2010

Agronomic and phenotypic responses to 75 years of recurrent selection for yield in the Iowa Stiff Stalk synthetic maize population

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by

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A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Crop Production and Physiology

Program of Study Committee:
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Iowa State University

Ames, Iowa

2010

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AWKNOWLEDGEMENTS

CHAPTER 1. GENERAL INTRODUCTION

Since the 1930's, average maize yields in the United States have increased by a factor

of nearly five, from an average of 1.5 Mg ha^{-1} to an average of 8.5 Mg ha^{-1} today (Duvick,

2005). In the United States, increases in average annual maize yields have ranged from 99 kg

ha^{-1} (Lee and Tollenaar, 2007) to approximately 109 kg ha^{-1} (Duvick, 2005).

These yield increases have occurred while average yield per-plant has remained

nearly constant, increasing by an average of just 0.05 kg plant^{-1} over the past 40 years

(USDA, 2010) while average planting density has increased by an average 720 plants ha^{-1} per

year over the past 44 years (USDA, 1965-2009). This indicates that increasing plant density

has driven increases in grain yield. Hammer et. al. (2009) found that yields maximized at

30,000 plants ha^{-1} in the 1960's, while today's hybrids can increase yield at densities two to

three times higher. Tollenaar (1989) noted that newer hybrids out yielded the oldest hybrid

by only 25% (6.97 vs. 8.78 Mg ha^{-1}) at 40,000 plants ha^{-1}, but by 190% (4.54 vs. 9.50 Mg ha^{-1}) at 130,000 plants ha^{-1}.

How has the corn plant changed to facilitate these yield increases? Changes in several

maize characteristics have been examined across several eras in hybrid maize (Duvick, 2005;

Meghji et. al., 1984; Russel, 1984; Russel, 1985; Sangoi, 2002). However, such changes have

not yet been examined as responses to recurrent selection for yield in a genetically closed

population such as the Iowa Stiff Stalk Synthetic maize population. With known genetic

relationships in a recurrent selection program, we can study the genetic basis for plant

characteristics and relate these changes directly to selection response.

Chapter two is a review of literature discussing these maize characteristics as they have occurred in hybrid maize. Chapter three addresses changes in characteristics including yield, grain moisture, grain test weight, stalk lodging, and root lodging that have occurred in concert with increased planting densities and recurrent selection for yield in the genetically closed Iowa Stiff Stalk Synthetic Maize population.

Chapter four addresses changes in characteristics including flag leaf angle, anthesis-silking interval (ASI), plant height, tassel branch number, and total number of leaves per plant that have occurred in concert with increased planting densities and recurrent selection for yield in the genetically closed Iowa Stiff Stalk Synthetic Maize population.

The objective of these experiments was to determine if direct selection for grain yield and agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density. This information will help progress our understanding of the ability of maize to adapt from a point where increasing plant density could not facilitate increases in yield to a point where increasing plant density is driving increases in yield.

Since long term demand for maize is continuing to grow worldwide it is important to ensure that maize yields can continue increasing in the years to come. In order to accommodate the need for continuing increase in yield, new and exotic genetics are being introduced to plant breeding programs and it is necessary to rapidly increase yields of these and genetic materials currently utilized. The long term goal of this project is to locate genetic markers for these important maize characteristics. Genetic markers could then be used to select for these characteristics and hopefully accelerate yield increase.

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CHAPTER 2. REVIEW OF LITERATURE

Iowa Stiff Stalk Synthetic

Contribution to commercial hybrids. Lines derived from this population have made major contributions to the maize industry. It has been estimated that BSSS inbred lines were parents of 19% of the total corn acres planted in the US in 1980 (Zuber and Darrah, 1980). BSSS is a good population to evaluate because, unlike hybrids, BSSS has remained a closed population with no introduction of new germplasm (Lamkey et. al., 1991).

BSSS. The Iowa Stiff Stalk Synthetic population (BSSS) was originally created in 1934 by inter-mating 16 inbred lines with greater than normal stalk quality (Lamkey, 1992). From this base population, two different recurrent selection programs were initiated using selection criteria focused primarily on yield increase (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995) with some consideration for low grain moisture and increased resistance to stalk and root lodging (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995).

BSSS(HT)C7. From the original BSSS population 7 cycles of half-sib selection took place utilizing testcrosses to IA13 to determine candidates for selection. The 7th cycle was denoted BSSS(HT)C7 (Lamkey et. al., 2001). Details of procedures used for cycle advancement were described by Eberhart et. al. (1973).

BS13(S)C0. Created from BSSS(HT)C7 by selecting 29 S_1 lines (Lamkey, 1992).

BS13(HI)C5. Starting with BS13(S)C0, Dr. Kendall Lamkey with USDA/ARS Ames, IA completed 5 cycles of half-sib selection utilizing testcrosses to B97 to determine candidates for selection. The most advanced cycle from this program is BS13(HI)C5 (Edwards, In-Press).

BSSS(R)C17. A second selection program was also initiated from the original BSSS population utilized recurrent reciprocal selection with Iowa State Corn Borer Synthetic #1 (BSCB1) (Holthaus and Lamkey, 1995). Details of procedures used were described by Penny and Eberhart (1973). The population resulting from the first cycle of selection with the BSSS population was named BSSS(R)C1. To date 17 selection cycles have been completed with current endpoint BSSS(R)C17.

Changes in BSSS population characteristics. Agronomic changes have been found in early cycles of BSSS. Yield increases in most selection cycles in the BSSS program were significant, with an average increase of 1.6% within the first 7 cycles of selection leading from BSSS to BS13(S)C0 and an average increase of 2.6% per cycle in the first 11 cycles of selection leading from BSSS to BSSS(R)C17 (Holthaus and Lamkey, 1995). Root lodging increased by an average of .44% per cycle and an average decrease in stalk lodging of 1.12% per cycle was found within the first 7 cycles of selection leading from BSSS to BS13(S)C0 (Lamkey, 1992). No change in grain moisture was seen in the first 7 cycles (Eberhart et. al., 1973).

Hybrid Maize Yield Increase

It is well known that maize yields have risen dramatically over the past 75 years. Since the 1930's, average maize yields in the United States have increased by a factor of nearly five, from an average of 1.5 Mg ha^{-1} to an average of 8.5 Mg ha^{-1} today (Duvick, 2005). In the United States, increases in average annual maize yields have ranged from 99 kg ha^{-1} (Lee and Tollenaar, 2007) to approximately 109 kg ha^{-1} (Duvick, 2005). Hybrids of central Iowa have increased nearly 79 kg ha^{-1} annually (Duvick, 1996).

In Ontario Canada increases in grain yield were as high as 1.6% per year during 1959-1988 (Tollenaar, 1989). The most notable yield increases were seen when comparisons were made between varieties planted at high and low densities. Tollenaar (1989) found that newer hybrids out yielded the oldest hybrid by only 25% (6.97 vs. 8.78 Mg ha^{-1}) at 40,000 plants ha^{-1}, but by 190% (4.54 vs. 9.50 Mg ha^{-1}) at 130,000 plants ha^{-1}.

Plant Density

Plant density has driven yield increase. These increases in yield have occurred while average Iowa maize yield per-plant has remained nearly constant, increasing from .34 kg plant[-1] in 1965 to 0.37 kg plant[-1] in 2008 (USDA, 2010) while average planting density has increased by an average 720 plants ha^{-1} per year over the past 44 years (USDA, 1965-2009). This indicates that increasing plant density has driven increases in grain yield. Hammer et. al. (2009) found that yields maximized at 30,000 plants ha^{-1} in the 1960's, while today's hybrids can increase yield at densities two to three times higher. Similar conclusions have been reached in a number of other studies. A comparison of yields of 1930's era corn to that of 1970's era hybrids found that the 1930's hybrids had their highest yields at 29,700 plants ha^{-1} while the 1970's hybrids had their highest yields at 59,300 plants ha^{-1}. The difference in yield

between the two eras was just 4.6% at the lowest density but 70.4% differences in yield were seen at the highest density (Russell, 1986). Similar results were observed by Duvick (1996). No yield increase was found when hybrids were compared at 30,000 plants ha^{-1} (1960's planting density), further emphasizing the ability of more advanced hybrids to produce high yields at high planting densities. Carena and Cross (2002) found an average yield advantage of 11% with a planting density of 56,000 plants ha^{-1} over 38,000 plants ha^{-1} but found no further yield advantage at densities higher than 56,000 plants ha^{-1}.

Optimum planting density. Researchers have examined the point at which increasing planting density fails to increase yield. Has et. al. (2008) compared seven hybrids at three plant densities. The optimum plant density for grain yield was 47,000 plants ha^{-1}. Increased coefficients of variation for yield, ear length, and number of kernel rows were found, indicating that higher densities may provide a less uniform stand of plants. Raymond et. al. (2009) reported a yield increase of 1141 kg ha^{-1} at a density of 86,000 plants ha^{-1} when compared to the low density of 49,000 plants ha^{-1}.

This, however, conflicts with other research indicating much higher optimum planting densities. A density study conducted by Widdicombe and Thelen (2002) indicated that even planting densities as high as 90,000 plants ha^{-1} were below potential maximum yield densities. This indicates an even higher tolerance to stand density may be possible in advanced hybrids. Although average planting density has increased by nearly 0.97 m^{-2} per year since the 1930's, Tollenaar (1989) found that plant density remained nearly constant until after 1950, indicating most of the increase in plant density has actually occurred in the past 60 years.

Changes in Maize Characteristics

Since the 1930's, yield has increased along with density. Researchers have examined how current maize hybrids have been able to increase yield at high densities where older maize hybrids could not. Characteristics including grain moisture, grain test weight, stalk lodging and root lodging, leaf angle, anthesis-silking interval, tassel branch number, plant height and total number of leaves per plant, among others, may have been impacted by increasing density and selection for higher yielding plants.

Past evaluation of these characteristics has been undergone in hybrid maize. Usage of hybrid maize introduces variation into experiments and has led to mixed findings for many characteristics. Such changes have not yet been examined as responses to recurrent selection for yield in a genetically closed population such as the Iowa Stiff Stalk Synthetic maize population. A genetically closed population facilitates research which could determine the genetic basis for morphological and physiological changes as information fully describing the selection program used to create the population is available.

Leaf Angle

Hesketh and Musgrave (1962) conducted research examining photosynthesis rates of individual leaves and determined that sunlight levels considerably less than 100% of maximum provided the most efficient usage of light energy. Specifically, maize leaves have the ability to attain 80% of maximum photosynthetic rate at just 50% of full sunlight (Mock and Pearce, 1975). When plants are arranged at high density, vertical leaf types allow light penetration deeper into the corn canopy (Williams et.al., 1968). This leaf arrangement allows

more leaves throughout the canopy to have access to at least 50% of full sunlight, increasing canopy photosynthetic levels. This is the foundation for the adaptation of leaf angles to high planting density observed over the past 75 years.

Impact of increasing plant density on leaf angle. Researchers have found a trend toward more vertical leaf angles at high density (Duvick, 1996; Duvick, 2005; Pepper et. al., 1977). This transition toward erect leaves was noticed more above than below the ear. While upright leaves may be more efficient light users, that doesn't necessarily translate into higher grain yield (Pepper et. al., 1977). Lambert and Johnson (1978) arrived at a similar conclusion that more vertical leaf types may be required in order for the plant to operate at optimal capacity in high planting densities. This again relates to the idea that since each leaf doesn't require 100% sunlight to maintain efficient photosynthetic rates, more leaves receiving less than 100% sunlight should lead to more photosynthetic activity on the canopy level.

Not all research supports the idea that higher plant densities lead to more erect leaf angles. Ariyanayagam et. al. (1974) compared hybrids with erect leaves to those with more horizontal leaves and found the same amount of light being intercepted by the upper canopy regardless of plant density. Leaf angle of the leaf above the ear was measured. Since Pepper et. al. (1977) indicated leaves higher on the plant tend to have more vertical leaf angles, the ear directly above the leaf may have been less effected by density than leaves higher on the plant and may explain these findings. Hammer et. al. (2009) also failed to detect any change in leaf angle at low plant density. If variation among hybrids used in this research were removed a clearer picture of any change in leaf angle might be obtained.

Leaf angle may impact grain yield. Research has indicated that plants with more erect leaf structures produce higher yields. Pendelton et. al. (1968) compared isogenic hybrids with more horizontal leaves to others with more erect leaves and found a yield advantage of 41% in hybrids with more erect leaves. Physical leaf angle manipulation was undergone to measure light radiation levels in the canopy. Hybrids with more horizontal leaves retrieved 99% of available radiation, mainly absorbed by the uppermost leaves. 90% light interception was found in hybrids with erect leaves above the ear and more horizontal leaves below the ear. This may be possible as more erect leaves toward the top of the plant would allow a larger quantity of light to pass through the canopy to be absorbed by the more horizontal leaves. Only 84% of the total light was intercepted by hybrids consisting of mainly erect leaves. This research led to the conclusion that the most efficient leaf canopy would contain feature erect leaves toward the top of the canopy transitioning to more horizontal leaves lower in the canopy. This relates to the idea of more leaves with less than 100% of maximum sunlight can maintain high photosynthetic efficiency and leads to greater canopy photosynthesis (Hesketh and Musgrave, 1962).

Pepper et. al. (1977) classified maize plants as upright, intermediate, or horizontal types and measured leaf area index (LAI) for each plant population. Leaf area index is the ratio of leaf surface area to soil surface area on which the plants are growing. Plants with upright leaf structures had higher increase in grain yield at the highest planting density than plants with intermediate or horizontal leaf types. Furthermore, erect leaf types demonstrated no yield advantage when compared to plants with more horizontal leaves at low densities and low LAI levels. Grain yield in plants with vertical leaf type increased as LAI increased to 5.5

while horizontal leaved plants with intermediate leaf type did not demonstrate a yield increase. Plants with erect leaf types were able to utilize light more efficiently than plants with horizontal leaves (Pepper et. al., 1977; Duncan et. al., 1971; Winter and Ohlrogge, 1973). Since leaf photosynthesis is more efficient when sunlight is less than 100% of maximum, erect leaf angles allow more leaves to have access to lower levels of light, allowing the potential for greater canopy photosynthesis.

However, Hicks and Stucker (1972) found a different result. Plants were organized by leaf angle and yield was compared across densities. Upright leaves had a more positive effect on yield at lower population densities than at higher densities. They also indicated a slight yield advantage for leaves with intermediate angles over horizontal and erect leaves. Plants with upright leaves did not yield greater than plants with less upright leaf angles as density increased. Hammer et. al. (2009) also failed to find changes in yield due to variation in angle.

Russell (1972) compared hybrids with erect and horizontal leaf angles at two row widths and three plant densities. All leaf types demonstrated a positive increase in yield as density increased when planted in wide (102 cm) rows however only the more horizontal leaves demonstrated a yield advantage when compared at the narrower (51 cm) leaf spacing. This may have resulted because one inbred parent commonly crossed to several of the erect leaf type inbreds consistently created the lowest yielding hybrids at high density. Since this inbred parent was used in about half of the erect leaf type hybrids, this may explain why erect leaf types had low yields.

Winter and Ohlrogge (1973) also used hybrid corn to test the effect of leaf angles on yield. Mechanical means were used to force hybrids to have upright leaf angles and the

treatment was not applied until the plant reached the 50% silk stage. Since leaf angle can affect photosynthesis rate (Pendelton et. al., 1968) waiting until 50% silk to apply this mechanism may have had a detrimental some effect on the outcome of the study. The authors concluded that "highest measured yield with upright leaves was virtually identical to the highest yield with normal leaves" and suggest this effect was caused by low kernel weight.

These experiments were conducted with hybrid plants from a wide variety of genetic background which likely explains the wide array of results found, again illustrating the need for experimentation using populations with less genetic variation.

Effect of leaf angle on photosynthesis. Pendelton et. al. (1968) measured apparent photosynthesis and discovered that while erect leaf types (19° from vertical) had as much as a 10% reduction in apparent photosynthesis compared to more horizontal leaves (90° from vertical), an increase in yield was found with more erect leaf types. This was likely due to increased relative efficiency per light unit that resulted from light penetration lower into the canopy, again relating to the idea that 100% of maximum sunlight is not required for maximum photosynthetic efficiency (Hesketh and Musgrave, 1962). Increased light penetration with erect upper leaves allowed more leaves to participate in the photosynthetic process, lowering fixation rates required of each individual leaf.

Duncan et. al (1971) studied leaf angle using simulations. This study supports the research of Pendelton et. al. (1968), suggesting that the highest apparent photosynthesis is attained with a combination of more erect upper leaves and more horizontal leaves lower in the canopy. Further, the study suggests an advantage of areas of distinct leaf angles in a canopy instead of a canopy that reduces angle from more erect to more horizontal gradually.

Other simulations analyzed combinations of leaf angles with the lowest rates of photosynthesis. Apparent photosynthesis at certain leaf angles seems to be related to leaf area index (LAI). A LAI of less than 3.0 with a canopy consisting of entirely vertical leaves caused the lowest photosynthesis rate and a LAI of 3.0 and above with a canopy containing horizontal leaves in upper layers and erect leaves in lower layers was had the worst rate (Duncan et. al., 1971). At a LAI of 3.0 or less, angle of the leaves (40° compared to 80°) has little to no effect on canopy photosynthesis (Duncan et. al., 1971). With a LAI of 3.0-5.0 leaf angle has some effect on photosynthesis but not a large effect and for canopies with LAI of 5.0 or more leaf angle had a large effect on photosynthesis.

Bunce (1990) compared photosynthesis in maize at a low density of 40,000 plants ha^{-1} and an intermediate density of 100,000 plants ha^{-1}. Lower photosynthesis rates were found at the higher density. The authors suggest this is may be due to increases in leaf shading at the higher density and cite lower leaf mass per unit area as evidence of this idea. They also suggest the reduced photosynthesis may be caused in part by water deficits to the individual plants in higher densities. This supports the idea that more upright leaf angles at high density has been an adaptation of the leaf to reduce shading and maximize total photosynthesis.

Experiments relating to leaf angle have also been conducted on barley. Tunglad et. al. (1987) found limited effect of leaf angle in barley. As population density increased, there were fewer negative effects of erect leaf angle on kernel weight and number.

Anthesis to Silking Interval (ASI)

Impact of increasing density on ASI. Russell (1985) found differences between eras of 1940-1980 in pollen shed, silk emergence, and pollen-silk interval (ASI). Seka and Cross (1995) compared ASI at densities of 24,000 and 48,000 plants ha^{-1} and discovered that ASI at the high density was an average of 3.7 days longer. Hammer et. al. (2009) associated this decrease in ASI to increased yields during stress at flowering. Another study also detected a linear increase in ASI as population density increased (Sangoi et. al., 2002). Although increasing density has been shown to increase ASI, more advanced hybrids appear to combat this better than older hybrids. In a comparison of hybrids across the 1930-1970 period, Duvick, (2005) discovered that ASI decreased in more advanced hybrids. The decrease in ASI was greater at higher plant densities. With a large impact of density on ASI found in hybrid maize, the reduced genetic variation in the BSSS population should provide an even clearer picture of the ability of more advanced populations to adapt to high density.

Effect of ASI on yield. Researchers have demonstrated that long anthesis-silking intervals (ASI) can result in mild to extreme yield losses and thus a short ASI is desirable. Bolanos and Edmeades (1996) used drought stress to induce varying ASI's. Drought stress didn't have a major effect on number of days from planting to anthesis, but did increase the number of days from planting to silking. Fischer et. al. (1989) also found increases in ASI during drought stress and through yield trials in drought stress conditions were able to reduce this increase. Drought and other stress during the flowering period can increase ASI. Grain yield of plants with an average increase in ASI of 5 was less than 20% of the grain yield of the control (Bolanos and Edmeades, 1996).

Many authors have observed lower yields as a consequence of drought stress during reproductive stages. This often results from an increase in ASI due to a delay in silk formation. Moss and Downy (1971) found delayed silking under drought stress in one case increasing ASI to as much as 16 days, meaning silks emerged 16 days after pollen began shedding. This was a large difference from the ASI of 2.6 for the un-stressed check. That stress caused 43% of the developing kernels to be severely disfigured. This disfigurement restricted fertilization of the kernel, resulting in a 90% reduction in dry matter.

Environmental effect on ASI. Struik et. al. (1986) tested factors that affect the length of pollen shed, desynchronization (of ear development and tassel development), and potential number of kernels. The potential number of kernels decreased with water stress and hot temperatures and increased with longer day length. Pollen shedding period is lengthened with short days, plenty of available water, and low heat. Desynchronization is increased with drought stress, longer day length, and warmer days.

Fischer et. al. (1989) compared lines of maize bred for improved drought resistance. They found little difference in days to anthesis between the more and less drought tolerant varieties at lower water stress, but did find differences at high water stress. This was attributed to lower ASI under the more drought tolerance varieties. They noticed a positive relation between shorter ASI and higher yields.

Causes of increased ASI. Several possible causes of increased ASI have been considered. Westgate and Boyer (1986) discuss how pollinations can be made at extremely low water potentials for pollen, but little to no pollinations were successful at low silk water potentials possibly due to a quantity of water needed on the silks for germination of the

pollen. Edmeades et. al. (1993) demonstrated time to 50% silking was affected by "changes in duration of silk growth phase and spikelet initiation phase." They also demonstrated that while stress had a severe effect on silking, it did not have an effect on the rate of development of the spikelet.

Hall et. al. (1992) tested pollen availability through stress applications. Pollen count was not impacted with stress at late development stages, but was impacted by stress just prior to tasseling. Tests showed that water stress did not decrease viability of the pollen, but stress before silking lengthened the period between anthesis and silking (Hall et. al., 1992). Specifically, the stress lowered the amount of pollen grains produced and shortened the time period in which they were released from the plant. Yield reductions were observed for all stressed cultivars. Research also reconfirmed that a four day window of pollen shed is ideal in making good pollinations (Hall et. al., 1992).

Plant and Ear Height

Plant and ear height has changed over time. Results of era studies conducted in hybrid corn varieties have found a reduction in plant and ear height (Meghji, 1984). When comparing hybrids across decades from 1940 to 1980, Russell (1985) found differences for plant and ear height across eras. Other research has found a reduction in ear height with no major reduction in plant height (Duvick, 2005). However, other research has failed to find any change in plant or ear height (Duvick, 1996), again providing reason for further examination of this characteristic.

Plant density naturally effects plant height. Plants at high density can sense neighboring plants by detecting a low red:far red ratio (Ballare et. al., 1990). This causes stem elongation (Ballare et. al., 1990), leading to an increase in plant height. Carena and Cross (2002) examined plant height at different planting densities. Plant height increased as plant density increased from 38,000 to 56,000 plants ha^{-1}, but did not find a plant height response to densities above 56,000 plants ha^{-1}. While selection for yield drove improvements in the era studies, plant height has also been affected when selecting for things other than yield. Fischer et. al. (1989) compared lines of maize bred for improved drought resistance. They found little difference in plant height at low water stress levels, but shorter plants were found at high levels of water stress.

Effect of plant density on plant and ear height. Tetio-Kagho and Garder (1988) showed plant height increased with increasing planting density until a certain point, followed by a decrease in height as plant density maximized (greater than 100,000 plants ha^{-1}). Sangoi et. al. (2002) conducted hybrid era studies which demonstrated a linear increase in plant height as density increased and also demonstrated that older hybrids were taller than the more advanced hybrids. Carena and Cross (2002) also discovered a linear increase in ear height as plant density increased.

Effect of plant and ear height on yield. Johnson et. al. (1986) conducted several cycles of recurrent selection for reduced plant height in maize and found that yields increased as plant height was reduced. Plants from the final selection cycle were shorter in height and took fewer days to reach 50% silking. The study attributed the yield increase under these conditions to shorter plants "being able to respond to higher plant densities without lodging,

while maintaining or reducing the level of barrenness". Fischer et. al. (1989) also discovered that shorter plants tended to have higher yields.

Acosta and Crane (1972) bred two populations representing an average ear placement of 82 and 170 cm. From these populations comparisons were made over several phenotypes. The authors found reduced yields of 14.5%-19.8% in the population with low ear placement when compared to the control population. They note this may have occurred due to selection for low ear height without considering overall plant height (possibly favoring plants with less vigor).

Tassel Size

Tassel size has changed over time. Reductions in tassel size have been observed during the past 75 years. These reductions have even been described as linear in nature (Duvick, 1996). A reduction in tassel branch weight and number was discussed by Meghji et. al. (1984). The study demonstrated that over the past 70 years reductions by as much as 2.5 tassel branches/decade have occurred along with a reduction in tassel weight by 0.5 grams/decade. This has occurred as yield in general has increased by 1,100 Mg ha^{-1} at 31,500 plants ha^{-1} and 3,000 Mg ha^{-1} at 58,800 plants ha^{-1}. Sangoi et. al. (2002) conducted research utilizing hybrids from different eras grown in Brazil and found also decreases in tassel branch number as hybrids became more advanced.

Effect of density on tassel size. Duncan et.al. (1967) found that tassel size varied depending on plant density. The experiment focused on how shadows of tassels by plants at 3 densities affected photosynthetic rates. Using the same genetic line of maize, they found the

shadow sizes at 34,000 plants per acre of 114cm^2 compared to larger shadows of about 150 cm^2 formed at 18,000 and 26,000 plants per acre. This suggests a decrease in tassel size at higher population densities. However, even though tassel size at higher densities were found to be smaller (Duncan et.al., 1967), simulations showed that higher plant densities lead to a greater reduction in yield due to reduced photosynthesis from the shadows. This likely results because at lower densities light is not a limiting factor of growth. At higher densities plants must compete more for light with neighboring plants and this likely results in the reduced yields shown (Duncan et.al., 1967).

Effect of tassel size on yield. Hunter et. al. (1969) found no effect on yield with tassel removal at standard populations. However, the experiment did find increased yield with tassel removal at high plant densities. This was attributed to an increase in the quantity of light available for the leaf to use in photosynthesis (Hunter et. al., 1969). Another possibility may be a lack of competition between sinks (tassel and ear) for carbohydrates (Hunter et. al., 1969).

Six cycles of recurrent selection for reduced tassel size were undergone by Fischer et. al. (1987). Their results, reduction in tassel size and tassel dry weight, were accompanied by reduced plant height and reduced ASI. The plants at cycle 6 had 13-14.6% higher yields. Lambert and Johnson (1978) found that removing tassels showed a trend of yield increase when averaged over several planting densities. Part of this yield increase was attributed to a reduced amount of the leaf shading that was noticed in varieties with larger quantities of tassel branches.

Reasons for reduced tassel size. After conducting drought research, Bolanos et. al. (1993) suggested that a possible underlying cause for reduced tassel size is a "repartitioning of biomass toward the female inflorescence at flowering". The authors suggested that drought, especially during reproductive stages, is an effective tool to use when selecting plants with a greater ability to focus their available assimilate on ear development.

Leaf Size and Number

Duvick (1999; 2005) reported no major changes in number leaves/plant while Meghji et. al. (1984) reports that more advanced hybrids have a higher number of leaves which are shorter in length. Seka and Cross (1995) found an increase of 0.35 leaves per plant as density was increased from 24,000 plants ha^{-1} to 72,000 plants ha^{-1}. Modeling experiments conducted by Hammer et. al. (2009) indicated reductions in leaf size above leaf 10 at high population densities. Hammer et. al. (2009) suggest that as plant density increases the amount of assimilate available per plant is reduced and this could be a possible reason for leaf size reduction.

Root and Stalk Lodging

Duvick (1996) found linear increases in resistance to root lodging. Others have seen variable responses to root lodging. Improvement and lack of improvement was seen depending on the severity of root lodging present in the experiment (Duvick, 2005).

Duvick (2005) did report increasing resistance to stalk lodging. These improvements were especially prevalent at high planting densities and were most noted upon comparison of oldest to most advanced varieties. Although increases were not seen among comparisons

between hybrids from every era, increase in resistance to lodging is thought to be trending toward increased resistance (Duvick, 2005).

Increases in stalk lodging response have been found as density increases. Response has ranged from a small increase of 0.6% as density increased from 56,000 plants ha^{-1} to 90,000 plants ha^{-1} to a larger increase of 6.5% as density increased from 38,000 plants ha^{-1} to 62,000 plants ha^{-1} (Widdicombe and Thelen, 2002; Carena and Cross, 2002).

There is little information in scientific literature indicating whether more advanced maize has reduced lodging at high plant density, indicating a need to research this characteristic further.

Grain Moisture and Test Weight

Although huge increases in grain yield have been noted over the past 75years, little change has been noted in grain moisture or test weight (Duvick, 1996). Increase has been shown in more advanced hybrids when experimentation subjected the hybrids to treatments of well watered and drought like conditions during flowering (Duvick, 2005).

In an experiment with various planting densities, Widdicombe and Thelen (2002) found differences in grain moisture for different hybrids. They also found that test weight varied according to planting density. While Seka and Cross (1995) found a linear response of grain moisture to increasing density, Carena and Cross (2002) also compared grain moisture at several planting densities and noticed no difference in grain moisture among densities but found higher grain moisture in genotypes requiring more days to initiate reproductive stages. They also found that genotypes with delayed flowering had lower test weights.

Other Considerations

     Leaf azimuth. Another potential phenotype that may affect other phenotypes of the plant is leaf azimuth. Leaf azimuth is the angle of leaf from the row it is planted in (Maddonni et. al., 2001). Researchers have shown that azimuth in corn plants tend to shift from a seemingly random state in early development to a more defined state in later stages of development (Girardin and Tollenaar, 1994) and (Drouet and Moulia, 1997). This may occur at stage V11-14 and is triggered by a change in red:far red ratios (Elmore et. al., 2005). Azimuthal position is also seemingly dependent on planting population (Girardin and Tollenaar, 1994) and may vary between successive plant leaves (Drouet and Moulia, 1997).

     Root structure. Hammer et. al. (2009) suggests that changes in rooting structures may also be a factor in the yield increases over the past several decades, especially in high stress conditions; specifically water stress at flowering. The modeling study indicated that rooting structures may be more important than canopy structure for increasing yield, specifically the ability of a rooting structure to acquire water necessary for higher yields that the canopy's ability to bring in sunlight to support higher yields. The authors thought this was possible due to the ability of the increasingly penetrating roots found in more advanced hybrids bringing water up to the plant from lower in the soil profile and increased "extraction capacity" of the roots (Hammer et. al., 2009). Although not covered in our research, root characteristics should be kept in mind as potentially affect above ground plant characteristics.

     Stress tolerance. Perhaps improvement in stress tolerance has led to some of the changes in plant characteristics observed in this research. Stress has been defined as "a factor that causes, either through its presence or absence, a reduction in grain yield" (Tollenaar and

Wu, 1999). Stress tolerance was defined as "a measure of the ability to mitigate the impact of stress on the physiological process involved in resource capture (nutrient uptake, absorption of incident solar radiation) and utilization (rate of leaf photosynthesis, rate of dry matter accumulation)", (Tollenaar and Lee, 2002).

Thus, modification of the plant or the environment in which plants are grown that may reduce stress or increases stress tolerance and could potentially lead to yield increase. Tollenaar and Lee (2002) attribute yield increase seen in more advanced hybrids grown in Ontario to increased planting densities which they indicate may be partially possible through "increased stress tolerance to weed interference, low soil N, low moisture and increased leaf photosynthesis in colder night temperatures during the grain fill period".

While "yield on a per plant basis has not increased in the absence of abiotic stress, stress tolerance has increased, and this increase in stress tolerance may be a result of the tendency of commercial breeding to test and develop hybrids for wide adaptation" (Tollenaar and Lee, 2002). Yield increases in advanced hybrids are often accentuated in poor yield environments (Tollenaar, 1989), suggesting an increasing ability for advanced plants to withstand stress.

Summary

Since the 1930's large increases in average plant density and grain yield have been observed in hybrid maize populations. In order for plants to successfully increase yields at higher planting densities, changes in several plant characteristics may have occurred. The objective of this experiment was to determine if direct selection for grain yield and

agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density, using changes in plant characteristics to measure this adaptation. This information will help progress our understanding of the plant's ability to adapt from a point where increasing plant density could not facilitate increases in yield to a point where increasing plant density is driving increases in yield.

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CHAPTER 3. INCREASED ADAPTATION TO HIGH PLANT DENSITY AS A RESULT OF SELECTION FOR YIELD IN THE IOWA STIFF STALK SYNTHETIC POPULATION: GRAIN YIELD, MOISTURE, TEST WEIGHT, ROOT AND STALK LODGING.

An article to be submitted to *Crop Science Journal*

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ABSTRACT

The plant density at which *Zea mays* L. hybrids achieve maximum grain yield has increased throughout the hybrid era while grain yield on a per plant basis has increased little. Changes in plant traits including grain yield, moisture, test weight, and stalk and root lodging have been well characterized in comparisons of commercial hybrids representing different eras of hybrid maize production but have yet to be examined in a recurrent selection program. The objective of this experiment was to determine if direct selection for grain yield and agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density. Material from an unselected base population, Iowa Stiff Stalk Synthetic (BSSS), was compared to the most advanced cycles of selection from two different recurrent selection programs at seven Iowa locations in 2008 and 2009.

Populations were compared at densities of 38,300, 57,400, 77,500, and 95,700 plants ha^{-1}. Treatments were replicated twice at each location and arranged in a split plot design. Root lodging has remained unchanged. Increasing density in advanced populations led to increased yield unlike the yield decrease seen in less advanced populations at high density, indicating an adaptation to high plant density. Increasing density in advanced populations did

not increase grain moisture, test weight, or stalk lodging supporting our hypothesis of increased adaptation to high plant density in more advanced populations.

INTRODUCTION

Since the 1930's, average maize yields in the United States have increased by a factor of nearly five, from an average of 1.5 Mg ha^{-1} to an average of 8.5 Mg ha^{-1} today (Duvick, 2005). In the United States, increases in average annual maize yields have ranged from 99 kg ha^{-1} (Lee and Tollenaar, 2007) to approximately 109 kg ha^{-1} (Duvick, 2005). Hybrids of central Iowa have increased nearly 79 kg ha^{-1} annually (Duvick, 1996).

These increases in yield have occurred while average Iowa maize yield per-plant has remained nearly constant, increasing from 0.34 kg plant^{-1} in 1965 to 0.37 kg plant^{-1} in 2008 (USDA, 2010) while average planting density has increased by an average 720 plants ha^{-1} per year over the past 44 years (USDA, 1965-2009). This indicates that increasing plant density has driven increases in grain yield. Hammer et. al. (2009) found that yields maximized at 30,000 plants ha^{-1} in the 1960's, while today's hybrids can increase yield at densities two to three times higher. Similar conclusions have been reached in a number of other experiments. Tollenaar (1989) noted that newer hybrids out yielded the oldest hybrid by only 25% (6.97 vs. 8.78 Mg ha^{-1}) at 40,000 plants ha^{-1}, but by 190% (4.54 vs. 9.50 Mg ha^{-1}) at 130,000 plants ha^{-1}. The plant density at which increasing density fails to increase yield has been rising steadily over time. Widdicombe and Thelen (2002) indicated that densities as high as 90,000 plants ha^{-1} were still below potential maximum yield densities, indicating even higher tolerance to stand density may be possible in advanced hybrids.

Although huge increases in grain yield have been noted over the past 75 years, little change has been observed for grain moisture or test weight (Duvick, 1996). While Widdicombe and Thelen (2002) found an increase in test weight at high density, the increase was small suggesting any impact of density is minimal. Seka and Cross (1995) found a linear response of grain moisture to increasing density and Widdicombe and Thelen (2002) found a decrease in moisture at higher plant densities. However, Carena and Cross (2002) did not find an effect of density on grain moisture.

Duvick (2005) found increased stalk lodging at higher plant densities. Response has ranged from a small increase of 0.6% as density increased from 56,000 plants ha^{-1} to 90,000 plants ha^{-1} to a larger increase of 6.5% as density increased from 38,000 plants ha^{-1} to 62,000 plants ha^{-1} (Widdicombe and Thelen, 2002; Carena and Cross, 2002). There is little information in scientific literature indicating whether more advanced maize has reduced lodging at high plant density, indicating a need to research this trait further.

Duvick (1996) analyzed changes in root lodging and found linear increases in resistance in advanced hybrids at high density while Duvick (2005) only found varied responses. Improvement or lack of improvement was dependent on the severity of root lodging present in the experiment, indicating that clearer results may be obtained using locations where higher levels of damage was present.

The Iowa Stiff Stalk Synthetic (BSSS) maize population has been undergoing two lineages of selection to increase grain yield since 1939 (Lamkey, 1992). One method of selection utilized was half-sib selection, which utilizes a common tester to create testcrosses for yield testing. The half-sib lineage consisted of 2 programs, HT and HI. Direct response

(yield of testcross) in the HT program increased 0.165 mg ha^{-1} per cycle (Eberhart et. al., 1973). Direct response of the second half-sib program (HI program) increased 0.13 mg ha^{-1} per cycle (Edwards, in-press). The second lineage of selection from BSSS was reciprocal selection which makes use of two populations using each as a target population and uses the other as the tester, advancing both populations simultaneously (Penny and Eberhart, 1971). The first eleven cycles of the reciprocal lineage had a direct response of 0.28 mg ha^{-1} per cycle (Keeratinijakal and Lamkey, 1993). The direct responses of these programs are proof of the success of the selection processes.

Lines derived from the Iowa Stiff Stalk Synthetic population have made major contributions to the maize industry. It has been estimated that lines from this population were parents of 19% of the total corn acres planted in the US in 1980 (Zuber and Darrah, 1980). The Iowa Stiff Stalk Synthetic population is ideal for examination because it has remained a closed population with no introduction of new germplasm (Lamkey et. al., 1991). With known genetic relationships in a recurrent selection program, we can study the genetic basis for plant characteristics and relate these changes directly to selection response.

The objective of this experiment was to determine if direct selection for grain yield and agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density. Our hypothesis is that recurrent selection for yield has caused an improved adaptation to plant density though modification of traits mentioned in this report.

Materials and Methods

Five breeding populations representing different levels of cycle advancement from three selection programs representing two separate lineages were the first of two treatments were used in this study.

BSSS. The Iowa Stiff Stalk Synthetic population was originally created in 1934 by inter-mating 16 inbred lines with greater than normal stalk quality (Lamkey, 1992). From this base population, three different recurrent selection programs were initiated using selection criteria focused primarily on yield increase (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995) with some consideration for low grain moisture and increased resistance to stalk and root lodging (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995).

BSSS(HT)C7. From the original BSSS population one lineage utilized half-sib selection. Seven cycles of half-sib selection took place utilizing testcrosses to IA13 to determine candidates for selection. The 7th cycle was denoted BSSS(HT)C7 (Lamkey et. al., 2001). Details of procedures used for cycle advancement were described by Eberhart et. al. (1973).

BS13(S)C0. This breeding population was base population of the second selection program within the half-sib lineage. The population was created from BSSS(HT)C7 by selecting the 29 best performing S_1 lines (Lamkey, 1992), thus having undergone the same 7 cycles of selection.

BS13(HI)C5. Starting with BS13(S)C0 (descendent of BSSS(HT)C7), 5 cycles of half-sib selection was completed utilizing testcrosses to B97 to determine candidates for selection. The most advanced cycle from the half-sib lineage is program is BS13(HI)C5 (Edwards, In-Press) representing a total of 12 selection cycles.

BSSS(R)C17. The second lineage was also initiated from the original BSSS population and utilized recurrent reciprocal selection with Iowa State Corn Borer Synthetic #1 (BSCB1) (Holthaus and Lamkey, 1995). Details of procedures used were described by Penny and Eberhart, (1971). The population resulting from the first cycle of selection with the BSSS population was named BSSS(R)C1. To date 17 selection cycles have been completed with current endpoint BSSS(R)C17. Table 2 summarizes the breeding populations used in this study.

The second treatment used in this study was planting density. Densities of 38,300, 57,400, 77,500 and 95,700 plants ha^{-1} were used.

Experimental Design

The experiment was arranged in a split plot design with planting density as the whole plot treatments and the five breeding populations as the split plot treatments. Treatments were replicated twice at each of three Iowa locations in 2008 and four Iowa locations in 2009. Locations included fields near Fairfield, Carroll, and Crawfordsville IA in 2008 and fields near Keystone, Ames, Crawfordsville, and Carroll Iowa in 2009. Soil type, planting date, and harvest date for all locations are listed in Table1. Since the greatest amount of variation in stalk lodging was at Crawfordsville and Carroll in 2009 and the greatest amount

of variation in root lodging was at Crawfordsville in 2008 and Keystone in 2009, data was

only analyzed for these traits at these locations. All other traits were analyzed over all

locations.

Each experimental unit consisted of four rows spaced 0.76 meters apart and 5.49

meters in length. The number of intact plants in each plot were recorded just before flowering

and converted to plants per hectare. Plots were harvested using a New Holland TR-88

combine modified for automatic acquisition of test weight, grain moisture, and grain weight

(Almaco Company, Nevada, IA). The number of plants visibly root-lodged and stalk-lodged

were counted and converted to percentages of final stand in the plot. Plants were considered

root-lodged if the base of the stalk was not visibly perpendicular to the ground. A plant was

considered stalk lodged if the stalk was broken below the node of attachment of the

uppermost ear.

Data Analysis

The following model was fit to the data:

$$y_{jkmn} = \alpha_i + \phi(\alpha)_{ij} + \eta_k + \delta(\alpha)_{ijk} + \tau_m + (\tau\eta)_{km} + (\alpha\tau)_{im} + (\alpha\eta)_{ik} + (\alpha\tau\eta)_{ikm} + \varepsilon_{ijkmn}$$

Where:

α_i = effect of environment i,

$\phi(\alpha)_{ij}$ = effect of replicate block j within environment i,

η_k = effect of planting density k

$\delta(\alpha)_{ijk}$ = split plot error A for planting density k, block j, environment i,

τ_m = effect of breeding population m,

$(\tau\eta)_{km}$ = interaction of planting density effect k with breeding population m,

$(\alpha\tau)_{im}$ = interaction of breeding population m with environment i,

$(\alpha\eta)_{ik}$ = interaction of planting density effect k with environment i

$(\alpha\tau\eta)_{ikm}$ = interaction of planting density effect k with environment i, breeding population m,

ε_{ijkmn} = residual error.

Interactions with environment were considered random effects. All other effects were treated as fixed effects. The model was fit using Henderson's mixed model equations (Henderson, 1984). Variance components corresponding to random effects were estimated using restricted maximum likelihood (Searle et al., 1992). Computations were done using SAS proc mixed (SAS Institute). Data were checked for outliers by computing the probability of obtaining a larger absolute value of each residual using the t-distribution. Individual p-values for residuals were adjusted with a Bonferroni correction and studentized residuals examined with quantile-quantile (QQ) probability plots. Based on QQ plots and Bonferroni adjusted pvalues, eleven observations were identified as outliers and were discarded. After removal of outliers, the following covariate model was fit to the final dataset:

$$\gamma_{jkm} = \alpha_i + \phi(\alpha)_{ij} + \delta(\alpha)_{ijk} + \tau_m + \beta_1 x_{ijkm} + (\tau\beta_1)_m x_{ijkm} + \beta_2 x^2_{ijkm} + (\tau\beta_2)_m x^2_{ijkm} + (\alpha\tau)_{im} + (\alpha\beta_1)_i x_{ijkm}$$
$$+ (\alpha\tau\beta_1)_{im} x_{ijkm} + (\alpha\beta_2)_i x^2_{ijkm} + (\alpha\tau\beta_2)_{im} x^2_{ijkm} + \varepsilon_{ijkm}$$

Where:

α_i = effect of environment i,

$\phi(\alpha)_{ij}$ = effect of replicate block j within environment i,

$\delta(\alpha)_{ijk}$ = split plot error A for density k, block j, environment i,

τ_m = intercept of breeding population m,

x_{ijkm} = observed plant density in environment i, block j, density k, breeding population m,

β_1 = linear effect of observed plant density,

$(\tau\beta_1)_m$ = interaction of linear density effect with breeding population m,

β_2 = quadratic effect of observed plant density,

$(\tau\beta_2)_m$ = interaction of quadratic density effect with breeding population m,

$(\alpha\tau)_{im}$ = interaction of breeding population m with environment i,

$(\alpha\beta_1)_i$ = interaction of linear density effect with environment i,

$(\alpha\tau\beta_1)_{im}$ = interaction of linear density effect with environment i, breeding population m,

$(\alpha\beta_2)_i$ = interaction of quadratic density effect with environment i,

$(\alpha\tau\beta_2)_{im}$ = interaction of quadratic density effect with environment i, breeding population m,

ε_{ijkm} = residual error.

Interactions with environment were considered random effects. All other effects were treated as fixed effects. The model was fit using Henderson's mixed model equations and estimators of variance components obtained by restricted maximum likelihood (Henderson, 1984; Searle et al., 1992). Computations were carried out using SAS proc mixed (SAS Institute, Cary, NC). Akaike's information criteria(AIC) was used to identify which random effects to retain in the model. Random effects with estimates of zero were eliminated. Subsequently, the set of random effects in the final model was chosen by fitting all combinations of random effects in the model. The model with the smallest AIC value was chosen as the final model. The possible random effects and those selected for use in the final model are listed in Table 3.

All fixed effects were initially included in the model and p-values were determined. For each trait, nonsignificant plant-density covariates and plant-density by population

interactions were dropped from the model beginning with the highest order terms. If a random interaction containing a plant-density covariate was retained in the model, the corresponding plant-density fixed effect was retained automatically. If there were no random interactions containing plant density interactions, the highest order terms were tested with F-tests using Satterthwaite approximate degrees of freedom. If the highest order term was not significant, it was dropped. The next highest level terms was then tested and dropped if not significant. All possible fixed effects and p-values for effects retained in the final model are listed in Table 4. When plant-density by breeding population interactions were retained in the final model, breeding-population specific covariates terms were fit in the model and tested individually. Covariate terms for individual breeding populations that were not significant were dropped from the model. Response curves corresponding to covariate terms were plotted for individual breeding populations. In cases that individual breeding populations had near identical plant density responses, covariate terms were compared statistically and if not different, the response curves were combined for populations have statistical indistinguishable responses.

Results and Discussion

Grain yield of the original BSSS population initially increased at 35,000 plants ha^{-1} but when density reached 50,000 plants ha^{-1} yield began to decrease (Figure 1). This is not the case with the more advanced populations where grain yield increased even at high density. Yield decrease at high density was eliminated in BS13(HI)C5 and grain yield of BSSS(R)C17 has yield to reach maximum yield potential even at 75,000 plants ha^{-1}. While the original BSSS population yielded less than BSSS(HT)C7 and BS13(S)C0 no difference

was found between BSSS(HT)C7 and BS13(S)C0. This is likely because BS13(S)C0 is actually 29 lines selected from BSSS(HT)C7 (Lmakey, 1992) and thus few differences between these breeding populations are expected.

Advanced BSSS(R)C17 yielded 62 Mg ha^{-1} at 70,000 plants ha^{-1}. This corresponds to an average 1.64% increase over the 17 cycles of selection. An average yield of 7 Mg ha^{-1} was found at the maximum stand density of 75,000 plants ha^{-1}, corresponding to an increase in grain yield of 2.3% per cycle over the original BSSS population. This means that an increase in yield at high density was found in the more advanced populations while less advanced populations showed decreased yields at high plant density. This confirms an adaptation to high plant density in advanced population and indicates that response to selection can depend on density.

Our findings also coincide with many hybrid studies showing increased yield across decades in Iowa (Duvick, 1996), the US (Russell, 1984; Duvick, 2005; Lee and Tollenaar, 2007) and Canada (Tollenaar, 1989). These data presented in Figure 1 also suggest that the most advanced breeding populations BSSS(HI)C5 and BSSS(R)C17 could maintain yield increase beyond 80,000 plants ha^{-1} as the yield curves are still increasing at 80,000 plants ha^{-1}. This information suggests future selection may need to be carried out at higher densities to if continued yield increase at high density is desired.

The less advanced populations BSSS, BSSS(HT)C7, and BS13(S)C0 had higher grain moisture than BS13(HI)C5 and BSSS(R)C17 and were not affected by plant density(Figure 2). BS13HI(C5) demonstrated a linear (P=0.057) decrease in grain moisture as planting density increased. At all stand densities (35,000, 50,000, 64,000 and 75,000 plants ha^{-1})

BSSS(R)C17 had lower grain moisture than the other populations, although the moisture level increased with density. No difference in moisture response to density was found for BSSS, BSSS(HT)C7 or BS13(S)C0.

The ability to remove genetic differences present in hybrids studies allows us to offer a much clearer picture of moisture response in advanced populations as hybrid studies have shown no change in grain moisture among genotypes (Duvick, 1996). While the lower grain moisture of BSSS(R)C17 did increase with increasing density, it remained consistently lower than the less advanced populations at all densities, supporting our hypothesis of improved adaptation to high plant density.

The original BSSS population had consistently lower test weight than all other populations at all densities (Figure 3). Both BSSS(HT)C7 and BS13(S)C0 have higher test weight than the original BSSS at all densities until 75,000 plants ha^{-1} is reached. BS13(HI)C5 had consistently higher test weight than any of the other populations at all densities. While test weight of BSSS(R)C17 remained higher than BSSS, BSSS(HT)C7, and BS13(S)C0 at most densities, a negative linear effect of plant density on test weight was found and at 75,000 plants ha^{-1} BSSS(R)C17 actually had lower test weight than BSSS(HT)C7 and BS13(S)C0.

The constant test weight of BSSS(HI)C5 across densities and generally higher test weight of BSSS(R)C17 demonstrates a trend toward increased test weight in more advanced populations, consistent with Widdicombe and Thelen (2002) whom also found variation in test weight among genotypes. The decrease in test weight as density increased in BSSS(R)C17 is consistent with Porter et. al. (1997), who also found a decrease in test weight

with increasing plant density. The high test weight of BS13(HI)C5 and BSSS(R)C17 and lack of density effect on the BS13(HI)C5 population indicate an increased adaptation of test weight to high plant density.

Increasing plant density caused an increase in stalk lodging for all populations except BSSS(R)C17 indicating a clear adaptation to reduced stalk lodging at higher densities. Less advanced populations BSSS, BSSS(HT)C7, and BS13(S)C0 have similar responses to density while BSSS(R)C17 had less stalk lodging response to density than all of the other populations (Figure 3). BS13(HI)C5 also showed a greater resistance to stalk lodging at 75,000 plants ha^{-1} than BSSS(HT)C7. Although our results do not indicate a reduction in stalk lodging within the first 7 cycles of selection leading from BSSS to BS13(S)C0, Lamkey (1992) found an average decrease of 1.12% per cycle. Our finding of reduced stalk lodging in the more advanced cycles are supported by increased resistance to stalk lodging that has been reported in hybrids (Duvick, 2005). Negative response of stalk lodging to increasing density on has also found in hybrid studies (Widdicombe and Thelen, 2002; Carena and Cross, 2002).

Increasing plant density led to increasing root lodging for BSSS(HT)C7, BS13(S)C0, and BS13(HI)C5. No difference was found at any density between the original BSSS population and BS13(HI)C5 or BSSS(R)C17, indicating that no progress has been made in resistance to root lodging. Lamkey (1992) also discovered an increase in root lodging of 0.44% within the first 7 cycles of selection leading from BSSS to BS13(S)C0. Some of the variation within the root lodging trait may be due to using small numbers of locations and limited environmental conditions causing lodging. If only one or two locations were to

receive lodging damage, those effects may be blinded by locations receiving no lodging damage. Edwards (in-press) found only marginally significant average lodging response, but upon examining specific location responses more significant p-values were discovered.

Conclusion

The BSSS program utilized yield and agronomic performance as an index to select for population advancement. This selection resulted in increased grain yield along with increases in test weight, resistance to stalk lodging, and decreased grain moisture (evaluated in populations not test crosses). Root lodging has remained unchanged. Pedigree by density interactions were observed for all traits. Increasing density in advanced populations led to higher yield without increases in grain moisture, test weight, or stalk lodging supporting our hypothesis of increased adaptation of more advanced cycles to high plant density. Selection of genotypes displaying increased test weight while maintaining low grain moisture and reduced lodging will continue to be important for continued adaptation to high plant density and future yield increase.

Acknowledgements

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Table 1. Soil type, planting date, and harvest date for all locations used in the study.

Location	Year	Soil type	Planting Date	Harvest Date
Carroll	2008	Nicollet loam	5/10/2008	10/28/2008
Crawfordsville	2008	Mahaska silty clay loam	5/6/2008	10/9/2008
Fairfield	2008	Taintor solty clay loam	5/10/2008	10/17/2008
Keystone	2009	Tama silty clay loam	4/24/2009	10/27/2009
Carroll	2009	Nicollet loam	5/4/2009	11/2/2009
Crawfordsville	2009	Mahaska silty clay loam	4/23/2009	10/13/2009
Ames	2009	Clarion loam	5/5/2009	11/4/2009

Table 2. Iowa Stiff Stalk Synthetic Maize populations used in this study. Populations are listed in order of advancement from original population to the most advanced population.

Population	Number of Cycles of Selection	Selection Method	Tester Used in Selection
BSSS		None – Original Population	
BSSS(HT)C7†	7	Half-Sib	IA 13
BS13(S)C0†	0 (7 total) – Base for BS13 Population	29 lines selected from BSSS(HT)C7	None
BS13(HI)C5	5 (12 total)	Half-Sib	B 97
BSSS(R)C17	17	Reciprocal Selection	BSCB 1 (Corn Borer Synthetic)

† Populations BSSS(HT)C7 and BS13(S)C0 are similar populations, differing only by population BS13(S)C0 being the compilation of the best performing lines from population BSSS(HT)C7.

Table 3. Combination of random effects with lowest AIC values which were used in final model.

Random Effects

Trait	plant density x replication (environment) †	breeding population x environment	plant density x environment	plant density quadratic x environment	plant density x breeding population x environment	plant density quadratic x breeding population x environment
Yield	NR††	X	NR††	X	NR††	NR††
Root Lodging	NR††	X	NR††	NR††	NR††	NR††
Stalk lodging	NR††	NR††	NR††	NR††	NR††	NR††
Grain Moisture	NR††	X	X	NR††	NR††	NR††
Test Weight	NR††	X	NR††	NR††	X	NR††

†Plant density x replication (environment) refers to plant density x environment nested in replication.

††NR = Not retained in the model because it was not part of the lowest AIC combination.

Table 4. P-values for fixed effects utilized in the statistical model.

Plant Trait	Breeding Population	Plant Density	Density x Breeding Population	Density Quadratic	Density Quadratic x Breeding Population
Yield	<.0001	<.0001	<.0001	0.0130	NS†
Root Lodging	0.3031	<.0001	0.0351	NS†	NS†
Stalk lodging	0.0749	<.0001	<.0001	NS†	NS†
Grain Moisture	<.0001	0.9195	<.0001	NS†	NS†
Test Weight	<.0001	0.1057	0.0337	NS†	NS†

† NS= Non-significant at P <0.05 level. Non-significant fixed effects were dropped from the statistical model.
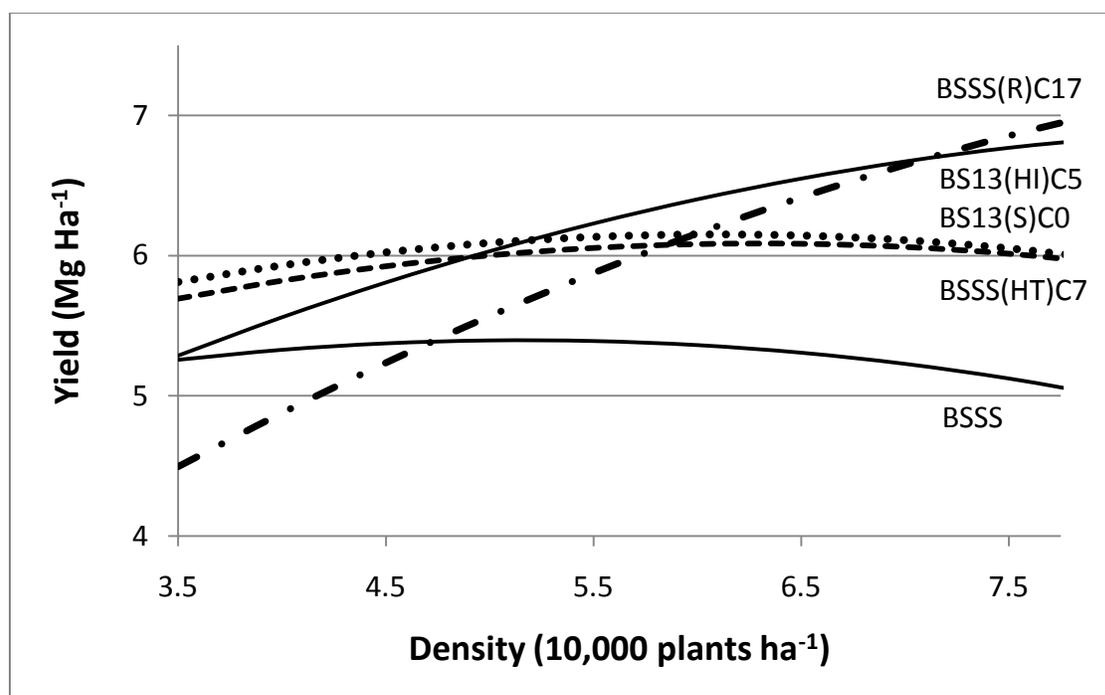
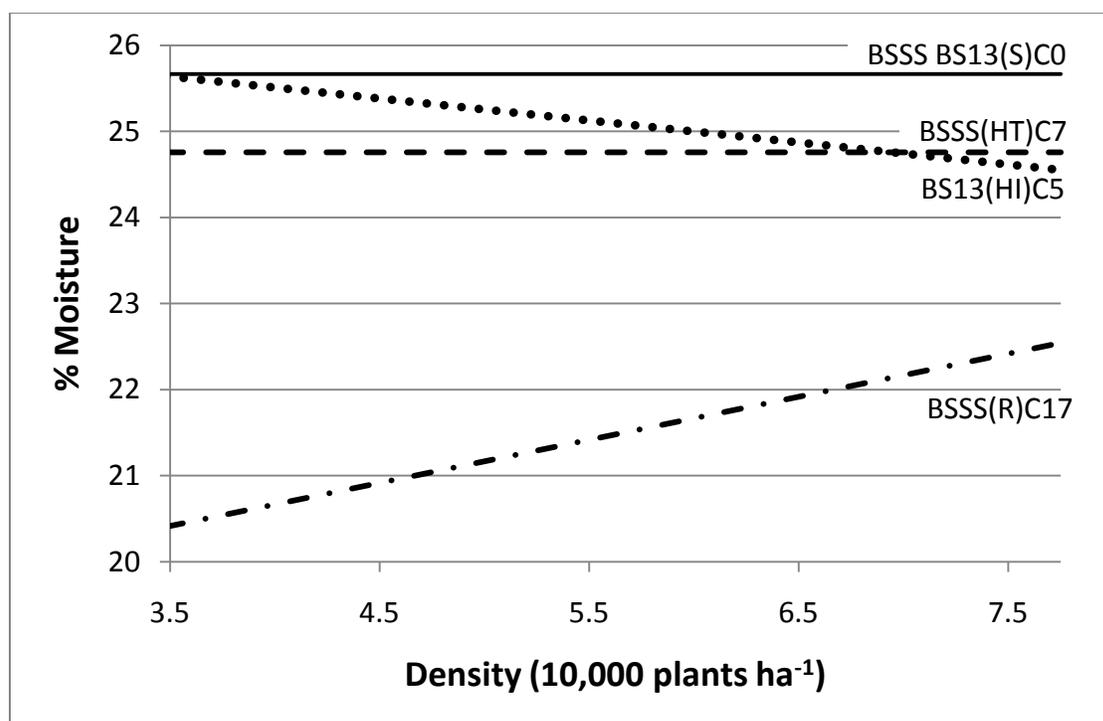**Figure 1. Grain yield in Mg ha⁻¹ at harvest.**
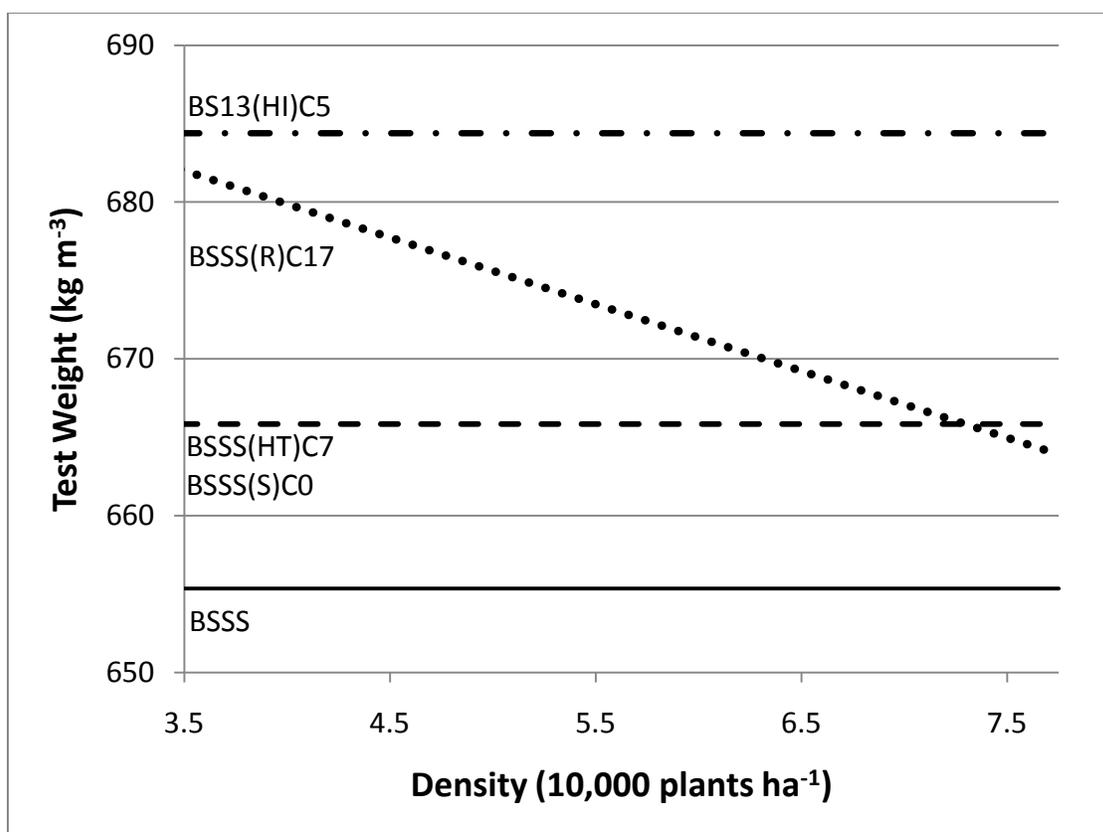
Figure 2. Grain moisture percentage at harvest.
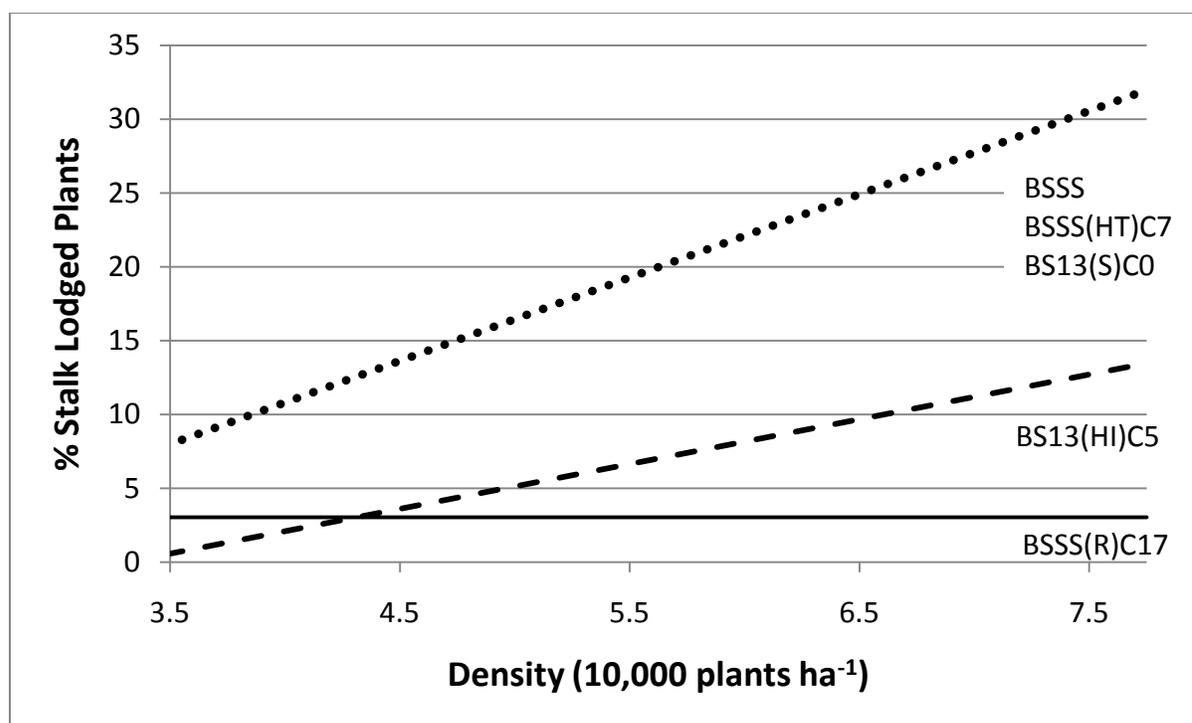
Figure 3. Grain test weight at harvest.

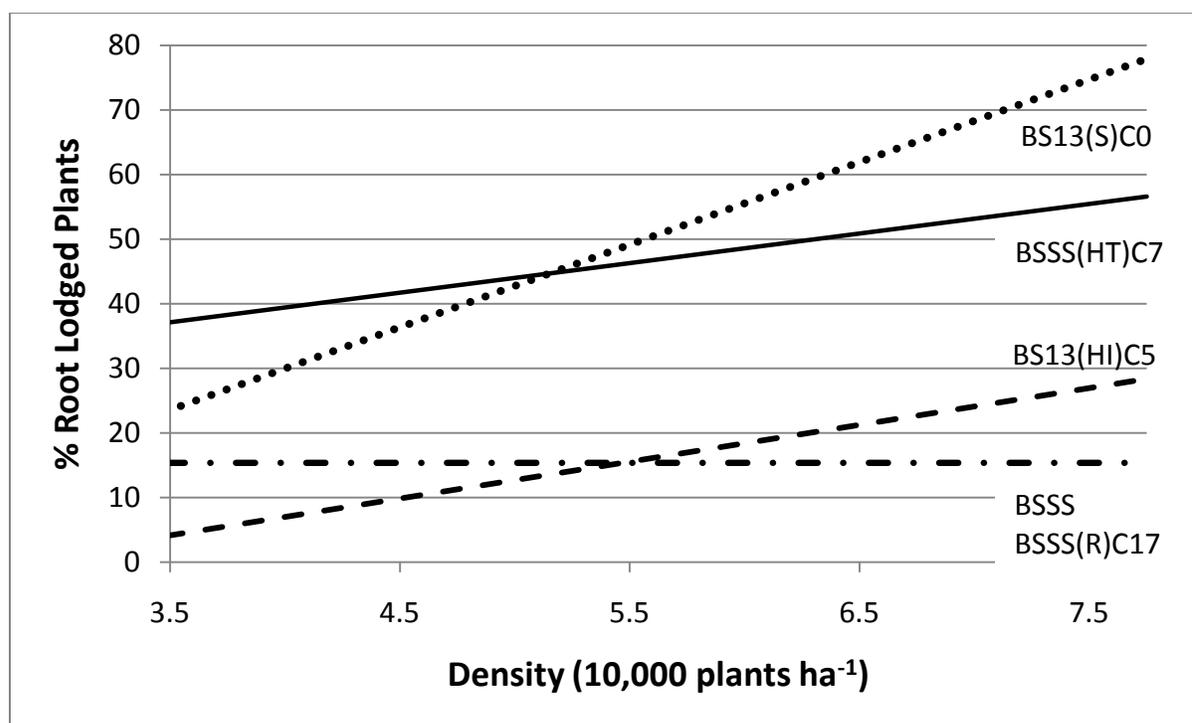**Figure 4. Percent stalk lodging.**

Figure 5. Percent Root lodging.

CHAPTER 4. INCREASED ADAPTATION TO HIGH PLANT DENSITY AS A RESULT OF SELECTION FOR YIELD IN THE IOWA STIFF STALK SYNTHETIC POPULATION: FLAG LEAF ANGLE, ASI, PLANT HEIGHT, TASSEL BRANCH NUMBER, AND TOTAL NUMBER OF LEAVES.

An article to be submitted to *Crop Science Journal*

Brent Brekke, Jode Edwards, and Allen Knapp

ABSTRACT

The plant density at which *Zea mays* L. hybrids achieve maximum grain yield has increased throughout the hybrid era while grain yield on a per plant basis has increased little. Changes in plant characteristics including flag leaf angle, anthesis-silking interval(ASI), plant height, tassel branch number, and total number of leaves have been characterized in comparisons of commercial hybrids representing different eras of hybrid maize production but have yet to be examined in a recurrent selection program. The objective of this experiment was to determine if direct selection for grain yield and agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density.

Material from an unselected base population, Iowa Stiff Stalk Synthetic (BSSS), was compared to the advanced cycles of selection from two different recurrent selection programs at 4 Iowa locations in 2008 and 2009. The advanced cycles and base population were compared at densities of 38,300, 57,400, 77,500, and 95,700 plants ha^{-1}. Treatments were

replicated twice per location and arranged in split plot design with plant density as whole plot and breeding population as sub-plot treatment. Advanced populations had reduced ASI. Plant density did not affect flag leaf angle which became more vertical in advanced populations. Increasing plant density in advanced populations increased plant height while not effecting ASI or tassel branch number; supporting our hypothesis of increased adaptation to high plant density.

INTRODUCTION

Since the 1930's, average maize yields in the United States have increased by a factor of nearly five, from an average of 1.5 Mg ha^{-1} to an average of 8.5 Mg ha^{-1} today (Duvick, 2005). In the United States, increases in average annual maize yields have ranged from 99 kg ha^{-1} (Lee and Tollenaar, 2007) to approximately 109 kg ha^{-1} (Duvick, 2005). Hybrids of central Iowa have increased nearly 79 kg ha^{-1} annually (Duvick, 1996).

These increases in yield have occurred while average Iowa maize yield per-plant has remained nearly constant, increasing from 0.34 kg plant^{-1} in 1965 to 0.37 kg plant^{-1} in 2008 (USDA, 2010) while average planting density has increased by an average 720 plants ha^{-1} per year over the past 44 years (USDA, 1965-2009). This indicates that increasing plant density has driven increases in grain yield. Hammer et. al. (2009) found that yields maximized at 30,000 plants ha^{-1} in the 1960's, while today's hybrids can increase yield at densities two to three times higher. Similar conclusions have been reached in a number of other experiments. Tollenaar (1989) noted that newer hybrids out yielded the oldest hybrid by only 25% (6.97 vs. 8.78 Mg ha^{-1}) at 40,000 plants ha^{-1}, but by 190% (4.54 vs. 9.50 Mg ha^{-1}) at 130,000 plants ha^{-1}. The plant density at which increasing density fails to increase yield has been rising

steadily over time. Widdicombe and Thelen (2002) indicated that densities as high as 90,000 plants ha^{-1} were still below potential maximum yield densities, indicating even higher tolerance to stand density may be possible in advanced hybrids.

In order to maintain yield increase at high plant density, certain phenotypes in hybrids maize have changed over time. One such phenotype studied in hybrid maize is leaf angle. Hesketh and Musgrave (1962) conducted research examining photosynthesis rates of individual leaves and determined that sunlight levels considerably less than 100% of maximum provided the most efficient usage of light energy. Specifically, maize leaves have the ability to attain 80% of maximum photosynthetic rate at just 50% of full sunlight (Mock and Pearce, 1975). When plants are arranged at high density, vertical leaf types allow light penetration deeper into the corn canopy (Williams et.al., 1968). This leaf arrangement allows more leaves throughout the canopy to have access to at least 50% of full sunlight, increasing canopy photosynthetic levels. This is the foundation for the adaptation of leaf angles to high planting density observed over the past 75 years.

Past authors have indicated that leaf angle in advanced hybrids have trended towards more upright angles (Duvick, 1996; Duvick, 2005; Pepper et. al., 1977) Williams et.al. (1968) examined the effect of leaf angle on light penetration through a maize canopy and found an increase in leaf angle as plant density increased suggesting that light was able to penetrate deeper into corn canopies with more vertical leaves. Others have observed similar results (Lambert and Johnson, 1978). Pepper et. al. (1977) indicated leaves higher on the plant tend to have more vertical leaf angles than leaves lower on the plant, again indicating a structure that allows light to penetrate into the canopy.

Research supports the effect of upright leaf angles and found that leaves with more upright leaf angles have higher yields at high densities. Pendelton et. al. (1968) compared isogenic hybrids with more horizontal leaves to others with more erect leaves and found a yield advantage of 41% in hybrids with more erect leaves. Pepper et. al. (1977) grouped hybrids using a leaf angle classification of upright, intermediate, or horizontal, and found that plants with erect leaf structures had a greater increase in grain yield at the highest planting density than plants with intermediate or horizontal leaf types. Other authors have also found that plants with erect leaf types were able to utilize light more efficiently than plants with horizontal leaves (Duncan et. al., 1971; Winter and Ohlrogge, 1973).

However, not all research has shown similar associations with yield and leaf angle. Hicks and Stucker (1972) compared hybrids of corn with varying leaf angles and found that upright leaves had a more positive effect on yield at lower population densities than at higher densities. Plants with upright leaves did not yield greater than plants with less upright leaf angles as density increased. Hammer et. al. (2009) and Pepper et. al. (1977) also failed to find changes in yield due to leaf angle. These studies may have failed to demonstrate clear advantages of upright leaves because of confounding with other phenotypes that also contribute to plant density response or because of dissimilar genetic backgrounds of hybrids compared.

Another such phenotype studied widely in hybrid maize is anthesis-silking interval (ASI). Increased plant density has created linear increases ASI (Sangoi et. al., 2002). Since density has increased at such a high rate (USDA, 1965-2009) the ability maintain low ASI at high density is especially important. Russell (1985) found differences between eras of 1940-

1980 in pollen shed, silk emergence, and pollen-silk interval (ASI). Seka and Cross (1995)

compared ASI at densities of 24,000 and 48,000 plants ha^{-1} and discovered that ASI at high

density was increased by 3.7 days. In a comparison of hybrids across the 1930-1970 period,

Duvick, (2005) discovered that ASI decreased in more advanced hybrids and also found

increasing plant density led to increases in ASI. Although increasing density has been shown

to increase ASI, advanced hybrids have the ability to maintain low ASI even at high

densities. This is likely possible because of a combination of things including more energy

supplied to the ear by higher photosynthesis rates due to erect leaf angles creating a more

efficient usage of light through the canopy (Duncan et. al., 1971; Winter and Ohlrogge, 1973)

and reduced tassel size potentially allowing for more assimilate availability to the ear sink

(Hunter et. al., 1969).

Research has shown that a large ASI can result in mild to extreme yield losses

making a short ASI more desirable. Bolanos and Edmeades (1996) used drought stress to

induce varying ASI's. Drought stress didn't have a major effect on number of days from

planting to anthesis, but did increase the number of days from planting to silking. Fischer et.

al. (1989) also found increases in ASI during drought stress. Grain yield of plants with an

average increase in ASI of 5 was less than 20% of the grain yield of the control (Bolanos and

Edmeades, 1996). Moss and Downy (1971) showed delays of silk emergence up to 16 days

after pollen shed resulted in a 90% reduction in plant dry matter compared to the check.

Plant height is another phenotype researcher have look at in hybrid maize. Several era

studies of hybrid corn have found changes in plant height over time (Meghji, 1984; Russel,

1985). Plant density also effects plant height. Plants at high density can sense neighboring

plants by detecting a low red:far red ratio (Ballare et. al., 1990). This causes stem elongation (Ballare et. al., 1990), leading to an increase in plant height. Sangoi et. al. (2002) found a linear increase in plant height as density increased and also demonstrated that older hybrids were taller than the more advanced hybrids. Carena and Cross (2003) found plant height increased as plant density increased from 38,000 to 56,000 plants ha^{-1}, but did not find a plant height response to densities above 56,000 plants ha^{-1}. Tetio-Kagho and Garder (1988) showed plant height increased with density to 100,000 plants ha^{-1}. Other research did not find a reduction in plant and ear height at all (Duvick, 1996). While average plant height has changed over the years, trending toward an average reduction in plant height, a consistent change in plant height response to density has not been observed. This variation in response illustrates the lack of certainty in the literature for plant height; perhaps a clearer picture could be gained if genetic variation was minimized.

Is there a yield benefit associated with shorter plant height? Johnson et. al. (1986) conducted several cycles of recurrent selection for reduced plant height in maize and found that as plant height decreased yields increased. Fischer et. al. (1989) also discovered yield advantage when shorter plants were compared to taller plants.

Large tassel reduce photosynthesis more at high density than low density. Duncan et.al. (1967) hypothesized that tassels intercept and block light from the canopy. Shadow size was measured to quantify the blockage. Tassels were removed from plants and placed in front of a projection lamp to determine shadow size. They discovered tassel size varied at different plant densities. Shadow size of 114cm^2 at 84,000 plants ha^{-1} was compared to reduced shadows of about 150 cm^2 formed at 44,000 and 64,000 plants ha^{-1}, suggesting a

decrease in tassel size at higher densities. Even though tassel size at higher densities were found to be smaller, simulations showed that high plant densities led to a reduction in yield due to reduced photosynthesis from the shadows. Photosynthesis reduction was calculated to be 19.4% at 98,800 plants ha^{-1} and just 4.1% at 17,209 plants ha^{-1}. At higher densities plants must compete more for light with neighboring plants, likely resulting in the reduced yields shown (Duncan et.al., 1967) indicating an advantage of smaller tassels at high density.

Tassel size responds to plant density and smaller tassels can lead to higher yield. Duvick (1996) found linear reductions in tassel size over the past 75 years. Meghji et. al. (1984) demonstrated that over the past 70 years reductions by as much as 2.5 tassel branches/decade have occurred along with a reduction in tassel weight by .5 grams/decade. Sangoi et. al. (2002) studied hybrids from different eras grown in Brazil and also found a decrease in tassel branch number more advanced hybrids. Tassel removal at high plant densities led to yield increase (Hunter et. al., 1969; Lambert and Johnson, 1978). Hunter et. al. (1969) attributed this to an increase in light available for photosynthesis or a lack of competition between sinks (tassel and ear). Advantages of smaller tassels have also been observed when hybrids were compared at a constant plant density. Six cycles of recurrent selection for reduced tassel size were undergone by Fischer et. al. (1987). A reduction in tassel size was accomplished and plants at cycle 6 had 13-14.6% higher yields.

Reduced leaf number has been shown in advanced hybrids (Sangoi et. al., 2002). However, others have reported no major changes in number leaves/plant (Duvick, 1996 and Duvick, 2005) and Meghji et. al. (1984) reported that advanced hybrids had a higher number

of leaves. Seka and Cross (1995) found an increase of 0.35 leaves per plant as density increased from 24,000 plants ha^{-1} to 72,000 plants ha^{-1}.

The Iowa Stiff Stalk Synthetic (BSSS) maize population has been undergoing two lineages of selection to increase grain yield since 1939 (Lamkey, 1992). One method of selection utilized was half-sib selection, which utilizes a common tester to create testcrosses for yield testing. The half-sib lineage consisted of 2 programs, HT and HI. Direct response (yield of testcross) in the HT program increased 0.165 mg ha^{-1} per cycle (Eberhart et. al., 1973). Direct response of the second half-sib program (HI program) increased .13 mg ha^{-1} per cycle (Edwards, in-press). The second lineage of selection from BSSS was reciprocal selection which makes use of two populations using each as a target population and uses the other as the tester, advancing both populations simultaneously (Penny and Eberhart, 1971). The first eleven cycles of the reciprocal lineage had a direct response of 0.28 mg ha^{-1} per cycle (Keeratinijakal and Lamkey, 1993). The direct responses of these programs are proof of the success of the selection processes.

Lines derived from the Iowa Stiff Stalk Synthetic population have made major contributions to the maize industry. It has been estimated that lines from this population were parents of 19% of the total corn acres planted in the US in 1980 (Zuber and Darrah, 1980). The Iowa Stiff Stalk Synthetic population is ideal for examination because it has remained a closed population with no introduction of new germplasm (Lamkey et. al., 1991). With known genetic relationships in a recurrent selection program, we can study the genetic basis for plant characteristics and relate these changes directly to selection response.

Therefore, the objective of this experiment was to determine if direct selection for grain yield and agronomic performance in the Iowa Stiff Stalk synthetic population has indirectly improved adaptation to high plant density. Our hypothesis is that recurrent selection for yield has caused an improved adaptation to plant density though modification of characteristics mentioned in this report.

Materials and Methods

Five breeding populations representing different levels of cycle advancement from three selection programs representing two separate lineages were the first of two treatments were used in this study.

BSSS. The Iowa Stiff Stalk Synthetic population was originally created in 1934 by inter-mating 16 inbred lines with greater than normal stalk quality (Lamkey, 1992). From this base population, three different recurrent selection programs were initiated using selection criteria focused primarily on yield increase (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995) with some consideration for low grain moisture and increased resistance to stalk and root lodging (Lamkey, 1992; Lamkey et. al., 1991; Holthaus and Lamkey, 1995).

BSSS(HT)C7. From the original BSSS population one lineage utilized half-sib selection. Seven cycles of half-sib selection took place utilizing testcrosses to IA13 to determine candidates for selection. The 7th cycle was denoted BSSS(HT)C7 (Lamkey et. al., 2001). Details of procedures used for cycle advancement were described by Eberhart et. al. (1973).

BS13(S)C0. This breeding population was base population of the second selection program within the half-sib lineage. The population was created from BSSS(HT)C7 by selecting the 29 best performing S_1 lines (Lamkey, 1992), thus having undergone the same 7 cycles of selection.

BS13(HI)C5. Starting with BS13(S)C0 (descendent of BSSS(HT)C7), 5 cycles of half-sib selection was completed utilizing testcrosses to B97 to determine candidates for selection. The most advanced cycle from the half-sib lineage is program is BS13(HI)C5 (Edwards, In-Press) representing a total of 12 selection cycles.

BSSS(R)C17. The second lineage was also initiated from the original BSSS population and utilized recurrent reciprocal selection with Iowa State Corn Borer Synthetic #1 (BSCB1) (Holthaus and Lamkey, 1995). Details of procedures used were described by Penny and Eberhart, (1971). The population resulting from the first cycle of selection with the BSSS population was named BSSS(R)C1. To date 17 selection cycles have been completed with current endpoint BSSS(R)C17. Table 2 summarizes the breeding populations used in this study.

The second treatment used in this study was planting density. Densities of 38,300, 57,400, 77,500 and 95,700 plants ha^{-1} were used.

Experimental Design

The experiment was arranged in a split plot design with planting density as the whole plot treatments and the five breeding populations as the split plot treatments. Treatments

were replicated twice at Carroll and Ames IA, in 2008 and 2009. Soil types and planting date

for both locations are listed in Table 1.

Each experimental unit consisted of four rows spaced 0.76 meters apart and 5.49

meters in length. Plots were planted at the densities given above with no thinning. The

number of intact plants in each plot was recorded just before flowering and converted to

plants per hectare. Measurements of flag leaf angle, plant height, tassel branch number, and

total number of leaves were taken after pollination on the center two rows of four row plots

from 5 plants per row starting with the fifth plant in from the outside of the plot, for a total of

10 plants measured per plot.

Leaf angle of the flag leaf was recorded using a digital protractor. First, vertical (0°)

was set by placing the protractor against the stalk. The flag leaf was removed from the plant

by cutting below the node under the flag leaf. The flag leaf and section of stalk was then held

vertically against the side of the remaining, intact, stalk. The digital protractor was calibrated

to 0° and the angle of the flag leaf was then recorded. Due to a late summer windstorm at the

Carroll location in 2008, flag leaf angle was only recorded at the remaining 3 environments,

for a total of 6 replicates.

Anthesis - silking interval (ASI) was established by taking daily recordings of the

number of plants with one or more visible silks and number of tassels shedding pollen. Plants

were recorded as shedding pollen when a single anther could be seen. Data was collected in

the center two rows of 4 row plots. This was recorded daily for approximately 2 weeks, or

until approximately 90% of the silks had emerged/plot. These recordings were only

performed at the Ames, IA location in both 2008 and 2009. Two replications were observed

each year for a total of four replicates. The percent of plants with pollen shedding or silks emerged was calculated as a percent of daily plants shedding/silking over final plot stand count. The date at which this reached 50% for each plot was recorded as used to determine the date of 50% silk/pollen shed for that plot. Anthesis-silking interval was calculated as number of days to silking minus number of days to pollen shed (Bolanos and Edmeades, 1996).

Tassel branch numbers were determined by removing the tassels from each plant after pollination and counting the number branches that branch directly off the main branch. Branches of other branches were not counted (Bolanos and Edmeades, 1996). Plant height was recorded after pollination as the height of the flag leaf collar (Maddonni et. al., 2001, Bolanos and Edmeades, 1996).

In order to be able to tabulate total number of leaves, it was important to track leaf number of each leaf throughout the growing season, as the lower leaves fall off prior to the growth of the uppermost (flag) leaf. Leaves were marked using an approach combining methods used by other researchers (Maddonni et. al., 2001; Maddonni and Otegui, 1996; Elmore et. al. 2005; Sangoi et. al., 2002). Leaves 5 and 11 on plants 6-10 in 2008 and 4-11 in 2009 were marked with spray paint in each plot. From these sets of painted leaves, the first 5 intact plants in each of 2 center rows were utilized for data collection, totaling a maximum of 10 plants per plot.

The 5th leaf on each plant was determined by counting vertically from the first true leaf. These leaves were marked at approximately V7. At approximately V14 the 11th leaf was marked. By this time leaves 1-4 had fallen off the plant in most cases. By marking the 11th

leaf, leaf numbers could still be determined after tasseling although, in most cases, the 8th

leaf and lower had already fallen off.

Data Analysis

$$y_{jkmn} = \alpha_i + \phi(\alpha)_{ij} + \eta_k + \delta(\alpha)_{ijk} + \tau_m + (\tau\eta)_{km} + (\alpha\tau)_{im} + (\alpha\eta)_{ik} + (\alpha\tau\eta)_{ikm} + \varphi_{ijkm} + \varepsilon_{ijkmn}$$

Where:

α_i = effect of environment i,

$\phi(\alpha)_{ij}$ = effect of replicate block j within environment i,

η_k = effect of planting density k,

$\delta(\alpha)_{ijk}$ = split plot error A for planting density k, block j, environment i,

τ_m = effect of breeding population m,

$(\tau\eta)_{km}$ = interaction of planting density effect k with breeding population m,

$(\alpha\tau)_{im}$ = interaction of breeding population m with environment i,

$(\alpha\eta)_{ik}$ = interaction of planting density effect k with environment i,

$(\alpha\tau\eta)_{ikm}$ = interaction of planting density effect k with environment i, breeding population m,

φ_{ijkm} = split plot error B for planting density k, block j, environment i, breeding population m,

ε_{ijkmn} = within plot error.

Interactions with environment were considered random effects. All other effects were treated as fixed effects. The model was fit using Henderson's mixed model equations (Henderson, 1984). Variance components corresponding to random effects were estimated using restricted maximum likelihood (Searle et al., 1992). Data were checked for outliers by computing the probability of obtaining a larger absolute value of each residual using the t-

distribution. Individual p-values for residuals were adjusted with a Bonferroni correction and studentized residuals examined with quantile-quantile (QQ) probability plots. Based on QQ plots and Bonferroni adjusted pvalues, fourteen observations were identified as outliers and were discarded.

After removal of outliers, the following covariate model was fit to the final dataset:

$$y_{jkmn} = \alpha_i + \phi(\alpha)_{ij} + \delta(\alpha)_{ijk} + \tau_m + \beta_1 x_{ijkm} + (\tau\beta_1)_m x_{ijkm} + \beta_2 x^2_{ijkm} + (\tau\beta_2)_m x^2_{ijkm} + (\alpha\tau)_{im} + (\alpha\beta_1)_i x_{ijkm} + (\alpha\tau\beta_1)_{im} x_{ijkm} + (\alpha\beta_2)_i x^2_{ijkm} + (\alpha\tau\beta_2)_{im} x^2_{ijkm} + \varphi_{ijkm} + \varepsilon_{ijkmn}$$

Where:

α_i = effect of environment i,

$\phi(\alpha)_{ij}$ = effect of replicate block j within environment i,

$\delta(\alpha)_{ijk}$ = split plot error A for density k, block j, environment i,

τ_m = intercept of breeding population m,

x_{ijkm} = observed plant density in environment i, block j, density k, breeding population m,

β_1 = linear effect of observed plant density,

$(\tau\beta_1)_m$ = interaction of linear density effect with breeding population m,

β_2 = quadratic effect of observed plant density,

$(\tau\beta_2)_m$ = interaction of quadratic density effect with breeding population m,

$(\alpha\tau)_{im}$ = interaction of breeding population m with environment i,

$(\alpha\beta_1)_i$ = interaction of linear density effect with environment i,

$(\alpha\tau\beta_1)_{im}$ = interaction of linear density effect with environment i, breeding population m,

$(\alpha\beta_2)_i$ = interaction of quadratic density effect with environment i,

$(\alpha\tau\beta_2)_{im}$ = interaction of quadratic density effect with environment i, breeding population m,

φ_{ijkm} = split plot error B for density k, block j, environment i, breeding population m,

ε_{ijkmn} = within plot error.

Interactions with environment were considered random effects. All other effects were treated as fixed effects. The model was fit using Henderson's mixed model equations and estimators of variance components obtained by restricted maximum likelihood (Henderson, 1984; Searle et al., 1992). Computations were carried out using SAS proc mixed (SAS Institute, Cary, NC). Akaike's information criteria(AIC) was used to identify which random effects to retain in the model. Random effects with estimates of zero were eliminated. Subsequently, the set of random effects in the final model was chosen by fitting all combinations of random effects in the model. The model with the smallest AIC value was chosen as the final model. The possible random effects and those selected for use in the final model are listed in Table 3.

All fixed effects were initially included in the model and p-values were determined. For each trait, nonsignificant plant-density covariates and plant-density by population interactions were dropped from the model beginning with the highest order terms. If a random interaction containing a plant-density covariate was retained in the model, the corresponding plant-density fixed effect was retained automatically. If there were no random interactions containing plant density interactions, the highest order terms were tested with F-tests using Satterthwaite approximate degrees of freedom. If the highest order term was not significant, it was dropped. The next highest level terms was then tested and dropped if not significant. All possible fixed effects and p-values for effects retained in the final model are listed in Table 4. When plant-density by breeding population interactions were retained in the

final model, breeding-population specific covariates terms were fit in the model and tested individually. Covariate terms for individual breeding populations that were not significant were dropped from the model. Response curves corresponding to covariate terms were plotted for individual breeding populations. In cases that individual breeding populations had near identical plant density responses, covariate terms were compared statistically and if not different, the response curves were combined for populations have statistical indistinguishable responses.

Results and Discussion

Increasing plant density had no effect on flag leaf angle for any breeding population. The most advanced population, BSSS(R)C17, had more upright leaf angles than all other populations (Table 5). BS13(HI)C5 had a more upright flag leaf angle than BSSS, BSSS(HT)C7, and BS13(S)C0. There was no difference in flag leaf angle between BSSS(HT)C7 and BS13(S)C0 most likely because BS13(S)C0 is actually 29 lines selected from BSSS(HT)C7 (Lamkey, 1992), and thus few differences between these breeding populations are expected. However both BSSS(HT)C7 and BS13(S)C0 had more vertical flag leaf angles than the original BSSS population.

These results coincide with hybrid studies indicating leaf angle in recent decades were trending toward vertical (Duvick, 1996; Duvick, 2005; Pepper et. al., 1977). Lambert and Johnson (1978) reported an effect of density on leaf angle while Ariyanayagam et. al. (1974) did not observe a density effect of leaf angle. Our experiment did not find a density effect of flag leaf angle. Since flag leaves are the shortest leaves on the plant they were likely by high density because flag leaves of neighboring plants would not overlap. If you were to

examine leaves further down in the canopy, leaves would begin to overlap and density may affect those leaves. The more erect leaf angle seen in the advanced populations indicates the ability of more advanced populations to allow light penetration deeper into the canopy, creating a more efficient photosynthetic system.

The original BSSS population had an ASI of 3 which increased to 5 as density increased while the advanced BSSS(R)C17 population was not affected by density and maintained an ASI of -0.56, meaning plants had silks available before pollen shed began (Figure 1). This indicates a dramatic improvement in ASI for the most advanced populations especially at high density. The synchronization of pollen and silk availability at high densities allows the plant to have a larger chance of successful fertilization of silks which would result in increased yield. This is consistent with hybrid maize studies also indicating decreases in ASI more advanced maize hybrids (Russell, 1985) and increases in ASI associated with increasing plant density in hybrid maize (Seka and Cross, 1995; Sangoi et. al., 2002). The reduction of ASI and lack of density response in advanced populations clearly supports our hypothesis of increased adaptation to high plant density.

At the low plant density of 35,000 plants ha^{-1} BSSS was taller than BSSS(HT)C7 and BS13(S)C0 while BS13(HI)C5 and BSSS(R)C17 were shortest in height (Figure 2). At the highest density of 75,000 plants ha^{-1} BSSS and BSSS(HT)C7 share the same tall plant height followed by a reduction in plant height between BSSS(HT)C7 and BS13(S)C0. Plant height also decreases from BS13(S)C0 to BS13(HI)C5 and from BS13(HI)C5 to BSSS(R)C17.

This reduction in plant height is consistent with findings of decreased plant height in advanced hybrids (Meghji, 1984; Russell, 1985; Sangoi et. al., 2002) and increases in plant

height at higher densities (Carena and Cross, 2002; Tetio-Kagho and Garder, 1988; Sangoi

et. al., 2002) although 17 cycles of selection may have led to some inbreeding depression

which may also have an effect on plant height. The ability of plants with more cycle

advancement to increase yield with shorter plants may relate to increased leaf angle. If light

can penetrate deeper into the canopy, neighboring plants may not be competing as much for

light, indicating increased adaptation to high plant density.

At low plant density each population had progressively fewer tassel branches as

populations progress from BSSS to BSSS(R)C17 except BSSS(HT)C7 and BS13(S)C0

(Figure 3). Tassel branches were reduced from 20 (BSSS) to just 7 branches in BSSS(R)C17.

These results confirm conclusions found in hybrid studies observing reductions tassel branch

number in more advanced maize hybrids (Duvick, 1996; Meghji et. al., 1984; Sangoi et. al.,

2002). Although only the original population demonstrated reduced tassel branch number at

high density that is consistent with hybrid studies suggesting an advantage of small tassels at

higher densities (Duncan et.al., 1967; Hunter et. al., 1969; Lambert and Johnson, 1978).

It is possible that more advanced populations may not have shown a reduction in

tassel branch number at high density due to the already low number of just 7 tassel branches.

The reduction in tassel branches in advanced populations may relate to decreased ASI since

less pollen would be needed by a plant with a shorter ASI because pollen production

wouldn't be need for as long a time period (a couple days instead of a week) since the silks

are ready to accept pollen as soon as it sheds instead of tassels having to shed pollen for up to

5 days as in less advanced populations. Reduced tassel branches may also make more

photosynthate available to the ear if repartitioned to that sink (Hunter et. al., 1969) which

could potentially contribute to increasing yield. Both of these ideas support adaptation

advanced populations to high plant density.

BSSS, BSSS(HT)C7, and BS13(S)C0 had a similar responses to density for total

number of leaves(Figure 4). BS13(HI)C5 had fewer leaves and BSSS(R)C17 had fewer

leaves than BS13(HI)C5. Similar results have been seen in hybrid maize. Sangoi et. al.

(2002) found fewer leaves on more advanced hybrids, although this has not been observed in

all studies as no change in leaf number (Duvick, 1996 and Duvick, 2005) or an increase in

leaves in advanced hybrids (Meghji et. al., 1984; (Seka and Cross, 1995) has also been

observed. This reduction in leaves in more advanced populations may be possible because

the increase in leaf angle provides a better method of light penetration to the canopy. This

would reduce interplant competition for light, raising the red:far red ratio which reduces the

signal for plants to grow taller, allowing fewer leaves to accomplish the same quantity of

photosynthesis. It may also be due to shorter plants simply having fewer leaves. Whatever

the cause, this change in leaf number clearly supports our hypothesis of increased adaptation

to high plant density as more advanced cycles planted at higher densities are achieving

increasing yields with fewer leaves.

Conclusion

The BSSS program used yield and agronomic performance as an index to select for

population advancement. This selection indirectly resulted in an increase in flag leaf angle

and decreases in ASI, plant height, tassel branch number, and leaf number (evaluated in

populations not test crosses). Increasing density in advanced populations led to increased

plant height while not having an effect on ASI, tassel branch number or flag leaf angle. The

ability of the advanced populations to be nearly immune to increases in plant density clearly confirms our hypothesis of an increased adaptation to high density. Selection of genotypes displaying reduced ASI, plant height, and tassel branch number will continue to be important for continued adaptation to high plant density and future yield increase.

Acknowledgements

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Industry Res. Conf. Chicago, IL. 9–11 Dec. American Seed Trade Association,

Washington, DC.

Table 1. Table 1. Soil type and planting date for all locations used in the study.

Location	Year	Soil type	Planting Date
Ames	2008	Clarion loam	5/22/2008
Carroll	2008	Nicollet loam	5/10/2008
Ames	2009	Clarion loam	5/5/2009
Carroll	2009	Nicollet loam	5/4/2009

Table 2. Iowa Stiff Stalk Synthetic Maize populations used in the study. List in order of advancement from original population to most advanced population.

Population	Number of Cycles of Selection	Selection Method	Tester Used in Selection
BSSS	None – Original Population		
BSSS(HT)C7†	7	Half-Sib	IA 13
BS13(S)C0†	0 (7 total) – Base for BS13 Population	29 lines selected from BSSS(HT)C7	None
BS13(HI)C5	5 (12 total)	Half-Sib	B 97
BSSS(R)C17	17	Reciprocal Selection	BSCB 1 (Corn Borer Synthetic)

† Populations BSSS(HT)C7 and BS13(S)C0 are similar populations, differing only by population BS13(S)C0 being the compilation of the best performing lines from population BSSS(HT)C7.

Table 3. Combination of random effects with lowest AIC values which were used in final model.

Random Effects

Plant Characteristic	plot(replication x environment) †	plant density x replication (environment) †	breeding population x environment	plant density x environment	plant density quadratic x environment	plant density x breeding population x environment	plant density quadratic x breeding population x environment
Angle of Flag Leaf	NR††	NR††	X	NR††	NR††	NR††	NR††
ASI	NR††	NR††	NR††	NR††	NR††	NR††	NR††
Plant Height	X	NR††	NR††	X	NR††	NR††	NR††
Tassel Branch Number	X	NR††	X	NR††	NR††	NR††	NR††
Number of leaves	X	NR††	NR††	NR††	NR††	NR††	NR††

† plot(replication x environment) refers to replication x environment nested within plot; plant density x replication (environment) = plant density x environment nested in replication

†† NR = Not retained in the model because it was not part of the lowest AIC combination.

Table 4. P-values for fixed effects utilized in the statistical model.

Plant Characteristic	Breeding Population	Plant Density	Density x Breeding Population	Density Quadratic	Density Quadratic x Breeding Population
Angle of Flag Leaf	<.0001	NS†	NS†	NS†	NS†
ASI	0.8367	<.0001	0.0553	NS†	NS†
Plant Height	0.0002	0.0102	0.0088	0.0637	0.0212
Tassel Branch Number	<.0001	0.3928	0.0387	0.6923	0.0527
Number of leaves	<.0001	0.0008	NS†	0.0070	NS†

† NS = non-significant at the P <0.05 level. Non-significant fixed effects were dropped from the statistical model.

Table 5. Angle of Flag Leaf

Breeding Population	Flag Leaf Angle†
	-------------Degrees from vertical-------------
BSSS	47.5
BSSS(HT)C7	30.4
BS13(S)C0	34.3
BS13(HI)C5	38.0
BSSS(R)C17	24.9

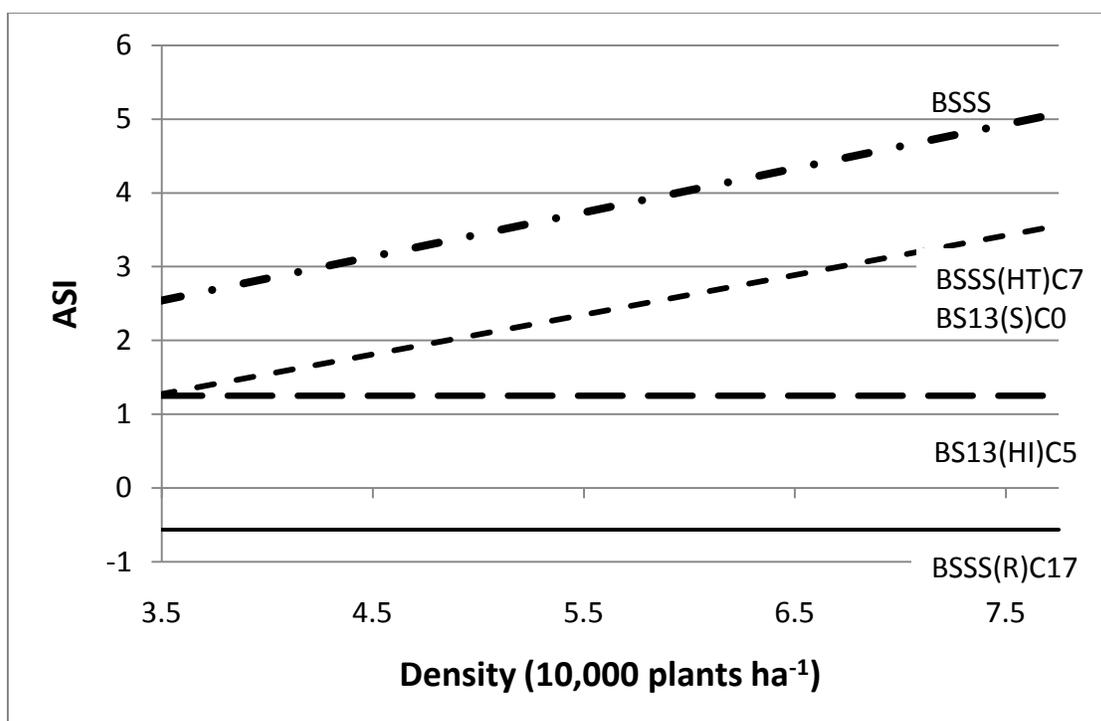† Density was non-significant for flag leaf angle.
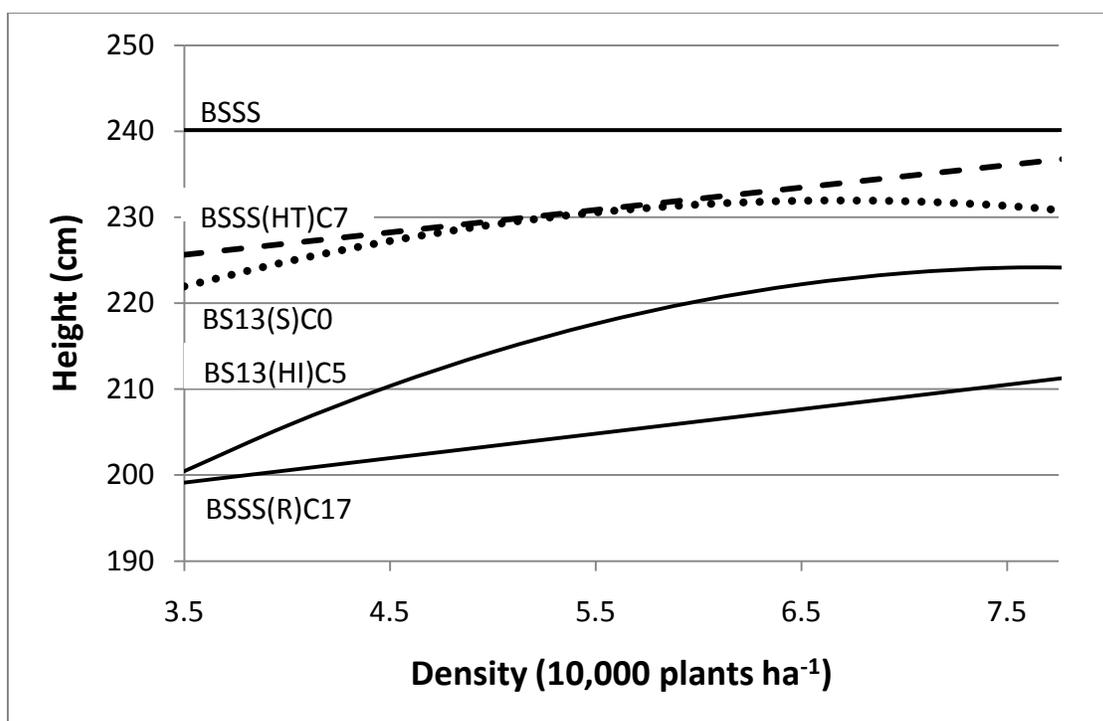
Figure 1. Anthesis silking interval.
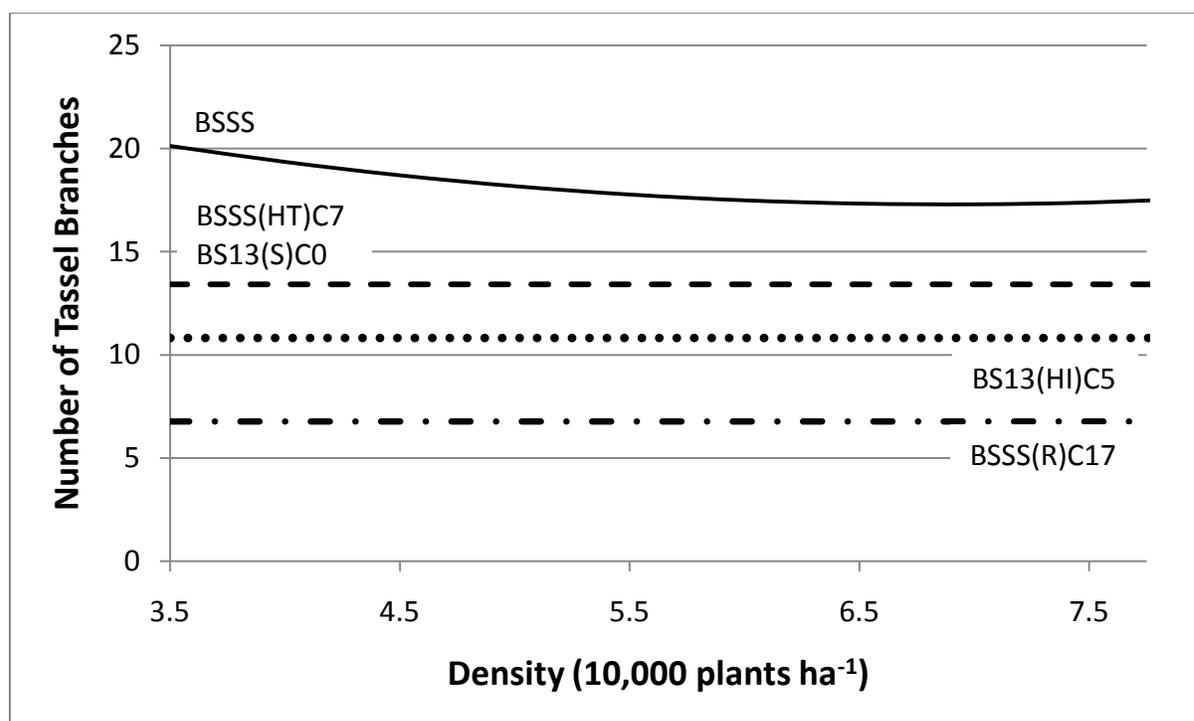
Figure 2. Plant height.

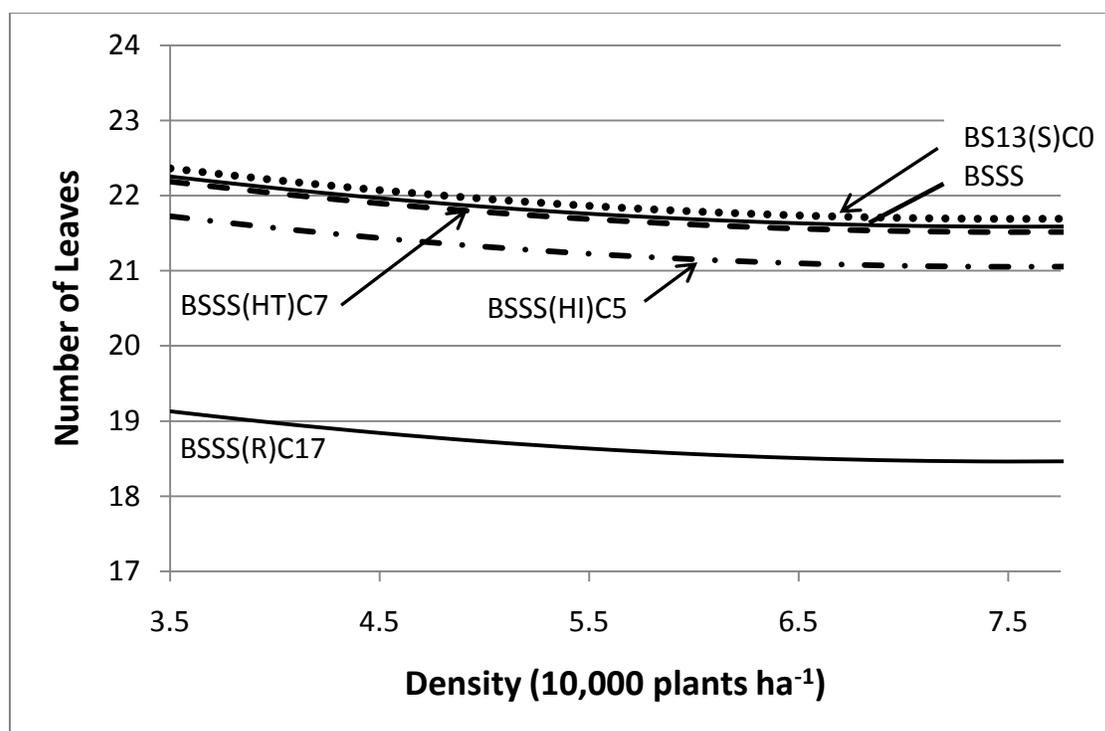**Figure 3. Tassel branch number.**

Figure 4. Number of leaves per plant.

CHAPTER 5. GENERAL CONCLUSION

The main conclusion of this research is an affirmation of our hypothesis that recurrent selection for yield has caused an improved adaptation to plant density though modification of characteristics mentioned in this report.

The BSSS program used yield and agronomic performance as an index to select for population advancement. This selection resulted in increased grain yield along with increases in test weight, resistance to stalk lodging, and decreased grain moisture. Root lodging has remained unchanged. This selection indirectly resulted in an increase in flag leaf angle and decreases in ASI, plant height, tassel branch number, and leaf number. Increasing density in advanced populations led to increased plant height while not having an effect on ASI, tassel branch number or flag leaf angle.

The ability of the advanced populations to be nearly immune to increases in plant density clearly confirms our hypothesis of an increased adaptation to high density. Selection of genotypes displaying these changes in plant characteristics will continue to be important for further adaptation to high plant density and future yield increase.