INVESTIGATION OF A XENIA EFFECT FOR YIELD CAUSED BY THE WAXY GENE IN GRAIN SORGHUM

A Thesis

by

LESLIE CHARLES KUHLMAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Plant Breeding

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Approved by:

Chair of Committee, William L. Rooney
Committee Members, Lloyd W. Rooney
Stephen R. King
Head of Department, Mark A. Hussey

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ABSTRACT

Investigation of a Xenia Effect for Yield Caused by the Waxy Gene in Grain Sorghum.

(August 2005)

Leslie Charles Kuhlman, B.S., Kansas State University

Chair of Advisory Committee: Dr. William L. Rooney

Sorghum grain with a waxy endosperm is more digestible and has a higher feeding efficiency compared to sorghum grain with a non-waxy (or normal) endosperm. However, waxy sorghums (*Sorghum bicolor* (L.) Moench) yield 10-15% less than normal sorghum and the cause of the yield reduction is unclear. The objective of this research is to determine if the yield decrease could be due to the waxy phenotype itself. The waxy phenotype is an example of a xenia effect, where the pollen not only contributes to the genotype of the resulting hybrid, but also immediately influences the phenotype of the resulting seed. Sterile hybrids under different pollination types, and different genotypes of fertile hybrids, both resulted in hybrids that produced different ratios of waxy phenotype seed. The effects on yield and 500 kernel weight were investigated in Weslaco, College Station, and Halfway, Texas over two years. Yields of sterile heterozygous waxy hybrids under waxy pollination, which produced an average 27% waxy seed over all environments, were nearly identical to homozygous nonwaxy hybrids that produced 0% waxy seed. Average 500 kernel weights for the same hybrids were not different. Grain yields for the fertile hybrid genotypes were significantly

different. Hybrids which produced 100% waxy seed yielded significantly less than hybrids which produced 25% waxy seed. Upon further examination, hybrids that were produced from waxy F_1 endosperm seed (*wxwxwx*) had significantly worse stand and panicle number per plot means than did hybrids from nonwaxy F_1 endosperm seed (*Wxwxwx*). Grain yields adjusted for panicle number showed no significant differences. The average 500 kernel weights between hybrids with different amounts of waxy phenotype grain did not significantly differ. The yield effect seen in this population was the result of waxy endosperm hybrid seed displaying significantly poorer stand establishment than nonwaxy endosperm hybrid seed. These data do not support a xenia yield effect due to the waxy gene.

DEDICATION

This thesis is dedicated to my wonderful wife and partner in all things, Kendra. "Let us laugh when life is humorous, let us cry when life is full of sorrow, let us push on when life is unbearable. But through all, let us celebrate life, together."

Also to my parents, Dennis and Carol, I wouldn't be the person I am today without all of your love, guidance, help, and support. To my brother and sister, Brock and Breanna, thank you both for your love and support.

To the future. Cheers.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF TABLES

TABLE Page

TABLE Page

TABLE Page

TABLE Page

TABLE Page

CHAPTER I

INTRODUCTION

Grain sorghum (*Sorghum bicolor* (L.) Moench) is a cultivated grass species whose origin traces to Africa. There is no definitive date as to when sorghum was domesticated, but some suggest 4000-6000 years ago (Kimber, 2000). The domesticated varieties first spread through parts of Africa before leaving the continent for India via migrating people and trade routes during the second millennium B.C. It then spread to China via trade with India. Beneficial phenotypes, arising from chance mutations and outcrossing, were selected by early farmers and constituted the earliest varieties. This form of selection and breeding began with the domestication of the species and continued throughout its history. Waxy endosperm sorghum grain was one of these phenotypes. It originated in Asia, and was likely selected based on specific cultural preferences about food appearance, texture, and taste (Fukunaga et al., 2002).

In the US, grain sorghum is the third leading production cereal crop in the US behind corn and wheat with a total harvest of 11,192,000 metric tons in 2003, worth an estimated $965,822,000. US acreage has fallen in recent years to 3,155,700 hectares in 2003 down from 6,791,400 hectares during the record harvest of 1985 (USDA, 2004). Sorghum is utilized mostly as a feed grain in the US with about 10% used in ethanol production and a small amount used in food applications (NGSP, 2004). Worldwide, sorghum ranks fifth in cereal crop production behind corn, rice, wheat, and barley with a

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total production of 59,442,000 metric tons in 2003 (FAO, 2004). It is a staple human food supply in many parts of the world including Africa, India, and Central America, where its importance in the everyday diet cannot be underestimated.

Expanding markets for sorghum is a primary goal for producer groups in the US and around the world, and waxy sorghum could provide some support. Waxy sorghum has generated interest from breeders due to its increased feeding efficiency (Brethour and Duitsman, 1965; Sherrod et al., 1969). This grain could be of use to animal finishing programs in that less grain can be fed to animals while maintaining the same weight gain. This would translate into a significant economic advantage for feeders that use waxy sorghum. However, waxy sorghums have traditionally not been competitive with normal sorghum in yield (Rooney et al., 2005). Sorghum producers are unwilling to grow waxy sorghums due to the significant yield drop associated with them. Without a price incentive from buyers, of which there have been none, producers will continue to avoid waxy sorghum production.

Sorghum breeding programs are the natural answer to alleviating the yield depression associated with the waxy phenotype, except the nature of the yield depression has never been confirmed. The waxy gene could affect sorghum yields in three ways: (1) the waxy phenotype grain could have altered seed characteristics that affect yield, such as lower seed weight or reduced germination of hybrid seed, (2) deleterious alleles, closely linked to the waxy allele, could be responsible for the yield decrease and, (3) the waxy allele could affect other physiological yield traits through pleiotrophy.

Information on the manner in which the waxy phenotype affects grain yield is necessary to inform breeders how to proceed with waxy sorghum development.

The two populations used here in were originally developed for a dissertation by Aydin in 2003 and the results later published by Rooney et al (2005). Those results clearly showed a yield decrease of approximately 17% across locations and populations due to the waxy phenotype per se in inbred lines (Rooney et al., 2005). In hybrid combinations, heterozygous waxy fertile hybrids, which produced 25% waxy grain, showed a statistical yield disadvantage compared to the nonwaxy hybrids in one environment and were numerically lower in the combined environments. Heterozygous waxy sterile hybrids serendipitously were pollinated mostly by nonwaxy pollen leading the hybrids to produce nearly 0% waxy grain. In this situation there was no yield difference compared with the nonwaxy hybrids. This lead the author to hypothesize that there may be a xenia effect due to the waxy gene causing the yield decrease, in that waxy phenotype seed produced in the panicle negatively impacts yield (Aydin, 2003).

This research will take up that hypothesis and attempt to determine whether a xenia yield effect could be the cause of the yield disadvantage associated with waxy sorghum. This will inform breeding programs how to proceed with developing competitive yielding waxy hybrids.

The research objectives are as follows:

1. Verify the yield decrease associated with the waxy gene in hybrid combinations, and investigate what yield parameters may be affected.
2. Determine if the waxy locus causes a xenia effect for yield.

CHAPTER II

LITERATURE REVIEW

Waxy Endosperm Origin and Phenotype

Kempton, in 1921, reported a waxy endosperm phenotype in two seed lots originating from China and the Philippine Islands (Karper, 1933). The seed endosperm was described to have a dull, waxy, opaque surface when cross sectioned as opposed to the crumbly starch grains found in the normal nonwaxy endosperm (Karper, 1933). The phenotype was controlled by a single gene in which the waxy allele is recessive to the dominant nonwaxy allele. Sorghum endosperm tissue is triploid, two genomes are derived from the female gamete and one from the male gamete, therefore, three waxy alleles are necessary to produce the waxy endosperm phenotype (*wxwxwx*). The waxy phenotype thus experiences a xenia effect, where the pollen parent has an immediate effect on the phenotype of the developing seed. As an example, a waxy plant contributing waxy female gametes will produce nonwaxy seed if fertilized by nonwaxy pollen.

The waxy phenotype is the result of a change in starch composition in the endosperm. Waxy endosperms (*wxwxwx*) contain starch comprised of 100% amylopectin and 0% amylose as opposed to normal nonwaxy (*WxWxWx*) endosperms that contain starch as 75% amylopectin and 25% amylose. Heterozygous waxy endosperms with one waxy allele (*WxWxwx*) and two waxy alleles (*Wxwxwx*) are both

phenotypically nonwaxy, but amylose content is reduced to about 23% and 17% respectively (Ellis, 1975). When nonwaxy endosperm starch is stained with an iodine potassium iodide solution, the iodine forms a close complex with amylose and the starch turns blue. Amylopectin does not associate with iodine in this way which is why waxy endosperm starch instead stains red in the presence of iodine.

Benefits

Waxy endosperm cereals have been of interest to researchers for several reasons. First, waxy grain has an enhanced nutritive value over nonwaxy grains. In feeding trials, waxy sorghum has an enhanced net energy value, and improved feed efficiency in yearling steers (Brethour and Duitsman, 1965; Sherrod et al., 1969). Dairy cattle produced more milk, and chicks, swine, and sheep all gained more weight when fed waxy corn versus nonwaxy corn (Akay and Jackson, 2001; Dinn et al., 1982; Camp et al., 2003; McDonald, 1973).

The increased feed efficiency is due to a higher dry matter digestibility of waxy endosperm over nonwaxy. The digestibility is increased through a combination of factors such as greater starch hydrolysis, greater protein solubility, and less dense protein matrix in the peripheral endosperm (Sullins and Rooney, 1975; Tovar et al., 1977; Walker and Lichtenwalner, 1977; Lichtenwalner et al., 1978).

This enhanced nutritive value translates into an economic advantage for animal production programs, in that less waxy grain is required to maintain the same weight gains in animals. This, along with the fact that waxy sorghums produce a superior steam

flaked product than nonwaxy sorghums (McDonough et al., 1998), would give animal feeding programs a clear advantage to use waxy sorghum.

As a second benefit, waxy sorghums have food applications as well. A granola breakfast cereal made from waxy sorghum was rated best over granolas made with nonwaxy sorghum and traditional oats by a sensory panel due to their superior physical attributes (Cruz y Celis et al., 1996). Waxy sorghum grits are also an adequate brewing adjunct for beer production (Barredo Moguel et al., 2001). These food products benefit from waxy sorghum's different starch composition. Other food applications would be recognized if a constant supply of waxy sorghum was available in the market. Waxy sorghum would offer food scientists another specialty tool for producing high quality food for consumers.

Wet milling waxy endosperm grain yields waxy starch which forms clear, low viscosity pastes in cold water and very high viscosities in hot water. Currently all the waxy starch is wet-milled from waxy corn, but waxy starch from sorghum was processed in the mid 1940s as a replacement for tapioca starch (Cushing, 1943), and could be milled again as an alternative to corn. Waxy starch is currently used for its special characteristics in frozen foods. On the industrial side, it is utilized by the papermaking, textile, and adhesive industries (Fergason, 2001).

These advantages in food, feed, and industrial applications generate interest in the development of competitive yielding waxy sorghum hybrids so its benefits can be utilized.

Waxy Gene

The waxy gene (*Wx*) codes for a protein product named starch granule bound starch synthase (GBSS1), which in normal endosperm synthesizes amylose (Nelson and Rines, 1962; Tsai, 1974; Preiss, 1991). The waxy phenotype is caused by a recessive mutant allele (*wx*) at the waxy gene locus. In rice, maize, barley, foxtail millet, and sorghum, the waxy allele results in translation of a nonfunctional mutant GBSS1 protein product (Wang et al., 1995; Varagona et al., 1992; Domon et al., 2002; Fukunaga, 2002). All these cereals show the same waxy phenotype, caused by various allelic mutations within the same homologous waxy gene. Without a functional GBSS1 protein, waxy endosperms develop no amylose. The nonwaxy allele is dominant because through transcription of normal GBSS1, it restores functionality to amylose production. There is a dosage effect as seen with the different levels of amylose in endosperms that contain one, two, and three nonwaxy alleles.

Developmental differences between waxy and nonwaxy endosperms may be a result of the nonfunctional protein and lack of amylose production. Creech (1965) reported the dry matter accumulation at 16 days post pollination between nonwaxy and waxy maize seeds was non significant, but at 20 days it was significantly different, as well as at 28 days. Other data suggests that by 18 days post pollination a size difference becomes significant and that it is the result of smaller starch granules in the mature waxy endosperm (Boyer et al., 1976). This may occur because amylose is synthesized later in endosperm development, and since waxy endosperms fail to produce amylose, they do not capitalize on the extra starch production and are physically smaller at maturity

(Boyer et al., 1976). This hypothesis is supported by the many reports that waxy endosperm sorghum seed are smaller in size than normal endosperm seed (Jones and Sieglinger, 1952; Ellis, 1975; Cruz y Celis, 1996), and in many cases, they are less dense as well (Cruz y Celis, 1996; Ellis, 1975). These physical changes may contribute to lower germination of waxy hybrid grain as well (Ellis, 1975).

Yield Consequence

Jones and Sieglinger (1952) made the first report of a yield depression in sorghum associated with the waxy endosperm phenotype. Their research showed a 9.2-10.9% yield deficit in waxy sorghums versus nonwaxy sorghums derived from multiple segregating populations. Seed weights of waxy phenotype grain were reduced approximately 3% as well. Karper and Quinby, (1937) had made similar observations in seed from segregating panicles.

While there were no further reports of yield differences between non waxy and waxy sorghums, sorghum breeders inherently determined that waxy sorghums did not yield competitively with normal sorghums since few have ever been released from breeding programs and none have been commercialized. From 1993-2003, the Texas Agricultural Experiment Station's Texas Grain Sorghum Performance Tests show waxy and heterozygous waxy hybrids consistently yielding below the mean of their nonwaxy counterparts in individual test locations (Pietsch et al., 2003). Recently, Rooney et al. (2005) reported an average yield deficit of 17% due to the waxy gene across environments and populations.

Research into endosperm mutants of corn has produced similar results. A xenia yield effect was first reported in corn where data showed a 34% increase in grain yield of sweet corn that was subjected to dent corn pollination (Kiesselbach and Leonard, 1931). Later it was shown that nonwaxy kernels from segregating ears were 3.2% heavier than the waxy kernels (Kiesselbach, 1944). Isogenic corn varieties, resulting from backcrossing, showed a 4.6%-7.4% yield depression for the waxy varieties over multiple years. The five year average was a 5.6% disadvantage for the waxy corn varieties with them never outyielding the nonwaxy isogenics over 13 location-years (Kiesselbach, 1948). Current waxy corn hybrid yields still seem to lag behind the conventional counterparts by approximately 5% (Fergason, 2001). Based on this information, the evidence is strong for a real yield decrease due to the waxy endosperm in both sorghum and corn.

Conclusions

The manner in which the waxy phenotype affects grain yield is necessary to inform breeders how to proceed with waxy sorghum development. The waxy gene could affect sorghum yields in three ways: (1) the waxy phenotype grain has altered seed characteristics that affect yield, such as lower seed weight or reduced germination of hybrid seed, (2) deleterious alleles, closely linked to the waxy allele, are responsible for the yield decrease and, (3) the waxy allele affects other physiological yield traits through pleiotrophy. The literature implicates reduced germination of waxy endosperms or lower kernel weight of waxy seeds as possibilities of the cause of the yield reduction. Determining how the waxy phenotype affects yield will inform breeders how to proceed.

CHAPTER III

R-LINE POPULATION

Introduction

Waxy endosperm sorghum hybrids would be very useful for food, feed, and industrial uses if they yield competitively with normal sorghum. However, homozygous waxy sorghum hybrids have never been commercially sold and few inbreds have been released from public breeding programs. The nature of the yield depression must be determined so breeders can make decisions on how best to develop competitive yielding waxy hybrids. The yield depression associated with the waxy endosperm could generally result from three mechanisms: (1) waxy endosperm grain has altered physical characteristics that reduce yield, (2) the waxy allele affects unknown traits in the plant through pleiotrophy that reduce yield, and (3) deleterious alleles tightly linked to the waxy allele reduce yield.

Specifically, a yield reduction associated with waxy phenotype grain must manifest itself in a measurable parameter of yield. The total weight of harvested grain in a production field is the product of four logical yield parameters: plants per unit area, heads per plant, kernels per head, and weight per kernel. The values of these parameters multiply to equal the total grain weight output of the field. Any measurable decline in yield associated with waxy grain must be also measurable in one of the four yield

parameters. The objective of this experiment is to verify the yield decrease associated with the waxy phenotype in hybrid combination. Four types of hybrids will be used that differ in the amount of waxy grain they produce and the F_1 endosperm genotype that they are grown from. Yield parameters will be measured to determine the source of the yield effect.

Materials and Methods

Population Development

A population, described in Rooney et al. (2005), was created from the cross between RTx2907 and RTx430. RTx2907 is a waxy sorghum germplasm released from the TAES sorghum breeding program (Miller et al., 1996). RTx430 is a nonwaxy inbred, also released from the TAES sorghum breeding program, which has been a parental line used commercially to produce hybrid seed (Miller and Kebede, 1984).

From this population, 120 individual F_2 progeny were randomly selected for advancement and self pollination. In each $F_{2:3}$ progeny row, a single panicle was randomly self-pollinated for advancement. Seed from each $F_{3:4}$ panicle was screened for endosperm type using the potassium iodide test (Karper, 1933). $F_{3:4}$ lines that were homozygous for either waxy or nonwaxy endosperm were grown and self pollinated to produce $F_{3:5}$ seed. No selection was done during the development of these inbreds except for endosperm type and against lines that were unacceptable in agronomic qualities like height and maturity. The waxy $F_{3:5}$ inbred lines were previously shown to

yield significantly less than the nonwaxy lines (Aydin, 2003). These $F_{3:5}$ lines, either homozygous waxy or nonwaxy, were crossed onto two male sterile testers, ATxArg-1 and ATx2928. ATxArg-1 is a waxy parental line and ATx2928 is a nonwaxy germplasm, both released from the TAES sorghum breeding program (Miller et al., 1992; Rooney, 2003).

Hypothesis

These crosses created four testcross hybrid types that varied in genetic composition at the *Wx* locus (Table 1). Grain yield comparisons between the four hybrid types will show any yield differences due to either the amount of waxy phenotype grain they produce or the F_1 endosperm genotype they were grown from. Measurements from the parameters of yield will identify the source of the yield decrease.

Individual kernel weight as a yield parameter contributes to total yield. A reduced individual kernel weight without increases in the other yield parameters will reduce overall yield. The different hybrid genotypes will produce different amounts of waxy F_2 grain in their panicles. Assuming complete self-pollination, nonwaxy (*WxWx*) hybrids will produce 0% waxy grain, heterozygous (*Wxwx*) hybrids will produce 25% waxy grain, and waxy (*wxwx*) hybrids will produce 100% waxy grain. Because the two testers used in this experiment are not isogenic, comparisons will only be made between genotypes within a tester. Five hundred kernel weights between hybrids that produce 100% and 25% waxy grain and 25% and 0% waxy grain, should be different if waxy grain weighs less than nonwaxy grain.

Table 1. Hybrid combinations, F_1 endosperm genotypes, hybrid genotypes, and percent waxy grain produced by hybrids between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Hybrid	F_1 Endosperm Genotype	Hybrid Genotype	% Waxy Grain
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	*wxwx*	100%
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	*Wxwx*	25%
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	*Wxwx*	25%
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	*WxWx*	0%

Plants per unit area and panicles per plant are two parameters of yield that if reduced, also negatively impact yield. The four hybrid types differ by F_1 endosperm genotype, from 3 waxy alleles to 0 waxy alleles. Stand is a subjective measurement of germination and early seedling vigor, which determine final stand establishment. Panicles per plot combines both yield parameters mentioned above and is influenced by stand establishment and the tillering ability of the plant. Comparisons within tester for F_1 hybrid endosperm genotype for these measurements are designed to determine if the dosage of the waxy allele affects early growth parameters such as germination and seedling vigor.

The hypothesis from previous research (Aydin, 2003) is that the waxy phenotype per se is negatively influencing yield; this research will attempt to resolve that hypothesis.

Experimental Design

A total of 50 hybrids (15 ATxArg-1/waxy $F_{3:5}$, 15 ATxArg-1/nonwaxy $F_{3:5}$, 10 ATx2928/waxy $F_{3:5}$, and 10 ATx2928/nonwaxy $F_{3:5}$ hybrids) were randomly selected and planted in two-row plots in a randomized complete block design with three replications. This experiment was planted on April 4, 2003 in College Station, Texas and irrigated once during the season, but was not harvested due to very poor stands. Plots at this location were 18 feet long on a row spacing of 30 inches. On May 21, 2003 it was planted in Halfway, Texas and needed irrigation three times before harvest on October 15. Plots at this location were 16 feet long on a row spacing of 40 inches. In 2004, this

test was planted on March 31 in College Station and harvested August 7, no irrigation was necessary. On May 24, 2004 it was planted in Halfway and was irrigated twice before harvest on October 26. Plot length and row spacing was consistent at the locations between the years. Hybrid seed was treated with a liquid mixture of Alliance, Concep, Apron, and Captan brand seed treatments prior to planting. All other agronomic practices were standard for grain sorghum production in the region.

Field Evaluation

Plant height, head exsertion, days to mid-anthesis, panicle number, and stand ratings were taken in the field for each plot. Plant height was measured in inches from the base of the plant to the tip of the panicle as an average for the plot. Exsertion was measured in inches from the base of the panicle at the flag leaf to the first panicle branch. Days to mid-anthesis was recorded as the Julian date when 50% of the plot reached 50% anthesis. Panicle number was recorded as the total number of panicles per plot. Stand was scored visually using a 1-9 scale, with 1 having a full stand and 9 having no stand. The plots were harvested with a modified John Deere 3300 plot combine equipped with a HarvestMaster HM-1000 weigh system. The combine measured plot weight, grain moisture and test weight. Random samples of three panicles were harvested by hand prior to combine harvesting to constitute a grain sample for each plot. These heads were measured for panicle length, then cut into thirds, bulked within third sections and threshed in a single head thresher. The middle one third panicle grain samples were sieved over a 6 ½ /64" round holed sieve to remove broken kernels and

any remaining foreign matter, and counted using an ESC-1 grain counter, into 500 seed lots and weighed. The sample grain was then decorticated using a TADD mill and visually separated into waxy and nonwaxy seed based on endosperm phenotype. The percentage of waxy seed was used to verify the correct phenotype of the entries.

Data Considerations

Hybrid entries that failed to produce the expected phenotype grain or had unexpected seed color were removed from the data set. Both these conditions indicate that the hybrid seed was somehow contaminated and thus did not represent the cross accurately. All individual plots with a stand rating of 8 and above were removed from the data set since a stand rating that poor cannot accurately reflect yield potential (Table 2).

Statistical Analysis

Individual environment analyses were conducted for grain yield, 500 kernel weight, days to mid-anthesis, height, exsertion, stand and panicle number. Data was analyzed using the GLM:Univariate procedure in SPSS v11.5 with replication as a

random component and all others as fixed effects (Table 3). The model term "Tester" refers to the female parent, either ATx2928 or ATxArg-1, and the model term "Genotype" refers to either waxy or nonwaxy $F_{3:5}$. Mean comparisons within environments were conducted using Fisher's least significant difference (LSD) procedure, with a probability level of 0.05.

Bartlett's test for homogeneity of error variances was used to test the validity of combining data from individual environments. Results indicated that the error variances were heterogeneous, but data from each environment was normally distributed and no appropriate data transformations were found. Therefore, combined analysis was conducted to make comparisons across environments. Combined analysis of variance was conducted using replication as a random factor and all other factors fixed (Table 4). Mean comparisons were conducted using Fisher's least significant difference (LSD) procedure, with a probability level of 0.05.

Table 2. Initial plot number and total plots removed from data analysis due to incorrect grain phenotype, stand rating greater than 8, or other considerations across environments for hybrids between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Location	Hybrid	Initial Plot Number	Number of Plots Removed			
			Grain Phenotype	Stand > 8	Other†	Total
03 Halfway	ATx2928/Nonwaxy $F_{3:5}$	30	0	0	0	0
	ATx2928/Waxy $F_{3:5}$	30	3	0	0	0
	ATxArg-1/Nonwaxy $F_{3:5}$	45	0	1	0	1
	ATxArg-1/Waxy $F_{3:5}$	45	9	2	0	11
04 College Station	ATx2928/Nonwaxy $F_{3:5}$	30	0	1	0	1
	ATx2928/Waxy $F_{3:5}$	30	3	0	0	3
	ATxArg-1/Nonwaxy $F_{3:5}$	45	3	16	2	21
	ATxArg-1/Waxy $F_{3:5}$	45	21	12	1	34
04 Halfway	ATx2928/Nonwaxy $F_{3:5}$	30	0	0	3	3
	ATx2928/Waxy $F_{3:5}$	30	3	0	0	3
	ATxArg-1/Nonwaxy $F_{3:5}$	45	3	0	3	6
	ATxArg-1/Waxy $F_{3:5}$	45	21	0	0	21

† These plots were removed from the data set due to errors during harvest.

Table 3. Analysis of variance model used for individual environments of hybrids, from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Source	df	Mean Squares	Expected Mean Squares
Replication	r-1	MS_R	$\sigma^2_e + gt\sigma^2_r$
Tester	t-1	MS_T	$\sigma^2_e + rg\kappa^2_t$
Genotype	g-1	MS_G	$\sigma^2_e + rt\kappa^2_g$
GenotypexTester	(g-1)(t-1)	MS_{GT}	$\sigma^2_e + r\kappa^2_{gt}$
Error	(r-1)(gt-1)	MS_e	σ^2_e
Total	rgt-1		

Table 4. Analysis of variance model used for combined environments of hybrids, from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Source	df	Mean Squares	Expected Mean Squares
Location	l-1	MS_L	$\sigma^2_e + rgt\kappa^2_l$
Replication(Location)	l(r-1)	MS_R	$\sigma^2_e + gt\sigma^2_r$
Tester	t-1	MS_T	$\sigma^2_e + rg\kappa^2_t$
Genotype	g-1	MS_G	$\sigma^2_e + rt\kappa^2_g$
GenotypexTester	(g-1)(t-1)	MS_{GT}	$\sigma^2_e + r\kappa^2_{gt}$
TesterxLocation	(t-1)(l-1)	MS_{TL}	$\sigma^2_e + rg\kappa^2_{tl}$
GenotypexLocation	(g-1)(l-1)	MS_{GL}	$\sigma^2_e + rt\kappa^2_{gl}$
GenotypexTesterxLocation	(g-1)(t-1)(l-1)	MS_{GTL}	$\sigma^2_e + r\kappa^2_{gtl}$
Error	(r-1)(gtl-1)	MS_e	σ^2_e
Total	rgtl-1		

Results and Discussion

Analysis by Environments

Halfway, Texas 2003

Analysis of variance for grain yield shows significant variation for both tester and genotype by tester (Table 5) and mean comparison detected differences between genotypes within both testers. ATxArg-1/waxy $F_{3:5}$ hybrids, which produce 100% waxy grain, yielded 22.1% lower than ATxArg-1/nonwaxy $F_{3:5}$ hybrids, which produce only 25% waxy grain (Table 6). Under the stated hypothesis, this is expected since waxy phenotype grain itself is supposed to be the cause of the yield reduction. ATx2928/waxy $F_{3:5}$ hybrids, which produce 25% waxy grain, yielded 11.7% more than the ATx2928/nonwaxy $F_{3:5}$ hybrids, which produce 0% waxy grain. This is contradictory to the hypothesis since the hybrid that produces more waxy grain should incur a yield penalty.

Analysis of variance was performed on 500 kernel weight and significant variation was detected for tester, but not genotype or genotype by tester interaction (Table 5). The 500 kernel weight means were significantly higher for ATxArg-1 hybrids over ATx2928 hybrids (Table 7). This alone is not relevant since the testers do not contain the same genetic potential, therefore differences between tester groups may be the result of these differences. Five hundred kernel weight was expected to be lower for genotype by tester hybrids that produce more waxy grain, but clearly the lack of

Table 5. Grain yield, stand, grain moisture, height, exsertion, panicle number, test weight, and 500 kernel weight analysis of variance for hybrids, from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003.

		Dependent Variables							
Sources of Variation	df	Yield	Stand	Moisture	Height	Exsertion	Panicle Number	Test Weight	500 Kernel
Replication	2	$1.8x10^{6}$	0.5	0.5	0.3	3.6	175	4.5	NA‡
Tester	1	$4.3x10^{8}$**	304**	8.4**	0.6	0.5	155281**	42**	35**
Genotype	1	$5.5x10^{4}$	8.9**	0.7	11	5.3	28	13	3.1
GenotypexTester	1	$2.7x10^{7}$**	24**	0.3	27**	33**	9432**	9.2	1.5
Error	129†	$1.0x10^{6}$	1.1	0.5	6.5	2.0	303	3.8	1.5

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error for 500 kernel weight equals 42

‡ 500 kernel weight means were unreplicated in this location

Table 6. Mean grain yields (kg/ha) by environment for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Hybrid	% Waxy Grain†	03 Halfway	04 College Station	04 Halfway	Combined
ATxArg-1/Waxy $F_{3:5}$	100%	3566[d]	1047[b]	5081[b]	3654[c]
ATxArg-1/Nonwaxy $F_{3:5}$	25%	4575[c]	988[b]	5626[b]	4125[b]
ATx2928/Waxy $F_{3:5}$	25%	8599[a]	3064[a]	9873[a]	7179[a]
ATx2928/Nonwaxy $F_{3:5}$	0%	7692[b]	3177[a]	9596[a]	6897[a]
	C.V. (%)	18	44	19	23
	LSD	478	522	629	309

Different superscript letters within locations are significantly different at α=.05 using Fisher's Protected LSD

† Percent waxy F_2 grain produced by the hybrid in the panicle

Table 7. Mean 500 kernel weights (g) by environment for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Hybrid	% Waxy Grain†	03 Halfway	04 College Station	04 Halfway	Combined
ATxArg-1/Waxy $F_{3:5}$	100%	14.55[a]	12.45[b]	14.42[a]	13.93[a]
ATxArg-1/Nonwaxy $F_{3:5}$	25%	13.63[a]	11.85[b]	13.85[a]	13.20[a]
ATx2928/Waxy $F_{3:5}$	25%	12.39[b]	13.23[a]	13.68[a]	13.30[a]
ATx2928/Nonwaxy $F_{3:5}$	0%	12.23[b]	13.12[a]	13.79[a]	13.29[a]
	C.V. (%)	9	9	10	10
	LSD	0.96	0.61	0.65	0.40

Different superscript letters are significantly different at α=.05 using Fisher's Protected LSD

† Percent waxy F_2 grain produced by the hybrid in the panicle

differences between the genotypes within tester indicates that there is no kernel weight difference for waxy phenotype grain.

Stand analysis of variance detected variation for tester, genotype, and genotype by tester interaction (Table 5). Hybrids from *wxwxwx* F_1 endosperms had significantly worse stands than hybrids which germinated from *Wxwxwx* F_1 endosperms within the ATxArg-1 tester (Table 8). There were no differences between mean stands of hybrids from *WxWxwx* and *WxWxWx* F_1 endosperms within the ATx2928 tester, but as a group they had better stands than the ATxArg-1 hybrids. Analysis of variance for panicle number shows variation for genotype by tester interaction (Table 5). Panicle number means are significantly different between all hybrids from all four F_1 endosperm genotypes. *WxWxwx* F_1 endosperm hybrids had more panicles than did *WxWxWx* F_1 endosperm hybrids within the ATx2928 tester. *Wxwxwx* F_1 endosperm hybrids had more panicles per plot than *wxwxwx* F_1 endosperm hybrids within the ATxArg-1 tester (Table 8). Stand is dependant on germination and seedling vigor and panicle number per plot is a direct result of stand and the tillering ability of the hybrid. This data shows a reduction

in stand and panicle number for the *wxwxwx* F_1 endosperm hybrids compared to those from a *Wxwxwx* F_1 endosperm. This indicates that stand establishment is reduced due to the waxy endosperm. Interestingly, it appears panicle number was improved with the presence of one waxy allele compared to no waxy alleles, in this location. The rank of the stand rating and panicle number means correlates exactly to the yield rank. Since, a reduction in stand and panicle number per plot will definitely reduce yield, the yield decrease in this environment seems to be the result of lower stand establishment due to the F_1 endosperm genotype.

Analysis of variance was performed on the same grain yield data, except the variable panicle number was used as a covariate to eliminate the yield variation due to the differences in stand between F_1 endosperm genotypes. The analysis of variance shows variation for tester, but genotype and genotype by tester variation is not significant (Table 9). The adjusted mean grain yields were lower for the ATxArg-1 hybrids compared to the ATx2928 hybrids, but there were no differences for genotypes

Table 8. Stand ratings and panicle number per plot means by environment for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1.

Hybrid	F_1 Endosperm Genotype	03 Halfway		04 College Station		04 Halfway		Combined	
		Stand	PN†	Stand	PN	Stand	PN	Stand	PN
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	4.94^{c}	38.0^{d}	5.42^{b}	29.8^{b}	2.54^{b}	67.3^{c}	4.20^{c}	44.0^{d}
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	3.57^{b}	54.0^{c}	4.94^{b}	34.1^{b}	1.87^{b}	85.0^{b}	3.27^{b}	56.6^{c}
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	1.04^{a}	123.9^{a}	2.56^{a}	70.0^{a}	1.00^{a}	162.0^{a}	1.53^{a}	118.6^{a}
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	1.37^{a}	106.0^{b}	2.52^{a}	71.1^{a}	1.04^{a}	156.6^{a}	1.64^{a}	111.3^{b}
	C.V. (%)	38	22	37	43	59	20	41	25
	LSD	0.45	7.7	0.90	12.8	0.48	11.1	0.31	5.6

Different superscript letters are significantly different at α=.05 using Fisher's Protected LSD

† PN = panicle number

within testers (Table 10). Adjusting for panicle number variation effectively eliminated the yield differences seen in the unadjusted yield data. This indicates that the yield differences at this location were due to variation in panicle number.

Analysis of variance for the other dependant variables showed height and exsertion to contain variation for genotype by tester interaction (Table 5). ATx2928/waxy $F_{3:5}$ hybrids were taller than the ATx2928/nonwaxy $F_{3:5}$ hybrids (Table 11) and ATxArg-1/nonwaxy $F_{3:5}$ hybrids had greater exsertion than the ATxArg-1/waxy $F_{3:5}$ hybrids. Grain moisture and test weight only showed variation for tester. Higher moisture means were present for ATxArg-1 hybrids over ATx2928 hybrids. Test weights were higher in ATx2928 hybrids than ATxArg-1 hybrids (Table 11).

The mean yield of the 100% waxy grain hybrid initially showed a significant drop in yield compared to the 25% waxy grain hybrid within the ATxArg-1 tester. This is what was expected assuming the yield effect for waxy hybrids is due to waxy phenotype grain. The 500 kernel weight data, however, shows no differences between waxy and nonwaxy grain. Stand and panicle number differences were found between F_1 endosperm genotypes, with the nonwaxy genotype (*Wxwxwx*) performing better than the waxy genotype (*wxwxwx*). When panicle number differences due to stand were adjusted for, the yield effect disappears. It appears the yield effect occurring in this location is due to a stand establishment effect, likely caused by the F_1 endosperm genotype.

Table 9. Grain yield analysis of variance, with panicle number as covariate, for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003.

Sources of Variation	df	Mean Squares
Replication	2	1280136
Tester	1	3212747*
Genotype	1	187317
GenotypexTester	1	354281
Error	131	491337
Panicle Number	1	77989112**

*, ** Significant at p<.05 and .01 respectively

Table 10. Mean grain yields (kg/ha) by environment for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, adjusted for panicle number.

Hybrid	F_1 Endosperm Genotype	03 Halfway	04 College Station	04 Halfway	Combined
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	5305[b]	1796[b]	6947[b]	4685[b]
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	5518[b]	1612[b]	6806[b]	4647[b]
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	6202[a]	2389[a]	8062[a]	5552[a]
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	6158[a]	2422[a]	7990[a]	5525[a]
	C.V. (%)	14	27	14	16
	LSD	348.3	288.3	497.5	221.6

Different letters within locations are significantly different at α=.05 using Fisher's Protected LSD

Table 11. Mean grain moisture (%), height (in.), exsertion (in.), and test weights (lb/bu) for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003.

		Dependent Variables			
Hybrid	F_1 Endosperm Genotype	Moisture	Height	Exsertion	Test Weight
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	14.94[a]	45.45[b]	2.15[c]	57.02[b]
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	15.21[a]	45.77[ab]	3.56[a]	56.92[b]
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	14.50[b]	46.48[a]	3.04[ab]	58.84[a]
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	14.54[b]	45.00[b]	2.44[bc]	57.57[ab]
	C.V. (%)	5	6	51	3
	LSD	0.33	1.09	0.63	0.85

Different letters are significantly different at α=.05 using Fisher's Protected LSD

College Station 2004

Analysis of variance for grain yield shows variation for tester (Table 12). The ATxArg-1 hybrids yielded lower than the ATx2928 hybrids but there were no differences between genotypes within testers (Table 6). The yield differences expected by the hypothesis did not exist in this location.

Analysis of variance was performed on 500 kernel weight and variation was observed only for tester (Table 12). ATx2928 hybrids had higher 500 kernel weights than ATxArg-1 hybrids (Table 7), but no differences were observed among genotypes within a tester. There was no evidence of an individual kernel weight difference between waxy and nonwaxy grain.

Stand analysis of variance shows variation for tester (Table 12). ATx2928 hybrids had better stands than ATxArg-1 hybrids (Table 8). Panicle number showed variation due to tester (Table 12) and ATx2928 hybrids had more panicles per plot than

did ATxArg-1 hybrids (Table 8).

Analysis of variance for grain yield adjusted for panicle number shows variation for tester even though variation for panicle number was controlled (Table 13). ATx2928 hybrids were higher in yield than ATxArg-1 hybrids (Table 11).

Significant variation for days to anthesis was detected among testers with ATx2928 hybrids being earlier than ATxArg-1 hybrids (Table 14). No differences existed between panicle length means.

Grain yield did not show any yield difference due to the amount of waxy grain a hybrid produces. The 500 kernel weights show no kernel weight difference between waxy and nonwaxy grain. Stand and panicle number differences were not statistically significant between F_1 endosperm genotypes within testers, but numerically they followed the same trend seen in Halfway 2003, with *wxwxwx* F_1 endosperm hybrids trailing *Wxwxwx* F_1 hybrids in stand and panicle number.

Table 12. Grain yield, stand, 500 kernel weight, days to mid-anthesis, panicle length, and panicle number analysis of variance for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in College Station 2004.

		Dependent Variables					
Sources of Variation	df	Yield	Stand	500 Kernel	Days	Panicle Length	Panicle Number
Replication	2	$6.6x10^{6}$**	14**	2.1	77**	5.4**	1091**
Tester	1	$7.0x10^{7}$**	144**	20**	472**	0.1	32470**
Genotype	1	$1.2x10^{4}$	1.2	1.6	0.8	0.7	130
GenotypexTester	1	$2.9x10^{5}$	0.9	0.8	30	0.3	42
Error	97†	$6.4x10^{5}$	2.2	1.4	7.6	0.6	445

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error in ANOVA for yield, stand, and 500 kernel weight equals 82

Table 13. Grain yield analysis of variance, with panicle number as covariate, for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in College Station 2004.

Sources of Variation	df	Mean Squares
Replication	2	294805
Tester	1	4337813**
Genotype	1	107864
GenotypexTester	1	225199
Error	96	237946
Panicle Number	1	35933637**

*, ** Significant at p<.05 and .01 respectively

Table 14. Mean days to mid-anthesis and panicle length (in.) for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in College Station 2004.

		Dependent Variables	
Hybrid	F_1 Endosperm Genotype	Days	Panicle Length
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	175.57[a]	10.79[a]
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	177.03[a]	10.71[a]
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	171.78[b]	10.98[a]
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	170.72[b]	10.66[a]
	C.V. (%)	2	7
	LSD	1.67	0.46

Different superscript letters are significantly different at α=.05 using Fisher's Protected LSD

Halfway 2004

Significant variation in grain yield was detected for tester (Table 15). ATx2928 hybrids yield more than ATxArg-1 hybrids (Table 6). There were no differences between genotypes within either tester. This environment did not show the hypothetically expected yield decrease for hybrids that produce more waxy grain.

No variation was detected in 500 kernel weight (Table 15). This environment showed no evidence of a difference in kernel weight for waxy and nonwaxy grain.

Stand data shows variation for tester (Table 15). ATx2928 hybrids have better stand ratings than ATxArg-1 hybrids (Table 8). Panicle number shows variation for tester and genotype by tester interaction (Table 15). ATx2928 hybrids have more panicles per plot than do the ATxArg-1 hybrids (Table 8). Hybrids from a waxy (*wxwxwx*) F_1 endosperm had fewer panicles per plot than nonwaxy (*Wxwxwx*) F_1 endosperm hybrids (Table 8). While the stand data could not show a significant effect due to F_1 endosperm genotype, there is a deleterious effect on panicle number due to the waxy F_1 endosperm in this data.

Adjusted grain yield, using panicle number as a covariate, shows variation for tester (Table 16). ATx2928 hybrids yield more than ATxArg-1 hybrids (Table 11).

Grain moisture analysis detected variation for tester (Table 15), with ATx2928 hybrids having higher moisture content than ATxArg-1 hybrids. For height, variation

for tester and genotype was detected (Table 15). ATx2928 hybrids were taller than the ATxArg-1 hybrids (Table 17). Variation was not detected in exsertion and panicle length. Variation for test weight was detected for tester and genotype as well as genotype by tester interaction (Table 15). ATx2928 hybrids had higher test weights than ATxArg-1 hybrids. Hybrids that produced 100% waxy grain had significantly lower test weights than hybrids that produced 25% waxy grain within the ATxArg-1 tester (Table 16).

The data from this environment does not support a yield effect due to waxy endosperm grain, nor a kernel weight difference due to waxy phenotype. It did show an effect on panicle number due to F_1 endosperm; hybrids from waxy endosperms (*wxwxwx*) produced fewer panicles per plot compared to hybrids from nonwaxy endosperms (*Wxwxwx*). Test weight was significantly reduced for 100% waxy grain hybrids compared to those that produced 25% waxy grain.

Table 15. Grain yield, stand, grain moisture, 500 kernel weight, height, exsertion, panicle length, panicle number, and test weight analysis of variance for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2004.

		Dependent Variables								
Sources of Variation	df	Yield	Stand	Moisture	500 Kernel	Height	Exsertion	Panicle Length	Panicle Number	Test Weight
Replication	2	$4.7x10^6$	0.1	0.7	15**	27*	2.6	1.1	8312	0.4
Tester	1	$4.3x10^8$**	40**	52**	4.5	799**	4.3	0.1	195574**	38**
Genotype	1	$4.0x10^5$	2.8	1.7	1.5	52*	1.9	1.4	1071	18**
GenotypexTester	1	$3.8x10^6$	3.5	0.7	3.3	25	2.5	0.0	3733*	7.6*
Error	111	$1.6x10^6$	0.9	7.2	1.9	8.6	1.7	0.5	570	1.5

*, ** Significant at p<.05 and .01 respectively

Table 16. Grain yield analysis of variance, with panicle number as covariate, for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2004.

Sources of Variation	df	Mean Squares
Replication	2	3980278*
Tester	1	7246049**
Genotype	1	246631
GenotypexTester	1	25944
Error	111	896306
Panicle Number	1	76001542

*, ** Significant at p<.05 and .01 respectively

Table 17. Mean grain moisture (%), height (in.), exsertion (in.), panicle length (in.), and test weights (lb/bu) for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2004.

Hybrid	F_1 Endosperm Genotype	Dependent Variables				
		Moisture	Height	Exsertion	Panicle Length	Test Weight
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	11.50[b]	51.21[b]	1.79[a]	10.00[a]	53.51[c]
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	11.91[b]	53.52[b]	2.35[a]	9.74[a]	54.91[b]
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	13.02[a]	57.52[a]	2.48[a]	10.02[a]	55.29[ab]
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	13.10[a]	57.93[a]	2.44[a]	9.83[a]	55.57[a]
	C.V. (%)	22	5	57	7	2
	LSD	1.25	1.37	0.60	0.34	0.56

Different letters are significantly different at α=.05 using Fisher's Protected LSD

Combined Environments

Grain yield showed variation for tester and genotype by tester (Table 18). Yields of hybrids that produced 100% waxy grain were lower than hybrids that produced 25% waxy grain (Table 6), and ATx2928 hybrids yielded more than ATxArg-1 hybrids. The combined data does show the yield effect that is concurrent with the hypothesis.

Analysis of variance of 500 kernel weights shows variation for tester (Table 18). No differences between either tester or genotypes within testers were found (Table 7). There is no evidence of a kernel weight difference due to the phenotype of the grain.

Combined stand data shows variation for tester and genotype by tester (Table 18). ATx2928 hybrids have significantly better stands than do ATxArg-1 hybrids (Table 8). Hybrids from *wxwxwx* F_1 endosperms have significantly worse stands than do hybrids from *Wxwxwx* F_1 endosperms. Panicle number data shows variation for tester and genotype by tester (Table 18) and mean comparisons show differences between all F_1 endosperm types. *WxWxwx* F_1 endosperm hybrids had more panicles per plot than *WxWxWx* hybrids. *Wxwxwx* F_1 endosperm hybrids had more panicles than *wxwxwx* hybrids (Table 8). The combined data clearly shows a stand establishment and panicle number per plot effect due to the F_1 endosperm genotype. The yield rank of the hybrids and the stand and panicle number mean ranks correlate exactly.

Grain yield, adjusted for panicle number, shows variation for tester (Table 19). ATx2928 hybrids yield significantly more than ATxArg-1 hybrids, but there are no differences within testers for genotype (Table 11). Adjusting grain yield, to reduce variation due to panicle number, effectively eliminates the yield difference between the genotypes within testers. It appears that the yield effect is due to stand establishment differences between the different F_1 endosperm genotypes.

Grain moisture and panicle length show no variation for genotype, tester or genotype by tester interaction (Table 18). Plant height showed variation for both tester and genotype by tester. ATx2928 hybrids were taller than ATxArg-1 hybrids, and ATxArg-1/waxy $F_{3:5}$ hybrids were shorter than ATxArg-1/nonwaxy $F_{3:5}$ hybrids (Table 20). Analysis of exsertion data detected variation for genotype by tester (Table 18), where ATxArg-1/waxy $F_{3:5}$ hybrids have significantly less exsertion than ATxArg-1/nonwaxy $F_{3:5}$ hybrids. Test weight analysis of variance detected variation for tester and genotype by tester (Table 18) and 100% waxy grain hybrids have lower test weights than 25% waxy grain hybrids within the ATxArg-1 tester (Table 20).

Table 18. Combined analysis of variance for grain yield, stand, 500 kernel weight, grain moisture, height, exsertion, panicle length, panicle number, and test weight for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003 , Halfway 2004, and College Station 2004.

Sources of Variation	Df	GY†	ST	500K	M	HT	EX	PL†	PN†	TW†
Location	2	$5.6x10^{8}$**	122**	38	325**	5278**	17	35*	107602**	412**
Rep(Location)	6	$4.3x10^{6}$**	4.9**	8.3**	0.6	14	3.1	3.3*	1699**	2.4
Tester	1	$7.5x10^{8}$**	411**	9.5*	8.9	453**	1.1	0.2	313590**	80**
Genotype	1	$1.6x10^{5}$	10**	6.2	2.2	9.2	6.6	2.0	519	0.2
GenotypexTester	1	$1.0x10^{7}$**	17**	4.7	1.0	52**	26**	0.1	7599**	17*
TesterxLocation	2	$2.2x10^{8}$**	29**	29**	51**	410**	4.0	0.0	9564**	0.0
GenotypexLocation	2	$1.1x10^{6}$	0.5	0.4	0.1	57**	0.3	0.0	380	30**
GenotypexTesterxLocation	2	$5.9x10^{6}$**	3.1	0.2	0.0	0.0	7.6*	0.3	1456*	0.0
Error	322	$1.1x10^{6}$	1.3	4.6	3.9	7.4	1.9	0.6	431	2.6

*, ** Significant at p<.05 and .01 respectively

† GY, ST, 500K, M, HT, EX, PL, PN, and TW = yield, stand, 500 kernel weight, grain moisture, panicle length, panicle number, and test weight

Table 19. Combined analysis of variance for grain yield, adjusted for panicle number, for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003, College Station 2004, and Halfway 2004.

Sources of Variation	Df	Adj. Grain Yield
Location	2	12021319**
Replication(Location)	6	1852295**
Tester	1	14318791**
Genotype	1	68813
GenotypexTester	1	1792
TesterxLocation	2	360224
GenotypexLocation	2	253110
GenotypexTesterxLocation	2	303288
Error	322	548683
Panicle Number	1	63316488**

*, ** Significant at p<.05 and .01 respectively

Table 20. Combined environment means for grain moisture (%), height (in.), exsertion (in.), panicle length (in.), and test weight (lb/bu) for hybrids from crosses between RTx2907/RTx430 $F_{3:5}$ lines and testers ATx2928 and ATxArg-1, in Halfway 2003, Halfway 2004, and College Station 2004.

Hybrid	F_1 Endosperm Genotype	Dependent Variables				
		Moisture	Height	Exsertion	Panicle Length	Test Weight
ATxArg-1/Waxy $F_{3:5}$	*wxwxwx*	13.22[a]	48.33[c]	1.97[c]	10.39[a]	55.30[c]
ATxArg-1/Nonwaxy $F_{3:5}$	*Wxwxwx*	13.56[a]	49.65[b]	2.96[a]	10.23[a]	55.91[b]
ATx2928/Waxy $F_{3:5}$	*WxWxwx*	13.76[a]	52.00[a]	5.76[ab]	10.50[a]	57.07[a]
ATx2928/Nonwaxy $F_{3:5}$	*WxWxWx*	13.82[a]	51.46[a]	2.44[b]	10.25[a]	56.57[a]
	C.V. (%)	15	5	54	7	3
	LSD	0.62	0.86	0.43	0.27	0.51

Different superscript letters are significantly different at α=.05 using Fisher's Protected LSD

Further Discussion

The hypothesis upon which this research is based is that waxy phenotype grain per se is reducing the yield of waxy sorghum. In this experiment, two related differences among hybrids are being compared: (1) does the amount of waxy grain produced by the hybrid affect yield, and (2) does the F_1 endosperm genotype of the hybrid seed have any effect on stand establishment. The first difference implicates lower individual kernel weight for waxy grain as the source of the yield reduction, which would be detected by a difference in 500 kernel weight. For the second to be true, the difference should appear in a factor related to germination and seedling vigor. Those parameters were measured as stand and panicle number per plot. A difference in total yield should be explained by at least one of the above effects.

Grain yield data showed hybrids that produce 100% waxy grain yielded significantly lower than 25% waxy grain hybrids in one individual environment and the combined environments, within the ATxArg-1 tester. Within the ATx2928 tester, 25% waxy hybrids yielded more than 0% waxy hybrids in one individual environment. No other differences were shown.

Five hundred kernel weights were not different within testers for hybrids that produced different amounts of waxy grain in any single environment nor in the combined environments. This clearly shows waxy grain does not weigh differently than nonwaxy grain.

Clear differences were found for stand and panicle number means due to F_1 endosperm genotype. Waxy F_1 endosperms (*wxwxwx*) had significantly poorer stands

than nonwaxy F_1 endosperms (*Wxwxwx*) in one environment and the combined environments, although numerically the trend was observed in every environment. Panicle number means showed *wxwxwx* means were significantly lower than *Wxwxwx* in two of three individual environments and the combined environments, with the fourth environment showing the same trend numerically. Stand ratings measure the final plot stand density prior to harvest but the factors most influential to it are germination and seedling vigor. Germination would be reduced for waxy endosperms if (1) they are more susceptible to grain mold or soil born pathogens, (2) germination reducing alleles are linked to the waxy allele, (3) physiologically waxy endosperms require stricter environmental conditions to successfully provide energy to the embryo. Of these three possibilities susceptibility to grain mold is a likely cause. Hybrid seed production for these hybrids occurred in Weslaco 2002 during the fall and College Station 2003 during the summer. Grain mold pressure in those locations can be high, and if waxy endosperms are more susceptible, could have shown evidence through lower germination.

Further proof that the yield effect seen in this research was due to variation in stand and panicle number, comes from the grain yield data adjusted for panicle number. Once adjusted, the previous yield effect is eliminated and no differences exist between yields of hybrids within tester in any environment.

Interestingly, combined test weight means indicated that 100% waxy hybrid grain samples were lower in test weight than 25% waxy hybrid grain samples. This is based on only two environments of data, since yields were too low to measure test

weight in the other environment. Test weight is a measure of bulk density, the weight of how many kernels can fit into a finite volume. If test weight truly decreases and 500 kernel weight does not change, one explanation would be that seed volume must be increasing and seed density be decreasing. This, however, would not affect yield since gross seed weight does not change.

Combined height data shows ATxArg-1/waxy $F_{3:5}$ hybrids are shorter than ATxArg-1/nonwaxy $F_{3:5}$ hybrids, while there is no such difference in the ATx2928 hybrids. This could imply that dominant height modification genes are linked to the nonwaxy allele, thus in ATx2928 hybrids no effect can be seen because each hybrid contains a dominant nonwaxy allele from ATx2928. In ATxArg-1 hybrids, it is apparent because the ATxArg-1 tester would carry the recessive alleles which would allow expression in only the ATxArg-1/nonwaxy $F_{3:5}$ hybrids.

Conclusions

The yield effect associated with the waxy gene in this experiment is due to stand establishment differences between F_1 endosperm genotypes and not due to individual kernel weight differences of waxy phenotype grain. Waxy endosperms (*wxwxwx*) have lower stand establishment probably from reduced germination which may be caused by higher susceptibility to grain mold.

CHAPTER IV

B-LINE POPULATION

Introduction

Waxy endosperm sorghum hybrids would be very useful for food, feed, and industrial uses if they yielded competitively with non-waxy sorghums. However, due to their reduced yields, waxy sorghum hybrids have not been commercially sold and relatively few waxy parental lines have been developed for hybrid seed production. Breeding programs have not devoted many resources to developing higher yielding waxy hybrids partially because the nature of the yield depression is unknown. The yield depression could result from three mechanisms: (1) waxy endosperm grain has altered physical characteristics that reduce yield, (2) the waxy gene affects unknown traits in the plant through pleiotrophy that reduce yield, and (3) deleterious genes tightly linked to the waxy allele reduce yield.

Based on previous research the waxy phenotype itself is suspected to cause the yield decrease. The waxy phenotype shows a xenia effect, in which the genotype of the pollen has an immediate effect on the phenotype of the developing seed. This characteristic will allow the phenotype of the grain developing on a hybrid to be changed by controlling the type of pollen it receives. Thus a comparison of identical hybrids under different pollination conditions, one resulting in the formation of waxy grain and

the other nonwaxy grain is possible. This situation allows the direct detection of an immediate yield effect due to waxy phenotype grain.

The objective of this experiment is to determine if a xenia yield effect exists for the waxy gene.

Materials and Methods

Population Development

A population, described in Rooney et al. (2005), was created from the cross between BTxArg-1 and BTx623. BTxArg-1 is a waxy parental line that was released form the TAES sorghum breeding program and has been used in the hybrid seed industry (Miller et al., 1992). BTx623 is a nonwaxy parental line released from the TAES sorghum breeding program in 1977, and has been widely used in commercial industry and the research community.

From this cross, 120 individual F_2 progeny were randomly chosen, self-pollinated, and advanced to the next generation. In each $F_{2:3}$ progeny row, a single panicle was self- pollinated. At this point, seed from each $F_{3:4}$ were phenotypically screened using the potassium iodide test (Karper, 1933). $F_{3:4}$ lines that were homozygous for either waxy or nonwaxy endosperm were then selected for advancement and self pollinated to produce $F_{3:5}$ seed. The waxy and nonwaxy $F_{3:5}$ lines were crossed as pollinators onto a nonwaxy tester, A3Tx436, to produce two testcross hybrid genotypes: heterozygous waxy (*Wxwx*) and homozygous nonwaxy (*WxWx*). A3Tx436 is

an unreleased line developed by the TAES sorghum breeding program. It is a version of RTx436 (Miller et al., 1992) that contains A3 cytoplasm, which results in male sterile hybrids in testcross combination with most lines, these hybrids are sterile as well.

Hypothesis

Genotypes were both planted into two blocks, one pollinated by waxy pollen and the other with nonwaxy pollen. The heterozygous waxy sterile hybrids segregate for the waxy allele, thus 50% of its female gametes contain the waxy allele. Fertilized with waxy pollen, it should produce 50% waxy grain. The nonwaxy sterile hybrid in the same pollination block will produce 0% waxy grain since it does not contain the waxy allele. When planted in the nonwaxy pollination block, both sterile hybrids will be fertilized by nonwaxy pollen resulting in both producing 0% waxy grain. This will set up a comparison between two hybrid types in two treatments. If the waxy phenotype is the source of the yield effect, heterozygous hybrids under waxy pollination should yield less than the nonwaxy hybrids, due to their production of 50% waxy grain, and under nonwaxy pollination they should yield the same, since both will produce 0% waxy grain.

A xenia yield effect in this manner can only be affecting yield by reducing the individual kernel weight of waxy grain compared with nonwaxy grain. In addition to yield, 500 kernel weight will be measured to detect a change in the kernel weights of grain samples containing higher amounts of waxy grain.

These two hybrid types are produced from two F_1 endosperm genotypes as well, *WxWxwx* for the heterozygous hybrid and *WxWxWx* for the nonwaxy hybrid.

Comparisons can be made between these two F_1 endosperm genotypes for their possible effects on stand and panicle number.

The hypothesis from previous research (Aydin, 2003) is that there is a xenia yield effect for the waxy gene. This research will attempt to determine if this is the case.

Experimental Design

From each endosperm type, 15 testcross hybrids were randomly selected and planted in two-row plots in a RCBD with 3 replications in two adjacent pollination blocks. The blocks were separated by four rows of tall hybrid corn to reduce pollen contamination between the blocks. A waxy pollinator mix, which consisted of 50% ATxArg-1/RTx2907, 25% RTx2907, and 25% BTxArg-1, was used to pollinate the waxy block. A nonwaxy pollinator mix, which consisted of 22% each of ATx623/RTx436, ATx378/RTx436, and ATx2928/RTx436 and 11% each of RTx436, BTx378, and BTx623, was used to pollinate the nonwaxy block. Pollinators were planted in two row plots the length of the field in the first two, middle two, and last two rows of the blocks. In this way, a pollinator was adjacent to every sterile hybrid plot.

The Halfway 2004 environment was planted differently than the other three environments due to available space. The two pollination blocks were not adjacent to one another, but since combined pollination block analysis is not performed this will not affect the analysis. Due to space restrictions the pollinators in each block were planted the length of the field in the first two, middle two, and last two rows, but instead of only four rows of hybrids between them, there were six. In this layout, there is a two row

hybrid that has sterile hybrid on either side instead of a pollinator. To compensate, a full range of pollinators were planted in front of the first and behind the last replication and in between each replication. This was to ensure sufficient pollen for fertilization. This should not affect the conclusions of the analysis since the replications were randomized, but seed set data will show how well the compensation worked.

This test was planted on February 12, 2003 in Weslaco, Texas and was irrigated twice during the growing season before harvest on July 1. Plots at this location were 25 feet long on a row spacing of 30 inches. On April 4, 2003 it was planted in College Station, Texas and irrigated once before harvest on August 10. Plots at this location were 18 feet long on a row spacing of 30 inches. In 2004, this test was planted on March 31 in College Station and harvested August 7, no irrigation was necessary. Plot size at this location was the same as the previous year. On May 24, 2004 it was planted in Halfway, Texas and irrigated twice during the season before harvest on October 26. Plots at this location were 16 feet long on a row spacing of 40 inches. Hybrid seed for all locations was treated with a liquid mixture of Apron, Captan, Alliance, and Concep brand seed treatments prior to planting. All other agronomic practices were standard for sorghum production in the region.

Field Evaluation

Plant height, head exsertion, days to anthesis, panicle number, seed set, and stand ratings were taken in the field for each plot. Plant height was measured in inches from the base of the plant to the tip of the panicle as an average for the plot. Exsertion was

measured in inches from the base of the panicle at the flag leaf to the first panicle branch. Days to mid-anthesis was recorded as the Julian date when 50% of the plot reaches 50% anthesis. Panicle number was recorded as the total number of panicles per plot. In College Station and Weslaco 2003, seed set was originally scored using a 1-4 scale, with 1 representing 75-100% seed set and 4 representing 0-25% seed set. This system was deemed inadequate so in College Station and Halfway 2004, seed set was scored on a 0-9 scale, with each number representing 10% seed set. A score of 0 indicates 0-10% seed set and a 9 indicates 90-100% seed set. Seed set data from 2003 College Station and Weslaco was converted to a corresponding 1, 3, 6, or 8 in the 0-9 scale, for analysis. Stand was scored visually using a 1-9 scale, with 1 having a full stand and 9 having no stand. The plots were harvested with a modified John Deere 3300 plot combine equipped with a HarvestMaster HM-1000 weigh system. The combine measured plot weight, grain moisture, and test weight. Random samples of three panicles were harvested by hand prior to combine harvesting to constitute a grain sample for each plot. These heads were measured for panicle length, then cut into thirds, bulked within sections and threshed in a single head thresher. The middle one third grain samples were sieved over a 6 ½ /64" round holed sieve to remove broken kernels and any remaining foreign matter, then counted using an ESC-1 grain counter, into 500 seed lots and weighed. The sample grain was then decorticated using a TADD mill and visually separated into waxy and nonwaxy seed based on endosperm phenotype. The percentage of waxy seed was used to verify correct phenotype and determine pollination efficiency.

Data Considerations

Plots in individual environments that had a stand rating of less than 8 were included in analysis. Those 8 and greater were removed because they contain too few plants to make yield data relevant. Other plots that contained plants with red seed color were removed since, in this population, this condition can only be caused by pollen contamination and outcrossing (Table 21).

Statistical Analysis

Analysis by individual environments was conducted for grain yield, stand, panicle number, seed set, height, exsertion, and 500 kernel weight. Data was analyzed using the GLM:Univariate procedure in SPSS v11.5. The ANOVA model used to analyze separate pollination blocks in individual environments partitions variation into replication, genotype, and entry within genotype with genotype as the only fixed term (Table 22). Mean comparisons were made using Fisher's LSD at an error rate of $P<.05$. Pollination blocks are not analyzed together because results from Bartlett's test for

homogeneity indicate error variances between adjacent pollination blocks are not equal. Disregarding the differences in error variances and analyzing them as combined blocks is an option, but doing so would confuse true effects from error effects due to an averaged error variance between blocks. There is no reason to use this analysis when the option of separate analysis can provide more accurate detection of the same information.

Bartlett's test for homogeneity of error variances was used to test the validity of combining individual pollen block data from individual environments. Results indicated that the error variances were heterogeneous but data from individual environments were normally distributed, no appropriate data transformations were found, and no alternative models exist. Therefore, combined analysis was conducted for separate pollination blocks. The analysis of variance for combined environments adds locations and their interactions to the existing model (Table 23). Mean comparisons were conducted using Fisher's LSD with a probability level of 0.05.

Table 21. Total plots remaining by genotype after removal due to high stand rating or incorrect seed color for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids in individual environment pollination blocks.

Location	Genotype	Pollen Block	Final n†
03 College Station	A3TX436/Nonwaxy $F_{3:5}$	NW	35
	A3TX436/Waxy $F_{3:5}$	NW	38
	A3TX436/Nonwaxy $F_{3:5}$	WX	35
	A3TX436/Waxy $F_{3:5}$	WX	37
03 Weslaco	A3TX436/Nonwaxy $F_{3:5}$	NW	45
	A3TX436/Waxy $F_{3:5}$	NW	42
	A3TX436/Nonwaxy $F_{3:5}$	WX	45
	A3TX436/Waxy $F_{3:5}$	WX	42
04 College Station	A3TX436/Nonwaxy $F_{3:5}$	NW	42
	A3TX436/Waxy $F_{3:5}$	NW	42
	A3TX436/Nonwaxy $F_{3:5}$	WX	42
	A3TX436/Waxy $F_{3:5}$	WX	42
04 Halfway	A3TX436/Nonwaxy $F_{3:5}$	NW	42
	A3TX436/Waxy $F_{3:5}$	NW	42
	A3TX436/Nonwaxy $F_{3:5}$	WX	42
	A3TX436/Waxy $F_{3:5}$	WX	42

† Initial plot number for all genotypes in each pollination block was 45

Table 22. Analysis of variance model used for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids in individual environment pollination blocks.

Source	Df	Mean Squares	Expected Mean Squares
Replication	r-1	MS_R	$\sigma^2_e + g\sigma^2_r$
Genotype	g-1	MS_G	$\sigma^2_e + r\sigma^2_{E(G)} + r\kappa^2_g$
Entry(Genotype)	g(e-1)	MS_E	$\sigma^2_e + r\sigma^2_{E(G)}$
Error	r(eg-1)	MS_e	σ^2_e

Table 23. Analysis of variance model used for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids in combined environment pollination blocks.

Source	df	Mean Squares	Expected Mean Squares
Location	l-1	MS_L	$\sigma^2_e + r\sigma^2_{E(g)l} + E\sigma^2_{r(l)} + rgE\kappa^2_l + rE\kappa^2_{gl}$
Rep(Location)	l(r-1)	MS_R	$\sigma^2_e + E\sigma^2_{r(l)}$
Genotype	g-1	MS_G	$\sigma^2_e + rl\sigma^2_{E(g)} + r\sigma^2_{E(g)l} + rEl\kappa^2_g + rE\kappa^2_{gl}$
Entry(Genotype)	g(e-1)	MS_E	$\sigma^2_e + rl\sigma^2_{E(g)}$
GenotypexLocation	(g-1)(l-1)	MS_{GL}	$\sigma^2_e + r\sigma^2_{E(g)l} + rE\kappa^2_{gl}$
Entry(Geno.)xLocation	g(e-1)(l-1)	MS_{EL}	$\sigma^2_e + r\sigma^2_{E(g)l}$
Error	r(egl-1)	MS_e	σ^2_e

Results and Discussion

Analysis by Environments

College Station 2003

Grain yield analysis of variance did not detect any differences due to genotype in either pollination block (Tables 24 and 25), but variation was present due to the entries within the genotypes. Stand, panicle number, and 500 kernel weight showed no differences for genotype in either pollination block (Tables 24, 26, 27, and 28).

The traits days to mid-anthesis, exsertion, and seed set show no differences due to genotype in the waxy pollination block. Nonwaxy hybrids were taller and had longer panicles in the waxy pollination block (Table 29).

In the nonwaxy pollination block height, exsertion, and panicle length showed no differences. Nonwaxy genotypes flowered two days later than heterozygous waxy genotypes in the nonwaxy pollination block, which may explain why heterozygous genotypes had significantly better seed set in the nonwaxy pollination block (Table 29).

Heterozygous waxy (*Wxwx*) hybrids produced an average of 37% waxy grain under waxy pollination compared to nonwaxy hybrids which produced 0% waxy grain (Table 25), there was no effect on grain yield or 500 kernel weight. Correct pollination ideally should have resulted in 50% waxy seed production for the heterozygous hybrid, but if a xenia yield effect exists there still should have been an effect.

Table 24. Grain yield, stand, and 500 kernel weight analysis of variance for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination block in College Station 2003.

		Pollination Blocks					
		Yield		Stand		500 Kernel	
		Pollination Blocks					
Source	df	WX	NW	WX	NW	WX	NW
Replication	2	3146057**	591450	0.5	2.7	0.1	0.3
Genotype	1	9603	1242676	0.6	6.5	2.5	1.9
Entry(Genotype)	26	343994*	926610**	2.2	2.9*	0.9	1.2
Error	42†	179394	410776	1.4	1.4	0.6	0.8

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error for 500 kernel weight equals 51

Table 25. Mean grain yields (kg/ha) for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by individual pollination blocks across environments.

	03 College Station		03 Weslaco		04 College Station		04 Halfway		Combined	
	Pollination Blocks									
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	$1310^{a}_{(37\%)}$	1873^{a}	$4700^{a}_{(23\%)}$	4872^{a}	$1670^{a}_{(25\%)}$	1001^{a}	$2690^{a}_{(23\%)}$	6426^{a}	$2635^{a}_{(27\%)}$	3583^{a}
Nonwaxy	1236^{a}	1628^{a}	4879^{a}	4930^{a}	1436^{a}	856^{a}	2337^{a}	5953^{a}	2569^{a}	3444^{a}
LSD	188	283	399	269	161	213	290	420	142	152
C.V. (%)	37	42	23	15	28	63	31	19	30	24

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Numbers in subscript parenthesis indicate average percent waxy phenotype seed produced; absence of parenthesis indicates 0%

Table 26. Mean stand ratings for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks across environments.

	03 College Station		03 Weslaco		04 College Station		04 Halfway		Combined	
	Pollination Blocks									
F_1 Endosperm Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
WxWxwx	5.16[a]	4.84[a]	2.67[a]	2.43[a]	2.14[a]	3.36[a]	1.19[a]	1.07[a]	2.72[a]	2.88[a]
WxWxWx	5.34[a]	5.23[a]	2.42[a]	2.31[a]	2.64[a]	3.50[a]	1.12[a]	1.14[a]	2.77[a]	2.94[a]
LSD	0.47	0.46	0.34	0.36	0.27	0.38	0.17	0.10	0.16	0.17
C.V. (%)	22	23	37	42	31	30	41	26	30	30

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Table 27. Panicle number means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks across environments.

	03 College Station		03 Weslaco		04 College Station		04 Halfway		Combined	
	Pollination Blocks									
F_1 Endosperm Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
WxWxwx	26.10[a]	32.97[a]	61.98[a]	65.14[a]	75.62[a]	47.05[a]	122.41[a]	125.64[a]	72.93[a]	68.55[a]
WxWxWx	26.57[a]	29.60[a]	66.56[a]	65.27[a]	68.26[a]	44.07[a]	117.86[a]	120.26[a]	71.59[a]	66.31[a]
LSD	3.30	4.62	3.36	3.10	4.53	3.92	6.64	5.22	2.38	2.12
C.V. (%)	32	38	14	13	17	23	15	12	18	17

Different letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Table 28. Mean 500 kernel weights (g) for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks across environments.

	03 College Station		03 Weslaco		04 College Station		04 Halfway		Combined	
	Pollination Blocks									
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	$15.62^{a}_{(37\%)}$	15.62^{a}	$14.63^{a}_{(23\%)}$	14.28^{a}	$14.03^{a}_{(25\%)}$	14.21^{a}	$15.59^{a}_{(23\%)}$	14.34^{a}	$14.97^{a}_{(27\%)}$	14.61^{a}
Nonwaxy	15.27^{a}	15.31^{a}	14.61^{a}	14.21^{a}	13.56^{b}	13.52^{b}	15.54^{a}	14.58^{a}	14.72^{a}	14.40^{a}
LSD	0.29	0.34	0.34	0.35	0.30	0.26	0.50	0.34	0.18	0.16
C.V. (%)	5	6	6	7	6	5	8	7	6	6

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Numbers in subscript parenthesis indicate average percent waxy phenotype seed produced, absence of parenthesis indicates 0%

Table 29. Height (in.), exsertion (in.), panicle length (in.), days to mid-anthesis, and seed set rating means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in College Station, 2003.

	Height		Exsertion		Panicle Length		Days		Seed Set	
	Pollination Blocks									
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	46.01[b]	46.89[a]	2.78[a]	4.04[a]	10.11[b]	11.28[a]	171.04[a]	169.73[b]	3.85[a]	4.98[a]
Nonwaxy	47.68[a]	47.79[a]	2.61[a]	3.38[a]	10.74[a]	11.62[a]	172.18[a]	171.95[a]	3.21[a]	2.36[b]
LSD	0.78	0.95	0.37	0.58	0.26	0.28	1.16	1.00	0.67	0.75
C.V. (%)	4	5	35	39	6	6	2	1	48	54

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Weslaco 2003

Grain yield and stand show variation for entry within genotypes in both pollination blocks, while 500 kernel weight shows variation for entry only in the waxy pollination block (Table 30). None of these traits are different between the hybrid genotypes (Tables 25, 26, and 28).

Panicle number, height, exsertion, panicle length, days to mid-anthesis, and seed set are not different between the hybrids in either pollination block (Tables 27 and 31).

This environment shows no differences anywhere between the two hybrid genotypes, so there are no differences due to percent waxy seed produced.

College Station 2004

Grain yield data shows no differences between the hybrid genotypes in either pollination block (Tables 25 and 32). Variation due to entry within genotype is present in both pollination blocks.

Stand analysis shows no variation between the F_1 endosperm genotypes in either pollination block (Table 26), while variation for entries is detected in both pollination blocks (Table 32).

Table 30. Grain yield, stand, and 500 kernel weight analysis of variance for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in Weslaco 2003.

		Yield		Stand		500 Kernel	
		Pollination Blocks					
Source	df	WX	NW	WX	NW	WX	NW
Replication	2	1798876	5949137**	2.2	0.7	0.8	14**
Genotype	1	550638	58880	1.3	0.3	0.0	0.1
Entry(Genotype)	27	2187369**	1001770**	2.1**	2.1**	1.5*	1.4
Error	56	974486	443896	0.9	1.0	0.9	0.9

*, ** Significant at p<.05 and .01 respectively

Table 31. Height (in.), exsertion (in.), panicle length (in.), days to mid-anthesis, and seed set rating means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in Weslaco, 2003.

	Height		Exsertion		Panicle Length		Days		Seed Set	
	Pollination Blocks									
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	49.07[a]	50.14[a]	5.60[a]	5.57[a]	10.18[a]	9.71[a]	82.67[a]	82.86[a]	6.62[a]	6.91[a]
Nonwaxy	48.96[a]	50.60[a]	5.60[a]	5.42[a]	10.31[a]	9.92[a]	82.78[a]	82.60[a]	6.13[a]	6.82[a]
LSD	0.45	0.43	0.32	0.32	0.23	0.23	0.40	0.38	0.61	0.49
C.V. (%)	3	2	16	16	6	7	1	1	27	21

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

The 500 kernels weight analysis of variance shows variation for genotype in both pollination blocks and entry within genotypes in only the nonwaxy block (Table 32). The means show heterozygous hybrids, which produced an average 25% waxy grain, were heavier than nonwaxy hybrids, that produced 0% waxy grain, in the waxy pollination block (Table 28). In the nonwaxy pollination block, where both hybrids produced 0% waxy grain, heterozygous hybrids were again heavier than nonwaxy hybrids. Since the increase in 500 kernel weight occurred in both pollination blocks for the heterozygous hybrid, it is independent of the amount of waxy seed the hybrid produces.

There were no differences due to hybrid genotype for days to mid-anthesis, seed set, or panicle length in either pollination block (Table 33). Seed set was quite low in both pollination blocks, likely due to extended wet weather during pollination. Height and exsertion data was not recorded in this environment.

Table 32. Grain yield, stand, and 500 kernel weight analysis of variance for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in College Station 2004.

		Yield		Stand		500 Kernel	
		Pollination Blocks					
Source	df	WX	NW	WX	NW	WX	NW
Replication	2	2845860**	591450	4.0**	2.3	0.3	1.7*
Genotype	1	920029	1242676	5.3	0.4	4.3*	8.4*
Entry(Genotype)	26	1138917**	926610**	1.6*	2.2*	0.7	1.4**
Error	54†	153558	410776	0.6	1.1	0.6	0.4

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error for 500 kernel weight were 50 and 45 for the waxy and nonwaxy pollination blocks respectively

Table 33. Panicle length (in.), days to mid-anthesis, and seed set rating means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in College Station, 2004.

	Panicle Length		Days		Seed Set	
	Pollination Blocks					
Genotypes	WX	NW	WX	NW	WX	NW
Heterowaxy	10.06[a]	10.14[a]	172.91[a]	175.41[a]	3.88[a]	2.24[a]
Nonwaxy	10.28[a]	10.31[a]	173.74[a]	176.12[a]	3.14[a]	1.45[a]
LSD	0.24	0.22	0.62	1.00	0.54	0.51
C.V. (%)	7	6	1	2	42	75

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

There were no yield effects observed in this environment caused by the amount of waxy grain a hybrid produces. The difference in 500 kernel weight is an environmental effect that is independent of waxy phenotype.

Halfway 2004

Grain yield analysis of variance detected variation for entry within genotypes in both pollination blocks but no differences between genotypes (Tables 25 and 34).

Stand and panicle number show no differences for F_1 endosperm genotype in either pollination block (Tables 26, 27, and 34).

The 500 kernel weight data shows no differences for hybrid genotype in either pollination block (Table 28 and 34). The two hybrid genotypes are not different for height, exsertion, panicle length, or seed set in either pollination block (Table 35).

This environment shows no differences due to the amount of waxy grain produced by the hybrids.

Table 34. Grain yield, stand, and 500 kernel weight analysis of variance for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in Halfway 2004.

		Yield		Stand		500 Kernel	
		Pollination Blocks					
Source	df	WX	NW	WX	NW	WX	NW
Replication	2	5468199**	14714943**	0.4	0.1	2.6	9.7**
Genotype	1	2094448	3735247	0.1	0.1	0.0	1.2
Entry(Genotype)	26	3465695**	1867233*	0.2	0.1	1.7	3.1**
Error	54†	498095	1044249	0.2	0.1	1.5	0.9

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error for 500 kernel weight waxy pollination block equaled 39

Table 35. Height (in.), exsertion (in.), panicle length, and seed set rating means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks in Halfway, 2004.

	Height		Exsertion		Panicle Length		Seed Set	
	Pollination Blocks							
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	57.62[a]	58.41[a]	4.05[a]	4.83[a]	10.06[a]	10.14[a]	5.42[a]	8.17[a]
Nonwaxy	57.07[a]	58.60[a]	3.69[a]	5.05[a]	10.04[a]	10.31[a]	4.31[a]	7.62[a]
LSD	0.66	0.58	0.40	0.49	0.48	0.21	0.44	0.26
C.V. (%)	3	3	28	27	13	6	25	9

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

Combined Environments

Combined analysis shows no difference between hybrids for grain yield or 500 kernel weight in either combined pollination block (Tables 25, 28, and 36). Stand and panicle number show no differences for F_1 endosperm genotype in either combined pollination block (Tables 26, 28, and 36). Days to mid-anthesis, height, exsertion, and panicle length show no differences for genotype in either combined pollination block (Table 37).

Heterozygous waxy hybrids have higher combined seed set in both pollination blocks (Table 37). An explanation for this effect is not readily apparent since the two hybrids did not differ in combined days to mid-anthesis.

The combined data shows no significant differences exist between hybrid genotype means for any yield trait regardless of the amount of waxy grain produced.

Further Discussion

In this experiment, the effect of the amount waxy grain produced, through different pollination treatments, on grain yield and 500 kernel weight, as well as the

Table 36. Grain yield, stand, and 500 kernel weight analysis of variance for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks combined across College Station and Weslaco 2003, and College Station and Halfway 2004 environments.

		Yield		Stand		500 Kernel	
		Pollination Blocks					
Source	df	WX	NW	WX	NW	WX	NW
Location	3	$1.7x10^{8}$**	$4.1x10^{8}$**	203**	209**	48**	35**
Rep(Loc)	8	$3.3x10^{6}$**	$5.5x10^{6}$**	1.8*	1.5	0.9	6.3**
Genotype	1	$1.0x10^{6}$	$2.9x10^{6}$	0.7	2.7	3.7	2.8
Entry(Genotype)	28	$2.6x10^{6}$*	$1.2x10^{6}$	1.8	2.4	2.2**	1.7
GenotypexLoc	3	$1.3x10^{6}$	$9.9x10^{5}$	2.4	1.9	0.9	2.9
Entry(Genotype)xLoc	77	$1.5x10^{6}$**	$1.2x10^{6}$**	1.4	1.7	0.8	1.8**
Error	206†	$4.7x10^{5}$	$5.5x10^{5}$	0.7	0.8	0.9	0.8

*, ** Significant at p<.05 and .01 respectively

† Degrees of freedom for error for 500 kernel weight waxy pollination block equals 196

Table 37. Height (in.), exsertion (in.), panicle length (in.), days to mid-anthesis, and seed set rating means for A3Tx436/(BTxArg-1/BTx623 $F_{3:5}$) hybrids by pollination blocks combined across Weslaco 2003, College Station 2003 and 2004, and Halfway 2004 environments.

	Height		Exsertion		Panicle Length		Days		Seed Set	
	Pollination Blocks									
Genotypes	WX	NW	WX	NW	WX	NW	WX	NW	WX	NW
Heterowaxy	50.90[a]	51.81[a]	4.14[a]	4.82[a]	10.10[a]	10.44[a]	142.20[a]	142.66[a]	4.95[a]	5.57[a]
Nonwaxy	51.18[a]	52.19[a]	4.00[a]	4.61[a]	10.28[a]	10.63[a]	141.50[a]	142.82[a]	4.23[b]	4.56[b]
LSD	0.35	0.37	0.21	0.26	0.19	0.12	0.42	0.46	0.28	0.26
C.V. (%)	3	3	24	26	10	6	1	2	34	28

Different superscript letters within pollen blocks are significantly different at a=.05 using Fisher's Protected LSD

effect of the F_1 endosperm genotypes on stand establishment and panicle number was investigated. The hypothesis that there is a xenia yield effect for the waxy gene is being tested.

The two hybrid genotypes produced different amounts of waxy phenotype grain under the two pollination regiments. The heterozygous waxy hybrid produced 27% waxy phenotype grain under waxy pollination. Perfect pollination should have resulted in 50% waxy grain, indicating that considerable pollen contamination occurred under these conditions. Regardless, there is no evidence that the heterozygous hybrid yielded lower when producing waxy grain than when yielding nonwaxy grain. Reinforcing this, 500 kernel weights, which detect differences in individual seed weight, show no differences or trends for hybrids with different amounts of waxy phenotype grain. The waxy phenotype does not reduce yield, nor does it reduce individual kernel weight. A xenia yield effect for the waxy gene did not occur.

The two hybrid types were planted with two genotypes of F_1 endosperm seed, *WxWxwx* and *WxWxWx*, which differ by one waxy allele. There is no statistical evidence that stand and panicle number are affected by either of these endosperm genotypes.

Conclusions

Waxy phenotype grain produced in the panicle does not affect grain yield. A xenia yield effect due to the waxy gene did not exist. From this experiment alone the nature of the yield effect associated with the waxy gene was not elucidated, but this evidence shows no support for the theory that waxy phenotype grain weighs less than nonwaxy grain, and thus is not the cause of the yield deficit.

CHAPTER V

SUMMARY

The nature of the yield decrease associated with waxy sorghum is important to know for breeders to determine how to address the problem. The waxy gene could affect sorghum yields in three ways: (1) the waxy phenotype grain has altered seed characteristics that affect yield, such as lower seed weight or reduced germination of hybrid seed, (2) deleterious alleles, closely linked to the waxy allele, are responsible for the yield decrease and, (3) the waxy allele affects other physiological yield traits through pleiotrophy.

The research reported here investigates parameters of grain yield that may be the cause of the yield decrease. The product of four parameters: plants per unit area, panicles per plant, kernels per panicle, and individual kernel weight equal the total yield output of grain per unit area. Changes must occur in one or more of these four areas for a yield deficit to occur due to the waxy gene.

Individual kernel weight was measured using 500 kernel weight of grain samples in both experiments. If waxy phenotype grain contained less starch, was physically smaller, or less dense, these comparisons would have detected those differences, they did not. Samples with high percentages of waxy grain weighed no less than samples with little to no waxy grain. Results from both of these separate populations agree that individual kernel weight of waxy phenotype grain was not less

than nonwaxy phenotype grain. Therefore, lower individual kernel weight was not the source of any yield difference due to the waxy gene.

Number of kernels per panicle as a yield parameter was not measured in this research so its effects on yield due to the waxy gene cannot be estimated. The yield parameters plants per unit area and number of panicles per plant were measured using stand and panicle number per plot. Comparisons were made in both experiments for the effects of the F_1 endosperm genotype on stand and panicle number. Clearly, waxy F_1 endosperms (*wxwxwx*) established thinner stands and produced fewer panicles per plot than did nonwaxy F_1 endosperms (*Wxwxwx*). This probably results from lower germination likely due to higher susceptibility to grain mold of the waxy endosperm. The net result of poorer stand establishment on yield is clear, waxy F_1 endosperm hybrids yield less. The yield effect associated with waxy sorghum is largely attributable to poor stand establishment of the waxy F_1 endosperm genotype.

Overall waxy F_1 endosperms establish thinner stands than nonwaxy endosperms, which cause the yield decrease associated with them. It remains to be seen whether the lower stand establishment is a result of higher susceptibility to grain mold, altered starch chemistry, or genes linked to the waxy allele, all reducing germination. It is likely to be due to grain mold pressure during seed production. This research should direct breeding programs to look at factors relating to germination and stand establishment in order to produce competitive yielding waxy hybrids. Future research should include the effect of grain mold on germination of waxy seed, and the genetic variance and heritability of germination and stand establishment within waxy populations. This will provide

breeders with enough information to determine whether developing high yielding waxy hybrids is feasible.

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