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Effects of recurrent selection for yield on plant growth across planting densities in maize (Zea mays L.)

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Effects of recurrent selection for yield on plant growth across planting densities in maize (Zea mays L.)

by

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A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

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CHAPTER I: GENERAL INTRODUCTION

Between the 1930’s and today, corn yields have increased from 1.5 to over 10 Mg ha\(^{-1}\) (Duvick, 2005b, Cooper et al., 2014). Grain yields per plant have failed to increase with time, meaning that increasing grain yields per unit-area is dependent on increasing planting densities (Duvick and Cassman, 1999). Between the 1930’s and today, planting densities have increased from 30,000 to over 81,000 plants ha\(^{-1}\) (Duvick, 2005a; Butzen and Burnison, 2014). Increased planting densities can have negative effects on plant growth, decreasing per-plant grain yields, increasing barrenness, decreasing plant biomass, and decreasing plant growth rates (Rutger and Crowder, 1967; Edmeades and Daynard, 1979; Fakorede and Mock, 1980).

In 1949, a reciprocal recurrent selection program was initiated between Iowa Stiff Stalk Synthetic (BSSS) and Iowa Synthetic Corn Borer #1 (BSCB1) with the goals of increasing grain yields and decreasing stalk and root lodging and grain moisture (Edwards, 2011). Numerous traits accompanied selection, including more upright leaves, smaller tassels, decreased plant height, and earlier pollen shed and silk emergence (Brekke et al., 2011; Edwards, 2011). Plants in the selected populations and population crosses are better adapted to growth at high plant densities, maintaining flowering times at increased densities and achieving higher yields at higher optimal planting densities (Brekke et al., 2011; Edwards, 2016).

Dissertation Organization. Chapter two of this dissertation contains an in-depth review of the literature concerning the development of BSSS and BSCB1 and the reciprocal recurrent selection program, the increase in grain yield in commercial agriculture, the history of planting density increases and the effect of density on plant growth and development, and the improved performance of modern maize varieties under increased stress.
Chapter three consists of a submitted manuscript to Crop Science. Chapter four consists of a manuscript in preparation for submission to Crop Science.

Chapter five contains a summary of stated conclusions from the studies performed in chapters three and four.

**Author Contributions:** In the manuscripts presented in Chapters III and IV, Michael Stein collected all data, ran all data analysis, and was the primary author. Fernando Miguez was heavily involved in non-linear data analysis and writing R code. Jode Edwards was the PI of both manuscripts, and was also heavily involved in designing the experiments, assisting in data analysis, and was involved in writing the manuscripts.

**Objectives.** The objectives of the studies found in this dissertation were to characterize the effects of recurrent selection for grain yield on the growth of plant organs under increasing planting densities. Five main traits: ear length, plant height, ear biomass, stalk biomass, and tassel biomass, were the organs examined in both studies described. The objectives for the study described in Chapter three were to characterize how increased plant density affected final phenotypes, growth timing, growth rates, and biomass partitioning across cycle 0 and cycle 17 per se populations. The study described in Chapter four was intended to characterize how increased densities affected BSSS/BSCB1 and BSSS(R)C17/BSBC1(R)C17 population cross performance, and to observe heterosis levels in final phenotypes, growth timing, growth rates, and harvest index.
References


CHAPTER II: REVIEW OF LITERATURE

Iowa Stiff Stalk Synthetic/Iowa Corn Borer Synthetic #1 Reciprocal Recurrent Selection

Recurrent selection. Recurrent selection is one of the most straight-forward methods of improving the performance of a population. The selection cycle consists of three major steps, the evaluation of a population for the desired trait, the selection of superior individuals, and the recombination of the selected individuals to reform the population in the next cycle (Fehr, 1987). The next cycle of selection is expected to have an increased population mean compared to the previous cycle, indicating improvement. In addition to improving the population phenotypically, a major goal in a recurrent selection program is maintaining genetic variety in the population (Martin and Hallauer, 1980).

While selection on individual plants is possible, commercially hybridized species often use test crosses to identify superior individuals to recombine (Hallauer and Darrah, 1985). These test crosses usually take one of two forms, half-sib selection using a single tester parent, and full-sib selection utilizing paired plant crosses (Hallauer and Darrah, 1985). Half-sib selection is a system well-designed to examine general combining ability, while full-sib selection is useful in calculating specific combining abilities (Comstock et al., 1949). General combining ability refers to a line or population’s ability to form a high-performing hybrid with a general tester, while specific combining ability refers to the superior performance of the combination of two specific genotypes above the general hybrid performance of the lines or populations (Sprague and Tatum, 1942).

Reciprocal Recurrent Selection. In 1949, Comstock et al. introduced the idea of a reciprocal recurrent selection program, in which two different populations would be selected
together, utilizing each other as hybrid testers. A recurrent selection program was developed to be superior to normal half-sib and full-sib programs by making use of both general and specific combining ability, with the added benefit of improving two genetically different populations at the same time (Comstock *et al.*, 1949).

The same year that Comstock *et al.* introduced their idea of a reciprocal recurrent selection program, G. F. Sprague began the BSSS/BSCB1 recurrent selection program (Penny and Eberhart, 1971).

**BSSS.** Iowa Stiff Stalk Synthetic (BSSS) was created in 1934 from the intermating of 16 inbred lines chosen for above-average stalk strength (Sprague, 1946). The population was developed from 10 Reid Yellow Dent lines and 6 non-Reid Yellow Dent lines, and was equal in yield to open-pollinated varieties of the time (Sprague, 1946; Messimer *et al.*, 1991)

**BSCB1.** Iowa Corn Borer Synthetic #1 (BSCB1) was created in the 1940’s from the intermating of 12 inbred lines chosen for resistance to the European Corn Borer (Penny and Eberhart, 1971).

**BSSS(R)C17** and **BSCB1(R)C17.** The seventeenth cycle of the reciprocal recurrent selection program. The program involved half-sib reciprocal selection from cycles 1 to 10, followed by full-sib reciprocal selection from cycles 11 to 17 (Edwards, 2011). The primary objective of the recurrent selection program was increased grain yield, while secondary objectives were decreased levels of stalk lodging, root lodging, and grain moisture (Penny and Eberhart, 1971; Edwards, 2011).

**Genetic gain in the BSSS/BSCB1 program.** The reciprocal recurrent selection program has achieved three of its four stated goals by increasing grain yield, decreasing grain moisture, and decreasing stalk lodging rates (Edwards, 2011).
Per cycle yield gain in the BSSS and BSCB1 populations has changed as the cycles progressed. Between cycle 0 and 3, BSSS had an average yield gain of 138 kg ha\(^{-1}\) cycle\(^{-1}\), while BSCB1 had a yield loss of 64 kg ha\(^{-1}\) cycle\(^{-1}\) (Penny and Eberhart, 1971). Between cycles 0 and 5, BSSS average yield gain was 24 kg ha\(^{-1}\) cycle\(^{-1}\), while BSCB1 average yield gain was 47 kg ha\(^{-1}\) cycle\(^{-1}\) (Eberhart \textit{et al.}, 1973). Between cycle 0 and cycle 7, no change in yield was observed in either BSSS or BSCB1 (Martin and Hallauer, 1980). Smith (1983) found a BSSS yield increase of 131 kg ha\(^{-1}\) cycle\(^{-1}\) between cycle 0 and 7, but also found no yield increase in BSCB1 between cycle 0 and 8. Between cycles 0 and 10, grain yield in BSSS increased on average by 137 kg ha\(^{-1}\) cycle\(^{-1}\) (Helms \textit{et al.}, 1989). Between cycles 0 and 11, grain yield in BSSS increased on average by 34 kg ha\(^{-1}\) cycle\(^{-1}\), while average BSCB1 grain yields were 50 kg ha\(^{-1}\) cycle\(^{-1}\) (Keeratinijakal and Lamkey, 1993a). Between cycles 0 and 17, the average grain yield increase in BSSS was found to be 2% per cycle (Brekke \textit{et al.}, 2011a). Overall, BSSS grain yield has risen from roughly 5 Mg ha\(^{-1}\) in cycle 0 to over 7 Mg ha\(^{-1}\) in cycle 17 (Brekke \textit{et al.}, 2011a). BSCB1 grain yield has essentially remained stagnant with selection, with both BSCB1 and BSCB1(R)C17 having grain yields of roughly 5.5 Mg ha\(^{-1}\) at optimal densities (Edwards, 2016).

Grain moisture decreased with selection (Edwards, 2011). Between cycles 0 and 5, grain moisture levels decreased in BSSS from 22.2% to 20.6%, while remaining unchanged in BSCB1 (Eberhart \textit{et al.}, 1973). Smith (1983) found that grain moisture in BSSS fell between cycles 0 and 4, while rising between cycles 4 and 8. This was corroborated by Helms \textit{et al.} (1989), who found that grain moisture decreased from 27% to 24% in BSSS between cycles 0 and 4, and rose again to 26% between cycles 4 and 10. Grain moisture in BSCB1 between cycles 0 and 8 remained unchanged, maintaining roughly 21% grain moisture level (Smith, 1983). Grain
moisture levels were the same at cycles 0 and 11 in both BSSS and BSCB1 (Keeratinijakal and Lamkey, 1993a). Edwards (2011) found that grain moisture in BSSS was at similar levels at cycles 0 and 11, but between cycles 11 and 17, moisture levels fell from roughly 25% to 21%.

Stalk lodging decreased with selection (Edwards, 2011). Between cycles 0 and 5, stalk lodging levels in BSSS decreased from 14.2% to 9.6%, while decreasing from 31.1% to 24% in BSCB1. Stalk lodging levels fell by 2.83% cycle\(^{-1}\) in BSSS (Keeratinijakal and Lamkey, 1993a). Reduction of stalk lodging in BSCB1 was much greater, with a 7.57% decrease per cycle, leading to stalk lodging levels falling from 40.4% to 9% between cycle 0 and 11 (Keeratinijakal and Lamkey, 1993a). Edwards (2011) found that stalk lodging levels fell from just over 8% to just under 2% between cycles 0 and 17.

Researchers are conflicted on the reciprocal recurrent selection program’s effects on root lodging. Smith (1983) found that root lodging levels decreased between cycle 0 and 4 in BSSS and between cycle 4 and 8 in BSCB1. Keeratinijakal and Lamkey (1993a) found that root lodging levels decreased between cycles 0 and 11 in both BSSS and BSCB1. On the other hand, Helms et al. (1989) and Edwards (2011) found no change in root lodging levels in BSSS between cycle 0 and cycles 10 and 17, respectively.

**Inbreeding in the populations.** BSSS and BSCB1 are closed populations, with no new genetic material being introduced since the populations were created 70-80 years ago (Lamkey et al., 1991). However, while this makes these populations ideal candidates to track genetic changes accompanying selection, it leaves them vulnerable to inbreeding.

Eberhart et al. (1973) were the first to estimate inbreeding in the populations. When grain yield increases in the *per se* populations were much lower than expected, Eberhart et al. (1973) identified loss of beneficial alleles via genetic drift as a main cause, and estimated
inbreeding levels in the populations at 22%. Martin and Hallauer (1980) estimated that the
inbreeding levels in the cycle 7 populations, estimated at 29%, were sufficient to cancel out the
effects of selection, leading to a lack of yield increase compared to cycle 0. Keeratinijakal and
Lamkey (1993a) found that yield increases in the BSSS and BSCB1 populations stagnated
between cycles 9 and 11, and estimated inbreeding levels at cycle 11 to be 37%. Between cycle
0 and cycle 11, the yield drag of heterozygosity loss was estimated at 12 kg ha\(^{-1}\) cycle\(^{-1}\) for both
BSSS and BSCB1 (Keeratinijakal and Lamkey, 1993b). In a large-scale, genome-wide genetic
study, Gerke \textit{et al.} (2015) found that large chromosomal sections became fixed between cycle 0
and cycle 16 in both BSSS and BSCB1. Observed heterozygosity levels fell from roughly 24%
to 14% in BSSS, and from roughly 27.5% to 16% in BSCB1, with the inbreeding level at cycle
16 exceeding 40% (Gerke \textit{et al.}, 2015).

Eberhart \textit{et al.} (1973), Martin and Hallauer (1980), Keeratinijakal and Lamkey (1993a,b),
and Gerke \textit{et al.} (2015) are all in agreement that the most likely cause of the loss of
heterozygosity in the populations was genetic drift caused by the low number of individuals
selected to form the next cycle. To try to alleviate genetic drift, the number of \(S_1\) lines used to
form new cycles was increased from 10 to 20 between cycles 8 and 9 (Helms \textit{et al.}, 1989).
However, while the increase in inbreeding levels was slowed, with a rise in only roughly 5%
inbreeding between cycles 11 and 16 compared to 15% between cycles 5 and 11, it also
negatively affected genetic gain by decreasing selection intensity (Keeratinijakal and Lamkey,
1993a,b).

**Heterosis in the population crosses.** Heterosis is the difference in performance between
an F1 individual and the parental mean (Penny and Eberhart, 1971). Just as inbreeding levels for
yield increased with time in the BSSS/BSCB1 program, so have heterosis levels in the test
crosses between the populations. Cycle 0 BSSS/BSCB1 crosses had grain yield heterosis levels calculated often around 14-15%, but some estimates are as high as 25%, with population cross yields 770 to 860 kg ha\(^{-1}\) higher than the *per se* population mean (Eberhart *et al*., 1973; Martin and Hallauer, 1980; Keeratinijakal and Lamkey, 1993a). By cycle 5, heterosis levels were 35%, and the population cross was out-performing the population mean by 1.92 Mg ha\(^{-1}\) (Eberhart *et al*., 1973). In cycle 7, while *per se* population yields had not changed, heterosis levels were nearly 42%, and the population cross was over 2 Mg ha\(^{-1}\) higher yielding than the mid-parent population average (Martin and Hallauer, 1980). Ears per plant increased in both BSSS and BSCB1 between cycle 0 and cycle 7, increasing from 0.89 to 1.02 ears plant\(^{-1}\) in BSSS, and from 0.97 to 1.04 ears plant\(^{-1}\) in BSCB1 (Martin and Hallauer, 1980). Between cycles 4 and 8, the population cross had a yield gain of an astounding 361 kg ha\(^{-1}\) cycle\(^{-1}\) (Smith, 1983). In cycle 11, heterosis levels had risen to 76%, and the population cross had a grain yield 2.92 Mg ha\(^{-1}\) higher than the mid-parent mean (Keeratinijakal and Lamkey, 1993a). Following cycle 11, heterosis levels no longer rapidly climbed, corresponding with a slowed inbreeding rate and resumed improvement in the parental populations, however, heterosis yields continued to rise. In cycle 14, heterosis levels had fallen back to 66.5%, with the population cross yielding 3.28 Mg ha\(^{-1}\) more than the mid-parent mean (Scheffler *et al*., 2008). In cycle 17, heterosis was roughly at 50% of the midparent values, and the population cross had a yield roughly 3 Mg ha\(^{-1}\) higher than that of the population mean (Edwards, 2016).

Grain yield is not the only trait that has seen an increase in heterosis with selection in the BSSS/BSCB1 program. In cycle 0, the heterosis level in plant heights was barely over 1% (Eberhart *et al*., 1973). In cycle 5, plant height heterosis had risen to over 7% (Eberhart *et al*., 1973). In cycle 7, plant height heterosis had risen to almost 10% (Martin and Hallauer, 1980).
In cycle 11, plant height heterosis continued to increase, as it was found that the heights of the per se populations continued to decrease, and the height of the population cross continued to increase (Keeratinijakal and Lamkey, 1993a).

There are two major hypotheses for the action of heterosis, dominance and over-dominance. The dominance hypothesis was introduced by Davenport (1908), and suggests that the superior performance of a hybrid individual over its parents is due to the covering of poorly-performing recessive alleles by superior-performing dominant alleles. The over-dominance hypothesis was introduced by Shull (1908) and greatly expanded upon by Hull (1946), and suggests that it is the heterozygous nature of hybrid alleles that induce superior performance, as long as the two parents are sufficiently genetically dissimilar. Heterosis in grain yield in the BSSS/BSCB1 program shows evidence of being determined through the dominance hypothesis. Keeratinijakal and Lamkey (1993b) found that dominance genetic distance increased with selection, increasing from 1.98 in cycle 0 to 6.97 in cycle 11. From this, it was hypothesized that increases in population cross yield and heterosis levels were due to the per se populations becoming fixed at complimentary loci (Keeratinijakal and Lamkey, 1993b). Gerke et al. (2015) found that while large segments of the BSSS and BSCB1 genome became fixed with selection, the fixed segments differed between the two populations, again suggesting that dominance, rather than over-dominance, was the cause of heterosis in the BSSS/BSCB1 program.

Changes associated with selection for yield in the BSSS and BSCB1 populations.

Selection for the four traits targeted in the recurrent selection program resulted in a large number of altered morphological and developmental traits in the populations.

Plant heights and ear heights decreased in both the BSSS and BSCB1 populations between cycles 0 and 11 (Keeratinijakal and Lamkey, 1993a). Plant heights continued to fall in
the later cycles, as BSSS(R)C17 plants were 30-40cm shorter than BSSS plants (Brekke et al., 2011b; Edwards 2011). Ear heights also continued to decline, decreasing almost 25cm between BSSS and BSSS(R)C17 (Edwards, 2011).

Canopy architecture was greatly affected by selection. Tassels had 10-13 fewer primary tassel branches in BSSS(R)C17 compared to BSSS (Brekke et al., 2011b; Edwards 2011). Flag leaves have become 17° more upright and 3cm shorter between BSSS and BSSS(R)C17 (Edwards, 2011). Plants in the cycle 17 BSSS population had 3 fewer leaves than cycle 0 BSSS plants (Brekke et al., 2011b; Edwards, 2011). Fakorede and Mock (1980) found that biomass accumulation for the plant, and ears in particular, increased until 111 days after planting in the BSSS/BSCB1 cross, while biomass accumulation continued past 125 days after planting in the BSSS(R)C7/BSCB1(R)C7 population cross.

Timing of plant growth and reproductive events changed with selection. Days to silking in the BSSS and BSCB1 converged in cycle 7, with silking time in BSSS decreasing from 76.8 to 73 days after planting, and silking time in BSCB1 increasing from 70.5 to 73.4 days after planting (Martin and Hallauer, 1980). Time to pollen shed also decreased in BSSS, falling from 79 to roughly 74 days after planting between cycle 0 and 17 (Edwards, 2011). Pollen shed and silk emergence timing converged around 73-74 days after planting in BSSS(R)C17, confirmed by Brekke et al. (2011b), who found that the anthesis-silking interval (ASI), the time between pollen shed and silk emergence, decreased from 2.5 days in BSSS to 0 days in BSSS(R)C17. Eichenberger et al. (2015) found that grain filling growth period increased between BSSS and BSSS(R)C17.

Changes in other BSSS recurrent selection programs. Although not examined in this dissertation, two equally-important recurrent selection programs have been initiated from BSSS.
The first of these is the BSSS(HT) program, which was initiated 10 years prior to the reciprocal recurrent selection program (Eberhart et al., 1973). The BSSS(HT) program was a half-sib selection program using the double-cross hybrid Ia13 as the tester (Eberhart et al., 1973). The second recurrent selection program was the BS13(HI) program, which is not an independent BSSS-based program, but rather a continuation of the BSSS(HT) program, with the BS13 population being developed from the intermating of 29 inbred lines developed from BSSS(HT)C7 (Edwards, 2010). The BS13(HI) was also engaged in a half-sib recurrent selection program with the inbred line B97 as a tester (Edwards, 2010).

Both the BSSS(HT) and BS13(HI) programs increased grain yield with selection. Grain yields in BSSS(HT)C7 were almost 1 Mg ha\(^{-1}\) higher than those of BSSS, while BS13(HI)C5 performed near BSSS(R)C17 levels at high density (Brekke et al., 2011a). Along with the rise in yield, BSSS(HT) and BS13(HI) saw many of the same unintentional responses to selection has the reciprocal recurrent selection program. Flag leaves were more upright and smaller in BSSS(HT)C7 compared to BSSS (Edwards, 2011) Tassel branch number and plant heights fell in both BS13(HI)C5 compared to BS13(S)C0 (Brekke et al., 2011b, Edwards, 2011). Stalk lodging and root lodging decreased in BSSS(HT)C7 compared to BSSS (Edwards, 2011).

**Importance of BSSS and BSCB1.** Several elite inbred lines have been developed from the BSSS/BSCB1 reciprocal recurrent selection program. The inbred lines B14 and B37 were developed from BSSS, while B97 and B99 were developed from BSCB1 cycles 9 and 10, respectively (Darrah and Zuber, 1986; Hagdorn et al., 2003). The famous inbred line B73 was derived from BSSS through the BSSS(HT) program, while the elite inbred B84 was derived from BSSS through the BS13 program (Darrah and Zuber, 1986; Hagdorn et al., 2003).
In 1980, 19% of the US commercial germplasm could trace some ancestry to BSSS (Lamkey et al., 1991). The percentages were higher in individual companies such as Pioneer Hi-Bred, which estimated that in the 1990’s, roughly 1 in 3 hybrids had BSSS in its ancestry (Smith et al., 2004).

**Grain Yield**

**Historic yield increase.** The average grain yield in the United States in the 1930’s was 1.5 Mg ha\(^{-1}\), and following 80 years of corn variety development through open-pollinated varieties, double-cross hybrids, and single-cross hybrids, grain yields are well over 12 Mg ha\(^{-1}\) today (Duvick, 2005b, Cooper et al., 2014). Average yield increases for US corn production have been calculated at 115 kg ha\(^{-1}\) year\(^{-1}\) between 1934 and 2004 (Duvick, 2005a). From the 115 kg ha\(^{-1}\) year\(^{-1}\) increase seen in grain yields, 65 to 75 kg can be attributed to genetic gains in commercial breeding programs (Duvick, 2005a).

**Agronomics.** Yield increases not attributed to genetic gain can be attributed to improved agronomic practices between the 1930’s and the present. Mechanization provided a large yield increase, standardizing planting timing and improving harvest efficiencies and allowing the timely removal of weeds and pests through large-scale tilling and chemical application (Cardwell, 1982). A dramatic increase in the use of synthetic fertilizers after World War II also helped facilitate large increases in grain yield. Application rates of nitrogen, potassium, and phosphorus in the Corn Belt increased for 40 years before plateauing in the 1980’s at 130, 60, and 80 lbs ac\(^{-1}\), respectively (Daberkow et al., 2000). One of the most influential agronomic practices was increasing planting density. As per-plant yields have remained virtually stagnant
over time, increasing density is necessary for increasing grain yield per unit of area (Duvick and Cassman, 1999).

**Plant Density**

**Historic increases in planting density.** Between the 1930’s and 2005, average plant densities for maize in the United States rose from 30,000 plants ha\(^{-1}\) to 80,000 plants ha\(^{-1}\) (Duvick, 2005a). Planting densities have continued to rise to the point that in 2014, one third of commercial planting densities in the United States and Canada were between 81,000 and 88,000 plants ha\(^{-1}\) (Butzen and Burnison, 2014). In Iowa, 68% of corn fields are planted above 81,000 plants ha\(^{-1}\) (Butzen and Burnison, 2014). While grain yield in maize has been increasing in a fairly linear fashion, planting densities did not rise in a consistent fashion. Planting densities increased approximately 333 plants ha\(^{-1}\) year\(^{-1}\) between 1930 and 1960 (Mansfield and Mumm, 2014). In 1960, when single-cross hybrids replaced double-cross hybrids, the planting density rate of increase accelerated to approximately 1000 plants ha\(^{-1}\) year\(^{-1}\) (Mansfield and Mumm, 2014). Between 1980 and 2012, the rate of increase slowed to approximately 625 plants ha\(^{-1}\) year\(^{-1}\) (Mansfield and Mumm, 2014).

In the BSSS/BSCB1 program, selection in cycle 0 was performed below 30,000 plants ha\(^{-1}\), while selection in cycle 17 was performed at 75,000 plants ha\(^{-1}\), closely matching commercial planting densities (Brekke et al., 2011a).

**Density effects on final phenotypes.** Increased planting density is known to affect the final phenotype of numerous traits in the plant. Ears are especially susceptible to high planting densities, and are often negatively affected. As planting densities increased from 50,000 to 100,000 plants ha\(^{-1}\), Edmeades and Daynard (1979) found that per plant yields fell from 134g to
36.2g, and kernel number per ear decreased from 640 to 473. As densities increased from 40,000 to 80,000 plants ha\(^{-1}\), ear lengths decreased by more than 3 cm (Rutger and Crowder, 1967). An increase in planting density from 30,000 to 120,000 plants ha\(^{-1}\) led to ears with almost 20 fewer kernels per kernel row (Hashemi-Dezfoulit and Herbert, 1992).

An increase in plant density not only affects the size of ears, but their number as well. As density increased from 66,000 to 100,000 plants ha\(^{-1}\), the number of ears per plant fell from 1.12 to 1.01, indicating a substantial loss of prolificacy (Jacobs and Pearson, 1991). When densities were increased from 50,000 to 150,000 plants ha\(^{-1}\), barrenness levels increased from 0% to 9.2% (Edmeades and Daynard, 1979).

Increases in density affect the vegetative and male structures of the plant as well. Tetio-Kahgo and Gardner (1988b) observed that increasing plant densities from 19,000 to 63,000 plants ha\(^{-1}\) decreased final stalk mass by over 20%. Tetio-Kagho and Gardner (1988a) and Brekke et al., (2011b) found that increasing plant densities led to an increase in plant height. Tetio-Kagho and Gardner (1988a) found that plant heights increased up to 90,000 plants ha\(^{-1}\), largely due to internode elongation. At densities higher than 90,000 plants ha\(^{-1}\), Tetio-Kagho and Gardner (1988b) observed that plant heights began to decrease. Increasing plant densities in BSSS from 35,000 to 75,000 plants ha\(^{-1}\) reduced the number of primary tassel branches, and decreased leaf number in BSSS and BSSS(R)C17 (Brekke et al., 2011b).

**Density effects on phenology.** Increased planting density can affect the timing of crucial developmental events in the growth of plants. Deynard *et al.* (1971) observed a 3 day delay in silking time when densities were increased from 44,700 to 124,300 plants ha\(^{-1}\). Edmeades and Daynard (1979) found that time to pollen shed was not affected by a density increase of 50,000 to 150,000 plants ha\(^{-1}\), while silking experienced a delay of almost 8 days, increasing ASI from 0
days to 6.5 days. When densities increased from 35,000 to 75,000 plants ha\(^{-1}\), ASI in BSSS increased from 2.5 days to 5 days (Brekke et al., 2011b). Observing four population crosses, including BSSS/BSCB1 and BSSS(R)C7/BSCB1(R)C7, Fakorede and Mock (1980) found that biomass accumulation in the plant, especially grain filling, ceased by 111 days after planting at 98,800 plants ha\(^{-1}\), while the biomass accumulation period extended well past 125 days after planting at 59,300 plants ha\(^{-1}\).

**Density effects on growth rates.** Increased planting density often negatively affects the ability of a plant to accumulate biomass. As densities increased from 90,000 to 120,000 plants ha\(^{-1}\), biomass accumulation in the ear decreased by 30\% in the 30 days surrounding flowering (Rossini et al., 2011). Echart et al. (2000) found that as densities increased from 50,000 to 120,000 plants ha\(^{-1}\), total biomass accumulation in the plant decreased by 30-50\%. Tetio-Kagho and Gardner (1988b), found that increasing plant densities decreased per-plant biomass accumulation rates for leaves and stems. Bos et al. (2000) observed that increased plant densities decreased the rates at which new leaves emerged during early plant growth. Fakorede and Mock (1980) found that growth rates in the stalk, ear, and grain were reduced as density increased from 59,300 to 98,800 plants ha\(^{-1}\). Per-plant kernel number and grain yields have been found to be highly correlated with plant biomass accumulation rates, and decreases in plant growth rates will lead to decreases in yield (Tollenaar et al., 1992).

**Density effects on biomass partitioning.** Increased planting density can affect how a plant partitions biomass between ears, stalks, and tassels; however, response is variable across genotypes. Li et al. (2015) found that as density increased from 15,000 to 180,000 plants ha\(^{-1}\), harvest index decreased from 0.65 to 0.4 in observed Chinese commercial germplasm. Cox
(1996) and Rossini et al. (2011) found that harvest index remained unchanged with increasing densities.

Ciampitti et al. (2013) found that density affected nutrient partitioning in plants. As density increased from 54,000 to 104,000 plants ha$^{-1}$, there was a decrease in the proportion of potassium, phosphorus, and sulfur partitioned to the ear (Ciampitti et al. 2013).

**Apical dominance.** Apical dominance is one of the most influential forces on a maize plant, transforming it from the bushy teosinte to the single-stalked grain familiar today (Doebley et al., 1995). Apical dominance in maize is controlled by the *teosinte branched1* (*tb1*) pathway, and a recessive mutation in *tb1*, or other loci located downstream in the gene pathway such as *grassy tillers1* (*gt1*), will produce a bushy plant resembling teosinte (Doebley et al., 1997; Hubbard et al., 2002; Whipple et al., 2011).

The intensity of an apical response is controlled by the balance between three main hormones, Indole-3 acetic acid (IAA), cytokinin, and strigolactones (Domagalska and Leyser, 2011). In apical responses, IAA and strigolactone inhibit axillary branching, while cytokinin promotes apical branching (Thimann and Skoog, 1933; Shimizu-Sato et al., 2009; Gomez-Roldan et al., 2008; Umehara et al., 2008). Due to the one-way movement of auxin inside a plant, cytokinin and strigolactone must be used as intermediaries to gain access to axillary buds in what is known as the second messenger model (Booker et al., 2003). Increased production of IAA by the plant inhibits production of cytokinin and promotes production of strigolactone, which can be transported to axillary buds (Shimizu-Sato et al., 2009; Gomez-Roldan et al., 2008; Umehara et al., 2008). In order for axillary buds to successfully initiate growth, sufficient levels of cytokinin must enter the bud to create a favorable cytokinin/IAA ratio (Cline, 1991; Tanaka et al., 2006). At the same time that cytokinin is entering the axillary bud, IAA is exiting via
efficient auxin transport pathways equipped with PIN proteins (Li and Bangerth, 1999; Balla et al., 2011). Strigolactone has been shown to destroy PIN proteins in axillary shoot buds, which can lead to inefficient removal of IAA, inhibiting growth (Crawford et al., 2010).

The presence or absence of apical dominance is genetically controlled in maize; however, environmental conditions can also affect the intensity of apical responses. Light, specifically the ratio of red to far-red light, is one of the major ways a plant can detect a high population density (Bellare et al., 1990). When plants are in a dense planting arrangement, the amount of high-quality red light from the sun is decreased by shading, while levels of far-red light, reflected from neighboring plants, increase (Bellare et al., 1990). Far red light is essential in initiating an increased apical response via detection by phytochrome B (PHYB) in plants (Kebrom et al., 2006). Increased levels of far-red light have been shown to increase expression levels of \textit{tb1} and \textit{gt1}, a gene in the \textit{tb1} pathway, in maize, teosinte, and sorghum (Whipple et al., 2011; Kebrom and Brutnell, 2015). Kebrom and Brutnell (2015) found that maize, represented by the field corn inbred B73 and the high-tillering sweet corn inbred P39, had \textit{gt1} and \textit{tb1} expression levels that did not change with increased far-red light; however, P39 did have a marked increase in expression levels of \textit{ZmDRM1}, which promotes axillary bud dormancy, as far-red light increased. High amounts of red light are known to decrease IAA concentrations inside maize seedlings (Fellner et al., 2006).

Maize seedlings exposed to far-red light exhibited behaviors associated with shade avoidance. Cell elongation and plant height increased with high levels of far-red light and in darkness (Fellner et al., 2006; Kebrom and Brutnell, 2015). At increased plant densities, maize axillary structures, tillers and ears, have both a drop in number per plant, and a decrease in size (Tetio-Kagho and Gardner, 1988a,b).
Adaptation to stress

**Optimal planting density.** Corn yields follow a negative parabolic curve with increasing plant densities (Prior and Russell, 1975). As plant densities increase, yield per plant decreases to a point where adding more plants no longer adds any further yield to a unit of area. Optimal planting density rose in both *per se* populations, and in the population cross in the BSSS/BSCB1 program. Between cycles 0 and 17, optimal planting densities rose from 58,000 to 77,000 plants ha\(^{-1}\) in BSSS, from 59,000 to 75,000 plants ha\(^{-1}\) in BSCB1, and from 59,000 to 81,000 plants ha\(^{-1}\) in the population cross (Edwards, 2016). An increase in planting density is necessary in maintaining grain yield increases in BSSS. Brekke *et al.* (2011a) found that while there was a 2% gain per cycle when plants were grown at 75,000 plants ha\(^{-1}\), there was a 1% grain yield loss per cycle when plants were grown at 35,000 plants ha\(^{-1}\).

**Morphological adaptations to density.** As commercial hybrids have been selected for high yields, unintentional morphological changes such as more upright leaves, shorter stature, and smaller tassels have been detected (Duvick, 2005b). These changes were also found in the BSSS/BSCB1 recurrent selection program (Brekke *et al.*, 2011b; Edwards, 2011). Upright leaf angles have been found to increase leaf area index and photosynthesis levels (Hammer *et al.*, 2009). Tetio-Kagho and Gardner (1988a) found that lower amounts of sunlight reached the lower canopy at increased plant densities, with 50% of sunlight being available at ear level at 19,000 plants ha\(^{-1}\) compared to 10% at 63,000 plants ha\(^{-1}\). At high planting densities, a more upright leaf angle will allow light to penetrate farther into the canopy (Williams *et al.*, 1968). As 50% sunlight intensity is enough to produce 80% of maximum photosynthesis rates, even a moderate amount of additional light entering the lower canopy will greatly increase
photosynthetic production in the plant (Mock and Pierce, 1975). Leaf area index, the amount of leaf area per unit of ground area, increased with selection between BSSS/BSCB1 and BSSS(R)C7/BSCB1(R)C7 population crosses (Fakorede and Mock, 1980). With more upright leaves, a higher leaf area index greatly increases the photosynthetic capacity of the plant canopy per unit area (Duncan, 1971).

A smaller tassel is one of the most recognizable traits accompanying selection for grain yield (Duvick, 2005b; Edwards, 2011). Smaller tassels are found to block less light from reaching the canopy (Duncan et al., 1967; Mickelson et al., 2002). Duncan et al., (1967) found that shading effects of tassels can decrease photosynthetic rates by as much as 19%. Sangoi and Salvador (1996) and Hammer et al. (2009) also hypothesized that small tassels benefited grain yield by providing a smaller competitive sink, allowing biomass to be partitioned to other parts of the plant, namely ears.

**Decreased response to environmental stimuli.** Modern commercial cultivars have been found to be less responsive to density-induced environmental conditions. Fellner et al., (2006), studying commercial hybrids from the 1930’s, 1960’s, and 1990’s found that modern hybrids were less responsive to growth in darkness than older hybrids. The modern hybrid had shorter coleoptiles and mesocotyls than older hybrids, and did not experience cellular depolarization when exposed to light (Fellner et al., 2006). Seyedin et al. (1980) found that commercial hybrids that were adapted to growth at high densities did not produce lower levels of IAA than hybrids that were density-sensitive. Fellner et al. (2006) found that modern hybrids did not see increases in mesocotyl length with externally applied auxins, while older hybrids did. As such, a lack of response to environmental stimuli is two-fold, a lack of recognition of poor light conditions, and a lack of response to internally-produced hormones.
Adaptation to density stress in final phenotypes. Modern maize varieties maintained higher kernel counts and larger ear masses at high planting densities than older varieties (Echarte et al., 2000; Sangoi et al., 2002). Sangoi et al. (2002) found that hybrids from the 1990’s maintained 0% barrenness from 25,000 to 100,000 plants ha⁻¹, while older hybrids had barrenness rates between 8 and 10% at high density. Sangoi et al. (2002) also found that older maize hybrids had stalk lodging levels that greatly increased with increasing density, while the hybrid from the 1990’s maintained a stalk lodging level near 0% across densities.

Adaptation to density stress in phenology. Modern maize varieties maintain higher growth rates and more consistent growth timing at high densities. Brekke et al. (2011b) found that while ASI increased by 2.5 days in BSSS between 35,000 and 75,000 plants ha⁻¹, ASI remained at 0 in BSSS(R)C17 across densities. Sangoi et al. (2002) also found that modern maize varieties maintained a lower ASI at 100,000 plants ha⁻¹ compared to older varieties.

Eichenberger et al. (2015) found that grain filling periods in BSSS increased with selection. Mock and Pierce (1975) postulated that high-yielding maize varieties should have the longest grain filling period possible, allowing plants to accumulate the maximum levels of biomass while still reaching physiological maturity before a killing frost.

Adaptation to abiotic stresses. Multiple abiotic stresses, from increased drought to nutrient deficiencies can affect the growth of a plant. Edmeades et al. (1993) observed that mild drought stress can delay silking dates, increase anthesis-silking intervals, and decrease plant growth rates at levels similar to high planting densities. Cooper et al. (2014) found that as time progressed, hybrid individuals had higher yields under drought stress than older hybrids. Drought tolerant hybrids were found to pull less water from the soil during peak growth periods,
and maintained rapid biomass accumulation in both the ear and the whole plant under water-stressed periods (Cooper et al., 2014).

**Plant growth modeling**

**Modeling of plant growth with logistic curves.** Multiple groups in the past have attempted to model plant growth throughout the entire growing season. The main method utilized has been to break the growing season into a series of pre-determined time points and simply model growth linearly between them. These time points have been days after planting as seen in Tetio-Kagho and Gardner (1988a,b) and Fakorede and Mock (1980), or plant vegetative and reproductive stages as seen in Rossini et al. (2011).

In biology, logistic curves are often well-suited to modeling growth, both for populations and, for the purposes of this study, individuals (Pinheiro and Bates, 2000). Logistic curves have successfully been used to model plant growth and biomass accumulation in other grass species, including durum wheat and rice (Villegas et al., 2001; Yu et al., 2002). Eichenberger et al. (2015) was able to use a general logistic curve to model grain filling in BSSS and BSSS(R)C17. The parameters of a three-parameter logistic curve are helpful in determining final phenotypes, the time point for 50% final phenotype, and the period of time required to move between 50% and approximately 73% final phenotype, enabling researchers to easily identify changes in phenology (Pinheiro and Bates, 2000).
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CHAPTER III:

EFFECTS OF PLANT DENSITY ON PLANT GROWTH BEFORE AND AFTER RECURRENT SELECTION IN MAIZE

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Abstract

Breeding for higher grain yield in maize (Zea mays L.), utilizing increased selection densities, has produced varieties that are adapted to grow at higher population densities. While the effects of density on final grain yield and plant phenotypes are well known, how density affects the early-season growth of the plant has been less studied. The objective of this experiment was to examine the effects of high planting density on the growth of stalk, tassel, and ear shoots in unselected Iowa Stiff Stalk Synthetic (BSSS) and Iowa Synthetic Corn Borer No. 1 (BSCB1), and in populations that have been selected for high grain yield via a reciprocal recurrent selection program. The selected and unselected populations were planted at four densities ranging from 3.23 to 12.92 plants m⁻² in six environments near Ames, IA over three years. Increased planting density affected plant organ growth timing differently in BSSS and BSCB1. High density delayed stalk biomass accumulation in BSCB1 but not BSSS, and delayed ear shoot and tassel biomass accumulation in BSSS but not BSCB1. Differences in biomass accumulation and partitioning between unselected populations were generally not observed between selected populations suggesting that selection caused a convergence in growth patterns between the populations. Increased density lowered maximum growth rates for all plant organs, but reduction in growth rate due to density occurred at higher density in selected populations than unselected populations.
Introduction

Iowa Stiff Stalk Synthetic (BSSS) and Iowa Synthetic Corn Borer #1 (BSCB1) have undergone 17 cycles of reciprocal recurrent selection. Selection was based primarily on a heritability index for increased grain yield, decreased stalk and root lodging, and decreased grain moisture, with primary emphasis on grain yield (Edwards, 2011). The reciprocal selection program was successful in increasing grain yield, decreasing stalk lodging, and decreasing grain moisture, but not in decreasing root lodging (Edwards, 2011). Grain yield increased both in the per se populations, and in the population cross. Keeratinijakal and Lamkey (1993) found an increase in grain yield of nearly 7% per cycle in the population cross over the first 11 cycles. Brekke et al. (2011a) found a 2% increase in grain yield per cycle between BSSS and BSSS(R)C17. The reduced improvement in the per se populations is largely due to inbreeding depression from genetic drift because of recombination of a finite number of lines in each cycle of selection without introduction of new germplasm (Keeratinijakal and Lamkey, 1993). Gerke et al. (2015) found that levels of heterozygosity in the populations fell drastically with selection, with BSSS and BSCB1 losing roughly 40% of their heterozygosity between cycles 4 and 16.

The secondary selection goals of reduced stalk lodging and grain moisture were also achieved, with stalk lodging decreasing from 8% in BSSS to 2% in BSSS(R)C17, and grain moisture decreasing from 25% in BSSS to 21% in BSSS(R)C17 (Edwards, 2011). Decreased root lodging was not achieved through selection, with a roughly 4% root lodging rate in all BSSS cycles (Edwards, 2011).

In addition to direct responses to selection, there were many indirect responses to selection in the BSSS/BSCB1 recurrent selection program, in particular with respect to plant architecture and flowering times. As selection progressed, flag leaves became more upright and
smaller, the number of primary tassel branches drastically decreased, plant and ear height decreased, leaf number decreased, and the node of primary ear attachment decreased in BSSS(R)C17 compared to BSSS (Edwards, 2011). Edwards also found that BSSS(R)C17 had fewer days to 50% silking and pollen shed, and the anthesis-silking interval (ASI), the time between pollen shed and silk emergence, decreased from 2 to 0 days. Fakorede and Mock (1980) found that in the second half of the growing season, the BSSS(R)C7/BSCB1(R)C7 population cross had significantly more leaf area per plant, and a larger leaf area index throughout the entire growing season than the BSSS/BSCB1 population cross. These changes with selection agree with results in commercial hybrid germplasm reviewed by Duvick (2005b), who noted that breeding for higher yields has either directly or indirectly lead to a variety of changes to plants including reduced plant height, more upright leaves, smaller tassels, fewer tillers, earlier silking, a smaller ASI, and a small increase in harvest index. Both Fakorede and Mock (1980) and Eichenberger et al. (2015) noted longer growth periods after selection. Fakorede and Mock (1980) found that while ear and grain biomass accumulation tended to level off around 111 days after planting in the BSSS/BSCB1 cross, biomass accumulation was still rapidly occurring at 125 days after planting in BSSS(R)C7/BSCB1(R)C7. Eichenberger et al. (2015) found that the biomass accumulation periods in kernels was larger in BSSS(R)C17 than in BSSS.

In the 1930’s, the commercial planting density of corn was 30,000 plants ha⁻¹, and that number has risen to 80,000 plants ha⁻¹ in the modern era (Duvick 2005a). Selection densities in the BSSS/BSCB1 reciprocal recurrent selection program have closely matched those of commercial agriculture, increasing from 30,000 plants ha⁻¹ in cycle 0 to 75,000 plants ha⁻¹ in cycle 17 (Brekke et al., 2011a; Mansfield and Mumm, 2014). As per-plant yields have barely
increased with time, this increase in planting density was crucial in raising corn yields from 1.5 Mg ha\(^{-1}\) in 1930 to well over 10 Mg ha\(^{-1}\) today (Duvick and Cassman, 1999; Duvick, 2005b; Cooper et al., 2014). Brekke et al. (2011a) found that when plants were grown at 75,000 plants ha\(^{-1}\), yield gains were 2\% per cycle in BSSS, but when grown at 35,000 plants ha\(^{-1}\), there was actually a 1\% loss in yield per cycle. However, when plants were subjected to higher densities, growth was often affected negatively. Brekke et al. (2011b) found that as planting densities increased from 35,000 to 75,000 plants m\(^{-2}\) in BSSS, tassel sizes decreased, leaf number decreased, and ASI was increased by 2.5 days. Brekke et al. (2011b) also found that final plant heights in BSSS(R)C17 were affected by density, with plants becoming taller as density increased. Eichenberger et al. (2015) found that final kernel weights were lower at 77,000 plants ha\(^{-1}\) than at 53,000 plants ha\(^{-1}\) in BSSS(R)C17. Fakorede and Mock (1980), studying BSSS/BSCB1 and BSSS(R)C7/BSCB1(R)C7 population crosses, along with two hybrids involving B14A, a line developed from BSSS, found that as densities increased from 59,300 to 98,800 plants ha\(^{-1}\), biomass growth rates and carbon assimilation rates decreased during the second half of the growing season, while leaf area and leaf area index were decreased throughout the whole growing season. Fakorede and Mock (1980) also found that as density increased, the masses of plant organs drastically decreased, with high density ears being roughly 100 grams lighter, and stalks and leaves being 10 grams lighter each, with a total plant biomass decrease of roughly 120 grams. Outside of the BSSS and BSCB1 populations, there is a plethora of evidence that high density negatively affects maize traits. After doubling population densities from 40,000 to 80,000 plants ha\(^{-1}\), Rutger and Crowder (1967) found that ears were 3cm shorter. Edmeades and Daynard (1979a) found that grain yield per plant decreased by almost 100 grams when planting densities increased from 50,000 to 100,000 plants ha\(^{-1}\). Hashemi-Dezfoulit and
Herbert (1992) found that quadrupling planting density from 30,000 to 120,000 plants ha\(^{-1}\) produced ears that had 20 fewer kernels per kernel row. Tetio-Kagho and Gardner (1988b) found that increasing densities from 19,000 to 63,000 plants ha\(^{-1}\) decreased final stalk mass by 20%. Echarte et al. (2000) also found that as planting densities increased from 50,000 to 120,000 plants ha\(^{-1}\), plant biomass accumulation fell by approximately 30-50%. Rossini et al. (2011), observed that as densities increased from 90,000 to 120,000 plants ha\(^{-1}\), the biomass accumulation rates for ear shoots fell by almost 30% in the 30 days surrounding flowering.

Corn varieties that have been selected for yield also show a marked ability to better perform under high plant densities. Brekke et al. (2011b) found that while stalk lodging rates and ASI increased in BSSS with increasing densities, there was no increase in BSSS(R)C17. Brekke et al. (2011b) also noted that while tassel branch number decreased with density in BSSS, there was no decrease in BSSS(R)C17. Prior and Russell (1975) showed that per-area yields versus planting density followed a negative parabolic shape, meaning that if density is continually increased, yields will hit a maximum, and then will begin to decrease, and that the density required to reach maximum yields varied among genotypes. Increased tolerance to high density allows modern maize varieties to reach maximum yields at much higher densities than older varieties (Sangoi, 2002). Brekke et al. (2011a) found that while BSSS reaches its maximum yield at roughly 50,000 plants ha\(^{-1}\), the maximum yield for BSSS(R)C17 is higher than 75,000 plants ha\(^{-1}\). Outside of the BSSS and BSCB1 populations, it has been found that newer varieties of maize better maintain ear phenotypes at high densities. When subjected to high densities, modern varieties maintained higher kernel counts and larger ear masses than older varieties (Echarte et al., 2000; Sangoi et al., 2002). Ear development is also greatly improved in
modern maize varieties, with decreases in the level of barrenness, earlier silking, and a smaller ASI at high densities (Tollenaar et al., 1992; Sangoi et al., 2002).

The populations used in this study, BSSS and BSCB1, have produced several elite inbreds including B14, B37, B73, B84, and B97 (Darrah and Zuber, 1986; Hagdorn et al., 2003). The elite inbreds produced from BSSS and BSCB1 have helped form the foundations of the Stiff Stalk and Non-Stiff Stalk heterotic groups and have become widespread throughout US commercial maize production, with nearly one in five hybrids produced in 1980 having BSSS at some point in its ancestry (Lamkey et al., 1991). In the 1990’s, the germplasm of individual companies contained as many as one in three hybrids that could trace some ancestry back to BSSS (Smith et al., 2004).

The objective of this experiment was to determine if reciprocal recurrent selection for grain yield changed how plant density affects rate and timing of biomass accumulation and partitioning in the stalk, ear, and tassel in populations per se.

Materials and Methods

Populations

Four synthetic populations representing two distinct levels of selection from the BSSS/BSCB1 reciprocal recurrent selection program were used in this experiment. Iowa Stiff Stalk Synthetic (BSSS) is a synthetic population developed in 1934 from the intermating of 16 inbred lines selected for high stalk quality (Sprague, 1946). Iowa Synthetic Corn Borer No. 1 (BSCB1) is a synthetic population developed in the 1940’s from the intermating of 12 inbred lines selected for resistance to the European corn borer (Penny and Eberhart, 1971). BSSS(R)C17 and BSCB1(R)C17 are the seventeenth cycles of a reciprocal recurrent selection.
program with the opposing population used as the tester. The populations were developed following 10 cycles of half-sib selection and 7 cycles of full-sib selection. Selections was based on a heritability index of increased yield, lower rates of stalk and root lodging, and reduced grain moisture levels (Edwards 2011).

**Experimental Design**

Each of the four populations were grown at four planting densities, 3.23, 6.46, 9.69 and 12.92 plants m$^{-2}$ (32,300; 64,600; 96,900 and 129,200 plants ha$^{-1}$). A precision SRES air planter was used to ensure proper densities and seed placements. The experiment was a split-plot design, with planting density as the whole plot factor and population as the split-plot factor. The experiment was grown at two locations near Ames, IA in the summers of 2012, 2013, and 2014, with a total of six different locations utilized in this study. Soils in the fields consisted largely of Clarion loam, Canisto clay loam, and Webster clay loam. All population by density combinations were replicated nine times at each location in 2012, and six times in 2013 and 2014. Subplots consisted of either three (2012) or four (2013 & 2014) rows 5.49 m long spaced 0.76 m apart. Temperatures in 2012 were above-average, with a severe drought occurring throughout the growing season. Temperatures were mild during the 2013 and 2014 seasons, and rainfall was heavy early in the season for both years, although the 2013 season was fairly dry July through September. Plots were grown without irrigation, using standard corn production practices for central Iowa. Due to a destructive hail storm in 2013, measurements could only be taken at one of the two locations. In the 2014 season, the 9.69 plants m$^{-2}$ density was not grown.
Data Collection

All data was collected on plants in the center rows of each plot, and plants at the ends of
plots were not tested due to decreased competition. The fifth and eleventh leaves were painted to
determine leaf number. Leaves were painted when they were sufficiently elongated and prior to
senescence of the first leaf when painting leaf 5 and prior to senescence of leaf 5 when painting
leaf 11. Plant height and leaf number measurements began when the plants had seven to eight
leaf collars visible. Plant heights were measured to the nearest centimeter from the ground to the
highest leaf collar. The leaf number value was the number of the highest leaf with a visible leaf
collar. Height and leaf measurements were taken weekly on the same plants throughout the
growing season, ending at anthesis. Beginning when eight or nine leaf collars were visible,whole plants were harvested from one or two randomly chosen replicates per field twice per
week, and continued several weeks after flowering when kernels were at the milk or dough stage
(Abendroth et al. 2011). A plant from each density by population combination was harvested
during each session. Plants were cut at ground level, including above-ground portions of brace
roots. The plant was divided into three parts. The ear portion consisted of the primary ear,
shank, and husks. The tassel portion consisted of all biomass above the final leaf node. The
stalk portion consisted of all remaining plant matter, including non-silked lower ear shoots, but
excluding tillers. Inflorescences were removed from the husks of the primary ear shoot under a
dissecting microscope. The length of ears from the tip of the ear to the attachment point with the
shank was measured using digital calipers. The three biomass components were dried for seven
days at 60°C (140°F), and were weighed using an electronic balance. Final ear length and
biomass were measured on mature ears at the end of the growing season. Mature ears were only
harvested from plants with neighboring plants to maintain density stress throughout the whole growing season.

Ear shoot length, leaf number, and plant heights were recorded during the 2012 and 2013 growing seasons, while biomass was recorded during the 2013 and 2014 growing seasons.

**Data Analysis**

Ear shoot length, plant height, and biomass were modeled with a three-parameter logistic curve (Pinheiro and Bates, 2000).

\[
y(ijkl)_{mt} = \frac{\phi_1_{ijkl} - G_t(ijkl) - \phi_2_{ijkl}}{1 + e^{-\phi_3_{ijkl}}} + \varepsilon(ijkl)_{mt}
\]

Observed data is represented as \(y(ijkl)_{mt}\) for plant \(m\) at environment \(i\) in replicate \(j\) in population \(k\) at density \(l\) at time point \(t\). The logistic parameters were \(\Phi_{ijkl}\) for the asymptote, \(\Phi_{2ijkl}\) for the inflection point, and \(\Phi_{3ijkl}\) for the scale. The residual error is represented as \(\varepsilon(ijkl)_{mt}\), and \(G_t(ijkl)\) is thermal time in growing degree days centigrade after planting. In a logistic curve, the asymptote refers to the final phenotype of the trait, with a larger asymptote implying a larger final phenotype. The inflection point is the value in thermal units for a trait to reach 50% final phenotype, with larger inflection points indicating a later developmental midpoint, and possibly a delay in development. The scale parameter is the time in thermal units necessary for the trait to move between 50% and ~73% final phenotype, with larger scale values indicating a longer developmental period.

Ear length, plant height, and the biomass logistic model parameters were modeled with a mixed linear model to account for design and treatment effects:

\[
\Phi(ijkl)_{pm} = \mu_p + \lambda_{pi} + \rho_{pj} + \tau_{pk} + \beta_{pk} + (\tau \beta \lambda)_{hijkl} + (\tau \beta \rho)_{ijijkl} + \delta_{pm(ijkl)}
\]
Parameter $\Phi_{ijklpm}$ was parameter $p$ in the logistic model, $\mu_p$ was the mean, $\lambda_{pl}$ was the effect of environment $i$ ($i=1..5$), $\rho_{ijpl}$ was the effect of replicate block $j$ ($j=1..9$ in 2012, $j=1..6$ in 2013 and 2014) nested within environment $i$, $\tau_{pl}$ was the effect of density $l$ ($l=1..4$ in 2012 and 2013, $l=1..3$ in 2014), $\beta_{pk}$ was the effect of population $k$ ($k=1..4$), $\tau_{\beta pkl}$ was the interaction between density $l$ and population $k$, $\tau_{\beta \lambda pkl}$ was the interaction of density $l$, population $k$, and environment $i$, $\tau_{\beta \rho ijkl}$ was the interaction of density $l$, population $k$, and replicate block $j$ which was the whole plot error term in the split plot design (Error A), and $\delta_{pm(ijkl)}$ was residual error. Environment, density, population, and density by population interactions were treated as fixed effects, while replicates and all replicate and environmental interactions were treated as random effects. The logistic models were fit using the nlme package in R. All random effects were added to the model, and effects with the smallest variance were removed one at a time. The final model was chosen as the model with smallest Bayesian Information Criterion (BIC). Values of the logistic parameters were estimated with maximum likelihood. Pairwise t-tests were performed to compare parameter values across densities and populations. The t-tests were performed in R, with variances estimated via restricted maximum likelihood.

Leaf number was fitted with a linear model:

$$y_{ijklm} = \pi_0 + \pi_1 x_{ijkl} + \pi_2 x^2_{ijkl} + \pi_3 w_{ijkl} + \pi_4 w^2_{ijkl} + \pi_5 x_{ijkl} w_{ijkl} + \pi_6 x^2_{ijkl} w_{ijkl} + \pi_7 x_{ijkl} w^2_{ijkl} + \pi_8 \gamma_{ijkl} + \pi_9 \delta_{pm(ijkl)} + \pi_{10} \epsilon_{ijklm}$$

$Y_{ijklm}$ represents leaf number, $\pi_0$ represents the y-intercept, and $\epsilon_{ijklm}$ represents the residual error. The subscripts $i, j, k, l,$ and $m$ all hold the same meanings as their previous uses, while the subscript $t$ represents the time point. The model contains two covariates: $x_{ijkl}$ for time measured...
in growing degree days, and \( w_{ijkl} \) for density measured in plants m\(^{-2}\). The time and density coefficients were associated with main effects, marked as \( \pi_i \) through \( \pi_8 \). The fixed effects of the model were \( \lambda_i \) representing environment, and \( \beta_k \) representing population. Combinations of symbols represented interactions between effects. Environment-specific effects of time were represented by \( \eta_i \) for the linear and \( \delta_i \) for the quadratic, while environment-specific effects of density were represented by \( \alpha_i \) for the linear and \( \chi_i \) for the quadratic. Population-specific effects of time were represented by \( \gamma_k \) for the linear and \( \nu_k \) for the quadratic, while population-specific effects of density were represented by \( \tau_k \) for the linear and \( \psi_k \) for the quadratic. Population-specific effects of time by environment were represented by \( \alpha_k, \eta_k, \varphi_k, \) and \( \kappa_k \). Environment-specific and population-specific interactions with time and density were considered fixed. Random effects were \( \rho_{ij} \) representing replicate, \( \rho \beta_{ij} \) as replicate by population interactions, \( w_{ijkl} \theta_{ij} \) as replicate-specific effects of density, \( w_{ijkl} \zeta_{ik} \) as the population by environment-specific effects of density, and \( w_{ijkl} \xi_{ij(ij)k} \) as the population by replicate-specific effects of density.

The maximum rate of increase in a logistic curve occurs at the inflection point, the midpoint of the curve on the X-axis. The maximum rate of growth was estimated by taking the first derivative of the logistic curve at the inflection point, which is 0.25 times the asymptote value divided by the scale value. A lower scale value will lead to a higher maximum growth rate, since a faster growth rate is needed to reach the same phenotype with a shorter developmental period. A smaller maximum growth rate will also produce a lower final phenotype, as the plant will not be able to grow to the full potential in the same developmental period. The maximum growth rate values were the fit to a linear additive model comprised of the significant variables from the logistic curve: \( Y_{ijkl} = \mu + \lambda_i + \pi_i + \beta_k + \tau \beta_{kl} + \tau \beta \lambda_{kl} + \epsilon_{ijkl} \), where \( Y_{ijkl} \)
represents ear shoot length increase, \( \mu \) is the mean, \( \lambda_i \) is the effect of environment \( i \), \( \tau_i \) is the effect of density \( j \), \( \beta_k \) is the effect of population \( k \), \( \tau\beta_{kl} \) is the effect of the population by density interaction, \( \tau\beta\lambda_{ikl} \) is the effect of the population by density by environment interaction, \( \epsilon_{ikl} \) and \( \epsilon_{ik} \) represents error. Environment, population, density, and the population by density interaction were treated as fixed, while the population by density by environment was treated as a random effect. The model for ear biomass maximum growth rates was 
\[
Y_{(kl)} = \mu + \lambda_i + \tau_j + \beta_k + \tau\beta_{kl} + \tau\beta\lambda_{ikl} + \epsilon_{ikl},
\]
with all variables the same as above. The models for stalk and tassel biomass growth rates were 
\[
Y_{(kl)} = \mu + \lambda_i + \tau_j + \beta_k + \epsilon_{ikl},
\]
as neither model had a significant population by density by environment interaction. The models were analyzed in SAS using the proc mixed procedure with variances estimated via restricted maximum likelihood.

**Results and Discussion**

Increasing density reduced maximum growth rates for ear length, ear biomass, stalk biomass, and tassel biomass in all four populations (Fig. 1, Table 5). Plant height maximum growth rates continuously decreased with increasing density for BSSS(R)C17 and BSCB1(R)C17, and decreased sharply from 3.23 to 9.69 plants m\(^{-2}\), before rebounding between 9.69 and 12.92 plants m\(^{-2}\) in BSSS and BSCB1 (Fig. 1, Table 5). Along with decreased growth rates, high density also led to decreased final phenotypes for most measured traits (Tables 1 & 2). Final ear lengths were roughly 20-30\% lower and final ear biomass was roughly 45-60\% lower was at 12.92 plants m\(^{-2}\) compared to 3.23 plants m\(^{-2}\) in all populations (Fig. 2, Table 2). Tassel biomass decreased by 45-50\% with density in BSCB1 and BSSS(R)C17 (Fig. 2, Table 2). Stalk biomass decreased with density in all populations, save BSCB1, with decreases being around 40-55\% (Fig. 2, Table 2). Plant heights decreased with density in only the BSCB1
population, with a reduction of about 8% between low and high density plants (Table 2). Reductions were verified statistically by pairwise t-tests on logistic parameters between high and low density and all comparisons were significant at $P<0.05$. The pairwise approach was used to provide a robust and straightforward statistical test for population-specific density effects on individual logistic parameters.

The three biomass traits, ear, plant, and tassel, were summed to obtain total above ground biomass, which was greatly reduced by increasing plant density (Fig. 3). Similar to our results, Edmeades and Daynard (1979a,b), observed that grain and stalk mass decreased when densities increased. Edmeades and Daynard (1979a,b) attributed much of this decrease to shading caused by competing neighbors. Deng et al. (2012) formalized the effect of light competition on total biomass per unit area, and showed that biomass per unit area increases linearly with plant density up to a point of maximum biomass per unit area. The model predicted a constant slope of logarithm of biomass per unit area versus logarithm of plant density for any crop species, a prediction supported empirically by data from several crop species (Deng et al., 2012). We compared the rate of increase of total above-ground biomass in our study to that of Deng et al. (2012). While we found a relatively large increase in total biomass per unit area with increasing plant density, it was generally less than predicted by Deng et al. (2012) (Fig. 4). Differences in slope of total-biomass response to density in Figure 4 may also be an indication of differential responses among our populations to resources other than light with increasing plant density. The lower rates of biomass accumulation in our study compared to results in Deng et al. (2012) were very likely due to limitations in resources other than light, since we did not take steps to ensure that water, nutrients or other resources were not limiting. Edmeades et al. (1993) observed that plants under mild drought stress perform similarly to those grown at high densities, showing
decreased biomass accumulation rates and delayed silking. As 2013 was fairly dry for the second half of the growing season, a mix of density and drought stress may have affected the plants, causing a deviation from Deng’s predicted biomass levels.

Significant decreases in harvest indices have been found to accompany increases in density in corn (DeLoughery and Crookston, 1979). While we did not study harvest index per se, we did study total ear biomass, and are able to compare it with the total above-ground biomass, which is very closely related to harvest index. BSSS(R)C17 had a higher proportion of final biomass partitioned to the ear at all three densities, suggesting that harvest index improved with selection in the BSSS population (Fig. 5). In the BSCB1 population, increasing plant density greatly reduced the proportion of biomass partitioned to the ear, whereas after selection, the proportion of biomass in the ear was relatively stable across densities (Fig. 5). As such, biomass partitioning to the ear appeared to improve in BSCB1(R)C17 primarily at high density. Density response for the proportion of biomass partitioned to ear (Fig. 5) was quite different for the cycle zero populations (BSSS and BSCB1) in contrast to the absolute ear biomass per plant versus plant density, which was quite consistent for the two cycle zero populations (Fig. 2).

When maximum growth rates are examined, BSCB1 does not exhibit a strong decrease in stalk growth rates between 6.46 and 12.92 plants m⁻², while the growth rate of the ear continues to decrease in a linear fashion, indicating that the difference in density response on a proportional basis was due to the fact that, while BSCB1 accumulated more total biomass at high plant density than the other three populations, it partitioned less to ear while maintaining biomass accumulation in the stalk (Fig. 1 & 4). Overall, BSSS accumulated less total biomass than BSCB1 at high plant density, but maintained partitioning to the ear comparable with other
organs. However, as mentioned previously, after selection both populations maintained relatively constant partitioning of biomass to the ear (Fig. 5).

In addition to growth rate and final phenotype, plant densities had a notable effect on the timing of biomass accumulation among plant, ear, and tassel. As planting densities increased from 3.23 to 12.92 plants m\(^{-2}\) in BSSS, the ear shoot and the tassel reached 50% biomass approximately 90 and 50 growing degree days later, respectively, and the biomass accumulation period increased for the ear shoot (Fig. 7 & 8, Tables 3 & 4). As planting densities increased in BSCB1, there was no change in the time point for 50% ear and tassel biomass, but the stalk reached 50% biomass 70 growing degree days later, and had a marginally increased (p=0.078) biomass accumulation period at high densities (Fig. 7 & 8, Tables 3 & 4). Differences in stalk growth characteristics between BSSS and BSCB1 can also be seen in the stalk maximum growth rates. Both BSSS and BSSS(R)C17 had very small reductions in stalk biomass maximum growth rates, 8.6% and 3.8% respectively between 3.23 and 6.46 plants m\(^{-2}\), compared to 37-42% found in BSCB1 and BSCB1(R)C17 (Fig. 2, Table 5). Across densities, BSSS produced leaves at a higher rate than the other three populations (Table 6). The difference in timing for organ biomass accumulation between BSSS and BSCB1 may be influenced by the lines used to create the populations. The lines used to create BSSS were chosen for superior stalk strength (Sprague, 1946). Because of this, BSSS plants may prioritize stalk growth to maintain stalk integrity. BSCB1, which was not selected based on stalk quality, experiences a large decrease in stalk growth rate at moderate densities, comparable to the decrease in ear growth rate (Fig. 1). After selection, planting density had much less impact on timing and rate of biomass accumulation. There was no change in the time it took to reach 50% ear or tassel biomass in BSSS(R)C17 like there was in BSSS, nor did the biomass accumulation period for the ear shoot
increase with density in BSSS(R)C17 (Fig. 7 & 8, Table 3). There was also no change in the
time it took to reach 50% stalk biomass or 50% plant height in BSCB1(R)C17, like there was in
BSCB1 (Fig. 7 & 8, Table 3).

Ear length and ear biomass maximum growth rates were less responsive to moderate
increases in density in BSSS(R)C17 compared to BSSS. Between 3.23 and 6.46 plants m\(^{-2}\), ear
length maximum growth rates dropped 26% in BSSS, while remaining unchanged in
BSSS(R)C17 (Fig. 9). At the same time, ear biomass maximum growth rates declined 55%
between 3.23 and 6.46 plants m\(^{-2}\) in BSSS, while remaining relatively stable in BSSS(R)C17
(Fig. 9). However, between 6.46 and 12.92 plants m\(^{-2}\), ear length maximum growth rates fell
31% and ear biomass maximum growth rates fell 47% in BSSS(R)C17, resembling the decrease
found in BSSS between 3.23 and 6.46 plants m\(^{-2}\) (Fig. 9, Table 5). Surprisingly, between 6.46
and 12.92 plants m\(^{-2}\), the maximum growth rates in BSSS did not continue to drastically
decrease, and ear length maximum growth rates effectively leveled out (Fig. 9, Table 5).
Between 3.23 and 12.92 plants m\(^{-2}\), both BSSS and BSSS(R)C17 had ear length growth rate
decreases of 30-35% (Table 5). Ear growth rates decreased by 55% in BSSS(R)C17 and by 70%
in BSSS (Table 5). The larger decrease in BSSS was mainly due to the growth rate decrease
found between 6.46 and 12.92 plants m\(^{-2}\), which, while more mild than the extreme drop seem at
moderate densities, was larger than the decrease found in BSSS(R)C17 at moderate densities.
Overall, the data shows that, while BSSS and BSSS(R)C17 exhibit similar maximum growth rate
decreases, the major decreases occur at a much higher density in BSSS(R)C17 than in BSSS.

Edwards (2011) and Brekke et al. (2011a,b) showed that populations in the advanced
cycles of the BSSS/BSCB1 reciprocal recurrent selection program were better adapted to growth
under high population densities. An increase in the anthesis-silking interval (ASI), the time
between pollen shed and silk emergence, has been noted in BSSS with increasing density, but not in BSSS(R)C17 (Brekke et al., 2011). In our study, we found that high plant density delayed ear biomass accumulation and lengthened the growth period for biomass accumulation in BSSS, but not in BSSS(R)C17 (Fig. 7 & 8, Tables 3 & 4). Additionally, Brekke et al. observed that even at the lowest density, there was a roughly 2.5 day ASI in BSSS, while BSSS(R)C17 had an ASI of zero. Edwards (2011) also observed that as selection in the BSSS program progressed, flowering dates became earlier, with BSSS(R)C17 reaching 50% silking 5 days earlier than BSSS. When ear length growth data was examined in this study, it was found that BSSS(R)C17 reached 50% ear length 55-110 growing degree days earlier than BSSS (Table 3). These findings suggest that improvement in ASI in selected populations may be due to earlier partitioning of biomass to the ear and improved proportional biomass partitioning to the ear.

With our examination of growth curves in the two populations before and after reciprocal recurrent selection, there was an apparent convergence of growth curve characteristics in the two populations with respect to proportion of biomass partitioned to the ear and with relative timing of biomass partitioning to stalk, ear, and tassel. Sprague (1946) pointed out that the lines intermated to form BSSS were chosen for stalk quality, but to the best of our knowledge no formal data was published on stalk quality of those lines compared to others. However, our study provides some physiological evidence that BSSS is quite different from BSCB1 with respect to stalk growth and plant density. Increasing plant density delayed biomass partitioning to the ear and tassel rather than the stalk in BSSS, whereas in BSCB1, the biomass partitioning was delayed to stalk, while ear and tassel biomass did not have any delays. We could infer that the lines chosen for BSSS by Sprague (1946) maintained partitioning to stalk at the expense of the ear.
References


Table 1. P-values of fixed effects for traits modeled with logistic growth curves.

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<th>Logistic Parameter</th>
<th>Effect</th>
<th>Ear Length</th>
<th>Plant Height</th>
<th>Ear Biomass</th>
<th>Stalk and Leaf Biomass</th>
<th>Tassel Biomass</th>
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^NS=Not Significant (p>0.05)
Table 2. Final phenotype values for all traits. The 9.69 plants m$^{-2}$ density was not tested with for biomass; blank spaces are used to represent missing values.

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‡SE=Standard Error
Table 3. Development midpoint values for all traits. Values below represent the inflection point in the respective logistic growth models, determining the timepoint post-planting for 50% final phenotype. The 9.69 plants m\(^{-2}\) density was not tested with for biomass; blank spaces are used to represent missing values. All time points are measured post-planting.

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\(^{\dagger}\)GD10=Ten growing degree days Celsius
Table 4. Developmental period lengths for all traits. Values below represent the scale value in the respective logistic growth models, indicative of the time required to move from the growth midpoint to roughly 75% final phenotype. The 9.69 plants m$^{-2}$ density was not tested with for biomass; blank spaces are used to represent missing values.

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\(^{\dagger}\text{GD10=Ten growing degree days Celsius}\)
Table 5. Maximum Growth Rates for All Logistic Phenotypes.

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GD10 = Ten growing degree days Celsius
Table 6. Slope Values and Mean Values for V Stage.
The quadratic coefficient for the growth models along with the standard error is listed, along with the mean number of leaf collars at 1000 GDD, the latest data collection point, and the value standard error. Models were estimated for a planting density of 7.97 plants m$^{-2}$, but results are expected to be similar across densities due to no significant density by population interaction.

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$^\dagger$GDD=Growing Degree Days Celsius
Figure 1. Maximum growth rate for plant organs for all populations versus planting density. Maximum growth rates decreased between 3.23 and 12.92 plants m\(^{-2}\) in all four populations for the traits shown.
Figure 2. Final phenotypes for all populations versus planting density. Final ear length and biomass were measured at harvest, while final stalk and tassel biomass were measured roughly 2-3 weeks after anthesis. Final phenotypes decreased between 3.23 and 12.92 plants m$^{-2}$ in all four populations for the traits shown.
Figure 3. Total per-plant final above-ground biomass versus planting density. Per-plant biomasses were calculated through the addition of final biomass measurements for stalks, tassels, and ear shoots. A) True values for total above ground biomass. B) Values are given as a proportion of the maximum biomass found in each population at the lowest planting density.
Figure 4. Log-transformed final organ and final total biomass per area versus planting log-transformed density. A line of the slope given for corn from Deng et al. (2012) is also plotted.
Figure 5. Final biomass partitioning to the ear, stalk, and tassel for all four populations versus density. Biomass partition to the ear decreased at high densities in BSCB1, while biomass partitioned to the stalk increased. Biomass partitioning was not changed in the other populations.
Figure 6. Proportion of final phenotype compared to maximum for all populations versus planting density.
Figure 7. Developmental midpoint for plant organs for all populations versus planting density. Ear length and ear biomass growth midpoints were later at high density in BSSS, while remaining unchanged in the other populations. The stalk biomass growth midpoint was later in BSCB1 at high density, while remaining unchanged in the other populations. The tassel biomass growth midpoint was later in BSSS and BSCB1(R)C17 at high density, while remaining unchanged in the other populations.
Figure 8. Proportional growth of the tassel, stalk, and ear shoot for all populations at the 3.23 and 12.92 plants m\(^{-2}\) densities versus growing degree days (Celsius) after planting. Ear and tassel biomass growth midpoints were delayed at high density in BSSS, while remaining unchanged in BSSS(R)C17. The stalk biomass growth midpoint was later and the stalk growth period was longer in BSCB1 at high density, while remaining unchanged in BSCB1(R)C17.
Figure 9. Maximum growth rate of ear length (mm 10GDD⁻¹) and biomass (g 10GDD⁻¹) for BSSS and BSSS(R)C17 versus planting density. Maximum growth rates did not decrease between 3.23 and 6.46 plants m⁻² in BSSS(R)C17.
CHAPTER IV:

EFFECT OF RECURRENT SELECTION FOR YIELD ON POPULATION CROSS PERFORMANCE FOR PLANT GROWTH ACROSS PLANTING DENSITIES IN MAIZE

Michael Stein, Fernando Miguez, and Jode Edwards

Paper in preparation for submission to Crop Science Journal. Formatting of the paper, references, tables, and figures is to journal specifications.

Abstract

Seventeen cycles of the BSSS/BSCB1 reciprocal recurrent selection program has produced high-yielding population crosses with greatly increased levels of heterosis for grain yield. While population cross performance in phenotypes such as grain yield and plant height has been examined across selection cycles, almost nothing is known about population cross performance in regards to growth rates and growth timing. The objective of this experiment was to examine the effect of recurrent selection on heterosis levels and population cross performance for biomass accumulation and growth timing in multiple plant organs in response to increasing plant densities. The selected and unselected populations, along with their population crosses, were planted at 3.23, 6.46, and 12.92 plants m$^{-2}$ in three environments near Ames, IA over two years. Heterosis was present in final phenotypes, growth midpoints, growing period length, and maximum growth rates. Heterosis levels for final phenotypes and maximum growth rates increased with selection. These increases in heterosis levels were often due to depressed per se population performance and slight increases in selected population cross phenotypes. The population crosses performed better at high density than the per se populations, being unaffected by high density in growth phenology, while BSSS had delays in tassel biomass and ear length growth. Ear length maximum growth rates were higher at medium and high densities in the
cycle 17 population cross. Densities required to initiate decreases in height and stalk biomass
growth rates were higher in the cycle 17 population cross compared to the cycle 0 cross. BSCB1
was often the dominant parent in population crosses in growth midpoint timing, maximum
growth rates, harvest indices, and density response.

**Introduction**

Beginning in 1949, Iowa Stiff Stalk Synthetic (BSSS) and Iowa Synthetic Corn Borer
No. 1 (BSCB1) have been subjected to a reciprocal recurrent selection program (Edwards, 2011).
The idea of a reciprocal recurrent selection program was presented in 1949 as a method to take
full advantage of both general and specific combining abilities (Comstock *et al*., 1949). The
primary goals of the selection program were increased yield and reduced levels of stalk lodging,
root lodging, and grain moisture (Edwards, 2011). The program has been successful in three of
its goals. Grain yield increasing from roughly 6.6 Mg ha\(^{-1}\) to nearly 9 Mg ha\(^{-1}\) between
BSSS/BSCB1 and BSSS(R)C17/BSCB1(R)C17 (Edwards, 2016). Between BSSS/BSCB1 and
BSSS(R)C11/BSCB1(R)C11, stalk lodging levels fell 5.12% per cycle and root lodging levels
decreased by 2.32% per cycle (Keeratinijakal and Lamkey, 1993a). Grain moisture levels did
not fall with selection between BSSS/BSCB1 and BSSS(R)C11/BSCB1(R)C11 (Keeratinijakal
and Lamkey, 1993a).

In studying yield increases in the BSSS/BSCB1 recurrent selection program, it was found
that *per se* population yields increased at a much lower rate than those of the population crosses.
Keeratinijakal and Lamkey (1993a) estimated an increase in grain yield of almost 7% per cycle
in the BSSS/BSCB1 population cross, while grain yield increases were nearly 2% per cycle in
BSCB1, and remained unchanged in BSSS. Early in the program, it was found that inbreeding
depression, likely caused by genetic drift, was significantly slowing yield gain in the per se populations. By cycle 5, inbreeding levels were already estimated at 22% (Eberhart et al., 1973). This number had risen to 29% by cycle 7, to 37% by cycle 11, and was over 40% by cycle 16 (Martin and Hallauer, 1980; Keeratinijakal and Lamkey, 1993b; Gerke et al., 2015). Between cycle 0 and cycle 16, Gerke et al. (2015) found that heterozygosity in BSSS had fallen from roughly 24% to 14%, while heterozygosity had fallen in BSCB1 from roughly 27% to 16%. The only way to remove inbreeding is to introduce new genetic material through outcrossing, which in commercial corn production is performed by creating hybrids. The superior performance of these hybrids over their mean of its inbred parents is known as heterosis (Penny and Eberhart, 1971). In the beginning of the 20th century, two competing hypotheses were developed to explain the genetic basis of heterosis. Charles Davenport (1908) proposed a system in which lower-performing or harmful alleles, if recessive to higher-performing alleles, would not be visible in a hybrid individual. This is known as the “dominance hypothesis.” The competing hypothesis was presented by George Shull (1908) and greatly expanded upon by Hull (1946), which stated that the crossing of two inbred lines produced an individual whose heterozygous genome actually gave it a superior phenotype compared to either parent, irrespective of dominant or recessive alleles. This has become known as the over-dominance hypothesis.

As cycles advanced in the reciprocal recurrent selection program, heterosis levels increased. Heterosis levels for grain yield in the BSSS/BSCB1 population cross have been estimated at between 14 and 15% (Eberhart et al., 1973; Martin and Hallauer, 1980). Heterosis in the BSSS(R)C5/BSCB1(R)C5 population cross had risen to 35% (Eberhart et al., 1973). By cycle 7, heterosis had risen to 41% (Martin and Hallauer, 1980). At cycle 11, heterosis was estimated at 76% (Keeratinijakal and Lamkey, 1993a). At cycle 14, heterosis was estimated at
66-72% (Rasmussen and Hallauer, 2006; Scheffler et al., 2008). Other agronomic traits have also shown increased heterosis as the cycles of selection progressed. Heterosis in plant heights increased from between 1-4% in the cycle 0 BSSS/BSCB1 cross to 7.19% in cycle 5 and 9.84% in cycle 7 (Eberhart et al., 1973; Martin and Hallauer, 1980). Keeratinijakal and Lamkey (1993a) noted that as plant heights continued to decrease with selection in the *per se* populations to cycle 11, plant heights in the population cross increased, indicating a continual increase in plant height heterosis.

Keeratinijakal and Lamkey (1993b) found very little evidence for an over-dominance explanation of heterosis in grain yield in the BSSS/BSCB1 recurrent selection program. Keeratinijakal and Lamkey (1993b) did find that dominance genetic distances between the BSSS and BSCB1 populations nearly quadrupled from 1.98 to 6.97 between cycle 0 and cycle 11. While the Keeratinijakal and Lamkey (1993b) study ended at cycle 11, Gerke et al. (2015) found that genetically, the populations continued to diverge genetically in cycles 12 and 16. From their findings, Keeratinijakal and Lamkey (1993b) hypothesized that the rapid rise in heterosis for the BSSS/BSCB1 recurrent selection program was due to large amounts of fixation for complimentary alleles between the populations, indicating that heterosis for grain yield followed the dominance hypothesis.

The objectives of this experiment were to verify any existence of heterosis and differential hybrid performance in BSSS/BSCB1 population crosses in regards to plant growth, and to characterize how heterosis for developmental phenotypes has changed with selection for grain yield. While heterosis for grain yield and other agronomic factors has been measured at multiple cycles throughout the recurrent selection program, data for heterosis in maximum growth rates and other growth characteristics is lacking.
Materials and Methods

Populations

Four distinct populations, representing two distinct cycles of a reciprocal recurrent selection program, along with two population crosses were present in this experiment. Iowa Stiff Stalk Synthetic (BSSS) is a synthetic population developed in 1934 from the intermating of 16 inbred lines selected for high stalk quality (Sprague, 1946). Iowa Synthetic Corn Borer No. 1 (BSCB1) is a synthetic population developed in the 1940’s from the intermating of 12 inbred lines selected for resistance to the European corn borer (Penny and Eberhart, 1971). BSSS(R)C17 and BSCB1(R)C17 are the seventeenth cycles of a reciprocal recurrent selection program with the opposing population used as the tester. The populations were developed following 10 cycles of half-sib selection and 7 cycles of full-sib selection. Selections was based on a heritability index of increased yield, lower rates of stalk and root lodging, and reduced grain moisture levels (Edwards 2011). The two population crosses consisted of BSSS/BSCB1 and BSSS(R)C17/BSCB1(R)C17. Full details of the selection program are given in Keeratinijakal and Lamkey (1993a). Selection densities in the BSSS/BSCB1 reciprocal recurrent selection program have closely matched those of commercial agriculture, increasing from 30,000 plants ha\(^{-1}\) in cycle 0 to 75,000 plants ha\(^{-1}\) in cycle 17 (Brekke et al., 2011a).

Experimental Design

Each of the populations and crosses were grown at three planting densities, 3.23, 6.46, and 12.92 plants m\(^{-2}\). A precision SRES air planter was used to ensure proper densities and seed placements. The experiment was a split-plot design, with planting density as the whole plot
factor and population as the sub-plot factor. The experiment was planted near Ames, IA at two locations in 2014 and one location in 2015. Soils in the fields consisted largely of Clarion loam, Canisto clay loam, and Webster clay loam. All population by density combinations were replicated six times at each location. Subplots consisted of four rows 5.49 m long spaced 0.76 m apart. Temperatures were mild during the 2014 and 2015 seasons, and rainfall was abundant throughout the season for both years. Plots were grown without irrigation, using standard corn production practices for central Iowa.

Data Collection

All data was collected on plants in the center rows of each plot, and plants at the ends of plots were not tested due to decreased competition. The fifth and eleventh leaves were painted to determine leaf number. Leaves were painted when they were sufficiently elongated and prior to senescence of the first leaf when painting leaf 5 and prior to senescence of leaf 5 when painting leaf 11. Plant height measurements began when the plants had seven to eight leaf collars visible. Plant heights were measured to the nearest centimeter from the ground to the highest leaf collar. Height measurements were taken weekly on the same plants throughout the growing season, ending at anthesis. Beginning when eight or nine leaf collars were visible, whole plants were harvested from one of two designated replicates in each field twice per week, continuing several weeks after flowering when kernels were at the milk or dough stage (Abendroth et al. 2011). A plant from each density by population combination was harvested during each harvest. Plants were cut at ground level, including above-ground portions of brace roots. The plant was divided into three parts. The ear portion consisted of the primary ear, shank, and husks. The tassel portion consisted of all biomass above the final leaf node. The stalk portion consisted of all
remaining plant matter, including non-silked lower ear shoots, but excluding tillers. Inflorescences were removed from the husks of the primary ear shoot, using a dissecting microscope when necessary. The length of ears from the tip of the ear to the attachment point with the shank was measured using digital calipers. The three biomass components were dried for seven days at 60°C (140°F), and were weighed using an electronic balance. Final ear length and biomass were measured on mature ears at the end of the growing season. Mature ears were only harvested from plants with neighboring plants to maintain density stress throughout the whole growing season. Mature ears were further dried at 60°C for several days and mechanically shelled. Grain yield per ear was measured with an electronic scale.

Data Analysis

Ear shoot length, plant height, and ear, stalk, and tassel biomasses were modeled with a three-parameter logistic curve (Pinheiro and Bates, 2000).

\[
y_{ijkl}^{mt} = \frac{\phi_1_{ijkl}}{1 + e^{\frac{\phi_2_{ijkl} - G_{t(ijkl)} - \phi_3_{ijkl}}{\phi_3_{ijkl}}}} + \epsilon_{ijkl}^{mt}
\]

Observed data is represented as \(y_{ijkl}^{mt}\) for plant \(m\) at environment \(i\) in replicate \(j\) in population \(k\) at density \(l\) at time point \(t\). The logistic parameters were \(\phi_1_{ijkl}\) for the asymptote, \(\phi_2_{ijkl}\) for the inflection point, and \(\phi_3_{ijkl}\) for the scale. The residual error is represented as \(\epsilon_{ijkl}^{mt}\), and \(G_{t(ijkl)}\) is thermal time in growing degree days centigrade after planting. In a logistic curve, the asymptote refers to the final phenotype of the trait, with a larger asymptote implying a larger final phenotype. The inflection point is the value in thermal units for a trait to reach 50% final phenotype, with larger inflection points indicating a later developmental midpoint, and possibly a delay in development. The scale parameter is the time in thermal units necessary for the trait to
move between 50% and ~73% final phenotype, with larger scale values indicating a longer developmental period.

Ear length, plant height, and the biomass logistic model parameters were modeled with a mixed linear model to account for design and treatment effects:

\[ \Phi_{ijklpm} = \mu_p + \lambda_{pi} + \rho_{ijp} + \tau_{pl} + \beta_{pk} + (\tau\beta)_{pikl} + (\tau\beta\lambda)_{ijkl} + (\tau\beta\rho)_{ijpikk} + \delta_{pm(ijk)} \]

Parameter \( \Phi_{ijklpm} \) was parameter \( p \) in the logistic model, \( \mu_p \) was the mean, \( \lambda_{pi} \) was the effect of environment \( i \) (\( i=1..3 \)), \( \rho_{ijp} \) was the effect of replicate block \( j \) (\( j=1..6 \)) nested within environment \( i \), \( \tau_{pl} \) was the effect of density \( l \) (\( l=1..3 \)), \( \beta_{pk} \) was the effect of population \( k \) (\( k=1..6 \)), \( \tau\beta_{pkl} \) was the interaction between density \( l \) and population \( k \), \( \tau\beta\lambda_{pikl} \) was the interaction of density \( l \), population \( k \), and environment \( i \), \( \tau\beta\rho_{lijk} \) was the interaction of density \( l \), population \( k \), and replicate block \( j \) which was the whole plot error term in the split plot design (Error A), and \( \delta_{pm(ijk)} \) was residual error. Environment, density, population, and density by population interactions were treated as fixed effects, while replicates and all replicate and environmental interactions were treated as random effects. The logistic models were fit using the nlme package in R. All random effects were added to the model, and effects with the smallest variance were removed one at a time. The final model was chosen as the model with smallest Bayesian Information Criterion (BIC). Values of the logistic parameters were estimated with maximum likelihood. Pairwise t-tests were performed to compare parameter values across densities and populations. The t-tests were performed in R, with variances estimated via restricted maximum likelihood.

The maximum rate of increase in a logistic curve occurs at the inflection point, the midpoint of the curve on the X-axis. The maximum rate of growth was estimated by taking the first derivative of the logistic curve at the inflection point, which is 0.25 times the asymptote
value divided by the scale value. A lower scale value will lead to a higher maximum growth rate, since a faster growth rate is needed to reach the same phenotype with a shorter developmental period. A smaller maximum growth rate will also produce a lower final phenotype, as the plant will not be able to grow to the full potential in the same developmental period. The maximum growth rate values were the fit to a linear additive model comprised of the significant variables from the logistic curve:

\[ Y_{(ikl)} = \mu + \lambda_i + \tau + \beta_k + \tau \beta_{kl} + \rho_{(ij)} + \tau \lambda_{il} + \beta \lambda_{ik} + \tau \beta \lambda_{ikl} + \tau \beta \rho_{(ijkl)} + \epsilon_{ijkl}, \]

where \( Y_{(ikl)} \) represents phenotypic increase, \( \mu \) is the mean, \( \lambda_i \) is the effect of environment \( i \), \( \tau \) is the effect of density \( j \), \( \beta_k \) is the effect of population \( k \), \( \tau \beta_{kl} \) is the effect of the population by density interaction, \( \rho_{(ij)} \) is the effect of replicate \( j \), \( \tau \lambda_{il} \) is the effect of the density by environment interaction, \( \beta \lambda_{ik} \) is the effect of the population by environment interaction, \( \tau \beta \lambda_{ikl} \) is the effect of the population by density by environment interaction, \( \tau \beta \rho_{(ijkl)} \) is the effect of the replicate by density by environment interaction, \( \epsilon_{ijkl} \) represents error. Environment, population, density, and the population by density interaction were treated as fixed, while replicates and population by density by environment interaction was treated as a random effect. As maximum growth rate is a function of the asymptote and scale logistic parameters, if effects were not significant for those parameters, they were removed from the model. The model for ear length maximum growth rates was \( Y_{(ikl)} = \mu + \lambda_i + \tau + \beta_k + \tau \beta \rho_{(ijkl)} + \epsilon_{ijkl} \). The model for plant height maximum growth rates was \( Y_{(ikl)} = \mu + \lambda_i + \tau + \beta_k + \tau \beta \rho_{(ijkl)} + \epsilon_{ijkl} \). The model for ear biomass maximum growth rates was \( Y_{(ikl)} = \mu + \lambda_i + \tau + \beta_k + \tau \beta \rho_{(ijkl)} + \epsilon_{ijkl} \). The model for stalk and tassel biomass growth rates was \( Y_{(ikl)} = \mu + \lambda_i + \tau + \beta_k + \tau \beta \rho_{(ijkl)} + \epsilon_{ijkl} \). The models were analyzed in SAS using the proc mixed procedure with variances estimated via restricted maximum likelihood. Pairwise t-tests were used to compare growth rates across densities and between populations.
Harvest index was calculated by dividing the final grain mass, measured from mature ears collected at the end of the season, by the total above ground biomass. Total biomass was the sum of the asymptotes of the ear, stalk, and tassel calculated in the logistic models.

The logistic parameters, maximum growth rates, and harvest indices were examined for heterosis. To do this, the average of the values of the *per se* populations was contrasted with the value for the population crosses. Heterosis contrasts for logistic parameters were conducted in R, while the contrasts for maximum growth rate and harvest indices were performed in SAS.

**Results and Discussion**

The final phenotypes of all measured traits were affected by increased plant densities (Table 1). All populations and population crosses had decreased final ear lengths and ear, stalk, and tassel biomasses at increased densities (Table 2). BSSS, BSCB1, the cycle 0 population cross ear lengths decreased 31-37%, while the cycle 17 population and population cross ear lengths decreased 24-29%, with all ear lengths at high density falling within the 120 to 145mm range (Table 2). The cycle 0 populations and population cross had ear biomass that decreased 62-75% between low and high density, while the cycle 17 populations and population cross decreased 42-60%, with all populations and crosses having a final ear biomass in the 55 to 90g range (Table 2). The cycle 0 populations and population cross had final stalk biomass decreases of 42-57% between low and high densities, while the cycle 17 populations and population cross decreased 33-47%, with all final stalk biomass falling within the 80 to 125g range (Table 2). Tassel biomasses for all populations and both crosses decreased 31-51%, with final tassel biomass falling between 2 and 7.5g at high density, with the cycle 0 populations and population cross having larger final tassels than their cycle 17 counterparts (Table 2). Plant heights
increased with density in the cycle 17 populations, with plants being 25-40cm taller at high
density, while no change in final height was noted in the cycle 0 populations or either population
cross (Table 2).

Increased plant density affected plant and organ growth periods and phenology. The
inflection point, the time to 50% final phenotype, was affected by increased planting densities in
ear length, plant height, ear biomass, and tassel biomass (Table 1). The scale value, the time
required to progress from 50% final phenotype to roughly 73% final phenotype, changed in plant
height and ear length and biomass with increasing planting densities (Table 1). The time
required to reach 50% ear length and tassel biomass increased in BSSS by roughly 50 growing
dergee days (GDD), while remaining unchanged in the other per se populations, and both
population crosses (Fig. 1&2, Table 3). Ear length growth period increased in BSSS, while
remaining unchanged in the other per se populations and population crosses (Fig. 1, Table 4).
This could mean that the later ear length midpoint in BSSS is not solely due to a delay in
growing point initiation, but also due to the fact that a longer growth period with the same
starting point will produce a later midpoint. The time required to reach 50% final plant height
increased in the high density plants in the cycle 17 populations by roughly 30 GDD, and in the
two population crosses by roughly 20 GDD, with no delay being present in the cycle 0 per se
populations (Fig. 1, Table 3). The time required to reach 50% final ear biomass decreased by
nearly 90 GDD in the cycle 0 population cross at high density, while remaining unchanged in the
per se populations and the cycle 17 population cross (Fig. 2, Table 3). Plant height growth
periods increased in the cycle 17 populations and in both population crosses, but not in the cycle
0 per se populations (Fig. 1, Table 4).
Heterosis in final phenotypes was only found in the cycle 17 population cross, and almost always occurred at the two lower densities (Table 7). The cycle 17 population cross had longer ears, taller plant heights, and more massive ears and stalks than the per se populations at 3.23 plants m$^{-2}$ (Table 2). Ear and stalk biomass was also higher and ear lengths were marginally higher (p=0.061) at 6.46 plants m$^{-2}$, while plant heights were also marginally taller (p=0.063) at 12.92 plants m$^{-2}$ in the cycle 17 population cross (Table 2). Final tassel biomass did not exhibit heterosis (Table 7).

Final phenotypes for ear length, plant height, and ear and stalk biomass were not different between the cycle 0 and 17 population crosses (Table 2). However, heterosis for final phenotypes was present in the cycle 17 population cross, but not the cycle 0 cross (Table 7). When the final phenotypes of the per se populations are examined, the cycle 17 phenotypes are significantly smaller than those of cycle 0, especially at low and moderate densities (Table 2). From this, we can see that the increase in heterosis between cycles 0 and 17 is entirely due to interbreeding between the populations eliminating the inbreeding in the advanced populations described by Keeratinijakal and Lamkey (1993b) and Gerke et al. (2015), and not increases in final phenotype with selection. The lack of improvement in ear size with selection agrees with the findings of Duvick and Cassman (1999), who found that per-plant grain yields have not increased with selection over time.

Heterosis in growth midpoints and growth period length was found in both the cycle 0 and cycle 17 population crosses, but most cases of heterosis were found at the lower plant densities (Table 7). Positive heterosis was present in the cycle 0 population cross for plant height at the lowest density and tassel biomass growth at the lowest and highest density (Table 7). At 3.23 plants m$^{-2}$, the population cross has a shorter plant height growth period than either
of the *per se* populations (Table 4). Negative heterosis was present in the cycle 0 cross for ear biomass at the lowest density (Table 7). The population cross has a marginally longer (p=0.0851) ear biomass growth period than the *per se* population mean, but not BSSS, at 3.23 plants m\(^{-2}\), while the growth period was significantly longer that both *per se* populations at 6.46 plants m\(^{-2}\) (Table 4). Negative heterosis was present in the cycle 17 population cross in marginal levels for stalk (p=0.0622) and ear biomass (p=0.0943) growth at the lowest density, and positive heterosis was present at significant levels for plant height growth at the lowest and highest densities (Table 7). The stalk biomass growth period displayed negative heterosis in the cycle 17 population cross at the lowest density, with a much longer growth period than the *per se* populations (Table 4). The delay found in stalk biomass accumulation is likely attributed to the increased length of the biomass accumulation time period leading to a later midpoint, rather than a general delay in biomass accumulation (Table 4).

The population crosses were more consistent in organ growth timing at high densities compared to the *per se* populations. While ear length and tassel biomass growth were delayed at high density in BSSS, there was no delay in the population cross (Table 3). There was also an increase in the ear length growth period in BSSS at high density that was not found in the population cross (Table 4). The cycle 0 population cross actually outperformed both *per se* populations in ear biomass accumulation, with the population cross reaching 50% biomass earlier and having a shorter growth period at high density than at low density (Tables c & d). This is largely due to the fact that maximum growth rates did not significantly decline in the population cross, so the decreased final phenotype found at high densities was achieved earlier and more rapidly (Table 5). An increased anthesis-silking interval (ASI) is a noted issue in BSSS, especially at high planting densities, where the time between pollen shed and silking can
be as long as 5 days (Brekke et al., 2011b). A lack of ear growth delay in the population cross may increase yield by ensuring more complete pollination of ears at high plant density.

Maximum growth rates were highly affected by density. All populations and both population crosses had a decreased ear length, stalk biomass, and tassel biomass maximum growth rate at high density compared to low density (Table 5). Plant height maximum growth rates were also lower at high densities in BSSS, BSCB1(R)C17, and the two population crosses, while growth rates were higher at high density in BSCB1 (Table 5). Ear biomass maximum growth rates were not statistically different between low and high density in any of the populations or either population cross (Table 5). This lack of differences in ear growth rates is not necessarily due to similar growth rates across densities in all populations, but rather due to large standard errors, which made all comparisons statistically insignificant.

Heterosis in maximum growth rates was more common than in final phenotypes, growth midpoints, and growing period length values (Table 8). Both the cycle 0 and cycle 17 population crosses had higher ear length maximum growth rates than the per se population average at all three densities (Table 5). Plant height maximum growth rates were higher than the per se population average at all densities in the cycle 17 cross, and at 3.23 and 12.92 plants m$^{-2}$ in the cycle 0 population cross (Table 5). Heterosis in ear biomass maximum growth rates was only found in the cycle 0 cross at 6.46 and 12.92 plants m$^{-2}$ (Table 8). The maximum growth rate in the population cross was below the per se average at 6.46 plants m$^{-2}$, and marginally higher (p=0.0707) than the average at 12.92 plants m$^{-2}$ (Table 5). Stalk biomass maximum growth rates were higher than the per se population average in the cycle 0 cross at 3.23 plants m$^{-2}$, and higher than the cycle 17 average at 6.46 plants m$^{-2}$, but lower than the cycle 17 average for 3.23 plants m$^{-2}$ (Table 5). Tassel biomass maximum growth rates were lower in the cycle 0 population cross
than *per se* population average at 12.92 plants m$^2$, and higher than the cycle 17 *per se* population average at 3.23 plants m$^2$ (Table 5).

Maximum growth rate heterosis levels increased with selection in ear length and ear biomass at 6.46 plants m$^2$, tassel biomass at 12.92 plants m$^2$, and plant height at all densities (Table 8). For these traits, either one or both cycle 17 growth rates in the *per se* populations was significantly lower than the cycle 0 counterpart; however, the cycle 17 population cross also had a higher growth rate than the cycle 0 cross (Table 5). This separates maximum growth rate heterosis from the increases seen in final phenotype heterosis, which was entirely due to decreased *per se* population performance. Stalk biomass at 6.46 plants m$^2$ is the only case where the increase in heterosis in cycle 17 can be completely attributed to an increase in maximum growth rate for the population cross (Table 5).

The cycle 17 population cross had a larger ear length growth rate at 6.46 and 12.92 plants m$^2$ than the cycle 0 population cross (Fig. 3, Table 5). The cycle 17 population cross also maintained a higher maximum growth rate at high density, decreasing 7% between 3.23 and 6.46 plants m$^2$, and 20.4% between 3.23 and 12.92 plants m$^2$, compared to 21.4% and 37.6% in the cycle 0 population cross (Table 5). The cycle 17 population cross had a higher plant height growth rate at 6.46 and 12.92 plants m$^2$, and a higher stalk biomass growth rate at 6.46 plants m$^2$, compared to the cycle 0 population cross (Fig. 3, Table 5). As planting densities increased from 3.23 to 6.46 plants m$^2$, maximum growth rates in the cycle 0 population cross declined by 4.6% for plant height and 57.7% for stalk biomass (Fig. 3, Table 5). In the cycle 17 population cross, there was no decrease in plant height growth rates, and stalk biomass growth rates increased by 13.8% between the same densities (Fig. 3, Table 5). Between 3.23 and 12.92 plants m$^2$, Plant height growth rates declined 11.5% in the cycle 0 population cross and 8.9% in
the cycle 17 population cross, while stalk biomass growth rates fell 51.8% in the cycle 0 population cross, and 54% in the cycle 17 population cross (Fig. 3, Table 5). While the cycle 17 population cross had similar overall decreases in plant height and stalk biomass maximum growth rates to the cycle 0 population cross, the density required to initiate growth rate decreases was higher in the cycle 17 cross.

Harvest indices were unaffected by density in BSSS, BSB1(R)C17, and the cycle 17 population cross (Fig. 4, Table 6). There was a marginal decrease in harvest index with increasing density in BSCB1 (p=0.0829) and in BSSS(R)C17 (p=0.0817), and a significant decrease in harvest index in the BSSS/BSCB1 population cross (Fig. 4, Table 6).

Harvest indices only displayed heterosis in the cycle 0 population cross (Table 8). The cycle 0 population cross had a marginally higher harvest index than the per se average at 3.23 plants m\(^{-2}\) (p=0.0648) and at 6.46 plants m\(^{-2}\) (p=0.0559) (Table 6).

Heterosis dominance values in the population cross increased with selection. In growth midpoints, dominance and additive values were similar in the cycle 0 population cross, with dominance values never exceeding twice those of additive effects (Table 9). In the cycle 17 population cross, additive values for growth midpoints were often very small as the BSSS(R)C17 and BSCB1(R)C17 phenotypes converged temporally, allowing dominance values in the selected population cross to reach as high as 77 times the additive values for the per se populations (Table 9). Growth period dominance values also remained under twice those of additive values in the cycle 0 population cross, while dominance values were 6.5 times additive values in the sole case of heterosis in growth period heterosis in cycle 17 (Table 9). In final phenotypes for the cycle 17 population cross, dominance values were often between 3 and 20 times higher than the additive values between the per se populations, but that number could climb as high as 97 times the
additive value in plant heights, and 122 times the additive value in stalk biomasses (Data not shown). The ratio of dominance to additive values for growth rates was not nearly as extreme, but was still larger in the cycle 17 population cross in both ear length and plant height when compared to the cycle 0 cross (Table 10).

The population cross, especially in cycle 0, appeared to more closely resemble BSCB1 over BSSS in many aspects. In the four heterosis events in growth midpoints of for the cycle 0 population cross, BSCB1 was the dominant parent in three, while BSSS was the dominant parent in one (Table 9). The population cross growth midpoints in ear length, plant height, and stalk and tassel biomasses were 20-70 growing degree days earlier than those of BSSS, but matched closely to the growth midpoints of BSCB1 (Table 3). In maximum growth rates, there are 12 occasions in which BSCB1 or BSCB1(R)C17 is the dominant parent, as opposed to six occasions where BSSS or BSSS(R)C17 is the dominant parent (Table 10). For the two occurrences of heterosis in harvest index found in the cycle 0 population cross, the population cross had the same harvest index as BSCB1, while being higher than that of BSSS (Table 6).

In density responses, BSCB1 was also the dominant parent in the population cross. Growth midpoints were delayed in BSSS for ear length and tassel biomass accumulation at high density, while midpoints were unchanged in BSCB1 and the cycle 0 population cross (Fig. 1&2, Table 3). A growth time period increase also existed in BSSS at high density for ear lengths, but not in BSCB1 or the population cross (Table 4). Harvest indices decreased in both the BSSS/BSCB1 population cross and marginally in BSCB1 (p=0.083), while harvest index was not affected by density in BSSS (Fig. 4, Table 6). In the cycle 17 populations, the harvest index of BSSS(R)C17 was marginally (p=0.082) decreased by density, while harvest indices for BSCB1(R)C17 and the cycle 17 population cross were unaffected (Fig. 4, Table 6). The fact that
the population cross is commonly phenotypically associated with one of the two populations suggests that developmental phenotypes of population crosses may be somewhat heritable from one of the parents. It is known that harvest index, where the population cross was associated closely with BSCB1, is highly heritable in maize (Hay, 1995). If other phenotypes, such as the increased density tolerance found in ear and tassel growth timing in both the population cross and its BSCB1 parent, are also found to be heritable, and a dominance/recessive pattern can be found for density tolerance and growth timing, it could be useful in creating commercial inbreds and hybrids that maintain proper growth and phenology under high population stress.

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Table 1. P-values of fixed effects for traits modeled with logistic growth curves. As the best-fit model did not include density, population, and density by population interactions for tassel biomass, they were listed as not applicable.

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<th>Stalk and Leaf Biomass</th>
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†NS=Not Significant (p>0.05)
†NA=Not Applicable
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\(^{\dagger}\text{SE=Standard Error}\)
Table 3. Development midpoint values for all logistic traits. Values below represent the inflection point in the respective logistic growth models, determining the timepoint of 50% final phenotype. Inflection points were measured as time after planting.

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<sup>1</sup>GD10=Ten growing degree days Celsius
Table 4. Developmental period lengths for all logistic traits. Values below represent the scale value in the respective logistic growth models, indicative of the time required to move from the growth midpoint to roughly 75% final phenotype. As tassel biomass was only included environment in the best-fit model, scale values were unaffected by population or density, and were not included in the table.

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GD10=Ten growing degree days Celsius
Table 5. Maximum Growth Rates for All Logistic Phenotypes.

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<th>Ear Biomass</th>
<th>Stalk Biomass</th>
<th>Tassel Biomass</th>
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<tr>
<td></td>
<td>plants m(^2)</td>
<td>mm GD(^{10})</td>
<td>cm GD(^{10})</td>
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\(^{1}\)GD10=Ten growing degree days Celsius
Table 6. Harvest indices for all populations and population crosses.

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Table 7. Tests for heterosis and heterosis percentages in logistic parameters. Heterosis levels were calculated by comparing the parameter value of the population cross against the mid-parent average of the *per se* populations. Negative heterosis values in inflection point indicate an earlier growth midpoint, while negative heterosis values in scale value indicate a decreased growth period length.

<table>
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<th>Logistic Parameter</th>
<th>Cycle</th>
<th>Density</th>
<th>Ear Length</th>
<th>Plant Height</th>
<th>Ear Biomass</th>
<th>Stalk Biomass</th>
<th>Tassel Biomass</th>
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<tr>
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<td>P-Value</td>
<td>%</td>
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<td>0.0634</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

‡Not Significant
Table 8. Tests for heterosis and heterosis percentages in maximum growth rates and harvest indices

| Cycle | Density | Ear Length | | P-Value | Plant Height | | P-Value | Ear Biomass | | P-Value | Stalk Biomass | | P-Value | Tassel Biomass | | P-Value | Harvest Index | | P-Value |
|-------|---------|------------|---------|-----------|-------------|---------|-------------|-----------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|---------|
| 0     | 3.23    | 6.61       | %       | 0.0001    | 12.60       | %       | 0.0001      | NS‡       | 16.68   | 0.0458      | NS      | 24.37       | 0.0648  |
|       | 6.46    | 0.94       | %       | 0.0236    | NS‡         | -51.94  | 0.0196      | NS        | 16.88   | 0.0458      | NS      | 24.37       | 0.0648  |
|       | 12.92   | 23.40      | %       | 0.0001    | 3.62        | %       | 0.0024      | 106.9     | NS -16.19| 0.0001      | NS      | 24.37       | 0.0648  |
| 17    | 3.23    | 3.00       | %       | 0.0001    | 25.85       | %       | 0.0001      | NS -18.98| 0.0027  | -5.33       | 0.023   | NS          |         |
|       | 6.46    | 5.01       | %       | 0.0001    | 24.25       | %       | 0.0001      | NS 48.42 | 0.0001  | NS          |         | NS          |         |
|       | 12.92   | 21.30      | %       | 0.0001    | 17.70       | %       | 0.0001      | NS        | NS      | NS          |         | NS          |         |

‡Not Significant
Table 9. Additive and dominance values for heterosis in growth timing parameters. Additive values were calculated by subtracting BSSS values from those of BSCB1. A positive d/a value indicates that the population cross phenotype more closely resembled BSCB1, while a negative d/a value indicates that the population cross more closely resembled BSSS. Ear lengths were not included in the table as there was no heterosis present in growth timing parameters. Tassel biomass scale values were not affected by population or densities.

<table>
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<th>Parameter</th>
<th>Cycle</th>
<th>Density Plants m(^{-2})</th>
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<th>d/a</th>
<th>Stalk Biomass a</th>
<th>d</th>
<th>d/a</th>
<th>Tassel Biomass a</th>
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<th>d/a</th>
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*Heterosis was present in the population cross
†The d/a value cannot be calculated due to dividing by 0
Table 10. Additive and dominance values for heterosis in maximum growth rates. Additive effects were calculated by subtracting BSSS growth rates from those of BSCB1. A positive d/a value indicates that the population cross phenotype more closely resembled BSCB1, while a negative d/a value indicates that the population cross more closely resembled BSSS.

<table>
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<th>Cycle</th>
<th>Density m⁻²</th>
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<th>Plant Height</th>
<th>Ear Biomass</th>
<th>Stalk Biomass</th>
<th>Tassel Biomass</th>
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<td></td>
<td></td>
<td>a  d  d/a</td>
<td>a  d  d/a</td>
<td>a  d  d/a</td>
<td>a  d  d/a</td>
<td>a  d  d/a</td>
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<tr>
<td>12.92</td>
<td>-0.71 1.32*</td>
<td>-1.87 -0.20 1.19* -5.95 -1.47 -1.21 0.82</td>
<td>-0.58 -0.80 1.38 0.10 0.04 0.40</td>
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*Heterosis was present in the population cross
Figure 1. Proportional growth of ear length and plant height for all populations at the 3.23 and 12.92 plants m\(^{-2}\) densities versus growing degree days (Celsius) after planting. Ear length growth was delayed at high density in BSSS, while remaining unaffected in the other populations and both population crosses. Plant height growth timing was not affected by density.
Figure 2. Proportional growth of ear, stalk, and tassel biomasses for all populations at the 3.23 and 12.92 plants m\(^{-2}\) densities versus growing degree days (Celsius) after planting. Tassel mass growth was delayed at high densities in BSSS, while remaining affected in the other populations and population crosses. The ear biomass growth midpoint was earlier at high density in BSSS/BSCB1 population cross, while remaining unaffected in the other populations and population cross. Stalk growth timing was unaffected by density.
Figure 3. Maximum growth rates for ear length, plant height, and stalk and tassel biomass for the cycle 0 and cycle 17 population crosses versus planting density. Ear length growth rates were higher in the cycle 17 population cross at 6.46 and 12.92 plants m$^{-2}$. Plant height growth rates were higher in the cycle 17 population cross at all density. There was no decrease in plant height growth rates between 3.23 and 6.46 plants m$^{-2}$. Stalk mass growth rates increased between 3.23 and 6.46 plants m$^{-2}$ in the cycle 17 population cross, while decreasing in the cycle 0 population cross.
Figure 4. Harvest indices for the per se populations and population cross for cycle 0 and cycle 17 versus planting density. Harvest indices declined with density in BSCB1, the BSSS/BSCB1 population cross, and BSSS(R)C17. Harvest indices in the BSSS/BSCB1 population cross were higher than those in BSSS at 3.23 and 6.46 plants m$^{-2}$, but were not different from BSCB1.
CHAPTER V: GENERAL CONCLUSIONS

The primary objective of these studies was to characterize the effects of recurrent selection for grain yield on plant organ growth rates and growth timing with regards to increased planting density. The second objective was to observe the effect of recurrent selection on heterosis levels and population cross performance for biomass accumulation and plant growth timing in response to increasing plant densities. This was accomplished by examining the populations BSSS and BSCB1, along with the seventeenth cycle of the reciprocal recurrent selection program between the two populations, and BSSS/BSCB1 and BSSS(R)C17/BSCB1(R)C17 population crosses at 3.23, 6.46, and 12.92 plants m$^{-2}$.

Phenotyping began at V7-8 and progressed the entire growth season, with plant height, stalk biomass, and tassel biomass being measured until pollen shed, and ear length and biomass being measured until physical maturity.

Final phenotypes for ear length and biomass, stalk biomass, and tassel biomass were decreased as planting density increased in all populations and population crosses. Plant height was unaffected by density in BSSS and the two population cross cycles, while decreasing with density in BSCB1 and increasing with density in BSSS(R)C17 and BSCB1(R)C17. Ear length, plant height, stalk biomass, and tassel biomass maximum growth rates decreased in both studies, while decreases in ear biomass maximum growth rates were found only in the study described in Chapter 3. The selected populations had similar growth rate decreases as the unselected populations, but required a higher population density to exhibit growth rate decreases. As densities increased from 3.23 to 6.46 plants m$^{-2}$, ear length and biomass maximum growth rates decreased by 26% and 55% respectively in BSSS, while remaining stable in BSSS(R)C17.
Between 6.46 and 12.92 plants m$^{-2}$, ear length and biomass maximum growth rates decreased by 31% and 47% respectively in BSSS(R)C17, while remaining stable in BSSS. In the BSSS/BSCB1 cross, plant height growth rates decreased 4.6% and stalk biomass growth rates declined 57.7% between 3.23 and 6.46 plants m$^{-2}$, while there was no decline in growth rates in the cycle 17 population cross. Between 6.46 and 12.92 plants m$^{-2}$, plant height growth rates in the BSSS(R)C17/BSSS(R)C17 population cross declined 8.6%, while stalk biomass growth rates declined 66%.

Delays in growth midpoints were present in ear length, plant height, and ear, stalk, and tassel biomass at high planting density in one or more populations. Increasing density affected plant growth differently between BSSS and BSCB1. High density delayed ear length growth and ear and tassel biomass accumulation and increased ear length growth periods in BSSS, while the traits remained unchanged in BSCB1. Stalk biomass accumulation was delayed in BSCB1 at high density, while remaining stable in BSSS. The delay in tassel biomass growth midpoints was not accompanied by a change in growth period length, meaning that the same amount of time was necessary to reach 50% and ultimately 100% tassel biomass. Because of this, we can hypothesize that the growth midpoint delay of tassels at high density is caused by a delay in organ initiation. Growth midpoint delays in ear length, plant height, and ear and stalk biomass were accompanied by an increase in the length of the growth period. Delays in organ initiation cannot fully explain growth midpoint delays in these organs. Plant height and stalk mass accumulation begins as soon as the seed germinates, and so cannot have delays in organ initiation. An increase in scale value alone is able to explain 38% of the stalk biomass midpoint delay, and as much as 60% of plant height midpoint delay. As scale value only covers the time from 27 to 50% final phenotype, an increase in time necessary to grow from 0% to 27% final
phenotype could explain the remainder of the growth delay. Ear length and biomass have large midpoint delays that are likely mostly due to a delay in organ initiation. Increases in scale values can explain 40% of ear length midpoint delay, and only 11% of the ear biomass midpoint delay.

In order to allot the maximum amount of photoassimilates to the growing ear shoot, stalk biomass accumulation should be completed as soon as possible to limit any overlap in growth periods with the ear shoot. As delays in stalk growth are due largely to a decreased growth rate lengthening the amount of time it takes to reach full height and stalk biomass, two possibly changes in plant physiology may be necessary in continuing yield gains. Either plants must be more efficient at producing and transporting photoassimilates to the stalk and leaves, or plants must be made shorter with less massive stalks in order to reach their final phenotype with decreased growth rates. The second option has been shown to work in the BSCB1 populations. BSCB1(R)C17 has a lower stalk mass at high density compared to BSCB1, and as such, does not require a longer growing period to reach its final phenotype, and there is no stalk biomass midpoint delay in the selected population. Ear length and biomass growth rates did not decline between low and moderate densities in BSSS(R)C17, while there was a growth rate decline in BSSS, and as a result, ears at 6.46 plants m\(^{-2}\) were significantly larger in the cycle 17 population compared to the cycle 0 population. Plants in BSSS(R)C17 not only did not have any ear length or biomass delays or changes in growth period length, but reached the ear length growth midpoint 55-110 GDD earlier than BSSS, regardless of density. In order to maintain larger ears, and therefore higher yields at increased plant densities, corn breeders should focus largely on maintaining assimilate movement into the ears and on breeding for plants with earlier primary ear shoot initiation and stable initiation times across densities.
BSCB1 was most often the dominant parent in the population cross. In 62.5% of growth midpoint heterotic events, 66% of maximum growth rate heterotic events, and 100% of harvest index heterotic events, BSCB1 was the dominant parent. Ear length, plant height, and stalk and tassel biomass growth midpoints were 20 to 70 growing degree days earlier in the population cross than BSSS, but were not different from growth midpoints in BSCB1. BSCB1 was the dominant parent in density responses as well. Ear length and stalk biomass growth was delayed in BSSS, while there were no delays in BSCB1 or the population cross. The proportion of biomass partitioned to the ear decreased with increasing density in BSCB1, and harvest indices decreased with density in BSCB1 and the population cross, but not BSSS. In the selected populations, harvest index decreased with density in BSSS(R)C17, but not BSCB1(R)C17 or the cycle 17 population cross. Population crosses maintained consistent growth timing at high densities in the same fashion as BSCB1. Tassel and ear length growth midpoint delays that accompanied high planting densities in BSSS were not present in the BSSS/BSCB1 population cross. If major loci can be identified as conferring density tolerance, as seen in tassel and ear shoot growth timing in BSCB1 or in harvest index in BSCB1(R)C17, and these loci are indeed found to dominant over loci that cause a plant to be susceptible to high density stress, then it would go a long way in creating corn hybrids that can thrive at higher densities.

Selection for grain yield has increased heterosis levels in final phenotypes and maximum growth rates. Heterosis levels were increased in final phenotypes for ear length, plant height, and ear and stalk biomass. The increased heterosis levels were due entirely to decreased performance of phenotypes in the selected *per se* populations. Heterosis levels in maximum growth rates were found in all traits measured, but where only present at all densities in ear length and plant height. Heterosis levels increased with selection in ear length, plant height, ear
biomass, and stalk biomass. Increases in maximum growth rate heterosis levels were mainly due to a combination of decreased final phenotypes in the selected per se populations and an increase in population cross performance in the selected cross. In heterosis levels in selected populations, dominance values are many magnitudes greater than additive values. In the cycle 0 population cross, dominance values were, at most, five times higher than additive values in final phenotypes and growth rates. In the cycle 17 population cross, dominance values can be 30, 50, or even 70 times greater than additive values.

The studies performed showed that selection for grain yield has affected how plants and plant organs grow. Selected populations are better adapted to growth at high plant densities, maintaining proper phenology, biomass partitioning and harvest indices, and higher growth rates as plant densities increase. Further studies will be needed to examine changes in allele frequencies between the populations that could have contributed to increased density tolerance in the selected populations. If the physiological and genetic basis for improved growth at higher plant densities can be identified, it would provide a major tool in helping breeders develop higher-yielding commercial varieties.
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