows ($2100) Puerto Rico 75 rows ($1875), Chile 127 rows ($3175), Florida 8 rows ($200). Total – $16,450.

2013 – North Dakota 397 rows ($9925), Cornell 80 rows ($2000), Ohio 100 rows ($2500), Puerto Rico 11 rows ($275), Florida 66 rows ($1650), Chile 168 rows ($4200), tests for transgene contamination of Red River population - $1405. Total – $21,955.

2014 – North Dakota 367 rows ($9175), Cornell 98 rows ($2450), North Carolina 24 rows ($600), Florida 161 rows ($4025), Chile 123 rows ($3075). Total – $19,325.

2015 – North Dakota 331 rows ($8275), Cornell 80 rows ($2000), North Carolina 40 rows ($1000), tests for transgene contamination - $416. Total – $11,691.

Justification – The number of rows used in New York, Ohio, North Carolina, Florida, Puerto Rico, and Chile largely represent rows for the project used as initially proposed, and that is where the bulk of the funds have gone in order to work with later maturing varieties and to include the off season. The great expansion in North Dakota after the first year was largely work to further refine and improve some of the early flint and dent populations and the blue lines which all really needed more work to be useful to organic farmers. As described in the results section above, I also added an additional population that appeared to have great merit, Zdrowie, and I wanted to include it with the rest as an eventual public release of "organic ready" corn once finished.

9. Leveraged Resources:

I did not seek other resources for this project, but did find partners willing to help at Cornell University, Miami University, North Carolina State University, and the University of Puerto Rico. We put in a great deal of time, especially for rows that needed to be redone, for populations that had to be increased in size due to pollination or weather problems, and for populations that really were not looking valuable to organic farmers. For a few years my son assisted in the fields and in 2015 I hired a niece and a nephew to help in the nursery. Major Goodman's offer to make crosses of North Dakota lines with his new source of gametophytic incompatibility from Mexican sweet corn was an awesome addition to the project and no doubt of great value for the future of organic corn. Everyone who helped is much appreciated!

10. References:

Evans, M.M.S., and J.L. Kermicle. 2001. Teosinte crossing barrier 1, a locus governing hybridization of teosinte with maize. Theor. Appl. Genet. 103:259-265. Available online: http://www.springerlink.com/content/6avefqvt9c1frcej/ (accessed 20 September 2011).

Hayes, H.K., R.R. Immer, and D.C. Smith. 1955. Methods of plant breeding, 2nd Ed. McGraw-Hill Book Company, New York.
Hayes, H.K., E.H. Rinke, and Y.S. Tsiang. 1944. The development of a synthetic variety of corn grown from inbred lines. J. Am. Soc. Agron. 36:998-1000.
Jones, D.F. 1918. The effects of inbreeding and crossbreeding upon development (statement found on page 59). Connecticut Agric. Exp. Stn. Bull. No. 207. New Haven, CT.
Kermicle, J.L., and M.M.S. Evans. 2010. The *Zea mays* sexual incompatibility gene ga2: naturally occurring alleles, their distribution, and role in reproductive isolation. Journal of Heredity doi:10.1093/jhered/esq090. Available online: http://jhered.oxfordjournals.org/content/early/2010/08/07/jhered.esq090.full.pdf (accessed 20 September 2011).
Kutka, F.J. 2009. Release of populations carrying Ga1s. Maize Genetics Cooperation Newsletter (http://www.agron.missouri.edu/mnl/84/PDF/20kutka.pdf).
Kutka, F.J., and M.E. Smith. 2007. How Many Parents Give the Highest Yield in Predicted Synthetic and Composite Populations of Maize? Crop Science 47:1905-1913.
Poneleit, C.G. 2000. Breeding white endosperm corn. Chapter 8 in Hallauer, A.R. (ed.). Specialty Corns, 2nd Ed. CRC Press, Boca Raton.
Lausser, A., I. Kliwer, K. Srilunchang, and T. Dresselhaus. 2010. Sporophytic control of pollen tube growth and guidance in maize. J. Exp. Bot. 61(3):673-682.
Thomas, W.I. 1955. Transferring the Ga^s factor for dent incompatibility to dent compatible lines of popcorn. Agron. J. 47:440-441.
Zeigler, K.E., and B. Ashman. 1994. Popcorn. Chapter 7, pp. 189-223 in, Hallauer, A.R. (ed.). Specialty Corns. CRC Press, Boca Raton.

11. Photos and Other Addenda:

2011 video about the trait - https://www.youtube.com/watch?v=WGTd3MsAl1g
2011 article in Non-GMO Report - http://www.nongmoreport.com/articles/july2011/organicreadycorntopreventGMOcontamination.php
2012 paper in OSA Conference Proceedings - http://www.seedalliance.org/uploads/2012%20OSGC%20Proceedings%20FINAL.pdf
2014 presentation at the Northern Grain Growers Conference in Vermont - http://www.uvm.edu/extension/cropsoil/wp-content/uploads/FKutka-Breeding-Corn-On-Farm.pdf
2014 article in Non-GMO Report - http://non-gmoreport.com/articles/september-2014/plant-breeder-save-organic-corn-from-GMO-contamination.php and reprinted via Harvest Media - http://harvestpublicmedia.org/article/breeder-working-%E2%80%98organic-ready%E2%80%99-corn-blocks-gmo-contamination. It spread widely that fall.
2014 article in the Organic Broadcaster - https://mosesorganic.org/farming/farming-topics/field-crops/organic-ready-corn/
2015 presentation to MOSES Organic Farming Conference - https://mosesorganic.net/product/protecting-organic-corn-2/
2015 presentation to FARRMS beginning farmers group in North Dakota - https://www.youtube.com/watch?v=tiI6H4pBxFE

2015 article in Organic Broadcaster about the Experimental Farm Network. The group attempted to collaborate with the project, but nothing resulted - https://mosesorganic.org/experimental-farm-network/

2015 radio spot on Minnesota Public Radio - http://www.mprnews.org/story/2015/03/17/genetically-modified-corn

2015 radio spot on Niijii Radio on the White Earth Reservation - http://niijiiradio.com/?page_id=324

Corn returning from the Florida nursery in spring of 2015.

One of the parents for the new Liberty (Cycle 0) population for the South

The droughty corn nursery in North Dakota in 2015